Marine and Inland Waters Research Symposium

former Panhellenic Symposium on Oceanography & Fisheries

2022

PROCEEDINGS

AKS Porto Heli Conference Center, Porto Heli, Argolida, Greece

16-19 September 2022

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TABLE OF CONTENTS

ENVIRONMENTAL QUALITY - POLLUTION

MARINE AND FRESHWATER BIODIVERSITY AND ECOSYSTEM FUNCTIONING

MANAGEMENT OF MARINE BIOLOGICAL RESOURCES

FISH, FISHERIES AND FISHERS

SPECIAL SESSION: MEDITERRANEAN SEA (AND FRESH WATER) LITERACY IN THE ERA OF 2030 AGENDA FOR SUSTAINABLE DEVELOPMENT AND DECADE OF OCEAN SCIENCE FOR SUSTAINABLE DEVELOPMENT (2021-2030)

EU MARINE POLICIES IMPLEMENTATIONINFORMATICS IN MARINE SCIENCES

INVASIVE ALIEN SPECIES

SPECIAL SESSION:
SPECIAL SESSION: VULNERABILITY OF FRESHWATER ECOSYSTEMS TO MULTIPLE STRESSORS IMPACTS

ECOLOGY AND MANAGEMENT OF INLAND WATERS

SPECIAL SESSION:
ARGO FLOATS CONTRIBUTION TO THE MARINE RESEARCH AND OPERATIONAL MONITORING OF THE MEDITERRANEAN SEA – EVOLUTION, ACHIEVEMENTS, AND FUTURE NEEDS

GENETIC APPROACHES IN AQUATIC SCIENCES

MARINE GEOLOGY-GEOPHYSICS I: GEOMORPHOLOGY - TECTONICS – SEDIMENTATION – MARINE AGGREGATES

MARINE GEOLOGY-GEOPHYSICS II: SEAFLOOR / HABITAT MAPPING - COASTAL DYNAMICS - OPTICS

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REVISITING THE HYDRODYNAMIC CIRCULATION REGIME OF THERMAIKOS GULF, GREECE

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Abstract

The hydrodynamic regime of Thermaikos Gulf (NW Aegean Sea) is investigated through a high resolution 4-year modelling study (2017-2020 period). Validated, state-of-the-art modelling tools are employed to simulate the atmospheric conditions over the study area, the major river discharges, and the resulting circulation of the coastal ocean. Field observations are used to assess the performance of the hydrodynamic model and supplement simulation findings. Results provide a detailed description of the interannual variability of water column stability structure, while circulation patterns and ocean processes such as coastal upwelling and dense water formation are quantified with a high-resolution tool.

Keywords: enclosed coastal ecosystem, numerical simulations, Delft3D, Aegean Sea.

1. Introduction

The availability of high quality and high-resolution oceanographic information is key to the apprehension of the functioning of marine ecosystems. This is particularly true for semi-enclosed coastal systems sustaining human activities and being heavily affected by them, such as the Thermaikos Gulf (TG, NW Aegean Sea; Fig. 1), where there is an urgent need to maintain its health and mitigate adverse effects of anthropogenic pressure and climatic shifts. To this end, the work at hand describes an integrated approach in quantifying in a detailed manner the 3-D coastal hydrodynamics in this microtidal environment driven mainly by the variability of atmospheric conditions and river outflows (Androulidakis *et al.*, 2021). It combines aspects of field monitoring of oceanographic variables (Fig. 1; Androulidakis *et al.*, 2021; Petala *et al.*, 2018), river runoff modelling, downscaled atmospheric modelling (Pytharoulis *et al.*, 2015), and fine resolution hydrodynamic modelling of ocean circulation with a Delft3D implementation (Fig. 1; Androulidakis *et al.*, 2021) for the 2017-2020 period. This multi-platform approach produces detailed information on how the TG system works and reacts to various forcing, providing for the first time a multiyear insight on sub-mesoscale and mesoscale processes, on seasonality and evolution of river plume spreading and water column structure, and on water mass exchange and renewal times.

2. Material and Methods

2.1 Atmospheric modelling of meteorological conditions

The meteorological forcing of the coastal hydrodynamic model was derived from the met-ocean weather forecast operational system Wave4Us (Krestenitis *et al.*, 2015; Androulidakis *et al.*, 2022a). Simulations of regional-scale, high-resolution, atmospheric circulation were conducted with the Weather Research and Forecasting model's Advanced Research dynamic solver (WRF-ARW-AUTh). The produced 3-hourly atmospheric datasets (wind velocities, sea level pressure, air temperature, relative humidity, cloudiness, precipitation) cover the finer scale domain with a resolution of 1.67 km (Pytharoulis *et al.*, 2015; https://meteo3.geo.auth.gr/WRF/home.html).

2.2. River basin modelling

The main freshwater input comes from four rivers (Gallikos, Axios, Loudias, and Aliakmon, Fig. 1) along with a complex system of irrigation canals and trench drains located at TG's western coast. The Hydrologic Modeling System (HEC-HMS) was used to simulate the hydrologic processes of the river basins (Makris *et al.*, 2022). This led to detailed simulations of stream networks, robustly estimating the freshwater outflows in the TG, after evaluation by comparisons against *in situ* measured flow rates. The freshwater inflows are used as lateral input for the hydrodynamic simulations in TG (see Section 2.3).

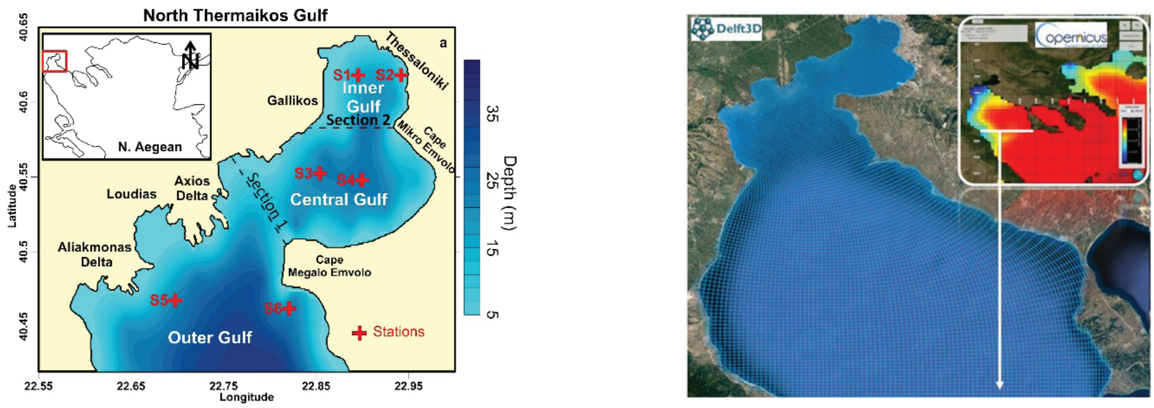

Fig. 1: Study area and monitoring stations S2, S3 and S6 (left), and hydrodynamic model computational domain, with open boundaries highlighted (right). At locations S1, S4 and S5 simulation output is also presented for a thorough insight of subbasin functioning.

2.3 Coastal ocean circulation modelling

The coastal circulation simulations were implemented with the FLOW module of the Delft3D (Delft3D-FLOW) modeling system in a 3-D, 15 sigma-layer configuration and a 110×126 curvilinear grid with a resolution step from 750 m offshore to less than 350 m in the inner gulf (Fig, 1.; Androulidakis *et al.*, 2021). The boundary conditions (open southern boundary) are derived from the Mediterranean Forecasting System model embedded into Copernicus CMEMS Mediterranean Sea Physical Reanalysis dataset (Simoncelli *et al.*, 2019). Androulidakis *et al.* (2021) discuss in detail the model setup (e.g., initial, boundary, and forcing conditions; parameterization and river input) and performance.

2.4 Observations

Field measurements of oceanographic parameters (currents, temperature, salinity) over a network of sampling stations (S2, S3, S6; Fig. 1) during the 4-year period from 2017 to 2020 are used to calibrate and validate the ocean circulation model and map the plume dynamics of river outflows. Data were recorded along the water column with a Conductivity-Temperature-Depth profiler (SBE 19plus V2 SeaCAT). A total of 36 CTD casts at a minimum of 3 field trips per year were conducted at stations S2, S3 and S6 (Fig 1). Locations S1, S4 and S5 correspond to model grid points, where simulation output is also presented for a thorough insight of subbasin functioning. Vertical distributions of horizontal currents were also frequently derived with the use of an Acoustic Doppler Current Profiler (ADCP; Workhorse Sentinel by TELEDYNE MARINE) in a moored mode. River outflow rates were derived from HEC-HMS modelling (Section 2.2.) and from available field observations (Hellenic Agricultural Organization "DEMETER" and TERNA S.A.). Satellite observations were also derived from the Sea Surface Temperature (SST) set by the JPL OUROCEAN product (Group for High Resolution SST project; GHRSST; ftp://ftp.nodc.noaa.gov/pub/data.nodc/ghrsst/L4/GLOB/JPL_OUROCEAN/G1SST).

3. Results

3.1 Model Validation

Much effort has been invested into validating the individual components of the integrated met-ocean, hydro-weather modelling system (Androulidakis *et al.*, 2021; Makris *et al.*, 2021). Herein we present aspects of the Delft3D-Thermaikos evaluation and finetuning covering the period 2017-2020. The spatially averaged (over the entire TG) SST from model simulations against satellite data by GHRSST, with and without the seasonal cycle (a and b respectively) during 2017-2020 are presented. Model prediction skill is quite high for the 4-year hindcsast period. The correlation coefficients for both seasonal and non-seasonal comparisons are high and statistically significant (p-value<0.001). The RMSE is 0.96°C for the seasonal timeseries and approximately 0.5°C for the timeseries without the seasonal cycle. Increasing SST trends were derived for all timeseries. The effect of different river inflow parameterizations on model performance is also evaluated (Fig. 2c and 2d). The two Taylor diagrams summarize the effect of different Axios river discharge parameterization on standard deviation, correlation coefficient and RMS error for temperature and salinity (c and d, respectively).

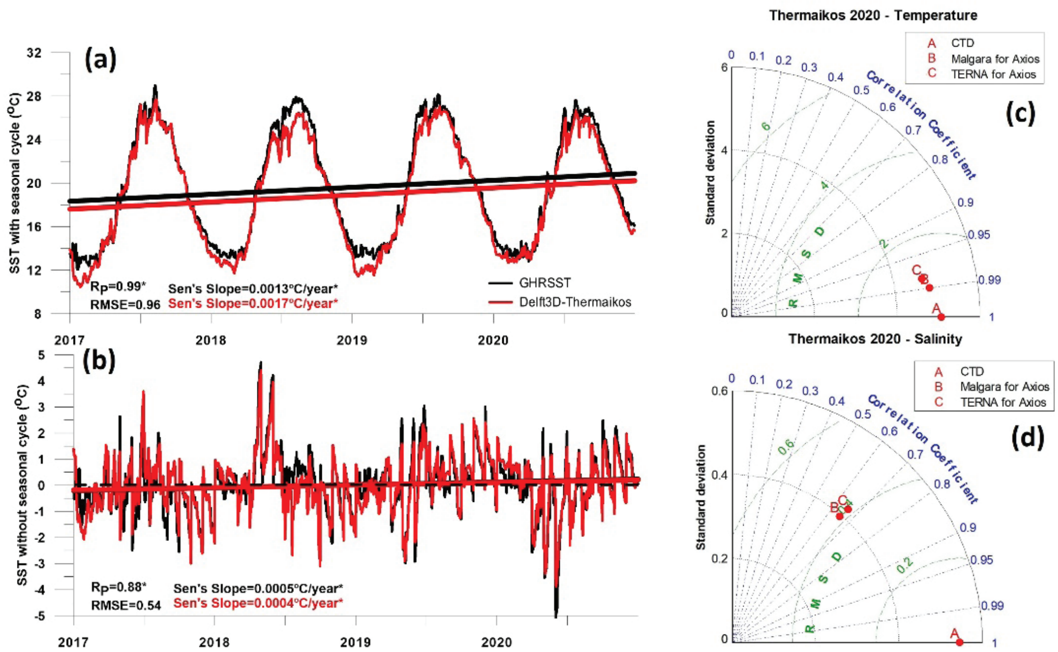

Fig. 2: Daily evolution of SST (a) with seasonal and (b) without seasonal cycle derived from GHRSST (black line) and Delft3D-Thermaikos, averaged over the model domain (2017-2020). Statistic metrics for each case are also shown. (c) Temperature and (d) salinity Taylor diagrams comparing different river inflow parameterizations.

3.2 Ocean circulation processes

The seasonality and interannual variability of water column structure together with the prevailing meteorological conditions are illustrated in detail in Figure 3.

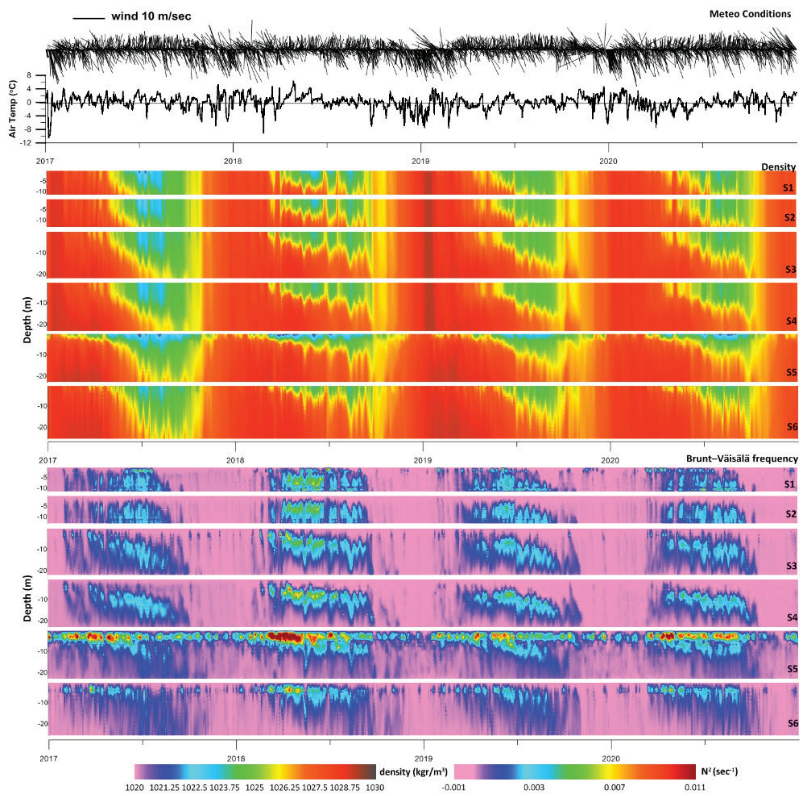

Fig. 3: Daily evolution of meteorological conditions (wind vectors, air temperature anomaly) derived at Station S3 from WRF-Thermaikos simulation, and Hovmöller diagrams of the temporal evolution of vertical density distribution and Brunt-Väisälä stratification frequency at S1, S2, S3, S4, S5, S6 as derived from Delft3d-Thermaikos simulation covering the 2017-2020 period.

The succession between mixing and stratification and its variability in time over different parts and subbasins of TG (locations in Fig. 1), are strongly depended on the atmospheric conditions and freshwater input. Notice the dense water formation incident at the beginning of 2019 that formed under strong northerly winds and low atmospheric temperature (deviating up to -6 or -8 ^oC from the mean monthly value). Water masses with density close to 1030 kg m^{-3} formed in Thessaloniki Bay and Central TG (S1, S2, S3, S4), gradually cascaded southward towards the outer parts of TG (S5, S6), occupying the deeper layers of the water column, during a 2–3-week period. Androulidakis *et al.* (2021), employing by and large the same modelling platform for the year 2017, established the association of eutrophication events (a major concern in TG), mainly with the dominance of southerly winds (not shown here). Northerly winds contribute on the renewal of the Gulf imposing a two-layer flow and cyclonic circulation, especially along the eastern coasts (Androulidakis *et al.* 2022b).

4. Conclusions

The implementation of a modelling system for the investigation of the hydrodynamic regime in Thermaikos Gulf is evaluated and discussed. The modelling platform consists of state-of-the-art and validated components that simulates the downscaled atmospheric conditions over the study area, the river discharges, and the coastal ocean circulation in a spatially high-resolution setup. The hydrodynamic model validation against satellite observations and field observations confirmed the good performance of Delft3d-Thermaikos in simulating both the seasonal cycle and the interannual variability of the physical properties. Results provide a detailed perspective on the evolution and interannual variability of water column stability structure as determined by meteorological forcing conditions and freshwater input, and new insight on the mesoscale oceanic circulation and the sub-mesoscale local hydrodynamic effects on marine eutrophication events.

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STORM SURGES DURING A MEDICANE IN THE IONIAN SEA

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Abstract

Medicane Ianos in September 2020 was one the most severe storms that formed over the Mediterranean Sea with Category 2 Hurricane characteristics. Ianos induced high storm surges along its pathway and led to significant increase of the sea level in the central Ionian Sea, at the Ionian Islands and along the western Greek coasts. We analyzed the characteristics of the storm surges and estimated the coastal inundation levels based on simulations of flooded areas and satellite ocean color images.

Keywords: sea level, Mediterranean Sea, coastal inundation, numerical modeling, remote sensing, Medicane.

1. Introduction

The intense atmospheric cyclones over the Mediterranean Sea with characteristics like tropical cyclones, with windless "eyes", spiral rain-bands, on the order of 300 km in diameter, and surrounding hurricane-force cyclonic winds (up to Category 1 Hurricane on the Saffir-Simpson scale; Emanuel, 2005) are termed as "Medicanes" (Cavicchia et al., 2014). A severe Medicane, named Ianos, with characteristics similar to a Category 2 Hurricane (recorded 1-min average winds of 44.1 m/s), affected central and eastern Mediterranean during 15-20 September 2020 (Lagouvardos *et al.*, 2021; https://marine.copernicus.eu/news/following-cyclone-ianos-across-mediterranean-sea). Ianos Medicane caused severe damages on both inland and coastal areas, especially in central and southwestern Greece, causing extensive flooding, infrastructure destructions and human casualties. The main motivation of this study is to examine the variability of the meteorological effects on the distribution of the induced storm surges and the consequent inundation along coasts of the central Mediterranean during this severe event.

2. Material and Methods

The Sea Level Anomaly (SLA) and circulation characteristics during the Ianos passage over the affected coastal regions are investigated with the use of a 2-D hydrodynamic model for barotropic circulation (High Resolution Storm Surge: HiReSS model) operating in both forecast (Operational System Wave4us; http://wave4us.web.auth.gr/; Krestenitis *et al.*, 2017) and hindcast (Androulidakis *et al.*, 2015) modes. HiReSS simulates the 2-D barotropic mode of the hydrodynamic circulation in large water bodies, enclosed seas, gulfs, and coastal areas over a rather shallow continental shelf, based on the shallow water equations. Atmospheric forcing of the hydrodynamic simulations was derived from operational Advanced Reasearch - Weather Research and Forecasting (WRF-ARW) simulations (Pytharoulis *et al.*, 2015; forecast mode: 15km x 15 km) and from the European Centre for Medium-Range Weather Forecasts (ECMWF) operational analysis products (hindcast mode: 10km x 10km) covering the study period of Ianos (September 2020). The coastal inundation has been estimated with two techniques: 1) the computation of the Normalized Difference Water Index (NDWI; Gao, 1996) derived from Sentinel-2 satellite images before (15/9) and after (20/9) the storm passage, and 2) the Coastal FLOODing (CoastFLOOD) model which is a 2-D horizontal, mass balance, coastal inundation model, based on the concepts of the established LISFLOOD-FP model for coastal (and river) plain flooding. Field measurements of sea elevation were collected by available tide-gauge sensors along the coasts (IOC/UNESCO; https://www.ioc-sealevelmonitoring.org/) to validate the performance of the numerical simulations and estimate the storm surge intensity during Ianos Medicane.

3. Results

3.1. Evaluation of Storm surge simulations

Herein, we extend previous evaluation of the HiReSS model (Krestenitis *et al.*, 2011; Androulidakis *et al.*, 2015; Makris *et al.*, 2021) to test the model's efficiency in simulating the storm surges during the Ianos event. The comparison between the simulated and in situ SLA at 8 coastal stations of the Ionian and Aegean Seas is presented in Table 1. The Pearson correlation coefficients between the hindcast simulated data and observations ranged between 0.62 and 0.86, with smaller Root-Mean-Square-Errors (RMSEs) in comparison to the forecast simulations. Although the usage of ECMWF operational analyses as meteorological forcing of the HiReSS significantly improved the SLA simulations (hindcast mode), the forecasts conducted by the operational system (WRF/ARW-fed HiReSS simulations) provide useful real-time daily predictions that improve in time, as they are forced by daily updated meteorological forecasts. The maximum SLAs were detected for the Ionian Sea coastal areas with the highest at western Peloponnese (e.g., Katakolo).

Table 1. Validation of HiReSS model performance in hindcast and operational forecast modes (left and right columns, respectively) against field observations, during September 2020 at 8 selected stations. SLA and RMSE in m.

	Sites	Hindcast Mode				Forecast Mode			
	STATION	SLA Max	Pearson Correlation	RMSE	Willmott Skill Score	SLA Max	Pearson Correlation	RMSE	Willmott Skill Score
1	Catania	0.13	0.79	0.028	0.80	0.02	0.72	0.047	0.40
2	Gokceada	0.06	0.80	0.026	0.94	0.04	0.70	0.042	0.89
3	Kalamata	0.12	0.63	0.025	0.80	0.10	0.64	0.039	0.65
4	Katakolo	0.29	0.86	0.030	0.90	0.21	0.87	0.028	0.92
5	Kiparisia	0.20	0.62	0.034	0.75	0.16	0.66	0.039	0.76
6	Otranto	0.12	0.73	0.021	0.86	0.04	0.64	0.044	0.50
7	Peiraias	0.12	0.70	0.027	0.83	0.09	0.66	0.041	0.67
8	Thessaloniki	0.08	0.71	0.033	0.91	0.08	0.65	0.049	0.84

The highest SLA (SLA Max_{Obs}=0.27 m; SLA Max_{Model}=0.29 m) were measured in Katakolo (west Peloponnesus; Fig. 1) on 18/09 00:00 UTC (Figure 4a). The core of the Ianos Medicane was over Cephalonia and Zakynthos Islands on 18/9 00:00, with very strong cyclonic winds (>20 m/sec) blowing towards the coast of Peloponnese (Fig. 4b). The onshore wind field led to the accumulation of seawater masses strengthening the storm surges along the western coasts.

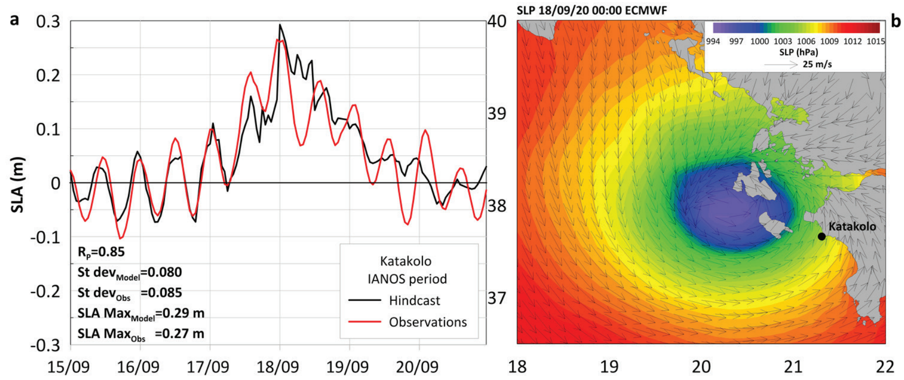

Fig. 1: (a) Variation of SLA derived from the hindcast simulation and the observed data at Katakolo (west Peloponnesus) during the Ianos passage (15/9-20/9) in the Ionian Sea. (b) Map of 2-D horizontal distributions of Sea Level Pressure (SLP) and winds, derived from the ECMWF analysis at the time of maximum SLA (18/9 00:00 UTC).

3.2. Analysis of marine weather conditions

The evolution of the marine cyclone, defined by the 10-cm SLA contour, is shown in Fig. 2. The sea level varied among high values between 17/9 and half of 18/9, when the storm reached the Greek coasts.

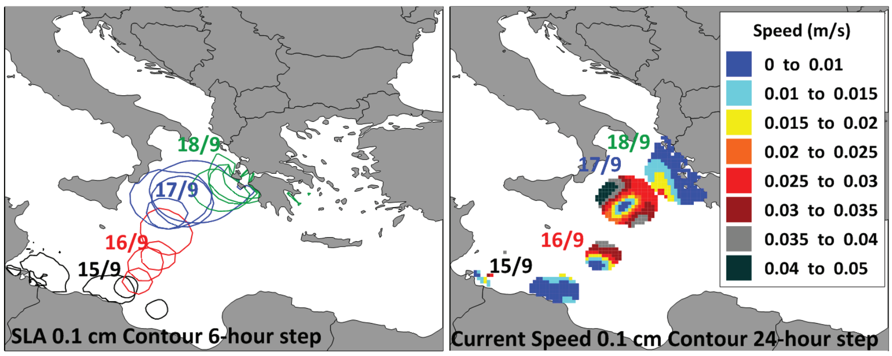

Fig. 2: Evolution of the marine cyclonic eddy (10-cm SLA contour) and distribution of the barotropic current speed from 15/9 to 18/9.

The spatially averaged current velocities inside the eddy revealed the highest values on 17/9. After the storm landfall on 18/9, the extension of the eddy and the respective storm surge signal significantly reduced from 100,000 km^2 to 40,000 km^2, when the SLA values above 10 cm spread along the western coastline of Greece.

3.3. Impacts on the coastal zone

The highest storm surges and the flooding impacts of the Ianos Medicane were mainly detected at the coasts of the central and southern Ionian Sea, especially around Cephalonia Island (0.25-0.30 m; Fig. 3). During the timespan of Ianos event, the inverse barometer effect was very strong over the Ionian Sea and the southwestern Aegean coasts (Pearson correlation coefficient>0.80; Fig. 3b). Although the storm surges over the Ionian Sea are mainly affected by the winds (Krestenitis *et al.*, 2011; Androulidakis *et al.*, 2015), in the case of a Medicane coming from the central Mediterranean, both wind and inverse-barometer contributed on the storm surge formation.

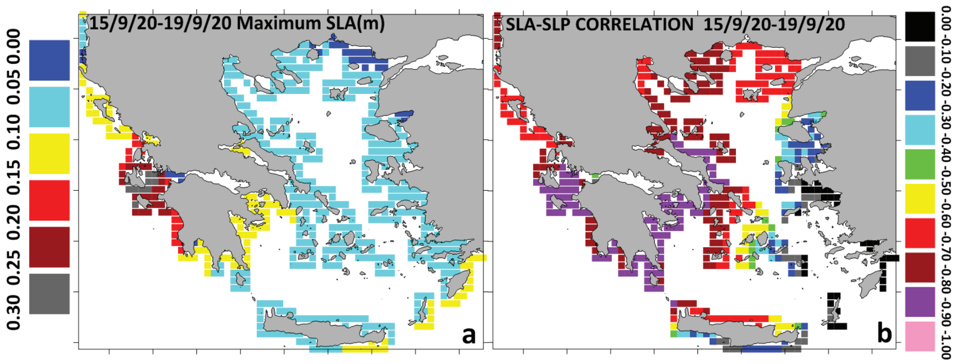

Fig. 3: (a) Maximum SLA and (b) Pearson correlation coefficients between SLA and SLP (ECMWF analysis) at the coastal cells of the Ionian and Aegean Seas during Ianos passage (15/9/20-19/9/20).

Eight coastal regions with extensive areas of land elevation lower than 1 m in the central Ionian Islands have been identified based on very high resolution (dx = 2 m) DEM data of the Hellenic Cadastre service (https://www.ktimatologio.gr/) (Fig. 4a).

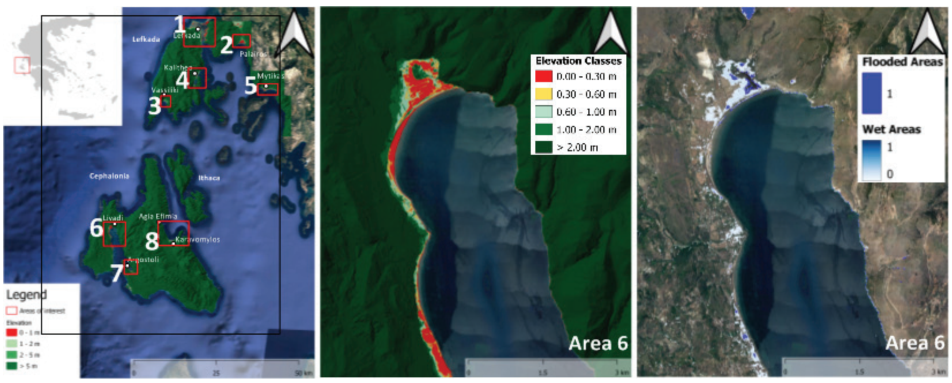

Fig. 4: Land elevation derived from the high-resolution DEM (2m) (a) over the central Ionian Sea and (b) over Area 6. (c) Flooded and Wet Areas derived from the NDWI difference between 15/9 and 20/9 for Area 6.

The largest extent of the coastal zone with land elevation lower than 0.30 m is identified at Area 6 in the Gulf of Argostoli, Cephalonia (Fig. 4b), where the potentially inundated area by a 30 cm sea level rise, is around 0.6 km^2. The mean NDWI difference in Area 6 is highest among the 8 selected coastal regions and is associated to the large number of “wet” cells presented in Fig. 4c (light blue areas). The increased levels of NDWI difference after the storm is a strong indication that the Ianos -induced surge might have boosted the inland moisture levels. A small portion of this area remained flooded (“flooded” cells) on 20/9 (blue areas in Fig. 4c). The area, still covered with water on 20/9, consisted of the 2.7% of the entire lowland (0-0.3 m) coastal zone of Area 6. The SLP dropped below 1000 hPa over the area (Fig. 5a), accompanied by strong surge-favorable southeasterly winds (Fig. 5c). Strong onshore currents accumulated waters inside the gulf of Area 6 in the evening of 17/9 (Fig. 5b), rising the SLA above 20 cm (Fig. 5d). In order to isolate the storm surge contribution on the observed flooding, which may also have other sources (e.g. precipitation, drainage basin runoff), we conducted numerical simulations with CoastFLOOD that used boundary conditions of the realistically simulated SLA (Fig. 5b) at the shoreline. Although, the simulated flooding is weaker than the observed, extensive flooding up to 200 m inland has been confirmed by both techniques (Fig. 5e).

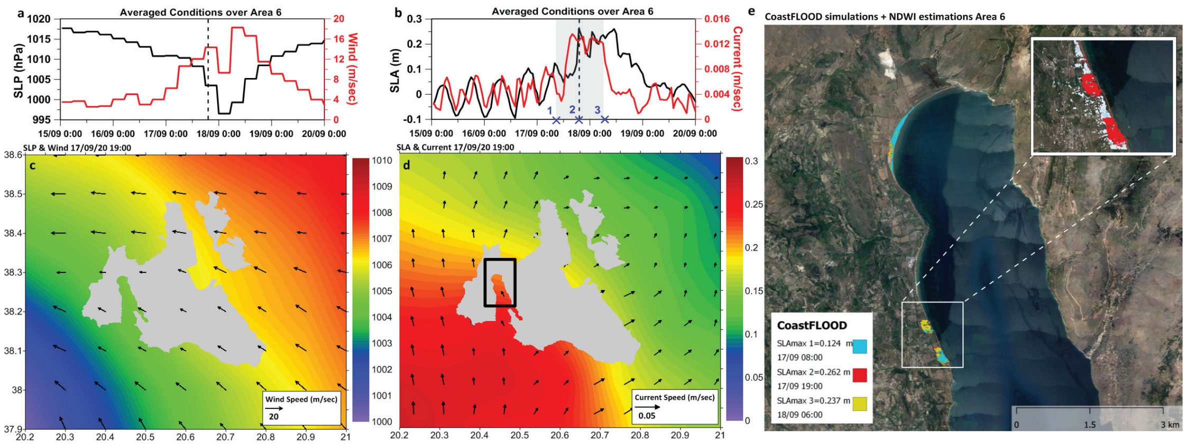

Fig. 5: Evolution of (a) SLP-Winds (ECMWF) and (b) SLA-Currents during 15/9-19/9 period averaged over Area 6. Maps of (c) SLP-Winds and (d) SLA-Currents over Cephalonia Island on 17/9 19:00 UTC. (e) Map of Flooded Areas derived from CoastFLOOD simulations during three characteristic dates (Fig. 4b). Insert in 4e: Estimations of simulated (red) and satellite ("Wet" areas with light blue) over the southwestern coastal region.

4. Conclusions

Ianos Medicane induced significant storm surges along its pathway and increased the sea level over the central Ionian basin, in the Ionian Islands' littorals and along the western Greek coasts. The prolonged stall of the Medicane over the central Ionian on 18/09 induced cyclonic onshore winds and respective currents along the entire coastline increasing the storm surge levels. Several coastal areas of the central Ionian Sea with land elevation lower than 30 cm were flooded during the storm surge. Ocean color images before and after the storm surge event (NDWI technique) in combination with coastal flooding modeling (CoastFLOOD model) provided a reliable estimation about the extension of the inundation.

5. Acknowledgements

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TOWARDS MORE REALISTIC EKMAN SPIRALS: TWO-LAYER PIECEWISE CONSTANT DIFFUSIVITY CASE

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Abstract

The Ekman spiral is one of the oldest and most famous results of dynamical physical oceanography, representing the response of the upper ocean to a steady wind forcing. In the classic solution, the ocean is considered spatially homogeneous and infinite and thus a constant eddy diffusivity is applied, which produces the familiar Ekman spiral characterized by the exponential decay of the velocity amplitude with depth. While a vast number of oceanographic observations certify the presence of Ekman spirals of the upper ocean, in most cases the shape of the spirals deviates from the classical solution, and this can partly be attributed to stratified upper water column. In this work a first approach to a solution compatible with stratified conditions is described. The stratification is introduced by describing the water column as a two-layer fluid: the upper layer represents a fully-mixed surface layer, while the lower layer exhibits a density increasing linearly with depth. The above conditions correspond to two layers with piece-wise constant eddy diffusivities, with the mixed layer diffusivity being orders of magnitude higher than the stratified layer. The Ekman solution is a deformed spiral, exhibiting low shear in the upper layer and high shear in the lower.

Keywords: Ekman spiral, upper-ocean, stratification.

1. Introduction

The response of ocean to the wind stress has been one of the earliest problems addressed by the physical oceanographers. It was during the 1893-1896 expedition of the his vessel "Fram" in the Arctic Ocean that marine explorer Fridtjof Nansen noticed that icebergs move at an angle of about 40° to the right of the wind. Still a graduate student at the University of Uppsala, Sweden, Vagn Walfrid Ekman was very impressed by Nansen's report, and several years later published his explanation, describing the response of an infinite and homogeneous ocean to steady wind forcing (Ekman, 1905). The solution, known since as Ekman currents, and the corresponding vertical structure as Ekman spiral, has become one of the pillars of modern dynamical oceanography, and the basis for understanding phenomena such as Ekman transport and pumping, coastal upwelling, and the impact of wind-forced currents on the deep ocean circulation. The presence of Ekman spirals has been certified on almost all suitably designed experiments, however in many of them deviations from the Ekman theory (regarding either the vertical structure or the angle between the surface currents and the wind) were identified (Stacey *et al*., 1986; Rio & Hernandez, 2003; Lenn & Chereskin, 2009). One of the most suspect failures of the hypotheses that the Ekman solution was based lies in the vertical homogeneity of the ocean. This hypothesis, valid within the surface mixed layer, may be acceptable in the winter for many parts of the ocean, but during stratified conditions, when the mixed-layer could be much thinner, it clearly fails. Eddy diffusivities in the stratified ocean tend to be inversely proportional to the stratification (Brunt-Väisälä) frequency (Gargett & Holloway, 1984), thus we expect this parameter to exhibit high values (of the order of 0.1 m^2s^{-1}) in the mixed layer and one or several orders of magnitude smaller in the stratified pycnocline below. Through this realization, the oceanographic community has addressed the role of the impact of vertically varying eddy diffusivities on the oceanic response to the wind (Madsen, 1977; Dritschel *et al*., 2020; Roberti, 2021; Guan *et al*., 2022), presenting solutions of varying complexities.

In this work we present the simplest (but expandable) approach, that of a surface mixed layer and a stratified pycnocline with a linearly increasing with depth density.

2. Material and Methods

2.1 The mathematical problem

Let there be a horizontally infinite ocean, comprised of two water layers, the upper being a fully mixed surface layer of thickness h_1 and the lower being a stratified pycnocline with the density linearly increasing with depth. Thus, for the upper layer, $0 \leq z < h_1$, the buoyancy (Brunt-Väisälä) frequency *N* is zero, while in the lower layer *N* is constant (Fig.). Thus, based on recorded relation of eddy diffusivity to stratification (Gargett, 1984), we can model the two-layer approach as a surface layer characterized of constant eddy diffusivity k_1 and a lower layer with a constant diffusivity k_2.

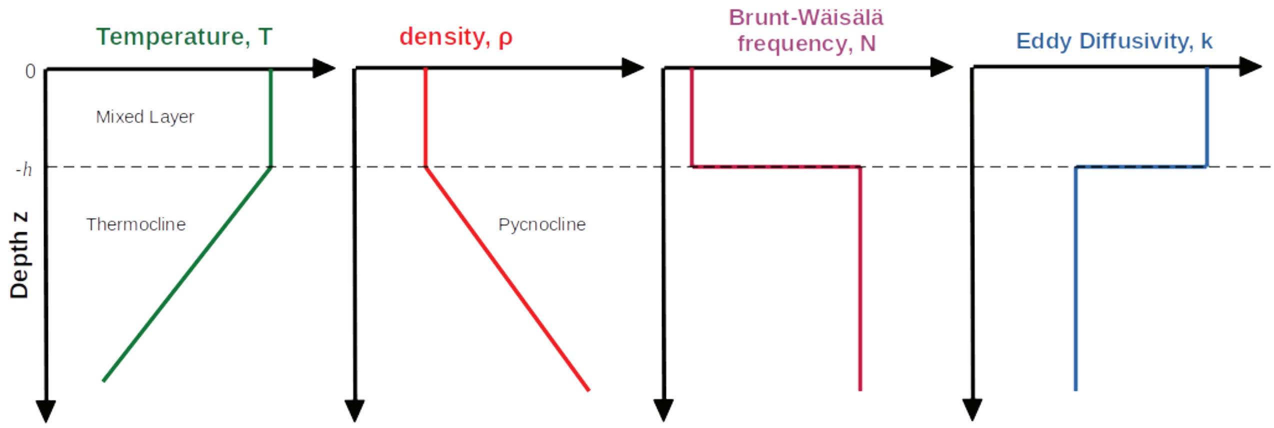

Fig. 1: Idealized vertical structure of the upper water column. From left to right: temperature, density, Brunt-Wäisälä frequency and eddy diffusivity. Figure modified from Tsakalaki, 2021.

Ekman dynamics can be described as a balance between the Coriolis acceleration and friction due to vertical shear. Thus, the governing Navier-Stokes equations in the two horizontal axes are:

$$xaxis: -fv = \frac{\partial}{\partial z}\left(k_i \frac{\partial u}{\partial z}\right)$$

$$yaxis: +fu = \frac{\partial}{\partial z}\left(k_i \frac{\partial v}{\partial z}\right),$$

where k_1 the eddy diffusivity of the i^{th} layer, *i*=1 denoting the surface mixed layer and *i*=2 the pycnocline, *u* and *v* the zonal and meridional velocity components and *f* the Coriolis parameter. Following the approach by Ekman, we choose the complex notation for the velocity: *U* = *u*+*iv* . Note that parameters in bold fonts denote vector quantities. The boundary conditions hereby selected are:

- At the surface, *z* = 0 the friction equals to the wind stress: $K\frac{\partial U}{\partial z}\Big|_{z=0} = \frac{\tau}{\rho}$
- At the interface $z = -h_1$, the solutions at the upper and the lower layer are identical: $U_1(-h_1) = u_2(-h_1)$, and
- As the depth increases, the Ekman velocity approaches zero: $U(-\infty) = 0$

2.2 The solution

The solution in the upper layer, $0 > z \geqslant -h_1$ is : $U_1(z) = U_0 e^{\frac{z}{\varepsilon_1}(1+i)}$,

where $\varepsilon_1 = \sqrt{\frac{2k_1}{f}}$ and $U_0 = \frac{\tau}{\rho\sqrt{2fK}}(1-i)$ and

The solution in the lower layer, $-h_1 > z$, is: $U_2(z) = U_0 e^{\frac{-h_1}{\varepsilon_1}(1+i)} e^{\frac{(z+h_1)}{\varepsilon_2}(1+i)} = U_1(-h_1) e^{\frac{(z+h_1)}{\varepsilon_2}(1+i)}$

where $\varepsilon_2 = \sqrt{\frac{2k_2}{f}}$, since $U_1(-h_1) = U_0 e^{\frac{-h_1}{\varepsilon_1}(1+i)}$

3. Results

The above solution corresponds to two Ekman spirals, positioned one on top of the other, with the layer with the highest eddy diffusivity (usually the surface mixed-layer) exhibiting the least velocity shear, while the pycnocline layer with the lower eddy diffusivity exhibiting high shear and very fast vector rotation with depth (Fig. 2).

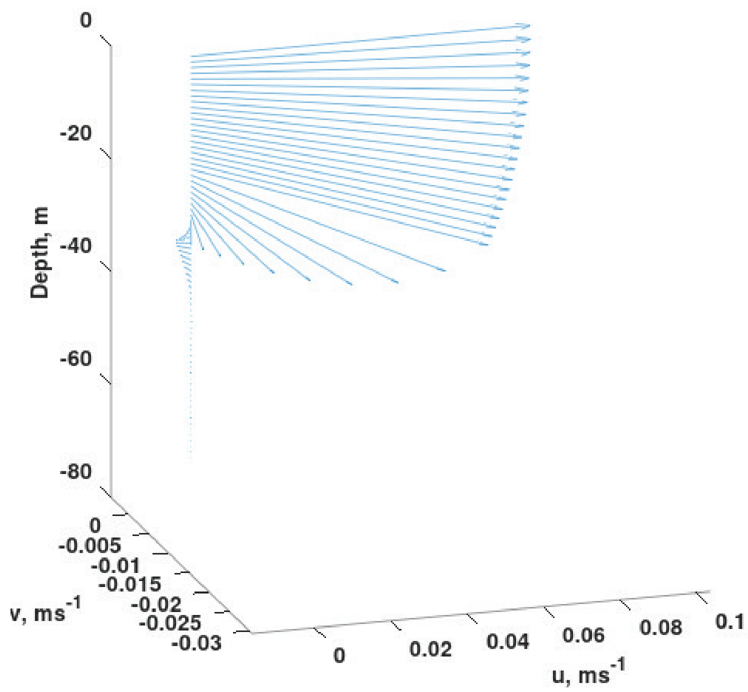

Fig. 2: Example of a two-layer Ekman spiral, computed for a latitude of 40° North, k_1 = 0.1 m^2s^{-1}, h_1=-20 m, k_2 = 0.001 m^2s^{-1} and U_0 = (0.1, 0) ms^{-1}. Current velocity vectors are plotted at every meter of depth.

4. Conclusions/Discussion

The above approach provides a simple and elegant solution to the problem of vertically in-homogeneous upper-ocean water columns, especially in cases when Ekman layers are thicker than the surface mixed layer. The work has already been expanded for many layers and also for a bottom Ekman layer, and is under submission.

A most striking result is that the surface current solution forms an angle of exactly 45° to the right of the wind in the northern hemisphere, exactly as Ekman predicted in his classical solution of 1905, and only the vertical structure of the Ekman spiral is modified depending on the eddy diffusivity values. This result is strikingly different than those of recent corresponding works (Constantin *et al.*, 2020; Ionescu-Kruse, 2021; Roberti, 2021; Guan *et al.*, 2022). Further research (and possibly intercomparison of different published solutions) is necessary to shed light to the question of validity of the analytical predictions of the various models of Ekman currents at environments with eddy diffusivities varying inwith depth.

5. Acknowledgements

This work was based on the approach developed in the framework of Kyriaki Tsakalaki's B.Sc. Thesis (Tsakalaki, 2022).

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PHOSPHORUS DYNAMIC AND PHYTOPLANKTON PHOTOSYNTHETIC RESPONSES IN THE NORTH AEGEAN SEA, EASTERN MEDITERRANEAN: THE FIRST RESULTS

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Abstract

The Eastern Mediterranean Sea is an oligotrophic marine area with a high Nitrogen to Phosphorus ratio (N:P), suggesting that P is the limiting factor of primary productivity. Phosphorus also seems to be the limiting nutrient of the offshore Aegean Sea, which is separated into the South (SAS) and North (NAS) parts, with the latter and most productive receiving additional nutrients from Black Sea and mainland rivers water. Therefore, the low phosphorus concentrations, which the ongoing climatic change may further affect, are central to understanding primary productivity changes in space and time. This study aimed to investigate the effects of hydrological and weather conditions supplying P to NAS by a review of existing nutrient and chlorophyll-a concentration data of different water types, e.g., river, coastal, offshore, and short-term nutrient amendment experiments. The results indicated that the supply of small, under the detection limit quantities of P can affect the nutrient regime of the area and explain the high productivity of NAS compared to SAS. Indeed, a significant ($p<0.05$) increase relative to the control of phytoplankton chl-a fluorescence variables was observed before but not after a winter storm filled the pool of P-deficient seawater in a representative of NAS sub-region site. However, this study should be regarded as preliminary, and therefore to understand P's role in NAS ecosystem functioning, further research is needed.

Keywords: Nutrients, chlorophyll-a, nutrient addition experiment, maximum quantum yield.

1. Introduction

The Eastern Mediterranean Sea (EMS) is an oligotrophic basin with high nitrogen (N) to phosphorus (P) ratio that indicates P-limitation of phytoplankton growth (Krom *et al*., 2010). The Aegean Sea, a part of the EMS, is divided into the North (NAS) and the South (SAS) parts, with different hydrographic characteristics mainly influenced by the Black Sea and Levantine Sea waters, respectively (Zervakis *et al*., 2000). The Black Sea and mainland rivers outflows, along with extensive continental shelf, are hypothesized to be the reason for higher primary (Ignatiades, 2005), secondary (Siokou-Frangou *et al*., 2002), and fish (Stergiou *et al*., 1997) production of the NAS vs. SAS. The contribution of BSW in the enhancement of NAS productivity is now evaluated (Souvermezoglou *et al*., 2014; Lagaria *et al*., 2017) and there is no doubt that NAS, regarding nutrient inputs, is among the most affected Mediterranean parts, having a mesotrophic to eutrophic trend (Karydis and Kitsiou, 2012). However, P may be more critical than N for NAS primary productivity, and therefore, its spatio-temporal variation is crucial to understanding the ecosystem's carrying capacity. Also, detrimental phenomena such as mucilage outbreaks of last year (2021) are linked in other regions with P-limitation and meteo-oceanographic conditions of reduced circulation (Totti *et al*., 2005). NAS offshore and coastal waters P-limited primary production may also be further affected by synergetic climatic change effects. It is well-known that global warming will increase ocean stratification, especially of the inefficient mixed water columns, as NAS (Sylaios, 2011) and eventually P availability. This study aimed to present preliminary results of the effects of hydrological and weather conditions supplying P to NAS by reviewing nutrient and chlorophyll-a literature data of different water types, e.g., river, coastal, offshore. Due to methodological limitations of P detection short-term nutrient addition experiments were also applied studying phytoplankton photosynthetic responses through chl-a fluorescence variables.

2. Material and Methods

2.1 Literature review

The review focuses on the primary water sources that contribute to the enrichment of NAS with nutrients, such as the Black Sea waters through the Dardanelles straits, the main North Greek rivers (Evros, Nestos, Strymon, Axios, Aliakmon, Spercheios), the majors gulfs (Kavala, Strymonikos, Ierissos, Thermaikos, Pagasitikos, Maliakos) as well as the Central Aegean Sea, where there is a continuous circulation and interaction with the NAS. The data were retrieved from fifty-four (54) scientific journals and relevant published technical reports, conducted under the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) for 1994 – 2021 (see at: https://www.dropbox.com/s/675jczl70c852c3/Lachanidou%20et%20al.%20reference%20list.xlsx?dl=0). The parameters that were selected and analyzed were total dissolved nitrogen (TDIN=N-NO_3, N-NO_2, N-NH_4), dissolved phosphorus (P-PO_4), dissolved silicon (Si-SiO_4), nitrogen to phosphorus ratio (N/P), total nitrogen (TN), total phosphorus (TP), and chlorophyll-a. All measurements covered different depths and seasons to represent the annual average of entire water columns of the indicative different sampling sites (Fig. 1).

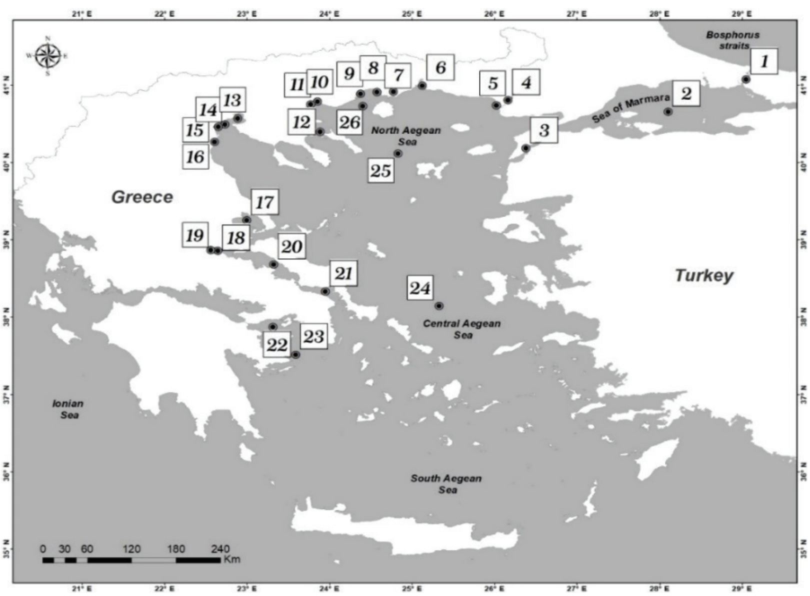

Fig. 1: Indicative sampling sites: 1) Bosphorus straits, 2) Sea of Marmara, 3) Dardanelles strait, 4) Evros River, 5) Evros River – delta, 6) Porto Lagos, 7) Nestos River, 8) Kavala Gulf – near lagoons, 9) Kavala Gulf – coastal, 10) Strymon River – delta, 11) Strymonikos Gulf, 12) Ierissos Gulf – Stratoni, 13) Thermaikos Gulf, 14) Axios River – delta, 15) Aliakmon River – delta, 16) Outer Thermaikos Gulf – Paralia Katerinis, 17) Pagasitikos Gulf, 18) Maliakos Gulf, 19) Spercheios River – delta, 20) North Evoikos Gulf, 21) South Evoikos Gulf, 22) Inner Saronikos Gulf, 23) Outer Saronikos Gulf, 24) Central Aegean, 25) North Aegean, 26) Kavala Gulf – offshore.

2.2 Nutrient addition experiments

Seawater was collected from 10 m depth from the offshore of Kavala Gulf (site 26) on 21/2, 3/3, and 30/3/2022 and was transferred within 2 hours to the laboratory. Noticeably, the winter storm "Bianca" affected the Macedonian coasts from 27/2 to 1/3/2022. In the laboratory the seawater was distributed into sixteen (16; n=4) 500 mL bottles consisting of an unamended control and three experimental groups, in which nitrate (3 μmol/L N-NO_3), phosphate (2 μmol/L P-PO_4), and nitrate and phosphate combined, were added. The bottles containing 300 mL aerated through air-pumps seawater were placed in Haake cryo-thermostat (±0.2°C) to maintain in situ temperatures (11.5-11.7°C) under controlled irradiance conditions (100 μmol photons m^{-2} s^{-1}, 12 h light per day). After 24 h, the maximum fluorescence (P_m) and maximum quantum yield (F_v/F_m) of seawater using a chlorophyll-a fluorometer (Mini-FiRe, USA) were measured to estimate the relative to control ($Treatment^{24h}$ - $Control^{24h}$) / $Control^{24h}$), in percent (%), rapid response in phytoplankton physiology to nutrient additions (see Sherman *et al.*, 2022). In three experiments, while P was under the detection limit in the sampled seawater, TDIN ranged from 0.021 in the 1st to 0.9 in the 2nd and 0.505 μmol/L in the 3rd.

3. Results

The first two axes of PCA analysis classified the sampling sites into five groups (Fig. 2). The first (Group A) and the second (B) groups included the sampling sites where potentially N was limiting, but in the first one, the sites were characterized by oligotrophic conditions, while in the second one by eutrophic conditions. The fourth (D) and the sixth (F) groups included those where the potentially P was limiting and characterized by oligotrophic and eutrophic conditions, respectively. The fifth group (E) included the sampling sites where there was no N or P limitation and was characterized by eutrophic conditions, while the Evros river was separated as a different category in the third group (C). All data ranges are presented in Table 1.

A significant ($p<0.05$) increase relative to control was observed by 210-264% of P_m and by 133-155% of F_v/F_m when phosphates and combined nitrates and phosphates added in the first experiment (21/2 before Bianca; Figure 3). While a significant decrease by 40% of Pm and by 27% was observed in the second experiment when phosphates was added, no significant change was observed in other conditions as well as in the third experiment.

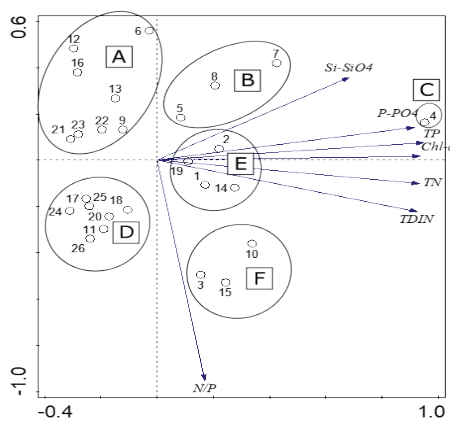

Fig. 2: Principal Component Analysis (PCA) that shows the correlations between the nutrient and chlorophyll-*a* variables in the North and Central Aegean Sea. The length of the arrows indicates the strength of representation and contribution of each parameter to the PC axes. The first axis-X accounted for 73.6% of the variation, while the second axis for 17.4%. TN=Total Nitrogen. TP=Total Phosphorus. TDIN=Total Dissolved Inorganic Nitrogen. Chl-*a*=Chlorophyll-*a*.

Table 1. Mean values range of different Principal Component Analysis (PCA) groups. For more information see Figure 1.

Group	TDIN (μmol/L)	P-PO4 (μmol/L)	Si-SiO4 (μmol/L)	N/P	Chl-a (μg/L)	TN (μmol/L)	TP (μmol/L)	Sites
A	0.37-2.89	0.06-0.29	1.20-54.65	6.17-12.04	0.30-1.14	4.07-20.07	0.07-1.11	6, 9, 12, 13, 16, 21, 22, 23
B	3.91-31.93	0.33-3.05	10.9-157.83	7.78-11.85	0.86-4.48	28.93-47-25	1.31-3.90	5, 7, 8
C	95.86	4.97	128.36	19.29	42.73	157.05	19.37	4
D	0.64-2.70	0.03-0.08	1.36-19.86	22.86-33.85	0.14-1.05	4.24-10.13	0.12-0.30	11, 17, 18, 20, 24, 25, 26
E	6.41-29.40	0.34-2.37	2.00-21.20	12.22-21.62	1.92-4.80	17.60-39.70	0.81-3.19	1, 2, 14, 19
F	27.2-29.18	0.67-0.69	3.81-25.78	40.62-43.55	1.60-3.60	33.54-98.80	0.86-3.94	3, 10, 15

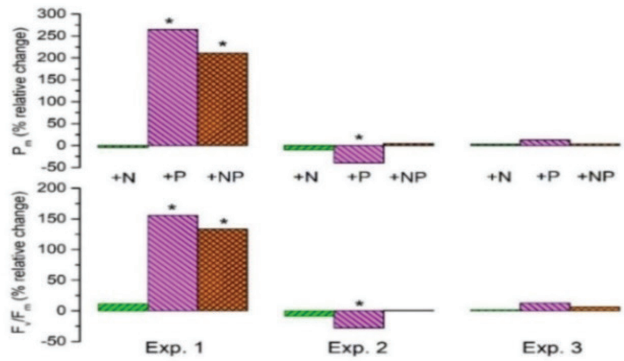

Figure 3. Relative changes in photophysiology 24 h after nutrient addition of Pm and Fv/Fm variables of the three experiments. Treatments included amendment with, nitrate (+N), phosphate (+P), nitrate, and phosphate combined (+NP). Exp. = experiment. * = statistical significance difference ($p<0.05$).

4. Discussion/Conclusion

This study confirms for first time experimentally that P was the limiting nutrient of primary productivity in Kavala Gulf offshore waters at least by the end of February 2022, in agreement with other monitoring studies in NAS (Simboura *et al*., 2016). Variable fluorescence measurements of Pm and Fv/Fm are widely used to rapidly assess nutrient limitation in situ worldwide (Kerkar *et al*., 2020; Sherman *et al*., 2022). Evidently, small and chemically undetected quantities of P supplied by the winter storm "BIANCA" were able to fill the pool of P-deficient seawater as indicated by the nutrient amendment treatments under laboratory conditions. Indeed, a significant ($p<0.05$) increase relative to the control of phytoplankton photophysiological variables (Pm and Fv/Fm) indicative of photosynthetic quantum yield was observed before but not after the storm (Figure 3). This result supports the Souvermezoglou *et al*. (2014) hypothesis that the supply of small quantities of nutrients is able to affect the nutrient regime of the area and explain the "enigmatic" high productivity of NAS in comparison to SAS (see also Lagaria *et al*., 2017). Although preliminary and focused on one study site, the results of this study could have a broader application because Central Aegean Sea (24), NAS (25) and Kavala Gulf offshore (26) sites were classified in group D of the PCA analysis (Figure 2). In agreement, the ratio of most biologically active forms of N (dissolved nitrate and ammonia) and P (dissolved phosphate) easily assimilated by phytoplankton ranged from 22.86 to 33.85.

Chl-a maxima was correlated with nutrient quantities (Fig. 1). Exceptionally high nutrient and Chl-a concentrations were noticed in Evros river waters, although the water quality status of the river system seems to be slightly improving, since Greece and Bulgaria are forced to implement the pollution controlling EU Water Framework Directive (Dimitriou *et al*., 2012). High nitrogen quantities but low N/P ratios noticed in Aliakmon, Strymon and Dardanelles strait, confirm the role of rivers discharges in nutrient inputs in the Aegean due to the remineralization of particulate matter. The outflows of Nestos River (group B) were nutrient enriched but N/P ratios were low even after the construction of hydropower dams (Kamidis *et al*., 2021). The N:P ratio decreases with increased eutrophication as for the costal sites of Group B.

The present study may be regarded as a first step in understanding the P-limitation of phytoplankton productivity contributing to a better comprehension of NAS ecosystem functioning.

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PARAMETRIC ANALYSIS OF THE WAVE TRAPPING PHENOMENON IN RHOMBUS-FORMING CYLINDRICAL ARRAYS

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Abstract

In the present work, the phenomenon of wave trapping will be presented, with the aim of exploiting the wave potential, which can be converted into electricity through a suitable system. The electricity generated can be transferred via underwater cables and stored in batteries on the shore or become part of the grid. In addition, the phenomenon can be exploited by an offshore structure, such as semi-submersible platforms, to reduce its dependence on fossil fuels to generate electricity that can lead to reduced operating costs. Furthermore, the wave energy converters that will be used can be installed on the bases of offshore wind turbines in order to improve the efficiency of the system and at the same time the total electricity generated. An open source program will be used to calculate the excitation forces and the elevation of the free surface in arrays of vertical floating cylinders, which form a rhombus arrangement.

Keywords: wave trapping, cylinder, wave energy, exciting forces, wave elevation.

1. Introduction

Wave trapping is a phenomenon where for certain frequencies hydrodynamic resonance occur, resulting in a large part of the wave energy being trapped within the local vicinity of the bodies instead of radiating to infinity. The wave trapping phenomenon was first detected by Ursell (1951) in a vertical fixed cylinder placed in the middle of an open channel. Maniar and Newman (1997) observed that there are certain wave numbers, where in an array of equal spaced cylinders the total force in the middle of the device increases as their number increases. Evans and Porter (1997) investigated circular arrangements of four to six vertically packed cylinders by varying the distance between them in order to observe how the phenomenon is affected. Chatjigeorgiou et al. (2018) dealt with the exploitation of the phenomenon to produce electricity in an arrangement of nine in a row floating cylinders. Manioudakis (2022) studied the wave trapping phenomenon in various cylinder configurations, solving both the diffraction and radiation problem.

2. Material and Methods

2.1 The open source code Nemoh

The open source code Nemoh is a numerical solver for computation of first order hydrodynamic coefficients such as added mass, radiation, damping and excitation forces (Babarit & Delhommeau, 2015) and mean second order drift forces (Mazarakos, 2010) in the frequency domain. The code was released in open source in January 2014. Nemoh is based on linear free surface potential flow theory with assumptions of an inviscid fluid and an incompressible and irrotational flow. The incident waves are of small steepness compared to their lengths in a finite water depth so that the linear wave theory may be used. Green's second identity and the appropriate Green function is applied. The resulting linear Boundary Value Problem (BVP) for the free surface flow around a body is of first order with assumptions of small motions around mean position and linearized free surface equations. In order to solve the linear BVP, Panel methods are applied in Nemoh.

Nemoh is composed of three different sets of programs which are intended to run in sequent order listed as:

1. Preprocessor: reads and prepare the mesh and calculation cases with stated body conditions
2. Solver: solves the linear BVP with potential theory for stated body condition and calculates hydrodynamic coefficients (wave exciting forces)
3. PostProcessor: processes the results and plot free surface wave elevation.

The excitation forces and moments are calculated from the following relation (Newman, 1951):

$$F_{e_j} = -i\rho\omega\frac{H}{2}\iint_{S_B}(\varphi_I + \varphi_D)\,n_j dS$$

Where ρ is the density of water, ω the wave frequency, $H/2$ the wave amplitude, φ_I the potential of the incident waves and φ_D the diffraction potential. For $j = 1, 2, 3$ the excitation wave forces are calculated and for $j = 4, 5, 6$ the corresponding wave moments.

Respectively, the free surface elevation is calculated using the following equation:

$$\zeta(x, y, t) = \frac{H}{2}e^{-ik(x_1\cos\beta + y_1\sin\beta)}e^{-i\omega t}$$

Where β is the wave propagation angle, k is the wave number and (x_1, y_1) are the coordinates of a point in space where we want to calculate the free surface elevation.

2.2 Description of the rhombus-forming cylindrical arrays

Consider two arrangements of nine vertical floating cylinders that form a rhombus, four of which are located at the corners of the rhombus, four at the middle of each side and one in the center of the array. The angles of each rhombus are the same regardless of the length *L* of each side (50 and 130 degrees, respectively). In the first layout the length of the rhombus side is 50m and in the second 80m. The cylinders are fully submerged, located at depth *d*, have radius *b* and draft *h*. The two structures are depicted in Figure 1. Details about the dimensions of the cylinders of the arrays are given in Table 1. The radius *b* and the draft *h* of each cylinder is 5 m and 20m respectively, and the water depth *d* is 200m.

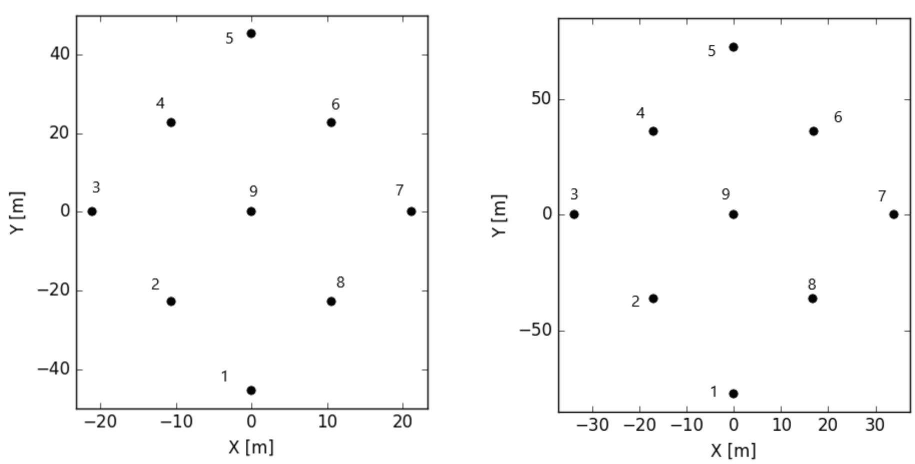

Fig. 1: Plan view of the rhombus-forming cylindrical arrays. Rhombus side equal to 50 m (left) and 80m (right). Rhombus angles 50 and 130 degrees.

Table 1. Coordinates of the rhombus-forming cylindrical arrays. Rhombus side equal to 50 m (left) and 80m (right).

Coordinates	x (m)	y (m)		x (m)	y (m)
Cylinder No. 1	0.000	-45.315		0.000	-72.505
Cylinder No. 2	-10.566	-22.658		-16.905	-36.253
Cylinder No. 3	-21.131	0.000		-33.809	0.000
Cylinder No. 4	-10.566	22.658		-16.905	36.253
Cylinder No. 5	0.000	45.315		0.000	72.505
Cylinder No. 6	10.566	22.658		16.905	36.253
Cylinder No. 7	21.131	0.000		33.809	0.000
Cylinder No. 8	10.566	-22.658		16.905	-36.253
Cylinder No. 9	0.000	0.000		0.000	0.000

3. Results

Figures 2 and 4 demonstrate the horizontal exciting wave forces for a range of angular frequencies for zero and 90 degrees wave heading. Figures 3 and 5 are dealing with the calculated wave elevation around the rhombus multi-body configuration for wave headings zero and 90 degrees.

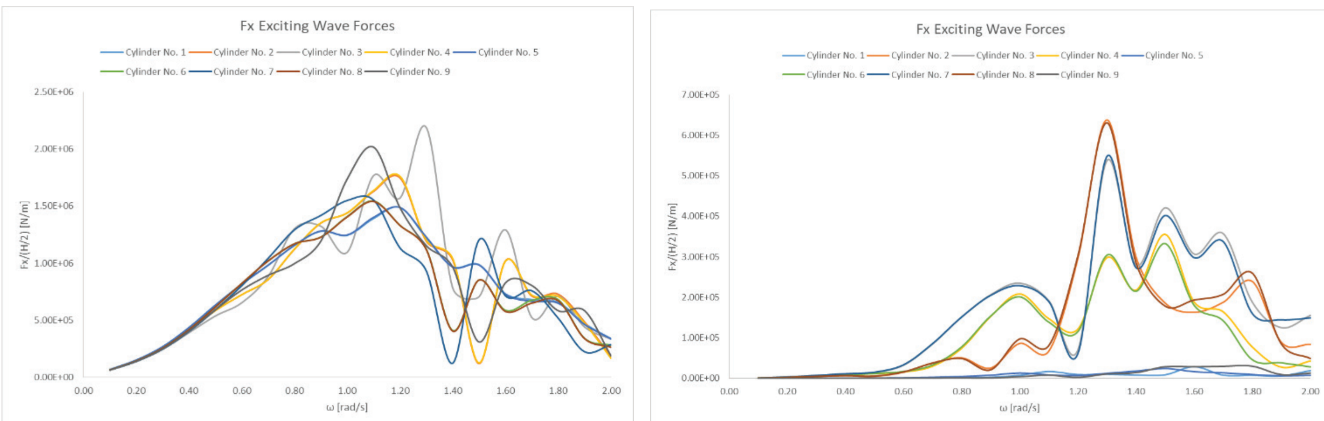

Fig. 2: Magnitude of the horizontal loads on 9 rhombus-forming cylindrical array of radius 5m. The distance between the axes of adjacent cylinders is 25m. Zero degrees (left) and 90 degrees (right) wave heading.

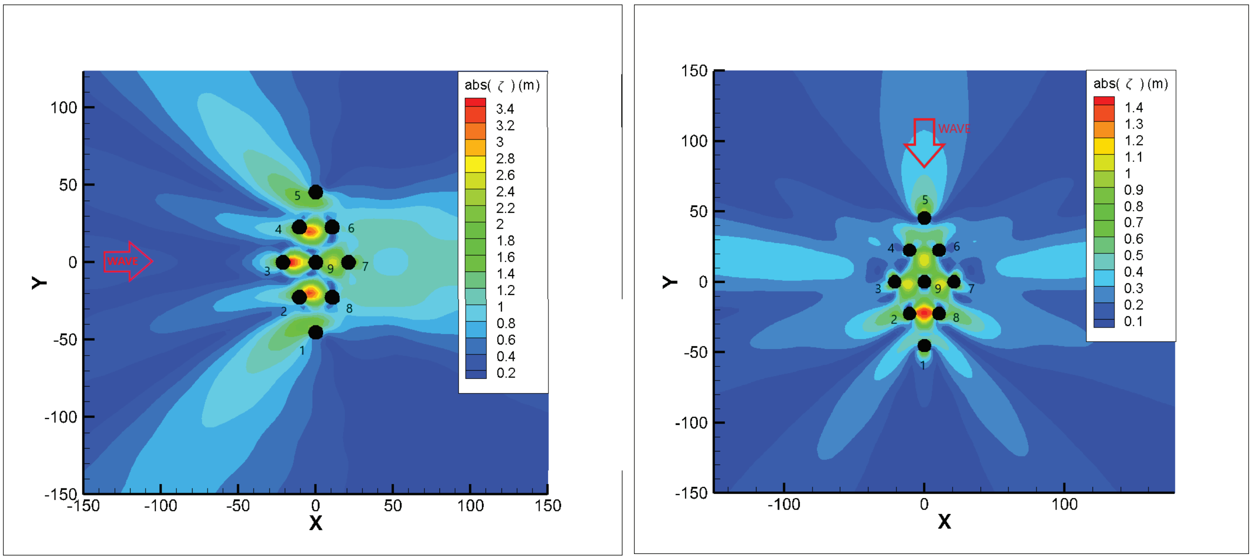

Fig. 3: Magnitude of the wave elevation on 9 rhombus-forming cylindrical array of radius 5m. The distance between the axes of adjacent cylinders is 25m. Zero degrees (left) and 90 degrees (right) wave heading.

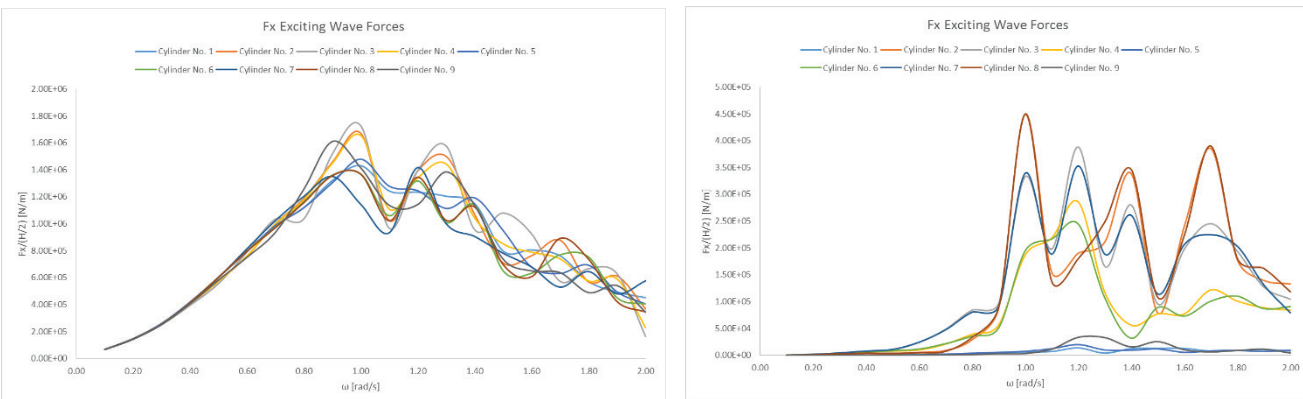

Fig. 4: Magnitude of the horizontal loads on 9 rhombus-forming cylindrical array of radius 5m. The distance between the axes of adjacent cylinders is 40m. Zero degrees (left) and 90 degrees (right) wave heading.

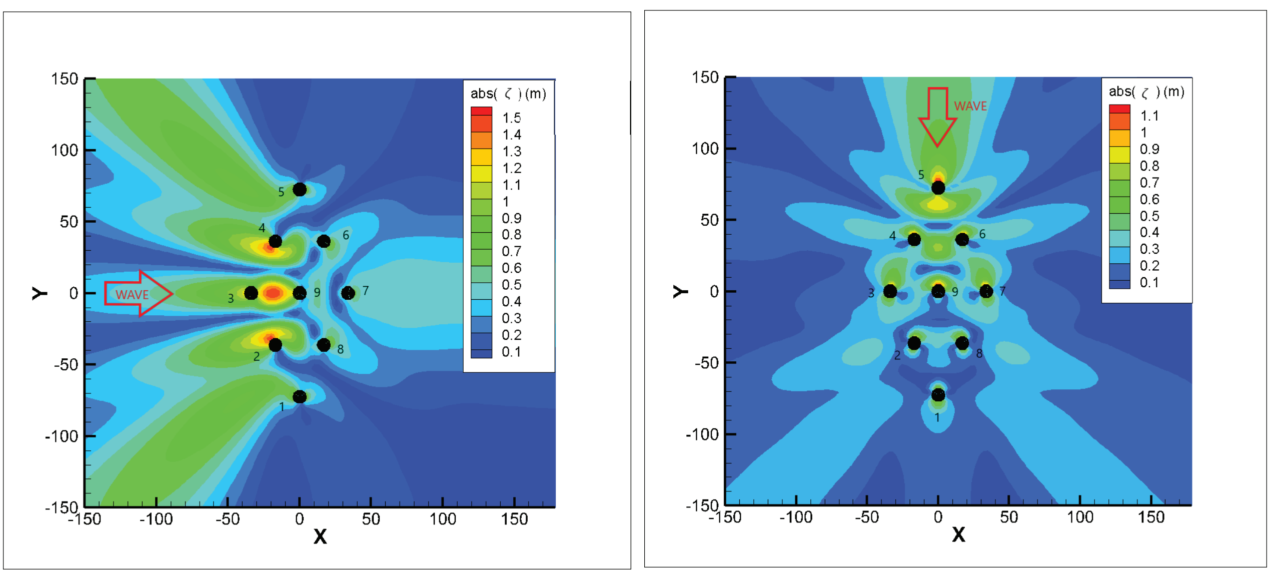

Fig. 5: Magnitude of the wave elevation on 9 rhombus-forming cylindrical array of radius 5m. The distance between the axes of adjacent cylinders is 40m. Zero degrees (left) and 90 degrees (right) wave heading.

4. Discussion/Conclusion

In the first arrangement we observe that the maximum exciting force appears on cylinder No. 3 at the frequency *ω =1.3 rad/s* with a value of *2190 kN/(H/2)*. Cylinder No. 9 also receives a large load in the circular frequency *ω =1.1 rad/s.* Due to the symmetry of the array we observe that in the cylinders No. 2 and No. 4, No. 6 and No. 8, No. 1 and No. 5 the same excitation forces are exerted. Similar observations can be made when the array accepts the action of sea waves at a propagation angle of 90 degrees. In this case the maximum force is displayed on the cylinders No. 2 and No. 8 in the frequency *ω =1.3 rad/s* with value of *638 kN/(H/2)*. In Figure 3 we can observe the distribution of the waves around the array for the frequencies where the maximum exciting forces were observed. For a propagation angle of 0 degrees, the cylinders No. 2, No. 3 and No. 4 show the highest elevation, while at 90 degrees this happens between the cylinders No. 2 and No. 8.

Similar observations can be seen in the second array where the maximum value of the force is presented in the frequency ω =1.0 *rad/s* for the angles of 0 and 90 degrees. It is worth mentioning that the closer the cylinders are to each other, the greater the forces and elevation we observe, i.e. the waves are trapped between the bodies. Therefore, knowing the positions of the maximum forces and the elevation, we can install suitable devices that will store energy in batteries or e.g. improve the efficiency of an offshore wind farm. The value of the installation area depth of 200m is a characteristic depth in the Aegean Sea, where strong wave phenomena prevail and therefore structures can be placed which will recover the wave energy.

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DESIGN AND APPLICATION OF UNDERWATER RADIOACTIVITY SYSTEMS FOR STUDYING INTENSE SUBMARINE SPRINGS IN GREECE

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Abstract

The measurement of radiotracers is recognized as a major tool for the investigation and characterization of submarine groundwater discharges (SGD). Moreover, the in-situ underwater gamma-ray spectrometry is proven a reliable solution for the tracing terrestrial – coastal interactions using key radiotracers. The capability of online continuous monitoring of SGD using direct instrumentation to estimate its velocity and discharge is a promising and cost effective tool since there is no need of traditional sampling and lab-based measurements. The quantification of SGD flux rate is based on radon progenies time-series provided by the spectrometers placed above the seabed and near the water surface, respectively. The proposed methodology has been applied in various sites in Greece during the last 15 years (Stoupa, Korfos, Chalkida, Anavalos). The activity concentrations of radio-tracers can provide estimations on the groundwater flux rates and residence time at the discharge area. The advantages and limitations of direct SGD estimation via underwater gamma-ray spectrometers are also discussed. Furthermore, in this work we describe the design and application of two autonomous in-situ systems for radioactivity measurement implementing low and medium resolution crystals, respectively. The low resolution sensor was deployed in two SGDs sites (Stoupa - Messinia and Anavalos - Argolida), while the medium resolution system was applied recently only in the site of Anavalos. Long-term monitoring of radon progenies concentration is presented by both systems. The radon progeny data were correlated with the flow rate during the deployments. The proposed platform can be easily applied to oceanographic survey activities (such as monitoring of radioactive gases emanation and fluxes on SGD, pockmarks, mud-volcanoes, submarine faults, as well as to the measurement of rainfall freshwater flux at the ocean surface).

Keywords: Submarine Groundwater Discharge, Radiotracers, Nuclear Instrumentation, Anavalos, Stoupa.

1. Introduction

The optimization of underwater autonomous detection systems for radioactivity is of important scientific priority nowadays, for marine sciences, security and safety issues and operational oceanography, due to continuous monitoring requests in order to use radiotracers in ocean applications. Such systems involve very important demands in the construction and calibration phase in order to produce reliable measurements for long time intervals. Special attention has also to be given to the tolerance of the system (e.g., impact of extreme ocean conditions), the power supply and system's autonomy. The low resolution spectrometer are mainly used on SGD studies for tracing water masses, since the discharged water is enriched in ^{222}Rn, in radiation levels well above the detection limit of such systems. SGD has been recognized as an important pathway for material transport between land and the marine environment. Groundwater often contains high concentration of pollutants and can play an important role for health of coastal ecosystems. Two are the main methods for quantitative assessment of SGD: direct physical measurement with seepage flux meter (measurement of the direction of the hydraulic gradient from land to coastal zone) and tracing techniques that measure geochemical species which are enriched in groundwater and present conservative behavior (Brunett *et al.*, 2006). Traditional methods of sampling water and then measuring the radon concentration demand laboratory facilities, manpower and a lot of expenses. In addition radon as inert and non-chemically reactive gas introduces many uncertainties due to its escape from the sampling holders and due to limited half-life of its decay products (^{214}Bi and ^{214}Pb). In case that SGD is investigated, the sampling procedure at depths of 20 to 50 m, are inconvenient due to the precision of position and to the labor costs.

This article describes autonomous detection in-situ instrumentation for marine radioactivity that was developed at the Hellenic Centre for Marine Research (Androulakaki *et al.,* 2020; Tsabaris *et al.,* 2018; 2019; 2021; Patiris *et al.,* 2021). The article presents a deployment of an underwater NaI(Tl) detector which is deployed above the seabed in order to perform measurements of radon on the SGD. The system was installed in a specific Remotely Operated Vehicle (ROV) and was leaded to 1 meter above the seabed in order to prevent the radon daughter's contribution from the seabed (Tsabaris *et al*., 2010; 2011). Furthermore, a second deployment has been made in the same area for 1-year of measurements providing significant data for the annual volume of discharge in Stoupa (Messinia). Recently, the low resolution and the medium resolution spectrometer were deployed for 5 months to estimate the flux of the water source as well as the residence time (Tsabaris *et al*., 2012).

2. Material and Methods

2.1 KATERINA II detection system

The Hellenic Centre for Marine Research (HCMR) has developed the detection system named KATERINAII mainly for radioprotection issues. However, this system was applied for gamma ray spectrometry marine applications like groundwater discharge, seismic hazards, and rainfall characterization. KATERINAII consists of a detector unit and a power unit shielded by an aluminum and polyester pressure tube (Tsabaris *et al*., 2018, Alexakis & Tsabaris, 2021). The detector unit is a 3"x3" NaI detection crystal with built in photomultiplier tube, preamplifier, an analog-digital converter, a high voltage controller and electronic modules for data acquisition, storage and transmission. Small housing and low power consumption are important factors for integrating the sensor with other submersible vehicles or systems. In order to keep the sensor's housing size as small as possible, high density electronics modules are fitted inside the sensor. The electronics designs allow keeping power consumption as low as 1.2 Watt. In order to use this system for ^{222}Rn progenies measurements the detector has been energy calibrated and tested for its stability to temperature variations and its energy resolution. Measurements of the detector marine efficiency and absolute calibration in Bq m^{-3} have also been performed. For this purpose, a calibration tank of 5.5 cubic meters volume, filled with drinking water has been used. The sensor was mounted in the middle of the tank in order to be surrounded by one meter of water, which is enough to imitate the real marine environment (the energy lines that are below 1400 keV), due to the high attenuation of the gamma rays in the water. At the bottom of the tank an electric pump was placed in order to circulate the water to avoid sedimentation, to mix the water with the appropriate reference radionuclides and to get homogenous conditions. After the various settings and calibrations the KATERINA II sensor was installed on a platform (mobile on the Super Achille ROV and stationary/fixed) and was placed in the region of south Peloponnese (Stoupa). The whole system is operated in autonomous mode logging on its internal data logger as it was designed to be used in inattentive systems like buoys, Argo floats, AUVs, landers, marine drones.

2.2 GeoMAREA detection system

The system described in this paper consists of a 2″ x 2" $CeBr_3$ detection crystal, connected with a photomultiplier tube, a preamplifier and a power supply unit, together with the electronics for signal amplification, data acquisition and storage (Androulakaki et al., 2020; Tsabaris et al., 2021; Alexakis & Tsabaris, 2021). The output of the preamplifier is connected to a shaping amplifier for low counting rate which is usually present during underwater operation. The output of the shaping amplifier is introduced to the Multi Channel Analyzer (MCA) and the output is then connected to a PC via the USB or Ethernet protocols.

The data logger consists of a compact stand-alone digital MCA with a fully controlled microprocessor to perform data acquisition using digital signal processing algorithms. The electronic modules operate in low power consumption (0.8–1.0 W) and are placed inside the detector enclosure which consists of a watertight cylindrical Acetal tube. The enclosure was designed to offer continuous functionality up to 400 m water depth. The data logger of the system was programmed accordingly in order to communicate with any typical computer system via USB interface and/or Ethernet communication for operating in standalone and sequential buffering mode. The detection system provides time series of data without connection to the personal computer. This function is performed by developing a software code where the end user gives as an input the time lag of the time series acquisition before the communication is initialized. Then, the buffering starts and the spectrum is cleared before the multichannel analyzer is enabled.

3. Results

3.1. Messinia (Stoupa, Kalogria bay)

The Kalogria Bay is located in southwestern Peloponnese, in the Messinia prefecture. In this area a lot of submarine groundwater discharges are identified where the intense SGD is located at around 180 m offshore and it is easily visible since groundwater is discharged into the sea through a submarine cave at 25 m depth. The KATERINA system was deployed for 1-year period of time (Tsabaris *et al.,* 2011) and the data were retrieved every month. The analysis of the in situ gamma-ray spectra from KATERINA underwater detector provided activity concentration of radon progenies at the emitted gamma-ray emitters (mainly at 351 keV -^{214}Pb, 609 keV –^{214}Bi). The averaged activity concentration during autumn for ^{214}Pb and ^{214}Bi activities are 580 ± 50 Bq m^{-3} and 625 ± 45 Bq/m^3, respectively. The activity of ^{40}K is 10800 ± 90 Bq m^{-3}, while the activity concentration of ^{228}Ac and ^{208}Tl exhibit values of 130 ± 20 Bq m^{-3} and 160 ± 15 Bq m^{-3}, respectively. According to an existing model in literature (Moore, 2006), the activity ratio of ^{208}Tl/^{228}Ac in the flux and inventory areas provide significant information about the residence time of the SGD which was around 4 ± 2 days.

3.2. Argolis (Anavalos)

Anavalos is a group of submarine springs located near Kiveri village on the West coast of the Gulf of Argolis at the NE Peloponnese peninsula in Greece (Eleftheriou *et al.,* 2020). The springs are clearly observed due to the mixing process between freshwater and seawater. The fact that the specific springs are discharged in an artificial closed system (surrounded by a dam) makes them more visible since the interaction with the sea is weak. In 1971, the construction of a concrete semicircular dam was finished capturing the groundwater discharge of eight coastal underwater springs. The dam separated the seawater from the groundwater flowing from the aquifer, achieving thereafter a decrease of salinity. Additionally, an intake system made possible the exploitation of the captured groundwater which was pumped directly inside the dam. Despite the groundwater quality deterioration during the overexploitation and drought periods, the salinity remains in expected levels and thus it still provides every summer period large mass of freshwater to the wider region of Argolis for different uses.

4. Discussion/Conclusion

The main process of ^{222}Rn enrichment into the groundwater is the diffusion through the soil of the aquifer and subsequently its decay to ^{214}Bi and ^{214}Pb. An inverse relation of ^{214}Bi and ^{40}K activity concentration was observed indicating that radon could be an ideal tracer for studying the quantity of the groundwater of the springs. The thoron progeny (^{208}Tl) is enriched into the groundwater and is a key tracer to study the invasion of seawater into groundwater of the aquifer by combining the increased salinity values with its activity concentration observed into the aquifer._The application of the in situ gamma-ray spectrometry method provides at the same time concentrations of radon progenies (^{214}Bi, ^{214}Pb), thoron progenies (^{208}Tl) and ^{40}K. The long term measurements provide direct information of the SGD characteristics and subsequently estimated data of annual activity concentrations of the radio-tracers related to quality and quantity of the groundwater and how these parameters may characterize potential radio-ecological hazards. Furthermore, the in-situ underwater detection systems can contribute significantly for developing and improving decision making applications providing in near real time mode data to an operational centre as well as potential alarm in case of enhanced activity concentrations. Such data sets will contribute to environmental variability study of marine ecosystems and they will improve decision policies and processes for studying SGD areas.

The detection systems acquired radioactivity for long periods in a special marine ecosystem with enhanced radon activity concentration. The ^{214}Pb and ^{214}Bi activity concentration was measured and analyzed through the typical gamma ray's energy lines which were clearly separated into the measured spectra. The in-situ detection systems offered indirectly salinity measurements (though ^{40}K) which can be converted to salinity taking into account radioactive and chemical parameters. KATERINA and GeoMAREA detection systems provide spatial and temporal measurements of radon, thoron progenies as well as for salinity.

In all cases, when a strong flow of freshwater takes place at the spring, the radioactivity instruments record low values of ^{40}K and high values of radon progenies. The activity concentration of radon progeny (^{214}Bi) is enhanced and at low salinity values. On the contrary, at high salinity values (25-35) the concentration of radon is low, thus strengthening the use of radon as tracer of SGD as well as adequate indicator for studying the interaction among groundwater and seawater. In previous work, the ratio of radon to radon progeny in groundwater springs was found to be up to a value of ~ 4, depending on the geomorphology of the aquifer (Tsabaris et al., 2010). Although the system does not provide radon concentrations but its progenies, we can assess the concentration of ^{214}Bi to ^{222}Rn taking into account the aforementioned ratio. So, the monthly average values of estimated ^{222}Rn is much lower in the area of Stoupa according to the commonly accepted limits of Commission (100 Bq l^{-1}) (Commission, 2001). However, in the area of Anavalos, it is close to the upper limit reference levels in drinking water (according to the WHO). Long term measurements are required in order to assess annually the radiological issues related to the high levels of radon activity concentrations (in the case of any potential exploitation of the water by humans).

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AEGIS: A PILOT COASTAL OBSERVATORY FOR SEMI-ENCLOSED BASINS

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Abstract

The first and most significant elements of a Coastal Environmental Observatory for the North Aegean Sea have been installed, focusing mostly on the semi-enclosed basin of Kalloni Bay, Lesvos, Greece. The Observatory aims to support both research and educational activities, as well as provide environmental information services to stakeholders ranging from national authorities to local fishermen and to become a focal point for coastal research and the development of instruments and methods for coastal oceanography. The Observatory, named AEGIS, consists of two main components: the observational component, focused at collecting meteorological and oceanographic time-series mainly from Kalloni Bay, and the forecasting component, which involves a chain of numerical models producing marine forecasts on a daily basis. The development, installation and gradual enhancement of the Observatory has been at the focus of our research strategy throughout more than a decade and has been supported by several research projects. Preliminary results of the operation of the AEGIS Observatory are hereby presented.

Keywords: coastal observations, oceanographic time-series.

1. Introduction

The coastal environment, while covering a relatively small fraction of the ocean, has a disproportionately high value regarding both its role in climate variability, ecosystem functioning, as well as a resource for human development. In terms of its climate role, recently it has recognized that most of the overturning circulation takes place along the boundaries, both on planetary (Ferrari *et al*., 2016) and basin (Waldman *et al*., 2018) scales. In terms their ecological value, coastal regions are usually characterized by higher productivity and biodiversity than the open sea, while serving as nursery grounds for offshore fish communities (Mendonça *et al*., 2018). Despite their high value, the proximity of coastal areas to land and human activities make them very sensitive: many coastal fisheries have been depleted and nutrient loads from land often lead to oxygen depletion in coastal regions (Millennium Ecosystem Assessment, 2005). Especially semi-enclosed coastal basins exhibit enhanced sensitivity, due to their small thermal inertia and enhanced role of riverine inputs. These basins can be very sensitive to human activities, especially in terms of nutrient or urban waste loading, or intervention with the water budget. Thus rises the need to record the oceanographic and environmental variability of semi-enclosed coastal regions, especially since coastal decorrelation scales are much shorter than oceanic ones, leading to a need for denser network of monitoring stations.

The Laboratory of Physical and Chemical Oceanography (LPCO) of the Department of Marine Sciences of the University of the Aegean has accordingly designed its research strategy to develop and establish a Coastal Environmental Observatory focused in the Bay of Kalloni, Lesvos island, North Aegean sea. The Coastal Observatory was also conceived as a potential synergetic infrastructure to the offshore operational system "Poseidon" (Soukissian *et al*., 2002; Korres *et al*., 2010), implemented successfully by the Hellenic Centre for Marine Research over the last two decades. Furthermore, a coastal system focused in the Bay of Kalloni can be viably operated with low requirements in infrastructure (research vessels and technicians) and associated costs, while at the same time can provide significant added value in the education provided by the Department of Marine Sciences.

The selection of the Bay of Kalloni was based on its great ecological (Papantoniou *et al.*, 2015) but also socioeconomic value (Oikonomou *et al.*, 2011). It should be noted that the great productivity and biodiversity of the Kalloni Bay provided the necessary material to Aristotle for his biology-founding work (Lerois, 2015). Hereafter, we describe the various components of the AEGIS Observatory, and proceed to present some preliminary results.

2. Material and Methods

2.1 Observational Component

The observational component of the AEGIS Coastal Environmental Observatory consists of:

- an oceanographic / meteorological observational platform moored in the middle of the Kalloni Bay. The platform incorporates a complete set of meteorological sensors, measuring wind speed and direction, humidity, atmospheric pressure and air temperature, as well as downwelling solar and long-wave radiation and precipitation. The set of oceanographic parameters include temperature and salinity, chlorophyll-a concentration, turbidity, dissolved oxygen, pH and NO_3 concentration at 4 m depth, as well as radioactivity levels in sea-water.
- two sea-level gauges, one at Skala Kallonis port (in Kalloni Bay) and another one at Skala Sykountos (Dipi, in Gera Bay), both in Lesvos island.
- a submarine telephone cable, suitable for the study of net exchanges through the Strait between Kalloni Bay and the open sea.
- the High-Frequency (HF) coastal radar "Dardanos", co-owned and co-managed with the Institute of Oceanography of HCMR, located on the eastern coast of Lemnos island, monitoring the sea-surface current formed by the Dardanelles outflow. This infrastructure provides information regarding the Black-Sea water inflow into the North Aegean, which is critical both for validation and data assimilation for the improvement of the North Aegean Sea circulation forecasts.
- *Periodic field campaigns at the Bays of Kalloni, Gera (Lesvos) and Moudros (Lemnos) also contribute with valuable information for model validation and intercomparison with the mooring data.*

2.2 Operational Forecasting Component

The forecasting component of the AEGIS Coastal Environmental Observatory consists of the two following based on ROMS – Regional Ocean Modeling System – (Shchepetkin and McWilliams, 2003, 2005) ocean models (Fig. 1.) : The main or donor model covers the central north Aegean Sea (CNAG) with a spatial resolution of 2.5 km in both horizontal directions and 20 sigma – coordinates vertical levels. The model runs on daily basis an overlap three (3) day assimilation cycle, using ROMS native 4DVAR scheme (Moore *et al.,* 2011) in order to produce optimal state initial conditions for a 4 days forecast. From each forecast cycle, high temporal boundary conditions (10 minutes) are extracted in order to used in a high resolution – 500 meters – model for Lesvos area (Petalas *et al.,* 2020) again for a 4 day forecast.

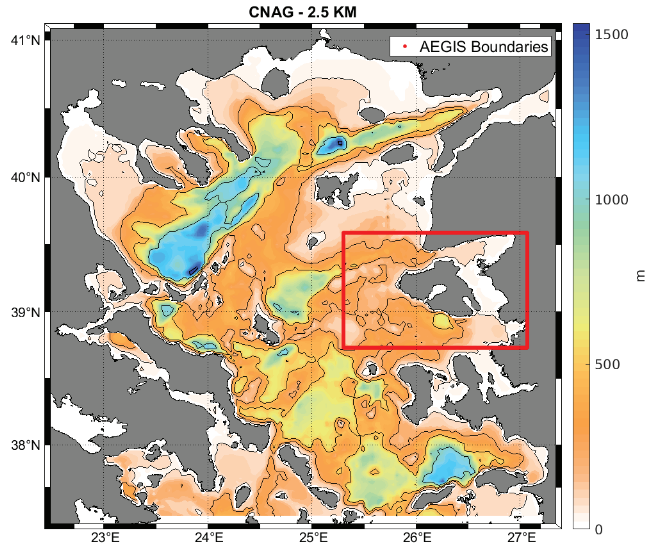

Fig. 1: Central North Aegean Sea (CNAG) geographical coverage. The red constant line denotes AEGIS – Lesvos area model.

3. Results

The observational component of the AEGIS Observatory has already provided time-series of several oceanographic and marine environmental parameters for Kalloni Bay. The initial mooring deployment took place on 28 Janury 2020, and the data flow lasted until 3 December 2020, when the loss of a solar panel resulted caused the interruption of the operation of the mooring. Since then, the instrumental tower of the mooring has been recovered and transferred to the premises of the Department of Marine Sciences in Mytilene, where it has undergone extensive maintenance and attachment of the marine radioactivity sensor and is planned for redeployment in May 2022.

The 2020 deployment provided almost a full year of data, for a wide set of parameters, which will shed light on various processes of the upper water column and the coastal regions. For example, a small sample of the record, comprised of the solar radiation, wind speed, clhorophyll-a fluorescence and dissolved oxygen concentration during February 2020 is presented at Figure 2.

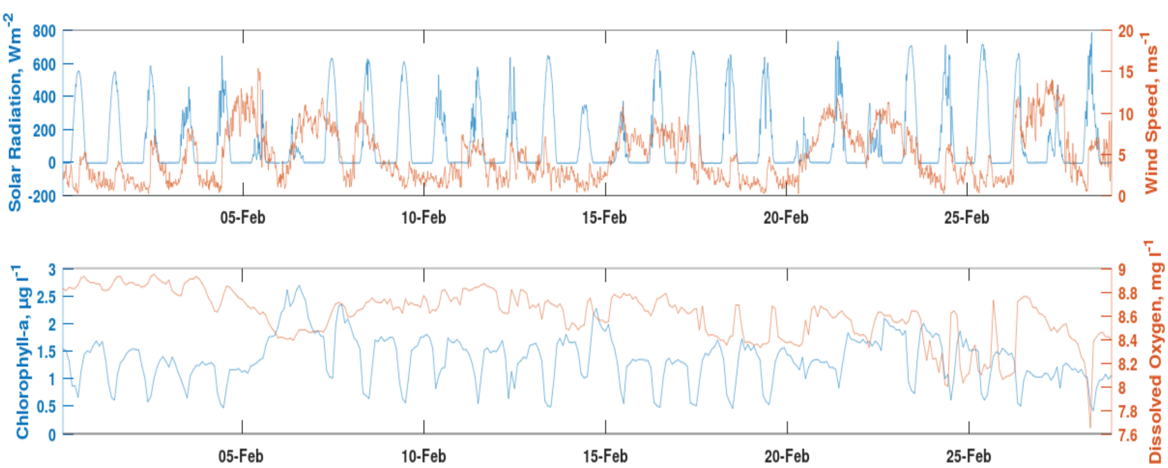

Fig. 2: Solar radiation (top, blue), wind speed (top, red), chlorophyll-a (bottom, blue) and dissolved oxygen concentration (bottom, red) as measured by the Kalloni Bay oceanographic/meteorological platform during February 2020.

The image reveals the response of near-surface chlorophyll to the daily cycle of solar radiation, as well as the impact of strong mixing due to wind.

The ocean/hydrodynamic component of the AEGIS system has been operational since January 2022.

An concise evaluation of the Lesvos area model performance via comparison to satellite observations for March 2022 is provided in Figure 3. The spatially integrated value for bias is equal to 0.104 ºC and for RMSE 0.331 ºC. The mean bias for SLA over all grid points is equal to -0.004 meters and RMSE 0.02 meters. The main pattern is similar but the crude spatial resolution of the altimetric observations cannot give us more information about the mesoscale features that are seen in model's results.

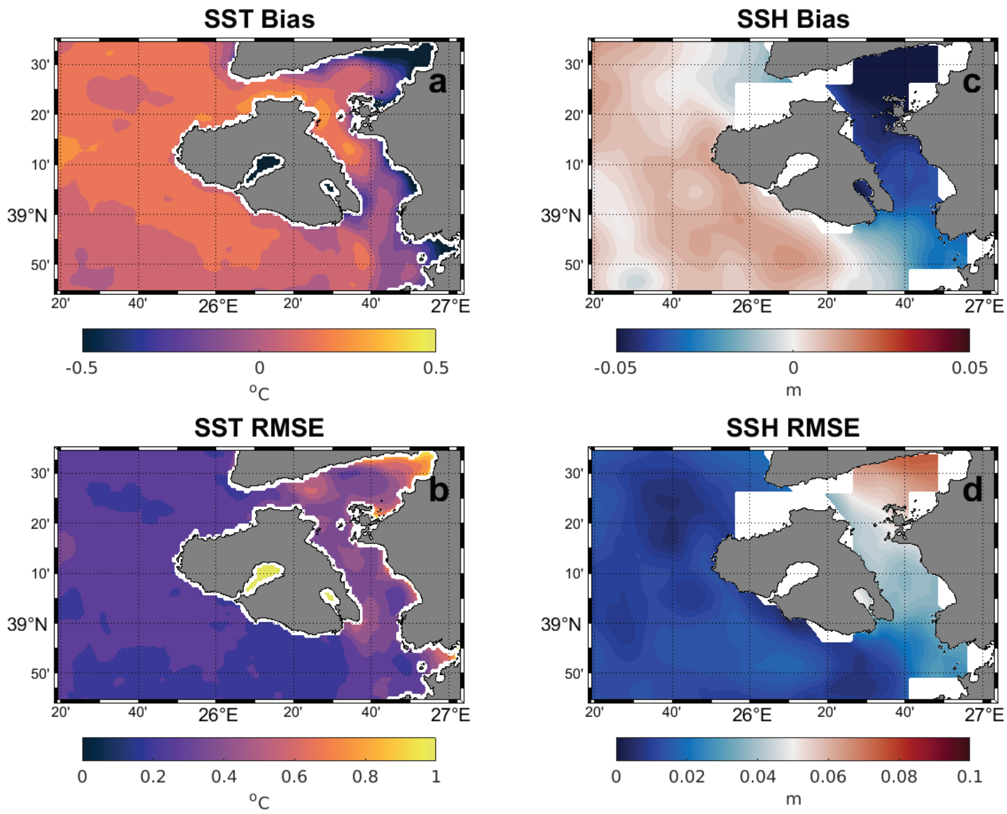

Fig. 3. Lesvos area model results for March 2022. Panels a and b show SST error in terms of bias and RMSE. Panels c and d

4. Discussion/Conclusion

The AEGIS Coastal Observatory is a strategic investment of the Department of Marine Sciences and has been implemented through several projects and a decade of development. Further evolution includes high-resolution forecasts of the Kalloni, Gera and Moudros bays, as well as the development and testing of biogeochemical models that will produce a valuable tool valuable for efficient management of the coastal resources of the North Aegean Sea.

5. Acknowledgements

The development, installation and implementation of the AEGIS Coastal Environmental Observatory is the result of a long-term effort of the LPCO, and thus has been supported by a series of projects, namely:

- The initial installation of the HF radar system "Dardanos" was carried out in 2009, in the framework of the project "Coastal Risk , Prevention and Management of Sea Originated Risks to the Coastal Zone (CORI)", funded by the Regional Development Plan of the North Aegean through the INTERREG IIIB Archimed programme.
- The oceanographic / meteorological platform in the Gulf of Kalloni, the two sea-level gauges at the bays of Kalloni and Gera and the development of the North Aegean forecasting model were implemented in the framework of the project "Infrastructure development to support Blue Growth in the

North Aegean: Coastal environment observatory AEGIS", funded by the North Aegean 2014-2020 Regional Operational Program, co-financed by 80% of the European Union's Structural Funds, European Regional Development Fund (ERDF) and European Social Fund (ESF).

- The upgrade of the "Dardanos" HF radar system took place through the period 2019 to 2021 within the project "Hellenic Integrated Monitoring, Innovation and Operational Forecasting of Seas and Internal Waters (HIMIOFoTS)", funded by the European Fund for Regional Development and the Hellenic State through the Operational Programme EPANEK 2014-2020.
- Finally the provision of a new coastal research vessel, an ocean glider for use in the North Aegean Sea, and the extension of the numerical simulations to detailed circulation models of the three island Bays and marine ecosystem modeling, are currently underway through the project "Coastal Environment Observatory and Risk Management in Island Regions AEGIS+" (MIS 5047038), implemented within the Operational Programme "Competitiveness, Enterpreneurship and Innovation" (NSRF 2014-2020), cofinanced by the Hellenic Government (Ministry of Development and Investments) and the European Union (European Regional Development Fund).

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CLIMATE CHANGE

MARINE HEAT WAVES AND SST VARIABILITY OVER AEGEAN, IONIAN AND CRETAN SEAS

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Abstract

The interannual and spatial distribution of Marine Heat Waves (MHWs) is investigated based on long-term satellite-derived Sea Surface Temperature (SST) data over the NE Mediterranean Sea (Aegean, Ionian, and Cretan Seas) for 14 years (2008-2021). The number of MHWs) showed an increase of approximately 1.7 event and 21 days of MHWs per decade; the spatially- and annually-averaged number of events was approximately 1 with less than 10 days duration on 2008 and increased to more than 3 events with almost 30 days by 2021. The SST trend of the mean annual values is mainly associated with the interannual increase of the lowest values (weaker minima during the cold seasons). MHWs were more frequent over the northern Aegean Sea, especially at Thermaikos Gulf which is characterized as "hot spot" for MHWs. Stratified upper-ocean, controlled by buoyant brackish plumes, such as the Black Sea Waters in the northern Aegean, may increase the heat storage capacity of surface water masses contributing to the further warming of the ocean. In particular, the warm year of 2021 showed significant high SST levels both in winter (January-February) and summer (July-August) during a prolonged period of an atmospheric heat wave.

Keywords: ocean warming, satellite observations, marine heat waves, barrier layer.

1. Introduction

Sea Surface Temperature (SST) is an important parameter of the natural environment strongly related to the earth's climate and the hydrological cycle, mainly due to exchanges between the atmosphere and the ocean. The Aegean, Ionian, and Cretan (AIC; Fig.1) Seas together with the Levantine Basin revealed the highest increasing trends of SST (>0.05° C/year) in the Mediterranean during 1982-2018 (Pisano *et al.*, 2020). Skliris *et al.* (2011) based on long-term satellite-derived (1985-2008) and in situ data (1950-2006), showed a small SST decreasing trend of the Aegean Sea until the early nineties and a rapid surface warming right after, analogous to the temperature rise observed on the global ocean scale. Ibrahim *et al.* (2021) have shown that over Eastern Mediterranean, Marine Heat Waves (MHWs) frequency increased by 1.2 events per decade between 1982 and 2020.

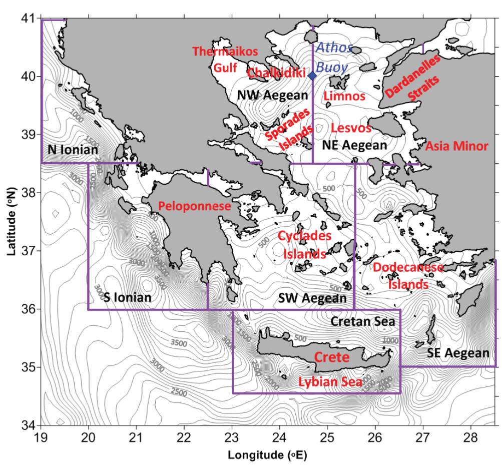

Fig. 1: Bathymetry of the study area divided in 7 sub-regions. The location of the Athos Buoy is also marked.

In this study, we focus on the spatial and temporal SST variability during the most recent 14-year period (2008-2021) using high-resolution satellite-derived data and field observations. We discuss the spatial differences between 7 sub-regions of the AIC Seas (Fig. 1). The main goal is to detect the interannual SST trend of each sub-basin and their spatial differences, focusing also on the formation of MHW and their interannual variability. In addition, we will examine the interannual evolution of the MHWs over the entire study region in comparison to the extreme conditions of 2021.

2. Material and Methods

The satellite data used in the study include an SST set, distributed by the E.U. Copernicus Marine Service (https://www.copernicus.eu/; Mediterranean Sea High Resolution and Ultra High Resolution Sea Surface Temperature Analysis). Observations collected at the Athos Buoy (Fig. 1) of the Poseidon System (https://poseidon.hcmr.gr/), were also used to define the physical conditions and structure of the upper 20 m. The study adopts the definition proposed by Hobday *et al.* (2017) to determine the MHW events, based on abrupt SST increases above a "climatologic" value (the baseline temperature) for a certain time period.

3. Results

3.1. Interannual variability of SST

The mean SST of the entire study region (Fig. 2) reveals a clear increasing trend derived from annual means (0.49°C/decade) with significantly high annual mean in 2021 (~20.9°C), while the respective annual mean was approximately 20°C in 2008. The increasing trend of the mean annual minima is stronger (0.38°C/decade) and statistically significant (p_{value}=0.026<0.05) in comparison to the trend of the maxima. This trend indicates that during the cold season when the lowest values usually occur, the winter-spring surface waters became warmer through the years.

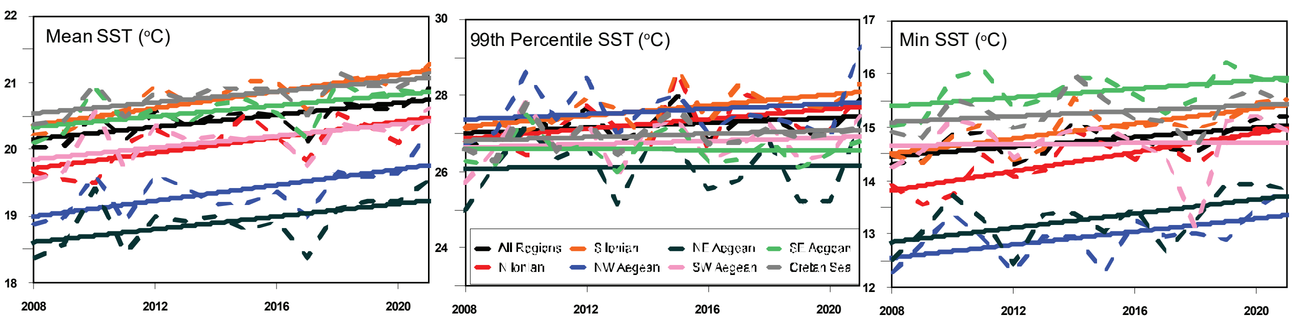

Fig. 2: Annual variability and trends of the mean SST, the 99th percentile of SST, and the min SST, averaged over 8 regions for the period 2008-2021.

The highest statistically significant (p_{value}<0.05; Fig. 3) Sen's slopes are observed at parts of the N Aegean (>0.75°C/decade) and central and southern Ionian (>0.65°C/decade).

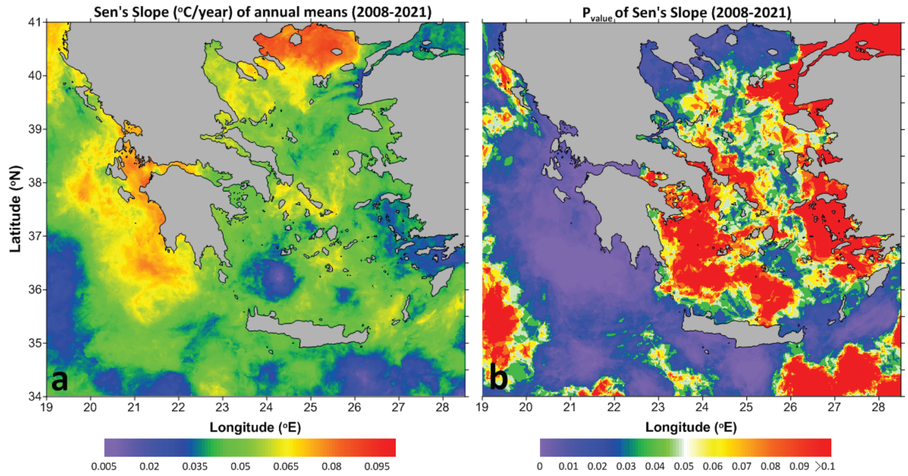

Fig. 3: Spatial distribution of (a) Sen's slopes of annual SST means and (b) statistically significance threshold (p_{value})

The eastern Aegean Sea and the SW Aegean revealed the weakest trends with very high p_{values}. The straits between the Peloponnesus and the northern Greek mainland are also characterized by strong SST trends. Especially a distinctive region between Peloponnesus and Crete showed trends less than 0.1°C/decade. Very low Sen's slopes (<0.35°C/decade) were also computed at the area southeast of Crete.

3.2. Formation of Marine Heat Waves

The largest number of MHWs was observed in the northern Aegean Sea (>35), where the accumulative period of all MHWs is more than 300 days; especially the entire northwestern region of the Aegean and Thermaikos Gulf can be characterized as a "hot spot" of MHWs affecting the respective coastal area (Fig. 4). The increase of the MHWs days is 21.3 days/decade and the respective increase of the MHWs events is 1.7 events/decade in agreement with the trend derived by Darmaraki *et al.* (2019) for the whole Mediterranean Sea using a different MHW definition and a longer period.

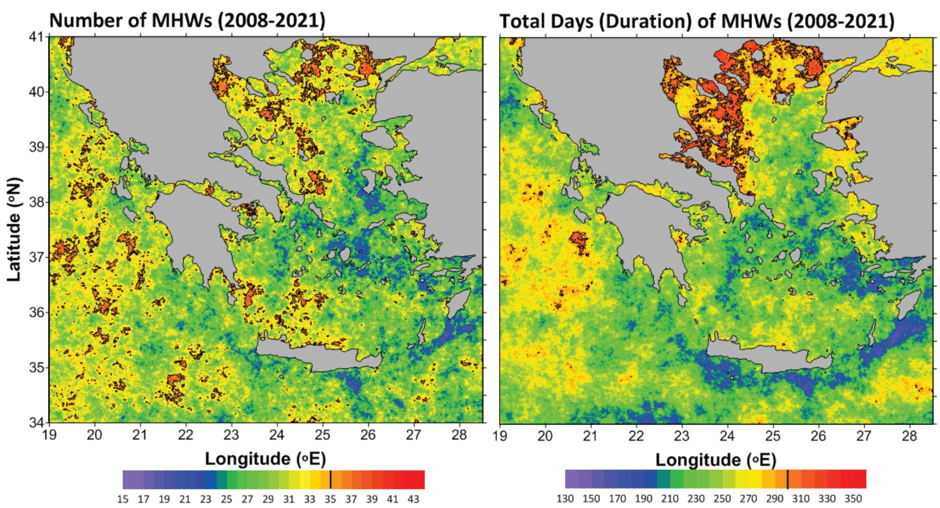

Fig. 4: Spatial distribution of the total number of MHWs events (left) and total duration (right) in days.

3.3. The warm year of 2021

The year of 2021 was characterized by successive atmospheric heat waves especially in August. (http://magazine.noa.gr/archives/4560). The highest values of the N Aegean in the summer of 2021 are mainly related to the MHWs that were formed in the western part of this region, and especially in the broader Thermaikos Gulf. The duration of the MHWs was more than 20 days in August 2021 and reached 30 days close to the western coasts of the central Gulf.

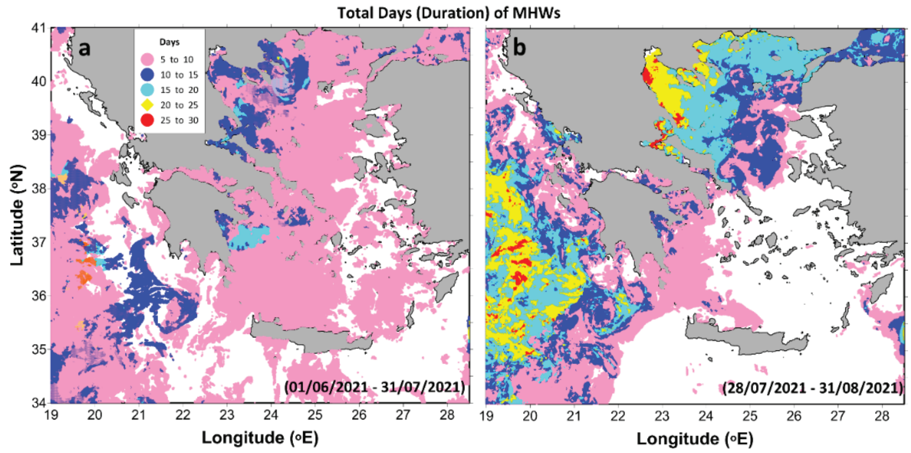

Fig. 5: Spatial distribution of the MHWs duration (days) for (a) June-July 2021 and (b) August 2021.

3.4. BSW effect over the N Aegean

We used the available salinity and temperature measurements at two layers of the Athos Buoy (1 and 20 m; ΔSalinity) to investigate the evolution of the upper-ocean stratification characteristics during the entire study period During 12 periods of high SST peaks, the surface salinity showed very low values while the deeper layer was characterized by more saline waters resulting to high ΔSalinity and peaks of the stratification frequency. The Ocean Heat Content (OHC), that represents the energy stored in the ocean revealed very high values during all 12 study events. Our results confirm the hypothesis that shallow mixed layers contribute to the warming of the surface layer in agreement with Amaya *et al.* (2021).

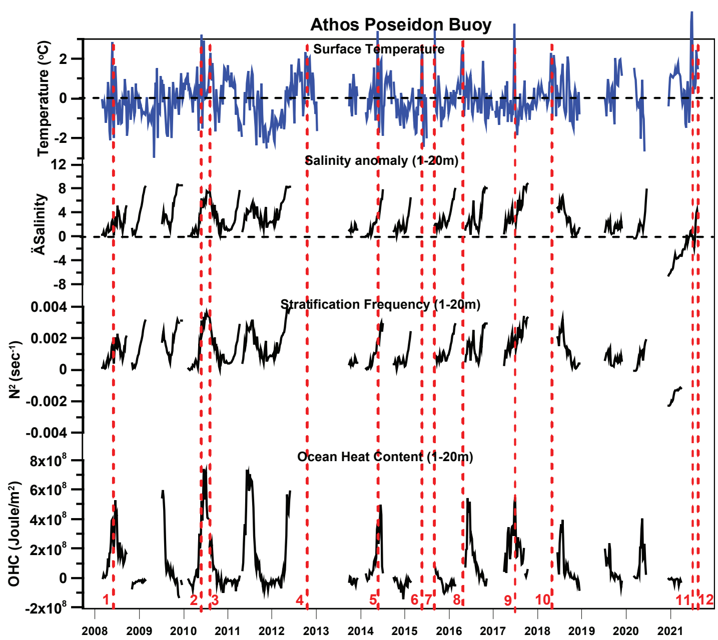

Fig. 6: Weekly means of SST, salinity anomaly between the two layers (1 m and 20 m) Brunt-Väisälä stratification frequency between 1 and 20 m, and OHC of the upper 20 m, derived from Athos Buoy daily values without the seasonal cycle. The red dashed lines represent 12 events when surface temperature was higher than 2°C.

4. Conclusions

The increase of the Marine Heat Waves (MHWs) total annual duration was 21.3 days/decade and the respective increase of the MHW events is approximately1.7 events/decade. The most-recent four years (2018-2021) revealed the highest number of MHWs with the longest durations, especially for the northern

Aegean Sea. The general increasing SST trend of over the whole AIC Seas is 0.49°C/decade with stronger local gradients over the southern Ionian and northern Aegean (0.6°C/decade). The strong upper-ocean stratification, related to buoyant barrier layers at the surface may increase the heat storage capacity of the upper layers contributing to the further warming of the surface water masses of the northern Aegean. This was the case over the northern Aegean in the summer of 2021, recording the highest maximum temperature levels among all years over the northern Aegean, characterized by intense MHWs in August.

5. Acknowledgements

The in situ temperature and salinity measurements were provided by the online Poseidon System service of Hellenic Centre of Marine Research (https://poseidon.hcmr.gr/).

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COASTAL HAZARDS UNDER CLIMATE VARIABILITY AND CHANGE THE CASE OF SANTORINI AND CRETE (AEGEAN SEA)

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Abstract

The objective of the present contribution is to examine some of the potential hazards and threats for the coastal zone, induced by Climate Variability and Change (CV&C), using as case studies two Greek islands: Santorini and Crete. Beach retreat/inundation due to mean and Extreme Sea Level (ESL) rise was assessed using cross-shore morphodynamic model ensembles. The results were used in conjunction with information (geo-spatial and human development features) from readily available satellite imagery to assess the impacts on the beach carrying capacity and the backshore assets/infrastructure. Potential impacts on critical transport infrastructure (e.g. airports, seaports) under ESL and heat waves were also discussed. By 2100 under a moderate emission scenario (RCP4.5) it is projected that up to 23% of Santorini beaches and 53% of Cretan beaches will be completely eroded under relative sea level rise (RSLR); the 100-year ESL event may overwhelm up to 93% of Santorini and 94% of Cretan beaches, causing damages to the backshore infrastructure/assets. The results show that both ESL_{100} and heat waves at the locations of 6 seaports and 3 airports in Crete will increase significantly in both magnitude and frequency, raising the potential for flooding, operational disruptions and possibly damages to the infrastructures.

Keywords: Beach erosion, Morphodynamic models, Extreme sea levels, Heat waves.

1. Introduction

Coastal areas, by their exposure to many hazards, such as rising mean sea levels, storm surges and waves, increasing temperatures, cyclones etc, are threatened by increasing land loss and flooding that can severely impact their ever-increasing populations, infrastructure, assets and economy. In Greece, coastal areas also form the pillar of tourism, a vital sector for the Greek economy. Tourism is increasingly associated with beach recreational activities according to the 'Sun-Sea-Sand-3S' model (Philips & Jones, 2006), at the same time beach erosion, which is projected to greatly increase under Climate Variability and Change (CV&C), poses a significant threat especially to island beaches; as they have limited dimensions and sediment supply (Monioudi *et al.,* 2017). Beaches are not only significant habitats in their own right, but they also provide protection from marine flooding to the coastal assets, infrastructure and activities they front. CV&C also poses significant challenges for the critical infrastructure located at the coastal front, such as airports and seaports, involving impacts from both flooding under extreme events and operational disruptions from e.g. the increasing magnitude and frequency of extreme heat waves. The objective of the present contribution is to examine some of the potential hazards and threats, induced by CV&C, for the island coastal zone, using as case studies two Greek islands: Crete and Santorini.

2. Material and Methods

2.1 Beach retreat/inundation projections

The geo-spatial characteristics (i.e. max. width, area, sediment type) and human development features (i.e., the density of backshore assets) of all the beaches of Santorini (30 were identified) and Crete

(828) were recorded on the basis of the images and other related optical information available in the Google Earth Pro application. The 'dry' (subaerial) parts of these beaches were digitized as polygons bounded on their landward side by either natural boundaries (vegetated dunes and/or cliffs) or permanent artificial structures (e.g. seawalls, roads, and buildings) and on their seaward side by the shoreline. Constraints in the approach can stem from the accuracy/resolution of the (not properly georectified) images and the varying hydrodynamic conditions during the image collection that can affect shoreline delimitation. These may introduce uncertainties which, however, cannot be avoided in regional studies.

Beach retreats under CV&C were projected using 1-D (cross-shore) morphodynamic model ensembles, following the methodology described in Monioudi *et al.* (2017). Specifically, beach retreat/inundation was assessed with regard to; (a) long-term relative sea level rise (RSLR) and (b) 1-100 year Extreme Sea Level (ESL, i.e. storm-induced water levels superimposed on RSLR and high tide), projected for the year 2100 under the IPCC RCP4.5 scenario. Projections of the RSLR, tide and ESL_{100} along the coasts of Santorini and Crete were abstracted from the JRC (Joint Research Centre) database (Vousdoukas *et al.*, 2017). Given the large scale of the application (Island scale), the input data of the models could not be based on in situ measurements. Therefore, the models were set up using a plausible range of environmental conditions (i.e. combinations of different beach slopes, wave conditions and sediment size) and they produced a range of beach erosion projections. The lowest and highest projections were compared with the recorded beach maximum width (BMW) to assess the impacts on the 'dry' beach width (and consequently the beach carrying capacity) under RSLR and the impacts on the backshore assets under ESL. It is noteworthy that the use of the reduction in BMW as the erosional impact indicator may result in conservative estimations.

2.2 Impacts on critical transport infrastructure

Transport infrastructure and related operations that are situated at the coast are likely to be seriously affected by the impacts of CV&C. Coastal flooding may inundate low-lying coastal airports/seaports, whereas higher mean temperatures and more frequent heat waves can affect runways (heat buckling) and aircraft lift, resulting in payload restrictions and disruptions. Also seaport/airport operations (and possibly, the infrastructure itself) could be seriously impacted due to increasing health and safety concerns and energy needs (and costs) for cooling (Monioudi *et al.*, 2018). Projections of heat wave changes in magnitude and frequency, under mean temperature increase scenarios of 1.5, 2 and 3 ^{o}C above the pre-industrial times (Special Warming Level – SWL), were abstracted from Dosio *et al.* (2016).

3. Results

3.1 Beach Erosion and impacts in Santorini

For the year 2100 under RCP4.5, it appears that Santorini beaches would be seriously affected due to the projected sea level rise (0.53 m); beach retreat is estimated between 5.5 m and 16.5 m causing 73% and 23 % (high prediction) of the beaches to be reduced by up to 50% and 100 % of their current BMW (Fig. 1a,b, Table 1). Many of these beaches lack the accommodation space to retreat landwards and, thus, will suffer coastal squeeze without appropriate replenishment.

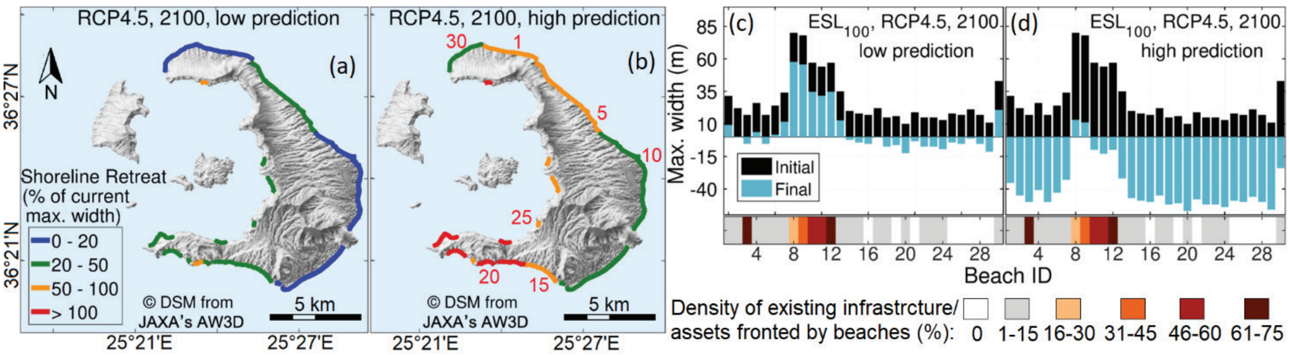

Fig. 1: The percentages of the current BMWs of the 30 Santorini beaches projected to be eroded under RSLR based on the (a) low and (b) high model projections. The current (initial) BMWs (black bars) are compared with those resulting from temporary inundation/retreat (blue bars) under ESL_{100} based on the (c) low and (d) high predictions and are shown together with the recorded density of the backshore assets (as a percentage of the beach length). The negative values indicate total beach inundation.

The 100-year ESL (ESL_{100}) in 2100 will result in storm beach inundation of up to about 22.3 and 66.9 m, under the RCP4.5 scenario based on the low and high model estimates respectively. The impacts could be devastating since 63 - 93% of all beaches will be completely (at least temporarily) inundated under the low and high projections, respectively. In terms of asset exposure, 50 - 91% of the beaches presently fronting assets are projected to be overwhelmed during the event (Fig. 1c, d, Table 1). These frontline backshore assets will sustain damages even in the case of a partial (or total) post-storm beach recovery as they are located within the beach erosion-recovery envelop.

Table 1. The low and high beach retreat/inundation estimates of Santorini beaches by the model ensembles. Percentages of the beaches that will be retreated/inundated more than 50% of their current BMW and more than their current BMW. Numbers (N) and percentages of beaches where backshore infrastructure and assets are projected to be impacted are also shown.

Sea Level Rise (m) under RCP4.5, 2100		**Retreat (R)/ Inundation(I) (m)**		**R/I to 50 % of max. width (%) N**	**R/I to max. width (%) %**	**Beaches with assets affected**	
RSLR	0.53	Low	5.5	7	0	0	0
		High	16.5	73	23	5	23
ESL_{100}	1.66	Low	22.3	83	63	11	50
		High	66.9	100	93	20	91

3.2 Beach Erosion and other coastal hazards in Crete

Under sea level rise of 0.55 m, projected for the year 2100 under RCP4.5, the model results show that the Cretan beaches would be seriously affected; beach retreat is estimated between 5.8 m and 19.6 m and 53 % of the beaches might see (high prediction) their BMW reduced by up to 100 % (Table 1). Similar to Santorini, many of these beaches will suffer coastal squeeze due to the absence of adequate inland accommodation space. Inundation due to the RCP4.5 ESL_{100} is predicted between 18 and 68.5 m and represents a catastrophic scenario, since 53-94% of all the Cretan beaches will be completely inundated causing damages to the backshore infrastructure/assets.

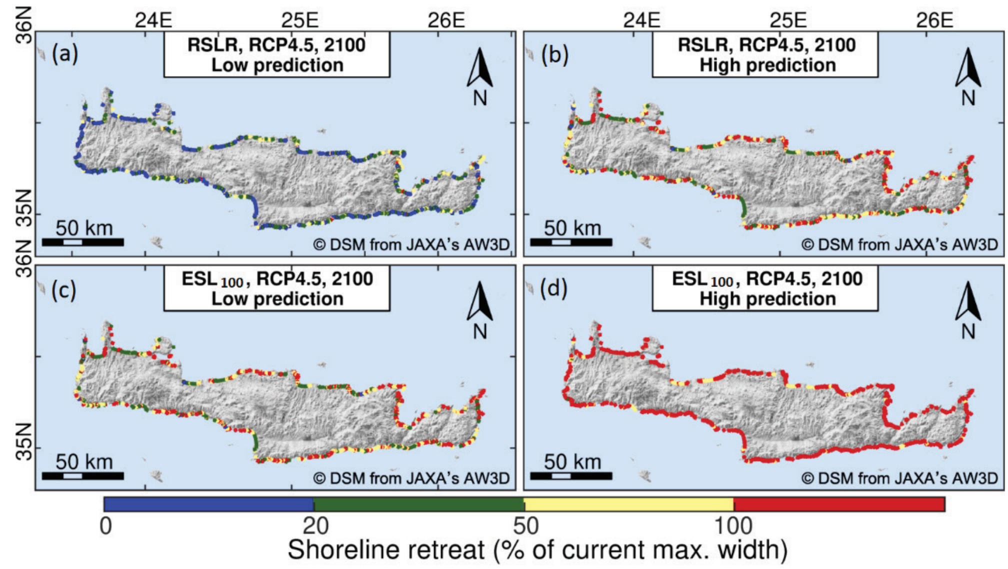

Fig. 2: The percentages of the current BMWs of the 828 Cretan beaches projected to be eroded under RSLR ((a) and (b)) and to be inundated under ESL_{100} ((c) and (d)) based on the low and high model estimates.

Regarding the flood hazard under extreme events, the results show that the baseline ESL_{100} (mean of the period 1980-2014) varies along the Cretan coastline, with the highest values found along the southern and eastern coast (up to 1.6 m above the mean sea level) (Fig. 3). By 2100, ESL_{100} at 6 seaports and 3 airports in Crete will increase on average by 45 cm under the RCP4.5 and medium ice melt scenario and by 93 cm under the high-end scenario (RCP8.5 and high ice- melt scenario) (Table 2). The assessment of future extreme heat waves for the same seaports/airports indicates that heat wave events will increase in both magnitude and frequency. Heat waves having the magnitude of the baseline 1 in a 100 years heat wave (the mean of the 1976-2005 period) are projected to occur on average every 12.8 years under SWL scenario of 1.5 °C (expected to be reached by the 2030s, see IPCC (2018)); every, 5.4 years under a SWL of 2 °C (expected by the 2050s), and every 1.4 years under a SWL of 3 °C (expected in the beginning of the next century) (Table 2). It is also projected that the 100-year heat wave will increase in magnitude on average by 2, 3 and 7 times respectively under the same scenarios.

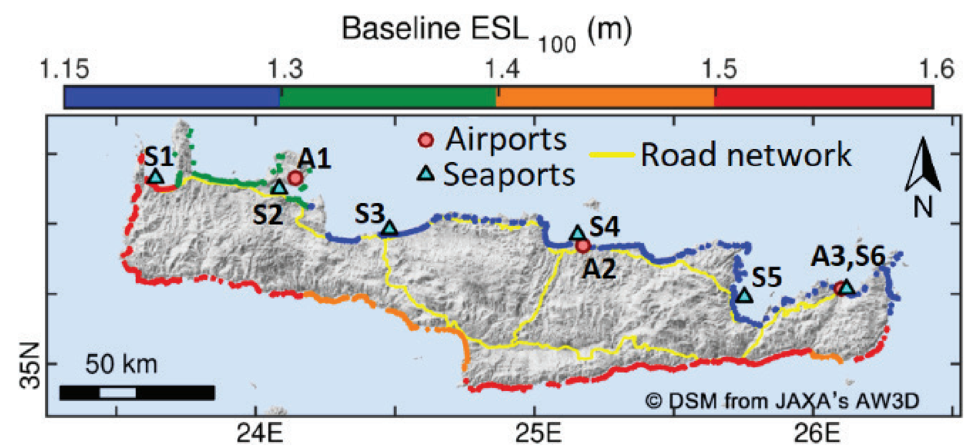

Fig. 3: Baseline ESL_{100} (mean of the period 1980-2014) along the coast of Crete. 6 seaports (S1: Kissamos, S2: Souda, S3: Rethymnon, S4: Heraklion, S5: Agios Nikolaos, S6: Sitia) and 3 airports (A1: Chania, A2: (current) Heraklion, A3: Lassithi).

Table 2. Future changes of ESL_{100} and 1-100 year Heat Wave at 6 seaports and 3 airports of Crete. Key: T_r = return period.

Seaports/ Airports ID	**ESL_{100} increase (cm) for 2100**		**1-100 year Heat wave**					
		Increase (times)			**New T_r (years)**			
	RCP4.5 med. ice	RCP8.5 high ice	1.5 °C	2°C	3°C	1.5 °C	2°C	3°C
S1	38	79	3	5	12	1	1	1
S2, A1	45	90	2	2	3	12	7	1
S3	46	92	2	3	8	4	1	1
S4, A2	49	102	1	2	2	36	15	3
S5, A3	46	99	2	2	3	19	8	1
S6	45	98	3	5	13	4	1	1

4. Conclusions

The results show that the coastal zone of Santorini and Crete faces significant threats from different hazards, which are projected to be exacerbated by CV&C. In the absence of adequate inland accommodation space for beaches to roll-over and/or effective coastal protection schemes, the RSLR will potentially have devastating effects on coastal natural and human systems. Tourism, for example, will be particularly affected by the projected large decreases in dry beach width, a critical control of the beach resilience and recreational use, and the long-term recreational value of the Santorini and Crete beaches and the value of associated assets may decrease considerably (e.g. Gopalakrishnan et al., 2011). Future extreme events will further exacerbate these impacts, with very severe flood damages and losses projected for the backshore infrastructure and assets, even if the fronting beaches would eventually recover. There will be also significant challenges for the critical infrastructure located at the coastal front, such as airports and seaports. The results show high and increasing potential impacts from climatic changes involving both flooding under extreme events and operational disruptions from the increasing magnitude and frequency of extreme heat waves.

5. Acknowledgements

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NEARSHORE HYDRODYNAMIC PROCESSES RELATED TO SHORELINE RETREAT DUE TO SEAL LEVEL RISE INDUCED BY CLIMATE CHANGE, ALONG THE NORTHERN COAST OF MESSINIAKOS GULF (IONIAN SEA)

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Abstract

In this paper we study the hydrodynamic and morphodynamic characteristics along the northern coast of the Messinian Gulf. The beach zone under investigation extends from Messini to Kalamata with a West – East trending shoreline a length of 21 km and an average width of 20 m. It is exposed to southerly wind-generated waves, with average significant wave height of 1.2 m and period of 7.6 s. The subaerial beach zone gentles smoothly, having rhythmic morphological features at its central and western part such as beach cusps, berms and crescentic / longshore bars, while low dune, river mouths and artificial coastal structures are also present. The central part is sandy with the westernmost beach sector being characterized by coarse-grained material due to wave energy concentration following the construction of the Kalamata port. In addition, shoreline retreat estimates due to 0.5m and 1m sea level rise until 2100, show that beach loss may account from 10% (SLR=0,5 m) up to 100% (SLR=1m).

Keywords: wave regime, nearshore morphodynamics, potential sediment transport, sea-level rise.

1. Introduction

The study area is the shore zone along the gulf of the northern Messinian gulf; a cuspateembayment, delimited by two natural headlands, Akrita and Tainaro (Fig. 1). The overall coastal geomorphology is the output of the interaction of geological processes, nearshore hydrodynamics and, riverine sediment supply (YPEKA, 2017). Continental shelf (<100 m) at its western part deepens gently with slopes <5%. In contrast, theeastern part is characterized by variable slopes exceeding 20%; the later morphology is associated with the presence of a submarine valley system (Pavlakis *et al.*, 1989).

The scope of the present study is to investigate the coastal hydrodynamic regime that affects beach evolution as well as to estimate the potential coastal retreat due to the anticipated sea level rise induced by climate change.

2. Material and Methods

2.1. Field sampling and laboratory analysis

The fieldwork conducted on 3rd – 4th and 24th of November, 2017, included subaerial and underwater sedimentological and geomorphological measurements on 19 shore-normal profiles (A to S, from east to west) (Fig.1). Surficial sediment samples were selected along each profile; one at the upper subaerial part of the shorezone (approximately 1 m above sea-level), one at the shoreline (beach face) and one seabed sample within the breaker zone (approximately 2 m depth). Sediment samples >63μm were subjected to dry sieving according to the Folk (1974) procedure, whilst sediment samples (<63μm) were analyzed with a Sedigraph-Micrometrics using X-rays (type III PLUS).

2.2. Wave data and analysis

For the need of the hydrodynamic analysis, the study area was divided into five sections (Fig. 1). The wave data (3-hour measurements) heights, period and direction were obtained from an offshore buoy (K2: 36° 45´N, 22° 6´E) for the period 1995-2004 (Poseidon/HCMR). The analysis of the wave data included wave frequency (f%), mean significant wave height (Hs), period (T) and median dominant direction (MWD). The aforementioned values were calculated for each section for both the average and extreme wave conditions. Wave parameters were calculated on the basis of Komar (1978), for intermediate depths (1/20 L < d < L/2) and for shallow water (d < 1/20 L) for each sector. Wave run-up was calculated using the Stockdon *et al.* (2006) equation, while the rate of longshore sediment transport was estimated following the equations of CERC (1974).

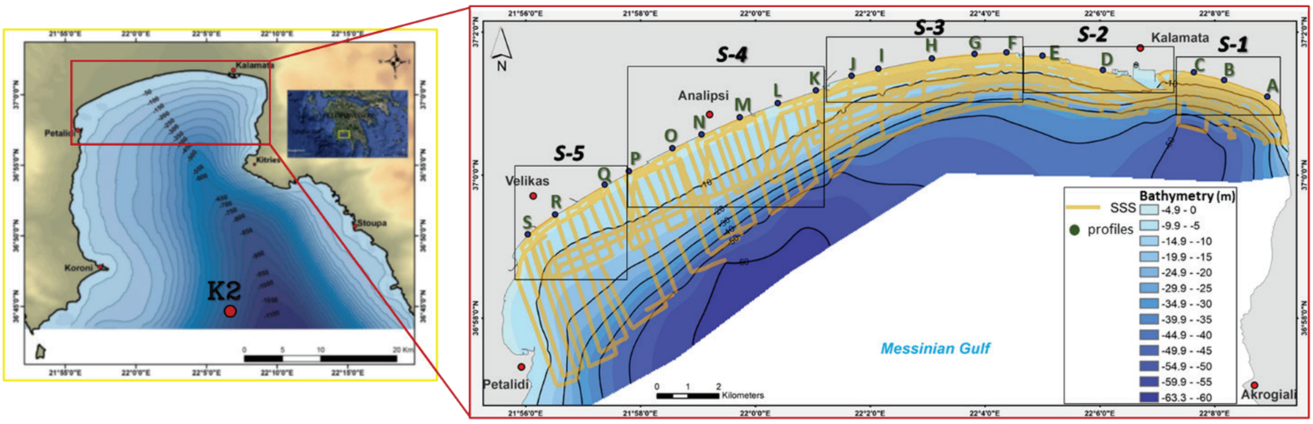

Fig. 1: Study area location (left: general bathymetry map of Messinian Gulf, right: shallow bathymetry map, green points showing the cross-shore profiles and squares showing each Sector coverage).

2.3. Estimation of shoreline future retreat due to potential sea-level rise

In order to quantify the consequences of a potential sea-level rise the arithmetic models of Bruun (1962), Dean (1991) and Kriebel & Dean (1985) were applied. The aforementioned models require the estimation of closure depth, defined based on Hallermeyer (1981, 1983) equation and breaker depth, for which breaking height and breakering wave index were calculated according to Battjes (2003) and McCowan's revised formula (1981) by Madsen (1976), respectively.

3. Results

3.1. Morphology – Granulometry

The length of the coastline is 21.3 km, having an average beach width ranging between 13 m and 27.1 m, and altitude between 1.4 m and 2.4 m. In addition, rhythmic beach formations (berms, cusps) with heights of 0.5-1.5m are located almost along the entire length of the swash zone, as well as dune fields of 2-3 m height (S3, S4); at the western and central sector. The topographic slopes range from 9% to 11% for the subaerial zone, 13% to 20% for the beach face, and 1-3% for the nearshore zone.

The longshore spatial variability of the sediment texture showed the predominance of sand [gS, (g)S], with some exceptions at the eastern part where gravelly sediments are present (sG, G) (Fig. 2). The shoreline material consists mostly of coarse sand [gS, (g)S, S] and in some locations of gravel (sG, G). The subaqueous part is divided in 3 zones (Z): Z-1 (up to a depth of 4-5 m) consisting of sand; Z-2 (depths from 5 to 18 m) consisting of muddy sand (mS) mud or sandy mud (M, sM); and Z-3 (depths > 18 m) consisting of mud (M).

3.2. Wave regime

The predominant offshore wave directions are southerly, while the analysis of the wave data showed that sections 3 and 4 are exposed to the highest frequency of the incoming offshore waves, at a rate of about 17.2% and 16.3%, respectively. The corresponding Hs is 1.3 m with T of 6.9 s. The maximum calculated wave height (storm waves) is 3.3m, with a frequency of occurrence ranging between 0.8% and 1.3% and periods of > 8s, approaching from 170° (Table 1). The breaking depth under average wave conditions is about 2 m, while for storm waves increase to about 4 m. The closure depth ranges 5.5 m and 6 m, at a distance of approx. 100 m (east of the Port of Kalamata) to more than 500 m at its central part with respect to shoreline. The maximum wave run-up (R_2%) for storm waves is estimated to be approximately 2 m.

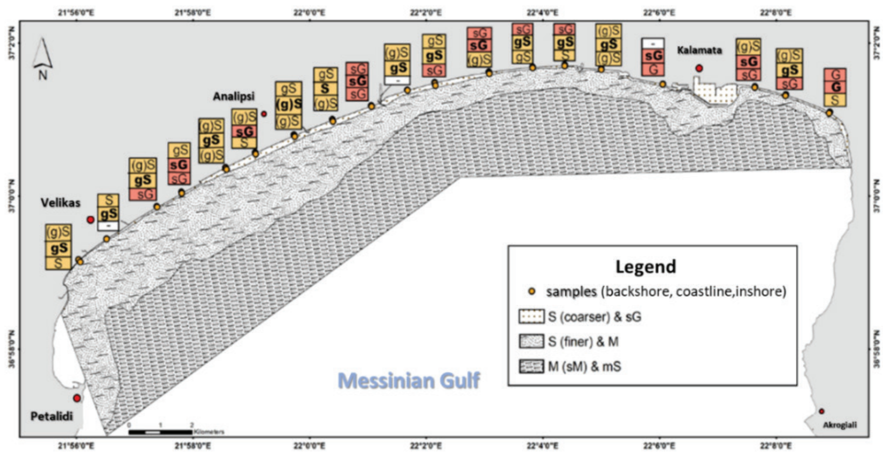

Fig. 2: Characterization of sediments (orange dots) in representative locations on the land, in the coastline and in the breaking zone, as well as the spatial distribution of the submarine samples (3 zones) of the study area.

Table 1. Physical characteristics of the incident waves [Hs, He, T wave period, hc closure depth, Hb wave breaking height, db breaking depth, $R_{2\%}$ maximum wave run-up.

Condition		*f(%)*	*H (m)*	*Tp (s)*	*hc (m)*	*Hb (m)*	*db (m)*	*R2% (m)*	*Retreat (m)*	*Beah Loss (%)*
T-1	Average	10,5	1,27	6.79		1.5	1.8			
	Extreme	1.3	2,74	8.01	5.43	3.0	3.5	1.8	7.0	31.1
T-2	Average	12,6	1,37	6.99		1.59	2.1			
	Extreme	1.1	2,86	8.53	5.74	3.11	4.1	2.5	14.4	88.4
T-3	Average	17.2	1.24	6.91		1.5	1.9			
	Extreme	0.8	2.87	8.59	5.77	3.1	4.1	2.3	17.1	69.6
T-4	Average	16.3	1.19	6.89		1.4	1.8			
	Extreme	0.8	2.82	8.50	5.67	3.1	4.0	2.1	21.4	79.2
T-5	Average	14.7	1.14	6.84		1.4	1.8			
	Extreme	0.9	2.70	8.96	5.52	3.0	4.0	1.9	35.4	100

3.3. Prediction of coastline retreat with accelerating Sea-Level rise

To quantify the consequences of a potential sea-level rise in the Petalidi-Messinia coastal zone, statistical models of Bruun (1962), Dean (1991) and Kriebel & Dean (1992) were applied, which are based on morphological alterations of the cross-shore beach profile due to the sea-level rise. The highest retreat values are calculated by the model of Bruun (1962), while the lowest by Kriebel & Dean (1993). Specifically, under the conservative scenario of 0.5m sea-level rise, the average potential shoreline regression, relative to the baseline year 2100, is projected to be 23.8 m and 12.4 m, respectively. With regard to the extreme scenario of 1 m, the reduction of beach width is estimated at about 48 meters on average by Bruun and 26 m by Kriebel and Dean. Eventually, the greater coastline retreat is estimated to occur at the western part of the coast, leading to 100% land loss (Table 1).

4. Discussion/Conclusion

The morphological characteristics along the beach zone are governed by the existing hydrodynamic processes (wave transformation and breaking) induced by the incoming southerly waves. Coastal infrastructures (port and marina of Kalamata, groynes and jetties) have a significant effect on the nearshore wave regime. The latter is characterized by significant waves heights of about 1 m, while the highest incoming waves exceed 2.5 m. Waves affect seabed morphology from a depth of about 6 m, breakat depths of 2-4 m and run-up to 2-2.5 m. The longshore spatial variability of the sediment texture showed finer sediment in the central part of the shore becoming coarser towards both ends, whilst the eastern end of the beach consists mainly of gravels. Seabed sediment from sand, to sandy mud Estimates for shoreline retreat at 2100 due to sea level rise, for the conservative scenario of SLR=0.5 m, show an average retreat of 24 m, whilst for the extreme scenario of SLR=1 m it reaches the 48 m (on average). Moreover, the most vulnerable sector is the western part that would be subjected to the greatest regression up to100% of the beach width.

5. Acknowledgements

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LATE HOLOCENE TO FUTURE SHORELINE RETREAT IN THREE TOURISTIC BEACHES OF MYKONOS ISLAND, BASED ON BEACHROCK POSITION AND UPCOMING SEA LEVEL RISE

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Abstract

The study of touristic beaches focusing on their response to the anticipated sea level rise is essential for the maintaining of their morphological characteristics and, consequently, the profitability from their use. In this work, a detailed survey was conducted by the use of both aerial and underwater vehicles mapping submerged beachrock formations, as well as other underwater morphological features of the highly touristic beaches of Agios Ioannis, Agios Sostis and Kalafatis, in Mykonos Island, central Aegean Sea, in order to plot relative sea level curves since Holocene and predict the beach area loss, due to the forthcoming sea level rise by 2100, that will have an immediate impact to the economy of the island.

Keywords: Sea-bottom topography, Sea level changes, Beach erosion, Holocene, Coastal morphology.

1. Introduction

The Mykonos Island is one of the most popular touristic destinations of the Mediterranean Sea. As stated by the Institute of Greek Tourism Confederation (INSETE, 2020), in 2019, that is the last pre-Covid year, about 3 million tourists, native and foreign, visited the island, mainly to enjoy its beaches. According to Velegrakis *et al.* (2005), the economic value of the average Greek touristically organized beaches is about €1400/m^2/year, whereas in some cases (including some of the most famous beaches of Mykonos) this worth can reach up to €60000/m^2/year. Therefore, it is crucial for the stakeholders to manage the beaches sustainably, not only for immediate profit, but also for future incomes. Hence, the study of the long-term past (last millennia) and future (till 2100) evolution of a beach, in particular its subaerial part, is of high importance. In order to study the shift of the beach areas, one has to look to the past (Holocene sea-level fluctuation) and to the future (sea-level rise). Beachrocks (compact coastal sedimentary lithified formations) are commonly used for dating past shorelines, on the aspect of sea-level alteration rates during Holocene (i.e., Saitis *et al.*, 2022; Alexandrakis *et al.*, 2021; Karkani *et al.*, 2017; Vousdoukas *et al.*, 2007). In addition, according to IPCC (2014), the mean global sea-level rise is projected to reach up to 30–80 cm above the present level by 2100.

In this study, high-resolution bathymetric data was combined with UAV imagery and future sea level rise (SLR) predictions to develop the evolution pattern of three touristic beach areas, Agios Ioannis, Agios Sostis and Kalafatis, in Mykonos Island (central Aegean Sea), from ~4.000 BP to 2100 AD.

2. Material and Methods

The detailed bathymetric maps of the beaches were recorded with the use of an R2Sonic2022 Multibeam echo sounder (MBES), at depths ranging from ~0.5 m to 10 m, with a total resolution of 0.2 m, in September 2019 (Fig. 1). The UAV imagery was acquired with a Phantom 4 Pro UAV. The wave regime has been calculated using the CERC (1984) semi-empirical equations, utilizing the local wind data (mean annual frequency of wind speed and direction) provided by the Wave and Wind Atlas of the Hellenic Seas of the Hellenic Centre for Marine Research (Soukissian *et al.*, 2007), nodal point H3 for the Agios Ioannis beach and nodal point M4 for the Kalafatis and Agios Sostis beaches. The sea-level rise predictions for 2100 was acquired from the Intergovernmental Panel on Climate Change (IPCC, 2014). For this study, emission scenarios RCP4.5 (intermediate scenario) and RCP8.5 (extreme but very possible scenario) were used, predicting an average SLR of 0.47 m (for RCP4.5) and 0.63 m (for RCP8.5), by 2100. The beach retreat was calculated according to Bruun's rule $R=L_{hc}/(B+h_c)S$, where R is the beach retreat, L_{hc} the cross-shore distance to the closure depth (h_c), B the elevation of the beach berm above the mean sea level, and S the SLR. (Bruun, 1962).

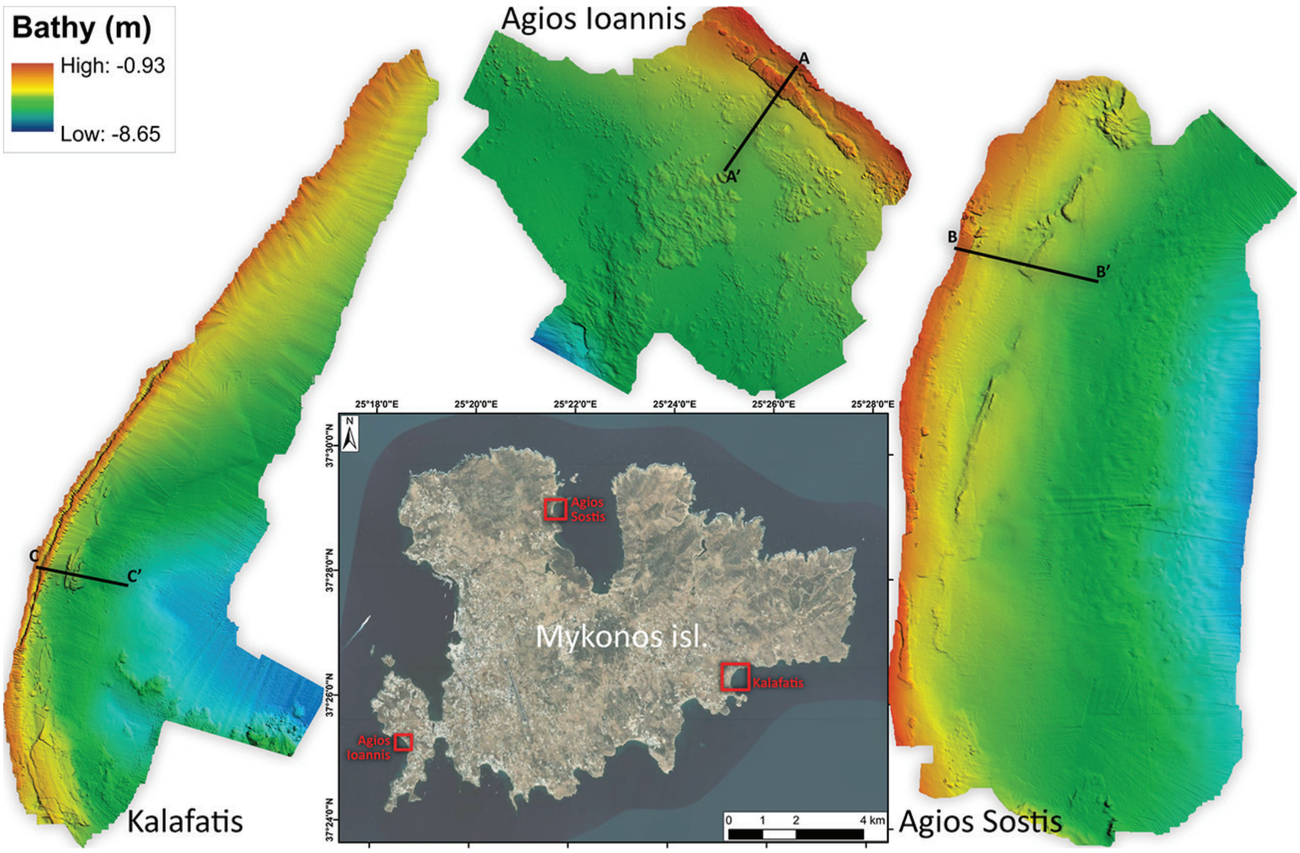

Fig. 1: Multibeam echo-soundings of the three studied beach areas and positions of topographic cross-sections.

3. Results

The detailed DTMs of the beach areas (Fig. 1) allowed the creation of cross-sections, vertical to the beach, for the identification of the three rows of beachrock formations previously studied and dated by Desruelles *et al.* (2004). As seen in Fig. 2, the younger beachrock (BR1) is located 5 – 15 m away from the shoreline, in an average depth of 1.3 m, having a width ranging between 7 and 20 m, with a dated age of ~1330 years BP, the second (BR2) is located 25 – 35 m away from the shoreline, in an average depth of 2.4 m, having a width varying between 5 and 30 m, with a dated age of ~3185 years BP and the older (BR3), which is present only in Agios Ioannis beach, is located 42 – 52 m away from the shoreline, in an average depth of 3 m, revealing a width 25 m, with a dated age of ~4860 years BP. All aforementioned dates are uncalibrated. All beachrock formations lie parallel to the present shoreline. According to the radiocarbon dating, the sea level rise of the beach areas of Agios Sostis and Kalafatis has been calculated to be 0.09 m / 100 ys, with a rather stable rate throughout the last 3500 years. On the contrary, the relative sea level curve of the Agios Ioannis beach has a relatively slower rise, at about 0.06 m / 100 ys. The latter is probably due to the tectonic regime of the SW peninsula of Mykonos Island, according to the tectonic map of the area (Avdis, 1983).

Regarding the annual climatic regime of the beach areas under investigation, the beach of Agios Ioannis is exposed to mild wind generated waves, as the adjacent island of Rinia provides a shadow to the SW part of Mykonos, with a fetch of ~ 4 km. The Agios Sostis beach is located at a similar environment, the western edge of the Panormos bay, in northern Mykonos, with a fetch of < 3 km towards NE. On the contrary, the Kalafatis beach is exposed to the open sea, with waves arriving from the SE. Due to its very long fetch (> 300 km), the waves arriving at the beach are ten times higher than the other beach areas under investigation. The beach of Kalafatis has waves with average height of 0.51 m, reaching up to 6.65 m, and corresponding wave periods of 3.6 s and 10.9 s, respectively (Table 2). The other two beaches receive waves with average heights between 0.1 and 0.3 m, and maximum heights of 0.62 m.

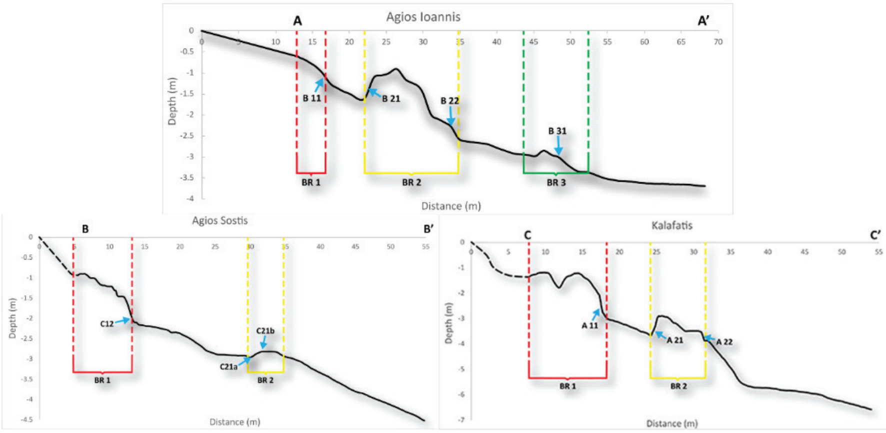

Fig. 2: Cross-sections of the beach areas and beachrock formations. For sections positions, see Fig. 1. Codes with arrows represent dated beachrock samples (Desruelles et al., 2004). B11: 1775 ± 40 ys BP; B21: 2465 ± 45 ys BP; B22: 2265 ± 30 ys BP; B31: 4860 ± 35 ys BP; C12: 970 ± 35 ys BP; C21a: 1750 ± 25 ys BP; C21b: 3745 ± 30 ys BP; A11: 1330 ± 35 ys BP; A22: 3185 ± 45 ys BP.

Table 2: Wind / wave annual regime of the studied beach areas. Grey cells: average conditions; white cells: max conditions.

MAIN WIND DIRECTION (%)	WIND SPEED U (*m/s*)	ANNUAL FREQUENCY %	FETCH (*km*)	WAVE PERIOD T_S (*sec*)	SIGNIFICANT WAVE HEIGHT H_S (*m*)
AGIOS IOANNIS	7-8	2.8	4.33	1.90	0.28
SW (25.3%)	14-16	0.3		2.52	0.67
AGIOS SOSTIS	4-5	1.3	2.73	1.33	0.12
NE (8.6%)	16-18	0.1		2.28	0.62
KALAFATIS	4-5	1.3	315.27	3.56	0.51
SE (8.6%)	16-18	0.1		10.91	6.65

According to the aforementioned sea level rise scenarios RCP4.5 (moderate scenario) and RCP8.5 (extreme but more possible scenario) (IPCC, 2014) and applying the Bruun's rule, the shoreline retreat at the end of the 21st century was calculated for each beach. Thus, the Agios Sostis beach presents the lower shoreline retreat (9.1 m for RCP4.5 and 13.3 m for RCP8.5), whereas Kalafatis presents the higher retreat (15.1 m for RCP4.5 and 22.1 m for RCP8.5). Agios Ioannis presents values between the aforementioned beaches (13.5 m for RCP4.5 and 19.8 m for RCP8.5), but due to its small beach area, 2358.3 km^2 in comparison with the 10146 km^2 of Agios Sostis and 15757 km^2 of Kalafatis, it will lose the least land area by 2100.

4. Discussion/Conclusion

The relative sea level curves of the last 4500 years at Agios Sostis and Kalafatis beaches, derived from the dating of the beachrock formations, match perfectly with the relevant general curve of the Attico-Cycladic region (Central; Aegean Sea) provided by Poulos et al. (2009), whereas the Agios Ioannis curve presents a gentler slope, due to the aforementioned tectonic regime of its surrounding area (see Fig. 3).

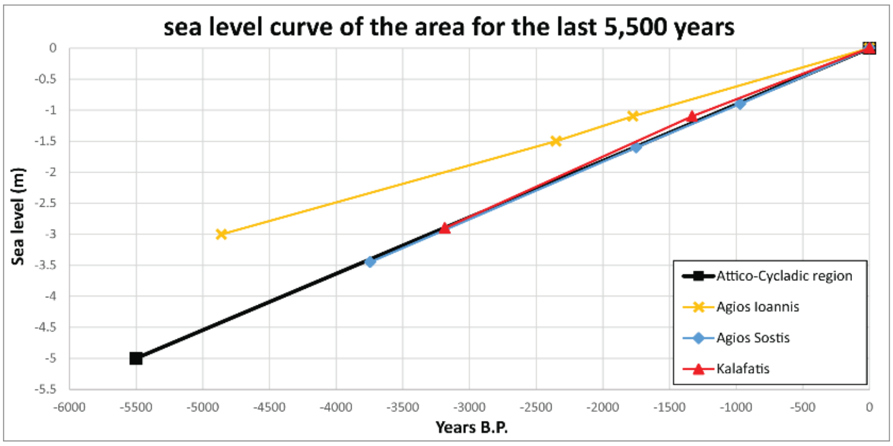

Fig. 3: Sea level curve of the Attico-Cycladic region after Poulos et al. (2009) and superimposed the dated beachrocks of the three beach areas under investigation.

Concerning the beaches' response to the anticipated sea level rise by 2100 (Table 2), Agios Ioannis may lose 84% of its initial area according to the RCP4.5 scenario, whereas according to the RCP8.5 scenario the whole beach area will be vanished. Similarly, Kalafatis beach will lose 50% and 3/4 of its initial area according to RCP4.5 and RCP8.5, respectively. On the contrary, Agios Sostis beach will lose 1/4 and 34% of its initial area with a SLR of 0.43 m (RCP4.5) and of 0.63 m (RCP8.5), respectively, by 2100. The beach area loss that is expected to occur due to the anticipated SLR will have a huge economic impact to the island's income, since beach areas are the main attractions of the island and, hence, its most important asset.

Table 2: Aggregated values of beach area losses in 2100, according to the RCP4.5 and RCP8.5 scenarios, (IPCC, 2014).

BEACH	INITIAL AREA (m^2)	SCENARIO	SLR IN 2100 (m)	RETREAT (m)	BEACH LOSS (m^2)	% OF BEACH AREA IN 2100
AGIOS IOANNIS	2358.3	RCP4.5	0.43	13.52	2010.3	16.3
		RCP8.5	0.63	19.81	2989.7	0.8
AGIOS SOSTIS	10145.8	RCP4.5	0.43	9.1	2404.9	76.6
		RCP8.5	0.63	13.33	3539.4	65.8
KALAFATIS	15756.7	RCP4.5	0.43	15.05	9360.5	43.1
		RCP8.5	0.63	22.06	13765.9	22.6

5. Acknowledgements

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ESTIMATES FOR BEACH RETREAT IN EIGHT POCKET BEACHES OF THE SW MESSINIA (IONIAN SEA, GREECE)

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Abstract

The present investigation estimates shoreline retreat in eight beach zones of the SW Peloponnese in response to sea level rise for the year 2100, according to the latest SSP scenarios of climate change (IPCC, 2021), considering beach zone hydrodynamics and morphodynamics. The analysis showed that beach retreat varies from 7 m up to 25 m for SLR=0.28 m, from 12 m to 53 m for SLR=0.60 m and from 21 m to 91 m for SLR=1.01 m; these values correspond to a minimum reduction of the maximum beach width of 9%, 20% and 33.5%, respectively. Thus, 5 from the 8 beaches may experience a total loss for the worst-case scenario of SLR=1.01 m (Voidokoilia, Divari, Gialova, Foiniki, Tsapi beaches), whilst Voidokoilia and Divari may be totally eroded even in the case of the moderate scenario.

Keywords: Ionian Sea, retreat, climate change, sea level rise.

1. Introduction

Coastal - marine and terrestrial - processes acting in different temporal (seconds to decades) and spatial (few meters to km) scales, are mainly defined by the incoming waves and their transformation and breaking conditions that induce nearshore currents and sediment transport; the latter refers to terrestrial sediment delivery (i.e., cliff erosion, river inputs), but also to various human interventions such as coastal works (e.g., ports, piers, coastal roads). In addition, sea level rise due to climate change and/or variability will have adverse effects on beach zone evolution and on their ecosystem services (i.e., coastal tourism). The scope of the present study is dual: (i) to describe the morphodynamic conditions in eight beach zone of the SW Peloponnese (Greece), with different morphological characteristics and degree of exposure to wave activity and (ii) to estimate their potential retreat in response to sea level rise for the year 2100, according to the latest SSP scenarios (IPCC, 2021).

2. Study Area

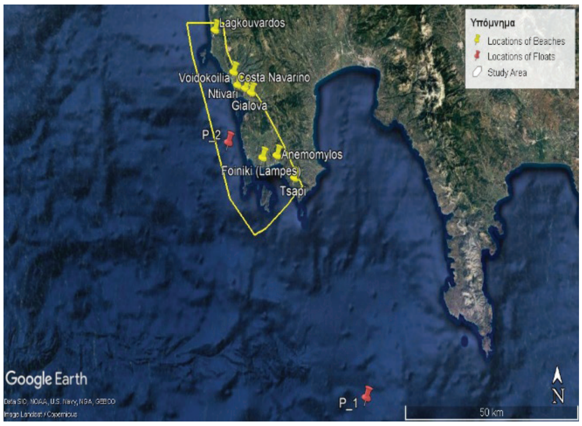

Fig. 1: Study area's map including the locations of beaches and of floats (HCMR).

The beach zones under investigation are located at the SW coast of Peloponnese facing the NE Ionian Sea (Fig. 1). The eight beaches are exposed to offshore incoming wind-generated (primarily) waves with westerly and southerly directions, apart from Gialova and Divari beaches that are located within the semi-enclosed embayment of Navarino bay, wherein depths exceeds the 60 m at its southern part. Tidal range, having amplitude less than 20 cm (Tsimplis, 1994), is not considered. Generally, Messinia landscape is characterized by complex landforms formed by tectonic activity, erosion and depositional processes. In the Pylia peninsula the main rivers and torrents, i.e., the Minagiotiko steam, the torrent of Methoni, the Xerias river and the Giannouzagas river are observed. In addition, vegetated sand dunes with significant signs of damage by human activity are evidenced in the beaches under investigation. In addition, a lagoon is present behind Divari and Voidokoilia beaches, whereas in Costa Navarino, Voidokoilia and Anemomylos beaches a river is observed.

3. Data collection and Methodology

Beach elevation was measured along shore-normal profiles with the use of a Leica Distance Meter (subaerial part) and a Portable EcoSounder Hondex bathymeter (subaqueous part). Along the profiles surficial sediment samples were collected and, subsequently, analysed granulometrically by dry sieving according to Folk's (1980) procedure. The locations of the sediment samples are presented on the topographic profiles of Figure 2. Reanalysis wave data obtained from the POSEIDON system of HCMR for two locations (see fig.1): P-1 (36° 17' N και 22° 05''E) and P-2 (36° 50''N και 21° 38' E) for the period 01/01/1993 - 31/12/2016. The data set included hourly values of significant wave heights (SWH in m), period (Tp in sec) and wave direction (MDW in degrees). Accordingly, the two-time series were analysed in terms of frequency of occurrence for the different wave directions, significant wave height ($H_{1/3}$), wave height of the highest 10% ($H_{1/10}$) and maximum (He) waves (H_{extr}) along with their associated periods ($T_{1/3}$, $T_{1/10}$ and Te) and the corresponding median wave direction. To estimate the potential retreat of all beaches to the various IPCC (2021) SSP scenarios (SSP1a-1: SLR = 0.28m; SSP2-4.5: SLR = 0.60m; SSP4-8.5: SLR = 1.01m) the Bruun (1962) and Edelman (1972) models were applied. The wave height at breaking zone (Hb) was calculated using Sakai & Battjes (1981) equations, the breaking parameter (γ) from the subaqueous beach slope based on Madsen (1976) equation the breaking depth according to McCowan (1894) equation ($\gamma = H_b / d_b$), whilst the depth of closure (d_c) according to Hallermeier's (1981) equation. Finally, the surf similarity parameter (ξ_o) was estimated using Iribarren & Nogales (1949) equation, assuming Ho' reduced by 10% due to shoaling.

4. Results and Discussion

4.1. Beach zone morphology

Lagkouvardos beach presents a gentle slope at the terrestrial part (2.3%) with the major berm at 1.80 m height at 58.50 m distance from the shoreline and a dune mapped at 1.90 m height (foot of the dune) at 80.50 m distance from the shoreline. Its submarine part slopes with 3.3%, whilst two bars are observed at 40 m and 60 m distance from the coastline. Costa Navarino beach presents a similar morphology to Lagkouvardos at its subaerial part with relatively smooth slope and dunes up to 2.4 m height with its foot at a distance of 61 m from the shoreline. The major berm (at 1.60 m height) was at 10 m distance from the shoreline. The submarine topography is quite steep (6%) up to 60 m (depth), where it changes to 2.2%), whilst it presents an extensive bar at about 120 m distance from the shoreline. The Voidokoilia beach presents a rather smooth -both subaerial and subaqueous- and uniform topography (slopes of 1%) with the major berm at 1.80 m height at distance of 23 m from the shoreline; its backshore zone hosts dunes of about 1.80 m height. Divari beach is part of a barrier island having subaerial slope of 3.8% and hosting dunes of about 1 m height. The major berm is formed at 1 m elevation and a distance of 26 m from the shoreline. Underwater slope is 1.5% while a bar appears at 80 m distance from the shoreline. The subaerial part of Gialova presents 7% slope, with the first major berm of 0.7 m height located at 10 m distance from the shoreline. Small dunes (tens of centimeters) are present at its backshore. The subaqueous slope is 2.2% includes a bar at 80 m distance from the shoreline. Foiniki (Lampes) beach has a much steeper terrestrial topography (14%), with the first berm at 0.80 m height, while dunes of 2.25 m height exist at 16.5 m distance from the shoreline. Its submarine part slopes with 2% and a bar being formed at 20 m from the shoreline. The terrestrial part of Anemomylos beach presents 6% slope, the major berm is at 1.50 m height and the foot of the dune at 2.1 m at a distance of 14 m and 35 m from the shoreline, respectively. The submarine slope is also rather steep (4%), having a bar at about 60 m from the shoreline. Tsapi beach presents terrestrial slope of 5% with the major berm at an elevation of 1 m and dunes at 1.80 m height at a distance from the shoreline of 31 m and 37 m, respectively. Underwater morphology is smooth (slope: 2%) with a bar at a distance of 15 m. Granulometrically, Lagkouvardos, Costa Navarino and Anemomylos beaches consist of three types of sediments (sG, gS, sgS), Voidokoilia, Divari, Foiniki and Tsapi beaches include two types of sediments ((g)S, S, mS), whilst Gialova beach has four types of sediments ((g)S, S, mS, sM). The observed differences in granulometry are the result of the different degree of exposure to incoming waves, nearshore bathymetry and the variable origin of beach sediment.

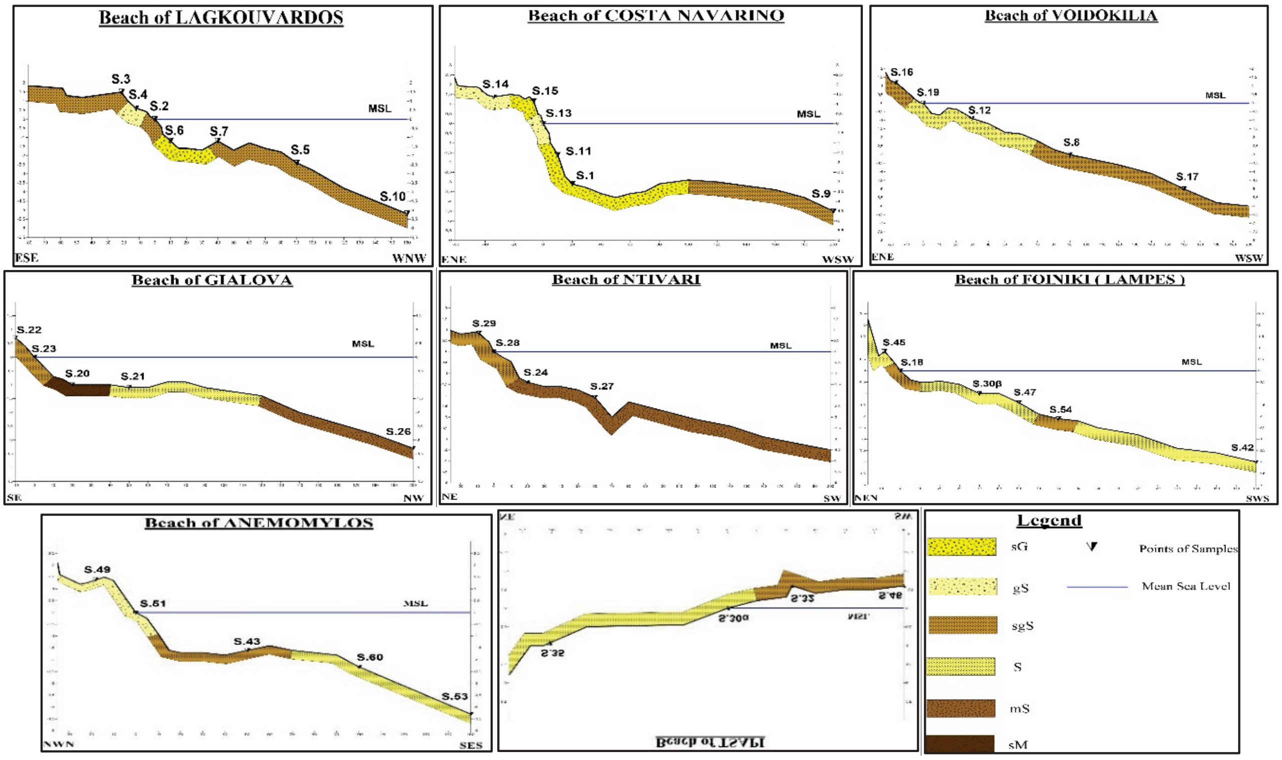

Fig. 2: Topographic profiles normal to shoreline with their granulometry (sG: sandy Gravel, gS: gravelly Sand, (sg)S: slightly gravel Sand, mS: muddy Sand, sM: sandy Mud).

4.2. Wave Regime

The extracted, by the two-time series wave regime (H: height, T: period and D: direction, f: frequency) is presented in Table 1, while in Table 2 the estimated key-morphodynamic parameters are given; the later has been used for the shoreline retreat estimates.

Table 1. Incoming (offshore) wave characteristics of the eight beaches.

Beaches	$H_{1/3}$ (m)	$H_{1/10}$ (m)	H_{extr} (m)	$T_{1/3}$ (s)	$T_{1/10}$ (s)	T_{extr} (s)	$f_{1/3}$ (%)	$f_{1/10}$ (%)	f_{extr} (%)	$D_{1/3}$ (deg)	$D_{1/10}$ (deg)	D_{extr} (deg)
Lagkouvardos	1.86	2.78	5.57	7.13	8.30	10.52	24.57	7.37	0.13	290	274	266
Costa Navarino	1.98	2.92	5.56	7.40	8.50	10.48	19.68	5.90	0.13	266	263	263
Voidokoilia	1.73	2.63	4.67	6.91	8.06	9.61	6.64	1.99	0.13	293	293	298
Divari	1.92	2.60	1.98	7.02	7.82	7.07	0.14	0.04	0.13	181	181	181
Gialova	2.20	3.01	3.25	7.55	8.19	8.39	0.66	0.20	0.13	205	205	205
Foiniki	2.22	2.98	3.38	7.34	8.10	8.54	0.94	0.28	0.13	201	202	201
Anemomylos	2.29	3.07	3.85	7.14	7.83	8.56	1.93	0.58	0.13	163	161	158
Tsapi	2.36	3.30	5.33	7.90	8.90	10.59	7.76	2.33	0.13	246	251	256

Table 2. The hydrodynamics and morphodynamic parameters of the eight beaches.

	Lagk.	C. Nav.	Void.	Ntivari	Gialova	Foiniki	Anemo.	Tsapi
H_b (m)	3.2	3.3	3.0	2.9	3.4	3.3	3.4	3.7
d_c (m)	12.5	12.5	10.5	4.5	7.3	7.6	8.6	12.0
ξ_0	0.21	0.14	0.07	0.10	0.13	0.12	0.24	0.13

4.3. Shoreline retreat due to sea level rise

The application of Bruun (1962) and Edelman (1972) models provided retreats of the shoreline for sea level rise of 0.28 m (scenario SSP1a-1), 0.60 m (scenario SSP2-4.5) and 1.01 m (scenario SSP4-8.5) the average values of which presented in Table 3.

Table 3. Estimated beach retreat (R in m) for the year 2100, induced by sea level rise due to climate change, together with their corresponding percentages of beach loss (BL%), relative to current beach width BW (m)

SLR		Lagk.	C. Nav.	Void.	Ntiv.	Gial.	Foin.	Anem.	Tsapi
0.28	Aver. R	6.8	10.5	21.8	15.2	11.6	12.3	5.5	12.4
	BW / BL	80.5/8.4	61.1/17.1	23.3/93.4	25.8/58.9	10.0/100	16.4/74.9	35.0/15.8	37.3/33.1
0.60	Aver. R	14.8	22.8	47.3	33.2	25.3	26.8	12.1	26.9
	BW / BL	80.5/18.4	61.1/37.3	23.3/100	25.8/100	10.0/100	16.4/100	35.0/34.5	37.3/72.0
1.01	Aver. R	25.4	39.1	81.1	57.3	43.6	46.2	20.8	46.2
	BW / BL	80.5/31.6	61.1/63.9	23.3/100	25.8/100	10.0/100	16.4/100	35.0/59.3	37.3/100

5. Conclusions

Beach retreat varies from 7 m up to 25 m for SLR=0.28 m, from 12 m to 53 m for SLR=0.60 m and from 21 m to 91 m for SLR=1.01 m; these values correspond to a minimum reduction of the maximum beach width of 9%, 20% and 33.5%, respectively. Thus, 5 from the 8 beaches may experience a total loss for the worst-case scenario of SLR=1.01 m (Voidokoilia, Divari, Gialova, Foiniki, Tsapi beaches), whilst Voidokoilia and Divari may be totally eroded even in the case of the moderate scenario. It should also be mentioned that the above estimates are based on the assumption that there will be no sediment input, including that from the adjacent sand dunes. Therefore, the actual retreat ranges are expected to be smaller.

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HABITAT SUITABILITY CURVES FOR BENTHIC MACROINVERTEBRATES FROM A LARGE NORTH AFRICAN RIVER (OUM ER-RBIA, MOROCCO)

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Abstract

Benthic macroinvertebrates have been widely used as biological indicators, in riverine ecosystems, to provide information on the ecological response to natural and anthropogenic disturbances. Arid and semi-arid north African countries are facing severe climatic pressures, intensified by human activities, but regional research on the biological response to such pressures is limited. In this work, we collected abiotic data and benthic macroinvertebrates (BMs) from the mid-reaches of the Oum Er-Rbia River, Morocco. We used generalized linear mixed-effects models to search for statistically significant relationships between variables, and to develop habitat suitability curves for BMs and major drivers of their community structure (water depth, flow velocity, water temperature, substrate type). BMs habitat suitability was highest in shallow (<0.2 m), fast-flowing (0.5-0.75 m/s), rocky habitats (large stones). The developed habitat suitability curves can be used for implementing ecohydraulic simulations as mitigation tools for the sustainable management and conservation of these vulnerable arid/semi-arid ecosystems.

Keywords: HSCs, GLMMs, Morocco, Semi-arid climate.

1. Introduction

Flow variability is a crucial driver of the distribution, abundance and diversity of aquatic communities in riverine ecosystems (Allan, 1995). Among the variety of organisms that have been used for the exploration of the status and the structural-functional characteristics of aquatic ecosystems, research has largely incorporated benthic macroinvertebrates (BMs) as bioindicators, since their immense biodiversity and relevant environmental response can provide valuable information, especially in cases of climatic and human pressures (Karaouzas *et al.*, 2019; Theodoropoulos & Karaouzas, 2021).

Studies have shown that ecosystems in semi-arid to arid regions, such as the North African landscapes, are critically sensitive due to their potential transition to desert-like zones (Kacem *et al.*, 2019; Bedoui, 2020), a phenomenon commonly called desertification (Safriel, 2009). However, research on the response of BMs in arid/semi-arid ecosystems, that would inform proactive and reactive ecological management strategies in these areas, is rather limited.

The purpose of this study was to develop habitat suitability curves (HSCs) for major environmental and hydrological variables that affect the structure and distribution of BMs, ultimately aiming to model the ecohydraulic properties of the largest Moroccan river (Oum Er-Rbia) using BMs as bioindicators. This study could also be used for the implementation of relevant ecohydraulic simulations elsewhere in Morocco, in river basins of similar hydro-ecological properties.

2. Material and Methods

2.1 Study area

Our study area is located upstream of the Al Massira Dam (32° 30'N, 7° 30›W; Morocco), the second largest national artificial reservoir (Bousseba *et al.*, 2020), in the middle reaches of the longest perennial Moroccan river Oum Er-Rbia. The regional climate is described as semi-arid to arid (Bousseba *et al.*, 2020). We sampled BMs, as well as environmental and hydrological variables in 59 microhabitats between the cities of Lamrapta and Kasba Tadla (reach length: 50km; mean elevation: 450 m a.s.l.), in December 2021.

2.2 Data collection and analysis

BMs sampling was carried out in 59 microhabitats, using a 0.25 m x 0.25 m sampler with a mesh size of 500 µm. All samples were preserved in bottles containing 70% ethanol and were transferred to the laboratory for analysis. At each microhabitat, hydraulic variables (V: flow velocity, m/s; D: depth, m) were measured with a OTTC2-1® discharge measurement meter, while temperature (T: °C), conductivity (C: µs/cm) and pH were recorded with a Hanna HI9828/4-01® water quality multi-parameter probe. Moreover, substrate type (S) was visually identified based on the categories shown in Table 1. Afterwards, microhabitat suitability was calculated using two alternatives (normalized and standardized) of a widely used BM-based habitat suitability index (SI; Theodoropoulos *et al.*, 2018 and references therein), as follows:

$$Ks_i = 0.4n_i + 0.3H_i + 0.2EPT_i + 0.1a_i \text{ [1]}$$

$$Kn_i = 0.4\frac{n_i}{n_{i[max]}} + 0.3\frac{H_i}{H_{i[max]}} + 0.2\frac{EPT_i}{EPT_{i[max]}} + 0.1\frac{a_i}{a_{i[max]}} \text{ [2]}$$

$$Ks_i = \frac{\sum_{i=1}^{N} K_i}{K_{i[max]}} \text{ [3]}$$

where K_i is the habitat suitability of the i^{th} habitat; is the number of the BMs taxa (families); denotes the Shannon's diversity index; is the number of Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa; is the abundance of BMs taxa; N is the total number of the i^{th} habitats; while Kn_i and Ks_i express the normalized and the standardized SI, ranging from 0 (unsuitable) to 1 (optimal habitat). Finally, we used generalized linear mixed-effects models (GLMMs) to search for statistically significant relationships between the dependent variable (Ks) and each explanatory abiotic variable (V, D, T, S, pH, C), by simultaneously excluding the effects of other abiotic variables (included as random effects). Significant relationships were identified by calculating the p-value and the Cox-Snell pseudo-R^2 for each model. All analyses were implemented in the non-parametric R package 'Generalized Additive Models for Location, Scale and Shape' (GAMLSS; Rigby & Stasinopoulos, 2005). Moreover, the GLMMs-based SI curves were depicted in the two-dimensional scale, and we additionally applied a smoother function (i.e., GAM) to detect minor local variations of the data. All analyses and visualizations were performed using the R 4.0.5 free software environment for statistical computing and graphics.

Table 1. Substrate type classification scheme applied during sampling (Scheider *et al.*, 2010).

Substrate type (Descriptor)	Grain size (mm)	ID
Silt	0.001-0.0625	1
Sand	0.0625-2	2
Small gravel	2-6	3
Medium gravel	6-20	4
Large gravel	20-60	5
Small stones	60-120	6
Large stones	120-200	7
Boulders	>200	8

3. Results

The Ks index was more robust for our dataset compared to Kn, since statistically significant and strong relationships were observed ($p<0.1$ and pseudo-$R^2>0.6$) for the majority of the abiotic variables (Table 2). As a result, we used the Ks to produce the HSCs with the integration of GLMMs for depth, flow, temperature and the substrate type.

Table 2. Statistical parameters (p-value and pseudo-R^2) of the generalized linear mixed-effects models for the multivariate response of the normalized and standardized suitability index (Kn; Ks) and the explanatory abiotic parameters. The asterisk (*) indicates the level of statistical significance (p**<0.05; p*<0.1).

Abiotic parameters	**Kn** (normalized SI)		**Ks** (standardized SI)	
	p-value	**pseudo-R^2**	**p-value**	**pseudo-R^2**
Flow* (V; m/s)	0.315	0.46	0.07*	0.72
Depth* (D; m)	0.036**	0.51	0.00005**	0.61
Temperature* (°C)	0.434	0.56	0.0545*	0.6
Substrate* (S)	0.0004**	0.63	0.000765**	0.78
pH	0.409	0.53	0.401	0.59
Conductivity (C)	0.0006**	0.18	0.283	0.73

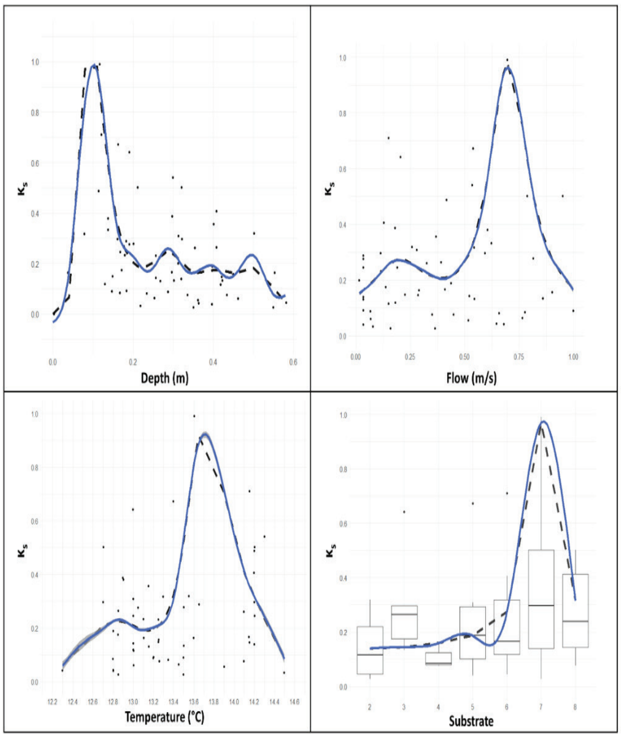

Fig. 1: Habitat suitability curves (HSCs; 0-1) for benthic macroinvertebrates in the Oum Er-Rbia River (Morocco; North Africa) based on the standardized suitability index (Ks) for water depth (m), flow velocity (m/s), water temperature (oC) and substrate type (S). The selected curves were produced and statistically validated using the generalized linear mixed-effects models (GLMMs, dashed black line; smoother, blue line; n=59).

BMs in the Oum Er-Rbia River had optimal habitat preferences in depths from 0 m to 0.2 m, while in deeper habitats there was a constant decrease of Ks (Fig. 1). Flow velocity suitability was optimal from 0.5 m/s to 0.75 m/s, while lower/higher velocity values indicated less-suitable preferences. Temperature suitability peaked at 13.6°C. Regarding substrate type, large stones (Table 1; ID=7) were characterized as the optimal suitable substrate type. All HSCs (Fig. 1) showed a unique peak ($Ks_{[max]}$), indicating a homogenous reaction to the environmental effects.

4. Discussion

We developed HSCs for BMs in Oum Er-Rbia, the largest perennial river in Morocco, setting for the first time the biological base for ecohydraulic simulations both across the specific river and in the wider North African region. Our comparative analysis between the investigated indices (Kn; Ks) showed that the standardized suitability index (Ks) fitted our dataset best. The response of BMs to depth, flow velocity and substrate is in agreement with previous works in Mediterranean basins (e.g., Greece: Theodoropoulos *et al.*,2018). More specifically, increased suitability was observed in shallow, fast-flowing habitats (Fig. 1), an expected outcome since this abiotic combination can facilitate ecological niches with adequate spatiotemporal supply of energy resources (e.g., detritus) and increased water purification rates (Khudhair *et al.*, 2019). Additionally, the large sized riffle-rocks (i.e., large stones; Table 1) was the optimal substrate, providing stable and constant conditions of substrate and flow, respectively, as well as protection from predators (Ramirez *et al.*, 1998; Theodoropoulos *et al.*, 2018). In accordance with the study of Karaouzas *et al.*, 2019, our analysis validated that BMs communities, and thus their habitat preferences, are highly influenced by hydrological-hydraulic drivers.

North African regions are facing climatic and human-exploitation related pressures, such as desertification (Kacem *et al.*, 2019; Bedoui, 2020), which may harm local human communities (e.g., increase poverty rates) and biodiversity as well (Reynolds *et al.*, 2007). Except for its ecological relevance, the outcome of this study can be used as the biological reference for the implementation of ecohydraulic simulations for mitigating the impacts of climatic pressures and/or for the formulation of conservation strategies for these highly vulnerable aquatic ecosystems.

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BLUE CARBON STOCKS IN SEAGRASS (*POSIDONIA OCEANICA*) MEADOWS OF THE SOUTH AEGEAN REGION

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Abstract

Seagrass meadows store large amounts of organic carbon in their underlying sediments, with meadows formed by the species *Posidonia oceanica* being considered among the largest blue carbon sinks. Lush *P. oceanica* meadows are found along the coasts of the South Aegean Region (Greece). However, little is known on the capacity of these ecosystems to store carbon. Here we assess the stocks of organic carbon in *P. oceanica* meadows of the South Aegean by studying a total of fourteen meadows extending around the Cyclades and Dodecanese Islands. The *P. oceanica* meadows of the South Aegean Region supported 8 Tg C_{org} in their top meter.

Keywords: Eastern Mediterranean Sea; climate change mitigation.

1. Introduction

Seagrass ecosystems rank among the largest blue carbon sinks (Fourqurean et al. 2012). Meadows of the iconic seagrass of the Mediterranean *Posidonia oceanica* (L.) Delile, in particular, are considered exceptional carbon sinks, storing up to 88 kg C_{org} m^{-2} in their top meter of sediment (Serrano *et al.*, 2016; Mazarrasa *et al.*, 2017; Apostolaki *et al.*, 2019; Monnier *et al.*, 2021). However, estimates are significantly biased towards the Western Mediterranean, while information from the Eastern Mediterranean meadows is lacking.

The South Aegean Region (Cyclades and Dodecanese Isalnads) hosts lush meadows, representing almost one-third of the total seagrass extent in Greece. (Panayotidis *et al.* submitted). The particular region is characterized by ultra-oligotrophic conditions and minimal riverine inputs, which result in exceptionally low turbidity and high light availability (Karageorgis *et al.*, 2008). Indeed, *P. oceanica* meadows located in this region are characterised as continuous and homogeneous, with high cover (> 80%) and shoot density (> 400 shoots m^{-2}), and generally exceed 30 m of depth or even locally reach down to 43 m (Gerakaris et al. 2021).

Here we measure dry bulk density and concentration of C_{org} down to 1 m of soil to determine the range of variability in C_{org} stocks across several seagrass meadows extending along the coasts of Cyclades and Dodecanese Islands.

2. Material and Methods

The study was conducted in fourteen meadows extending at 3 to 14 m water depth in the Region of the South Aegean. A total of nine sites were sampled along Cyclades Islands in August 2018 (Akrotiti-Santorini, Perivolos-Santorini, Keros, Milos, Serifos, Amorgos, Koufonisi, Ios, Kythnos) and five sites along Dodecanese Islands in September 2021 (Pefki-Rhodos, Apollakia-Rhodos, Kalami-Rhodos, Gyali, Nisiros). At each site, triplicate cores (PVC pipe with an inner diameter of 7 cm) were collected using manual percussion and rotation. The cores were inserted down to 1 m of sediment. Compression was estimated by measuring the outer, inner, and total length of the core. Prior to any calculation, the thickness of each sediment slice was corrected for compression.

In the laboratory, the cores were sliced into 1 cm intervals, and each slice was dried at 60 ^{o}C for 48 - 72 hours. A sub-sample (5 g DW) of each slice was milled to fine powder and was used for the determination of elemental concentration of organic carbon (C_{org}) using an Elemental Analyser. Prior to the analysis, sediments were acidified (HCl, 2N) to eliminate carbonate contents.

Dry bulk density (DBD, g cm^{-3}) was estimated by dividing the dry weight of sediment by the volume of the corresponding wet sample. C_{org} stocks were estimated as the cumulative product of C_{org} concentration, DBD and decompressed sediment slice thickness and were standardized at 1 m of sediment thickness.

3. Results

The mean areal extent of *P. oceanica* was obtained from Panayotidis *et al.* (submitted) and has been estimated at 25,762 ha along Cyclades Islands and 37,152 ha along Dodecanese Islands. Stock of C_{org} ranged between 9.3 and 32.1 kg m^{-2} in 1 m thick soil deposits, with a mean of 15.0 ± 0.9 kg m^{-2} in Cyclades and of 11.4 ± 0.5 kg m^{-2} in Dodecanese Islands. Overall, the meadows of South Aegean supported in total 8 Tg C_{org}.

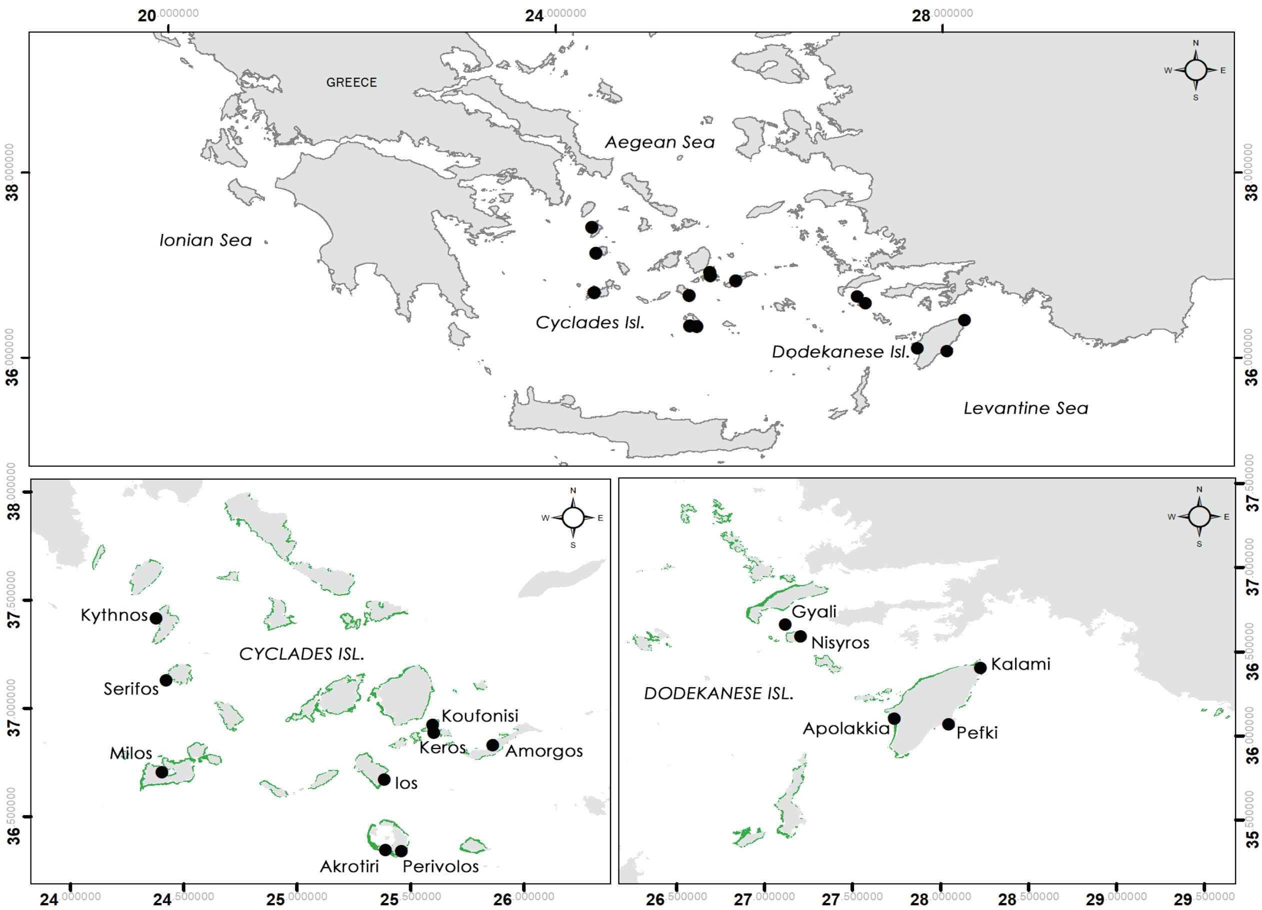

Fig. 1: *P. oceanica* meadows along the coasts of Region of South Aegean. Samplings stations at Cyclades and Dodecanese Islands are also indicated.

4. Discussion/Conclusion

Our data analysis revealed that seagrass meadows of the South Aegean Region store significant amounts of C_{org} in their underlying sediments. Available literature suggests that *P. oceanica* meadows support variable stocks depending on local environmental factors and geochemical conditions that determine the magnitude of C_{org} sink. Data from the West Mediterranean report variable 1 m stocks ranging from 20.3 ± 4.6 to 37.5 ± 6.5 kg m^{-2} (Mazarassa *et al.*, 2017; Serrano *et al.*, 2016). Our data are similar to that range, albeit slightly lower, possibly pointing to the general lower organic matter availability towards the south-eastern part of the Mediterranean Basin that may lead to lower storage. Nevertheless, the meadows of the South Aegean Region accumulated more carbon than other seagrass species around the world (Röhr *et al.*, 2018), verifying the exceptional role of *P. oceanica* meadows to sequester carbon and contributing to mitigate climate change.

5. Acknowledgements

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TYPES OF THERMAL AND OSMOTIC INTERACTIONS IN *CYMODOCEA NODOSA* OF MACEDONIAN AND THRACIAN COASTS, GREECE

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Abstract

Cymodocea nodosa is a key habitat forming seagrass species of the Mediterranean coasts, considered as favored from climate change. The combined effect (additive, antagonistic or synergistic) of global warming and extreme weather events along with local anthropogenic stressors on shallow marine ecosystems, make the study of interaction between key environmental factors imperative in order to fully comprehend the underlying mechanisms leading to meadow shifts. In the present study, interaction types of thermal (15°C, 34°C) and osmotic (salinity 15 and 50) stress on *C. nodosa* shoots from three meadows (Imeros, Nea Karvali and Cape Vrasidas) of the Eastern Macedonian and Thracian coasts were determined during two sampling periods (2013, 2015-16) by measuring **Δ**F/Fm′, leaf Chl-*a* content, leaf Chl-*b* content, leaf Chl *a*/*b* ratio and leaf elongation rate. Most of the interactions were found to be additive, while the HT/HS (high temperature, 34°C - high salinity, 50) interaction was found to be antagonistic for shoots from Cape Vrasidas in 2013. Noteworthy, the LT/HS (low temperature, 15°C-high salinity, 50) was mostly found to be additive in most of the response variables examined. These results confirm that *C. nodosa* is a ‘winner' of climate change as thermal and osmotic interactions do not magnify and occasionally dampen its effects locally.

Keywords: additive, antagonism, synergism, seagrass.

1. Introduction

Seagrasses are key foundation species of shallow coastal ecosystems and are considered important habitats, offering a variety of functions and services, such as providing food and nursery, supporting food webs, limiting coastal erosion, sequencing carbon, etc. (Nordlund *et al.*, 2016). Climate change is affecting marine ecosystems, including benthic macrophytes, both due to ocean warming, but also the increasing frequency and intensity of extreme weather events. Simultaneously, local anthropogenic stressors such as saline brine discharges aggravate seagrass degradation. Temperature is the most important limiting factor affecting seagrasses' photosynthetic performance and growth and therefore, their geographical distribution and seasonality (Lee *et al.*, 2007). Salinity is also a key stressor for the performance of seagrasses, but its influence is local.

It has been repeatedly proven that the interaction of two or more stressors can differ significantly from the theoretical sum of the single stressors (additive interaction), and as a result the interaction of two stressors cannot be predicted by studying them independently. In general, non-additive effects can be antagonistic, i.e., the effect size (ES) of the interaction being less than the sum of the two individual ES, or synergistic, i.e., the ES of the interaction being greater than the sum of the two individual ES. However, researchers have not yet come up with a specific method for determining the interaction types (IT) to date. As a result, determining the nature of IT is crucial, in order to produce effective management plans (Brown *et al.*, 2013). *Cymodocea nodosa* (Ucria) Aschers is often considered as a "winner" of climate change (Tsioli *et al.*, 2019; Schäfer *et al.*, 2021), but the IT of global warming with local stress factors such as salinity is still under consideration (Tsioli *et al.*, 2022).

This study aimed to investigate the thermal and osmotic stress interactions on *C. nodosa* to detect possible impairments scenarios on Eastern Macedonian and Thracian species populations. For this purpose, the IT of thermal (15°C, 34°C) and osmotic (salinity 15 and 50) stress in *C. nodosa* along three meadows (Imeros, Nea Karvali and Cape Vrasidas) of the Eastern Macedonian and Thracian coasts were determined for the response variables studied (ΔF/Fm', leaf Chl-*a* content, leaf Chl-*b* content, leaf Chl *a*/*b* ratio and leaf elongation rate).

2. Materials and Methods

2.1 Experimental setup

Intact shoots of *C. nodosa* were collected using scuba diving from 0.8 m to 3 m depth at Cape Vrasidas- CV (Gulf of Kavala; 40'49'37.53''N, 24'19'8.78''E) in the summer 2013, at CV and Nea Karvali- NK (Gulf of Kavala; 40°57'28.15"N, 24°31'10.29"E) in the summer 2015 and NK and Imeros- IM (Municipality of Rhodopi; 40°55'42.83"N, 25°16'9.56"E) in the summer 2016 (Fig.1).

Shoots were transported in plastic containers containing seawater to the laboratory of the Benthic Ecology & Technology Laboratory (FRI – Kavala), were cleaned from epiphytes and then left to acclimatize for 10 days in 5 l well-aerated aquaria at 21–22 °C, at 60 μmol photons m^{-2} s^{-1}, 14 h light per day. The aquaria contained 4 l artificial seawater medium (33.5 salinity, 0.3 μM N-NO_3^-, and 0.02 μM P-PO_4^-) produced by Münster™ Sea Salt (Meersalz) diluted in resin-filtered tap water (<5 μS/cm).

The experimental design is presented in Table 1, showing the temperatures and salinities used.

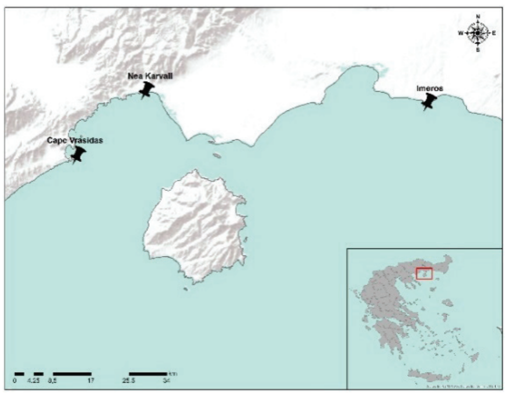

Fig. 1: Map of the sampling sites.

Table 1. The meadows, experimental treatments and abbreviations that were used at shoots of *Cymodocea nodosa*.

Meadow	Salinity	Temperature (°C)	Abbreviation	Abbreviation in full
Vrasidas 2013	50	23	HT/HS	High Temperature/ High Salinity
Vrasidas 2015, Nea Karvali 2015, Nea Karvali 2016, Imeros 2016	15	15	LT/LS	Low Temperature/ Low Salinity
		23	LS	Low Salinity
	33.5	15	LT	Low Temperature
		23	CONTROL	
	50	15	LT/HS	Low Temperature/ High Salinity
		23	HS	High Salinity

The experiment of CV 2013 lasted 20 days and samplings of shoots were conducted at 1st, 3rd, 5th, 10th and 20th day. All other experiments lasted 18 days. The treatments of 23°C were performed in 1 L glass vessels holding one plant each, submerged in Haake™ (ThermoFisher Scientific, Waltham MA, USA) cryo-thermostats (±0.2°C). Six replicates (n=6) were assigned per treatment and light conditions were 60–90 μmol photons m^{-2} s^{-1}, 14 h light per day. The aerated medium was the same as the one used during acclimation and was changed every other day. The salinity treatments were kept in a constant temperature chamber (21–22 °C), using 2 l plastic aquaria and the medium was measured using a portable conductivity meter (WTW, Weilheim, Germany). Each plastic aquarium was filled with 1 l of medium and contained one shoot.

2.2 Response variables

The parameter used to assess the condition of the photosynthetic apparatus, as a measure of the proportion of the light absorbed by the chlorophyll associated with PSII that is used in photochemistry, hence, of plant fitness, was the effective quantum yield (ΔF/Fm'). ΔF/Fm' (Fm'-F'/Fm') was measured in the morning every other day, using a diving PAM fluorometer (Walz, Germany) inside a Haake cryothermostat (regulated to experimental temperatures; ±0.2°C). ΔF/Fm' was performed at a standard position on the second leaf approximately 2 cm from the plant meristem (Ralph, 2000; Papathanasiou *et al.*, 2015). The part of the leaf that was sampled for measuring ΔF/Fm' (about 2 cm) was cut at the end of each experiment and stored at -75°C until leaf Chl-*a* content analysis (Granger & Lizumi, 2001). Based on the same methodology, leaf Chl-*b* content and leaf Chl *a*/*b* ratio were calculated. Leaf elongation rates (cm/shoot/day) were measured using in-situ leaf mark technique (Short & Duarte, 2001).

2.3 Statistical analysis

To determine the type of interaction (additive, synergistic or antagonistic) of *C. nodosa* for the response variables used, the Observed (Obs) effect sizes (ES) were compared with the Expected (Exp) additive interaction. The ES were calculated per sampling day per response variable, in the case of CV 2013 and per response variable for the rest of the treatments. The Expected ES were calculated based on the sum of ES of each stressor, using a multiplicative risk model, which was compared with the Observed combined ES. If Obs was greater than the Exp (with no overlap of the 95% Confidence Intervals-CI), then the interaction was classified as synergistic. If Obs was less than the Exp (with no overlap of the 95% CI), then the interaction was classified as antagonistic. If the 95% CI of the Obs overlapped with the 95% CI of the Exp, the interaction was classified as additive (Darling *et al.*, 2010). If the ES of a single stress is negative and the other positive and the Obs is greater than Exp with no overlap of 95% CI, then the interaction is classified as antagonism, while when the Obs is less that Exp, the interaction is classified as synergistic (Crain *et al.*, 2008).

3. Results

3.1 The interaction of high thermal and high osmotic stress

The IT for the HT/HS treatment per sampling day for the response variables ΔF/Fm' and leaf Chl-*a* content were calculated for shoots of CV 2013 (Table 2). The HT/HS interaction for ΔF/Fm' (Fig.2) was found to be additive (with strong antagonistic trend) on the 1st day, additive on the 3rd day, additive (with strong antagonistic trend) on the 5th and 10th day and antagonistic on the 20th day. The HT/HS interaction for the leaf Chl-*a* content was additive (with antagonistic trend) on the 1st day, additive on the 3rd, 5th and 10th day and additive (with synergistic trend) on the 20th day. In the majority of the cases examined, OS effect size was greater that the HS effect size, showing that salinity stress affected the response variables more that the heat stress.

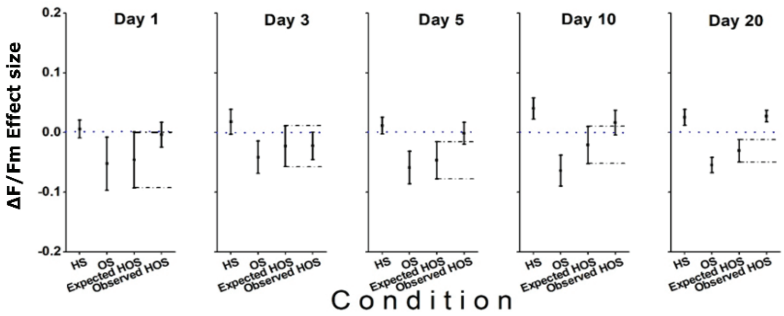

Fig. 2: Effect sizes (± 95% CI) per treatment day for effective quantum yield (ΔF/Fm') of *C. nodosa* under heat stress (HS, salinity 35 - temperature 34°C), osmotic stress (OS, salinity 35 - temperature 22°C), the Expected additive and Observed of the combined heat and osmotic stress (HOS; salinity 50 - temperature 34°C).

3.2 The interaction of low thermal with low and high osmotic stress

The IT of low thermal stress (15°C) and high (50) and low (15) osmotic stress were calculated at the end of the 18-day experimental period. The results are presented in Table 2 per meadow (IM 2016, NK 2016, NK 2015, CV 2015 and CV 2013), per year and per response variable. Most of the interactions were found to be additive, while some antagonisms and a case of synergy were found.

Table 2: Summary of the interaction types in the present study. LT/LS: low temperature, 15°C - low salinity 15, LT/HS: low temperature 15°C - high salinity 50.

Experimental condition	Response variable	Imeros 2016	Nea Karvali 2016	Vrasidas 2015	Nea Karvali 2015	Vrasidas 2013
LT/LS: 15°C – Salinity 15	ΔF/Fm'	Additive	Additive	Antagonistic	Additive	
	LER*	Additive	Additive	Additive	Additive	
	Chl-*a*	Antagonistic	Additive	Additive	Additive	
	Chl-*b*	Additive	Additive	Additive	Additive	
	Chl *a*/*b*	Additive	Additive	Antagonistic	Additive	
LT/HS: 15°C – Salinity 50	ΔF/Fm'	Additive	Additive	Additive	Additive	Antagonistic (HT/HS)
	LER*	Additive	Additive	Additive	Additive	
	Chl-*a*	Additive	Additive	Additive	Additive	Additive (HT/HS)
	Chl-*b*	Additive	Additive	Additive	Synergistic	
	Chl *a*/*b*	Additive	Additive	Antagonistic	Additive	

*Leaf elongation rates

4. Discussion/Conclusion

Most of the IT were found to be additive on *C. nodosa*, as it has also been found for other seagrass species (Egea *et al.*, 2018; Moreno-Marín *et al.*, 2018), showing that thermal and osmotic interactions do not magnify and occasionally dampen the effects of climate change. Although additive interactions are considered to be non-interactions, it has been suggested that stressors whose reactions follow similar biochemical/ cellular pathways, ultimately are found to be additive (Christensen *et al.*, 2006; Crain *et al.*, 2008). Malandrakis *et al.* (2017) found no overlap between the transcriptors of thermal and osmotic stress in *C. nodosa*. Remarkably, the HT/HS interaction (High Temperature 34°C – High salinity 50) indicated an antagonistic interaction on ΔF/Fm' of CV 2013, while the LT/HS interaction (salinity 50-15°C) was found to be additive, i.e., no interaction was found. These results suggest that increased salinities might dampen the adverse effects of high temperatures, thus buffering, to some extent, the impact of marine heatwaves in the context of climate change. Furthermore, the IT depend on the response variable used, the duration and dose of the stressor and the species under study. The almost complete absence (except Chl-*b*) of synergisms in the present study was also found in other studies of primary producers, emphasizing that synergisms are more scarse than it was previously thought (Crain *et al.*, 2008; Bansal *et al.*, 2013).

5. Acknowledgements

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INTERNATIONAL AND EU POLICIES AND LEGISLATION FOR COASTAL FLOODS

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Abstract

In recent decades, there has been an escalation of the environmental and socio-economic impacts of coastal floods caused by extreme events, prompting introduction of several policy and legislation instruments for their prevention/management. Here, we present a brief analytical overview of the fast-evolving international and EU policies and legislation pertaining to the assessment and reduction of the coastal flood risk in the EU. Interacting international regimes have been identified that aim at the coherence and mainstreaming of resilience-building across sectors and policy domains, such as the 2015 Sendai Framework for Disaster Risk Reduction (SFDRR), the 2015 Paris Agreement and the UN 2030 Agenda for Sustainable Development. At the EU level, there has been also a flurry of recent policies, plans and mechanisms aiming at Disaster Risk Reduction (DDR), Climate Change Adaptation and civil protection. There is also specialized EU legislation (e.g., the 2007 Floods Directive) aiming at coherent response at the EU level to (coastal) floods; however, the review of its implementation has found fragmentation and diverse approaches across the EU Member States. There has also been an increase in policy and legal requirements for: research and development/use of knowledge, approaches and tools for reducing coastal flood risks including the development of integrated Early Warning Systems.

Keywords: coastal floods, disaster risk reduction, environmental policy and legislation.

1. Introduction

In recent decades, there have been a worsening of the impacts of coastal floods caused by extreme events (e.g., tropical and extratropical cyclones and storm surges/waves) on many coastal ecosystems, populations, infrastructure, assets and socio-economic activities (WMO, 2021). Over the course of the century, coastal floods are projected to become more disruptive due to climatic changes (IPCC SROCC, 2019); a recent study assessed the total value of assets exposed to episodic coastal flooding by 2100 as 12 – 20% of the global GDP without effective adaptation (Kirezci *et al.*, 2020). This makes the prevention/mitigation of flood impacts (disaster risk reduction) a particularly urgent case.

Flood risk reduction in the coastal zone requires development and availability of tools and data that can provide pertinent, evidence-based information (UNFCCC, 2020). It also needs to be underpinned by strong and synergetic policy and legal frameworks. Policies define and formulate ambition, objectives and commitments, whereas legal instruments are powerful tools for the implementation of policy objectives. They can both provide economic incentives to support DRR efforts, improve coastal resilience, promote technology transfer and contribute to the collection, availability and accessibility of indispensable climatic data at different spatio-temporal scales.

Here, we present a brief analytical overview of the fast-evolving policy and legislation framework pertaining to the assessment and reduction of the coastal flood risk in the EU.

2. Materials and Methods

The present study reviewed primary sources (e.g., official policy and legislation instruments) pertaining to coastal floods, as well as relevant commentaries and academic publications. The review follows the hierarchy of norms, i.e., considering first the most relevant international policy and legal instruments to which EU Member States have decided to adhere to, and then the EU policies and legislation.

Due to space constraints, only a brief overview of this analysis can be presented.

3. Results

International and EU Policy and Legislation Framework
3.1. International policies and legislation

There has been a fast-evolving policy development to facilitate DRR and Climate Change Adaptation (CCA) in coastal areas. This is reflected expressly in the Sustainable Development Goals (SDGs) and Targets (SDTs) that collectively make up the UN 2030 Agenda for Sustainable Development. These include in particular: SDG 1 that envisages to '*…build the resilience of the poor and those in vulnerable situations and reduce their exposure and vulnerability to climate-related extreme events…*' (SDT 1.5); SDG 2, that asks the Parties to '*… help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters…*' (SDT 2.4); SDG 9 that envisages to '*… develop quality, reliable, sustainable and resilient infrastructure…*' (SDT 9.1); SDG 11, which requires to '*significantly reduce the number of deaths and the number of people affected and substantially decrease the direct economic losses … caused by disasters, including water-related disasters…*' (SDT 11.5); SDG 13, that requires to '*strengthen resilience and adaptive capacity to climate-related hazards*', '*integrate climate change measures into national policies and planning*', and '*improve education, awareness-raising and human and institutional capacity on climate change mitigation, adaptation, impact reduction and early warning*' (SDTs 13.1, 13.2 and 13.3); and SDG 14 that asks Parties to '*sustainably manage and protect marine and coastal ecosystems…*' and '*conserve at least 10 % of coastal and marine areas based on the best available scientific information*' (SDTs 14.2 and 14.5).

The 2015 SFDRR provides the overarching post-2015 policy framework for disaster risk reduction, management and response. It identifies climatic factors as potent disaster drivers (para. 13), and highlights the importance of implementation and coherence of DRR instruments/tools, including those on biodiversity, climate change and sustainable development (paras 19(h), 28(b)). The SFDRR focuses on disaster risk drivers (paras. 6 and 19(i), (j)) and embraces a multi-hazard approach (paras. 15 and 19(g)). It emphasizes the importance of governance noting that '*Strengthening disaster risk governance for prevention, mitigation, preparedness, response, recovery and rehabilitation is therefore necessary…*'. (para. 26). The SFDRR advocates for substantial reduction of the disaster risk and losses and establishes a comprehensive goal to: '*Prevent new and reduce existing disaster risk through the implementation of integrated and inclusive […] measures that prevent and reduce hazard exposure and vulnerability, increase preparedness for response and recovery, and thus strengthen resilience*'.

To this end, relevant priorities for action include understanding the risk, strengthening the governance, investing in resilience and enhancing disaster preparedness for effective response and 'build back better' in the DRR recovery, rehabilitation and reconstruction phases. Coastal flood risk assessment/mapping and Early Warning Systems (EWSs) are amongst the tools required in these actions. The SFDRR specifically promotes '*mainstreaming of disaster risk assessment, mapping and management into the management of, inter alia, … coastal flood plain areas, […]*' (para. 30(g)). Global measurable targets have been agreed for 2030 to assess the achievement of the SFDRR objectives. These include targets/indicators for the reduction in: mortality, the number of affected people, direct economic losses, damages to critical infrastructure and the disruptions of basic services. There are also targets for monitoring the number of countries with national/local DRR strategies (by 2020), the increase in international cooperation, and the availability of and access to EWSs and disaster risk information and assessments.

Other international instruments of high relevance include the international Agreements to which the EU Member States are Contracting Parties (CPs), notably the 1992 UN Framework Convention on Climate Change (UNFCCC) and the 2015 Paris Agreement. The commitment of the CPs to the UNFCCC for integrated coastal zone management (ICZM) and the protection/rehabilitation of flood-affected areas, as well as for impact assessments and the promotion of scientific research, systematic observation and data archiving for the climate system (Art. 4 (1)), are of direct relevance for coastal zone flood resilience and DRR.

Amongst others, an objective of the 2015 Paris Agreement is to increase the adaptation ability to climatic impacts and foster climate resilience through adaptation planning/implementation and strengthening the scientific knowledge, research, systematic climatic observations and EWSs (e.g., Arts. 7(7) and 8(4)).

There are also international Conventions on the conservation of coastal ecosystems that could be flooded. Contracting Parties to the 1971 Ramsar Convention have obligations to designate (coastal) wetlands on account of their international environmental significance (RAMSAR sites) and formulate/implement conservation plans. The 1992 Convention on Biological Diversity (CBD) establishes a global legal regime for the conservation of biological diversity, including of coastal ecosystems that could be threatened by floods. Contracting Parties shall (amongst others): develop relevant conservation strategies, plans and programmes; restore degraded ecosystems; and promote research on biodiversity conservation. There are also regional international legal instruments, such as the 1995 Barcelona Convention (particularly its 2008 ICZM Protocol) and, to a lesser extent, the 1992 HELCOM Convention. The 2008 ICZM Protocol (in force, 12 Mediterranean CPs including the EU) provides a common framework for ICZM implementation, addressing also coastal erosion and flooding. Art. 8 prescribes establishment of a 100 m setback zone '*as from the highest winter waterline … where construction is not allowed*' subject to limited exceptions. Contacting Parties shall develop policies for the *'prevention of natural hazards, undertake vulnerability and hazard assessments of coastal zones and take prevention, mitigation and adaptation measures to address the effects of natural disasters…*' (Art. 22). They shall endeavour to improve knowledge on the state, development and impacts of coastal erosion (Art. 23), and '*coordinate the use of the equipment for detection, warning and communication at their disposal…*' (Art. 24).

3.2. EU Policies and Legislation

Concerning EU-level policies, plans and mechanisms, this review has found that the 2021 EU Climate Change Adaptation Strategy (EU-CCA Strategy), the EU Action Plan on SFDRR 2015–2030, and the Union Civil Protection Mechanism (UCPM) are of particular relevance for the assessment/management of coastal flood risks. The 2021 EU CCA Strategy, which specifically mentions coastal floods, promotes mainstreaming of climatic adaptation into all EU policies, increasing of infrastructure resilience, improved data availability and usability from EU scientific lighthouses, and reinforcement of adaptive capacity and reduction of vulnerability (in line with the 2015 Paris Agreement and the 2021 European Climate Law). The EU SFDRR Action Plan: recognises the importance of monitoring to assess progress (in line with the UCPM); asks for systematic disaster data; suggests National Risk Assessments (NRAs) as the basis for DRR; and promotes research/innovation and co-design, co-development and co-evaluation of the EU climate services. It also envisages specific actions to strengthen the UCPM and the EU Copernicus Emergency Management Services (CEMS). The UCPM (Decision (EU) 2019/420 amending Decision 1313/2013) states that flooding constitutes an increasing risk and requires EU Member States to carry out multi-hazard risk assessments and assessments of risk management capability, and to develop/refine disaster risk management planning (Art. 6). It also establishes various mechanisms that shall aim to, *inter alia*, enhance all phases of disaster management, taking into account adaptation to and mitigation of climate change (Art. 13).

There are various European legal instruments which explicitly, or implicitly, address issues of coastal flood risk prevention, management and response. The recent 2021 EU Climate Law (Regulation (EU) 2021/1119) is of particular relevance, as it envisages strong action on CCA and resilience-building, as well as stocktaking, assessment and review procedures from 2023. The Regulation is directly applicable and effective in all EU Member States and is expected to benefit resilience building and CCA, including by facilitating improved coastal flood risk assessments and management. Other EU Regulations that require sectoral responses to coastal flooding (and related tools/services) include Regulation (EU) 1315/2013 for the development of the trans-European transport network (TEN-T) that includes seaports and other coastal transport infrastructure, and Regulation (EU) 2018/1999 on Governance of the Energy Union and Climate Action which *inter alia* focuses on the resilience of energy systems, significant components/nodes of which are located along the EU coastal zone.

The most relevant Floods Directive (FD) 2007/60/EC establishes the framework for flood risk assessment and management, including in coastal zones. The FD imposes a general duty to EU Members States to assess the coastal flood risk, map the flood extent, assets and humans at risk and take adequate risk management measures. It requires Member States to draw up comprehensive flood hazard and flood risk maps (FHRMs) and flood risk management plans (FRMPs) in recurring implementation cycles. Its first implementation cycle was completed in 2015. The FD required reviewing of FHRMs by 2019 (and every 6 years thereafter) and updating of FRMPs, as necessary, by the end of 2021. Several issues were identified in the first FD implementation cycle, such as a 'patchy' record in the coverage of the coastline, the FD-prescribed flood probability scenarios and flood characteristics, as well as considerable diversity in the geo-spatial information and the oceanographic and coastal flood models used. Although the subsidiarity principle allows for 'policy space' and, thus, diversity in national approaches, it is submitted that in respect of the flood risk assessment and management, such diversity might not be conducive to coherent action across the EU. In the latest Member States' updates (2021) of their preliminary flood risk assessments (Arts 4, 5, 14 and 15) there have been improved data collection and risk assessment methodologies, but there is room for significant improvements (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52021DC0970&from=EN).

Another Directive the implementation of which requires (implicitly) coastal flood risk monitoring/assessment is the Water Framework Directive (WFD) 2000/60/EEC. The WFD is generally considered together with the FD (https://water.europa.eu/), as coastal flooding can affect adversely the quality of the coastal surface and ground waters (e.g., Mishra *et al.*, 2021) which the WFD aims at protecting and improving. Other relevant EU legislation includes the amended Environmental Impact Assessment (EIA) Directive 2014/52/EU that requires coastal flood assessments with a view to facilitate coastal infrastructure resilience and environmental protection. There is also environmental EU legislation aiming at the protection of (coastal) ecosystems, which although not dealing explicitly with the coastal flood risk, still necessitates its assessment and management. For example, the Habitats Directive 92/43/EEC requires implementation of measures for the conservation of coastal habitat types, vulnerable species habitats and designation of sites forming an EU wide network (Natura 2000) for which there are particular conservation requirements; thus, there is an implicit obligation to develop integrated flood risk cycle monitoring services that can improve the flood preparedness, response, recovery and prevention of the natural ecosystems under protection. Finally, there are also EU Directives dealing with the right of access to and the coherence of essential information for effective coastal flood risk assessment and management: Directive 2003/4/EC (implements the 1998 Aarhus Convention) and the INSPIRE Directive 2007/2/EC that aims to establish compatible and usable spatial data infrastructures (SDIs) in a EU-wide and transboundary context.

4. Conclusions

This brief review of the international and EU policies and legislation pertinent to coastal flood risk has revealed fast-evolving, interacting regimes which try to address the strategic importance of and keep pace with the urgency of flood resilience building for coastal populations, infrastructure/assets, services and environments. They aim at the coherence and mainstreaming of climate resilience-building across sectors and policy domains, which hopefully are going to enhance flood risk resilience, response and recovery as well as stimulate adaptation action at local levels. This will depend, amongst others, on policy and legal instruments that remain to be developed to implement on the ground both the international DRR and the EU policies/agreements. Effective reduction and management of the coastal flood risk require concrete actions that, in many cases, can be only mandated by national legislation due the 'policy space' afforded by the EU Member States.

Despite the slow-pace improvement in the implementation of the relevant EU legislation (i.e., the FD), there has been fragmentation and diverse approaches in terms of the coastal flood risk coverage, characteristics and management plans across the EU Member States that may lead to challenges in the effective implementation of common policy objectives. Nevertheless, a common thread has been identified: there is an apparent increase in policy and legal requirements for the expansion, coherent development and use of knowledge, approaches and tools for reducing coastal flood risks, including monitoring, assessment, management, information sharing, strengthening co-ordination, public participation, improved research and technologies, and the development of integrated EWSs.

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ENVIRONMENTAL QUALITY - POLLUTION

MONITORING THE UNDERWATER SOUNDSCAPE OF A "QUIET" SHALLOW-WATER COASTAL MARINE ENVIRONMENT IN THE AEGEAN SEA

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Abstract

The continuous increase in marine human activities has led to growing concerns regarding the effects of the underwater anthropogenic acoustic footprint on the marine ecosystem. Research on this topic requires challenging in-situ monitoring missions for the estimation and assessment of the underwater soundscape in targeted marine areas. In this work, we present the design and results from the deployment of a custom-made underwater hydrophone-recorder system performed in a shallow-water coastal area of the Argolikos Gulf, Central-Eastern Aegean Sea. The operation provided 6 days of quasi-continuous recordings in the area. The data analysis revealed relatively low levels of sound energy in a soundscape that is dominated by Sound Pressure Levels (SPL) maxima within a narrow frequency band around 4 kHz associated with the diurnal cycle of biological sound emissions (shrimps). Vessels activity was also apparent within the frequency band from 50 Hz to about 2 kHz or even higher in certain cases. In general, the outcomes from this study underline the feasibility of coastal monitoring and its importance for the description and assessment of the Greek Seas' soundscape status. The monitoring methodology proposed here will both serve marine research and the implementation of environmental policies for ecosystem protection.

Keywords: Underwater soundscape, Hydrophones, Sound Pressure Level, Shipping noise, Marine environment.

1. Introduction

Anthropogenic noise in the seas and oceans is related to several activities and has been officially listed as a source of pollution for the marine environments and ecosystems. At a European level, underwater anthropogenic noise is amongst the parameters that need to be monitored for the achievement of Good Environmental Status (GES) under the Marine Strategy Framework Directive (MSFD). Such monitoring is acknowledged as a demanding procedure that involves appropriate instrumentation and challenging deployment procedures. This is reflected by the limited number of studies at a European scale that describe efficient monitoring techniques of the marine soundscape in the context of operational observations and identification procedures (see, e.g., Garrett *et al.*, 2016; Merchant *et al.*, 2016). The Hellenic Centre for Marine Research (HCMR) has been utilizing hydrophones for passive acoustic monitoring of the Greek Seas since 2008 (Nystuen *et al.*, 2015). However, such activities were restricted due to the lack of sustainable funding schemes, and thus, there are considerable knowledge gaps regarding the underwater soundscape of the Greek Seas. During the last five years, sound monitoring activities have been re-initiated and strengthened on the grounds of HCMR's designation as a responsible Greek organization for implementing underwater noise monitoring under MSFD requirements (Prospathopoulos *et* *al.,* 2017). The formation of HCMR's LITTUS (LIsten To The Underwater Soundscape) team followed, and a close collaboration with other institutes and organizations has been established for building an underwater soundscape monitoring infrastructure. Under this new framework, the Laboratory of Underwater Acoustic Measurements of the IACM-FORTH designed and constructed a set of new custom-made and low-cost hydrophone-recorders instrumentation based on previous experience (Papadakis *et al.,* 2017; 2018). The devices were delivered to HCMR in 2021 and a targeted coastal operation was designed with a twofold aim: their testing as well as assessing the underwater soundscape of a "quiet" shallow water marine environment, taking into account previous experience from coastal soundscape monitoring research (Haxel *et al.,* 2013; Hermannsen *et al.,* 2019; Bittencourt *et al.,* 2020). In this study, we present the experience and outcomes from the recent underwater quasi-continuous sound recordings operation in Argolikos Gulf. We provide technical information on the instrumentation and the configuration used, and analyse the acquired data, in an attempt to assess the information extracted regarding the soundscape of a coastal area, which was expected to exhibit low sound levels.

2. Material and Methods

2.1 Instrument technical specifications and configuration

The underwater acoustic listener deployed in Argolikos is designed and constructed at the Laboratory of Underwater Acoustic Measurements of the IACM-FORTH. The device is one of four similar instruments that have been delivered to HCMR to cover its needs for the monitoring of underwater ambient noise under the framework of the MSFD D11 assessment. The instruments have been constructed in the context of low-budget underwater soundscape monitoring systems and are specified for coastal operations with recording autonomy of approximately 6 months (duty cycle of 1% with sampling frequency of 50 kHz). The hydrophone-recorder system consists of the following main components: ERTACETAL waterproof case to withstand pressure up to 200 bars; hydrophone (Benthowave model BII-7121) with dynamic range from 10 Hz to 20 kHz; data acquisition card (MCC USB-1608FS Plus); CPU unit (Raspberry Pi 3 Model B+); Real-time Clock and Power Management (UUGear Witty Pi 3); Hard disk (512 Gb); Lithium rechargeable battery pack (110 Ah at 7.4V). For the test mission the instrument was configured to sample quasi-continuously (50 min of recording - 10 min of storing data per hour) under its maximum sampling frequency (50 kHz). With this setup, we estimated the maximum operation period to be approximately one week.

2.2 Deployment mission and data analysis

The instrument was deployed from the R/V AEGAEO (Greek Water Framework Directive autumn cruise). The location (Fig.1) was selected to test the device under "quiet" sea-environment conditions. For the deployment, a joint scuba-diving operation was performed in 22/11/2021 by HCMR scientific diving team. The instrument was placed on the seabed and secured with pegs and struts at a depth of 16 m, 250 m approximately off the coast. Although recovered on the 01/12/2021, the device was powered by the battery pack until 05:00 of 28/11/2021. After recovery, sub-samples of the acoustic recordings were extracted, and further analyzed. The analysis included the production of the associated full-band spectrograms which were produced using time step of 1 sec and frequency step of 15.625 Hz up to 24 kHz. Additionally, the Cumulative Distribution Function (CDF) of Sound Pressure Level (SPL) relevant to the 1/3 octave bands of 62.5 and 125 Hz where produced in order to be in line with the minimum MSFD requirements for continuous noise assessment. Regarding vessel's noise, records from the Automatic Identification System (AIS) system (www.marinetraffic.com) were also investigated for the wider area of Argolikos Gulf to associate real-time ship traffic activity with noise signals recorded by the instrument.

Fig. 1: Deployment location (Lat: 37.45, Lon: 23.09) of the hydrophone-recorder system in 22/11/2021.

3. Results

We applied a preliminary analysis to the acquired dataset that referred to almost 6 days of 50 min per hour recordings on-site (~ 7000 min). From our analysis, we noticed that the ambient noise levels were very low throughout the spectrum for the full time period of operation, reflecting a limited anthropogenic activity in the area during the mission. The occasional passage of vessels was obvious throughout the spectrograms and altered temporarily the ambient noise levels mainly at the low frequencies, a fact that was further corroborated by AIS records (Fig. 2). In the absence of individual vessels, the ambient noise level at the frequency range of 20 - 1000 Hz ranged in very low levels of the order of 50 dB re 1μPa/Hz. On the contrary, in the event of a boat-engine presence, these levels may exceed 100 dB re 1μPa/Hz within this frequency range (Fig. 2). Natural sound signals were also captured with a snapping shrimp activity to be dominant in the 3 - 5 kHz frequencies band and observed throughout the period of the recordings. This activity is more prominent during the evening/night hours presenting SPLs that exceed 80 dB re 1μPa^2 whilst, during the morning time this is reduced to less than 70 dB re 1μPa^2. Other sound patterns such as breaking waves and wind are also apparent especially during the last days of operation (not shown here). In general, more analysis is needed to describe the environmental soundscape after omitting mechanical or electronic self-noise introduced by the device such as a monochromatic signal at about 19 kHz (Fig. 2).

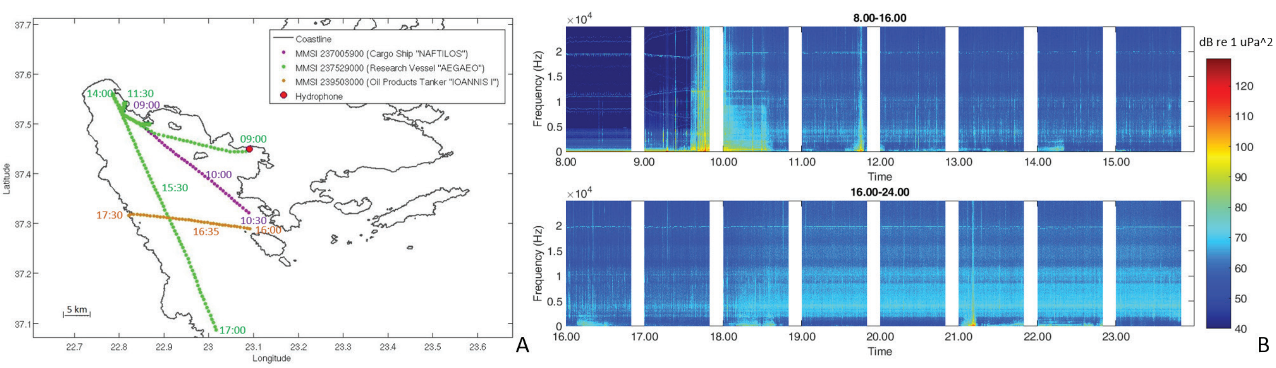

Fig. 2: A: Ship routes recorded by the AIS system in Argolikos Gulf (8:00 - 23:59 on the 22/11/2021, hydrophone position is marked with red dot). B: Spectrogram from hydrophone recordings during the same period.

We further estimated the SPL distribution for the 1/3 octave bands with central frequencies at 62.5 Hz and 125 Hz (Fig. 3). The results confirm what the spectrogram indicated, i.e. low SPL levels with median values slightly above 60 dB re 1μPa^2 whilst, the 95% of records do not exceed 76 dB re 1μPa^2 in both cases (Fig. 3).

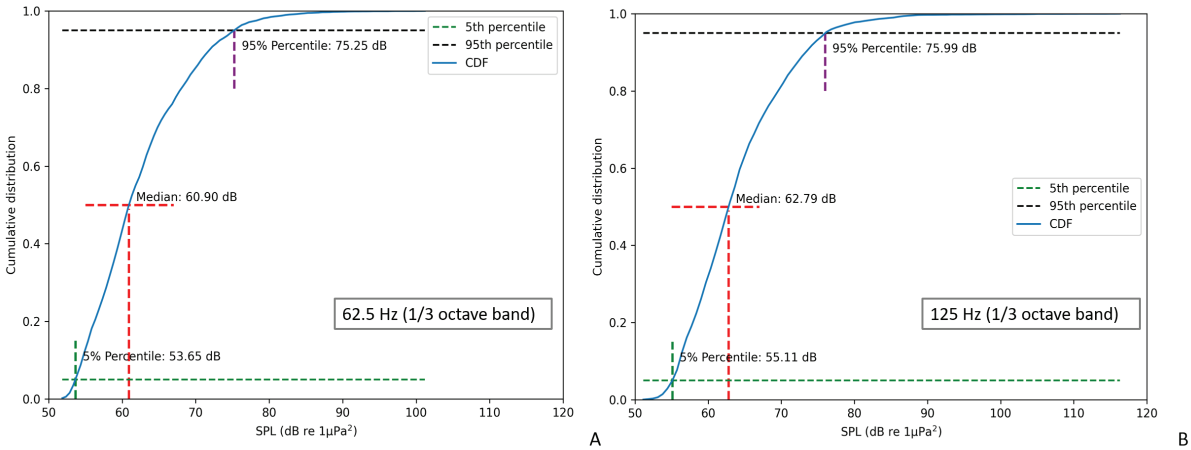

Fig. 3: Cumulative distribution of SPL along with statistical indicators associated with the 1/3 octave bands at 62.5 Hz (A), and 125 Hz (B) derived from all recorded data at the deployment location.

4. Discussion/Conclusion

The preliminary results show a satisfactory performance of the tested hydrophone-recorder system. The instrument managed to capture several sound events and signals under the continuous-mode recording configuration, providing an almost full 6-day dataset for a broadband frequency range. The quality of the data and the derived SPL resolution is good, with only drawback being the monochromatic signal related to electronic self-noise. Regarding the soundscape, interesting information is extracted highlighting a particularly "quiet" coastal area that is characterized by a dominant natural sound field, which is occasionally disrupted by vessels' activity. In terms of natural sound signatures, the effect of crustacean species is dominant, and characterized by a strong diurnal variability. Furthermore, sound sources such as wind induced breaking surf are also captured in frequencies below 100 Hz and their signatures are in-line with previous similar studies in coastal areas (Haxel *et al.,* 2013). Regarding vessel's noise, the events recorded where few but exclusively distinctive, characterized by strong sound energy signals. At this point, it should be noted that only a sub-set of the vessel activity captured could be associated to AIS records. Previous research has shown the strong contribution of motorized vessels that are amongst the most widespread sources of anthropogenic underwater noise especially in coastal areas (Hermannsen *et al.,* 2019). We assume that there will be a strong additional noise input in the case of Argolikos Gulf during the summer period due to the increased presence of recreational boats. In general, the presented work has shown the feasibility of monitoring the soundscape in coastal areas with autonomous hydrophone-recorder systems. Such missions in the near future can shed light to the functioning of sensitive ecosystems, reveal their soundscape picture, and assess the pressures related to anthropogenic noise inputs and marine organisms.

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DEEP LEARNING TECHNIQUES FOR THE DETECTION AND CLASSIFICATION OF SPERM WHALE AND STRIPED DOLPHIN BIOACOUSTIC PATTERNS

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Abstract

In this work, we implement Neural Network based Deep Learning techniques to recognize and categorize biosignals generated by two cetacean species: sperm whales (*Physeter macrocephalus*) and striped dolphins (*Stenella coeruleoalba*). The dataset consists of a total of 607 characteristic cetacean vocalizations (clicks and whistles) recorded with passive acoustic monitoring systems from two geographic areas near Pylos in the Ionian Sea and in the Bay of Sougia in Southern Crete, Greece. We convert the audio signals into time-frequency representations, applying the Short-Time Fourier Transform (STFT) and generate an image dataset consisting of manually labeled spectrogram images. We show that applying fine-tuning over a pre-trained ResNet-101 deep network architecture, typically used for image classification, training and validity accuracies of 84% and 88% respectively and a testing accuracy of 91% can be obtained. The above results demonstrate that deep learning techniques are capable of learning patterns in meaningful time-frequency representations originating from complex and noisy bioacoustics signals. Further research needs to be carried out in order to evaluate the relative performance of alternative spectro-temporal or time-scale representations such as mel-spectrograms and/or wavelets respectively.

Keywords: Machine learning, neural networks, pattern recognition, supervised learning, cetacean vocalization.

1. Introduction

Deep Learning (DL) is a subfield of machine learning widely used today in applications to various scientific domains delivering state-of-the art performances on tasks such as classification, image segmentation and speech recognition, natural language understanding, medical applications etc. (LeCun *et al*., 2014). DL methods are representation-learning methods that allow a machine to automatically discover intrinsic representations of raw input data. In the domain of cetacean bioacoustics, ML has made significant progress in recent years and it has been demonstrated that a plethora of different machine learning architectures can achieve promising results in species detection and identification. Traditional Gaussian Mixture Models (GMMs) and Support Vector Machines (SVM) algorithms have been used to construct a species classifier (Roch *et al*., 2008). It has also been demonstrated that Convolutional Neural Networks (CNN) models are able to classify spectrograms generated from sperm whale acoustic data constructing thus a click detector (Bernant *et al*., 2019). In the same study the authors implement long short-term memory (LSTM) and Gated Recurrent Unit Recurrent Neural Networks (GRU-RNN) models to classify sperm whale coda types, categorize a plethora of different vocal dialects, and recognize individual whales.

Sperm whales vocalizations are made up mostly of broadband short duration impulsive signals named clicks involved in echolocation (Mohl *et al*., 2003). Usual clicks are highly directional with regular interclick intervals ranging between approximately 0.5s and 2s (Zimmer *et al*., 2004). The duration of a usual click may reach 20 to 30 ms and its centroid frequencies are of 15 kHz (Mohl *et al.*, 2003). Sperm whales emit also stereotyped patterns of repetitive series of clicks, termed 'codas', which are less directional with centroid frequency at 5 kHz (Huggenberger *et al.*, 2016) and are assumed to be used for communication within the groups or individual whales while at surface (Frantzis & Alexiadou, 2008). Sounds generated by delphinids have in generally been categorized into three distinct types: Echolocation clicks, burst pulse clicks and whistles. Echolocation clicks are broadband signals the frequency of which can vary from few tens of kHz to well over 100 kHz (Oswald *et al.*, 2007), while burst pulse clicks are closely-spaced broadband clicks. Whistles generated by striped dolphins are continuous narrowband signals with harmonics the fundamental frequency of which ranges from 2 to 30 kHz (Oswald *et al.*, 2007). Whistles are characterized by geographic variability while their durations range between 100ms and just over 4 s (Papale *et al.*, 2013).

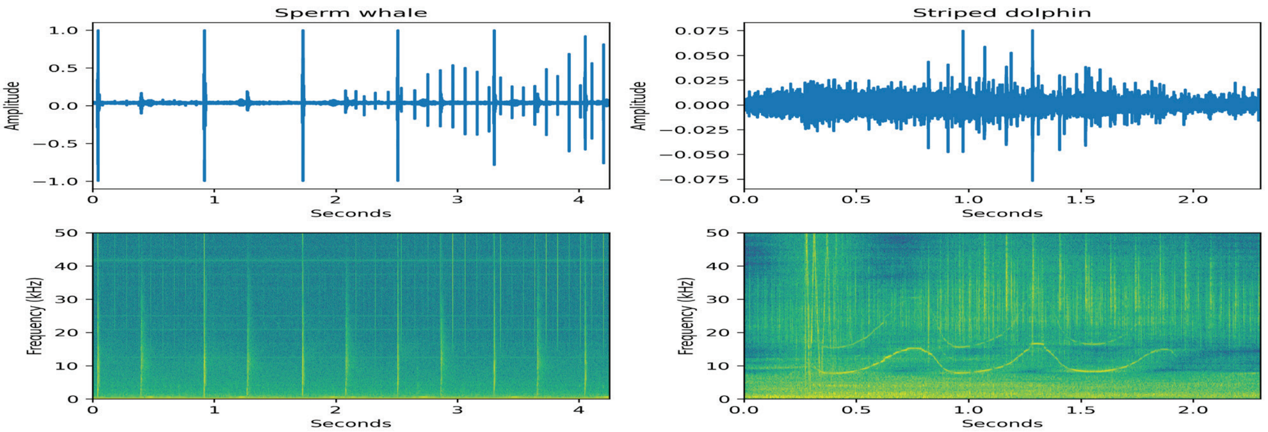

Fig. 1: Waveform (top panels) of sperm whale usual clicks (left), delphinid clicks and whistles (right) and corresponding spectrograms (bottom panels; 1024-point FFT, Hanning Window, overlap = 50%) recorded by a Passive Acoustic Listener at Pylos site.

In Figure 1 a waveform of sperm whale usual clicks and a waveform of striped dolphin whistles and clicks are plotted. The spectrograms reveal the broadband character of the clicks as well as the continuous nature and narrowband frequency width of a dolphin whistle and its harmonics.

The main purpose of this study is to enhance our understanding about the efficiency of deep networks on detecting bioacoustics patterns. Reinforcing a network's learning ability seems however to depend on factors associated with the choice of data preprocessing techniques applied. Thus a major task during digital signal processing is to preserve patterns of cetacean clicks on the denoised audio signal after filtering or/and dimensionality reduction take place. Consequently training the network on the dataset consisting of spectrograms and testing its generalization ability to new data are the concluding tasks on a multi-step procedure affecting the performance and robustness of the network.

2. Material and Methods

2.1 Origin of data

The data reported here were collected at different geographic areas and time periods. A Passive Aquatic Listener (PAL) was deployed at Pylos station of the POSEIDON buoy network in the Ionian Sea at 500-m depth from November 2008 to March 2009 approximately 10 km off the west Peloponnese coast (Nystuen *et al.*, 2015). Further acoustic data was collected in summer 2020 and 2021 from the 'SAvE Whales observatory' (System for the Avoidance of Collisions with Endangered Whales) consisting of three acoustics stations with one hydrophone each suspended at a depth of ~100 m, and deployed in the Bay of Sougia, Southern Crete ~2 km offshore and 1-2km apart in an area of water depth ~500 m. Both acoustic recordings were carried out with a sampling frequency of 100 kHz.

2.2 Signal Processing

Raw acoustic data contain a leading silent part which is variable in time. We discriminate the silent signal, which is of zero informative content from the main signal by thresholding on its short-time energy rate. Given that audio recording is realized in a polyphonic environment, raw signals inevitably incorporate noise. Thus, we apply a high-pass Butterworth filter with a low-frequency cutoff at 1 kHz. This enables us to remove a large part of noise coming from sources other than marine mammals. Finally, a spectral noise gating filter is applied to the signals in order to separate coherent features in the spectrogram structure from background noise. We use the denoised signals to construct a spectrogram image dataset applying the windowed Fourier transform (STFT) to a manually labeled dataset consisting of distinct audio files. The spectrogram is obtained by framing and windowing the signal, then computing the discrete Fourier transforms (DFT) over every windowed signal. We use a Hanning window with a size of 512, corresponding to a window of 0.5ms duration to align with the impulsive structure of clicks, while overlap of 50% is used.

2.3 Short analysis in the framework of a residual Deep Learning architecture

We use a pre-trained on ImageNet dataset residual network (ResNet-101), typically used for image classification and object detection. ResNet deep CNN architecture consists of multiple blocks that are connected to each other in series, while each block develops shortcut links parallel to the normal convolutional layers. The main advantage of using ResNet architectures is their ability to overcome drawbacks of traditional deep CNN, such as the problem of vanishing gradients. In our experimental setup, the dataset was divided into a training set, validation set, and test set with the proportions of 80%, 10%, and 10%, respectively. Thus, we generate a training dataset consisting of 486 spectrograms as well as a validation set and a test set comprising of 121 spectrograms in total. For this study, the model is trained for 150 epochs with a batch size of 32. During the first 90 epochs we train exclusively the top layer of the network (2049 parameters) while for the next 60 epochs we apply fine-tuning in order to extend training over 1 million parameters. We use an adaptive learning optimization algorithm ('adam') with initial learning rate set at 0.0001 while the binary cross-entropy function is used as loss function. A dropout regularization of 0.25 was employed to prevent from potential overfitting.

3. Results

Under the above parametrization, the deep ResNet-101 transfer deep learning model achieves training and validation classification accuracy of 84% and 88% respectively. We investigate the network's generalization ability using 60 unseen spectrograms and observe that the trained model achieves accuracy of 91%. The results are considered as promising given that the dataset is not equally balanced. In fact the initial dataset of 609 spectrograms includes 242 sperm whale vocalizations vs 365 striped-dolphins vocalizations (clicks and/or whistles). In this framework, we calculate that a precision performance indicator (fraction of detections reported by the model that were correct) is relatively lower when detecting sperm whales (88%) while the same metric characterizing the classification of striped dolphins reaches a level of 94%. In Figure 2, model training results are shown.

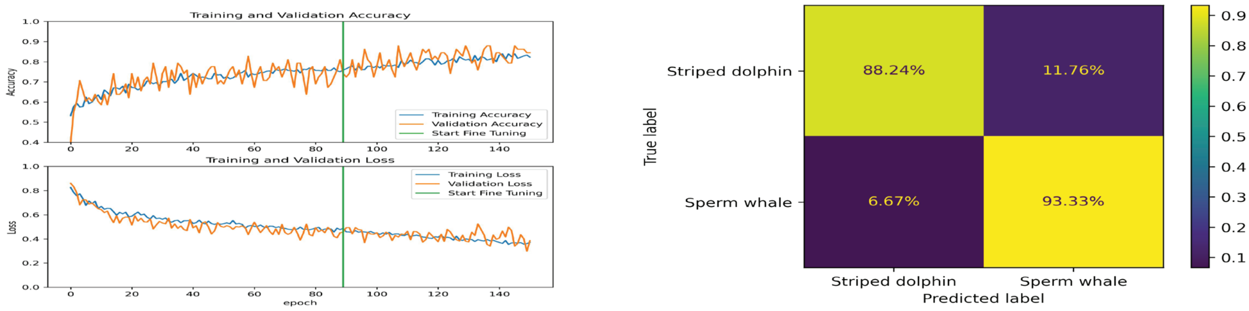

Fig. 2: Plot of Model accuracy on Training and Validation Datasets (left top panel) and plot of Model loss on Training and Validation datasets (left bottom panel). The ResNet-101 model generalizes well without overfitting problems. At the right panel the confusion matrix describes the accuracy on the test set.

We observe that despite the deepness of the residual network, overfitting is efficiently prevented during both phases of training, before and after fine-tuning as long as we maintain learning rate at a low level and apply sufficient drop out regularization. In fact, we note that the behavior of the network remains satisfying for hyper-parameters tuned closely and around the ones described here above, and its training efficiency as well as its long term stability strongly depend on the choice of the learning rate.

4. Discussion/Conclusion

The study confirms recent findings on the efficiency of supervised learning techniques for spectrogram-based pattern recognition when applied to bioacoustics. In fact, pre-trained deep architectures in computer vision tasks seem to provide a remarkable bioacoustic pattern recognizer despite the significant differences between spectrograms and standard ImageNet image samples. CNN finally prove strong learning ability over localized complex patterns in spectrograms and verify that time-frequency representations provide remarkable input candidates for supervised neural networks. Another aspect comes from the fact that Deep Learning is a data driven set of algorithms and the results have to be seen with care in relation with the nature, quantity and structure of the available dataset. The number of samples used in this study is not considered sufficient and the dataset is characterized by strong heterogeneity, firstly due to the fact that the available wav files are of variable sound length, and secondly because the dataset is unbalanced. From this perspective, the results of the actual study seem even more promising while a further enrichment of the dataset will most probably improve network's accuracy and stability. We should also keep as a conclusion that ResNet model reduced efficiently training and test errors managing to preserve the information flow into the network efficiently. Moreover, alternative neural networks architectures such as long short-term memory (LSTM) networks should be designed and tested in order to compare performances of supervised techniques on the problem here examined. Actually, further experiments are underway testing deep networks on alternative time-frequency or time-scale representations such as mel-spectrograms or wavelets respectively and will help in further understanding the relative impact of input representations on network's performance. We could then proceed to richer multiclass experiments incorporating a third class where clicks or whistles are absent or interestingly include richer vocal behaviors (for example, treating dolphin whistles and clicks as distinct classes). Ideally, the aim of this research is to include more cetacean species living along the Hellenic Trench or in the Greek Seas in general –a possibility that today cannot be implemented due to limited data availability– in order to build a species recognizer tool which could be used in a long-term project for real-time detection and classification of cetacean vocalizations.

5. Acknowledgements

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COMBINING IN-SITU AND SATELLITE DATA FOR TURBIDITY MONITORING IN COASTAL WATERS: VOULIAGMENI BAY, GREECE

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Abstract

Water quality information is important for the Good Environmental Status of coastal and marine waters which are influenced by several anthropogenic activities. A combination of remote sensing and in situ data contributes to continuous monitoring of water quality in such environments, enabling to develop the most appropriate water quality management plans. This study investigates the use of PlanetScope high-resolution satellite data to monitor water turbidity in the Vouliagmeni Bay (Attica, Greece). Based on multiple field measurements and concurrent satellite imagery a new regional model has been developed that allowed the monitoring of turbidity dynamics after a severe storm. The results showed that turbidity in the Bay was affected by physical factors such as waves and rainfall, as well as human activities (i.e., dredging operations). Although turbidity presented high values in the shallow area close to the Vouliagmeni beach a day after the storm, a remarkable reduction was recorded in the following days at the whole study area, revealing a highly dynamic system with rapid water renewal due to the prevailing circulation inside the bay.

Keywords: water quality, PlanetScope, high-resolution imagery, dredging operations, storms.

1. Introduction

Water quality monitoring is crucial in order to ensure human health and marine life. According to Clark and Wilber (2008), sea water turbidity values of less than 25 FTU are characterized as "tolerant" by marine organisms and do not cause irreparable damage. Based on numerous of relative studies (Ruiz & Romero 2003; Houngnandan *et al.*, 2020; Li *et al.*, 2021) the following categories were adopted for the water quality concerning the turbidity levels: (a) ≤ 1 FTU "excellent"; (b) 1-5 FTU "normal"; (c) 5-15 FTU "satisfactory"; (d) 15-30 FTU "tolerant"; (e) > 30 FTU "non-tolerant".

Field measurements provide accurate information about turbidity levels; however, they are not always feasible due to acknowledged weaknesses, such as proximity to land, human activities etc. On the other hand, remote sensing can be a powerful complementary to in situ observations tool, towards the continuous monitoring of the coastal and marine environment (Gohin *et al.*, 2020). Recently, Pleiades high-resolution (Luo *et al.*, 2020), Landsat-8 and Sentinel-2 (Kuhn *et al.*, 2019) satellite images have been successfully used to retrieve water turbidity and examine its dynamics. Landsat-8 observations were also utilized by Kanellopoulos *et al.* (2019), to evaluate water quality changes due to dredging operations in a semi-enclosed environment.

In this study, high-resolution satellite data obtained from PlanetScope multispectral sensor were correlated with simultaneous in situ turbidity measurements, to develop a regional turbidity model in the Vouliagmeni Bay in Attica. A severe storm event was investigated, occurring at the broader area of the Vouliagmeni Bay on December 11, 2021, which led to high turbidity levels. By exploiting satellite data and using a new model, the monitoring and evaluation of the spatio-temporal turbidity changes in the study area during this event was feasible. The results suggest that PlanetScope high-resolution satellite images can provide significant information about turbidity levels in coastal waters, forming a successful tool for water quality monitoring.

2. Material and Methods

2.1 Study area

Vouliagmeni Bay is located in the Western part of the Attica peninsula, about 40 km SE of Athens. It covers an area of ~1.5 km^2 and is a typical pocket beach, of N – S orientation, with the 800 m long sandy beach being located at the northern part of the Bay. The sea-bottom deepens gradually to the Southern border of the Bay, at about – 40 m, with a steady slope of ~ 2% (Fig.1a). It consists mainly of sand, with the exception of the eastern part of the Bay, where a rocky substrate is present. *Posidonia oceanica* meadows cover almost the entire seafloor, from the depth of ~9 m and deeper, to the southern edge of the Bay.

The Vouliagmeni marina is located at the SW part of the Bay and is under radical reconstruction of the whole facility since the summer of 2021, with heavy onshore and offshore dredging activities, including deepening of the marina seabed, elongation of piers and construction of buildings for port and leisure activities (Fig. 1b).

The factors that affect the turbidity dynamics in the Vouliagmeni Bay are the following: a) the waves and coastal currents' action (physical factor), b) discharges through surrounding streams on a regular basis or after high precipitation events (physical factor) and c) dredging operations in the marina (human factor).

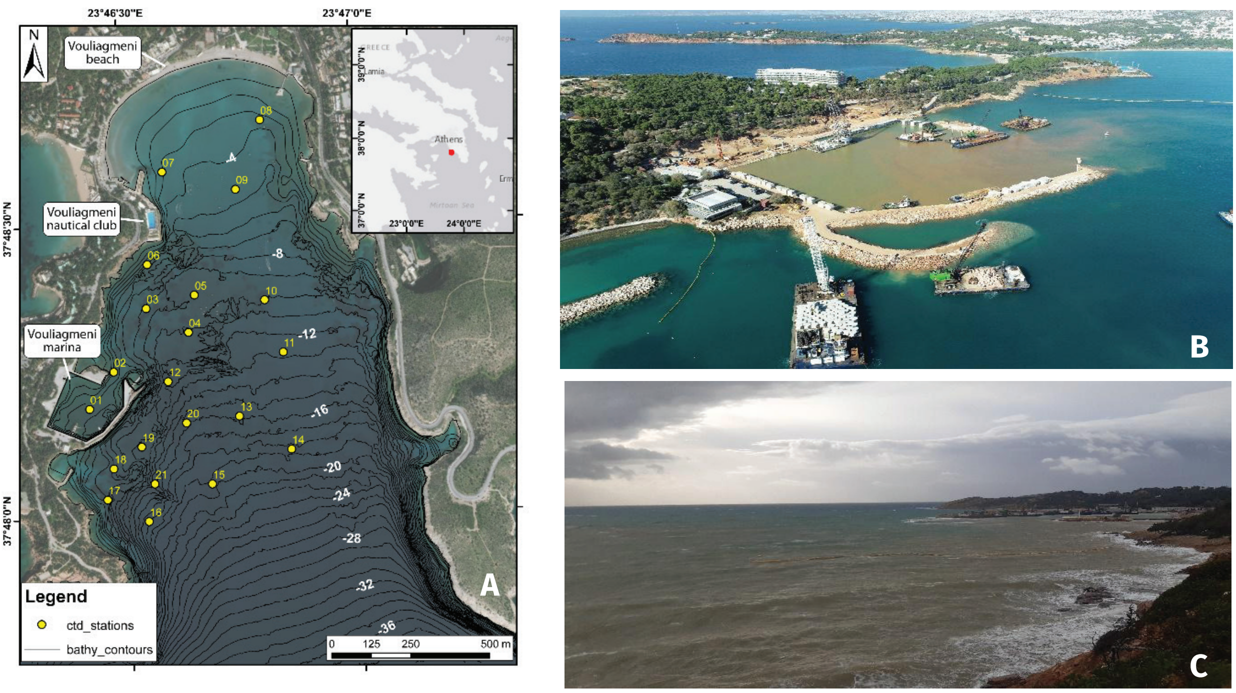

Fig. 1: a) Bathymetry of the Vouliagmeni Bay and representative CTD stations; b) Vouliagmeni marina and adjacent infrastructures; c) the storm of 11/12/2021.

2.2 In situ Data collection

The in situ turbidity data were collected weekly, for a period of 10 months (June, 2021 to March, 2022), using the RBRsolo[3] turbidity sensor (measuring range 0-1250 FTU, deviation ± 2%). Vertical recordings were performed in the water column (from the depth of ~ 1 m to the sea-bottom) in various locations, inside the marina and around the Bay.

2.3 Satellite Images collection and processing

Based on the collected in situ observations, the corresponding PlanetScope (Planet) and Sentinel-2 (S2) satellite images were acquired from PlanetExplorer and Copernicus websites, respectively. PlanetScope provides almost daily satellite images at a 3 m spatial resolution, and Sentinel-2 provides multispectral data at 10 m resolution with a revisit time of 5 days. Specifically, the top of atmosphere Planet and S2 level1C data were obtained and processed with ACOLITE atmospheric processor to extract surface reflectance values.

Regarding the validation part, diffuse attenuation coefficient (Kd) was first extracted from the closest available S2 data to Planet observations using ACOLITE. Kd values were then used to estimate the optical depth for each sampling location. Based on linear regression analysis, a new model was developed to calibrate the Red band surface reflectance values with the in situ turbidity measurements. The Red band (B3) reflectance was used, since there is a good relationship between this band and turbidity (Nechad *et al.*, 2009, Dogliotti et al., 2015). The regression model was obtained using 127 pairs (field and satellite) of data (see equation 1) with $r = 0.74$ and p-value <0.05.

$$\text{Turbidity} = 200.6 * B3 - 2.4808 \qquad (1)$$

2.4 Weather data collection

The wind and wave data during, and a few days after, the major storm event were obtained from the HCMR's Poseidon System. During the event of December 11, 2021, strong, southern winds of 8 Beauforts created waves with heights more than 4 m, for a period of ~ 6 hours, that struck the Vouliagmeni Bay (Fig.1c). The next day the wave heights were reduced to 1.5 m, whereas the storm gradually calmed on December 15. The waves caused destruction to the marina area, by dislocating accropodes off the piers and ripping the floating silt curtains put to retain the dispersed dredged plume from reaching the northern part of the Bay.

3. Results

In this chapter, the satellite-derived outcomes on December 12, 2021, one day after the storm event, and on December 13, 2021 are described, when PlanetScope images were available. To further evaluate the impact of the storm in the study area, turbidity levels in Vouliagmeni Gulf were compared with the extracted values in the adjacent Varkiza Gulf, where no added turbidity from dredging activities was present.

Figure 2 demonstrates the PlanetScope RGB composites, and the corresponding turbidity maps based on the new model. On December 12, 2021 the mean turbidity value in the Vouliagmeni marina (area A) was 12,7 FTU, while in the northern part of the Gulf (area C) the recorded value was 29 FTU. In the rest of the Gulf (area B) including the southern-open part of the Gulf, the mean turbidity value was 8 FTU. Comparing the mean turbidity values over Vouliagmeni Gulf and nearby Varkiza Bay (area D), it is noticed that they are very similar, with the latter being slightly higher (i.e., 12 FTU in Vouliagmeni, and 14 FTU in Varkiza).

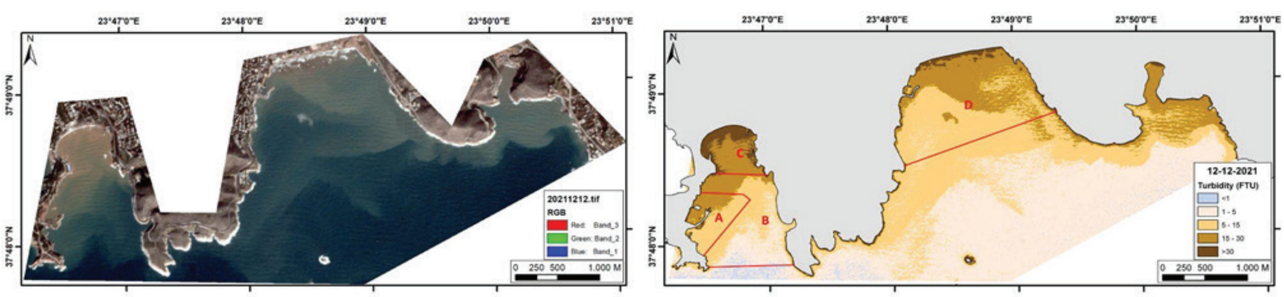

Fig. 2: Turbidity on December 12, 2021. Left: PlanetScope RGB image; Right: turbidity map.

Regarding the condition of December 13 (Fig. 3), both RGB images and turbidity maps show that two days after the storm, the turbidity levels have been significantly reduced revealing the strong impact of the dominant currents. More specifically, in area A the mean turbidity levels were 3 FTU, in area C 7 FTU, and in area B 2 FTU. The highest turbidity values (i.e., 18 FTU) were recorded in the area of the marina where dredging operations took place until December 10. In the Varkiza Bay (area D), turbidity again exhibits slightly higher values than in Vouliagmeni Bay (i.e., 3,7 FTU in Varkiza and 3,1 FTU in Vouliagmeni). It is also highlighted that in Varkiza Bay, discharges from a small stream (Skobi stream) led to a plume of higher turbidity values than the rest of the Gulf (Fig. 3).

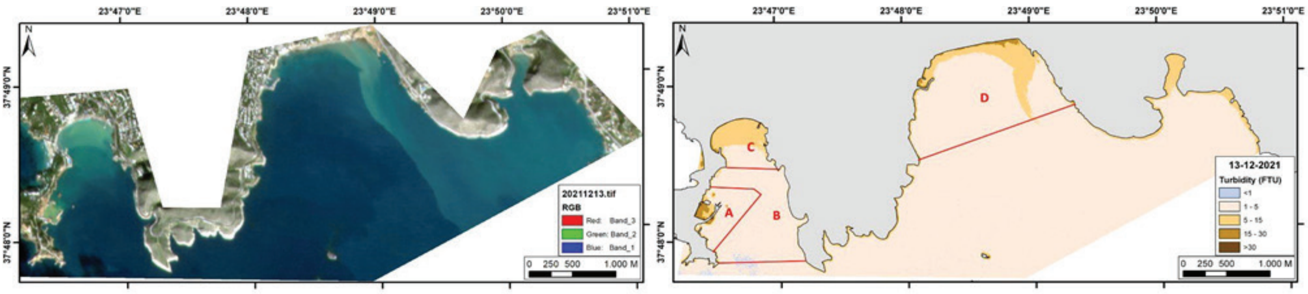

Fig. 3: Turbidity on December 13, 2021. Left: PlanetScope RGB image; Right: turbidity map.

4. Discussion/Conclusion

During a nine-month period, in situ observations were acquired and correlated with simultaneous PlanetScope satellite images over Vouliagmeni Bay, to develop a new linear-regression model for turbidity monitoring. Satellite-derived results indicated that the severe storm lasted until December 13, 2021, resulting to 'non-tolerant' conditions on December 12, 2021 at the shallow northern part of Vouliagmeni Bay (area C). The maximum values of 36 FTU are attributed to the seabed sediment resuspension partly originated from the marine area through the dredging activities due to waves and currents, as well as to the accumulation of dead *Posidonia Oceanica* leaves in the surf zone.

On December 13, the mean turbidity value in the whole Vouliagmeni Bay was 74% lower than the recorded mean value on the previous day, leading to "normal" seawater conditions. The resuspension of fine-grained sediments due to the prevailing southern waves action again led to high values (i.e., 23,5 FTU) in the shallow sector (area C) of Vouliagmeni Bay.

To validate our new model, we evaluated its performance in satellite/ field data that were not used for its development. The results indicated that the model tends to overestimate the very low turbidity values <0.5 FTU. More low turbidity values need to be collected in order to improve the developed model. Additionally, the atmospheric correction technique needs to be improved, since, in some cases, the estimated values are affected by the sun glint.

Based on the analysis described above, we conclude that both physical and human factors affect the seawater particle dynamics in Vouliagmeni Bay, leading to high turbidity values after severe storm events in the shallowest areas. The combination of satellite and in situ observations can contribute to mapping turbidity changes and assessing their impact on the marine environment. The applied methodological approach can be further validated with additional in situ observations and extended to monitor and better understand seasonal turbidity fluctuations in coastal areas.

5. Acknowledgements

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MUTLIDISIPLINARY INVESTIGATION IN A SUBMARINE GUSHING, GULF OF GERA, LESVOS ISLAND

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Abstract

The combination of different tracers to investigate and characterize submarine groundwater discharges has been proved adequate in such complex systems. The determination of the biochemical load due to the freshwater input in the coastal ecosystem of Gera's Gulf is presented. Moreover the radioactivity concentrations in the sediment and water were utilized to estimate the radiological risk to marine biota. The results showed that the biochemical load and radiological risk is negligible. However, further radiological assessment must be performed for phytoplankton and zooplankton.

Keywords: Natural Radioactivity, Submarine Groundwater Discharge (SGD), Organic Carbon (OC), major elements.

1. Introduction

The Submarine groundwater discharge (SGD) phenomenon involves the flow of fresh groundwater into the sea and the re-circulation of seawater. The chemical composition of SGD depends on the coastal aquifer though which infiltrates in the sea (Moore, 2010). This hydrogeological process has an impact to the coastal zone due to the transfer of biochemical constituents, such as carbon, metals, nutrients and radionuclides. The complex SGD system can be studied by a combination of methods, such as natural geochemical tracers and radiotracers. Usually the radionuclides of radon (^{222}Rn) and thoron (^{220}Rn) can be used in *in-situ* measurements to localize and quantify the SGD flux. In this work organic carbon (OC), major element and radionuclide concentrations were measured as combined tracers to study a gushing point of the SGD spring at Gera's Gulf, Lesvos Island and determine the contaminants' load. Natural radioactivity was measured in the matrices of sediment and seawater. Additionally, granulometry determination and radiological risk assessment were performed to investigate possible biochemical constituents' variations in the sediment and to estimate the dose rate received by marine biota.

2. Material and Methods

2.1. Study area and field work

The sediment sampling and the *in-situ* measurement were performed in a small submarine water gushing which is located in the center of a "crater" in the northeast part of Gera Gulf at Lesvos Island (Fig. 1). The studied gushing point was the one with the most intense outflow among the gushing points of the SGD spring. Seven small sediment cores 5-cm-long each, were collected. Four of them were located in (THS2) and near (THS1, THS3, THS4) the gushing point, one in the crater (THS5), one outside the crater (THS6) and one in the center of Gera Gulf (THS7), far away from the crater (Fig.1). The *in-situ* measurement regarding natural radioactivity in the water took place directly above the gushing point and was performed via the submarine gamma spectrometer KATERINA (Tsabaris *et al.*, 2008). The duration of the in situ measurement was 24h to observe possible variations in the radionuclides concentrations during the local hydrological cycle. The surface area of the gushing source where the THS1-THS4 samples were collected and the *in-situ* methodology was applied, has an almost circular shape of 16 m^2 area and 2.25 m radius.

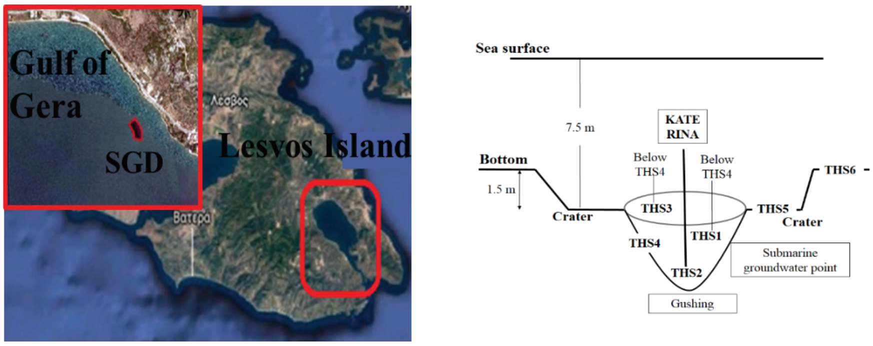

Fig. 1: The sampling area (left) and the sampling points (right).

2.2. Sample preparation and measurement methodology

Initially, the small cores were divided into two pieces, the “top” near the sea-sediment interface and the “bottom” below the seabed to observe possible differences in the biochemical and radiological load. Then, the sediment samples were oven dried to remove the moisture and were sieved to remove the coarse-grain material (namely, gravel, shells of calcareous organisms and their fragments, etc), then they were grounded and homogenized. The estimation of mud (<63 μm) and sand (63 μm -2mm) fraction of the sediments was performed through dry sieving. The methodologies of OC, major elements and radioactivity measurements have been described in detail in Gaudette *et al*. (1974), Karageorgis *et al*. (2005), Pappa *et al*. (2016), respectively. The major element content was determined via fused beads which were analyzed by X-Ray fluorescence (XRF) spectrometry. The natural radioactivity concentration in the sediments was measured with gamma-ray spectrometry using High Purity Germanium (HPGe) detector, while the natural radioactivity in the water was measured *in situ* with a low resolution gamma-ray spectrometer, as mentioned in section 2.1.

3. Results

3.1. Granulometry and organic carbon (OC) content

The collected samples, near and in the gushing source, were found mainly sandy, while those in the center of Gera’s Gulf were found to be muddy (Fig. 2). This observation was attributed to the strong-mixing environment at the SGD compared to the center of the gulf, as well as to the different sampling depths (approximately 6 m and 16 m, respectively). The continuous gushing suspends constantly the fine material and only the coarser sandy material is deposited. However, fine-sediment was found on the slope compared to the rest of the surface samples, indicating a process which accumulates the fine-grain material.

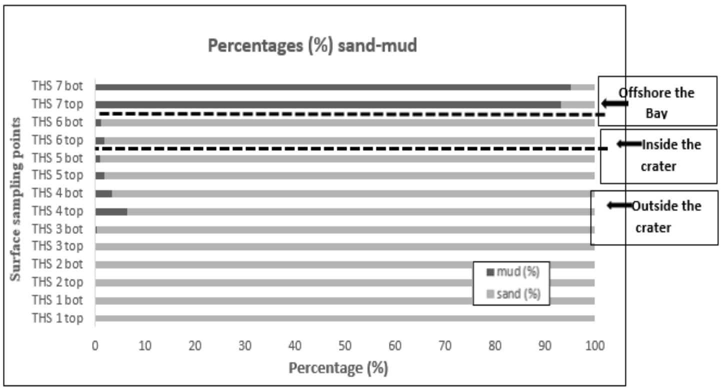

Fig. 2: The mud and sand fraction (%) of the surface sediments. The dash lines are depicted to guide the eye.

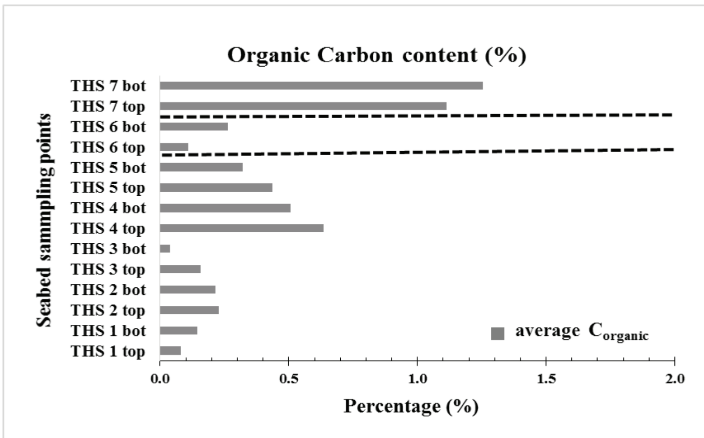

Fig. 3: The OC content (%) of the surface sediments. The dash lines are depicted to guide the eye.

The muddy sediments in the center of Gera Gulf (THS7) are rich in OC, in accordance with the general ‘rule of thumb’ that organic matter has affinity for fine-grain material (e.g., Keil *et al*., 1994). The combination of the distance from the gushing point with the muddy content seemed to form the OC distribution near (THS6, THS5) and in the gushing (THS1-THS4) area. Again the intense mixing environment in the gushing removes away or suspends the fine grain material in which the organic carbon could be sorbed hence in the gushing area the OC content is minimum except from the slope (THS4) where the muddy content resulted in the enriched OC concentration.

Concentrations of major elements and natural radionuclides

The majority of the major elements’ concentrations showed elevated values at the sampling points of fine grain content and OC, thus the center of Gera’s Gulf (THS7) and the slope of the gushing (THS4). The Si and Ca concentrations (Fig. 4) showed an opposite spatial distribution, meaning their concentrations were not connected to the fine grain and OC content but maybe to the gushing process itself. The maximum concentrations of Si were found in and near the gushing area indicating a Si-transport processes from the Earth’s crust, rich in Si, to the surface. Ca concentrations exhibited elevated values near the SGD (THS5, THS6) indicating a possible deposition route due to the water gushing out.

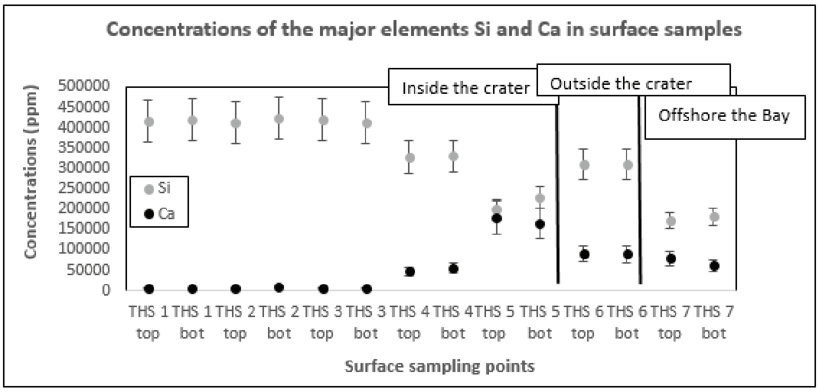

Fig. 4: Major element concentrations of Si and Ca in surface sediments.

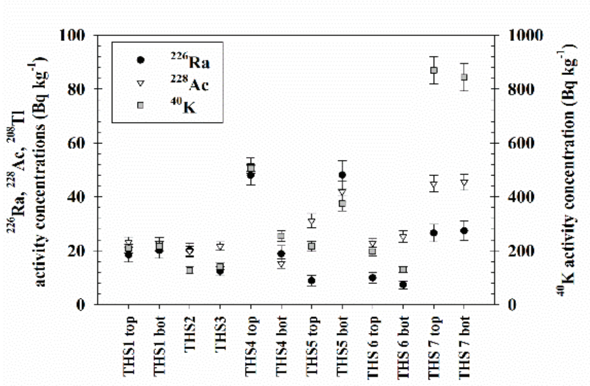

Fig. 5: The natural radioactivity concentrations in the sediment.

In this work, the natural radionuclides of ^{228}Ac, ^{208}Tl, ^{214}Bi and ^{40}K were measured in the sediment (Fig. 5) and the seawater. The ^{228}Ac and ^{208}Tl exhibited similar values within uncertainties in both matrices. The maximum activity concentrations in the sediment were also found in the sampling points characterized with elevated fine grain material. The activity concentrations of ^{228}Ac, ^{208}Tl, ^{214}Bi and ^{40}K in the seawater were almost constant during the in situ measurement exhibiting values of 500 Bq m^{-3}, 520 Bq m^{-3}, 220 Bq m^{-3} and 18000 Bq m^{-3}, respectively, probably indicating a constant flux of radionuclides gushing from the vent.

4. Discussion/Conclusion

The obtained activity concentrations in the different matrices (sediment, seawater), were used to estimate the radiological risk of marine biota via the software ERICA (Brown *et al.*, 2008). The methodology of the dose rates determination (μGy h^{-1}) was similar to Pappa *et al.*, (2016) regarding the default parameters of concentration factors. The default solid-water distribution coefficients (Kds) were not used and the experimental data were utilized instead. The screening levels proposed by IAEA (1992) and UNSCEAR (1996) are 400 μGy h^{-1} and suggest that in the case of chronic exposure, dose rates below these adopted values will not result in measurable population effects.

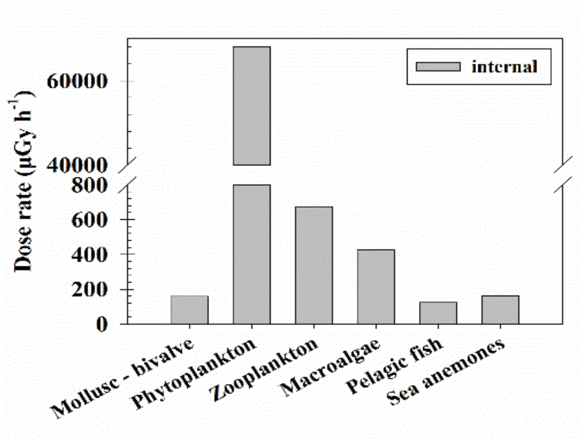

Fig. 6: The internal dose rate (μGy h^{-1}) of characteristic marine biota.

In this work the total dose rate was mainly attributed to the internal dose rate. The internal dose rate was found orders of magnitude higher than the external one, thus only the previous is depicted in Fig. 6. The biota with maximum dose rates were phytoplankton and zooplankton. Generally, the estimated dose rates of all biota were below the proposed levels indicating negligible radiological risk, except from phytoplankton and zooplankton. In these two cases the radiological assessment must be improved by obtaining experimental data. The high phytoplankton and zooplankton dose rates are mainly attributed to the alpha radionuclide emitters which ERICA takes into consideration assuming secular equilibrium between ^{208}Tl and its parent nuclide (^{228}Th).

The concentrations of major elements were within the ranges of the continental crust (Wedepohl, 1995) and the OC values were low indicating the absence of human impact despite the spatial OC variations and the close to the coast sampling points. The activity concentrations in the sediment were found similar with other radioactivity values obtained in different Greek gulfs (Pappa *et al.*, 2016). The activity concentrations of ^{228}Ac, ^{208}Tl and ^{214}Bi in the seawater were well above, similar and well below the measurements of the aforementioned radionuclides held in a similar SGD ecosystem, isolated from the sea and located at Anavalos, Gulf of Argolis (Eleftheriou *et al.*, 2020). The ^{40}K activity concentrations in this work could not be compared with those at Anavalos SGD flux, as the later were below the detection limits of the used method. Nevertheless, ^{40}K values at Gera's Gulf were found well above ^{40}K activity concentrations (350 Bq m^{-3}) of an inventory site of a freshwater environment (Eleftheriou *et al.*, 2013) showing the presence of seawater, rich in ^{40}K, near the freshwater flux. To conclude, the radiological risk for all the biota was found to be negligible, however the high dose rates, above the screening levels, for phytoplankton and zooplankton, suggest a further assessment for these specific organisms.

5. Acknowledgements

The authors would like to thank Dr. Kalfas C. for providing them with the SPECTRW program for the analysis of the gamma-ray spectra.

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ELEMENTAL CONTENT OF COMMERCIAL AND NON-COMMERCIAL FISH FROM THERMAIKOS GULF: NUTRITIONAL BENEFITS AND PUBLIC HEALTH RISKS

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Abstract

This research provides an evaluation of the quality of commercial and non-commercial fish species from the Eastern Mediterranean Sea. The concentration of 21 metals and elements (Li, Mg, Al, P, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Pb, and U) were investigated in the muscle of 28 commercial and non-commercial marine fish species from Thermaikos Gulf. Various parameters such as estimated daily intake, metal pollution index, hazard quotient, carcinogenic risk, maximum safe consumption and safety standards were estimated to assess the potential risks of metal content from the consumption of commercial and non-commercial fish. The nutritional benefits were calculated based on established Nutrient Reference Values. The analysis indicated great benefits regarding intake of Se and P for both commercial and non-commercial fish species; whereas the inorganic As content was below safety limits. Some commercial fish species had higher content of Pb than safety limits. In total, both commercial and non-commercial fish species should be considered as safe for human health and are a good source of essential nutrients.

Keywords: metals, major and trace elements, non-commercial fish, health risks, nutritional benefits.

1. Introduction

Until recently, non-commercial fish species were discarded from fishing vessels. Enforced measures by the European Commission have reduced the practice of discarding (Tsagarakis *et al.*, 2017) causing economic and logistic problems to fishing vessels obliged to retain and preserve unwanted catches on board. In addition, the demand for fish products has increased the interest in valorization of non-commercial fish species, such as the use of previously discarded species in newly developed markets, the use of low-value specimens for aquaculture and for seafood, pharmaceuticals, or cosmetics (Antelo *et al.*, 2012). However, before considering the possible use of some species, it is important to assess the health of their populations and to rule out the possibility for risk from a health perspective (Casadevall *et al.*, 2016).

There are numerous health benefits to consuming fish, however, with the presence of toxic metals and matalloids, and when ingested in toxic amounts, effects can be harmful to the human body (Bosch *et al.*, 2016). Fish discards contain contaminants which may be transferred to their valorized products, leading to possible long-term bioaccumulation and thus subsequent adverse health effects (Antelo *et al.*, 2012). The European Commission (Commission Decision 78/2005) and other international authorities have recommended some restrictions concerning the consumption of marine species and have regulated the maximum levels of toxic elements in seafood (Casadevall *et al.*, 2016).

Although the levels of contaminants in non-commercial, non-targeted and/or discarded fish species are not usually assessed, the sustainable management of discards involves the evaluation of these levels (Antelo *et al.*, 2012; 2016). The aim of this study is to assess the public health risks and benefits deriving from the total metal content (both toxic and essential metals and elements) of commercial and non-commercial fish species in Mediterranean countries.

2. Material and Methods

2.1 Sampling

Commercial and non-commercial fish were collected by means of a bottom trawler in the eastern region of the Thermaikos Gulf, in the coastal zone of the Northern Aegean Sea, Greece (Fig. 1). A total of 89 demersal, 13 benthopelagic and 17 pelagic fish specimens representing 17 commercial and 11 non-commercial marine fish species were collected. Specimens were sorted, measured in length, weighed, photographed, and rinsed with distilled water before dissection. Muscle was sampled above the abdominal area, on the left side of the fish. Samples were stored at -20° C until further analysis.

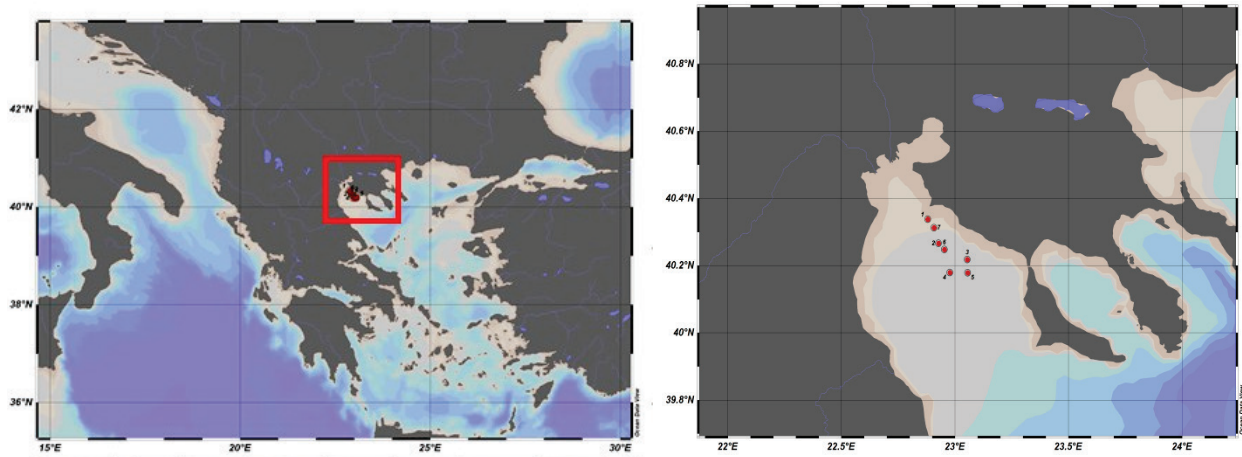

Fig. 1: Map of sampling area in Thermaikos Gulf, Greece.

2.2 Samples analysis

Trace element and metal concentrations were determined using the Method 3052 USEPA for digestion and trace metal analysis. An Inductively Coupled Plasma – Mass Spectrometer (Thermo Fischer Scientific, Winsford, United Kingdom; Plasma lab software) was used to measure element concentrations in the digests according to Method 6020A USEPA. The protocol is described in detail in Kalantzi et al. (2013) and Kalantzi et al. (2016). The precision and accuracy of the applied analytical procedure were assessed using certified reference materials (CRMs): fish protein (DORM-3) and non-defatted lobster hepatopancreas (LUTS-1) certified by the National Research Council of Canada and mussel tissue (BCR-668) certified by the Joint Research Centre of European Commission. The limit of detection (LOD) was calculated by multiplying the standard deviation of the blanks by three.

2.3 Human health risks and benefits assessment

Several parameters were considered to assess the human health risks from fish consumption: (1) estimated daily intake (EDI, ug/kg/d); (2) metal pollution index (MPI, ug/g ww); (3) hazard quotient (HQ) and total hazard quotient (THQ); (4) carcinogenic risk (CR); (5) maximum safe consumption (MSCA, kg ww/d); (6) comparison of mean concentrations of metals and elements with permitted limits for edible fish tissues. The nutritional benefits of marine fish were estimated by comparing the mean concentrations of essential nutrients with the daily reference intakes for vitamins and minerals (Nutrient Reference Values, NRVs) established by the European Union (Kalantzi *et al.*, 2013, 2016; Sofoulaki *et al.*, 2019). In all risk assessment estimations, a content of 3% of the total As was assumed present in inorganic, potentially toxic form (Sofoulaki *et al.*, 2019).

3. Results

Mean concentrations per species of Cr, Fe, Ni, Cu, Zn, As, Se, Cd, and Pb were well below the Safety Standards established by the European Union and various other international authorities while mean concentrations of Pb in some cases exceeded them for both commercial and non-commercial fish species. Mean Pb concentrations for three commercial species, *E. encrasicolus, P. acarne, T. trachurus*, (0.56-1.72 mg/kg ww) and Pb concentrations for three non-commercial species, *G. niger, S. cabrilla, P. blennoides*, (0.31-0.50 mg/kg ww) were found to be higher than the permitted limit of 0.30 mg/kg ww. If a content of 3% of the total As was assumed to be in its toxic inorganic form (that would be 0.04–0.98 mg/kg considering commercial and non-commercial fish species), the safety limit of 1.3 mg/kg would not be exceeded.

Regarding the nutritional value of commercial and non-commercial fish species, mean concentrations of Se can cover the 126% and 112%, respectively, of the recommended daily intakes established by the European Union. Significant amounts of P dietary requirements are also covered by commercial (33-104%) and non-commercial (20-102%) fish. Regarding K and Cr, 11 to 44% of these recommended daily intakes are covered.

4. Discussion

Results of this study show that consumption of both commercial and non-commercial fish species should be considered as safe for human health and provide a significant source of essential nutrients. In addition, non-commercial fish species could add value to fishery products in food, pharmaceuticals, and cosmetics. However, the high Pb levels found in muscle of some commercial fish species may be attributed to traces of Pb in the sediment of the Thermaikos Bay. Increased level of Pb result from anthropogenic perturbations, notably at the inner part of the bay. This could have a negative effect on marine biota (Violintzis *et al.*, 2009). The concentrations of the measured elements in muscle tissues (wet weight) were compared to studies conducted in the Adriatic Sea, the Mediterranean Sea and the Black Sea. In most cases, element levels were in range or below reported levels, except for As and Pb levels (Storelli, 2008; Tuzen, 2009; Yilmaz *et al.*, 2010; Aydın & Tokalıoğlu, 2015; Yabanli & Alparslan, 2015; Sofoulaki *et al.*, 2018; Varol *et al.*, 2019) which were greater in some fish species.

5. Acknowledgements

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TROPHIC STATUS EVOLUTION IN THERMAIKOS GULF DURING 1995-2007

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Abstract

In this work, nutrient and chlorophyll-a data were used, to assess the trophic status in Thermaikos Gulf (North Aegean Sea), which is impacted by both natural and anthropogenic pressures. The Eutrophication Index and the Chl-a classification scheme were applied at two sites, one in the inner part of the gulf and the other near the estuaries of Axios river, for the period 1995 - 2007. According to the Eutrophication Index, during 1995 to 2000 both areas were classified into Poor trophic status, while in the inner part of Thermaikos an improvement was recorded after 2000, as the trophic status shifted from Poor to Moderate. On the other hand, Chl-a concentrations in the inner Thermaikos Gulf were higher than those near the estuaries of Axios and the N:P ratio always revealed N- limitation under the study period. At the area near Axios estuaries, the trophic status did not show a clear declining trend, although a general improvement was obvious after 2000, indicating the complexity of the functioning of Thermaikos Gulf marine ecosystem in relation to the different pressures.

Keywords: Eutrophication Index, Chl-a classification scheme, Trophic status, Thermaikos Gullf, Anthropogenic pressures.

1. Introduction

Thermaikos Gulf is a marine coastal ecosystem of major importance, not only environmentally, but also due to the various socioeconomic activities associated with the area, also being an important marine coastal area in the Northern Greece because of the natural harbor of the city of Thessaloniki. Thermaikos Gulf is a typical, river-fed coastal inlet susceptible to several anthropogenic pressures, strong river discharges and variable meteorological and ocean (met-ocean) conditions. Among the rivers affecting the hydrology and functioning of the area is Axios River, which assumed to be the second most polluted river in Greece in terms of nutrient pollution (Androulidakis *et al.,* 2021; Nikolaidis *et al.,* 2006; Alexandra Pavlidou *et al.,* 2015; Simboura *et al.,* 2016; Skoulikidis, 1993, and references therein)river-fed, microtidal, semi-enclosed, coastal inlet of the east-central Mediterranean Sea. It is an important coastal ecosystem susceptible to several anthropogenic pressures, strong river discharges and variable meteorological and ocean (met-ocean. The anthropogenic pressures in the watershed area that drains into the Gulf include the urban effluents from the city of Thessaloniki, the discharges of the watersheds of Axios, Aliakmon, Loudias and Gallikos Rivers, where more than 250 industrial units operate (e.g., Androulidakis *et al.,* 2021; Karageorgis *et al.,* 2005; Krestenitis *et al.,* 2012).

In this work we have selected two sites in the marine area of Thermaikos Gulf, in order to assess their trophic status during the period 1995-2020 in relation to the different pressures, as Thessaloniki harbor, industrial activities, treated or partly treated sewage discharges (Site in the inner Thermaikos Gulf - stations TP10), agricultural discharges from the heavily polluted Axios River and mariculture (Site near to Axios estuaries - station TP16) (Androulidakis *et al.,* 2021; Pavlidou *et al.,* 2015).

Regarding eutrophication, Thermaikos Gulf has faced extended eutrophication phenomena (red tides, mucilaginous aggregates), especially over the Northern Thermaikos Gulf (Pavlidou *et al.,* 2015; Nikolaidis *et al.,* 2006). Several methods have been developed in the EU, in order to assess the trophic status and evaluate its trends under the umbrella of EU Directives (e.g., WFD; 2000/60/EC) (Pavlidou *et al.,* 2015 and references therein). In this work we used the Eutrophication Index (E.I.) as a methodological tool to assess the eutrophication status of Thermaikos Gulf in relation to the various pressures that impact the marine environment.

2. Material and Methods

Data were obtained during multiple oceanographic campaigns in Thermaikos Gulf fro the period 1995 - 2007 in the framework of several projects: MAST-III Metro-Med; the HCMR monitoring project "EYATH" funded by the 'Thessaloniki Water Supply and Sewerage Company S.A"; FATE project "Transfer and Fate of Harmful Algal Bloom (HAB) Toxins in European Marine Waters. Two stations were selected, TP10 and TP16, the first representing Thessaloniki bay and the latter the area near the estuaries of Axios river (Fig. 1). Nutrients and Chlorophyll-a (Chl-a) measurements were based on standard methods which are described in Pavlidou *et al.* (2020).

The annual concentrations have been derived from the mean integrated values per sampling. Only data available at least at seasonal scale were considered for the assessment. More specifically, we considered data from January to March as winter period data, April to June as spring data, July to September as summer data and October – December as autumn data (MedRegion Project, D6.6, 2021). We used data for the periods: 1995-1996; 1997-1998; 2000; 2001; 2003-2004 and 2006-2007.

Nutrients and chlorophyll-*a* annual concentrations were log-transformed prior to statistical treatment.

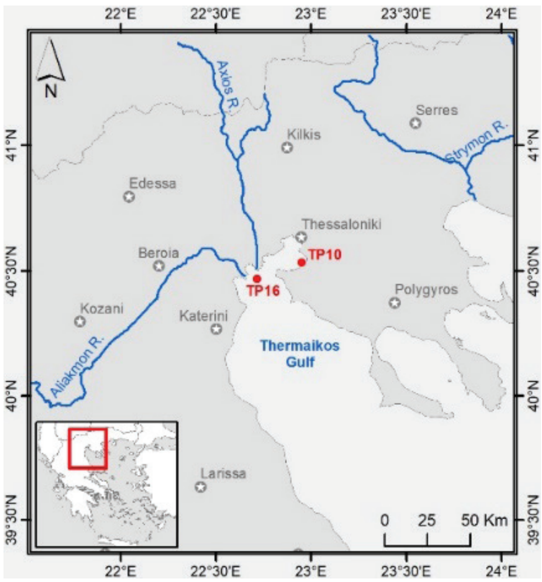

Fig. 1: The study area of Thermaikos Gulf, indicating the sampling stations and the main rivers.

In addition, the multiparametric method E.I. (Primpas *et al., 2010)* was used for the assessment of the trophic conditions of Thermaikos Gulf.

3. Results

Figure 2 presents the distribution of nutrients and Chl-a in TP10 and TP16 from 1995 until 2007. Continuous plot was used for the years that data were not available. At TP10 the highest values were observed in 2000 for nitrates (ranged from 0.33 to 0.96 μmol/L) and in 1997-1998 for silicates (ranged between 2.28 to 3.57 μmol/L). At station TP16, nitrates ranged from 1.08 μmol/L (in 2001) to 2.90 μmol/L (in 2004-2005) and silicates from 3.12 in 2001 to 9.36 μmol/L in 2004-2005. Ammonium ranged between 0.30 to 1.33 μmol/L at TP10 and from 0.33 to 1.15 μmol/L at TP16 (the highest values were measured in 1995-1996 at both stations). Phosphates ranged between 0.25 to 0.46 μmol/L at TP10 and from 0.17 to 0.55 μmol/L at TP16. The N:P ratios were always below 16. At station TP10, the N:P ratio ranged from 3.07 to 7.02 and at station TP16 between 5.34 and 9.58. Chl-a at TP16 ranged from 0.25 to 1.74 and at TP10 from 0.83 to 3.24; this high value was measured in 1995-1996. The Eutrophication Index (E. I.) is presented in Fig.3. Both stations were classified in Poor trophic conditions during 1995-2000, while TP10 was in Moderate trophic status in 2001-2007.

4. Discussion/Conclusion

In this study data from two stations located at Thermaikos Gulf are presented, the first (TP10) representative of the area at Thessaloniki Bay and the other (TP16) of the area near the estuaries of Axios river. At station TP16, the temporal distribution of nitrate and silicate presented common pattern, probably implying a common source, possibly from the land through the river. This is also supported by the significant correlation (0.853*, at the 0.05 level) of nitrate with silicate. Moreover, silicate and phosphate correlate significantly (0.945**, at the 0.01 level). The mean annual values of nitrate, phosphate and silicate at TP16 fluctuated a lot, probably following the fluctuation of the Axios flow-discharges.

Fig. 2: Mean annual integrated values of nitrate, ammonia, silicate, phosphate, N/P ratio and Chl-a at TP-10 and TP-16 stations from 1995 to 2007.

At station TP10, nutrients concentrations were generally lower than those at TP16, with the exception of ammonia during the period 1995 - 2000. On the other hand, Chl-a concentrations were higher at TP10 than those at TP16 in almost all the study period, with most values ay TP10 exceeding 1 μg/L. There is no significant correlation among nutrient concentrations, while Chl-a correlates significantly with ammonia (0.883*, at the 0.05 level). The N:P ratio was constantly calculated below 16 (always below 10), highlighting the strong N-limitation in the inner part of the Gulf, also highlighted in other studies (e.g., Androulidakis *et al.*, 2021; Nikolaidis *et al.*, 2006; Pavlidou *et al.*, 2015; Simboura *et al.*, 2016)river-fed, microtidal, semi-enclosed, coastal inlet of the east-central Mediterranean Sea. It is an important coastal ecosystem susceptible to several anthropogenic pressures, strong river discharges and variable meteorological and ocean (met-ocean.

An improvement in the trophic status during the study period is obvious, as the status was Poor till

2000, while from 2001 to 2007 both stations were classified in Moderate status with exception in the period 2004-2005 at TP16 which was in Poor status (Fig. 3). In addition, there is an indication of possible improvement after 2012 in both sub-areas, based on the data obtained from the monitoring Project for the implementation of the Water Framework Directive (data are not presented in this work since they refer to only two sampling periods per year), which, if confirmed, it could be driven mostly by the very low phosphate levels and the decreasing trends of Chl-a and nitrates.

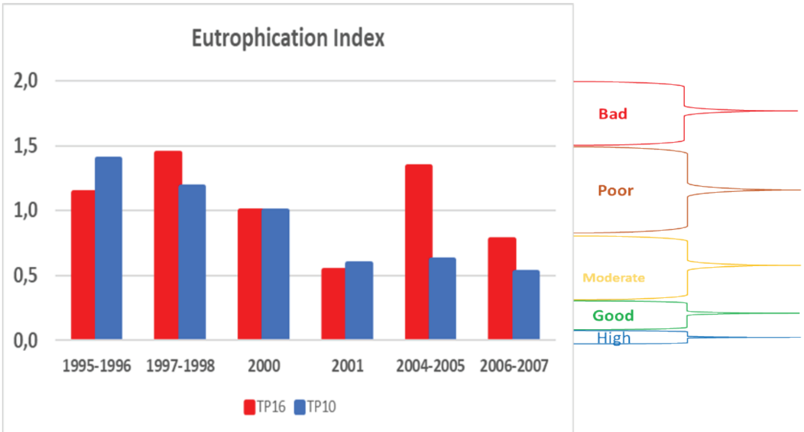

Fig. 3: Mean annual values of the Eutrophication Index at TP-10 and TP-16 stations from 1995 to 2007.

The study stations seem to differentiate as nutrients and Chl-a distributions and correlations demonstrated. Further evaluation of the data will examine the relationships between abiotic drivers, such as, continental inputs, the physical properties of the marine waters and the bay hydrodynamic status towards nutrients and Chl-a distributions, as well as algal species blooms and biomass.

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INNOVATIVE QUALITY MONITORING OF INLAND WATERS IN GREECE WITH THE USE OF UNMANNED BOATS AND HYDRO-TELEMETRIC STATIONS

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Abstract

Unmanned boats and their integrated sensors have been operated from EYDAP's R&D Department since 2019 in order to evaluate this innovative cost-effective tool for environmental monitoring in surface water bodies. Current monitoring programs produce water quality data based on periodical, often spot sampling, from specific points of interest following by laboratory analysis and thus, often give an insufficient temporal picture of the water quality on catchment scale. Automatic, telemetric stations for specific parameters installed by HCMR-IMBRIW in several Greek rivers and lakes offer significant improvements in continuous, temporal monitoring. An extra tool is the application of the unmanned boats that offer high temporal and spatial coverage over specific timescales that can be focused on sites or events of interest. The aim of the present study is to highlight the importance of an improved monitoring strategy, that leads to more reliable datasets and, hence, allowing the prediction of water quality in a large area of a catchment with higher frequency than traditional sampling methods. Here, we present the spatially fluctuations of chl-a and electrical conductivity during a sampling campaign in Lake Koumondourou and Kifissos river. The analysis shows that the use of unmanned boats, using commercially available sensors, can contribute to traditional monitoring programs of inland waters, detecting in time, possible pollution from anthropogenic activities or natural processes.

Keywords: remote sensing, Chlorophyll-a, electrical conductivity, Lake Koumoundourou, Kifissos river.

1. Introduction

Current water quality monitoring strategies are highly susceptible to discontinuous and/or unpredictable precipitation and hazardous events, such as direct discharge of sewage, combined sewer overflows or surface run-off from agricultural areas into drinking water resources or water bodies used for recreational purposes (Katsouras *et al.*, 2021). Such events occur occasionally and are usually not reflected by traditional monitoring approaches that are temporal and spatial specific, putting obstacles in assessing the water related pressures, and, thus, deriving effective countermeasures (Moustaka-Gouni *et al.,* 2019). Automatic monitoring stations offer the potential for near-real time monitoring of basic water quantity and quality parameters but they also perform point measurements and thus large investments are necessary to achieve the desirable spatial resolution (Mentzafou *et al.*, 2019). This points out the great importance of an improved monitoring strategy leading to more reliable datasets with higher spatial resolution and, hence, allowing the prediction of water quality on catchment scale as well as paving the way to water quality improvements (Mantzouki *et al.*, 2018; Warner *et al.*, 2018). As part of the activities undertaken by EYDAP and IMBRIW, the aim of the study was to demonstrate that water quality data, obtained from telemetric stations and the more novel unmanned boats, would add value to present monitoring strategies.

2. Material and Methods

2.1 Operation and description of the unmanned boats

The system architecture consists of an unmanned boat, the electronic components that allows to control the boat and to read data from the sensors (Fig. 1a). The unmanned boat can be directly tele-operated through an RC device or controlled with a tablet that provides high level instructions to the system, i.e.: monitor a pre-defined area using autonomous navigation (Steccanella *et al.*, 2019). The data are streamed online to a cloud-based information system (Knutz, 2020). Data stored in the cloud-based information system can be downloaded or visualized via a web-based application. Moreover, data can be visualized during the data collection campaign via a mobile application (Bloisi, 2018; Calisi, 2018). For the visualization of the results, a specific color pattern is followed, which includes the colors Blue, Green, Yellow, Orange and Red (indicating increasing values). The unmanned boat is equipped with sensors for standard parameters monitoring such as Dissolved Oxygen (DO), Temperature (T), pH, Electrical Conductivity (EC) and Chlorophyll–a (Chl–a).

a) b)

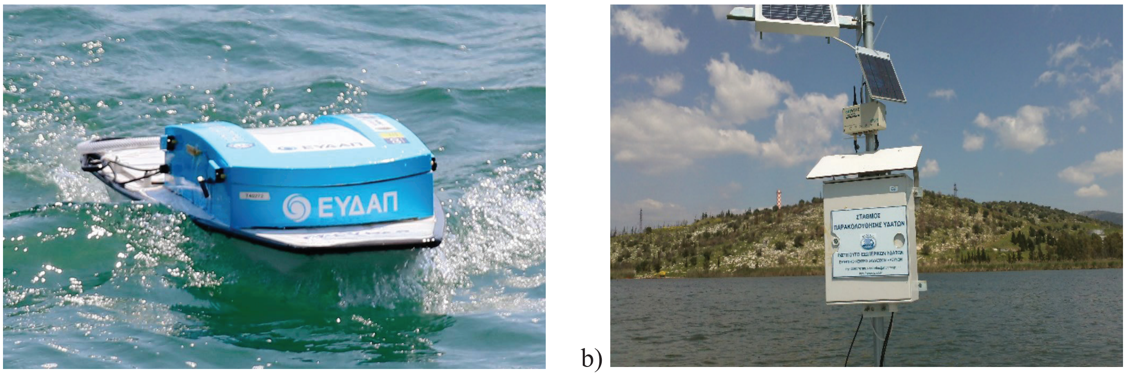

Fig. 1: a) Boat equipped with the basic sensor Kit, **b)** Hydro-telemetric station at Koumoundourou lake.

2.2 Hydro-telemetric stations

The Institute of Marine Biological Resources and Inland Waters (IMBRIW) of the Hellenic Centre for Marine Research (HCMR) has developed a network of automatic telemetric stations in lakes and rivers of Greece (https://hydro-stations.hcmr.gr/). Two of these stations have been installed in Koumoundourou lake and Kifissos estuaries and provide high-frequency information concerning water level, pH, EC, T, DO, and ORP, and at Koumoundourou lake also salinity (Table 1).

Table 1. Automatic telemetric stations of IMBRIW -HCMR.

Station	Latitude	Longitude	Altitude	Installation date	Site
Koumoundourou lake	38.0235	23.6018	0.83	28/03/2011	https://hydro-stations.hcmr.gr/koumoundourou-station-koumoundourou-lake/
Kifissos estuaries	37.9472	23.6727	3.35	15/07/2020	https://hydro-stations.hcmr.gr/kifissos_ekv-station/

2.3 Study areas

The campaign with unmanned boats in Lake Koumoundourou took place on 28th May 2019. The lake is located in Attica, close to the municipalities of Elefsis and Aspropyrgos, the main industrial area of Athens. It is a lagoon with mean depth 3.5 m, as it lies upon the sea level, very close to the coasts of the Elefsina Bay. Kifissos River is the main river of Attica region and its last part flows parallel or under the highway 1 linking Athens and Thessaloniki until it reaches the Faliro Bay in the Saronic Gulf. On 16th of

July 2020, a campaign was organized by EYDAP and IMBRIW of the Hellenic Centre for Marine Research (HCMR).

3. Results

3.1 Lake Koumoundourou

Within an hour, the unmanned boats covered much of the inner perimeter of the lake. The concentration of Chl-a ranged from 10.0 to 17.5 μg/l and was elevated in the northeast part of the lake, north of the gate (Figure 2a), probably due to the circulation of water in this direction and the influx of groundwater (Katsouras *et al.,* 2020). The conductivity ranged from 2800 to 3050 μS/cm (Fig. 2b) with its values continuing the steady decreasing trend recorded by HCMR studies from 1984 until today (Dimitriou *et al.,* 2012; Kousouris, 2014; Mentzafou *et al.,* 2016). On 28/05/2019 the telemetric station of Koumoundourou Lake recorded 10820 μS/cm. It should be noted that the monitoring sensors of the telemetric station have been installed near the lake bottom, while the sampling conducted by unmanned boats is surficial. This may explain the difference in the recorded values since the lake's bottom is below the sea level and is mostly affected by seawater while the freshwater coming from springs at the periphery of the lake forms a surface layer which is separated from the underneath saltwater due to their density difference. The maximum values were measured in northeast, indicating a small inflow of seawater mixing through the underground inlets.

Fig. 2: a) Chl-a and b) Conductivity in Lake Koumoundourou, 28/05/2019.

3.2 Kifissos river

During the campaign, the unmanned boat covered almost 2 km under the highway, reaching the mixing zone of the river with the sea, identifying interesting profiles of the physicochemical parameters that were monitored. Chl-a concentrations ranged from 2.0 to 15 μg/l (mean 5.7 μg/l), while two high groups of chlorophyll concentrations were found upstream, the first near Piraeus Avenue and the second near lines of the Electric Urban Railway ISAP (maximum value 15 μg/l). The concentration of conductivity ranged from 1400 μS/cm to 3500 μS/cm, indicating a significant inflow of the sea front. On 24/07/2020, few days after the Kifissos estuaries telemetric station installation and full function, EC was reported to be 3369 μS/cm which is very close to the recorded values with the unmanned boat.

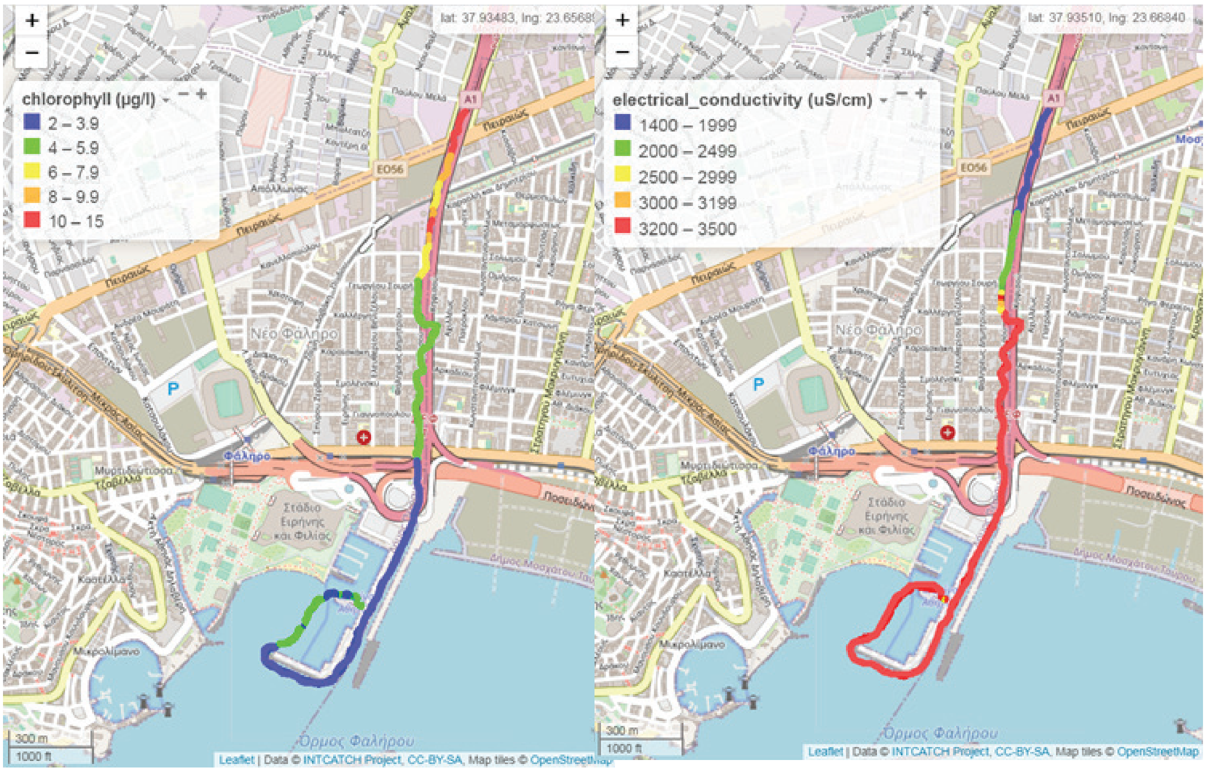

Fig. 3: a) Chl-a and b) Conductivity in Kifissos river, 16/07/2020.

4. Discussion/Conclusion

As reflected by the results obtained by the unmanned boats and the telemetric stations there is a clear trend of steady decrease of conductivity in Lake Koumoundourou while the eutrophic state of the ecosystem is also in accordance with previous studies (Dimitriou *et al.*, 2012; Kousouris, 2014; Mentzafou *et al.*, 2016). Additionally, the application of unmanned boats in Kifissos River provided valuable insights into the distribution of conductivity and chl-a related to environmental pressures.

The use of unmanned boats in parallel with hydro-telemetric stations achieves a thorough monitoring coverage both in space and in real-time providing significantly higher amount of water quality data, even from inaccessible sampling areas, without requiring costly monitoring schemes and subsequently raising alerts in time to take action to protect end users. The systematic, full scale application of unmanned boats and hydro-telemetric stations could support the investigative monitoring programs and represent a valid rapid tool/approach in case of emergencies (e.g., in relation to climate changes events such as flooding). The vision is that EYDAP and HCMR-IMBRIW will provide a service to authorities and organizations interested in assessing water quality in relation to catchment management and the traditional sampling protocols.

5. Acknowledgements

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COLLAPSING COASTAL REEFS: THE CASE OF GYAROS MARINE PROTECTED AREA AND THE NORTH CYCLADES ISL., AEGEAN SEA, GREECE

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Abstract

To explore possible effects of marine protection (no-take fishing zones) on shallow sublittoral reef systems, an ad hoc study was designed and implemented in the North Cyclades, Aegean Sea, where the Marine Protected Area (MPA) of Gyaros island has been established since 2016. Sessile macrobenthic community structure and composition at 5 m depth was monitored through: a) a 3-year period (2019-2021) to investigate short-term interannual changes, and b) a cross-comparison between Gyaros and unprotected rocky coasts of five surrounding islands to investigate interspatial differences in 2021. Our findings detected a general and widespread shallow reef degradation across sites and years studied, as indicated by extremely low occurrence and abundance of late-successional habitat-forming canopy algae. An abrupt shift from shrubby to turf-dominated communities was further recorded in Gyaros between 2019 and 2020, suggesting an ongoing decline. Cross-comparison between sites inside and outside protected (no-fishing) zones in 2021 indicate that against this general deteriorating trend, Gyaros shallow reefs present a more resilient state and still maintain potential for recovery, which should however be timely and actively supported by concrete restorative interventions.

Keywords: marine forests, canopy algae, ecological phase-shifts, marine conservation.

1. Introduction

The Natura 2000 (SCI/SPA) site GR4220033 “Gyaros island and surrounding sea” (hereafter Gyaros MPA) has been established as recently as 2016 to protect the largest monk seal population in the Mediterranean Sea (Dendrinos *et al.*, 2008; Karamanlidis *et al.*, 2016). At the time of designation, the area was characterized as “pristine” by virtue of its geographical remoteness, non-anthropized landscape, and a rather obscure fishing restriction due to its use as a firing range by the Hellenic Navy until 2001. In the frames of the “Gyaros MPA” and “Gyaros Co-Managed NTZ/MPAs” projects, an extensive study of the infralittoral reefs of Gyaros and adjacent islands was conducted with a view to explore possible short-term interannual (2019-2021) and interspatial (inside and outside the MPA) effects of protection.

2. Material and Methods

2.1 Study sites

Benthic community structure and composition of shallow infralittoral (5 m) rocky reefs were systematically studied in June 2019, July 2020, and June 2021 by means of non-destructive scuba diving surveys at six (6) sites within Gyaros MPA (“inside”: G1, G2, G6, G8, G11, G14). Another fifteen (15) sites beyond the MPA (“outside”) were additionally surveyed in June 2021 along the adjacent rocky coasts of Kea (KE1, KE2, KE3), Kythnos (KY1, KY2, KY3), Syros (SY1, SY2, SY3), Tilos (TI1, TI2, TI3) and Andros (AN1, AN2, AN3) islands, facing Gyaros (Fig. 1). All “outside” sites were carefully selected to avoid areas affected by pollution or other direct anthropogenic pressures but for commercial and recreational fishing activities.

2.2 Data collection

At each site, five systematic-randomly placed photo-quadrats (21 x 30 cm) were obtained by SCUBA diving at equal distances along three replicate 30 m-length transects, resulting in a dataset of 15 photo-quadrats per site and year studied. Permanent marking of transects on Gyaros reefs allowed the exact replication of the sampling effort at each site during consecutive sampling years.

2.3 Data Analysis

Conspicuous taxa were identified to the lowest possible taxonomic level or morphological group, and mean percentage cover per species (or higher taxon) was estimated using a superimposed digital grid in the Adobe Photoshop CS5 image editing environment. Following the classification scheme proposed by Thibaut *et al*. (2017) for the Multicellular Photosynthetic Organisms (MPOs), macroalgal taxa were assigned to four stratum categories: canopy, shrubby, encrusting and turf, while sessile invertebrates were collectively grouped in a fifth category (Invertebrates). To explore effects of marine protection on shallow reefs biotic patterns, statistical comparisons of benthic cover estimates were performed across years studied (2019-2020-2021) within Gyaros MPA, as well as between Gyaros MPA ("inside") and five non-protected adjacent islands ("outside") in June 2021. Since data did not satisfy the normality and homoscedasticity assumptions (Shapiro-Wilk test and Levene's test, respectively), the statistical significance of the differences over time and between sites was defined by non-parametric tests, including the Mann-Whitney's U, and the Friedman's tests (more than two related samples).

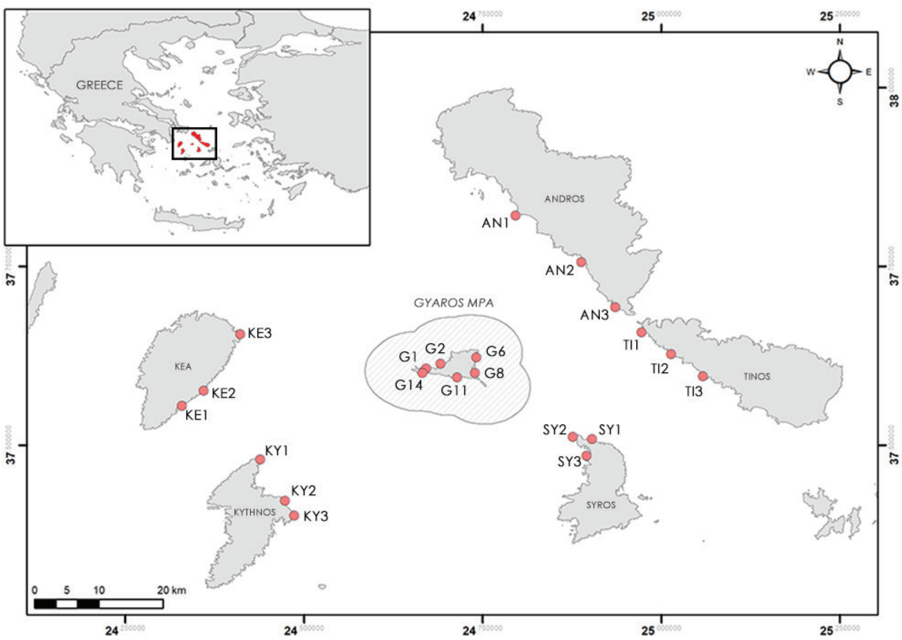

Fig. 1: Study sites in Gyaros MPA and surrounding islands in the North Cyclades.

3. Results

3.1 Short-term interannual changes (Gyaros MPA, 2019-2021)

Out of a total of 270 photo-quadrats sampled at 5 m depths (90 photoquadrats per year 2019-2020-2021), 22 phytobenthic taxa were identified, among which 11 down to species level, 4 to genus level and the rest within higher taxonomic or morphological / functional groups (e.g., *Laurencia* spp. complex, turf algae, encrusting calcareous algae, etc.). Benthic cover was largely dominated by macroalgae (> 95 %), while invertebrate cover was persistently low (1.1 % with 95 % CI [0.64 – 1.52]) at all cases (Fig. 2).

Statistical analysis revealed a clear dominance of the shrubby stratum (here mainly consisted of *Padina pavonica* and various Dictyotales) in 2019, which decreased by a remarkable 30.3 % in cover (95 % CI [8.2 - 52.4], $\chi^2_F(2)=7$, $p<0.03$) within the following years, giving way to 30 % (95 % CI [11.0 – 48.9], $\chi^2_F(2)=10.3$, $p<0.006$) higher cover of low ephemeral turf. This shift seems to have taken place rather abruptly between 2019 and 2020 and remained stable one year after (2021). Canopy algae only accounted for hardly detectable cover values (0.3 % with 95% CI [0.04 – 0.61] in 2019, 0.1 % with 95 % CI [0 – 0.12] in 2020, and 0.1 % with 95 % CI [0 – 0.15] in 2021) across the 5 m depth contour throughout the studied period.

3.2 Interspatial differences (Gyaros MPA vs surrounding islands, 2021)

Interestingly, no significant differences in benthic community structure and composition were detected between Gyaros MPA and its surrounding islands in 2021 (Fig. 3). The turf layer predominated across samples and sites, followed by shrubby and encrusting vegetation. Canopy algae were only present in low cover values, with a notable local exception for site KE3 where the abundance of various species of Fucales (*Cystoseira s.l.* spp. and *Sargassum* sp.) was 49.6 % with 95 % CI [29.5 – 69.7]. When excluding this outlier from further analysis, shrubby vegetation, although generally in moderate cover values appeared significantly more abundant ($U(6, 14)=18$, $p<0.048$) within Gyaros MPA as compared to sites beyond (Inside Gyaros: 26.2 % with 95 % CI [7.0 – 45.4], Outside Gyaros 14.2 % with 95 % CI [8.1 – 20.3]).

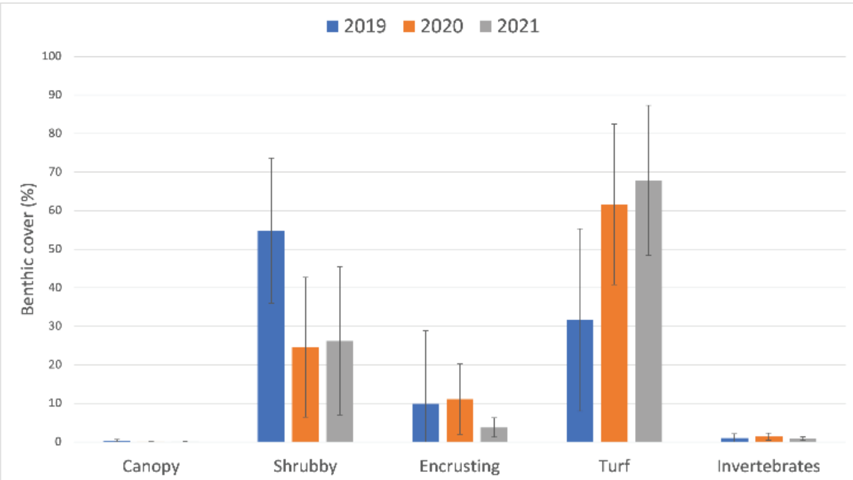

Fig. 2: Benthic community structure (percent cover) of Gyaros MPA rocky reefs per year. Error bars denote 95 % CI.

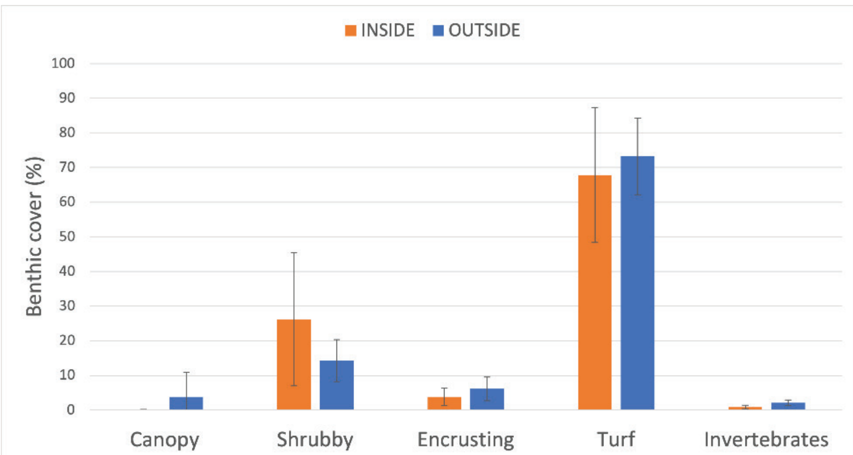

Fig. 3: Benthic community structure (percent cover) inside and outside Gyaros MPA in 2021. Error bars denote 95 % CI.

4. Discussion/Conclusion

Contrary to the wider established notion of Gyaros MPA being pristine due to its remoteness and uninhabitedness, our findings revealed that the islands' infralittoral reefs are practically devoid of climax arborescent (forest-like) canopy algae, which are historically known to characterize healthy shallow Aegean reefs (Pérès & Picard, 1964; Montesanto & Panayotidis, 2011; Bianchi *et al.*, 2014). Although well-developed fucoid stands still fringe the upper (0-1 m) as well as much of the lower infralittoral and upper circalittoral (~ 15-55 m) zone of Gyaros (Salomidi *et al.*, 2021), intermediate depths appear highly degraded both in terms of habitat-forming as well as associated species.

Even more alarmingly: a) the significant increase in dominance of ephemeral low turf over shrubby (*pseudo-canopy*) algae within the 3-year study period signals an ongoing phase shift (sensu Montefalcone *et al.*, 2011) in the island's benthic communities, and b) this degradation seems to be a widespread phenomenon, hereby shown to concern the entire area of the N. Cyclades islands. These findings sadly corroborate similar studies in the Aegean Sea and the Eastern Mediterranean basin (e.g., Dimitriadis *et al.*, 2021; Giakoumi *et al.*, 2012; Giakoumi, 2014; Sala *et al.*, 2011; Salomidi *et al.*, 2016; 2021; Verges *et al.*, 2014), indicating a long-term and ongoing decline of these once highly productive and diverse ecosystems, driven by the combined effects of rising seawater temperatures, native predator fish depletion, and overabundance of various herbivores (mainly for the area of Cyclades, the non-indigenous rabbitfish *Siganus* spp., the Mediterranean parrotfish *Sparisoma cretense*, and the sea-urchins *Arbacia lixula* and *Paracentrotuts lividus*).

Despite this general trend, shallow reefs within Gyaros MPA still present a more resilient state when cross-compared with unprotected sites, insofar as: a) the shrubby layer is found to fare significantly better in the former, and b) scattered presence of several perennial fucoid thalli (i.e., *C. compressa*, *C. corniculata*, *Sargassum vulgare*), though overgrazed and low in cover, suggest the area still maintains potential for recovery. All things considered, climax canopy communities of Gyaros and the wider Cyclades shallow reefs, along with their multiple functions and ecosystem services, are currently seriously jeopardized. Immediate restoration actions within the 5-10 m depth zone should therefore be employed to help enhance adult *Cystoseira s.l.* recruitment capacity and ensure these valuable brown algal forests replenishment and conservation.

5. Acknowledgements

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SETTING AN ECOLOGICAL BASELINE FOR REGIONAL-SCALE MONITORING OF *POSIDONIA OCEANICA* MEADOWS IN THE GREEK SEAS (NE MEDITERRANEAN)

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Abstract

Following long-term and extensive monitoring efforts carried out in over 100 locations along the coastal areas of the E. Ionian and Aegean Seas, an ecological baseline (sensu Reference Conditions; RCs) has been set up for *Posidonia oceanica* meadows' main features. Field data collection and sampling of *Posidonia* meadows was conducted based on several methodological protocols relying on scientific diving, among which extensive underwater surveys using ten (10) different metrics (i.e., meadow, plant and associated flora/fauna descriptors) and ad hoc biotic indices. RCs' determination was based on the composition of a "reference network" consisting of sites with high ecological status as determined through a stepwise screening process analysing anthropogenic pressure and multiple ecological criteria. Data analysis highlighted significant differences in the features of *Posidonia* meadows located in coastal areas under high natural environmental stress (e.g., mainland or insular areas with increased turbidity levels due to various causes such as large river outflow) as compared to those located in areas under low or moderate natural stress (areas with low turbidity). Aiming at a scientifically sound assessment of *Posidonia* meadows' ecological and conservation status, two different RC sets are proposed based on two virtual ("optimal") sites (i.e., expressing the "next best" conditions for meadows' growth) depending on their spatial location along the Greek coastline.

Keywords: seagrass, reference values, spatial variation, favorable conservation status.

1. Introduction

Seagrass meadows constitute elements of high ecological and economic importance for temperate and tropical coastal ecosystems (Short and Wyllie-Echeverria, 1996). However, despite their great importance to the marine environment and coastal human communities, seagrass meadows suffer a pan-Mediterranean (Telesca *et al.*, 2015), but also global decline (Orth *et al.*, 2006).

Effective assessment of seagrass ecological state initially relies upon the comparison of their present state against a well-defined ecological baseline, i.e., the reference conditions (Gatti *et al.*, 2015). Indeed, the determination of Reference Conditions (RC) is considered a key element for detecting ecological changes (e.g., degradation) and identifying associated anthropogenic stressors.

This study aims to investigate natural variability of *P. oceanica* reference conditions in the NE Mediterranean across i) various spatial scales, i.e., national, regional (sub-ecoregions: Eastern Ionian, South Aegean, or North Aegean Seas), ii) different physiographic characteristics (i.e., mainland vs insular areas), and iii) locally varying key environmental factors (i.e., trophic status, water turbidity), with a view to identifying and setting robust reference values for meadows in the E. Ionian and Aegean Seas that will allow the implementation of sound national or regional-scale monitoring programmes and conservation or restoration schemes.

2. Material and Methods

2.1 Study sites

A total of 105 sites were studied from 2012 to 2021 (Fig. 1). Data collection and sampling activities were conducted during the period from April to October. Across the study area, sites were selected to be evenly distributed: (i) between mainland and insular coastal areas, (ii) within and beyond the Natura 2000 Greek network of protected areas, and (iii) among areas presenting different levels of anthropogenic pressures. The identification of the most suitable study sites was further based on land-use mapping data, satellite image analysis, and field observations.

2.2 Environmental Factors

Four regulating factors (i.e., light, hydrodynamic conditions, salinity, and temperature) of *P. oceanica* meadows' growth dynamics and distribution patterns were examined using remote sensing techniques (i.e., analysis of satellite and modelling data), namely: KdPAR (Photosynthetically Active Radiation), Chl-a (Chlorophyll a), Salinity, SST (Sea Surface Temperature) and significant wave height (Hs). The monthly average of all variables was calculated for the period 2012-2021.

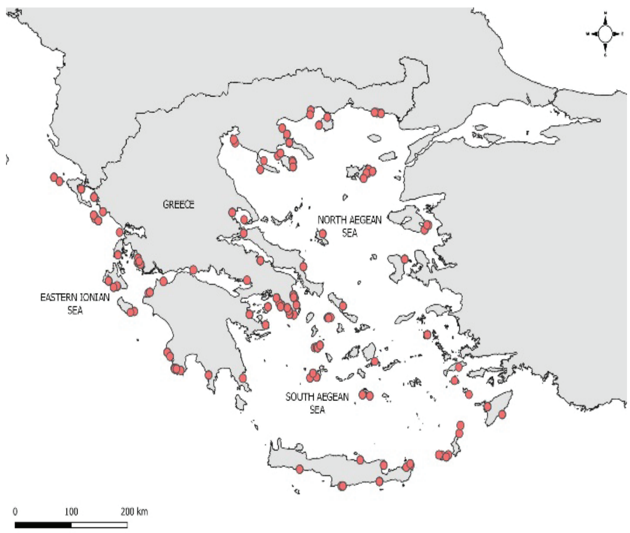

Fig. 1: Location of study sites in the Eastern Ionian, North Aegean and South Aegean Seas.

2.2 Data collection (sampling design)

In total, ten (10) metrics assigned to three different *P. oceanica* biotic levels were selected: "population" (meadow descriptors), "individual" (plant descriptors), and "community" (associated flora/fauna descriptors) (Table 1). Field data collection was carried out by SCUBA diving at standard depth of 15 ±1 m, and the lower (deeper) limit of each meadow.

Table 1. Studied metrics classified into three different levels of biological organisation.

Biotic level	Metrics	Method /Analysis
Population	Type of Lower Limit Lower Limit Depth (m) Meadow Cover (%) Dead Matte Cover (%) Shoot Density (shoots/m^2) Plagiotropic Rhizomes (%)	In-situ (non-destructive)
Individual	Shoot Leaf Surface (cm^2/shoot) Shoot Length (cm/shoot) Leaf Biomass (g/shoot)	In the laboratory, using a minimal number (20) of shoots
Community	Epiphytic Biomass (g/shoot)	

2.3 Anthropogenic Pressures

Anthropogenic pressures were evaluated in each study site using the Land Uses Simplified Index (LUSI) based on land use analysis (Flo *et al.*, 2019). For the LUSI index, we used the LUSIsg version that takes into account both indirect (terrestrial: urban, commercial, industrial and agricultural land use) and direct anthropogenic pressures (marine: aquaculture, urban wastewater, ports) within a radius of three (3) km around each study site (MedGIG, 2013).

2.4 Setting of Reference Conditions

Reference conditions (RC) for the examined *P. oceanica* features were determined sensu the Water Framework Directive (WFD), where RC are considered to be a description of biological quality elements (BQE) that exist or would exist under a "high" ecological status, i.e., with none or minor disturbance by human activities (EC, 2003). To this end, a "reference network" of sites presenting meadows of high ecological status was selected through a stepwise screening process, analyzing anthropogenic pressures and multiple ecological criteria.

2.6 Data Analysis

Statistical analysis was performed in the R Environment (R Core Team, 2021). Statistical significance of spatial factors effect ("Site", "Location", and "Region") was determined by a nested Analysis of Variance (ANOVA) for each metric. "Region" a factor with three levels (sub-ecoregions: E. Ionian Sea, North Aegean Sea, and South Aegean Sea), "Location" a factor with two levels (insular and mainland areas) nested within "Region", and "Site", nested within "Location". Principal Component Analysis (PCA) was performed on both the biotic variables (biotic metrics) as well as the abiotic variables (sea water variables) to check the existence of common patterns. The capacity of abiotic variables (i.e., constraints) to explain the differences between sites was explored by using Redundancy Analysis (RDA). Before the ordination, a Kendall's Tau correlation matrix of abiotic variables was produced to highlight and exclude variables with high correlation.

3. Results

Data analysis highlighted significant statistical differences for all metrics across all spatial scales, i.e., “Site”, “Location”, “Region”. The mean values of all metrics were significantly higher at *P. oceanica* meadows studied in the Ionian and S. Aegean Seas than those in the N. Aegean, as well as at meadows located in insular rather than mainland coastal areas.

The analysis of abiotic factors revealed that high levels of turbidity, hereby clearly pronounced within enclosed gulfs and bays, and coastal areas which are under the influence of large river outflow, cause significant environmental stress and directly shape and affect the *P. oceanica* meadows.

This is particularly the case of study sites in the Thracian Sea and the gulfs of Ierissos, Strymonikos, Pagassitikos, Evoikos, Korinthiakos, and Kavala, as also revealed through the PCA analysis, which highlighted a clear clustering of the studied *P. oceanica* meadows under different levels of natural environmental stress (Fig. 2). Indeed, *P. oceanica* meadows in coastal areas under high natural stress presented significantly different values for most metrics (e.g., Meadow Cover, Shoot Density, LL depth, Shoot length).

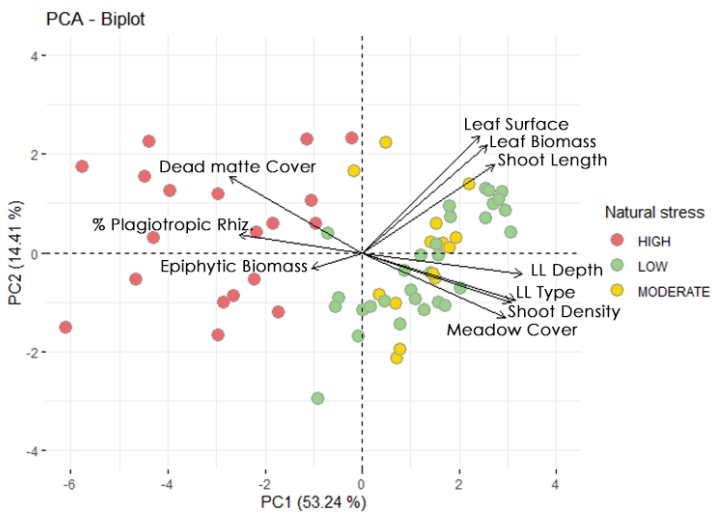

Fig. 2: Principal component analysis (PCA) biplot of studied sites and *P. oceanica* metrics. The biplot shows the PCA scores of the *P. oceanica* metrics as vectors (in black) and sites as circles (red = high natural stress, yellow = moderate stress, green = low stress).

According to the analysis of anthropogenic pressures, the number and geographical distribution of study sites that could be considered suitable as “reference sites” were not sufficient to form a “reference network” as intended. Further evaluation of anthropogenic pressures based on additional screening criteria identified a final set of 10 alternative benchmark sites (i.e., sites presenting the lowest possible anthropogenic pressures; sensu WFD) selected from all Greek ecoregions.

In this light, RCs were reconsidered based on two virtual sites, an “optimal” site for coastal areas under low/moderate natural stress, and another “optimal” site for coastal areas under high natural stress, representing the “next best” conditions for meadows’ growth in each case (Fig. 3).

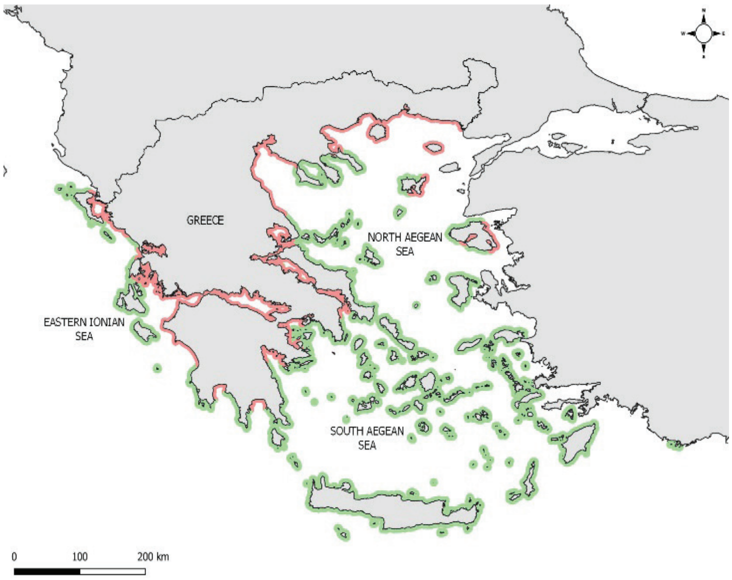

Fig. 3: Map showing the coastal areas under low/moderate (green) and high natural environmental stress (red).

4. Discussion/Conclusion

In the context of this work, the determination of *P. oceanica* meadows Reference Conditions based on the use of existing data from a specific set of alternative benchmarks (i.e., "next best" conditions) was considered the most appropriate option, which is also in line with the practice followed by most Member States (MedGIG, 2013). However, the recorded significant differences in the features of *P. oceanica* meadows located in coastal areas under different natural environmental stress (e.g., mainland or insular areas with increased turbidity levels due to various geophysical causes such as confinement and large river outflows), in contrast to the other Member States, indicate the need to resort to different RCs. This proposed distinction in two different RC sets based on two virtual ("optimal") sites depending on naturally varying environmental stress across large spatial scales, is expected to contribute significantly to a scientifically sound assessment of *Posidonia* meadows' ecological status along the Greek coasts. At the same time, these findings further confirm the ocean's *shifting baseline* (Pauly, 1995) and emphasize the immediate need to engage in effective restoration of degraded *P. oceanica* and other key marine ecosystems.

5. Acknowledgements

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USE OF SEDIMENT GEOCHEMISTRY AND IMAGING TECHNIQUES FOR HABITAT CHARACTERIZATION OF *HOLOTHURIA POLI* (DELLE CHIAJE, 1823*)*: PRELIMINARY RESULTS

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Abstract

The rapid exploitation of sea cucumbers for decades has raised global concerns regarding their important role in bioturbation and nutrient recycling in marine benthic ecosystems. Recently, *Holothuria poli* (Delle Chiaje, 1823), became one of the most exploited sea cucumbers in the Mediterranean Sea. However, few stock assessment studies have been done and little is known about the ecology of the species. Therefore, in this study, evaluation on sediment quality variables influencing the population dynamics of *H. poli* and the species influence on macrofaunal community and geochemical properties were investigated through imaging techniques (Remotely Operated Vehicle–ROV) and sediment geochemistry. Two stations were sampled in Agios Nikolaos of Crete (South Greece) with distinct characteristics (seafront-S1 and enclosed-S2). Results showed that in S1, where sandy oxic environment with low organic concentration was recorded, population density was low (0.036±0.01 ind m^{-2}) but with heavier individuals (70.42±17.31g) and higher macrofaunal diversity. Conversely, silty low redox regime and high organic matter load environment was found in S2, population density was higher (2±2 ind. m^{-2}) with lighter individuals (43.92±13.49g) and low macrofaunal diversity. Seemingly, topography, seasonal variations and sediment geochemical properties influenced the differences on the results of population parameters of *H. poli*.

Keywords: *Holothuria poli*, population density, redox potential, organic matter, sediment geochemical properties.

1. Introduction

Sea cucumbers are known for their important ecological roles including bioturbation, bioremediation, nutrient recycling in oligotrophic waters, local buffering through feeding and excretion as well as symbiotic associations in the trophic chain, which are significant for the health of marine ecosystems (Purcell *et al.,* 2016; Zamora *et al.,* 2018). *Holothuria poli* lives predominantly in soft bottom sediments, particularly in areas where seagrass detritus is intensively accumulated and is recognized as a deposit feeder playing a significant role in bioturbation and nutrient recycling (Boncagni *et al.,* 2019).

Recently, *H. poli*, one of the 37 sea cucumber species widely distributed in the Mediterranean Sea (Rakaj *et al.,* 2019; Aydin 2016)"ISSN":"00448486","abstract":"Holothuria polii (Delle Chiaje, 1823, became one of the most targeted and commercially exploited for the global seafood trade (Friedman *et al.,* 2017) social, and economic impacts. The illness produces a complex array of gastrointestinal, neurological and neuropsychological, and cardiovascular symptoms, which may last days, weeks, or months. This paper is a general review of CFP including the human health effects of exposure to ciguatoxins (CTXs. The significant exploitation of this species, and of sea cucumbers in general, have raised global concerns hence they are important segment in benthic dynamics and productivity.

Unregulated Overfishing harvesting of *H. poli* can impose a risk for the natural stocks if no management actions is taken regarding its fishery (Rakaj *et al.,* 2019), similar to what has already happened in several fishing grounds in Asia

Despite the potential value of sea cucumbers, little is known about population and ecology of the *H.*

poli species in the Mediterranean basin, as only few stock assessment studies of natural populations have been done over the past years (Mezali *et al.,* 2006). Understanding knowledge is also limited on certain habitat characteristics, such as sediment geochemical properties and their interaction with the population dynamics of the species. This study aims to determine the environmental variables, population density and influence of *H. poli* on sediment geochemical properties and macrofaunal community, at the Lasithi area of Crete, at two locations with different envrionmental characteristics.

2. Material and Methods

2.1 Study area

Study area was carried out in two stations: station 1 was an open sandy beach (S1) and station 2 a muddy bay (S2 in a touristic area with popular beaches. Both stations are known to have local populations of *H. poli* and seagrass *Cymodocea nodosa* with random distribution of meadows and patches at shallow water depth.

S1 is located in an exposed shore 50m away from the coastline, 1 km away from a river inlet with sandy bottom substrate. A control site was sampled in S1 from an adjacent area of same depth and sediment characteristics but with absence of the holothurians. Thus, in S1, sediments were taken in the presence (Hol) and absence (Ctrl) of *H. poli*. S2 is situated 20 m from the coastline in an enclosed bay with numerous small islands with narrow passages in-between. No control site was sampled in S2 as species population was observed to be abundant and widely distributed across the entire bay.

2.2 Environmental variables

Sediment biogeochemical variables (LOI, Granulometry, Redox) and macrofaunal community were measured monthly using corer samplers placed 10 cm depth within the sediment by scuba diving. Macrofauna organisms were identified at the family level. Also, monthly measurements of water salinity and sediment temperature was taken using a refractometer and a thermometer respectively.

2.3 Population structure and density

Population density of *H. poli* in the sampling stations was analysed mainly through underwater video techniques, using a mini Remotely Operated Vehicle – ROV (Blue Robotics ROV2) equipped with two video cameras. The HD ROV camera was used mainly for operation of the ROV while a Parallenz dive 4k resolution camera recorded the videos used for the analysis. The Paralenz + camera features a 140° angle of view and was constantly focusing up to 0.5m ahead on the sediment, therefore the field was 0.84 m^2 for all transects.

The establishment of the transects was done through deployment of the ROV with an attached floating GPS devise able to take high precision locations (±5m). At the start of the transect, the coordinates were recorded and the ROV started moving at few centimetres from the substrate, at maintained velocity of 0.1-0.3 m/s, with a constant heading for a distance of at least 50 m. At the end of the transect the exact coordinates were recorded again. Video outputs were then analysed through a High definition (HD) screen, using the VLC program for quantification of all individuals in every video transect, and for characterization of habitat structural characteristics. Tracks and transects were plotted on Google Earth and Quantum Geographic Information System (QGIS) – Version 3.14, in order to determine the exact transect length. With the different transect lengths covered per sampling, the explored area was determined through the camera visual field calculated above. The determination of the population distribution pattern was done by dividing each transect into four sub-sections of equal length, in order to see whether the population density of holothurians was similar thought the transect.

2.4 H. poli size measurement

Characteristics of the body size measurements of *H. poli* in the study area was determined by collecting of at least *n* = 10-15 individuals via scuba diving in each sampling station during every sampling event. After the collection, the animals were left out on the ground for few minutes to remove the excess sea water and then when the body finally shrinks, samples were measured using a portable weighing balance (weight, gr) and a calliper (length, cm).

2.5 Statistical analyses

The determination of the variation between months for environmental variable station was done through one-way analysis of variance (ANOVA), using the SigmaStat 3.5 software, sinse the variables met the assumptions for parametric analysis. Significant differences between means were further analysed through pairwise comparisons, using Tukey Test. For the presence/absence of *H. poli* data in each month the non-parametric Kruskal-Wallis test was selected since the data failed the assumptions for parametric analysis. For macrofaunal community data, the Shannon-Wiener's diversity index was calculated, and non-metric Multidimensional Scaling (MDS) with Bray–Curtis similarity and ANOSIM were made using PRIMER-E v7.

3. Results

3.1 Sediment quality parameters:

The sediment biogeochemical parameters among the two studied stations were statistically different ($p<0.05$). Comparison of % labile OM showed that S2 containing higher organic matter concentration than S1 (Fig. 1a) and redox potential Eh were relatively lower in S2 compared to S1. Eh values in S2 started to drop negative after depth sediment of -2.5 cm, on contrast with Eh in S1 which was positive until -5 cm depth (Fig. 1b). For sediment samples taken from S1, sand fraction was 94.51%, while silt & clay fraction was 5.49%, respectively. On the contrary, 69.09% was attributed by silt and clay, while remaining 30.96% was by sand in sediment of S2. Cntl and Hol stations in S1 were no statistical different in any parameter.

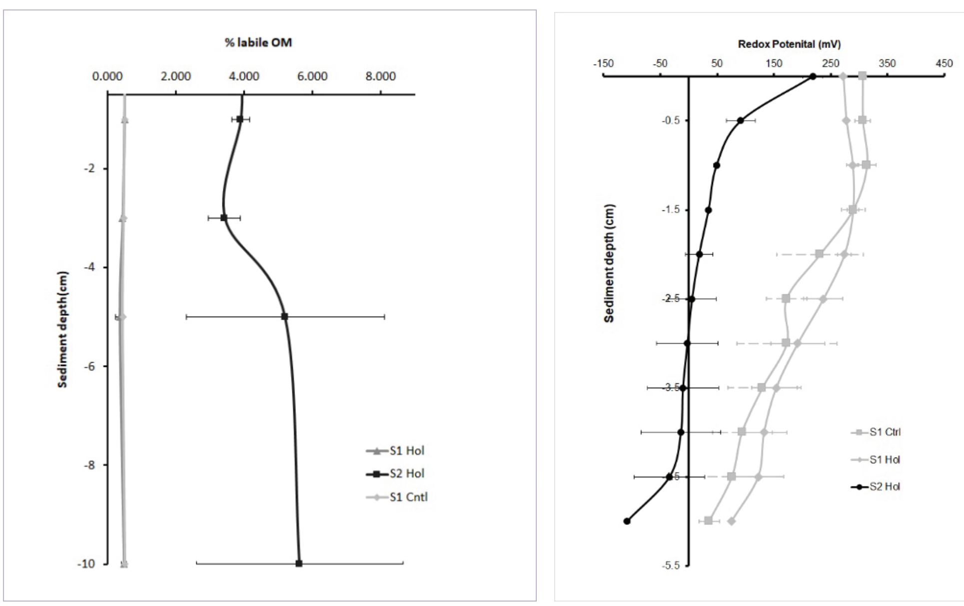

Fig. 1: a) Graph showing the variation of % Labile OM relative to sediment depth (0 to -10 cm) at S1 and S2 and on Cntl and Hol site. b) Graph showing the variation of Redox Eh relative to sediment depth (0 to -5cm) at S1 and S2 and on Cntl and Hol site. No statistical differences were observed in Cntl and Hol site in S1.

ANOSIM and MDS plot of S1 indicated significant differences ($p<0.05$) for the macrofaunal community composition with the presence/absence of *H. poli*. ANOSIM revealed a value of $r=0.83$($p<0.01$), indicating significant differences in macrofaunal community composition between sampling stations (Fig. 2).

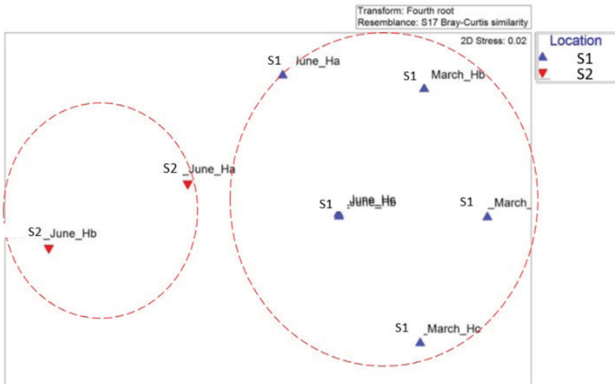

Fig. 2: MDS plot of macrofaunal community composition at S1 and S2. On S2 data was missing for the month June. Circle represents similarity 80.

3.2 Holothuria population

Holothurian populations of the studied stations were found significant different ($p<0.05$) regarding density, distribution and holothurians body size. At S1 and S2 average population density was 0.0361±0.01 ind $m^2$and 2±2 ind.m^{-2}, respectively. Overall, mean weight of *H. poli* individuals were 70.42±17.31g in at S1, which was heavierwhile in compared to S2 thewith a mean weight wasof 49.35±11.89 g. Also, population-population of *H. poli* at S1 showed a patchy distribution based in an visual assessment , while at S2 a random distribution pattern was observed.

4. Discussion/Conclusion

According to the results of this study, *H. poli* seems to thrive in a silty enclosed area with low a minimal wave action and abundant high concentrations of organic material input, which was found (mainly at S2). Seemingly, topography, seasonal variations and sediment geochemical properties influenced the differences recorded on morphology and density population parameters of *H. poli* populations between sampling among studied stations. Mediterranean finfish commercial mariculture, which often situated in semi-enclosed areas sheltered from strong currents and wave action (Karakassis *et al.,* 2013), could use *H. poli* as a capable extractive species for its wastes offload. Holistic Understanding the sediment dynamics, empirical approach on the intricate underlying and the factors that affecting population dynamics on annual base, and all year round research sampling and monitoring would mitigate the gaps of knowledge on biology and ecology of *H. poli*. Additionally, comprehensive analysis on its diet composition and how this factor is related to it influences species growth, could be addressed in future studies to facilitate aquaculture production given the active fisheries of this species. Nevertheless, outputs generated from this study will provide the baseline information fundamental in developing plans on species conservation, fishery management, restocking and facilitating mariculture activities of *H. poli* including for integrated multi-trophic aquaculture(IMTA) system perspectives in Mediterranean aquaculture industry.

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METRICS OF BENTHIC COMMUNITIES IN THE IONIAN SEA

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Abstract

Monitoring a marine ecosystem is a multifactorial operation to which multiple ecological parameters and factors that impact benthos, its structure and its evolution, have an effect on. The use of benthic indices for ecological status classification and creating a better visual understanding of the ecological functions of benthic organisms is widely used for monitoring processes. This study aims at examining the correlations of benthic indices used in assessing the ecological status of a benthic environment in some areas of the Ionian Sea, resulting in a better understanding of the interaction between ecological status, diversity and ecosystem function.

Keywords: marine ecology, Greece, benthos, biodiversity.

1. Introduction

The marine environment is influenced by a plethora of factors both physiochemical and biological which affect the species of said environment, as well as their habitats (Reiss *et al.*, 2015). Changes also occur due to the extensive impact that human activities have on a global level. Benthos, as part of the marine environment and the species that inhabit it, namely benthic organisms which are mostly invertebrates, are widely used as indicators of ecological conditions since the response to human disturbance is highly variable (Harrison *et al.*, 2007). Benthic organisms and especially benthic macrofauna, which corresponds to animals retained on a 0.5 - 1mm sieve, is widely used to estimate different types of pollution. The diversity at species level is substantially high, the mobility of those species is reduced compared to other species groups and their tolerance to disturbance has considerable variation (Sanz-Lázaro & Marín, 2011). The latter, is currently used in numerous benthic indicators used to evaluate ecosystem ecological status (ES) in the context of the EU Water Framework Directive.

Moreover, infaunal invertebrates are responsible for bioturbation which is essentially the reworking of sediment by mixing and transporting sediment particles due to the activities of those organisms that include, for example, feeding, burrowing etc. Bioturbation is a key for many biogeological processes due to its influence on oxygen, pH, redox gradients and other variables and thus reduces the negative impact of organic matter accumulation (Queiros *et al.*, 2013) and, therefore, it is an essential information to be obtained from benthic monitoring. The functional identity of macrofaunal species is important and grouping them into functional groups by their respective role in the ecosystem, (using physiological and behavioral traits), can lead to better understanding of the structure of benthic communities and better monitoring of biodiversity levels and anthropogenic impacts (Tsikopoulou *et al.*, 2021).

The aim of the present study was to assess the status of benthic habitats in the Ionian Sea using different biodiversity indices and ES indicators. By using different indicators, (biodiversity indices, ES and functional indicators) it is possible to examine similarities and differences between locations and communities while exploring the complexity of the factors that impact the benthic environment.

2. Material and Methods

The samples were collected in the context of the ECODISC project, whose primary objective was to assess the effect of fish discards in different components of the marine ecosystem in the Ionian Sea. The sampling took place in 13 transects with the purpose of covering an area of the largest geographical scale possible in the Ionian Sea (Fig. 1) with varying fishing effort (although this is not discussed in the present study). The samples were collected from various depths from 20 to 300 m, by means of a Smith-McIntyre sampler (0.1 m^2) using the RV Philia in May 2014. From each grab, environmental variables, namely temperature (T °C), depth and redox potential (Eh) were measured. The rest of each sample was sieved on site consecutively using an upper 1.0 mm and a lower 0.5 mm mesh size sieve, stored and preserved using 10% Formalin. In the laboratory, the samples were sorted and macrofaunal individuals identified to the species level (or lowest possible taxon). This work is still in progress and therefore only the samples from Kefalonia and Ithaka (ED_KEF1 to ED_KEF6 and ED_ITHAKA1 to ED_ITHAKA6) are presented here.

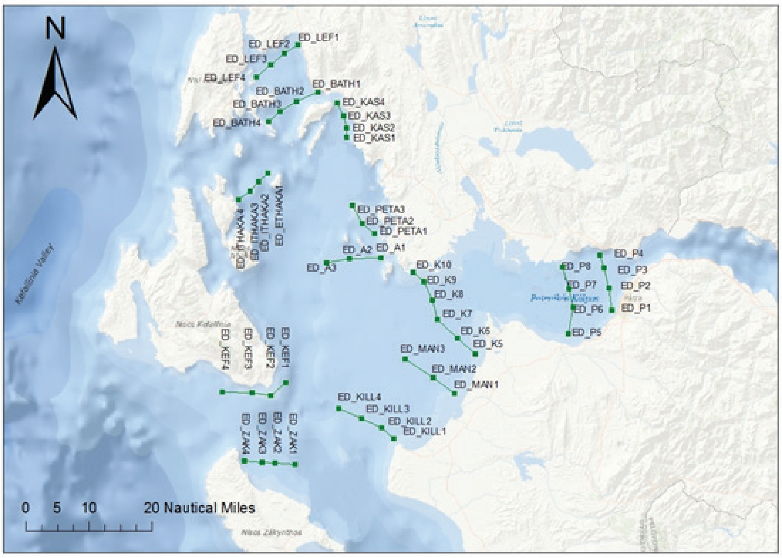

Fig. 1: Sampling area and stations of the ECODISC project.

The number of individuals (N) and species (S) from each species was counted and the biomass was measured with a high precision scale (0.0001 g). Indices that were calculated include, the ES indicators BQI_Family (Dimitriou *et al.,* 2012), the BQI (Rosenberg *et al*., 2004) and BENTIX (Simboura & Zenetos, 2002), the W index (Warwick, 1986), the Bioturbation Potential Index (BPc) (Queiros *et al*., 2013), and the diversity indices Shannon-Wiener *H'*, the Simpson Index D', the expected number of species ES(10). Additionally, the W index, calculated in abundance-biomass curves (ABC curves) measures the extent to which the biomass curve lies above the abundance curve. Biomass fractionation Index (BFI) (Lampadariou *et al*., 2008) was calculated by dividing the biomass of <1mm specimens at each station retained on the 0.5mm sieve by the total (0.5mm and 1mm) biomass of each station. Similarly, an abundance fractionation index (Abund fr) was calculated by dividing the abundance of <1mm specimens at each station retained on the 0.5mm sieve, by the total (0.5mm and 1mm) abundance of each station. A non-metric Multi-Dimensional Scaling (nMDS) and a second stage MDS plot were made using the PRIMER-E V7 software as well as an Analysis of Similarities (ANOSIM) using depth as a factor. Spearman correlation was used to find significant correlations between all possible pairs of indices.

3. Results

It is important to note that all the stations were well oxidized as the Eh values ranged from +360 to +444. As it may be observed (Table 1), in the shallow stations (<120m), the biodiversity was high with the maximum number of species counted being 82 in Kefalonia (ranging from 24 to 82). In the deeper stations the number of species decreased significantly and ranged from 9 to 22. Similar pattern was observed in all diversity indices i.e. *H'*, *D'* and ES (10). The ecological status of the samples according to BQI and BQI family indices, decreased as the depth reaches high values (around 200+ meters) but the overall ES of the shallow areas that were examined is classified as Good. On the other hand, BENTIX classified all samples as Good or High in all depths.

STATIONS	Depth (m)	S	H'	D'	ES(10)	W	BFI	Abund fr	BPc	BQI_Family	BQI	BENTIX	ES BQI_ Family	ES BQI	ES BENTIX
ED_ITHAKA4	27.5	38	4.75	0.96	8.48	0.41	0.02	0.54	10.8	16.07	19.40	5.14	G	H	H
ED_KEF4	35.5	82	5.46	0.96	8.58	0.42	0.01	0.30	31.8	20.65	26.08	4.54	G	H	H
ED_KEF3	50	57	5.18	0.96	8.57	0.44	0.02	0.33	17.8	23.10	23.97	4.66	H	H	H
ED_ITHAKA3	62.8	24	4.31	0.96	8.67	0.39	0.02	0.09	4.45	13.44	15.63	3.53	G	G	G
ED_KEF2	73	32	4.69	0.97	8.76	0.33	-	-	2	16.46	16.81	4.69	G	G	H
ED_ITHAKA2	87	55	5.22	0.97	8.74	0.49	0.02	0.26	12.8	15.59	22.04	4.52	G	H	H
ED_KEF1	110	42	4.31	0.91	7.33	0.25	0.02	0.44	14.9	16.73	21.63	4.48	G	H	G
ED_ITHAKA1	112	25	4.48	0.98	9.01	0.50	0.12	0.58	3.23	13.67	19.29	4.59	G	H	H
ED_KEF5	190	22	4.19	0.95	8.27	0.50	0.13	0.67	4.54	11.70	12.51	4.23	G	M	G
ED_ITHAKA5	197	9	2.99	0.92	7.20	0.59	0.09	0.50	3.54	5.77	6.76	4.77	M	P	H
ED_ITHAKA6	315	10	3.03	0.90	6.76	0.02	0.46	0.74	1.02	6.11	8.05	4.11	M	P	G
ED_KEF6	320	14	3.65	0.96	8.32	0.20	0.55	0.60	1.53	7.63	9.04	4.32	M	P	G

Table 1 . Values of different indices and indicators of the 12 stations from Kefalonia and Ithaki (Ecological Status Classification: G= GOOD, H= HIGH, M=MODERATE, P=POOR).

A resemblance matrix was created using the Spearman rank correlation from all possible pairs of indices (Table 2). In general, similar types of indices were significantly correlated with each other. It is important to note that there was a strong, significant correlation of both the BPc and BFI index with BQI, BQI_Family, *H'*, Abundance fractionation and S (Table 2).

Table 2. Spearman rank correlation between environmental indices. ns = not significant * = p<0.05, ** = p<0.01

	W	BQI_Family	BQI	BENTIX	*H'*	BPc	BFI	Abund fr	S	ES(10)
BQI_Family	ns									
BQI	ns	0.923**								
BENTIX	ns	0.336*	ns							
H'	ns	0.825**	0.916**	0.329*						
BPc	ns	0.741**	0.832**	ns	0.678*					
BFI	ns	-0.587*	-0.748**	ns	-0.657*	-0.881**				
Abund fr	ns	ns	-0.545*	ns	-0.476*	-0.699*	0.769**			
S	ns	0.944**	0.993**	ns	0.923**	0.825**	-0.720**	ns		
ES(10)	ns	0.448*	0.497*	ns	0.692*	ns	ns	ns	0.49*	
D	ns	ns	ns	ns	0.664*	ns	ns	ns	0.434*	0.993**

The second stage MDS (Fig. 2a) visualizes the similarities between the correlation of the indices calculated previously with the resemblance matrix. Similarly to the indices, macrofaunal community structure reflected a strong depth gradient. From the ANOSIM, it was found that the benthic community structure differed between depths (R=0.53, p<0.001) with the cluster 0-100 being significantly different from the other two (p<0.05). This was also reflected on the MDS plot (Fig. 2b).

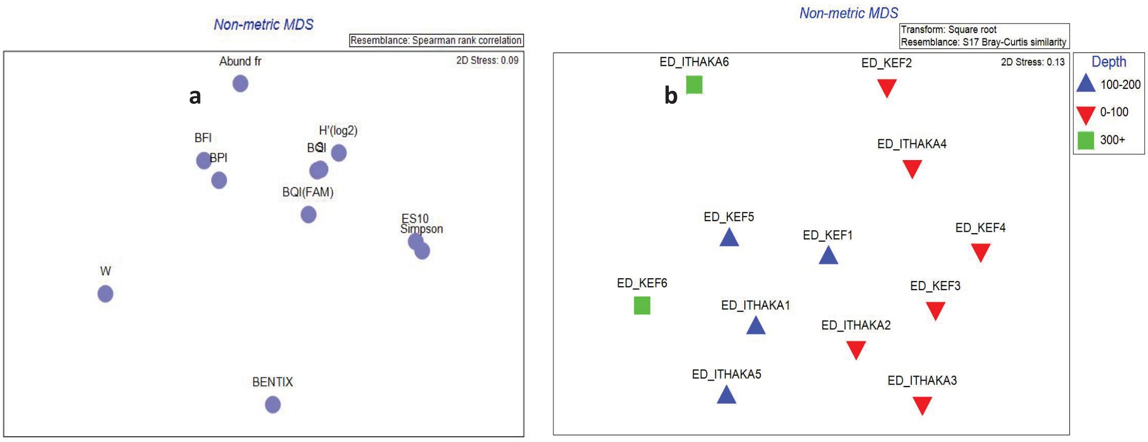

Fig. 2: (a) Second-stage ordination plot **(**MDS) between community metrics for all the sampling stations analyzed. (**b)** MDS plot for the grouping by depth of macrofaunal samples.

4. Discussion

As previously mentioned, the Bioturbation Index (BPI) and the Biomass fractionation index (BFI) show a strong and statistically significant correlation to other ecological status evaluation indices and between them. The BPI index calculates bioturbation from abundance and biomass data (at the species level) as well as a functional classification of organism traits associated with sediment reworking and mobility. Its correlation to BQI and BQI family indices, which evaluate ecological status with the classification of species by their dominance indicates that the process of bioturbation is connected to k dominant species mobility (Quieros *et al.*, 2013). The BFI, also correlated negatively to both BQI indices and BPI, indicates that individuals that are heavier have a stronger effect on the ecological status of a sample. BQI and BFI are also correlated to the number of species S, to Shannon, which is a diversity index that measures evenness in a community, and to abundance fractionation as the abundance of species directly impacts the bioturbation process. It becomes evident that the process of reworking the sediment reflects the richness, evenness and diversity of benthic communities and is vital for the ecological status of the environment.

The highly oxic conditions (high Eh values) found in all stations are reflected on the Ecological Status, which was found to be generally either good or high. Samples with a similar depth are also similar to each other as depicted in the MDS plot (Fig.2). However, in regards to stations with the highest depth values, a conflicting observation on the ecological status classification arises as the ecological quality score of the stations decreases, indicating poor or moderate status. A possible cause for this is likely attributed to the low number of species (S) which is significantly smaller in those stations and significantly correlated to both BQI and BQI family indices. In the context of monitoring studies it is important for an ES indicator to be effective in distinguishing disturbance (Labrune *et al.*, 2012). In the present study BENTIX was not affected by the low biodiversity in the deep stations and classified them as Good or High ES. On the other hand, BENTIX was not significantly correlated to any of the other indices, except for Shannon and BQI_Family therefore for an in-depth ecological study the BQI and BQI_Family indicators would be preferable, as they seem to incorporate more relevant information to biodiversity than BENTIX.

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FUNCTIONAL PATTERNS ACROSS MEDITERRANEAN BENTHIC HABITATS

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Abstract

Investigating functional patterns in coastal waters is crucial in assessing impacts beyond the simple decrease of species diversity, as a growing number of environmental policies request to address the rapid loss of biodiversity and ecosystem services. The present study aims to comprehend the role of habitat structure on functional variation across 11 common habitat types of the Mediterranean Sea. Four main clusters were identified (dense seagrass beds, coarse detritic substrates, infralittoral/circalittoral muds, bathyal muds), where all types of rigid biogenic structures/detritus showed similar functional profiles and significantly higher Functional Richness, probably favored by the three-dimensional topography and microhabitat heterogeneity. The results are expected to aid in promoting habitat types, often not regarded as priority, in the management consideration of an area's ecosystem functionality.

Keywords: Biological Traits Analysis, zoobenthos, polychaetes, FRic, ecosystem functioning.

1. Introduction

Disentangling the complex relations between environmental conditions, biodiversity and ecosystem functions is the next challenge of ecology, amid rapid loss of habitats, biota, and services. In order to assess environmental ramifications beyond the simple decrease of species diversity, Biological Traits Analysis (BTA), has emerged in terrestrial and freshwater ecology (Hewitt *et al.*, 2008). BTA is based on what organisms do in a community and thus, how they mediate to important ecosystem functions, through their morphological, behavioral, and life history traits. Despite coastal waters being historically threatened by multiple human activities , relative applications to benthic communities are still limited (Lam-Gordillo *et al.*, 2020) and we lack even basic knowledge on the functional role of most benthic habitats (Bremner, 2008). At the same time, a wide spectrum of sedimentary and biogenic habitats sustains a remarkable proportion of macrofaunal diversity in coastal waters. The physical and biochemical conditions that vary across these habitat favor or exclude the presence of certain functional traits (Olenin & Ducrotoy, 2006), which in turn play a crucial role for numerous vital ecosystem functions. Most often, benthic habitats are designated based on their seascape features, nevertheless, they are ecologically open systems, where the important functions extend across their physical boundaries and the health of a community in a habitat type may be dependent on processes occurring elsewhere (Frid *et al.*, 2005). Therefore, defining functional spatial patterns may facilitate the comprehension of ecosystem functioning in a coastal area and its vulnerability to specific human pressures.

Such understanding may be extremely useful in environmental management, where a growing number of habitat-based legislative agreements(e.g., Convention on Biological Diversity, European Marine Strategy Directive, Habitats Directive) have started to consider human impacts in ecosystem functioning and require management schemes to address their assessment (Frid *et al.*, 2005). The first steps to use BTA in the conservation of habitats and their associated processes, would require describing the reference conditions and defining the key functional traits (Bremner, 2008). To this end, this study conducts a BTA on 11 common coastal benthic habitat types of the Mediterranean Sea, aiming to provide insights into the functional variation across coastal waters, as well as into the importance of habitat structure for the observed patterns. Polychaetes have been selected as target-organisms, since studying a community component with the same body plan and environmental perception strengthens the detectability in environmental gradients (Verberk *et al.*, 2013), while they also constitute a dominant benthic group with crucial role in most benthic functions and a remarkable variety of functional types (Hutchings, 1998). The results may aid several stages of assessment and management, such as in redefining the traditional all-purpose management objectives to monitoring impacts in specific functions or defining future boundaries of MPAs in respect to complementary functions among habitat types.

2. Material and Methods

The study area stretches across all Greek Seas (Eastern Mediterranean Sea). The classification into habitat types was based on depth zone, granulometry, presence/type of biogenic detritus and seagrass species. The habitat types constitute a *sensu lato* of the definitions used in the European Nature Information System (EUNIS; EAA, 2019). In total, the 11 habitat types considered herein were: Bathyal Muds (referred as BM), Circalittoral Muds (CM), Circalittoral Muddy Detritic (CMD), Infralittoral Muds (IM), Infralittoral Muddy Detritic (IMD), Rhodolith Beds (RB), Circalittoral Sandy Detritic (CSD), Infralittoral Sandy Detritic (ISD), *Cymodocea nodosa* (Ucria) Ascherson, 1870 Beds with scarce density (CBS), *C. nodosa* Beds with dense density (CBD) and *Posidonia oceanica* (Linnaeus) Delile, 1813 Beds (PB). Biogenic detritus was accounted when the surface cover and/or volume proportion exceeded 10%. Scarce *C. nodosa* beds had shoot density of < 100 shoots/m^2.

In total, 84 samples were collected from 40 undisturbed sites (0.2 – 431m depth), during the period 2010-2019. The criteria for selecting undisturbed sites were: (a) GOOD/HIGH Ecological Quality Status (Bentix index), (b) Total Organic Carbon percentage > 1.2% (empirical threshold), and (c) no recent ecological crisis. Three replicate samples per site were collected by scuba-diving, using hand-held corer (0.018m^2) in seagrass beds (PB, CBD). For the remaining sites, two replicate samples were collected by R/V AEGAEO, using a box corer (0.1m^2). Sediment samples were subsampled and frozen in -20 °C. Macroinvertebrate samples were sieved through 1mm sieve-size and fixed with 10% formalin, stained with Rose Bengal. In the laboratory, polychaetes were identified and counted (standardized to m^2). A total of 20 traits (related to the categories of dispersal, morphological adaptation, habitat engineering, nutrient cycling, stability and resilience) were assigned using fuzzy coding (0 – 4) and a traits-sites matrix was created after weighting with abundance. A nMDS with Bray-Curtis similarity was plotted after square-root transformation. Significance of difference between habitat types was tested by one-way ANOSIM. The best explanatory environmental variables were identified with BIOENV. Functional Richness (FRic) was calculated and statistically significant differences ($p < 0.05$) were tested between habitat types (ANOVA and Tukey HSD post-hoc tests), after testing for normality (Shapiro-Wilk), homogeneity (Barlett) and transforming where needed . All analyses were made in R. Details on stations and traits used can be found in Katsiaras (2021).

3. Results

Four clusters with similarity over 60% were distinguished in the nMDS plot (A to D, Fig. 1). Namely, dense seagrass beds (Cluster A), coarse detritic substrates (Cluster B), muddy substrates (Cluster C) and BM (Cluster D). However, several muddy substrates were grouped with Cluster B, as well as few coarse detritic samples were included in Cluster C. Samples from CBS were distributed along Clusters A to C. ANOSIM pairwise comparisons showed significant differences of the dense seagrass beds (PB, CBD) with all the other habitat types ($R = 0.7 – 1$), including scarce beds (CBS). CBS were quite similar to detritic bottoms (CSD, ISD, CMD, IMD). Coarse detritic substrates (RB, CSD, ISD) showed differences, but with community overlap with muddy substrates (CM, IM, $R = 0.31 – 0.49$) and overlap was increased with the muddy detritic habitats (CMD, IMD $R = 0.06 – 0.26$). BM was found to be different from all the other habitat types ($R = 0.52 – 1$).). BIOENV showed that the environmental variables best explaining the functional patterns were depth, granulometry, and seagrass density (0.651 correlation). FRic was found different between the habitat types ($F = 8.363$). It was significantly higher in PB, compared to CBS ($p = 0.02$) and most of the muddy substrates (BM, CM, CMD, IM; $p = 0.000004 – 0.04$). Likewise, it was significantly higher in CBD and the coarse detritic substrates (RB, CSD, ISD), compared to the deep muddy substrates (BM, CM, CMD $p = 0.000009 – 0.04$), but not the shallow (IMD, IM).

4. Discussion/Conclusion

Distinct functional spatial units were found along the habitat types examined, which may indicate different mechanisms by which polychaetes mediate ecosystem functions in each unit. Prevailing functional trait modalities per habitat type can be found in Katsiaras (2021). A summary of our results showed: (a) strong differences between dense seagrass beds and unvegetated substrates, (b) high similarities among coarse detritic substrates, including either RB or sands with shell debris, (c) differences but with community overlap between infralittoral/circalittoral muds and coarse detritic substrates, and (d) strong differences between BM and all the shallower habitat types.

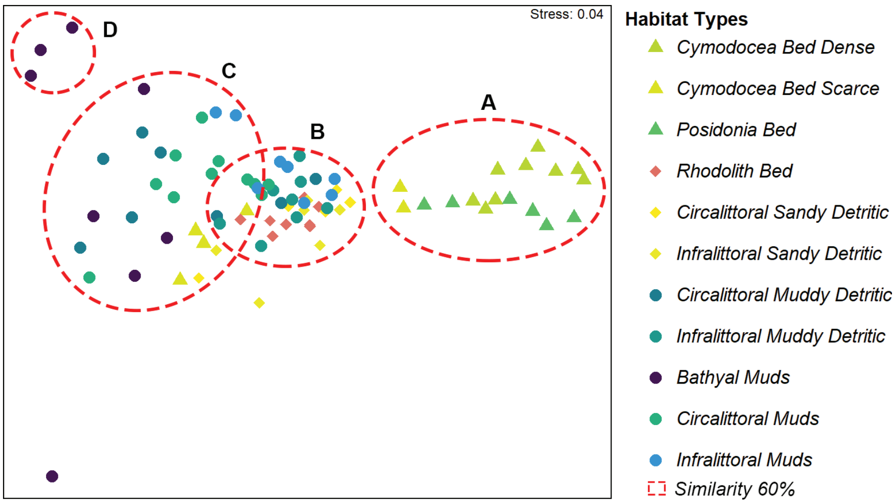

Fig. 1: MDS analysis of polychaete communities between different habitat types (highlighted with different color and shape), based on trait modalities. Similarity circles of > 60% are derived from the relative Cluster Analysis.

Dense seagrass beds differed in part due to the remarkably higher abundances of individuals found, since the offered properties, such as high food availability, shelter from predation, and stable substrate can attract high abundance of macroinvertebrates (Como *et al.*, 2008). In fact, meadow density was found to be an important factor in functional patterns, since CBS was mostly clustered together with either coarse detritic, or muddy substrates, probably depending more on the local sediment grain-size, rather than the presence of *C. nodosa*. Regarding the coarse detritic substrates, the present study showed similarities in their functional profiles despite the variation in morphology and origin of the biogenic structure/detritus, even though only RB are considered hot points of Mediterranean biodiversity in dim light conditions and are currently recommended for protection (Basso *et al.*, 2015). In general, habitats with rigid biogenic structure showed dominance or relatively increased abundances of trait modalities fit for complex topography (vagile species, carnivores/herbivores, high sensory ability) and were related to analogous transfer pathways of nutrients and energy to the local environment. These habitats are characterized by multi-layered structures, where the unique conditions of each layer may favor different sets of trait modalities and functions, adding up to a remarkable spatial heterogeneity and microhabitat differentiation (Como *et al.*, 2008). Therefore, the presence of microhabitat heterogeneity may extend the total functional range (Biswas *et al.*, 2019) and was linked with significantly higher Functional Richness in the present study.

On the other hand, a completely different set of trait modalities was dominant in muddy substrates (tube/galley-dwellers, detritus feeders, low sensory ability), which create heterogenous structures in rather physically homogenized habitats and evidently affect diversity of smaller-sized species (Jumars & Gallagher, 1982). The functional overlap between muddy and coarse detritic substrates may be explained by the mixed gravel and biogenic detritus in the former and the interstices filled with mud in the latter, a mosaic-nature repeatedly linked with species assemblages overlaps in the past (Somaschini *et al.*, 1998; references therein). A major obstacle in studying the relation between functional composition and structural complexity of detritic bottoms is the lack of typology on morphology and proportion of the biogenic detritus, which range from mollusc shells to bryozoan/coral skeletons, or scarce rhodoliths (see EAA, 2019). Therefore, classifying the complexity of the biogenic detritus is crucial in understanding relative functional variability and vulnerability. Finally, the significant differentiation of BM possibly depends on the community adaptations to the local extreme conditions (e.g., oligotrophy, low oxygen, darkness).

Differences across functional units emphasize the importance of habitat heterogeneity in ecosystem functioning, affecting the recycling and processing of energy and matter (Hewitt *et al.*, 2008). Investigating habitat diversity along with functional diversity can play a key role in understanding the ecological drivers of biodiversity and energy flow. The present study highlights the importance of traditionally spotlighted habitat types in ecosystem functioning, but may also aid in changing the management perspective over several types of habitats, that are often seen as hosting poor species assemblages, to be considered important functional units in the wider ecological system.

5. Acknowledgements

The present study was funded by the Ministry of the Environment for implementing the WFD National Monitoring Network.

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PHYTOPLANKTON DIVERSITY INDICATORS IN THE OPEN WATERS OF THE AEGEAN AND LEVANTINE SEAS (EASTERN MEDITERRANEAN) TOWARDS PELAGIC HABITAT ASSESSMENT FOR MSFD

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Abstract

Many phytoplankton metrics are known to provide valuable insights on population dynamics, however they are not operational for the pelagic habitat assessment yet. In the Mediterranean Sea, not all eight European member states have defined Good Environmental Status (GES) targets for plankton communities and pelagic habitats till now in the context of the EU Marine Strategy Framework Directive (MSFD). Phytoplankton diversity indicators in particular are under ongoing research of their sensitivity against environmental pressures. In the present study, phytoplankton diversity indices were calculated on the basis of phytoplankton communities' structure and abundance from open waters of the Aegean and Levantine Seas during MSFD monitoring campaigns. The behavior of the tested indices in such oligotrophic waters and their distinctiveness along spatial and seasonal scales are discussed.

Keywords: phytoplankton diversity indicators, plankton communities, population dynamics, pelagic habitat assessment, MSFD monitoring.

1. Introduction

Many metrics for phytoplankton communities have been demonstrated to provide valuable insights on population dynamics, however they are not yet operational for the pelagic habitat assessment. In the Mediterranean Sea, a recent revision of approaches for Good Environmental Status (GES) definitions and environmental targets (Articles 9 and 10 of the EU-Marine Strategy Framework Directive and the Commission Decision EU 2017/848) in the eight Mediterranean member states showed that GES for plankton communities and pelagic habitats has not been defined in all member states yet (Varkitzi et al.,2018a). The GES determination should consider changes in the spatial heterogeneity of the regional sea characteristics, and therefore depends on the spatial and temporal characteristics of the marine area under study (Magliozzi et al., 2021).

The pelagic habitats in Eastern Mediterranean (EMed) are characterized by oligotrophic conditions, whereas some coastal areas present pollution pressures, eutrophication problems and Harmful Algal Blooms (HABs) (Varkitzi et al. 2018b, 2020). A previous study reports for a set of phytoplankton diversity indices tested against pressure levels defined by expert knowledge (such as point and non-point pollution, industry, ports and fisheries) that most of them were able to distinguish between the highest level of impact and the rest of the impact categories, while they maintained this sensitivity across latitudinal and longitudinal gradients in the Adriatic, Ionian and Aegean Seas (Francé et al., 2021). In the present study, phytoplankton diversity indices were tested on the basis of the structure and abundance of phytoplankton communities from open waters of the Aegean and Levantine Seas during MSFD monitoring campaigns. Our scope was to study the behavior and performance of phytoplankton diversity indices in such oligotrophic waters and look into their distinctiveness along spatial and seasonal scales.

2. Materials and Methods

Seawater samples for the analyses of phytoplankton diversity (community composition and abundance) and chlorophyll-a concentrations were taken during oceanographic campaigns of the MSFD monitoring program in the Aegean (central and south) and Levantine Seas (Fig. 1). Field sampling and laboratory analyses (inverted microscopy by Utermöhl 1958, and imaging) were performed according to standard methodologies (see for details Varkitzi *et al.*, 2020). Nanoflagellates were not included in this analysis. The set of phytoplankton diversity indices was computed according to Cozzoli *et al.* (2017).

3. Results

The distributions for a set of phytoplankton indices (the number of species and other diversity indices, evenness indices, total abundance, abundance of groups and total biomass as chlorophyll-a) across sampling stations in open waters (surface layer 2-20m) of the Aegean and Levantine Seas during two seasonal sampling campaigns of the MSFD monitoring program (warm and cold period of the year 2019) are presented in Fig. 2 and 3. The phytoplankton total abundance ranged between 4280-30280 cells L^{-1} during the cold period (March 2019). The number of species, total abundance and chlorophyll-a maximized in the waters off Rhodes island in the Levantine Sea (stations MSFD6 and MSFD7) whereas in the other stations expected margins for this season were reported. During the warm period (August 2019), number of species, total abundance and chlorophyll-a decreased at lower levels than in the cold period (793-2040 cells L^{-1}), as expected.

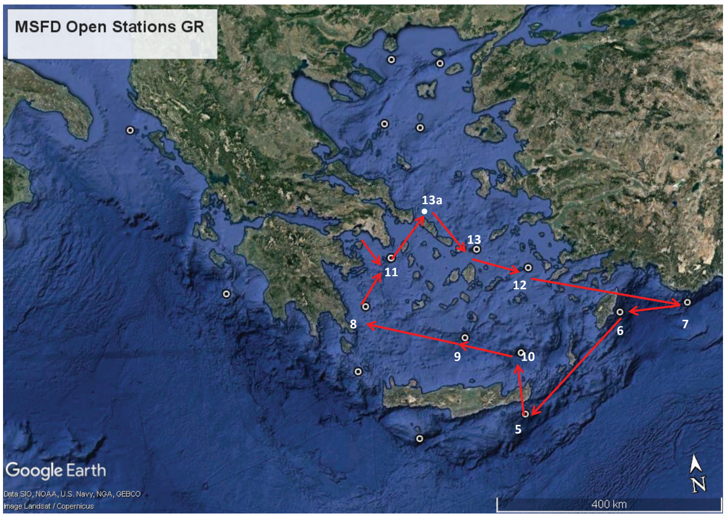


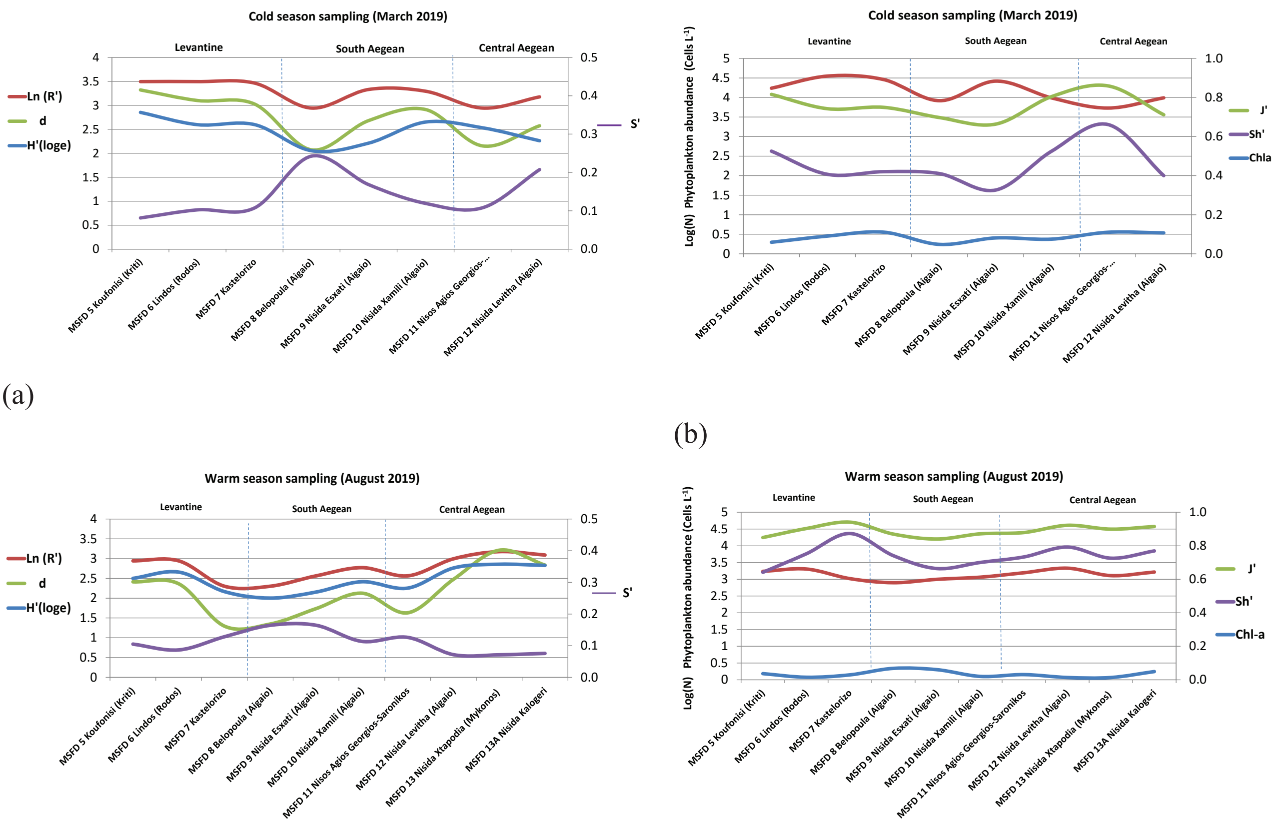
Fig. 1: Sampling stations of the MSFD monitoring network in the Levantine, Central and South Aegean Seas.

When analyzing the distribution of phytoplankton groups during the cold period (Fig. 3), the dominance of diatoms over all other groups was evident in most MSFD stations, whereas the most prominent signal of diatoms dominance (95%) was identified in the broader Rhodes marine area (MSFD7). Coccolithophores were the second most dominant phytoplankton group, reaching 56% in Central and South Aegean waters (MSFD12 and MSFD8), whereas dinoflagellates were the third dominant group in most cases. During the warm period, diatoms dominated again the phytoplankton communities in all stations, whereas dinoflagellates were ranked as the second dominant group. Silicoflagellates were the third dominant group in most of the stations.

Phytoplankton diversity indices presented high levels in most stations during the cold period (Fig. 2). Particularly the diversity indices R' number of species, d' Margalef and H' Shannon followed similar increasing trends as total abundance and chlorophyll-a towards the Levantine Sea, whereas S' Simpson index did not present such distribution. J' Pielou and Sh' Sheldon evenness indices formed inverse patterns to those of total abundance N, presenting higher scores in Central Aegean. During the warm period, all diversity metrics presented lower scores due to the lower number of species and abundance, whereas their distributions were similar to those of the cold period. J' and Sh' evenness indices ranged at scores higher than in the cold period.

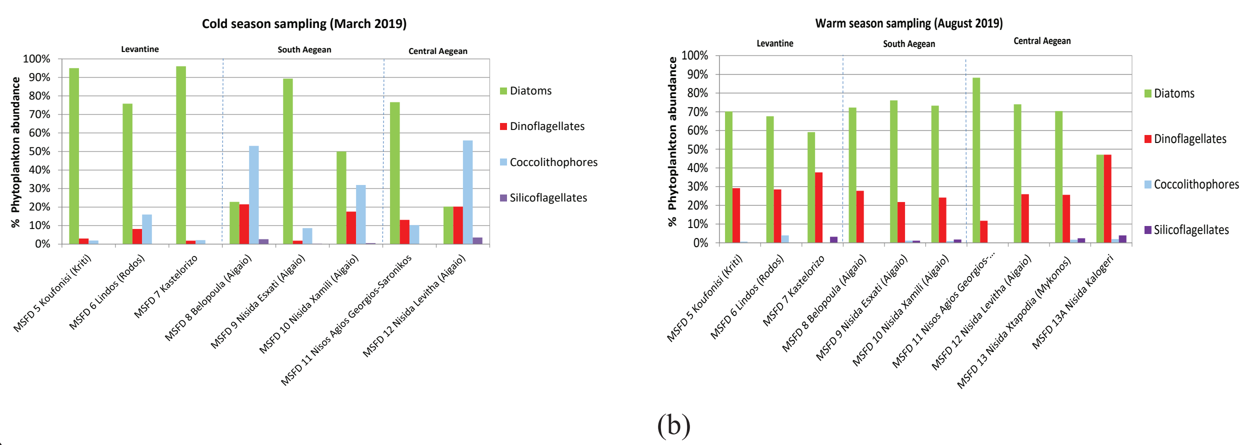
4. Discussion/Conclusion

In the Aegean and Levantine open waters examined in the present work, the phytoplankton total abundance and chlorophyll-a were found to range within margins well documented from previous studies (Varkitzi *et al.*, 2020; Ignatiades *et al.*, 2002; 2009). However, higher levels were reported from open waters off Rhodes Island (Levantine Sea) during the cold period. This might be linked to the impact of the Rhodes gyre which is in the vicinity of the sampled water masses. Rhodes gyre is one of the most persistent mesoscale structures in EMed, shaping the distribution of nutrients and biological activity (Protopapa *et al.*, 2020). Strong winter mixing episodes in Rhodes gyre have been shown to result in significant nutrient pumping into the euphotic zone from the deep, leading to unusual peaks of phytoplankton biomass (chlorophyll-a >1 μg L^{-1}) especially in March (D'Ortenzio *et al.*, 2021). Phytoplankton total abundance and chlorophyll-a decreased significantly during the warm period in the present study. This is a well documented pattern in the Aegean and Levantine Seas during the stratified period of summer-autumn due to the trapping of nutrients below the euphotic zone which creates unfavorable conditions for phytoplankton growth (Varkitzi *et al.*, 2020; Siokou *et al.*, 2010; Ignatiades *et al.*, 2002; 2009).



(a) (b) (c) (d)

Fig. 2: Phytoplankton diversity indices of R' number of species, d' Margalef, H' Shannon, S' Simpson indices (a and c), and phytoplankton evenness indices of J' Pielou and Sh' Sheldon against total phytoplankton abundance Log(N cells L^{-1}) and total phytoplankton biomass with chlorophyll-a (μg L^{-1}) as proxy (b and d) across MSFD monitoring stations in open waters of the Aegean and Levantine Seas (surface layer 2-20m) during the cold (March 2019) and the warm period (August 2019).



(a) (b)

Fig. 3: Relative abundance of phytoplankton groups (%) across MSFD monitoring stations in open waters of the Aegean and Levantine Seas (surface layer 2-20m) during the cold (March 2019) and the warm period (August 2019).

Previous studies in the Aegean and the Levantine Seas have shown that almost 60-70% of autotrophic biomass and primary production is performed by small phytoplankton cells <3.0 μm, whereas larger phytoplankton cells (>5 μm) increase their contribution during the seasonal mixing (Ignatiades *et al.,* 2002; Varkitzi *et al.,* 2020; Psarra *et al.,* in press). In the present study, diatoms (>5 μm) clearly dominated over all other phytoplankton groups in most MSFD stations. Diatom and coccolithophore species numbers and abundances are known to be higher during the mixing period in the Aegean and Levantine Seas, when nutrients are fueled to the euphotic zone from deeper masses (Ignatiades *et al.,* 2002; Varkitzi *et al.,* 2020). An unprecedented spring bloom was documented for the first time in South Aegean by Varkitzi *et al.* (2020), comprising mostly of diatoms and other large sized phytoplankters (77%), as a result of a strong deep mixing event. Phytoplankton abundances during that bloom event were > 3 times higher than those reported by Ignatiades *et al.* (2002).

In the present study, the scores of the diversity indices R' number of species, d' Margalef, H' Shannon and S' Simpson were high inter-seasonally and comparable with previous studies (Ignatiades *et al.* 2002; Varkitzi *et al.,* 2020). R', d' and H' diversity indices followed closely the total abundance and chlorophyll-a distributions, whereas J' Pielou and Sh' Sheldon evenness indices formed inverse patterns in cases when the number of species was lower. Overall, the scores of J' and Sh' evenness indices were indicative of balanced communities which are not under pressure. Varkitzi *et al.* (2020) reported comparable high levels of H' Shannon diversity (3.65–4.36) which were constant in different seasons or stations in the Aegean, Levantine and Ionian Seas. In conclusion, diversity is at high levels in the Aegean and Levantine open waters despite their oligotrophic conditions. In this respect, oligotrophy is the main driver for phytoplankton distribution there, but these water masses can occasionally support higher phytoplankton biomass and blooms, e.g. in gyres and deep mixing events.

5. Acknowledgements

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SPATIO-TEMPORAL VARIABILITY IN MESOZOOPLANKTON DIVERSITY PATTERNS IN THE AEGEAN SEA

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Abstract

Mesozooplankton dynamics and the underlying mechanisms shaping their variability are a central issue of oceanographic research in our days, as mesozooplankton plays an important role in oceanic carbon flux for being the primary biological mechanism responsible for the carbon transferred from the surface to deeper waters and in higher trophic levels. In the current study an extensive analysis of mesozooplankton alpha and beta diversity was conducted based on published species composition data from 1991 till 2017 in the Aegean Sea. Our aim was to highlight the spatio-temporal variability of the communities composition in relation with biotic/abiotic gradients. Our results indicate a clear-cut pattern in alpha diversity along the North-South axis, with the South Aegean being characterized by lower total abundance and higher species richness and the North Aegean being characterized by the opposite. On the study area three assemblages were identified: 1) an autumn assemblage of the North Aegean 2) a spring assemblage of the North Aegean and 3) a South Aegean assemblage with no distinct differentiation between the seasons. Temperature and geographical distance were identified as the main variables affecting compositional turn over, suggesting that mesozooplankton communities of the Aegean Sea may be vulnerable to the higher sea water temperatures predicted by climate change scenarios.

Keywords: mesozooplankton, alpha diversity, beta diversity, Aegean Sea, community composition.

1. Introduction

Plankton communities' high diversity regulates some of the most important marine ecosystem services (Stocker, 2015). The far exceeding number of species in comparison with the number of limiting resources was described as the 'paradox of plankton' (Hutchinson, 1961). A possible explanation could be attributed to the cyclic processes occurring in the water column, e.g., upwelling events, terrestrial inputs (Sakavara *et al.*, 2018). For zooplankton in particular seasonal and spatial community patterns seem to be influenced by hydrology in complex environments as the Aegean Sea (Zervoudaki *et al.*, 2020). Most zooplankton species are short-lived, ectotherms so there can be tight coupling between population dynamics and environmental fluctuations (Hays *et al.*, 2005).

The aim of this study was to investigate the potential effect of biotic and abiotic variables in mesozooplankton surface assemblages' composition of the Aegean Sea and highlight the variables responsible for beta diversity changes in space and time.

2. Material and Methods

In this research, published mesozooplankton community data coming from 7 oceanographic cruises conducted by the Hellenic Centre for Marine Research (HCMR) during the period from 1991 till 2017 were analyzed (Fig. 1). Overall, 182 taxa found in 32 stations in the upper water column layer 0-100m, were tested in relation to all available parameters that could be affecting community composition. Namely the stations' sampling season, geographical position and bottom depth, integrated sea water temperature, salinity, and concentrations of dissolved oxygen and Chlorophyll a. All statistical analysis, data manipulations and presentation mentioned below were performed with the R programming language (R Core Team, 2021).

A thorough analysis of 17 ecological indices, expressing diversity (e.g., Menhinick index), evenness (e.g., Pielou's evenness J) and dominance (e.g., Berger-parker index), was performed to investigate structural changes of mesozooplankton communities. To recognize and interpret underlying patterns of community composition the Non-metric Multi-Dimensional Scaling (NMDS) method was used. The information on the potential parameters shaping that dissimilarity between samples were extracted by correlating vector and factor variables into the NMDS ordination. Finally, the generalized dissimilarity model (GDM) was applied as a method for analyzing the variation in biodiversity between pairs of geographical locations (Ferrier et al., 2007). The GDM is a powerful method used in ecological research for characterizing and predicting beta diversity (Mokany *et al.,* 2022), by relating dissimilarity in species composition between sites to their environmental conditions' differentiation (environmental distance) and how isolated they are with each other (geographical distance) (Tuomisto *et al.,* 2016). Geographic distance is incorporated as the Euclidean distance between pairs of sites based on their coordinates. Two types of composition data were tested with this method, presence/absence data taking values 1 or 0 if a species is present or not and species abundance data. For GDM and NMDS analysis a distance matrix is required as an input and in our case we chose the Bray-Curtis dissimilarity. Prior to the analysis the Hellinger's transformation method was applied to the abundance data to prevent the "double zero" problem. Also, a number of permutations was used in order to evaluate the significance of each fitted variable in the two methods.

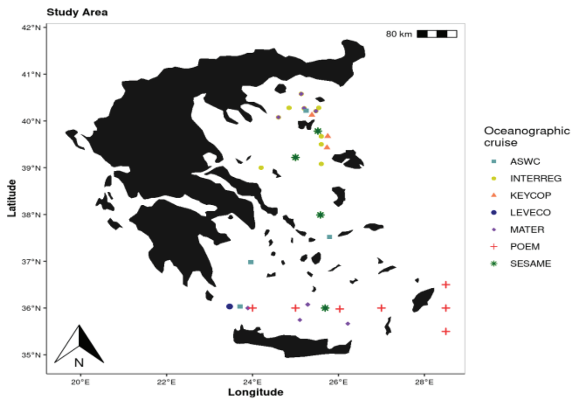

Fig. 1: Study Area: Mesozooplankton sampling stations in the Aegean Sea during the period 1991 till 2017, in spring and/or autumn from seven oceanographic cruises conducted by the HCMR.

3. Results

A clear-cut pattern in alpha diversity was observed along the North-South axis of the Aegean, mainly due to differences in species richness (around 60 in the stations of the North Aegean (NA) and above 70 in the South Aegean (SA)), evenness (Pielou's J generally below 0.7 in the NA stations and above 0.75 in the SA stations) and total abundance. Total abundance expressed a clear trend with generally higher values being recorded during autumn in the NA (mean = 1971 ind/m^3) and spring (mean = 1337 ind/m^3) and lower in SA in both seasons (autumn mean = 415 ind/m^3 and spring mean = 465 ind/m^3). Dominance was generally low (% of the most abundant species below 50), although higher in North Aegean waters.

The non-metric multidimensional scaling (NMDS) analysis showed that the mesozooplankton communities analyzed can be clustered into two main groups based on their location: the NA communities in which distinct dissimilarities are observed between spring and autumn and the SA community in which season although plays a role in species composition, the differentiation is not as prominent. Temperature was found to have a statistically significant effect on assemblages' distribution in the 2D NMDS ordination with squared correlation coefficient r^2 = 0.69 (p-value = 0.001), followed by stations' bottom depth and salinity with r^2 = 0.49 (p-value = 0.001) and r^2 = 0.37 (p-value = 0.001) respectively. Generally, autumn is characterized by higher temperatures in the upper water column layer in comparison with spring. Moreover temperature was identified as the main driver responsible for the seasonal assemblages' differentiation in the NA. Dissolved oxygen and chlorophyll a concentrations were found to have no important effect on beta diversity in the Aegean Sea (Fig. 2, Table 1).

The fitted generalized dissimilarity model (GDM) for the abundance data, included the sea water temperature, the geographical distance, station's bottom depth and salinity. The above predictive variables explained approximately 47% of the observed stations dissimilarities (p-value < 0.001). The temperature was identified as the most important environmental variable causing beta diversity turn over. The GDM model trained with the binary species data (presence/absence) explained only 32.3% of the observed variance in the dissimilarity matrix (p-value <0.001). Statistically significant as predictive variables were the sea water temperature and the geographical distance. On this analysis geographical distance was statistically more important than sea water temperature (Table 1).

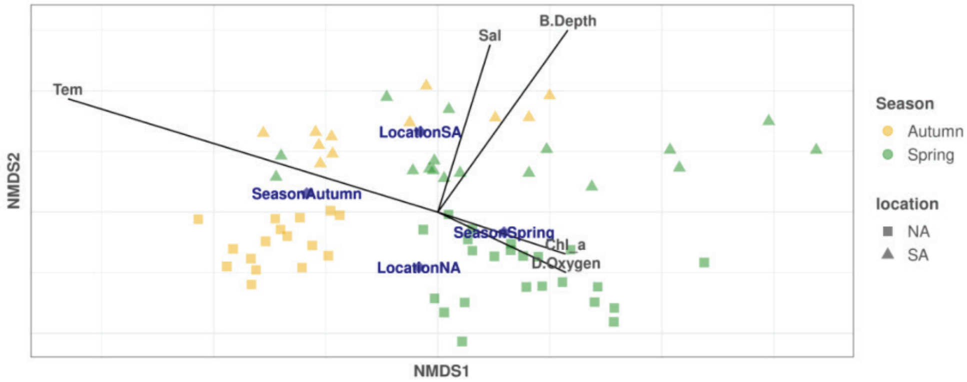

Fig. 2: Distribution of stations according to the NMDS ordination analysis (stress value ≈ 0.18). The stations are color coded according to season and the shapes represent the stations location. The centroid values for all samples belonging to a certain category are plotted as well. The lines represent regression vectors for environmental variables with correlation to the mesozooplankton assemblages. Line length represents correlation strength and the line angle shows the direction of sample's increase with respect to that variable.

Table 1. Results of the significance level for each explanatory variable from the fitted models identified by the NMDS and GDM analysis.

	NMDS		**GDM**			
	Abundance data		**Abundance data**		**Binary data**	
Environmental variables	r^2	P-value	Variable Importance	P-value	Variable Importance	P-value
Temperature	0.69	0.001 ***	39.03	0.000 ***	32.91	0.000 ***
Salinity	0.37	0.001 ***	3.64	0.02 *	-	-
Chlorophyll a	0.08	0.073 .	0.25	0.646	-	-
Bottom depth	0.49	0.001 ***	4.15	0.030 *	-	-
Dissolved oxygen	0.11	0.032 *	0.33	0.43	-	-
Geographic distance	-	-	9.19	0.000 ***	65.74	0.000 ***

Signif. Codes: 0'***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1' '1 (Number of permutations: 999)

4. Discussion/Conclusion

In this research, alpha diversity was generally higher in the more saline and oligotrophic waters of the South (SA) compared to the North Aegean (NA), whereas seasonal differentiations in the α-diversity patterns were more prominent for the NA, which is in accordance with previews research for this study area (Zervoudaki *et al.*, 2020).

Generally, mesozooplankton communities were dominated by copepod species in both the NA and SA. Although, during autumn in the NA a shift in the community composition was recorded, mostly caused by three cladoreran species (*Penilia avirostris*, *Evadne spinifera* and *Pseudevadne tergestina*). That shift is correlated with the increase in sea water temperature. For the SA the communities are mostly shaped by the salinity and seasonal differentiations in species composition were not as clear.

The geographical position was identified from the GDM analysis to be the most important variable for the presence/absence data, while temperature was identified as the variable having the outmost effect on the communities' composition and beta diversity turn over when abundance data were used. This contradiction indicates that the amount of common species between two stations is correlated by the stations distance though temperature is the main variable controlling the abundance of the species within the communities.

This study is the first step into understanding how the biotic and/or abiotic parameters affect mesozooplankton alpha and beta diversity in the complex physicochemical environment of the Aegean Sea. Further research will shed more light to the mechanisms shaping mesozooplankton community structure. The potential use of mesozooplankton in ecological assessments that has been proposed in the context of the Marine Strategy Framework Directive (Ndah et al., 2022), is making the understanding of mesozooplankton dynamics and the parameters effecting their variability necessary.

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THE MESOZOOPLANKTON FAUNA OF THE NORTH AEGEAN SEA: A LITERATURE REVIEW

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Abstract

Zooplankton species' presence, abundance, and biogeography for the North Aegean Sea (NAS) are reviewed in the present study. One-hundred-eighty species were recorded in total. The most abundant taxon is Copepoda represented by 134 species (74.3%), Mollusca group follows with 12 species (6.7%), and Appendicularia and Cladocera with 6 species (3.4%) respectively. Regarding their biogeographical affinities, cosmopolitans dominated in all zooplankton groups (84.4%), followed by the Atlanto-Mediterraneans (10.6%). Two ctenophores, *Mnemiopsis leidyi* and *Beroe ovata*, the cyclopoid copepod *Oithona davisae* and the calanoid copepod *Pseudodiaptomus marinus* are non-indigenous and invasives in the Mediterranean Sea. Finally, a synopsis of quantitative and qualitative characteristics of the zooplankton communities in the NAS is presented. This study establishes a useful knowledge base to guide future research and assessments on the zooplankton fauna of NAS.

Keywords: mesozooplankton, diversity, abundance, North Aegean Sea, sub-regions.

1. Introduction

One of the most distinct, complex and, dynamic sub-ecoregion of the Mediterranean Sea is the North Aegean Sea (NAS). It is a relatively small, semi-enclosed sea with high habitat diversity and important fishing grounds of high economic value (Tsagarakis *et al*., 2010). The special characteristics of NAS are mainly shaped by: a) the inputs of Black Sea Waters (BSW) that and have a maximum runoff during the warm periods (Poulos *et al*., 1997; Zervakis & Georgopoulos, 2002); b) the outflow of the regional rivers (Axios, Strymon, Nestos and Evros) the period December – May, further enhancing the coastal productivity (Kehayias *et al*., 2005); c) the unique, diverse topography, including the extensive continental shelf (average width 60 km) of Thracian Sea, Lemnos Isl. Basin and the "Gorge of Mount Athos" Basin; and d) the annual summer north winds (Zervakis & Georgopoulos, 2002). The hydrological complexity and the strong depth heterogeneity, which characterize the area are reflected in spatio-temporal composition and in the distributional patterns of the zooplankton species (Isari *et al.*, 2006). Zooplankton plays a pivotal role in marine environments through its function as a driver of carbon and nutrient cycles, a grazer of primary production, and prey for commercial fishes (Keister *et al*., 2012). Furthermore, zooplankton can incorporate a wide range of environmental information, thus long-term changes in biomass, species composition and communities' structure can be used as indicators of the environmental changes and potential impacts associated with anthropogenic pressures. This study aims to review information on the presence, abundance, and biogeography of NAS zooplankton species, identifying the research gaps and revealing the challenges that still exist. The present analysis based on twenty-five relevant published papers, including review and research articles with species catalogues and information on biodiversity and abundance of the mesozooplankton fauna.

3. Zooplankton characteristics

3.1 Species presence and biogeographical affinities

A total of 180 zooplankton species belonging to 60 families and 109 genera have been recorded in the NAS. One hundred and seventy-six species are holoplanktonic (97.8%), while the remaining are meroplanktonic (2.2%). The holoplanktonic group consists of 86% cosmopolitan species, 8.6% Atlanto-Mediterranean and 3.4% endemic, while four species, the ctenophores *Mnemiopsis leidyi* and *Beroe ovata*, the cyclopoid copepod *Oithona davisae* and the calanoid *Pseudodiaptomus marinus* are non-indigenous in the Mediterranean. From the meroplanktonic group, all species are Atlanto-Mediterranean.

3.2 Species diversity

NAS can be divided into four sub-regions based on hydrological features: **a) the thermal front of the north-eastern Aegean Sea (NEA).** In surface layers (0-50 m), during the warm period, some predominant species are the cladoceran *Penilia avirostris*, the copepods *Clausocalanus furcatus*, *Paracalanus* spp., *Temora stylifera*, *Oithona plumifera*, *Oncaea media*, *Calocalanus* spp., Larvacea and Thaliacea, while in the deeper layers (> 50 m) the copepods *Ctenocalanus vanus*, *Clausocalanus jobei*, *Oithona plumifera* and Larvacea predominate (Siokou *et al*., 2009; Zervoudaki *et al*., 2006). During the cold period, the most abundant species are *Clausocalanus* spp., *Oithona* spp., *Calocalanus* spp., *Paracalanus spp.*, *Centropages typicus*, *Ctenocalanus vanus*, *Evadne nordmanni*, Larvacea and Chaetognatha (Siokou *et al*., 2014); **b) the anti-cyclonic zone of Samothrace.** During the warm period, *Penilia avirostris* numerically dominates, followed by *Pseudevadne tergestina*, *Evadne spinifera* and Thaliacea -*Oikopeura* spp.. Larvacea and Copepoda appear in low densities and diversity, while *Temora stylifera* is the only abundant copepod species. Heterotrophs dinoflagellates, prey of mesozooplankton, which consist an important presence in the microheterotrophs biomass, are observed as peripherals to the anticyclonic zone lower levels. Also, an increase of the small size copepods [e.g. *Acartia* (*Acartiura*) *clausi*, *Paracalanus* spp., *Oithona* spp. and *Clausocalanus* spp.] is observed, while Cladocera follows with a lower contribution (Isari *et al*., 2010); **c) the pelagic zone**. In the lower epipelagic (50 - 100 m) layer of the area, both in warm and cold periods, *Clausocalanus paululus*, *Oithona setigera setigera*, *Oncaea media* and *Farannula rostrata* dominate (Siokou-Frangou *et al*., 2014). In the mesopelagic layers, both in warm and cold periods, the predominant groups are Copepoda and Ostracoda, which are capable of consuming organic detritus (Siokou *et al*., 2013); **d) the Macedonian coasts (Thermaikos Gulf Strymonikos Gulf, Kavala Gulf)**. In Thermaikos Gulf, copepods and cladocerans are by far the most abundant, with remarkably high numbers of copepods in the inner part of the gulf during the cold period. The dominant species are *Acartia* (*Acartiura*) *clausi* followed by *Oithona nana* and *Podon polyphemoide* in cold period as well as *Centropages typicus* and *Evadne spinifera*, while in warm period the cladocerans *Evadne tergestina, Evadne spinifera, Penilia avirostris* and copepods *Paracalanus parvus parvus* and *Temora stylifera* dominated (Siokou-Frangou & Papathanassiou, 1991). In Strymonikos Gulf, the chaetognaths *Flaccisagitta enflata, Mesosagitta minima*, *Parasagitta setosa* and *Serratosagitta serratodentata* have been recorded by Kehayias *et al*. (2005) from March to September. Moreover, in Kavala Gulf, *Penilia avirostris*, *Acartia (Acartiura) clausi*, *Oithona plumifera*, the tunicates *Oikopleura*, *Fritillaria* and Doliolida species have been recorded by Karagianni *et al*. (2019) in the warm period. Depending on the season, the area plays the role of nursery grounds for meroplankton.

3.3 Species abundances

Mesozooplankton total biomass and abundance estimations are almost one order of magnitude higher in the BSW layer than in the Levantine Waters (LW) layer (Siokou *et al*., 2014). The seasonal fluctuations in the abundance of zooplankton in the NAS are characterized by two peaks, one in spring and one in late summer/early autumn. In coastal areas, which are strongly affected by human activities and rivers outflows, the spring peak corresponds to the water temperature and phytoplankton fluctuations

(Kovalev *et al*., 2003). On the other hand, the second peak, often during the period of June – September, corresponds to the areas mainly affected by the BSW (Siokou-Frangou & Papathanassiou, 1991; Karagianni *et al*., 2019). Higher abundance values of the predominant mesozooplankton species, in the surface layer (0-50 m) during April, reaches 2.039 ind. m^{-3} of *Centropages typicus*, 371 ind. m^{-3} of *Paracalanus* spp. and 346 ind. m^{-3} of *Clausocalanus* copepodites only in the deeper layer (50-100 m) (Siokou *et al*., 2014). During the warm period, the corresponding values reach 367 ind. m^{-3} of *Ctenocalanus* spp., 117 ind. m^{-3} of *Clausocalanus* copepodites and 121 ind. m^{-3} of apendicularias. Mesozooplankton presents an important gradual increase in total abundance values as approaches the coastal zone (Ramfos *et al*., 2005). Characteristic abundance values of the representative sub-areas of NAS, are given in Table 1.

Table 1. Mesozooplankton abundance (ind. m^{-3}) in different sub-regions of NAS, per sampling year, period and discrete layers of the water column.

Sub-region of NAS		Year	Period	Layer (m)	Abundance (ind. m^{-3})	Citation
NEA	Thermal front	1997	cold	10-20	14157	Siokou-Frangou *et al.*, 2009
		1998	warm	0-50	1233	Siokou-Frangou *et al.*, 2009
		1999	warm	0-20	3083[1]	Zervoudaki *et al.*, 2006
		1999	warm	20-50	2140[1]	Zervoudaki *et al.*, 2006
NEA	Thermal front	1999	warm	50-100	17141	Zervoudaki *et al.*, 2006
		2000	cold	0-20	2798[2]	Zervoudaki *et al.*, 2006
		2000	cold	20-50	1104[2]	Zervoudaki *et al.*, 2006
		2000	cold	50-100	6432	Zervoudaki *et al.*, 2006
	Anticyclonic	1997	cold	50-100	188	Siokou-Frangou *et al.*, 2009
	zone	2004	warm	20-50	2473	Isari *et al.*, 2010
		2004	warm	0-20	13157	Isari *et al.*, 2010
		2004	warm	20-50	14316	Isari *et al.*, 2010
		2004	warm	0-20	26823	Isari *et al.*, 2007
Pelagic	All	1997	cold	0-50	1975	Siokou *et al.*, 2013
zone		1998	warm	20-50	12244	Siokou-Frangou *et al.*, 2009
		1999	warm	20-50	1602	Zervoudaki *et al.*, 2006
		1999	warm	50-100	522	Zervoudaki *et al.*, 2006
		1999	warm	0-20	1257	Zervoudaki *et al.*, 2006
		1997	warm	0-50	2545	Siokou *et al.*, 2013
		2004	warm	0-20	939	Isari *et al.*, 2007
	Mesopelagic	1997	cold	500-700	41	Siokou *et al.*, 2013
		1997	warm	500-700	94	Siokou *et al.*, 2013
Macedonian	Thermaikos	1984	cold	0-45*	8328[3]	Siokou-Frangou & Papathanassiou, 1991
coasts	Gulf	1985	cold	0-45*	7022[3]	Siokou-Frangou & Papathanassiou, 1991
		1985	warm	0-45*	1956[3]	Siokou-Frangou & Papathanassiou, 1991
	Kavala Gulf	2002	warm	0-45*	103004[4]	Karagianni *et al.*, 2019
		2003	warm	0-45*	46128[4]	Karagianni *et al.*, 2019
Other coasts	Pagasitikos Gulf	1998	warm	0-60*	1738	Ramfos *et al.*, 2005

Assumptions have been made for total zooplankton abundance values to be included in the table: [1,2]Average abundances of sampling stations with confirmed positions within the thermohaline front; [3]Average abundances of all sampling stations in semi-enclosed study area; [4]Average abundances of all stations, as no significant differences were observed. *Water column where vertical hauls took place.

4. Discussion/Conclusion

Zooplankton species' composition in NAS is, in general, mixed neritic-pelagic (Isari *et al.*, 2006), with distinct species assemblages appearing in areas with BSW and LW (Zervoudaki *et al.,* 2006; Siokou-Frangou *et al.*, 2009). Copepoda is the dominant group of zooplankton communities, except for some coastal areas and surface layers (0-50 m); in the warm front of the NAS. In the Anticyclone of Samothrace, during the warm periods, neritic Cladocera and small-sized Copepoda prevail (Zervoudaki *et al.*, 2006). BSW seems to create ideal conditions for the filtrate-eating organisms, such as *Penilia avirostris,* which probably exploit directly microbial biomass as they feed with small organic items (Isari *et al.*, 2006; Siokou *et al.*, 2014). Notably, copepods, decapods and cladocerans are the main food source of small pelagic fishes (Karachle & Stergiou, 2013). Mixed Trophic Impact (MTI) analysis, which provides quantification of direct (predation) and indirect (competition) feeding interactions between compartments in an ecosystem (Ulanowicz & Puccia, 1990), revealed that mesozooplankton holds an important ecological role in the NAS as well as in other Mediterranean Sea parts, e.g., the Adriatic Sea because it constitutes the main prey of many organisms (Tsagarakis *et al.*, 2010). Although mesozooplankton community structure is an important element in defining the Good Environmental Status within MSFD, a need for more systematic biomonitoring of zooplankton of NAS has been raised. Filling this gap could provide a valuable asset, adding to the existing knowledge that could be used to refine and expand the monitoring of the ecosystem that has already been developed for the area. Accordingly, various zooplankton indicators, focusing on zooplankton size, abundance, community structure, and distribution, need to be developed to cover the hydrological complexity of the area. In conclusion, most studies on zooplankton in the NAS region are focused on mesozooplankton recordings, especially on copepods. Nevertheless, other taxa, such as chaetognaths, amphipods, euphausiids, and particularly Decapoda play an important role in the trophic web of the area (Karachle & Stergiou, 2013), with a significant contribution to the overall zooplankton biomass. Although up to date, these taxa, as well as the important trophic link between classical and microbial trophic web of small metazooplankters (e.g. nauplii and small copepod species), have been overlooked in the relevant literature, regarding detailed and informative classification studies, there is an arisen necessity to fill this important gap (Frangoulis *et al.*, 2017).

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TEN YEARS OF MARINE TURTLES STRANDING EVENTS IN THE GREEK SEAS

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Abstract

In the present study, a decade of data on stranding events of the two most common species of marine turtles in the Mediterranean, *Carreta carreta* (Linnaeus, 1758) and *Chelonia mydas* (Linnaeus, 1758), were analyzed. Analysis involved categorization of events depending on the cause of stranding, seasonality, analysis of spatial distribution, as well as an assessment of the possible correlation with environmental/climatic patterns. Most stranding events due to "anthropogenic impact" involved predominantly injured animals, either by ship collision or intentional wounds, and secondary by fishing gear entanglement. Both species indicated significantly higher number of strandings in the period of "mild weather" (from April to September), a period with amplified ship traffic in the coastal zone and increased fishing effort of the small-scale fishery. Time series analysis was also applied in order to assess the relationship of "Unknown" cause of stranding with environmental/climatic patterns. Results underlined the importance of temperature for both species, large scale climate pattern during summer for *C. carreta* and productivity index for *C. mydas*. Finally, spatial analysis underlined certain high-density areas in the Greek Seas in terms of stranding events.

Keywords: marine turtles, loggerhead turtle, green turtle, stranding events, climate change patterns, Greek waters.

1. Introduction

Loggerhead turtle *Carreta carreta* (Linnaeus, 1758) is the most common marine turtle species in the Mediterranean Sea; it nests mainly in the eastern part, from where juveniles disperse widely throughout the basin (e.g., Casale & Margaritoulis, 2010). Regarding green turtle *Chelonia mydas* (Linnaeus, 1758), foraging habitats have been identified in the Aegean and Ionian Seas (Casale *et al.*, 2018 & references therein) but our knowledge on species distribution and movements in the Mediterranean is very limited in comparison to that of *C. carreta*. Both species do not come ashore normally, except the adult females for nesting, unless they are dead, seriously wounded or ill, as a consequence of natural or anthropogenic factors. Stranding events data i.e., the occurrence of a sea turtle (dead, ill or healthy) immobilized on the beach, can provide auxiliary information on marine turtle spatial distribution (e.g., Casale *et al.*, 2010; Türkozan *et al.*, 2013), improve our understanding of the causes of mortality especially those related to human activities (i.e., vessel strikes or fishery interactions), may affect their population and eventually support public awareness, policy, and management decisions. Here, we analyzed a decade of stranding data, aiming to retrieve information on the seasonality of events, the cause of death, identify hot-spots of interaction between species and anthropogenic activities like small-scale fisheries and finally assess possible correlation between environmental/climatic patterns and stranding events.

2. Material and Methods

Stranding data of marine turtles derived from port authorities reports addressed to the National Network for Monitoring Stranding Events during the period 2010 to 2020, and were stored at IMBRIW-HCMR database. Data documented over 6000 stranding events of *C. carreta* and *C. mydas*. Analyses of stranding events included only those cases where identification of marine turtle species was confirmed (photographs and/or trained observers were used to confirm species identification). All stranding events were georeferenced based on toponym information and mapped using ArcGIS (ESRI, 2015). Stranding events were categorized initially into “Dead” or “Alive” and then based on the cause of stranding into: “Unknown” (in case of no information about the cause of stranding), “Human induced” (in case the stranding event was related directly or indirectly to human activity). “Human induced” cause of stranding was further categorized as into “Marine litter ingestion”, “Oil pollution”, “Fishing gear entanglement”, and “Injured” (representing injuries caused by other pressures, such as ship collision or intentional wounds). Seasonal stratification was applied separating the year into “rough weather” period (i.e., October to March, when low temperatures, heavy storms, and rough sea conditions are more likely to occur) and “mild weather” period (i.e., April to September). Analysis of variance (ANOVA) was applied to detect annual differences in the number of stranding events between “rough weather” and “mild weather” periods per species and cause of stranding. Furthermore, Minimum/maximum Auto-correlation Factor Analysis (MAFA, Solow 1994; Zuur *et al.*, 2007) was applied to identify any correlation between stranding events of “Unknown” cause (dependent variable) and temporal environmental/climatic patterns (independent variables) by species. Specifically, the time series of (i) sea surface temperature (SST, www.oceancolor.gfc.nasa.gov): mean annual SST, 3-month running average values at Mediterranean basin and Greek seas, (ii) North Atlantic Oscillation (NAO, https://www.ncdc.noaa.gov/teleconnections/nao/): annual bimonthly values iii) Mediterranean Oscillation Index (MOI) (https://crudata.uea.ac.uk/cru/data/moi/), iv) sea surface chlorophyll (Chla, www.oceancolor.gfc.nasa.gov): annual values and annual seasonal values over the continental shelf of Greek Seas. Finally, regarding the spatial analysis, the Kernel Density analysis tool in ArcGIS (ESRI, 2015) was applied to identify hotspot stranding areas per species in Greek waters.

3. Results

The recorded stranding events were 5858 for *C. carreta* and 472 for *C. mydas*. The evolution of the stranding records showed a significant temporal trend with an increasing number of strandings at the latest years for *C. caretta*, whereas, for *C. mydas,* no significant temporal trend was observed. Most of *C. caretta* records (73%) included stranded animals due to “Unknown” cause and 23% were assigned as “Human induced”. The majority of the “Human induced” stranding events were related to “Injuries” (70.2%), followed by “Fishing gear entanglement” (19%). In the case of *C. mydas*, most records involved stranded animals due to “Unknown” cause (63%) and 32% of “Human induced” cause. The latter included “Injuries” (81.9%), followed by “Fishing gear entanglement” (16.8%). Regarding seasonal differences, total strandings of *C. caretta* were significantly higher when the weather was mild (F-ratio=98.39, p-value<0.01). Similarly, stranding events were higher during “mild weather” in the case of “Unknown” and “Human induced” cause of stranding (F-ratio=71.54, p-value<0.01; F-ratio=23.69, p-value<0.01, respectively). In the case of *C. mydas*, total strandings were significantly higher during “mild weather” period (F-ratio=19.66, p-value<0.01). Likewise, stranding events were higher during “mild weather” period in the case of “Unknown” (F-ratio=9.69, p-value<0.01) and “Human induced” cause of stranding (F-ratio=31.84, p-value<0.01).

MAFA results revealed differences in response to environmental/climatic patterns per species. MAFA1 axis was found significant for *C. caretta* (r=0.604, p-value=0.005) revealing a positive correlation with SST patterns at Mediterranean scale (i.e., mean annual SST, average SST from December to February & the average SST from January to March) denoting higher number of stranding events upon higher temperatures in winter period. Similarly, a positive correlation was found with the average MOI index for the “mild weather” period, the bimonthly NAO index from May to June, and the 3-month moving average ENSO index from April to June and May to July, denoting higher strandings when strong positive climatic patterns occur in the spring-summer period. Likewise, MAFA1 axis was found significant for *C. mydas* (r=0.07, p-value=0.289) revealing a positive correlation with SST patterns at Mediterranean scale (i.e., mean annual SST, 3-month moving average SST from September to November & October to December) denoting higher stranding events upon higher temperatures, especially during the autumn period. Contrary, a negative correlation was observed between the common trend of MAFA1 and Chla patterns (i.e., mean annual Chla & mean annual Chla in the "rough weather" period over the Greek continental shelf) denoting higher stranding events at lower productivity years over the Greek potential feeding grounds, especially during the winter period.

Spatial analysis underlined certain areas presenting high density of *C. caretta* and *C. mydas* stranding events, such as Amvrakikos Gulf and Zakynthos Island in Ionian Sea, Saronikos Gulf, Thermaikos Gulf, and the coastal part of Thracian Sea in Aegean Sea (Fig. 1). Analysis of "Human induced" and "Fisheries related" strandings of *C. carreta* pointed out high density of stranding events at the north-east part of Thracian Sea, Saronikos Gulf, Zakynthos Island, Patraikos Gulf, Amvrakikos Gulf and Paros-Naxos islands (Fig. 1). For *C. mydas*, high density areas for stranding characterized as "Human induced" and "Fisheries related" also pointed Patraikos Gulf, Saronikos Gulf, South Evoikos Gulf, Thermaikos Gulf and the north-east part of Thracian Sea (Fig. 1).

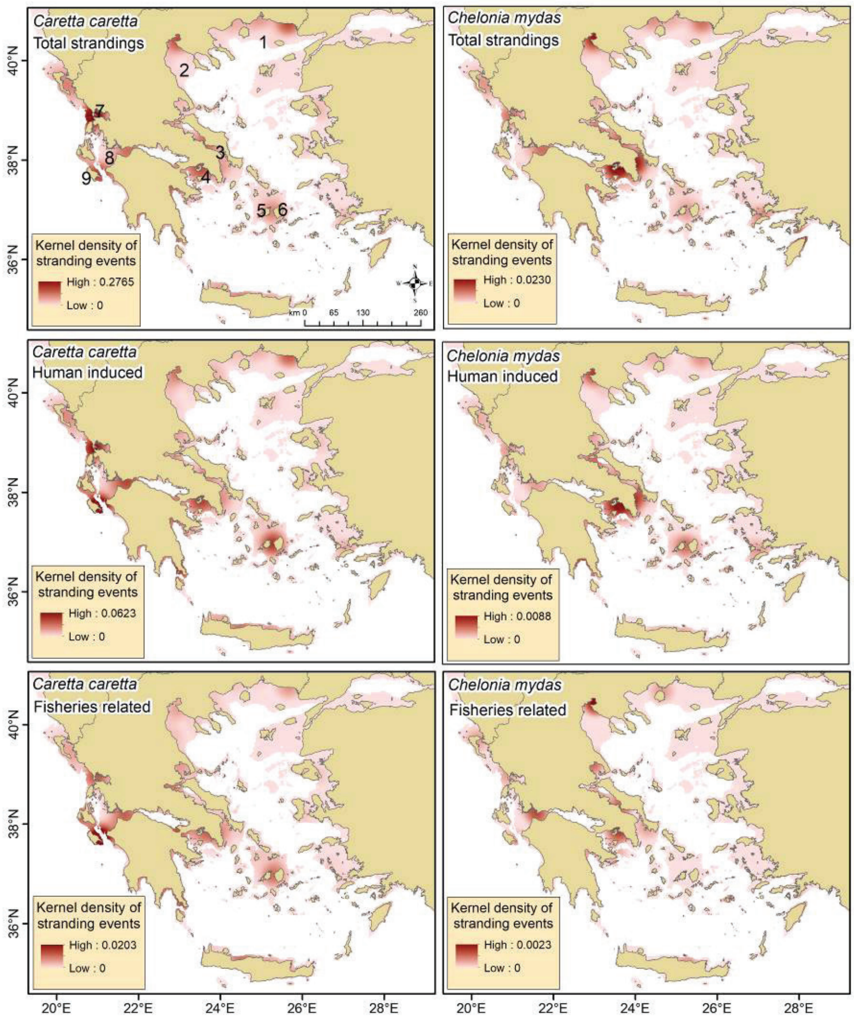

Fig. 1: Kernel density of total, human induced and fisheries related strandings of Carreta carreta and Chelonia mydas for the period 2010-2020. 1 Thracian Sea; 2 Thermaikos Gulf; 3 South Evoikos Gulf; 4 Saronikos Gulf; 5 Paros Island; 6 Naxos Island; 7 Amvrakikos Gulf; 8 Patraikos Gulf; 9 Zakynthos Island

4. Discussion

The main threats to the Mediterranean subpopulations of *C. carreta* and *C. mydas* include fishery bycatch, foraging and nesting habitat degradation due to coastal development (Casale & Margaritoulis 2010). Stranding events analysis largely reflected that "anthropogenic impact" holds a significant part of the identified incidents as it represented 23% and 32% for *C. carreta* and *C. mydas*, respectively. However, these percentages can be underestimates of the actual situation as additional info from necropsies is often lacking and it may alter our perception. Necropsies are often not possible due to the large number of events and the encountered difficulties accessing the stranded animal. The majority of "anthropogenic impact" involved predominantly injured animals (70 to 80%) either by ship collision or intentional wounds and secondary (around 20%) fishing gear entanglement. Both species also indicated significantly higher number of incidents in the period of the "mild weather" from April to September. This period corresponds to the nesting period of *C. carreta*, the amplified ship traffic in the coastal zone due to tourism activities, and the increased fishing effort period of the small-scale fishery.

For the first time, we attempted to assess the "Unknown" fraction of the stranding events with environmental/climatic patterns over the 11 years study period. Sea turtles, including *C. carreta*, are susceptible to climate and ecosystem changes, particularly those associated with temperature. Our results highlighted the importance of temperature in both species, denoting an increase in stranding events with higher temperatures during the foraging winter period and the autumn period for *C. carreta* and *C. mydas*, respectively. SST is a variable known to be well associated with *C. carreta* suitable habitat (Patel *et al.*, 2021). The increase in stranding events upon higher temperatures might be related to marine disease dynamics (Tracy *et al.*, 2019) and the subsequent animals' vulnerability to infections. Warming temperatures can also affect the composition of prey communities or deterioration of foraging habitats like seagrass meadows (Poloczanska *et al.*, 2016). As marine turtles perform energy consuming migrations over thousands of kilometers, the reduced accessibility to suitable foraging habitat can lead to poor body condition, thus more vulnerable animals and higher possibility of a stranding event. The latter may be the explanation behind the negative correlation found for the herbivore *C. mydas* and the productivity index, denoting increased stranding events in years of low productivity over the neritic Greek waters, where overwintering foraging grounds are known to occur (Casale & Margaritoulis, 2010).

Despite the short time series available, a significant relationship with large scale climatic patterns was identified for *C. carreta*, denoting higher number of stranding events with the positive phase of northern hemisphere large scale climatic patterns during late spring-summer. Such climatic patterns were expressed by the MOI index and the bimonthly NAO index, with higher summer NAO phases being related to colder temperatures and cloudiness over the Greek peninsula and the surrounding waters (NAO: Chronis *et al.*, 2011; MOI: Criado-Aldeanueva & Soto-Navarro, 2013). In the same direction, Báez *et al.* (2011) found increases in stranding events of Mediterranean juvenile and adult *C. carreta* in Spanish waters associated with regional decreases in SST, resulting from an increasing frequency of NAO positive phases.

Finally, important stranding areas, as highlighted by the density maps, largely coincide with important foraging grounds for *C. carreta* such as Amvrakikos Gulf, Patraikos Gulf, the north-east part of Thracian Sea, Saronikos Gulf, Thermaikos Gulf, and certain breeding areas such as Zakynthos Island. Similarly, for *C. mydas* stranding events largely coincide with known foraging grounds such as Patraikos Gulf, Saronikos and South Evoikos Gulfs, Thermaikos Gulf and the north-east part of Thracian Sea.

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STRANDING EVENTS OF SMALL CETACEANS IN THE GREEK SEAS: HOW MANY, WHERE AND WHY

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Abstract

An 11-year dataset on stranding events of four small cetaceans in Greek waters is analyzed. Species include *Tursiops truncatus* (Montagu, 1821), *Delphinus delphis* (Linnaeus, 1758), *Stenella coeruleoalba* (Meyen, 1833), and *Phocoena phocoena* (Linnaeus, 1758). Analyses involved data categorization based on the likely cause of stranding event, seasonality, as well as identification of hotspot stranding areas. Time series analysis was applied to assess the relationship among stranding events and environmental/climatic patterns. The majority of stranding events were due to "Unknown cause", followed by stranding events related to injured animals and to a lesser degree due to entanglements by fishing gear. Time series analysis results pointed out the importance of temperature for both *S. coerueloalba* and *P. phocoena,* although the two species presented opposite temperature effects. Moreover, a higher number of *T. truncatus* stranding events was observed upon higher chlorophyll concentrations over Greek *waters.* Finally, spatial analysis highlighted certain high-density areas of stranding events in the Greek Seas.

Keywords: small cetacean, stranding events, MAFA, spatial distribution, climatic patterns, Greek waters.

1. Introduction

Stranding event is the occurrence of a cetacean (dead, ill or healthy) immobilized on the beach. Information on stranding events is often ignored, although it can provide a unique opportunity to learn more about the population dynamics, improve our understanding of the causes of morbidity and mortality in cetaceans (ACCOBAMS 2021). Such datasets can be one of the primary sources of evidence of how human activities, i.e., vessel strikes or fishery interactions, may affect marine mammals. Spatial analysis of marine mammal stranding events can be a useful tool for identifying existing patterns that may support policy and management decisions. Moreover, it can identify correlations between stranding patterns and human influences such as fishing effort (Crosti *et al.*, 2017) or even climate change effects. Here, we analyze a 11-year period of stranding data based on port authorities reports, focusing on four common species of small odontoceti in the Greek Seas, i.e., bottlenose dolphin *Tursiops truncatus* (Montagu, 1821), common dolphin *Delphinus delphis* (Linnaeus, 1758), striped dolphin *Stenella coeruleoalba* (Meyen, 1833), and harbor porpoise *Phocoena phocoena* (Linnaeus, 1758). Analyses are focused on the seasonality of events, the categorization of the cause of stranding, the identification of hotspot stranding areas and finally, the identification of possible correlation between environmental/climatic patterns and stranding events.

2. Material and Methods

Stranding data on cetacean derived from port authorities reports addressed to the National Network for Monitoring Stranding Events during the period 2010-2020 and stored at a dedicated database including over 900 stranding events of clearly identified and/or reported as *T. truncatus*, *D. delphis*, *S.*

coeruleoalba and *P. phocoena* stranding. Analyses of stranding events included only those cases where identification of the aforementioned four species was confirmed (photographs and/or trained observers were used to confirm species identification). All stranding records were georeferenced based on toponym information. An ArcGIS (ESRI, 2015) point shapefile was produced. Stranding events were categorized initially into "Dead" or "Alive" and then based on the cause of stranding into: "Unknown" (no information about the cause of stranding) and "Wounded animals" (one or more wounds are apparent). "Wounded animals" were further categorized as "Fishing gear entanglement" or "Injuries" based on official reports and/or photo identification. Seasonal stratification was applied separating into "rough weather" period (i.e., October to March, when low temperatures and rough sea conditions are more likely to occur) and "mild weather" period (i.e., April to September). Analysis of variance (ANOVA) was applied to identify annual differences in the number of stranding events between "rough weather" and "mild weather" periods per species and cause of stranding. Furthermore, Minimum/maximum Auto-correlation Factor Analysis (MAFA, Solow, 1994) was applied to identify any correlation between stranding events of "Unknown" cause (dependent variable) and temporal environmental/climatic patterns (independent variables) by species. Specifically, the time series of (i) sea surface temperature (SST, www.oceancolor.gfc.nasa.gov) as 3-month running mean at Mediterranean basin level and at Greek seas level, (ii) North Atlantic Oscillation (NAO, https://www.ncdc.noaa.gov/teleconnections/nao/): bimonthly mean values, and (iii) sea surface chlorophyll (Chla, www.oceancolor.gfc.nasa.gov): annual values and seasonal values over the continental shelf of the Greek Seas. Finally, regarding the spatial analysis, the Kernel Density analysis tool in ArcGIS was applied to identify hotspot stranding areas per species in Greek waters.

3. Results

The recorded stranding events were 419 for *T. truncatus*, 70 for *D. delphis*, 400 for *S. coeruleoalba*, and 12 for *P. phocoena*. Most of the records were assigned as "Unknown" cause (~67% to 78% depending on the species), whereas 20-30% were assigned as "Wounded animals". The majority of the "Wounded animals" were related to "Injuries" (i.e., 57% to 76%), followed by "Fishing gear entanglement" (19% to 39%). The latter involved "Nets" (i.e., 16 records of *T. truncatus*, 3 records of *D. delphis*, 7 records of *S. coeruleoalba*, 1 record of *P. phocoena*) and to a lesser degree "Longlines" (1 record of *T. truncatus*, 4 records of *S. coeruleoalba*). It should be noted that any estimates based on stranding reports are likely to be underestimates of the actual entanglement rate as it is solely necropsy that can provide accurate information. However, this is often not feasible upon a stranding event. Regarding "Injuries", this is a general category as injuries can be assigned to anthropogenic effects or natural causes like predation. However, this discrimination is also not feasible in most of the cases. No significant differences in the "total strandings" were observed between "mild" and "rough" weather period, besides *D. delphis,* which showed significantly higher stranding events in the "rough weather" (F-ratio=8.58, p-value=0.0083).

MAFA results showed that no overall trend was found significant for any species in MAFA1 axis, however different responses to environmental/climatic patterns were revealed depending on the species. Specifically, results showed that *T. truncatus* trend in MAFA1 axis presented a statistically significant positive correlation with the annual and seasonal values of Chla in the Greek Seas, denoting higher number of *T. truncatus* stranding events upon higher productivity in the Greek waters. Similarly, for *S. coeruleoalba* a positive correlation in MAFA1 axis was found significant with the annual average NAO index and the "mild weather" NAO index as well as the SST in the Greek waters from May to July, and the 3-month running mean of SST at Mediterranean basin from March to July. This means higher number of *S. coeruleoalba* stranding events with higher temperatures in the March to July period. For *P. phocoena,* a negative correlation in MAFA1 axis was found significant with the average annual SST over the Greek Seas and especially the 3-month running mean of SST from May to August underlining higher stranding events upon lower SST in Greek waters. No trend or correlation with environmental variable was found significant for *D. delphis* trend in MAFA1 axis.

Density maps (Fig. 1) showed that certain areas presented higher number of stranding events. For *T. truncatus*, areas with high number of total strandings were the coastal area of Thracian Sea, Thermaikos

Gulf, Lesvos Island, and Amvrakikos Gulf. Hotspots of "Wounded animals" were also identified in Thermaikos Gulf and Lesvos Island, as well as Kerkyraikos and Amvrakikos Gulfs in Ionian Sea. For *D. delphis*, areas with high number of total and "Wounded animals" were Limnos Island, the coastal part of Thracian Sea and Pagasitikos Gulf. For *S. coeruleoalba*, areas with high number of both total and "Wounded animals" were the wider area of Thracian Sea, Strymonikos Gulf, Cyclades and Sporades islands, and Corinthiakos Gulf. Finally, for *P. phocoena* results highlighted exclusively the importance of the coastal part of Thracian Sea.

4. Discussion

Marine mammals have been found ashore over the years, for reasons that are not always well understood. Current work involves the analysis of eleven years of stranding events of four of the most common small cetacean species in the Greek Seas. Most of the stranding events were assigned as "Unknown" and secondary as "Wounded animals" i.e., "Injuries" and "Fishing gear entanglement".

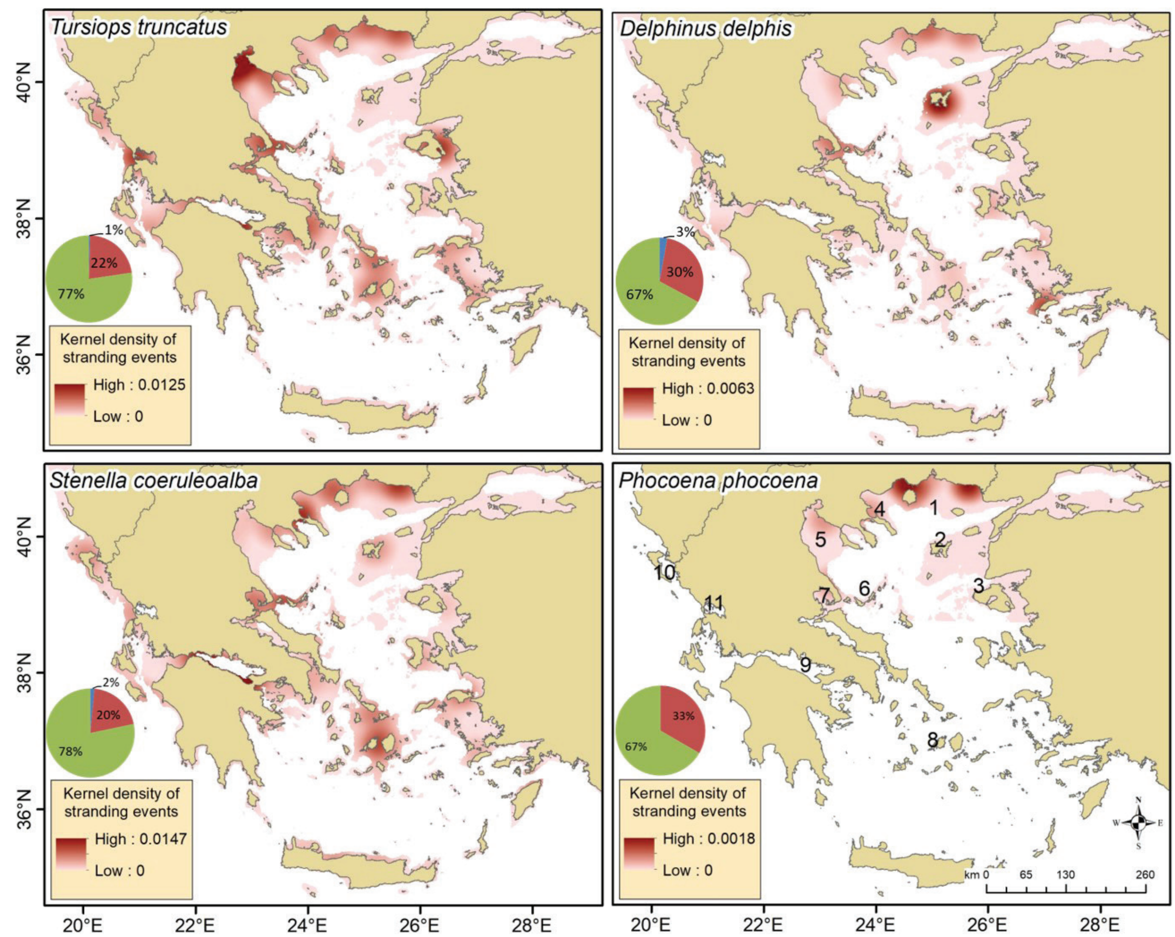

Fig. 1: Kernel density of total stranding events of small cetaceans for the period 2010-2020 in Greek Seas. 1 Thracian Sea; 2 Limnos Island; 3 Lesvos Island; 4 Strymonikos Gulf; 5 Thermaikos Gulf; 6 Sporades islands; 7 Pagasitikos Gulf; 8 Cyclades islands; 9 Corinthiakos Gulf; 10 Kerkyraikos Gulf; 11 Amvrakikos Gulf. Colors in the pie charts represent the different causes of stranding (blue: "Disease"; red: "Wounded animals", green: "Unknown").

"Injuries" to a large extent can be due to natural causes, like predation from scavengers or be the result of deliberate actions by fishermen, as a result of depredation, gear damage and catch loss (Jog *et al.*, 2022). *T. truncatus* entangled strandings were higher compared to the other three species, and mostly associated with "Nets" as a result of gillnet depredation (Bearzi *et al.*, 2019). The latter is underlined by the coastal character of this species population in Greek waters. *S. coeruleoalba* entangled stranding events were associated mainly with "Nets", but also with "Longlines". As *S. coeruloalba* is known of a preference for open waters beyond the continental shelf (Aguilar, 2000), entanglement events could be accidental (Frantzis & Alexiadou, 2003).

Special interest presents the attempt to assess the “Unknown” fraction of the stranding events with environmental/climatic patterns. The coastal nature of *P. phocoena* and *T. truncatus* populations was underlined by the time series analysis results. *T. truncatus* stranding events were associated with the productivity of the Greek waters, indicating higher stranding events upon higher productivity values. The latter could be related to higher probability of harmful algal blooms events, known to have a direct negative impact on *T. truncatus*, frequently causing illness and death (Fire *et al.,* 2020). Alternatively, high productivity areas may be preferred by the bottlenose dolphins due to prey availability, and high number of stranding may be the result of increased abundance of the species in those areas.

The presence of *P. phocoena* in the northern Aegean Sea was first reported almost three decades ago by a sighting in 1993 and a live stranding in 1997 (Frantzis *et al.*, 2001). Recently, it was confirmed by a dedicated survey (Cucknel *et al.,* 2016). The negative correlation of the species strandings with SST in Greek waters, implying higher stranding events upon lower SST, could be related to seasonal changes in prey abundance in the area deteriorating animals’ health condition, making them more susceptible to ectoparasitic infestation (Cucknel *et al.,* 2016). SST appears as a crucial factor affecting also *S. coeruleoalba* stranding events. Contrary to *P. phocoena*, the offshore species *S. coeruleoalba* showed association with SST estimates at Mediterranean scale, presenting higher stranding events upon higher temperatures from March to July. Higher temperatures are known to foster the development of pathogens (Albouy *et al.*, 2020) like the morbili virus increasing animals’ vulnerability to infections. Warming temperatures can also affect the composition of prey communities and subsequently species diet. Moreover, despite the short time series available, a significant relationship with large scale climatic patterns was identified for *S. coeruleoalba* denoting higher number of stranding events with the positive phase of NAO index (i.e., April to September). This could also be related to fluctuations of fish populations (Katara *et al.*, 2011) and possibly reduced food availability during late spring-summer, the energy-demanding reproduction period of the species.

Finally, hotspot stranding areas were identified within the gulfs, the coastal waters of Thracian Sea and over the Cyclades plateau. Despite the difficulties of interpreting stranding data as stranded carcasses can be found on the beach as a result of many processes (e.g., buoyancy, drift, and detection probability), the higher number of strandings within the high productivity areas might be indicative of their importance as potential feeding grounds of the species in question. This is even more pronounced, as areas of high productivity are clearly localized in the general oligotrophic pattern of eastern Mediterranean. Identifying such areas can support existing or future spatial management conservation plans.

5. Acknowledgements

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IDENTIFYING POTENTIAL AREAS FOR SEAGRASS TRANSPLANTATION IN TRANSITIONAL WATERS

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Abstract

As evidenced in multiple locations around the globe, seagrass transplantation is an effective practice for lagoonal habitat restoration, and the practice is capable of improving the ecological status of transitional waters. The main aim of LIFE TRANSFER (LIFE19 NAT/IT/000264) project is to improve the conservation status of habitat 1150 through seagrass transplantation in Natura 2000 sites in Greece, Italy and Spain and further enforce restoration activities in several sites in the Mediterranean. Results from a four-year monitoring program of the Venice lagoon restoration initiative (LIFE SERESTO) indicated that certain conditions are more likely to encourage seagrass sod survival, plant rooting, and growth. Using these results, and data from the monitoring program of the Greek Water Framework Directive network, we attempt to identify suitable conditions and potential locations in Greece for seagrass restoration (*Zostera noltei*, *Cymodocea nodosa*, and *Ruppia cirrhosa*). Five parameters (reactive phosphorus of the water column, dissolved inorganic nitrogen, water transparency, pH of the water column and salinity) were used to prioritise suitable locations for successful transplants. Subsequently, species-specific parameters were assessed for each of the three target seagrass species. In total six candidate lagoons were identified as having similar conditions to the successful transplant sites in Venice, and of those, three lagoons (Korission, Messolonghi, and Lefkada) appear optimal. The results are expected to assist with the effective resource allocation for the management and ecological conservation of Greek transitional water bodies and as an aide for the fledgling practice of seagrass transplantation in Greece.

Keywords: Habitat restoration; Seagrass; Transitional waters, Greek lagoons, Ecological status.

1. Introduction

Habitat restoration techniques such as seagrass species transplantation in lagoons have been successful in multiple locations around the globe [e.g., Australia: Paling *et al.,* (2001); New Zealand: Matheson *et al.,* (2017); the Netherlands: Giesen and van Katwijk, (2011) and most recently in Italy: Sfriso *et al.,* (2019)]. The transplantation of seagrass sods from healthy flourishing seagrass meadows to sites that have experienced a relative decline over the last decades (due to various human-induced pressures) is an essential tool for the goal of protection and conservation of the health of lagoonal ecosystems. Aside from the Logarou lagoon in the Amvrakikos Gulf (lifetransfer.eu), such restoration techniques have not yet been implemented in Greece, a country whereby, in parallel to most European Member states, the conservation status of its transitional water bodies (WBs) has been evaluated as inadequate. According to the most recent river management plans published for the Water Framework Directive (WFD; 2000/60/EC), only 5% of Greek transitional WBs meet the threshold for a *Good* ecological status (GES), with 42.5% failing to meet GES in a *Moderate*, *Poor* or *Bad* status (the ecological status of 52.5% of the Greek transitional water sites is currently unknown) (EEA, 2018).

Seagrass restoration practices aim to improve the environmental quality of such sites, whereas the expansion of seagrass meadows throughout the lagoonal ecosystem increases the ecological connectivity of key flora and fauna species; however, implementing such techniques is often time and resource consuming. Here we assess whether it is possible to identify suitable locations to support a transformative change towards large-scale coastal restoration in order to assure the recovery of biodiversity, ecosystem services and climate mitigation.

Lagoons of the Northern Adriatic share many common species with those of Greece and the rest of the Eastern Mediterranean. Seagrasses used for transplantation inside the Venice lagoon include *Zostera marina* Linnaeus, 1753, *Zostera noltei*, *Cymodocea nodosa* (Ucria) Asch. and *Ruppia cirrhosa* (Petagna) Grande, 1918, of which only *Z. marina* is absent from Greek lagoons (Christia *et al.*, 2018). Recent results from a four-year monitoring program of the seagrass transplantation efforts in the Venice lagoon noted that certain sites have a higher probability of transplantation success than others. In particular, sites with a low nutrient load and those that are well connected to the sea encouraged plant rooting and growth (Sfriso *et al.*, 2021). Based on these results and environmental data collected in Greek lagoons and transitional waters since 2013 under the guidance of the WFD, we aim to test and validate the principles and criteria to identify potential sites in order to upscale ecological restoration for three seagrass species in Greek transitional waters.

2. Material and Methods

Five environmental criteria were identified by the Venice Lagoon seagrass restoration initiative that were found to favour plant rooting and spreading of all seagrass species (Sfriso *et al.*, 2021). The parameters were related to the nutrient concentration (level of reactive phosphorus, dissolved inorganic nitrogen), water transparency, and the water renewal rate of the lagoon (by using pH and salinity as a proxy for identifying hypo-anoxic conditions). The threshold values for each parameter identified by Sfriso *et al.* (2021) (Table 1) were used to filter out theoretically unsuitable locations from the Greek WFD transitional waters monitoring network.

Table 1. Envirinmental targets for seagrass transplantation site suitability based on the Venice Lagoon restoration initiative results

	Reactive Phosph.	DIN	Transparency	pH	Salinity
Threshold values	< 0.2 μM	< 15 μM	>1.0 m	>8.15	>27 psu

The WFD database curated by the Hellenic Centre for Marine Research holds biotic and abiotic data from the 1st (2012-2015) and 2nd implementation period (2018-2023). All transitional WBs throughout Greece included in the network (n = 33) are systematically sampled in Spring and Autumn every two years. The threshold values stated in Table 1 were used to filter out possible locations. Only sampling events that measured all the above criteria were considered. Reactive phosphorus (PO_4^{3-}), pH, and salinity were all recorded in the WFD database (for methodology see Simboura *et al.*, 2014). Dissolved inorganic nitrogen (DIN) was calculated as nitrate (NO–**3**) + nitrite (NO^-_2) + ammonium (NH_4^+). Where stations were less than 1m in depth, and transparency measurements (Secchi disk) were equal to water depth were included in the list of candidate sites.

3. Results

Out of 314 sampling events covering 39 lagoons and river mouths in the WFD database, 262 sampling events were missing at least one parameter of the suggested criteria (Table 1) as they were not measured at the time, and excluded from the analysis. Of the remaining 52 sampling events (28 lagoons), only six separate locations were identified as matching the suitability criteria (Table 2). Three lagoons (Korisson in SW Corfu, the lagoon of Lefkada, the Messolonghi lagoonal complex), met the environmental targets multiple times from separate sampling years.

Table. 2: Recorded values for each suitability criterion. Season (A= Autumn, S = Spring) and Year of the specific sampling event are also included, along with the water body type category used for WFD reporting.

Lagoon Name	Year	Season	Depth (m)	Transparency (m)	pH	Salinity	Reactive Phosphorus	DIN	Lagoon type
Kitros, Pieria	2018	A	0.5	0.5	8.83	34.7	0.05	0.79	Choked
Klissova, Messolongi complex	2015	S	0.75	0.75	8.58	35.8	0.09	0.90	Restricted
Korission, Corfu	2021	S	0.6	0.6	8.5	27.37	0.08	1.14	Restricted
Korission, Corfu	2015	S	0.9	0.9	8.2	30.86	0.13	3.46	Restricted
Lefkada (Stenon)	2015	S	0.6	0.6	8.3	36.53	0.03	0.79	Restricted
Lefkada (Stenon)	2021	S	0.3	0.3	8.47	37.96	0.05	3.74	Restricted
Messolonghi	2015	S	0.75	0.75	8.85	34.68	0.07	0.94	Leaky
Messolonghi	2021	S	0.6	0.6	8.23	29.53	0.10	1.95	Leaky
Xirolimni, Rodopi	2018	A	0.3	0.3	8.66	41.43	0.03	2.34	Choked

4. Discussion/Conclusion

Except for Kitros lagoon in Pieria, Northern Greece, all the potential restoration sites have been recorded as containing small patches of seagrass species (including *Cymodocea nodosa, Zostera noltei, Ruppia cirrhosa*) during the WFD sampling campaigns and in the literature (e.g., Cabana *et al.*, 2017). However, apart from the restricted lagoon of Lefkada, most of the potential sites require supportive actions to improve their ecological status. Since 2013, the restricted lagoon of Korission (Corfu island) and the choked lagoons of Xirolimni and Kitros in North Greece have not met GES. Although seagrass restoration techniques, including sod transplantation, are not a "fix-all" solution, it is quite possible that encouraging the healthy growth of seagrass meadows in lagoons will positively increase the ecological status of a water body (Zoffoli *et al.*, 2021).

According to Sfriso *et al.*, (2019), certain seagrass species prefer different lagoon and estuary typologies regardless of the nutrient load and physico-chemical values. *Cymodocea nodosa* prefers areas that are strongly influenced by marine waters (e.g., near the lagoon mouths or high-flow canals), but it can also be present in choked areas, whereas the species *Z. noltei* and *R. cirrhosa* prefer shallow choked areas. The former grows mainly along saltmarsh edges and the latter on any shallow bottom, provided the waters are clear. To this end, *C. nodosa* would be a more appropriate target species for the Messolonghi lagoon (classified as a "leaky" lagoon and well connected to the Ionian Sea). *Zostera noltei* appears most suitable for the "restricted" lagoons of Klissova, Korission, and Lefkada, whilst for the two "chocked" lagoons (Xirolimni and Kitros), *R. cirrhosa* seems more preferential. It is important to note however, that the environmental targets obtained from Venice may not be wholly appropriate for Greece, and thorough baseline surveys and pilot projects are needed for any seagrass restoration initiatives.

Poor definition of restoration goals, as well as the criteria used to verify their achievement, are widely reported as common limitations for restoration effectiveness (Borja *et al.*, 2010; Wortley *et al.*, 2013). As presented here, the use of data to enhance modeled scenarios (aimed at assessing the ecological potential of restoration initiatives) increases the probability of large-scale restoration. Identifying restoration potential is essential for clarifying the priorities of action plans and for the assessment of their effectiveness in terms of biodiversity and ecosystem recovery, and ecosystem services. While pilot restoration actions for ecosystem restoration are currently available in the lagoonal complex of Amvrakikos Gulf, they are neither up-scaled nor incorporated in a wider management framework. The tested methods could be up-scaled to other candidate lagoons in Greece that are identified here, to improve replicability, and as a way to strengthen the conservation policy of priority habitats in Natura 2000 sites. The ecological emergency that transitional water bodies currently face throughout Europe highlights the limits of current management approaches and demands investment in innovative, sustainable, and effective ecosystem restoration actions that could trigger transformational change.

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INTEGRATING POSIDONIA DISTRIBUTION MAPS IN THE NAUTICAL CHARTS OF THE HELLENIC NAVY HYDROGRAPHIC SERVICE

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Abstract

Intending to enhance both human and environmental safety at sea, cartographic data of *Posidonia oceanica* distribution and other Habitat types (mainly sandy bottoms) of the Annex I of the Habitats Directive (92/43/EEC) initially produced in 2001 for selected Natura 2000 Network SCI sites, are for the first time integrated after critical processing and synthesis into the national nautical charts of the Hellenic Navy's Hydrographic Service (HNHS). The purpose of this work is to provide clear and easy-to-use spatial information to help boat-users avoid damaging sensitive and protected biodiversity, particularly the *Posidonia oceanica* seagrass meadows, of EU conservation priority (Habitat Type 1120*), by pursuing alternative anchorage areas.

Keywords: *Posidonia oceanica* beds, Cartography, Marine Protection, Sites of Community Importance, anchoring.

1. Introduction

In pursuit of preserving the endemic to the Mediterranean *Posidonia oceanica* (L.) Delile seagrass beds, recognized as sensitive (*sensu* the Mediterranean Fisheries Regulation, 1967/2006), and priority for conservation (*sensu* the Habitats Directive 92/43/EEC) marine Habitats, urgent limitation of direct harmful activities is required. Despite the wide acknowledgment of their ecological significance, assessments by Telesca *et al*. (2015) revealed a regression of up to 34% of *Posidonia* meadows extent in the Mediterranean these last 50 years, with coastal construction, destructive fishing gear and uncontrolled anchoring being the most acute direct pressures.

According to field data collected within the scope of assessing the state of the Greek Natura 2000 Network (2014-2015), in a total of 30 marine Sites of Community Importance (SCI) with recorded presence of *Posidonia* beds, scarring from boat anchors and chains was as frequent as 66% (HCMR unpub. data). Drakopoulou *et al*. (2019), in their analysis of available satellite images for 21 marine SCIs or Spatially Protected Areas comprising *Posidonia* beds, pinpointed to the widespread, locally and temporally intensive pressure of uncontrolled anchoring by vessels of various types and sizes. Gerakaris *et al*. (2019) used *ad hoc* biotic indices to show significant degradation of the vitality and functioning of *Posidonia* meadows even in the uninhabited islet of Gyaros, where a Marine Protected Area has been recently established.

Although various efforts have been invested by several EU countries to address the anchoring pressure on *Posidonia* beds, the problem remains pertinent and even escalates along most of the Mediterranean coastal zone, as was recently presented and discussed during the "Anchors Away Networking Event" (Athens, 21-22 November 2019) (Salomidi *et al*., 2020). Incorporating the available knowledge on *Posidonia* distribution within official Nautical Charts to help navigate boat-users away from sensitive seabeds, has therefore been put forth as a critical step towards meaningful marine conservation in the Mediterranean Sea, which ranks among the most popular nautical tourism destination in the world (Venturini *et al*., 2016).

For the case of Greece, thematic layers of *Posidonia* beds were originally produced in 2001 for several marine sites of the Natura 2000 Network (Panayotidis *et al*,. 2001). In order to incorporate these layers in a cartographic background fit for purpose for the Nautical Charts, however, meticulous spatial adjustment to the shoreline shapefile currently used in the official environmental geospatial data was required. This study presents the steps taken to achieve this goal.

2. Materials and Methods

A polygon layer of Annex I marine Habitat Types at a 1:10,000 mapping scale, originally obtained in 2001 (Panayotidis *et al*., 2001) was used for this study. It included the spatial distribution of the Habitat Types (Annex I Habitat Directive 92/43/EEC). To accommodate more seabed types not properly addressed within the Habitat Directive typology, two more soft-bottom Habitat types were used, namely: a) Soft substrate with the presence of marine vegetation other than *Posidonia* (Code 119A), and b) Soft substrate without vegetation (Code 119B). For the recognition and imprinting of Habitat types there was a combination of data from high-resolution color aerial photos, field observations, analysis of phytobenthos samples and scientific echo sounder discrimination system "RoxAnn". The original high-resolution color aerial photographs (1:5,000 scale), taken in the second half of 1999, and used for this Habitat mapping, were also used to produce the vector dataset of the coastline which in combination with field observations helped the visual analysis of Habitat Type polygons that are distinct in the 0-10 m depth zone (Panayotidis *et al*., 2002)

To include layers of the mapped *Posidonia* beds in the Nautical Charts, spatial adjustment of their vectors to the shoreline shapefile that is currently officially in use was performed. Despite the fact that the official freely available Greek coastline vector is the one produced by the Hellenic Hydrographic Service (scale 1: 90,000), this was not used for adaptation due to its smaller scale in relation to the scale of the Habitat Types (1:10,000). Granted the geospatial thematic level of the Coastal Water Bodies as defined by the Water Framework Directive (2000/60/EC) has been built on the coastline of the Hellenic cadastre (scale 1:5,000), this vector dataset was considered more suitable for spatial adaptation and integration of marine Habitat Types.

The following particularity was however observed in relation to these two-vector coastline thematic layers; the coastline of the cadastre covers the whole of mainland Greece but only the largest Greek islands. Most small islands and rocky islets are not covered by the 1:5,000 scale and even show a significant spatial deviation in relation to the coastline of the Hydrographic Service but also to the available High-Resolution Online Satellite Data (ArcGIS online: World Imagery – imagery ranges from 0.3 m to 0.03 m resolution). In this case, the spatial adaptation was carried out on the basis of the coastline of the Hydrographic Service but it was not incorporated into the polygons of the Habitats due to scale.

The Polygon Feature Class vector that was delivered to the Hellenic Navy Hydrographic Service in the form of a geodatabase to be included in the Nautical Charts, includes a total of 50 marine Natura 2000 sites featuring occurrence of extensive *Posidonia* beds. Surface areas of Habitat Types for these sites are presented in Table 1.

In terms of coding (SITECODE), the attribute table of the vector set retains the code of the Natura 2000 site as officially assigned in 2001. The connection with the updated codification and naming of the areas in Greek and English (Natura 2000 End 2019, EEA), was included in the geodatabase and pre-defined through Relationship Class of the relevant table, with the attribute table of the Habitat Types vector.

Table 1. Area in hectares (ha) per Habitat type according to data of Panayotidis *et al*., 2001.

1110	1120	1130	1140	1150	1160	1170	119A	119B	8330
15,877.83	35,681.21	142.49	368.67	6,878.94	7,299.38	4,539.16	85,510.97	9,854.70	3.84

3. Results

The geospatial data which derived from the processing of the existing Natura 2000 cartographic data for 50 Natura marine sites (Panayotidis *et al*., 2001) were incorporated accordingly in the national Nautical Charts of the Hellenic Navy's Hydrographic Service with a scale of 1:50,000. These data concern the adaptation to the coastline of the Greek cadastre of scale 1:5,000 with emphasis on the polygons of Habitat Type 1120*Posidonia* beds, along with the geospatial total of the boundaries of these areas.

This map series, its first item (code 323/3, HNHS) shown in Figure 1, includes the names and boundaries of the SPA (Marine Protected Area in Natura 2000 Network under the Birds Directive (2009/147/EC) and/or SCI (Marine Protected Area in Natura 2000 Network under Habitat Directive 92/43/EC) sites, indicated as “Marine Environmentally Sensitive Areas” under Notes 1 and 2 respectively for each site with green color. Note 3 refers to *Posidonia* meadows, indicated as “*Posidonia* seagrass” (Habitat Type 1120*), with clear and easy-to-use information to sailors along with a prompt to avoid anchoring on or near these areas to protect these sensitive marine Habitats (Fig. 1).

4. Discussion/Conclusion

Although this initiative was originally conceived as an opportunity to help expand our awareness of and actively avoid harm to valuable *Posidonia* beds and other sensitive marine biodiversity, it further enhances safety at sea since *Posidonia* beds are not considered strong anchor holding grounds as compared to the much more suitable sandy and silty bottoms. At the same time, compiling, updating, adapting and making better available any existing Habitat Type maps further serves multiple other environmental purposes, such as those put forward by the Marine Strategy Framework (2008/56/EU) and the Maritime Spatial Planning (2014/89/EU) Directives. In addition, it partially meets the requirements of the Mediterranean Fisheries Regulation (1967/2006), provided that they are taken into account in relevant fisheries planning and conservation processes.

This map series, its first item shown in Figure 1, includes the names and boundaries of the SPA and/or SCI sites, indicated as “Marine Environmentally Sensitive Areas” under Notes 1 and 2 respectively; Note 3 refers to *Posidonia* meadows, indicated as “*Posidonia* seagrass”, with a prompt to avoid anchoring on or near them.

This map series greatly contributes to the EU nature restoration targets of the EU Biodiversity Strategy for 2030. To achieve these environmental and social benefits however, a legislative ban on anchoring over sensitive seabeds within at least the Greek Natura 2000 Network is required, following examples offset by other pioneering Mediterranean countries, such as France and Spain (Bardolet, 2019; Villers, 2019). Meanwhile, broad and effective communication of this series to all relevant local or national stakeholders (port and local authorities, MPA managers, boating associations/companies, concerned citizens, etc.) can significantly augment the series use and support on a voluntary basis.

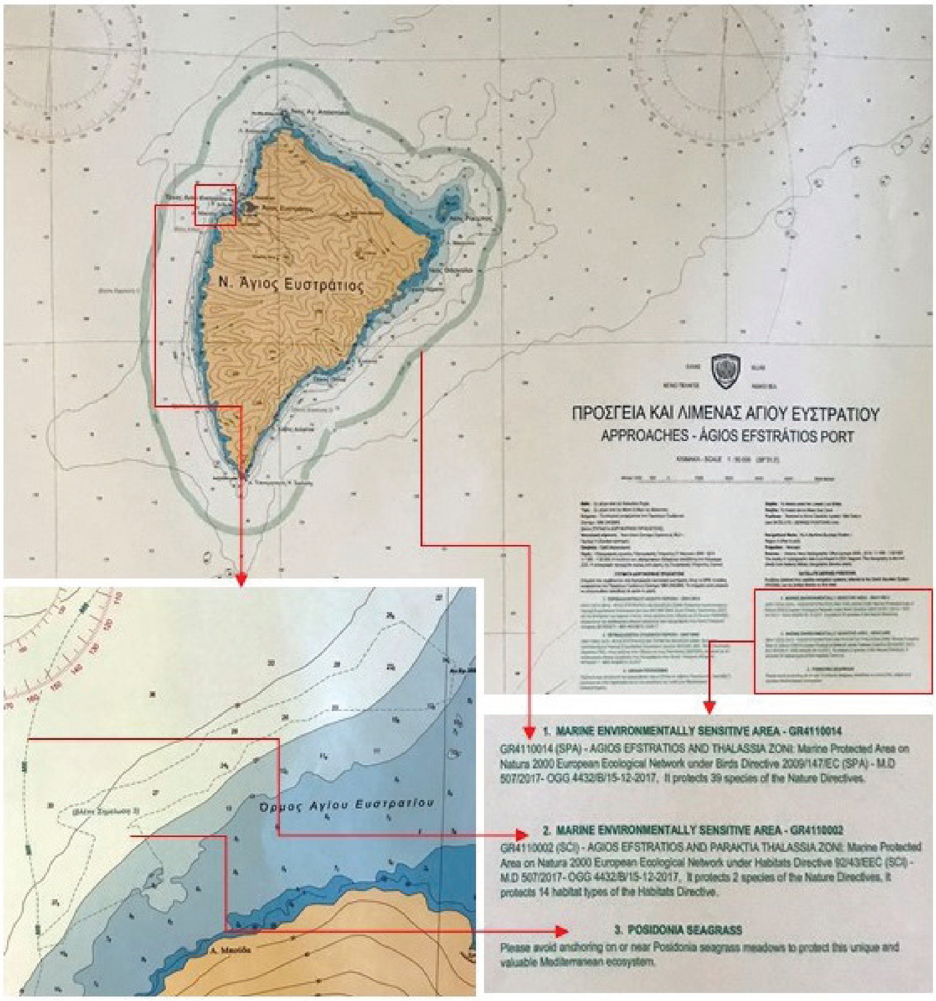

Fig. 1: Nautical chart of the Hellenic Navy Hydrographic Service including Marine Environmentally Sensitive Areas and *Posidonia* seagrass beds.

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AGE AND GROWTH OF *BOOPS BOOPS* (L., 1758), IN THE IONIAN AND SOUTH AEGEAN SEAS

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Abstract

Boops boops is a species of the family Sparidae for which information on its age and growth in the Ionian and the South Aegean Seas is generally lacking. This work aims to gather information on the above-mentioned topics. Five (0 to 4) and seven age groups (0 to 6) were defined in the Ionian Sea and the South Aegean Sea, respectively. Von Bertalanffy parameters did not reveal significant differences between the two study areas.

Keywords: Bogue, otoliths, age-length key, Von Bertalanffy parameters, Mediterranean.

1. Introduction

The bogue, *Boops boops* (Linnaeus, 1758), is a demersal and semi pelagic species of the family Sparidae (Cannizzaro *et al*., 2001). Its distribution extends in the Eastern Atlantic and the Mediterranean and Black Seas (Bauchot & Hureau, 1986) at depths down to 350 m. It is of low commercial value even though it is one of the top 13 most landed demersal fish species in the Mediterranean Sea and the most landed of the family Sparidae (Lleonart & Maynou, 2003).

The age and growth of *B. boops* has been studied broadly in the Eastern Atlantic (Gordo, 1996; Monteiro *et al.,* 2006; Abecasis *et al*., 2008;) and the Central, Western and South Mediterranean Sea (Hernandez, 1989; Cannizzaro *et al*., 2001; Allam, 2003; El-Haweet *et al*., 2005; Khemiri *et al*., 2005; El-Okda, 2008; Ramdane *et al*., 2013; Mehanna *et al*., 2014; Kherraz *et al*., 2016; Azab *et al*., 2019) using otoliths and scales. More scarce is the information on the age and growth of the bogue in the Eastern Mediterranean (Saronikos Gulf: Tsangridis & Filippousis, 1991; Cretan Sea: Kallianiotis, 1992; Eastern Ionian: Isaias & Sinis, 2006; Eastern Aegean: Kara & Bayhan, 2015; Soykan *et al.,* 2015). The objective of the present work was to study the age and growth of *B. boops* in the Ionian and the South Aegean Seas (Greek waters) and compare the growth pattern of the species between the two study areas. This information is necessary in stock assessment studies and in fisheries management.

2. Materials and Methods

The samples were collected in the framework of the National Fisheries Data Collection program from 2020 in the Ionian and South Aegean Seas. Overall, 555 individuals of *B. boops* were examined; 405 in the Ionian Sea (total length *TL*: 91 - 243 mm) and 150 in the South Aegean Sea (total length *TL*: 66 - 297 mm).

For each specimen, the left and the right sagitta otolith were removed from the cranial cavity, cleaned, and photographed with the Image-Pro Plus software (Version 4.5.0.29). Age estimation was based on counting the annual growth rings macroscopically along the left sagittal otolith axis, from the core to the post-rostrum edge (Fig. 1), and the 1st of January was considered as the date of birth for all the individuals according to the spawning period (mainly winter and early spring months) of the species in the Greek waters and Central Mediterranean Sea (Kallianiotis, 1992; Bottari *et al*., 2014). Otoliths were read twice by two readers to reduce subjectivity. When readings differed, a re-reading was made from the two readers to obtain a common decision.

The age-length key was constructed per area, and growth parameters (L_∞, k and t_0) were calculated using the Von Bertalanffy (1957) model and the growth performance index ($\Phi' = \log_{10}k + 2\log_{10}L_\infty$). The growth parameters were compared between the two areas through two sample t-test. Differences were considered at the significance level α = 0.05.

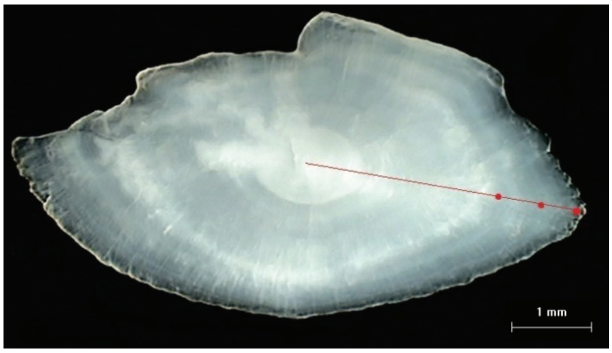

Fig. 1: Otolith of *Boops boops* with three annual growth rings (red dots); date of capture February.

3. Results

Five (0 to 4) and seven (0 to 6) age groups were defined in the Ionian and South Aegean Seas, respectively. The age length keys for both areas are presented in Table 1. The results for the growth function and *Φ'* are presented in Table 2 for both areas of this study. The Von Bertalanffy growth parameters did not differ between the two sampling areas (L_{∞}: P-value = 0.52; *k*: P-value = 0.84).

Table 1. Age-length key for *Boops boops* from the Ionian and Aegean Sea (TL: total length; N: total number of individuals); Length classes: TL, mm.

	Ionian Sea							**Aegean Sea**							
Length classes		**Age classes**													
	0	1	2	3	4	**N**		0	1	2	3	4	5	6	**N**
60-69								2							2
70-79								2							2
80-89								2							2
90-99	6					6		4							4
100-109	21					21		2							2
110-119	43					43		5							5
120-129	27	24				51		3	1						4
130-139	10	24				34			5						5
140-149		38				38			13						13
150-159		38	6			44			9						9
160-169		40	11			51			7	3					10
170-179		24	22			46			2	9					11
180-189		1	23			24				12					12
190-199			18	3		21				13					13
200-209			4	7		11				11	2				13
210-219			2	5		7				3	6				9
220-229				4		4					6				6
230-239				1	2	3					8	1			9
240-249					1	1					2	2			4
250-259											2	4			6
260-269												6			6

Length classes	Ionian Sea							Aegean Sea							
	Age classes														
	0	1	2	3	4	N		0	1	2	3	4	5	6	N
270-279												1			1
280-289													1		1
290-299														1	1
N	107	189	86	20	3	405		20	37	51	26	14	1	1	150

4. Discussion/Conclusion

Based on the results concerning the Von Bertalanffy growth parameters it seems that *B. boops* is a species of medium growth rate. Growth did not differ between the two study areas, which may be attributed to their close environmental conditions (Kontoyiannis *et al.*, 2005). Furthermore, the values of L_∞ and k as well that of Φ', estimated in this work, are included within the range of the values mentioned in the published literature (Table 2). Descriptive statistics (box-plot not shown here) indicated that the growth parameters of the species in the Ionian Sea were situated in the interquartile range of the values published in the literature; L_∞ and Φ' of the south Aegean Sea were located in the upper third quartile, while k was in the interquartile range. Therefore, it seems that the results of this study are similar with the results found in other regions of the Eastern Atlantic and Mediterranean waters inhabited by the species. The results could be useful in fishery management. However, further investigation along with genetic studies are necessary to verify the homogeneity of the two stocks.

Table 2. Growth parameters of *Boops boops* from different study areas, the aging method that was used; O: otoliths; LF: length-based analysis; S: scales; and the samples source; E: experimental fishing; C: commercial; B: both (from experimental and commercial source); ND: not defined; L_∞: the mean theoretical asymptotic length in mm; k: a growth rate parameter in year^{-1}; t_0: the theoretical age at zero length in years; Φ': the growth performance index.

Reference	Area	Method	Samples source	L_∞ (mm)	k (year^{-1})	t_0 (year)	Φ'
Hernandez (1989)	Central Adriatic Sea	O	E	332	0.17	-1.48	2.28
Tsangridis & Filippousis (1991)	Saronikos Gulf	LF	E	360	0.40	-0.91	2.71
Kallianiotis (1992)	Cretan Sea	O	B	237	0.42	-0.24	2.37
Gordo (1996)	South Portugal	O	ND	435	0.14	-2.40	2.42
Gordo (1996)	West Portugal	O	ND	424	0.15	-2.29	2.43
Cannizzaro *et al.* (2001)	Strait of Sicily	O	ND	450	0.13	-1.90	2.42
Allam (2003)	Alexandria	S	C	317	0.15	-1.78	2.19
El-Haweet *et al.* (2005)	Saloum Bay (Egypt)	S	C	319	0.15	-1.53	2.18
Khemiri *et al.* (2005)	North Tunisian Coast	O	C	287▪	0.20	-1.41	2.22
Khemiri *et al.* (2005)	Gulf of Tunis	O	C	243▪	0.23	-1.65	2.13
Khemiri *et al.* (2005)	East Tunisian Coast	O	C	267▪	0.22	-1.43	2.20
Khemiri *et al.* (2005)	South Tunisian Coast	O	C	235▪	0.21	-1.98	2.06
Isaias & Sinis (2006)	Eastern Ionian Sea	S	C	274	0.20	-2.20	2.18
Monteiro *et al.* (2006)	South Portugal	O	B	280	0.22	-1.42	2.24
Abecasis *et al.* (2008)	South Portugal	O	C	281	0.20	-1.90	2.21

Reference	Area	Method	Samples source	L_∞ (mm)	k (year^{-1})	t_0 (year)	Φ'
Abecasis *et al.* (2008)	South Portugal	S	C	268	0.34	-1.27	2.39
El-Okda (2008)	Alexandria	O	C	301	0.15	-1.51	2.14
Ramdane *et al.* (2013)*	East Coast of Algeria	O	C	270	0.24	-1.53	2.24
Ramdane *et al.* (2013)**	East Coast of Algeria	O	C	275	0.28	-1.20	2.32
Mehanna *et al.* (2014)	Alexandria	O	C	272	0.54	-0.33	2.60
Kara & Bayhan (2015)*	Izmir Bay	O	C	299	0.24	-0.98	2.34
Kara & Bayhan (2015)**	Izmir Bay	O	C	308	0.24	-0.90	2.37
Soykan *et al.* (2015)	Izmir Bay	O	E	296	0.27	-1.14	2.37
Kherraz *et al.* (2016)*	Western Algeria	O	C	268	0.38	-0.75	2.43
Kherraz *et al.* (2016)**	Western Algeria	O	C	341	0.26	-1.50	2.48
Azab *et al.* (2019)	Alexandria	O	C	307	0.28	-0.16	2.42
This study	Ionian Sea	O	B	318	0.24	-1.57	2.38
This study	South Aegean Sea	O	B	355	0.26	-1	2.51

*: Males; **: Females; ▪: Fork Length

5. Acknowledgements

The present work was carried out based on samples collected by the Hellenic Centre for Marine Research in the framework of the National DCF Programme 2020, Ministry of Rural Development and Food of Greece.

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MORPHOMETRIC MEASUREMENTS OF *PAGELLUS BOGARAVEO* (BRÜNNICH, 1768) OTOLITHS IN THE GREEK SEAS

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Abstract

Pagellus bogaraveo is a member of the family Sparidae for which information on otolith morphometrics is generally scarce. In the present study, the relationship of the total length with ten morphometric characteristics of the otoliths of this species was examined in samples collected from the Aegean and the Ionian Seas. Linear regressions were found to fit well the data between the total length and seven otolith characters (radius, length, width, area, perimeter, rectangularity and ellipticity) in both areas. Differences in the slope for the first five of these regressions were detected between the two geographical areas indicating potential differences between the two stocks of the species.

Keywords: Blackspot seabream, otolith morphometrics, Mediterranean.

1. Introduction

The blackspot seabream, *Pagellus bogaraveo* (Brünnich, 1768), is a demersal fish, which belongs to the family Sparidae. It is common in the eastern Atlantic and the western Mediterranean, less common in the eastern part of the basin, while absent from the Black Sea (Bauchot & Hureau, 1986; Spedicato *et al.,* 2002; Mytilineou *et al*., 2019). This species inhabits shallow waters with sandy and muddy bottoms when young, while its bathymetric distribution extends at depths down to 700 m when adult (Bauchot & Hureau, 1986; Mytilineou *et al*., 2005; Mytilineou *et al*., 2013). The biological characteristics of the species (protandrous hermaphroditism), and the intensive gill net fishing of its stocks in the Greek waters the last decades, have resulted in their depletion (Mytilineou & Machias, 2007; Mytilineou *et al*., 2013). These facts combined with the lack of the related scientific information of this species, may explain the limited information on the biology and ecology of this species in the Greek waters.

Otolith morphology is a low-cost technique used in phylogenetic and stock identification studies (Lombarte & Lleonart, 1993; Tuset *et al*., 2006) providing important information for fisheries management. Although, several studies have been carried out on the otolith morphometrics of the congeneric species *Pagellus erythrinus*, only two studies on this topic have been conducted for *P. bogaraveo* in the North Aegean Sea and the Tyrrhenian Sea (Altin & Ayyildiz, 2017; D'Iglio *et al*., 2021); the former investigating three otolith characters only, while the latter studying the comparison of otolith characters among three *Pagellus* species. The objective of this work was to study for the first time the relationship of ten otolith morphometric characters with the total length of *P. bogaraveo* in the South Aegean and the Ionian Seas (Greek waters) and define differences between the two areas.

2. Materials and Methods

Data on the otolith morphometrics were collected from 198 individuals of *P. bogaraveo* caught by bottom trawl from July to September of 2018 and 2019; in total, 88 in the South Aegean Sea (total length TL: 56 - 227 mm) and 110 in the Ionian Sea (total length TL: 55 - 249 mm). For each specimen, the left and the right sagitta otolith were removed, cleaned, and photographed. Only the left otolith was used for the measurements using the Image-Pro Plus software (Version 4.5.0.29). Broken and damaged otoliths were not included in the analysis (7 from the Aegean Sea and 4 from the Ionian Sea).

The following otolith morphometric parameters were recorded (Fig. 1): Radius (*RA*, mm), Otolith Length (*OL*, mm), Otolith Width (*OW*, mm), Otolith Area (*OA*, mm^2), Perimeter (*PE*, mm), Roundness (*RD*) according to Ponton (2006) and Circularity (*CI*) according to Tuset *et al.* (2003). Additionally, the following shape factors were calculated: Form Factor (*FF*), Rectangularity (*RC*), and Ellipticity (*EL*) according to Tuset *et al.* (2003). The relationship of *TL* with each morphometric parameter was examined using simple linear regression. Furthermore, the significance of the differences in regression lines was checked through Analysis of Covariance. The level of significance used was α = 0.05.

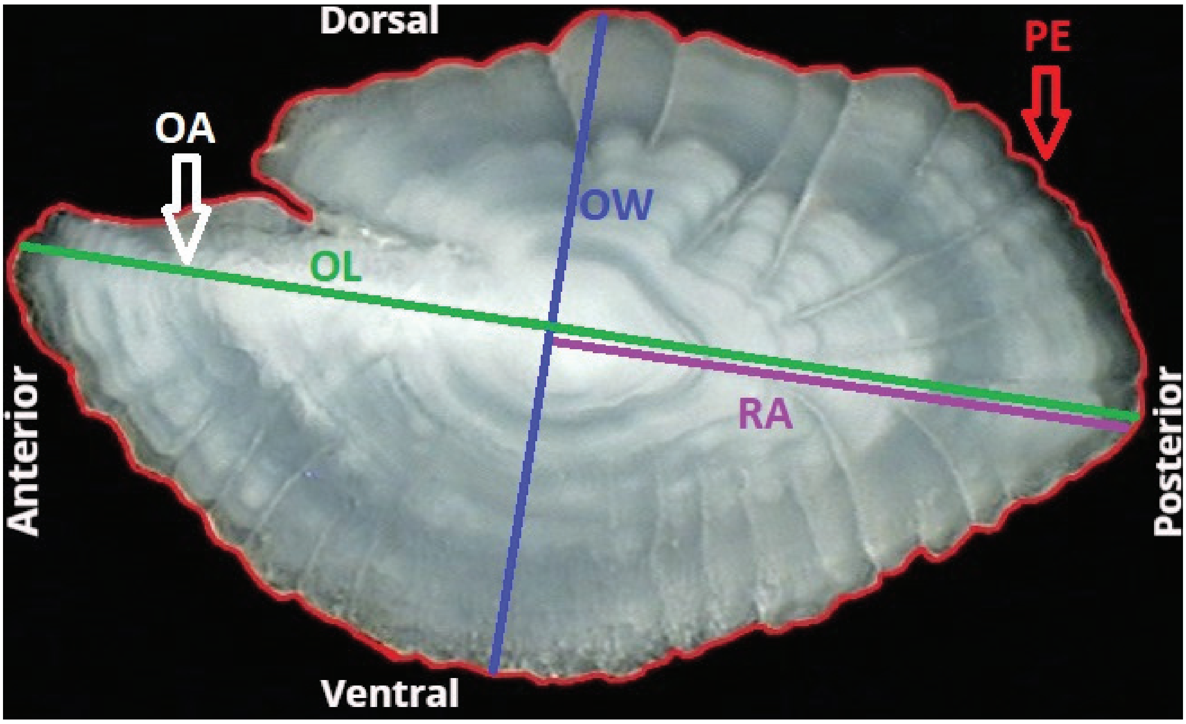

Fig. 1: Left otolith of *Pagellus bogaraveo*, illustrating the measurements analyzed RA: Radius (mm); OL: Otolith Length (mm); OW: Otolith Width (mm); OA: Otolith Area (mm^2); PE: Perimeter (mm).

3. Results

Statistically significant relation (P-value < 0.05) was found between *TL* and the examined characters *RA*, *OL*, *OW*, *OA*, *PE*, *RC* and *EL* in both the South Aegean and Ionian Seas. There was no statistically significant relationship (P-value > 0.05) in both areas between *TL* and the factors *RD*, *CI*, and *FF*. Significant differences (ANCOVA, P-value < 0.05) were detected for the slope *b* of the regression lines of *RA*, *OL*, *OW*, *OA* and *PE* with TL between the South Aegean and the Ionian Seas. This was not the case for the slope of the regressions of the *RC* and *EL* with TL (P-value > 0.05) (Table 1).

4. Discussion/Conclusion

The regressions of *RA*, *OL*, *OW*, *OA* and *PE* with *TL* showed that these characters followed a linear relationship. Although the relation of *TL* with *RC* and *EL* were statistically significant, these, because of the very low R^2, did not seem to fit quite well the data indicating that the otolith of *P. bogaraveo* increases in rectangularity and ellipticity with size, but probably not linearly. The factors *RD*, *CI* and *FF,* which show the closeness of *P. bogaraveo* otolith shape with a circle (Tuset *et al.*, 2003), did not reveal any correlation with *TL*. Our results concerning the relationship between the *TL* and the *RA*, *OL* and *OW* in *P. bogaraveo* coincided with those of Altin & Ayyildiz (2017) who also reported a high correlation between them.

The significant differences detected for the relationships of *TL* with *RA*, *OL*, *OW*, *OA* and *PE* between the two areas may indicate differences between the two stocks of the species. These could be ascribed to the combined effects of genetics and environmental factors, such as temperature, salinity, and food availability, features known to affect otolith morphometry (Lombarte & Lleonart, 1993; Avigliano *et al.*, 2014). More specifically, Lombarte & Lleonart (1993) claim that genetic factors influence the otolith form, and environmental factors (particularly temperature in carbonate-saturated waters) control the amount of material deposited during the creation of the otolith. Avigliano *et al.* (2014) mentioned that variations in the otolith morphometric of a fish species in two geographical areas might be attributed to differences in the water chemistry and salinity of these areas. Velaoras *et al.* (2013) found differences in the temperature and salinity levels between the Aegean and Ionian Sea with the former area presenting higher values for both environmental parameters. These differences may explain the differences in the otolith morphometrics of *P. bogaraveo* detected in the two study areas. However, further work and genetic studies are needed to prove this hypothesis.

Table 1. Parameters of the linear regression of the total length (TL, mm) of *Pagellus bogaraveo* with the morphometric characters of the otoliths RA (Radius), OL (Otolith Length), OW (Otolith Width), OA (Otolith Area), PE (Perimeter), RC (Rectangularity), and EL (Ellipticity) in the Aegean and Ionian Seas. a: intercept; b: slope; R^2: coefficient of determination; r: correlation coefficient. The P-value of the regressions and the P-value of the comparison of the slope b between the regression lines of the two areas, are also shown (ANCOVA). *: statistically significant difference.

Morphometric character	Area	a	b	R^2	r	Regression, P-value	ANCOVA, P-value for b
RA	Aegean	0.99	0.02	0.81	0.90	<0.05*	<0.05*
	Ionian	0.36	0.02	0.98	0.99	<0.05*	
OL	Aegean	1.54	0.03	0.89	0.94	<0.05*	<0.05*
	Ionian	0.70	0.04	0.99	0.99	<0.05*	
OW	Aegean	1.11	0.02	0.85	0.92	<0.05*	<0.05*
	Ionian	0.63	0.02	0.98	0.99	<0.05*	
OA	Aegean	-5.09	0.15	0.92	0.96	<0.05*	<0.05*
	Ionian	-7.48	0.17	0.99	0.99	<0.05*	
PE	Aegean	4.15	0.08	0.90	0.95	<0.05*	<0.05*
	Ionian	2.36	0.09	0.99	0.99	<0.05*	
RC	Aegean	0.71	0.00	0.07	0.27	<0.05*	0.47
	Ionian	0.71	0.00	0.11	0.33	<0.05*	
EL	Aegean	0.24	0.00	0.12	0.35	<0.05*	0.36
	Ionian	0.23	0.00	0.44	0.66	<0.05*	

5. Acknowledgements

The present work was carried out based on samples collected during the MEDITS surveys of 2018 and 2019 conducted by the Hellenic Centre for Marine Research in the framework of the national DCF programme funded by the Ministry of Rural Development and Food of Greece.

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COMPARATIVE SHAPE MORPHOLOGY OF THE SAGITTAL OTOLITHS BETWEEN COMMON AND EGYPTIAN SOLES FROM CENTRAL GREECE

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Abstract

Although *Solea aegyptiaca* and *Solea solea* have been distinguished based on meristic counts and molecular markers, little information exists about their otolith morphology and its potential use for species identification. In recent years, the application of otolith shape analysis has yielded good results in the separation between species, stocks and populations. Here, we showed the separation of the two cryptic flatfish species from Central Greece by means of wavelet transformation with the use of the Rpackage, ShapeR. Forty - eight standardised wavelet coefficients were extracted from a total of 164 right otoliths from both species and were used to reconstruct mean otolith outlines. Outline variation was evaluated with an ANOVA-like permutation test and multivariate analysis which indicated that the mean otolith outlines were significantly different.

Keywords: flatfish, Maliakos Gulf, wavelet, outline, R.

1. Introduction

Soleidae is a large family of flatfishes currently including 180 valid species worldwide (Fricke *et al.*, 2022). Soleidae flatfish are characterized by highly dorsoventrally compressed and elongated bodies with both eyes on the right side. They are demersal and inhabit muddy and sandy bottoms of the continental shelf and slope (Quéro *et al.,* 1986). Many species within the family have important commercial value and are common fisheries targets. The two species are easily confused due to great similarity in their external morphology. However, they can be differentiated based on the higher numbers of vertebrae, dorsal and anal fin rays present in *S. solea* compared to *S. aegyptiaca* (Vachon *et al.*, 2008; Sabatini *et al.*, 2018, personal observation) as well as molecular data (Borsa & Quignard, 2001; Boukouvala *et al.*, 2012). To date, the Egyptian sole has been recorded in the Northern coasts of the Mediterranean in the Gulf of Lions and the Adriatic and Aegean Seas (Quéro *et al.*, 1986; Charmpila *et al.*, 2016; Sabatini *et al.*, 2018, Masnadi *et al.,* 2020). However, because *S. aegyptiaca* can be easily misidentified as the more abundant *S. solea*, there may not be reliable stock assessments for the populations of the two species in European waters. Therefore, it is difficult to develop frameworks for their stock management within the geographical subareas of the Northern Mediterranean Sea. Moreover, the study of the Egyptian sole biology from the European Mediterranean waters has been mostly overlooked so far, even though several studies have been published for the species from the African coasts of the Basin (e.g. Mehanna, 2007; Chanet *et al.*, 2012). Conversely, the common sole has been widely studied from European waters (e. g. Ofelio *et al.*, 2020).

This paper investigates the use of otolith shape analysis, in particular the wavelet transformation, in separating between the sympatric *S. aegyptiaca* and *S. solea* found in Maliakos Gulf and provides robust evidence for the importance of otoliths in species discrimination.

Otoliths are calcium carbonate increments inside membranous sacs of the inner ear of bony fishes (teleosts). They form three pairs of mineralized structures termed sagittae, lapilli and asterisci which are located respectively in three distinct otolithic end organs (Parmentier *et al.,* 2007). Fish otoliths serve as a tool to sense balance and sound and in effect they help stimulate the sensory hair cells of the otolithic organ (Popper *et al.,* 2005). Sagittae are the largest of the three otolith pairs in most fish taxa and their structure is usually species-specific (Tuset *et al.,* 2008). To date, otolith morphology has been applied successfully to separate between species, populations or stocks with methods ranging from simple descriptive to quantitative such as morphometric landmark analysis and otolith outline shape analysis (Libungan *et al*., 2015; Chakour & Elouizgani, 2018).

2. Material and Methods

2.1 Sampling area and specimen identification

Specimens were collected from 14 samplin,g stations in the inner and outer part of Maliakos Gulf from May 2014 to August 2015 within the framework of "KRIPIS - *Development of an integrated management system for basin, coastal and marine zones*", and in 2016 and 2017 within "Maliakos 2017 – 2020" (Fig. 1). Specimens were identified based on the mitochondrial cytochrome b gene marker (Charmpila *et al*., 2016) or solely on meristic counts and otolith morphology.

2.2 Otolith extraction and imaging

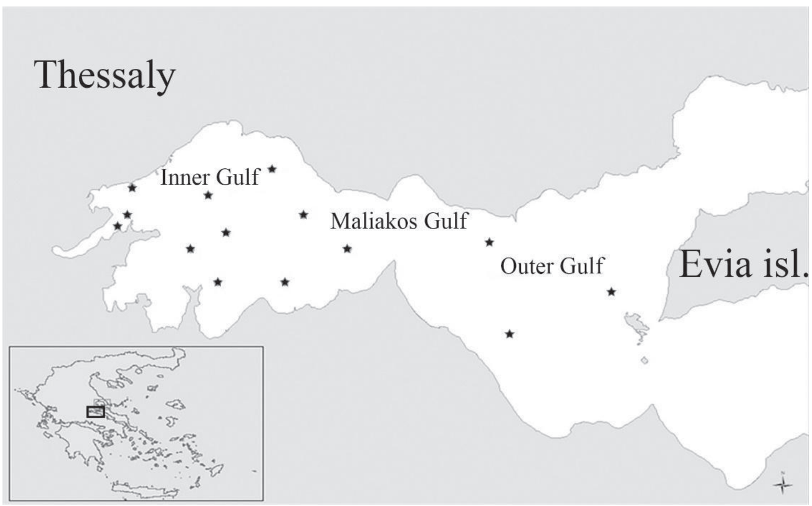

Fig. 1: Sampling stations (stars) in Maliakos Gulf. Inset indicates location of Maliakos Gulf.

Left and right saccular otoliths (referred as otoliths thereafter) were extracted, cleaned with distilled water to remove residual tissue and stored dry. Digital images of the otoliths were taken with a stereomicroscope with an attached digital camera and the software Image-Pro Analysis 4.5. Otoliths were placed with their distal surface showing, immersed in water, against a dark background and under direct light. Shape analysis was conducted on calibrated otoliths images.

2.3 Otolith shape analysis and statistical processing

Otolith outlines were generated with the ShapeR 0.1 – 5 package (Libungan & Pálsson, 2015) from digital images. Images were previously stored in JPEG format (*.jpg) with increased brightness and contrast between otolith and background, and rotation with their rostrum to the left. Equally spaced radii were drawn out from the otolith centroid to the otolith outline acting as univariate shape descriptors and x and y coordinates were collected from the drawn radii (Libungan & Pálsson, 2015). The polar coordinates were returned as wavelet coefficients after implementation of the wavelet transformations. Built-in function in ShapeR removed the effect of allometric growth on otolith size using a normalization technique based on regression (Lleonart *et al*., 2000); the Wavelet coefficients were scaled with standard fish length; coefficients which showed significant interaction between species and fish length ($P < 0.05$) were omitted automatically from the following analyses (Libungan & Pálsson, 2015). Mean otolith shapes of smoothed out contours were reconstructed for each species. All statistical analyses were conducted with RStudio v 1.4 (RStudio Team, 2021). Canonical Analysis of Principal Coordinates (CAP) of the standardized for fish length wavelet coefficients was applied to analyse shape variation between species. An ANOVA-like permutation test was used to find the significance in shape differentiation between species. Linear Discriminant Analysis (LDA) was applied on standardized coefficients to demonstrate classification of individual otoliths. Classification success rate based on LDA was estimated with leave-one-out cross-validation (Libungan *et al.*, 2015; Libungan & Pálsson, 2015).

3. Results

In total, right otoliths from 164 (78 *S. aegyptiaca*, 86 *S. solea*) individuals ranging between 9.1 – 33.5 cm in standard length (mean 21.21 cm ± 5.10 standard deviation) were used for shape analysis. Comparison of otoliths from female (= 39) and male (= 34) individuals of *S. aegyptiaca* indicated no significant difference (P = 0.924 > 0.01, F-value = 0.3585), therefore otoliths from both sexes were pooled. Although information on sex was not available from individuals of *S. solea*, otoliths were pooled together as well. Forty – eight wavelet coefficients were produced after standardization for fish length to express the overall otolith shape. Most variation between species was traced at 180 – 210° which roughly corresponded to the rostrum and excisura angle areas of the otolith (Fig. 2). An ANOVA-like permutation test showed that otoliths from *S. aegyptiaca* differed significantly (P = 0.001 < 0.01, F-value = 0.3585) from those from *S. solea* based on 1000 permutations. All shape variation between the two species was expressed with one principal coordinate (CAP1). The classifier based on LDA with leave-one-out cross-validation gave a good overall score of correct classification (82 %) with 87.2 % (68/78 otoliths) of *S. aegyptiaca* otoliths being correctly assigned to species, while in the case of *S. solea*, 78 % (67/86 otoliths) were classified correctly.

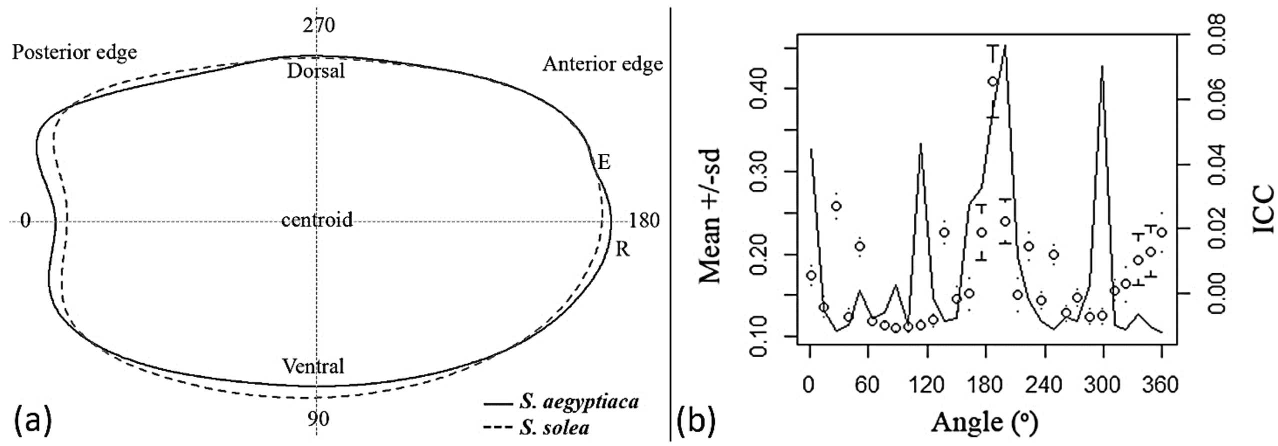

Fig. 2: Mean otolith shapes based on wavelet reconstruction for *S. aegyptiaca* and *S. solea*, the numbers 0, 90, 180 and 270 indicate angle in degrees (°) on the outline (a). Mean and standard deviation (sd) of the wavelet coefficients for all otoliths combined and the proportion of variance among species or intraclass correlation (ICC, black solid line). Horizontal axis shows angle based on polar coordinates where the centroid of the otolith is the center point of the polar coordinates (b). E, excisura angle; R, rostrum.

4. Discussion/Conclusion

Both *Solea aegyptiaca* and *S. solea* were collected from the 14 stations within Maliakos Gulf. The two sympatric species are quite similar in external morphology which has been the cause for false synonymisation of *S. aegyptiaca* with the more abundant *S. solea* in European Mediterranean waters (Quéro *et al.*, 1986; Borsa & Quignard, 2001; Vachon *et al.*, 2008; Chanet *et al.*, 2012). Additionally, the two species present elongated otolith contours with flattened posterior edges and poorly defined excisura angle, however the otoliths of *S. aegyptiaca* are elliptical, whereas the otoliths of *S. solea* are more discoidal with sometimes a pointed anterior edge (Fig. 2a). Therefore, the two species can be suitable candidates to test the ShapeR package for otolith shape analysis to identify subtle overall shape differences. Otolith shape analysis of *S. aegyptiaca* and *S. solea* demonstrated a significant difference between the two species ($P < 0.01$) based on the ANOVA-like permutation test. Multivariate analysis also confirmed the distinction between the otolith contours from the two species with good classification success after cross-validation (~ 80 %). This study provides robust evidence of the distinct morphology between the otoliths of the Egyptian and the common sole from Central Greece. Our results also indicate that the ShapeR package is suitable for the study of otolith shape variation among species, extending the previous application of the package from comparisons among fish populations or stocks (Libungan *et al.*, 2015; Sadeghi *et al.*, 2020). In conclusion, the outcome of this study reinforces the distinction between the two soleids and adds robust evidence for the importance of otolith shape morphology in species identification.

5. Acknowledgements

Fish samples were collected within the framework of the research project "Development of an integrated management system for basin, coastal and marine zones, (KRIPIS)" and "Maliakos 2016 – 2017".

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UPDATED GAP ANALYSIS ON THE BIOLOGY OF MEDITERRANEAN MARINE FISHES

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Abstract

We updated the investigation of the knowledge gap in the biological characteristics of Mediterranean marine fishes, five years after the initial gap analysis was published. We extracted literature containing information on eight characteristics, namely, length-weight relationships, growth, maximum age, mortality, spawning, maturity, fecundity and diet, for the 722 fish species of the Mediterranean Sea listed in FishBase. The results showcased a considerable knowledge gap as 37% of the species have no information at all, while 13% have information on only one characteristic. Out of all the biological characteristics, the smallest gap was found in the length-weight relationships (studied for 51% of the species), while the least studied characteristic was mortality (studied for 10% of the species). The most studied species were European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*) European anchovy (*Engraulis encrasicolus*), European pilchard (*Sardina pilchardus*), common pandora (*Pagellus erythrinus*) and annular seabream (*Diplodus annularis*), which are all highly commercial. The gap seems to have shrunk compared to the previous gap analysis five years ago and it appears that research has slightly shifted towards species that have been traditionally neglected, e.g., the elasmobranchs. The effort needs to continue in this direction as many of these species are particularly vulnerable in the everchanging Mediterranean region.

Keywords: growth, mortality, diet, knowledge gap, Mediterranean Sea.

1. Introduction

The Mediterranean Sea has been identified as an area with a large information gap on biological characteristics of many fish species (Dimarchopoulou *et al.*, 2017). The lack of biological information impedes the efforts of scientists working in the fields of Fish Ecology and Fisheries Management, as this information is needed for a variety of models. Fish stock assessment models alone require an array of biological information such as length- weight relationships, maximum age, size at maturity and growth parameters (Chrysafi & Kuparinen, 2016). Moreover, life history traits such as maximum age, growth rate and age at maturation, affect species resilience against overexploitation (Russ & Alcala, 1998) and climate change (Wang *et al.*, 2020), therefore playing an important role in modelling the resilience of a particular species. Additionally, ecosystem based fisheries management (EBFM) needs all ecosystem components, in addition to the characteristics of individual stocks, to be taken into consideration for the decision-making process (Garcia *et al.*, 2003). The more holistic approach of EBFM requires life history knowledge of both commercial and non-commercial fish species of the ecosystem in question (Claudet *et al.*, 2010). Granting the importance of acknowledging and highlighting the existing gaps, Dimarchopoulou *et al.* (2017) published a gap analysis on the biological traits of Mediterranean marine fishes. Considering that five years have passed since this publication and given the everchanging profile of the Mediterranean region (Turley, 1999), we revisited the topic to monitor the existing gap and identify any progress made.

2. Materials and Methods

An updated gap analysis was carried out on the Mediterranean marine fish species, which followed the existing methodology (Dimarchopoulou *et al*., 2017) regarding the missing biological information in the Mediterranean as a whole, and on a subregional basis (W: western; C: central; E: eastern, Mediterranean). The gap stems from the difference between the current status and the ideal scenario in which all biological characteristics -or as many characteristics as possible- are known for every species -or at least for as many species as possible- (Dimarchopoulou *et al*., 2016).

We updated the gap analysis now covering the years 2016-2021 to report the number of fish species and their related biological characteristics that has been fully or partially studied and to compare the results with the ones previously published (Dimarchopoulou *et al*., 2017). To do that, we collected data on all fish species which have been recorded in the Mediterranean Sea large marine ecosystem as they are listed in FishBase (Froese & Pauly, 2021). Altogether, 758 Mediterranean marine fishes are currently listed in FishBase, of which 36 fish species were excluded as misidentified and questionable records. For each of the 722 remaining species, the available information on length-weight relationships, growth parameters, maximum age, mortality rate, spawning period, size at maturity, fecundity and diet composition was extracted from FishBase and published papers found in SCOPUS. For length-weight relationships (LWR) records we registered species with both the slope (b) and intercept (a) of the equation, for somatic growth (G) records with the asymptotic length (L_∞) and the rate at which L_∞ is approached (K) and for lifespan, records with the maximum age (t_{max}). Concerning the reproduction parameters, we considered the onset and duration of spawning (Spawn) and length at maturity (L_m) to identify spawning and maturity related information, respectively. Additionally, we regarded absolute and relative number of oocytes for fecundity (Fec). Lastly, we used prey items, stomach content and feeding preferences as records for diet, while the natural mortality rate was used as natural mortality (M), no matter the estimation method.

Moreover, we recorded the protection status (IUCN Red List of Threatened Species) of each species based on the IUCN categories (LC: least concern; EN: endangered; DD: data deficient; NE: not evaluated; NT: near threatened; VU: vulnerable; CR: critically endangered), alongside their commercial value (Val), which was shown as “price category” in FishBase (VH: very high; H: high; M: medium; L: low). Lastly, we extracted the Mediterranean landings averaged for the years 2015-2019 from the FAO-GFCM database (FAO, 2021).

3. Results

Within the data we collected for the 722 Mediterranean fish species, there is no information on any biological characteristic for 270 species (37%), while for 95 (13%) of them there is information for only one characteristic. Regarding the biological characteristics separately, the gap is smaller for the most common characteristic, i.e., length-weight relationships, as they have been studied for 366 (51%) species, followed by spawning (312 species; 43%), diet (284 species; 39%), growth (211 species; 29%), maturity (192 species; 27%), maximum age (184 species; 25%) and fecundity (142 species; 20%). The largest gap occurs in natural mortality (70 species; 10%) for which information is sparse. Concerning the studied species that are listed under the categories near threatened (NT), vulnerable (VU), endangered (EN) and critically endangered (CR) of the IUCN Red List (n = 91), the percentages for all biological characteristics except mortality and growth were higher compared to the total of the species. In terms of commercial value, the species listed under the categories high (H) and very high (VH) (n = 167), had higher percentages in every biological characteristic, compared to all species listed.

With respect to the studied characteristics, the record distribution pattern is consistent among the western, central and eastern Mediterranean, with the exception of some spatial variations within each biological characteristic. Spawning, maturity, fecundity and diet were most extensively studied in the western subregion, length-weight relationships and maximum age were studied mostly in the eastern subregion. Growth and mortality records were rather evenly distributed among the three subregions (Fig. 1).

In terms of number of records, the most studied species were European hake *Merluccius merluccius*

(227 records in total with 46 new records in the last five years), red mullet *Mullus barbatus* (163 records in total with 34 new), European anchovy *Engraulis encrasicolus* (142 records in total with 55 new), European pilchard *Sardina pilchardus* (125 records in total with 43 new), common pandora *Pagellus erythrinus* (124 records in total with 26 new), annular seabream *Diplodus annularis* (115 records in total with 17 new), surmullet *Mullus surmuletus* (115 records in total with 15 new) and bogue *Boops boops* (100 records in total with 24 new). Furthermore, we identified a newfound interest for elasmobranchs as records for a lot of elasmobranch fish species were published in the last five years. The least studied species were those belonging to the Gobiidae, Blennidae, Myctophidae, Carangidae, Labridae, Gobiesocidae and Syngnathidae families.

Concerning the Mediterranean fish species with at least one studied biological characteristic and with available landings data, the number of records had a weak positive correlation to total landings ($n = 125$, Spearman $\rho = 0.31$, $P < 0.001$) and maximum reported length ($n = 451$, Spearman $\rho = 0.20$, $P < 0.001$). In opposition to total landings, the trophic level ($n = 451$, Spearman $\rho = 0.086$, $P = 0.068$) did not appear to correlate with the number of records.

Regarding the distribution of the records amongst the three Mediterranean subregions, spawning, maturity, fecundity and diet were most extensively studied in the western subregion, while the majority of the length-weight relationships and maximum age records came from the eastern subregion. The central part of the Mediterranean Sea holds the least number of records compared to the other two regions. Growth and mortality records were rather evenly distributed among the three subregions (Fig. 1).

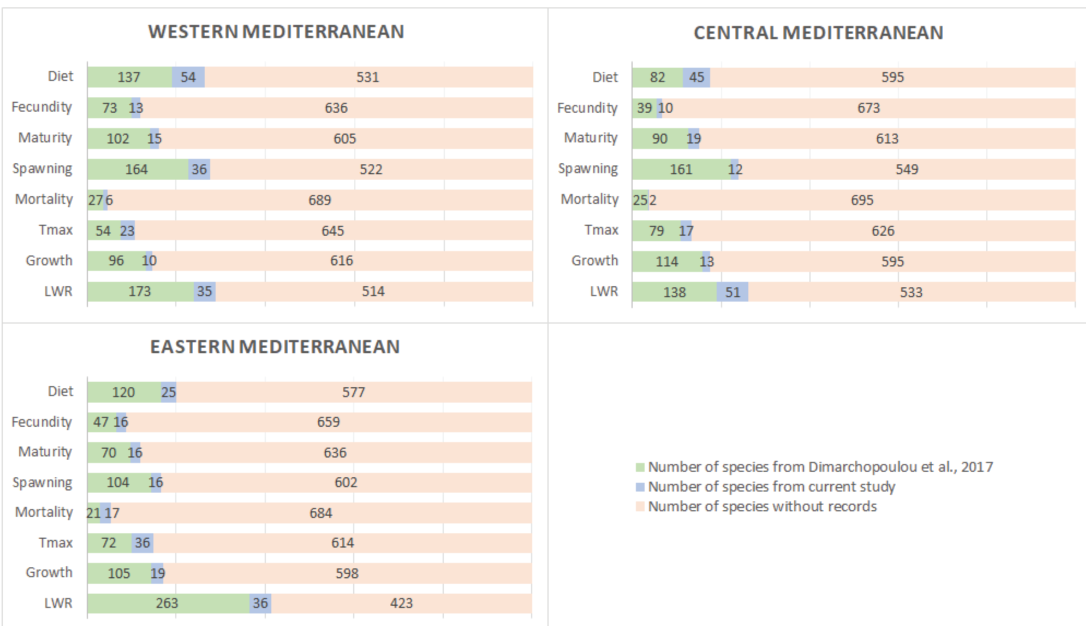

Fig. 1: Percentage of fish species with (green, from Dimarchopoulou et al., 2017 & blue, from the present study) and without (pink) information on length-weight relationships (LWR), growth parameters (G), maximum age (tmax), mortality rate (M), spawning period (Spawn), size at maturity (Lm), fecundity (Fec) and feeding preferences (Diet), across the western, central and eastern Mediterranean Sea.

4. Discussion/Conclusions

The results showed that during the last five years (2016-2021) the knowledge gap on the biology of Mediterranean marine fishes was slightly reduced, as the percentage of fish species without any biological characteristics has dropped from 43% (Dimarchopoulou *et al.*, 2017), to 37% in 2021. The scientific interest is still focused on length-weight relationships, spawning and diet with 124 new diet records, 122 new length-weight relationship records and 64 new spawning records. Mortality records are still very few and exist for only 10% of the Mediterranean fish species. For the species of high commercial value and of poor protection status, the percentages were, overall, higher for all biological characteristics in comparison with all the species. Focusing on the biological characteristics, the least studied remain mortality and fecundity, the same as previously reported (Dimarchopoulou *et al.*, 2017).

With respect to the number of records per species the most studied ones were the same as in the 2017 gap analysis: European hake, red mullet, European anchovy, European pilchard, annular seabream and common pandora, which are all highly commercial species and information on them exists for all the biological characteristics. The minimization of the gap is also evident from the new records of elasmobranchs (Tsikliras & Dimarchopoulou, 2021) that were published since 2017. This is particularly hopeful as elasmobranchs are, generally, vulnerable species due to the fact that they have slow growth, late maturity, produce only a few oocytes and a lot of them are of poor protection status (Bradai *et al.*, 2012), so they are priority species to be studied. Apart from the apparent rise in the records regarding fish biological characteristics, knowledge about species belonging to the Gobiidae, Blennidae, Myctophidae, Carangidae, Labridae, Gobiesocidae and Syngnathidae families is still missing.

In conclusion, the knowledge gap seems to be getting smaller as the papers published the last five years fill in a considerable part of the gap that was previously identified (Dimarchopoulou *et al.*, 2017). Regarding the scientific effort, although the number of records of non-commercial species has increased, the focus remains on the more commercial species and needs to be expanded to the less commercial, as well as to the more vulnerable species of the list. With the constant influx of new species entered from the Red Sea, the effort to fill in the existing knowledge gaps needs to be intensified.

5. Acknowledgements

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SEASONAL DISTRIBUTION OF ICHTHYOPLANKTON ASSEMBLAGES IN MALIAKOS GULF (EASTERN MEDITERRANEAN)

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Abstract

This study presents the seasonal distribution and abundance patterns of ichthyoplankton in Maliakos Gulf based on monthly surveys carried out from September 2018 to September 2019. Bongo nets were used to sample ichthyoplankton and a CTD profiler was used to collect environmental information. The taxonomic composition of fish eggs and larvae is described as well as its relationship with the seasonal changes in temperature. A total of 49 taxa belonging to 30 families were identified. Multivariate analysis revealed a clear distinction of ichthyoplankton into two main seasonal groups. Determining the temporal patterns of ichthyoplankton composition is important for understanding spawning strategies and predicting potential effects of climate change on cooccurring fish species.

Keywords: Fish eggs, fish larvae, Bongo net, multivariate analysis, Mediterranean Sea.

1. Introduction

By studying the distribution and abundance of ichthyoplankton and relating it to various abiotic and biotic factors we can extract important information about the biology and ecology of the early life stages of fish (Heath, 1992). Seasonal and annual fluctuations of environmental factors influence directly the adult fish, by affecting their reproductive output (period, duration, fecundity and spawning areas) as well as the eggs and larval abundance, distribution and mortality (Bakun *et al.*, 2006). Small and medium scale oceanographic structures (currents, gyres, fronts, etc.) directly affect either the retention of fish larvae in nutritious areas or their dispersion, thus helping to reduce competition for food and enhancing survival (Cushing, 1974). Since ichthyoplankton abundance and species composition largely reflect the population dynamics of adult fish, understanding the factors that shape the ichthyoplankton assemblages is key for developing management strategies (Shuai *et al.*, 2016).

2. Material and Methods

In order to study the distribution and abundance of fish eggs and larvae in the Maliakos Gulf, a network of 7 ichthyoplankton stations was sampled (Fig. 1). Thirteen monthly cruises were carried out from September 2018 to September 2019. A Bongo net was used to collect ichthyoplankton, which consists of two joined wreaths 60 cm in diameter each (fitted with 250μm- and 500μm-mesh nets respectively). Due to the shallow depth of the research area (~20 meters) the sampler was lowered and retrieved successively from the surface to near the bottom for about 5 minutes while the speed of the ship ranged between 2 to 2.5 knots. Vertical profiles of temperature and salinity were also sampled using a CTD. The identification and classification of fish eggs and larvae reached the level of species, genus or family where this was possible. Multivariate methods were applied to investigate changes in the composition of the ichthyoplankton community using the Primer 7 e-Permanova data analysis program. Abundance values were logarithmically transformed [$\log_{10}(x + 1)$] in order to reduce the effect of the most abundant taxa.

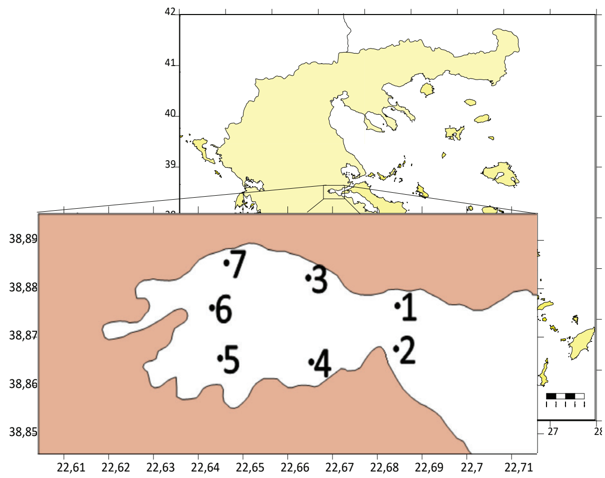

Fig. 1: Map of the survey area (Maliakos Gulf) showing the sampling stations (1-7).

3. Results

During the 13 monthly cruises (September 2018-September 2019), 20217 eggs and 3322 larvae were collected. In total, 49 taxa belonging to 30 families were identified. Larvae of *Engraulis encrasicolus* (22,5%), Gobiidae (17,7%), *Serranus hepatus* (9,2%), *Pagrus pagrus* (6,2%) and *Caranx rhonchus* (5,3%) and eggs of *Engraulis encrasicolus* (6,8%), *Sardina pilchardus* (4,4%), Mugilidae (2,3%), *Buglossidium luteum* (2,1%) and *Sardinella aurita* (1,5%) were the most abundant groups. As shown in Figure 2, the surface temperature was ~25°C in September 2018 and decreased during the winter months, reaching a minimum in January 2019 (~13°C). In the subsequent summer months, temperature increased again reaching a maximum in August 2019 (~27°C). The highest concentrations of eggs were recorded in May and June 2019, whereas the lowest, in autumn and winter (from October 2018 till February 2019). The concentration of larvae exhibited two different peaks, the first in September and the second in May-June (Fig. 2). The highest concentrations of eggs were recorded in the 250 μm-mesh net, especially in June 2019, mainly due to the high concentration of *E. encrasicolus* eggs. The anchovy eggs are small and have elliptical shape, so they easily escape from the 500μm-mesh net. Similarly, the highest abundances of fish larvae were also recorded in the 250μm-mesh net (Fig. 2). Regarding the total number of taxa, the lowest numbers (<7 taxa) were recorded from November to January (Fig. 2).

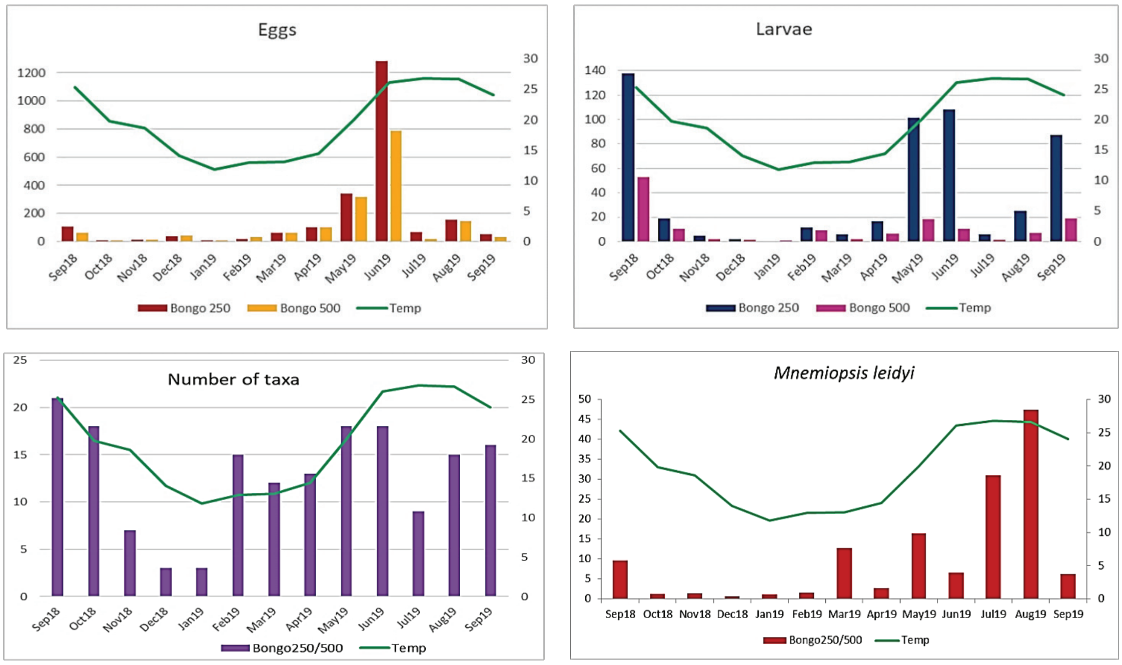

Fig. 2: Upper panel: Histograms of monthly mean concentration of eggs (eggs/10m ^{3}) (left) and larvae (larvae/10m^3) (right) caught with Bongo 250- and 500-mesh nets. Lower panel: Histograms of total number of ichthyoplankton taxa (left) and concentration of the ctenophore *Mnemiopsis leidyi* (individuals/10m^3) (lower panel, right) per month from both Bongo nets (250- and 500-mesh). The average sea surface temperature (°C) is also presented (green line, right axis).

The lower number of larvae in the 500 μm-mesh net is due to the extrusion of certain taxa through the net mesh, e.g., larvae with tube-like (e.g., anchovies and sardines) or elongated bodies (e.g., gobies) whose actual abundance is much higher. In addition, it should be mentioned here that in August and July 2019, the concentrations of larvae were unexpectedly low. This can be attributed to the increased abundance of the alien ctenophore species *Mnemiopsis leidyi*. As shown in Figure 2, this species occurred in Maliakos Gulf throughout the year, with low concentrations in autumn and winter, especially from October to February, but its concentration increased substantially in July and August 2019.

According to the Heat Map Diagram of Cluster Analysis (Fig. 3) there are two major groups of both stations and species. The first group consists of stations sampled during winter and early spring, between December 2018 and April 2019. This group included eggs and larvae of species like *D. labrax, Solea solea, S. pilchardus* and *Buglossidium lutem*. The second group consisted of stations sampled during summer and early autumn, in September and October 2018 and between May and September 2019. This group included eggs and larvae of species such as *E. encrasicolus*, *S. hepatus*, *C. rhonchus*, *P. pagrus* and *Solea aegyptiaca*.

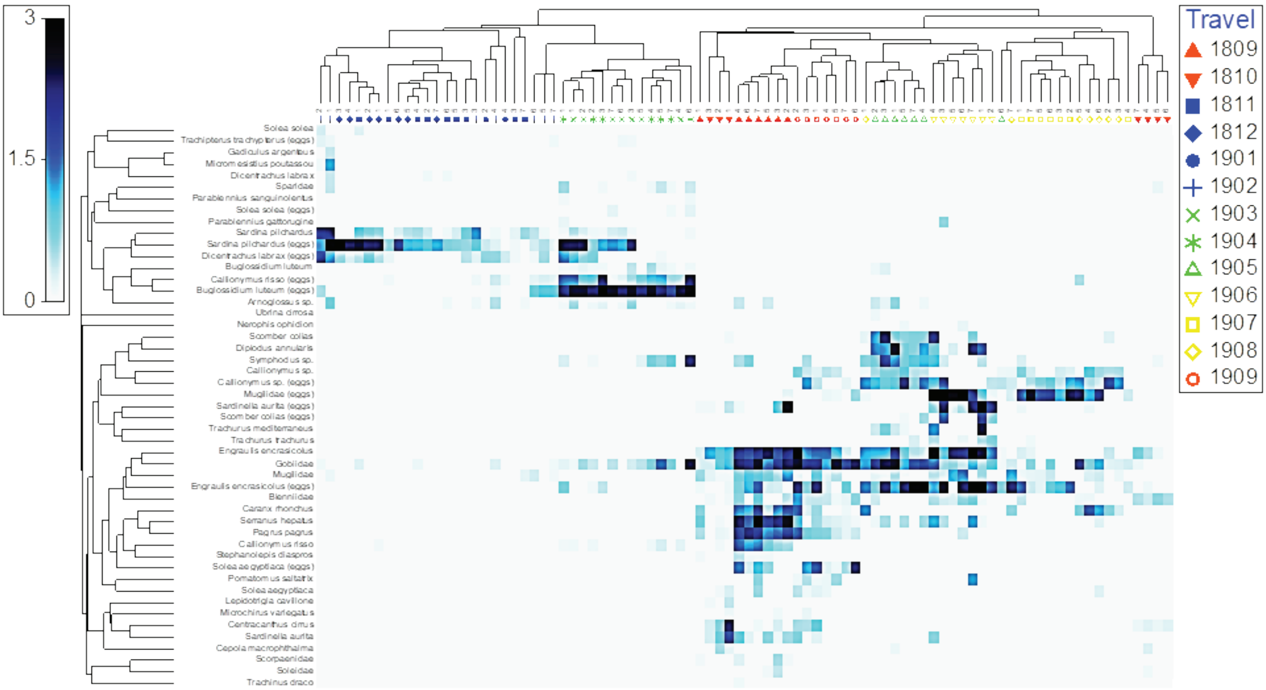

Fig. 3: Heat map diagram of a two-way hierarchical analysis of the most abundant egg and larval taxa and the sampling stations.

4. Discussion/Conclusion

It is well known that temporal patterns of ichthyoplankton occurrence vary between different environments and depend on local environmental factors. In the Mediterranean most fish species breed in late spring/early summer (Tsikliras *et al.*, 2010). Strong water supplies from river during the beginning of summer are followed by stratification of the water column, due to the heating of surface waters by the increase of day-length and the increase of atmospheric temperature. The higher water temperatures favor the rapid development of the critical early life stages of fish (from egg to juvenile) and thus, they reduce the risk of predation. The reproduction of most adult fishes seems to be synchronized with the spring zooplankton bloom which follows the late winter phytoplankton bloom. Subsequently, species that reproduce during the late spring / early summer seem to be benefited, since the high concentrations of microzooplankton in that period serves as food for fish larvae (Fernandez de Puelles *et al.*, 2003). High concentrations of microzooplankton (such as nauplii, copepodites and small and medium sized copepods) have been recorded in Maliakos Gulf during late spring/early summer (Kosmarikou *et al.*, 2019), which presumably serve as food for fish larvae and contribute to their survival.

In addition to food availability, another crucial factor affecting the concentration of ichthyoplankton is the presence of predators. In recent years, high abundances of the alien ctenophore *M. leidyi* have been observed in the Maliakos Gulf and according to the results of this study, the species showed an exceptional bloom in the area during the summer months of July and August. This bloom most likely affected the composition of ichthyoplankton at that period.

The Maliakos Gulf is an important spawning area with high concentration of many fish species. The two groups that emerged from the multivariate analysis of ichthyoplankton data implied a clear seasonal pattern in the occurrence and abundance of eggs and larvae of the different species. Similar seasonal differentiation was observed in the same area from April 2014 to January 2016 (Siapatis *et al.*, 2016). In the Hellenic seas, two of the most important commercial small pelagic species reproduce in different periods. Sardine spawns in winter and anchovy, in summer. In terms of egg and larval abundances, both species dominate the ichthyoplankton assemblages, therefore, a common breeding season would probably lead to competition between their larvae. Additional differentiation in seasonal spawning was also observed among three carangid species, namely *Trachurus trachurus, Trachurus mediterraneus* and *C. rhonchus*. *T. trachurus* reproduces mainly from winter to early summer, whereas *T. mediterraneus*, mostly in summer. *C. rhonhus*, on the other hand, spawns towards the end of summer and beginning of autumn. Segregation of spawning periods is also important for other sympatric species such as *S. solea* and *S. aegyptiaca*. *S. solea* reproduces during winter and early spring (January to April) while *S. aegyptiaca* spawns from the end of summer to the end of autumn (August to November). The increase in temperature observed in the Mediterranean over the last two decades, probably favors the thermophilic species (*S. aegyptiaca*), due to the prolongation of the warm period and the reduction of the cold period which coincides with the spawning period of *S. solea*.

In conclusion, this study provides evidence that differences in breeding season are advantageous for coexisting species with similar ecological roles, and that future sea warming may affect differently species that spawn in summer vs species that spawn in winter. Ichthyoplankton surveys provide information on a range of factors that affect population dynamics and given the different spawning period of species, it is important that such surveys be carried out at regular (e.g., monthly) intervals. In a period of changing climate, it is important to understand the differences in reproductive strategies of fish species, and the study of seasonal changes in ichthyoplankton is essential towards this direction.

5. Acknowledgements

The authors would like to thank the fishermen of ''Agia Marina'' fishing boat for their help in field sampling. This work was part of the project ''Maliakos 2018'' supported by HCMR (Hellenic Center of Marine Research) and funded by the Region of Central Greece.

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A MÉTIER-BASED CHARACTERISATION OF LONGLINE FISHERIES IN GREECE: PRELIMINARY RESULTS

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Abstract

The present study has identified the main métiers practiced using longlines in three subareas (northern, central, southern) of the Ionian Sea. The data used were gathered from the Greek Data Collection Framework program, during 2013-2020. A three-step procedure was followed in each subarea: the first step involved setting the trip ID, haul number and hook size as key trip variables for distinguishing fishing operations, the second step created landing profiles and applied Principal Component Analysis to reduce the complexity of the dataset, and the third step implemented a hierarchical cluster analysis to identify potential métiers. In all three subareas, two major métiers were identified, and although in the north and central Ionian most of the species targeted were Sparidae, in the south Ionian *Merluccius merluccius* and *Galeus melastomus* accounted for the biggest part of the landings. Additionally, the catch composition, by-catches and discards showed differences between the métiers, and subareas, reflecting their distinct ecosystem characteristics. Overall, our outcomes suggest the need to analyze the impacts of métiers at aggregation level 6 (hook size) at subregional/local scale, with the aim to shed further light on the fishing practices that may potentially affect the sustainability of the natural resources.

Keywords: cluster, mediterranean fisheries, Ionian, landings, discards.

1. Introduction

Successful implementation of the Ecosystem Approach to Management (EAM), used within the Common Fishery Policy (CFP) and the Marine Strategy Framework Directive (MSFD), requires solid information on the spatial dynamics of the different types of fisheries. The latter includes knowing where fishing is occurring, what gear is being used and what is being caught, and hence, active engagement with fishers in this approach is of crucial importance. The vast majority of the Greek fishing fleet (96.5%) comprises small scale fishery (SSF) vessels (Anon., 2020), most of which are below 8 meters. SSF fisheries are characterized by numerous vessels that target a variety of species under a complex scheme of fishing effort allocations and interactions. The overall contribution of vessels that operate as longlines to the annual small-scale landings is about 20% (Anon., 2020), and hence they have significant socio-economic importance, as they contribute to sustaining the livelihoods of fishery-dependent coastal/island communities. Métier analysis is commonly used in mixed fisheries to identify common patterns in individual fishing trips with respect to species composition, target species, fishing gear, time, location, and season (Castro *et al.*, 2011; Falsone *et al.*, 2020; Katsanevakis *et al.*, 2010; Pelletier & Ferraris, 2000; Tzanatos *et al.*, 2005). In this study, we tried to identify the main métiers practiced using longlines in three subareas (the northern, central, and southern parts) of the Ionian Sea (GSA 20), distinguished following administrative and geo-ecological criteria.

2. Material and Methods

The data used were collected as part of the Greek Data Collection Framework (DCF) program in the Ionian Sea by trained scientific observers on board commercial SSF vessels from 2013 to 2020. To conduct our analysis, we had at our disposal the following sources of data: trip-ID, haul number, catch composition (i.e., landings in total weight by species, distinguishing between commercial and discards), hook size, and month. The dataset was split into three subareas within the Ionian Sea (the northern, central, and southern parts), to explore differences in fishing practices that may reflect the different environmental characteristics, e.g., South Ionian fishing grounds comprise more deeper, open sea sites as compared to the other two areas of the Ionian Sea (Katsanevakis et al., 2010). Moreover, within each subarea, data were collected from different vessels, which can exhibit different exploitation patterns.

A multivariate clustering framework was applied to identify groups of homogeneous métiers, following the approach taken by Pelletier & Ferrari (2000) and applied by Katsanevakis *et al.* (2010) and Falsone *et al.* (2020). For each subarea, we conducted a separate analysis. First, the trip ID, haul number and hook size were used as key trip variables for grouping and distinguishing fishing operations. Then, landings were organized into a data matrix with fishing operations as rows and landings per species as columns. The weight of the landings was transformed into landings per 500 hooks to reduce biases related to fishing effort, and a landing profile (relative species composition) was defined by dividing the weight of the landings per species by the total weight of the landings derived from their fishing operation. Landing profiles were then log-transformed to make their distribution symmetrical. Very low landings, i.e., species caught below 0.05% of the fishing operations, were excluded from the analysis. A principal component analysis (PCA) was applied on the data matrix to account for a lower dimensional summary of the original variables. A hierarchical agglomerative cluster analysis (Ward, 1963) was applied on retained principal components to create clusters of different landing profiles amongst the fishing operations. The optimal number of clusters was determined using the silhouette coefficient (Rousseeuw, 1987), as in Castro *et al.* (2009). Silhouette measures the internal homogeneity of each cluster and its external separation with the other clusters, providing a more objective selection of number of clusters rather than a visual separation. It ranges between -1 and 1, with higher values indicating a better separability of clusters. Each cluster was then considered as representing a potential longline métier.

3. Results

In all subareas, the highest values of Silhouette coefficients identified two clusters for grouping homogeneous fishing practices. In the north Ionian Sea, as hooks used in most cases recorded by observers were smaller than size No 11, both identified métiers Sparidae specimens of the species *Sparus aurata*, Pagellus *erythrinus* and *Diplodus sargus* in their landing profiles, while Epinephelus *aeneus* and *Pteromylaeus bovinus* were grouped in métier 2 (Fig. 1, upper panel). Indeed, almost 70% of fishing operations in metier-1 used hook sizes of 15, whereas in metier-2 the main hook sizes used were 13, 14, 15 (~70% of operations) (Fig. 2). Fishing activity in metier-1 was higher mainly during month July and November (~30% of operations), whereas in métier-2, activity was rather uniformly distributed between months May-November. The average±sd catch (Kg) per fishing operation was 32.4±34.4 for métier 1 and 51.7±38.5 for métier 2, while the average number of discards species per fishing operation was 28 in métier 1 and 71 in métier 2. In the central Ionian Sea, the landing profiles between the two identified métiers found different in terms of the key species; *Epinephelus aeneus* and *Dentex dentex* were the most abundant species in métier 1, and *Sparus aurata* and *and Diplodus sargus* were the top-2 commercial species in métier 2 (Fig. 1, upper panel). Hook size 14 was the dominant size for métier-1 (100% of operations), while for métier-2, the main hook sizes were 11,12 and 14, covering the 60% of fishing operations. Fishing activity in metier-1 peaked in February and July (~35% of operations), whereas fishing activity in métier-2 was rather uniform during April-November. The average±sd catch (Kg) per fishing operation was 184.6.1±184.2 for métier 1 and 22.3±50.4 for métier 2, while the average number of discards species per fishing operation was 20 in métier 1 and 10 in métier 2. In the southern part of the Ionian Sea, *Merluccius merluccius* and *Galeus melastonus* were found as the main species in the landing profiles of both métiers, showing, however, differences in the catch composition of the other commercial species (Fig. 1, upper panel). The dominant hook size was 7 in métier 1 (~80% of operations) and 6 in métier 2 (~55% of operations).April and November showed the highest activity in métier 1 (~30% of operations), and March,May,October for métier 2 (~%40 of operations). The average±sd catch (Kg) per fishing operation was 155.7.1±301.9 for métier 1 and 19.2±41.3 for métier 2, while the average number of discards species per fishing operation was 10 in métier 1 and 8 in métier 2.

The catch composition of discards within identified métiers per subarea are shown in Fig 1 (lower panel). In the north Ionian Sea, *Pteromylaeus bovinus* and *Dasyatis pastinaca* were the most abundant discards in métier-1, whereas *Conger conger* and *Murarena helena* dominated discards in métier-2. In the central Ionian Sea, *Conger conger, Diplodus annularis and Serranus scriba* were the key discards in métier-1, and *Diplodus annularis* and *Pagellus erythrinus* were the most representative discards in métier-2. Accordingly, in the south Ionian Sea, *Hexanchus griseus* specimens formed the bulk of the discarded portion in métier-1 and *Conger conger* was the dominant discarded species in métier-2.

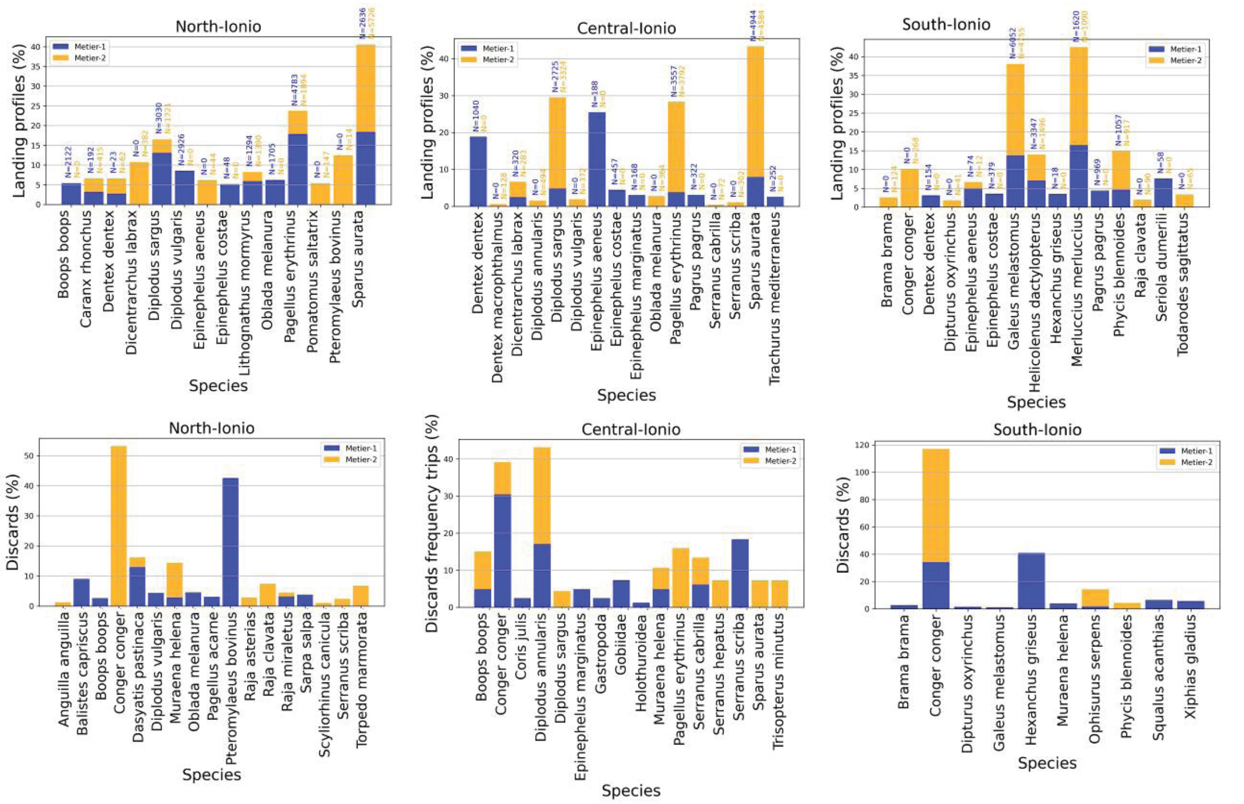

Fig. 1: Upper panel: Landing profiles of key commercial species, calculated as percentages of biomass, per métier and subarea in the Ionian Sea. Legend values show the number of fish individuals captured. Lower panel: Discards of key species, calculated as percentages of biomass, per métier and subarea in the Ionian Sea.

4. Discussion/Conclusion

Despite the partial overlap in catch composition found within the identified métiers, distinct patterns in fishing activity were also noticed within them. Two major métiers were identified in all three subareas, their catch composition, by-catches and discards showing different patterns. Although Sparidae species were dominating in the central and north Ionian longline métiers, where fishers used relatively small hooks, in the S. Ionian Sea, an open sea area with deeper waters, fishers used large hooks and mainly targeted large specimens of *Merluccius merluccius*. The latter is a species of major commercial importance considered however as not being fished sustainably (Fernandes *et al.*, 2017), and hence it is important to identify specific areas and métiers where large hakes are caught, particularly during spawning periods.

What is more, the presence of vulnerable species in the catches of a particular métier should be considered with caution; in the case of *Pteromylaeus bovinus*, a data-deficient species, there were 14 specimens caught in the north Ionian Sea, that in métier-1 were all discarded, while in métier-2 they were all landed, suggesting a different behavior by fishers considering the value of their overall landings. In future work, we aim to explore fishing activities at a finer scale by separating fishing operations between large and small hook sizes in longlines. It is important to investigate practices that may have an impact on commercial stocks, either by catching undersized fish, or spawning individuals of particular species, as well as on species of conservation importance, considering also those with commercial interest (e.g., *Epinephelus marginatus*). Overall, outcomes of the present study suggest the need to analyze the impacts of gears/métiers at aggregation level 6 (mesh/hook size) at subregional/local scale, with the aim to shed further light on the fishing practices that may take place within each GSA and may potentially affect the sustainability of the natural resources, requiring thus suitable spatio-temporal management measures.

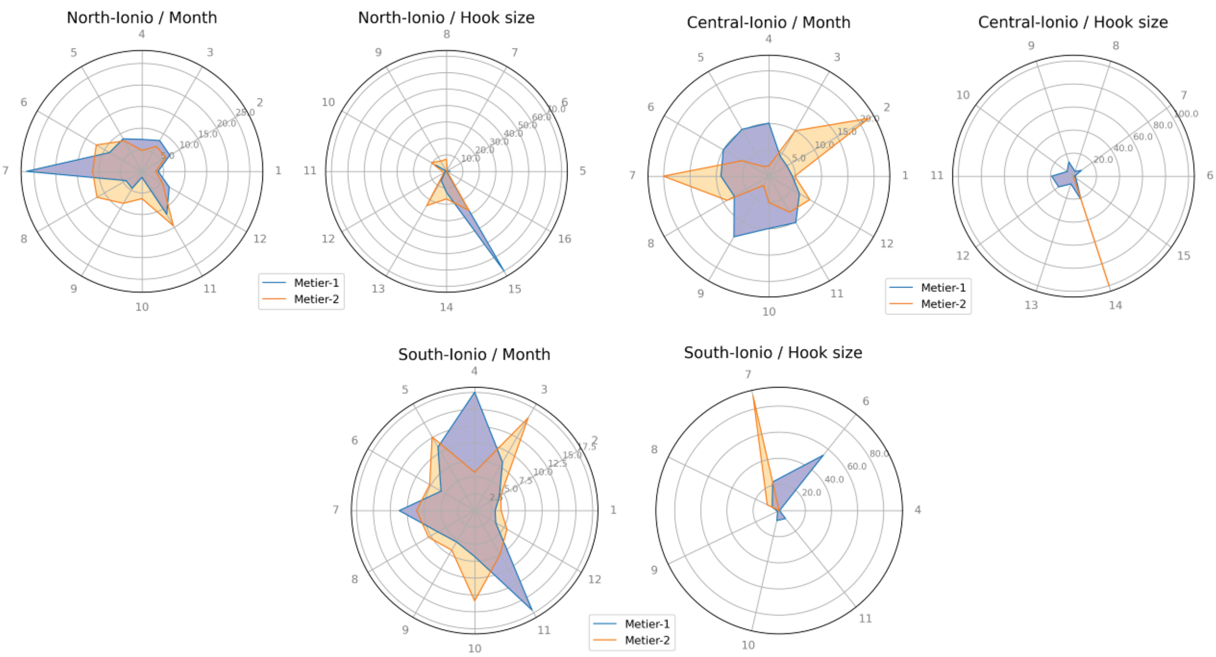

Fig. 2: Distribution of fishing operations per month and hook size per identified métier and subarea.

5. Acknowledgements

This work has been supported by the Greek Data Collection Framework Program and the implementation of monitoring under the Marine Strategy Framework Directive in Greek waters.

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USE OF AIS DATA TO ASSESS BOTTOM TRAWL FISHING EFFORT IN INTERNATIONAL WATERS OF THE AEGEAN SEA

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Abstract

Bottom trawling is totally prohibited in Greek waters during the summer months of August to September, however, fishing in international waters of the Aegean Sea is selectively allowed to Greek trawlers. Fishers from other countries also operate concurrently in the same waters and time period. In this work, we use data from the Automatic Identification System (AIS) and report that during the summer fishing ban of September 2020, bottom trawl fishing effort exceeds 4500 hours, including fishing vessels of non-Greek nationality.

Keywords: Automatic Identification System, fishing activity, fisheries management.

1. Introduction

Bottom trawling in Greece is a multispecies fishery (Stergiou *et al.*, 2003) that is regulated by several national laws, Royal and Presidential decrees, and European Union (EU) regulations (Kapantagakis, 2007). Notwithstanding exclusion zones near the shore, in closed bays or over *Posidonia oceanica* meadows, the main restriction for this gear is that bottom trawling is prohibited in Greek waters from August to September, as most demersal fish species in this region are summer spawners (Tsikliras *et al.*, 2010).

However, fishing licenses can be selectively granted to Greek trawlers during the summer fishing ban, on the condition that bottom trawling will occur in the international waters of the Aegean Sea (i.e., at a distance > 6 miles from the shore) and east of the 25th meridian. This contradictive regulation has raised scientific concern (Karachle, 2013; Tsikliras *et al.*, 2013) for maintaining fishing effort during a period that stocks are meant to be protected from fishing. The concern is further exacerbated by the fact that bottom trawlers from other countries are also operating concurrently in the same waters and time period.

The activity of fishing vessels can be monitored *via* data from the Vessel Monitoring System (VMS), but confidentiality issues hinder their use for scientific purposes (Hinz *et al.*, 2013; Natale *et al.*, 2015). As of May 2014, EU fishing vessels of overall length > 15 m are fitted with an Automatic Identification System (AIS) transceiver (EU, 2011), which is a short-range VHF navigational aid that broadcasts near real-time state vector (e.g., position, speed) and static (e.g., vessel identifier) information about a vessel.

In this study, we use AIS-based data to assess the fishing effort in the international waters of the Aegean Sea during August and September 2020, estimating fishing activity based on AIS vessel identifiers, speed profiles and patterns of movement.

2. Materials and Methods

Our study areas were the General Fisheries Commission for the Mediterranean (GFCM) geographical sub-areas 22 and 23, i.e., the Aegean Sea and the Sea of Crete. From September 1st to September 30th 2020, we used the publicly accessible online platform www.marinetraffic.com to manually monitor the AIS messages of bottom trawling vessels with an overall length greater than 15 m. Logging was performed daily and on an hourly basis, from 06:00 AM to 16:00 PM. Ship type and flag state were identified according to the Maritime Mobile Service Identity (MMSI) of each vessel, cross-referenced with the EU fishing fleet register database (DG MARE, 2017). Whenever this information was not available, ship type was determined empirically based on its photograph registered on www.marinetraffic.com and its overall movement pattern during the study period.

The vessels performing bottom trawling are usually moving in repetitive straight-line courses reaching limited small velocities (2-5 kn) (Ferrà *et al*., 2020). Based on a series of studies based on the subject, we used the same method in order to track and monitor the general movement of the fishing vessels located in the study area (e.g., Russo *et al*., 2016; Vespe *et al*., 2016; Ferrà *et al*., 2018). The activity of each vessel was estimated according to this specific moving pattern. Using spreadsheet software, we defined two possible states for the fishing vessels a) active fishing named YES and b) different form of movement named NO. We estimated one hour of fishing effort when two consecutive active fishing indicators occurred within the same 24h period, and half an hour when an active fishing indicator was followed by a different form of movement indicator.

Using QGIS, we created buffer zones representing the Greek territorial waters. We divided the vectors based on their imprint being spotted within the Greek territorial waters or outside of them. Thus, we calculated the fishing effort hours for every monitored nationality as well as their summary for the international and Greek waters.

Working towards data enrichment, older vectors were also used, for August 2020, which were obtained via packets available for sale from the platform www.marinetraffic.com. The final calculation of the fishing effort for August 2020 was completed following the same principle and method as we did for September.

3. Results

Overall, 5929 AIS messages were recorded in the study area during September 2020, originating from 119 bottom trawlers that were registered to Greece (GR, 24 vessels), Turkey (TR, 63 vessels), and Italy (IT, 32 vessels); vessel total length ranged from 20 to 35.5 m (Table 1).

Table 1. Bottom trawl fishing vessels observed during September 2020, tabulated by flag state.

Flag state	Number of vessels	Number of AIS messages	Vessel total length (m)	
			Mean	Range
GR	24	938	26.6	20 – 34
TR	63	664	23.4*	16 – 36*
IT	32	4327	30.4	26 – 35.5

* Length information was not available for 3 vessels.

Following the methodology which was described earlier we calculated the fishing effort (hours) per nationality and in totality for both months under investigation, dividing them by fishing effort within Greek waters and in international waters.

Table 2. Estimated bottom trawl fishing effort during September 2020.

Flag state	Number of vessels		Fishing effort (hr)	
		Greek waters	International waters	Total
GR	24	47	658.5	705.5
TR	63	123.5	295.5	419
IT	32	95	3321	3416
Total	119	265.5	4275	4540.5

Table 3. Estimated bottom trawl fishing effort during August 2020.

Flag state	Number of vessels		Fishing effort (hr)	
		Greek waters	International waters	Total
GR	2	2	395.5	397.5
TR	2	15	37	52
IT	2	6	368.5	374.5
Total	6	23	801	824

The visualization of data which can be seen in tables 1 and 2 are represented below in map format where it is easier to distinct the Greek territorial waters and each vessel's vectors, divided by nationality of origin and activity.

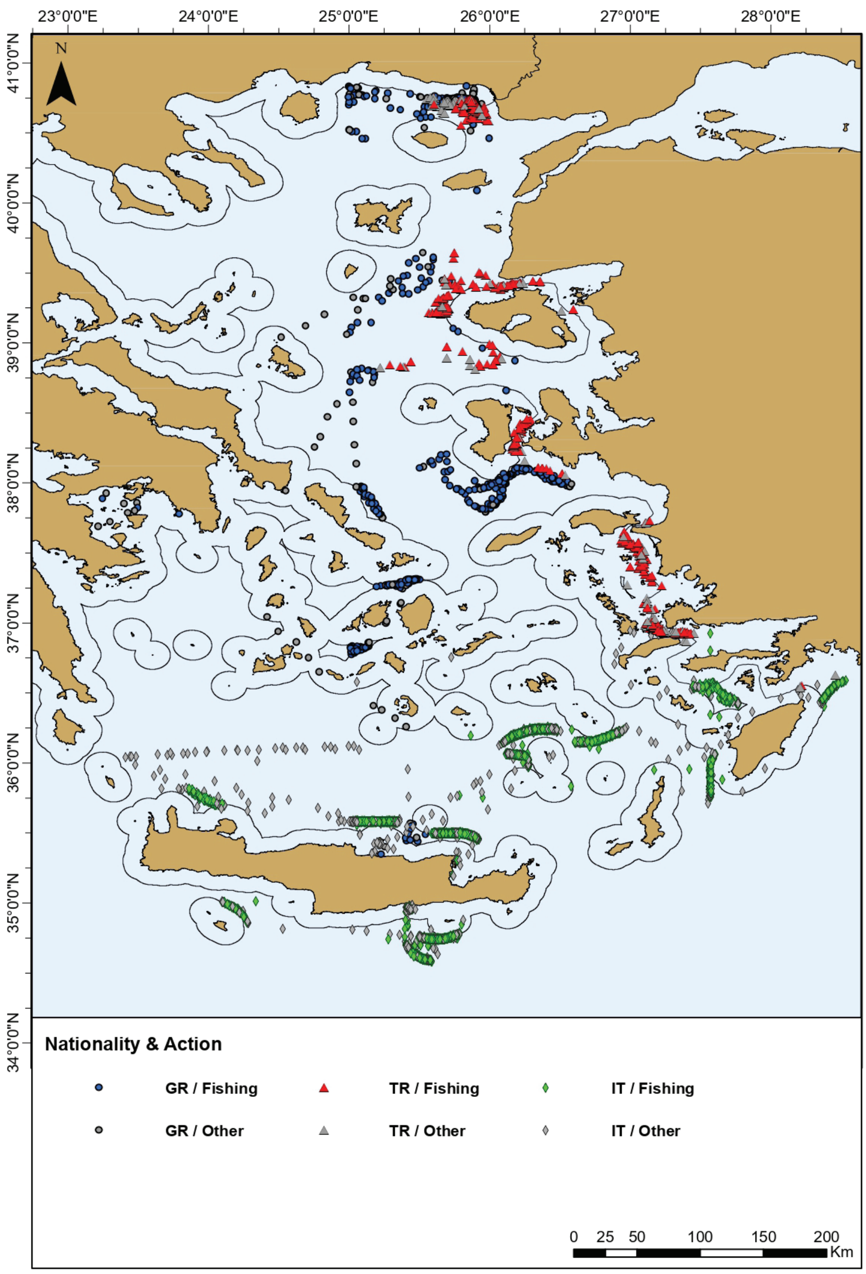

Fig. 1: AIS-based bottom trawler tracks during September 2020, color-coded by flag state. Each nationality is represented by its distinctive color (Blue for Greek vessels, Red for Turkish and Green for Italian) when engaged in fishing. All vessel activities besides fishing (steaming, anchored) are represented by the color gray regardless of the vessel's flag state. The black lines denote the Greek national waters.

4. Discussion/Conclusion

The legislative directive is instituted in order to protect the population of fisheries that are reproducing during the summer period. Concurrently it is easily observed that fishing is taking place on the border limits of the Greek territorial waters as well as some minor breaching of their confidentiality. The fluid interpretation of the legislative directive combined with the serious lack of monitoring of vessel activity by the enforcers responsible makes matters worse for an already intractable situation. Considering how manageable the monitoring of fishing vessels is, as shown by this study, maybe the time has come for a sincere and constitutive endeavor of fishing vessel tracking and monitoring. If we focus on an essential outline of the pragmatic instead of the hypothetical vessel activity, we can actually put the current legislative directive to the test while at the same time enriching our scientific models and data. Even if the method presented in this study is currently in an early stage it can in fact produce a clear image of the tremendous pressure placed upon the study area by fishing activities during a period which mandates restrictions on fishing effort to preserve fisheries population. Further research is needed on methods of monitoring to optimize the current methodology, further data gathering and the production of optimized scientific models.

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BOTTLENOSE DOLPHIN DEPREDATION IMPACTS ON THE THERMAIKOS GULF GILLNET FISHERY BASED ON EXPERIMENTAL FISHING AND QUESTIONNAIRE DATA

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Abstract

Dolphin depredation is a long-standing source of human-wildlife conflict in the Mediterranean, but its impacts on small-scale fisheries can be varied and difficult to quantify. The aim of this study was to measure catch loss due to depredation in a bottom-set gillnet fishery in the inner Thermaikos Gulf (northern Aegean Sea). We conducted experimental trials over two seasons, in 2020 and 2021, and performed questionnaire interviews in 2021. Catch weight and number of damaged individuals were calculated per haul and modelled to assess the impact of depredation. Self-reported catch weight from the questionnaire data was also run through a similar model. Bottlenose dolphins depredated both experimental (RV) and questionnaire (GN) hauls with similar frequency (RV: 32.5% of 80 hauls; GN: 27.5% of 40 hauls). Depredation significantly reduced intact catch weight by 231% in RV hauls and 194% in GN hauls, while the number of damaged individuals in RV hauls increased significantly from 10.4% when dolphins were not present to 21.8% when they were. Besides quantifying the direct loss in catch, our findings highlight the similarity between self-reported and directly observed effects of depredation in coastal gillnet fishing.

Keywords: cetacean depredation, catch size, gear damage, coastal fisheries.

1. Introduction

Cetaceans taking fish from nets has long been a source of human-wildlife conflict in the Mediterranean (Bearzi, 2002). This behaviour is termed depredation and can have serious financial impacts on small scale fishers (e.g., Bonizzoni *et al*., 2016; Gonzalvo *et al*., 2015). Fishery observers or questionnaire surveys are the most frequently used approaches to quantify the scale and likelihood of such interactions (e.g., Garagouni, 2013; Milani *et al*., 2019; Pardalou & Tsikliras, 2020), but have not often been tried concurrently. Questionnaire surveys also generally include one interview per fisher and can include questions referring to periods of a year or more. For small scale fishers, even a small financial loss can be very serious, thus, when an interviewer asks about depredation rates or the magnitude of income loss, there may be a tendency on the part of the interviewee to overestimate (Garagouni, 2013). Repeated questionnaires, however, referring to shorter time periods, could potentially remove some of the personal bias from such interviews.

The present study fulfilled two objectives, namely: the quantification of catch size reduction because of cetacean depredation; and the comparison of this reduction measured in experimental hauls with the self-reported catch changes in concurrent questionnaire surveys.

2. Material and Methods

Two types of data were collected in this study, the first through a series of experimental hauls on a chartered fishing vessel (RV) and the second through a questionnaire survey of coastal fishers. Both the fishing and interview efforts took place in the inner Thermaikos Gulf in the northern Aegean Sea, one of the most important fishing grounds in Greece, both for medium- and small-scale fisheries. The experimental hauls were conducted outside the port of Nea Michaniona, while questionnaire surveys were conducted there and in the neighbouring port of Angelochori.

Fishing surveys took place over two seasons, from May to October in 2020 and 2021. We chartered a coastal fishing vessel (8 m, 2.3 GT, 43 hp) for the experimental part of the study and used bottom-set nylon gillnets, with a stretched mesh size of 36 mm. Each net panel measured 100 m in length and 1.8 m in height and was attached to a head rope with floaters and a lead-cored ground rope. Three net panels were attached to form a 300 m long fleet, and three fleets (that is, a total of 900 m) were deployed in each haul. Nets were set just before sunrise, in depths shallower than 20 m, and allowed to soak for approximately 1.5 hours. Throughout each fishing effort, two observers on board visually monitored the area for cetaceans and other predators. If cetaceans were sighted, the species was identified, their number estimated, and their behaviour around the fishing gear was recorded (Fig. 1a).

All fish and invertebrates caught in the nets were removed from the gear and subsequently taken to a wet lab for processing. Every individual was identified to as low a taxonomic level as possible and morphometric measurements, as well as weight, were taken. The catch was also visually assessed for damage due to depredation, such as bite marks or missing body parts, and individuals were labelled as intact or damaged (Fig. 1b, c).

Interviews were conducted in 2021, almost always on the same dates as experimental trials, with fishers who had just returned from their own hauls. The same fishers were approached as frequently as possible in order to get consistent information. Questions were asked regarding that day's fishing effort (including hr at sea, location, depth, type & length of fishing gear), catch size and composition (including kg and species caught), and whether or not dolphins interacted with their gear. To gain as complete a picture of depredation rates as possible, we also asked about fishing effort and cetacean interactions on the days preceding the questionnaire.

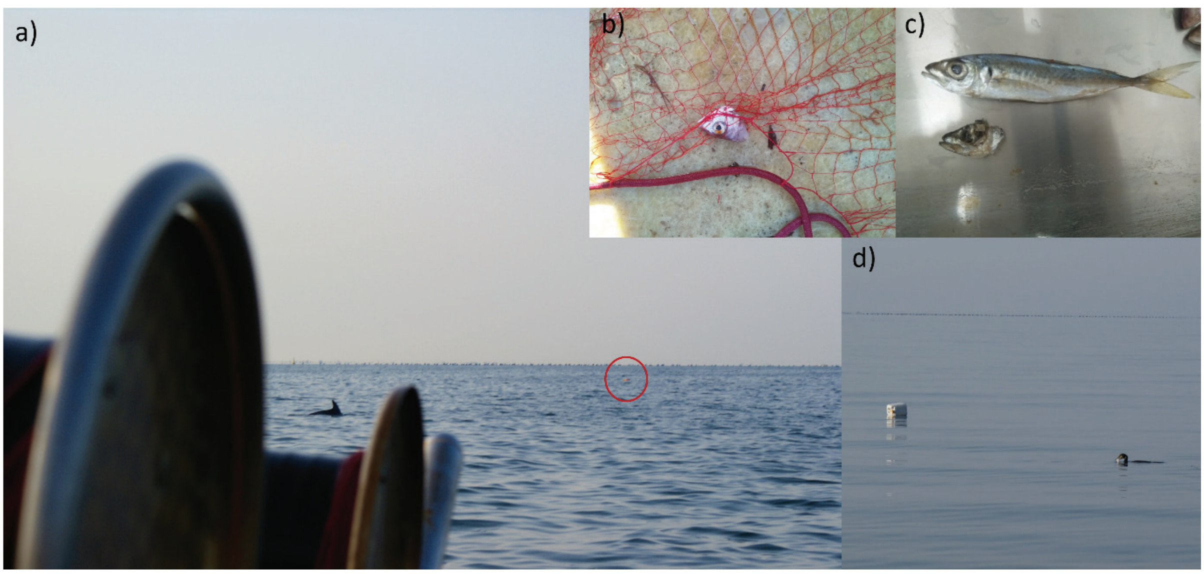

Fig. 1: a) Bottlenose dolphin surfacing in front of fishing vessel as gillnet is being hauled (surface buoy in red circle marks the end of that net). b) Head of a *Pagellus* sp. found on net following a depredation event. c) Intact (top) and damaged (bottom) *Trachurus* sp. identified in the lab. d) Cormorant surfacing near gillnet surface buoy with a fish in its mouth.

Depredation frequency was compared between the experimental hauls and the questionnaire fishing effort using a chi-squared test. The effect of depredation on catch size in the experimental hauls was assessed by modelling catch per effort (kg/haul) against dolphin presence, during the months when dolphins were most active (June to September). Only intact fish and cephalopods were used in this analysis, as the damaged individuals are essentially catch lost due to depredation. Because we observed a seasonal fluctuation in catch sizes, a mixed effects generalised linear model (GLMM) was used, with Month as a random effect and a Gamma error distribution family (log link).

A similar model was run on the self-reported catch size in the questionnaire data, which referred only to intact fish and marketable cephalopods. In order to be fully comparable with our experimental findings, we only included data from gillnet fishing effort that took place in depth < 20 m and when dolphins were reported most frequently (May to September).

The number of damaged individuals in our experimental catch was also compared between days when dolphins were the observed predators and days when they were not, using a chi-squared test. To test whether the pattern is consistent across all months despite the catch size fluctuations, we ran a GLMM on the number of damaged individuals per haul versus dolphin depredation occurrence, with Month as a random effect, total catch size (number of fish and cephalopods per haul) as an offset, and a Poisson (log link) error distribution.

3. Results

We conducted 36 experimental hauls (RV) in the first season and 44 in the second. Bottlenose dolphins (*Tursiops truncatus*) were observed depredating our gear during 11 hauls in 2020 and 15 in 2021, or 32.5% of all hauls. A total of 40 interviews pertained to shallow-set gillnets (GN, in 35 of which catch size was reported) with a 27.5% depredation rate. The chi-squared test confirmed that depredation frequency was similar between RV and GN fishing effort ($\chi^2 = 0.173$, $df = 1$, $p = 0.677$). Depredation events peaked in July and August and tapered off towards the end of both seasons. Catch for all cases is shown in Table 1.

Table 1. Catch sizes in kg and number (n) of individuals of intact and damaged fish (where applicable), for two seasons of experimental hauls (RV) and one season of questionnaire data (GN), in relation to dolphin depredation.

	Dolphins absent		Dolphins present	
	Intact fish (n; kg)	Damaged fish (n)	Intact fish (n; kg)	Damaged fish (n)
RV 2020	3134; 152.2	191	575; 25.8	80
RV 2021	3419; 158.7	246	405; 17	132
GN 2021	- ; 252.6	-	- ; 30	-

Both RV and GN catch was significantly lower in the presence of dolphins than in their absence, in months when cetaceans were most active (Fig. 2a, b). Indeed, both GLMMs resulted in similar estimated coefficients for the effect of depredation (RV: -2.31, $SE = 1.2$, $p < 0.001$, *conditional* $R^2 = 0.397$; GN: -1.94, $SE = 1.24$, $p = 0.003$, *conditional* $R^2 = 0.407$).

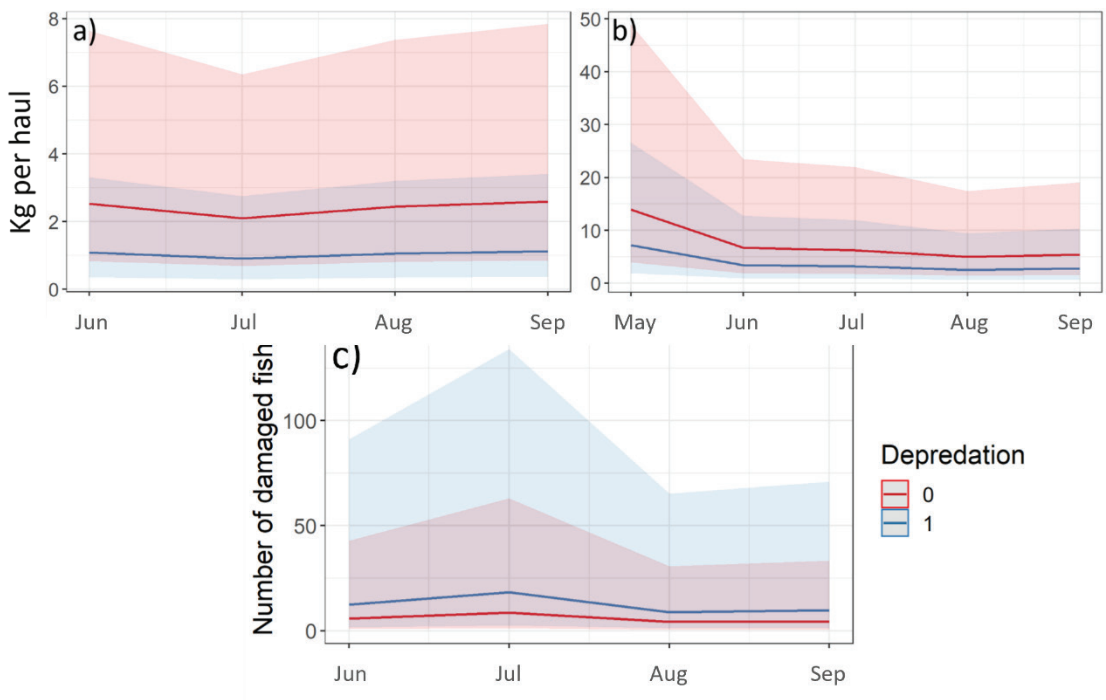

Fig. 2: Monthly GLMM predictions depending on whether dolphin depredation occurs (1) or not (0): (a) catch (kg) per haul based on experimental data; (b) catch (kg) per haul based on questionnaire data; (c) number of damaged individuals per haul.

Within the months when dolphins were most active (June to September in both years), the percentage of damaged fish per RV haul increased significantly from 10.4% when dolphins were absent to 21.8% when depredation occurred (χ^2 = 59.96, *df* = 1, *p* < 0.0001). The GLMM showed that this pattern remained consistent across all months (*estimated coefficient* = 2.12, *SE* = 1.11, *conditional* R^2 = 0.64; Figure 2c).

4. Discussion/Conclusion

Both experimental and questionnaire data showed clear changes in catch size due to dolphin depredation. Not only were the overall interactions with bottlenose dolphins in the area as frequent in our directly observed hauls as in the questionnaire surveys, but the magnitude of catch loss was also similar between the two. This is in contrast with other studies, which have shown that fishers overstate the negative effects of depredation in questionnaires (e.g., Bearzi *et al.*, 2011). This increased accuracy is a direct result of the repetitive nature of the interviews covering the most recent daily or biweekly records, as opposed to monthly or yearly estimates of depredation frequency and intensity.

Regarding the number of individuals damaged by depredation, we confirmed that such damage can happen even when dolphins are not the perpetrators. Indeed, during the experimental hauls we often observed cormorants diving near our gear and surfacing with fish in their beaks (Fig. 1d), on some occasions even damaging a very large proportion of the catch. So other marine megafauna do contribute to catch loss, and it is possible that because they are less noticeable than the dolphins, the latter are blamed by the fishers even when they (and associated gear damage) are not directly observed (Garagouni *et al.*, in press). Of course, the higher magnitude of the damage attributable to dolphins is clearly evident from our own data, and in fact highlights another reason that catch loss is difficult to quantify. That is, the difference in catch size is much larger than the number of damaged fish when dolphins are present—dolphins clearly remove large amounts of fish biomass either from the gear or the surrounding water column without leaving a measurable trace.

Our findings highlight the importance of conducting concurrent surveys of both directly observed and self-reported fishing effort, as a means of ground-truthing the accuracy of the latter and gaining a clearer bigger picture. Repetitive interviews are crucial to reducing personal bias in the estimation of depredation impacts. Finally, bottlenose dolphins do remove substantial and consistent amounts of biomass from the gillnets in the area, which should be a strong incentive to develop new mitigation techniques.

5. Acknowledgements

We wish to thank the fishers who graciously participated in the questionnaire surveys, as well as Theofanis Karydas, for skippering our chartered vessel and sharing his insights. The present work was performed within the framework of CETA_NET project funded by the Greek Operational Programme for Fisheries and Sea (2014-2020), under the "Innovation for Fisheries" call [MIS 5030544].

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SPECIAL SESSION: MEDITERRANEAN SEA (AND FRESH WATER) LITERACY IN THE ERA OF 2030 AGENDA FOR SUSTAINABLE DEVELOPMENT AND DECADE OF OCEAN SCIENCE FOR SUSTAINABLE DEVELOPMENT (2021-2030)

Marine and Inland Waters Research Symposium
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2022

MAPPING THE OCEAN LITERACY MOVEMENT: EXPERIENCES FROM THE PAST, PRESENT INITIATIVES, AND FUTURE EXPECTATIONS

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Abstract

This study portrays in brief the history of marine education movement from its beginning in the late ’60s till the present day. Information is provided regarding how it started in the US, its parallel development with Environmental Education during the next two decades, its “fall” in the ’90s and rebirth in the dawn of the new century. More details are given for the actions taken over the last 15 years with the communication and acceptance of the novel Ocean Literacy framework outside the boundaries of its birthplace, as well as the first attempts of introducing and adapting Ocean Literacy to national contexts and the specificities of certain regions; the Mediterranean Sea Literacy guide is presented as a pioneering example of such an adaptation. Finally, recommendations are provided to meet the requirements of contemporary Agendas towards a sustainable protection and conservation of the blue planet.

Keywords: Ocean Literacy, Mediterranean Sea Literacy, Principles, Environmental education.

The volume of water and the surface it occupies is so vast that, if our predecessors knew, they would have called our planet *Ocean* and not *Gaia* (Earth). This is exactly the reason that made this planet unique in terms of life, as it is the marine environment where life first appeared 3.5-4 billion years ago and where it exclusively evolved for the next 3 billion years. The global ocean produces almost half of the oxygen and absorbs huge amounts of atmospheric carbon dioxide, regulates weather and climate, supports a great diversity of life, and provides food and minerals (Cava *et al.*, 2005). At the same time, however, it is treated as a place of easy deposition of the modern societies’ wastes, while a complete lack even of basic knowledge regarding its functions and values has come to light.

Side by side with the birth and development of Environmental Education (EE), another type of education, focused on the marine world, arose in the late ’60s. It was the time when we received the first photos of the Earth from outer space, revealing the magnitude of how blue our planet is, and at the same time how limited its capabilities can be. It was only then when the British writer Arthur C. Clark pointed out *how inappropriate is to call this planet Earth when it is quite clearly Ocean*. Moreover, space exploration events had become a daily routine to the modern world to such a degree that Slonin (1977 in Fortner & Wildman, 1980) elegantly described that *we know more about the backside of the moon than we do about the drop of water upon which each of us, and all living organisms, depend for survival*.

In light of the increasing pressures on the aquatic world, mainly from human activities, the need to understand this environment and how it functions became imperative and should concern not only the scientists and/or the maritime sector, but also the general public. Goodwin and Schaadt (1978) used the term “Marine and Aquatic Education” which was generally adopted for the next couple of decades. In parallel with the progress in EE, Marine and Aquatic Education followed a similar path of development and maturation in the ’70s and ’80s through a series of grassroots publications (e.g., Charlier & Charlier, 1971; Schweitzer, 1973; McFadden, 1973; Goodwin & Schaadt, 1978; Fortner & Wildman, 1980; Madrazo & Hounshell, 1980; Picker, 1980; Dresser & Butzow, 1981; Rakow, 1983/1984; Picker *et al.*, 1984; Fortner, 1985;

Fortner & Lyon, 1985; Fortner & Mayer, 1989). The conditions were ideal for its rapid establishment and development, basically on the west side of the Atlantic. Towards this direction, important decisions were taken such as the foundation of the National Marine Educators Association (NMEA) and the launching of the ambitious Sea Grant College Program.

However, in the mid-'90s it was highly marginalized, and when the new National Science Education Standards were launched in 1996 in the USA, an unjustified absence of ocean-related issues was revealed. That said, a new systematic crusade began, originally from the National Geographic Society (NGS) and the College of Exploration (CoE) to develop a guide with detailed ocean content that could be used to teach Geography, triggering all the upcoming actions and events that led to a new era of marine education. An in-person workshop in 2000 sponsored by the National Science Foundation (NSF) from which the Centers of Ocean Sciences Education Excellence (COSEE) emerged, and a virtual workshop that took place in 2004, co-organized by NGS, CoE, NMEA, the National Oceans and Atmospheric Administration (NOAA) Office of Education, the Lawrence Hall of Science, University of Berkeley, the COSEE and the NOAA's Sea Grant College Program, brought together educators and scientists to share their ideas regarding the important concepts everyone should know about the ocean (e.g., Mokos *et al.*, 2022; Payne *et al.*, 2022).

These ideas were the basis for conceptualizing the new framework, called *Ocean Literacy* (OL), organized under some essential principles and fundamental concepts. This framework contained (a) the definition of OL as *the understanding the ocean's influence on you and your influence on the ocean*; (b) the description of an ocean-literate person as the one who *understands the essential principles about the ocean, can communicate about the ocean in a meaningful way and is able to make informed and responsible decisions regarding the ocean and its resources* (Cava *et al.*, 2005); (c) a set of seven principles (Table 1) and 44 concepts (revised to 45 in March 2013); (d) a Scope and Sequence, a pedagogical tool to serve as conceptual guidance as to what students should be taught along the K-12 school grades (NMEA, 2010; NOAA, 2013). This huge attempt coincided with the respective reports released by the Pew Oceans Commission in 2003 and the US Commission on Ocean Policy in 2004, revealing, among others, the Americans' low level of content knowledge about the ocean (Payne *et al.*, 2022).

Table 1. Ocean Literacy principles (OLp).

OLp1: The Earth has one big ocean with many features
OLp2: The ocean and life in the ocean shape the features of the Earth
OLp3: The ocean is a major influence on weather and climate
OLp4: The ocean makes Earth habitable
OLp5: The ocean supports a great diversity of life and ecosystems
OLp6: The ocean and humans are inextricably interconnected
OLp7: The ocean is largely unexplored

The OL guide proved to be so successful in terms of acceptance by several actors, that became a model for other literacy guides, in the United States and all over the world, not only within the context of the water world (e.g., Great Lakes Literacy, Estuarine Literacy, Mediterranean Sea Literacy), but within other targeted environmental contexts such as Climate, Energy, Earth science, and Forest literacy. NMEA with a history that goes back more than 40 years now, under the prism of the new OL framework helped to spread the word for the creation of respective associations and networks around the world that could facilitate the integration and enhancement of OL guide to the specificities of the various regions. As such, the International Pacific Marine Educators Network (IPMSN) was formed in 2007, while the first association outside the US appeared in Europe, where the European Marine Science Educators Association (EMSEA) was founded in 2011. Soon after that, the Canadian Network for Ocean Education (CaNOE) was formed in 2014, a year later the Asia Marine Educators Association (AMEA), and the Latin American

Education Network for the Ocean (RELATO) followed. In parallel to the above, several corresponding initiatives have taken place from 2016 till the present day at the regional and/or national level, such as the Korean Marine Education Association, the EMSEA regional groups (EMSEA-Baltic, EMSEA-Atlantic, EMSEA-Northern Seas, EMSEA-Med), and the Ocean Literacy Italia (Mokos *et al.*, 2022; Payne *et al.*, 2022). The EMSEA-Med serves as a pioneering example of the efforts to adapt the OL guide to the specific features of a regional sea, resulting in the development of the Mediterranean Sea Literacy guide, that comprises seven principles and 43 concepts (Table 2) (Mokos *et al.*, 2020) (see also www.emsea.eu/regional-groups/mediterranean-sea, www.emsea.eu/ocean-literacy/publications).

Special thrust towards the direction of mobilizing the modern societies around marine issues was given by several political initiatives such as the "Galway Statement" signed in 2013 by the EU, USA, and Canada to promote transatlantic ocean research cooperation; the Atlantic Ocean Research Alliance (AORA) was also launched during the same period to coordinate research efforts, while in 2014 the Rome Declaration reinforced the concept of responsible research and innovation. In 2017, the Belem Statement was launched with the aim of uniting research in the north and south Atlantic through enhancing cooperation between Brazil, South Africa, and the EU. This is the time when the United Nations declared the Decade of Ocean Science for Sustainable Development (2021-2030), seeking to contribute to the achievement of the Sustainable Development Goal 14 of the UN Agenda 2030 and to extend its scope to fill the gap from "the ocean we have" to "the ocean we want"

Table 2. Mediterranean Sea Literacy principles (MSLp).

MSLp1: The Mediterranean Sea, semi-enclosed by land of three continents, is part of one big ocean and has many unique features
MSLp2: The Mediterranean Sea and its living organisms shape the features of the Mediterranean region and its adjacent landmasses
MSLp3: The Mediterranean Sea has a major influence on the climate and weather of the Mediterranean region
MSLp4: The Mediterranean Sea made the Mediterranean region habitable through its richness of life thus becoming the cradle of western civilization
MSLp5: The Mediterranean Sea is a marine biodiversity hotspot, with a high level of endemism
MSLp6: The culture, history, economy, lifestyle, health, and well-being of the peoples of the Mediterranean region are inextricably interconnected
MSLp7: Although the Mediterranean Sea has been explored for centuries, it still remains largely unknown

Despite all the above major actions undertaken basically during the last decade at national, regional, and international levels, what remains a big challenge is the financial support for OL research, initiatives and activities. Long experience from the US provides useful morals, as Payne *et al.* (2022) beautifully describe *what was once a well-funded initiative with a strong network of partners, now falls on the shoulders of few members of NMEA, and the financial and institutional support once provided has now waned tremendously*. This becomes even harsher for most of the cases in which OL receives no legal validation or serious endorsement of its existence. Towards this direction the European Union, for example, became active in 2014 when, under the Horizon 2020 programme, funded two large and ambitious multinational projects, ResponSeable and SeaChange to enhance OL, as well as the EU4Ocean Coalition since 2020. Moreover, in 2019 the European branch of the Global Ocean Observing System (EuroGOOS) of the Intergovernmental Oceanographic Commission (IOC) of UNESCO launched the EuroGOOS OL Network, emphasizing OL as a strategic activity area in oceanography (Eparkina *et al.*, 2021). Most recently another promising initiative launched by the EU Commission, but still in pursuit of financial support, is the establishment of the European Blue Schools Network engaged in OL.

Regarding the information presented earlier, no one can reliably evaluate the progress that has been achieved in OL over the last couple of decades without turning to the research efforts realized so far. Paredes-Coral *et al*. (2021), in their attempt to map global research on OL from 2005 to 2019, portrayed the existence of a rather low number of relevant publications with a slow-growing pattern especially during the first years, indicating that this field is not yet adequately cultivated. This could probably be attributed to the absence of common measurement tools which have to take into consideration not only the knowledge/awareness factor but, according to Santoro *et al.* (2017), the multi-perspective dimension of OL as well. In any case, important baseline studies to portray some of these perspectives, namely content knowledge, attitudes, and behaviour have been made so far on a global scale, while good practices of regional collaborations have also emerged, such as the EMSEA-Med group being the leader with its publications (e.g., Boubonari *et al*., 2013, Mogias *et al*., 2015; 2019, Realdon *et al*., 2019; Mokos *et al*., 2020; Koulouri *et al*., 2022).

In conclusion, important steps have already been made towards disseminating OL, but many others remain to be achieved. Translations of the OL guide into several national languages are on the way (see www.marine-ed.org/ocean-literacy/translations), while respective guides focusing on the specificities of certain sea basins have to be developed, according to the example of the MSL guide. Moreover, systematic collaborations among different actors (i.e., scientists, educators from both formal and non-formal educational settings, other stakeholders, policy and decision-makers) coming from governmental and non-governmental agencies are considered imperative for jumping to the next step of actually meeting the requirements of the UN Agenda 2030 and the Decade of Ocean Science for Sustainable Development (2021-2030).

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THE BLUE CHALLENGE FRAMEWORK: A GUIDE FOR THE DEVELOPMENT AND IMPLEMENTATION OF BLUE CHALLENGES AT SCHOOLS

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Abstract

The core principles of the Erasmus+ BlueS_Med project, namely co-construction and co-design of the Blue Challenges, interaction and proactivity, inclusiveness, and sustainability, guided the drafting of its first deliverable, The Blue Challenge Framework. This framework, based on the collection and validation of existing Ocean Literacy experiences and practices in "bringing the sea" to school, serves as guidance for the process of defining the Mediterranean Blue Challenges. Priorities, resources, and initiatives already available at the EU and international level are considered. Criteria for monitoring and evaluating the Blue Challenges to bring out their added value and benefits are proposed.

The application of this framework critically reflects on the pros and cons that Mediterranean teachers/schools consider before choosing, whether or not, to join the EU Blue Schools Network (EUBSN). Main obstacles that should be removed to facilitate the process of schools joining EUBSN were identified, while recommendations on how to eliminate them were provided. Finally, a first analysis of the resources and tools currently available at the European level was made. The EUBSN, based on this framework, is expected to encourage and help schools join in, and to consolidate an educational path to foster Ocean Literacy within the next 10 years and thus the ongoing Ocean Decade*.

Keywords: Ocean Literacy, EU Blue Schools Network, Marine Education, Mediterranean Sea, Sustainable development.

1. Introduction

To achieve the goal of bringing Ocean Literacy (OL) into the European school system in the short and long run, teachers' involvement is necessary. In order to get and keep European teachers engaged, the EU citizens, through the DG MARE, should increasingly support them, recognize their efforts and give them something in return. Schools in the EU in general, and in some Mediterranean countries in particular, are more and more charged with additional requests, commitments, and educational activities, often without adequate investment in human and economic resources, as well as without enough time to cope with all these new overall challenges and tasks. Incentives, professional development training, educational resources, and recognition for them are therefore needed.

The Erasmus+ project entitled "Supporting the development of socially-inclusive Blue Challenges in schools in the Mediterranean Sea basin" (BlueS_Med) aims to develop, implement, and evaluate innovative approaches to integrate ocean/marine sciences, issues and challenges into school curricula, through educational activities, in four Mediterranean countries (i.e., France, Greece, Italy, and Malta), working side by side with the teachers of 14 pilot "blue" schools. Through these projects, the pupils (and teachers) will acquire the knowledge and skills enabling them to understand the complexity of the ocean/sea issues related to sustainable development, to think about their responsibilities, to propose and implement concrete actions. These responsibilities and actions will be presented and shared by delegates (i.e., the Blue Ambassadors) from the pilot school during project meetings, training and public events.

Combining expertise in education, marine sciences and policy, together with a long experience in the development of OL initiatives, the project foresees four phases (1/conception, 2/development of Blue Challenges, 3/ implementation, 4/ evaluation and dissemination). This paper describes and summarizes the results of its first phase, which focused on the conception of the Blue Challenges Framework (BCF) which guides the subsequent development, implementation and evaluation of these Blue Challenges at schools. The collaborative work accomplished during this phase also helped to consolidate the consortium and to share experiences and practices among the partners of Mediterranean countries.

2. Material and Methods

A series of dedicated meetings, both online and on-site, between the partners of the consortium as well as between partners and teachers, was organized during the first year of the project to discuss and define the aspects linked to the Blue Challenges Framework (BCF), listed below and in Table 1.

2.1. Conceptual steps to guarantee a successful implementation

Based on the *Handbook for Teachers* (Copejans *et al.*, 2020) published by the EUBSN, first steps concerned the elaboration of the framework and getting in touch with the (pilot) schools involved, in order to clarify the meaning of a “Blue Challenge”, and how to develop such at school. In line with the OL movement, each Blue Challenge should in fact be designed to: 1) increase pupils’ understanding of the ocean/sea and the relevant impacts, challenges, and risks; 2) develop responsibility in order to facilitate to take action for its protection and sustainable management. Through the Blue Challenge, pupils, teachers, and the whole school community, shall be able to explore how they are connected to the ocean/sea, whether they live near or far from it.

The second aspect concerned the definition of methods and criteria to be used by the BlueS_Med consortium to monitor and evaluate the Blue Challenges at school, as well as to start identifying best practices that will be communicated at the end of the BlueS_Med project to enrich the EUBSN. Each BlueS_Med partner, with their specific competencies, has been called to help understand: 1) which strategy is most effective to bring OL into the school environment; 2) what can be the useful/necessary methods and tools for a teacher to bring OL into classrooms; 3) why and how these methods/tools are useful/necessary in order to continue the OL efforts; 4) and help the teachers to get started.

As a third aspect, teachers involved in the project were asked to tell the BlueS_Med partners their needs (and difficulties encountered) to begin or improve their Blue Challenge and/or to build pathways in the pilot “blue” schools. Therefore, meetings were held to encourage discussions, share ideas and facilitate the process for teachers to choose an appropriate Blue Challenge for their school. Moreover, a survey was developed and undertaken to collect information and to allow an appropriate reply via an operational tool that will be developed by the BlueS_Med consortium (see below, 2.2).

The last aspect then concerned the practical definition of the “logical path” to follow in order to bring the Blue Challenges to school. After the first phase, this conceptual framework will be tested throughout the duration of the BlueS_Med project. Based on the results of this test, a revised version of the framework will then be made available at the end of the project.

2.2. Operational tool to facilitate implementation

In order to facilitate the implementation of OL aspects as described above, the partners’ consortium creates an interactive and evolutive internet-based platform, integrated into the project website. This will be used to collect the framework elements, educational resources, and make such information easily accessible to blue schools. The platform will also progressively include upcoming innovative/new resources, instructional materials, and best practices as school projects are implemented.

3. Results

The main results of the project's first phase were to have:

A. outlined the conceptual framework, the so-called Blue Challenges Framework (BCF; corresponding to the deliverable "Intellectual Output 1"), and in particular have clarified the meaning of what is a Blue Challenge. In addition, methods and criteria were proposed by the BlueS_Med consortium in order to monitor and evaluate the achievement of the Blue Challenges at school;
B. identified four main steps and associated actions to be set up and implemented during the BlueS_Med project to ensure the subset of the EUBSN will operate in a structured and efficient way and to guarantee its evolution (see Table 1);
C. collected a series of Blue Challenges and "blue path experiences" suggested by the partners, which can be used by teachers as best practices and an outline on which to develop their own "blue path experience" at school;
D. summarized teacher's feedback as for example many (especially those who want to undertake a blue path at school for the first time) prefer to have a defined pre-set reference scheme, with clear rules of participation and transparent evaluation.
E. fostered interaction between partners and teachers from different schools (and including different school levels) and Mediterranean countries through online and on-site meetings.

Table 1. Main steps, associated actions to be set, and identified activities to be implemented by the BlueS_Med project partners within the context of the EUBSN to ensure its development with a common structure and efficiency.

Step number	***Specific actions at the EUBSN level***	***Proposed activities (examples) identified in the framework for implementation and focusing on a national/local and/or school level / involved actors etc.***
Step 1	EUBSN proposes to the Blue Schools (BS) a structured path to follow based on objectives and results that EUBSN/DG MARE wants to achieve in the next 3-5 years, with monitoring and evaluation criteria and awards that are fixed, transparent, and defined from the beginning.	Definition of the path scheme (e.g., logical framework); definition of evaluation criteria & grid (i.e., participation and achievements); definition of awards (e.g., funds to build an OL lab at school, and/or to participate in a summer camp dedicated to pupils and/or teachers, and/or consumables for toolkits, and/or to participate in EU and MED events, and/or to help organize the Open Day at school, etc.) / DG MARE; EUBSN; scientific institutions and schools.
Step 2	Based on the steps of the BS relevant to the planned path, EUBSN/DG MARE assesses what has been achieved and how. Then, DG MARE awards progressive Blue School commitment by recognising increasing efforts at the end of the year at an official European event dedicated to the ocean (e.g., European Maritime Day, MED Coast Day, etc.).	Every year the BS can improve, take another step, extend the internal participation and/or the topics, involve more stakeholders and/or experts, organize a dedicated event, etc. / DG MARE; EUBSN; scientific institutions and schools.

Step 3	EUBSN will guarantee: a) independent evaluation of Blue School projects and achievements; b) supervision of the teacher training about marine sciences and related topics; c) advice on paths and priorities; d) innovation in the marine science teaching, etc. e) inclusion and accessibility; f) bridge towards ongoing OL research.	The EUBSN should create dedicated expert committee(s) with representatives of sciences from various disciplines including education, dissemination within the European countries, etc. / DG MARE; EUBSN; independent science and educational experts.
Step 4	DG MARE, or other dedicated EU or intergovernmental institutions, should stimulate EU and MED teachers and their pupils to a) learn more about ocean/sea issues in order to be able to compete with their peers in Europe and worldwide; b) to implement their “blue” OL laboratory at school.	Organisation of Ocean Literacy Olympics rewarding personal implication by taking into account various categories, and also offering adapted equipment, material and tools, etc. to strengthen a further development of BS / DG MARE; EUBSN; independent science and educational experts; schools.

4. Discussion/Conclusion

The key role of the BlueS_Med consortium is to identify and propose the most suitable framework to bring Blue Challenges in (Mediterranean) schools, as well as to identify best practices and applicability for the EUBSN. Bearing in mind the general objective of the project, namely to bring OL into school curricula, Blues_Med consortium proposes a path that takes into account a number of specific objectives, such as: train Blue School teachers (and science animators) on OL principles and concepts in general, and specifically on Mediterranean Sea issues; foster multi-level networks within the school environments, i.e. in the local community and among Mediterranean schools; create shared OL resources and materials to be used by teachers at school on EU and international level; develop a series of approaches to encourage critical thinking and bring closer the scientific method(s) as well as empower the pupils with a sense of citizenship and transversal skills (e.g., via problem solving, team building, argumentation and confrontational skills, authentic learning, constructivist approaches, discovery learning); promote the multi- and inter-disciplinarity approach; promote participatory approaches to include the whole school community (i.e., teachers, pupils, principals and families/parents); build a local learning “blue” community; visualize the “big picture” (i.e., the spatial-temporal context) in which the chosen Blue Challenge fits; align the educational pathways with the Agenda 2030 of the UNESCO Sustainable Development Goals.

We also help and recommend teachers with/to read/consult/share the selected EU publications (e.g., EU legislation, white papers, reports) and other reference material from intergovernmental organizations (e.g., UNESCO-IOC), as well as international scientific initiatives (e.g., IODP, GEBCO, Seabed 2030, EMSEA, EuroGOOS, IPCC) dealing with EU ocean/sea regulation, socio-economic agenda and priorities, and environmental research and challenges (Mokos *et al.*, 2020; Cappelletto *et al.*, 2021; Eparkhina *et al.*, 2021). They can be a source of inspiration for the choice of the Blue Challenge at school and a way to keep updated with what is going on in society.

Moreover, to implement a BS project, it is necessary to define a topic that makes it possible to establish a link between the pupils and their territories/local context. The pupils will be involved in choosing the research topic(s) while taking into account their interests as well as the relevance of the topics in their local context. Each classroom should also organize itself as a crew where the master is democratically elected by peers, where roles (e.g., delegate/reporter) and tasks among team members are defined and recognized based on skills and abilities, by ensuring gender balance/equity and inclusiveness, by rewarding merit and effort, by sharing responsibility, respect for rules, etc.

The EUBSN furthermore aims to encourage dialogue and discussion among young Europeans on sustainable development issues in order to strengthen their capacity for action and commitment and to move towards sustainable societies. Since the BlueS_Med project is in line with the goals of education for sustainable development and those of international citizenship, it will empower young people to consider global vs. local contexts, while it will prepare them to discuss important issues and values in such a way that they can take enlightened positions and implement thoughtful actions.

Since we have just entered the Decade for the Education for Sustainable Development dedicated to Ocean Sciences, within all the European funds planned from now on (e.g., Recovery Fund, Green Deal, Next Generation EU, etc.) we strongly suggest to dedicate funding schemes directly to schools that partner with scientific institutions and want to join the EU Blue Schools Network. Some suggestions about possible EU incentives, among others, are a) an annual funding scheme to schools that have undertaken the Blue School pathway, commensurate with their commitment (e.g., number of involved classes/pupils/teachers); b) an annual/biennial/triennial recognition (economic and/or academic) to teachers who are proactive within this path, commensurate with their commitment and innovation; c) a one-time award to the country/European Marine Regional Area that has the highest number of Blue Schools.

5. Acknowledgements

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Links * https://www.oceandecade.org/

A BLUE PEDAGOGICAL INITIATIVE FOR HIGH SCHOOL STUDENTS AND EDUCATORS IN MEDITERRANEAN REGION

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Abstract

In the framework of Erasmus+, a marine education related project titled MEDEDUC was developed during 2018-2021. The project aimed to the creation of a series of European level marine educational resources for middle and high school educators and students. These resources provide educational material covering the Mediterranean Basin and are consisting by 3 intellectual outputs: an educational reference system for educators, an activity guide for educators and students, and an online resource center for students, educators and public. Furthermore, these educational resources concerned the discovery and valorization of the coastal and marine environment in the Mediterranean Sea, engaging various stakeholders, citizens and youth in an open and participatory dialogue to share knowledge, forge partnerships and empower actors on societal and marine issues. The pedagogical material produced through the MEDEDUC project is intended for teachers and educators who are open to innovative teaching models based on experiential and holistic learning approaches. MEDEDUC was also contributed to enrich the field of citizenship education for young Europeans in the Mediterranean Basin.

Keywords: Ocean literacy, marine education, Mediterranean Sea.

1. Introduction

Ocean literacy is an understanding of the ocean's influence on society and the society's influence on the ocean (Schoedinger *et al.*, 2005; Fauville *et al.*, 2019). An ocean literate individual understands fundamental concepts about ocean functioning, is able to discuss ocean issues in meaningful ways, and is also capable of making informed and responsible decisions in regards to the ocean and its resources (Cava *et al.*, 2005; Fauville *et al.*, 2019; Mogias *et al.*, 2019). Marine education was generally considered to be complex for teachers to deal with due to its integrated nature (Awkerman *et al.*, 1974). Bibliography suggested that there was a need to provide quality resources to educators at different levels supporting ocean science. (Caste *et al.,* 2010). According to literature, many national school curricula worldwide, lack ocean literacy-related issues (Caste *et al.*, 2010, Visbeck, 2018). Concerning Mediterranean European countries bibliography indicates the fact that marine sciences do not constitute a basic part of the educational system in Italian, Croatian, and Greek national curricula, even if many subjects related to marine environments are appeared in their programs in inconsistent and discontinuous manner (Fauville *et al.*, 2015). There is scarce literature regarding the teaching and learning of ocean and aquatic science topics at secondary (high) school level. Consequently, promotion of ocean literacy in elementary and secondary education is vital (Visbeck, 2018), as children represent the future citizens and consumers, who will develop attitudes and make decisions that will inevitably affect the environment.

MEDEDUC was based on the recognition of a lack of environmental education resources specifically focused on the Mediterranean Sea analyzing the issues and environmental problems under the prism of human/nature cohabitation. MED EDUC was funded by Erasmus+ framework under the theme: Marine Education related to the Mediterranean Sea for high school students and educators from countries of the Mediterranean Basin.

2. Materials and Methods

The MED EDUC aimed to the creation of a series of European educational resources for middle and high schools, focused on educational activities of the coastal and marine processes in the Mediterranean Sea. It recommends new educational tools focused on the specific issues of environmental protection in the Mediterranean Sea, helping to better understand environmental processes, the identification of the future challenges and the contribution of the collective search for solutions. All the pedagogical resources are addressing the issue of environmental education skills and competencies from multiple focus: by contributing to reinforce the emergence of multidisciplinary educational projects (one of the flagship issue of formal education), by proposing activities integrating different themes and different pedagogical approaches, by dealing with environmental issues from the perspective of sustainable development, human / nature coexistence in the Mediterranean Sea. During the development of the pedagogical resources, the partners mobilized experts, educators and/or teachers (internal or external to partner structures) on a voluntary basis in order to strengthen the educational content of the resources. Experts reviewed the chapters of the pedagogical framework.

Concerning the activity guide, the production steps have been accompanied by classroom tests carried out by 15 volunteer teachers, with 372 students. In addition, nine (9) activities were tested online during the transnational training with 27 participants of different profiles (teachers, educators, school heads, and representatives of environmental education structures). The seven (7) final dissemination events involved 392 people, including teachers, school leaders, academic representatives, representatives of resources of the educational world, elected officials and technicians of local authorities, parents' students' associations, environmental education associations and journalists. Finally, questionnaires were carried out and submitted to external experts and volunteer teachers during the testing and proofreading phase of the activities from the pedagogical guide to improve this educational tool.

3. Results

3.1 MED EDUC educational resources

Pedagogical Framework: With the creation of the pedagogical framework as a reference system, the project was reinforcing the integration of environmental education, focused on the Mediterranean Sea combining fundamental scientific disciplines, proposing activities and content for all age groups from high school to college and for all subject areas (science, humanities, literature, arts, science education, civic education).

By analysing the distribution of individual sea related content, according to the curricula of all partner countries, the content was grouped and presented it to educators enabling them to teach and the students to learn effectively about the Mediterranean Sea and the littoral life. The final sub-topics were the following: Waste and pollution, Seawater quality Coastline artificialization, Energy: production and resources, Maritime Economy, Politics and governance, Cultural heritage, Natural Hazards in the Mediterranean Basin, Biodiversity and Climate change. Each of these topics can be addressed through one or more school disciplines multidisciplinary or not, to students of different ages (11-17 years). The scope of each topic is determined by key concepts which are often integral component of a particular school discipline (e.g., Science, Geography, Physics, Chemistry, History, Art), but they can be also used in processing tools of other disciplines (e.g., Mathematics, Mother language, Foreign language, ICT). In this way, multiple school disciplines connect in multidisciplinary teaching, as recommended by recent pedagogical theories. The pedagogical framework includes an introduction, ten (10) thematic chapters, as well as a section on the place of the Mediterranean Sea in education taking also into account the problematic of the subject and the use of the tool. The introduction presents generalities of the Mediterranean Sea and the problem of sustainable development. Then, each chapter includes a synthesis of the topic, tables on the main concepts and transversal skills that can be developed by teaching the themes, defining keywords, introducing the subject and the problematic of the subject, the position of the subject in the school programs and resources used for the writing of the chapter. The pedagogical framework was translated into five (5) languages and presented at dissemination events.

Pedagogical Guide: The Pedagogical Guide has been prepared to teach, implement, inform, sensitize and enable educators, teachers and secondary school level students from the Mediterranean countries to learn more about the Mediterranean Sea addressing it from an environmental, societal, cultural and economic point of view. It contains 44 interdisciplinary and multidisciplinary learning activities and it has been designed to primarily serve youth aged from 11 to 18 years old, but can be used also by educators outside the formal schooling system. Each activity is tailored to specific grade levels and learning objectives and includes detailed step-by-step instructions, academic correlations, time and material requirements, and corresponding student worksheets. The proposed activities provide an opportunity to introduce students to the diversity of life in the marine environment of the Mediterranean Sea, to make them understand the key marine processes, to discuss with them about ocean health and safety applying adequate monitoring tools, and in general to excite and inspire students about marine sciences by developing among all students' critical thinking and problem-solving skills.

Online Resource Center: The MED EDUC Resource center capitalizes on the project's intellectual outputs, making them available interactively and extending them by visual and bibliographic contributions as well by complementary expertise. The resource center, is hosted on the project website (mededuc.eu), is launched in six (6) languages (English, French, Italian, Greek, Croatian, and Spanish) and consists of six (6) sections: the pedagogical framework; the activity guide; the self-assessment tests for students; activity search (search engine facilitating the selection of activities according to the criteria of discipline, age, thematic and type of activity); the forum; and a section entitled "Share your experience" to improve the guide and propose new activities. The resource center allows in particular to consult the experiences collected in the guide in an interactive way, download them and collect the testimonies of teachers and students.

4. Discussion and Conclusions

Mediterranean basin is today a major subject of social, ethnic and religious tensions. MED EDUC project contributed to the emergence of a Mediterranean citizenship of young people based on exchange and mutual understanding. Within the MED EDUC project, multiple expertise from the world of formal and non-formal education and the economic, social and science sector, were brought together in a mutual learning, consultation process and joint action, to work on key issues and develop challenging resources to strengthen marine education for sustainable management of marine ecosystem services by European citizens.

The MED EDUC project has been highly effective, not only in its introduction of marine life in the Mediterranean Sea and the vital importance of the ocean to middle schools teachers /educators and students, but also in its promotion of ocean literacy to the wider public in Mediterranean Basin through parents, relatives and the community at large.

Assessing marine education programs is necessary for refining and improving ocean literacy efforts in formal and informal education (Guest *et al.*, 2015). Understanding their successes and challenges is critical for a coordination of approaches to advance Ocean Literacy in the Mediterranean Basin. Overall, the examples of proposed educational resources which have been described in this paper provided for all participants valuable experience in ocean literacy. In particular, the gained experiences are related to the following aspects: a) The ability to discuss marine and environmental problems from different perspectives; b) the involvement of Youth people in activities within and outside of the classroom; c) The establishment of a connection among the students/educators and their environment through the use and implementation of the Pedagogical framework and the Educational Activities Guide; d) the understanding of the coexistence needed between the society and the marine environment throughout history; e) the understanding of the knowledge needed to sustain the marine management; f) the learning about the marine environment and the need to coexist with it; g) the learning and understanding of the phenomena in the environment that result from the degradation of marine resources; i) the learning and understanding of the consequences of the degradation of the marine environment; j) the understanding of the need to change the humans' habits and behavior to protect the sea; k) Providing students with a specific knowledge about the marine environment that is otherwise not sufficiently represented in the school curricula of the Mediterranean countries; l) the development of student competences; the preparation of the students for having responsible citizenship and coexistence with the sea; m) Strengthen students' motivation for learning and better understanding of teaching content.

Such pedagogical resources can provide a common framework for the curriculum designers, textbook authors, and education officials, contributing to design and put into effect up-to-data science curricula across the Mediterranean countries, while preserving the most valuable characteristics of their diverse societies and cultures.

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EMSEA EDUCATIONAL ACTIVITIES PROMOTING OCEAN LITERACY IN THE MEDITERRANEAN REGION

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Abstract

In 2015, within European Marine Science Educators Association (EMSEA), the Regional Group for the Mediterranean Sea (EMSEA-Med) was established. Since then, this group has been working on the diffusion of the Ocean Literacy (OL) in the Mediterranean region. As a result, educational activities, for primary and secondary school students, consisting of lectures and workshops, were held at international events in three Mediterranean countries (Croatia, Greece and Italy), from 2018 to 2021. The activities outcome was assessed based on the observation of students' behaviour, comments, drawings and handcrafts, while for 465 students of 1,300 in total a pre- and post-test on OL content knowledge, pro-environmental attitudes and behaviour were applied. The results evidenced improvement in knowledge and awareness in most of the involved groups, suggesting that this approach can provide a positive sustained effect on students and possibly on their families, friends and local communities, thus contributing to the protection, conservation and sustainable use of marine resources and consequently to the wellbeing of future generations in the Mediterranean Sea region.

Keywords: primary school, secondary school, Mediterranean Sea Literacy, educational activities, EMSEA-Med.

1. Introduction

Ocean Literacy (OL) is *"...a tool, a framework and, more broadly, a mindset that forefronts the ocean in all aspects of life on Earth. As an approach for society as a whole, OL catalyzes actions to protect, conserve and sustainably use the ocean..."* (IOC-UNESCO, 2022). Acknowledging the importance of OL, a declaration in the high-level conference "Our Ocean", in 2017, which reads "*Support plans to foster ocean-related education, for example as part of education curricula, to promote ocean literacy and a culture of conservation, restoration and sustainable use of our ocean*", was issued to support the implementation of Sustainable Development Goal 14 on the ocean, along with the United Nations Agenda 2030. Towards the achievement of this goal, ocean-literate citizens are a pre-requisite and therefore, the promotion of OL in education is vital (Santoro *et al.,* 2017).

In the early 2000s, an OL movement was born in the United States to bridge the existing knowledge gap about the two-way relationship between cause and effect of ocean deterioration and human wellbeing, evident in both school students and citizens. In 2011, a European Marine Science Educators Association (EMSEA) was established, followed by the EMSEA Working Group for the Mediterranean Sea (EMSEA-Med) at EMSEAs' annual conference held in Crete (Greece) in 2015, to disseminate OL principles and concepts in the Mediterranean region (Previati *et al.*, 2018). Since then, this group: a) organized many educational activities for primary and secondary school students and general public at international events; b) translated OL principles and concepts into several languages of the Mediterranean countries (see https://www.marine-ed.org/ocean-literacy/translations); c) carried out educational research (e.g. Mogias *et al.*, 2019; Koulouri *et al.*, 2022); and d) developed the regionally-specific Mediterranean Sea Literacy (MSL), based on OL principles and concepts (Mokos *et al.*, 2020a), a guide to better understand the important role of the Mediterranean Sea in peoples' lives in the region and therefore the need for sustainable management of its depleted marine resources (Coll *et al*., 2012).

The aim of this article is to present educational activities, concerning OL and MSL in the Mediterranean region, performed by the EMSEA-Med group, mainly in three Mediterranean countries (Croatia, Greece, Italy), in primary and secondary education, and to discuss their possible outcomes on forming and training "ocean/sea citizenship".

2. Material and Methods

The activities were held in primary and secondary schools in three Mediterranean countries (Croatia, Greece, Italy) at the European Maritime Day (EMD) and Mediterranean Action Day (MAD) events from 2018 to 2021 - involving 1,300 students - addressing different principles of OL and MSL (Table 1). They consisted of lectures and workshops including hands-on labs (e.g., "Mediterranean Sea is under "plastic siege". Be a part of the solution...Recycle!"), field work (e.g., "Meet your local sea"), role-play/game/competition (e.g., "Can not take your class to a Mediterranean seashore? We take the Mediterranean Sea into your classroom!"), drawing and handcraft activities (Table 1). In addition, intermediate sessions of questions and answers from educators to children and vice versa were performed, to ensure the understanding of each addressed topic. The duration of didactic interventions ranged between 1.5 and 5 hours.

Most of the activities have been assessed based on observation of students' behaviour, especially by asking questions during each activity, eliciting students' comments and by means of drawings and handcraft activities at the end of each session. Several of these activities, in which 465 students participated, included an assessment, pre- and post-activity, through the same questionnaire, to investigate students' knowledge and attitudes (Realdon *et al*., 2019; Mokos *et al*., 2020b), as well as their behaviour (Cheimonopoulou *et al*., 2019), regarding ocean-related topics, while ensuring students' anonymity. Teachers' feedback was obtained through telephone calls after the activity.

Along with the activities under the umbrella of MAD 2021, a brochure was developed based on the MSL content (Mokos *et al*., 2020a) in English and then translated into Croatian, Greek and Italian.

Table 1. List of educational activities of EMSEA-Med group concerning primary and secondary school students in Croatia, Greece, and Italy at EMD and MAD events.

Year	Event	OL/MSL principles addressed	Country	City	Student's age	Title of the activity	Type of activitiy
2018	EMD	1, 5, 6	Greece	Veria	8-10	A "sea" of plastic: How plastic wastes threaten our seas and therefore our lives.	Lecture, workshop
2018	EMD	3, 4, 5, 6	Croatia	Zadar	7-10	Sea and me	Lecture, workshop
2018	EMD	6	Italy	Monfalcone	6-14	Promoting Ocean Literacy	Practical labs
2019	EMD	1, 4, 5, 6	Greece	Veria	10-12	Hatching marine scientists on action! Learning about sea floor, currents and mountain fossils!	Lecture, workshop
2019	EMD	1, 2, 3, 4, 5, 6	Greece	Veria	13-15	Mediterranean Sea is under "plastic siege". Be a part of the solution... Recycle!	Lecture, workshop
2019	EMD	5, 6, 7	Croatia	Zadar	8-12	Little marine explorers	Workshop
2019	EMD	6	Italy	Monfalcone	6-18	EMD in the Extreme Northern Corner of the Mediterranean	Practical labs
2021	EMD	1, 2, 4, 6	Greece	Veria	8-12	Exploring a drop of sea water and not only...	Online lecture, workshop
2021	EMD	4,5	Croatia	Zadar	7-12	Wonders of the Adriatic Sea	workshop

Year	Event	OL/MSL principles addressed	Country	City	Student's age	Title of the activity	Type of activitiy
2021	MAD	1, 2, 4, 6	Greece	Veria	7-12	Marine photosynthetic organisms. How would our lives be without them?	Online lecture, workshop
2021	MAD	5, 6, 7	Croatia	Zadar	8-9	Meet your local sea	workshop
2021	EMD	6	Greece	Crete	10-11	Planet vs Plastic	Online lecture, handcraft activities
2021	EMD	6	Italy	Monfalcone	6-10	EMD at the extreme northern corner of the Mediterranean Sea	Online workshop
2021	MAD	5, 6	Italy	Monfalcone	10-11	"Can not take your class to a Mediterranean seashore? We take the Mediterranean Sea into your classroom!"	Practical labs, role-play

3. Results

The activities were successful according to students' comments, evidencing understanding, awareness and willingness to behave sustainably in order to protect the sea. Teachers expressed satisfaction and willingness to address OL and MSL topics, during school activities throughout the year, and explicitly requested to participate in similar activities, including fieldwork, in the future. Repeated collaborations with the same schools and teachers for several years, is indicative of the satisfaction and fruitful collaboration established between schools and EMSEA-Med members. Additionally, several schools that participated in these events are candidates for becoming European Blue Schools.

Concerning the activities with pre- and post- assessment, the results were encouraging. Most of them revealed a statistically significant increase in students' knowledge level and behaviour and an increase in positive attitudes towards the marine environment (Cheimonopoulou *et al.*, 2019; Realdon *et al.*, 2019; Mokos *et al.*, 2020).

MSL brochures were distributed among students and teachers to spread the word of MSL, while they are also available online on the EMSEA website (https://www.emsea.eu/ocean-literacy/publications). In January 2022, after the online presentation of the MSL brochure to the EMSEA community several volunteers started to translate the brochure into other Mediterranean languages such as Catalan, Maltese, Slovenian, Spanish, as well as non-Mediterranean languages, such as German.

4. Discussion/Conclusion

Students of elementary and partly of secondary school show an innate curiosity about nature; therefore, they are interested in acquiring new knowledge (US Commission on Ocean Policy, 2004) and building pro-environmental attitudes and behaviour (Hartley *et al.*, 2015). This is evidenced by the positive comments of students participating in the above-mentioned activities, their eagerness to participate in new ones and their increased knowledge, pro-environmental attitudes, and behaviour, even weeks or months after the interventions, (Cheimonopoulou *et al.*, 2019; Realdon *et al.*, 2019; Mokos *et al.*, 2020b), suggesting that this approach could represent a valuable strategy for building "ocean/sea citizenship". Moreover, children are potential agents of social change as they influence knowledge, attitudes, and behaviour of their families and friends (Hartley *et al.*, 2015). This way, students participating in such educational activities, can help in changing the perception of their community on environmental issues, at present, and most certainly in the future, as they will be the ocean/sea citizens who can understand the "dynamic balance" between their rights and duties on the ocean and in particular on the Mediterranean Sea.

The lack of ocean/sea topics in the school curricula of the three countries (Croatia, Greece, Italy), also evident in other Mediterranean countries (Mokos *et al.*, 2021; Pocze *et al.*, 2020), and of specialized teacher training on marine issues, probably are the main factors for inadequate level of ocean/sea knowledge among students (Mokos *et al.*, 2020b). **E**ducational activities, combining collaboration among schools and local scientists/marine research centres can complement the limited ocean/sea knowledge, provided by national curricula, and empower the diffusion of OL and MSL. A noticeable finding is that teachers also acquire substantial ocean/sea knowledge from these activities, especially the ones that had a lasting collaboration with EMSEA-Med members, and keep disseminating their new knowledge to their students all along the school year. Currently, several of these schools collaborating with EMSEA-Med members are candidates for becoming European Blue Schools, an initiative launched by EU4Ocean coalition in 2020 to actively engage teachers and students in ocean/sea education and citizenship.

Effective outreach and dissemination of ocean/sea knowledge to the general public (e.g., understanding of long-term impacts of human activities, historical ecology) is also important, as most people seem unable to recognize the impacts of their activities on the marine environment (Vincent, 2011; Stoll-Kleeman, 2019). EMSEA-Med members have participated in activities aimed at enhancing OL and MSL of the general public at international events, e.g., the Mediterranean Coast Day, the World Ocean Day and the UN Ocean Decade Laboratories.

Promotion of OL and MSL, in education, requires an integrated and holistic approach (IOC-UNESCO, 2022), including flexible and hybrid teaching and learning methods, new curricula and textbooks, teacher training and ready-to-use teaching materials, extracurricular activities and field trips, as well as spreading European Blue Schools initiative around the Mediterranean. All these actions will enhance pro-environmental attitudes and behaviour, contributing to the sustainable use of marine resources and, consequently, to the wellbeing of future generations in the Mediterranean region (Realdon *et al.*, 2019; Mokos *et al.*, 2020a, b; IOC-UNESCO, 2022). Emotional engagement of children, and people in general, with the ocean, is a key element to that direction (Vincent, 2011).

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SCIENTISTS FOR OCEAN LITERACY - EMPOWERING SCIENTISTS AS OCEAN ADVOCATES IN THE UN DECADE OF OCEAN SCIENCE FOR SUSTAINABLE DEVELOPMENT 2021-2030

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Abstract

The presentation will showcase the EuroGOOS 'Scientists for Ocean Literacy' project endorsed by the UN Decade of Ocean Science for Sustainable Development 2021-2030. The 'Scientists for Ocean Literacy' project aims to empower scientists as ocean advocates to their communities, countries, and regions, and help transform ocean knowledge and awareness into actions for sustainability. The project has several objectives: (i) support blue careers and education, (ii) promote activities in the land-freshwater-sea continuum, (iii) help share best practices and impact assessment guidelines among ocean literacy practitioners, and (iv) embrace multi and trans-disciplinarity and inclusiveness through connecting science with art and culture. The project was developed from the work of the EuroGOOS Ocean Literacy Network which has been promoting the role of the European (including Mediterranean) oceanographic and meteorological agencies in educating and engaging with the general public towards a more ocean-literate society. The 'Scientists for Ocean Literacy' project will raise public awareness about the importance of sustained publicly-funded information on the ocean state, natural and anthropogenic-induced phenomena, and requirements for sustainable ocean management. The 'Scientists for Ocean Literacy' project is connecting science with society not only through conferences and publications, but through outreach and engagement with the public in the cities, at the coast and far away from it, in schools and hospitals. The activity will follow the strategic outlook delivered by EuroGOOS in its policy brief, titled 'Ocean Literacy in European Oceanographic Agencies – EuroGOOS recommendations for the UN Decade of Ocean Science for Sustainable Development 2021-2030'.

Keywords: environmental education, public awareness, societal engagement, oceanography, Ocean Decade.

1. Introduction

Ocean Literacy is a cross-cutting priority area of the UN Decade of Ocean Science for Sustainable Development 2021-2030 (Ocean Decade). The European Global Ocean Observing System, EuroGOOS, has engaged its network of oceanographic and meteorological agencies to upscale national and regional efforts in increasing societal and policy awareness of the ocean's importance. Almost any societal objective of the Ocean Decade requires ocean observing and services. Ocean Literacy is becoming a new strategic activity area in scientific institutions as a key enabler to achieve societal and policy support towards sustained oceanographic activities and sustainable blue economy.

Developed in the early 2000s through the work facilitated by the US College of Exploration, the international Ocean Literacy framework has since been embraced by various scientific sectors in oceanography and marine research. Today, Ocean Literacy is seen as an enabler for engaging with stakeholders and determining common understanding and joint identification of solutions towards sustainability. In addition to strengthening dialogue and engagement at the science-policy interface, Ocean Literacy also helps reach out to society at large as well as the sectors and disciplines outside of the traditional domains of marine sciences or maritime economy and management, from art and culture to sport and recreation.

EuroGOOS has emphasized that Ocean Literacy should become an integral part of the activities of the oceanographic and meteorological agencies. In Europe, ocean observing, marine science, modelling and forecasting activities are maintained predominantly by public funding. Increasing the public engagement and the awareness of the ocean's paramount importance for all lives on the planet, will help achieve sustained operations and additional funding sources of these activities, while helping to reach the objectives of the European Green Deal and the Biodiversity Strategy, and develop a sustainable Blue Economy (Eparkhina *et al*., 2021).

The Mediterranean Sea is recognized as a key component in the development, economy, and culture of European, North African, and Middle East countries as well as an agent for the 'transformative change' needed to achieve a sustainable future in this region (Cappelletto *et al*., 2021). The Ocean Decade 'Scientists for Ocean Literacy' project and the activities of the EuroGOOS Ocean Literacy Working Group will help and support relevant actions across the Mediterranean Sea region.

2. Material and Methods

The 'Scientists for Ocean Literacy' project of the UN Ocean Decade builds on the activities of the EuroGOOS Ocean Literacy Network, which was formed in 2019 and transferred into a long-term EuroGOOS Ocean Literacy Working Group in 2021. Over twenty organizations in Europe (including EU Mediterranean countries) conducting marine science and delivering oceanographic services, have come together under this initiative to mainstream Ocean Literacy as a strategic area of activity and a research area in its own right. The EuroGOOS Ocean Literacy Working Group has filled a niche currently existing within the area of oceanographic and meteorological research. It upscales the efforts of publicly-funded oceanographic and meteorological agencies as ocean stewards for society, recognizing the importance of their Ocean Literacy work, and creating new opportunities for partnerships.

Since 2019, many activities have been coordinated by EuroGOOS allowing the Ocean Literacy practitioners to share best practice, jointly contribute to events, organize workshops and trainings, and finally deliver a community submission for a project in the UN Ocean Decade framework. In 2019-2020, the group conducted its first survey of national Ocean Literacy activities in European oceanographic and met agencies, based on five years of operations. The survey helped to identify the initiatives conducted in Europe (including EU Mediterranean countries) and analyze them. It was structured in five main parts, including: i) description of the activity (i.e., topic, Ocean Literacy principles covered, type of activity, type of resource, target audience), ii) details of the activity (e.g., title of activity, edition year, duration in time, language, available resource link), iii) impact (e.g., statistics on the participants and their uptake, iv) details of the leading organisation (e.g., name, country, number of people involved in the activity, staff time allocation), and v) general information on the reference person completing the survey.

3. Results

The results of the survey displayed 300 activities across 11 organizations, conducted between 2016 and 2019 and showing a diversity of approaches. The activities were primarily addressed to the general public and students, with a few initiatives focusing on multipliers such as teachers. The topics covered spanned biodiversity and ecosystems, marine pollution, environmental management and climate, as well as promotion of gender balance and diversity in marine science. A number of multi-topic events and activities have emerged, demonstrating the effort of the scientific community to provide a broader view of ocean science. The way in which these activities took form included multi-annual programmes, experimental projects combining efforts from different disciplines, festivals and competitions, school workshops, and the production of resources, such as, cartoons, smartphone apps, videos, toolkits, games, and books, with a number of other type of resources and artworks.

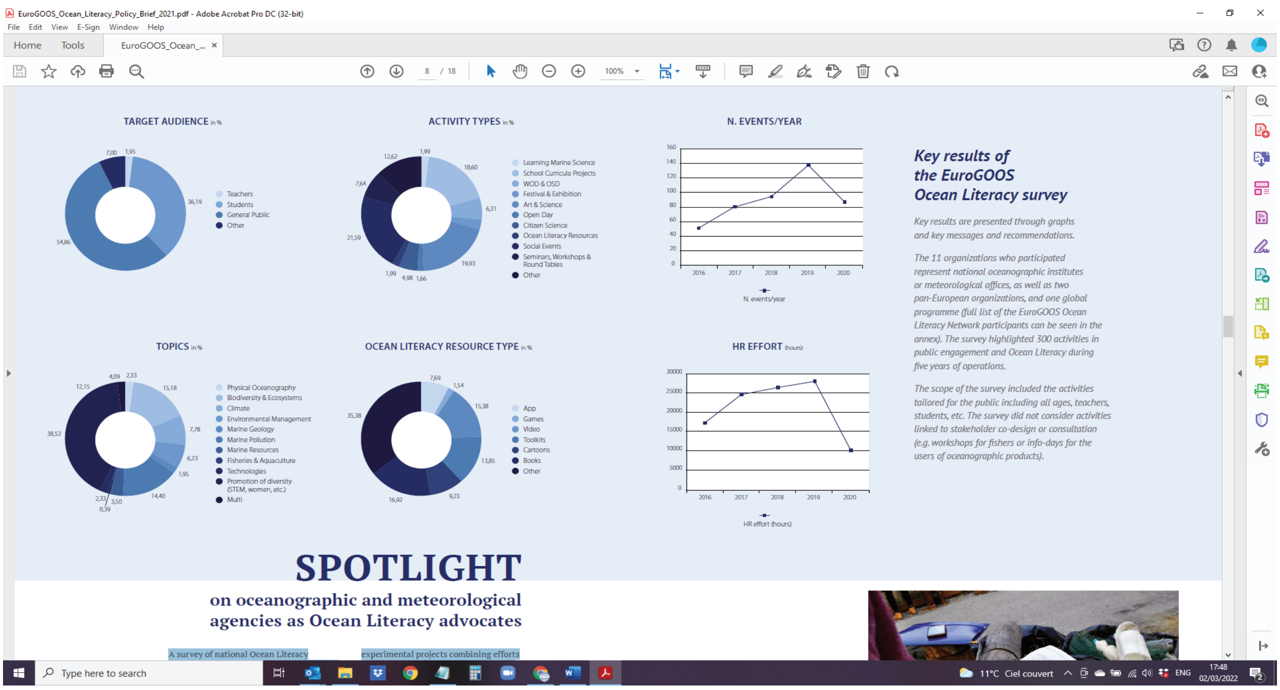

Fig. 1: Key results of the EuroGOOS Ocean Literacy survey conducted in 2019-2020.

A continuous update of the survey is one of the objectives of the EuroGOOS Ocean Literacy Working Group. The updates will reveal the contribution and extent of the online activities organized during the COVID-19 pandemic. The online format offered new opportunities to reach the audience and exchange knowledge, as discussed at the CommOCEAN 2020 conference.

By making visible the information on the Ocean Literacy activities within the EuroGOOS network, the survey has stimulated the sharing of best practices and lessons learnt, awareness of the competences and resources, identification of trends and gaps, and new opportunities for dissemination. The survey has also allowed assessing the status of the activities at the national levels. Such information can help managers better capitalize on the Ocean Literacy opportunities and support these activities going forward.

4. Discussion

A discussion is proposed after the special session on the Mediterranean Sea (and freshwater) Literacy in the Era of the Agenda 2030 and the UN Decade of Ocean Science for Sustainable Development 2021-2030 of the HCMR Symposium to address the way forward for the Euro-Mediterranean Ocean Literacy. Such discussion will be based on the EuroGOOS policy brief recommendations, the activities of 'Scientists for Ocean Literacy' Ocean Decade project, the EMSEA-Med, and the plans of the SciNMeet programme of the UN Ocean Decade.

5. Acknowledgements

The authors acknowledge the EuroGOOS Ocean Literacy Network and Working Group for their contributions to EuroGOOS Ocean Literacy survey (2019-2020) as well as to the design and implementation of EuroGOOS 'Scientists for Ocean Literacy' project endorsed by the UN Decade of Ocean Science for Sustainable Development 2021-2030.

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A CITIZEN SCIENCE STUDY ON MARINE MAMMALS IN PAGASITIKOS GULF (GREECE); PRELIMINARY RESULTS

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Abstract

Citizen science, was firstly used in 1989 as data collection method involving public engagement. In Greece, citizen science has been used in several marine research projects. However, in Pagasitikos Gulf citizen science projects regarding marine mammal has not been conducted before. Pagasitikos Gulf is a busy marine area regarding anthropogenic activities and home to essential marine mammal fauna including the species bottlenose dolphin (*Tursiops truncatus*), striped dolphin (*Stenella coeruleoalba*) and, short-beaked common dolphin (*Delphinus delphis*). The project aimed to gather marine mammal scientific information via public engagement, enhance marine conservation literacy and push for development of marine mammal conservation policy and management. The 23.7%. (n=177) encountered marine mammal including bottlenose dolphin (*Tursiops truncatus*) with the majority of sightings (85%) occurring over day time and mostly in summer time whilst, the animals expressed Normal Swimming. The majority of the respondents stated that 3 different types of threats impact the dolphins in the area with Environmental Pollution being the main one. This project could indicate possible important habitats via marine mammal indicator species within Pagasitikos Gulf. Hence, policy towards marine mammal conservation and effective marine spatial planning could be achieved and possibly mitigate conflicts between humans and the marine mammal fauna.

Keywords: dolphins, citizen science, Pagasitikos Gulf.

1. Introduction

Recent studies indicated citizen science to be especially assisting in large scale scientific research and to promote ocean literacy and marine citizenship by providing data that would otherwise be costly and not time efficient (Kelly *et al*., 2019).

The marine area of Pagasitikos Gulf is home to an essential marine mammal fauna including the species common bottlenose dolphin (*Tursiops truncatus*), striped dolphin (*Stenella coeruleoalba*) and, short-beaked common dolphin (*Delphinus delphis*) (Pardalis *et al*., 2021), with the first species mentioned being protected by several legislative frameworks. Also, in several occasions the Mediterranean monk seal (*Monachus monachus*) has been observed (Panou *et al*., 1999; personal observations). However, no citizen science project has been conducted on marine mammals in Pagasitkos Gulf.

Due to lack of scientific information on marine mammals in Pagasitikos Gulf, and their unidentified migration patterns, citizen science is a complimentary, time and financially efficient approach along with the traditional monitoring methods already ongoing in the gulf (Aceves-Bueno *et al*., 2015).

The Marine Mammal Monitoring Unit of the Department of Ichthyology and Aquatic Environment, University of Thessaly, utilised the research method of citizen science in University of Thessaly Marine Mammal Citizen Science project within the framework of the Pagasitikos Gulf Marine Mammal Monitoring Programme. The aim of the project is focused in gathering sightings information via public engagement, invite the public to participate in actions that serves public awareness and education and push for development of conservation policy and management, not only for the local marine mammal fauna but, in the entire Greek marine areas where marine mammals are recorded.

2. Material and Methods

The questionnaire preparation was initiated in March 2021 by the Marine Mammal Monitoring Unit of the University of Thessaly, aiming in face - to - face interviews and online distribution in order to include a wide range of professionals, stakeholders and members of the general public from different backgrounds, age, sex and lines of work that are involved with marine activities in the area of Pagasitikos Gulf either regularly or, occasionally. However, due to Covid19 pandemic, there were delays in finalising the questionnaire's context whilst, health restrictions rendered the in - person interviews impossible.

The online questionnaire was launched February 2022 and enabled the creation of the database of the project. It involved 23 basic questions that were divided in 4 main different sections. These sections were: Demographics, Marine Mammals Observation/Sightings (map of the gulf was included with the opportunity of pinning location), Environmental Management and Evaluation of the questionnaire. In order to point out the environmental interest of the people towards the local marine ecosystems of Pagasitikos Gulf, a question correlating members of the public and their interaction with the marine ecosystem was set. Additional questions on how they perceive environmental Non-Governmental Organisations (NGOs) and their interference in such actions were also included. A separate section was attached, should the respondees wished to share potential photographic or video material from their encounters with the dolphins including the opportunity the team to contact them.

3. Results

In terms of marine mammals' observation and sightings, out of 177 participants, 42 responded positively in encountering a marine mammal making up the 23.7%. All responses referred to cetacean species. The seasonality of observations was mainly over spring - summer time (89%) (Fig. 1). with the great majority of them (85%) occurring over day time hours (06:00am to 06:00pm). The 52% of the sightings involved more than 3 dolphins whilst, 48% of the encounters involved 1 or 2 individuals (Fig. 2).

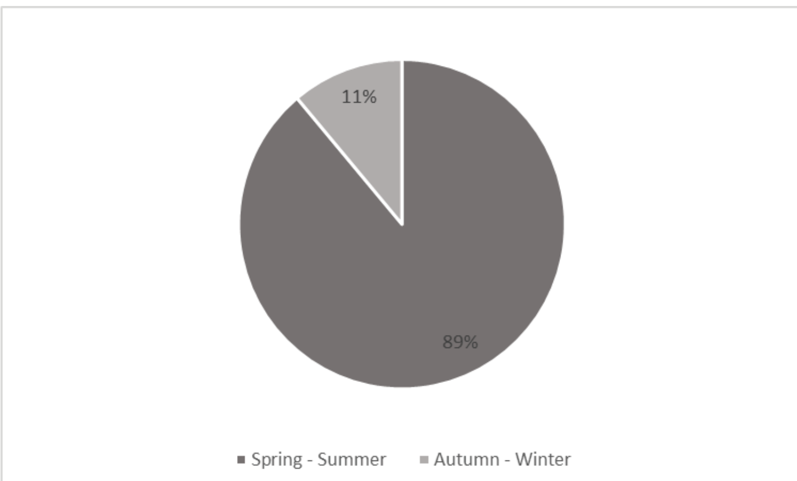

Fig. 1: Seasonality of sightings (proportions %)

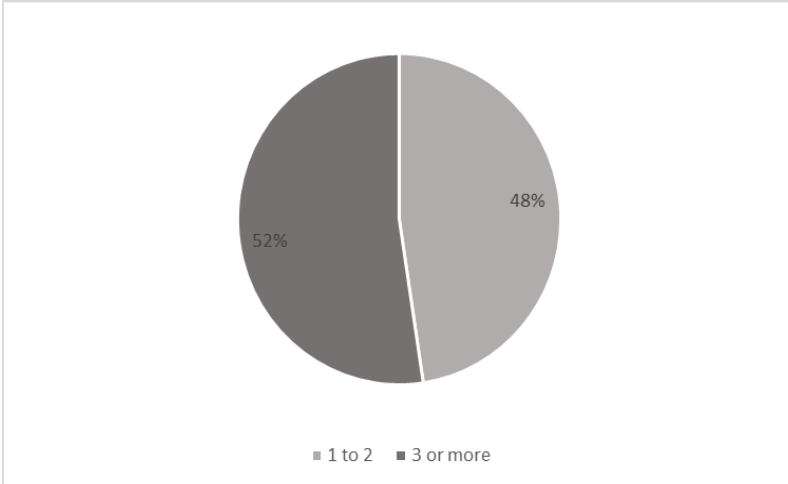

Fig. 2: Number of dolphins in each sighting (proportions %).

The behavioural response of the participants when encountered the animals was mainly moving away from them (57.1%) followed by those approaching the animals (23.8%). Finally, other behavioural responses such as "did nothing/continued my on my route", "turned off the engine" "observed them from distance" made up the 19%. Regarding the dolphins' response towards boat activity, the most prevalent behavioural responses expressed by the animals were, Normal Swim (32.5%), Breaching/Leaping (28.6%), Socialising (15.6%), Bow-riding (11.7%), Fast Swim (5.8%) and Tail Slap (2.6%). Other behaviours like Foraging, Approaching and Milling consisted the 1.3%.

Regarding to species ID, only responses where the participant was strongly confident on his ID skills was taken under consideration. The rest of the species, where the participant was apprehensive with his species ID skills or the ID was dubious, was enlisted in a second group as 'other species'. Species such as striped dolphin (*Stenella coeruleoalba*) and short-beaked common dolphin (*Dephinus delphis*) were included in the second group. In overall, the main cetacean species encountered was the bottlenose dolphin (*Tursiops truncatus*) making up the 35.7% whilst, the rest of the species/unsure responses made up 64.3%.

The 23.2% of the respondents stated that the dolphins in the area of Pagasitikos Gulf are possibly impacted by 3 different types of threats whilst, 20.3% stated 4 different types of threats. Lastly, 19.2% and the 16.4% of the participants believe that 2 different types of threats and more than 4 different types of threats impact the dolphins' wellbeing in the gulf respectively. Among the threats, Environmental Pollution was the main (26,0%) followed by Ecosystem Degradation (21.8%), By Catch (17.3%) and Boat Traffic (15.2%).

4. Discussion

The species of bottlenose dolphin (*Tursiops truncatus*) was mainly observed by the participants alongside, in smaller proportions, with the striped dolphin (*Stenella coeruleoalba*) and short-beaked common dolphin (*Dephinus delphis*). As far as concerned the species using the gulf, the bottlenose dolphin is the most prevalent species found in the gulf but also the other two have been confirmed in the past. However, due to the uncertainty of the participants' skills in species identification, the actual numbers may fluctuate in reality.

However, seasonality and time patterns are indicated of referring to spring-summer and over day time (06:00am-6:00pm) which can be backed up by the sightings the Marine Mammal Monitoring Unit has recorded in boat - based surveys in the gulf and by the general biological and behavioural patterns of the species observed in the gulf.

The majority of the respondents, observed more than 3 dolphins with main behavioural response towards the animals to be moving away from them (57.1%) whist 23.8% of them choosing to approaching them. That behaviour from the observers may indicate that regardless not being efficiently familiar with the code of conduct when encountering a marine mammal at the sea, there is a tendency to realise what is a better practice or not to apply.

On the contrary the dolphins appeared to mainly to swim normal, breaching/leaping or socialising when a boat was in the area expressing minimum levels of stress. However, other common behaviours like foraging, approaching and milling were found in considerable minimum proportion (1.3%). That result could stress the hypotheses that either the number of participants encountering cetacean in the gulf is low and more time and interaction is needed, or the participants' knowledge on marine mammals' different behavioural cues is poor or, maybe the animals are not as used to anthropogenic interaction and boat traffic within the gulf as perceived. In terms of number of threats and challenges the marine mammals in the gulf face the most, the participants acknowledged mainly 3 different types of threats including mainly Environmental pollution, Ecosystem degradation, By Catch and Boat Traffic. Marine mammal By Catch has not been reported before in scientific literature for Pagasitikos Gulf. That response can be attributed to the participants facing difficulty to specialise on the specific area of Pagasitikos Gulf and made their responses more generic as to the threats impacting dolphins (although the question clearly stated Pagasitikos Gulf). Possibly the proportion of Boat Traffic might have received a higher proportion since it is a regular activity.

Regardless the great majority of participants perceived the questionnaire as easy to understand, there is a possible indication of some confusion especially, in the question of threats. However, even if this is not the case, the questionnaire needs further update in order to achieve higher efficiency and robustness of responses.

In overall, important information was yield from the preliminary results of this citizen science project including new information about the marine mammals of the gulf, the usage of the gulf by man and dolphin and, social aspects such as perception of the public towards the marine mammal fauna of the area. Areas of opportunities for sustainable development are identified and there is great need for ocean literacy in the area.

The existing conservation framework for marine mammals is characterised as inadequate. Hence, active engagement of the public has the ability to influence policy development on that matter. Citizen science application on marine conservation projects could provide deeper understanding of the topic to the participants and thus inspire public action towards sustainable natural resource management (Kwan, 2016).

Citizen science in marine research could function as avenue to form public perceptions towards the local marine mammal fauna in Pagasitikos Gulf, since no other similar project has been conducted in the past. This project has brought to attention challenges that needs to be tackled such as environmental and social challenges. These challenges involve fisheries in the gulf and the antagonizing aspects with the dolphins over the natural resources, especially since one third of the professional fishermen perceives a very negative view of the dolphins (Pardalis, 2021).

Pagasitikos Gulf is a very busy marine area with human activities spreading in the wide breadth of its coastal marine areas. If bottlenose dolphin (*Tursiops truncatus*) and Mediterranean monk seal (*Monachus monachus*) and/or any other suspected marine mammal species using the gulf function as indicator species, the data from this project could indicate possible important habitats for them within Pagasitikos Gulf. Hence, understanding of how crucial habitats of important species and the spatial distribution overlap with human activities, pressure and impacts will be revealed (La Manna *et al*., 2020). Therefore, policy towards marine biodiversity preservation and effective marine spatial planning could be achieved and possibly mitigate conflicts between humans and the marine mammal fauna (La Manna *et al*., 2020). Finally, as umbrella species, and based on their large marine area requirements the whole marine ecosystem would benefit and some areas within the gulf could receive protection status.

5. Acknowledgements

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IMPLEMENTING OPEN SCHOOLING FOR POLYMER PLASTICS SCIENTIFIC LITERACY

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Abstract

In the context of an educational scientific action scenario (Open Schooling framework), students explored the variety of polymers and especially plastics, discovered the properties and structure of the material universally used as well as its environmental impacts. They also explored the possibilities to reduce it (limited use, replacement of non-recyclable plastics from new materials, conscious recycling, etc.). The scenario consists of activities aimed at making the sciences more affordable to students, showing them how scientific research and innovation can affect their lives, and how they can use natural sciences to have benefits as young scientists. It was built in three stages based on the relevant worksheets and activities (Care, Know, Do) as well as the discussion of students with scientists specializing in each subject (material, pollution, sustainable management). The Open Schooling was introduced mostly with regards to science education and public engagement; there was a strong collaboration between academic/research members (universities, research centres), non-academic/research members (schools, local communities) and teaching staff. The design and implementation of the science action "targets all three components of student scientific literacy: motivation (Care), knowledge (Know) and skills and attitudes (Do) for preparing students to engage and learn with real problems".

Keywords: material, pollution, recycling, sustainability, Open Schooling, Scientific Literacy.

1. Introduction

According to the Open Schooling framework, schools in cooperation with other stakeholders, become agents of community well-being (European Commission, 2015); by building innovative ecosystems, where school projects meet the real needs of the community, for which leaders, teachers, students and the local community share responsibility, over which they share authority, and from which they all benefit through (…) the development of responsible citizenship (Sotiriou & Chevouris, 2017). Open schooling has a lot of dimensions and the most known indicators for monitoring are the following ones: science education, public engagement, open access, gender equality, ethics, and governance. "CONNECT: Inclusive Open Schooling through Engaging and Future oriented Science" is a Horizon 2020 project that suggests a sustainable model for enabling more secondary schools to adopt open schooling. The project embeds Science Actions in school curriculum through fun participatory approaches. Science action" is a type of educational scenario that targets all three components of student scientific literacy: motivation (Care), knowledge (Know), skills and attitudes (Do) for preparing students to engage with and learn real problems (Okada & Sherborne, 2018). In this study, we have designed and implemented a science action to teach "Polymers-Plastics".

The plastic polymer is a material with properties (resistant, lightweight and supple, etc.) (Qinfei & Gotsis, 1999) that make it ideal for many industrial products and everyday items (Schäfer *et al*., 2010). It is a driving force of the modern economy and its use has helped industrial development over the last 60 years, with its global production rising rapidly (Caterbow & Speranskaya, 2019). However, about 40% of plastic products are thrown into the garbage in less than a month of their life (WWF, 2021). This constantly inflated pile of plastic waste causes serious environmental problems as almost one third of all plastic packaging leaks into the sea (Pinto da Costa *et al*., 2020). Effective meta-use of plastics, adopting recycling, reuse, and controlled biodegradation for specific applications as naturally controlled plastic production gives a coexistence of valuable material and nature.

The scenario that was implemented for three months during the school period of 2021 (March-May) with the participation of 22 students of the second grade of the General Lyceum of Kolymvari (Chania, Crete) was included in the course of Organic Chemistry of which the syllabus contains the teaching of the modules “Polymerization” and “Polymers-Plastics”. It was structured in three stages of six steps, incorporating a realistic challenge into an existing thematic framework aiming at science education and scientific literacy. The method followed promotes the active participation of students and their families through collaborative activities and insists on the exploratory nature of learning and student-scientist interaction as well as initiatives to solve the problem-target. Students need to acquire skills such as to: a) understand how to deal with a topic - a challenge; b) recognize that often there is a conflict of interest and therefore different approaches in a given problem; c) formulate proposals - recommendations to the organizations involved and citizens. They also need to work on their attitude and values in relation to research and documentation of views, teamwork and collaboration in drawing conclusions, and the active role for protecting the environment and its sustainability. The scenario is also in compliance with Sustainable Development Goals 12, 14 and 17.

2. Material and Methods

On 8th of March 2021 and before the assignment, the scenario and its six steps were discussed with the students (framing), signaling the start of the science action. Since then and until the end of May 2021, every week, for an hour, online, we had been implementing the following stages:

CARE: The learning objectives were first to arouse their interest and then to recognize the existence of the scientific framework. They were first asked to identify and photograph polymers they use every day, watch videos on marine pollution of the seas by plastics, and recognize the consequences of their overuse and wasteful disposal. Then (research-discussion with the family), they discussed with their family the use and disposal of the material after its life cycle. They were asked to explore the properties of seemingly irreplaceable material and the most environmentally friendly ways to manage it. Their tasks were: to detect polymer plastics in their environment and to photograph them (1st worksheet); to discuss on their own the reasons that pushed them to purchase and use these products; to take a walk and collect pictures of the plastic garbage on the beach and elsewhere as they live in a coastal area. In the discussion that followed in the class (plenary), many materials such as construction, transport, clothing, agricultural tools, etc. that belong to the category of polymer plastics were not recognized by students.

KNOW: The questions asked after the plenary debates were: a. Why is the use of plastics so widespread? b. How are they made and what properties make them necessary in everyday life? c. What uses or related activities are harmful to the environment and cause pollution? d. Are they a threat to our health and why? e. Can they be replaced by new materials? Is there research aimed at replacing them? f. Could we continue to use them without causing an environmental burden? g. Are there ways to manage them sustainably (circular economy)? The students were divided into groups. Each group acquired an electronic file (e-class) which contained bibliography, videos, simulations based on which they had to: a) find information on one of the three sub-topics (material, pollution, sustainable management) b) meet and discuss. The purpose of this step was for students to prepare questions for the expert researcher and stakeholder group and to discuss possible solutions with the committee.

Introduction to the material (creation of plastic polymers, material properties): The learning objective (2nd-3rd worksheets) was to realize the involvement of science in the creation of new materials and the improvement of the quality of life. Initially, a course on polymerization was implemented by the teacher with monomer-polymer simulation software and reference to the uses of each. Research-Material: The team undertook the bibliographic search on: a) the historical background of discovery and use; b) the marking and the information it provides; c) features that make a new material valuable. A discussion took place with an expert scientist of the Department of Mineral Resources Engineering (Technical University of Crete) (4th-5th worksheets).

Introduction to pollution: They monitored the result of the reckless use of plastic in the pollution of the seas and looked for the waste stains. The learning objective was to acquire the ability to "look at different perspectives". They also practiced the ability to consider the economic, social and environmental consequences of this chemical invention. They learned about marine pollution and its threat to the life of the planet. Research-Pollution: The team undertook the bibliographic search on: a) the continuation of the life of the plastic waste that ends up in the water; b) the entry of the plastic into the food web; c) the effects of their presence on marine organisms; d) experiments looking for the existence of microplastics in materials such as toothpaste, exfoliating cosmetics, etc. A discussion with an expert scientist of the Hellenic Centre for Marine Research took place based on questions that the students had co-decided (6th worksheet).

Introduction to plastic waste management: The learning objective was to coordinate scientific knowledge and skills in a performance appraisal. Research-Recycling, sustainable management: The team undertook the bibliographic search on: a) marking and mapping of recognized everyday materials; b) ways to manage plastic waste (circular economy, recycling, incineration); c) policies and commitments to solve the problem. A discussion took place with an expert scientist, who is the Director of Alternative Management Municipal Corporation (DEDISA SA), for the proper management of plastic waste based on questions co-decided by students.

DO: After a discussion-consultation, students came to the following conclusion: "The active citizen deals with these problems daily and, with his/her own behaviour, persuasion and assertion, tries to change the consumer's behaviour and to point out the indifference of the state. So, the "enemy" is not the material, but the citizens that behave and live as if the others do not exist". Students chose the following actions in order to disseminate this message: a) to do a beach cleaning project that would give them the opportunity to discuss the findings; b) to discuss with family and friends about modifying consumer's behaviour and sustainable development that meets the requirements of the present without undermining the ability of future generations to meet their own needs; c) to send a letter to a local newspaper informing about their experience concerning the scenario and stating their views and conclusions; d) to make a presentation for their school website where they could also chat in a short movie (publicity).

Students were given an anonymous online questionnaire before and after the intervention. The questionnaires used a 5-point Likert scale, were designed in Google forms using mostly closed questions and delivered online to "*provide a significant overview of students' mindsets, interpreted through the lenses of students' science capital and aspirations (Archer et al, 2015), epistemic beliefs and fun in learning (Okada & Sheehy, 2020) and science identity with recognition (Carlone & Johnson, 2007)*" as stated by Okada & Gray (2022). Through those questionnaires we have tried to find out whether the intervention had any impact on students' beliefs, perceptions and feelings regarding real world Science and Science as a school subject. Those beliefs, perceptions and feelings are considered fundamental for students' learning outcomes and career aspirations (Archer *et al.*, 2015).

3. Results

Based on the answers of the 10 students that filled both pre- and post- questionnaires, after implementation of the scenario the students didn't feel more confident about their scientific knowledge and their ability to discuss scientific issues, nor had they changed their career aspirations. However, before its implementation only 2 out of the 10 students had been searching for extra information -apart from the school book- related to science activities, while after implementation 5 more students had started searching and using extra information. Furthermore, 5 out of 10 students changed their perceptions during the implementation, finally deciding that science is not about memorizing terms and equations. Finally, 5 out of 10 students changed their opinion during the implementation, concluding that scientists do not work alone.

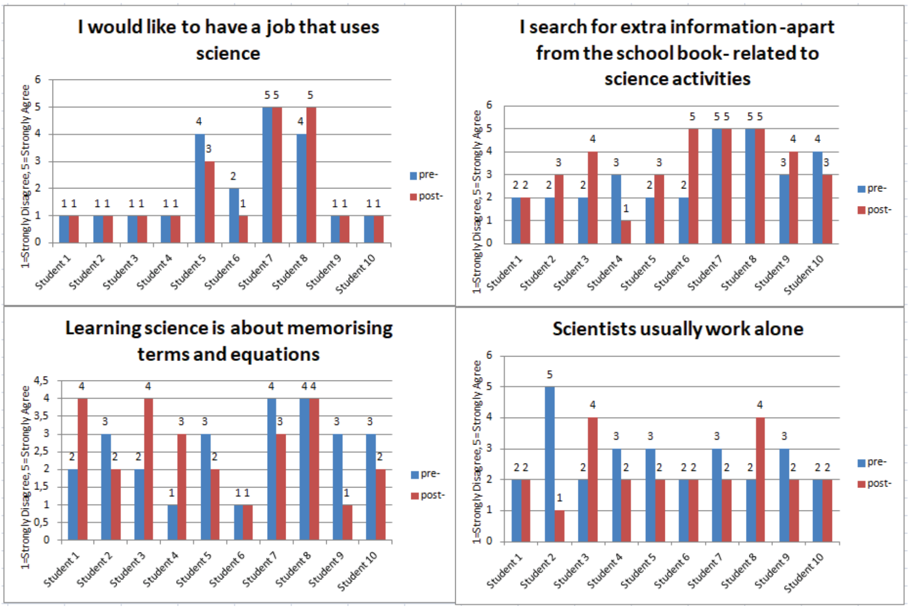

Fig. 1: Students' responses before and after the implementation of the scenario.

4. Discussion/Conclusion

The course of the implementation of the scenario encountered many difficulties as a large part of it took place, due to the pandemic, through teleconferencing. As it is obvious by the results, most of the students that participated in the study had low science capital in the dimension of "how they feel about Science". The intervention hasn't changed this feeling, maybe due to emergency remote teaching circumstances but also due to the short term of the intervention (Archer *et al.*, 2015; Okada & Gray, 2022). Nevertheless, the students experienced teaching and learning activities with the teacher and the scientist that made half of them change their epistemic beliefs of how they learn, a result that is in line with the result regarding all students of the project as presented by Okada & Gray (2022). Finally, in terms of science identity with regards to whether Science is an individual or collaborative activity the intervention had a great impact on students' perceptions.

The particular intervention in the context of CONNECT project has provided the opportunity for those ten (10) students -most of them with low science capital- to work with STEM professionals in a collaborative way despite the emergency remote teaching. The students became actively involved in a diversified form of teaching which helped them make the scientific method accessible. They collaborated creatively and pondered, discovering that a target topic can usually be subject to different perspectives. The last stage enabled them to seek their role as active citizens with substantiated positions and views.

Though our conclusions cannot be generalized due to the small number of students, we aim at continuing that open schooling science learning project, this time face to face, providing more opportunities to students (a) to communicate with experts and other community stakeholders and (b) to be engaged in a participatory research context.

5. Acknowledgements

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ATHENS WATER FORUM AS A PARADIGM SHIFT FOR PUBLIC AWARENESS

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Abstract

As the global population is rising rapidly, so is the demand for water. However, without the right knowledge and awareness, the depletion of water will soon become even more evident and achieving a sustainable future will seem futile. That is why we need to start engaging and cultivating the people of all ages to participate in this chance of preserving this vital resource and extending its lifetime. Public awareness and education on environmental and even more importantly, water, is a crucial method for a combined effort to make a change. One way to achieve this is through engaging citizens in water issues, e.g. through citizen science projects. In 2021, EYDAP together with 10 other major stakeholders in the water sector, created the Athens Water Forum, under the European program Fiware4Water, also supported by the United Nations WWQA Social Engagement Program. Using targeted sampling a questionnaire was widely distributed among the stakeholders' contacts in order to get a better understanding of what interests' citizens in the water sector. We got 348 responses back. To proceed with this initiative, we carried out a mass digital public event during World Water Day 2022, called "Water for City and City for Water" with different sub-themes, which fostered dialogue on water, the environment, climate change and other issues, identified while also enhancing the active participation and cooperation of citizens.

Keywords: citizen science, green technologies, smart technologies, public awareness, communities, stakeholder, survey.

1. Introduction

Nowadays, lack of adequate scientific principles and lack of public awareness of environmental or other environmentally-related issues that have great influence on the implementation of sustainable practices are barriers to controlling environmental degradation. As much as laws and policies can do to control environmental and water pollution, it is still a matter of individual actions and behaviors, which root from education and knowledge that could be an obstacle in achieving a sustainable future for the humankind on local, national and global level (Rahmani *et al.,* 2021). Water awareness, like public awareness, includes the recognition of water quality threats such as that freshwater is a limited resource and engaging citizens in adopting environmentally responsible behavior towards the protection of this resource (Seelen *et al.,* 2019).

Raising awareness for water issues is a way to build a common understanding on water issues and to create shared values on how water should be used and managed, so raising awareness surrounding water resources is seen as increasingly important (GWP, 2017). There are two different areas that activities to raise awareness should cover. One is the more general public awareness, which involves wide-spread acknowledgment and understanding of issues surrounding water. The other one is self-awareness, which means understanding the relation between personal water use and natural and societal impacts of human actions. The aim of rising public awareness for water issues is to engage the public in topics such as: water conservation, hygienic water use; and preservation of ecosystems amongst others. Ideally, public awareness is not a one-way communication, but an interaction of many active stakeholders, who influence each other and provide social control by mutually reinforcing agreed sets of values (GWP, 2017).

The initiative of the Athens Water Forum is coordinated by the Research & Development Department of EYDAP, in collaboration with EYDAP's Public Relations Department in the framework of the European research project Fiware4Water (which explores the use of smart digital applications in the field of water).

EYDAP joined forces with 10 major Athenian stakeholders that participated actively in the design of this initiative and jointly opened the dialogue. Its aim was to open the wider debate on the most important natural asset, the protection of which is at the top of the modern challenges facing the planet. It was also supported by the UN World Water Quality Alliance, which builds networks of people and organizations from around the world. Two activities were carried out; the first was a targeted survey aimed at looking into the interests of the citizens around water in Athens, water quality, different types of consumption, water reuse, new technologies and water sustainability. Based on the results of the research, the Athens Water Forum planned a digital public event for March 22; World Water Day, with different sub-topics, cultivating the dialogue around the water, the environment, climate change and other issues identified while enhancing the active participation and cooperation of citizens.

2. Material and Methods

2.1 Athens Water Forum-Survey Questions

Background knowledge and expertise were the main drivers for the completion of the survey questions. As this survey was for the internal use of the department, social media and any forms of mass media were not used. The survey was distributed to personal contacts of the participating stakeholders. Apart from the more general questions which included age, gender, educational background and place of residence, the more advanced questions comprised the following:

- Which of the following topics for **water supply** in Athens interest you (Water Reuse, Urban Water Cycle, Water Quality & Distribution Network)?
- Which of the following topics for **water use** in Athens interest you (Domestic consumption, recreational use, agricultural use & energy production)?
- Which of the following topics for **water sustainability** in Athens interest you (reduce in plastic use, low water footprint, water & climate change and water management for circular economy)?
- Which of the following topics for **water technologies** in Athens interest you (smart water systems, blue-green infrastructure, reduction of water consumption in buildings and digitalization of water utilities)?

2.2. Athens Water Forum- Digital Public Event

On the 22nd of March 2022, a digital event was hosted where water professionals, scientists, businessmen, artists, nature lovers and citizens who were interested in the future of the planet and wanted to act locally were invited to discuss and share ideas around the water. Representatives from EYDAP, the Municipality of Athens, the National Technical University of Athens, the National Kapodistrian University, the Hellenic Center for Marine Research, the Cultural Center of Stavros Niarchos, United Nations WWQA Social Engagement Program and the Joint Research Center of the European Commission, participated in this discussion (14 speakers), while public participation was encouraged using digital participatory tools, such as live discussions and interventions from the audience, online quizzes to engage the public and a Q&A section.

3. Results

3.1- Survey Questions Analysis

The questionnaire of the Athens Water Forum was shared to targeted contacts by the networks of the members of the Forum, from 11 to 21 January 2022, addressed to residents of Attica who are interested in water and the future of the planet and wish to engage locally.

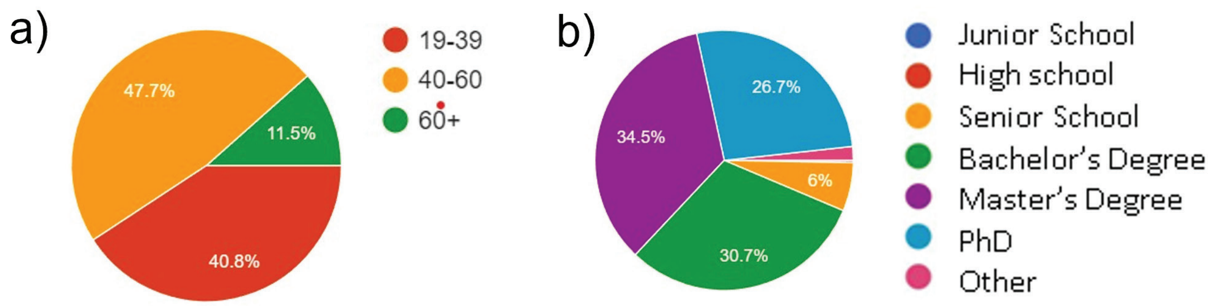

Fig. 1: Survey results for a) Age Group, b) Educational Background.

The aim of the research was to collect and classify the interests of the citizens in relation to water, which will determine the topics of the next actions of the Athens Water Forum, but also to gather the first community of people who are interested in participating in the Forum. In 10 days, we received 348 completed questionnaires, keeping the research at a low communication level (social media and other traditional promotions were not used). On Figure 1 and Tables 1, 2 we present the results based on age and educational background.

Table 1. Survey results per Age Group for targeted questions

Topics	Per Age Group			
	Up to 18(Total 0)	19-39 (Total 142)	40-60 (Total 166)	60+ (Total 40)
Water Supply				
Water Reuse		110	125	27
Urban Water Cyle		113	130	25
Water Quality		113	126	29
Water Distribution Network		45	53	12
Water Use				
Dometic Consumption		128	154	38
Recreation		53	55	16
Agricultural Use		43	44	14
Energy Production		98	88	25
Sustainability				
Reduction of plastic consumption		109	113	27
Low water footprint		106	124	31
Water & Climate Change		116	117	26
Circular Economy Water Management		101	117	27
Technology				
Smart Water Systems		110	115	23
Bue-Green Infrastructure		107	111	25
Reduce consmption in buildings		115	123	32
Digitilization of water utilties		78	83	17

For the age group 19-39, it can be observed that the urban water cycle and water quality have the highest scores, with water reuse being the third option. The same applies for the age group 40-60. The age group 60+ there was a higher interest in water quality, water reuse and then the urban water cycle. Regarding water supply, all the age groups showed a higher interest in domestic water supply, followed by energy production and recreational use. In relation to sustainability, agegroup19-39 showed a higher interest in water and climate change, followed by reduction in plastic. Age group 40-60 preferred a low water footprint, followed by water and climate change and then the circular economy of water. Interestingly, age group 60+ had the highest interest in low water footprint and the lowest in climate change. Could this be a generational difference? Finally, all three age groups showed high interest in decreasing water consumption in buildings, followed by blue-green infrastructure and smart-water technologies.

Table 2. Survey results per Educational Background for targeted questions.

THEMES	PER EDUCATIONAL LEVEL				
	Senior Schol (Total 21)	Bachelors Degree (Total 107)	Masters Degree (Total 120)	PhD (Total 93)	Other (Total 6)
Water Supply					
Water Reuse	14	80	92	71	4
Urban Water Cyle	13	85	89	77	4
Water Quality	13	85	94	74	3
Water Distribution Network	7	37	34	30	2
Water Use					
Dometic Consumption	19	96	115	85	4
Recreation	7	37	48	30	2
Agricultural Use	7	33	29	29	3
Energy Production	11	66	77	52	5
Sustainability					
Reduction of plastic consumption	15	83	84	64	2
Low water footprint	12	77	91	77	4
Water & Climate Change	12	86	91	65	4
Circular Economy Water Management	10	74	85	72	4
Technology					
Smart Water Systems	12	83	85	67	4
Bue-Green Infrastructure	10	76	88	65	3
Reduce consmption in buildings	14	86	93	73	4
Digitilization of water utilties	9	51	62	54	2

Looking at the educational backgrounds, people with a bachelor's degree and PhD opted for the urban water cycle, whilst people with a master's degree preferred water quality and reuse of water was mostly chosen by people who solely received a senior school diploma. With reference to water usage, all levels of education primarily were interested in domestic water consumption, and if observed closely had quite high differences than the rest of the options. As can be seen, for sustainability there was a variety of answers depending on the level of education. Could this variety illustrate a general importance to the sustainability of water? If we look at all the categories (age, location and education), there is a standard variance in interest. All levels of education showed primarily interest in decreasing water consumption in buildings.

3.2 Outcome of Athens Water Forum Digital Event

During the event, discussion revolved around the history of water in Attica, the challenges of water management, the issue of quality, the possibilities of water reuse, but also the utilization of non-drinking water through the presentation of best practices. Emphasis was placed on the sustainability dimension, actions to reduce the environmental footprint and the rational use of water through modern smart technologies and emphasized on the social character of water. 210 citizens attended this online digital event, 18% of which wish to participate in similar open discussions, 47% urged for more activities in the city engaging citizens and 35% would like to engage in raising awareness campaigns.

Fig. 2: Banner for the Athens LWF (Source: EYDAP).

4. Discussion/Conclusion

The world faces increasing freshwater supply challenges, fueled partly by rising demand as well as mismanagement of existing resources. This necessitates an aware and an engaged public as well as sound water resource management practices, supported by data-driven scientific knowledge. Citizen science offers a powerful tool to tackle both these challenges by providing efficient and effective ways to obtain useful data while educating citizens about water issues and drive them towards water sensitization (Araya, 2020).

The Athens Water Forum was a successful initiative aimed at educating and encouraging citizens to improve their water use efficiency through presentations and dialogue. Still, there needs to be a more consistent and constant engagement for citizens to adopt certain water management practices, but also to obtain deeper knowledge and understanding on the different water-related sectors. Therefore, having once recruited its initial membership representing the Athenian Quintuple Helix, Athens Water Forum, will proceed to raise awareness on water issues, supported by the United Nations WWQA Social Engagement Program and the Joint Research Center of the European Commission.

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IDENTIFICATION OF STRENGTHS, WEAKNESSESS, OPPORTUNITIES AND THREATS FOR FISHING COMMUNITIES IN THE IONIAN SEA: INTEGRATION OF STAKEHOLDERS' PERSPECTIVES STEMMING FROM RESEARCH PROJECTS

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Abstract

The purpose of this study is to present important elements stemming from interactions with fishing communities in the Ionian Sea under three research projects (ECOAST, ARIEL, SEAwise). The objective was to explore characteristics of the Ionian Sea small scale fisheries (SSF) communities using participatory approaches, and then identify e strengths, weaknesses, opportunities and threats of this social-ecological system (SES) by applying SWOT analysis. Outcomes have captured the broad picture of the SSF SES in the study area, highlighting persistent elements stemming from all three projects. Indeed, all three participatory studies have pointed out similar structural issues and consistent deficiencies that should be addressed while aiming to promote the sustainable development of the fisheries sector, and particularly the SSF in Greek waters. Then, perspectives f related to the specific focus of each of the three projects have also revealed complementary elements, which may be considered as adding up parts to the broad picture of the SSFSES. The study highlights the necessity for organizing integrated research efforts embracing the participatory approach, as they provide a more comprehensive understanding of the challenges faced by place-based fishing communities, and enable the identification of crucial building blocks of the respective SSF SES.

Keywords: small-scale fisheries; participatory approaches; experiential knowledge.

1. Introduction

Fisheries research heavily relies upon fisheries-dependent data from statutory obligations (e.g., catch and effort data). However, the integration of experience and knowledge from local stakeholders and particularly fishers into marine science are becoming more and more important for a range of research topics, such as the evaluation of fisheries management strategies, analysis of fishers' behavior and gathering of local ecological knowledge (LEK) for filling data monitoring gaps. The latter becomes even more important as in recent decades there has been a shift in small-scale fisheries (SSF) management from centralized government systems to decentralized ones that include the local communities. What is more, persistent challenges facing SSF, such as social inequality and unclear property rights, have a strong impact on the livelihoods and well-being of coastal fishing communities, emphasizing the importance of place-based understanding of particular contexts and challenges that influence social and ecological systems (SES) (Andrachuk et al., 2018). Indeed, interactions between the social, ecological and governance characteristics of a SES seem to be those influencing resource management outcomes, as well as the achievement of sustainability goals (Osuka et al., 2020). However, despite the growing literature showing that participatory approaches can greatly support gaining knowledge pertinent to local fishing communities and the respective SES, traditional scientific processes are often incapable of collecting and analyzing such information.

The purpose of this study is to present elements stemming from interactions with SSF communities in the Ionian Sea, that took place during consultations foreseen within three research projects two completed and one ongoing, which were analyzed using SWOT analysis to identify enabling conditions and bottlenecks for creating pathways towards operational ecosystem-based co-management approaches supporting the sustainability of coastal SES.

2. Material and Methods

In the following section, a brief description of consultations that took place in the two completed research projects (the COFASP ECOAST and the Interreg ADRION ARIEL) is presented. Outcomes from those interactions have provided feedback for organizing stakeholder engagement under the recently initiated Horizon 2020 project SEAwise, in the frame of which a number of targeted workshops and events are also foreseen in the near future, while herein, findings from a scoping exercise are included.

2.1 ECOAST

The project was held from 2016 to 2019. The main objective for organizing the stakeholder events was to gather information regarding spatial interactions among human uses, as well as to collect the information required to evaluate existing spatial management practices. Hence, in the Ionian Sea, interviews were held in the summer of 2017, during which, participatory GIS was carried out; small-scale fishers were asked to provide more explicit information regarding their fishing grounds considering target species and métiers, to address conflicts with the existing aquaculture farms in the study area and identify potential future ones from the future development of the aquaculture sector, as foreseen in the officially ratified Special Spatial Framework for Aquaculture (SSFA). (Anon, 2019)

2.2 ARIEL

The ARIEL project was held from 2018 to 2020 and has focused on the SSF and aquaculture sectors in the Adriatic-Ionian Region. Its main objective was to promote innovative solutions for small-scale fishery and aquaculture in the Adriatic-Ionian basin. In September 2018, two consultation meetings took place with local stakeholders from SSF and AQ, respectively, where outcomes from the ECOAST project were also discussed. After those events, innovation audits were held, outlining the innovation state of play and future development scenarios, taking into account research, policy and economic aspects. Local knowledge, gathered during the consultation meetings and the audits, was combined with elements stemming from literature review, and were then elaborated using SWOT analysis addressing the sustainable development of the two sectors (Anon, 2018). In this study, findings related to interactions with the SSF representatives have been considered.

2.3 SEAwise

The SEAwise project began in October 2021 and will last till 2025, aiming to provide a fully operational approach for Ecosystem Based Fisheries Management (EBFM) in EU waters based on stakeholder networks and co-designed innovation. The study area is once again the Ionian Sea, where targeted interactions with key actors are planned during the life course of the project. In February 2022 a preliminary scoping exercise with selected stakeholders took place, during which, diverse stakeholders were asked to indicate the main elements that each one felt should be taken into account while developing an operational EBFM. In this study, elements stemming from the scoping exercise with small scale fishers have been considered.

2.4 SWOT analysis

A SWOT analysis is a simple and effective framework for identifying strengths, weaknesses, opportunities, and threats that a sector, a company or an industry face. In this case, the SWOT analysis was applied for assessing Strengths (S), Weaknesses (W), Opportunities (O), and Threats (T) for the SSF SES in the Ionian Sea on the basis of the elements derived from the aforementioned projects.

Strengths and weaknesses are derived from the internal environment of the sector, that is usually consists of the human and natural resources, the local stakeholders and infrastructure in the local area that constitute the sector. In the internal environment, the purpose of the analysis is to report which of the characteristics of the sector empower its position (Strengths), and which hinder its performance and development (Weaknesses) and thus they need to be improved. On the other hand, the external environment is a source of opportunities and threats, including factors that cannot be controlled, such as political, economic, social, legal and environmental. Based on this analysis, the analyst can build a strategy by identifying ways of utilizing strengths and opportunities in such a way that weaknesses and threats can be circumvented or outweighed (Sigurðardóttir *et al.*, 2015).

3. Results

Based on each project's specific objectives, important knowledge was derived related to the characteristics of the coastal fishing communities SES in the Ionian Sea; each project has revealed a specific set of factors that are significant for the Ionian Sea fisheries. Based on the collected information from the projects, a SWOT analysis was performed and the elements of the SWOT table are presented in Table 1. In particular, Table 1 indicates which element of the SWOT analysis have emerged from which of the three projects, providing insight on persistent matters that were revealed during all consultations, indicating also some complementary building blocks of the Ionian Sea SSF SES picture that were identified while addressing specific points of concern related to spatial conflicts (ECOAST) and innovative solutions (ARIEL).

Table 1. SWOT elements comparison for SSF among the three research projects.

Strengths (S)	**ECOAST**	**ARIEL**	**SEAwise**
Access to foreign markets	Ö	Ö	Ö
Basic/Descent networking among research/academia and SSF actors (still not fully utilized)	X	Ö	Ö
Good and close collaboration with regional authorities	X	Ö	Ö
High importance for local societies (main source of income and employment)	Ö	Ö	X
Low environmental impact	Ö	Ö	Ö
Positive public perception	X	Ö	Ö
Successful cooperation cases	X	Ö	X
Weaknesses (W)	**ECOAST**	**ARIEL**	**SEAwise**
Financial issues/ High costs	X	Ö	Ö
Lack of information flow	Ö	Ö	Ö
Low human capital (aged fishermen unable to handle and utilize modern equipment)	Ö	Ö	Ö
Low level of entrepreneurship	X	Ö	Ö
Low succession rate	X	Ö	Ö
No active role of SSF sector in policy-making process	Ö	Ö	Ö
No cooperative spirit	X	Ö	Ö
Poor technology level / Aged vessels	X	Ö	Ö
Spatial overlap between Aquaculture and SSF	Ö	X	Ö

Strengths (S)	**ECOAST**	**ARIEL**	**SEAwise**
Outdated legislation and outdated existing management measures / Absence of co-management approaches	Ö	Ö	Ö
Opportunities (O)	**ECOAST**	**ARIEL**	**SEAwise**
Clean environment	X	Ö	X
SSF friendly EU-Policy/Recognition of SSF services on the society	X	X	Ö
Growing consumer demand for fisheries products	X	Ö	Ö
Important research/academia actors in the area	X	Ö	Ö
SSF research is at the center of international attention	X	Ö	Ö
Threats (T)	**ECOAST**	**ARIEL**	**SEAwise**
Not encouraging financial environment due to crisis, COVID-19 pandemic etc./ Low access to credit	X	Ö	Ö
High bureaucracy	X	Ö	Ö
Low fish prices	X	Ö	Ö
Meeting consumer’s demands becomes more and more of a difficult task	X	Ö	X
No consistency in policy design / lack of long-term planning	Ö	Ö	Ö
Not well-adjusted EU-policy driven measures/actions	X	Ö	Ö
Overexploitation from other métier / recreational fishing	X	Ö	Ö
Strong global competition	X	Ö	Ö
Non-effective control of illegal fishing / undermanned local	X	Ö	Ö

Out of the 31 elements of the SWOT table, the 23% of them (7) were identified during all the three projects. These elements are mainly related to structural deficiencies of the SSF sector such as low human capital and lack of information flow as well as common and regular issues that affect the fisheries sector, such as non-targeted policy making and inefficient governance mechanisms.

The majority of the SWOT Table elements (20 out of 31 or about 68% share) are issues that have been identified by the two out of three projects. Then, four more issues were identified by only one project, indicating the different specific focus of each project, exploring the Ionian Sea SSF SES from a different perspective and for a different scope, revealing thus more elements that may be considered as adding up parts of the overall picture of the SSF SES in the Ionian Sea.

4. Discussion/Conclusion

This study presents important elements stemming from interactions with fisher communities in the Ionian Sea conducted under three research projects. Structural deficiencies existing in the SSF sector, as well as issues related to the lack of effective governance for tackling SSF needs were mentioned in all cases. As each project had a different specific focus, more elements for the Ionian Sea SSF SES have emerged, highlighting the necessity for integrating existing knowledge stemming from participatory approaches and on the basis of lessons learned, organize future research efforts (i.e., within SEAwise).

What is more, the identification of research scenarios while exploring potential alternative solutions for the sustainable development of the SSF sector should be conducted in a truly transparent participatory process. Indeed, the participation of fishers, as well as other key stakeholders, in research activities can enhance gaining local knowledge and can contribute to more targeted and efficient research outcomes supporting effective decision-making for inclusive sustainable development. However, there is still a high level of uncertainty related to the knowledge and status of the SSF SES at a Mediterranean scale (Villasante et al., 2021) which can be tackled through institutional support for improving the science-policy-stakeholders interface in order to design meaningful research actions aiming towards the protection of the marine environment, which is a prerequisite of the Marine Strategy Framework Directive (MSFD), and hence the resilience of the marine and coastal ecosystems and the communities depending on them. The latter are in line with Blue Economy (BE) concepts, closely linked with the Sustainable Development Goals (SDGs) of the United Nations' Agenda 2030, and particularly with the principle of "ensuring that no one is left behind" which is particularly relevant for people involved in SSF (CoFi, 2016).

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ASSISTING THE INTEGRATION OF MSFD & MSPD IN THE MEDITERRANEAN

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Abstract

Alignment and coordination of the Marine Strategy Framework Directive (MSFD) with other key policies is essential to attain its objectives. To ensure that economic activities do not pose additional pressures on the marine environment, it is imperative to streamline the MSFD with the Maritime Spatial Planning Directive (MSPD), the policy item regulating maritime activities. To assist the integration of MSFD and MSPD, a workshop was organised in the framework of the MEDREGION project focusing on the needs and challenges encountered by Mediterranean Member States' Competent Authorities (CAs) in the coordination of the directives. The results of the survey conducted in this workshop have led to certain recommendations concerning the integration of the two directives, provided herein, in an effort to support CAs in tackling the various issues and therefore, facilitate the alignment of the two directives. Overall, although MSPD implementation considers MSFD provisions, there are still major challenges. Moreover, the analysis of the MEDREGION Programmes of Measures (PoMs) catalogue for descriptors 1, 4 and 6 highlights that the MSFD PoMs constitute a concrete basis for the MSP process as they address human activities which relate to all MSP sectors. Thus, they contribute to the integration of the two directives.

Keywords: Marine Policy, MSP, EU directives.

1. Introduction

In the last few decades there has been effort in Europe to streamline existing policies and adopt a more holistic, ecosystem-based approach to management (Gorjanc *et al.*, 2020; Boyes & Elliott, 2014). Thereof, the EU's Integrated Maritime Policy (IMP) aspires to provide a more coherent approach to maritime issues, with increased coordination between different policy areas (Schultz-Zehden, 2019). The

Marine Strategy Framework Directive (MSFD) constitutes an overarching framework directive setting out environmental objectives to be applied across all European marine regions, ensuring the sustainable use of EU waters and marine resources (EU, 2008) and as such, it is closely linked to other longstanding, key policies. Maritime Spatial Planning (MSP) has been recognised as the optimal process to manage marine resources in space and time (Ehler, 2020), while considered by IMP as a key instrument for the sustainable development of marine and coastal areas. In this setting, the present paper intends to contribute to the challenge for policy integration, showing that the MSFD implementation can support the MSPD process (Paramana *et al.,* 2021a) as well as the importance of their complementarity.

Concerning the Maritime Spatial Planning Directive (MSPD) implementation in the EU, the 22 coastal EU Member States (MSs) had to produce national maritime spatial plans identifying all existing human activities and the most effective way of managing them by March 2021, establishing appropriate cross-border cooperation (EU, 2014). However, none of the Mediterranean EU MSs managed to release plans by the official deadline (WWF, 2021). Currently, in the Mediterranean, approved maritime spatial plans exist in France, Malta, and Slovenia. In Croatia, various spatial plans exist comprising both terrestrial and marine areas. Italy and Spain are about to finalise their plans. Finally, sectoral plans are in place in Greece, whereas in Cyprus a pilot plan has been developed for the coastal and marine area of Limassol.

MSFD is due to conclude its second implementation cycle in 2023, and MSs try to coordinate their actions at regional scale to ensure coherent assessments of the marine environment and coordinated measures in the cycle to follow, to lead towards the achievement of Good Environmental Status (GES).

2. Methodological Approach

Information on the challenges encountered by Competent Authorities (CAs) regarding the implementation and integration of MSFD and MSPD as well as feedback regarding their needs and experiences were gathered in a web-workshop organized on 20th April 2021 in the framework of the MEDREGION project. The workshop was attended by 39 participants, 13 of whom were CAs representatives from Italy, Greece, Slovenia, Malta, Cyprus and Croatia as well as UNEP/MAP. The MSP CAs of Cyprus, France, Spain, Malta and Greece were also addressed in the aftermath of the workshop. A set of questions was provided;

Q1	In case more than one CAs are involved in the implementation of MSFD and MSPD, is there adequate communication/ collaboration?
Q2	Regarding planning, who is responsible within administration for MSP components?
Q3	How are transboundary issues tackled?
Q4	To which extent are stakeholders involved? How influential are they?
Q5	To which extent has MSFD been considered in the process of MSP planning?
Q6	If plans have not been delivered so far, what is the intention?
Q7	What has been the biggest challenge for your MSP process?

In addition, the PoMs Catalogue compiled in the framework of the MEDREGION project and populated with EU Mediterranean MSs' measures for Descriptors 1, 3, 4, 6 was analysed to identify human activities which are related to MSP sectors and highlight the valuable contribution of MSFD PoMs to MSPD implementation.

3. Results

The Competent Authorities (CAs) that responded to the survey were mainly related to MSFD, with the exception of Cyprus and Spain; Ministry of Ecological Transition (Italy), Ministry of the Environment and Spatial Planning (Slovenia), Ministry of Economy and Sustainable Development (Croatia) as well as Ministry of Ecological Transition/ DG for the Sustainability of the Coast and the Sea (Spain), and Shipping Deputy Ministry (Cyprus). The CAs responsible for the implementation of MSFD and MSPD are in most Mediterranean MSs different, except for Spain, Slovenia and Greece, which commonly generates various problems of objectives and administrative competences. However, Interministerial Committees (IT, CY) and working groups (SP) are in place supporting the implementation of the directives. Therefore, closer collaboration is needed between the MSPD & MSFD CAs to align the two directives and facilitate competences & resources.

Overall, the maritime plans are to be defined by the technical Committees and/or the Inter-ministerial Committees and approved by the relevant Ministry (IT, CY). In Spain, the plan is elaborated by the CA and approved by the Council of Ministries with the participation of several sectorial administrations. In Slovenia and Croatia, the Government has the final approval of the plans.

All EU Mediterranean MSs claim to consider the MSFD framework and requirements in the MSP process.

Concerning transboundary consultation, it is obligatory in the Strategic Environmental Assessment in Slovenia, whereas in Croatia, the work of river basin commissions addresses transboundary issues. In Spain, official consultation takes place with neighbouring MSs as well. The CA and Interministerial Committee are to cooperate with bordering EU MSs and third countries in Cyprus. In Italy, political guidelines and cross-border planning activities could be strengthened, however, Trilateral meetings among Italy, Slovenia and Croatia address the issue and the Coastal Area Management Programs of UNEP/ MAP enhance cooperation.

Stakeholders are involved in all national processes through public participation and consultation, although force majeure (Covid-19) hindered processes. In Croatia, all MSFD relevant stakeholders are present in the National Expert Committee, whereas in Italy, stakeholder involvement can be extended further as the plans are elaborated at a sub-regional level.

The biggest challenges in Italy lie in combining the various needs of a broad range of actors and institutions, as well as the complexity generated by competencies of different administration levels (i.e., central and local), which impede an overall, synthetic vision. In Cyprus, the biggest challenge for the MSP process is cooperation with third countries, whereas in Slovenia, compromising development interests of already existing activities and uses with very strict protection requirements. Finally, in Spain coordinating CAs and enabling the deployment of new sectors (e.g., offshore wind farms) raise challenges.

The analysis of the MEDREGION Programmes of Measures (POMs) catalogue showed that all MSFD activities are addressed with measures; amongst the 638 measures included in the catalogue the majority relate to fishing activities, professional or recreational (284), while these MSFD measures are related to all MSP sectors (apart from Offshore wind energy for which there aren't direct measures) (Figure 1). The number of measures indicates the level of the potential contribution of MSFD knowledge to MSPD implementation, as it denotes that there is information relating to the state of the environment and pressures generated by the MSFD activity.

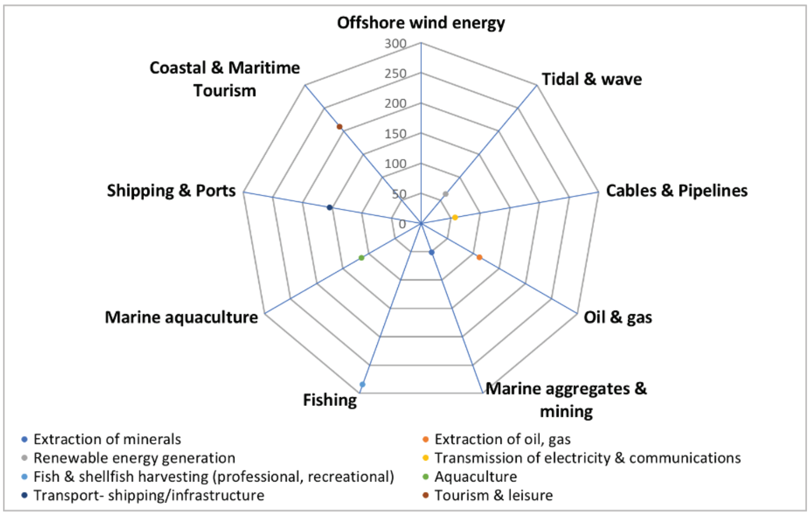

Fig. 1: MSFD established measures relevant to MSP sectors.

4. Discussion/Conclusions

Streamlining MSFD and MSPD is crucial to ensure that the introduction or expansion of economic activities do not generate additional pressures on the marine environment (Gilbert *et al.,* 2015), while the IMP objectives can only be achieved through their synergy (EU, 2011). MSFD and MSPD are adaptive processes, they act complementarily and have determinative commonalities that guide their coherence; Sustainable development and management of marine resources; Ecosystem based approach; Cross-border cooperation; Land-sea interactions; Engagement of stakeholders & public participation; Sound data for decision making (Paramana *et al.,* 2021b).

The results of the survey conducted in the framework of the workshop have led to certain recommendations aiming to assist the integration of the two directives. Thus, it is proposed to;

foster the interaction between the goals of the MSFD and MSPD.

implement the two directives under a common CA to ensure common competences and objectives and enhance governance. Conversely, establish an active inter-ministerial Committee to facilitate the coordination among different CAs and administrations, ensure sound governance, sharing of knowledge, competences and resources.

consider ecosystem boundaries and not only administrative or national borders, or economic activities, in order to implement the directives in accordance with the Ecosystem Approach.

identify the appropriate geographical scale for the definition of planning and management units, mapping environmental resources and protection needs, maritime activities as well as their impacts and interferences. The nested approach could ensure more spatially- explicit plans (e.g., local, national, subregional).

use the best available data, information and tools relying on concrete scientific knowledge. Create a compatible geo-data base for the two directives to provide for transboundary implementation.

actively and effectively engage relevant stakeholders having direct or indirect interest at an early stage with a common vision, to enable better understanding of policy processes as well as sectors/ activities goals.

enable regional/ subregional cooperation regarding human activities, functions of highly transboundary nature, definition of common planning objectives and measures.

establish a transnational Committee for transboundary issues, engaging all levels of administration, considering existing institutional and legal transnational cooperation frameworks, and macro-regional strategies.

Overall, the integration of the MSFD and MSPD facilitates MSP, which can make use of the marine environment assessments, monitoring and well positioned adopted measures necessitated by the MSFD.

Equally, MSFD is substantially assisted as the marine environment is considered in the planning process entailed in MSPD, and consequently the Ecosystem Based Approach is strengthened.

5. Acknowledgements

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BENTHIC COMMUNITIES WITHIN THE EU MARINE STRATEGY FRAMEWORK DIRECTIVE: MEIOFAUNA AND MACROFAUNA PATTERNS FROM THE DEEP SEA

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Abstract

For the protection of the European seas several directives are implemented at national scale, such as the Marine Strategy Framework Directive (MSFD). According to MSFD, marine ecosystems are assessed and monitored on the basis of 11 descriptors by applying an ecosystem-based approach. Macrobenthos is one of the components traditionally used for assessing good environmental status within descriptors D1, D2 and D6 of MSFD implementation in Greece. Nonetheless, meiofauna - the most abundant and phyletically diverse component of marine sediments - has not yet been considered in monitoring programmes, despite the fact that meiofauna may be abundant in a range of marine habitats, such as the deep sea, from where macrofauna are scarce. In this context, we study patterns of meio- and macrofaunal communities from stations of the Greek MSFD, and explore the potential use of meiofauna as an indicator of environmental quality status in the eastern Mediterranean deep sea. First results suggest that meiofauna abundance patterns correspond to those of macrofauna; yet there is no mach in patterns when richness is considered, probably due to the different level of taxonomic analysis between the two components. Future analysis, including more samples and variables will help further address our questions.

Keywords: environmental assessment, monitoring, Mediterranean Sea, Ionian Sea, benthos.

1. Introduction

European seas are increasingly vulnerable to the cumulative impacts of anthropogenic activities. As we are now fully aware that human well-being is closely linked to ocean health, sustainability and protection, a strong focus is set on environmental impact assessment, monitoring and management. Over the last decades, this has been mightily highlighted through the establishment of European directives, such as WFD and MSFD. According to these directives, the environmental status of marine ecosystems is assessed and monitored on the basis of a holistic, ecosystem-based approach, which requires the study of several descriptors that address multiple aspects of marine ecosystems. To this end, a wealth of tools and indicators have already been developed and available for ecological quality assessment (Borja *et al.*, 2014).

Benthic invertebrates have long been used for studying environmental quality and ecological disturbance in the marine realm, as their communities are sensitive to changes in sediment and water quality and they tend to reflect the cumulative effects of present and past conditions. For this, the study of the macrofaunal component of marine benthos is already required for assessing good environmental status (GES) within descriptors one (D1, biological diversity), two (D2, non-indigenous species) and six (D6, seafloor integrity) of the MSFD implementation in Greece. Yet, integrating meiofauna - the most abundant and phyletically diverse component of marine sediments - in a set of tools for ecosystem assessment and monitoring remains a challenge, not only regionally but worldwide. This is due to a number of reasons that mainly relate with existing gaps in their taxonomy, lack of experts, as well as with the more sophisticated laboratory procedures required for their analysis. Nonetheless, this will need to change, as meiofauna may not only be an ideal, but perhaps the key tool for environmental monitoring in a wide range of marine habitats from where the commonly used macrofauna are scarce, such as the deep sea

(Ingels *et al.*, 2021).

In this context, we take off to investigate and compare patterns of both meio- and macrofaunal communities from stations of the Greek MSFD. Apart from contributing to a holistic approach in environmental assessment, we aim to explore the potential use of meiobenthic features as indicators of environmental quality status that may apply to the oligotrophic eastern Mediterranean deep sea. In this study, we report first insights on benthic metazoan communities from the Ionian and the Libyan seas.

2. Material and Methods

During cruise 4MSFD onboard R/V AEGAEO (26 February-2 March 2020) sediment samples were collected from four deep-sea stations of the MSFD monitoring network extending from the north Ionian Sea to the west Libyan Sea (Fig. 1, Table 1). For the estimation of benthic environment variables and for meiofaunal community analysis, samples were collected using a Bowers and Connelly multiple-corer with eight cores of 9 cm inner diameter. The sampling device was deployed 1–2 times at each station in order to collect an adequate number of sediment samples. For meiofaunal community structure analysis, the top 5 cm of three sediment cores were used. Meiofaunal samples were preserved in plastic containers by adding 10 % formalin until laboratory processing.

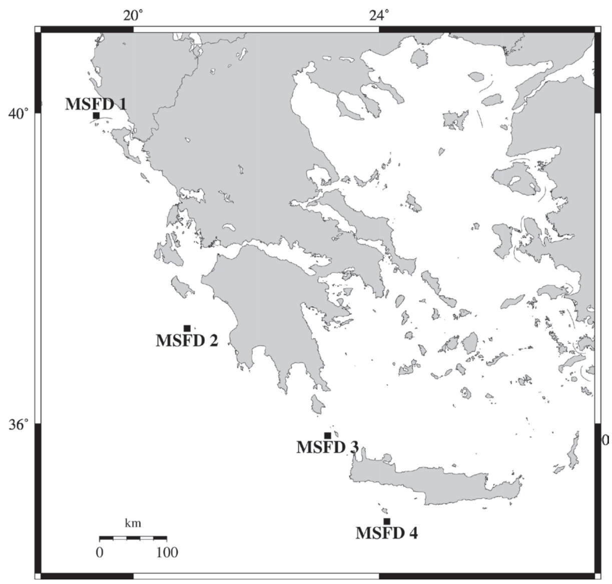

Fig. 1: Map indicating the study area and the four sampling stations.

Table 1. Location of the study stations. Coordinates are given in DD MM,MMM; Chla: average chlorophyll a concentrations in the sediment.

Station	Area	Region	Type	Depth (m)	Latitude	Longitude	Chla (μg/g)
MSFD 1	Othonoi	Ionian Sea	Deep-sea mud	290.8	39° 58.0920	19° 23.8289	0.12
MSFD 2	Strofades	Ionian Sea	Deep-sea mud	1218.9	37° 14.9937	20° 52.5698	0.01
MSFD 3	Antikythira	Ionian Sea	Deep-sea mud	942.0	35° 50.7090	23° 09.7560	0.01
MSFD 4	Gavdos	Libyan Sea	Deep-sea mud	1069.5	34° 41.9394	24° 07.2684	0.04

Macrofaunal samples were collected by using a standard 0.1 m^2 USNEL box corer that was deployed twice at each station. Each sediment box was sliced into two layers of 5 cm, each of which was washed through a set of sieves with 300 and 500 μm mesh size. Each sediment fraction was kept separately and preserved in 10 % formalin.

In the laboratory, standard macrobenthic and meiofaunal techniques were used for the extraction of the animals from the sediment and their classification in major taxa (Eleftheriou & Moore, 2013). For meiofauna, samples were first washed through a 32 μm mesh sieve followed by the density gradient separation method proposed by de Jonge and Bouwman (1977). After extraction, rose bengal solution was added in each sample. Both meio- and macrofaunal organisms were sorted and identified under a stereoscopic microscope.

3. Results

3.1 Meiofauna

Overall, 14 meiofaunal taxa were encountered at all stations. Of these, only nematodes, copepods, polychaetes, and tardigrades were found in all stations, whereas gnathostomulids and bryozoans were found in just one sample. Nematodes predominated in all stations with an average percentage contribution ranging from 80 % (MSFD4, south of Crete) to 90 % (MSFD1, north Ionian Sea), while copepods reached up to 18 % at station MSFD4 (Fig. 2).

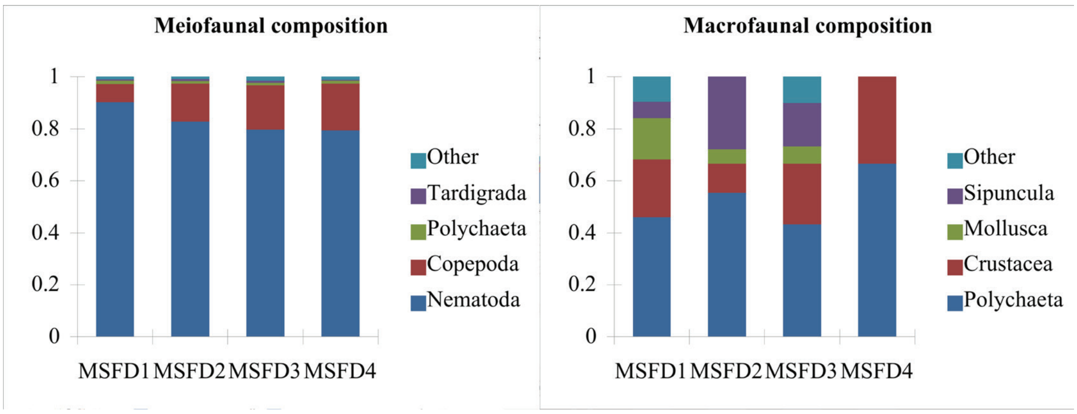

Fig. 2: Meiofaunal and macrofaunal abundance composition by sampling station.

Average densities ranged from 39 ind/10 cm^2 at the deepest MSFD2 station west of Peloponnese to 410 ind/10 cm^2 at the shallowest, northest MSFD1 station in the Ionian (Fig. 3), where sedimentary pigments concentrations were higher (Table 1). In contrast, the overall number of meiofaunal taxa was lower where abundance peaked (MSFD1, 8 taxa) and slightly higher in south Ionian Sea, near Antikithyra (MSFD3, 11 taxa) (Fig. 3). Nevertheless, one-way analysis of variance confirmed statistically significant differences among stations only when abundance is considered ($p < 0.001$), with Tuckey HSD test further indicating the significantly higher meiofaunal densities at the shallowest, more food-rich MSFD1 station.

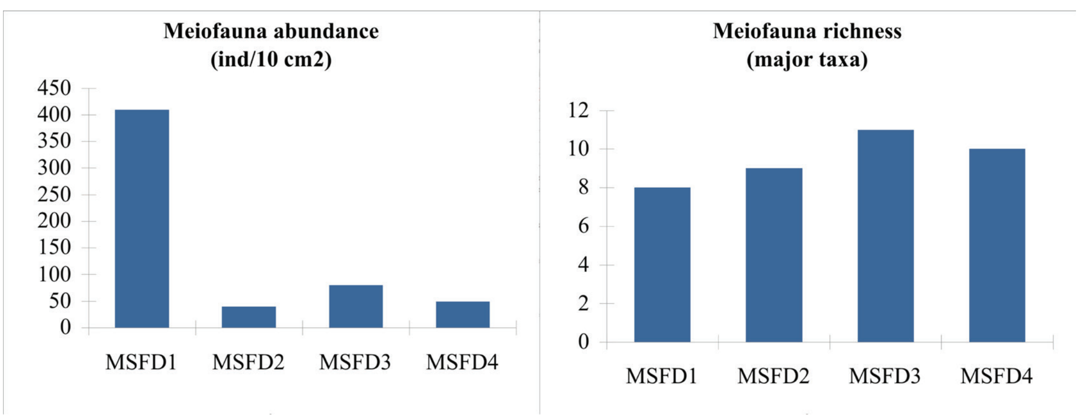

Fig. 3: Average meiofaunal abundance and total number of meiofaunal major taxa by sampling station.

The application of the nematodes/copepods ratio (Raffaelli and Mason, 1981), that has been suggested in the past as a potential indicator of benthic disturbance, was also applied in this study (results not shown) and though it also differentiated the shallower station MSFD1 from the rest, it did not appear to provide any discernible pattern for the deeper stations.

3.2 Macrofauna

In total, 58 macrobenthic species/taxa belonging to nine phyla were identified from the collected macrofaunal samples, most of which were polychaetes. Polychaetes dominated also in terms of abundance in all stations (43-67 %), followed by either crustacea (11-33 %) or Sipuncula (6-28 %) (Fig. 2). Macrobenthic abundance was rather low, ranging from 15 ind/m^2 at the deep station MSFD4 in the Libyan Sea to 315 ind/m^2 at the shallowest station MSFD1 in the north Ionian Sea (Fig. 4). Species richness exhibited an identical pattern, with very low numbers of species reported from the two deepest stations of the area (MSFD4, 3 species; MSFD2, 12 species) and a much higher number appearing at the much shallower northern station MSFD1 (39 species) (Fig. 4). Similar to meiofaunal results, one-way analysis of variance confirmed statistically significant differences among stations ($p < 0.001$), with Tuckey HSD test suggesting that differences were due to the highest macrofaunal densities and richness at MSFD1 station but also due to the significantly lower macrofaunal densities at the deep MSFD4 station located in the Libyan sea.

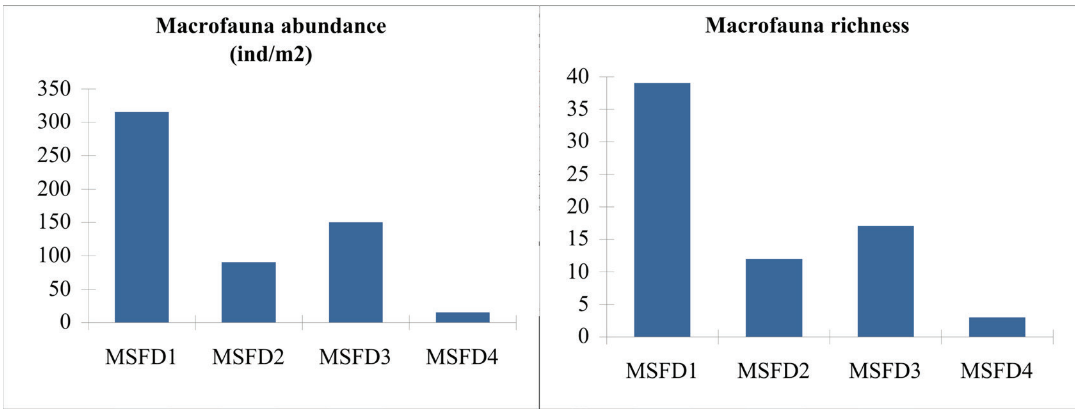

Fig. 4: Average macrofaunal abundance and total number of macrofaunal species by sampling station.

4. Discussion/Conclusion

First results of our investigation suggest that both meiofauna and macrofauna thrive in the shallowest station of the study area, where sedimentary chlorophyll *a* concentration is also much higher. More importantly, meiofaunal abundance exhibits the same spatial pattern with both macrofauna density and macrofauna richness in the deep-sea stations of the MSFD sampling grid in the Ionian and Libyan seas; this may well be an indication that the use of meiofauna in the deep sea as an assessment tool of environmental quality may not only be complementary but also a promising quality element.

On the other hand, the observed mismatch in spatial patterns between meiofauna and macrofaunal richness may be due to the different taxonomic level that was employed for each benthic component. Nevertheless, further analysis of meiofaunal samples and organisms in order to include more diversity indices, community structure analyses, and more variables, such as selected key taxa/populations (e.g., kinorhynhs, copepod nauplii), will shed further light to whether meiofauna may comply, complement or even outweigh macrofauna as a potential indicator of environmental status for a range of environments and pressure types in the deep sea.

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EMODNET INGESTION AND SAFE-KEEPING OF MARINE DATA

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Abstract

Easy access to high-end data is fundamental for different target users and applications related to the understanding and the efficient management of the marine environment. The access to marine data is a key issue for the EU Marine Strategy Framework Directive and the EU Marine Knowledge 2020 agenda and includes the European Marine Observation and Data Network (EMODnet) initiative. There is a wealth of marine data collected in Europe by public authorities, researchers, private operators of coastal and offshore facilities or citizens science. EMODnet, a DG-MARE initiative, is a network of 120 organizations that work together to assemble, harmonize marine data from diverse sources and make them discoverable and accessible in a uniform way. However, numerous data sets remain hidden or unusable. The 'EMODnet Ingestion and safe-keeping of marine data' project, tackles this problem by reaching out to data holders, explaining the benefits of sharing their data and providing a support service to assist them in releasing their data for subsequent processing, quality control, long term storage, and inclusion in EMODnet data products.

Keywords: data network, submission service, data management, data sharing, data centre.

1. Introduction

Many data collected by public authorities, researchers and private operators of coastal or offshore facilities still do not arrive to national or regional repositories and are thus unavailable to potential users. This creates additional costs for those working on marine issues who will either have to accept lower confidence in their analysis and research than would otherwise be the case, or be compelled to needlessly repeat observations at the sea. There is therefore the need to streamline the data ingestion process so that data holders from public and private sectors can easily release their data for safekeeping and subsequent distribution through EMODnet or other means.

In 2016, the Executive Agency for Small and Medium-sized enterprises (EASME) concluded a contract for developing a service for ingestion and safe-keeping of marine data. In that context, a Data Ingestion Portal has been developed, which facilitates data managers to ingest their marine datasets for further processing and publishing as open data. In 2019, a follow-up project was established with the same consortium for continuing the Data Ingestion service for another two years. Recently, in March 2022, the European Climate, Infrastructure and Environment Executive Agency (CINEA) renewed the successor project for two more years under the same coordination group (MARIS as coordinator, HCMR as scientific coordinator). In the current third phase, EMODnet Data Ingestion will built on the work done under the previous phases and keep the operational network over Europe with the additional task to integrate the current ingestion service into the EMODnet Central Portal.

The overall aim of EMODnet Data Ingestion project is to facilitate and streamline the process whereby marine data from whatever source (including national monitoring programs, research projects and private companies) is delivered on a voluntary basis for safekeeping to data repositories from where it can be freely disseminated.

More analytically the objectives are:

- To identify and reach out to organizations from public, research and private sectors who are managing marine datasets for bathymetry, geology, physics, chemistry, biology and/or human activities and who are not yet connected and contributing to the existing marine data management infrastructures.

- To motivate and support those potential data providers to release their datasets for safekeeping and subsequent freely distribution through EMODnet.
- To facilitate the inclusion of those marine datasets by means of a data ingestion service and subsequent communication with expert data repositories to work up the metadata documentation for direct publishing and, in a second stage, for making the submitted datasets fit for inclusion in the EMODnet data services and products.

The EMODnet Data Ingestion portal provides a range of services such as:

- a Submission service for easy ingestion of marine data packages.
- a View Submissions service to oversee submitted data sets 'as is' and further elaborated as entries in major European marine data management infrastructures such as SeaDataNet, EurOBIS, and others which feed into EMODnet and CMEMS.
- a Data wanted service to post requests for specific data types.

The EMODnet Data Ingestion is undertaken by a European consortium of overall 43 organizations (marine research institutes, governmental agencies, and SME's) from 27 coastal countries covering all European seas, and are members of pan-European marine data management infrastructures such as SeaDataCloud, EurOBIS and EGDI, and in international organizations such as IODE, ICES, EuroGeoSurveys, EuroGOOS, and IHO. The network includes also the coordinators of all the EMODnet thematic projects for ensuring the connections with their thematic networks for processing the incoming data submissions.

2. Material and Methods

The EMODnet Data Ingestion portal with its integrated services has been launched early February 2017. The Data Submission service is the core service of the portal and has been technically developed, operated and maintained by HCMR. The on-line submission form is based on ISO 19115-2 standard and is compliant with the INSPIRE model and makes extended use of vocabularies and controlled terms.

We made distinction between 2 phases in the life cycle of a data submission:

- Phase I: the ingested data set is published 'as is' at the project View service. To simplify the process as much as possible, the completion of the submission form is split in 2 steps:
  - Step 1: Data submitter only completes a number of key fields of the submission form and uploads a zip file with the datasets and related documentation.
  - Step 2: The submission is then assigned to competent Data Centre depending on the country of the data provider and the type of EMODnet theme who reviews and completes the submission form with additional Thereafter, those completed submissions are published with their data packages 'as-is' at the portal in the Summary Submissions service, where users can search, browse and download the data packages.
- Phase II: As a next step, the assigned data centres elaborate selected submissions further to make (subsets of) the data fit for population into national, regional, European and EMODnet thematic portals. Elaboration includes activities like review, validation, conversions to standard formats, and further population to the relevant European infrastructures such as SeaDataNet, EurOBIS, EGDI, CMEMS, and others, depending of the theme, which then feed into EMODnet data portals.

The use of best practices, standards and vocabularies is a key activity throughout the process to make the submitted data and metadata interoperable, accessible and re-usable, improving thus the data FAIRness across and within the EU and the global data infrastructures such as EMODnet and Copernicus.

The EMODnet Data Ingestion process is indicated in the following image.

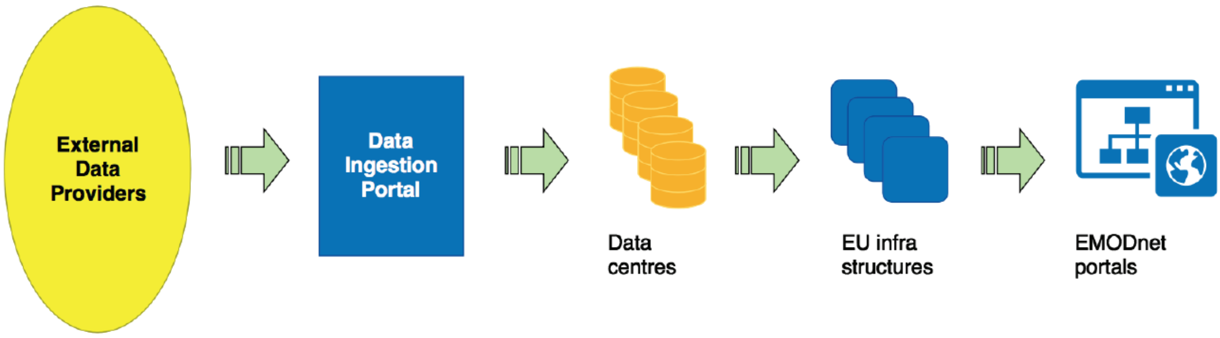

Fig. 1: Flow of data sets from external data providers to EMODnet portals.

3. Results

The project outreach approach (refined over time and based on lessons learned) has been successful as there is a steady increase of data submissions since the launch of the portal and its submission service in 2017.

Currently, there have been more than 1100 data packages submitted of which circa 1000 have been completed and published ''as-is' and more than 450 have been elaborated in standard formats and are now available in leading European marine data management infrastructures, feeding EMODnet thematic portals and their products (Fig. 2a). Around 60% of the published submissions originated from Research Institutes and Universities, 20% from Governmental agencies, 16% from private companies and 4% from NGOs (Fig 2b). These are excellent outcomes, which demonstrate that EMODnet Data Ingestion has established its place in the European marine data landscape, performing roles as promotor of data sharing, educator for how to adopt common standards, and facilitator for publishing and elaborating a wide range of data sets for various disciplines and from all sectors.

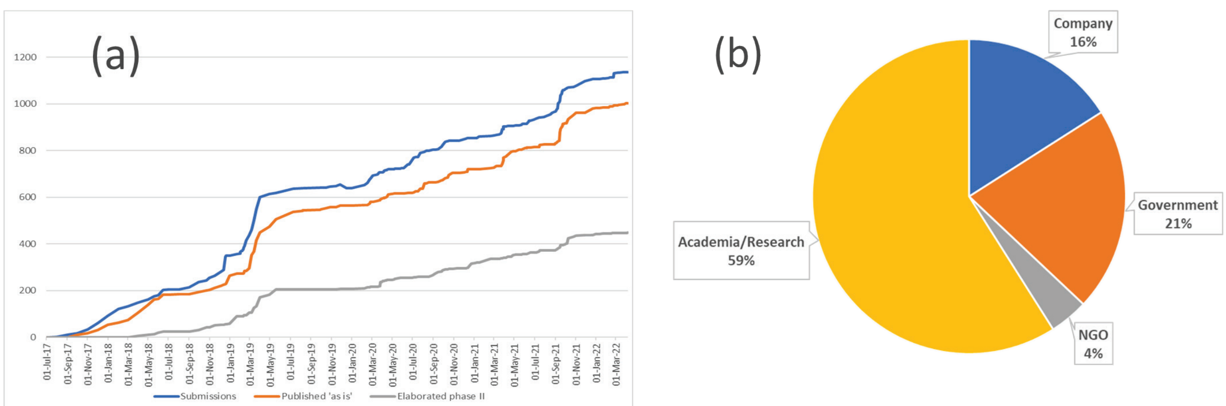

Fig. 2: (a) Submissions and processing in time, (b) Published submissions per type of Organization.

Furthermore, a (semi-)automatic coupling was established between SeaDataNet/SEANOE data citing service and EMODnet Data Ingestion. SEANOE, operated by IFREMER, is a SeaDataNet service, inviting European scientists to publish their scientific papers and associated data collections in return for a DOI which will facilitate their wider citation. The coupling facilitates that (selected) scientific submissions from SEANOE are harvested by EMODnet Data Ingestion for further metadata completion, publishing 'as-is', and elaboration of data sets for inclusion and publishing in national, European and EMODnet portals. The cooperation between SEANOE publication service and EMODnet Data Ingestion is addressing issues related with restricted data policies impeding the free sharing and data exchange because SEANOE service allows embargos besides the free and open access. Once data become freely available (e.g., finalization of publications, other commercial reasons) are exchanged with EMODnet Data Submission service for further uptake as publicly open data.

EMODnet Data Ingestion, besides the handling of archived marine data sets, is supporting also the integration into EMODnet of the Near Real Time (NRT) and even Real Time (RT) oceanographic data that are collected by fixed and moving platforms such as fixed stations, moorings, buoys, tide gauges, surface drifters, ferryboxes, Argo floats, gliders, HF radars and other platforms. In cooperation with EMODnet Physics, operators of operational oceanography networks and platforms are motivated and given guidance for making their datasets part of the European oceanography data exchange as managed by Copernicus CMEMS-INSTAC, EuroGOOS, and SeaDataNet, which are pillars under EMODnet Physics. Currently, more than 300 platforms with thousands of data sets are connected. In addition, EMODnet Data Ingestion jointly with EMODnet Physics is promoting the uptake of Sensor Web Enablement (SWE) standards for operational oceanography data streams. Using the SeaDataNet SWE toolkit as developed by 52North, a SWE pilot has been set-up and is maintained. This pilot gives discovery and access to data streams from real time oceanographic monitoring systems, covering a range of operators and platforms, and allowing direct standardized access to selected data types from selected monitoring instruments (currently more than 1500 sensors). In the current phase, faster availability of NRT and RT data will be achieved by machine to machine exchanges. Existing technologies for operational data connectivity will be expanded with ERDDAP (developed and managed by NOAA) and also by making use of DAB brokerage technology for connecting various web services and APIs. In addition, the project will continue the SWE adaptation for European research vessels in synergy with the H2020 Eurofleets+ project for improving underway data flows from research vessels to shore.

4. Discussion/Conclusion

The EMODnet Data Ingestion Portal streamlines the data ingestion process, allowing data holders from the public and private sectors who are not yet connected to existing marine data management infrastructures or who do not share data on a regular basis to easily release their data for safekeeping and subsequent distribution through EMODnet. There are still many data providers who are not aware of the European and international standards and infrastructures for making their data interoperable and re-useable for other applications. This strengthens the mandate of the EMODnet Data Ingestion to continue with its mission and operation for making more and more data providers aware and informed about the European marine data management and the larger benefits of sharing their data with the wider marine data community. Data sharing helps to eliminate duplication of ocean data collection work, waste of resources, and reduce environmental impacts. All data sources are properly acknowledged giving greater visibility and recognition to data owners. Scientists who share their data can increase their citation scores as digital object identifiers (DOIs) can be allocated to their data submissions. The increase of data availability in European data repositories and infrastructures results to improved data products and information of higher confidence, for example, digital terrain models (DTM), seabed habitat maps and subsurface, assessment of the environmental state or ocean forecasts that the private sector can use and benefit from. More data providers with additional data can contribute to increasing productivity of those working on marine issues, stimulating innovation in the blue economy and reducing uncertainty in our knowledge of the behavior of the sea.

A series of successful cases of data holders who joined EMODnet and shared their data can be found at the project website. To discover how to become part of our success story, visit us at emodnet-ingestion.eu.

5. Acknowledgements

The European Marine Observation and Data Network (EMODnet) is financed by the European Union under Regulation (EU) 2021/1139 of the European Parliament and of the Council of 7 July 2021 establishing the European Maritime, Fisheries and Aquaculture Fund and its predecessor, Regulation (EU) No. 508/2014 of the European Parliament and of the Council of 15 May 2014 on the European Maritime and Fisheries Fund.

EMODnet Ingestion and safe-keeping of marine data current phase is funded by the European Maritime, Fisheries and Aquaculture Fund (EMFAF): https://ec.europa.eu/oceans-and-fisheries/funding/emfaf_en

Websites

European Marine Observation and Data Network (EMODnet) Data Ingestion portal, 2022, https://www.emodnet-ingestion.eu/ (Accessed 18 April 2022)

A MARINE MONITORING SYSTEM OF THE HELLENIC SEAS USING REMOTE SENSING AND IN-SITU DATA: THE MARRE PROJECT

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Abstract

The research project MARRE (2018-2022) aimed at developing an open source GIS system for monitoring the marine environment by using satellite remote sensing data combined with field measurements (historical and real-time data). The development of such a system for Greece stems from the need to support national policies in areas of high interest such as the environmental status of the coastal zone and the wider marine environment. Innovative products were developed and new knowledge obtained providing information on water quality using free satellite observation data and in-situ measurements of the participating institutions as well as from available data at European repositories. Existing empirical algorithms for the estimation of water quality from satellite data were evaluated and improved. As a result, the derived products were adapted to local conditions and the specific features of Greek seas. Particular attention was given to the monitoring of the marine biodiversity through the spatial and temporal monitoring of the Posidonia meadows.

Keywords: oceanography, remote sensing, satellite data, marine water monitoring, Marine Strategy Framework Directive.

1. Introduction

Continuous and accurate monitoring of both coastal waters and open seas is necessary to preserve ecosystems, safeguard public health and protect economic and social activities related to the sea. The core target of MARRE project was the development of a new service that would combine the scientific knowledge with innovative technologies for providing data, products and information easily accessible and usable by a broad range of users and stakeholders, from the research community to decision-making bodies and the general public. Furthermore, the availability of high quality and accurate data and products can effectively contribute to the information system being developed by HCMR in support of the implementation of the Marine Strategy Framework Directive 2008/56/EC in Greece.

The main and innovative elements of the project are: i) the combination of field measurements with multi-scale remote sensing data (from aerial photographs with a spatial resolution of a few centimeters to satellite remote sensing data with a spatial resolution of tens of meters) and, ii) the combination of information extracted from different types of data into an information system for monitoring the state of the coastal and marine environment.

The project was undertaken by a consortium of three partners:

- GET company for the development of a new innovative geospatial information management and dissemination platform (acting as project coordinator),
- University of Aegean, Department of Marine Sciences, for the development of the algorithms that were used to create the new satellite products e.g., Seagrass mapping and potential fishing zones (acting as scientific coordinator),
- HCMR, the largest marine Research Institute and data provider in Greece, for the provision of data that were used for the creation of new products through their combination with satellite data.

2. Methodology

Free earth observation data were used. The new products provide information on water quality and were derived from data of the participating bodies, real-time data from the HCMR Poseidon system, in-situ measurements from the new MARRE cruises conducted by HCMR as well as from data available in European repositories such as SeaDataNet, EMODnet. In particular, using high-resolution satellite data such as Sentinel 2 and 3 data in combination with in-situ measurements at the Hellenic seas, essential environmental parameters such as chlorophyll concentration and turbidity were assessed.

The combined use of satellite data and in-situ measurements led to the evaluation and improvement of existing empirical algorithms (such as the MedOc4). As a result, the derived are adapted to the local conditions and specific characteristics of the Hellenic seas, which is not the case for products produced with the aim of monitoring wider geographical areas such as the whole Mediterranean region.

The products generated by the automated process of satellite data acquisition and analysis are archived in a central geospatial database and a series of services were created to enable their exploitation. These services are based on open standards and architectures such as the OGC catalogue services, view and download services or the INSPIRE metadata specifications. The products are then made available through the project geoportal. The portal also offers selected HCMR in-situ data and products that are provided through web services while the Poseidon real-time data through an API. The portal also is making use of free, open data from other sources, such as the Marine Copernicus, SeaDataNet and EMODnet repositories, thus making it a central service for monitoring the marine environment in Greece (Figure 1).

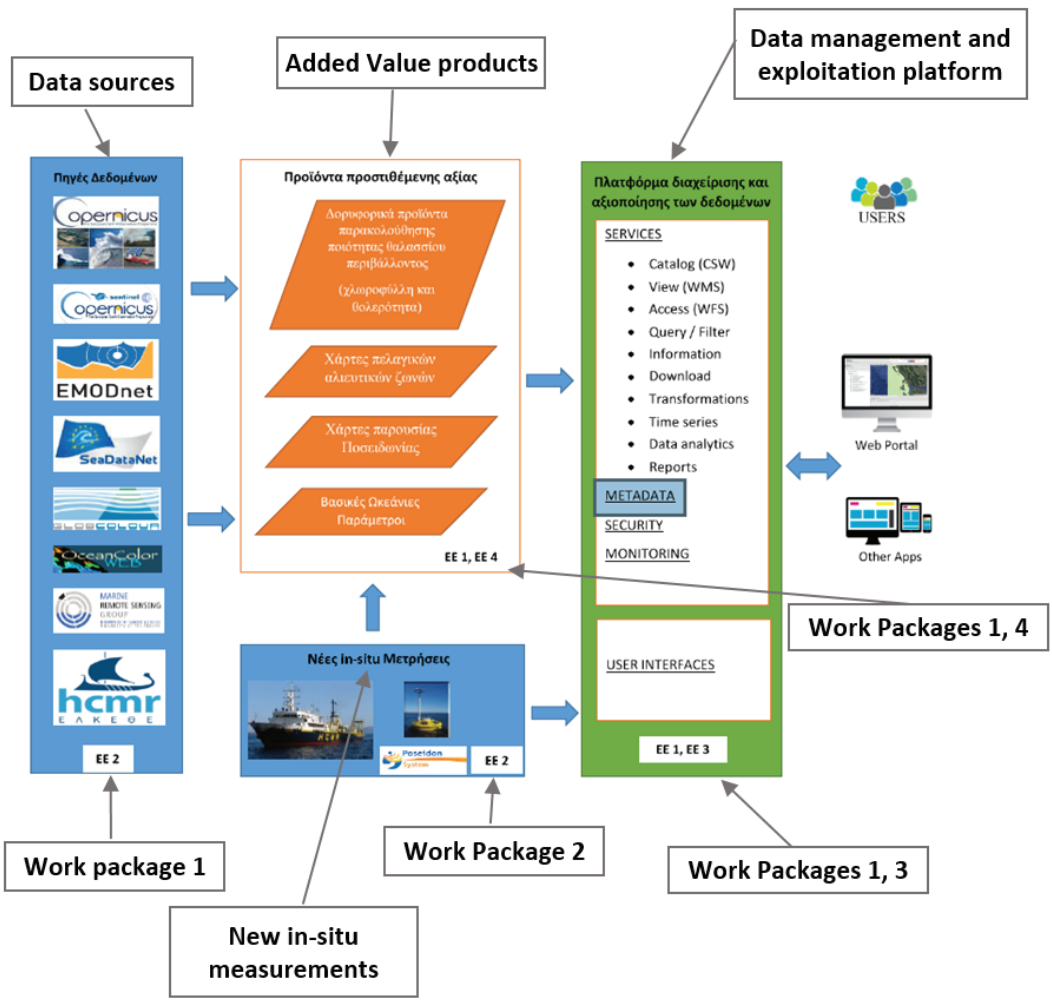

Fig. 1: The MARRE project data flow: satellite and in-situ data (data sources - left) and new in-situ measurements from new MARRE cruises and Poseidon data (New in-situ measurements - middle bottom) that are compiled within Work Package 2, are used for the generation of added value products - middle top) within Work Packages 1, 4. Data and products are

feeding the MARRE platform that provides several services for data exploitation and access as developed within Work Packages 1, 3 (New Environmental Monitoring System for the Greek Seas – left).

3. Results

The project delivered a series of products, services and new knowledge to monitor the status of the marine environment and understand its complexity. MARRE platform (Figure 2) is a user friendly environment that allows users to choose among thematic layers, area and time period of interest, search, view and download the respective data.

- The thematic layers concern basic water quality parameters such as:
- chlorophyll concertation maps,
- suspended matter concentrations maps,
- marine habitats (Posidonia meadows),
- potential fishing zones based on Sentinel-2 and Sentinel -3 data,

developed by the University of Aegean, and essential ocean parameters such as:

- temperature, salinity and transparency of the sea water,
- caesium-137 measurements at the water column,
- time series of the Poseidon system,
- climatic indices of temperature and salinity of the sea water,

provided by HCMR.

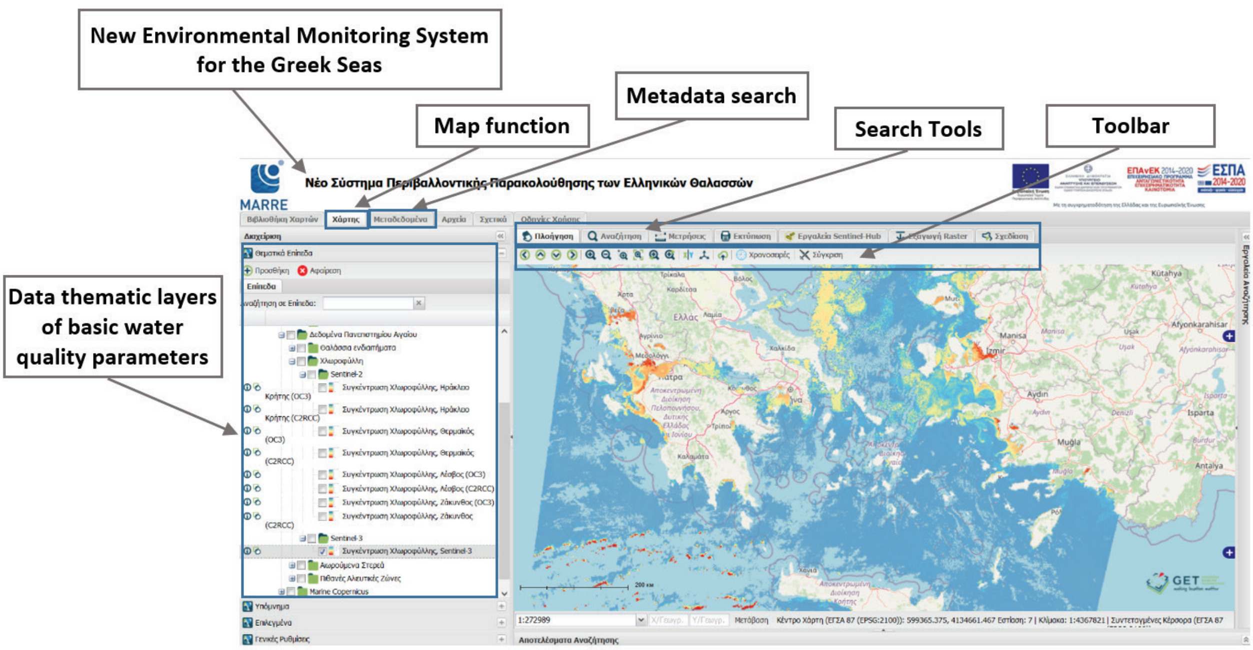

Fig. 2: The MARRE platform for the monitoring of the Greek Seas – Chlorophyll concertation (Sentinel -3) map example: an open Web-GIS application with a map-centric interface for data navigation, search and download. It makes use of OGC web services from the project database and several external data repositories and provides an integrated environment for viewing and analysing spatial marine environmental data.

Besides the final products served by the MARRE platform, another important output is the new knowledge obtained by the new in-situ measurements on the optical properties in the Eastern Mediterranean region and the evaluation of remote sensing methods with respect to ocean colour. Moreover, the analysis of the radioactivity measurements gathered by the coupling of a detection system with a Poseidon fixed monitoring platform resulted to an improvement of the calculation of low concentrations of 137Cs in the marine environment using in situ gamma-ray spectroscopy. This approach can be used as a monitoring solution for radioactive contaminants in the Greek seas.

4. Discussion/Conclusion

The produced maps from satellite observations combined with historical and new in situ measurements contribute significantly to providing information on the quality of coastal and marine ecosystems through the systematic recording of a number of parameters such as chlorophyll, suspended matter, Posidonia meadows, radioactivity and the mapping of fishing zones. The data, maps and all information are available, through a user-friendly interface (MARRE platform), to interested users and stakeholders, covering a wide range of applications and needs. MARRE products are able to significantly support the country's national programmes for monitoring the quality of the marine environment by improving the spatial/temporal scale and quality of the ocean products generated. The project results also confirm that remote sensing offers a cost-effective solution for monitoring the quality of large areas of pelagic and coastal waters even in areas where access is difficult or impossible. However, the results of the MARRE project suggest that multiple field measurements are needed to draw a firm conclusion on the effectiveness of estimating chlorophyll-a and suspended matter concentrations and of recording habitat and fishing zones from satellite data in the study area. It is important to note that field data in future cruises should be obtained at the same time as the satellite images are acquired. In conclusion, the MARRE data and products combined with the collection of new future field measurements, as well as the methodology followed for both satellite observations and field measurements, can serve as a model for future work aiming at continuous and reliable monitoring of Greek water quality, as well as the adaptation and development of new ocean color algorithms using satellite imagery.

5. Acknowledgements

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THE SMART DRIFTER CLUSTER: A NEW CHALLENGE FOR MARINE MONITORING

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Abstract

MARTA Smart Drifter Cluster is an EU granted project aimed at creating an integrated system of data acquisition on marine currents and pollutants, much cheaper and logistically simpler than the present ones. Using the latest software and hardware technologies in IoT, pattern recognition and sensing, we have developed newly conceived oceanographic drifters that can be used for mesoscale monitoring of marine currents and other applications (recovery of people and materials at sea, pollutant spills, marine litter (ML) dispersion). The floating structure that supports the electronics is designed to be environmentally friendly, as well as having specific buoyancy and drogue requirements depending on the use. The entire system, including low level control of thrusters, navigation algorithms, radio/satellite communication systems, drifter trackers and motion predictive control has been successfully implemented using the popular middleware ROS2 (Robot Operating System). Performances of the operational system will be further evaluated during summer 2022. In conclusion, by introducing the concept of clusters in this context and using, in an innovative way, modern 'consumer' electronic technologies for wireless communication, a marine monitoring system is achieved with low hardware costs, almost no maintenance and capable of self-generating the needed energy.

Keywords: marine monitoring, smart drifters, drifter' cluster, wireless communication, IoT, Digital Twin.

1. Introduction

The EU granted MARTA project[1] is a brainchild of CNR-ISMAR, which brings together the long experience of the Institute in the study of marine currents, and the specific skills of some researchers in the field of technology for ecological and electric mobility and integrated electronics and consumer electronics, particularly in the marine sector. The basic idea is to use the latest software and hardware technologies in the field of IoT (Internet of Things), pattern recognition and sensing, to create an integrated system of "real time" acquisition of data on marine currents, monitoring of pollutants (in addition to micro and macro-plastics, hydrocarbons), in a much cheaper and logistically simpler than the present ones. It promotes an alliance between research centers, universities, small and medium industries and foundations that, working together and sharing infrastructure and resources, aim to improve the quality of marine research and services provided in Europe. The major innovation is the introduction of the cluster concept (the Smart Drifter Cluster) in the mesoscale monitoring of marine currents, with also further possible applications (recovery of people/materials lost at sea and pollutant spills, etc.). Today, in the study of marine currents, passive buoys (*drifters:* Poulain & Zambianchi, 2007; Poulain et al., 2009, Subbarya *et al.,* 2016) are used to be transported by surface movements of water or currents of the first meters. The knowledge of marine/oceanographic data is essential and strategic, but today a strong obstacle to the improvement of this knowledge is given by the high cost of equipment and management of measurement campaigns.

1 "SVILUPPO DI UNA INNOVATIVA MULTIPIATTAFORMA SMART DRIFTER – UMV – SAPR PER INDAGINI MARINE" – (M.A.R.T.A.), POR CreO FESR Toscana 2014-2020, Call R&S 2020, Activity 1.1.5.a1. Partners: SIGMA INGEGNERIA S.R.L. (coordinator), MDM TEAM S.R.L., DMG ENGINEERING S.R.L., CNR- Istituto di chimica dei composti organometallici (CNR-ICCOM), CNR– Istituto per i processi chimico-fisici (CNR-IPCF),

The European Commission has indicated, in the context of the Integrated Maritime Policy inaugurated by the Blue Book (COM2007(575)), the need (COM2009(544)) for an integrated sea observation and forecasting system for intermediate and end users that provides data, products and services to facilitate the management of the coastal and marine environment, intervene in the presence of risks, implement the security of sensitive areas, provide visibility on the quality of the coastal and marine environment (EMODNET - European Marine Observation and Data Network). So, expanding and optimizing the communication capabilities between oceanographic measurement instruments can bring significant improvements in the monitoring of ocean currents and search and rescue activities in the Mediterranean Sea.

2. Material and Methods

The smart drifters cluster is composed of two different floating units: the slaves and the masters. The first type is the cheaper, and it is designed to acquire GPS data and optional marine data (e.g. salinity, temperature) and transmit them via a LoRa (Long Range radiofrequency wireless technology) system. It also equipped with photovoltaic (PV) panels to be energy-independent. The second one is very similar to the previous one, but is also equipped with a pair of underwater thrusters and long range (e.g., satellite) communication.

2.1. Conceptual step to be implemented

The project is developed along the following 6 main lines:

1. R&D on low-cost wireless communication technologies, to extend the range and energy efficiency, also through 'intelligent' management optimized for the specific case;
2. IT (Informatic Technology) for intelligent management of cluster elements and drifter data processing;
3. R&D on biodegradable materials for the physical support of the drifter;
4. design of the propulsion system of the master elements;
5. IT (with digital twin technology) for the master elements, to forecast long term motion and future cluster fragmentation and take a certain optimization policy, also based and the quality and quantity of collected data.
6. Energy efficiency and energy harvesting by PVpanels to make the smart drifters cluster virtually energy autonomous.

2.2. Existing technologies and materials used

The technologies that are currently considered for the hardware and software development of the project are the following:

- For hardware: GPS receivers with low consumption and low cost, PSOC (programmable system onchip) Cypress microcontroller with very low consumption. LoRa technology, which is a sub gigahertz wireless transmission system designed to cover long distances at a modest bit rate and low power consumption. The rather wide diffusion of this standard among the various manufacturers allows to have a low production cost compared to other less established standards. An SD (Secure Ditital) flash memory enables to record data from the same drifter element and data from the other drifters of the connected cluster. Possible additional hardware components will be digital sensors (e.g, temperature and hydrophones). Small PV panel with MPPT (Maximum Power Point Tracker) DC/DC-converter and battery charger.

- For software: communication and control strategies with mesh-based architecture and methods of 'dynamic election' of the 'contact point', solid-state mass storage for measurement history with 'dynamic reset' for transmission management.

- The floating frame: bio-degradable materials and a design functional to the requirements of buoyancy and drogue according to the needs for the specific use (Fig. 1). Corn flour thickeners processed are promising, through which it is possible to control the final porosity of the product, and therefore the characteristics of buoyancy and durability.

Fig. 1: The smart drifter prototype under construction. The modular structure allows fast assembly of parts in different possible conformations. The diameter is about 40cm.

2.3. Network Architecture

The elements of the cluster exchange with each other data related to geo-location (mandatory) and possibly other data obtained from environmental devices and sensors installed onboard. Each element has the dual role of generating and transmitting data related to the same element and bridging the transmission of data from the elements connected to it. Thus, for the Smart Drifter Cluster to remain connected it is enough for each element to be able to communicate with its first neighbors.

3. Results

So far, following what is described in Material and Methods section, a few prototypal Smart Drifters have been developed that will make up what we call “MARTA Smart Drifters Cluster”. The suffix “smart” indicates the ability of the drifter to make decisions about different communication strategies (frequency of data sending, type of data sent, signal strength, synchronization process with other elements etc..) according to different occurrences (rough sea, very expanded cluster etc.), so as to maximize the communication distance among the drifter elements. An important role, for the cluster to be effective, is played by the “master” drifter elements.

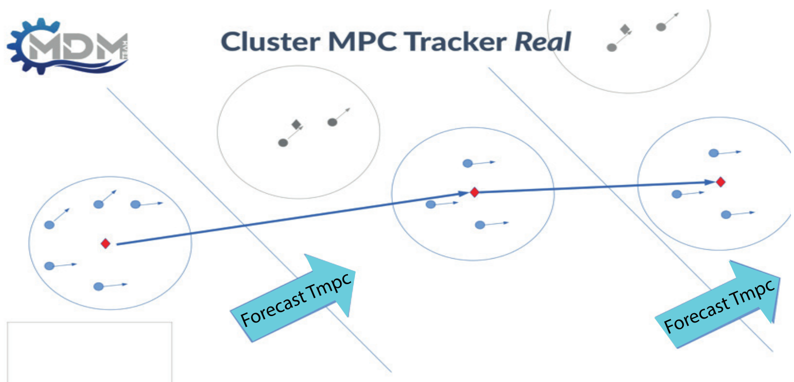

Fig. 2: The red rhombus indicates the active drifter (master) relative to the corresponding connected sub-cluster (the blue circles inside the large circle that includes the master). Based on the previous position and drift data, the master elaborates a strategy for the possible activation of the thrusters in order to follow the “assigned” sub-cluster.

Unlike the other elements, this is also equipped with a light propulsion system, has a more advanced data processing capacity and a long range (e.g., satellite) communication. In the case of fragmentation of the initial cluster of drifters into smaller un-connected (by LoRa communication system) sub-clusters, the masters, equipped with active thrusters, will play a fundamental role in maintaining communication coverage with the fragmented clusters (see Fig. 2).

The entire system has been successfully implemented using the popular middleware Robot Operating System 2 (ROS2, https://docs.ros.org/en/foxy/index.html).

We are working to optimise communication capabilities to make Smart Drifter Clusters effective for monitoring sea currents and for search and rescue activities at sea. We look forward to evaluating the performance of the operating system during the summer of 2022. In parallel, the mechanical design and material realization of the drifter frame, has also been puzzling. We designed a modular shape for the smart drifter (Fig 1), which allows different configurations: from the classic donut shape (similar to the CARTHE drifters, Novelli et al. 2017) to other types (e.g., catamaran, see Fig. 1). The material we are testing is a particular blend of biodegradable polymers (corn flour thickeners), which can have degradation times (in the marine environment) of no more than 6 months. This is a considerable challenge, as recent studies of the degradability of the most common so-called biodegradable materials (PLA, PBAT) show that they degrade rapidly in an environment such as industrial compost, but do not behave in the same way when placed in the marine environment, either in the sea or on the beach (De Monte et al. 2022).

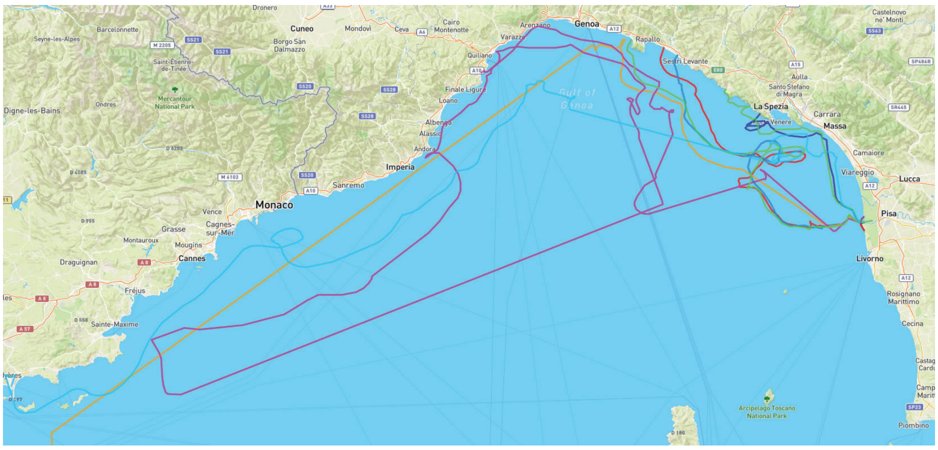

Fig. 3: Some traces obtained with test-launches, in the area of Pelagos Sanctuary (Italy), to track the movements of ML exiting the Arno river. Drifters of different shapes (PET bottles, wooden boards, PVC tanks), equipped with MARTA tracking system, were launched at the mouth of the river, and followed during days, weeks and months, depending of drifter' type and season. The map reports tracks obtained during different launch (from April to December 2021). Completely straight tracks are areas where data were not recorded (lost signal).

In the meantime, some tests have been made to investigate the feasibility of using the wireless communication technology adopted for MARTA smart drifters to study the diffusion (in the sea) and the distribution of accumulation points (on the beaches) of marine debris (Fig. 3). This topic is considered, according to ISPRA and the Marine Strategy Framework Directive, one of the main indicators to be monitored in order to define the ES (Environmental Status) of our seas and coastal areas. Early results have shown a strong dependence of marine litter on the direct wind component, rather than on the surface sea current, depending on its shape (Locritani et al. 2022, in press; Merlino et al. 2022, in preparation). This also implies a considerable dispersion of anthropogenic objects transported by rivers once they are released into the sea, and this must be taken into account in the design of smart drifter clusters dedicated to this specific study.

4. Discussion/Conclusion

MARTA Smart Drifter Cluster is a R&D project that aims at defining the specifications, designing and realizing a cluster of a new type of drifters, where the elements communicate to each other via IoT technologies. The project constitutes an important innovation in the field of marine surface currents monitoring as it introduces in this context the idea of the cluster of drifters, while currently the drifters used are independent elements that communicate data directly to the satellite (or via GSM phone network). Our idea is to take advantage of the modern IoT 'consumer' technologies to obtain a marine monitoring system with low hardware costs, almost no maintenance costs and energy-independent. Smart Drifter Cluster therefore introduces a paradigm shift in the field of marine monitoring whose success and effectiveness the project itself proposes to verify.

The versatility of this new conception of drifters proves to be appropriate also in environmental emergency situations. In fact, in these cases it is essential to have the most accurate predictions possible of the trajectories of floating objects. The logistical ease of use and the low cost of purchase and operation open up new opportunities for the benefit of organizations and institutions, including those in economically disadvantaged countries.

One of the main tasks of the project is to define a sort of 'standardization' in the development of the Smart Drifter Cluster, in order to make the system modular. This means that other instruments and sensors can, in the future, complete the spectrum of possible measurements and predictions of the cluster, integrating new software 'plugins' for data analysis and processing. This standardization is also aimed at making the system easily replicable at low cost, in order to propose it on the market at a massive level.

5. Acknowledgements

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INVASIVE ALIEN SPECIES

RISK ASSESSMENT OF NON-INDIGENOUS SPECIES' INTRODUCTION INTO THE SARONIKOS GULF

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Abstract

Availability of accurate data and scientific effort are directly related to impact studies, especially concerning introduction of Non-Indigenous Species (NIS) into a region. The risk of transporting alien species to the Saronikos Gulf via maritime transport has been ranked at ports around the world, with emphasis in the Mediterranean Sea. Our preliminary findings indicate that some of the high-risk NIS are likely to be present in Saronikos Gulf.

Keywords: impact study, alien species, maritime activity.

1. Introduction

To prevent introduction of Non-Indigenous Species (NIS) in an area via maritime transport it is important to study the factors that favor their survival, settlement, and dispersal. In a recent study by Kalyvioti & Zenetos (submitted), different ports around the world have been ranked in terms of their degree of risk of transporting alien species to the Saronikos Gulf. The ports that are most likely to transfer primarily alien species to the Saronikos Gulf, according to the last departure port of vessels, were Singapore, Malaysia Port Klang and Vietnam Cai Mep port from the Central Indo-Pacific and Jeddah port and Aqaba Industrial port (Jordan) from the Western Indo-Pacific.

Mediterranean ports that were assessed as high risk for secondary introduction of NIS in the Saronikos Gulf were also categorized by eco-region (Kalyvioti & Zenetos, submitted). Specifically in the Levantine, ports of Turkey (Nemrut, Mersin, Iskenderun), Israel (Ashdod, Haifa), Egypt (Alexandria, Port Said, El Dekheila), Cyprus (Limassol, Larnaca, Vassiliko), Lebanon (Beirut) and Syria (Latakia) were evaluated as high risk. The Aegean Sea includes only Turkish ports, such as Izmir, Gemlik, Ambarlı, Aliaga, Asyaport, Derince, and Diliskelesi. In the Ionian Sea the ports of Augusta and Taranto in Italy and Marsaxlokk (Malta) were assessed as of high risk also. The Western Mediterranean holds the larger number of ports in Italy (Salerno, Livorno, Gioia Tauro, Napoli, Genova, Vado Ligure), Spain (Barcelona, Valencia, Tarragona), France (Fos Sur Mer), Algeria (Alger) and Tunisia (Rades).

A risk assessment of NIS reported from the aforementioned ports is essentially the first step for early detection of species that can potentially be transported through cargo and commercial vessels. This study is expected to contribute to proposals for timely management measures, in line with the Marine Strategy Framework Directive and the EU Commission Regulations alongside prevention of the spread of new NIS in anthropogenic areas with high maritime activity such as the Saronikos Gulf.

2. Material and Methods

2.1 Data compilation

A literature survey was conducted including scientific papers, books, PhD theses, technical reports, conference proceedings, either in print or online form. All NIS records of the aforementioned ports were reported in all sectors of the Mediterranean Sea. From the total sum of species, firstly those that have been transported through maritime activity were selected. Thus, Lessepsian immigrants were excluded, as well as fish and also species not established in ports. Then, NIS that are already recorded in the Saronikos Gulf according to the most recent literature (Zenetos *et al.*, 2020; Kondadakis *et al.*, 2021; Crocetta *et al.*, 2021; Koulouvari & Zervoudaki, submitted) were excluded from the dataset. The resulting species were finally classified into high, moderate and low risk, taking into account the sensitivity of the recipient area, the similarity with the origin area and the invasive or non-invasive character of species.

3. Results

Excluding the already reported NIS in the Saronikos Gulf, 160 NIS have been recorded as transported via maritime activity, in the 24 ports of all the Mediterranean ecoregions. Among those species, 44 NIS (Table 1) are very likely to be secondarily imported into the Saronikos Gulf.

Table 1: Alien species with moderate to high risk to be imported in Saronikos Gulf through maritime activity concerning all Mediterranean ports.

Taxonomic group	Species
Ascidiacea	*Aplidium accarense* (Millar, 1953)
Ascidiacea	*Polyandrocarpa zorritensis* (Van Name, 1931)
Ascidiacea	*Symplegma brakenhielmi* (Michaelsen, 1904)
Ascidiacea	*Ascidiella aspersa* (Müller, 1776)
Ascidiacea	*Diplosoma listerianum* (Milne Edwards, 1841)
Ascidiacea	*Microcosmus squamiger* Michaelsen, 1927
Ascidiacea	*Styela plicata* (Lesueur, 1823)
Bryozoa	*Celleporaria aperta* (Hincks, 1882)
Bryozoa	*Licornia jolloisii* (Audouin, 1826)
Bryozoa	*Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985
Bryozoa	*Celleporaria brunnea* (Hincks, 1884)
Bryozoa	*Watersipora subtorquata* (d'Orbigny, 1852)
Chlorophyta	*Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini, 2013
Crustacea	*Anoplodactylus californicus* Hall, 1912
Crustacea	*Eucrate crenata* (De Haan, 1835 [in De Haan, 1833-1850])
Crustacea	*Sphaeroma walkeri* Stebbing, 1905
Crustacea	*Synidotea variegata* Collinge, 1917
Crustacea	*Amphibalanus eburneus* (Gould, 1841)
Crustacea	*Pilumnopeus vauquelini* (Audouin, 1826)
Crustacea	*Mesanthura romulea* Poore & Lew Ton, 1986
Crustacea	*Paracerceis sculpta* (Holmes, 1904)
Crustacea	*Paranthura japonica* Richardson, 1909
Crustacea	*Caprella scaura* Templeton, 1836
Echinodermata	*Ophiactis savignyi* (Müller & Troschel, 1842)
Mollusca	*Afrocardium richardi* (Audouin, 1826)
Mollusca	*Anadara natalensis* (Krauss, 1848)
Mollusca	*Diodora ruppellii* (G. B. Sowerby I, 1835)
Mollusca	*Finella pupoides* A. Adams, 1860
Mollusca	*Marmorofusus verrucosus* (Gmelin, 1791)
Mollusca	*Oscilla jocosa* Melvill, 1904

Taxonomic group	Species
Mollusca	*Paratapes textilis* (Gmelin, 1791)
Mollusca	*Theora lubrica* Gould, 1861
Mollusca	*Trochus erithreus* Brocchi, 1821
Mollusca	*Voorwindia tiberiana* (Issel, 1869)
Mollusca	*Zafra selasphora* (Melvill & Standen, 1901)
Mollusca	*Arcuatula senhousia* (Benson, 1842)
Phaeophyta	*Dictyota cyanoloma* Tronholm, De Clerck, A.Gómez-Garreta & Rull Lluch, 2010
Polychaeta	*Ficopomatus enigmaticus* (Fauvel, 1923)
Polychaeta	*Hydroides brachyacantha* Rioja, 1941
Polychaeta	*Spirorbis (Spirorbis) marioni* Caullery & Mesnil, 1897
Polychaeta	*Branchiomma bairdi* (McIntosh, 1885)
Polychaeta	*Hydroides dirampha* Mörch, 1863
Porifera	*Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004
Rhodophyta	*Womersleyella setacea* (Hollenberg) R.E.Norris, 1992

More specifically, out of the 143 NIS occurring in the eastern Mediterranean ports, three species (*Hydroides dirampha*, *Pilumnopeus vauquelini*, *Styela plicata*) with a presence in at least three ports have a very high probability of being transported into the Saronikos Gulf (high risk). Subsequently, 15 species with presence in at least two ports of the Eastern Mediterranean (Table 2), are considered very likely to be transported into the Saronikos Gulf (moderate risk).

Table 2. Alien species established in the Eastern Mediterranean with presence in at least two (2) ports (moderate risk).

Taxonomic group	Species
Ascidiacea	*Symplegma brakenhielmi* (Michaelsen, 1904)
Ascidiacea	*Paracerceis sculpta* (Holmes, 1904)
Ascidiacea	*Polyandrocarpa zorritensis* (Van Name, 1931)
Ascidiacea	*Microcosmus squamiger* Michaelsen, 1927
Bryozoa	*Celleporaria brunnea* (Hincks, 1884)
Bryozoa	*Watersipora subtorquata* (d'Orbigny, 1852)
Crustacea	*Pseudodiaptomus marinus* Sato, 1913
Crustacea	*Mesanthura* cf. *romulea* Poore & Lew Ton, 1986
Crustacea	*Paranthura japonica* Richardson, 1909
Crustacea	*Caprella scaura* Templeton, 1836
Mollusca	*Arcuatula senhousia* (Benson, 1842)
Phaeophyta	*Undaria pinnatifida* (Harvey) Suringar, 1873
Porifera	*Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004
Rhodophyta	*Solieria filiformis* (Kützing) P.W.Gabrielson, 1985
Rhodophyta	*Womersleyella setacea* (Hollenberg) R.E.Norris, 1992

In the Western Mediterranean, a total of 40 NIS was recorded in at least three ports and are considered high risk to be introduced and established (high risk). These are (in a descending order) the ascidians *Microcosmus squamiger* and *Styela plicata*, crustaceans *Caprella scaura*, *Paranthura japonica*, *Mesanthura* cf. *romulea*, polychaete *Hydroides dirampha*, phaeophyte *Dictyota cyanoloma* and *Arcuatula senhousia*. Moreover, eight species (Table 3) with presence in at least two ports, are considered very likely to enter via shipping in the Saronikos Gulf (moderate risk).

Table 3. Alien species (moderate risk) established in the Western Mediterranean with presence in at least two ports.

Taxonomic group	**Species**
Ascidiacea	*Symplegma brakenhielmi* (Michaelsen, 1904)
Ascidiacea	*Polyandrocarpa zorritensis* (Van Name, 1931)
Bryozoa	*Celleporaria brunnea* (Hincks, 1884)
Bryozoa	*Watersipora subtorquata* (d'Orbigny, 1852)
Crustacea	*Paracerceis sculpta* (Holmes, 1904)
Phaeophytes	*Undaria pinnatifida* (Harvey) Suringar, 1873
Rhodophytes	*Solieria filiformis* (Kützing) P.W.Gabrielson, 1985
Rhodophytes	*Womersleyella setacea* (Hollenberg) R.E.Norris, 1992

Finally, out of the 28 NIS that have been recorded in Central Mediterranean ports three species are considered of high risk to be introduced and settled in the Saronikos Gulf. These are the tunicate *Styela plicata*, the polychaete *Branchiomma bairdi* and the chlorophyte *Caulerpa taxifolia var. distichophylla*. One hundred twelve (112) species that have a single observation in only one Mediterranean port are considered as low risk for introduction.

4. Discussion/Conclusion

Impact studies are directly associated to the availability of accurate data and scientific effort undertaken in each eco-region. Records of NIS for high-risk ports for primary transfer were not found and therefore possible introduction and risk priority cannot be assessed at present. Moreover, regarding high-risk ports of secondary introduction, no data were reported for six ports of Turkey (Gemlik, Ambarli, Asyaport, Derince, Diliskelesi, Nemrut), two ports of Italy (Gioia Tauro, Vado Ligure) and also for the ports El Dekheila (Egypt), Vassiliko (Cyprus) and Fos Sur Mer (France).

Generally, the majority of records either originate from extensive surveys targeted specifically in ports and/or marinas, such as the example of Spain in the port of Barcelona (Ulman *et al.*, 2017) or for the port of Tarragona (Lopez-Legentil *et al.*, 2014) or are either focused on research for specific species such as for *Dictyota cyanoloma* (Steen *et al.*, 2016; Aragay 2016). Also, several species like the high-risk tunicate *Styela plicata* are easier to recognize and record.

Our preliminary results suggest that some of the high-risk NIS are likely present in port areas of the Saronikos Gulf. These are currently under study by expert taxonomists based on both morphological characters and molecular analyses.

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MODELLING THE SPATIAL DISTRIBUTION OF THREE RED SEA SPECIES IN THE GREEK SEAS

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Abstract

Red Sea species have entered the Mediterranean Sea since the opening of the Suez Canal. As they rapidly establish local populations and increase their abundance they form a potential threat for local biodiversity and fisheries. Here, we focus on three invasive alien, demersal fish species, *Pterois miles*, *Siganus luridus* and *Siganus rivulatus.* Georeferenced records from online databases, published scientific literature and questionnaires were assembled within a Species Distribution Modelling approach aiming to map the suitable habitat of the target species over the Greek Seas. Using the Maximum Entropy modelling approach, we constructed a habitat suitability model correlating species occurrence data from the Eastern Mediterranean basin with environmental and topographic explanatory variables. Results highlight the strong coastal nature of all three species and their association with the presence of *Posidonia oceanica* meadows. Probability maps were constructed based on the selected models for the wider Greek Seas area, evidencing high presence probabilities mainly in southeastern coasts and the Ionian Sea for all the species, and a probable expansion everywhere in the study area with the exception of the Thracian Sea for *P. miles* and *S. luridus*, as this sea currently seems to exhibit unfavorable to the species environmental conditions.

Keywords: maximum entropy, habitat suitability, alien species, invasive, marine.

1. Introduction

Introductions of non-indigenous species have been considered a major driver of change in the Mediterranean Sea (Golani, 1998) and the majority consists of thermophilic Red Sea species introduced through the Suez Canal (Karachle *et al.*, 2018). Here, we focus on three demersal fish species, the venomous *Siganus luridus* (Rüppell, 1829) (dusky spinefoot), *Siganus rivulatus* (Forsskål & Niebuhr, 1775) (marbled spinefoot) and *Pterois miles* (Bennett, 1828) (lionfish). All three species have succeeded in establishing populations and expanding their distribution throughout the eastern Mediterranean. *P. miles* although native to the Indian Ocean, has established a population in the Mediterranean in the last decade (Dimitriadis *et al.*, 2020). Both *S. luridus* and *S. rivulatus* are originally distributed in the Western Indian Ocean and count more than fifty years of occurrence in the eastern Mediterranean (Golani, 1998). All three species are edible and seem to slowly enter the commercial fisheries in Greece. In the current work, we aim to assess the potential spatial distribution of the three species over the entire Greek Seas using Species Distribution Modelling quantifying the distribution of the species along environmental gradients. Subsequently, the selected models were used for forecasting and assessing habitat maps to indicate those geographic areas where environmental variables, in the absence of explicit biotic interactions (such as competition or predation), are considered suitable for the presence of the particular species.

2. Material and Methods

A spatial dataset on species occurrences was constructed based on georeferenced observations along the Eastern Mediterranean coastal waters, dating from 2010 to present, originated by various sources of information: databases (i.e., offline ELNAIS database, Zenetos *et al.*, 2015; www.gbif.org), published scientific literature and questionnaires filled by local fishers (Margaritis *et al.*, 2021). Data were filtered using the spThin R package (Aiello-Lammens *et al.*, 2015) to retain the highest number of records being at least 9km apart in order to condense any spatial autocorrelation issues in modelling. Subsequently, Species Distribution Modelling (SDM) was applied to georeferenced information of species' presence to quantify the distribution of the species along physical and environmental gradients. Satellite environmental data were used as explanatory variables to model the suitable habitat of the target species. Sea surface temperature (SST in ^{o}C; oceancolor.gsfc.nasa.gov) of the warmest month, SST of the coldest month, SST range, sea surface salinity (SSS in psu; marine.copernicus.eu) of the least saline month and sea surface chlorophyll (CHL in mg/m^3; oceancolor.gsfc.nasa.gov) of the most productive month were downloaded from the respective databases for the period 2010-2020 and used in SDM. These environmental variables are considered important either as a direct influence on the distribution of fish (e.g., SST, CHL) or as proxies for causal factors. Bottom depth was derived from the GEBCO portal (General Bathymetric Chart of the Oceans GEBCO_2021 Grid, DEP in m; www.gebco.net) along with, bottom slope. Finally, a model-based quantification of *Posidonia oceanica* meadows was downloaded from the EMODNET portal (https://emodnet.ec.europa.eu/en). All monthly-averaged satellite images from daily measurements were processed as regular grids at a spatial resolution of 400 x 400 m^2 under a GIS (Geographic Information Systems) environment using ArcInfo GRID software.

Maximum Entropy, an SDM approach (Phillips *et al.*, 2017) specialized for modeling species distributions from presence-only records, was subsequently applied using the MaxEnt ver. 3.4.4 software (Phillips *et al.*, 2017). Models were trained using pooled data from the wider Eastern Mediterranean Sea, while background points for modeling were selected solely within the current confirmed boundaries of the distribution of each species. A 5-fold cross-validation method was selected and the accuracy of the models was evaluated using the Receiver Operating Characteristic curve and the Area Under Curve (AUC) metric along with True Skill Statistics (TSS). Finally, the predictor importance was evaluated with jackknife analysis and a logistic output was selected because of its extensive use in bibliography and ease in interpretation. In order to examine the possibility of extrapolation beyond training conditions, we used MESS analysis (Multivariate Environmental Similarity Surface) and produced the corresponding maps.

3. Results

The most important variable was bottom depth for all species, followed by the presence of *P. oceanica* meadows. On the contrary, annual SST range contributed the least to the models for all species. SSS of the least saline month had very small contribution on *Siganus* species' models but was moderately important for *P. miles*. SST of the coldest month and bottom slope seem to have low to moderate contribution to all models, while SST of the warmest month play a limited role for *P. miles* but was more important for the two *Siganus* species. Chlorophyll of the most productive month is more or less equally important to all species with higher probabilities of presence within 0.20 – 2.5 mg/m^3, besides *S. rivulatus* which exhibits high probabilities of occurrence in higher chlorophyll concentrations too. MaxEnt map predictions for all three species are depicted in Figure 1, along with jackknife analyses tables. Maps produced confirmed the known extended presence of *P. miles* in the southern part of the Greek Seas (i.e., Crete, Cyclades, Dodecanese), the coastal part of the eastern Ionian Sea and the Gulf of Patras. The species seems to be able to expand its suitable habitat as north as the Chalkidiki peninsula and Limnos island in the North Aegean. Areas with a low probability of presence of *P. miles* include North Evoikos Gulf, Korinthiakos Gulf and the Thracian Sea. Respective maps were very similar for *S. luridus*, while *S. rivulatus* exhibits relatively higher probabilities of presence in the Thracian Sea. According to MESS analysis, the values of the environmental variables fall outside the range of the training conditions in the Thracian Sea and partially at Thermaikos Gulf, as indicated by the negative values of the analysis in the above areas. Consequently, we should be particularly cautious with interpretations in the aforementioned areas. Prediction accuracy proved to be high as AUC was >0.92 for all species, and the threshold-dependent metric of TSS scored satisfactory high values as well (>0.76 for all species).

4. Discussion

Areas favoring the occurrence of all three species the most consist of shallow waters with high probability of *P. oceanica* meadows presence. Specifically, all three species exhibit high probabilities of pres-

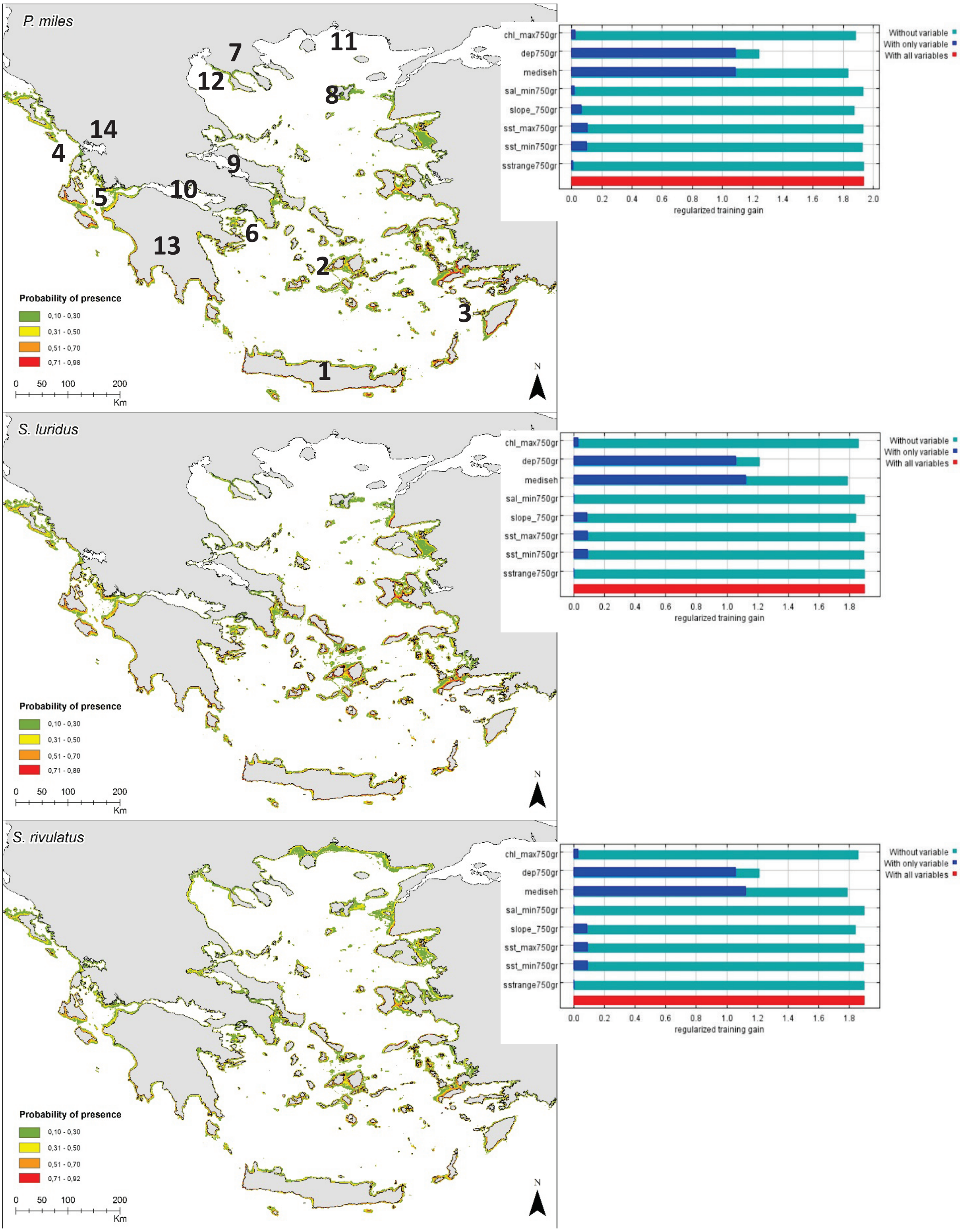

Fig. 1: Probability of occurrence, ranging from 0 to 1, for P. miles, S. luridus and S. rivulatus in the Greek Seas as predicted by maximum entropy modeling approach and the corresponding jackknife analysis tables. Probabilities lower than 0.1 are not depicted on the maps. 1 Crete; 2 Cyclades; 3 Dodecanese; 4 Ionian Sea; 5 Gulf of Patras; 6 Argosaronikos Gulf; 7 Chalkidiki; 8 Limnos; 9 North Evoikos Gulf; 10 Korinthiakos Gulf; 11 Thracian Sea; 12 Thermaikos Gulf; 13 Peloponnese; 14 Amvrakikos Gulf.

ence (>50%) in depths ranging from 0 to approximately 40 m when combined with *P. oceanica* meadows. Both *Siganus* species are herbivorous, feeding on the epiphytes associated to phanerogams and to a lesser extent on the phanerogams (e.g., Stergiou, 1988 and references therein), while *P. miles* is likely to feed on the fish and crustacean species associated with the meadows (e.g., Zannaki *et al.*, 2019). Furthermore, *P. miles'* probability of presence increases with the increase in SSS of the least saline month, an outcome anticipated by the fact that most records are found in the southern parts of Greek waters, where saline waters with very limited freshwater inflows occur. The low contribution of SST of the warmest month to the selected model of *P. miles* was an outcome much expected by its tropical origin. SST temperatures of the warmest month do not seem to restrict the species' expansion. On the contrary, *S. luridus* and *S. rivulatus* are affected by SST of the warmest month reaching their higher probability of occurrence between 26 and 28 ^{o}C. Regarding SST of the coldest month, *P. miles* has higher probabilities of presence close to 17 ^{o}C, while *S. luridus* and *S. rivulatus* exhibit their peaks at colder waters, close to 14 ^{o}C. As alien species usually shift their niche when located away from their native range (D' Amen & Azzurro, 2020) this makes the prediction of their future expansion a challenging procedure. Our results predict the probable expansion of the three species over the largest part of the Greek Seas, especially for *S. rivulatus*. Dimitriadis *et al.* (2020) predictions for *P. miles* are quite similar to current findings, opposed to Poursanidis *et al.* (2020) and D'Amen & Azzurro (2020), who limit the expansion of the species in the southeastern coasts of Greece.

Modelling approaches could probably be enhanced by the inclusion of variables arising from biotic interactions between alien and native species. For example, predation rates, interspecific competition and feeding habits could, among other factors, explain the rapid expansion and spatial distribution of some alien species in the Mediterranean (e.g., Azzurro *et al.*, 2007; Ulman *et al.*, 2021).

5. Acknowledgements

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NON-INDIGENOUS SPECIES IN GREEK MAJOR PORTS AND MARINAS

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Abstract

Among the complex and potentially fundamental shifts that are already reported in the Mediterranean Sea, is the increasing number of non-indigenous species (NIS), affecting the structure and functionality of the marine environment and the consequent supply of resources and marine-related services. Ports and marinas are considered as hotspot sites for NIS. Within the framework of a research project, a number of different protocols were used to reveal the marine diversity of marinas focusing on alien species. Results showed that a great benthic biodiversity and NIS are hosted in marinas. Destructive and non-destructive methods are equally reliable, regarding the detection of NIS. However, a protocol needs to be implemented on a regular basis, in selected areas, to monitor the NIS populations, especially during their early stages of succession, preventing their dispersal to natural habitats.

Keywords: Non-indigenous species, Photoquadrats, Polychaeta, Benthic communities.

1. Introduction

The number of NIS in the Mediterranean Sea has dramatically increased over the last decades (Zenetos *et al*., 2017). NIS s can significantly change the structure of the Mediterranean habitats by altering the distribution of native species via competition, degradation of the local biodiversity and alteration of the habitat functionality. Polluted or physically degraded environments are more prone to invasion than pristine sites (Ardura *et al*., 2016). Ports and marinas are considered hotspot habitats for NIS (Lehtiniemi *et al.,* 2015), since they act not only as gateways but also as reservoirs for alien species (Zenetos & Galanidi, 2020). Such habitats host a great number of transnational vessels (Tempesti *et al*., 2020) and more specifically Greek ports, where various domestic and transnational vessels are co-located. As a result, NIS can potentially spread in nearby natural habitats by recreational vessels that perform local routes (Ulman *et al.,* 2019). Although it is known that artificial structures, such as docks and floating pontoons found in ports and marinas, provide suitable habitats to host opportunistic fouling species (Ferrario *et al*., 2017), still there is not even a pilot-scale monitoring network implemented in Greek ports. Within the framework of the AlienPorts project, a common protocol was applied in selected ports and marinas of the Southern Hellenic Seas in order to reveal the biodiversity patterns and record the presence of NIS.

2. Material and Methods

2.1 Location

Four significant Greek ports and / or marinas (Heraklion (port), Rhodes (marina), Patra (marina) and Zea (marina), Fig. 1) were investigated on a seasonal scale from September 2020 to October 2021. At each site three sampling stations were established, according to the distance from the port entrance (outer, intermediate and inner station).

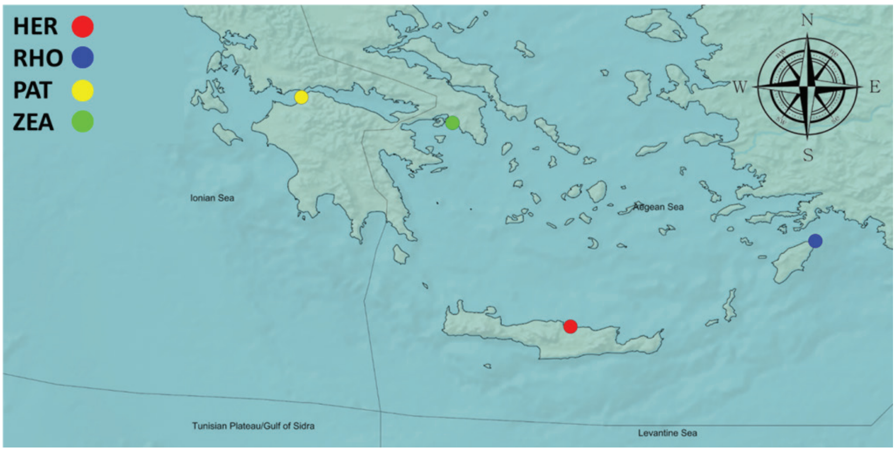

Fig. 1: Map of Southern Greece, showing the four sampling sites.

2.2 Sampling design

Destructive and non-destructive protocols were applied in order to collect scraped and photographic samples, respectively, from benthic assemblages over the artificial hard substrates of these areas. Five replicates of photographic samples and three replicates of destructive samples were collected from each sampling station.

Five random replicates of quadrats were photographed on each station per study area for two sampling events, resulting in a total of 120 photos (30 for each port). Photos were collected from the vertical and horizontal concrete surfaces in each port / marina. In order to calculate the coverage of each taxonomic category in the photoquadrats, PhotoQuad was used (Trygonis & Sini 2012); a free software, specialized for underwater ecological applications integrating various methods and tools for analysis. In each photo sample, 100 random points were spawned in the 25 x 25 cm photo frame. Regarding the destructive samples, three replicate padded quadrats 25 x 25 cm were randomly scraped and collected from each station with a MaNOSS suction sampler (Chatzigeorgiou *et al.*, 2013). The qualitative samples were preserved in 97% ethanol, sorted and so far only the taxon of Polychaeta were identified up to the species level, when possible, with the use of the most recent literature. In addition, an observatory at 2m depth was installed, at the intermediate station of each sampling site, recorded temperature, salinity, pH and dissolved oxygen every two hours.

2.3 Statistical analysis

Statistical analysis was performed on species abundances with PRIMER (ver. 7) software package (Clarke & Gorley, 2006). To mitigate the contribution of the most abundant species, data were fourth-root transformed and a triangular similarity matrix was created based on Bray-Curtis similarity index (Clarke *et al.*, 2001). Non-metric multidimensional scaling (nMDS) analysis was performed to reveal the spatial patterns in the community structures from photo-samples and from fauna samples. The significance of the multivariate results was assessed using a one-way analysis of similarity test (ANOSIM) on the transformed data within and between sampling sites. The analysis of abiotic parameters is still in progress.

3. Results

3.1 Community structure

From the visual analysis a total of 70 taxa were identified (45 to the species level, 12 to the genus level and 13 to higher taxonomic or morphofunctional groups). More specifically, sessile taxa were represented by 13 Macroalgae (4 Chlorophyta, 6 Rhodophyta and 3 Phaeophyta), 12 Porifera, 4 Cnidaria, 2 Polychaeta, 8 Mollusca, 7 Bryozoa, 14 Tunicata. Additionally, 10 motile benthic species were identified. In each port /marina different varieties of taxa were recorded. The greatest diversity was found in the port of Heraklion with 45 taxa followed by Rhode's marina with 37 taxa, while Zea's and Patra's marinas shared the same number of taxa (25).

From the analysis of the destructive samples, 73 Polychaeta species were identified. The highest number of Polychaeta with 48 species was recorded in Heraklion, followed by Zea with 28, Patra with 25 and Rhodes with 24 species.

3.2 Diversity patterns

The nMDS plots from both destructive and non-destructive samples showed differentiation among the four sampling sites (Figures 2 & 3). Due to the large number of photo-samples, the produced patterns of nMDS analysis for each sampling site, are more scattered than those derived from destructive samples. However, one-way ANOSIM showed a discrimination among the four study areas ($R = 0.48$, $p < 0.01$ and $R= 0.691$, $p < 0.01$ for photographic and scrapped samples respectively). Pairwise tests for non-destructive samples were statistically significant in all cases (for both sampling protocols).

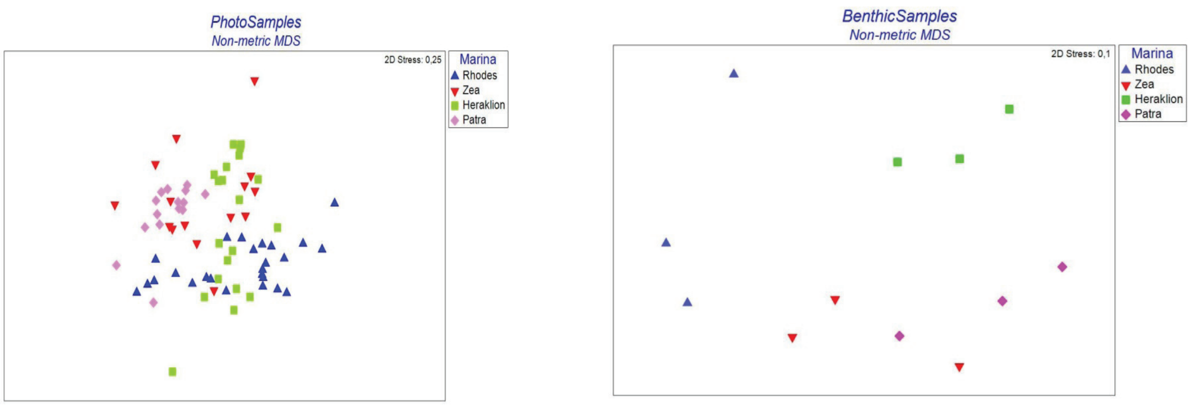

Fig. 2 & 3: nMDS plots from non-destructive (left) and from destructive samples (right).

3.3 Non-Indigenous Species

A great number of NIS was recorded with both sampling protocols. In photo-samples, 17 NIS (13 sessile and 4 motile) were recorded. In Heraklion port, 11 NIS were identified, 6 of which were exclusively found in this marina, while 5, 3 and 8 NIS were recorded in Patra, Zea and Rhodes areas respectively. Four of the abovementioned NIS (*Dendostrea folium, Amathia verticillata, Bugula neritina* and *Herdmania momus*) were widely distributed among sampling sites (3 out of 4). Regarding the destructive samples, 19 NIS of Polychaeta were identified, most of which are located in Heraklion (14). Patra and Rhodes followed with 10 species each, while the lowest number was recorded in Zea with 7 representatives. Four NIS seem to have been distributed in all sampling sites (*Dodecaceria capensis*, *Hydroides dirampha*, *Hydroides elegans* and *Syllis crassicirrata*)

4. Discussion/Conclusion

Ports and marinas are highly disturbed environments due to a number of anthropogenic activities. Within the framework of AlienPorts project, we were able to identify a great number of benthic species, including NIS, in four major ports and marinas of Greece. As indicated by our results, Heraklion seems to host the greatest number of species, presumably because it is the single study site that jointly includes marina and a port sector.

Based on the ANOSIM results, destructive and non-destructive samples revealed differences among the benthic communities of the different ports and marinas. However, for the non-destructive samples, the MDS plots do not show clustering for the different areas due to the high presence of turf recorded in the photoquadrats. On the other hand, the destructive samples showed a scattered distribution of the sampling stations and a good discrimination among the locations, confirming the ANOSIM results. For future analysis, a more detailed description of the turf may provide important information about the species that compose a significant part of the hard substrates. The unique physical characteristics of every sampling site led to a unique macro-habitat formation which was reflected in Polychaeta communities. The abiotic characteristics of each port and / or marina will be further investigated, taking into account the records of various physical parameters (seawater temperature, pH and salinity) that were simultaneously compiled in the framework of AlienPorts project. Regarding the number of NIS species, in both sampling protocols, reached 25% of the total species pool, in contrast to natural habitats where this percentage does not exceed 10% (Ardura *et al.*, 2016). This validates the fact that ports and marinas host a great number of NIS, already stated in previous studies (Tempesti *et al*, 2020). In addition, polychaete communities seem to be a good descriptor for whole benthic communities at these man-made habitats, as it is in natural habitats (Chatzigeorgiou *et al.*, 2012).

To our recollection, this study was the first comprehensive attempt to record and monitor NIS in Greek ports and marinas. The results of this study, although it is a snapshot of the benthic fauna hosted in these types of habitats, highlight the importance of a constant monitoring protocol at least in one port or marina of every Hellenic marine sector.

5. Acknowledgements

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THE IMPACT OF *LAGOCEPHALUS SCELERATUS* (GMELIN, 1789) ON SMALL-SCALE FISHERIES IN CRETE: PRELIMINARY RESULTS

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Abstract

The aim of the present study was to investigate the impacts of *Lagocephalus sceleratus* on small-scale fisheries in Crete. Based on onboard and port samplings, the by-catch of *L. sceleratus* per vessel was estimated at approximately 858 kg during the sampling period (June 2020-August 2021), with the highest by-catches recorded in trammel nets operating during spring in eastern Crete. The daily catch loss due to *L. sceleratus* predation on commercial species that had been caught by the nets or longlines was higher in spring. The longline fisheries were the most seriously affected. Gear damages to both nets and longlines were higher in spring and summer. Our results demonstrate that the establishment and subsequent population increase of *L. sceleratus* in Crete cause serious economic impacts on small-scale fisheries.

Keywords: eastern Mediterranean, silver-cheeked toadfish, catch loss, gear damages.

1. Introduction

The toxic *Lagocephalus sceleratus* (Gmelin, 1789) was first recorded in the Mediterranean Sea in 2003 (Akyol *et al.*, 2005) and it was soon characterized as a “major nuisance” for small-scale fisheries due to the damages it inflicts on fishing gears (cutting off nets and lines), baits and commercial catches (Nader *et al.*, 2012). Although the interactions between *L. sceleratus* and small-scale fisheries have been recognized as important in eastern Mediterranean countries (Galanidi *et al.*, 2018), quantitative data for the negative impacts of the species on local fisheries are scarce (Ünal & Bodur, 2017). During the last decade, the abundance of the silver-cheeked toadfish has been continuously increasing in the seas around the island of Crete (Peristeraki *et al.*, 2013). This is especially true for its eastern parts, which is considered as the starting point for the expansion of lessepsian migrants around the island (Peristeraki *et al.*, 2015; Skarvelis *et al.*, 2015). The increase in abundance of *L. sceleratus* has been accompanied by numerous complaints from Cretan fishermen regarding the damages caused to fishing gears and commercial catches. Yet, no assessment of such damages has been carried out. The present study aims at providing some first quantitative information on the impacts of *L. sceleratus* on coastal fisheries in Crete.

2. Material and Methods

The catch of *L. sceleratus* was estimated using data from 90 fishing trips of 23 vessels of the small-scale fishing fleet of Crete in which captures of the species were recorded. These trips were assigned into 5 subareas (northwest, north central, northeast, southeast and south central Crete) during the period June 2020-August 2021. The main gears sampled were static nets (trammel nets and gillnets) and bottom longlines. For each fishing trip, the catch volumes (weights) of commercial, species and *L. sceleratus* were recorded. Additional data concerning the depredation of *L. sceleratus* on commercial species (biomass loss, recorded by the scientific personnel) and the gear damages (number of holes in nets and missing hooks in longlines, reported by the fishermen), were collected from 35 out of the 90 fishing trips. The fishing days of each vessel in which *L. sceleratus* was caught, was estimated per season and fishing gear through interviews with the fishermen. For the analysis, four seasons were considered: winter (January to March), spring (April to June), summer (July to September) and autumn (October to December).

The catch of each species, as well as the weight of damaged catches per vessel and fishing day were estimated (CPUE). The mean CPUE of *L. sceleratus* (LS_CPUE_{mean}) by season, fishing gear and area was estimated as follows:

$$LS_{CPUE\,mean} = \frac{1}{N_{vessels}} \sum_{i=1}^{N_{vessels}} LS_{CPUE\,i}$$

where $N_{vessels}$ *is* the number of small-scale fishing vessels sampled.

The mean fishing effort (FE_{mean}), in fishing days with *L. sceleratus* catches (F.d.), by season, fishing gear and area was estimated as follows:

$$FE_{mean} = \frac{1}{N_{vessels}} \sum_{i=1}^{N_{vessels}} F.d._{i}$$

The total catch per vessel of *L. sceleratus* (Total B_{lago}) by season and area was estimated as:

$$TotalB_{lago} = LS_{CPUE\,mean} \times FE_{mean}$$

The percentage of damaged fish (due to *L. sceleratus* predation on the catches) per fishing day was estimated by gear and season, as follows:

$$\%\ Commercial\ Catch\ Loss = \sum_{i=1}^{N_{vessels}} \frac{B_{damaged}}{B_{commercial}} \times 100$$

The total catch loss of commercial species due to *L. sceleratus* depredation was also estimated by species and gear. Finally, the number of gear damages (holes in nets and missing hooks in longlines) was estimated by 10 kg of catch, by gear and season:

$$Damages\ per\ 10\ kg = \frac{N\ of\ Gear\ damages}{Total\ commercial\ catch} \times 10$$

3. Results

The mean vessel catch of *L. sceleratus* for the whole sampling period (June 2020-August 2021) was 858 kg corresponding to 13% of the vessel's total catch. Regarding the employed fishing gears the highest contribution on *L. sceleratus* catch was provided by trammel nets (744 kg) followed by gillnets (83 kg) and longlines (31 kg). The catch was highest in spring in all areas except S and SE Crete, where the catch was highest in autumn. The seasonal and spatial distribution of estimated *L. sceleratus* catches is illustrated in Figure 1.

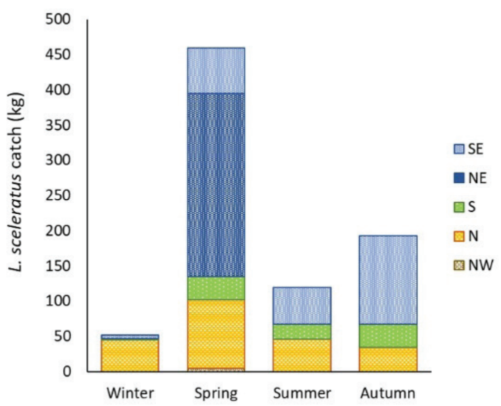

Fig. 1: Estimated catch per vessel of *L. sceleratus* in Crete during June 2020-August 2021 by season and fishing area (SE: southeast Crete, NE: northeast Crete, S: south central Crete, N: north central Crete, NW: northwest Crete).

Regarding the commercial catch damaged by *L. sceleratus*, the highest loss per fishing day was recorded in longlines (13.3 %), whereas lower losses were recorded for trammel nets (2.5 %) and gillnets (1.9 %). On a seasonal base, catch loss for all gears collectively was higher in summer (11.2 %) and spring (7.2 %), compared to autumn (1.1 %) and winter (1.6 %). The highest daily catch loss was recorded for longlines during spring (17.3 %) (Fig. 2). The mean catch loss per fishing day and vessel was estimated at 0.8 ± 1.6 kg and the main species damaged included the red porgy *Pagrus pagrus* (Linnaeus, 1758) and the white seabream *Diplodus sargus* (Linnaeus, 1758) in longlines, and the common octopus *Octopus vulgaris* (Cuvier, 1797) and stripped red mullet *Mullus surmuletus* (Linnaeus, 1758) in nets (Table 1).

Fishing gear damages were higher during the summer and spring months. For every 10 kg of commercial catches landed by netters, a mean of 14 and 11 damages (holes) from *L. sceleratus* bites were calculated for summer and spring respectively. The corresponding damages to longlines (missing hooks) were 32 in spring and 5 in autumn.

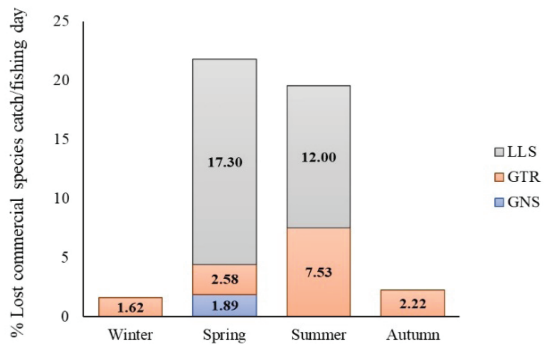

Fig. 2: Percent catch loss of commercial species due to *L. sceleratus* by fishing day, gear and season. (LLS: longlines, GTR: trammel nets, GNS: gillnets).

Table 1. Estimates of commercial species' catch loss (kg), due to *L. sceleratus* depredation, in 35 fishing trips of small-scale fisheries vessels in Crete. Blue bars indicate the percentage contribution of each species to total catch loss by fishing gear (GTR= trammel nets, GNS= gillnets, LLS= longlines).

2020-2021	GNS	GTR	LLS	Total
Boops boops			1.20	1.20
Dasyatis pastinaca		3.60		3.60
Diplodus annularis		5.20		5.20
Diplodus sargus		3.48	49.00	52.48
Diplodus vulgaris		4.65	14.00	18.65
Lithognathus mormyrus		2.60		2.60
Liza aurata		2.42		2.42
Mullus barbatus		4.50		4.50
Mullus surmuletus		17.81		17.81
Oblada melanura	0.80			0.80
Octopus vulgaris		29.00		29.00
Pagrus pagrus		3.90	63.00	66.90
Sarpa salpa		6.50		6.50
Serranus scriba			14.00	14.00
Siganus luridus		1.44		1.44
Solea solea		0.44		0.44
Sparisoma cretense			1.28	1.28
Sphyraena sphyraena		0.30		0.30
Spicara maena		0.96		0.96
Trachurus mediterraneus		3.15		3.15
Uranoscopus scaber		2.86		2.86
Total	**0.80**	**92.81**	**142.48**	**236.09**

4. Discussion/Conclusions

Results of the present study revealed, for the first time, the magnitude of damages caused by *L. sceleratus* to fishing gears and commercial catches of the coastal fisheries in Crete. The total mean by-catch of the species was approximately 858 kg in June 2020-August 2021. However, this is probably an underestimated value since many individuals that are being caught by the fishing gears eventually escape from capture through biting and cutting off the nets or lines. The high number of gear damages per 10 kg of landed catch observed during the on-board sampling corroborates to this conclusion.

Present results show that the main species damaged by *L. sceleratus* were the commercially valuable *P. pagrus* and *D. sargus* in longlines and *O. vulgaris* and *M. surmuletus* in nets. Based on damaged catches left on the gears, it was estimated that fishermen in Crete lose, on average, almost 1 kg of valuable species per fishing day, which corresponds to an average of 15-25 euro. In fact, the economic damage is probably much higher considering that a number of fish and cephalopods caught by the fishing gears might have been consumed entirely by the pufferfish with no leftovers on the gears, and these have not been included in the estimation of daily catch losses. The overall economic impacts of *L. sceleratus* on small-scale fisheries, including gear repair costs, lost fishing days, extra manhours will be assessed in a future report.

The seasonal pattern in *L. sceleratus* catches, catch losses and gear damages indicate a higher feeding activity of the species during spring and summer probably related to its increased metabolic rate during that period, caused by the sea temperature rise (Volkoff & Rønnestad, 2020). It could also be related to the spawning period of the species, which, in Crete, extends from late spring to early summer (Peristeraki *et al.,* 2010). However, the possibility of seasonal migrations of the species in the area cannot be excluded. Further investigation is required in order to discern the causes of seasonality in pufferfish-fisheries interactions as well as of the observed seasonal differences between areas, such as the high abundance of *L. sceleratus* in S and SE Crete during autumn.

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FISHERS PERCEPTIONS ON THE STATUS AND IMPACT OF THE ALIEN FISH *SIGANUS LURIDUS* AND *S. RIVULATUS* IN THE AEGEAN AND IONIAN SEAS

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Abstract

Marine biological invasions are considered as a threat for biodiversity and ecosystem services. The two spinefoot species, *Siganus luridus* and *S. rivulatus* (*Siganus* spp.), are among the dominant alien fish species that have been established in the eastern Mediterranean Sea. In this work we used a questionnaire-based survey addressed to small-scale fishers operating in the Aegean and Ionian Seas, regarding the statuses of these two species and any perceived impact on the environment. Our analyses revealed a positive temporal shift in the frequency of occurrence of the species during 2016 to 2021. Moreover, fishers from the Aegean Sea stated that both species affect the commercially targeted species, a fact that can be attributed to the successful establishment of the species in the region and their impact on the environment.

Keywords: Biological invasions, Small-scale Fisheries, *Siganus* spp., Greece, Mediterranean.

1. Introduction

Marine alien species are considered as one of the most important ecological threats at an international scale since they negatively affect the local biodiversity and ecosystem services. This phenomenon is rapidly expanding in the Mediterranean Sea due to anthropogenic disturbances and activities related to shipping and aquaculture (Katsanevakis *et al.*, 2014). Besides the ecological aspect, there is a huge economic impact caused by biological invasions, which has been estimated at $27.3 billion, or $3.6 billion when only realized costs are considered, over the last three decades for the Mediterranean basin (Kourantidou *et al.*, 2021).

The two herbivore species of the genus *Siganus* (Dusky spinefoot *S. luridus* and Marbled spinefoot *S. rivulatus*; hereafter *Siganus* spp.) have been established in the eastern Mediterranean since the last century (Ben-Tuvia, 1964; Dulčić *et al.*, 2011). During the last decade their distribution has gradually expanded in the Greek coasts of the Aegean and the Ionian Sea (Karachle *et al.*, 2021), and their effects on the environment and ecosystem services have been well documented (Giakoumi 2014; Katsanevakis *et al.*, 2014; 2020). The aim of this study is to record and analyse the perceptions of the Greek fishers on the status and the impacts of *Siganus* spp.

2. Material and Methods

2.1 Data collection

A questionnaire was compiled within 4ALIEN project, and a total of 250 in-person interviews were conducted with professional fishers in 2021 throughout Greece (for details see Margaritis *et al.*, 2021). However, information on the two *Siganus* spp. was extracted only from 93 interviews from the Aegean Sea and 50 from the Ionian Sea. Here we present and analyse responses in six questions focusing on the current status and impact of the species on the ecosystem and commercially exploited species (Table 1).

2.2 Data analysis

The responses in the first four questions concerning the frequency of encounters and the contribution to the catch were classified in ordinal interval classes (IC): "1" (0-5%), "2" (5-20%), "3" (20-50%), "4" (50-75%), "5" (75-100%). The other two questions' replies where of "Yes/No" nature, accompanied by a short free-text reply, and thus quantified accordingly. Initially, bar plots were employed to highlight potential spatial and temporal patterns; however, they did not allow the efficient interpretation of the multi-dimensional features of the data. Moreover, comparisons cannot be implemented based on individual-based changes since the data were pooled. Hence, with the use of the delta difference [Δ_{yi}: frequency of occurrence (Δ_{Freq}); proportion of the catch share (Δ_{Prop})] of each ordinal interval class variable (*y*) we attempted to highlight the temporal change (*c*=5 years) based on the individual-based observational shifts of each fisher (*i*) regarding the past (2016; *t*) and the present (2021; *t+c*) as follows (Eq.1):

$$\Delta_{yi} = y_{i,t+c} - y_{i,t} \quad [1]$$

$$\Delta_{y\mu} = \frac{\sum_i^{n_y} \Delta_{yi}}{n_y} \quad [2]$$

where $\Delta_{y\mu}$ (Eq.2) denotes the mean (μ) value of each variable (*y*) and *n* the total number of individuals. As a result, in case of $\Delta_{y\mu} > 0$, the difference exhibits that there is a proportional increase compared to five years before and if $\Delta_{y\mu} < 0$, the opposite. The data have been depicted in a two-dimensional scale with a random local variation assessed to the discrete data points to avoid visually overlapping. Finally, with the use of the non-parametric Kruskal-Wallis (KW) rank sum test we validated if the Boolean answers of the fishers from the two marine regions derived from the same population, while we applied the chi-square test (χ^2) to the yes/no answers against the equal ratio 1:1.

Table 1. List of the questions addressed to fishers from the Aegean and Ionian Seas on the status and the impact of *Siganus luridus* and *S. rivulatus*. IC=interval class. For details see text and Margaritis *et al.* (2021).

A/A	Question	Data type
1	"How often did you catch the species during the last year (i.e., 2021)?"	[Frequency of occurrence: IC]
2	"How often did you catch the species five years ago (i.e., 2016)?"	[Proportion of the catch share: IC]
3	"What was the species' proportional share of the catch during the last year?"	[Frequency of occurrence: IC]
4	"What was the species' proportional share of the catch five years ago?	[Proportion of the catch share: IC]
5	"Have you observed environmental changes due to the presence of the species?"	[Yes | No]
6	"Do you consider that the species affect the commercially targeted species?"	[Yes | No]

3. Results

According to the answers of the fishers, trammel nets (GTR) and gillnets (GNS) were characterized as the predominant fishing gears for the catch of the species in both Seas (GTR: Aegean=72%, Ionian=50%; GNS: Aegean= 22%, Ionian=22.5%) and thus, were included in the analysis (Fig. 1). It appears that there was a temporal increase from 2016 to 2021 in the frequency of occurrence during the use of GTR in the Aegean (red bars) and in the Ionian (blue bars) Sea (Fig. 1), whereas the proportion of catch share exhibited only a relatively minor temporal increase in both seas. In the case of GNS, the pattern is analogous since there was an increase in the frequency of occurrence and in the proportional shift in the Aegean

Sea. However, the proportion of catch share of *Siganus* spp. observed by the fishers in the Ionian Sea indicated a reduction during the period of 2016 to 2021.

The temporal difference of the mean frequency of occurrence ($\Delta_{Freq\mu}$) showed that the $\Delta_{Freq\mu}$ of *Siganus* spp. resulted in a positive mean in both fishing gears in the Aegean ($\Delta_{Freq\mu}$= 0.86 and 0.82, for GTR and GNS respectively) and in the Ionian Sea ($\Delta_{Freq\mu}$= 1.09 and 0.55, for GTR and GNS respectively). On the contrary, the mean values regarding the observation shifts on the proportion of catch share ($\Delta_{Prop\mu}$) were lower compared to the $\Delta_{Freq\mu,}$ in the two marine regions [Aegean: (GTR: $\Delta_{Prop\mu}$= 0.17; GNS: $\Delta_{Prop\mu}$= 0.11); Ionian (GTR: $\Delta_{Prop\mu}$= 0.11; GNS: $\Delta_{Prop\mu}$= -0.5). The calculated positive means (Δ_{Freq}) in the y axis mean (triangles) validated the positive temporal shift detected in the frequency of observation on the species in both fishing gears and marine regions (Aegean: red polygon; Ionian: blue polygon), respectively (Fig. 2). Apparently, there was a higher variance in the values of the x axis (Δ_{Prop}) since the polygons were allocated in both positive and negative values. In the exceptional case of the Δ_{Prop} in the Ionian Sea, there was a general trend towards negative values.

Regarding the observed environmental changes, the majority of the fishers reported that the species do affect the environment (Yes: Aegean = 49, Ionian = 28; No: Aegean = 44, Ionian = 22). However, no statistical difference between the answers of each region (χ^2, $p > 0.05$) was found, neither a statistical difference between the answers of the Aegean and Ionian Seas (KW, $p > 0.05$). Finally, with respect to the effects on targeted species, the same pattern was observed with generally a positive response (Yes: Aegean = 56, Ionian = 29; No: Aegean = 29, Ionian = 31). The positive answer of fishers from the Aegean was statistically significant (χ^2, $p < 0.05$) compared to the Ionian that was not (χ^2, $p > 0.05$), along with a non-statistically significant effect between the two marine regions (KW, $p > 0.05$).

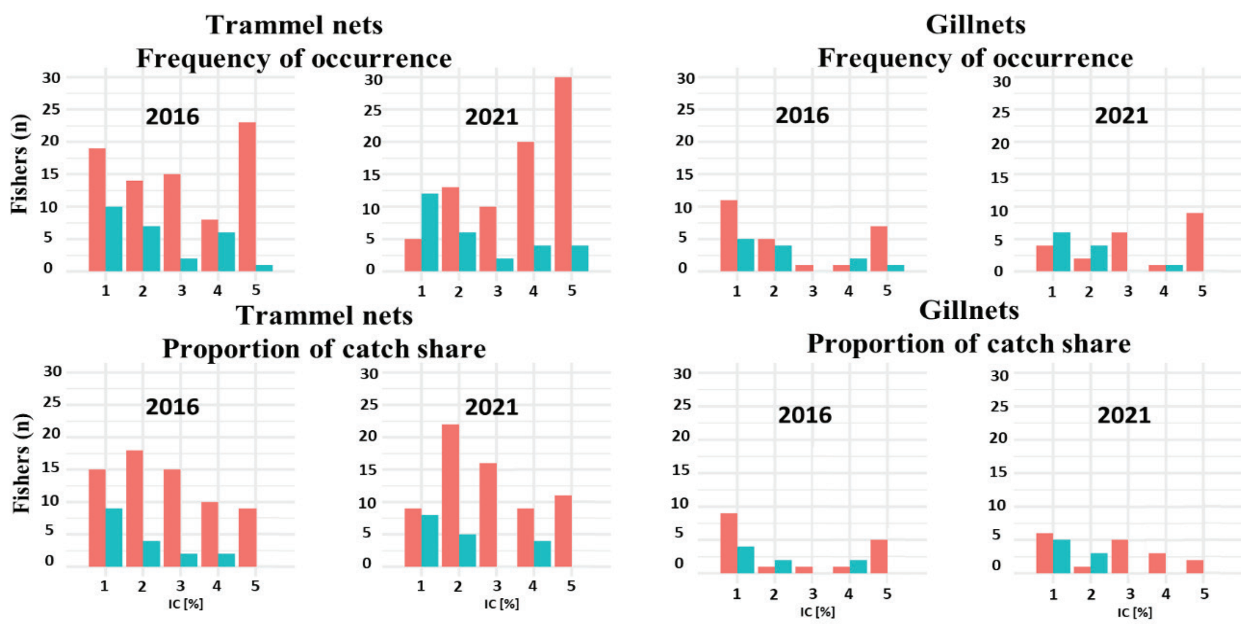

Fig. 1: Bar plots indicating the 5 interval classes (IC; 0-5%, 2: 5-20%, 3: 20-50%, 4: 50-75%, 5: 75-100%) of the frequency of occurrence (top; IC) and the proportion of catch share out the total catch (bottom; IC) of *Siganus luridus and S. rivulatus* with the two major fishing gears (Trammel nets, left; Gillnets, right) based on answers of fishers (n) in the Aegean (red bars) and Ionian (blue bars) Seas.

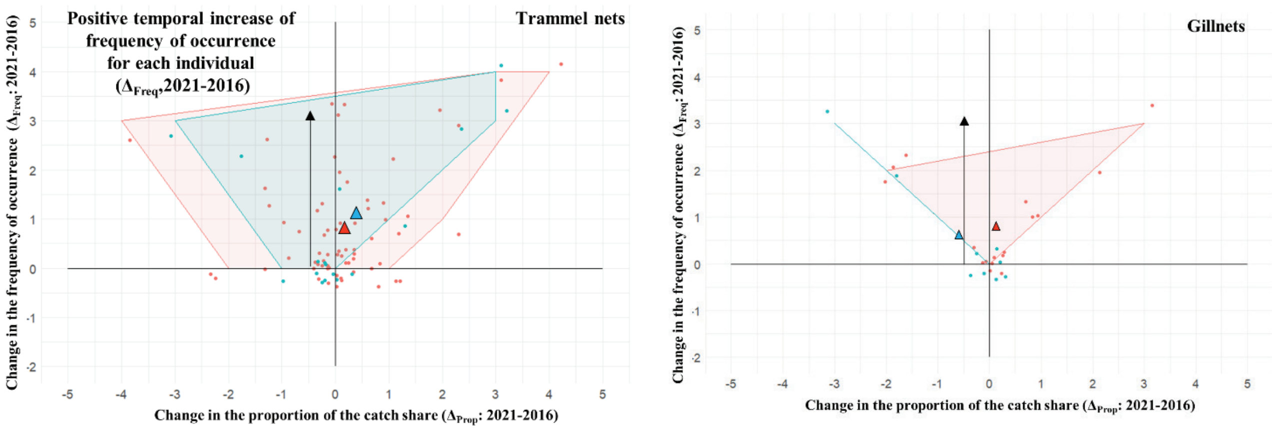

Fig. 2: Two-dimensional depiction of the temporal difference of the frequency of occurrence (*Δ_{Freq}*; y axis*)* and the proportion of *Siganus luridus and S. rivulatus* out of the total catch share (*Δ_{Prop}*; x axis*)* for both fishing gears (Trammel nets: left; Gillnets: right) based on answers of fishers in the Aegean (red polygon) and Ionian (blue polygon) Seas. The triangles indicate the mean values of each subset (Aegean: red; Ionian: blue).

4. Discussion

The present study recorded and analysed the perceptions of Greek fishers on the current status and the impact of the alien fish species *Siganus luridus* and *S. rivulatus.* Based on their answers, a positive temporal shift (2016-2021) was highlighted in the frequency of occurrence of both species in the Aegean and Ionian Seas, in both trammel nets and gillnets. On the contrary, the distribution of the values regarding the proportion out of the total catch was scattered and a temporal shift was not that clear. In the case of *S. rivulatus*, a possible explanation on the positive temporal shift of the frequency of occurrence can be attributed to the fact that the invasive species has the ability to cover larger distances in the eastern Mediterranean (i.e., larger home range, lower site fidelity), compared to its native range in the Red Sea (Pickholtz *et al.,* 2018).

Fishers' responses regarding whether the species change the marine environment were positive in both marine regions, while in the question on the effect of the species on commercially target species there was a statistically significant positive answer for the Aegean Sea. Indeed, it has been previously recorded that increased abundance of the herbivorous *Siganus* spp. can lead to benthic algal communities with extremely low biomass and the creation of barrens (Sala *et al.,*2011; Giakoumi, 2014), and this was clearly perceived by the fishers. These barrens have multiple effects on the algal communities, the rocky infralittoral food webs, but can also lead to habitat loss for a wide variety of animals, loss of spawning and recruitment grounds for fishes, even extirpation of fish species (e.g., Katsanevakis *et al*., 2020).

The results of this study further underline the importance of the collaboration between the fishers and the scientific community. These types of collaborations enhance the mutual transfer of ecological information, such as the fundamental fishers ecological knowledge based on their observations (Holm, 2003), which will contribute to practices focusing on the management and the protection of the marine environment.

5. Acknowledgements

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TECHNICAL QUALITY AND NUTRITIONAL VALUE OF FOUR INVASIVE FISH SPECIES

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Abstract

The present research aimed at evaluating the technical quality and the nutritional value of four invasive to the Mediterranean fish species, which however, have a potential for commercial exploitation for human consumption in order to ease their pressure and to enrich the seafood market. In total 145 individuals of *Etrumeus golanii*, 26 of *Siganus rivulatus*, 26 of *Siganus luridus*, and 96 of *Pterois miles* were obtained, measured their somatometry, and analyzed for their proximate composition and fillet fatty acid contents. Among them, *S. rivulatus* was the one with higher fat contents. All species are rich in their total PUFA content. The ω3 eicosapentaenoic (EPA) and docosahexaenoic (DHA) prevail. Both *Siganus* species were rich in total ω6 PUFA and ARA in particular, thus exhibiting low ω3/ω6 ratio (1.0), while on the contrary this ratio was extremely high for *P. miles* (4.0) and *E. golanii* (9.3). All species, based on their total ω3, EPA and DHA contents can be characterized as exceptional sources for these PUFA that are essential for human nutrition. Their dressing and filleting yields and their proximate and fatty acid compositions indicate a very strong potential for their exploitation for human consumption.

Keywords: nutritional value, fatty acids, composition, invasive fish.

1. Introduction

The present research deals with four common invasive species with strong environmental and social impact in the Mediterranean, which however, have a potential for commercial exploitation for human consumption in order to ease their pressure and to enrich the seafood market. These four species, namely the Golani round herring *Etrumeus golanii* DiBattista, Randall & Bowen, 2012 (with potential impacts in purse seine fisheries and species competition with anchovies and sardines), the lionfish *Pterois miles* (Bennett, 1828), (which based the Caribbean example can have detrimental ecosystem effects), and the two spine-foot species, *S. luridus* (Rüppell, 1829) and *S. rivulatus* Forsskål & Niebuhr, 1775 (with proven ecological impacts by barrens' formation, and impacts to the artisanal fisheries). The aim of this study was to assess the technical quality and nutritional value of these four species, since no similar data occurs.

2. Material and Methods

The four species were collected from different locations in the Greek waters based on their availability, throughout the whole year. In total 145 individuals of *E. golanii*, 26 of *S. rivulatus*, 26 of *S. luridus*, and 96 of *P. miles* were obtained. They were transferred fresh, their somatometry took place and subsequently they were filleted and frozen until analysis. Proximate composition took place according to standard analysis (AOAC, 2005) and fatty acids were obtained after lipid extraction and direct methylesterification (Lepage & Roy, 1984). The statistical analysis was done by the GraphPad Prism v. 8.4.2. software. After normality check, one way ANOVA was applied with Tukey test used for comparing the means.

3. Results and Discussion

The somatic characteristics and technical yields of the four species appear in Table 1. It has been observed that *E. golanii* has a dressing yield and a filleting yield higher than the rest species. The lowest filleting yield was observed in the two *Siganus* species. The total composition of the four species fillets is presented in Table 2. The *S. rivulatus* was the species with the higher lipid content among all.

Table 1. Mean (± standard deviation) of total length (TL), weight (W), eviscerated weight (Wev), filleting yield (FY), condition index (CI) and dressing yield of the four species.

	S. rivulatus	*S. luridus*	*P. miles*	*E. golanii*
TL (mm)	2226 ± 168.6	2058 ± 112.4	2463 ± 258.0	1992 ± 379.4
W (g)	148.3 ± 32.55	155.5 ± 25.17	216.4 ± 83.31	77.2 ± 38.20
Wev (g)	120.9 ± 25.23	123.8 ± 20.41	188.8 ± 71.68	70.7 ± 35.36
FY (%)	35.01 ± 2.56 a*	31.00 ± 2.66 a	29.06 ± 1.34 a	44.26 ± 1.43 b
CI	1.3 ± 0.08 b	1.8 ± 0.18 c	1.4 ± 0.19 b	0.9 ± 0.04 a
DY (%)	81.8 ± 3.26 a	79.7 ± 4.74 a	87.4 ± 3.86 b	91.2 ± 2.91 b

*Different letters stand for statistically significant differences ($p < 0.05$)

Table 2. Mean (± standard deviation) of proximate composition (%) of the four species.

	S. rivulatus	*S. luridus*	*P. miles*	*E. golanii*
Protein	20.5 ± 0.5 a	18.9 ± 0.1 a	18.1 ± 2.8 a	23.0 ± 0.1 b
Fat	2.2 ± 0.1 c	1.7 ± 0.2 b	1.7 ± 0.1 b	1.2 ± 0.0 a
Moisture	75.6 ± 0.3	77.6 ± 0.2	76.8 ± 0.4	73.8 ± 0.8 a
Ash	1.5 ± 0.0 a	1.5 ± 0.0 a	1.4 ± 0.0 a	1.7 ± 0.1 b

*Different letters stand for statistically significant differences ($p < 0.05$)

The fatty acid composition, presented as main fatty acid group content and main polyunsaturated (PUFA) content in mg/100g fillet (Fig. 1) showed that all species are rich in their total PUFA content. The ω3 eicosapentaenoic (EPA) and docosahexaenoic (DHA) prevail as well as the essential ω6 arachidonic acid (ARA, 20:4ω6). Both *Siganus* species and *S. rivulatus* in particular were the species rich in total ω6 PUFA and ARA. The ω3/ω6 ratio was 1.0 for the two *Siganus*, 4.0 for *P. miles* and 9.3 for *E. golanii*.

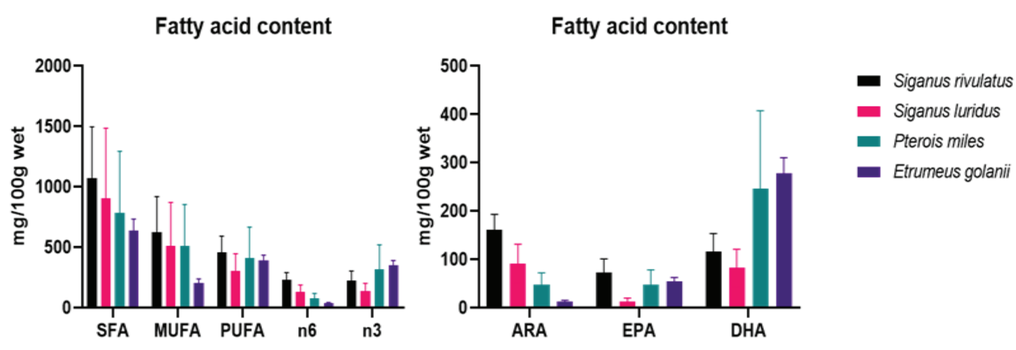

Fig. 1: Fatty acid composition (in mg / 100g fillet): Saturated (SFA), monounstaturated (MFA), total polyunsaturated (PUFA) fatty acids and arachidonic (ARA), 20:5ω3, 22:6ω3 (DHA) fatty acids. Bars represent standard deviations.

4. Discussion/Conclusion

The differences in dressing and filleting yields can be attributed to the morphometric characteristics of the fillets. Thus, the small size head and the plumpy body shape justify the particularly high DY and FY in *E. golanii*. In all cases the FY was satisfying and similar to those of many marine species that are commercialized as filleted (Borderías & Sánchez-Alonso, 2011).

The proximate composition of the four species has a typical protein content of around 20%, observed for most Mediterranean marine fish while they can all be characterized as low fat species (Grigorakis, 2017).

Out of the four species, the two *Siganus* ones exhibited a low ω3/ω6 ratio, almost half than the ideal for human that should be higher than 2. This is clearly due to their high ARA content, which, unlike the plant-derived ω6, mainly constituting of the low carbon-chain linoleic fatty acid (18:2ω6), is highly beneficial for the human health.

The nutritional importance of ARA for the growth and immune function of the herbivore species *S. rivulatus* has been previously shown (Nayak *et al.*, 2017) and this justifies the high concentration of this fatty acid in this species fillets. Besides the physiological importance of this fatty acid, the high concentration in *Siganus* spp. mirrors the natural dietary fatty acid profile for these two species deriving from the macroalge that mainly constitute their diet (Grigorakis, 2011).

On the other side so *P. miles* as *E. golanii* -the latter in particular- have very high ω3/ω6 ratios, much higher than several wild and farmed Mediterranean species (Grigorakis, 2017), a fact that dignifies that they are exceptional sources of ω3 PUFA. From the scarce available literature data, *E. golanii* show similar high ω3 PUFA to those found in a previous study of the same species in the Mediterranean (Kucukguimez *et al.*, 2010). The sum of ω3 PUFA and of EPA και DHA in *P. miles* and *E. golanii* are particularly high, comparing to those of species with much higher lipid contents like mackerels and the Australian amberjack *Seriola lalandi* (Chen & Liu, 2020).

Conclusively, the results of this study prove the excellent quality and high nutritional value of the four studied species, a fact that classifies them as very promising candidates for human consumption. Their technical yields are similar to those of many commercialized species, thus making their commercial exploitation and processing highly feasible in technical aspects. Since data of the present study derive from sporadic samplings throughout the year and seasonal sample collection is still undergoing, no seasonal effects in composition and fatty acids were evaluated herein.

The particularly high ω3/ω6 ratio of *E. golanii*, is an interesting observation and can be used as a strong point and advantage towards promoting the species in the food market.

A similarly important individuality, is the extremely high ARA content of both *Siganus* species, a fatty acid that is essential for human brain development and function. This can also serve as a strong feature for potential advertisement for human consumption of these two species. The potential commercialization of these species can largely facilitate the reduction of the ecological and social pressure applied by their presence in the Mediterranean.

5. Acknowledgements

This work has been conducted within the frame of the project *"4ALIEN: Biology and the potential economic exploitation of four alien species in the Hellenic Seas"*, funded by NRSF 2017-2020 (MIS (ΟΠΣ): 5049511).

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SPECIAL SESSION: MEDITERRANEAN SEA (AND FRESH WATER) LITERACY IN THE ERA OF 2030 AGENDA FOR SUSTAINABLE DEVELOPMENT AND DECADE OF OCEAN SCIENCE FOR SUSTAINABLE DEVELOPMENT (2021-2030)

IDENTIFICATION OF RIVERS' RESPONSE TO CLIMATE RELATED STRESSORS USING A SIMPLE STATISTICAL APPROACH

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Abstract

Climate stressors on riverine ecosystems, such as floods and droughts, are expected to intensify due to anthropogenic induced climate change. Deepening our understanding of the rivers flow response to precipitation variation can be a valuable tool in decoding the riverine ecosystems' complex operation. In this study, a cross-correlation analysis of daily precipitation and water level time-series covering the period between September 2019 and January 2022 was performed, so as to investigate the response of water level to precipitation events at Pinios River in Greece. Based on the results, there is a one to two days time lag of the peak discharge appearance between the upstream and downstream stations. The upstream area is affected one day and the downstream area three to four days after precipitation events. Additionally, precipitation is attenuated during its passage through the system. The understanding of the response of rivers to precipitation can be a valuable tool towards flood protection and warning system implementation. The methodological approach proposed when applied on a dataset with higher temporal resolution will provide a more detailed aspect of the system's functioning and can therefore be used as an early warning system for flood protection.

Keywords: water level, precipitation, time-series analysis, cross-correlation analysis, Pinios River.

1. Introduction

Lotic ecosystems sustain riverine, terrestrial, and marine biodiversity and contribute to the global biogeochemical cycles (Palmer & Ruhi, 2019). Riverine ecosystems in particular are highly biodiverse and are characterized by high spatial but also temporal heterogeneity (Ward, 1989), while their structure, functioning and processes are controlled in great extent by the rivers' flow regime (Poff *et al*., 1997). Apart from the river catchment's geophysical characteristics and the water management practices of the area, the quantitative state of rivers' is mainly controlled by the climatic elements of the area, especially precipitation (Zeiringer *et al*., 2018). Climate stressors on riverine ecosystems, such as floods and droughts, are expected to be intensified due to anthropogenic climate change (Settele *et al*., 2014). Therefore, deepening our understanding of the rivers flow response to precipitation variations can be a valuable tool in decoding the riverine ecosystem's complex operation and can be used in flood protection management plans.

In the present study, daily time-series of water level from two telemetric stations along Pinios River in Thessaly, Greece, and precipitation from five weather stations upstream the catchment area, were statistically elaborated. The final scope of this effort was to investigate the spatial riverine system's response to the temporal meteorological variations.

2. Material and Methods

2.1 Study area-Monitoring program

The investigated monitoring stations are located at Pinios catchment, in Thessaly, Greece. The study area is flood prone since the antiquity and several flood protection structures along the Pinios River and its tributaries had been constructed over the last 2500 years (Mimikou & Koutsoyiannis, 1995). The water level monitoring stations are part of the automatic monitoring network of the Hellenic Centre for Marine Research (HCMR), Institute of Marine Biological Resources and Inland Waters (IMBRIW), Department of Inland Waters and have been installed through **HIMIOFoTS** National project (https://www.himiofots.gr/). For the present study, daily water level measurements, covering the period between September 2019 and January 2022 were employed. Nomi station is located upstream, while Tempi monitoring station is located in the downstream part of Pinios River, close to its mouth to the Aegean Sea and about 100 km downstream of Nomi station (Fig. 1; Table 1). At Tempi Pinios River courses through a narrow valley that favors flood genesis (Bathrellos *et al.*, 2018).

Daily precipitation measurements were available from the National Observatory of Athens (NOA) network of meteorological stations (Fig. 1; Table 1). For the present study, precipitation data from stations upstream the water level telemetric station were only used.

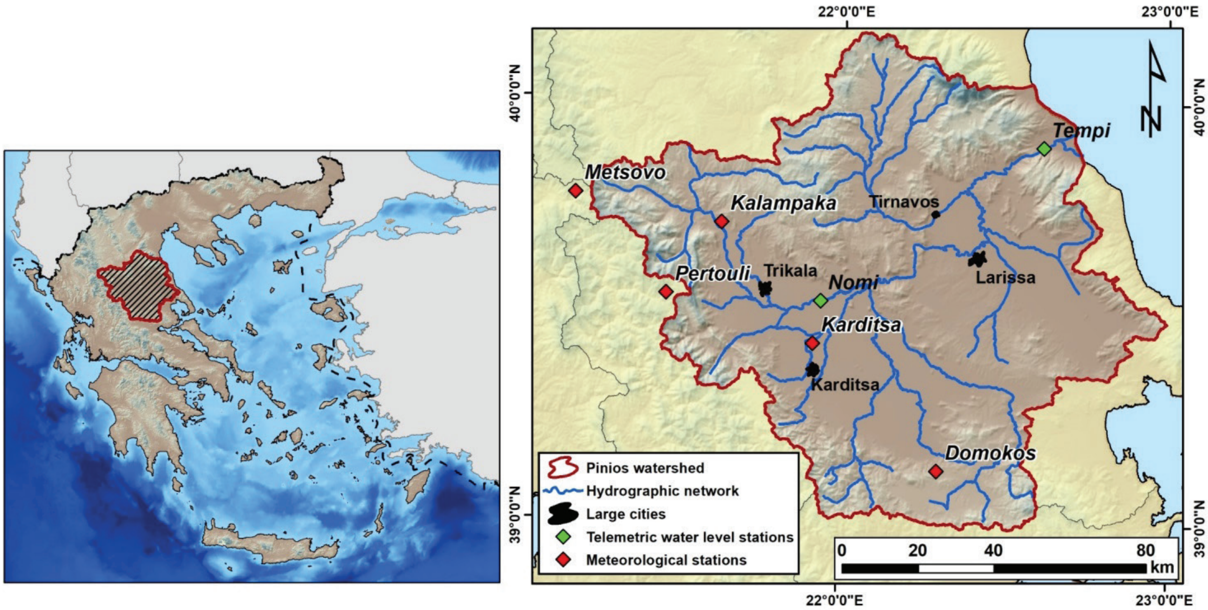

Fig. 1: Study area and location of monitoring stations.

Table 1. Summary of the characteristics of monitoring stations.

Station	Latitude	Longitude	Elevation (m)	Parameter	Owner	Website
Nomi	39.52660	21.93830	91.2	Water level	IMBRIW	https://hydro-stations.hcmr.gr/nomi-station/
Tempi	39.89680	22.61520	3.5	Water level	IMBRIW	https://hydro-stations.hcmr.gr/tempi-station-pineios-river/
Metsovo	39.77162	21.17698	124.0	Precipitation	NOA	https://penteli.meteo.gr/stations/metsovo/
Pertouli	39.53853	21.46445	1170.0	Precipitation	NOA	https://penteli.meteo.gr/stations/pertouli/
Kalampaka	39.70899	21.62846	238.0	Precipitation	NOA	https://penteli.meteo.gr/stations/kalampaka/
Karditsa	39.42535	21.91524	91.0	Precipitation	NOA	https://penteli.meteo.gr/stations/karditsa/
Domokos	39.12756	22.30020	570.0	Precipitation	NOA	https://penteli.meteo.gr/stations/domokos/

2.2 Statistical analysis

The statistical tools employed to examine the time variations and the interactions between water level and precipitation of the selected monitoring stations were descriptive statistics, cross-correlation analysis and cross-correlograms. This approach allows the determination of the extent to which two data series exhibit oscillations, differing by a distance of *k* units in time (Legendre & Legendre, 2012). The time lag between lag 0 and the lag of the maximum value of the cross-correlation function (CCF) gives an estimation of the response of the system against an unitary impulse (Benavente *et al.*, 1985)cross- correlation and spectral analysis methods are applied to daily rainfall and discharge sequencies - over the 1974—81 period - in a mediterranean karstic system (Alicante province, Eastern Spain. Cross-correlation coefficient *)* can be defined as:

$$r_{xy}(k) = \frac{C_{xy}(k)}{\sigma_x \sigma_y},\ C_{xy}(k) = \frac{1}{n}\sum_{t=1}^{n-k}(x_t - \bar{x})(y_{t+k} - \bar{y}),$$

where is the cross-correlogram, and σ_x and σ_y are the standard deviations of the time-series. The overbar represents the temporal mean value of the signal (Larocque *et al.*, 1998).

3. Results

Daily water level at Nomi monitoring station ranged between 0.03 and 4.79 m (mean 0.52 m and median 0.27 m) and at Tempi station between 0.02 and 4.49 m (mean 0.54 m and median 0.36 m) for the period September 2019 - January 2022. The mean daily precipitation was 3.9 mm, 4.8 mm, 2.4 mm, 1.9 mm, and 1.9 mm for Metsovo, Pertouli, Kalampaka, Karditsa, and Domokos weather stations respectively for the investigated period (Table 2).

Table 2. Descriptive statistics of the monitoring stations.

Parameter	**Water level***		**Precipitation**				
Station	**Nomi**	**Tempi**	**Metsovo**	**Pertouli**	**Kalampaka**	**Karditsa**	**Domokos**
Descriptive statistics	**(m)**		**(mm)**				
# of measurements	829	878	884	884	884	884	884
Mean	0.52	0.54	3.93	4.78	2.42	1.92	1.91
Median	0.27	0.36	0.00	0.00	0.00	0.00	0.00
Std. Deviation	0.75	0.59	10.25	14.49	8.29	6.63	6.38
Minimum	0.03	0.02	0.00	0.00	0.00	0.00	0.00
Maximum	4.79	4.49	86.40	238.80	93.40	90.60	77.40

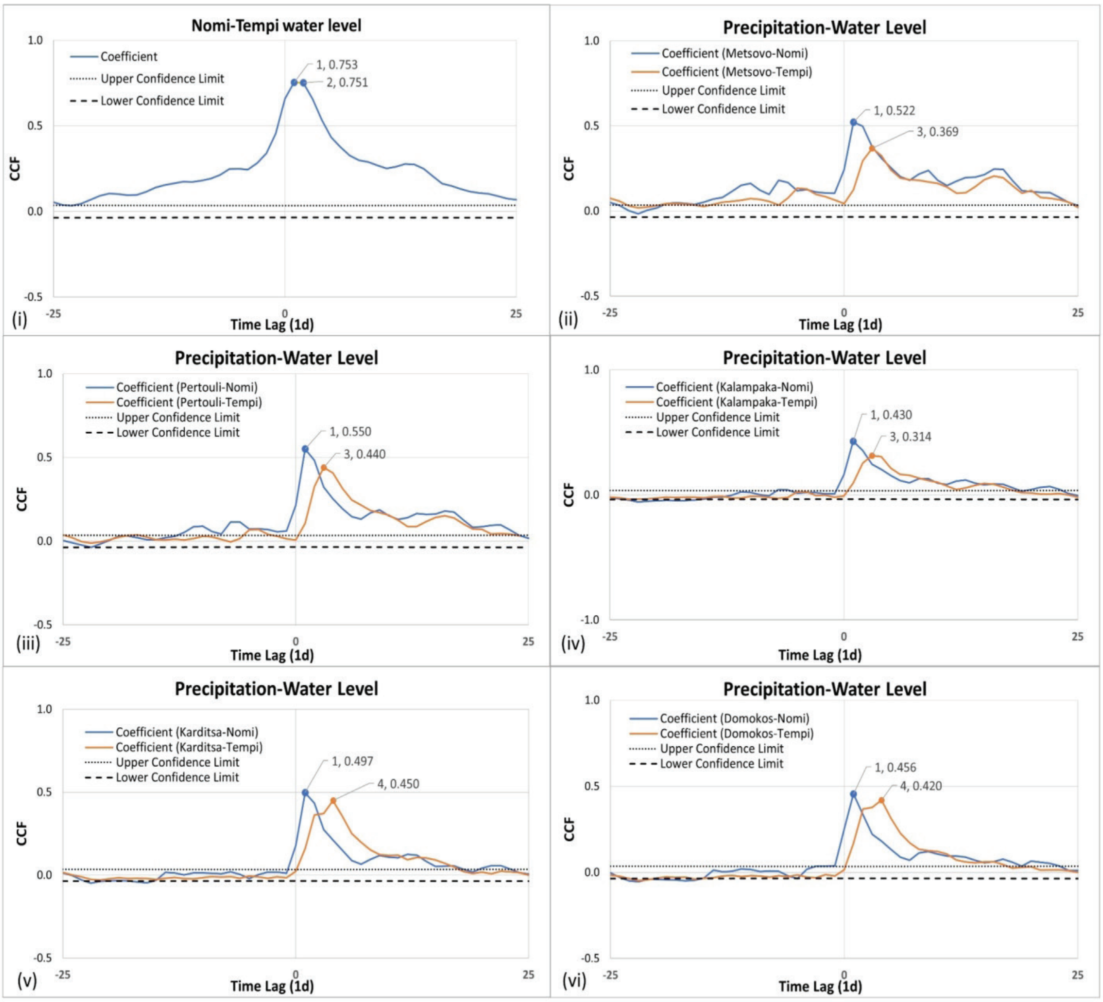

Fig. 2: Cross-correlograms of (i) water level between the two telemetric stations, and between weather station (ii) Metsovo, (iii) Petrouli, (iv) Kalampaka, (v) Karditsa, and (vi) Domokos, and Nomi and Tempi water level stations.

The cross-correlogram of water depth between Nomi and Tempi telemetric stations was asymmetrical. The highest CCF was achieved after 1-2 days, while the values were high (0.753 and 0.751 respectively), indicating the significant delay in peak discharge appearance between the two stations (Fig. 2i). The cross-correlograms between water level measurements at Nomi station and precipitation of the upstream weather stations, showed a dissymmetry towards the positive values and a time lag of one day. The highest value was achieved between Nomi water level station and Pertouli weather station (0.550). Likewise, the cross-correlograms between water level measurements at Tempi station and precipitation of the upstream weather stations showed a dissymmetry towards the positive values, while the cross-correlation coefficient was lower in all cases, indicating that the input signal from precipitation is attenuated during its passage through the system. The time lag between Tempi water level station and precipitation of the upstream weather stations varied between three and four days (Fig. 2ii-vi).

4. Discussion/Conclusion

In the present study a water level-precipitation dataset obtained from two monitoring stations at Pinios River and five weather stations upstream the catchment, were statistically elaborated. Based on the results, the delay in peak discharge appearance between the two stations is one to two days. The rainfall events taken place on the mountainous areas of the catchment affect the upstream station Nomi after one day, while the downstream station Tempi is affected after three to four days.

Riverine ecosystems are sensitive to climate stressors such as precipitation that impact the hydrological regime, water quality and the structure, composition, and distribution of riparian vegetation (EcoAdapt, 2021). The understanding of the response of rivers to precipitation can be a valuable tool towards flood protection and warning system implementation. Towards this scope, a simple yet effective and low-cost approach based on the statistical analysis of automatic monitoring station data of water level and precipitation has been proposed. This methodology when applied using a dataset with higher temporal resolution (for example hourly measurements) will be able to provide a more detailed aspect of the system's functioning and can therefore be used as a valuable early warning system for flood protection.

5. Acknowledgements

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ECOLOGICAL STATUS AND TRENDS OF LAKE KASTORIA: TEN YEARS OF BIOLOGICAL MONITORING

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Abstract

Lake Kastoria, in northwestern Greece, is a shallow lake, impacted mainly by pollution. The objectives of the research are to assess the ecological status and trends of Lake Kastoria during the ten-year operation of biological monitoring and to present the classification of its ecological status, according to the WFD provisions. The biological quality elements of phytoplankton, aquatic macrophytes and littoral zoobenthos were monitored during 2012-2021 and three national assessment systems (i.e., HeLPhy-phytoplankton, HeLM-aquatic macrophytes and HeLLBI-littoral zoobenthos) were used in order to classify the ecological status of the lake. Phytoplankton biovolume values varied considerably during the first monitoring period (2012-2015); while lower variations were recorded during the second monitoring period (2016-2021). Lake Kastoria is classified as Moderate according to HeLPhy and HeLLBI assessment systems and Good according to HeLM. Overall, it is classified as Moderate. HeLM and HeLLBI indicated some improvement in the littoral zone of the lake in 2018-2021 compared to the previous periods. However, the status of the pelagic zone of Lake Kastoria remained relatively stable, in moderate status during the 10 years. Nutrient inputs from the lake catchment, causing eutrophication, should be further controlled in order to improve the lake ecosystem's status.

Keywords: WFD, EQR, HeLPhy, HeLM, HeLLBI.

1. Introduction

Most lakes suffer from degradation in the last decades, causing decline in their water quality, due to anthropogenic pressures (Reid *et al.*, 2019). The need to monitor their status and trends, in order to design effective management measures and to reduce those impacts are now being highlighted worldwide. The national monitoring network for lakes, in the context of the Water Framework Directive (WFD) (EU, 2000), became operational in 2012 with the Joint Ministerial Decision 140384/2011 (JMD, 2011). Lake Kastoria, in northwestern Greece, is a shallow urban lake, situated by the town of Kastoria. It belongs to the Natura 2000 network as a Special Area for Conservation according to the Habitats Directive and as a Special Protection Area according to the Birds Directive (codes GR1320001 and GR1320003, respectively). In the 2nd round of the River Basin Management Plan of Western Macedonia (RBMP, 2017), eutrophication from point and non-point sources has been identified as a significant pressure to Lake Kastoria. Therefore, the lake is subject to operational monitoring (JMD, 2011). The lake has been designated as a heavily modified water body, according to WFD (EU, 2000), due to hydromorphological modifications (code GR000900030073H). The objectives of this research are to assess the status and trends of Lake Kastoria during the ten-year operation of biological monitoring and to present the classification of its ecological status, according to the WFD provisions.

2. Material and Methods

According to WFD (EU, 2000), samplings for phytoplankton analysis are carried out annually and samplings for aquatic macrophytes and zoobenthos analyses, every three years. In Lake Kastoria, water samplings for phytoplankton analysis took place during the warm period (June – October) of each year from 2012 to 2021. All samples were collected from the deepest part of the pelagic zone, from the euphotic zone of the water column (2.5 × Secchi disk depth), using a Nansen type sampler, according to the Hellenic Lake Phytoplankton (HeLPhy) assessment system (Tsiaoussi *et al.*, 2017). Phytoplankton samples were preserved with lugol's solution. Thirty-two phytoplankton samples were analysed. Quantitative analysis was performed using the Utermöhl sedimentation method (ELOT EN 15204:2006) and phytoplankton biovolume was estimated based on ELOT EN 16695:2015. Chlorophyll a concentrations were determined according to standard methods (10200 H, APHA, 2017). Regarding aquatic macrophytes sampling process, the belt transect-mapping method was applied and sampling within each transect followed the Hellenic Lake Macrophyte (HeLM) monitoring and assessment system (Zervas *et. al.*, 2018). To apply this method, full surveys of twenty permanent transects per lake were undertaken during the vegetative period, in 2014, 2017 and 2020, corresponding to the triennia 2013-2015, 2016-2018 and 2019-2021 respectively. The abundance, frequency and depth distribution data of all taxa encountered were recorded. Additionally, the maximum colonization depth of species per lake was measured annually. Regarding benthic macroinvertebrates, sampling and analysis followed the specifications of the Hellenic Lake Littoral Benthos (HeLLBI) assessment method (Mavromati *et al.*, 2021). In particular, samplings were undertaken using the three-minute kick/sweep method with standard hand net (500 μm mesh size) at the littoral zone of the lake (up to 1.2 m depth of water). Five selected sites were sampled during spring 2017 and 2021, corresponding to the triennia 2016-2018 and 2019-2021 respectively. Samples were sieved on site and sorting, identification and counting were carried out in the lab (Mavromati *et al.*, 2021). The Ecological Quality Ratios (EQRs) for each Biological Quality Element (BQE) were calculated according to the respective WFD compliant national assessment methods (EC, 2018). The overall ecological status classification for Lake Kastoria was determined with the application of the one-out-all-out principle, i.e. by the lowest of the values for the biological monitoring results of BQEs (EU, 2000).

3. Results

The mean annual (mean value of the warm season) total phytoplankton biovolume values in Lake Kastoria for the period 2012-2021 are shown in Figure 1. In particular, high variations were recorded during the first monitoring period (2012-2015); the highest phytoplankton values were measured in 2014, and the lowest in the following year (2015). During the second monitoring period (2016-2021), lower variations in phytoplankton biovolume values were recorded. The mean annual total phytoplankton biovolume ranged from 7.3 mm^3/L in 2015 to 24.8 mm^3/L in 2020, with the exception of 2014. Specifically, in the last warm months of 2014, exceptionally high phytoplankton biovolume values were measured, due to an extensive bloom of cyanobacteria in the lake; thus, the mean total phytoplankton biovolume of the whole warm season was calculated to 83.8 mm^3/L. The organisms that contributed mostly to total phytoplankton biovolume values, with the exception of 2015, were cyanobacteria, ranging from 64.9% to 100% of total biovolume in 2018 and 2013 respectively.

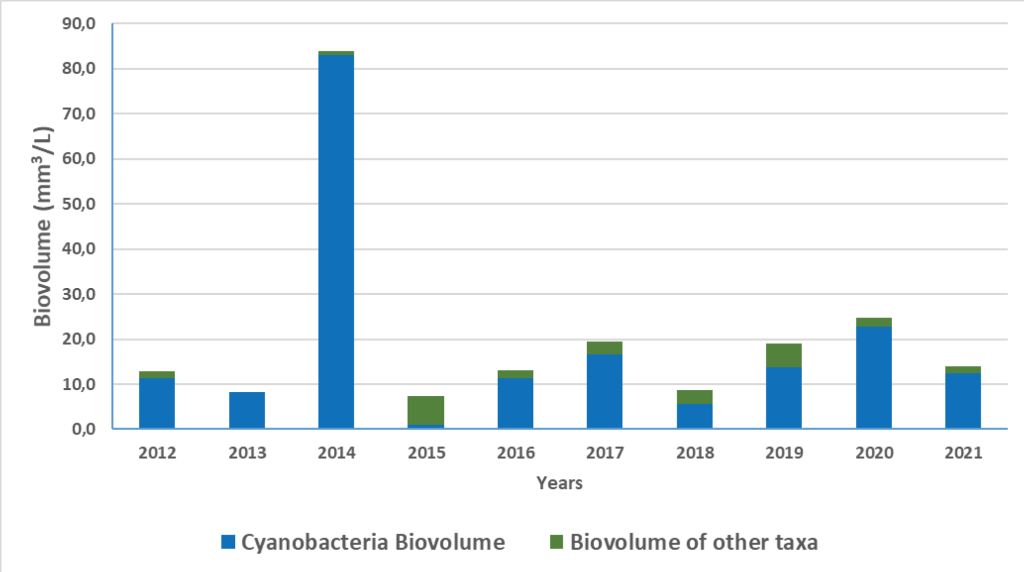

Fig. 1: Values of mean annual phytoplankton biovolume, in Lake Kastoria (2012-2021).

The annual values of the EQRs of the Hellenic Lake Phytoplankton (HeLPhy) assessment system for phytoplankton, in Lake Kastoria, for ten years (period 2012-2021) are shown in Figure 2. The mean EQR was 0.45, thus, the ecological quality of the lake was assessed as Moderate during the last decade. In particular, during the first monitoring period (2012-2015), the mean value of the EQR was 0.47 (± 0.19), ranging from 0.24 (in 2014) to 0.71 (in 2015) and during the second monitoring period it was 0.44 (± 0.03), ranging between 0.40 and 0.49.

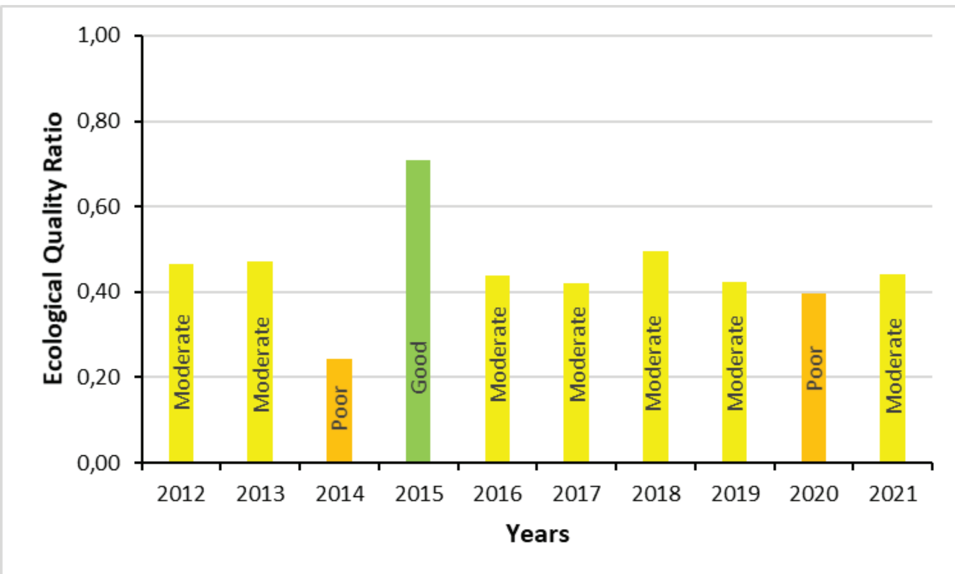

Fig. 2: Annual EQR values of the HeLPhy assessment system in Lake Kastoria (2012-2021).

The EQR values of each of the three assessment systems; HeLPhy, HeLM and HeLLBI in Lake Kastoria, for the three triennia (period 2013-2021) are shown in Fig. 3. The mean EQR value of the HeLPhy assessment system for phytoplankton was 0.45 (± 0.03), indicating the Moderate ecological quality of the lake. The mean EQR value of the HeLM assessment system for aquatic macrophytes was 0.71 (± 0.06), evaluating the lake as Good, throughout the examined period. Notably, an increase in these values has been observed in the period 2019-2021 compared to the previous two triennia (2013-2015, 2016-2018). The mean EQR value of the HeLLBI assessment system for littoral zoobenthos was 0.52 (± 0.07), classifying the lake as Moderate. An increase in the EQR values of HeLLBI has been observed in the period 2019-2021 compared to the period 2016-2018. All three assessment systems were constructed to respond to the pressure of eutrophication, whereas HeLLBI also responds to morphological modifications (Tsiaoussi *et al.*, 2017; Zervas *et al.*, 2018; Mavromati *et al.*, 2021).

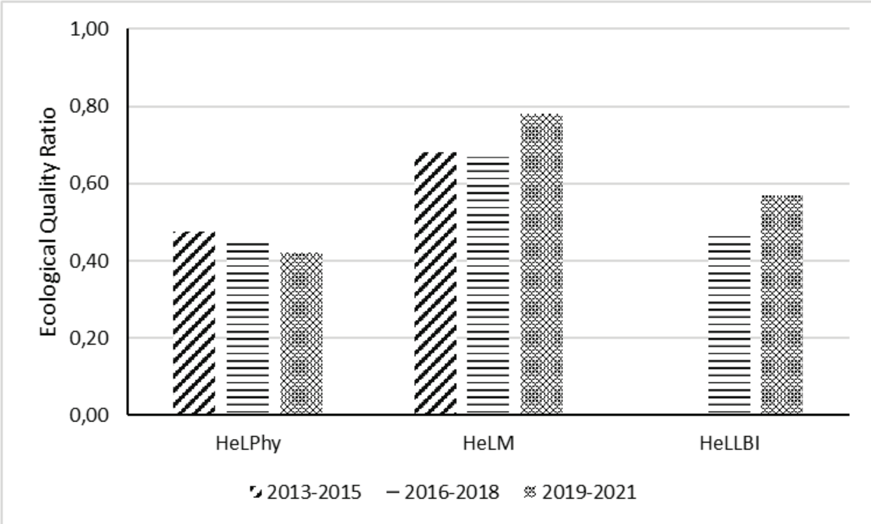

Fig. 3: EQR values of the HeLPhy, HeLM and HeLLBI assessment systems in Lake Kastoria.

4. Discussion/Conclusion

Based on the results from the National Monitoring Project, and with the application of the one-out-all-out principle (EU, 2000), Lake Kastoria is classified overall as Moderate, during both the first and the second monitoring periods. The HeLM assessement system classified the lake consistently as Good, unlike the other two assessment systems (HeLPhy and HeLLBI). Results from aquatic macrophytes and zoobenthos suggest that there has been an improvement in the biological quality of the littoral zone in the period 2018-2021, compared to the previous years. Results from phytoplankton show that the pelagic zone of the lake is at moderate status, with no signs of improvement throughout the decade. Human activities have been affecting the lake, which is a recipient of urban wastewater and fertilizers from agricultural areas. Although some actions are underway, such as the reconstruction of certain sewage pumping stations of the town, more actions that minimize nutrient loading from all pollution sources of the lake catchment are necessary, in order to reduce eutrophication and to improve the overall status of the lake ecosystem. Biological monitoring should continue in the future, in order to document changes in the state and trends of the lake, and thus evaluate the effectiveness of management measures applied.

5. Acknowledgements

The present study was conducted in the frame of the Greek National Water Monitoring Network, according to the JMD 140384/2011, implemented by The Goulandris Natural History Museum, Greek Biotope/Wetland Centre (EKBY). The network is supervised by the General Directorate for Waters of the Ministry of Environment and Energy. EKBY’s personnel conducted samplings. D. Zervas contributed to sampling and analysis of aquatic macrophytes. M. Bozatzidou contributed to zoobenthos identification. Part of phytoplankton analysis was commissioned to AUTH. The research was funded from Act MIS 5001204 financed by the European Union Cohesion Fund (Partnership Agreement 2014-2020) and from Act MIS 371140 financed by the European Regional Development Fund (National Strategic Reference Framework 2007-2013).

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ETHOLOGY AS A TOOL FOR FRESHWATER FISH CONSERVATION: THE BEHAVIOURAL EFFECTS OF TURBIDITY

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Abstract

Animal behaviour plays a key role in gene-environment interactions, and is especially important within the interplay of anthropogenic pressures and animal fitness. Thus, ethology, the scientific study of animal behaviour, can be useful in conservation efforts aiming to mitigate the impact of these pressures and prevent biodiversity loss. Here, we describe four basic behavioural tests that can be utilized to examine fish responses to acute or prolonged exposure to stressors, and we argue they can offer valuable insight for future conservation efforts under different stress scenarios. These tests are the emergence test, the open field test, the sociability test and the predator response test. We provide methodological information and present the application of a combination of these tests in examining the impact of water turbidity, an important environmental stressor, often exacerbated by human activities, on native and alien freshwater fish species. The implications of our findings for more targeted future conservation actions are discussed.

Keywords: refuge, boldness, thigmotaxis, shoaling, anti-predator behaviour.

1. Introduction

Behaviour refers to an animal's array of responses to environmental stimuli, and thus acts as a bridge between physiological and ecological processes. Often, these behavioural responses are reactions to human-induced disturbances or stressors. Conservation aims to measure the impacts of the anthropogenic stressors on biodiversity, and subsequently to mitigate any detrimental effects; toward this aim, animal behaviour can thus factor into successful conservation and eco-management efforts. There are several studies highlighting the successful application of animal behaviour in conservation biology (see Berger-Tal *et al*., 2011), as well as evidence of previous conservation programs failing due to lack of knowledge of key behavioural characteristics of the target species (see Knight, 2001). This integration of behavioural sciences into biodiversity conservation has resulted in a discipline termed 'conservation behaviour', and although the theoretical relevance of behaviour to conservation is now well-established, its practical implementation is still lagging behind (Knight, 2001; Berger-Tal *et al*., 2011).

Here we present four basic fish behavioural tests that can provide valuable insight into key fitness-related traits of the target species under various environmental conditions. These tests do not require specialized equipment or software, and can be conducted in relatively short time scales. Combinations of these tests can be conducted in succession for time economy and avoidance of excess fish stress due to repeated handling (Leris *et al*., 2022). These tests are the emergence test, the open field test, the sociability test and the predator response test. Performance in the emergence test measures an individual's boldness and offers a good estimation of its temperament within the boldness-shyness spectrum (Brown *et al*., 2005). The open field test provides insight into the subject's anxiety- and stress-related behaviours and overall risk aversion, but also into its general activity levels and exploratory tendencies, when encountering a novel environment (Godwin *et al*., 2012). Sociability tests are designed to measure the propensity of an individual to be near a group of conspecifics, i.e., its grouping (or shoaling) tendency; hence they are often called grouping or shoaling tests. They are especially important for social species living in groups (e.g., minnows) as they offer information about the subject's reliance on social interactions for foraging information or predator avoidance (Cote *et al*., 2012). Lastly, the predator response test, examines the behavioural reactions of an individual presented with visual and/or chemical cues of a predator or a simulated predation event (see Brown, 2003).

2. Methods

2.1 Emergence test

The emergence test typically begins with the placement of the subject inside a safe area, chamber or compartment of the test tank, in which it is allowed to acclimate for a designated time interval (Fig. 1A). This safe zone can be covered/shaded and may include plants and gravel for shelter. After acclimation, the fish is allowed to exit the refuge, usually by remotely lifting a divider or opening a 'door', and to enter a novel and potentially dangerous area of the test setup (e.g., Brown *et al.*, 2005). The main behavioural measure that can be obtained by this test, is the time required by the fish to exit the refuge ('latency to exit' – typically measured in seconds). This test could potentially run indefinitely but usually a pre-designated waiting period is used, and if the subject does not leave voluntarily within it, it is given a max score (equal to that period) and is considered censored. A Kaplan-Meier survival-type analysis or a similar approach taking into account censored data, is usually employed for data analysis (Wing *et al.*, 2021; Leris *et al.*, 2022).

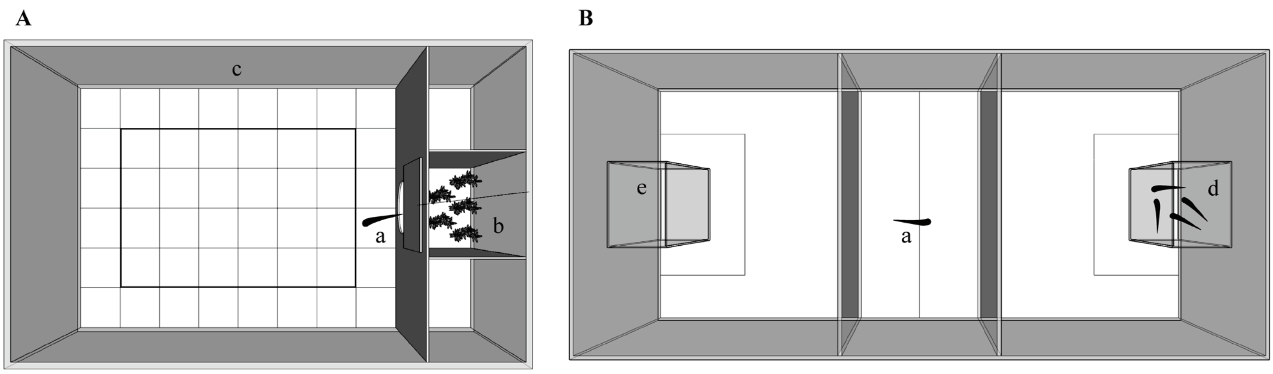

Fig. 1: A, Emergence test setup where the subject (a) exits an artificial shelter with plants (b), followed by the open field test in a novel arena (c). B, Sociability test setup in which the subject (a) can choose to spend time near a shoal of conspecifics (d), or near an empty container (e).

2.2 Open field test

Open field tests were initially designed for rodents but they have been adapted for usage with fish (e.g. Godwin *et al.*, 2012). The test typically involves the introduction of the subject in a novel tank (open field arena), where it is allowed to swim freely and explore its surroundings. The walls of the tank are covered to avoid external stimuli, and the bottom is usually light coloured to aid with video recording from above. In addition, water level is typically kept low, to confine the subject's movement to two dimensions (Godwin *et al.*, 2012). The main behavioural measures that can be obtained from the basic open field test are 'time spent moving'/'time immobile', and 'time spent in the periphery'/'time in the centre' of the arena (Godwin *et al.*, 2012; Wong *et al.*, 2012), with most animals showing a preference to stay near the walls ('thigmotaxis'). Wall-touching may also be scored, as well as more pronounced stress-related behaviours, such as darting events or bouts of erratic swimming. Using automated tracking software, one can obtain more sophisticated measures, such as 'total distance travelled' and 'swimming velocity'. However, with slight modifications in the apparatus design (e.g. squares drawn at the bottom, Fig. 1A), we can obtain additional behavioural data reflecting e.g. exploratory tendency ('number of unique squares visited') or general activity levels ('total number of squares visited'), without the need of automated tracking (e.g. Leris *et al.*, 2022).

2.3 Sociability test

Sociability tests, typically offer two choices to the subject (dichotomous tests), i.e., a tank compartment (or a container) holding a group of conspecifics ('stimulus shoal') on the one side of the tank and an empty compartment/container on the other side (Fig. 1B). However, different or additional choices can be offered, e.g. between different-sized groups of conspecifics, or between familiar and unfamiliar individuals, kin and non-kin members, etc (e.g., Cote *et al*., 2012). Usually, a single subject is placed in a starting location (behind a removable partition or inside a tube that can be remotely lifted) and is allowed to acclimate, before being released. The fish is then free to swim to any of the choice areas and to interact with the fish shoal or the empty container (see Ward *et al*., 2004; Cote *et al*., 2012). Designated choice zones drawn on the bottom or the side of the tank, aid with the scoring of the shoaling behaviour, i.e. the amount of time the fish spends near the shoal or near the empty container. These shoaling zones (commonly measured in body lengths) are dependent on the tested species and its specific social behaviour. 'Absolute shoaling time' (time spent near the shoal minus time spent near the empty container) and 'shoaling preference' (proportion of time spent shoaling to total time) are typical behavioural measurements obtained from these tests.

2.4 Predator response test

There are many variations of predator response tests which may involve the exposure of the subject to visual and/or chemical cues of a predator, or to a simulated predator attack. Visual cues may be simply presented by removing a partition and revealing a predator in an adjacent compartment/tank, or by showing images of a predator, either from live video recordings or from artificial 3D models. Chemical cues can be alarm cues from conspecifics or predator odours (kairomones) (see Brown, 2003). A simulated predator attack is a sudden event in the tank, e.g. the fall of a plastic rod, or a 'flyby' of a plastic object above it (e.g., Ward *et al*., 2004; Leris *et al*., 2022). In this test, a pre-stimulus testing period is required, to assess the baseline behaviour of the subject. After the predator presentation or the simulated attack, the post-stimulus behaviour of the fish is also recorded. Typical before/after measures in these tests are 'swimming activity', 'time spent moving/immobile', 'foraging activity' and stress-related behavioural events. Latency to resume activity may also be analysed in this context (Ward *et al*., 2004).

2.5 Behavioural responses to turbidity in native and alien freshwater fishes

We used a combination of the open field and sociability tests, conducted in succession, in order to assess the potential effects of rising turbidity on the behaviour of the native threatened killifish *Valencia letourneuxi*, and the alien Eastern mosquitofish *Gambusia holbrooki*. We used 36 killifish (18 females and 18 males) and 44 mosquitofish (26 females and 18 males) from HCMR aquaria, and tested them in clear (0 NTU) or turbid water (30 NTU). For the latter, we used suspended bentonite clay. Each subject was placed individually in a vertical plastic tube for a 2-min acclimation. The tube was remotely lifted, the fish was allowed to explore the novel arena for 5 min (open field test), before being placed back in the tube. During a 2-min settling period, we placed two transparent containers in the other end of the tank, one holding a pair of conspecifics and the other being empty. The tube was lifted once again and the fish was allowed to choose and interact with either container (sociability test). Main behavioural measures in the open field test were 'activity', 'exploration', 'time in the centre' and 'time immobile' (see Leris *et al*., 2022). In the sociability test, we measured the 'absolute shoaling time' and 'shoaling preference'. As data from the sociability test are currently being analyzed, in the following section the main findings from the open field test are presented.

3. Results and Discussion

We detected several effects of turbidity on both species behaviour in the open field test. First, turbidity decreased the activity of all fish irrespective of species or sex (3-way ANOVA: $F_{1,71}$ = 10.89, P < 0.01; Fig.

2A). There was a significant interaction effect between 'species' and 'sex' regarding exploration, irrespective of treatment (3-way ANOVA: $F_{1,71}$ = 9.82, P < 0.01; Fig. 2B), with mosquitofish males displaying higher exploratory tendency than mosquitofish females and killifish males (Dunett t post hoc tests: P < 0.05). There was also a significant interaction effect between 'treatment' and 'species' for both 'time immobile' (Kruskal-Wallis test: H_3 = 24.38, P < 0.001; Fig. 2C) and 'time in the centre' of the open field arena (Kruskal-Wallis test: H_3 = 12.85, P < 0.01; Fig. 2D). Killifish spent more time immobile than mosquitofish within each turbidity treatment (Kruskal-Wallis tests: P < 0.01), and killifish tested in clear water spent less time in the centre of the arena compared to both species tested in turbid water (Kruskal-Wallis tests: P < 0.05).

For prey fish species, turbidity can either increase perceived risk (e.g. Leris *et al.*, 2022), or act as a refuge and decrease perceived risk (see Utne-Palm, 2002). In the current study, turbidity decreased overall activity for both species (an indicator of increased perceived risk) but increased time spent in the centre (an indicator of decreased perceived risk) specifically for the native killifish. These conflicting findings could reflect a more complex role of turbidity with differing effects on risk perception, depending on the source of the risk (e.g. aquatic versus aerial predators). In addition, our results suggest that turbidity may act differently upon the behaviour of the two studied species, which often compete in the wild. Thus, elevated turbidity can be considered a stressor under specific circumstances for the native killifish *V. letourneuxi*, but may not impact the mosquitofish at the same degree. Siltation and suspended sediment, from human activity and from natural events (e.g. floods) are expected to increase in the future, especially under current climate change scenarios. Thus, mitigation plans should aim also to prevent/ameliorate such increases in sediment or algal turbidity, in the target native species' habitats, especially where alien species and aquatic predators are present. Moreover, in planned fish translocations, turbidity levels at the release habitats should also be considered. Lastly, further work is required on the behavioural effects of turbidity on other threatened native species (and their co-occurring alien species) towards the design of more successful future conservation plans.

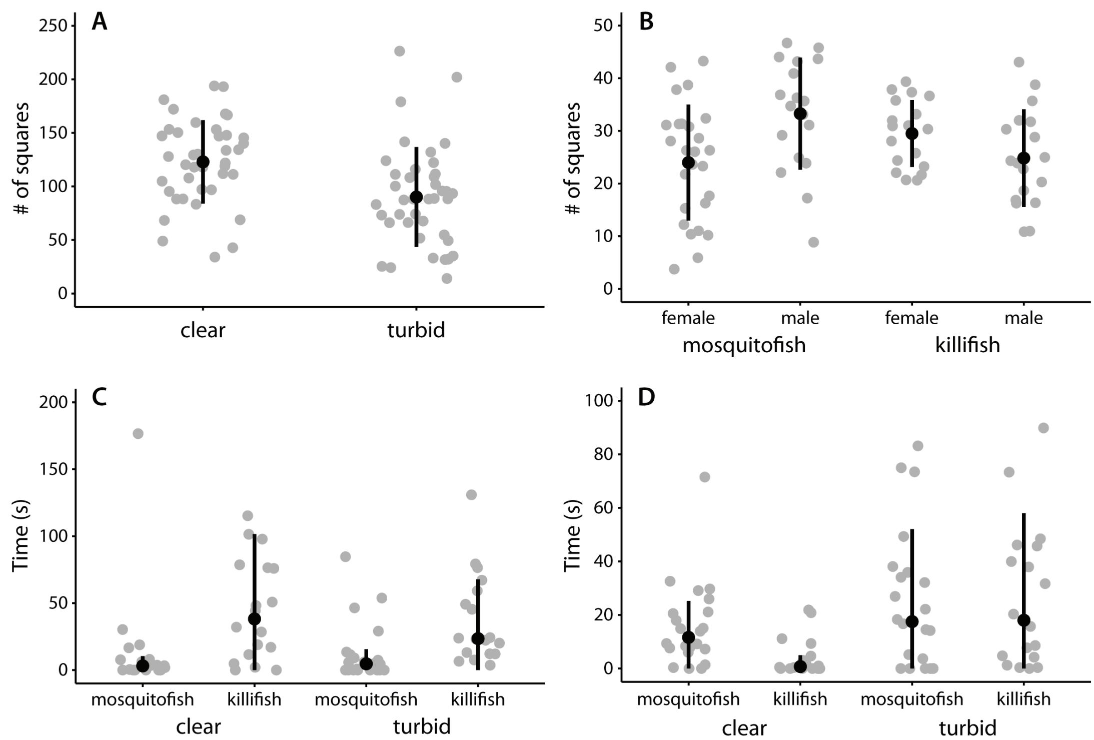

Fig. 2: A, Mean activity and B, mean exploration in the open field test. Black dots represent the mean, black lines the standard deviation and grey dots individual data points. C, Median time immobile and D, median time in the centre of the arena. Black dots represent the median, black lines the interquartile range and grey dots individual data points.

4. Acknowledgements

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A SHORT REVIEW OF HYDRAULIC-HABITAT MODELLING FOR ENVIRONMENTAL FLOWS

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Abstract

This research is embedded into the FlowTech project which focuses on the estimation of river environmental flows. Human interventions to natural streamflow are rising; most of the time these disruptions do not consider environmental needs. Several methods of environmental flow estimation have been developed in different parts of the world addressing different goals and levels of protection. Due to the large number of streams involved and money-manpower limitations, methods developed and used must be simple and require little field investigation. The integration of in-stream fish habitat and hydraulic modelling is among the most advanced approaches for estimating environmental flows based on biological criteria. In this study we reviewed the current knowledge and literature developed to estimate fish habitat availability for environmental flow requirements. Among our main findings are that parameters such as the selection of fish species and their habitat preferences during their life cycles, their relationships within ecosystems, the spatial and temporal scales that represent habitat, and the validation strategies, are considered crucial for the estimation of the water required to sustain aquatic habitats and that those methods need to be used within a wider decision support framework to define a flow regime for both the environment and human health.

Keywords: flow regime, habitat, fish, hydraulic, modelling, ecohydraulic.

1. Introduction

Hydromorphological pressures alter the natural flow regime of rivers and can be directly linked to impacts on their physical and chemical attributes and processes. The environmental flow regime is a key element in determining river processes, affecting ecosystem health. Methods addressed to estimate environmental flows are classified into four different categories (Tharme, 2003): hydrological methods (e.g., Karimi *et al.,* 2012), hydraulic methods (e.g., Gippel & Stewardson, 1998), physical habitat methods (e.g., Papadaki *et al.,* 2016) and holistic methods (e.g., McClain *et al*., 2014).

A river ecosystem is affected by many parameters, so the delineation of different habitat types will facilitate comprehending the mechanisms by which the river operates as an ecosystem. Habitat simulation methods for environmental flow assessment and estimation attempt to interpret the importance for fish of these patterns of flow within channels, primarily resulting from the geometry of the channel. Simulated discharge through the means of a hydraulic model, aims to find an optimal flow such that the amount of physical habitat for the selected fish species does not decline beyond a subjectively determined conservation level (Linnansaari *et al.,* 2013).

Among the most widespread approaches for exploring the relationship between stream flow and physical habitat is the Physical Habitat Simulation System (PHABSIM), the Instream Flow Incremental Methodology (IFIM) and the Ecological Limits of Hydrologic Alteration (ELOHA) framework for determining and implementing environmental flows at the regional scale (Poff *et al*., 2010). IFIM methodology was designed by a multidisciplinary United States Fish and Wildlife Service team of biologists, hydrologists, engineers and computer scientists working together in Colorado in the 1980s. PHABSIM quantifies an aquatic habitat in terms of physical variables such as flow depth, velocity, and substrate (Bovee 1982). Apart from PHABSIM habitat hydraulic modelling methods have been applied by many countries either as physical habitat modelling or as independent approaches (Acreman & Dunbar, 2004). Instream physical habitat models were firstly introduced and developed to estimate flow requirements below dam constructions in North America (Bovee 1982). Later, those methods were applied to various relevant problems such as flow alteration caused by manmade hydromorphological changes, including instream channelization, and hydropeaking (Boavida *et al.,* 2012). Additionally, environmental flow or minimum flow was also estimated using physical habitat simulation (Heggenes *et al.*, 1996; Katopodis, 2005; Dunbar *et al.*, 2012; Papadaki *et al.*, 2016; Nikghalb *et al.*, 2016; Armas-Vargas *et al.*, 2017; Stamou *et al.*, 2018).

2. Important Parameters for applying habitat hydraulic simulation for environmental flow estimations

2.1 Ichthyofauna and habitat suitability

The fish fauna is considered as the most sensitive Biological Quality Element (BQE) to river flow changes and is a key factor of the inter-relations among many BQEs. The target species for conducting ecohydraulic modelling should reflect the environmental constraints on the overall community and their abundance should be dependent on environmental changes that affect specific habitat variables related to important life cycle events (e.g., spawning season). Many previous studies have focused on migratory species (Ferguson *et al.*, 2011; Mendes *et al.,* 2021), headwater (rhithron) (Meyer *et al.,* 2007) and/or endangered species or particular fish species (e.g., salmonids) which have an important economic value and attract public attention. Techniques which have traditionally been used for fish habitat measurements are direct observations from the river banks, snorkeling, electrofishing (Heggenes *et al.,* 1991; Martínez-Capel *et al.,* 2009; Papadaki *et al.,* 2016), and various fish telemetry methods, including the use of acoustic or radio transmitters and Passive Integrated Transponder methods (Linnansaari *et al.,* 2009).

2.2 Relationships within ecosystems

All components of the flow regime are important for aquatic organisms. Relationships among streamflow, habitat conditions, temperature, and the distribution, growth, and abundance have been most extensively studied for summer months (Barbieri *et al.*, 2021). Reference to anthropogenic pressures is also a rising research topic; and most sampling work is also during summer flows (Tachos *et al.,* 2022; Vavalidis *et al.,* 2021). Summer months represent the main growing season for most fishes, and changes to stream temperature may affect fish metabolism, feeding, and growth (Zorn *et al.*, 2012). Moreover, previous studies have shown that high discharge is correlated with larger and more frequent movements, and that extreme low discharge could also induce movement if habitat is being de-watered (e.g., Taylor & Cooke 2012).

2.3 Spatial and temporal scales to represent the fish habitat

The spatial patterns of depth, velocity, and substrate are important factors for the habitat of river fishes. Research studies have aimed at quantifying and establishing hydro-physical variables in both microhabitats and a larger spatial scale (mesohabitat), such as the number of reaches or an entire drainage basin. In spite of technological advances that allow for more comprehensive data collection, hierarchical scaling procedures are often required to optimize the field work and data collection protocol. As well, upscaling is often required to extend results produced for single species to an entire community, and to select a regime associated with the proper time scale (Parasiewicz, 2003; Duel *et al.*, 2003). Selection of representative river reaches are crucial for the estimation of environmental flows when applying the aforementioned methods, considering them to represent samples of healthy aquatic habitats. Delimitation of a section of a river down to smaller river sections, within which a representative river reach will be defined including important habitat types and Hydro-Morphological Unit types (HMU), according to their depth and flow characteristics. In situ studies conducted for fish habitat selection covering all likely spatial and temporal heterogeneity are not possible. Therefore, the ecohydraulic modelling at spatial and temporal scales relevant to the fish, and based on presumably representative samples of stream sections (Bovee, 1982; 1986). Moreover, habitat availability estimations of aquatic organisms in a natural, non-regulated river act as the reference condition for comparing the degree to which an environmental flow scenario deviates from the natural flow regime (Boavida *et al.*, 2012).

2.4 Modell and Validation

Validation is usually recognized as an essential step in any modelling effort. Model validation consists of ensuring that both the biological parameters in habitat models and the numerical modeling in the hydraulic models is adequate by comparing model results to observed values (Leclerc *et al.*, 2003). Inevitably modelling involves several sources of uncertainty such as the input data the model structure and the internal model parameters (Papadaki *et al.*, 2017; Papaioannou *et al.*, 2021). Moreover, previous studies showed that, in complex river topographies where flows have local variations, the use of a 2D model is important in the assessment of flow alteration effects associated with water resource projects and habitat modelling (Leclerc *et al.*, 1995; Jowett & Duncan, 2012) and the investigation of spatiotemporal variability of available habitats using 2D models can be affected by the data set and the methodology followed for the digital terrain model (DTM) generation, which therefore affects the hydraulic characteristics and the habitat availability.

4. Discussion/Conclusion

Incorporating fish species and their associations with the hydrologic regime, may provide flow prescriptions which can meet human water requirements. Ecohydraulic models provide researchers and managers with important tools for the evaluation of flow regime alterations on many aspects of the riverine environment, including riparian ecosystems, river connectivity, and managing flows according to either reference physical habitat conditions, thresholds, rates of change or minimum values (Dimitriou & Papadaki, 2021). Aspects that need improvements include: the range of abiotic variables considered; modelling of habitat preference; modelling strategies in the context of multi-specific use of rivers; fish behavior related to winter conditions and model validation strategies. Finally, recent improvements of technology can provide the necessary support to overcome the obstacles and produce reliable environmental information for the estimation of environmental water needs. Incorporation of cutting-edge technology (e.g., high-resolution bathymetric; habitat types and habitat availability maps) will significantly improve the amount and accuracy of information that is necessary for a scientific understanding of how changes in the natural flow regime affect ecological conditions (Papadaki, 2021). Additionally, possible human interactions and resource competition within aquatic ecosystems and water should be analyzed within a holistic approach. In conclusion, future research on environmental flows and ecohydraulic modelling should be carried out within an integrated approach in which instream flow will benefit the overall understanding of the ecological functioning of river systems, as well as the potentially changing environmental conditions such as climate change effects.

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METHODOLOGICAL FRAMEWORK AND IMPLEMENTATION OF CONSERVATION ACTIONS FOR TWO THREATENED CYPRINIDS IN AN INTERMITTENT RIVER

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Abstract

In this study, we designed a methodological framework for habitat restoration and fish translocation for the conservation of threatened freshwater fishes in Mediterranean-type rivers. We implemented this framework for the conservation of the endangered Evrotas chub (*Squalius keadicus*, Stephanidis 1971) and the critically endangered Evrotas minnow (*Pelasgus laconicus*, Kottelat & Barbieri, 2004) native at the Evrotas/Vassilopotamos River basin, within the Natura 2000 GR2540003 site. *In-situ* conservation actions included: (a) habitat rehabilitation through deepening and extending the most important pools in a selected section of Evrotas River that would act as summer refugia for the targeted species, following the application of hydrological modeling, (b) native species' translocation (reintroduction/supplementation) actions following a translocation feasibility assessment and population viability modeling and (c) pilot alien species' reduction/eradication, as well as (d) *ex-situ* actions to create aquaria safety stocks of the two threatened species. This framework can have wider application for other freshwater fish species inhabiting intermittent streams in peri-Mediterranean countries. The success and effectiveness of these actions, however, need to be evaluated by post-implementation monitoring.

Keywords: Restoration, translocation, eradication, threatened fish, intermittent.

1. Introduction

Although representing a large part of Europe's hydrographic network, intermittent rivers have only recently begun to be the subject of targeted research (Leigh *et al.*, 2016). In the Mediterranean region, a biodiversity hotspot hosting a large number of endemic and threatened species, rivers and streams of intermittent flow regime are the predominant aquatic ecosystems (Skoulikidis *et al.*, 2017). In Greece, intermittent rivers account for 50% of the hydrographic network, due to unsustainable water resources management that, coupled with the gradual reduction of precipitation, have altered the natural flow regime of most of these rivers (Skoulikidis *et al.*, 2017). The Evrotas river basin is a profound example of this process, as its discharge has decreased by almost 85% in the last three decades, mainly due to water over-abstraction for field irrigation (Karaouzas *et al.*, 2017). The Evrotas River hosts two threatened cyprinids with restricted geographical distribution and declining population trends, included in the IUCN Red List (2022), the endangered Evrotas chub *Squalius keadicus* which is found exclusively in the Evrotas basin and the critically endangered Evrotas minnow *Pelasgus laconicus* which is found exclusively in the Evrotas/Vassilopotamos River basin and the upper Alphios River basin. This study outlines the methodological framework and the conservation actions implemented to improve the conservation status of *S. keadicus* and *P. laconicus* at the Evrotas/Vassilopotamos River basin, within the Natura 2000 GR2540003 site.

2. Material and Methods

In the framework of this study, three main actions were designed and implemented during 2020-2021 with the aim to: (a) improve the hydromorphological conditions of a selected section of the Evrotas River that can act as a summer refugia for the target species (Evrotas chub and Evrotas minnow), (b) implement native fish translocation (reintroduction/supplementation) actions, combined with a pilot reduction/eradication of the alien invasive Eastern mosquitofish *Gambusia holbrooki* (Girard, 1859) to increase the self-sustainability of the target species in the translocation water bodies, and (c) create an *ex-situ* stock of the target species.

2.1 Improvement of the hydrological regime and the environmental conditions of the Evrotas summer refugia

A methodological framework was developed for the assessment of the hydromorphological conditions during the dry season of the refugia, in a section of the Evrotas main stem that retains water throughout the year. For this purpose, hydraulic-hydrodynamic models were setup and applied. HEC-RAS model were selected for flow routing and the simulation of depth and velocity at the various parts of the river. Evaporation and infiltration rates were estimated through *in-situ* water level measurements from probes. Data from Unmanned Aircraft Systems (UAVs) were also used to record with high accuracy the bathymetry of the river, at the section of the river that could, after our restoration intervention, store significant amounts of water during the summer season. Hydraulic simulations were realized, including the infiltration and evaporation processes, as well as several scenarios of interventions, i.e. rehabilitation through deepening and extending the most important pools. To select the optimal scenario, habitat suitability curves for the target species were used and the results of the hydraulic simulations (water depth and velocity values) were converted into suitability indicators. Detailed restoration guidelines (excavation volume, elevation differences, estimated volume of retained water, estimated water depth) were then formulated within the frame of the optimal scenario for the threatened fish fauna.

2.2 Implementation of fish reintroduction/supplementation actions and alien species' reduction/eradication

Seasonal fish samplings were performed in summer-autumn 2020-2021, with a concomitant collection of water, macroinvertebrate and diatom samples to assess the physicochemical status and the biological status of a series of sites in the Evrotas/Vassilopotamos basin, based on standardized methodologies. Based on the field data, the water bodies for fish translocation actions and alien invasive fish control actions were selected, and the specifications for the translocation of the two threatened native fish species, as well as for the pilot reduction/eradication of the alien species were formulated. Specifically, we developed and applied a tool for assessing the feasibility of native fish translocation in Mediterranean-type river basins. The feasibility assessment tool consists of two main elements assessing: (a) whether the potential release water bodies can support a sustainable population of the target species in terms of carrying capacity, habitat suitability, the species' ecological requirements, anthropogenic pressures, presence of alien species, and current structure of their fish assemblage, and (b) whether the potential source water bodies can provide a sufficient number of genetically related individuals of the species, without compromising the viability and robustness of the source populations. The tool was applied at five spring-fed water bodies that form the upper part of the Vassilopotamos basin. The reintroduction of the Evrotas chub and the supplementation of the Evrotas minnow were simulated by applying a Population viability analysis (PVA) using the VORTEX model (Lacy, 1993) for the assessment of the optimal size of the transferred populations (application of three reintroduction/supplementation scenarios, i.e., translocation of 100, 75 or 50 individuals per water body), through modeling. At the same time, the eradication/reduction of the alien species was also simulated, for the complete restoration of the fish fauna at the Vassilopotamos water bodies. Native fish collection for translocation was conducted using a high-power shore electrofishing device with the minimum possible fish handling to avoid fish stress and mortality. Fish were measured in the field (Total length) and placed in aeriated tanks with aquatic plants added, for transport. Prior to their release, fish were acclimatized. Alien mosquitofish eradication was conducted at five streams at the Vassilopotamos basin in March and July 2021, prior to native fish translocation in three of these streams. Eradication was conducted using a high-power shore electrofishing device and nets. Mosquitofish were euthanized with high dose anesthetic, after recording their size and sex.

2.3 Actions to create aquaria fish stocks

Fish were collected to create a stock of the two threatened Evrotas species. The collection was carried out using an onshore electrofishing generator at an undisturbed location on the upper Evrotas R. and the fish were transported the same day to the facilities of HCMR in Anavyssos.

3. Results

The implementation of the optimal scenario for restoration interventions at the Evrotas summer refugia entailed the removal of approx. 900m^3 of sediment, deepening a large part of the intervention area, by an average of approx. 70 cm. The restoration actions were implemented in October 2021 and entailed excavation and dredging at an approx. 120 m section of the Evrotas River (Fig. 1).

The application of the fish translocation feasibility tool demonstrated the high potential of four of the five streams in the upper Vassilopotamos basin for the implementation of Evrotas chub reintroduction and Evrotas minnow supplementation (Fig. 2). Based on the PVAs, no significant differences were observed between the scenarios for both targeted species and all scenarios showed population growth in all recipient streams indicating viable populations after fish transport, with the source water bodies not affected demographically.

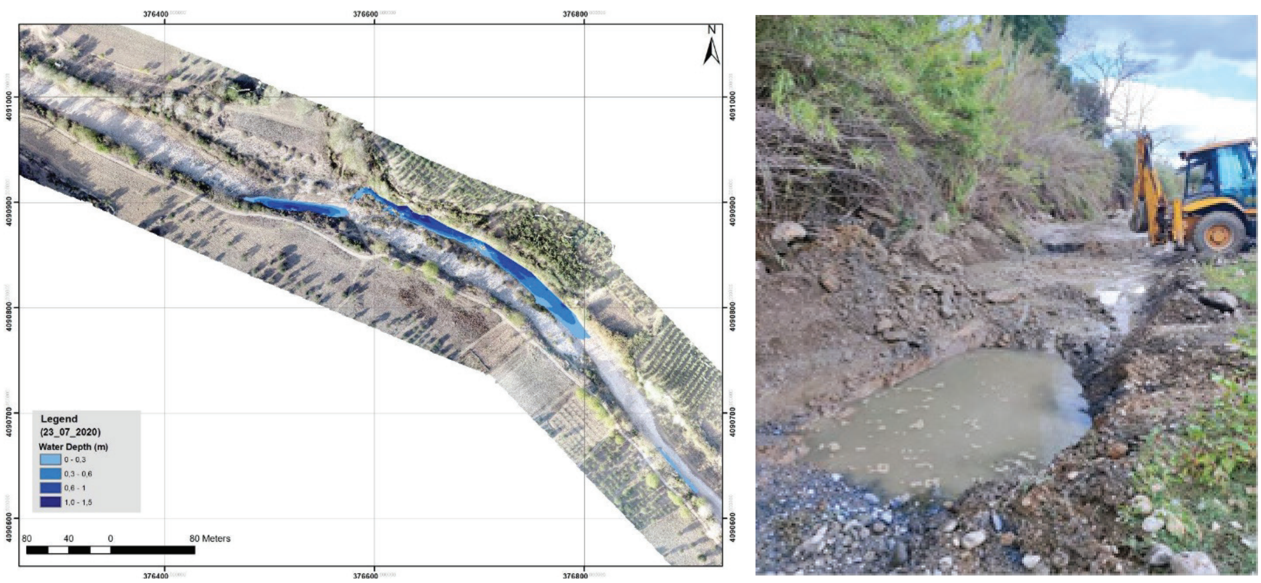

Fig. 1: Orthophoto map of the study area depicting remaining pools during July 2020 (left) and excavation and dredging interventions during October 2021 (right).

Concerning the eastern mosquitofish, PVAs showed that in the case of the scenario of removal of 75% of the original alien fish population in all streams this will lead to a short-term reduction of its populations.

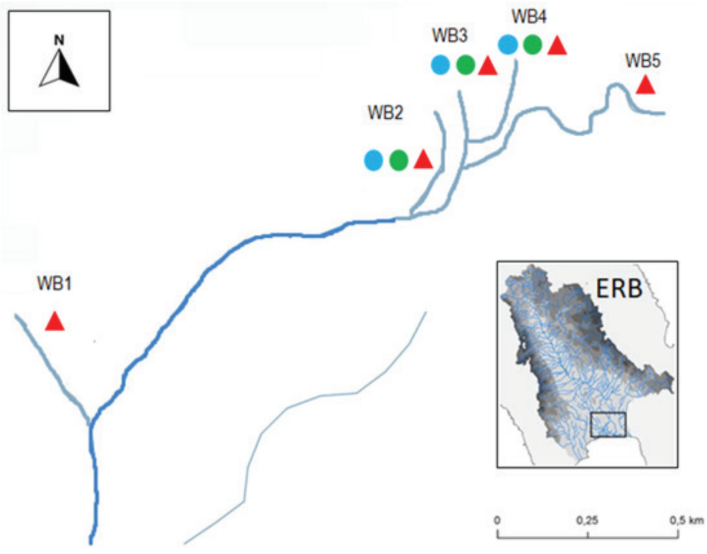

Fig. 2: Map of the upper course of Vassilopotamos River depicting the water bodies (WB) where conservation actions of the targeted species where implemented. ●: Reintroduction of *S. keadicus*, ●: Supplementation of *P. laconicus*, ▲: Elimination / drastic reduction of *G. holbrook*i. ERB: Evrotas River Basin.

In total, 515 individuals of the targeted species (265 inds of Evrotas chub and 250 inds of Evrotas minnow) were collected from two sites at the main stem of the Evrotas river and released in three streams at the Vassilopotamos basin (WB2, WB3 and WB4) in October 2021 (Fig. 2). At the same time, a total of 4059 individuals of the eastern mosquitofish collected from all five water bodies (Fig. 2) were euthanized in March and July 2021, prior to the translocations. Finally, a total of 36 Evrotas chub individuals and 31 Evrotas minnow individuals were transported, without any mortalities to HCMR aquaria, to create fish stocks *ex-situ* (Fig. 3). The success of the conservation actions implemented will be monitored during 2022.

Fig. 3: The targeted species in aquarium at the facilities of HCMR in Anavyssos (Greece).

4. Discussion/Conclusion

This study provides a methodological framework for the design and implementation of a series of *in situ* and *ex situ* conservation actions to improve the conservation status of endangered freshwater fish species, by applying hydrological and ecological modelling to pre-assess their effectiveness. This framework can have wider application for other freshwater species; the success of such conservation actions, relies however also on post-implementation monitoring with a standardized methodology, as well as on local community awareness and consent of stakeholders, local authorities' cooperation and securing the necessary funds.

Fish conservation actions are essential for the protection of imperiled species, especially when these species inhabit ecosystems displaying intense seasonal environmental variations and invasive species. In Greece, only one project, targeting the critically endangered cyprinid *Ladigesocypris ghigii* (Gianferrari, 1927) implemented *in situ* concrete conservation actions that combined habitat enhancement and fish translocation similar to our actions (Corsini Foka et al., 2002), while translocation has been also attempted for two other threatened freshwater species, i.e., *Acipenser naccarii* (Bonaparte, 1836) and *Pungitius hellenicus* (Stephanidis, 1971) (Koutsikos *et al.*, 2021). Alien species eradication is attempted for the first time in Greece in the current project.

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TAXONOMIC VS FUNCTIONAL PATTERNS IN BENTHIC DIATOMS IN GREEK RIVERS-IMPLICATIONS FOR BIOMONITORING

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Abstract

Benthic diatoms are the dominant part of phytobenthos and key primary producers in rivers. Apart from being one of the most common and important BQEs for the purposes of Water Framework Directive (WFD), their distribution patterns across Greek rivers have not been studied so far. The scope of this study was to describe taxonomic and functional patterns of benthic diatoms in Greek rivers and to identify the relationships between environmental parameters and diatom functional traits. To achieve this goal, we compiled benthic diatom and environmental data from three years of the National Monitoring Programme (2018-2020) and we formulated both a taxonomy-based and a functional trait-based dataset. We tested spatial patterns based on biogeographical areas formerly suggested for freshwater fish. Taxonomic data suggested a strong geographical pattern, where diatom assemblages from islands were distinct from assemblages in the mainland. On the other hand, functional patterns could be better explained based on environmental variables' variation. The results discussed herein could have implications for biomonitoring, as taxonomic quality indices applied so far could be biased of biogeographical patterns.

Keywords: biogeography, spatial patterns, isolation, WFD.

1. Introduction

Benthic diatoms are unicellular algae that live attached to various substrates (phytobenthos). They are key primary producers in river ecosystems (Peres *et al.*, 1997), responsible for up to 20-25% of organic carbon fixation in the planet and due to their short life cycle, they respond quickly to chemical and physical changes that occur in the environment (Round *et al.,* 1990). Although diatom ecology has been studied extensively in freshwater ecosystems, there is a lack of ecological studies regarding diatom biogeography, as microorganisms have been considered cosmopolitan (Finlay, 2002; Oikonomou, 2021). Over the last decade, awareness has been drawn to the macroecological patterns of microbes, demonstrating that passively dispersed taxa (e.g., diatoms) may be strongly influenced by spatial factors (Liu *et al.*, 2013) such as biogeographic barriers (e.g., mountain ranges, islands) that can shape their assemblages (Soininen *et al.*, 2016). Despite the ecological importance of benthic diatoms in Greece, they are exclusively used for assessing the ecological quality of rivers. Benthic diatoms' distribution and their relationships with environmental variables are understudied; yet, large-scale β-diversity studies are still lacking.

Apart from the taxonomic composition, diatom functional traits have been increasingly considered as drivers of ecosystem functioning as well as useful tools for biological quality assessment (Masouras *et al.*, 2021). Common functional traits described in benthic diatoms are cell size and adherence to substrate (ecological guilds), and have been successfully related to environmental stressors (i.e., pesticides–herbicides, heavy metals, nutrients and organic pollution) (and are increasingly used in ecological studies, but are only very recently considered for freshwater riverine microalgae. Here, we iRimet *et al.*, 2011).

The aim of the study was to test for taxonomic β-diversity patterns of benthic diatoms in Greek rivers, using previously defined faunal (i.e., freshwater fish) biogeographical areas and to compare them with patterns based on functional traits. We hypothesize that barriers formulating freshwater fish communities may also shape benthic diatom assemblages, albeit to a lesser extent, whereas functional trait distribution would be more affected by environmental variables.

2. Material and Methods

2.1 Study site and sampling strategy

Greece is located in Southeastern Balkan Peninsula, being characterized by impressive geomorphology, presenting distinctive geographical barriers (e.g., large mountain ranges, island chains) and a highly fragmented hydrographic network. These characteristics led to the formation of biogeographical regions with high dissimilarity (Oikonomou *et al.*, 2014).

During the Greek National Water Monitoring Programme over the past three years (2018-2020), benthic diatoms and water samples were collected from 227 sites, including islands, and have been assigned to large faunal biogeographical areas based on freshwater fish (Table 1).

Table 1. Biogeographical Areas in Greece based on freshwater fish (based on Oikonomou *et al.*, 2014).

Biogeographical Areas	
BA_1	East Macedonia, Thrace
BA_2	Central and West Macedonia
BA_3	West Greece
BA_4	East Greece, Attica, Thessaly, Euboea
BA_5	Islands of the Aegean Sea
BA_6	Island of Crete

2.2 Sampling and laboratory procedures

Diatom sampling and sample preparation was based on European standards (EU 2003, 2004). Samples were collected from stones (wherever possible) or plants from a lit area away from the river shore and were preserved with 70% ethanol. In the lab, samples were treated with hot hydrogen peroxide to remove organic matter and obtain clean frustules, used for diatom species identification (Battarbee, 1986). Clean frustules were mounted with Naphrax. 400 frustules per sample were identified to species level with a light microscope, at 1000X magnification. For the taxonomy, the work of Cantonati *et al.* (2017) was mainly used.

Conductivity, salinity, total dissolved solids (TDS), turbidity, dissolved oxygen (DO), pH and temperature (T) were measured in the field with a probe. Nutrients and chloride were measured in the laboratory following standard procedures (APHA, 1995).

2.3 Data analysis

Functional traits used were size classes based on length to width ratio (L/W) and ecological guilds based on species attachment to substrate (Rimet & Bouchez, 2012). We created a dataset where functional traits are being assigned to each species observed. Afterwards, all similar functional traits were summed per site, thus creating a trait abundance x site matrix (instead of a typical species x site matrix). Even though the functional traits were assigned to species, the overall functional trait matrix, was different from the species matrix (e.g., different trait combinations between species, same traits for different species)

Diatom samples were grouped within the six defined biogeographical areas (Oikonomou *et al.*, 2014). To test for possible differences among biogeographical areas we used two datasets with diatom data (species presence/absence and functional traits abundances) and a dataset with physicochemical data. To test for diatom assemblage similarity between the different biogeographical areas we performed hierarchical clustering analysis using Sorensen similarity index for presence-absence data and Bray-Curtis similarity for abundance data, using group average. To show the relationships between diatoms traits and environmental variables Pearson correlation tests were conducted. For statistical analysis Primer 6 and R (v. 3.6.2) package corrplot were used.

3. Results

Based on taxonomic presence/absence data, biogeographical areas were separated in two main groups (Fig. 1a). The first group included sites on islands of the Aegean Sea and Crete (BA_5, BA_6) that presented more than 60% similarity and the other one (BA_1-BA_4) included sites in the mainland with 70% similarity. On the contrary, in the case of functional traits, biogeographical areas presented even higher similarity (Fig. 1b) and no distinction between the islands and the mainland was apparent. Despite this overall similarity, a grouping seems apparent, separating BA_1 and BA_2 (Northern Greece) from the other areas, a grouping that is not associated to any geographical barrier. This indicates that geographical factors do not affect functional traits composition and environmental variables may be responsible for observed similarities.

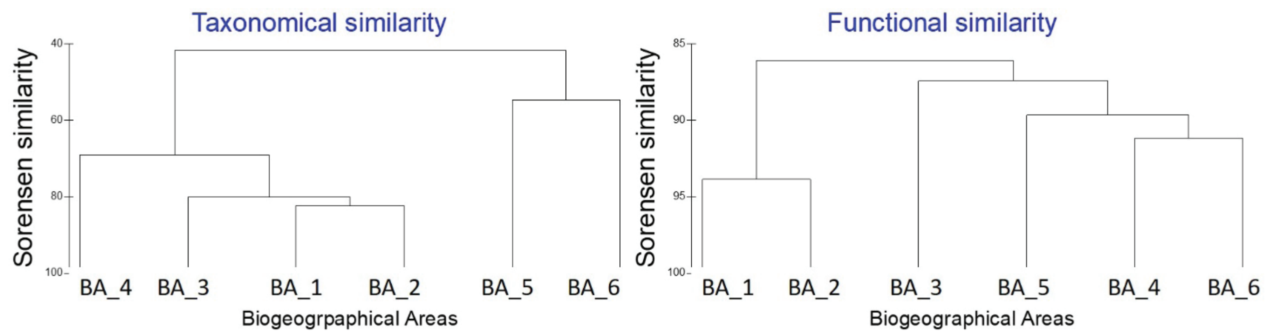

Fig. 1: a) Taxonomical similarity, b) Functional similarity. Hierarchical clustering analysis with (Sorensen and Bray-Curtis similarity) and the clustering method (group average) depicting similarity of biogeographical areas in Greece based on diatom species presence/absence (BA_1-BA_4>70% similarity) and functional traits (BA_1, BA_2>90% similarity and BA_3-BA_6>80% similarity).

The environmental variables that correlated strongly with functional traits were physicochemical parameters (T, Conductivity, TDS, Salinity, pH, Turbidity) and nutrients (Cl, SIO_4, NO_2, NO_3, TN, NH_4, PO_4, TP) (Fig. 2). Diatoms with intermediate L/W (S3) presented strong positive correlation (r>0.7) with pH, Turbidity, NO_2, NH_4 and negative correlation (r<-0.8) with Conductivity, Salinity and Cl, whereas diatoms with high L/W (S5, S6) were strongly positive correlated (r>0.6) with T, TDS and Salinity. Furthermore, diatoms of high-profile ecological guild were correlated (r>0.63) with pH, NO_2, NO_3 and TN. On the other hand, diatoms of low-profile guilds presented positive correlation (r>0.63) with T, TDS, Salinity and negative correlation (r<-0.71) with Turbidity. Finally, planktonic diatoms presented negative correlation (r<-0.61) with T, pH, NO_3 and TN.

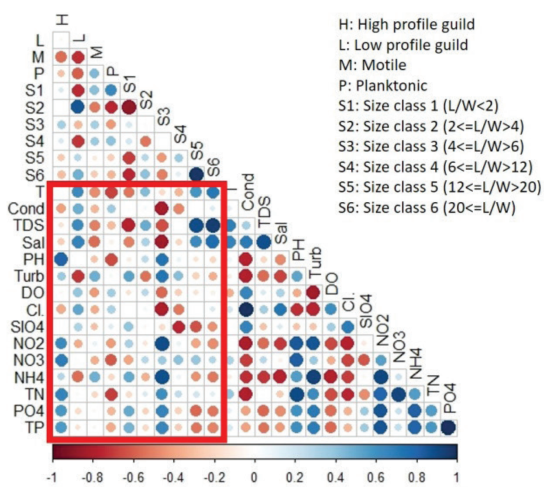

Fig. 2: Pearson's Correlation test between functional traits, physicochemical variables and nutrient concentrations. The color of the cycles shows the correlations (red color-negative correlation, blue color-positive correlation) between two variables. The size of cycle's diameter shows strong correlation. Blank cells show insignificant correlation.

4. Discussion/Conclusion

The Aegean sea, is one of the major sea basins in the Mediterranean sea, with a large number of islands with complex paleogeographical history and topographical and environmental heterogeneity (Hupało et al., 2020) which resulted in geographic isolation from the mainland (Dermitzakis, 1990). In our study, this geographic isolation of islands is confirmed for microorganisms, where spatial and temporal (historical) factors seem to play a major role in the observed differences in the composition of benthic diatoms between islands and mainland. In the mainland, mountains do not seem to be efficient barriers for diatom dispersal. However, further investigation towards this scope is needed, as the weak spatial relationships of microorganisms have been linked to sampling effort (Woodcock *et al.*, 2006). On the contrary, functional traits are related more to environmental variables whereas spatial factors do not seem significant in shaping assemblages. Strong correlations between functional traits and environmental factors reinforce this conclusion.

Different drivers of taxonomic and functional patterns could have important implications for biomonitoring. As quality indices based on taxonomy could present a geographic bias, quality indices based on functional traits could be a more promising tool in river biomonitoring when considering benthic diatoms, as already suggested (Masouras *et al.*, 2021).

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A COMPARATIVE ANALYSIS OF FOOD WEB MODELS IN LENTIC ECOSYSTEMS

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Abstract

Food web models (e.g., Ecopath) allow the assessment of trophic interactions in aquatic food webs and given the interconnectedness between populations, environment and human activities, represent efficient tools for ecosystem management. The application of Ecopath models in freshwater ecosystems is limited compared to the respective marine, despite their ecological importance and increasing vulnerability due to environmental and anthropogenic pressures. The main objective of this work was to review and screen published Ecopath models developed for lentic ecosystems on a global level, detect similarities and differences among their functioning and identify the main parameters responsible for the observed patterns. To this end, we performed multivariate analyses (e.g., k-means, PCA) on the output variables of 10 Ecopath models developed for lake ecosystems that shared similar topologies. Our results highlighted that the two major clusters identified coincided with the trophic state of the lakes while total system throughput (TST) was found to be the main variable responsible for the dissimilarity (45.5%; SIMPER) between the clusters, confirming the positive relationship of ecosystem size and productivity.

Keywords: Lakes, Ecopath, trophic state, ecosystem functioning.

1. Introduction

Freshwater ecosystems provide various valuable ecosystem goods and services; however, they are vulnerable to ecological degradation and biodiversity loss due to a dramatic increase in resource use and environmental stress (Winfield *et al.,* 2016). Lake ecosystem structure and functioning can be affected by multiple parameters, among others, physicochemical properties of the water, primary production, nutrient flows between trophic levels, species richness and abundance (Hoverman & Johnson, 2012). Considering the interconnected and interdependent nature of the aforementioned parameters, improving our understanding of their interactions is fundamental for the implementation of sustainable management measures. Ecopath models, are mass-balanced food web models that provide quantitative representations of ecosystem structure and functioning in terms of trophic flows and biomasses (Christensen & Walters, 2004) and constitute useful management tools. Contrary to the large number of Ecopath models that have been developed in various marine regions, e.g., the Mediterranean Sea (Papantoniou *et al.,* 2021 and references therein), there is only a limited number of Ecopath models concerning lentic ecosystems (Petriki *et al.,* 2021a).

This work attempts to review published Ecopath models for lentic ecosystems on a global scale, screen their topologies, analyse the output variables of selected models and identify the main parameters responsible for the similarities and differences among the structure and functioning of these ecosystems. The collected information and the produced results offer a comprehensive framework for the ecological interpretation of food web model outputs in lake ecosystems.

2. Material and Methods

2.1 Data collection

After the reviewing and screening process of the published literature we decided to include in our analyses the output parameters of 10 Ecopath models that were developed following similar procedures and shared relatively comparable topologies (e.g., absence of microbial loop, similar top predators). These models concerned three Mediterranean, two African and five Asian lentic ecosystems (Table 1). In order to avoid structure-related pitfalls in the comparison procedure, we only used robust output variables that are not influenced by the model's structure (e.g., statistics and flows, fish exploitation indices) (Coll & Libralato, 2012). These output variables that provide information on the key structural and functional traits of the ecosystems consist of total consumption (TQ), total export (E), mean trophic level of the catch (mTLc), total system throughput (TST), the sum of total respiration (TR), the sum total flow to detritus (TFD), the net of primary production (NP) and the ratio of the primary production to respiration (P/R). Finally, the lakes were classified as eutrophic (Eu) and oligotrophic (O) according to the published information concerning the trophic status of the ecosystems, while oligo-mesotrophic lakes (QC, TG) were classified as oligotrophic (Table 1).

2.2 Data analysis

A multivariate comparison of the output variables of the 10 Ecopath models was performed using ordination and clustering methods in an attempt to elucidate the similarities and dissimilarities among the lake ecosystem functioning. Principal Component Analysis (PCA) was performed on the data in order to reduce the dimensionality of the system. With the use of the silhouette algorithm, the optimal clusters in the dataset were detected and the k-means unsupervised clustering method was applied for the extraction of the major clusters. Moreover, the clusters were visualized through an enhanced scatterplot that integrates points and vectors for two-dimensional depictions of the structure and functioning of the systems. Additionally, the Similarity of Percentages (SIMPER) assessed the contribution effect of dissimilarity among the variables. All the aforementioned analyses were performed using R 4.0.5 free software for statistical computing and graphics.

3. Results

The PCA routine reduced the eight numeric variables to a two-dimensional system that explained 87.5% of the variability (PC_1: 64.2%; PC_2: 23.3%). All the variables appeared to be positively correlated among them except for mTLc which was found to be inversely related to E, NP and P/R. The average silhouette width was maximized at k_{max}=0.51 through 999 Monte-Carlo simulations, revealing the presence of two (=2) optimal clusters (Fig. 1a). The between cluster sum of squares and the total sum of squares was estimated at 68%, implying the presence of two coherent and separated clusters.

Table 1. The output variables of the Ecopath models of the 10 lentic ecosystems. The asterisk (*) indicates that the variable is standardized (i.e., $t*km^{-2}*y^{-1}$). TJ: L. Tōya, Japan; KIA: L. Kivu, Democratic Republic of Congo-Rwanda; QC: L. Qiandaohu, China; TG: L. Trichonis Greece; VG: L. Volvi, Greece; AWA: L. Awassa, Ethiopia; ANF: L. Annecy, France; SAIN: L. Sasthamkotta, India; KLAIN: L. Kelavarapalli, India; TACH: L. Taihu, China, TQ: total consumption E: total export, TR: total respiration, TFD: total flow to detritus, mTLc: mean trophic level of the catch, TST: total system throughput, NP: net primary production, P/R: total primary production/total respiration, O: Oligotrophic; Eu: Eutrophic.

ID	TQ*	E*	TR*	TFD*	mTLc	TST*	NP*	P/R	Trophic State	References
TJ	34.5	2.85	20.46	14.08	3.78	72	-2.15	0.90	O	Hossain *et al.*, 2010
KIA	2190.04	1499.85	1233.99	1762.12	2.9	6686	1499.85	2.21	O	Villanueva *et al.*, 2008
QC	5337.54	3083.13	1131.54	5990.3	2.65	15543	3083.13	3.72	O	Liu *et al.*, 2007
TG	1501.87	4.28	811.66	2149.28	2.52	4467.1	1659.64	3.04	O	Petriki *et al.*, 2021a
VG	9062.46	3913.92	3584.68	6803.82	2.97	23364.88	3888.92	2.08	Eu	Petriki *et al.*, 2021b
AWA	2382.14	6808.06	1442.69	7583.63	2.57	18217	6974.1	5.83	Eu	Fetahi & Mengistou, 2007
ANF	787.43	635.53	314.46	973.01	3.17	2710	705.69	3.24	O	Janjua & Gerdeaux, 2009
SAIN	2560.88	2743.25	1405.07	2786.07	3.08	9495	2743.25	2.95	Eu	Regi *et al.*, 2020
KLAIN	4133.08	10655.07	1571.6	1190.87	2.21	28264	10655.07	7.78	Eu	Feroz Khan & Panikkar, 2009
TACH	3629.8	3616.77	1130.61	5208.73	2.92	13586	4350.15	3.85	Eu	Yunkai-Li *et al.*, 2009

The application of a convex polygon in the PCA grouped the lakes according to their trophic state and revealed that the trophic state groupings almost coincided with the k-means clusters. More specifically, the oligotrophic (blue convex) and eutrophic groups (green convex) demonstrated an almost identical pattern to clusters 2 and 1, respectively (Fig. 1b).

As indicated in Figure 1, the centroids of the ordination analysis (triangle: oligotrophic models; circle: eutrophic models) based on their trophic state were positioned along the PC_1 axis with the oligotrophic lakes positioned on the left side of the graph and the eutrophic on the right. Furthermore, based on the prevalence of autotrophic or heterotrophic flows the lakes were placed along the PC_2 axis with systems correlated with autotrophic and heterotrophic processes being distinguished in the upper right and bottom right quadrant respectively (Fig. 1b).

Finally, SIMPER analysis highlighted that the three variables that contributed to more than 70% to the total dissimilarity between clusters were TST (45.5%), E (13.3%) and TFD (13.1%).

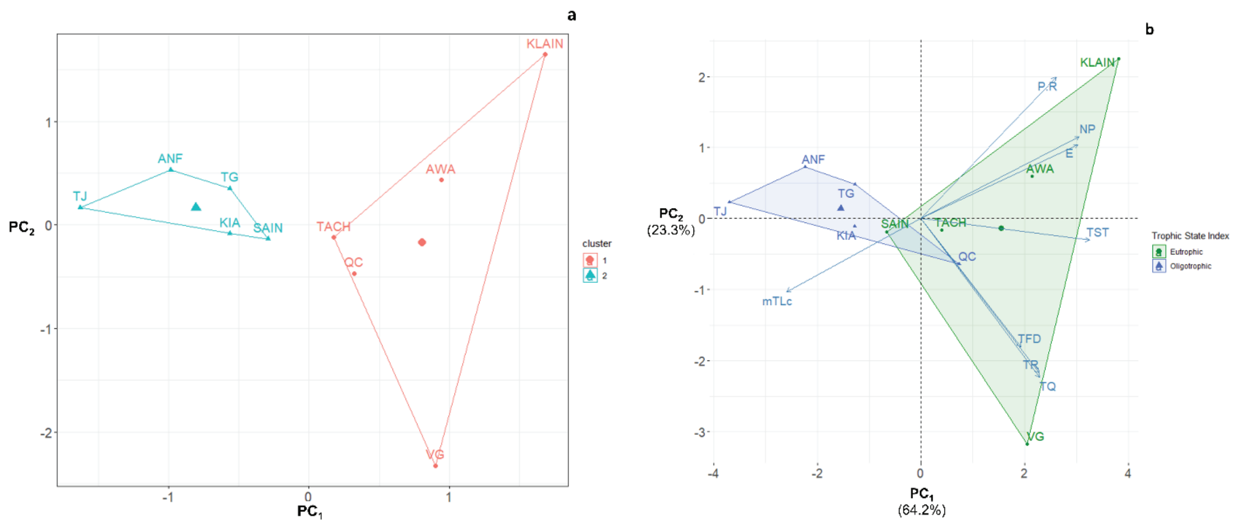

Fig. 1: Visualization of (a) the k-means clustering and (b) the Principal Component Analysis (PCA) of the examined Ecopath models (points) and the output variables (vectors) in a two dimensioned system PC_1-PC_2). TJ: L. Tōya, Japan; KIA: L. Kivu, Democratic Republic of Congo-Rwanda; QC: L. Qiandaohu, China; TG: L. Trichonis Greece; VG: L. Volvi, Greece; AWA: L. Awassa, Ethiopia; ANF: L. Annecy, France; SAIN: L. Sasthamkotta, India; KLAIN: L. Kelavarapalli, India; TACH: L. Taihu, China, TQ: total consumption E: total export, TR: total respiration, TFD: total flow to detritus, mTLc: mean trophic level of the catch, TST: total system throughput, NP: net primary production, P/R: total primary production/total respiration.

4. Discussion

The main focus of our work was to present and analyse the output variables of 10 Ecopath models developed for lake ecosystems using multivariate approaches widely used in ecological research for determining similarity patterns. Although environmental parameters such as water depth, volume, pH, nutrient concentrations greatly influence the trophic status of lentic ecosystems (Skoulikidis et al., 1998), here we address the relationship between the structural and functional traits of lentic food webs with their trophic status. According to our results, the clustering of the lentic ecosystems was primarily related to their trophic state, clearly separating oligotrophic from eutrophic lakes (Fig. 1b). Accordingly, Qiandaohu Lake (QC) in China was placed in between clusters and trophic states, successfully reflecting the reported oligo-mesotrophic state of the system (Sun *et al.*, 2021). In the case of Trichonis Lake (TG), even though it has been characterized as oligo-mesotrophic (Kehayias & Doulka, 2014), based on its structural and functional traits it was clearly ordinated with the group of oligotrophic lakes and closely located in the centroid of the PCA analysis (Fig. 1b). The eutrophic group of lakes presented higher variability in respect to the dominant autotrophic (e.g., Lake Kelavarapali) or heterotrophic processes (e.g., Lake Volvi) in relation to the oligotrophic group that presented less diverse functional attributes. Finally, TST, which serves as an indicator of ecosystem size in terms of flows, was highlighted as the major variable related to the dissimilarity between oligotrophic and eutrophic ecosystems. This finding confirms the relationship between ecosystem size and system productivity whereby high and low TST values indicate productive and oligotrophic ecosystems respectively. The current work presented a robust classification of lakes based on Ecopath output variables, providing a comprehensive framework for the interpretation of various functional aspects of lentic ecosystems.

5. Acknowledgements

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ASSESSING THE TROPHIC LEVELS OF FISH SPECIES AT A TEMPERATE LAKE USING STABLE ISOTOPE ANALYSIS

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Abstract

Stable isotopes can be used to assess food webs and species' trophic levels. This study aimed to estimate the trophic levels of the six most abundant fish species found in Lake Volvi, a temperate lake in Northern Greece, by analysing carbon (**δ**^{13}C) and nitrogen (**δ**^{15}N) isotopes. The isotopic values of nitrogen were further used for fish trophic level assessment. Isotopic values differed among species. *Perca fluviatilis* was assessed as the top predator in the system while similarities were observed among our results and those based on stomach content analysis from previous studies and FishBase.

Keywords: Aquatic ecosystems, freshwater, food web, carbon, nitrogen.

1. Introduction

In aquatic ecosystems, trophic relations can be described with food webs and species' trophic levels, which express the relative position of an organism within the food web, starting from the lower (producers) to the upper (consumers) levels. The concept of fractional trophic level has become widely used in ecology and fisheries management (Pauly *et al.*, 1998). Moreover, knowledge of trophic relationships through isotope analysis has gain research attention and contribute to the emergence of new aspects in issues such as trophic species interactions and food preferences (Jafari *et al.*, 2020).

The synthesis of carbon isotopes in a consumer's tissue reflects its diet (DeNiro & Epstein, 1978). Carbon is related with photosynthesis and has separate isotopic signatures in energy sources, i.e., producers. Animals receive also the required nitrogen by consuming other living organisms (Post, 2002), with δ^{15}N being related with nutritional levels. The isotopic signal changes from prey to consumer, by enriching it approximately 0.4-1 ‰ for C and 2.5-3.4 ‰ for N between successive trophic levels (Post, 2002; Perkins *et al.*, 2014). Nitrogen range (NR) and Carbon Range (CR) are quantifications of trophic structure representing food chain length and breadth of sources, respectively (Perkins *et al.*, 2014).

Food web analysis is based on the assumption that autotrophic producers are the base and their trophic level equals to 1. Therefore, each heterotrophic organism is dependent on the producers and its trophic level is greater than 1, depending on the prey it consumes.

The main purpose of this research was to estimate the trophic levels of the most common and abundant fish species from Lake Volvi. The method of stable isotopes analysis was used for the first time for such estimations in Greece, contributing, through a new approach, to the investigation of trophic relations in a lake. Furthermore, comparisons were made with the estimated fish trophic levels known from FishBase (Froese & Pauly, 2022), and previous studies based on stomach content analyses conducted in the same lake.

2. Material and Methods

2.1. Study Area

Lake Volvi, the second largest natural lake in Greece, located in the northern part of the country, is a warm, eutrophic, monomictic lake, with moderate ecological quality. It is protected as a "Natura 2000" (GR1220009) and a Ramsar Convention site.

2.2. Sample Collection, Preparation and Stable Isotope Analysis

Water samples were collected for the evaluation of particulate organic matter (POM) that was used as baseline of the food web. The water was filtered through 1 μm Whatman GF / F filters (dry biomass was weighted before and after). For fish samples, adult individuals of *Alosa macedonica* (N=8), *Carassius gibelio* (N=8), *Cyprinus carpio* (N=6), *Lepomis gibbosus* (N=4), *Perca fluviatilis* (N=8) and *Rutilus rutilus* (N=8) (Table 1) were collected with the assistance of professional fishers. A small part of muscle, without bones and scales, was removed from the lower part of the body of each fish (between dorsal fin and tail). In the tissue samples the lipids were removed with chloroform-methanol solution (Elliot *et al.*, 2017). Samples were dried and weighted in a precision scale. About 0.8 mg of each sample was packed in a tin capsule and sent for Stable Isotope Analysis (SIA) to the Friedrich-Alexander University Hydrogeology Laboratory (FAU Erlangen-Nuremberg) for estimating the ratios of $\delta^{13}C$ and $\delta^{15}N$. All results of the isotopic analysis refer to the standard size δ (delta) per thousand (‰) according to the relation: $\delta X = (Rsample / Rreference - 1) \times 1000$, where *R* is the ratio ^{15}N / ^{14}N or ^{13}C / ^{12}C in the sample and the reference (Coplen, 2011). Sample values were compared with atmospheric nitrogen (air-N_2; $\delta^{15}N$ by USGS-40 and USGS-41) and VPDB standard for $\delta^{13}C$.

2.3. Trophic position assessment and Nitrogen Range

The trophic position assessment (TrPo: Trophic Position) was performed using the formula (Meerhoff *et al.*, 2019): TrPo (consumer) = [$\delta^{15}N$ (consumer) -$\delta^{15}N$ (baseline) / 3,4] +2, where: *consumer* = fish sample and *baseline* = POM. We estimated the trophic position of each sample separately to distinguish the range of values into each sample type. We used the average $\delta^{15}N$ of POM as baseline. We also calculated Nitrogen Range (NR): NR = maximum $\delta^{15}N$ – minimum $\delta^{15}N$ of each sample type.

2.4. FishBase

We used FishBase for the trophic level values of each species. References with only adult individuals were preferred, but if it was not possible references with both juveniles and adults were chosen. We calculated the mean value of trophic level wherever the number of references we used was greater than 1 (N>1).

3. Results

Ratios of $\delta^{15}N$ differed among fish species. Nitrogen ratio for POM ranged between 4.8 ‰ and 4.9‰, and for the fish samples between 8.1 ‰ and 13.9 ‰ (Fig. 1).

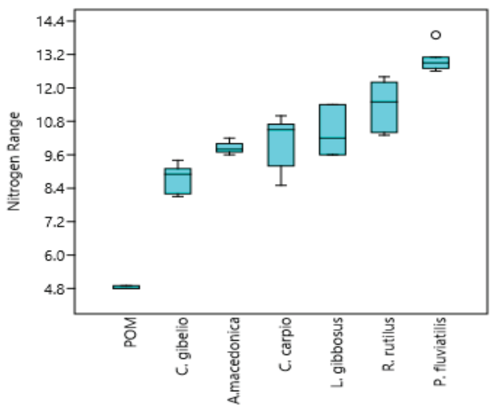

Fig. 1: Nitrogen range for each sample type at lake Volvi.

To understand even better the differences in the isotopic synthesis of fish species and POM inter-specific and intraspecific, we depicted isotopes as paired values of C and N (Fig. 2). Comparisons of our results for trophic levels with other available showed similarities for *P. fluviatilis* and *A. macedonica* (Table 1).

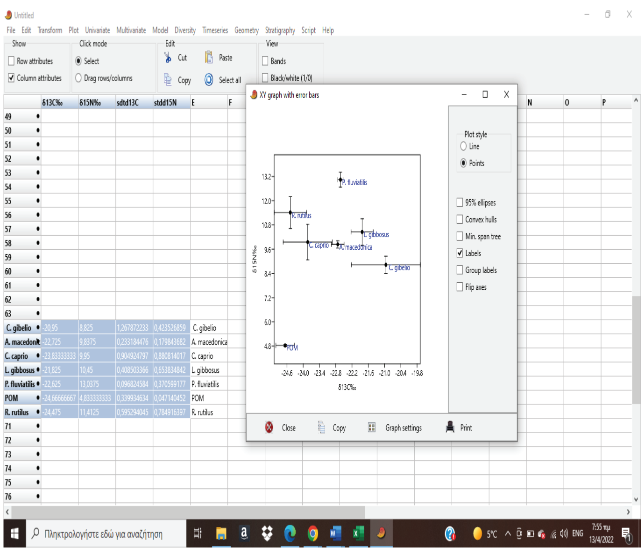

Fig. 2: Mean δ^{13}C and δ^{15}N of samples analyzed from Lake Volvi and their standard error.

Table 1. Trophic levels of fish species from Lake Volvi assessed using stable isotope analyses (SIA; present study) compared with the trophic levels via Stomach Content Analysis (SCA) (Salvarina, 2006)[1] and FishBase (Froese & Pauly, 2022). For the trophic level (FishBase) the mean value from the references used is given. The range of the values used and the standard error of the mean value is given in brackets. * studies with both adult and juvenile fish.

FISH SPECIES	Number of samples analyzed	TROPHIC LEVEL (SIA) Lake Volvi (Greece)	TROPHIC LEVEL (SCA) Lake Volvi (Greece)	TROPHIC LEVEL (FISHBASE) Number of references used Study areas
Alosa macedonica	8	3.47 (0.05)	-	3.71 (0.64) (N=1) Lake Volvi (Greece)
Carassius gibelio	8	3.17 (0.12)	2.5 [1] (0.22)	2.5 (2.2-2.8 , 0.21) (N=4) Lake Balaton (Hungary)
Cyprinus carpio	6	3.51 (0.26)	3.38 [1] (0.36)	3.1 (0.00) (N=1) Lake Balaton (Hungary)
Lepomis gibbosus	4	3.64 (0.2)		3.2 (2.6-3.4 , 0.25) (N=7*) Lake Albufera (Spain), Lake Opinicon (Canada)
Perca fluviatilis	8	4.41 (0.11)	4.28 [1] (0.74)	4.4 (0.00) (N=1) Loch Kinord, Loch Davan (UK)
Rutilus rutilus	8	3.93 (0.23)	-	3.0 (0.00) (N=1) Lake Balaton (Hungary)

4. Discussion

Perca fluviatilis seems to be the top predator in Lake Volvi, as its trophic level was the highest among the species examined (Figs 1, 2; Table 1). This is mainly a piscivorous fish, confirmed by previous studies in the lake (Bobori *et al.*, 2013). The rest of the fish species prey on different food items (phytoplankton, zooplankton, macroinvertebrates, etc.), resulting in their lower trophic position, a fact also indicated by the differences found between the levels of $\delta^{13}C$. Indeed, *C. carpio* and *C. gibelio* tend to prey more on arthropods and detritus, respectively (Salvarina, 2006). *Carassius gibelio* preys also partly on zooplankton and its trophic position appears to be near to *A. macedonica* (Fig. 1), an endemic species of Lake Volvi, which is mainly zooplanktivorous (Kleanthidis & Sinis, 2001; Bobori *et al.*, 2013). Trophic levels obtained from FishBase seemed to be close to the results of our study, especially for *A. macedonica* and *P. fluviatilis* (Table 1). Differences with the FishBase values are probably due to differences in the ecosystems they are referred to, geographical or seasonal differences (Table 1). Furthermore, they may reflect differences in the methodology used, as most studies use stomach content analysis (SCA). This technique allows the identification of the prey consumed and gives a "snapshot" of species recent diet. On the other hand, we used muscle samples for analysis of stable isotopes that give a long-term diet analysis (Davis *et al.*, 2012). Trophic levels assessed via SIA usually accord with the ones assessed by stomach content analysis (Gao *et al.*, 2017). However, differences can also be observed (Davis *et al.*, 2012), and thus isotopic results should be interpreted carefully, considering also those deriving from the other methods (Davis *et al.*, 2012).

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SPECIAL SESSION: ARGO FLOATS CONTRIBUTION TO THE MARINE RESEARCH AND OPERATIONAL MONITORING OF THE MEDITERRANEAN SEA – EVOLUTION, ACHIEVEMENTS, AND FUTURE NEEDS

Marine and Inland Waters Research Symposium
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MONITORING TARGETED SHALLOW/COASTAL WATERS OF THE MEDITERRANEAN SEA WITH ARGO FLOATS

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Abstract

Under the Euro-Argo RISE (H2020) project, we have performed special Argo float operations in targeted coastal areas of the Mediterranean Sea. The Argo platform capability was pushed towards its limits to investigate the feasibility of such operations and to build the complementarity with other existing coastal monitoring systems. We deployed floats in four different sites, from the western to the eastern Mediterranean Sea (Balearic Archipelago, Ligurian sub-basin, north Adriatic Sea, north Aegean Sea) and specific setting configurations were tested according to the missions targets. The most critical configuration parameters are the parking depth and the cycle length that were preferably set deep enough to limit the platform drift and between 1 and 5 days, respectively. In addition, we deployed some national floats to further improve the best configuration settings of our case studies. Shallow/coastal operations with Argo floats have required the tailoring of the existing monitoring systems, the in-house development of warning procedures, and the need of real-time data decoding. These test missions showed that Argo can be used for operations in shallow/coastal areas and, if properly managed, the floats life expectancy can be larger than one year (with more than 100-150 profiles acquired).

Keywords: operational oceanography, Argo Float, marginal seas, coastal monitoring.

1. Introduction

Mediterranean coastal waters have important socio-economic impacts and Argo floats were tested in targeted missions to monitor such areas in the framework of the European H2020 Euro-Argo Research Infrastructure Sustainability and Enhancement (Euro-Argo RISE) project (Notarstefano *et al.*, 2021a). There are specific reasons behind the use of Argo floats: Argo is an autonomous platform that can perform a great number of profiles; it is programmable and potentially recoverable. The Argo system presents a high degree of efficiency thanks to the homogenous spatio-temporal distribution of its profiles. The latter means that Argo provides more data and a wider coverage with respect to other platforms like research vessels (Figure 1). In Euro-Argo RISE we are pushing the existing Argo technology to move towards the coast for targeted missions with the aim of building the complementarity with other networks to close spatial and temporal gaps in shallow/coastal waters (Euro-Argo ERIC, 2017). Argo extension in these areas is essential for operational oceanography services, climate, and environmental studies. Argo is also more cost-effective and less environmental-harmful than R/Vs and this extension is useful for the

Euro-Argo ERIC objectives because it helps to engage with neighbouring countries and develop regional partnerships.

In this work, we describe the tests of Argo platforms during targeted missions in selected coastal areas of the Mediterranean Sea (Kassis *et al*., 2021a), and the associated monitoring tools tailored for such operations. We further provide recommendations for the selection of the optimal configuration setup and the best practices for platform monitoring in similar future activities in the European marginal seas.

2. Material and Methods

The optimization of the Argo sampling in targeted coastal regions consists of adjusting and tuning the float configuration in order to remain in the area of interest and to acquire an adequate number of profiles. In parallel, it is important to avoid risky events like stranding, being stuck at the sea bottom, or moving close to coastlines and islands. A set of controlling and monitoring tools provided by Euro-Argo and OceanOPS is used in addition to other tools and systems. In-house developed tools for quick Argo data decoding and for provision of notifications in near real time were generated. This is needed to have important information such as the platform location, bathymetry, and distance from the coastline. Moreover, weather information, accurate sea charts, enhanced bathymetry data, and model current data are fundamental tools for the estimation of floats trajectories and the achievement of optimal mission performances. Four Euro-Argo RISE platforms and additional national floats have been deployed in the Mediterranean Sea since the end of 2019 (Table 1) in targeted areas for missions close to the coast (Fig. 2). We have tested several configurations, and selected the most useful monitoring tools, according to the characteristics of the area and the specificity of the mission. The final goal is to be able to operate Argo floats in shallow/coastal waters and to achieve a good life expectancy of the platforms (Kassis *et al*., 2021a).

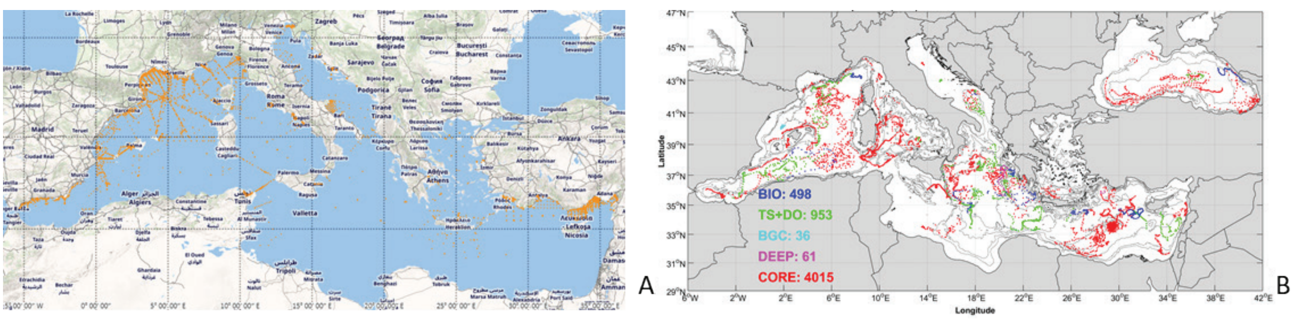

Fig. 1: (A) Profiles' location of CTD profiles (approx. 8700) from R/V in the last 10 years in the Mediterranean Sea Source: https://cdi.seadatanet.org/search#. (B) Number of CTD profiles (approx. 5000) from Argo floats in 10 months (Jan-Oct 2021) in the Mediterranean Sea. Profiles are color-coded per platform type (core-Argo carries CTD sensor; TS+DO carries CTD and dissolved oxygen sensors; BGC-Argo carries the full biogeochemical sensors suite: oxygen, pH, nitrate, chlorophyll fluorescence, suspended particles, downwelling irradiance; Bio-Argo carries some of the BGC sensors; Deep-Argo is a deep float capable of reaching 4000 m).

For the Adriatic case, two floats were deployed in the north Adriatic Sea off the Italian city of Senigallia (Fig. 2). The floats were standard core-Argo models and the deployments were performed in shallow waters (maximal depth 70 m) and quite close to the coast. The main configuration parameters that were tuned during the mission are the cycle length (time between consecutive profiles) and the parking depth (depth where the float spend most of its time before it starts acquiring the profile). The cycle length was set to 5 days and the parking depth was deep enough to reach the sea bottom. The Euro-Argo monitoring tool provided a complete set of technical information, graphs and alerts. In addition, in-house developed tools were used to perform a faster Argo data decoding. CMEMS model of the daily mean sea water velocity near the float's parking depth were utilized to try to predict the direction of the float displacement. In the Aegean, two floats were deployed in its north basin (Fig. 2). For the first, a 2-day frequency sampling and a drifting/profiling depth of 800 m were selected whilst, for the latter the drifting/profile depth is set to 450/600 m and the sampling period is set 5 days (Table 1). The Euro-Argo monitoring tool and an in-house developed automatic alerting system to check the platforms' location, bathymetry, distance from the coast, and data transmission were used. In the Balearic Sea, one float was deployed south of Palma in the Balearic Archipelago in March 2020, to investigate the potential of Argo in such coastal areas. In this mission it has been used the Western Mediterranean OPerational forecasting system (WMOP, an in-house developed tool) in conjunction with the sea currents model provided by the AVISO satellite derived data to try to predict the float displacement and hence to facilitate the changing of the platform configuration settings when needed. Enhance bathymetry contoured maps were also used in support of float parking depth planning decisions. A national Spanish float has recently approached (February 2022) the Balearic Archipelago and it will be used to complement the Euro-Argo RISE experiments. One float was deployed in the Ligurian Sea in December 2019 to sample the Ligurian Current and to complement measurements from the MOOSE network. This float was programmed with a parking depth of 1000 m and a cycle length of 3 days.

Table 1. Main information of float deployments in targeted missions close to the coast.

WMO	Deployment Date	Deployment location	Sub-basin	Cycle period (days)	Parking/profile depth (m)	Total Cycles	Status
6902899	11/12/2019	43.41 N 7.86 E	Ligurian	3	1000	194	Inactive
6903288	09/02/2020	40.42 N 25.42 E	North Aegean	2	800/800	120	Inactive
6903298	13/11/2021	38.87 N 26.42 E	North Aegean	5	450/600	26	Active
6903783	31/07/2020	44.05 N 13.70 E	North Adriatic	1/2/5	Bottom (~70)	40	Inactive
6903800	04/05/2021	44.05 N 13.62 E	North Adriatic	1/5	Bottom (~70)	34	Inactive
6904065	14/08/2020	39.28 N 1.98 E	Balearic	1/2	180/300	127	Active
6901278	12/03/2020	39.37 N 2.52 E	Balearic	1/2/4	100/300/1000	198	Active

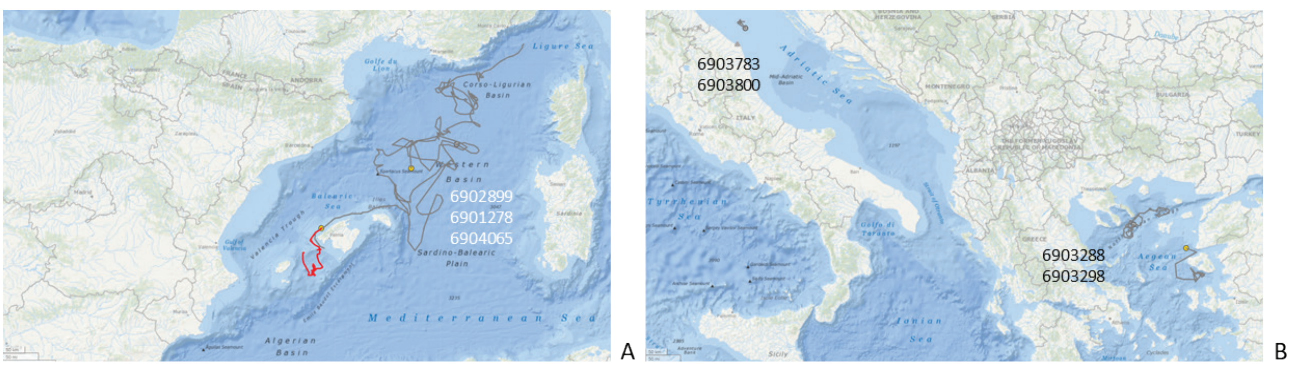

Fig. 2: Trajectories of floats in targeted mission in shallow/coastal waters of the Mediterranean Sea. (A) Western Mediterranean (B) Eastern Mediterranean. Source: https://fleetmonitoring.euro-argo.eu.

3. Results

3.1 North Adriatic experiment

A preliminary quality control of the float salinity data was performed. Despite the reference dataset used is scarce, a comparison between the θ-S diagram of Argo profiles and most recent (closest in time and space to Argo profiles) historical data is made (not shown here). The analysis reveals that there is quite a good agreement in the deepest layers of the water column. Both the floats (6903783 and 6903800) performed well and the displacements from the deployment locations were strongly limited thanks to the adopted configurations. This allowed to keep the floats within the targeted area, on the shallow shelf of the north Adriatic Sea and at a quite constant distance from the coastline (Fig. 2 B). Unfortunately, the floats stopped transmitting prematurely and the cause in still unknown.

3.2 North Aegean experiment

The 6903288 float remained near the deployment location for the first 30 profiles. Then it drifted south-westward and continued sampling in another deep plateau until it stopped transmitting, unexpectedly, in October 2020 (Fig. 2 B). All 120 profiles transmitted by the float are of good quality and have provided interesting oceanographic information. The other mission, currently ongoing in the Aegean Sea, for testing the Argo coastal monitoring is the one of the 6903298 float (Table 1, Fig. 1). The float, which was deployed in an enclosed deep coastal basin of the northeaster Aegean, provided 20 profiles before exiting the area and it is now moving north providing good quality profiles.

3.3 Balearic experiment

The float 6901278 was mantained in the targeted shallow waters close to the coast for about 5 months and then it drifted away from the archipelago. The best sampling strategy consisted of setting a relatively long cycle length and a deep parking depth (Table 1) to limit the time spent in surface layers where currents are stronger. Regarding the 6904065 experiment, the float is currently drifting over the 200-meter isobath, and first results re-confirmed that the combination of a deeper parking depth and longer cycle length is the best strategy to maximize the permanence time of the float in the area of interest.

3.4 Ligurian experiment

The float 6902899 was deployed in the deep water area quite close to the coast and it quickly drifted south-south west at an average speed of 3 km per day. The float sampled the Ligurian current during 6 months, the deep-water formation zone in the center of the Gulf of Lions during 8 months, and finally the Balearic front during 5 months. This float was recovered on 27 June 2021 after 194 cycles.

4. Discussion/Conclusion

The experiments conducted show that targeted coastal float operations are possible. Such operations however, require a high level of interactivity between the operator and the float, much higher than what is usually needed for standard operations. Hence, an intense monitoring activity is also required to track the floats and change their configurations when needed. The tests done in different coastal areas of the Mediterranean Sea seem to demonstrate that the platforms' configurations used are adequate to achieve the objectives of the missions. In particular, we have shown that 1) it is possible to reduce the float drift from the targeted area; 2) a great number of profiles can be acquired; 3) repeated grounding events are manageable; 4) Argo is a powerful monitoring platform that can complement coastal measurements acquired by other systems. Two main configuration parameters seems to be crucial: a) the parking depth, and b) the cycle length. The coastal test missions revealed that a relatively deep parking depth (in some cases close to the sea bottom) is probably the best setting to keep the float within the targeted area. Regarding the cycle length, tests showed that setting the sampling frequency in the range 1 to 5 days allows limiting the platform displacement between consecutive profiles, acquiring a large number of profiles near the coast, and checking more frequently the platform location. The set of monitoring tools tailored for such kind of operations and additionally tuned for these experiments, are indeed the key to succeed in coastal Argo missions. In particular, the Euro-Argo and OceanOPS monitoring tools combined with sea state information, detailed bathymetry maps, near-real time notification systems, and a fast data decoding system are fundamental to reach the best float performance. Despite it is early to define the life expectancy of Argo floats in shallow/coastal areas, results are quite satisfactory. The mean half-life of Argo floats for standard operations in the Mediterranean Sea is about 700 days and 150 cycles. Shallow/coastal missions exhibit a slightly lower performance on average, both in terms of time and cycles performed but there are also examples showing similar performances. However, a comparison between the two statistics is perhaps not fully pertinent since targeted missions close to the coast are different and in general more risky in terms of survival rate. Nevertheless, we can conclude that most of the coastal Argo operations reached the planned targets.

5. Acknowledgements

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ARGO FLOAT MISSIONS IN TARGETED COASTAL AREAS OF THE AEGEAN SEA

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Abstract

Over the last decade, the expansion of Argo into marginal seas has provided unpreceded oceanographic information for previously under-sampled areas. For the European Seas, this expansion had accentuated recently, and has been focused on targeting shallow-coastal areas under the joint efforts of the Euro-Argo Research Infrastructure community. This work presents the experience and outcomes from three such Argo missions undertaken by the Greek Argo Research Infrastructure during 2020 – 2022 in the North Aegean, a basin with an intriguing coastline and challenging bathymetry. An assessment of each float's performance and dataset is presented, taking into account the special configuration setup used in each case and the oceanographic characteristics of the covered area. The data analysis presents interesting oceanographic features such as the spatio-temporal distribution of distinct water-masses, intermediate circulation patterns, air-sea interactions and their effect on the upper-layers' physical properties. Furthermore, of specific importance is the identification of deep homogenization and vertical mixing within the water-column, possible convection events, and strong spatial thermohaline gradients over the deep layers of the examined areas. The results highlight the benefit of such missions and underline the potential of Argo to contribute to the environmental and climatic monitoring, along with the operational oceanographic coastal monitoring.

Keywords: Drifting profilers, Operational Oceanography, Boundary Currents, Thermohaline Properties, Mixed Layer.

1. Introduction

The use of Argo floats has radically enhanced the operational oceanographic monitoring of the physical and biogeochemical parameters of the oceans and seas worldwide over the last twenty years (Riser *et al.*, 2016). Although the initial design of the Argo platform was originally for monitoring the oceanic environment, its contribution is continuously expanding, expressed by the extension of float missions into previously under sampled marginal seas and ice-covered areas (Jayne *et al.*, 2017). With regards to the Mediterranean, the joint efforts of national infrastructures and the Euro-Argo European Research Infrastructure Consortium (Euro-Argo ERIC), which timely adopted a strategic monitoring plan for the European Sea (Euro-Argo ERIC, 2017), have produced a marked increase in float coverage (Kassis & Korres, 2020), which in turn is expanding rapidly to peripheral seas and sub-basins such as the Aegean (Kassis & Korres, 2021). However, in sea basins such as the Aegean Archipelagos, Argo float operators have to deal with several difficulties mainly related to the rapidly changing bathymetry (shallow plateaus, deep trenches) and the complicated coastline. Thus, specially designed missions are required that will allow for better monitoring and increased float lifetime. Within the Euro-Argo RISE H2020 project, Argo operators are undertaking activities to serve the extension of Argo into targeted coastal waters with increased oceanographic interest (Notarstefano *et al.*, 2021). Under this framework, several floats have been deployed since 2020, focusing on the under-sampled but important North Aegean basin (Kassis *et al.*, 2021). These missions have been implemented by the Greek Argo Research Infrastructure (www.greekargo.gr/) which is a component of the Hellenic Integrated Marine Inland water Observing, Forecasting and Offshore Technology System (HIMIOFoTS) and member of Euro-Argo ERIC.

The North Aegean is an irregular bottom topography that incorporates shallow plateaus and deep troughs along a weaving coastline. An important feature of the basin is that it communicates from the northeast with the Black Sea through the Dardanelles Straits through which, cold and fresh water inflows south and west into the Aegean. This inflow forms the Black Sea Water (BSW), a characteristic water mass recognized by a strong low salinity - temperature signal in the upper layers of the water column. The high salinity Levantine Surface Water (LSW) is another dominant water mass in the area's upper layers. The LSW, and the Levantine Intermediate Water (LIW), which occupies the underline layers, create strong thermohaline fronts with BSW masses (Zervakis & Georgopoulos, 2002). The role of BSW inflow in the area is of particular importance since it can modulate the hydrography of the whole Aegean Sea (Kassis & Korres, 2021). As shown in previous studies, weak BSW inflow in combination with increased winter atmospheric cooling and LSW intrusion triggers Dense Water Formation (DWF) events which result in the formation of very dense water in the deep horizons of the sub-basin (Zervakis *et al.*, 2000).

In this work, three Argo missions in targeted areas of the North Aegean are presented in an attempt to assess the floats' performances and highlight important hydrographic features revealed by the acquired profiles and trajectories. Oceanographic processes of the coastal zone are investigated, with a focus on the water-column physical properties distribution, the water-masses structure and interaction, and the intermediate circulation.

2. Material and Methods

2.1 Floats' configuration, deployment, and monitoring

All floats were standard CTD floats and were tested and configured in the HCMR laboratory before the deployments. The configuration used in each case differed according to the mission requirements and tests to be applied. For the 6903288 case, 2-days mission cycles were chosen whilst the drifting and profiling depth of the float were both set to 800 m. This configuration was tested in order for the float to remain near the deployment location within the deep trench north of Limnos Island shelf break (Fig. 1). Similar settings were chosen for the 6903298 float that was deployed in a relatively deep but narrow plateau south of Lesvos Island (Fig 1). In this case, a 5-days cycle was set to avoid frequent float surfaces in an area of intense shipping activity. On the contrary, for the 6903297 that was deployed in the deep plateau of the central part (Fig.1), the standard settings of MedArgo (Poulain *et al.*, 2007) were kept. Details on the deployments and the basic configuration settings used for this mission are summarized in Table 1.

Table 1. Information on the deployment and configuration of the 3 missions in the North Aegean.

Float type	WMO	Deployment date	Deployment location / Depth (m)	Cycle Period (Days)	Drifting depth (m)	Proling Depth (m)
APEX 11	6903288	9 February 2020	40.42 N, 25.42 E / 820	2	800	800
ARVOR I	6903297	17 October 2021	39.84 N, 24.42 E / 1250	5	350	1000
ARVOR I	6903298	13 November 2021	38.91 N, 26.30 E / 690	5	450	600

All missions were closely monitored after deployment to assess the float's performance in the targeted areas and under the specific configurations settings. For this scope, the Euro-Argo monitoring tool (available at https://fleetmonitoring.euro-argo.eu/dashboard) which provides enhanced information regarding technical and functional parameters of the floats' performance has been utilized. Furthermore, the POSEIDON's automatic alerting system (http://poseidonsystem.gr/alerts/?m=2) has been also used for the monitor of each platforms' location and data transmission.

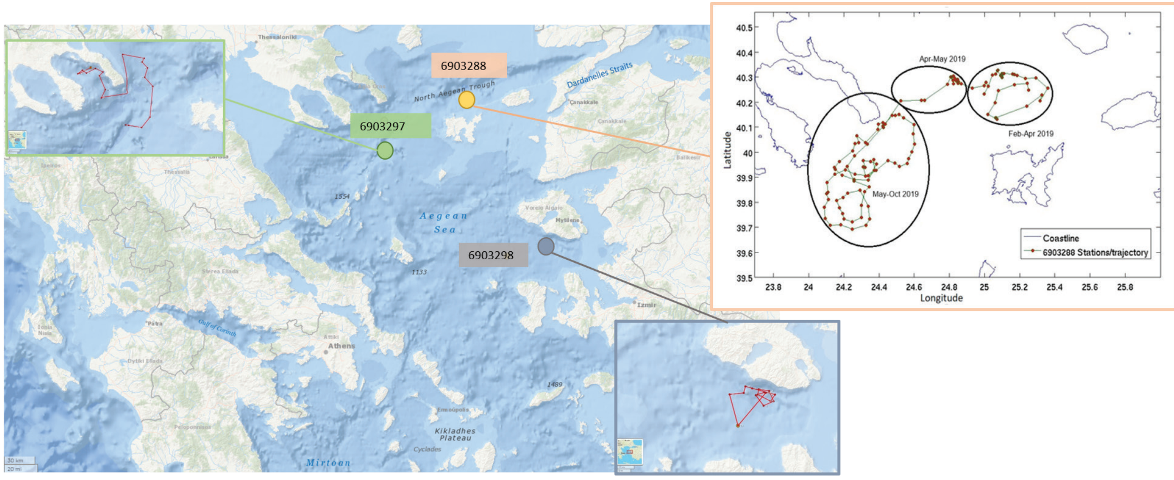

Fig. 1: Deployment locations and trajectories of the 3 floats in the North Aegean.

2.2 Data assessment and analysis

In total, 142 profiles, acquired by the floats, have been processed for the scopes of this study. The float 6903288 performed 120 profiles in its lifetime that lasted until 5/10/2020. The other two floats (6903297-8) are still operational and have performed 19, and 13 profiles as of January 2022. The first data assessment showed that the vast percentage of recorded values (> 98 %) have successfully passed the automatic quality control procedure and were flagged as "good". These data were used for further analysis that included additional threshold tests and visual inspection, to exclude dubious data (Kassis & Korres, 2020). Several parameters such as the potential temperature (θ) and density (σ_θ), the Mixed layer Depth (MLD), and the Brunt-Väisälä Frequency (BVF), were calculated for each profile as described in Kassis & Korres, 2020 and Kassis & Korres, 2021.

3. Results

3.1 Floats' performance and trajectories

The floats' missions can be characterized as successful until now. The 6903288 float managed to acquire 120 valid profiles operating for 8 consequent months, exceeding the average time period of the Argo life expectancy in the area. This fact can be partly assigned to its high sampling frequency along with its deep parking depth that prevented the float from drifting ashore. The other 2 floats (6903297-8) are still operational and transmit valid profiles from the coastal areas around their deployment locations. In the southeastern part, 6903298 float has remained "trapped" in the deep plateau performing a cyclonic path (Fig. 1). In the central part, 6903297 float has drifted north and then moved back to enter the Gulf of Agio Oros months after its deployment. Interesting results regarding its trajectory are provided by the 6903288 float which remained in the deep trench north of Limnos under a cyclonic drift for almost 2 months (Fig. 2) before drifting westwards through a shallow passage to enter a second deep plateau southwest of the deployment location. In this area, it remained under the effect of a deep anti-cyclonic system in the west and an adjacent cyclonic in the east (Fig. 2).

3.2 Hydrographic features

The profile data analysis from the 6903288 float has revealed an interesting gradient for both temperature and salinity along the deep layers of the North Aegean observed between the eastern and the central part of the sub-basin (Fig. 2). This is expressed by warmer (> 0.5 °C) and more saline (> 0.08 psu) water masses in the eastern part (Figs. 1, 2A, 2B), possibly reflecting variable DFW events in the area. The salinity of the upper-layers also presents an interesting span, with a decrease of more than 4 psu between February (eastern part) and October (central part) (Figs. 1, 2C) depicting a strong BSW signal in summer-autumn 2020.

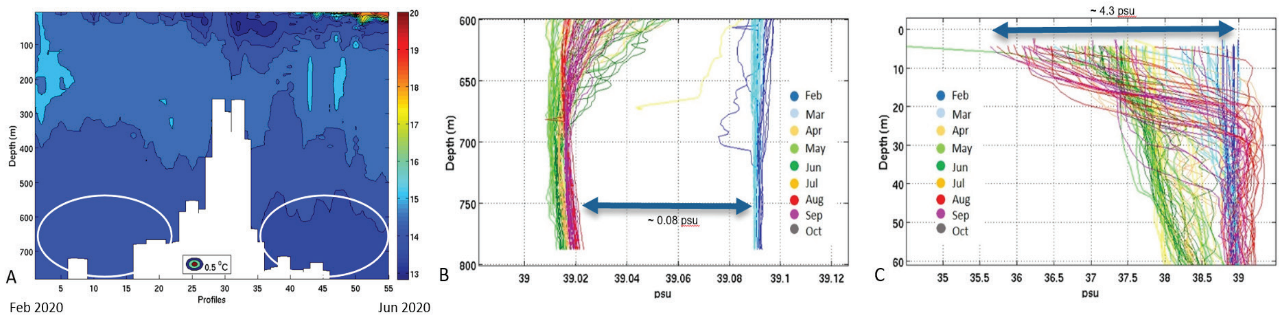

Fig. 2: Hovmöller diagram of potential temperature (first 55 profiles) from 6903288 float (A), Practical salinity profiles for the period Feb-Oct 2020 in the depth zones 600 – 800 m (B), and 5 – 60 m (C) recorded from the 6903288 float.

The 6903297 float has also recorded a strong BSW signal one month after its deployment and while moving north of Athos Peninsula (Fig. 1). However, after entering the Agio Oros bay, BSW gradually disappears and LSW dominates (not shown here). For the 6903298 case, the domination of Levantine water masses is evident. As the atmospheric cooling advances, a fast homogenization of the water-column is observed with an abrupt deepening of the MLD in January 2022. This process seems to have been accentuated at the beginning of February producing dense water signals along the water-column (not shown here).

4. Discussion/Conclusions

Although still in its preliminary phase, the data analysis has revealed interesting hydrographic features. The depicted inhomogeneous salinity distribution of the upper-layers seems to determine the observed thermohaline differences in the bottom layers of the different coastal sub-basins of the North Aegean. Concerning the BSW presence, it seems to be restricted with the exception of the central part of the basin. However, this was the only case of available profiles in the spring-summer period when the BSW presence is intensified (Kassis & Korres, 2021). The absence of BSW signals north of Limnos Isl. during February-March 2020 is another interesting feature concerning previous studies that describe a strong BSW-LSW front across the northeast of the island in winter that is transferred southeast of the island in the summer due to the Etesian winds (Zervakis & Georgopoulos, 2002). According to the data analyses in this work, in the two areas where LSW dominates, deep waters masses seem to have been recently produced from convention events (north of Limnos trench), or DWF will be most probably triggered due to the preconditioning status and the intense winter surface cooling (south of Lesvos plateau). The latter has been identified as an area where convention events were favoured due to the deep homogenization observed during 2015-2017 (Kassis & Korres, 2021). Of specific interest is moreover the observed thermohaline gradient between the northeastern and the southwestern part of the north Aegean trough, areas sampled by 6903288 float in early 2020, and late 2020, accordingly. The deep-water masses recorded in the southwestern case seem to have not been ventilated recently probably due to the re-occurrence of BSW signals in the area. As described in previous studies, strong BSW may act restrictively over convection processes (Zervakis *et al.*, 2000).

Summarizing, the points that are highlighted in this study are a) the strong variable spatial distribution of thermohaline properties across the coastal areas of the North Aegean, b) the strong presence of Levantine waters in both surface and intermediate depths and its association to DWF events, c) the role of BSW presence and LSW-BSW fronts that determine deep homogenization and convention processes, d) the existence of mainly cyclonic mesoscale circulation systems at the bottom layers of the targeted areas, e) the successful operations from Argo floats in targeted coastal areas and their potential to provide high quality, and spatio-temporally dense datasets in previously under-sampled regions.

5. Acknowledgements

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PRELIMINARY DELAYED-MODE QUALITY CONTROL ACTIVITY OF DEEP ARGO DATA IN THE MEDITERRANEAN SEA

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Abstract

In 2016 OGS has started to deploy some Deep-Argo floats in the Mediterranean Sea to observe the deeper (below 2000 m) water masses of this marginal sea. We have recently begun performing the delayed-mode quality control (DMQC) of temperature and salinity data acquired by these floats, following the Deep-Argo Team's recommendations. The DMQC analysis of Deep-Argo floats requires a different approach with respect to the Core-Argo floats, due to the pressure dependent salinity bias. Hence, the correction for pressure effects on conductivity, called CPcor correction, is necessary before applying the OWC procedure, that is a statistical method that relies on accurate reference dataset for checking any sensor drift and offset in salinity. Three different CPcor values are applied. The three re-computed salinities are compared to a CTD reference profile to estimate the most reliable CPcor value. Then, the OWC method and additional qualitative analysis are applied to evaluate any potential sensor drift and obtain high quality data. In order to do this quality control, an adequate reference database is required: OGS reviews and improves on a regular basis the high-quality ship-based CTD reference dataset from the near surface layers to deeper than 2000 m.

Keywords: Euro-Argo RISE, MedArgo, salinity, CPcor correction, Mediterranean Sea.

1. Introduction

Deep-Argo is a new generation of floats that play an important role in the systematic sampling of the deep layers of the ocean (between 2000 and 6000 m). In the framework of the MedArgo Regional Center, 6 Deep-Argo floats (4000 m Deep-Arvor model manufactured by NKE, France) have been deployed by OGS (as part of Argo-Italy contribution) in the Hellenic Trench and in the Rhodes Gyre areas that represent some of the deepest bathymetries of the Mediterranean Sea. Deep-Argo floats are equipped with a SeaBird SBE41CP conductivity-temperature-depth (CTD) sensor. OGS started to perform the DMQC of temperature and salinity data of these Deep-Argo floats as official responsible for the DMQC activity of physical Argo data in the Mediterranean and Black Seas and as part of the work planned in task 3.2 of the European H2020 Euro-Argo Research Infrastructure Sustainability and Enhancement (Euro-Argo RISE) project. The DMQC analysis is an advanced methodology that allows to obtain high quality data for scientific purposes and operational oceanography. It is essential that also data send back on land from Deep-Argo floats undergoes the delayed-mode quality control in order to provide the scientific community with accurate data of the deepest part of the water column. Before checking the conductivity sensor drift of Deep-Argo floats, it is necessary to apply a correction due to a salinity bias, dependent on pressure. The potential effect of the pressure dependency on the salinity is adjusted using a correction term called CPcor value. In this work we describe the process to check and correct the Deep-Argo salinity, based on the delayed-mode procedures proposed by the Argo community (Cabanes *et al.*, 2016; Wong *et al.*, 2022). In addition, in-house developed qualitative checks are also used in complement of the main analysis. In this work, we present the results obtained for the WMO 6903203 as an explicative example ot the DMQC analysis on Deep-Argo floats in the Mediterranean Sea.

2. Material and Methods

2.1 Reference dataset

The reference dataset plays an important role in the DMQC analysis since the accuracy of the float data is assessed by comparison with high-quality shipboard measurements. Moreover, in the Deep-Argo float analysis, a reference profile (preferably collected at the float deployment time and location) is used to obtain the optimized estimate of the CPcor correction. OGS Argo team reviews and improves on a regular basis the high-quality ship-based CTD reference data from the near-surface to deeper than 2000 m, for QC purposes of Core and Deep-Argo float data in the Mediterranean and Black seas (Gallo *et al.*, 2021). The OGS Argo team collects CTD data in complement of the official CTD reference dataset, provided by the Coriolis Global Data Assembly Center (GDAC), from the main European Marine Services and several research institutes at regional level. In this way, a high-quality ship-based CTD reference data is obtained. Figure 1 shows the up-to-date (2021) spatial and temporal distribution of the CTD dataset deeper than 2000 m, used in his work.

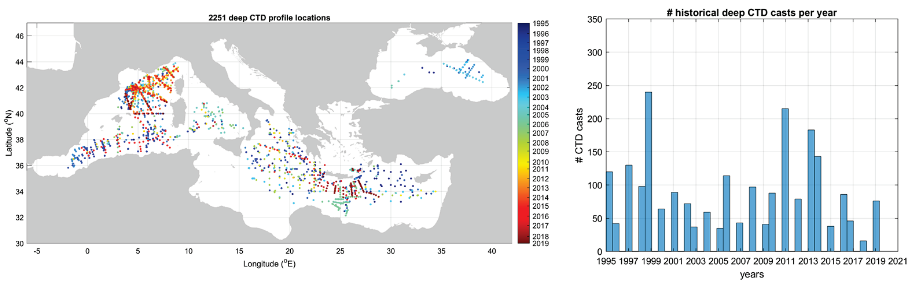

Fig. 1: Spatial distribution, color-coded for time, (left panel) and temporal distribution (right panel) of the CTD profiles deeper than 2000m in the final version of the CTD reference dataset of the Mediterranean and Black Seas.

2.2 DMQC analysis method

Following the Deep-Argo team's recommendations, the OGS team performed the DMQC analysis of the Deep-Argo floats in the Mediterranean Sea. First, the CPcor correction for pressure effects on conductivity is applied. Three CPcor values are used:

- •the nominal CPcor value from Sea-Bird;
- •the CPcor_new default value obtained by the Argo deep team (recommended value);
- •the optimazed CPcor value obtained in delayed-mode by comparing a deep float profile to a reference profile.

To obtain the optimized value it is important to use the CTD cast done at the float deployment location in order to have a reliable comparison between the profiles. As recommended by the Deep-Argo Team, the robustness of the refined optimized CPcor_new value is defined by the difference from the reference levels which must be within +/- 0.5 e–08 dbar–1 (Wong *et al.*, 2022). The CPcor correction is applied to original conductivity from which new salinity is computed. After this correction, the OWC method (Cabanes *et al.*, 2016) is used for checking the conductivity sensor calibration drift or offset. It is a statistical method based on the comparison between float salinity profiles andan accurate historical reference dataset. When the detected salinity difference is higher than the required accuracy (0.004 psu), the computed OWC adjustment is applied to float data. In conjunction with OWC method, additional procedures are used to provide the best quality control analysis: 1) the visual inspection of profiles diagrams: pressure versus temperature (P-T), versus salinity (P-S), and versus density (P-Rho); potential temperature versus salinity (θ-S). This help in detecting salinity anomalies and spikes. 2) The analysis of the θ-S curves of deeper profiles, where the water column exhibits a uniform θ-S relationship, that allow to have an indication of the potential conductivity sensor drift, that is quite easily detectable by a systematic full vertical shift in the θ-S measurements. 3) The comparison of selected float salinity profiles with the nearby historical CTD profiles and 4) the comparison of the θ-S curves of the float and of the reference data. The last two points can help in detecting a potential sensor calibration offset when there is a systematic deviation between the float salinity data and the CTD profiles.

3. Results

3.1 DMQC WMO 6903203 deep float

The WMO 6903203 float was deployed in the Ionian sub basin within the Hellenic trench area (Fig. 2) in December 2016 and performed 81 cycles. The real-time QC flag applied to pressure, temperature and salinity is 1 for all cycles. The theta-salinity (θ-S) diagram of the float was analyzed and in particular the area where the θ-S relationship is the tightest (Fig.

2). A potential salinity drift was observed. Before applying the OWC method, CPcor corrections are applied and compared. The optimized CPcor value was estimated using the CTD nearest in time and space (Fig. 3).

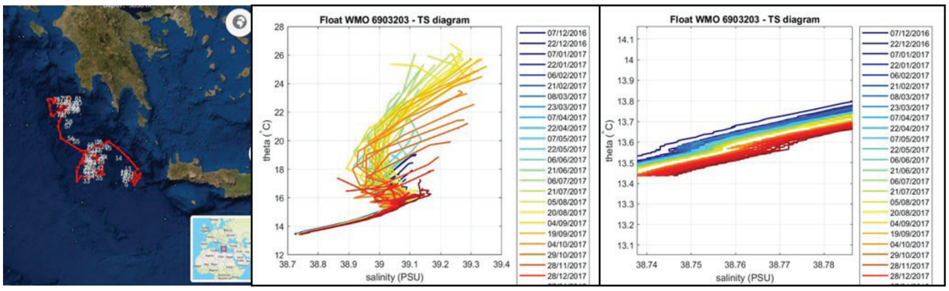

Fig. 2: Trajectory (color-coded per cycle number) of float WMO 6903203 (left panel). θ-S diagram color-coded per cycle number and where the θ-S relationship is more uniform on the central and right panels respectively.

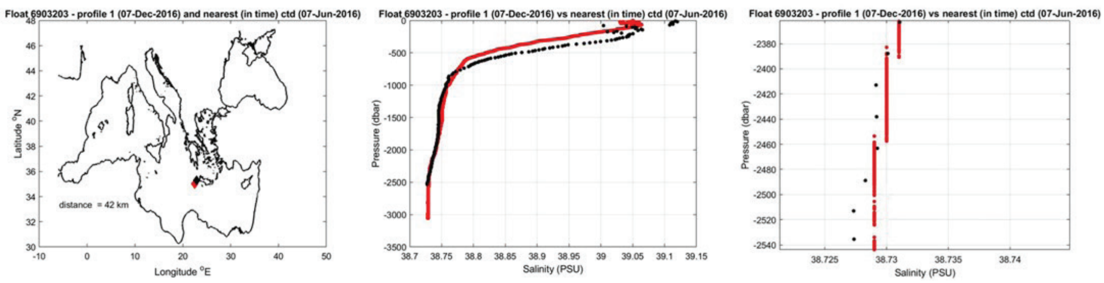

Fig. 3: The salinity float profile number 1 (black dots) are compared to the nearest in time reference profile (red dots) in the central and right panels. The locations of the two profiles and their distance are given in the left panel.

Comparing the float salinity deviation from the CTD cast at deployment obtained using the three different CPcor corrections (Fig. 4), the best result was obtained using the CPcor_new default value. The theta-salinity (θ-S) diagram is built again after applying the CPcor correction and the OWC adjustments (Fig. 4, right panel). The optimized value was not taken in account, because it represents the worst result (not shown). The delayed-mode adjusted salinity obtained with the CPcor default value (green profiles in Figure 4) shows a better agreement with the salinity of CTD reference data (red profiles in Figure 4).

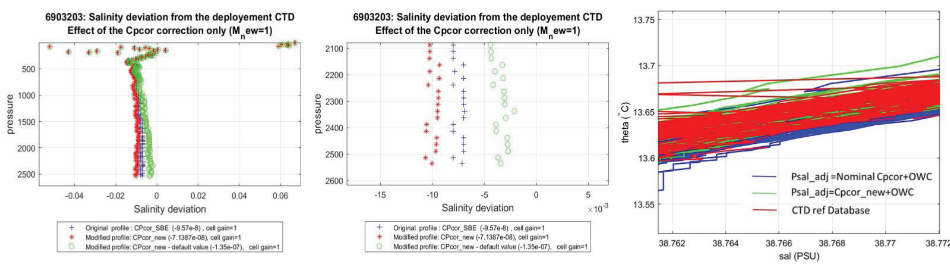

Fig. 4: Salinity deviation from the deployment CTD due to the CPcor correction using three values (left and central panels). CTD reference dataset (red) compared with θ-S diagram obtained used the three CPcor correction plus OWC correction in the right panel.

The OWC analysis showed a positive salinity drift. The correction proposed is shown in figure 5a that presents the evolution of the suggested adjustment with time (green curve). The red line indicates the reliability of the fit. Also it can give an idea of the spatial and temporal variability due not only to the sensor malfunction but also for example if the float crosses different water masses. The correction suggests that the sensor started to drift at cycle 30 and that such correction (extended up to 0.015) is larger than the Argo requested accuracy (0.004). Figure 5b shows the evolution of salinity in time along with selected θ-levels with the minimal salinity variance. A positive drift starts from cycle 30 and increases with time. Moreover, the comparison between float salinity data and mapped historical data evidences the drift pointed out by the OWC method (Fig. 5c). The salinity data of the Deep-Argo float WMO 6903203 needs a delayed mode correction applied to cycles from 30 to 81.

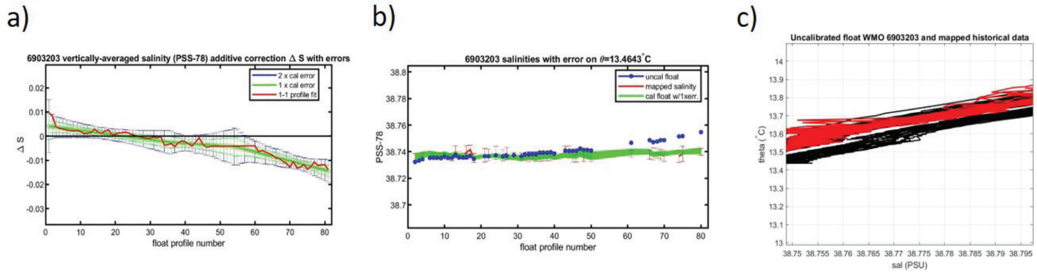

Fig. 5: a) Evolution of the suggested adjustment with time. b) Evolution of salinity with time along with selected theta levels with minimum salinity variance. c) Uncalibrated float salinity profile (black lines) and mapped historical data (red lines) in the most uniform part of the θ-S curve.

4. Discussion/Conclusion

The DMQC analysis of Deep-Argo floats deployed in the Mediterranean Sea by OGS was performed following the recommendations of the Deep-Argo Team. This quality control provides high quality data in the deepest layers of the water column (2000-4000 meters in the Mediterranean Sea). The reference dataset that is crucial for the QC of float data has been reviewed and improved. CTD data from Copernicus Marine Environment Monitoring System (CMEMS) and CTD profiles acquired by European colleagues from different research institutes are integrated into the CTD reference dataset provided by Coriolis Global Data Assembly Center (GDAC). The salinity data of Deep-Argo floats show a bias that is dependent on pressure. Before applying the OWC procedure, the correction for pressure effects on conductivity, called CPcor correction, was applied. This analysis highlights the importance to have a reference CTD cast taken at the float deployment location and time to better evaluate the most robust value of CPcor correction. In this work the analyses conducted on the WMO 6903203 Deep-Argo float deployed in the Hellenic Trench area was described. Three CPcor values are used to compute a reliable pressure dependent correction on salinity: one provided by the sensor manufacturer, the second estimated by the Deep-Argo Team, and the third is a refined and optimized estimate of the CPcor_new value obtained in delayed-mode by comparing the first Deep-Argo float profile to a reference profile close in space and time. The best result in the correction for pressure effects on conductivity was obtained using the CPcor_new default value. From the corrected conductivity, the float salinity is recomputed and the OWC method was applied. The OWC results suggested a positive salinity drift that extends up to 0.015. Additional qualitative checks as the visual inspection of the deepest portion of the θ-S diagram and the comparison of selected float salinity profiles with the nearby historical CTD profiles, were performed in support of OWC analysis. These investigations confirmed that salinity drifted towards positive values. Hence, the salinity data of WMO 6903203 Deep-Argo float was corrected in delayed-mode applying the correction proposed by the OWC method.

5. Acknowledgements

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VENTILATION PROCESSES IN THE SOUTH ADRIATIC PIT CONVECTION AREA

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Abstract

Ocean oxygenation dynamics is a particularly important topic, especially in those areas where ocean processes connect the surface with the deep layers, therefore affecting dark ocean ecosystems. Among these areas, the deep-water formation site of the south Adriatic Sea represents an interesting “case study” since the Dissolved Oxygen (DO) concentration increases with depth in contrast to the general reduction of oxygen content observed in the last few decades in the global ocean. In the last decade, the South Adriatic area has been intensely monitored using Argo floats, ocean gliders and other traditional in-situ methods. This present study, for the first time, analyses a 7-year long time series of DO concentrations showing its significant interannual variability. Our results highlight that the variability of DO concentration in the surface layers is mainly trigged by convection events and primary production, however, advection from adjacent sub-basins also play a key role. In addition, in the intermediate and bottom layers the water advection and convection processes are the main mechanisms regulating the DO concentration. Periodic reversals of the surface circulation in the Northern Ionian Gyre, well known to affect the salinity distribution in the south Adriatic, also play a key role in the DO enrichment of this area, potentially influencing the ventilation mechanism of the whole Eastern Mediterranean Sea.

Keywords: Dissolved oxygen, multi-dataset, chlorophyll a, Bi-OS, Mediterranean Sea.

1. Introduction

Dissolved oxygen measurements in south Adriatic Pit (SAP) represent a topic of primary interest for the knowledge of the ventilation processes in the central and eastern Mediterranean Sea as the water masses formed in the Pit condition the deep circulation of the whole Mediterranean. Oceanographic and biogeochemical characteristics in the SAP are strongly influenced by the advection of different water masses (Fig. 1a): the oxygenated, cold and fresher water from the north Adriatic and poorly oxygenated, saltier and warmer, water masses originating from the south and influenced by surface circulation periodic reversal of the Northern Ionian Gyre (NIG), from anticyclonic to cyclonic and vice-versa (Menna *et al.*, 2019).

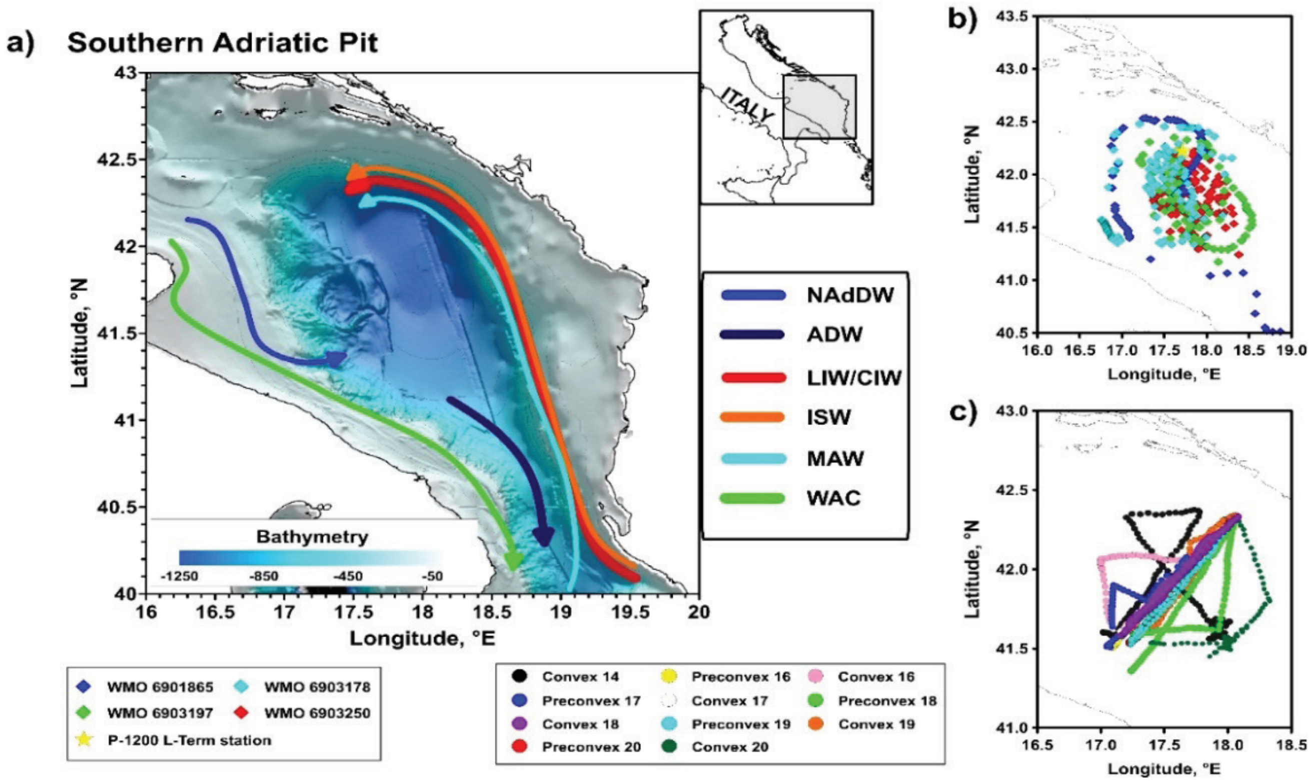

Fig. 1: Location of the study area (a), site in the Adriatic Sea, Central Mediterranean, with schematic representation of the main water masses (NAdDW: north Adriatic dese water, ADW: Adriatic dense water, LIW: Levantine intermediate water, CIW: Cretan intermediate water, ISW: Ionian surface water, MAW: modified Atlantic water, WAC: western Adriatic current, from Manca *et al.* (2006)). Geographical position (b) of the Argo floats (6901865 blue diamond; 6903178 cyan diamond 6903197 green diamond and 6903250 red diamond) and P-1200 L-Term station (yellow star). (c) Glider surfacing locations during the glider missions listed in the legend.

The cyclonic phase implies an increase in the advection of salty LIW/ CIW into Adriatic Sea, while the anticyclonic phase implies advection of less saline Ionian water, diluted by MAW, (Civitarese *et al.*, 2010), the latter circulation additionally causes an intensification of the eastern coastal current in the Ionian Sea favouring the inflow of the highest salinity waters in the intermediate layers (100-200m) (Menna *et al.*, 2019, 2022).During the winter season, northerly wind generating a favorable condition for deep water formation (Manca *et al.*, 2006). Occasionally, the SAP receives the North Adriatic Dense Water (NAdDW) (Querin *et al.*, 2016), that ventilates the deepest layers.

Vertical temperature and salinity profiles derived by float in the SAP showed a pronounced interannual variability in the period 2014 – 2020 (Mauri *et al.*, 2021), with low salinity intermediate water in 2015 (Kokkini *et al.*, 2019) and higher salinity records observed between 2018 and 2020 in the sub surface layer (100-200 m) (Menna *et al.*, 2022). Since 2017, strong salinization has been occurring throughout the water column, the causes and possible effects of which were recently described by Mihanović *et al.* (2021) and Menna *et al.* (2022). During the investigated period, the surface circulation in the northern Ionian alternated between anticyclonic (negative vorticity; 2012-2013, 2017-2018) and cyclonic (positive vorticity; 2014-2016) phases (Menna *et al.*, 2022). Despite the thermohaline variability in the SAP has been well investigated over the last decades, the long-term oxygen dynamics were never studied with in-situ data.

2. Material and Methods

The SAP oxygen concentration dataset spans from 1 January 2014 to 31 December 2020 and includes data from different platforms. In particular, the area was explored by four autonomous profiler floats which remained inside the pit (colored diamonds in Fig. 1b) for almost 7 years. The SAP was also investigated during eleven short term autonomous glider missions mainly along the transect Bari-Dubrovnik (colored dots in Fig. 1c). A few ship surveys were also conducted in the area during the study period along with in-situ Winkler samples. Moreover, a L-term station (Batistić *et al.*, 2014) close to the Croatian coast (yellow star in fig. 1b) has collected oxygen data mainly between 2016 and 2018.

In order to have a robust and consistent dataset (Gerin *et al.,* 2020, 2020b), all the oxygen data collected with the autonomous instruments (floats and gliders) were calibrated (or inter-calibrated) and validated by using the Winkler sample results (whenever available) or the data of any platform (mainly the floats) previously Winkler-calibrated as suggested in Queste *et al.*, (2015). We apply a direct like-with-like comparison using least square minimization between different DO profiles (model Y=X+A, where A has to be optimized). The shapes of the different DO profiles are quite similar and the minimization method highlights a very high coefficient of determination (R^2), between 0.85 and 0.95, and about 0.82 for glider.

MLD was calculated using only the float dataset and following the method explained in Kokkini *et al.* 2019.

3. Results

3.1. Dissolved Oxygen variability

The diagram in Figure 2 describes the DO variability in the SAP for the 2014-2020 period. In the first 100 m depth of the water column each year, during the winter convection period (i.e. February - May) the DO content reaches values of 5.5 ml/l within the MLD. From about May to November (stratification period) the ~ 25 – 70 m deep layer is characterized by the shallow oxygen maximum (SOM), where DO concentration was larger than 6 ml/l. This high oxygen layer remained stable until the occurrence of the next winter mixing.

In the intermediate and deep layer (200 and 1000m) DO distribution is strongly related to depth of the mixed layer. An interannual variability was clearly visible. In 2014, the lowest oxygen concentration (<5 ml/l) was observed between 200 m and 400 m depth, and the highest between 400 m and 1000 m depth (5.3 ml/l). In 2016, an increase of DO down to 400 m occurred. This increase was due to the 2016 convection (MLD= 400) and to the inflow of the NAdDW (reduction in salinity), leading the Oxygen Minimum Layer (OML) down to 700m. Below this layer, between 800m and 1000m depth, DO gradually decreased until 2017, reaching 4.5 ml/l. The severe winter 2017 triggered a deep convection (MLD reached 800m; Figure 2- grey line) that mixed the water column destroying the previous pattern. After the convection, a sharp decrease in oxygen concentration occurred between 400 m and 800 m depth, concurrently with a decrease in salinity (38.8 isohaline sloping upward in Figure 2). An evident increase of oxygen concentration in the deep layer occurred only after the spring, concurrently with a deepening of the 38.8 isohaline (dashed line in Figure 2). In 2018, the deep convection reached again 800m with a similar water messes distribution. In the deep layer (800m-1000 m) DO concentrations increased again during the late spring (as in 2017), following the salinity decrease. This high oxygenated water lasted over two years (2018-2019) and the DO (5.4 ml/l) remained nearly unperturbed until the summer of 2019. From then on, oxygen started gradually to decrease to 5.2 ml/l reaching in late 2019-early 2020 concentration of 4.9 ml/l in the 300 and 1000 m layer. The water column structure changed after the 2020 convection event, when the MLD reached 500m, while in the intermediate and deep layers a reduction in DO concentrations occurred as well as an increase in salinity.

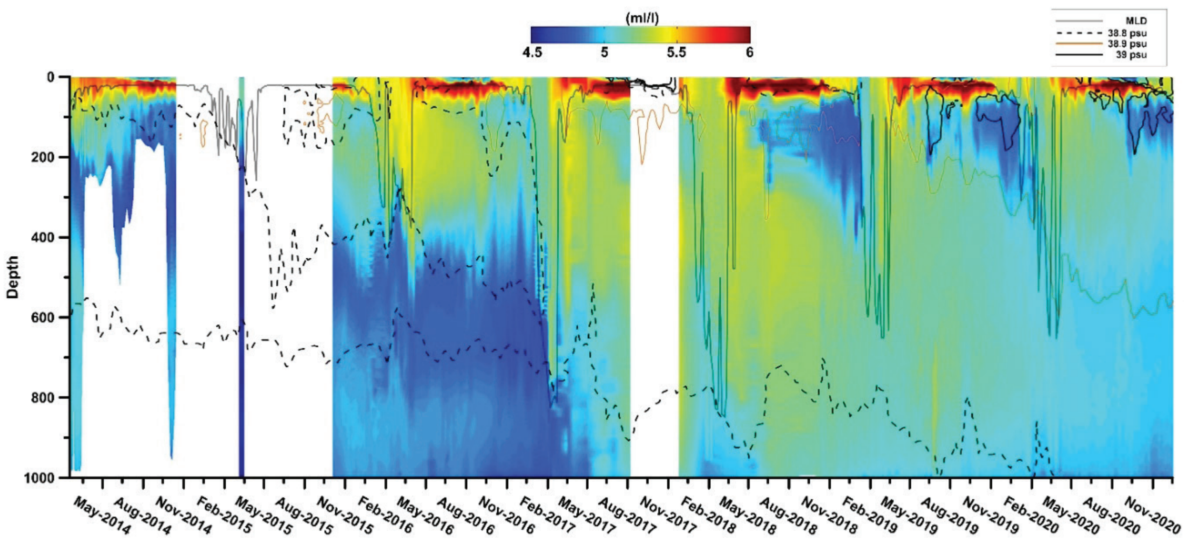

Fig. 2: Dissolved oxygen time series (ml/l) in the SAP between 2014 and 2020 color coded derived from float, glider and CTD data. The dashed black line corresponds to 38.8 (39) isohaline. The solid grey line indicates the mixed layer depth.

4. Discussion/Conclusion

In the surface layer of the SAP, DO variability is mainly triggered by annual primary production and convection events. The high oxygen concentrations observed in the SOM are the result of biological activity and vertical mixing occurring during winter (Fig. 2). The highest concentrations observed between 2017 and 2019 are correlated to the primary production (Gačić *et al.*, 2002). The increase in chlorophyll (Chla) and the consequent production of oxygen are the result of different processes (Lévy *et al.*, 1998) such as mixing, that make available the nutrients present in the deep layers, which are in turn conditioned by the biogeochemical characteristics of the water masses advected by the rotation of the NIG (Civitarese *et al.*, 2010). The intermediate layers present the highest interannual variability and are characterized by the OML, generally associated with the high salinity waters of Levantine origin (Manca *et al.*, 2006), that disappears during convection events. In 2014, the OML was observed between 200 m and 400 m (Kokkini *et al.*, 2019, Manca *et al.*, 2006), instead during 2015 and 2016 the OML sank down to 800 m depth, where the LIW (Kokkini *et al.*, 2019, Mihanović *et al.*, 2021) was pushed down by the intrusion of lighter and oxygenated water masses. This intrusion led to a double salinity (Kokkini *et al.*, 2019) and oxygen pattern, in which oxygen maxima were out of phase with respect to salinity maxima. After 2017 the OML was shallower (extending from 100 m down to 350m) and was associated with salinities larger than 39 (Mauri *et al.*, 2021; Menna *et al.*, 2019, 2022).

The inflow of the high salinity waters is linked to the intensification of the eastern coastal current in the Ionian Sea, occurring during the anticyclonic NIG phase (Menna *et al.*, 2019, 2022).

In the SAP aphotic layer (200-1000 m) the DO, besides being conditioned by physical processes (mixing and inflow of waters from the Ionian Sea) and biological activity (Santić *et al.*, 2019), it is also conditioned by ingression of cold, dense and new formed water coming from the northern Adriatic (Querin *et al.*, 2016). High oxygen concentration is also visible between 2017 and 2019, strongly related to the exceptional air-sea interaction that occurred in 2017 (Kokkini *et al.*, 2019, Mihanović *et al.*, 2021) and to the ingression of the oxygenated NAdDW. These years (2012-2014 and 2017-2019) are characterized by the highest DO concentrations and also by the reduction of the less oxygenated LIW inflow due to the anticyclonic NIG (Menna *et al.*, 2022). On the contrary, years with low oxygen are interested by strong LIW inflow (e.g. cyclonic phase of the NIG) as the Levantine waters mix with the Adriatic waters, reducing the oxygen content (between 2014 and 2016, and in 2020). Our result highlights that the observed DO variability is triggered by convection events due to severe winters, by the annual primary production, and by the advection of waters both from the northern Adriatic and from the Ionian Sea. In particular, the NIG reversals appear to play a key role not only in salinity, but also in the oxygen enrichment occurring in the southern Adriatic Sea. Oxygen maxima are observed during the anticyclonic phase (which brings more nutrients and fresher water) of the NIG while oxygen minima take place during the cyclonic phase (which brings less nutrients and salty water). This study helps to better understand the dynamics of DO in the south Adriatic convection area, and allows to increase the knowledge of the ventilation mechanism of the Central-Eastern Mediterranean Sea.

5. Acknowledgements

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COMBINING GLIDER DATA WITH SATELLITE DATA TO STUDY MESOSCALE COHERENT VORTICES IN SARDINIA CHANNEL

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Abstract

Sea straits and channels are peculiar areas of the World Ocean as they represent a connection of relatively small spatial extension between different oceanic basins. This often implies that some gross oceanographic features of the surrounding zones can exert a deep influence on the channel dynamics, and vice versa. In this context, an investigation including deployment of the Glider and satellite data was carried out in the Sardinia-Tunisia Channel between March and April 2018. The comparison of the Temperature/Salinity (T/S) vertical distribution and θ/S diagrams allows us to assess mixing processes occurring within water masses. In spring, we noticed that Sardinia-Tunisia Channel STC is characterized by a cooler T in its Northern part and a warmer one in its Southern one. The situation starts to be reversed at the end of April. The Sea Surface Salinity SSS revealed the dynamics of eddies in the STC with a cyclonic eddy in the North and an anticyclonic one following the geostrophic flow in the south. Eddies generation and Upwelling along the western coast of Sardinia combined with a southward geostrophic flow within the upper layers play a key role in their formation process.

Keywords: Mesoscale eddies, Spatio-temporal variability, Glider data, Satellite, data analysis.

1. Introduction

Mesoscale eddies are omnipresent in the global ocean (Cheleton *et al.,* 2011) and play a key role in multiple ocean processes but are not yet fully analyzed and monitored at global scale due to their relatively small size. Unlike linear waves, nonlinear dynamics in mesoscale eddies can transport water mass with their heat content as well as chemical (e.g., salt) and biological properties (e.g., nutrients and biomass) over large distances. Their crucial role in the transport of heat fluxes has been shown in many studies (Jayne S. R. & Marotzke J. 2002). The surface circulation in the WMED is mainly cyclonic around the basin with several areas where strong eddy activity has been observed. Along the Algerian shore, baroclinic instabilities of the Algerian Current generate meanders and, eventually, eddies that detach from the main current (Olita *et al.,* 2011). In the upper layer, the Atlantic Water (AW) enters the Mediterranean Sea through the Strait of Gibraltar. Circulating through the whole oceanic basin, AW becomes saltier through atmospheric and oceanic processes. Below the surface layer, there is the Levantine Intermediate Water (LIW), which is formed in the EMED (Millot,1999). However, the Sardinia Channel remains one of the regions where the dynamical processes and water exchanges are not yet clearly identified. Within the framework of the GETSCh project under the JERICO-NEXT TNA EC funded programme. Glider cruises were carried out in the Sardinia-Tunisia Channel (STC here after) from 16th March to 27th of April 2018 as a contribution to increase the knowledge on the STC exchange system and its connexion with the Algerian Basin circulation (Ben Ismail, 2018).

2. Material and Methods

Along the Algerian coast, the AW is transported mainly by the Algerian current from which the anticyclonic Algerian eddies (Puillat *et al.,* 2002) often involving surface and intermediate waters, are generated by baroclinic instabilities of the AC itself. The AEs generally remain more or less included in the main AC flow. In order to clarify some of these processes, including the behavior of the Algerian current and associated eddies, our methodology is based on a combined approach using glider observations and sea surface features observed by satellite.

2.1. The observations of bonpland glider on getsch deployment

GETSCh is a second step in order to incorporate glider-based oceanography sampling at the Tunisia Sardinia section on a regular basis (Fig.1). For each mission, the glider was deployed off Sardinia towards Tunisia (or off Tunisia toward Sardinia) and back several times to investigate the variability in an area highly interested in mesoscale activity. We have a temporal extent of data from 14 March 2018 till 27 April 2018. Indeed, as shown on figure 12, the glider was deployed along SARAL track n°887 highlighted in red. Bonpland glider took slightly over 9 days each way, from March 16 to May 04.

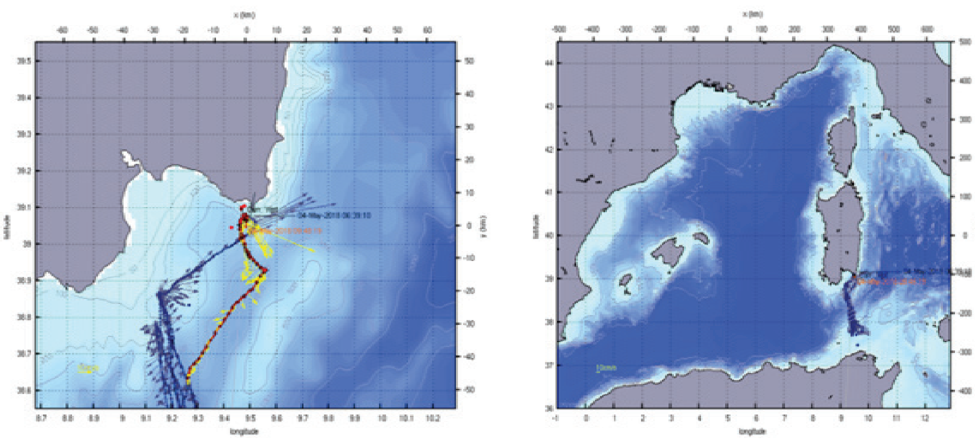

Fig.1: Glider tracks across the Sardinia Channel in 2018.

2.2. Spatial discretization

As a first step, after a data analysis, the idea was to divide the data collected along the Glider trajectory relative to a spatial discretization, based on the latitude in the western Mediterranean Sea. A deep water glider (up to 1000m) was deployed in the Sardinia Channel and has carried out 3 return trips during the period spanning from the 14th of March 2018 to the 27th of April 2018. We chose to discretize the Sardinia-Tunis trajectory into six legs. Each zone will be treated apart as follow: Leg 1: from 14/03/2018 to 22/03/2018, the starting Glider deployment off-coasts Sardinia and heading to Tunisia, which is a forward. Leg 2: from 23/03/2018 to 28/03/2018, when the Glider will return back from Tunisian coasts to Sardinia trajectory it is a backward. Leg 3: from 29/04/2018 to 03/04/2018, when the Glider arrives at the Sardinian coasts and heads back to Tunisia. Leg 4: from 04/04/2018 to 10/04/2018. The Glider is in Tunisian coasts and will move forward to Sardinian coast. Leg 5: from 11/04/2018 to 16/04/2018 when the Glider is heading to Tunisian coasts. Leg 6: from17/04/2018 to 27/04/2018 returning back to Sardinia.

2.3. Satellite data analysis

We studied four key parameters of the marine environment in our study area, where the Glider crosses the water bodies of the Western Mediterranean Sea: CHL, SST, Salinity and Sea Water coupled hydrodynamic-wave model. Regarding the CHL analysis, NRT multi-sensor products (MODIS+VIIRS+OLCI) incorporating OLCI in the processing chain have been used. For SST, the CNR MED Sea Surface Temperature provides daily gap-free maps (L4) at high (HR 0.0625°) and ultra-high (UHR 0.01°) spatial resolution over the Mediterranean Sea. Remotely-sensed L4 Sea Surface Temperature (SST) datasets have been used. To investigate the temporal behavior of the Mesoscale Eddies in the STC, we used the Salinity and Geostrophic velocity fields, produced by CMEMS. The physical component of the Mediterranean Forecasting System (Med-Currents) is a coupled hydrodynamic-wave model implemented over the whole Mediterranean Basin. The model horizontal grid resolution is 1/24˚ and has 141 unevenly spaced vertical levels.

3. Results and Discussion

The objective is to compare four main parameters: Temperature, Salinity, Dissolved Oxygen and Chlorophyll-a from Glider 2018 mission. We analyzed θ/S diagrams to detect mesoscale eddies afterwards, finally we analyzed CHL, SST, Wave model and a coupled hydrodynamic-wave model from CMEMS website for Satellite data validation. To verify the CHL, Temperature and Salinity vertical distribution found through Glider, we generated satellite images of daily surface CHL, SST, SSS and Velocity to show the mesoscale dynamics in STC.

3.1. Comparison along glider tracks for 2018 getsch mission

3.1.1 Salinity vertical distribution

The salinity vertical distribution up to 200m depth is displayed in Figure 2. Near the surface layer, lenses of fresher water are observed till 100m depth at about 37.6°N (Fig.2-A), and it corresponds to meandering of AW, which is advected from West to East by the Algerian current. The suspected existence of the upwelling in the surface layer is confirmed by the shape of the isohalines in (Fig.2-B). Consequently, the mesoscale eddy generates an upwelling, which, in turn, brings salty water to the surface (38.4) (Fig.2-C). In-situ glider data described the hydrological situation by giving an indication that the structure on which we are focusing on is a cyclonic eddy. The AW follows a cyclonic course in the western basin (Fig.2.A). It is directly subjected to the atmospheric forcing which will modify its characteristics under the action of heat and water flows (Damien, 2015). AW enters the Algerian basin forming the Algerian Current. A part of it turns back, skirts Sardinia to the south. From Leg#4 and Leg#5 in Figure 2.D-E, when the cyclonic eddy brings saltier and colder waters to the upper layers, the upwelling occurs, the salty water mass in Figure 2-D and for temperature clearly describe the upwelling of the water mass, which signature is salty (38.4) and cold (14.2) between 60 and 90m depth.

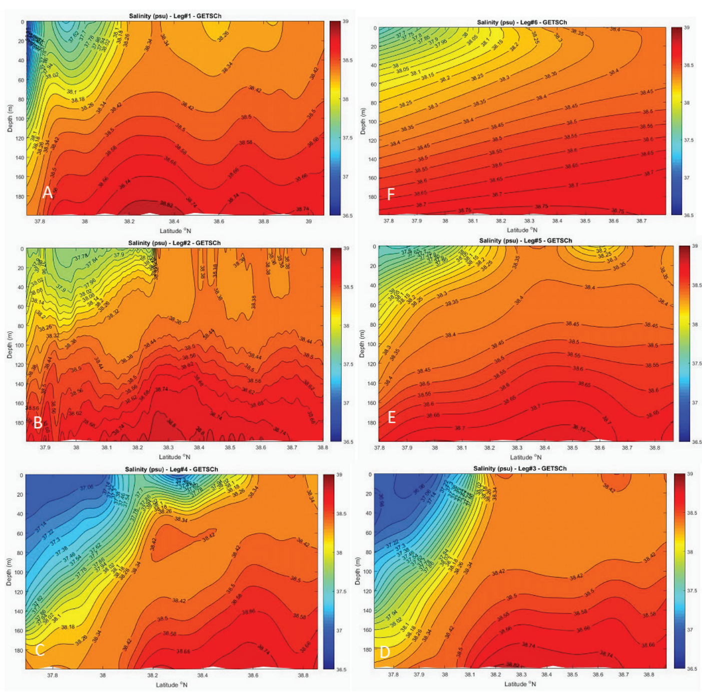

Fig. 2: Vertical distribution of Salinity in all legs from 0-200m depth for GETSCh mission: A: Leg#1, B: Leg#2, C: Leg#3, D: Leg#4, E: Leg#5 and F: Leg#6.

3.1.2. Eddies characterization from θ/S diagrams

In March 2018, we can clearly detect the signature of AW highlighted in grey, in Leg#2 period, only deep AW was detected. We noticed an interesting event occurred in Leg#3, which is the intrusion of waters masses (highlighted in Yellow rectangle) characterized by 15<θ<16.2 °C and S > 38.3, these water masses lead to mixture in Spring, we also have the presence of LIW, confirmed by in situ data (Fig.3-A-C) describing the vertical distribution of water masses where we recognized LIW with θ=14.6°C and S=38.8. An event between Leg#4 and Leg#5 occurred, AW changed its water properties, after a mixture between water masses, AW masses temperature increased to reach 16°C, Salinity changed from 37<S<37.8 to 37.5<S<38. This could be explained clearly in Leg#6 during 17-27 Apr 2018, when water masses of LIW characterized by a relative maximum of temperature (~18°C) and absolute maximum of Salinity (~38.9 ‰) (Rixen et al, 2005) mixed with AW waters during the upwelling period. These water masses dynamics are mainly related to Mesoscale Eddies generated to advect them.

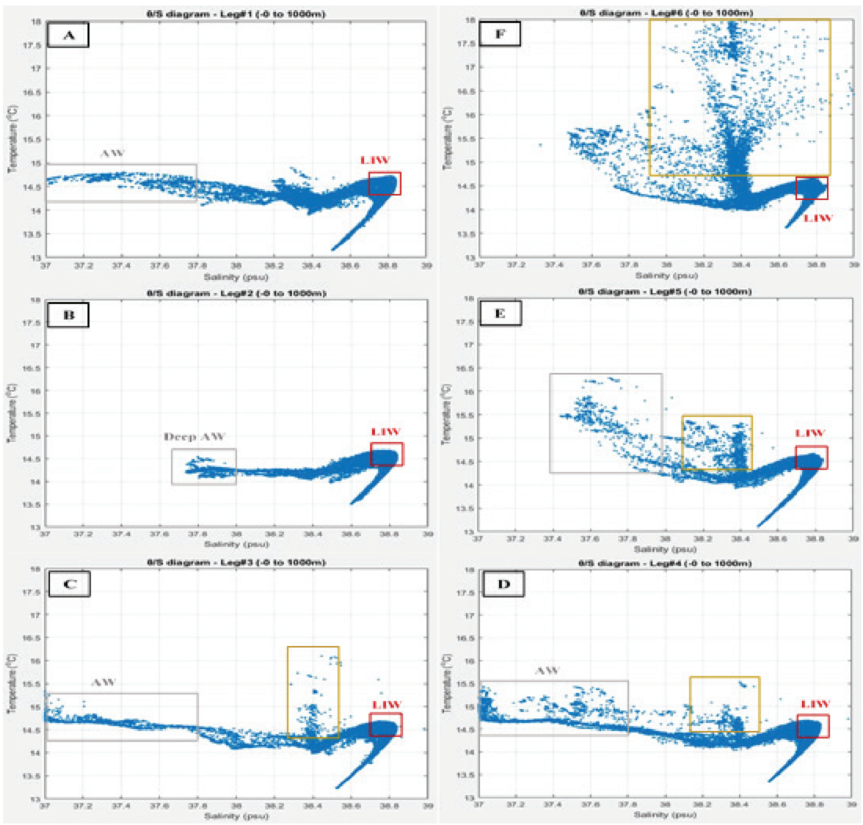

Fig. 3: Glider θ/S diagrams during GETSCh mission in all legs: A: Leg#1, B: Leg#2, C: Leg#3, D: Leg#4, E: Leg#5 and F: Leg#6.

3.2. Comparison with satellite data

3.2.1. Horizontal distribution of sea surface salinity SSS in stc

The corresponding average sea surface salinity map is obtained from the Mediterranean Sea Physics Analysis Model in CMEMS website (Fig.4). These data are interpreted in relation with the Glider data acquired during the same period in the same area. We can clearly confirm through the Glider hydrologic vertical distributions, that Sea Surface Salinity is higher in Sardinian coasts compared to Tunisian coasts. Basically, we have a generated mesoscale eddy already detected in the map of Surface CHL in 02/04/2018, which is leading to a Cyclonic shape in the North of STC. This map clearly shows the shape generated near the Sardinian coasts at about 38.5°N and 37.8°N by anticyclonic eddies near the Tunisian coasts.

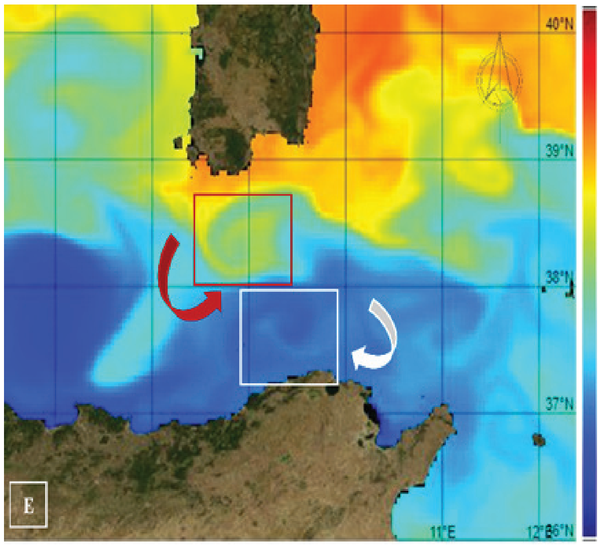

Fig. 4: Sea Surface Salinity in 21/04/2018.

3.2.2. Eddy detection and tracking using remote sensing products

To investigate the temporal behavior of the Mesoscale eddies in the STC, we used the geostrophic velocity fields in order to follow the complete trajectory of the surveyed cyclone and anticyclone eddies as well as their interaction with neighboring eddies. It quantifies the dynamical characteristics of the detected eddies and especially the mean velocity profile. Eddy tracking revealed that eddy-eddy interactions play a role in north-south mass exchanges. The high-resolution in situ Glider observations highlight small-scale processes embedded in Mesoscale eddy dynamics. In fact, *in situ* measurements are in good agreement with the analysis of the geostrophic velocity field given by CMEMS. The trajectory of the anticyclonic eddy (highlighted in white rectangle in Figs 5, 7, 8) shows that it emerged from a meander of the Algerian Current, which detached from the coast at 8°E and traveled up to the Sardinian coast. We can clearly see that this eddy generated anticyclonic eddies in the northern edge of their trajectory. We might notice that near Algerian and Tunisian coasts, this southward geostrophic flow within the upper layers seems to play a key role in the formation process of Mesoscale eddies. Through the southward geostrophic flow, the eddy-eddy interactions generated anticyclonic eddies in the South of STC and this vein deflected currents in the Sardinian coasts which generated a Cyclonic eddy already confirmed in the previous profiles.

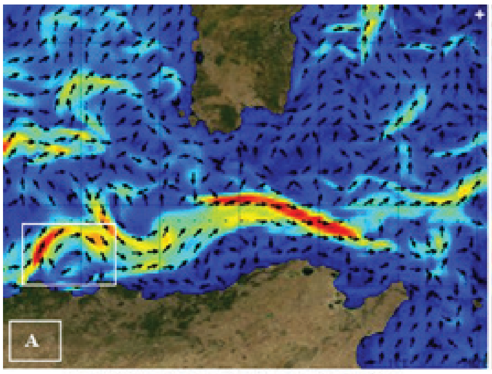

Figure 5. Mean Sea Water velocity (m/s) in 15/03/2018

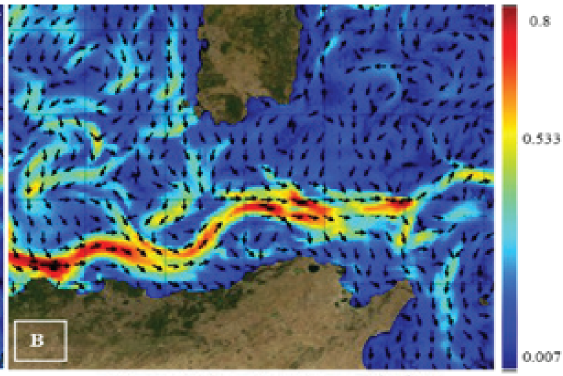

Figure 6. Mean Sea Water velocity (m/s) in 27/03/2018

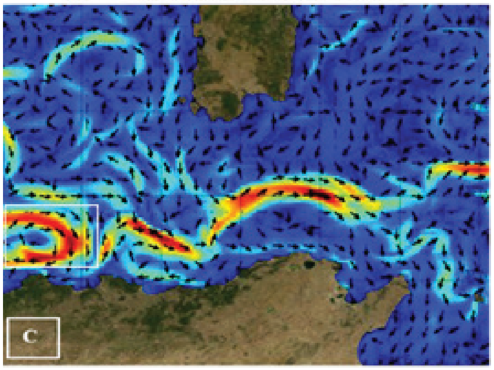

Figure 7. Mean Sea Water velocity (m/s) in 06/04/2018

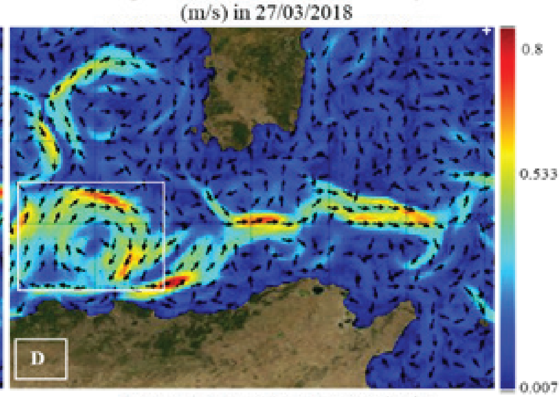

Figure 8. Mean Sea Water velocity (m/s) in 27/04/2018

4. Conclusion

Glider and satellite data revealed a significant finding in the horizontal distribution of Sea Surface Salinity (SSS), allowing us to detect the presence of the cyclonic eddy in the north of STC, generating an upwelling and bringing salty water to the surface, which had been suspected from the start (38.4). Indeed, the Algerian Current's meandering, which is advecting AW first eastward through the Algerian slope, and later along the Sardinia-Tunisia Channel, produces freshwater lenses near the surface (STC), a few lenses detach from the Algerian slope and propagate along the Sardinian one. This appears to be another kind of mesoscale structure that has been observed. Close to the Sardinian continental slope, the LIW and TDW coming from the STC, flow northward in a well-marked vein and interactions between this vein and the Algerian Gyre are supposed to produce these Sardinian Eddies (SEs), which has been previously suspected by (Testor *et al.*, 2005).

5. Acknowledgements

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GENETIC APPROACHES IN AQUATIC SCIENCES

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PARENTAGE ANALYSIS AND GENETIC PARAMETER ESTIMATION AT DIFFERENT AGES IN MEAGRE *ARGYROSOMUS REGIUS*

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Abstract

Meagre is a sciaenid species with high commercial interest for the aquaculture industry. Due to its recent history in culture conditions, the study of genetic parameters on economically important traits is limited. The aim of the present work was to investigate the family structure and parental contribution of thirteen meagre broodstock and 1,200 offspring from a mass spawning event, as well as to estimate genetic parameters for body weight and length in different ages. Parentage analysis was conducted with a multiplex of 12 microsatellite loci and achieved 71,8% assignment rate. The offspring distribution in families was unbalanced emerging from an unequal contribution of broodstock in both sexes. Genetic parameter estimation was conducted using the *Restricted Maximum Likelihood* method, resulting in high estimated values of heritability for the studied traits, indicating the suitability of the studied traits to be selection objectives in a breeding program with a high expected genetic gain. Furthermore, the high estimated genetic correlation between body weight and length suggests the possibility of selection using only one of the two traits, as selection criteria. A major setback in the present study was the high fish mortality which led to limited data from the latter studied ages and resulted in high standard errors. Thus, the repetition of the study using a larger population is proposed in order to acquire more accurate estimates of traits of economic interest in meagre.

Keywords: *Argyrosomus regius*, microsatellite markers, parentage analysis, heritability, genetic parameters.

1. Introduction

Meagre (*Argyrosomus regius*) is a species of the *Sciaenidae* family with great potential for aquaculture due to its fast growth, good feed conversion ratio and relatively easy management in culture conditions (Duncan *et al.,* 2013). The employment of DNA markers to infer relationships between parents and offspring was revolutionary for the aquaculture industry, allowing the implementation of more elaborate breeding schemes, such as family-based selection, in an easier and more affordable way. Microsatellites have been the markers of choice for such endeavors, due to their abundance and high polymorphism especially in fish genomes (Chistiakov *et al*., 2006). The ability to acquire pedigree information in aquaculture is of great importance, since it makes broodstock management for controlled inbreeding and parental contribution easier, while also enabling the estimation of genetic parameters for economically important traits. The estimation of such parameters is crucial in the design of breeding programs, as they provide critical information for the traits that are set as breeding goals and are expected to render the breeding strategy more efficient and therefore profitable. In this context, very little is known about meagre, with only two published studies, until today, investigating the genetic parameters of important traits such as body weight, length, flesh composition etc. (Nousias *et al.,* 2020; Vallecillos *et al.,* 2021), even though there is a clearly growing interest in the aquaculture for fast-growing species like meagre, with plenty of studies aiming at the development of molecular markers for the *Sciaenidae* family. In the present study, we attempted to assign offspring from a mass spawning event to their putative parents using a multiplex of 12 microsatellites and later combined the acquired pedigree information with phenotypic data of body weight and length at two and three different ages, respectively, in order to estimate the genetic parameters of these traits.

2. Material and Methods

2.1 Rearing conditions, DNA sampling & data collection

Thirteen broodstock, 7 females and 6 males, coming from three different stocks (one fish from stock 1, nine fish from stock 2, and three fish from stock 3) were used; all fish, except one male, were injected with GnRHa (18/01/2019) to induce spawning and were allowed to spawn spontaneously in a tank. Hatching occurred at 24/01/2019 and the larvae were reared until 84 DPH (*Days Post Hatching*), when they were transferred to a round cage. Around 297 DPH the juveniles were graded and the bigger fish (mean weight ~550 gr.) were transferred to cage 1, while the smaller fish (mean weight ~370 gr.) were transferred to cage 2. At 394 DPH, 600 fish from each cage were randomly selected, individually tagged in the abdominal cavity with a Passive Integrated Transporter (PIT-tag) and finally transferred all together into a rectangular cage. Fin clips were sampled from all broodstock fish at the beginning of the experiment, as well as from the 1,200 randomly chosen offspring at 394 DPH. Weight was measured on all the surviving offspring at 394 DPH (BW1), 770 DPH (BW2) and 978 DPH (BW3). Additionally, length was measured on all surviving offspring at 770 and 978 DPH (Len2, Len3, respectively).

2.3 DNA extraction, PCR & genotyping

DNA was extracted from all fish fin clips using a protocol developed by Miller *et al*. (1988) with minor modifications. A multiplex of 12 microsatellite loci from Nousias *et al*. (2020, 2021) was used and later resolved by capillary electrophoresis. Allele identification was performed using the software STRand v. 2.4.110 (https://vgl.ucdavis. edu/STRand).

2.4 Data analysis

Offspring were assigned to their putative parents with the exclusion-based computation method, through Vitassign software (Vandeputte *et al*. 2006), which was firstly used to simulate the assignment power of the microsatellite multiplex. Analyses were run, gradually increasing the number of accepted mismatches up to 2, from which point on the assignment rate reached a plateau.

Heritability estimates as well as genetic and phenotypic correlations were obtained using the Restricted Maximum Likelihood method (REML), using a bivariate animal model (Equation 1) in AIREMLF90 (Misztal *et al*. 2018) for all pairs of the studied phenotypes and the cage as a fixed effect:

$$Y = Xb + Zu + e \qquad (1)$$

where, Y corresponds to a matrix of the observations (weight, length), b is a vector of the fixed effect, u is the additive genetic effect using a pedigree relationship matrix, X and Z are incidence matrices relating observations with b and u, respectively and finally e is a vector of the residual variance.

3. Results

3.1 DNA extraction & genotyping

DNA extraction was conducted successfully for all broodstock and 1,187 offspring samples. The extracted DNA quality was medium to low, with many samples presenting an extensively degraded image. Genotyping was also successful for all parents (13) and 1,179 out of 1,187 offspring. The unsuccessful genotyping of 8 samples was attributed to their poor DNA quality.

3.2 Parentage assignment, family distribution & parental contribution

The assignment power of the multiplex used in the present study was simulated by Vitassign at 65.9%. The final assignment rate of the parentage analysis was 71.8%, *i.e.*, 847 out of 1,179 offspring were successfully assigned to their putative parents with 0 to 2 mismatches allowed. The 847 offspring were distributed in 24 out of the 42 expected families (6 males by 7 females), forming 3 big families (>15%) and 3 smaller families (5-9%), as is presented in Table 1. The rest of the formed families represented a very small percentage (< 2% each). All broodstock were identified as possible parents with the exception of one female (F904). Parents of both sexes presented an unequal contribution in spawning with males M896, M900, M902 and females F903, F852, F895 and F905 presenting a dominant role (indicated with blue and red, respectively, in Table 1).

Table 1. Distribution of the 24 formed families from the crossing of 13 broodstock in *A. regius*. The 3 biggest families are indicated by dark yellow, while the 3 smaller families are indicated by light yellow. Indicated with blue and red respectively, the male and female broodstock with higher contribution. Also, underlined the only male broodstock which was not injected with GnRHa.

	Males						
Females	**M894**	**M896**	**M900**	**M901**	**M902**	**<u>M907</u>**	**Sum**
F852	0.24	0.35	24.32	0.00	0.12	0.00	25.03
F895	1.30	1.89	0.00	0.00	14.64	0.00	17.83
F898	0.59	4.96	0.00	0.12	1.77	0.12	7.56
F903	1.18	30.22	0.47	0.24	1.89	0.12	34.12
F904	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F905	0.94	9.09	0.00	0.00	3.90	0.00	13.93
F906	0.00	0.00	0.35	0.24	0.94	0.00	1.53
Sum	4.25	46.52	25.15	0.59	23.26	0.24	**100**

3.3 Quantitative genetic analysis

Heritability estimates for body weight (BW) and length (Len) ranged from 0.32 to 0.80 and 0.58 to 0.72 (Tables 2, 3), respectively. Heritability estimates tended to increase with age and were in general quite high for all the studied traits, except for body weight at 394 DPH (Table 2) which is within a medium range (0.20 – 0.40). Genetic correlation estimates between body weight and length were high at both 770 and 978 DPH (Table 3). Genetic correlation estimates between body weight at different ages were lower (Table 2). Finally, standard errors were quite high both for heritability and genetic correlation estimates. Most estimates presented statistical significance and are pointed out with (*) on the following tables which summarize the aforementioned results.

	BW1	**BW2**
BW1	**0.32** (0.18)	0.56 (1.05)
BW2	0.63 (0.07) *	**0.68** (0.27) *

Table 2. Heritability (bold, diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlation estimates for body weight at 394 DPH (BW1) and at 770 DPH (BW2) on the left and for body weight at 394 DPH (BW1) and at 978 DPH (BW3) on the right. Standard errors are in parentheses.

	BW1	**BW3**
BW1	**0.32** (0.18)	0.46 (1.01)
BW3	0.52 (0.09) *	**0.80** (0.33) *

*Statistically significant results

Table 3. Heritability (bold, diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlation estimates for body weight (BW2) and length (Len2) at 770 DPH on the left and for body weight (BW3) and length (Len3) at 978 DPH on the right. Standard errors are in parentheses.

	BW2	Len2
BW2	**0.69** (0.28) *	0.91 (0.54) *
Len2	0.88 (0.028) *	**0.58** (0.25) *

	BW3	Len3
BW3	**0.80** (0.35) *	0.94 (0.52)
Len3	0.90 (0.03) *	**0.72** (0.32) *

*Statistically significant results

4. Discussion/Conclusion

Generally, higher assignment rates (>90%) have been recently achieved in meagre (Nousias *et al.*, 2020; Vallecillos *et al.,* 2021) as well as in other sciaenid species (Liu *et al*., 2013). The medium assignment power of the multiplex used in this study can probably be attributed to high kinship within the broodstock, as most of them came from the same stock. Moreover, there is an indication of possible elevated inbreeding, since a low number of alleles in the microsatellite loci of the multiplex was observed (<6 alleles/locus). The unbalanced family distribution and consequent unequal parentage contribution has previously been reported in meagre (Nousias *et al.,* 2020; Vallecillos *et al.,* 2021) and other fish species (Liu *et al*., 2013). These findings can be viewed as serious indications towards the need for better broodstock management, in order to avoid inbreeding and consequent genetic variability loss, which, between other important issues, leads to difficulties in parentage assignment using molecular tools.

The estimated heritability for body weight and length in this study was quite high and fell in the same range as the estimates in the study of Nousias *et al.* (2020) but it was generally higher than other studies in meagre (Soula, 2012; Vallecillos *et al.,* 2021) and other species of the *Sciaenidae* family. These heritability values suggest that selection for these traits could be highly profitable in meagre. The observed increase in the estimates of heritability with increasing age was also reported by Soula (2012) in meagre and is generally observed and in other fish species. As for genetic and phenotypic correlations between the studied traits, the estimated values were very high between body weight and length at 770 and 978 DPH suggesting a possible pleiotropy and/or linkage among the genes affecting these traits, indicating the possibility of selection using only one of these traits in selective breeding programs. The respective genetic correlation estimates for body weight between different ages were lower.

Finally, the very high standard errors of the majority of the aforementioned estimates can be attributed to the sample size in the present study, which was progressively smaller, due to high mortality in the studied population of fish. Consequently, the future study of a larger sample would be essential, in order to estimate these genetic parameters in meagre more precisely.

5. Acknowledgements

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QUALITATIVE COMPOSITION OF BIOFOULING ORGANISMS: A STUDY ON COMMERCIAL VESSELS FROM SARONIKOS GULF

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Abstract

Underwater vessels' cleaning is considered necessary for their efficient operation due to the extensive biofouling of organisms attached to their hull. Marine organisms developing at the hulls of ships are very likely to be released, survive and become alien species. This study is based on sampling of biofouling organisms on commercial ships from different destinations in the Saronikos Gulf. Qualitative and semi-quantitative analyses will lead to conclusions on the biodiversity of the biofouling material. Combination of taxonomic and molecular techniques can provide more reliable results on new alien species' identification and origin. Preliminary results indicate the presence of three polychaete species never reported from the area before, two of which are hitherto unknown from the Greek Seas and the Mediterranean.

Keywords: introduced species, commercial shipping, hull biodiversity.

1. Introduction

Shipping (Transport-Stowaway) is considered the main pathway of marine alien species' transfer worldwide. In European waters it is estimated to be responsible for 43.8% of all alien species introduction after 1950 (Korpinen *et al.*, 2019). According to Zenetos *et al.* (2018), 36.7 % of introduced species have been transferred to Greek waters via maritime activities. Saronikos Gulf is a known hotspot area for the introduction of alien species in the Hellenic Seas (Zenetos *et al.*, 2020).

Molecular tools are now used systematically to track alien and/or invasive species (Nagarajan *et al.*, 2020) and methods based on molecular analyses are a useful tool for species-level identification. Among the existing molecular approaches, DNA barcoding is the most commonly used technique for identifying unknown samples (Hebert *et al.*, 2003). The method involves sequencing a small DNA fragment from a specific gene region that is suitable for species identification (Hebert & Gregory, 2005). The technique is particularly useful in research aimed at identifying species from different geographical locations (Bergsten *et al.*, 2012), as in the case of alien species.

The purpose of this study is the identification of organisms that adhere on cargo/commercial ships and the spread prevention of new NIS. Early detection of species that can potentially escape to the environment is of major importance for protection from new introductions, providing information that could ultimately lead to a prominent management plan.

2. Material and Methods

2.1 Sampling procedure

Biofouling samples were collected from twenty-four (24) ships from September 2020 until March 2022. More cleaning events are expected until the end of 2022. The collection of samples has been conducted by experienced divers of the 'DIVING STATUS UNDERWATER SERVICES'. Qualitative samples were taken mainly from the sides of the ship and/or from various points, depending on the fouling coverage observed and stored in containers with 99% alcohol to be subsequently sorted into the main taxonomic groups and photographed. Information on the routes of each ship including last ports of departure is retrieved by the online platform Marine Traffic.

2.2 Genetic analysis of organisms - DNA barcoding & sequencing

The organisms selected for molecular identification from each ship hull belong to groups that are potentially alien and/or invasive, are often found on ships and/or in ports or have shown great coverage on the sampled ships. In order to reveal putative cryptic NIS, organisms that have similar morphological characteristics and are usually confused with native species were also analysed. Molecular identification at species level will be finalized within the year 2022.

Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) for animal tissues according to the manufacturer's protocol with minor adjustments. The primers used for amplification and sequencing are the mitochondrial Folmer primers (Folmer *et al.*, 1994). Sanger sequencing was performed in-house, using an ABI 3730XL DNA sequencer (ThermoFisher).

2.3 Taxonomic classification of fouling organisms

All samples from each ship were initially divided into main taxonomic groups such as Mollusca, Crustacea, Polychaeta, etc. Then, taxonomic identification was performed by experts according to the type of organisms up to species level, where possible. Many of the organisms are difficult to measure due to their morphology and the way they form colonies, such as Cirripedia, which are measured with qualitative characteristics (i.e., few, enough, a lot, single-species coverage).

3. Results

3.1 Evidence of newly recorded NIS in the Saronikos gulf

Among the species identified, the polychaetes *Hydroides elegans* (Haswell, 1883), *Hydroides dirampha* Mörch (1863) and the bivalve *Brachidontes pharaonis* (P. Fischer, 1870) are amongst the most common NIS in European seas (Katsanevakis *et al.*, 2014). *Hydroides elegans* and *Brachidontes pharaonis* have already been reported from the Saronikos Gulf (Zenetos *et al.*, 2020), while the species *Hydroides dirampha* is reporded for the first time (Fig. 1). Additional species collected from the ship hulls only but according to Faulwetter *et al.* (2017) never reported from Greek waters are: *Neanthes vaalii* Kinberg, 1865 (Fig. 2) and *Neanthes gisserana* (Horst, 1924) (Fig. 3).

Hydroides dirampha secretes a calcareous tube and a feathery crown of modified prostomial palps (radioles). The operculum is roughly funnel-shaped, with 28-33 radii, each with pointed tips, a concave distal surface and with a circular row of six outwardly curved terminal spines. The thorax consists of seven segments while the abdomen has approximately 72 segments.

Both *Neanthes* species (*N. vaalii* & *N. gisserana*) are recognizable by their anterior appendages, including two prostomial palps and four peristomial tentacular cirri. Prostomium is short, broad and not as long as peristomium. The peristomium is apodous and asetigerous. The pharynx bears a distinct eversible proboscis. The everted proboscis has two rings, oral (or proximal) and distal (or maxillary) and terminates with two fangshaped jaws. Each ring is equipped with many papillae and conical paragnaths and their patterns are taxonomically relevant.

Fig. 1: 1a. The polychaete *Hydroides dirampha* Mörch, 1863, found on the ship “CELESTYAL OLYMPIA”. Photos 1b, 1c show in magnification the operculum, one of the species’ basic taxonomic features.

Fig. 2: 2a. The polychaete species *Neanthes vaalii* Kinberg, 1865 found on the ships “GINCO” and “ST MARSEILLES”. Magnification in photos 2b and 2c show the jaws of the ventral dorsal sides, respectively.

Fig. 3: The polychaete species *Neanthes gisserana* (Horst, 1924) found on the ship “CELESTYAL OLYMPIA”. Magnification in photos 3a and 3b focuses on the jaws of the dorsal and ventral sides, respectively.

4. Discussion

4.1 Indication of NIS transportation via shipping

Regardless of the preliminary nature of our study, five NIS were already discovered in the samples that have been analyzed so far, including two first records for Greece and one new record for Saronikos gulf. Combination of classical taxonomy and DNA barcoding methods can provide more accurate results, especially in unraveling cryptic species and difficult-to-identify specimens (Cross *et al.* 2011). Molecular identification at species level is currently in progress and will be completed within the year 2022, expecting to reveal more NIS, since genetic analyses can unveil cryptic species which are currently considered indigenous

4.2 Origin of species and ship routes

The polychaete species *Neanthes vaalii* was found on the ship "GINCO", whose native distribution includes Australia and Tasmania (Read & Fauchad, 2022a). As our results from marine traffic indicate, before anchoring in Lavrio, Greece, ''GINCO'' had been in the Suez Canal (Egypt), in Sri Lanka, in India and South Africa. The same species was also interestingly discovered on the ship ''ST MARSEILLES'', which route included the port of Rotterdam, anchorage on the Gibraltar canal, Marsaxlokk port and also Bulgaria's Varna port. *Neanthes gisserana* with type locality in Indonesia (Read & Fauchad, 2022b), was found during a cleaning event of ''CELESTYAL OLYMPIA'' which was not en route for at least 10 months before it was cleaned in Greece. Further analysis of the routes of ships, in parallel with endemicity of the abovementioned species in each anchorage area, will provide a better understanding of the biogeographical path that NIS follow upon introduction into new areas.

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MOLECULAR IDENTIFICATION OF A PARASITIC MITE FOUND IN THE RESPIRATORY SYSTEM OF A STRANDED MEDITERRANEAN MONK SEAL IN THE AREA OF PAGASITIKOS GULF

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Abstract

The aim of the current study was the molecular identification of a parasitic mite found in the cardio – respiratory system of a sub-adult Mediterranean monk seal individual, that was found stranded in the area of Pagasitikos Gulf, Greece in 2019. The body of the seal was transferred to the Laboratory of Hydrobiology and Ichthyology for a post-mortem examination where analysis of the lungs revealed the presence of 12 intact parasitic mite individuals. Standard protocols were followed as DNA extraction of the parasites was conducted and PCR amplification of the ribosomal DNA internal transcribed spacer [ITS] regions was implemented. Sequencing analysis displayed a 87.18% similarity of the screened mites to the *Halarachne halichoeri* species. A Maximum Composite Likelihood bootstrap tree (10,000 replicates) was constructed with the use of the Kimura -2- parameter (K2P). The phylogenetic tree revealed a clear genetic similarity between our parasitic mite individuals named as Sequence and *Halarachne halichoeri* (bootstrap value: 0,95) which is indicated for the first time as such, to be found in the waters of the Mediterranean Sea and also in the lungs of a Mediterranean monk seal.

Keywords: Eastern Mediterranean, *Halarachne halichoeri*, rDNA, *Monachus monachus.*

1. Introduction

The Mediterranean monk seal (*Monachus monachus*) belongs to the Phocidae family and is considered an endangered species. Today the biggest population is found in the eastern Mediterranean basin (Dendrinos *et al.*, 2008). In recent years there has been a rise in insightful research around the relationships between the Mediterranean monk seal and its environment as well as the interactions between its abiotic and biotic factors. It is well known that the species is threatened by habitat destruction and fragmentation, negative interactions with fisheries, and pollution, while lack of information regarding basic aspects of its biology is considered to hinder the development of effective conservation strategies (Johnson *et al.*, 2006). In addition, due to climate change invasions of non – indigenous and indigenous species that have gone undetected through the years have been recorded and in this particular case, parasites. The appearance and establishment of a parasitein the population of the *M. monachus species* could potentially threaten its health, already imperiled by anthropogenic and abiotic factors, and alter the structure of its community (Formigaro *et al.*, 2017; Karamanlidis *et al.*, 2008; Langwig *et al.*, 2015). Furthermore the low genetic diversity of the species should also be considered as a factor that affects the relationship between the parasite and the host species, as it could hinder the population susceptible to novel infections (Karamanlidis *et al.*, 2016).

2. Material and Methods

During the anatomy of the lungs of the Mediterranean monk seal, 12 individuals 9 from the right lung and 3 from the left were detected and afterwards preserved in a 70% ethanol solution. DNA extraction was carried out with the Phenol – Chloroform protocol, PCR fragments were amplified using a universal primer pair targeting the ITS rDNA subunit 5.8S (Nadler *et al*., 2000). PCR reactions were performed in 20 μl containing 1 μl Template DNA, 5μl Buffer 10^X, 2μl MgCl2 25mM, 1.5 μL each of forward and reverse primer (10 μM), 0,2μl dNTP's 10mM, 0,2μl Taq polymerase 5U/μL and ddH2O 8,6μl. A PCR was then optimized for a number of cycles and annealing temperature at 48°C. In order to infer phylogenetic relationships a Maximum Composite Likelihood phylogenetic tree was constructed with 10.000 repeats by the program MEGA-X. The sequences were aligned with the program AliView (Alignment Viewer and Editor) and analyzed by Bootstrap and with the Kimura -2- parameter (K2P).

3. Results

The morphological analysis of the parasites revealed the presence of 12 intact parasites. Blast analysis through the NCBI GenBank established a 87.18% similarity with the species *Halarachne halichoeri*. A phylogenetic tree was constructed by utilizing 10 sequences from several parasitic species, 1 sequence as the outgroup *Uncinaria hamiltoni* and the studied sequence "Sequence". The evolutionary relationships between the specimens and the other species used for the construction of the phylogenetic tree are shown in the phylogenetic tree that was constructed (Fig. 1).

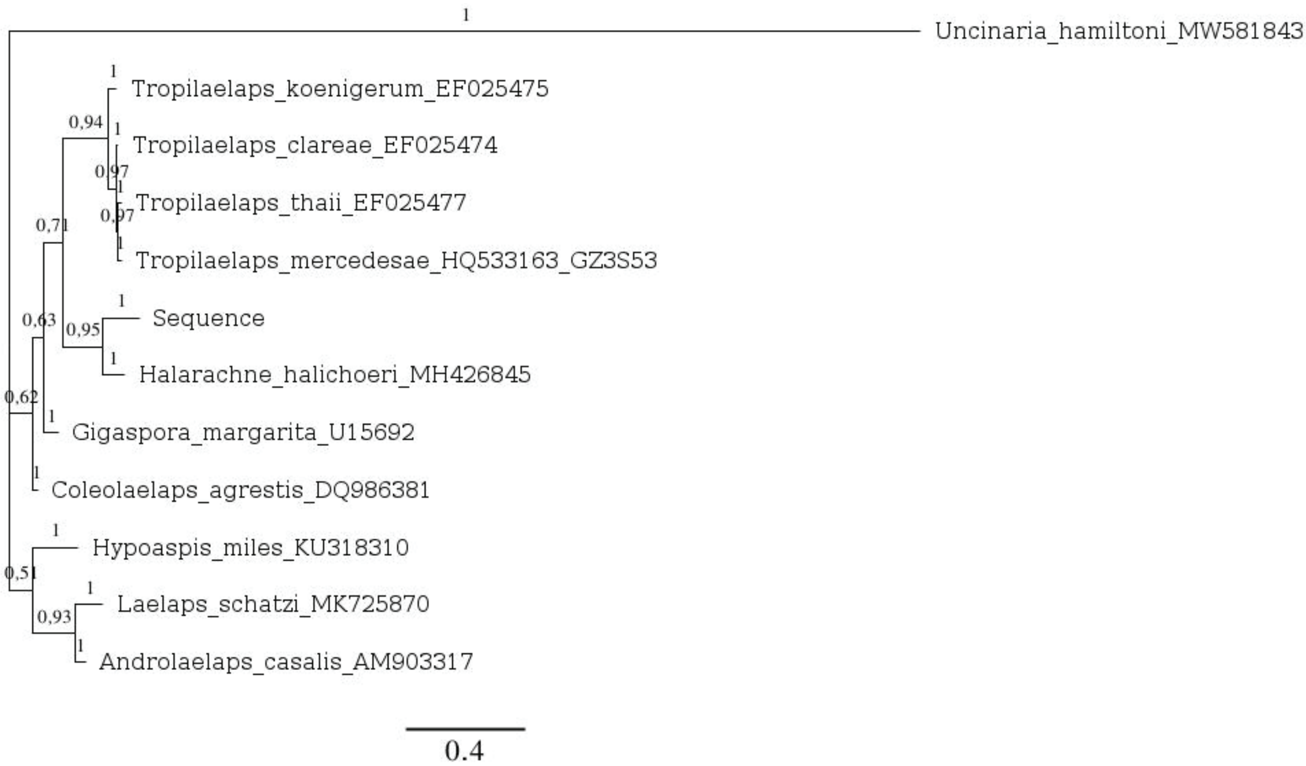

Fig. 1: Phylogenetic tree generated with MEGA X software using the Maximum Composite Likelihood analysis. Scientific names and accession numbers of the used species are indicated on the tree branches, along with the posterior probabilities.

4. Discussion/Conclusion

The close evolutionary relationship between the specimen species and *Halarachne halichoeri* indicates the possibility of the species being present in the Mediterranean Sea. The genera *Halarachne* occurs typically in members of the family Phocidae and in North Atlantic European waters, the nasal mite *Halarachne halichoeri* has been described to have Grey seals (*Halichoerus grypus*), as its main host (Alonso-Farré *et al.*, 2012). The life cycle includes a free-living hexapod, larva that is responsible for the propagation of infections since it is highly resistant and mobile (Alonso-Farré *et al.*, 2012). In the possible presence of *H. halichoeri* in the Mediterranean many questions arise regarding its appearance. One possible explanation is that it was transferred through social interactions. Monk seals in Greece have been recorded to travel long distances, (Adamantopoulou *et al.*, 2011). During their movements, it is possible that seals often come in close contact with other species and thus might become infected by pathogens possibly due to species-level potential mechanics behind transmission and intermediate hosts (Pool *et al.*, 2019). In addition the Mediterranean Sea has been exposed to new marine animal species that mainly migrate from the Red Sea through the Suez Canal (Golani, 1998) although recent studies have brought to light new migratory pathways that appear to originate from the Atlantic ocean. The first recorded pathogens could bring disastrous consequences on the health of the indigenous population. This was evident in the research of Papadopoulos *et al.*, 2010) where two nematodes belonging to the species *Acanthocheilonema spirocauda* were found in the right ventricle of the heart of a Mediterranean monk seal (Komnenou *et al.*, 2021) where the presence of the hookworm *Uncinaria hamiltoni* was recorded for the first time in rehabilitated Mediterranean monk seal pups in Greece; and (Koitsanou *et al.*, 2022) where the presence of a nematode of the genus *Pseudoterranova* was recorded for the first time in the stomach of a *M. monachus*. It is evident that urgent conservation efforts must be made to protect the *M. monachus* subpopulation, by enhancing existing protection methods and enlisting new molecular practices that could potentially be of use in rehabilitation, and parasitological surveys that are essential for preventing the emergence of novel diseases, thus consequently helping the survival of the species (Komnenou *et al.*, 2021).

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THE FINDING OF THE MINUTE CHROMOPHYTE ALGAE *SCHIZOCLADIA ISCHIENSIS* (SCHIZOCLADIOPHYCEAE, OCHROPHYTA) AT A CORALLIGENOUS SITE OF THE NORTHEAST COAST OF RHODES (GREECE) RAISED BY GERMLING EMERGENCE FROM SUBSTRATUM

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Abstract

The biodiversity and ecology of coralligenous communities in the Eastern Mediterranean is still largely unknown. Substratum samples collected during 46 person-dives down to 55 m depth off the Greek island of Rhodes (Dodecanese, SE Aegean) in late 2015 were incubated in the laboratory. Culturing of germling emergence from substratum samples and DNA barcoding and bioinformatics was implemented for the identification of the minute algae. Stereoscopy and microscopy were used for preliminary algal identifications, using keys specific for the region. Among the emerging macroalgal germlings there was the second-ever record and isolate of the small benthic multicellular alga *Schizocladia ischiensis* of the poorly known monotypic Schizocladiophyceae, the sister group of the brown algae (Phaeophyceae). This new strain formed branched upright thalli attached to the substratum by an amorphous substance around the bottom of the basal cell. *S. ischiensis* is probably a common member of the circalittoral community in the Mediterranean and generally overlooked because of its minute size. Germling emergence appears to represent the method of choice to reveal cryptic circalittoral benthic algae invisible during visual or photographic diving surveys. Significant gaps remain in the knowledge of Mediterranean seaweed diversity and deeper waters are still a promising field for biodiversity research.

Keywords: Maërl beds, DNA sequencing, PCR.

1. Introduction

Much of the world's algal biodiversity and biogeography remains unknown due to undersampling of the world's euphotic benthos and the widespread occurrence of genetically or morphologically cryptic species. The seaweed flora of the Eastern Mediterranean, where Rhodes Island is located, has been studied, nevertheless many gaps remain, especially regarding the circalittoral macroalgal flora and minute algae. Brown algae (Phaeophyceae) are the most abundant group in the euphotic zone and findings of brown algae in deeper waters (80-100m) might be because the oligotrophic waters of the Mediterranean display high transparency (Küpper *et al.*, 2019), enabling photosynthesis deeper. To this day, more than 270 records of brown algal species are reported in the Mediterranean (Ribera *et al.*, 1992) but there are significant gaps in the knowledge of Mediterranean seaweed diversity from deeper waters, therefore, this zone is still a promising field for biodiversity research (Ni Ni *et al.*, 2011). Coralligenous communities or maërl beds have been characterized as priority habitats by EU (EU 1992) having a high importance for fisheries and are considered as diversity hotspots, harbouring 20% of the Mediterranean species (Garrabou & Harmelin, 2002, Ballesteros 2006; Piazzi *et al.*, 2010; Canovas-Molina *et al.*, 2016). These habitats occur from 15 m in the western part to more than 120m in the East. They are common at 40 - 60m depth in dim light conditions. Recent reviews highlight large gaps in the coverage of the Mediterranean seaweed flora by DNA barcode sequences and other molecular data (Bartolo *et al.,* 2020). Furthermore, the Mediterranean seems to host taxa which are key to a better understanding of brown algal evolution, compared to other parts of the globe, such as the genera *Choristocarpus* and *Discosporangium* that have only been recorded in the Mediterranean and the genus *Schizocladia*, which constitutes a sister group to the brown algae as a whole and of which only one collection has ever been made from Ischia Island (Kawai *et al.,* 2003). The Germling Emergence Method, which consists of obtaining algal isolates from incubating substratum samples, followed by DNA barcoding and bioinformatics, has significant potential for new discoveries (Peters *et al.,* 2015) enabling the detection and characterization of algae existing in cryptic microstages, invisible during visual or photographic diving surveys.

2. Materials and Methods

2.1 Diving Surveys

Sampling was conducted by 46 person-dives down to 55m depth, targeting the circalittoral zone near Vodi on the northeast coast of Rhodes, in October-November of 2015 (Fig.1). During the survey, macroalgal samples were collected, as well as substratum samples in 15 ml Falcon™ sterile tubes.

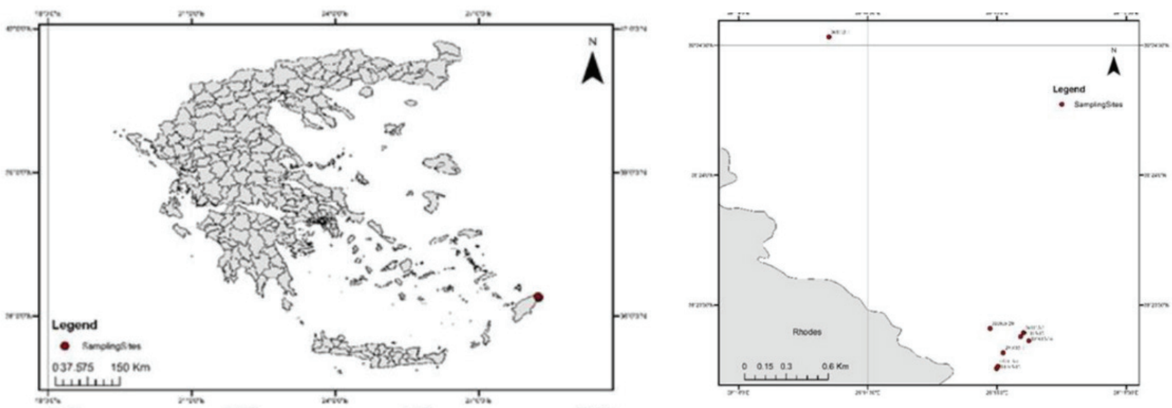

Fig. 1: Sampling sites.

2.2 Macroalgae Identifications and Germling Emergence Method

Macroalgae were identified morphologically using keys. Culturing of germling emergence isolates from substratum samples was implemented following the protocol established by Peters et al. (2015). Samples were incubated at 15°C under natural light with 35ml of Provasoli-enriched natural autoclaved sea water (Starr and Zeikus 1987) enabling examination under a stereomicroscope. Following a period of 1-3 months, monoclonal strains of filamentous brown algae were isolated, and each culture yielded 1-5 individuals that exhibited different morphologies. Ectocarpoid algae clones were isolated directly from field material. In several of these, as in a few isolates from emerging germlings, identification was possible based on morphological characters.

2.3 DNA extraction, amplification, and sequencing

The CTAB-based DNA extraction protocol for macroalgae was modified from the one reported (Gachon et al. 2009) . Few milligrams of algal biomass were transferred into Eppendorf tubes containing a stainless bead and CTAB buffer (65°C). Following, the samples were ground mechanically and incubated to prepare for DNA extraction using the Dneasy Plant Mini Kit. DNA concentration and purity was measured in an Implen Nanophotometer™. PCRs were performed, and different PCR programs were used for amplification for each primer pair. Cycling conditions included an initial denaturation step followed by

33-35 cycles of extension-elongation, one cycle of final elongation and, finally the final hold. PCRs were realized with primers for the following DNA regions: i) 5'-COI gene (cox1, cytochrome oxidase, subunit I – 5' end), ii) rbcL gene (Ribulose bisphosphate carboxylase large chain), iii) rbcL-rbcS spacer, vi) SSU partial gene (ribosomal small subunit).

The diluted purified PCR samples were sent to be sequenced to a commercial sequencing provider. The amplified sequences were obtained by the sequencing company together with the chromatograms rom forward and reverse primers. The obtained sequences were checked for quality, aligned using AliView software (GNU General Public License, editor version 1.23) to produce single consensus sequences for each strain/DNA locus investigated in the present study. Taxonomic and nomenclatural aspects (Guiry and Guiry 2022) were confirmed through AlgaeBase. The generated sequences were run through the nBLAST tool of NCBI to find the closest similarity with known sequences.

3. Results

Germling emergence method isolates

The germling emergence method yielded a total of 56 isolates including a minute chromophyte alga. The thalli had the shape of a minute bush of up to ca. 500μm in height, consisting of cylindrical cells of 15-20μm in length and 4-5μm in diameter, which showed sub-dichotomous or whorled branching. The basal cell of the thallus was wedge-shaped and attached to the petri dish by amorphous material secreted at the lower end of the basal cell. Plastids were brown and of elongated shape, without pyrenoid.

(**a**) (**b**)

Fig. 2. *Schizocladia ischiensis* from laboratory culture. (a) Strain RH15-53 from Rhodes. Note attachment by amorphous extracellular material (arrow) at lower end of wedge-shaped basal cell (b) Strain KU-333 from Ischia. Note similar dimensions and overall morphology.

4. Discussion/Conclusion

Based on sequences and morphology, isolate RH15-53 from 24m depth was identified as *Schizocladia ischiensis* (Rizouli et al. 2020). This finding constitutes the second-ever record and isolate of *Schizocladia ischiensis* of the poorly known monotypic Schizocladiophyceae, sister group of the brown algae (Phaeophyceae). Our new isolate differed slightly from the previous isolate of the species because it showed an entirely upright habit consisting of cylindrical cells, lacking specialized cells that would serve to fix them to the substratum. Instead, attachment of the thallus was by an amorphous, apparently sticky substance. This kind of attachment reminds of species of the Stylonematophyceae (Rhodophyta; West *et al.*, 2016) or of colonial benthic diatoms such as *Berkeleya rutilans* (Trentepohl ex Roth) Grunow (Hamsher and Saunders 2014) or *Licmophora flabellata* (Greville) C. Agardh (Ravizza and Hallegraeff 2015). It is possible that this likely rather weak attachment of *Schizocladia*, Stylonematophyceae and colonial benthic diatoms is one of the reasons why larger thalli, which would resist to stronger drag forces, have not evolved in these groups.

Our isolate was from the lower infra littoral zone site (24 m depth) and possibly is a common member of the community of macroalgae occurring in the deeper sublittoral. In such habitats, phycologists are often constrained by limited diving time and visibility, which is exacerbated with increasing depth. Also, in many Mediterranean and tropical coral reef locations, much of the actual macroalgal diversity may not be conspicuous during diving surveys due to the intense grazing activities (Tsirintanis *et al.*, 2018). It is not by coincidence that both the original Ischia isolate of *Schizocladia* and ours from Rhodes emerged in the laboratory from cultivated substratum and were only detected by meticulous scrutiny of the incubated dishes. Several Ectocarpales were recorded over a depth range from 24 to 52m as expected for the region. The most diverse sample was the one collected at 43.2m depth with Ectocarpales being the dominant isolates of the sample. At the shallowest sampling point (24m), there were Ectocarpales as well as Ulvales. Highest diversity was detected at 42m depth with 6 different phyla identified in this sample and Ectocarpales being the dominant phylum.

The surveys in the present study provide substantial complementary data to already established species checklists for the area. Possibly, the species observed here have existed in these communities without yet been recorded either because they emerge in cryptic stages or because there were not enough surveys to capture them or both. It is also possible that since Rhodes and the southeast Aegean are a focal point for the establishment of invasive species (Tsiamis 2012) new records will continue to appear. In this context, the development of the Germling Emergence Method has enabled numerous new records and discoveries of macroalgal taxa around the world.

5. Acknowledgements

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A DETAILED EXAMINATION OF ALGAE SPECIES FROM A CORALLIGENOUS SITE OF RHODES ISLAND (DODECANESE, GREECE)

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Abstract

Coralligenous assemblages or maërl beds have been characterized as priority habitats from the EU, however their biodiversity and distribution in the Eastern Mediterranean is still unknown. Through our extensive collections and photographic surveys around Rhodes, we here present a detailed ecological and taxonomic examination of the algae on a coralligenous site on the NE coast of the island. Sampling was conducted by 46 person-dives down to 55 m depth. Stereoscopy and microscopy were used for the macroalgal identifications, while detailed SEM examination was performed for the identification of the coralligenous algae. In total, 2 phanerogams and 36 algal species were identified, 10 of them belonging to green, 8 to brown and 18 to red algae respectively. One of the red algae identified, *Ptilophora (Beckerella) mediterranea*, is endemic for the south Aegean. The surveys revealed the presence of 7 non-indigenous species which include 3 *Caulerpa* species and the red seaweed *Womersleyella setacea*, one of the worst invaders of the Mediterranean.

Keywords: coralligenous, circalittoral, rhodoliths, electronic microscopy.

1. Introduction

The Mediterranean is a crossroad of biodiversity and at least 315 species of macroalgae have been reported from the coralligenous ecosystem (Boudouresque, 1973; Ballesteros, 2006). Coralligenous assemblages are multispecific habitats, hosting many different species such as coralline algae, bryozoans, cnidaria, serpulids and other organisms growing on top of them, the majority of them slow growing, therefore prone to climate change and other stress factors such as turbidity, sediment deposition, biological invasions, ocean acidification and climate change (Piazzi *et al.,* 2012). Coralligenous communities and maërl beds have been characterized as priority habitats by the EU (EU 1992) having a high importance for fisheries as fishing grounds, diversity hotspots, harboring 20% of the Mediterranean species (Garrabou & Harmelin, 2002, Ballesteros 2006; Piazzi et al., 2010; Canovas-Molina *et al.,* 2016) and being significant carbon storages (Ballesteros 2006; Salomidi *et al.,* 2012), while at the same time they are considered highly vulnerable to long-term climate change. These habitats occur from 15 m in the western part to more than 120m in the East. They are common at 40-60 m depth in dim light conditions. The exact location of coralligenous habitats and their algal diversity in Greece remains unconfirmed, especially due to undersampling but also due to the lack of funding for such scientific expeditions. Algal species that inhabit the twilight zone are difficult to collect by man-dives due to the increasing depth. This is especially the case for the Eastern Mediterranean, where water transparency allows photosynthesis deeper (Küpper *et al.,* 2019).

The shallow-water seaweed flora of Rhodes Island (SE Greece) has been studied rather intensively over the last 20 years, especially because of its high interest on the finding of new species arriving via the Suez Canal (Tsiamis, 2012), nevertheless many gaps remain and therefore, deep waters are still a promising field for biodiversity research.

2. Materials and Methods

2.1 Diving Surveys

A coralligenous habitat was spotted on the northeast coast of Rhodes Island (SE Greece) by Multi-Beam scanning and random deep water grab sampling. Based upon this, in late 2015, sampling was conducted by 46 person-dives down to 55 m depth, targeting the circalittoral zone (Fig. 1).

Fig. 1: Sampling sites at the NE coast of Rhodes Island, Vodi Area.

Seaweed specimens were collected by SCUBA diving, as entire thalli, where feasible in triplicates, which were subsequently conserved as herbarium specimens on Bristol paper or (for smaller specimens, <1 cm) on microscope slides using acetocarmine as fixative and dye and 50% Karo™ syrup as embedding medium (Müller and Ramírez 1994). Photos of the habitat were taken at each sampling station. Working at such depth required larger than usual air cylinders, independent bail-out cylinders, extra air for the decompression stops and a bottom-reel for the divers to find their way back to the decompression lines.

2.2 Macroalgal Identifications and Scanning Electron Microscopy (SEM)

Species were identified down to species level immediately after collection using their morphological features and keys specific for the area (Guiry and Guiry 2021) as well as publications on newly reported species, especially those arriving through the Suez Canal (ELNAIS 2012). Calcified red algae and small rhodoliths were left at the lab to air dry. Anatomical analysis of coralline algae was achieved by scanning electron microscopy (SEM). To this end, fragments of samples were mounted on aluminum stubs with acrylic adhesive and sonicated using a Vitec sonicator (Carlsbad, CA, USA) to remove sediments. Prior to SEM observation, samples were coated with gold (Sputter Coater K550X, Emitech, Quorum Technologies Ltd, Laughton, East Sussex, UK). Observation was carried out using a Quanta250 SEM (FEI Technologies Inc, Hillsboro, OR, USA).

3. Results

3.1 Qualitative analysis at species/phyla level

A total of 36 macroalgal species were detected, 10 of them belonging to green, 8 to brown and 18 to red algae respectively. Also, 2 species of Phanerogams where identified, *Posidonia oceanica* and *Halophila stipulacea*, the latter being a non-indigenous species arriving through the Suez Canal. The surveys revealed the presence of another 6 non-indigenous species, *Asparagopsis armata*, *Caulerpa lamorouxii*, *Caulerpa racemosa* var. *cylindracea*, *Caulerpa taxifolia* var. *distichophylla*, *Womersleyella setacea* and *Stypopodium schimperi*. One of the red algae identified, *Ptilophora (Beckerella) mediterranea* (Huvé 1962) is endemic for the south Aegean.

3.2 Coralline red algae

Among the specimens collected, six taxa of coralline algae were identified (Fig. 2). On hard bottom the reef builder *Lithophyllum stictiforme* was found, while *Lithothamnion corallioides*, *Lithothamnion minervae*, *Spongites fruticulosus* and *Titanoderma* sp. were found as rhodoliths (i.e., free-living thalli composed mostly of calcareous red algae. The epiphyte *Pneophyllum confervicola* was observed colonizing the surface of some of the rhodoliths.

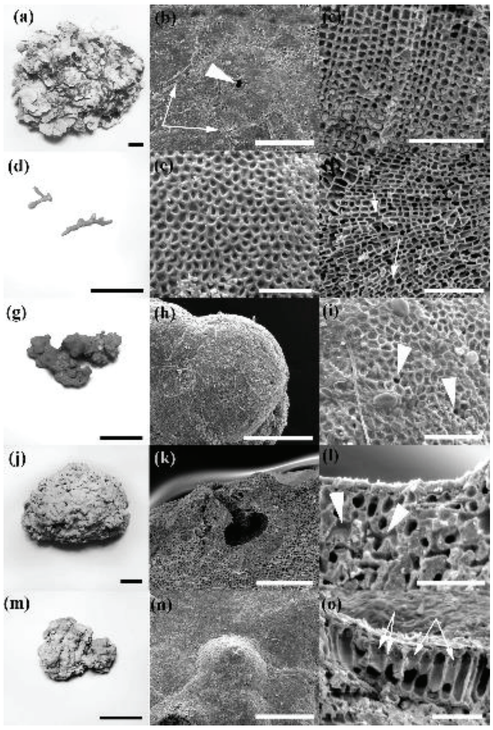

Fig. 2: *Lithophyllum stictiforme* (301015-I): (**a**) external morphology (scale bar 1 cm); (**b**) uniporate conceptacle (arrowhead) and epithallus in surface view overgrown by thalli of *Pneophyllum confervicola* (arrows) (scale bar 200 μm); (**c**) section with perithallium (scale bar 100 μm). ***Lithothamnion corallioides*** (0311152-C): (**d**) unattached branches (scale bar 1cm); (**e**) epithallus in surface view (scale bar 50 μm); (**f**) section of a branch (scale bar 100 μm), with filamanets linked by lateral cell fusions (arrows). ***Lithothamnion minervae*** (031115-C): (**g**) unattached praline (scale bar 1cm); (**h**) multiporate conceptacle in surface view (scale bar 300 μm); (**i**) detail of the pores of the conceptacle (arrowheads) (scale bar 50 μm). ***Spongites fruticulosus*** (031115-D): (**j**) unattached thallus (scale bar 1cm); (**k**) section of an uniporate conceptacle (scale bar 300 μm); (**l**) section of the epithallus and perithallium with cells of neighbouring filaments linked by lateral cell fusions (arrowheads) (scale bar 50 μm). ***Titanoderma* sp.** (031115-D): (**m**) unattached praline (scale bar 1cm); (**n**) uniporate conceptacle in surface view (scale bar 500 μm); (**o**) section of the thallus with cell joined laterally by secondary pit-connections (arrows) (scale bar 50 μm).

4. Discussion

The results of this study provide new insight into the circalittoral macroalgal diversity in the Aegean Sea down to 52 m. A total of 36 algal species were identified, namely 10 Chlorophyta, 8 Phaeophyta and 18 Rhodophyta respectively. A higher representation of Rhodophyta in our samples was expected for this habitat firstly because of the sampling depth and due to the Wastewater Treatment Plant (WWTP) of the island located near the sampling site, offering suspended organic matter in the water column, therefore producing dim light conditions shallower. Two species of Phanerogams where identified, *Posidonia oceanica*, a habitat building species, and *Halophila stipulacea*, an introduced species of Indo-pacific origin. *Womersleyella setacea*, a red alga, is considered one of the worst invaders of the Mediterranean (Streftaris and Zenetos 2006) with unknown effects on coralligenous communities to date. Two of the red algae identified, *Ptilophora (Beckerella) mediterranea* and *Osmundaria volubilis* were previously recorded in the south Aegean but recently also from the Ionian Sea (Athanasiadis 1987, Tsiamis et al. 2013). On the contrary, important species of coralligenous assemblages (e.g *Corallium rubrum, Paramuricea clavata*) were not observed, maybe because of the diving depth limitation. These species might be encountered in deeper waters. The surveys in the present study provide substantial complementary data to already established species checklists for the area. Possibly, the species observed here have existed in these communities without yet been recorded either because they emerge in deeper waters or because there were not enough surveys to capture them or both. Furthermore, use of SEM is a key for coralline algal identifications and more species records.

The coralligenous flora of the Western Mediterranean shows higher diversity but this could be attributed to the fact that coralligenous assemblages (e.g., in southern France) are much easier to study than in SE Mediterranean due to the much shallower depth at which they occur there (15 m as opposed to > 40 m off Greece). In this context, the location of Rhodes in the southeast Aegean makes it a focal point for the establishment of invasive species (Tsiamis 2012), therefore new records and discoveries of macroalgal taxa await.

5. Acknowledgements

This research was funded by Greece and the European Union (European Regional Development Fund - ERDF) through the Operational Programme “Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK)” (MIS 5045792) “DRESSAGE”. This project was also supported by SAMS and NFSD core funding (Oceans 2025 WP 4.5 from the UK Natural Environment Research Council) and the TOTAL Foundation (Paris; Project “Brown algal biodiversity and ecology in the Eastern Mediterranean Sea”).

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AN IMPROVED GENOME ASSEMBLY FOR THE EUROPEAN SEA BASS *DICENTRARCHUS LABRAX* USING LONG READ SEQUENCING DATA

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Abstract

The European sea bass is a species of high significance for European and Mediterranean aquaculture. The availability of a high-quality reference genome for the species would provide a valuable resource for follow up genetic or physiological studies and improving breeding selection efforts. Here, we present a new highly complete chromosome level genomic assembly for the European sea bass. We use a combination of in-house short and long read data to construct a high-quality assembly, which is then scaffolded to chromosome level based on the existing assembly. The new assembly includes almost 20 megabases of missing information and integrates more than 96% of the total length of the genome in 24 chromosomes, an integration increase of more than 93 megabases. This level of contiguity and completeness will allow for better SNP discovery and comparative work, empowering downstream applications.

Keywords: genomics, aquaculture, minion.

1. Introduction

The European sea bass (*Dicentrarchus labrax*) is one of the two most important species for the Mediterranean aquaculture, along with the gilthead bream (*Sparus aurata*), accounting together for a yearly production of over 212,977 metric tonnes (APROMA, 2019). Hence, commercial interest in the species has spurred a large number of studies in various fields such as nutrition, immunology, reproductive physiology, and genetics(Vandeputte *et al.*, 2019). Detailed genome sequence information would greatly boost both fundamental and industrial research, as shown by the high citation impact of the paper reporting the first genome draft for European sea bass (Tine *et al.*, 2014). However, the contiguity and completeness of this draft assembly may not be nowadays sufficient for various demanding analyses, such as uncovering the spatial organization of genes or gene clusters, linking regulatory elements to protein coding genes, and sliding-window analysis of genetic variation. Furthermore, highly contiguous genome assemblies are extremely important for the identification and optimal choice of SNPs to create high-throughput SNP assay tools (SNP-chips or SNP-arrays). Alternatively, high quality genome assemblies will ensure the best strategy to map SNPs obtained through genotyping by-sequencing (RAD, ddRAD, 2bRAD, Pool-Seq, low coverage whole-genome resequencing) (Robledo *et al.*, 2018) and for missing data imputation (Tsai *et al.*, 2017). The present study aimed at using long DNA sequences to improve the existing European sea bass genome assembly, in order to obtain a nearly complete genome for high quality annotation and the establishment of a genome browser for organizing future European sea bass genomic resources. Overall, this will improve genomic selection and selective breeding programmes, supporting the growing number of hatcheries that are employing genetic methods to increase aquaculture production in the species (Chavanne *et al.*, 2016).

2. Materials and Methods

2.1 Genomic DNA Isolation

High molecular weight genomic DNA (>30 kb) was isolated using the Genomic-tip Kit (Qiagen) from double-haploid sea bass biological samples. Samples were treated with RNAase A and eluted in the provided elution buffer. DNA quantity and quality were assessed using a Qubit®ds DNABR Assay Kit (Invitrogen–Thermo Fisher Scientific), a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and by loading an aliquot on an Agarose Megabase gel (Biorad) with an extended range DNA ladder.

2.2 Sequencing Data Acquisition

Long-read sequencing was carried out using the Oxford Nanopore Technologies (ONT) MinION platform that provides contiguous long reads. The short-read data was previously produced from a second generation (Illumina HiSeq4000) platform, while libraries were prepared using the Kapa Hyper Prep DNA kit. A total of 6 flowcells were sequenced on the MinION platform (Oxford Nanopore Technologies). The Illumina data used was retrieved from a larger set of previously published genomic data from pooled *Dicentrarchus labrax* DNA (Peñaloza *et al.,* 2021), selecting populations of three different fish farms in Greece. These populations that represent farm populations, are expected to have much lower levels of genetic variation compared to wild populations.

2.3 Genome Assembly

A containerized pipeline for *de novo* genome assembly built in-house (Angelova *et al.,* n.d.) (https://github.com/genomenerds/SnakeCube) was used to assemble the genome from MinION long read data and polish the resulting assembly using Illumina pool-sequencing short read data. The assembly pipeline integrates various software to carry out all of the assembly and polishing steps. First, Trimmomatic (Bolger *et al.,* 2014) is used for read trimming, while Nanoplot (de Coster *et al.,* 2018) and fastqc (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) are used for MinION and Illumina data quality control respectively. Subsequently, the Flye assembler (Kolmogorov *et al*., 2019) builds the initial assembly of the long read data, while Racon (Vaser *et al.,* 2017) and Medaka (https://nanoporetech.github.io/medaka/) perform an initial polishing round using the long read data. Then, Pilon (Walker *et al.,* 2014) uses the short read data in the final polishing step to improve the polished long read assembly. Quast (Gurevich *et al.,* 2013) and Busco(Simão *et al.,* 2015) (BUSCO v4.1.2, actinopterygii_odb10 database) were used between polishing steps to assess the quality of produced assemblies in terms of contiguity and completeness, respectively. Merqury (Rhie *et al*., 2020) was also used to assess the completeness of the final assembly, based on kmer counting and the Illumina data. The final assembly obtained from this process was then scaffolded to a near chromosome level guided by the published assembly by Tine et al. (Tine *et al.,* 2014), using RagTag (Alonge *et al.,* 2019) to map and scaffold our assembly contigs based on the published scaffolds.

3. Results

The long-read assembly produced was composed of 574 contigs, with an N50 (a statistic defined as the sequence length of the shortest contig at 50% of the total genome length) of 12,698,854bp, a BUSCO score of 95.4% and 105 missing BUSCOs. Polishing with the short-read data provided an improved assembly with a total length of 694,870,088bp and an N50 of 12,677,075bp, increased the BUSCO score to 98.7% and gave a final Merqury completion score of 96.6%. Further scaffolding of the contigs of this polished assembly, guided by the 24 chromosomes of the previous draft assembly by Tine *et al.* (2014) provided a final assembly composed of 302 scaffolds, with a total length of 695,892,153bp and an N50 of 29,885,359bp. Furthermore, the 24 largest scaffolds contain 96.669% of the total length of the assembly. Hence, the current assembly is at near chromosome level, with the 24 largest scaffolds corresponding to the 24 chromosomes of the species. This new assembly provides a substantial increase in total sequence information and contiguity, increasing the total length of the genome from 675,917,103bp to 695,892,153 bp (19,975,050 bp difference), reducing the amount of missing data (from 1,133.75 Ns/100kbp previously to 146.87 Ns/1,000kbp) and greatly improving contiguity, with 24 scaffolds containing 96.6% of the total length compared to 85.6% reported previously. Metrics related to the sequencing data used and the final assembly produced are detailed in Table 1. The assembly has been submitted in the European Nucleotide Archive (ENA) under the accession GCA_905237075 (https://www.ebi.ac.uk/ena/browser/view/GCA_905237075.1).

Table 1. Sequencing and Assembly Metrics.

Sequencing Metrics		
	MinION	**Illumina**
Number of reads	4,209,595	482,323,460
Mean read length	10,284.1	150
Mean read quality	12.8	37.9
Read length N50	14,173	-
Total bases	43,291,702,904	72,348,519,000
Coverage	54.5	30.9
Assembly Metrics		
Length	695,892,153bp	
Number of contigs	302	
N50	29,885,359bp	
N75	25,354,164 bp	
L50	11	
L75	17	
GC%	40.38	
#N'sper100kbp	146.87	
% of length in 24 Scaffolds	96.60%	
Merqury Completeness Score	96.60%	
Assembly BUSCO		
BUSCO Score	98.50%	
Single-copy BUSCOs	97.40%	
Duplicated BUSCOs	0.70%	
Fragmented BUSCOs	0.40%	
Missing BUSCOs	1.50%	
total BUSCOs searched	3,640	

4. Discussion

The new genome constitutes a considerable improvement over the previous version, adding a large amount of missing sequence and integrating the vast majority of data in 24 chromosomes. In addition, completeness metrics highlight that the present assembly is highly complete, while missing data has been significantly reduced. Undoubtedly, this new resource will consist a valuable tool for follow up studies on various aspects of European sea bass biology and greatly facilitate practical work on the species, including SNP discovery and selection for breeding effort improvement.

5. Acknowledgements

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SPECIAL SESSION:
ANTHROPOGENIC LITTER AND PLASTICS POLLUTION

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2022

LEVERAGING ARTIFICIAL INTELLIGENCE FOR TACKLING MARINE LITTER POLLUTION: A SURVEY AND A WEB DATABASE

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Abstract

A literature survey was conducted to explore the state of knowledge regarding the use of Artificial Intelligence (AI) on marine litter research with the aim to provide a resource for scientists who want to apply AI techniques for their case study and discuss limitations, together with possible directions for further research. Our analysis showed that AI has the potential to provide automatic, rapid, and scalable solutions for tackling marine litter pollution. However, the geographic distribution of real-world applications combining AI and litter is still scarce, publications are mainly restricted to technical implications, and onsite image datasets are limited. This highlights the need to adopt a more strategic and sustainable approach that will engage AI in a more efficient way. A web database is also presented. The user can query papers based on categories and tags, as well by bibliometric information. The database is operational and new publications will be uploaded regularly, aiming to become a resource for marine scientists that want to follow the state-of-the-art progress of AI on the theme. The database can be found here.

Keywords: marine litter, survey, artificial intelligence, deep learning.

1. Introduction

Anthropogenic Marine Litter (AML) constitutes one of the most pervasive and persistent threats in our planet (Bergmann *et al.*, 2015). Marine litter is commonly found on the sea surface, beaches and seafloor, or washed up on shorelines, after entering the seas from land and marine-based sources (UNEP, 2016; NOAA, 2016). Marine litter can injure or kill marine fauna, degrade coastal habitats and the quality of life in coastal communities, and threaten human health. Monitoring the marine environment is the foremost step to assess the type, abundance and distribution of collected marine litter items with the aim to assess the state or level of pollution and, in turn, design mitigation measures and clean-up activities (Kershaw *et al.* 2019). However, litter characterization, especially in images and videos is a challenging task. Litter experts need to process thousands of images to locate litter objects and harmonize tons of schemas and metadata of data reported, a process that is time-consuming and costly. Concurrently, the volume of marine litter data is growing and is expected to grow further in the future. This signifies the need for automated tools (Canals *et al.*, 2021). Modern artificial intelligence (AI) technology called deep learning offers the opportunity to make this automation feasible. AI is one of the branches of computer science and information technology that attempts to create machines to behave as intelligent as humans (LeCun *et al.*, 2015). For researchers whose expertise is outside of computer science, becoming familiar with the applications of AI on marine litter research and keeping track of new developments is valuable. The aim of this study is to provide a resource for marine litter scientists who want to apply AI techniques for their case study. As such, we conducted a systematic literature review of peer-reviewed papers that use AI to resolve marine litter tasks. Additionally, we present a web database from which marine litter scientists can seek publications of interest based on a list of categories and tags, providing an easy way to follow the progress on the theme.

2. Material and Methods

Document collection was conducted using the Scopus and Google Scholar databases to access peer-reviewed key papers that implement AI techniques for conducting marine litter research. The terms used as search criteria were: “deep learning + marine litter/debris”, “machine learning + marine litter/debris” and “artificial intelligence + marine litter/debris”. The search returned 41 documents with a digital object identifier (DOI). A content analysis was adopted to examine the selected documents. The content categories defined to explore the progress of AI on marine litter were ‘Litter deposit’, ‘Dataset’, ‘Data type’, ‘Goal’, ‘Region’, ‘Approach’, and ‘Implications’ (Table 1). For each category, tags are defined to describe specific topics and associate further related content.

Table 1. Content categories and tags used to explore the progress of AI on marine litter research.

Content category	Tags
Litter deposit	Floating, Seafloor, Beached
Dataset	Open, Onsite
Data type	Image, Orthophoto, Tabular
Goal	Classification, Detection, Segmentation, Quantification
Region	(Longitude, Latitude)
Approach	Convolutional Neural Networks (CNN), Object Detection (OD), Feed Forward Neural Network (FFNN), Machine Learning Algorithms (MLA), Generative Adversarial Networks (GAN).
Implications	Technical, Practical

An online database with the collected papers was also created. Particularly, the database includes a map with reported applications around the globe and an interactive table from which the user can query papers based on the adopted content categories and tags, as well by auxiliary bibliometric information, such as scientific journal, title, year, author and DOI link. The database was built with Python using the web app framework Streamlit, and it’s available at the URL address: https://share.streamlit.io/dimpolitik/ai-marine-litter/main/app.py

3. Results

From 2004 to March 2022, the rate of yearly publications referring to AI and marine litter did not follow a linear pattern (Fig. 1). Before 2016, only one paper exploring the theme was published. After 2018, we notice an increased interest in the topic, which exploded in 2020 and 2021, with more than twelve paper per year. Four publications were reported till March 2022 (end of current review), indicating a growing annual rate of publications in the years to come. Although marine litter has a worldwide presence, there is currently a limited use of AI to tackle it. No study has been carried out on the African continent and Australia, and just one study was developed in the coastal waters of America (Fig. 2). In Europe and Asia continents, nine and seven studies were reported.

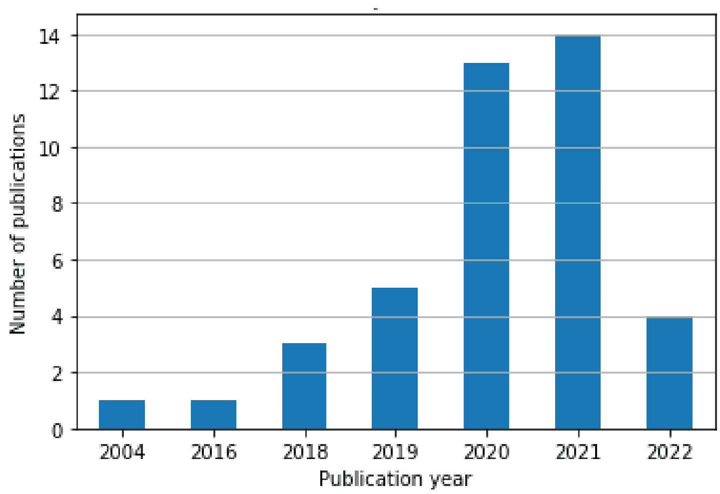

Fig. 1: Number of peer-reviewed publications that implement AI techniques on marine litter research between 2004 and March 2022.

The number of publications per content category is shown in Figure 3. The analysis of floating and beached litter was slightly higher than seafloor litter in the reviewed papers. Most studies set as a goal the classification (n=33) and detection (n=21) of litter items found in the datasets and less quantification (n=7) and segmentation (n=7); one study (n=1) calculated the volume of litter items (Kako *et al.*, 2020). OD (n=18) and CNN (n=11) algorithms were mainly adopted by scientists for their analysis, being in accordance with the goal of their study. MLA were used in n=8 cases, whereas FFNN and GAN were the less used methods with n=3 and n=2 cases. The use of open and onsite datasets was rather balanced with n=22 and n=19, respectively. Finally, images (n=31) or orthophotos (n=9) were the majority data type, with only one study (n=1) using tabular data (Kaandrop *et al.*, 2022).

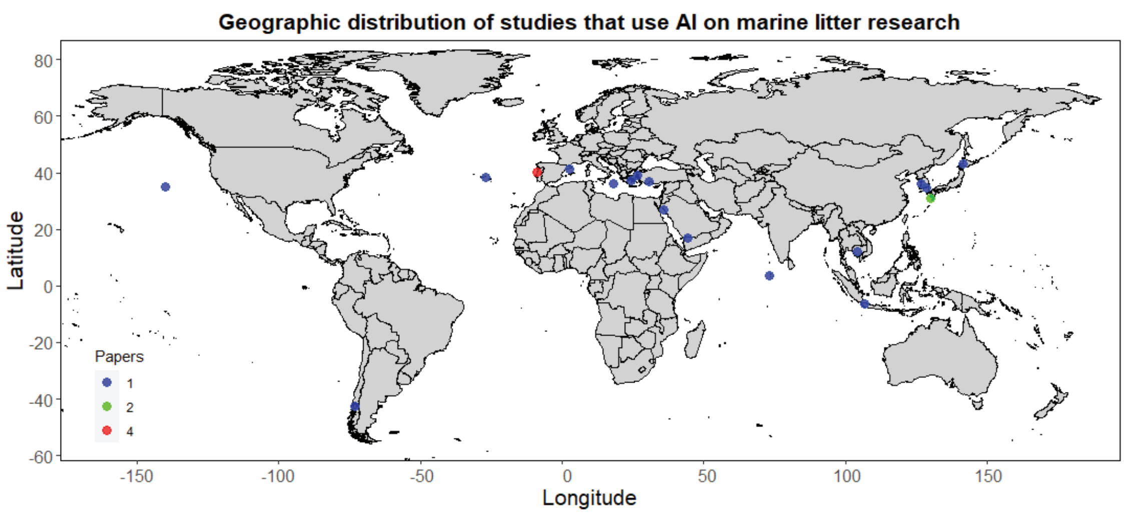

Fig. 2: Geographic distribution of regional case studies that implement AI techniques for marine litter research with onsite datasets during 2004 – March 2022. In total, n = 22 papers used an onsite dataset for a regional case study.

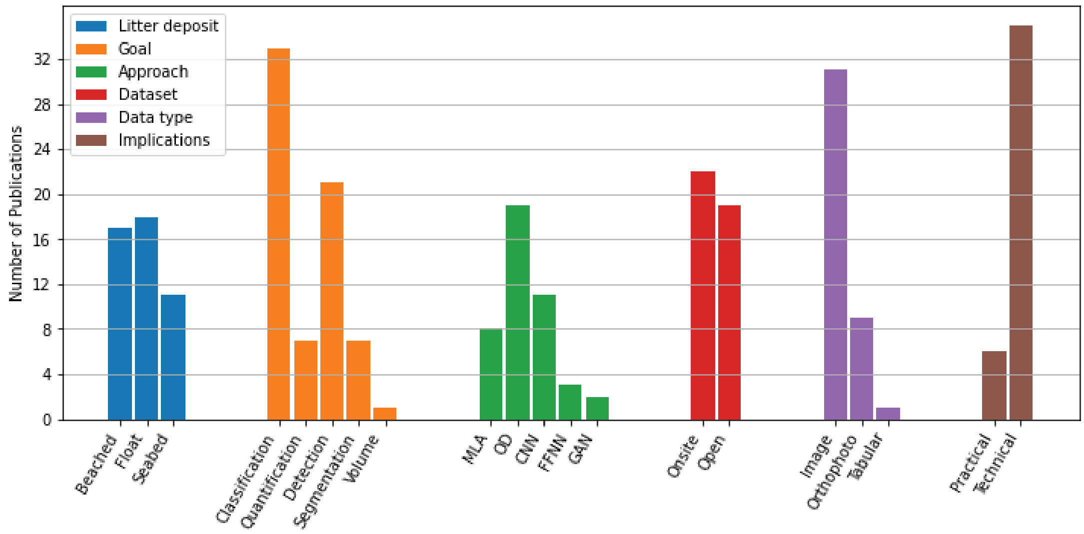

Fig. 3: Number of publications per content category. We note that multiple options were possible for the categories 'Litter deposit', 'Goal' and 'Approach'. Total number of surveyed papers was n= 41.

4. Discussion/Conclusion

From the total documents gathered in the search, almost half of the publication used open image datasets for their analysis (e.g., Córdova *et al.*, 2022; Hong *et al.*, 2020). Although open image datasets have the benefit that the expensive data collection and labelling of images have already done, however, they don't have practical implications to process big datasets from litter monitoring programs, since most of them were built in controlled setups, i.e., with only one instance of litter per image or taken in indoor scenarios. Accordingly, most publications (36/41) focused on technical aspects aiming to achieve high performance and compare algorithms and did not automate processes for practical use (e.g., Fulton *et al.*, 2018; Kyllili *et al.*, 2019). Exception was the studies that had as a goal the quantification of marine litter (de Vries *et al.* 2021; Gonçalves *et al.*, 2020; Papakonstantinou *et al.*, 2021), which provided an automatic estimation of litter density. Additionally, the geographic distribution of real-world applications is still scarce, with onsite image datasets being limited.

Our analysis showed that AI has the potential to provide automatic, rapid, and scalable solutions in marine litter (e.g., Acuña-Ruz *et al.* 2018; Martin *et al.*, 2021; Pinto *et al.*, 2021). However, close collaboration of computer scientists with marine litter experts through working groups and projects is an important step to build an innovative, strategic, and sustainable approach that will engage AI in a more efficient way. Additionally, systematic collection of litter images and videos from realistic environments and more effective algorithms are crucial steps to build reliable and operational tools for marine litter stakeholders.

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PRELIMINARY RESULTS ON THE VERTICAL DISTRIBUTION OF MICROPLASTICS IN THE AEGEAN AND LEVANTINE SEAWATERS. INSIGHTS FROMTWO DIFFERENT SAMPLING NETS

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Abstract

We investigated microplastics' vertical distribution in the surface and subsurface water layer in the oligotrophic East Mediterranean Sea. The Manta net, a well established microplastic sampling method was deployed at the surface simultaneously with a WP3 plankton net at the subsurface layer. The microplastics' abundance in volume, maximum item length in mm and shape were determined at the surface and subsurface of the Aegean & Levantine Seas. Zooplankton biomass was measured at the subsurface samples. Microplastic concentrations at the subsurface waters were recorded for the first time in the Greek sea waters. Of all microplastics shape types, fragments and filaments had the highest abundance in both layers. The majority of fragments at the surface had sizes of 1-5mm whereas at the subsurfacemost fragments fell in the 300μm – 1mm size range. Filaments in both layers were found more abundant in the 1-5mm size range.Our results present, the so far unknown microplastic contamination, in the subsurface layer of the Aegean& Levantine waters and provide preliminary insights on the co-existence of microplastics and zooplankton inthe same ecological niche.

Keywords: Manta net, plankton net WP3, microplastics, zooplankton.

1. Introduction

Pollution by microplastics, plastic items ≤ 5mm, is a threat to marine ecosystems. Great concern has been raised, on their global occurrence and their potential impacts on the marine environment including their uptake by various organisms (Thompson *et al*., 2004; Barnes *et al*., 2009). Microplastics are classifiedin two categories, primary and secondary microplastics. Primary ones are plastic resin pellets used as raw materials andplastic items used intentionally in products, i.e., cosmetics.Secondary ones originate either from physical fragmentation of larger plastic itemsand are produced on land and/or on beaches, or during the use of products (i.e.synthetic fibers deriving from wastewater treatment plants)and are further transported to the marine envi.ronment through runoff. Their low density comparedto seawater, allows them to float for long periods in surface waters before being washed off on beaches and/or deposited on the seafloor. The universal risks derived by marine plastic to marine and coastal life, ecosystems and potentially human health are widely acknowledged. The EU Marine Strategy Framework Directive (MSFD) (2008/56/EC) addresses directly the marine litter problem by Descriptor 10 (Properties and quantities of marine litter – including microplastics- do cause harm to the coastal and marine environment). In particular, microlitterin the marine environment is included in the primary criteria (D10C2) (2017/848/EU) andseasurface floating microlitterisoneof the parameters studied. Microplastic contamination has been mainly investigated in the Western and Central Mediterranean Sea (Collignon *et al*., 2014; Cózar *et al*., 2015),while fewer studies exist in the Eastern part (van der Hal *et al.,* 2017; Gundoglu *et al*., 2017; Adamopoulou *et al*., 2021). Although zooplankton and floating microplastics share the same ecological niche there is still lack of information on their coexistence in the Mediterranean Sea (Collignon *et al*., 2012; Collignon *et al*., 2014). The present study aimed to investigate the presence of subsurface microplastics contamination in the East Mediterranean Sea. Also, to gain insights on the zooplankton biomass and the microplastics abundance. The study was carried out at standard monitoring sampling locations as part of the national monitoring program of the MSFD, and Manta net, which is used in the vast majority of surface microplastics surveys (Pasquier *et al*., 2022) was deployed simultaneously with the plankton net WP3.

2. Material and Methods

2.1 Study Area and Microplastics sampling

The study area covered parts of the Aegean and Levantine Sea. In total sixteen samples (16) were collected during the sampling campaign in March 2021. Samplingswereconducted with the research vessel R/V Aegaeo. Eight (8) samples, were collected with each net, the Manta net and the plankton net WP3, both equipped with a flow meter (HydroBios).The Manta net washorizontally towed for 30min, from the side of the vessel and beyond the vessel's wake. The WP3 was horizontally towed for 20 min. The nets were towed simultaneously. The Manta net samples approximately the top 15 cm of the water column and its dimensions are: W60 × H24 cm rectangular frame opening; net 3m length and mesh size of 330 μm. The WP3 net samples between 0.5 – 2m below the sea surface, and has the following dimensions: diameter of 113cm, conical bag length of 200cm and mesh size of 300μm. The vessel speed was always kept <2 knots. At the end of the tows, the netswerewashed, and therefore all particles were gathered in the cod end.

2.2 Microplastics and zooplankton analysis

The samples were filtered through Glass Microfibre Filters GF/C filters under a laminar flow bench (HN14) to avoid contamination. Then dried at 40 °C, and the dry weight was obtained. The dried sample was then examined under a stereomicroscope. The counting of microplastics, identification of shape type and size was performed using the OLYMPUS SZX10 stereomicroscope, equipped with a digital camera and the INFINITY ANALYZE software was used to measure the microplastics dimensions. For the characterization of the microplatics polymer type, ATR-FTIR spectroscopy was used (Agilent Cary 630; Perkin Elmer Spectrum Two with (UATR) accessory with a 9-bounce diamond top-plate). Furthermore, fiber free (tyvek) lab coats were used and airborne contamination was estimated by using blank filters at all stages of the analyses. For the zooplankton biomass the samples were filtered, dried and then weighted.

3. Results

3.1 Microplastic concentrations

The microplastics concentrations, measured at the Manta net samples (Fig. 1A), showed increased variability among the samples.The concentrations ranged from 6.62 items/m^{3}to 0.60 items/m^3. Whereas the concentrations, measured at the WP3net samples (Fig. 1B), had highervariability among the samples, specifically the range was 0.28 items/ m^3 to 0.01items/m^3 .

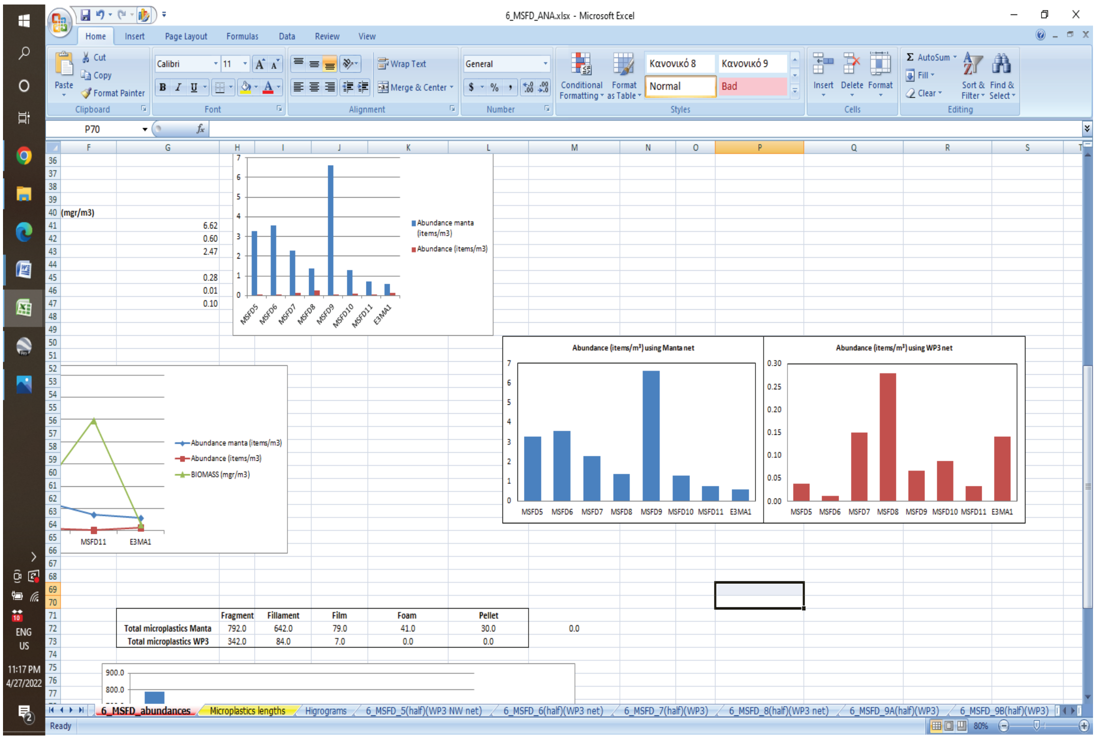

Fig. 1: Microplastics concentrations(items/m³) per sampling location(x'x), for samples collected with Manta net (A) (left- blue) and WP3 net (B) (right- red).

3.2 Microplastic propertiesand Zooplankton Biomass

The microplastics were categorized according to their shape types: fragments, filaments, films and foam. In both Manta and WP3 nets fragments had the highest percentage contribution reaching 81% and 79%, respectively. Filaments presented the second higher shape type, in both netscontributing 10% and 19%, respectively. Films had 5% presence and foams 4% presence in Manta net, whereas in WP3 films had 2% presence and there was no foam observed.Image analysis was used to measure the longest dimension of each microplastic. The frequency diagram (Figs 2.A and B) presents the microplastics size distribution forboth sampling nets. Specifically,96% of the fragments collected with Manta net had lengths between 1-5mm, peaking at 1.3mm and 2mm, while 73% of those collected with the WP3 net had lengths between 300μm – 1mm peaking at 1mm. About 95% of the filaments collected byboth nets had lengths between 1-5 mm. Forthe Manta net samples the filaments length peaked at 2mm and forthe WP3 net samples each of the lengths 1.6mm, 2.5mm, 3.2mm, 4mm and 4.5mm had equally high contribution of 13%. Furthermore, the most abundant microplastics polymer type was Polyethylene. Additionally, biomass values were determined for the subsurface layer ranged from 0.23 mg DW m^{-3} to 6.11 mg DW m^{-3}.

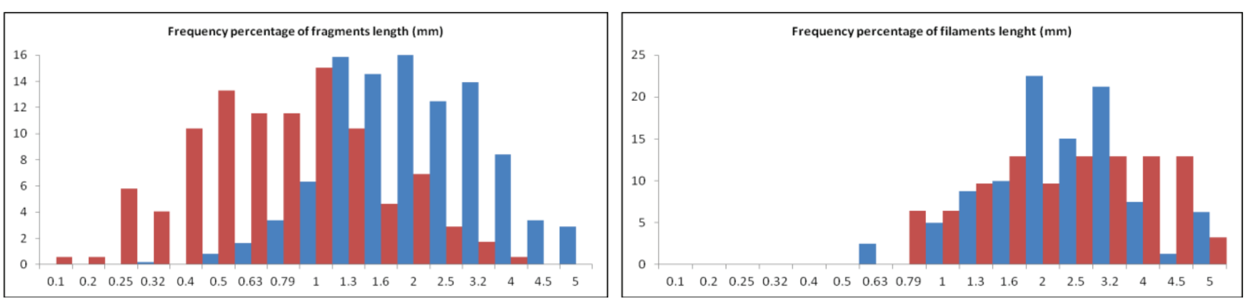

Fig. 2: (A) Histogram of the frequency distributionof fragments maximum length in mm (left). (B) Histogram of the frequency distributionof filaments maximum length in mm (right). In both (A) and (B) charts the blue columns represent the items sampled with Manta net and the red with WP3 net, also x'x presents the maximum microplastic lenght in mm and the y'y thefrequency perecentage.

4. Discussion/Conclusion

The data obtained from the present sampling campaign allow us to have a better overview of the state of microplastic contamination in the surface and subsurface water column of the Aegean&Levantine Seas. Microplastics were found in both sampling layers at all sampling stations. Our data obtained at the surface layer werewithin the range of microplastic concentrationsreported previously in the East Mediterranean Sea (Adamopoulou *et al.*, 2021; Gundoglu *et al.*, 2017). The concentrationobtained at the surface layer was highercompared to the subsurface layer, more specific 23 and 60 times of the maximum and minimum values, respectively. Furthermore, all four microplastic shapes i.e. fragments, filaments, foam and films were found at the surface. In contrast, at the subsurface there was total absence of foams. Foams include trapped air that improves their buoyancy, therefore their occurrence at the surface layer is expected. The shape fragments accumulated larger particles at the surface compared to the subsurface;for instance 96% had lengths between 1-5 mm whereas in the subsurface only 26%of fragments fell into this size range. The filaments shape in both layers, had lengths between 1-5 mm (96% of particles). The microplastics present different buoyancy based on their density, shape size and biofouling degree (Kooi *et al.*, 2016, 2017; Kaiser *et al.*, 2017). It is observed that small sized and elongated items have the tendency to float deeper as a result of the increased water friction forces (Reisser *et al.*, 2015; Kooi *et al.*, 2016). This observation matches our results which show a higher share (73%) of small sized particles (0.3 – 1mm) in the subsurface layer and also a relatively elevated contribution of filaments (19%) in comparison to the surface layer.

Further zooplankton and statisticalanalysis are ongoing, in order to gain an integrated understanding of microplastics vertical distribution and zooplankton abundance by using different sampling methods such as Manta and WP3 nets.

5. Acknowledgements

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BEACH SEDIMENT MICROPLASTICS IN THE N. AEGEAN SEA, GREECE

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Abstract

Beach sediment was collected from four sub-regions of the N. Aegean Sea, Greece, and microplastic abundance and typology was studied. The most highly impacted sub-region was found to be the Thermaikos Gulf (mean=14.10±2.33 items/50ml). Higher abundances were recorded in the beaches of Peraia, Agria and Alexandroupoli West and lowest in Agios Efstratios and Alexandroupoli East. Large scale fibers dominated in all sampled stations except Agria, a beach close to an industrialized area, where particles were more fragmented. The presence of microplastics in every site, irrelevant to the proximity to large cities, indicates the magnitude of their distribution.

Keywords: Fibers, plastic, particles, litter.

1. Introduction

It was back in the 1972 when Carpenter & Smith were describing for the first-time plastic particles of 0.25 to 0.50 centimeters diameter, found on the Sargasso Sea surface. They were then predicting that the increasing production of plastics, combined with referring to that era waste-disposal practices, will undoubtedly lead to increases in the concentration of those particles. Almost fifty years later microplastics (MPs) have been found in almost every marine habitat around the world (Lusher, 2015) and within the biota (Thompson *et al.*, 2004).

MPs are defined as plastic particles smaller than 5 mm (Arthur *et al.*, 2008). Primary MPs are plastics manufactured to be of a microscopic size (Cole *et al.*, 2011), used with some airblast cleaning media (Gregory, 1996) and present in numerous personal care and cosmetic products such as lotions, soaps, facial and body scrubs and toothpastes (Carr *et al.*, 2016). Microbeads are also used in cleaning agents, coatings and paints, drilling fluids in the oil and gas industry, and as precursor resins and pellets for the manufacture of finished plastic products (Hale *et al.*, 2020). Secondary MPs describe tiny plastic fragments derived from the breakdown of larger plastic debris at sea and on land (Ryan *et al.*, 2009). MPs in surface waters weather, biofoul, aggregate, and sink, are ingested by organisms and redistributed by currents (Hale *et al.*, 2020). Because of the tendency of both types of MPs to settle, ocean sediments are likely the ultimate destination, known to be one of the most impacted environmental matrices (Hale *et al.*, 2020; Bellasi *et al.*, 2021).

This work aimed to study the presence of MPs in marine sediments of beaches neighboring three metropolitan, maritime areas of Greece, as well as to measure their distribution, abundances and composition.

2. Material and Methods

2.1 Study area and field works

The study was conducted on seven sites of the N. Aegean Sea, representing four sub-regions, Thermaikos Gulf (Peraia and Kitros), Pagasitikos Gulf (Agria and Pagases), Thrakiko Pelagos (Alexandroupoli East and West) and the NE Aegean Sea (Agios Efstratios). Six of the sites were selected due to their proximity to three of the most populated coastal urban regions of Greece, in a close distance of the cities of Thessaloniki, Volos and Alexandroupoli. The seventh site was located on (AE), a sparsely populated and the most isolated island of the Aegean Sea, chosen to be used as a reference site.

Sediment samples covered the entire extent of the beach from the lower to the higher tidal zone, distributed in a stratified random manner. Five replicates of the top 5 cm of sediment were collected, each separated by at least 5 m, from a representative part of the beach as recommended from the MSFD technical subgroup (2013). A metal spoon and a stainless-steel corer of 5 cm long and 12 cm diameter were used. The tube was introduced into the sediment and the enclosed sand was collected and preserved in glass containers with metal lids, wrapped with aluminum foils. Samples were transported back to the Laboratory of Benthic Ecology and Technology of the Fisheries Research Institute and placed at stable room temperature.

2.2 Laboratory analyses

Each sample was dried at 50 °C for one week to remove the moisture according to Constant *et al.* (2019). A volume of 50 ml of sediment was added to a beaker using a metal spoon and afterwards 200 ml of saturated NaCl solution (1.2 g cm^{-3}) were added to perform density separation as proposed by the MSFD technical subgroup (2013). The NaCl solution was previously filtered through a 10 μm filter. The mixture was stirred for 2 minutes and then left settling for another 2 minutes. The supernatant was added to a glass separating funnel, transferred to a suction filtration device and passed through retention glass fiber filter with a pore size of 10 μm. The filters were introduced in glass Petri dishes and dried at room temperature for 48 h before their examination under an Olympus BX60 microscope (magnification 5x). Three repetitions were performed on each sample to ensure full extraction.

2.3 Mitigation of cross-contamination

Measures to prevent contamination were taken by following the five rules of Prata *et al.* (2019): 1) glass and metal equipment were used, 2) operators were wearing cotton lab coats, 3) surfaces were cleaned with 70% ethanol and paper towels while every sampling tool and equipment was washed and rinsed with distilled water, 4) open glass petri dishes and replicates to control for airborne contamination were used, 5) samples kept covered with aluminum foil as much as possible and handled in a clean room with controlled air circulation and limited access. Every lab procedure was conducted in a glovebox fume hood.

2.4 Statistical Analysis

Analysis was conducted within the R environment. The statistical difference between regions, shapes and classes (3 levels for each factor) were examined using the adonis() function from the "vegan" package, according to the model adonis(formula = Number of Items ~ Sub-region * Class * Type, permutations = 1500, method = "euclidean", contr.unordered = "contr.sum", contr.ordered = "contr.poly"), since parametric assumptions weren't met. All figures were produced with the "ggplot" package.

3. Results

The analyses revealed the presence of microplastics in all sampled regions with a mean value of 11.97 ± 1.26 items per 50ml of sediment. While Thermaikos Gulf was most heavily impacted by microplastics (mean=14.10±2.33 items/50ml), there was no statistically significant difference between sub-regions (Table 1). Three stations (Peraia, Agria, Alexandroupoli West) from three different sub-regions were the ones with the highest microplastic concentrations(Fig. 1A), further highlighting the absence of a geographical pattern in our results. The two less impacted stations were Agios Efstratios and Alexandroupoli East (7.40±1.81 & 7.00±1.10 items/50ml respectively).

Microplastic shapes and classes were unevenly represented and statistical differences between both levels were found. Fibers of larger size were the most dominant shape of microplastics (Fig. 1), that was significantly higher than all other shapes and sizes (Table 1). Thermaikos Gulf had the largest number of large fibers while NE Aegean Sea the lowest (Fig. 1B). All other categories were poorly represented in our data, with almost no fragments and spheres, or items of medium to small size. An exception can be seen in Agria station of Pagasitikos Gulf, which is characterized by high number of small and medium fragments (26 and 11 items were measured respectively).

Table 1. Analysis of number of items between regions (Thermaikos Gulf, Pagasitikos Gulf, Thrakiko Pelagos), shapes (Fibers, Fragments, Spheres) and size classes (10-100μm, 101-400μm, 401-1000μm).

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
`Sub-egion`	3	21.3	7.110	1.396	0.006	0.252
Class	2	300.4	150.184	29.496	0.090	0.001
Type	2	521.5	260.727	51.206	0.156	0.001
`Sub-egion`:Class	6	20.9	3.483	0.684	0.006	0.674
`Sub-egion`:Type	6	47.5	7.913	1.554	0.014	0.171
Class:Type	4	952.4	238.089	46.760	0.285	0.001
`Sub-egion`:Class:Type	12	53.2	4.431	0.870	0.016	0.579
Residuals	279	1420.6	5.092		0.426	
Total	314	3337.7			1.000	

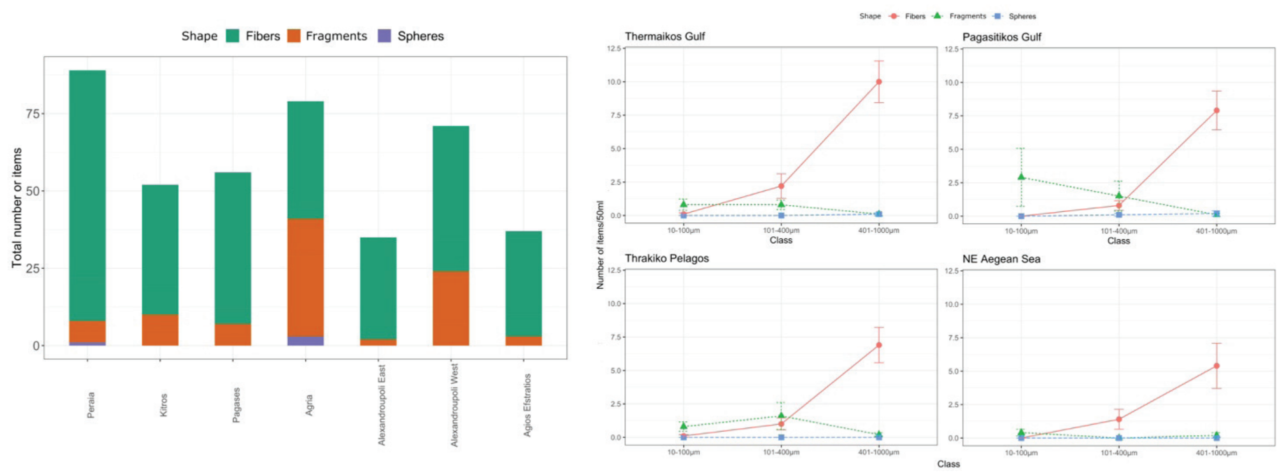

Fig. 1: A) Total number of items per shape and B) mean number of items/ 50ml±se per size class and shape in the three study regions.

4. Discussion/Conclusion

Our findings show the significant concentration of microplastics in the N. Aegean Sea. A connection could be made between nnumber of and urbanization level, with more items found close to the larger city of Thessaloniki, while much lower items were found in the remote island of Agios Efstratios. However, this connection isn't statistically significant, highlighting the wide distribution of microplastics in the wider region of the N. Aegean Sea. This result is also in agreement with the analysis of macro-litter in the sea bottom from the N. Aegean Sea (not-published) that show a wide distribution, correlated more with water currents than the major litter sources.

Fibers were the main shape found, dominating in all stations. Thompson *et al.* (2004), Browne *et al.* (2010), Mathalon & Hill (2014) and Nel & Froneman (2015) are also mentioning that microplastic fibers were the predominant shape appearing in their beach sediment samples. Domestic laundering of clothes may constitute the main source, as a single garment can release more than 1,900 synthetic fibers per washing circle (Browne *et al.*, 2011), that will survive the sewage treatment process (Habib *et al.*, 1998) and reach the marine environment. Shen *et al.* (2021) estimated that microplastics deriving from washing machines may be almost the same order of magnitude as fibers released from discarded cigarette butts, since every smoked filter, consisted of more than 15,000 plastic strands, detaches around 100 fibers per day (Belzagui *et al.*, 2021). Fibers may also originate from tyres got eroded, washed off the road by rain or spread by the wind (Boucher & Friot, 2017) and from lost or discarded objects from fishing activities such as ropes, lines and fishing nets (Cesa *et al.*, 2017). Finally, fibers could also be driven by the wind either from local sources or from remote areas (González-Pleiter *et al.*, 2020). Verschoor *et al.* (2014) prioritized fibers with a 7/9 score as a microplastic source, indicating the urgent need for emission-reduction measures.

Small and medium fragments were only found in Agria, a beach close to industrial facilities. This record agrees with the work of Abbasi *et al.* (2018), which found more fragmented MPs in the industrial region and more fibrous MPs in the street dust of the urban areas of their study area, even though the difference was no significant.

Mainly microplastics of larger size were found. Nel & Froneman (2015) recorded consistently tangled clumps of even larger fibres (≈5 mm). However, even though according to Mathalon & Hill (2014) no correlation has been found between grain size and microplastic concentration, more research is needed to interpret the spatial and vertical patterns of distribution of microplastics in the environment and in the sediment specifically.

The wide distribution of microplastics should be considered as something alarming. Their distribution and abundance should be closely monitored, while more research fully understand main sources and pathways for the Greek seas should be conducted. Especially in beaches, microplastics are a more robust measure of anthropogenic litter than macro-litter, as the second's distribution and abundances can be easily shifted, either by natural (i.e., storms, floods) or anthropogenic (i.e., organised beach cleanings, coastal constructions) events. As such, frequent monitoring efforts are needed to acquire confident data. On the contrary, microplastics trapped in the sediment are not as easily removed, offering more trustworthy timeseries on the level of litter pollution.

5. Acknowledgements

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INGESTED LITTER BY SEA TURTLES ACROSS THE GREEK COASTLINE: AN ASSESSMENT ON THE TYPES, SHAPES AND POSSIBLE SOURCES

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Abstract

Marine litter is nowadays a global phenomenon, which evidently affects the marine environment and wildlife. Sea turtles are known to ingest plastic debris globally; however, information from Greek waters is scarce. In this study, 56 stranded dead sea turtles *(Caretta caretta and Chelonia mydas)* were collected from the Greek coastline area and grouped into the four main regions of the Greek Seas: North Aegean, South Aegean, Levantine, Ionian. The gastrointestinal contents of the turtles were analysed and the results showed that 38 individuals (68%) had ingested litter, at an average of 9 ± 3.79 (SE) items per turtle. In total, 497 items were counted and categorised by size, shape, colour, and polymer type. More than half of the ingested items were plastic sheets (i.e., plastic bags and packaging) items linked exclusively to single-use products. The highest level of ingested plastic sheets was detected in turtles from Levantine, in agreement with model simulated floating plastic bags distribution. Fourier Transform Infrared Spectrometry revealed that polypropylene and polyethylene were the dominant polymer plastic types found in all regions. This study provides evidence of plastic ingestion by sea turtles in Greek waters and gives preliminary results for the possible sources and high-risk locations for this vulnerable species.

Keywords: Anthropogenic debris, plastics ingestion, single-use products, Mediterranean Sea, Greece.

1. Introduction

Marine litter is considered as one of the most severe human-caused threats for the marine environment. It is reported that 80% of the litter observed offshore and on beaches is made of plastic. Plastic items are ubiquitous in the marine environment and accumulate continuously. A huge range of marine organisms has been reported to interact with litter either by entanglement (Kühn, 2015) or by ingestion (Digka *et al.,* 2018; Lusher *et al.*, 2018; Nelms *et al.,* 2016). Evidence on sea turtles show that ingestion of plastic debris affects all 7 species (Kühn, 2015). Both loggerheads and green turtles have been classed as Annex IV Priority Species for conservation by the EU Habitats Directive (HD), requiring a strict protection regime applied across their entire natural range (both within and outside Natura2000 sites) (**92/43/EEC)**. Unfortunately, in the 2013 – 2018 HD reporting period, both species were classified as being in an "Unfavorable" conservation status by EU Member states in the Mediterranean (https://nature-art17.eionet.europa.eu/article17/).

In alignment with the HD but covering a much broader scope, the EU Marine Strategy Framework Directive (MSFD) (2008/56/EC as revised according to 2017/845/EC) aims to achieve Good Environmental Status (GES) in marine regions. Among the 11 descriptors set by the MSFD, descriptor 10 (D10) states that GES is achieved when "properties and quantities of marine litter do not cause harm to the coastal and marine environment", and it is considered that sea turtles may be suitable indicator species to monitor the level of harm on the marine ecosystem. Turtle population numbers are closely associated with the health of their environment, and the scientific community has been working on developing a standardized methodology to measure the amount of debris ingested by the sea turtles. Sea turtles have wide geographic range (Atlantic and Mediterranean), and utilize different habitat compartments (from neritic to pelagic), thus they are a good indicator species for large scale regions. Today, the methodological standard procedures needed for the use of this indicator species urgently must be implemented in Greece to support its inclusion in the MSFD monitoring programs, in order to meet the regional MSFD requirements. This study aims to provide an assessment of the levels, types, shapes and possible sources of litter ingested by sea turtles in Greek waters following a standardized approach according to the INDICIT protocol (Matiddi *et al.,* 2019).

2. Material and Methods

2.1 Sample collection

A total of 56 sea turtles, *C. caretta* and *C. mydas*, were necropsied between October 2017 and October 2021, found stranded along the Greek coastline (Table 1). A full necropsy was performed by trained veterinarians, and their gastrointestinal tract (GI) was dissected, divided into three different sections (oesophagus, stomach, intestine), and stored at -20°C. Frozen GI sections were then transferred to the HCMR laboratories for further analysis. Prior to necropsy, various biometric data, i.e., curved carapace length (CCL), straight carapace length (SCL), curved plastron length (CPL), curved carapace width (CCW), age stage, sex and weight were recorded.

2.2 Plastic analysis and identification

The gastrointestinal contents of the sea turtles were analyzed for ingested plastic items. Each GI section was individually measured (weight, length, width, fullness) and its content was emptied and washed on top of 3 stacked metallic sieves of different mesh size (5 mm, 1 mm and 300 μm). Each sieve was thoroughly washed and inspected for litter. All items were counted, weighed, and categorised by size, shape, colour, and polymer type. Litter items were categorised by shape (sheets, filaments, foams, fragments and others) according to the MSFD Technical Subgroup on Marine Litter guidelines (JRC, 2014). All items were separated into three colour groups (white/transparent, dark, coloured) and three size categories: microplastics (300 mm - 5 mm), meso- plastics (5 - 25 mm), and macroplastics (> 25 mm). Microplastics smaller than 300 mm were not counted, in order to prevent miscounting due to airborne or other types of contamination (in particular fibres). Fourier Transform Infrared Spectroscopy (Agilent Cary 630 FTIR) was used primarily to confirm the synthetic polymer origin of all the extracted items and then used to identify their exact polymer type, using a self-generated polymer library (i.e., spectra of reference polymer types provided by industry), with a level of certainty set to 80%.

2. 3 Data analysis

Sea turtles were categorized depending on their stranding location in four regions of the Greek coastline (North Aegean, South Aegean, Levantine, Ionian) (Table 1). The results are presented as mean ± standard error of the mean (SE). As data did not comply with the assumptions of normality and homogeneity of variance (Shapiro- Wilk test and Levene's test, respectively), the Mann-Whitney U test was applied to determine differences in ingested plastic numbers among regions. Frequency of occurrence, e.g., the percentage of animals with plastic debris in their gastrointestinal tract, was calculated for all individuals and each region separately. The likelihood ratio Chi-square test was used to compare types of ingested plastics (size classes, shapes, colours, polymer types) amongst regions. Pearson correlation coefficient was applied to detect correlation between ingested sheets and floating plastic bags in the same four regions using data from Tsiaras *et al.* (2021). Statistical analyses were performed with SPSS Statistics Version 20.

2.4 Model simulated plastics concentrations

Simulated concentrations of macroplastics were obtained from a basin-scale (Mediterranean) model simulation (Tsiaras *et al.*, 2021) that was used to track the pathways of plastics from major land-based (rivers, cities) sources, taking into account of the most important processes (ocean currents, wind/waves drift, sinking due to biofouling etc).

3. Results

3.1 Litter ingestion in all turtles

A total of 56 sea turtles were analyzed from which 38 were found with ingested plastic in one or more of the GI sections (68%). A total of 497 litter items were detected with a total dry weight of 26 g. All ingested items originated from plastic apart from one metal hook. The vast majority of the items (88%) were found in the intestine, 11% were found in the stomach and 2% in the oesophagus. The average ingested plastic for each sea turtle was 8.84 items/individual. Mesoplastics (5- 25 mm) was the most abundant size category (42%) among all the plastics recovered from the turtles, followed by macroplastics (38%) and microplastics (20%). Among all ingested items, sheets (52%) were the most common with 258 items, followed by filaments (28%) with 141 items, fragments (15%) with 74 items, foam (4%) with 19 items, pellets (1%) with 3 items and 4 unclassified items (1%). Among all plastics detected, the amount of white/transparent plastics (62%) was significantly higher than the dark (21%) and the coloured (17%) plastics (Mann- Whitney U test, $p < 0.05$). Out of all plastic items extracted, the most common polymers identified were polypropylene (PP) (46%), followed by polyethylene (PE) (41%), nylon (5%), thermoplastic elastomer (TPE) (4%), polystyrene (PS) (2%) and polyethylene terephthalate (PET) (2%).

3.2 Litter ingestion per region

Higher occurrence of ingested litter was detected in sea turtles stranded at the coastline of Levantine (80%) and the higher average litter items per turtle was also detected in these turtles (33.8 items/ turtle) (Table 1), although differences were not significant (Chi-square, $p>0.05$).

Table 1. Information on litter ingestion by sea turtles by region in the Greek coastline.

Regions	Number of sea turtles	Occurrence %	Average litter items/ individual	Average sheets/ individual
North Aegean	28	68	9.11 (± 4.94)	3.36 (± 1.5)
South Aegean	11	64	5.09 (± 2.62)	3.18 (± 2.4)
South Cretan	5	80	33.80 (± 15.12)	25 (± 11.18)
Ionian	12	67	1.36 (± 0.48)	0.58 (± 0.34)
All	56	68	9 (± 3.76)	8.03 (± 10.87)

Plastic sheets were significantly higher in sea turtles stranded in Levantine compared to those stranded in Ionian coasts (Mann Whitney, U test, $p < 0.05$) (Fig. 1a). Data obtained by modeled results of Tsiaras *et al.* (2021), on floating plastic bags in the same 4 regions correlate positively with the ingested plastic sheets (Pearson correlation, $r= 0.97$, $p < 0.05$). Filaments and fragments were mostly abundant in North Aegean. Turtles from the Ionian seem the least affected from litter ingestion with an average of 1.36 items/ turtle (Table 1).

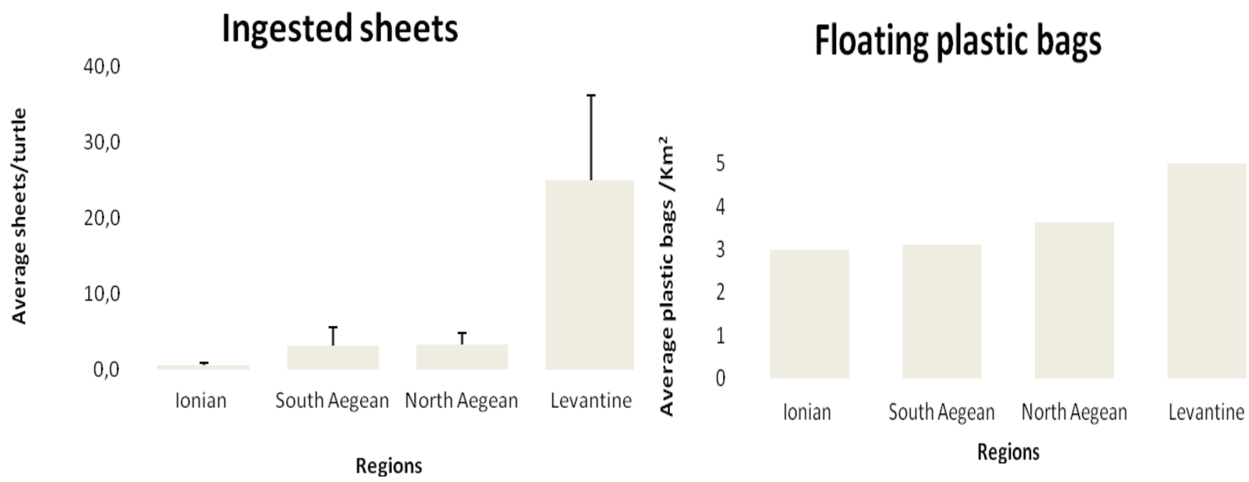

Fig. 1: Concentration of plastic sheets in different regions in Greece: (a) found ingested in sea turtles; (b) modeled results on floating plastic sheets in sea surface. * Significant difference between regions (Mann Whitney, U test, $p < 0.05$).

4. Discussion/Conclusion

The analysis of the gastrointestinal tract of 56 turtles along the Greek coastline indicated an occurrence of 68% of the turtles with ingested plastic. This percentage varied among the various regions (Table 1), with the South Cretan presenting the highest occurrence (80%).

Across all regions, analysis of the different shapes of the ingested litter, indicate that plastic sheets of various sizes, represent 52% of the total ingested plastic items. Plastic sheets originate from fragmented pieces of plastic bags and packaging, which are exclusively connected to single-use products. Consequently, the results of this study indicate that more than half of the ingested debris originates from products used only once, which has reported recently in the western Mediterranean sub-region (Cammeda *et al.*, 2022). The model simulated distribution of floating bags, obtained from Tsiaras *et al.* (2021) indicates a higher concentration of items/km^2 in the Levantine, followed by North Aegean, South Aegean and finally by Ionian (Fig. 2b). Interestingly, the ingestion of plastic sheets by sea turtles of the present study follows the same pattern across the different regions (Pearson correlation, $r= 0.97$, $p < 0.05$), which suggests that the selection of sea turtles as suitable indicator species for plastic pollution for a large-scale monitoring, is on the right direction.

According to the European Union, plastic constitutes 80 to 85 % of beach litter, with single-use plastic items representing 50%. Single-use plastic products are therefore a particularly serious problem in the context of marine litter; pose a severe risk to marine ecosystems, to biodiversity and to human health and negatively affect activities such as tourism, fisheries and shipping (EU, 2019). In Greece 45% of beach litter consists of single-use plastic products (i.e., plastic bags, plastic straws, plastic cups) (unpublished HCMR data 2015-2020). Single-use plastic products include a diverse range of commonly used fast-moving consumer products that are discarded after having been used once for the purpose for which they were provided, are rarely recycled, and are prone to becoming litter. These products are mainly made by polyethylene and polypropylene. These polymer types represent the 80% of the ingested plastic found in the collected sea turtles, in agreement with other studies in the Mediterranean Sea (Cammeda *et al.*, 2022). However, Greek legislation has recently banned specific single-use products in accordance to the European legislation (Directive (EU) 2019/904) concerning the reduction of the impact of certain plastic products on the environment. These measures along with those of MSFD achieving GES are likely to have a positive impact to the marine environment and especially on larger marine organisms such as sea turtles.

The results of this study suggest that more than half of the ingested litter items, found in the gastrointestinal tracts of the sea turtles stranded in Greek coastline, are related to single-use plastic items (i.e., fragmented pieces of plastic bags and packaging). The analyses on the polymer types of the ingested items strengthen this approach, as polyethylene and propylene, major representatives of single-use products, together consist the 86% of the total plastics. Further investigation is needed to shed light on the potential harmful effects of ingested litter on the sea turtles and eventually on the marine environment they inhabit.

5. Acknowledgements

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MICROPLASTIC CONTAMINATION IN CULTURED MUSSELS AND PEARL OYSTERS OF EVOIKOS GULF: PRELIMINARY RESULTS

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Abstract

This study aims to investigate the microplastic (MPs) particles contained in the soft tissue of mussels (*Mytilus galloprovincialis*) and pearl oysters (*Pinctada imbricata radiata*) samples. The two species were collected from the wider Evoikos Gulf, from an Integrated Multi-Trophic Aquaculture (IMTA) unit with the help of divers and they were of various sizes. A total of 145.09 gr of mussels and 231.04 gr of pearl oysters, wet mass, were used, from which 5,17 ± 2,56 particles/gr for mussels and 2,93 ± 1,27 particles/gr for pearl oysters were extracted. The MPs found in the samples were divided into 5 types: fibers, fragments, microbeads, foams and films. While the samples of mussels and pearl oysters were similar in age, the difference found in the concentrations of MPs appears to be due to their different culture methods. This study indicates the infiltrative abilities of the two species and shows once again that both species can be characterized as bioindicators of marine microplastic pollution.

Keywords: Microplastics, Soft tissue, *Mytilus galloprovincialis, Pinctada imbricata radiata*, IMTA.

1. Introduction

In the last decades, microplastics (MPs) have become a major concern for the scientific community, as they are rapidly increasing in the oceans, with various ecological consequences (Aldrady, 2011). Many scientists have approached the term microplastics, referring to plastic particle <5 mm that are not visible to the bare eye (Rocha-Santos & Duarte, 2015). Apart from size, MPs are also characterized by their shape and are divided into categories such as microbeads, microfibers, microfoam, microfragments and microfilms (Wu *et al.*, 2019). MPs enter the marine environment with many ways for example, from the terrestrial environment through surface water runoff or plastic degradation, where they remain for long periods of time (Dris *et al.*, 2016), affecting the existing aquacultures. MPs have been found in a variety of aquaculture environments, such as fishing farms and rice-fish coculture systems etc. (Chen *et al.*, 2021; Piperagkas *et al.*, 2019).

Aquaculture directly affects and is affected by the environment and its processes. It depends on the various ecosystem processes and is vulnerable to any changes that may be caused by anthropogenic or other activities. Aquaculture releases large amounts of nutrients and organic wastes, capable of causing eutrophication phenomena in nearby coastal and aquatic systems (Sarà *et al.*, 2018). Therefore, there has been an increased interest in alternative sustainable practices such as Integrated Multi-Trophic Aquaculture (IMTA) (Alexander *et al.*, 2016). IMTA focuses on the sustainability of aquaculture through the integrated production of species coming from different trophic levels, which can minimize the energy losses and environmental degradation (Chopin *et al.*, 2012). The present study was based on IMTA, in which there were fish coexisting with mussels and pearl oysters to reduce energy losses, as they are water-filtering organisms and are often considered as bioindicators of marine pollution. The scope of the study was to detect the microplastic particles contained in the soft tissue of mussels (*Mytilus galloprovincialis*) and pearl oysters (*Pinctada imbricata radiata*).

2. Material and Methods

2.1 Study site

The study was part of the Innovative Development of Multitrophic Aquaculture (IDMA – www.idma.uoc.gr) project that included a pilot IMTA culture in Northern Evoikos gulf area. The Northern Evoikos Gulf is a semi-enclosed and extended sea basin, with a shallow bathymetry (maximum depth 100 m), which is connected to the western Aegean and the Southern Evoikos Gulf (Voutsinou, 1988). The pilot IMTA included mussels (*Mytilus galloprovincialis*) and pearl oysters (*Pinctada imbricata radiata*) near Sea bass fish cages. The IMTA culture methodology was based in the typical mussel culture methodology: mussel seeds were collected from ropes within the fish farm and were put in elongated plastic cylindrical tubing nets (pergolaris) of 6 m in length and net eye of 80 mm. The pergolaris were made using polyvinylchloride cylindrical tubes with a diameter ranging from 4–7 cm, which were then submerged around a fish cage in the center of the fish farm. Pearl oyster juveniles were also collected from ropes within the fish farm and were cultivated in baskets used in oyster culture made by SEAPA© for oyster growers. Although these baskets are designed for longline oyster farming, they can be adapted to suit a range of alternative farming systems and methods such as IMTA. The baskets were tied to ropes around another fish cage in the center of the farm. Cultivation duration was 9 months during which both bivalves reached commercial size.

2.2 Sample collection

Mussel and oyster samples were collected at 07/2021. A total of 12 individuals of cultivated *Pinctada imbricata radiata* with a total flesh weight of 231.04 gr and 30 individuals of cultivated *Mytilus galloprovincialis* with a total flesh weight of 145.09 gr were collected with the help of divers. All the collected samples were measured on the field and placed in glass jars, where they were refrigerated to prevent distortion until further analysis.

2.3 Mussel and oyster sample preparation and digestion

The procedure to extract the microplastic particles from the soft tissue of mussels and pearl oysters was based on the methods of Patterson *et al.* (2021), Yozukmaz (2021), Wakkaf *et al.* (2020) and Munno *et al.* (2018) and was the same for both species. The samples were rinsed with distilled water and measured for total wet flesh weight and separated to nine subsamples, with weight of about 10 gr. Each sample of soft tissue was placed in conical flasks and digested with a 200 ml KOH (10%, 1:20 $^{w}/_{v}$) and 2 ml H_2O_2 (30%) mixed solution. The samples were carefully covered with aluminum foil and placed at 60°C for 48 h, with regular 40 sec shaking every 8 h. After 48 h, 2 ml of H_2O_2 (30%) solution was added approximately every 4 h when the foam was gone and the samples were carefully stirred for 40 sec. This procedure was repeated until there was no more organic material present and the solution was clear yellow in the case of mussels and clear green in the case of pearl oysters. A saline solution (NaCl) with 1,2 gr cm^{-3} density was then added to each sample in concentrations twice as high as the KOH (400 ml saline solution), to induce flotation of the microplastic particles contained in the sample. The samples were well stirred and left for 24 h, allowing any organic material remaining in the conical flask to settle. The supernatant solution was then transferred to a new clean conical flask, very careful not to disturb the material at the bottom of the flask. At the end of the above procedure, the samples were filtered with 0.8 μm diameter Whatman™ cellulose nitrate filter papers and transferred to glass Petri dishes with lids where they were stored until further analysis.

2.4 Microscopic inspection of microplastics

The filtered samples were analyzed visually using a stereoscope (magnification x1.0 to x5.0), to identify the type of MPs larger than 0.8 μm according to their physical characteristics (Hidalgo-Ruz *et al.*, 2012) and then they were counted. The confirmation of the presence of MPs was performed by the 'needle test'. This method involves the use of a red-hot needle, which, upon coming into contact with the presumed microplastic, melts it (De Witte *et al.,* 2014). The MPs found in mussels and oysters were classified into 5 types (fibres, fragments, microbeads, foam and film). In order to enable a correct count of the particles, the filter was carefully marked near them with the tip of a needle.

2.5 Quality control and contamination precautions

All of the equipment used in the experiment were either glass or metal and were properly rinsed with HCl solution before use and benches were cleaned regularly with acetone. The samples were covered with foil throughout the experiment to avoid airborne contamination of MPs from the background. All liquid solutions (KOH, H_2O_2, NaCl) used in the experiment were filtered with 0.8 μm filter paper before use. While conducting the experiment, blind samples were created to estimate the air contamination from the laboratory background and to remove the error from the sample analyses.

2.6 Data analysis

The measured numbers of MPs for each sample (total and each subcategory) were transformed to counts per 1 gr of biomass. The analysis is still ongoing and here the data of 30 mussels and 12 oyster samples are presented. T-Test was used to check if there were significant differences in the counted MPs between the different species.

3. Results

The size of the MPs found was greater than 0.8 μm. The counted particles showed that mussels had more total MPs in their tissue per gr (5.17 ± 2.56) compared to pearl oysters (2.93 ± 1.27) consisting of more fibers (2.20 ± 0.99 vs 0.96 ± 0.64). Detailed counts are presented in Figure 1.

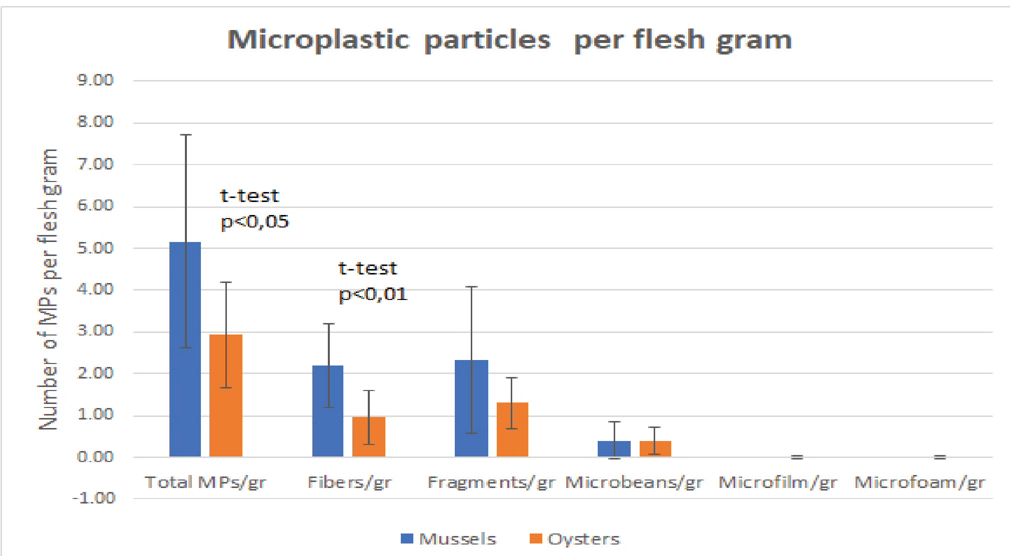

Fig. 1: *Average number (± SD) of* MPs *(total and subcategories) in 1 gr of mussel and pearl oyster flesh.*

Results of t-Test confirmed that there were significant differences in total MPs and fibers between mussels and pearl oysters (F = 4,812, $p < 0.05$ and F = 9,298, $p < 0.01$ distinctively). No other significant differences were detected. Total mussel MPs consisted mainly of microfibers in percentage of 49.91% (Figure 2a), while total oyster MPs consisted mainly of fragments 48,46% (Figure 2b).

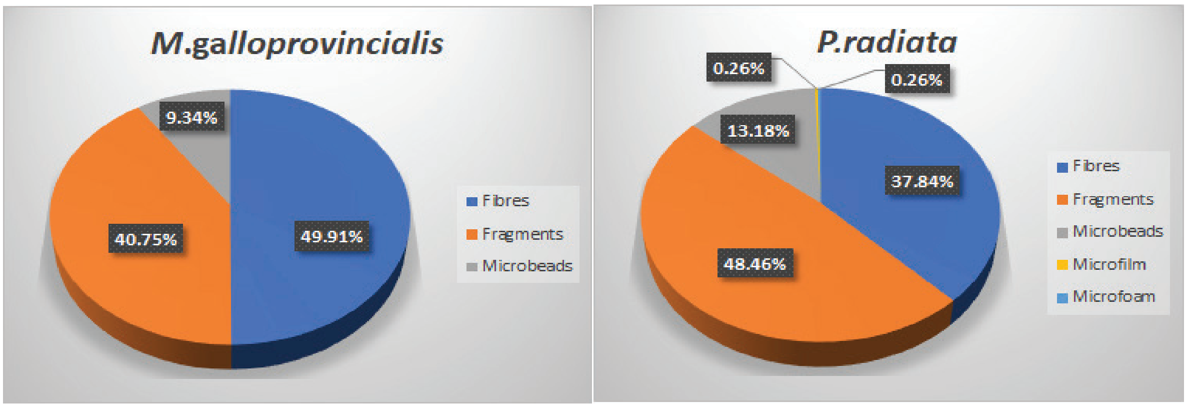

Fig. 2: *Percentage of each microplastic type found within all mussel (a) and pearl oyster (b) samples.*

4. Discussion/Conclusion

This study provides information on microplastic pollution and their widespread presence in the soft tissue of marine cultured organisms. The presence of MPs in the soft tissue of mussels and pearl oysters indicates that the MPs released from an aquaculture farm may end up in the food chain, especially in the case of filter feeding organisms that may be considered as bioindicators.

The main MP type found within mussels is microfibers while microplastic fragments were found within pearl oysters. Microplastic fibers probably originate from the plastic nets used in the pergolaris culture methodology, since nets are primarily made of fiber plastic. On the other hand, the SEAPA oyster cage is made of sturdy plastic which is less likely to break into smaller pieces. Breaking microplastic from these cages is more likely to form fragments and fibers.

The mussels MP results of the present study agree with others conducted in the Aegean Sea [Izmir Bay, Marmara Sea] (Yozukmaz, 2021; Gedik *et al.*, 2022), where fibers were the main type of MPs found in their soft tissues. However, studies conducted on wild mussels (Yozukmaz 2021; Gedik *et al.*, 2022) showed higher concentrations of MPs. More investigation should definitively be done to determine whether the lower concentration of MPs found in this study is related to the study site or to the mussel culture.

For the case of pearl oysters, no similar studies were found in the literature, and therefore further analyses should be conducted on wild populations, to determine whether culture is a factor that affects the concentration of microplastic particles in the soft tissue of these organisms.

5. Acknowledgements

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PRELIMINARY ASSESSMENT AND RESULTS ON MICROPLASTICS INGESTED BY TWO COMMERCIAL FISH FROM THE NORTHERN AEGEAN SEA

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Abstract

This study assesses microplastic ingestion occurrence in *Mullus barbatus* and *Boops boops* in the Northern Aegean Sea. Two hundred thirty-one (231) fishes (128 *M. barbatus* and 103 *B. boops*) were sampled and analyzed from four different locations (Alexandroupoli, Thermaikos Gulf, Pagasitikos Gulf, and Agios Efstratios Island), in 2021. In total, microplastics were present in 38 *M. barbatus* and 28 *B. boops*. Frequency of microplastic occurrence was 29.7% for *M. barbatus* with an average microplastic abundance of 0.37 ± 0.62 SE per individual, while microplastic items were found in 27.2% of *B. boops* with 0.34 ± 0.63 SE items per specimen. Fibers between 0.1 mm and 1 mm in size were the most common microplastic type and size category. Occurrence and number of microplastics were statistically significant only for the interaction between species and location, suggesting that the nature of different species in interaction with the location have an effect on the occurrence and number of microplastics ingested by fish. These results support *M. barbatus* and *B. boops* as bioindicators of microplastic pollution in the context of the Marine Strategy Framework Directive.

Keywords: Marine litter, microlitter, edible species, bioindicator.

1. Introduction

Plastic particles smaller than 5 mm in size are defined as microplastics and constitute a major global environmental problem (Razania *et al*., 2018). Microplastics are widely spread in the marine environment and are nowadays found in all marine matrixes (Digka *et al*., 2018). Therefore, they can be ingested by, and potentially affect, a variety of marine organisms (Lusher, 2015). About 700 species have been reported so far to ingest marine litter, with the number rapidly increasing (Gall & Thompson, 2015), while plastic in general is representing 92% of all ingestion incidents between marine organisms and marine litter (Phillips & Bonner, 2015).

Chemical additives used in plastic manufacturing are likely to bring about potential toxic effects when being ingested by aquatic organisms (Anastasopoulou *et al*., 2018). These harmful elements can be released in the gastrointestinal fluids and consequently transferred to edible tissues (Wright *et al*., 2017). The potentially increased concentration of harmful chemicals by species destined for human consumption, such as fish, raises major concerns on human health (GESAMP, 2016). Thus, considering that fish constitute significant levels into food chain towards higher trophic levels (including human consumers) along with the increased fish consumption (FAO, 2021) makes the imperativeness of such studies crucial.

Due to abovementioned concerns, international agencies and legislative frameworks prioritized microplastic ingestion by marine biota as an urgent necessity for assessment and monitoring. The European Union (EU) and Commission (EC) address microplastic ingestion by marine organisms among the elements to be used in order to achieve a Good Environmental Status (GES) set by Marine Strategy Framework Directive (MSFD) (2008/56/EC). However, common indicator fish species for monitoring microplastic ingestion have not been established yet.

The aim of the present study is to assess microplastic ingestion (frequency of occurrence, number of ingested microplastic items, type and size category) from two commercial fish species living in different habitats, the demersal red mullets (*Mullus barbatus* Linnaeus, 1758) and semi-pelagic bogues (*Boops boops* Linnaeus, 1758), in Northern Aegean Sea (Mediterranean Sea), using a common methodology for these potential bioindicator-species of marine pollution.

2. Material and Methods

2.1 Data collection

The species *M. barbatus* and *B. boops* were collected from four Northern Aegean Sea locations (Table 1), distributed across different environmental conditions, e.g., semi-closed gulfs, island. All specimen were sampled within the framework of the MSFD project and caught by local commercial fishing vessels in 2021 using various fishing gears (Table 1). Whole fish individuals were stored at -20 ^oC until further laboratory analysis. Then, fishes were thawed at room temperature, measured (Total Length – TL) and weighed (Total Weight – TW) before dissection. Dissected gastrointestinal tracts (GIs) were weighed (wet biomass) and placed in sealed bags and re-frozen before the next processing step.

2.2 Microplastic extraction, observation and quantification

Fish GIs were thawed at room temperature and separately placed into glass beaker in 1:20 (*w/v*) hydrogen peroxide (15% H_2O_2, Chem-Lab, Germany) and heated on a hot plate at 60 ^oC for the digestion procedure, according to Digka *et al*. (2018b). Samples were then diluted with 100 ml of purified water (Milli-Q), stirred and filtered under vacuum on fiber glass filters (pore size 1.2 μm, GF3, CHMLAB Group, Spain) which were placed in glass Petri dishes and dried overnight at room temperature.

Dried filters were examined under a stereomicroscope (Olympus BX60) for items resembling microplastics. Using a digital camera (Basler PowerPack) and the pylon Viewer software, items with no organic structures were photographed, counted and categorized according to maximum length and type (fiber, fragment, sphere). All filters containing microplastic items (MPs) were preserved for polymers identification in the future using the Fourier transform infrared (FT-IR) spectroscopy technique. Two metrics were counted: (1) Occurrence (presence/absence) and (2) number of MPs (NoMPs) per individual examined. Based on these metrics, three indicators were calculated (Table 1): (1) The frequency of occurrence of ingested MPs (FO%) was calculated for each species and location as the percentage of individuals containing MPs out of the total examined fish. (2) Microplastic abundance was expressed as the average NoMPs per individual in all fish examined (MAb). (3) The average NoMPs per individual in fish ingesting MPs was also calculated and is referred in this study as microplastic density (MDe).

In order to reduce and monitor airborne contamination, the Torre *et al*. (2016) methodology was followed and procedural blank samples (clean filters exposed to air in the laboratory) were used in all steps (Galgani *et al*., 2013). Procedural contamination was always < 10% of the mean microplastic number in all samples.

Table 1. Number (N), sampling gear (OTB: Bottom trawl; PS: Purse seine; GTR: Trammel net, and LLS: Longline) mean total length (TL ± SE), mean total wet biomass (TW ± SE), frequency of occurrence (FO%), microplastic abundance (MAb) and microplastic density (MDe) of M. barbatus and B. boops sampled at four locations (AXD: Alexandroupoli; THG: Thermaikos Gulf; PAG: Pagasitikos Gulf, and AE: Agios Efstratios) of the Northern Aegean Sea.

Area	Species	S. gear	N	TL (mm)	TW (g)	FO (%)	MAb	MDe
AXD	*M. barbatus*	OTB	32	173.34 ± 8.90	60.52 ± 9.03	50.00%	0.69 ± 0.81	1.38 ± 0.60
	B. boops	PS	32	206.75 ± 17.07	96.51 ± 20.75	15.63%	0.22 ± 0.60	1.40 ± 0.80
THG	*M. barbatus*	OTB	32	132.09 ± 8.34	24.80 ± 5.36	12.50%	0.13 ± 0.33	1.00 ± 0.00
	B. boops	OTB	32	180.22 ± 18.40	58.78 ± 20.24	37.50%	0.44 ± 0.66	1.17 ± 0.55
PAG	*M. barbatus*	GTR	32	174.25 ± 29.67	67.30 ± 39.47	31.25%	0.38 ± 0.60	1.20 ± 0.40
	B. boops	GTR	32	205.59 ± 15.28	90.98 ± 19.58	31.25%	0.41 ± 0.65	1.30 ± 0.46
AE	*M. barbatus*	OTB	32	143.78 ± 13.62	31.20 ± 8.07	25.00%	0.28 ± 0.51	1.13 ± 0.33
	B. boops	LLS	7	245.14 ± 20.62	151.03 ± 33.97	14.29%	0.14 ± 0.60	1.00 ± 0.00

2.3 Data analysis

The R Environment (R Core Team, 2021) was used for all statistical analysis. Data of occurrence and NoMPs were analysed using a General Linear Model (GLM) with binomial and negative distributions respectively, to account the absence of a normal distribution and the overinflation of count data. The model used was Metric (occurrence/MP) ~Species (2 levels) * Area (4 levels), with the glm() and glm.nb() functions from the “MASS” package. The function glht() from the multcomp package was used for post hoc analysis.

3. Results

A total of 128 *M. barbatus* (TL: 155.87 ± 25.34 SE mm, TW: 45.95 ± 27.69 SE g) and 103 *B. boops* (TL: 200.76 ± 18.40 mm, TW: 86.78 ± 31.90 g) GIs were analyzed for microplastic ingestion (Table 1). Occurrence was statistically significant only for the interaction between species and location (Table 2). In total, MPs were found in 38 *M. barbatus* and in 28 *B. boops*. Maximum individuals with MPs were measured for *M. barbatus* in AXD (occurrence=16) while minimum for the same species in THG (occurrence=4) and in the case of AE where MPs were found only in one out of the 7 *B. boops* studied. From occurrence, FO% was calculated as 29.69% and 27.18% for *M. barbatus* and *B. boops*, respectively (Table 1).

NoMPs per individual was statistically significant only for the interaction between species and location (Table 2). In total 82 MPs were identified, 47 in *M. barbatus* and 35 in *B. boops,* ranging between 0.1 mm and 1 mm size class, categorized mainly as fibers (95.1%) and fragments (4.9%). In both species the number of ingested MPs ranged from 0 to 3 items. Only 3 individuals (2 *B. boops* and 1 *M. barbatus*) were found containing 3 MPs, while two of them were from AXD and one from THG.

The MAb was 0.37 ± 0.62 SE MPs/individual in *M. barbatus* and 0.34 ± 0.63 MPs/individual in *B. boops*. The MDe was 1.24 ± 0.48 SE and 1.66 ± 1.04 MPs/individual for *M. barbatus* and *B. boops,* respectively. FO%, MAb and MDe were highest for fish from AXD (FO%: 32.81%, MAb: 0.45 ± 0.75 and MDe: 1.38 ± 0.65 MPs/individual), followed by those from PAG (FO%: 31.25%, MAb: 0.39 ± 0.63 and MDe: 1.25 ± 0.49 MPs/individual). Fish from THG were third in order (FO%: 25%, MAb: 0.28 ± 0.54 and MDe: 1.13 ± 0.48 MPs/individual), while fish from AE seem to ingest less MPs in terms of both FO% and NoMPs (FO%: 23.08%, MAb: 0.26 ± 0.49 and MDe: 1.11 ± 0.31 MPs/individual).

When exploring the size (TL, TW) correlations with the measured parameters (Occurrence, NoMPs), a weak but significant correlation was found only between both species TL and occurrence (Pearson’s $r_{M.\ barbatus} = 0.21$, $p = 0.016$, Pearson’s $r_{B.\ boops} = -0.2$, $p = 0.038$).

Table 2. GLM analysis of microplastic occurrence and NoMPs ingested by *M. barbatus* and *B. boops* sampled from four locations of the Northern Aegean Sea.

Parameter	Level	Deviance	Resid. Df	Resid.Dev	Pr(>Chi)
Occurrence	NULL		230	276.400	
	Species	0.176	229	276.230	0.675
	Location	2.016	226	274.210	0.569
	Species*Location	14.427	223	259.780	**0.002**
NoMPs	NULL		230	211.560	
	Species	0.118	229	211.440	0.731
	Location	4.221	226	207.220	0.239
	Species*Location	13.852	223	193.370	**0.003**

4. Discussion/Conclusion

This study provides the first report of microplastic ingestion from the Northern Aegean Sea. Our results suggest that the nature of different species in interaction with the location have an effect on the occurrence and NoMps ingested by fish. Major differences were identified between AXD and THG locations, with *M. barbatus* individuals from AXD being significantly more prone to ingesting MPs than those from THG (post hoc analysis *M. barbatus* AXD-THG: $P_{occurrence}$ = 0.045, P_{NoMPs} = 0.034). This fact could possibly be related to the differences between mean TL of the species in the two locations, with *M. barbatus* sampled from AXD being bigger than their counterparts of THG, however more study is needed towards this goal.

The microplastic ingestion values in *M. barbatus* (FO%: 29.69%, MAb: 0.37 ± 0.62 and MDe: 1.24 ± 0.48 MPs/individual) are in line with values reported by similar studies from other parts of the Mediterranean Sea. Specifically, in western Mediterranean coasts, Bellas *et al.* (2016) found MPs in 19% of *M. barbatus*, with MAb 0.35 and MDe 1.9 MPs/fish. A more recent study by Güven *et al.* (2017) reported a high FO% (66%) and a MAb and MDe of 1.39 and 2.12 MPs/fish, respectively, for *M. barbatus* in the eastern Mediterranean. On the contrary, Anastasopoulou *et al.* (2018) found MPs in only 7% of the *M. barbatus* caught in the Adriatic Sea, with a MAb and MDe of 0.09 and 1.29 MPs/individual. Concerning the Greek Ionian waters, a study carried out on this species reported a FO% of 16% and a MAb and MDe of ingested MPs/specimen of 0.21 and 1.33, respectively (Digka *et al.*, 2018).

Regarding microplastic ingestion values in *B. boops* of the present study (FO%: 27.18%, MAb: 0.34 ± 0.63 and MDe: 1.25 ± 0.57 MPs/individual), these are lower than those reported from the Balearic Islands (FO%: 58%, MAb: 2.17 ± 0,63 and MDe: 3.78 MPs/individual; Nadal *et al.*, 2016) and the Italian coastal waters (FO%: 56%, MAb: 1.8 and MDe: 3.2 MPs/individual; Sbarna *et al.*, 2020). Values found in our study are similar though to those acquired by Rios-Fuster *et al.* (2019) around the Iberian Peninsula coast and the Balearic Islands (FO%: 27%, MAb: 0.46 and MDe: 1.69 MPs/individual). In Greek Ionian waters, Tsangaris et al. (2020) reported that 22.9% of *B. boops* found to have ingested MPs, with a MAb of 0.28 and a MDe of 1.29 MPs/individual.

Consistent with most of the abovementioned scientific papers our results show that the majority of ingested microplastic items are fibers, which is probably related to synthetic clothes through wastewater discharges (Cesa *et al.*, 2017).

It should be mentioned that comparisons, either between species or different regions are difficult and, in many cases, biased, due to the different methodology approaches. Thus, the need to establish a common methodology harmonized with the needs that will serve the purpose of the MSFD is considered as imperative (Lusher *et al.*, 2017). In conclusion, the obtained results of this assessment could provide support to the existing studies for the two selected species and their potential use as small-scale bioindicator species for microplastic and marine litter pollution in general.

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PRELIMINARY RESULTS OF PLASTIC POLLUTION IN *ARISTAEOMORPHA FOLIACEA* (RISSO, 1827) IN THE EASTERN IONIAN SEA

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Abstract

Deep seafloor is the ultimate sink of plastics as with other forms of marine litter. In this study the occurrence of ingested plastics was investigated for the first time in the deep-sea shrimp *Aristaeomorpha foliacea* in the eastern Ionian Sea (Greek waters). A total of 352 individuals were collected from five stations during a MEDITS sampling survey in 2019. The results showed that 11.93% of individuals had ingested plastics. Three different plastic polymers were identified by FT-IR analysis. Polyester presented the highest occurrence among all identified polymers. The vast majority (78.80 %) of the filaments were <25 mm in size belongs to males. This study is the first study looking on deep sea commercially valuable benthic animals and potential effects of plastics ingestion on their stock.

Keywords: plastics, decapods, deep sea, Ionian Sea.

1. Introduction

It has been estimated that 5–8 Mt of plastic moves from land to oceans every year, and reaching even to some environments such as polar regions and the deep ocean seafloor (Bergmann *et al.,* 2017). Therefore, plastics are globally recognized as one of the most concerning threats to oceans' wildlife (Jamieson et al., 2019). The Mediterranean Sea has been described as one of the areas that is most polluted by plastics worldwide (UNEP/MAP, 2015). The high values of microplastics in this enclosed basin are probably linked to the intense anthropogenic activity alongside the hydrodynamics (Eriksen *et al.*, 2014). Various animals including benthic and demersal animals are known to be susceptible to plastic pollution. However, when considering the deep oceans (>200m depth), such knowledge is very limited (Filgueiras *et al.*, 2019).

Aristaeomorpha foliacea is commonly trawled in waters down to 700m depth and it is one of the most important species of the deep-water ecosystems in the Mediterranean Sea.

This study presents preliminary results on the occurrence of ingested plastics in *A. foliacea* in the Greek Ionian Sea. The aim of this study was to analyse the occurrence, size and composition of anthropogenic filaments ingested by this red shrimp from different stations in their essential habitat, to investigate possible differences in the plastic ingestion between juvenile and adults and to highlight any potential effects on the stock of this valuable species.

2. Materials and Methods

Individuals of *A. foliacea* were collected from five stations in the eastern Ionian Sea (eastern Mediterranean Sea). All samplings were carried out within the framework of MEDITS survey (An international bottom trawl survey in the Mediterranean Sea) in August 2019 (Figure 1). Individuals were collected, immediately frozen on board and kept at −20 °C until further inspection. In the laboratory, shrimps were sexed and measured (cephalothorax length, CL (mm); body weight, BW (g); Stomach weight, STW (g) and Stomach content weight, STCW (g)) and dissected. The identification of stomach content was carried out under a microscope (Nikon SMZ 745 T) and the stomachs with ingested plastic were recorded and placed on clean glass petri-dishes. Ingested plastics counted and classified into single-piece or ball- forming filaments (tangled). The colour and size of each piece of plastics was also recorded. Plastics were then classified into micro- (<5 mm), meso- (5-25 mm) or macro-sized items (>25 mm). In order to minimize the risk of airborne contamination, all equipment was washed with purified water (Milli-Q), alcohol and all precautional measures were taken. Fourier transform infrared spectroscopy (FT-IR) equipped with a single-reflection diamond attenuated total reflectance (ATR) unit (Nicolet iS50 FTIR spectrometer, Thermo Scientific) was used to detect the synthetic origin and identify the polymer composition of the ingested plastic (Torre *et al*., 2016).

GLM and Multifactor ANOVA tests were used to investigate possible differences on plastic ingestion by the red shrimps among various stations, sex and type of plastics. BW and CL were used as a covariant factor, followed by Tukey's HSD post-hoc pairwise comparisons.

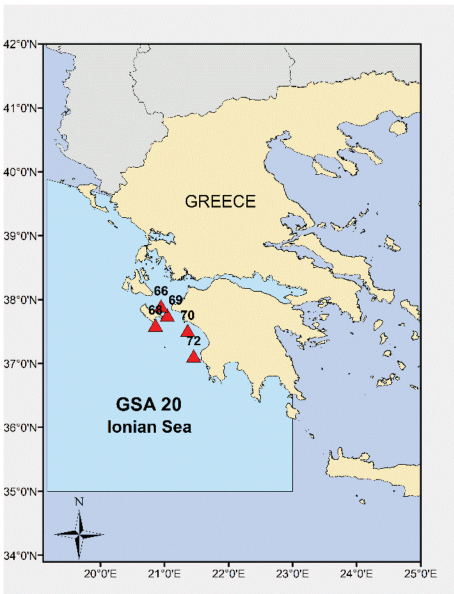

Fig. 1: Sampling stations, depth and the coordinator of each station.

Table 1: Depth and coordinator of each sampling station.

Stations	Depth (m)	Latitude (N)	Longitude (E)
66	533	3754.06	2056.68
68	590	3736.11	2051.53
69	580	3745.49	2102.57
70	646	3731.18	2121.68
72	746	3707.21	2127.33

3. Results

A total of 352 individuals (187 females and 165 males) with CL range between 19.65 and 62.36 mm was collected from 5 stations, from which 42 (13 females and 29 males) of them were found with ingested plastics. The stations were located in the Essential habitat for red shrimps in the Greek Ionian Sea (Mylitineou, personal communication). Plastic sizes ranged from 0.75-110.59 mm and were of different color. The number of plastics per stomach ranged from 1 to 23 pieces of plastics in form of single piece or tangled. The 12.5 % of stomachs with ingested plastics showed tangled form of plastics. Among those shrimps with ingested plastics only 10 of them were mature males (stage II) and 2 of them mature females (stage IV). An additional important finding of this study is that 38.46% of the shrimps with ingested plastics were juveniles (CL<25 mm). The largest ingested plastics (more than 10 cm) came out of a mouth of one mature female with spermatophore and it seems the individual was chocked due to this big plastic filament. The vast majority (78.42%) of plastics filaments were less than 25 mm in size belongs to males. Figure 2 showed the percentage of ingested plastics per size category. Plastics were found in different colors such as green, red, black, blue and transparent and categorized in three groups of poly-acrylonitrile, Polyacrylate and Polyester. Polyester found to be the dominant ingested material (highest percentage) in both sex (Fig. 3). Multifactor ANOVA and GLM did not show any significant differences (p<0.5) among sex, stations and the polymers. SIMPER analysis showed, 87.53% of similarities among the stations and sex of those shrimps with ingested plastics.

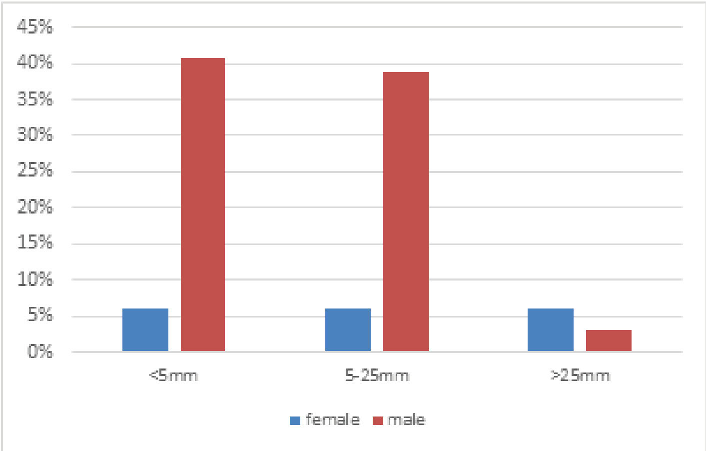

Fig. 2: Percentage of ingested plastics by size category for females and males. Blue is indicating female and red is indication male shrimps. Filaments <5mm are Microplastics, <5-25>mm are Mesoplastics and >25mm are Macroplastics.

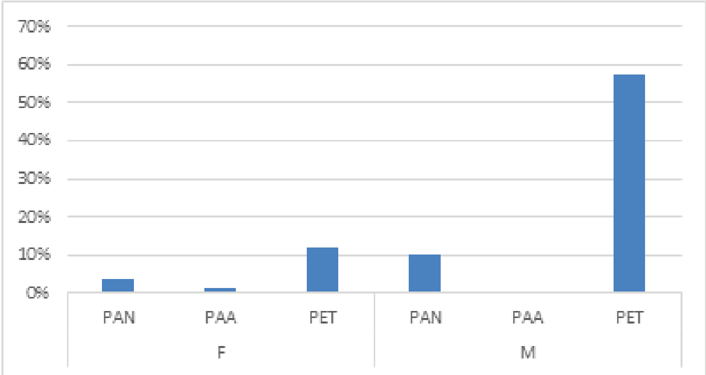

Fig. 3: Percentage of different polymers in females and males. PAN – polyacrylonitrile, PAA – polyacrylate, PET- polyester.

4. Discussion

Benthic animals are very susceptible to plastics ingestion. This study highlights the preliminary results of the presence of plastics in the stomachs of the shrimp *A. foliacea.* Similar studies have been carried out on the *Aristeous antennatus* (same family) in Spain and Italy (Cau *et al.*, 2019; Carreras-Colom *et al.*, 2020). In these studies, the percentage of ingested plastics were 84.6% off the Catalan coast (Carreras-Colom *et al.*, 2020) and 67% around the Sardinia Island (Cau *et al.*, 2019), which is higher than the plastic occurrence of 11.93% found in *A.foliacea* in this study. The differences in the percentage of ingested plastics in these red shrimps inhabiting similar environments may be attributed to the feeding strategies and interactions with the sea floor. *A. foliacea* displays a highly diversified diet, preying primarily on zooplankton and micronektonic and benthic organisms (Kapiris *et al.*, 2010), while *A. antennatus* has a great ability to root in the mud, preying on endobenthic and epi- benthic small invertebrates (Carreras-Colom *et al.*, 2018). An additional important finding of this study is that 38.46% of the shrimps with ingested plastics were juveniles (CL<25 mm). Despite the limited number of plastics in this species in comparison with those found in the other part of Mediterranean Sea, *A.foliacea* may represent an appropriate sentinel species to assess the impact of plastics in deep-sea environments and therefore future research effort should be focused on the stock of this considerable market price species and their essential habitats.

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BLUE ECONOMY AND MARINE LITTER: A SYSTEMS THINKING APPROACH

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Abstract

In the Mediterranean Sea, a region identified as a "plastic trap", advanced efforts are required to enhance coordination in order to reduce plastic pollution. The BlueMed Initiative aims to advance a shared vision for a healthier Mediterranean Sea, promoting the citizens' social well-being and boosting economic growth. In June 2020, the BlueMed CSA organised an innovative series of seminars exploring the multiple connections of the blue economy sectors to the plastic litter issues in the Mediterranean aiming to identify methods enabling sustainable transformations of the blue sectors, while also considering the role of citizen science. Through the collection of the lectures' material and using a systems thinking approach, is feasible to connect the dots of the different economic sectors to the possible impacts and actions and identify potential interventions in policy or technological areas. Furthermore, group mental mapping workshops with a coastal community in the NE Mediterranean (COASTAL, H2020 project), provided the opportunity for a multi-scaled approach to the litter issues, from Mediterranean regions to local communities. By integrating Marine Litter (ML) within the blue economy systems framework, we aim to increase understanding through visualization of the complexity. Such a process could help design more effective solutions for addressing waste minimization through a circular economy approach.

Keywords: plastic pollution, Mediterranean Sea, citizen science, concept mapping, Blue Economy.

1. Introduction

The Mediterranean basin has been recognized as one of the most affected area for marine litter (ML) and an accumulation hotspot (Cozar *et al*., 215) due to the semi-enclosed morphology, numerous anthropogenic activities in the coastal zone of its countries, maritime traffic, as well as the high discharges that receives from large rivers. ML of different sizes is found along the coastline, floating both on the surface and in the water column, down to the seafloor (Bergmann *et al*., 2015). Mediterranean plastic pollution represents today a serious risk for the local environment and human health, but also for the key economic sectors that rely on sea resources and health as for Fisheries and Tourism. Although Mediterranean economy is strongly based on the healthy coastal and marine resources, the current level of knowledge concerning the ecological, social and economic impacts of ML at basin-level is insufficient, due to the high complexity of the problem (Beaumont *et al*., 2021). The number of stakeholders involved affects also the transition to circular and bio-economy which have both been recognised by industries, scientists, and policy makers as promising approaches to improve sustainability of the blue economy sectors, given their ability to minimize waste generation (reduce, reuse, recycle) and to produce low-impact biodegradable products, respectively (ten Brink *et al*., 2016). In 2021, the European Commission published a Communication document on a new approach for a sustainable blue economy in the EU, which integrates the Marine Strategy Framework Directive and targeted actions of SDG 14 for ocean protection, promoting research and innovation actions and collaborations for implementing the circular economy approach and uses the plastics strategy in order to reduce ML and microplastics throughout the lifespan of products[1].

The main goals of the analysis to: i) foster a better understanding of the socio-economic impacts

1 European Commission (2021), COM/2021/240 final: Communication on a new approach for a sustainable blue economy in the EU Transforming the EU's Blue Economy for a Sustainable Future

from ML pollution at regional scale; ii) guide the national industry, decision-makers or managing authorities towards meaningful research and innovation actions for plastic-free oceans; and iii) improve marine policy for ML issues. Furthermore, we aim towards setting the foundations for designing an integrated socio-technical behavior change concept for ML management in Mediterranean Regions, in accordance with the ‘polluter-pays’ principle and the risk of loss of ecosystem services due to ML pollution. Through a systemic analysis, this study identifies the blue economy sectors, which are entangled in a reinforcing feedback loop with ML and need further measures, helping to prioritise actions and the most affected sectors, disseminate innovative practices and design the optimal approach to problem-solving, according to the specific characteristics of each area.

2. Methods

2.1. A systems thinking approach and development of concept map

Through systems thinking and systems analysis the world is presented as a framework of connected components which can be analysed in a holistic synthesis fostering an understanding between the relationships and the interactions of the components, rather than knowledge on the development of each distinctive component (Skyttner, 2005). A system can be represented descriptively through the use of conceptual models. These are graphical representations of the basic constructs of the system being analysed, offering the basic system feedback structure including concepts or state variables including the causalities existing between these variables. To systematically describe the components of the different aspects of the ML issue we used: *a)* the distinctive components analysed during the BlueMed e-training course to describe the ML - Blue Economy system in the Mediterranean as a whole, which was designed to inform on the impacts of litter at sea but also to be complementary to the enhancement of blue skills and the blue component of the economy; *b)* outcomes from stakeholders workshops in SW Messinia, in relation to litter and waste management for connecting with local scale of Mediterranean communities (Tiller *et al*., 2021); and *c)* literature, where gaps still existed such as for example the connections to the Global Plastic Production and Consumption (Driesbach, 2020). We used Vensim software to graphically visualise the results.

3. Results

3.1 Mental model build up – Causal Loop Diagram

Using the material provided by the experts during the course, we were able to identify possible connections and interactions of each component and hence produce a conceptual map of ML in the Mediterranean Sea, integrated in the blue economy system of the region (Table 1).

Table 1. ML from the Blue Economy sectors: Sources, Impacts and Reduction.

	Sources	Biodiversity Impact	Socioeconomic Impact	Cleaning/ Reduction
Tourism	beach litter	entanglement ingestion	beach attractiveness, destination dissatisfaction	waste management
Fisheries	derelict fishing gear, discarded boats	entanglement, benthic habitat damage, ghost fishing	revenue loss, ghost fishing, CPUE[2] decrease, equipment damage	trawling intensity & fishing for litter, port collection points, financial incentives
Aquaculture	gears, nets, tags, cages, ponds	fauna trapping, benthic habitat damage, microplastics ingestion	economic impact, revenue loss	gear collection points, legislation, financial incentives
Shipping (incl. ship building repair)	Packaging operation. waste, accidents, discarded boats	transfer biofouling, plastic additives by leaching, toxicity[3]	costs to cover damaged equipment (e.g., propelles)	legislation, - on board waste management, new modular design

The developed concept map of ML and Blue Economy in the Mediterranean is shown in Figure 1. The produced map reveals the links and feedback loops between the presence of ML, the blue economy sectors (i.e., shipping, fisheries, aquaculture, and tourism in the blue boxes), and how these are impacted by the presence of ML. There are four categories of waste reaching the sea from the Blue Economy sectors (orange boxes in Fig. 1), *Production Waste* that is generated from the production phase (nets, fishing gear, gloves etc.), and *Packaging waste* generated as a byproduct of consumption, both related to Fisheries and Aquaculture. A third category is *Beach Litter* associated with the coastal population and Tourism Economy. *Discarded boats* and *Accidents*, form separate categories not included in the *Production waste* variable due to the size of the wasted material, and the need for dedicated management actions. Each of the 4 main sectors creates feedback loops with the presence of ML which are either reinforcing (i.e., activities that add to the issue – lost or destroyed gear creates the need for more gear, which will also be lost or destroyed, or balancing – tourist dissatisfaction causes the tourism sector to consider more advance waste management).

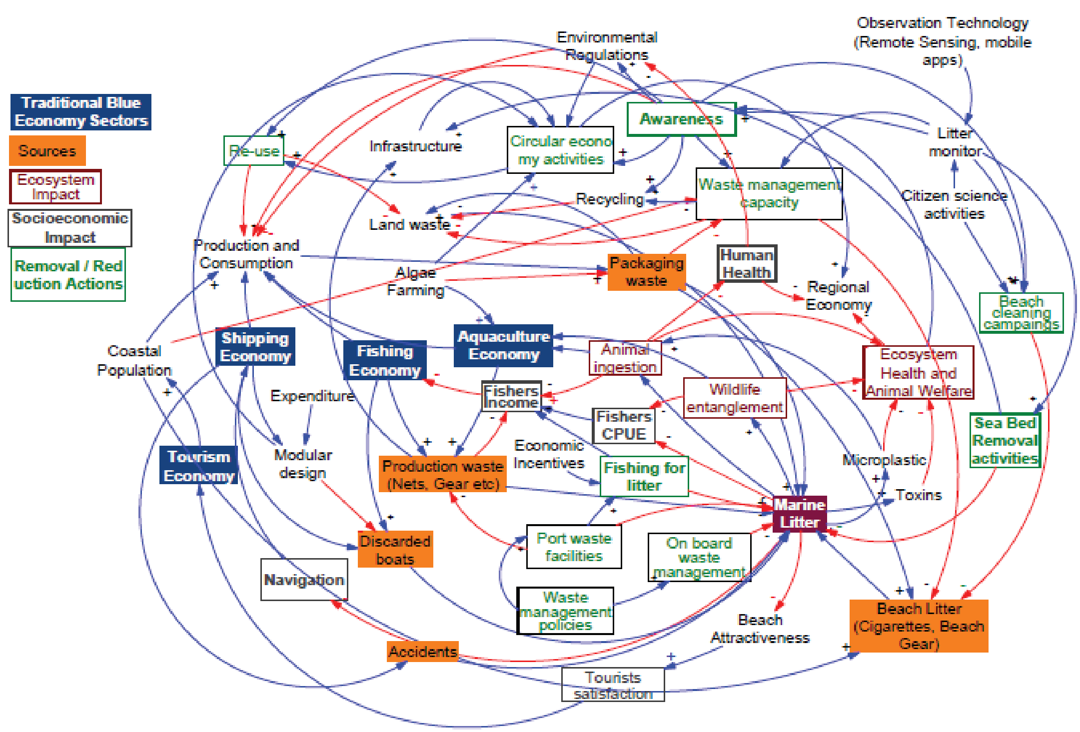

Fig. 1: ML Concept Map (Blue arrows for positive - reinforcing connections and red arrows negative -weakening connections).

2 Cost Per Unit Effort
3 These impacts are true for all categories

In most cases, for the balancing feedback to be activated, there is the need to provide infrastructure (port waste facilities) or economic incentives to the actors (fishers and aquaculture farmers). At the moment, the recognized litter removal activities are focusing on citizen beach clean-up campaigns, volunteer divers and NGOs who have increased awareness of the ML issue. Through the map (Fig. 1), we can also identify possible balancing loops i.e. activities that are currently removing ML from the sea (i.e., cleaning campaigns at the beach of fishing for litter) and how these could be potentially be re-enforced if available infrastructure is in place to allow for circular economy activities.

Fig. 2: Beach clean ups in Greece (2018-2020)

3.2 Mapping actions of beach-underwater waste removal, re-suing and education

The BlueMed project also initiated a Mediterranean pilot on Healthy Seas, which includes hubs in several Mediterranean countries to monitor actions related to ML. For the Greek hub on ML, we recorded several activities related to clean-ups, circular economy and education organized during the period 2018 to 2020. A representative map of beach clean-ups taken place in Greece is demonstrated in Fig. 2. Clean-ups were primarily conducted on coasts and ports of large coastal cities (e.g., Athens, Thessaloniki, Heraklion), on touristic islands such as Mykonos, Naxos, Zakynthos as well as on known polluted areas (e.g., Saronikos Gulf, Andros isl.). In most cases (~68%), the weight/volume of the collected waste is estimated, as for few cases (~13%) a further classification of collected ML plastic types is achieved following the Marine Strategy Framework Directive guidance. Very few of the actions reported connections to circular economy initiatives, such for example upcycling of the retrieved items. The extent of the mapped actions shows the importance of volunteers and citizen scientists in removal of ML from the environment and addressing the plastic pollution problem.

4. Discussion/Conclusions

The integrated concept map can inform the design of a mental model for ML in the Mediterranean, which can be used to visualize a socio-technical behavior change system for addressing the social and economic costs from plastics pollution. Based on the analysis described above, we observe that social and economic costs are often experienced by coastal communities, especially those relying on fishing and tourism.

The most important result from the systemic analysis shows that fishers are entangled in a reinforcing feedback loop where they need the nets to conduct their activities, food packaging for storing their products but the discards are reducing their income and harming their product. The complexities identified show that there are not straightforward solutions. It is argued that ML threatens all main sectors of the Mediterranean blue economy, mainly fisheries, tourism and maritime transport. At the same time, these sectors are major contributors of sea-based ML in the area. Fisheries and a large percentage of the tourism sector in the Mediterranean are based on small and medium enterprises (SME), which may not be able to afford strict mitigation measures (e.g., environmental regulations and taxes for plastic products in their operation, replacement with expensive plastic-free products) resulting in social exclusion. With the current level of knowledge, circular economy practices can be adopted in business models of tourism and maritime sectors.

The valorization of seafood processing by-products for the production of biodegradable, food packaging, is considered as an interesting solution for replacing single use plastics (Gianakourou *et al.,* 2021). For the case of the Mediterranean, it seems feasible only if regional or national blue economy enterprises and research institutions work in a synergistic and complementary way. To achieve that, future research should further examine and evaluate the potential for blue biotechnology plastic replacement in the Mediterranean and there is a need to raise funds for a circular plastic economy and awareness in the fishery, aquaculture and tourism industries. Most of the solutions identified through this study are expensive, and production is insufficient in volume hence there is a need for policy and financial incentives to support the transition. We are referring to new business models and new value chains, but also new social practices and initiatives that promote and ensure the viability of alternatives capable of transforming current sectors and systems and opening up new horizons for development or replacing them with others that can generate greater value (economic, social and environmental).

5. Acknowledgements

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THE PHYSIOLOGICAL RESPONSES OF TWO FUCALEAN SPECIES TO INCREASING AMMONIUM CONCENTRATIONS: ECOLOGICAL AND AQUACULTURE CONSIDERATIONS

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Abstract

The perennial fucalean species under increasing nutrient conditions are often replaced by congeneric, semi-perennial species worldwide, endangering the maintenance of essential ecosystem services. Such a process, also typical in the Mediterranean Sea, is shifting, for example, of *Ericaria barbatula* forests to *Cystoseira compressa*. Thalli apices of the two species were compared under laboratory conditions to assess the effects of ammonium, a common of human activities product, on chlorophyll-a and growth physiological parameters. *Cystoseira compressa* showed higher than *E. barbatula* mean RGR values only within the first days of cultivation in all ammonium concentrations, while *E. barbatula*, in general, did not show significant differences under different ammonium concentrations over time. *Ericaria barbatula* photosynthetic apparatus was also more tolerant than *C. compressa* at higher ammonium seawater concentrations, as evidenced by higher maximum PSII quantum yield (φ_{P0}), and low specific energy flux for dissipation per RC (DI_0/RC). TR_0/RC represents the maximal rate by which an exciton is trapped by the reaction center (RC) increased under high ammonium treatments only for *C. compressa*, confirming photodamage rather than a photoacclimation of the RC complexes. Understanding the interplay between species photosynthesis, growth, and ammonium is critical to understanding physiology and predicting fitness in species distribution and aquaculture applications.

Keywords: Brown algae, photosynthesis, growth, JIP-test.

1. Introduction

Human activities affect fundamental ecological processes, such as seaweed competition, shaping their community's structure and function (Viaroli *et al*., 2008). For example, the perennial fucalean species under gradually increasing nutrients are often replaced by congeneric, semi-perennial species worldwide, endangering the maintenance of essential ecosystem services. In the Mediterranean Sea, *Cystoseira* s.l. forests have undergone a significant decline in the last decades, with *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, for example, replacing *Ericaria barbatula* (Kützing) Molinari & Guiry forests (Panayotidis *et al*., 2004). Although mariculture and urbanization seem to be the main stressors of this process (Orfanidis *et al*., 2021), mechanistic studies for testing these hypotheses are missing. On the other hand, they are known for their biomedical and pharmaceutical potential (De La Fuente *et al*., 2021), and therefore, their cultivation could diversify aquaculture products and ameliorate environmental impacts.

A standard human activities product, ammonium is a vital nitrogen source for seaweed growth and enriches coastal waters via rain or sewage inputs and/or animal-fish farming. It can also be released from particulate organic matter decomposition or excretion by marine fauna, particularly by herbivores such as zooplankton and/or benthic grazers or fish aquaculture (Hurd *et al*., 2014). However, its optimal, inhibitory, or toxic effects on seaweeds and especially on fucalean species are less studied.

Fast chlorophyll-a fluorescence analysis is a simple and non-destructive method for plants to estimate the photosynthetic apparatus function under different types of stress (Kalaji *et al*., 2016). OJIP fluorescence kinetics give information about the structure and function of photosynthetic apparatus (Tsimilli-Michael, 2020).

This study aimed to investigate the effects of ammonium in photosynthetic apparatus and the growth of the brown algae *E. barbatula* and *C. compressa*. The results will provide information about (1) species' physiological and growth responses in habitats under increasing anthropogenetic pressure, and (2) the acclimation strategies to survive under stressful environments in biotechnological applications, such as the Integrated Multi-Trophic Aquaculture (IMTA).

2. Material and Methods

Ericaria barbatula and *C. compressa* were sampled from Cape Vrasidas (40°49'42.52"N, 24°20'2.00"E) and Palio rocky coasts (40°53'46.52"N, 24°20'17.91"E) of the Kavala Gulf, respectively, on June 11th and on July 3rd, 2018. Two factorial experiments (n=6) of healthy 0.11 to 0.14 g wet biomass (wb) specimens were carried out and lasted 16 days, in which individual and combined effects of species (two levels: *E. barbatula* and *C. compressa)* and ammonium (four levels: 0.6, 30, 60, 90 **µmol**/L N-NH_4^-) were tested. For logistically and space reasons, the conditions C1 (0.6 **µmol**/L N-NH_4^-, 2 **µmol**/L P-PO_4^-) and C2 (30 **µmol**/L N-NH_4^-, 2 **µmol**/L P-PO_4^-) were tested in the first experiment, and the C3 (60 **µmol**/L N-NH_4^-, 2 **µmol**/L P-PO_4^-) and C4 (90 **µmol**/L N-NH_4^-, 2 **µmol**/L P-PO_4^-) were tested in the second experiment. Two hundred milliliters of the medium were renewed daily inside small glass jars covered by transparent glasses (2 mm) to avoid evaporation. All glass jars used for the experiment were placed on shakers to avoid medium stratification. The temperature (22-24 °C), salinity (34.5-36), and pH (7.1-7.4) were monitored every morning. Irradiance was provided for 14 h per day by LED Fyto-Panels (81 x 27 cm; Photon Systems Instruments, Drasov, Czech Republic). pH was monitored with a portable pH meter (WTW pH 3210).

Chlorophyll-a fluorescence measurements were performed using the Handy-PEA fluorometer, a continuous excitation plant efficiency analyzer (Hansatech Instruments Ltd, UK). Measurements were carried out on days 1, 5, 12, and 16, and JIP-test parameters were calculated using the OJIP transients (Tsimilli-Michael, 2020). Chlorophyll-a (Chl-a), Chlorophyll-c (Chl-c), and Fucoxanthin (Fx) were estimated on the replicates used for JIP-test based on Evans's (1988) method. Wet biomass (wb) was measured for the six (6) replicates in each condition on experimental days 1, 5, 8, 12 and 15. The relative growth rate (RGR) was calculated from 1-5, 5-8, 8-12 and 12-15 days according to the equation: RGR (day^{-1}) = $(LnWBt_2 - LnWBt_1)/(t_2 - t_1)$, where WBt_2 and WBt_1 were the wet biomasses in the end and beginning of measuring periods, respectively.

Two-way ANOVA analyses and post-hoc tests were performed for each measuring period by using STATISTICA (version 7.1; StatSoft and TIBCO Software, USA) software and Principal Component Analysis (PCA) by using Canoco 5.1 (Microcomputer Power, USA) software. For the samples without a normal distribution and homogeneity of variance (periods 8-12 and 12-15 days) the significance level was set at 0.01 instead of 0.05.

3. Results

There was a significant effect of species on RGR during the period 1-5 days and interaction between species and ammonium concentrations on RGR ($p < 0.001$) for the other periods (Figure 1). *Cystoseira compressa* showed higher than *E. barbatula* mean RGR values within the 1-5 days of cultivation (Cc=0.059-0.077 day^{-1}; Eb=0.034-0.046 day^{-1}) in all ammonium concentrations (Figure 1). RGR values of *E. barbatula* did not present significant differences under different ammonium concentrations at other times, except under C2 during the 5-8 days period where the maximum mean value was measured (0.088 day^{-1}). However, RGR values in *C. compressa* have dropped acutely after the 8th day under C3 and C4 concentrations, indicating death.

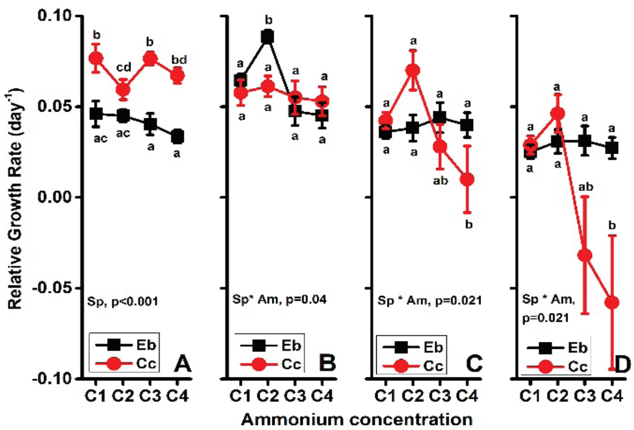

Fig. 1: Effects of time and ammonium concentration on species (Ec=*Ericaria barbatula*, Cc=*Cystoseira compressa*) relative growth rate (RGR; means ± standard error, n = 6). Time period (days): A=1-5, B=5-8, C=8-12, D=12-15. C1=0.6 **µ**mol/L, C2=30 **µ**mol/L, C3=60 **µmol**/L, C4=90 **µmol**/L. Statistical differences from the post hoc tests (p-value <0.05 for A and B, p-value < 0.01 for C and D) are represented using different Latin letters.

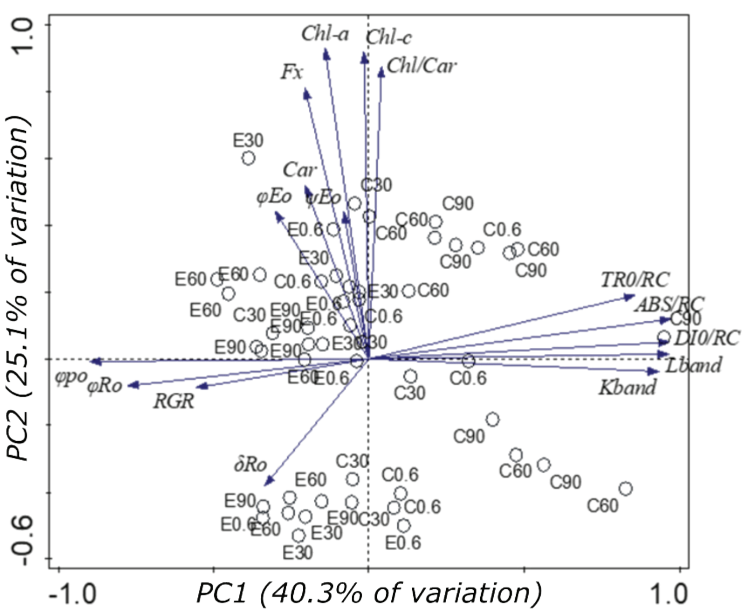

Fig. 2: Principal Component Analysis (PCA) that shows the correlations between the JIP-test parameters, photosynthetic pigments concentrations and relative growth rate (RGR) of *Ericaria barbatula* and *Cystoseira compressa*. The length of the arrows indicates the strength of representation and contribution of each parameter to the PC axes. E = *E. barbatula*, C = *C. compressa*. 0.6 = C1, 30 = C2, 60 = C3 and 90 =C4 condition. The first axis accounted for 40.3% of the variation, while the second axis for 25.1%. $ABS/RC= M_0 \times (1/V_J) \times (1/\phi_{P0})TR0/RC= M_0 \times (1/V_J)$, $DI_0/RC= ABS/RC – TR0/RC$, $RC/ABS= (TR_0/ABS) \times (TR_0/RC)^{-1}$, $\varphi_{P0}= TR_0/ABS = [1 - (F_0/F_M)]$, $\psi_{E0}=ET_0/TR_0 = (1 - V_J)$, $\varphi_{E0}= ET_0/ABS = [1 - (F_0/F_M)] \times (1 - V_J)$, $\varphi_{R0}= RE_0/ABS = [1 - (F_0/F_M)] \times (1 - V_I)$, $\delta R_0= RE_0/ET_0 = (1 - V_I)/(1 - V_J)$, K-band= $V_{300\mu s}$ (variable fluorescence), $\Phi_{PSII}= (F_M'-F_S')/F_M'$, $qP= (F_M'-F_S)/(F_M'-F_o')$, $qL= qP \times (F_0'/F_S)$, $qN= (F_v-F_v')/F_v$, $NPQ= (F_M/F_M') - 1$, $qI= (F_M-F_M'')/(F_M-F_0')$.

The responses of two species to ammonium at photosynthesis and growth levels were studied using multivariate analysis (Figure 2). The PCA showed differentiation between the two species. The parameters L-band, DI_0/RC, K-band, ABS/RC, and TR_0/RC were positively correlated with Axis 1 (40.3% of the total variance), which were influenced by high ammonium concentrations of *C. compressa*. In contrast, the parameters φ_{P0} and φ_{R0} were negatively correlated with Axis 1, which were influenced by high ammonium concentrations of *E. barbatula*. Chl-*a*, Chl-*c*, F_x, and Chl/Car were positively correlated with Axis 2 (25.1% of the total variance) and were influenced by low ammonium concentrations of both species.

4. Discussion

Ericaria barbatula and *C. compressa*, i.e., a perennial vs. a semi-perennial species, responded differently to ammonium (Figures 1 and 2), underpinning different environmental and biotechnological considerations. Surprisingly, *E. barbatula*, a late-successional species, and indicator of pristine, high ecological status, conditions in the Mediterranean Sea showed higher tolerance to ammonium than *C. compressa*, a late-successional species indicator of pristine to moderately degraded status (Panayotidis *et al*., 2004; Ballesteros *et al*., 2007; Orfanidis *et al*., 2021). Therefore, in agreement with an existing ecological status assessment model (Orfanidis *et al*., 2011), nutrient and light resource allocation strategies and not toxic effects are mainly involved in species adaptation across a coastal water pollution gradient. On the other hand, a high ammonium tolerance could be valued under IMTA or other biotechnological practices.

During the first studied period (1-5 days), under all ammonium concentrations *C. compressa* grew better than *E. barbatula* (Figure 1), indicating a higher uptake affinity that can scavenge any limiting molecule, a typical feature of opportunistic species (Hurd *et al*., 2014). In agreement, thallus shape is an important factor influencing nutrient-uptake rates since nutrient uptake occurs through the surface membrane of the thallus, *C. compressa* shows a higher surface-area to volume (SA:V) ratio. Also, higher RGR values mean better fitness and competitive ability under moderate degraded coastal waters, which may explain the well-known pattern of replacement of perennial fucalean species by relatively fast-growing *C. compressa* under stressful conditions (Falace *et al*., 2010; Kletou *et al*., 2018).

The higher sensitivity of *C. compressa* than *E. barbatula* to ammonium concentrations was evident at growth and the photosynthetic levels (Figures 1 and 2). At higher ammonium levels (C2, C3) *C. compressa* died but *E. barbatula* remained alive. Increased NH_4^+ significantly affected the absorbance flux per reaction center (ABS/RC) in *C. compressa*, which indicated that PSII RC's and plastoquinone (PQ) pool were overloaded with electrons (Lepedus *et al*., 2020) that cannot be transferred over the transport chain, and the excessive excitation energy is dissipated as heat (DI_0/RC) in order to prevent the over reduction of electron transport chain and consequently a photooxidative damage. Electron's accumulation in RCs TR_0/RC values increased at C3, C4 only in *C. compressa* which showed a higher maximal rate by which an exciton is trapped by the RC, confirming photodamage rather than a photoacclimation of the RC complexes (Stirbet & Strasser, 1996).

Increased ammonium levels also affected the quantum yields φ_{P0} and ψ_{E0}. In *C. compressa*, the maximum quantum yield for primary PSII photochemistry (φ_{P0}) and the efficiency/probability that an electron moves further than Q_A^- (ψ_{E0}) were decreased indicating limitation of the redox state of Q_A and thus decreased the electron transport between Q_A and Q_B electron acceptors (Zhang *et al*., 2020). *Ericaria barbatula* presented higher quantum yields φ_{P0} and ψ_{E0} which indicated the increase of electron transfer from Q_A and Q_B electron acceptors at C3, C4 conditions. The quantum yield of reducing end electron acceptors at the PSI acceptor side (φ_{R0}) was at the same levels for both species at low ammonium levels (C1, C2). On the contrary, as ammonium levels increased *E. barbatula* showed higher $\boldsymbol{\varphi}_{R0}$ values than *C. compressa*, indicating higher energy supply from PSI towards the Calvin cycle, and therefore, higher RGR values (Malea *et al*., 2021).

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FIRST IN VITRO STUDY OF EARLY DEVELOPMENTAL STAGES OF *GONGOLARIA MONTAGNEI (= CYSTOSEIRA SPINOSA)* (FUCALES, PHAEOPHYTA)

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Abstract

An ongoing decline of the climax communities of canopy-forming algae along the Mediterranean shallow rocky reefs is increasingly documented, reportedly driven by a multitude of stressors. Apart from alleviating local or global pressures to reverse this alarming trend, basic knowledge of these key species' reproductive phenology, embryology and growth is a crucial stepping stone towards successful restoration strategies, as put forward by the EU Biodiversity Strategy for 2030. *Gongolaria montagnei* is one of the most common canopy algal species presenting a wide horizontal and vertical distribution along the Greek coasts. Mature receptacles of the species were collected from Saronikos Gulf (Aegean Sea) and germlings were cultivated in mesocosms for 34 days, reaching up to 1.30 mm length. Divisions and early developmental stages of *G. montagnei* are for the first time documented and described, confirming the species assignment into Group I in Guern's embryonomic classification study.

Keywords: marine algal forests, cultivation, embryology, germination.

1. Introduction

Cystoseira sensu lato (hereafter referred to as *Cystoseira*), principal formers of Marine Algal Forests in the Mediterranean, provide a wide range of crucial ecosystem services, including food provision, biodiversity enhancement, nutrient cycling, gas and climate regulation, bioremediation, and leisure and recreation, to name a few (Dayton, 1985; Chapman, 1995; Küpper & Kamenos, 2018). In the last decades, an ongoing decline of *Cystoseira* forests has been observed throughout the Mediterranean, including the Greek coasts. Canopy forming algae are known to be highly susceptible to natural and human disturbances (Montesanto & Panayotidis, 2001; Sales & Ballesteros, 2009; Orfanidis *et al.*, 2021). Furthermore, low growth rates (Orfanidis, 1991) and low dispersal capacity of *Cystoseira* zygotes (Verdura *et al.*, 2018) renders the species' natural recovery highly unlikely. Notwithstanding, under suitable temperature and light conditions *Cystoseira* species have shown high reproductive potential, thus methods for developing laboratory cultivation of *Cystoseira* are increasingly being investigated (Cebrian *et al.*, 2021 and references therein). In this study, we present the first attempt to document and describe early developmental stages of *Gongolaria montagnei (= Cystoseira spinosa)* (J. Agardh) Kuntze, one of the most common canopy algal species along the Greek coasts.

2. Material and Methods

The reproductive period of *Gongolaria montagnei* was followed in-situ, in the upper infralittoral zone (0-1 m), at three sites from February to May 2021. Mature fertile apices were detected from early April until the end of May, when sea surface temperature ranged from 16 to 18° C. In early May, *G. montagnei* apices (n=200) were carefully harvested from Saronida (Saronikos Gulf, Aegean Sea, Greece). Apices were immediately transferred to the HCMR laboratory, where they were rinsed with sterile seawater and stored in dark and cold conditions (5° C) for 24 h to induce zygote emission. The following day, apices were placed on microscope slides in polypropylene tanks containing 2 L of 0.2 µm filtered and UV-sterilized natural seawater. The tanks were set in an environmentally controlled room, where temperature (19 °C), photoperiod (12:12) (PHILIPS LED tube lamps cool white) and light intensity (125 µmol m^{-2} s^{-1}) were adjusted to simulate seasonal conditions in the natural environment. To minimize nutrient limitations, the seawater was enriched with Von Stosch's solution (VSE), which was renewed every four days (Falace *et al.*, 2018). Germanium dioxide (GeO_2) was added to prevent diatom growth (Falace *et al.*, 2006) and air pumps were installed in the tanks to keep seawater aerated.

The experiment lasted 34 days, during which the different stages of zygote and germling development were observed with a light microscope (OLYMPUS BX43, Japan). Microscopy photographs were taken using the Image Pro Premier 9.3.

3. Results

Due to the shock induced by the temperature difference (5° C to 19° C), zygote release occurred shortly (3-6 h) after the receptacles were placed on the slides (Fig. 1A). The first equatorial division was observed 18-24 h after zygote release (AZR) (Fig. 1B). The second division was perpendicular to the first (Fig. 1C), while in some zygotes the second division was parallel to the first (Fig. 1D) and it occurred within 22-42 h AZR. Rhizoids began to develop from 36 h AZR (Fig. 1E) and continued to increase in length over the following days (Fig. 1F). Up to day 6, the embryos maintained their spherical shape; they were still surrounded by the fertilization membrane and their surface remained quite stable. From day 7 AZR, the fertilization membrane detached from the embryos (Fig. 1G), they took on a more elongated shape and their surface gradually increased. From day 12 AZR, hyaline hairs were observed growing from the apical region of the embryo (Fig. 1H). On day 34, the length of the germlings ranged from 0.88 to 1.30 mm (Fig. 1I).

In the fourth week of cultivation, a proliferation of microalgae belonging to the genus *Nannochloropsis* (Trebouxiophyceae) began in the tanks. In the fifth week, the mortality of *G. montagnei* germlings increased due to the *Nannochloropsis* outbreak, and at the end of the experiment (day 34) only 10 individuals had survived.

4. Discussion/Conclusion

In Guern's (1962) most comprehensive embryonomic classification study, *Cystoseira s.l.* species are distinguished into three groups based mainly on zygote segmentation, oogonia shape and number of rhizoids. *Gongolaria montagnei*, although not studied at the time since the species had reportedly disappeared from sampling stations around Banyuls (France), was tentatively classified in Group I. Our results indeed confirm that, as predicted, *G. montagnei* early developmental stages present Group I traits, in close resemblance with *Ericaria mediterranea*, *Ericaria crinita* and *Gongolaria barbata* (Guern, 1962).

In the present study, the final length of *G. montagnei* germlings (0.88-1.30 mm) after 34 days of cultivation was quite smaller compared to other studies on different *Cystoseira* species (Falace *et al.*, 2018; Savonitto *et al.*, 2019, 2021; Orlando-Bonaca *et al.*, 2021). Although such differences in seedling lengths among *Cystoseira* species could be species-specific, this cannot be confirmed with certainty in the timeframe of this study. In addition, microalgal proliferation after one month of cultivation may have prevented germlings from reaching their maximum potential length.

Microalgal, bacterial and diatom proliferations are notoriously favoured during prolonged mesocosm cultures (e.g., Orlando-Bonaca *et al.*, 2021). Possible ways to prevent these outbreaks could be to thoroughly rinse fertile apices, shorten the overall duration of the culture and to adjust accordingly the cultivation protocols (e.g., culture medium).

The improvement of cultivation protocols could provide a high number of larger germlings suitable for active restoration initiatives. In a rapidly changing marine ecosystem, information on the reproductive phenology, embryology and growth of *Cystoseira sensu lato* juveniles will set a scientifically-sound basis for contributing to the efficient restoration of valuable and largely degraded infralittoral reefs, in line with the nature restoration targets of the EU Biodiversity Strategy for 2030.

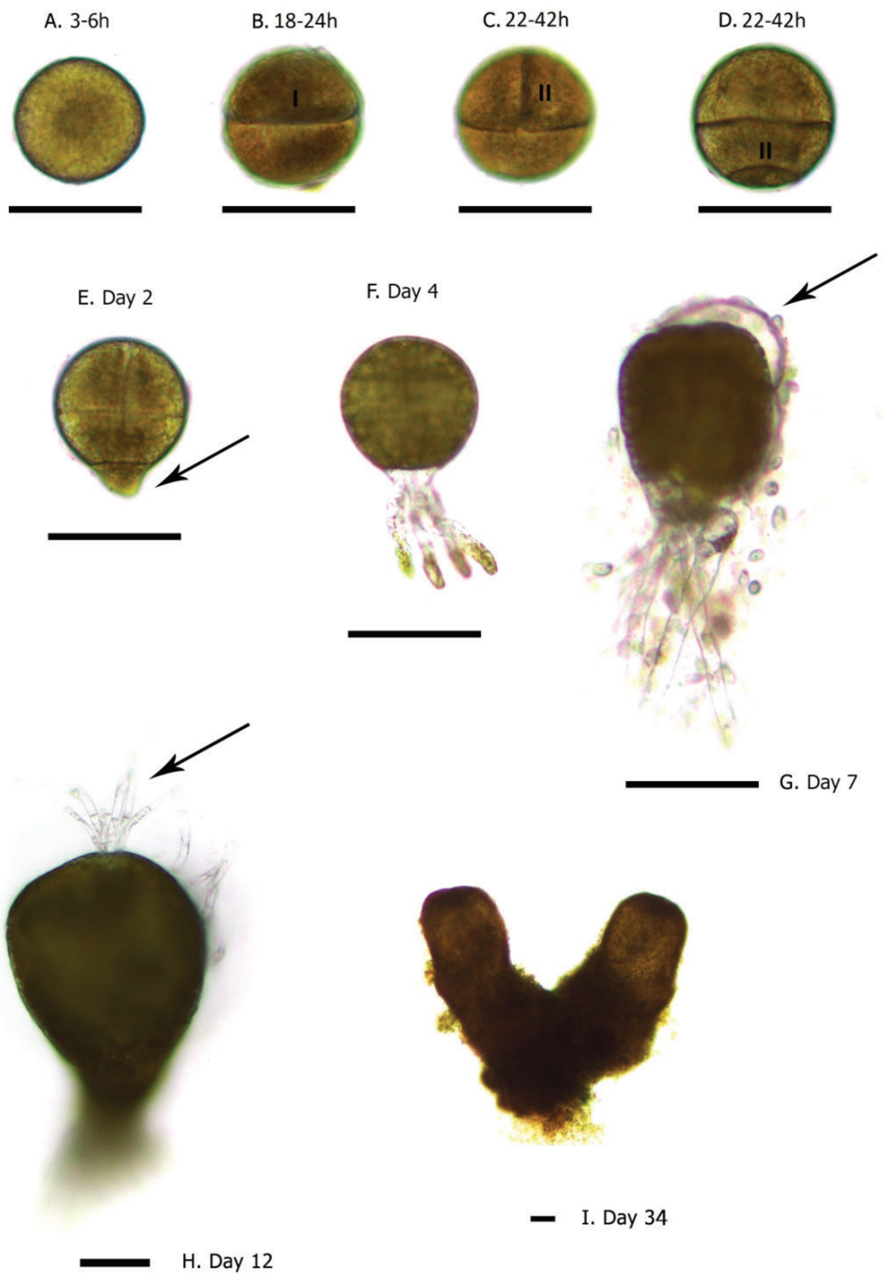

Fig. 1: Divisions and developmental stages of *Gongolaria montagnei*. A. Zygote after release from the conceptacle. B. First division. C. Second, most common, division. D. Second, rare, division. E. Development of the rhizoids (arrow). F. Elongation of the rhizoids. G. Fertilization membrane (arrow). H. Hyaline apical hairs. I. Germling after 34 days of culture. Scale bar = 100 **μm**.

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MORPHOLOGIGAL AND PHYSIOLOGICAL PLASTICITY OF TWO NORTH AEGEAN SEA, GREECE, *CYMODOCEA NODOSA* MEADOWS

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Abstract

Seagrass meadows are some of the most ecologically important habitats declining globally due to anthropogenic activities. To monitor their status and feed predictive models the study of their background phenotypic plasticity is needed. In this study, key morphological and physiological metrics, including growth description of the plastochrone interval (PI), for *C. nodosa*, the second most abundant seagrass in the Mediterranean, were conducted in two genetically uniform meadows (Cape Vrasidas-CV and Fanari-FN) in the N. Aegean Sea. According to morphological metrics, the two studied meadows being in good ecological status were different. Shoots were larger in FN, with higher biomass but lower density in comparison to CV ($p<0.05$). A slightly higher PI value was also measured in CV (5.8±0.9), compared to FN (7.8±1.7), but the values were lower than in other geographical regions. On the other hand, while maximum quantum efficiency (Fv/Fm) was higher in CV (0.79) than in FB (0.76), their photosynthetic performance (rETRmax, a) did not differ significantly. The morphometric, growth and photosynthetic performance results fall in agreement with the high phenotypic plasticity of the species, which can acclimate to different good environmental conditions.

Keywords: Seagrass growth, climate change.

1. Introduction

Seagrass is a unique group of plants that act as ecological engineers, creating dense meadows of high biodiversity, providing shelter and nursery fields for numerous species (Larkum *et al.*, 2006). Their ability to trap large quantities of carbon for extensive chronic periods, and their global distribution, are some of the reasons they have been targeted as key ecosystems for nature-based solutions for climate change mitigation (Chausson *et al.*, 2020). However, these habitats are declining globally, at 0.4-2.6% per year, due to anthropogenic emissions (Pendleton *et al.*, 2012) ,while their protection and recovery are key environmental policy aims.

Cymodocea nodosa (Ucria) Asch. is the second most important seagrass species in the Mediterranean Sea in terms of distribution and abundance (Pergent *et al.*, 2014). At the North Aegean Sea coasts, it grows in the upper sub-littoral zone and creates mosaic-type meadows that reach a depth of 5-10 m. Key species characteristics are its high growth rates and phenotypic plasticity (e.g., leaves can range from 10cm to 1m long). In areas were the prevailing sensitive seagrass *P. oceanica* is declining, the new empty space created is quickly claimed by the *C. nodosa*, which is characterized by higher growth rates (Sghaier *et al.*, 2011) and significant resilience to stress (Papathanasiou *et al.*, 2015).

Exploring such a habitat substitution is crucial for environmental management and protection. It is important to monitor such cases in order to be able to predict changes and take the appropriate measures in order to stop them. The creation of relevant ecological models of high confidence is based on continuing quality data. As such in this effort we have described key *C. nodosa* metrics from two meadows in the N. Aegean Sea. At the same time, for the first time we have calculated the growth rate of the species using the plastochrone interval method for Greece. Since environmental and local conditions highly influence species morphology and physiology, knowledge of the regional growth characteristics is important for any further research.

2. Material and Methods

2.1 Study Area

Cymodocea nodosa growth was studied in 2 locations in the North Aegean Sea, Cape Vrasidas-CV in N. Peramos, 22 km from the city of Kavala, and Fanari-FN, Vistonikos Gulf, in the Southeastern of Rhodope Prefecture. Both locations belong to the Natura 2000 network with codes GR1150009 and GR1130009, respectively. Fishing and tourism are the key anthropogenic pressures in both areas, with Fanari being further impacted by the extensive agriculture in the surrounding area and the water circulation from the adjacent mouth of Vistonida lagoon. Meadows in both areas are shallow (0-3m), with significant spatial coverage.

2.2 Data collection

Plastochrone interval was measured in both areas during two subsequent periods of seven days each during late July to early August 2021. In each sampling, abiotic parameters (temperature, salinity, dissolved oxygen concentration and pH) were measured. Shoot density was measured in 20 random 25x25 metallic quadrates in each area. Fifteen *C. nodosa* shoots were randomly selected, labelled and marked at the shoot node using a needle, according to the methodology described by Short F.T. & Duarte C.M. (2001). After seven days shoots were collected with their rhizomes and roots. In the laboratory each shoot was separated to leaves and rhizomes together with roots, leaf length and width, stem length and number of leaves per shoot were measured. Pierced leaves were classified as Old, while leaves with no apparent mark from the needle were characterized as New. Leaf area per shoot was calculated as the total leaf length/shoot * mean leaf width. Samples were then oven dried in 50°C for 24 hours and total shoot Dry Biomass was measured using a precision scale. Plastochrone interval (PI) was calculated using the formula: PI = (T1 - T0) / N where: T1: day the samples were collected, T0: day the samples were placed, N: new leaves.

During the second experiment, physiological measurements were measured using the DIVING-PAM (Walz). Five Rapid Light Curves (RLC) from five random shoots in each meadow were taken. Leaves were first shaded for 15 minutes and then RLC was conducted using varying Photosynthetically Active Radiation (PAR) from 0 to 654 μmol photons $m^{-2} s^{-1}$. To quantitatively equate RLC, rETRmax, α and Fv/Fm variables were assessed by fitting data from each RLC to the Eilers & Peeters (1988) photosynthesis-irradiance model using R.

Differences between the two studied areas for key metrics were explored using Permutation Multivariate Analysis of Variance – PERMANOVA, since data didn't follow multivariate normality according to the model Metrics Matrix (total leaf length and width, number of leaves/shoot, shoot biomass)~ Meadow*-Experiment and the adonis function from the vegan package. Shoot density and RLC parameters were analyzed separately using ANOVA, since data were normal according to the shapiro-wilk test and the quantile plots. All figures were prepared using the ggplot package.

3. Results

Environmental conditions didn't vary between the two periods, with water temperature ranging from 29°C in the morning to 32.6°C in the midday. Salinity in CV was 34.3±0.1, D.O. 111.1±10.6% and pH 9.10±0.06, while the same values in FN were 34.7±0.3, 129.5±1.7% and 9.00±0.07. The two meadows had significant differences on morphological and growth metrics measured (Table 1). The impacted meadow of Fanari was characterized by larger shoots, with longer and wider leaves and higher total biomass (Figure 1A). Vrasidas meadow had significantly higher ($F_{(1,20)}=193.1$, $p<0.001$) shoot density (1140.8 ± 31.56 shoots/m^2) than Fanari (498.67 ± 32.95 shoots/m^2), while number of leaves per shoot was similar between the two meadows (mean=40±0.08 leaves/shoot). Plastochrone interval in Vrasidas, was 4.9 days during the first experiment and 6.2 days during the second. Fanari, had higher PI values and specifically 6.7 and 9.5 for the two experiments, respectively.

Effective quantum yield (ΔF/Fm’) decreased with increasing PAR in a similar manner between shoots from the two meadows, ranging from 0.79±0.01 to 0.08±0.01, while in Fanari values were always lower than Vrasidas at each RLC step (Figure 1). rETR increased faster and higher by the irradiance in Vrasidas meadow than in Fanari, reaching to 24.48±1.52 and 22.10±2.32, respectively(Figure 1). ANOVA of the variables showed that there was statistically significant difference only between Fv/Fm and not between rETRmax and *α*(Table 2). Mean Fv/Fm in Vrasidas was 0.793± 0.006, with maximum value of 0.808, while in Fanari it was 0.765±0.006 with a maximum value of 0.784.

Table 1. Permanova of the two studied meadows and the two studied periods.

Level	**Df**	**SumsOfSqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**
Meadow	1	4.663	4.663	123.780	0.157	0.001
Experiment	2	0.074	0.037	0.979	0.002	0.422
Meadow:Experiment	1	-0.014	-0.014	-0.383	0.000	0.999
Residuals	661	24.899	0.038		0.841	
Total	665	29.621			1.000	

Table 2. One way ANOVA for the RLC parameters between the two studied meadows.

Parameter	***Level***	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Fv/Fm	Meadow	1	0.002	0.002	10.810	0.013
	Residuals	7	0.001	0.000		
rETRmax	Meadow	1	34.160	34.160	1.893	0.211
	Residuals	7	126.320	18.050		
a	Meadow	1	0.000	0.000	1.645	0.241
	Residuals	7	0.002	0.000		

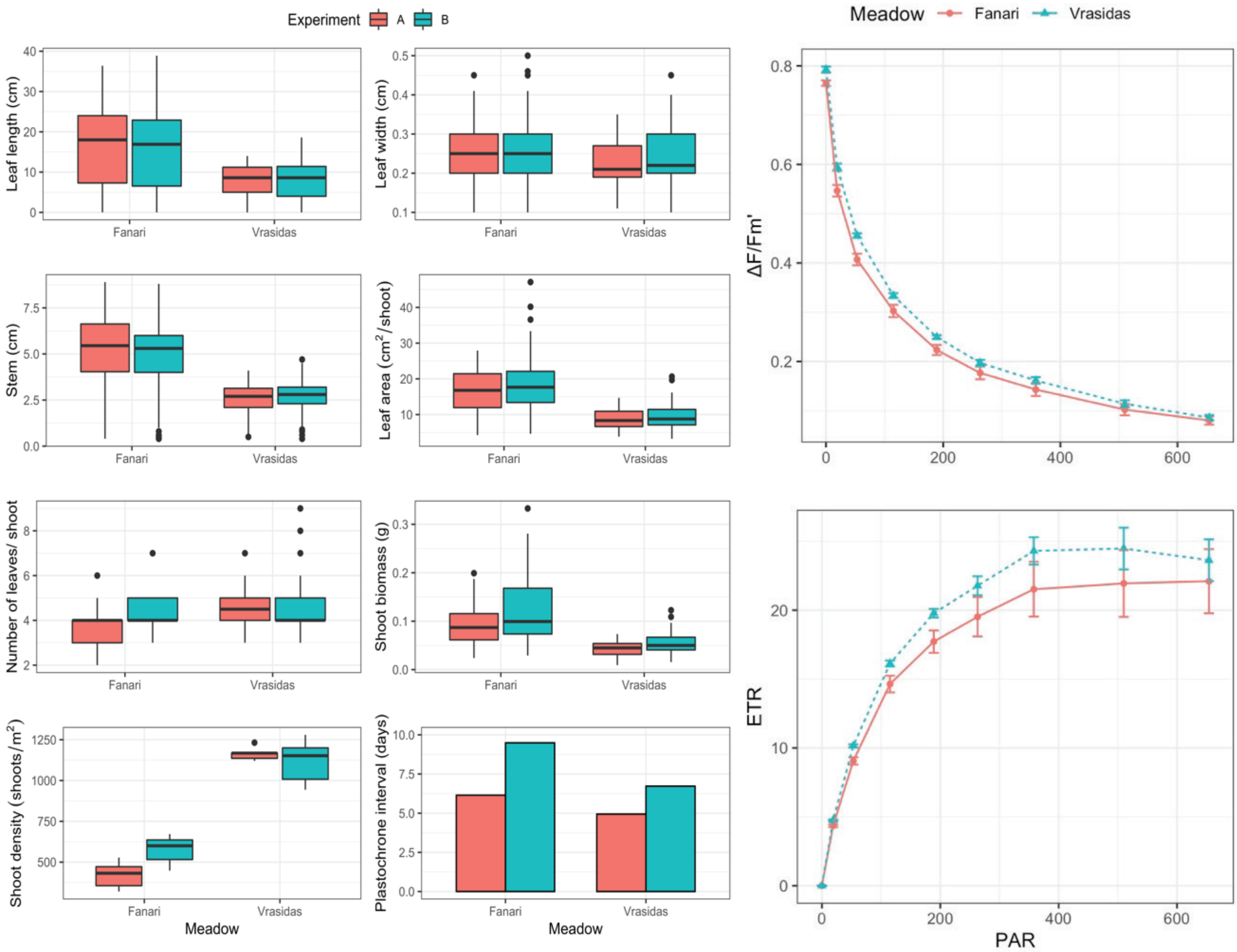

Fig. 1: A. Plots of key C. nodosa metrics and B) Rapid Light Curves collected in situ from the two studied meadows.

4. Discussion/Conclusion

The two sampled meadows were different according to morphological, growth, and maximum quantum efficiency metrics, with differences reflecting the varying natural and anthropogenic conditions. High nutrient availability leads to larger leaf size (Leoni *et al.*, 2006). This is the case of Fanari, an area with significantly higher nitrate concentrations (Papathanasiou & Orfanidis, 2018) due to the extensive agriculture of the surrounding area and increased turbidity caused by the water circulation from the Vistonida lagoon mouth. While nutrients are essential for plant growth, light deprivation can trigger a response in seagrasses, that invest in longer leaves, reaching closer to the surface and capturing more light (Longstaff & Dennison, 1999).

Despite shoot density being higher in Vrasidas, a common attribute of non-impacted seagrass meadows (Burkholder *et al.*, 2007), shoot size was enough to explain the significantly larger shoot biomass, as well as leaf area measured in Fanari (Fig. 1). This contradicting result, together with the absence of profound differences in all metrics, reflect the species phenotypic plasticity, which can be significant even between meadows of similar ecological status (good ecological quality based on CymoSkew; Orfanidis *et al.*, 2020), belonging to the same genetic population (Exadactylos, 2015).

Photophysiological metrics of seagrasses have been linked to stress (Bhagooli *et al.*, 2021). However, the physiological performance of PSII of two meadows was not different, but the Fv/Fm which was slightly higher in Vrasidas than in Fanari meadow. Growth was significantly higher in Vrasidas, with a new leaf forming every 5.8 days, in comparison to Fanari where a new leaf was formed every 7.8 days. Light deprivation has been reported to lead to significant declines in leaf production (Kelaher *et al.*, 2013). Even so, the values reported here are significantly lower than those from other research studies, that range from 12 (Sghaier *et al.*, 2011) to 45 (Olesen *et al.*, 2002). Indeed, PI is a varying parameter influenced by a series of local factors. Measurements were taken during late July- August, when seawater temperature was significantly high (29-32^{o}C), possibly causing increased growth for the two meadows, as has been reported in other cases (Lee *et al.*, 2007). In order to properly describe the growth dynamics of *C. nodosa* meadows, under different climate scenarios, seasonal and spatial PI calculations are needed. In general, the morphometric, growth and photosynthetic performance results fall in agreement with the high phenotypic plasticity of the species, which can acclimate to different good environmental conditions.

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PHYTOMYXID PARASITE PRESENCE IN A HALOPHILA STIPULACEA MEADOW NEAR A FISH-FARM IN SOUTH EVOIKOS GULF (GREECE)

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Abstract

Halophila stipulacea is a tropical seagrass native to the Indo-Pacific and Red Sea. Nevertheless, it has invaded the Mediterranean due to the Suez Canal opening. It is also a known host for phytomyxids which cause a distinct gall formation in the leaf petiole. In the Mediterranean Sea, until now, two records of the parasite infection in *H. stipulacea* are reported and molecular data revealed that the phytomyxid parasite found, belongs to a new species (*Marinomyxa marina*), forming a novel genus. In the present study we collected samples of *H. stipulacea* during July 2021 and October 2021 from a meadow located near a fish farm unit in the Southern Evoikos Gulf (Aegean Sea, Greece). We state the third record of phytomyxid parasitism in the Mediterranean, with the higher infection rates to be recorded in June 2021. Some morphological and anatomical traits of the formed galls were also studied, and the phytomyxid residing spores had a larger diameter to the already reported, mostly found in dyads. More research is required in order to elucidate the phytomyxid parasitic nature.

Keywords: Aegean Sea, invasive species, *Marinomyxa marina,* Phytomyxea.

1. Introduction

The Mediterranean Sea is home to four autochthonous marine seagrass species (*Cymodocea nodosa* (Ucria) Asch., *Posidonia oceanica* (L.) Delile, *Zostera marina* L. and *Z. noltii* Hornem) but also to the alien *Halophila stipulacea* (Forsskål) Ascherson, 1867, one of the first Lessepsian migrants to cross the Suez Canal, originating from the Indo-Pacific (Winters *et al.*, 2020). Since its first Mediterranean sighting, (Rhodes Island, in 1894) (Fritsch 1895) *H. stipulacea* has been colonizing the Mediterranean basin, while -since 2002- *H. stipulacea* was also found to another biogeographic area: the Caribbean Sea (Winters *et al.*, 2020). Over the last years, the species is expanding increasingly, possibly due to its superior ability to tolerate a wide range of environmental conditions (Lowe *et al.*, 2000). However, its spread has not yet been considered to be related to the decline of the native seagrasses species, with only a handful of studies to report a competition with *C. nodosa* (Sghaier *et al.*, 2014).

H. stipulacea is a known host of an obligate endobiotic parasite of the Phytomyxea class (SAR: Rhizaria, Endomyxa) recently categorized as a new species, namely *Marinomyxa marina* Kolátková, Čepička, Hoffman et Vohník, (Kolátková *et al.*, 2021). *M. marina* causes the formation of characteristic galls in the leaf petioles of *H. stipulacea*. Intracellular phytomyxid infection has been reported in native populations of *Halophila* species (Gulf of Aqaba) and in other native seagrass species like *Zostera* (Kolátková *et al.*, 2020, 2021; and references therein). In *H. stipulacea* populations of the Caribbean Sea –up until now– there is only one report of the parasite (Maitz *et al.*, 2021). In the Mediterranean Sea there are only few reports stating the presence of the parasite: one is from the Strait of Messina (Sicily, southern Italy) (Marziano *et al.* 1995; Kolátková *et al.*, 2020) and one from the Aegean coast of Turkey (Vohník *et al.*, 2017).

Given the argument that *M. marina* infecting *H. stipulacea* is most likely a species-specific parasite (Kolátková *et al.*, 2021), in the present study, we focused on the presence of the phytomyxid parasite in an *H. stipulacea* meadow in the Aegean Sea, near a fish farm, during two seasons. Some of the morphological/anatomical traits of the galls formed were also described. Our results further confirm the above argument while a season specific pattern of infection was revealed. Given the scarcity of data, recording the existence of phytomyxea parasites is of importance, especially in an invasive seagrass like *H. stipulacea* (Vohnik *et al.*, 2017).

2. Materials and Methods

Halophila stipulacea plant sampling was carried out during July 2021 and October 2021 in the Southern Evoikos Gulf (Aegean Sea, Greece) in a meadow located near a fish farm (Fig. 1). The study was conducted at three distinct sites of the meadow in the proximity of the fish farm having different depths and light intensity. Specifically, site C; average depth: 14.4 m, located directly under the cages and sites A and B both located at a distance of 100 m from the farm edge, and in average depths of 7.8 m and 8.8 m, respectively.

Five replicates of seagrass were collected from each site with scuba diving, using quadrats. Samples were stored in plastic jars filled with sea water under constant aeration until they were transported to the laboratory for further examination and analysis. All samples were checked for the presence of phytomyxid infection and the number of galls from each sample was documented. In order to investigate the morphology and anatomy of the parasite-derived galls, photographs were taken with a stereoscope and a microscope.

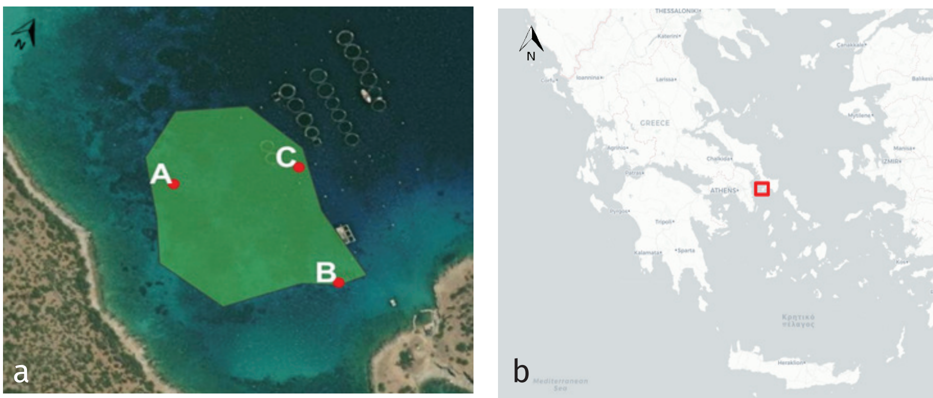

Fig. 1: The studying site. The Halophila stipulacea meadow studied is located in the Southern Evoikos Gulf, Aegean Sea (red rectangle) (a). The meadow covers an area of approximately 3.26 ha, (green area) and the three distinct sites (A,B,C) studied are marked with red points.

3. Results

H. stipulacea samples were visually checked for phytomyxid infection (described as enlargements of the leaf petiole; galls filled with white, brown and dark material; Vohník *et al.*, 2017) (Fig. 2a) and the number of galls was documented. In July 2021, gall numbers were greater than in October and did not seem to differ among the sites (Fig. 2c). The infection was only restricted to the leaf petioles. Different developmental stages of the reported phytomyxid infection could be distinguished on the screened seagrass specimens, differing in their color (white and black) (Fig. 2a). Transverse sections of matured galls (black) of either fresh (Fig. 3a) or fixed material (Fig. 3c, 3d) showed that infected cells were filled with mature resting spores, which were arranged mostly in dyads and had an average diameter of 6 μm (Fig. 3b). Infected galls comprised cells with a different density of spores: those with densely packed spores and those bearing a smaller number of them.

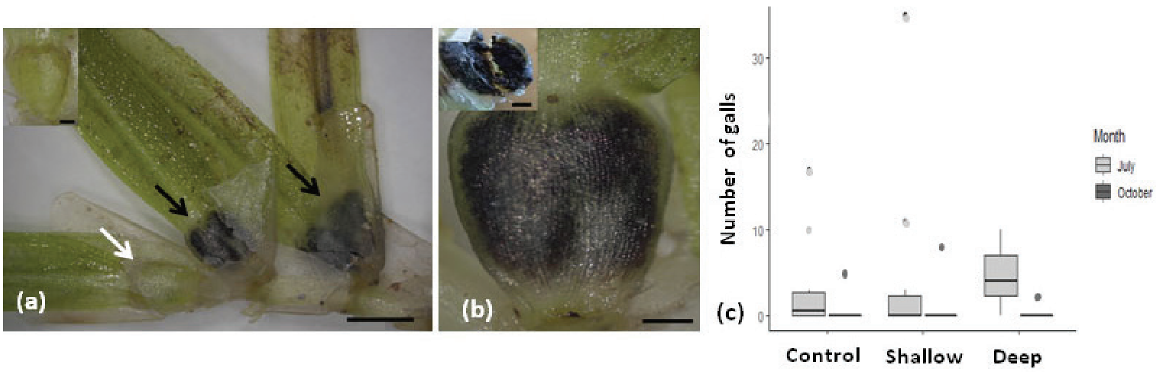

Fig. 2: Phytomyxid infection observed in *Halophila stipulacea* petioles showing developmental stages. Immature white colored galls, white arrow (and inset in a) and mature spores, black colored galls, black arrows (a). Increased magnification of a mature gall (b), inset: a cracked opened mature gall. Dot plot represent the observed number of galls in the different sites and periods (July and October) (mean ± SE), in the different sites and periods (July and October). No statistically significant differences were observed between the sites. Scale bars: a =1 cm; inset in a = 0.3 cm; b = 0.3 cm; inset in b = 0.3 cm.

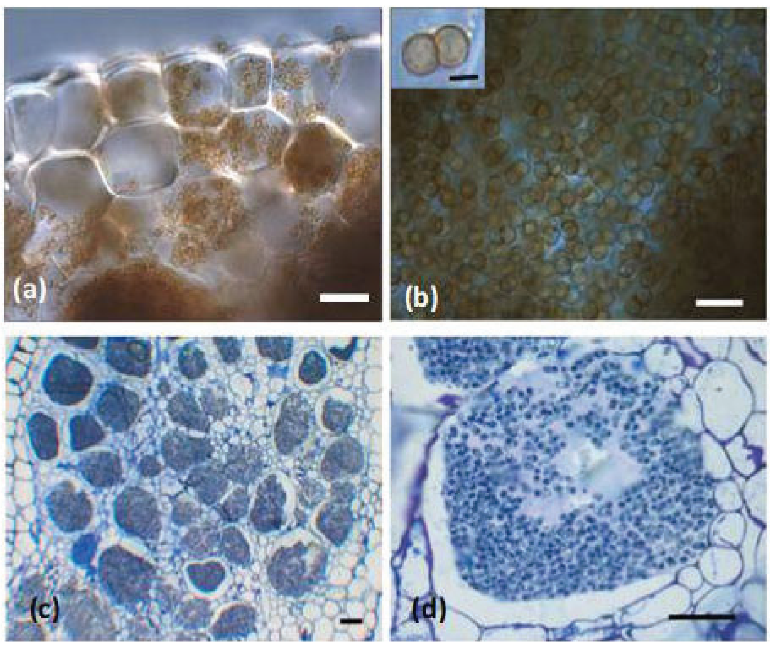

Fig. 3: Micrographs of phytomyxid infection in H. stipulacea. Transverse section of a petiole gall, fresh material (a) Mature resting spores inside the galls, almost all of the spores are arranged in dyads (insert in b, larger magnification of the residing spores) (b) Transverse semi-thin section of a fixed gall, stained with toluidine blue (c) Host cells filled with spores (d). Scale bars: a = 200 μm; b = 50 μm; scale bar in inset = 5 μm; c = 100 μm; d = 50 μm.

4. Discussion

Here we present the third record of this phytomyxid infection in the Mediterranean Sea, and its northernmost location in the Aegean Sea. The galls formed in *H. stipulacea* showed a similar morphology to those already reported (Vohnik *et al.*, 2017) and a color dimorphism (white and black ones) probably indicating the different stage of maturity of the resting spores (Fig. 2), was also recorded. The residing spores inside the plants tissue had a mean diameter of 6 μm, somewhat larger to those previously reported, formed dyads and the infested cells showed a different spore density (Fig. 3), inducing similar anatomical malformations to what was previously stated. One interesting finding was that the increased gall numbers were found in July while in the October specimens their number declined (Fig. 2c). Parasitism recorded in Sicily (Marziano *et al.*, 1995), showed an opposite seasonal pattern to that which was observed in the *H. stipulacea* population of Evoikos Gulf (Fig. 2c). Moreover, parasitism did not seem to differ between the different sites (A,B,C) showing that the parasite is present to a *H.stipulacea* meadow in the proximity of a fish farm unit, northernmost of its first presence in Aegean, Marmaris bay, Turkey (Vohnik *et al.* 2017) .

Considering their roles as parasites of important marine primary producers, the nature of their parasitic interactions and their potential impacts on ecosystem stability and dynamics in marine and estuarine plant communities, must be further evaluated.

5. Acknowledgements

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THE ASSESSMENT OF THE HABITAT TYPE LAGOONS' CONSERVATION STATUS USING BENTHIC MACROPHYTES IN EAST MACEDONIA AND THRACE NATIONAL PARK, GREECE: A SPATIAL SCALE-BASED STUDY

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Abstract

Benthic Macrophyte communities were studied to assess the Conservation Status (CS) of the Habitat type lagoon (code 1150) at two spatial scales (reference grid-10x10km, Site Natura 2000), as required in Article 11 of the Habitats Directive (92/43/EU). East Macedonia & Thrace National Park includes "Nestos Delta and Keramoti Lagoons" (GR1150010) and "Lakes and Lagoons of Thrace" (GR1130009) Natura 2000 Sites. Seventy-four random quantitative samples (relevé) of benthic macrophytes from sixteen stations were collected during (June-September) 2013-2014, and the synthesis and abundance of benthic macrophyte communities were studied. The CS was estimated by biotic index methodology MATECS (ranges from 0 to 1), which combines the use of structural and functional metrics of the benthic macrophytes to take into account the effects of anthropogenic stresses. At the reference grid-cell level the CS was estimated as satisfactory (A, 2 cells), unsatisfactory-sufficient (B, 1 cell) and unsatisfactory-bad (B, 4 cells). The DC was non-satisfactory-sufficient (B) for GR1150010 and unsatisfactory-bad (C) for GR1130009 Sites.

Keywords: coastal lagoons, benthic vegetation, Natura 2000, Ecological Evaluation Index.

1. Introduction

Marine benthic macrophytes (macroalgae, angiosperms) are key structural and functional primary producers of many Mediterranean coastal lagoons (Agostini *et al.,* 2003; Orfanidis *et al*. 2008) providing habitat for commercially valuable species of fish, crabs, and shellfish (Hansen *et al.* 2008; Snickars *et al.,* 2009). With the exception of late-successional angiosperms, which tend to respond more slowly to environmental changes, macroalgae and phytoplankton respond directly to physiochemical fluctuations (nutrients, and irradiance availability). Therefore, they can be used as bioindicators (Orfanidis *et al*. 2011, 2014). Indeed, angiosperms predominate in lagoons that are in reference (pristine) conditions, while in those subjected to anthropogenic deterioration, e.g., eutrophication, tend to be replaced by opportunistic (fast-growing) macroalgae species and Cyanobacteria. In intermediate conditions, there is a conjugation of angiosperms and micro-, macroalgae (Viaroli *et al.,* 2008).

Habitats Directive (92/43/EE) ensures the conservation of natural and semi-natural European habitat types, including coastal lagoons, through periodically monitor and report of their Conservation Status (CS; Evans & Arvela, 2011). Therefore, this study aimed to assess the CS of East Macedonia & Thrace National Park lagoons based on the benthic macrophyte communities in contribution to the management of anthropogenic stress at two spatial scales (reference grid-10x10km, Site Natura 2000).

2. Material and Methods

2.1 Study area and sampling methods

The studied lagoons are located in the Natura 2000 Sites "Nestos Delta and Keramoti Lagoons-GR1150010" (Vasova, Erateino, Agiasma, Keramoti) and "Lakes and Lagoons Thrace-GR1130009" (Vistonida, Lafri, Lagos, Mesi, Elos, Fanari) of the National Park of East Macedonia & Thrace.

Based on benthic vegetation and salinity, two main types of lagoons were recognised: (1) Vistonida, a freshwater affinity lagoon (salinity ranged from 3.1 to 7.6 psu) characterised by the presence of *Stuckenia pectinata* assemblages, (2) the rest saline lagoons (salinity ranged from 16 to 41.9 psu) characterised by *Ruppia maritima* macrophyte assemblages. Seventy-four random quantitative samples (relevé; 17x17cm) of benthic macrophytes were collected from sixteen stations during the hot period, June-September, of 2013-2014.

2.2 Analysis of data

The benthic macrophyte communities' syntheses (species and abundance) were studied in the laboratory under stereoscope and light microscope. While the ecological assessment at a sampling site scale was assessed for Vistonida lake by expert's judgment based on Cyanobacteria blooms and *S. pectinata*' presence, twelve metrics related to community structure [multidimensional scaling plot of Bray–Curtis similarity, species number, Shannon–Weaver index (log2), Pielou evenness, percentage of total coverage] and function (ESG IA, ESGIB and ESGIC percentage coverage, ESG IIA and ESGIIB percentage coverage, and EEI-c) for the saline lagoons were estimated. The CS at cells of the EE reference grid (10km x 10km) and in the Natura 2000 Site was estimated by MATECS (ranges from 0 to 1; Orfanidis *et al.* 2013) methodology, which combines the use of structural and functional metrics of the benthic macrophytes to take into account the effects of both large-spatial scale, e.g., alien species, and local stresses, e.g., eutrophication.

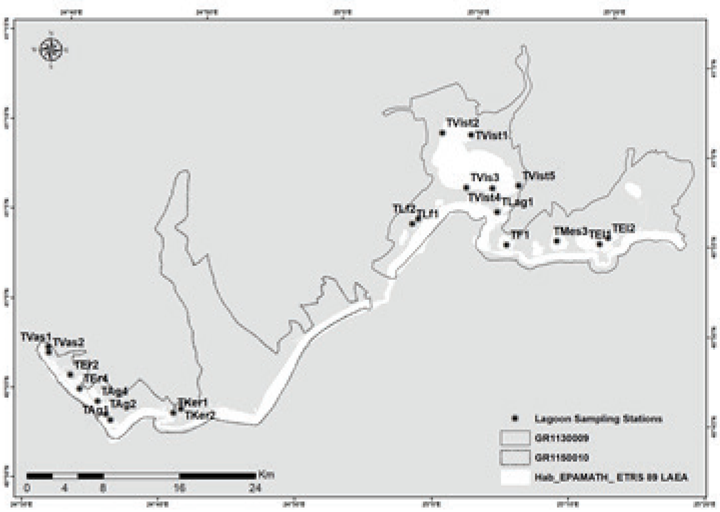

Fig. 1: Map of the habitat type 1150 sampling stations at the National Park of East Macedonia & Thrace

3. Results

A total of twenty-six (26) benthic macrophytic taxa were recorded: Eight (8) Chlorophyceae, two (2) Phaeophyceae, nine (9) Rhodophyceae, Cyanobacteria spp., one (1) seagrass species (*Ruppia maritima*), and three (3) species of freshwater angiosperms (*Stuckenia pectinata, Potamogeton crispus, Ceratophyllum* sp.). The seagrass *Ruppia maritima* was quantitatively dominant at Erateino (30,5%), Agiasma (31,9%), and Fanari (24%) stations, whereas the Chlorophyceae *Umbraulva dangeardii* dominated in Elos (79,7%), the Rhodophyceae *Gracilaria bursa-pastoris* in Vassova (29,4%) and Cyanobacteria colonies in Lagos (100%), Lafri (55,3%) and Mesi (65%) stations. One angiosperm (IA) and 22 (2 IIA, 20 IIB) were classified in ESG I and II, respectively.

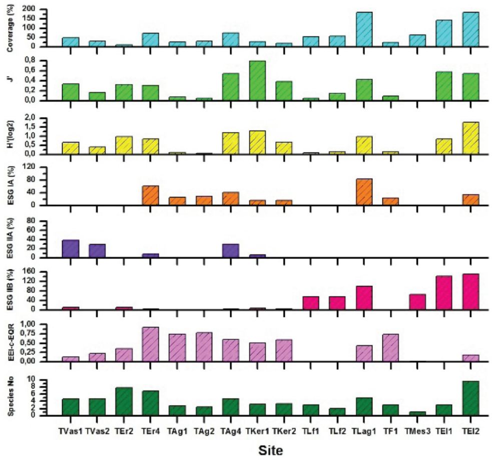

Fig. 2: Mean values (±SE) of different metrics of the benthic macrophyte communities (macroalgae, angiosperms) at sampling stations.

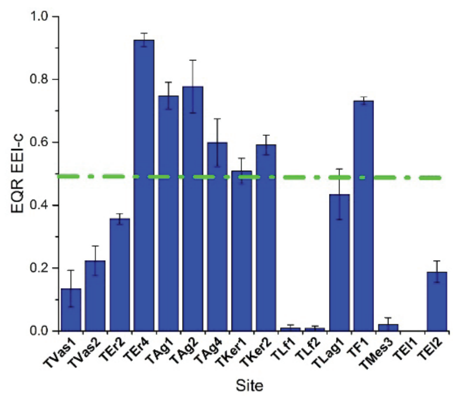

Fig. 3: Mean values (±SE) of the Ecological Evaluation Index (EEI-c) at sampling stations.

The number of species per sampling station ranged from 2 (TLf2) to 10 (TEl2). Total coverage (%) values per sampling station ranged from 10.4% (TEr2) to 184.3% (TEl2). The total coverage of ESG IA (%) ranged from 0% (Vas1, Vas2, Er2, TLf1, TLf2, TMes3, El1) to 83.3% (TLag1), values of ESG IIA (%) ranged from 0 (TLag1, TF1, TMes3) to 37.9% (Vas1) and the values of ESG IIB (%) ranged from 0.18% (TAg1) to 149.9% (TEl2). The lowest values of the Ecological Evaluation Index (EEI-c) EQR's (Figure 3) were observed in the stations TEl1 (0), TMes3 (0,021), TLf1 (0,01), TLf2 (0,01) while the highest values characterized stations TEr4 (0,92), TAg2 (0,78), TAg2 (0,75) and TF1(0,73).

At 50% similarity, Bray–Curtis multidimensional scaling ordination grouped the sampling stations into five clusters (not shown), the majority of stations grouped with the reference stations (Ref1, Ref2), except for the stations where opportunistic macroalgae species predominate and/or angiosperm species are absent.

Based on the Table 1 data, the CS of the habitat type 1150 at the reference grid-cell level in EPAMATH is estimated as satisfactory (A, 2 cells), unsatisfactory-sufficient (B, 1 cell) and unsatisfactory-bad (B, 4 cells). Conservation Status of habitat type 1150 in Natura 2000 protected Sites GR1150010 was judged as unsatisfactory-sufficient (B; 0,572), while in GR1130009 as unsatisfactory-bad (C; 0,342).

Table 1. Assessment of the Conservation Status (CS) of the habitat type 1150 at two spatial scales (reference grid-10x10km, Natura 2000 Site).

Natura code	Cell code	Station Code	Station CS	Cell CS	Natura 2000 CS
GR1150010	10kmE554N209	TEr2	0,4875	0,613	0,572
		TEr4	0,725		
		TAg4	0,625		
	10kmE554N210	TVas1	0,375	0,40625	
		TVas2	0,4375		
	10kmE555N209	TAg1	0,575	0,625	
		TAg2	0,6		
		TKer1	0,656		
		TKer2	0,669		
GR1130009	10kmE558N211	TLf1	0,4125	0,342	0,342
		TLf2	0,4125		
		TVis3	0,2		
	10kmE559N211	TLag1	0,65	0,395	
		TF1	0,575		
		TMes3	0,35		
		TVist4	0,2		
		TVist5	0,2		
	10kmE560N211	TEl1	0,325	0,35625	
		TEl2	0,3875		
	10kmE558N212	TVist2	0,2	0,2	
		TVist1	0,2		

4. Discussion/Conclusion

The results of this study indicate a considerable difference in the CS of the two investigated Natura Sites, with the Lakes and Lagoons of Thrace appearing more affected by anthropogenic stress than Nestos Delta and Keramoti Lagoons. That is because Vistonida, the largest lagoon in the region categorized as hypereutrophic, a status mostly related to the phosphorus release from the bottom sediments (Gikas, 2002; Markou *et al.*,2007) and the particularly high concentrations of heavy metals (Pb, Cd, Cr, and Cu) (Fytianos et al. 1987). Anthropogenic eutrophication has also degraded the ecological status of the Vistonida-Porto Lagos estuarine system in recent decades, leading to algal blooms, consequently causing anoxia and fish mortality (Moustaka *et al.,* 2016). Excessive algal blooms due to nutrient inputs were also recorded in Lafri lagoon, also categorized as a hypereutrophic lagoon (Orfanidis *et al.,* 2019). Less impacted lagoon in the site was Fanari Lagoon, low concentration of nutrient inputs combined with low water turbidity and extensive growth of the angiosperm *Ruppia maritima* allows the use of Fanari as a least impacted or in pristine condition lagoon (Orfanidis *et al.*, 2011).

Based on the synthesis of macrophyte communities, the majority of Nestos Lagoons seem moderately impacted by anthropogenic stress, in agreement with other studies that report them subjected to increased nutrient and pollutant inputs, due to agricultural and domestic activities (Sylaios & Koutroumanidis, 2002). The absence of annual/perennial angiosperms such as *Ruppia maritima* and strong presence of opportunistic macroalgae species of the genera *Ulva*, *Gracilaria*, as well as Cyanobacteria, as a result of eutrophication (Viaroli *et al.*, 2008) were studied in the most degraded areas, such as Vassova lagoon. Vassova is a typical eutrohicated, from fresh agricultural waters outflows, lagoon (Orfanidis *et al.*, 2005) combined with extensive fish cultivation. Coastal lagoons are dynamic ecosystems with differences not only among them but also spatially within the same lagoon (Pérez-Ruzafa *et al.*, 2011), a phenomenon observed in Erateino lagoon where a strong species diversity and abundance gradient between central and eastern parts was observed.

The high natural variability of the lagoons adds to the difficulty in distinguishing human induced from natural stresses generating a need for a comprehensive outline of dynamics in the macrophytic communities based on long-term datasets of these ecosystems. Nonetheless, long-term studies on the spatial and temporal dynamics of benthic macrophyte communities in the lagoons of the National Park of East Macedonia and Thrace are scarce in literature.

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MARINE GEOLOGY-GEOPHYSICS I:
GEOMORPHOLOGY - TECTONICS – SEDIMENTATION – MARINE AGGREGATES

Marine and Inland Waters Research Symposium
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GEOMORPHOLOGICAL FEATURES OF THE EAST MEDITERRANEAN SEA

Sakellariou D., Drakopoulou V., Rousakis G., Livanos I., Loukaidi V., Kyriakidou Ch., Morfis I., Panagiotopoulos I., Tsampouraki-Kraounaki K. and Manta K.

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Abstract

Sea bottom geomorphology is widely considered a promising research field of interdisciplinary interest, since understanding the general structure of the ocean terrain can promote knowledge in several scientific fields (e.g., Geology, Biology, Physics, etc.). Hence, identifying submarine landforms is of significant importance. This paper presents the main morphological structures encountered in the Eastern Mediterranean. By making use of available bathymetric data obtained from various resources, as well as existing literature, we have identified hundreds of morphological features, grouped into five major types: shelf, basins, positive features (like seamounts, ridges or guyots), volcanoes, as well as canyons. A more detailed investigation of the seafloor supported by integrated analysis of the bathymetric, morphological, seismological etc. data will unveil the origins of these morphological features and openly correlate their formation to active geological processes.

Keywords: Eastern Mediterranean Sea, offshore geomorphology.

1. Introduction

Mapping of the seafloor has long uncovered the fascinating complexity of the Eastern Mediterranean geomorphology as a geologically active system that has evolved in the zone of convergence between the Eurasian and African plates (Emery *et al*., 1966; Maley & Johnson, 1971). But as mapping technology advances, more seafloor morphological details come to light. High-resolution swath bathymetry obtained through various oceanographic expeditions throughout the years has been compiled against an almost 130m-resolution bathymetric data publicly distributed by the EMODnet Bathymetry Lot (release year 2018) in order to reveal a more detailed and accurate sea bottom morphology. Thus, as seabottom surveys progress, they gradually reveal a complex mosaic of various other mid-scale morphological features described or highlighted for the first time, such as shallow shelves, flat-bottomed and variably deep basins, seamounts or shallow ridges, submarine canyons, mud volcanoes, banks, plateaus and steep slopes associated with active faults. This information, along with already existing literature or previous relevant works, has been lately compiled into an updated atlas of geomorphological features of the East Mediterranean Seas, that will be published by IUCN in the framework of the “State of the knowledge of deep-water vulnerable species and habitats in the Eastern Mediterranean” project.

2. Identification of major underwater landforms

The Eastern Mediterranean Sea consists of three main composite basins: the Aegean back-arc basin north of the Hellenic Arc, the Ionian basin west and southwest of the Hellenic Trench and the Libyan-Levantine basin that occupies the rest of the Eastern Mediterranean. It is a geological hotspot, since it belongs to the most active areas on the Earth in terms of plate tectonics and seismicity and is characterized by immense geomorphological complexity Hundreds of morphological features have been identified and grouped into five categories, as described below.

2.1 Shelf

An almost continuous shelf runs around the Eastern Mediterranean. With the edge of the shelf located at roughly -120m, shelf portions usually never exceed 15km of width. Wide shelf areas are usually located around the northern (e.g., Thermaikos Gulf) or eastern margins of the Aegean Sea (e.g., Limnos Plateau or Dodecanese Plateau), as well as the central section (e.g., Saronikos Gulf or the isolated Cyclades Plateau, the latter dividing the Aegean Sea into North and South section and hosting the Hellenic Volcanic Arc). The eastern or southern Levantine Sea is also a host to remarkably wide (over 70km-wide) shelf portions (e.g., Mersin and Iskenderun gulfs or off the Nile Delta). On the contrary, narrow shelf portions are more prominent at the southeastern margins of the Ionian Sea, notably around SW of Peloponnese or south of Crete. The edge of the shelf marks the location of the palaeo-shoreline during the last glacial period, when the sea-level had dropped by roughly 120m below the present sea-level.

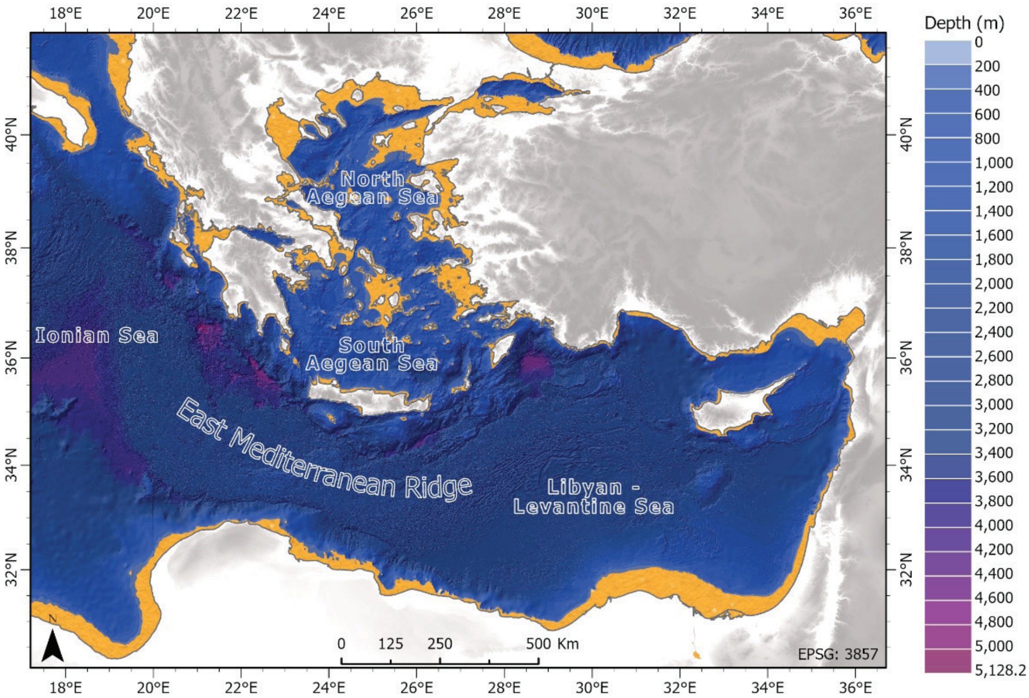

Fig. 1: Shelf occurrence (in yellow) along the boundaries of the East Mediterranean Sea.

2.2 Basins and abyssal plains

The Aegean Sea hosts numerous, smaller or larger, flat-bottomed basins of various shapes (rhomboid, elliptical, trapezoid or spindle). Most of them are less than 1,000 m deep of depth, especially in the North Aegean. An exception would be the North Aegean Trough (NAT), a 300km long feature with a depth of 1,600 m, developed along the prolongation of the North Anatolian Fault in the Aegean Sea. Several other relatively shallow basins occur between the NAT and the shallow plateau of the Central Aegean (e.g., N of Ikaria, N of Mykonos, etc.). Few basins located in the South Aegean reach depths greater than 2,000m (e.g., NW of Karpathos). Basins of more than 3,000 m of depth constitute abyssal plains and are located along the NE boundaries of the Ionian Sea (Ionian Trench) and / or the NNW boundaries of the Lebantine Sea (Pliny and Strabo Trenches). They are elongated or spindleshaped and relatively small in size, but as a compound amalgam they give shape to the Hellenic Trench. Oinousses Deep, with 5,130 m depth, is located SW of Pylos (Peloponnese) and marks the deepest part of the Mediterranean Sea. All the aforementioned basins are bounded by steep slopes, whose strike is either NE-SW to ENE-WSW or NW-SE to WNW-ESE, and are separated from each other by shallow ridges and seamounts. The formation and ongoing subsidence of these basins is fundamentally controlled by tectonic activity (Sakellariou *et al.*, 2021). Notable are also two extensive abyssal plains situated far off the Trench; the almost triangularly-shaped Ionian abyssal plain that sides the western part of the East Mediterranean Ridge, as well as the long but narrow Herodotus abyssal plain that is located between the Mediterranean Ridge and the Nile Deepsea Fan.

2.3 Seamounts, ridges and guyots

In the East Mediterranean Sea there are more than 240 positive features like seamounts, bank rises, mounds, knolls or spurs. Most of them are to be found in the Aegean Sea and along the Hellenic Trench, while few mentionable seamounts (e.g., Herodotus or Cyrene, Battos, Anaximander and Eratosthenes) are located in the Libyan-Levantine Sea. However, the Mediterranean Ridge is the most spectacular submarine mountainous feature of the Eastern Mediterranean. Small mounts or mount-like features are usually located along the shelf, thus form rises of a few tens or hundreds of meters, although some shallow mounts may occur at the very tip of steep slopes footed at a depth of more than 900m (e.g., the seafloor of the East Cretan Strait, between Crete and Kassos Island). On the contrary, it is not uncommon for individual seamounts or ridges to display an elevation span of a few thousands of meters (e.g., almost 4,000m at Argostoli Ridge, the most prominent rise in the Ionian Sea). In addition, complex seamount formations with more than one summits are also common (e.g., Strophades Seamount or Nestor Ridge, both located WSW of Pylos). Volcanic processes control the formations of seamounts located along the volcanic arc (e.g., Milos, Thera or Nisyros islands). Tectonic processes are the main mechanism of formation of seamounts in the Aegean Sea and along the Hellenic Trench. Ridges usually occur between large fault zones, where the basement is uplifted. Next to the East Mediterranean Ridge, significant ridges occur along the western and eastern parts of the Hellenic Trench and in the Aegean Sea. Finally, a less common type of mountainous areas with a remarkable geological history has marked its presence in the East Mediterranean: it is the Eratosthenes guyot, located between Cyprus and the Nile Deepsea Fan, an isolated, continental block that has experienced several phases of emergence and submergence in the geologic times.

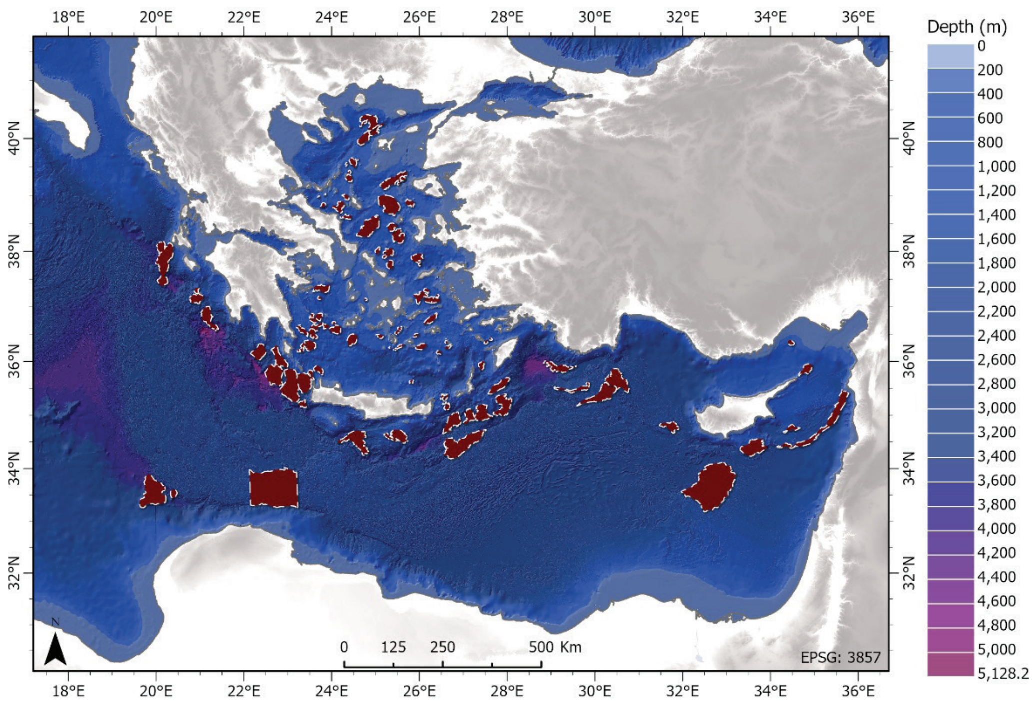

Fig. 2: Positive features (in red) in the East Mediterranean Sea.

2.4 Volcanoes

Submarine volcanoes are directly associated with the Hellenic Volcanic Arc. The most notable submarine volcano system is the NE-SW trending Kolumbo chain of volcanic cones and domes, located NE of Thera island. Kolumbo, a circularshaped cone with a 500 m deep caldera at the centre is the largest among them. On the contrary, mud volcanoes are most commonly found along distinct belts on the East Mediterranean Ridge, the Anaximander Mountains and the Nile Deep-Sea Fan. Their occurrence is highly connected to active tectonics as has been documented in the broader eastern Mediterranean Sea.

2.5 Canyons

Despite the multiple fragmentation of the seafloor into several heterogenous blocks with differences in their origin and evolution, numerous canyons or gullies are identified on the bottom of the Eastern Mediterranean Sea. The vast majority of them originates from the outer slopes of the Hellenic Arc and ends in the basins that form the Hellenic Trench or other deep basins or abyssal plains of the Ionian or of the Levantine Sea (e.g. Rhodes abyssal plain). This systematic occurrence of well-formed canyons hosts some of the longest seabottom incisions, with one located at the NW of Corfu actually exceeding 150km in length. Numerous others may be exclusively hosted by steepsloped basins, such as the North Aegean Trough, the Corinth Gulf, the North Evoikos Basin etc. while only a few may originate from shallow parts of the Greek shelf (e.g. canyons beginning at -100m south of Nisyros or SW of Tilos end up in Karpathos Basin at -2,000m) or the long canyons that incise the Nile Deepsea Fan and end up in the Herodotus Basin). Some canyons constitute the underwater prolongation of existing rivers, as are the examples of the canyons off Samaria Gorge of Crete or Pineios and Neda rivers of Peloponnese. Their underwater sections (South of Crete and Kyparissiakos Gulf, respectively) have a length of more than 50km. The total length of the identified canyons is estimated at 9,000 km.

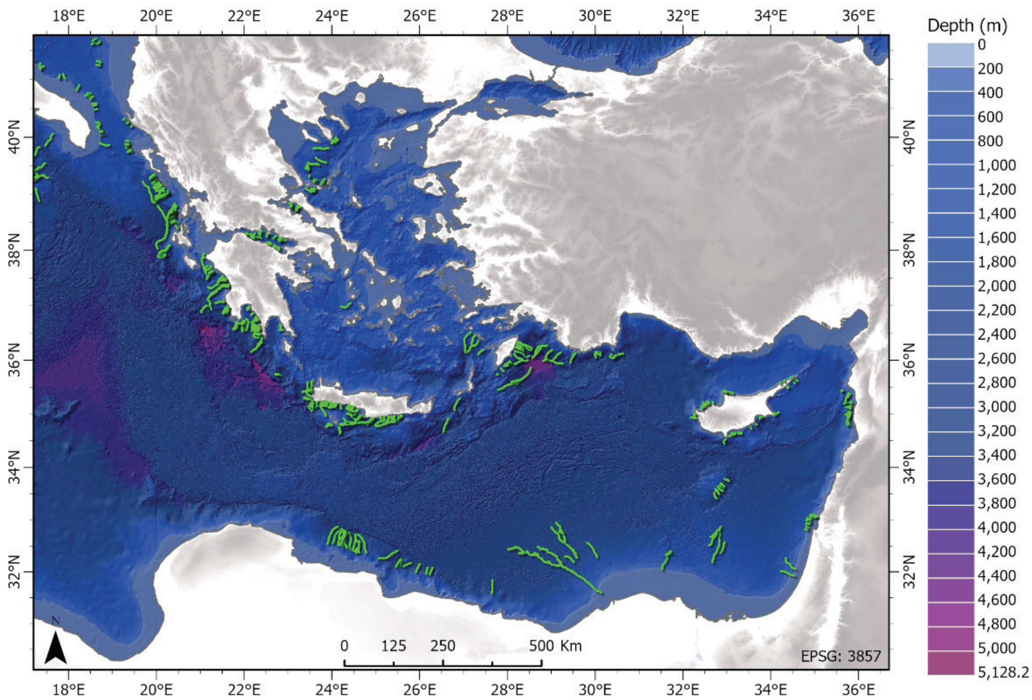

Fig. 3: Canyons (in green) in the East Mediterranean Sea.

3. Conclusion

Detailed analysis of data collected both through field research and literature has shed light into the intriguing diversity of the seafloor. It is evident that the East Mediterranean Sea is a highly complex system that engulfs impressive geomorphological features such as shelves, canyons, seamounts, active volcanic structures, abyssal plains and basins. These rapid changes in the morphology of the seafloor trigger further discussion regarding the underlying relief forming processes, especially if considered under the prism of long-term and recent tectonic activity. But systematic swath bathymetry, geophysical, seismic profiling and geological data are available only for limited areas. In contrast to the Ionian or the Levantine Sea, most of the Aegean seafloor still remains poorly surveyed in terms of swath bathymetry coverage.

4. Acknowledgements

The authors would like to acknowledge EMODnet Bathymetry Consortium (2018): EMODnet Digital Bathymetry (DTM) (https://doi.org/10.12770/18ff0d48-b203-4a65-94a9-5fd8b0ec35f6) for providing the bathymetric tiles (version of September 2018, which has a resolution of 1/16 * 1/16 arc minutes) for the East Mediterranean region. This work has been partly funded by IUCN.

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SEISMIC STRATIGRAPHY AND STRUCTURE OF MYRTOON BASIN: PRELIMINARY RESULTS

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Abstract

The current study presents the preliminary results of a marine geological survey of Myrtoon Basin, on the western part of the Hellenic Volcanic Arc. The processing and interpretation of recently acquired swath bathymetry and seismic data, along with older relevant information, led to the improvement of our knowledge of the geological structure, the fault network, and the seismo-stratigraphy of the area. The understanding of the geodynamic evolution of Myrtoon Basin will provide insights into the evolutionary pattern of the broader South Aegean. Normal faults, striking almost W-E to WNW-ESE, control the recent subsidence of the southern part of the basin. Faults, striking NE-SW and with a significant horizontal sense of movement, dominate in the SE (South of Serifos) and NW parts of the basin. NW-SE striking faults control the eastern margin of the basin (west of Serifos). Two major unconformities have been identified within the sedimentary infill. They can be correlated with similar major unconformities described previously from other basins in the South Aegean and may represent major tectonic events.

Keywords: South Aegean, basin evolution, active faulting, Hellenic Volcanic Arc, fault kinematics.

1. Introduction

Myrtoon Basin occupies the area west of the Cyclades Plateau, west of Kithnos, Serifos, and Sifnos islands, and north of Milos Island (Fig. 1). Attica peninsula defines its northern boundary. Numerous highs and mounds (Falkonera, Velopoula, Karavi islets) form the natural boundary of the basin with the neighboring Argolikos Basin. It is located in the western part of the Hellenic Volcanic Arc, northwest of the Milos volcanic group that includes the islands of Milos, Kimolos, Polyaegos, and Antimilos (Nomikou et al., 2013). Anastasakis et al., (2006) proposed that Myrtoon Basin was a Messinian lake. They also argued that the erosional surface, which they have mapped at about 3sec depth below sea level, represents the Messinian exposed land surface.

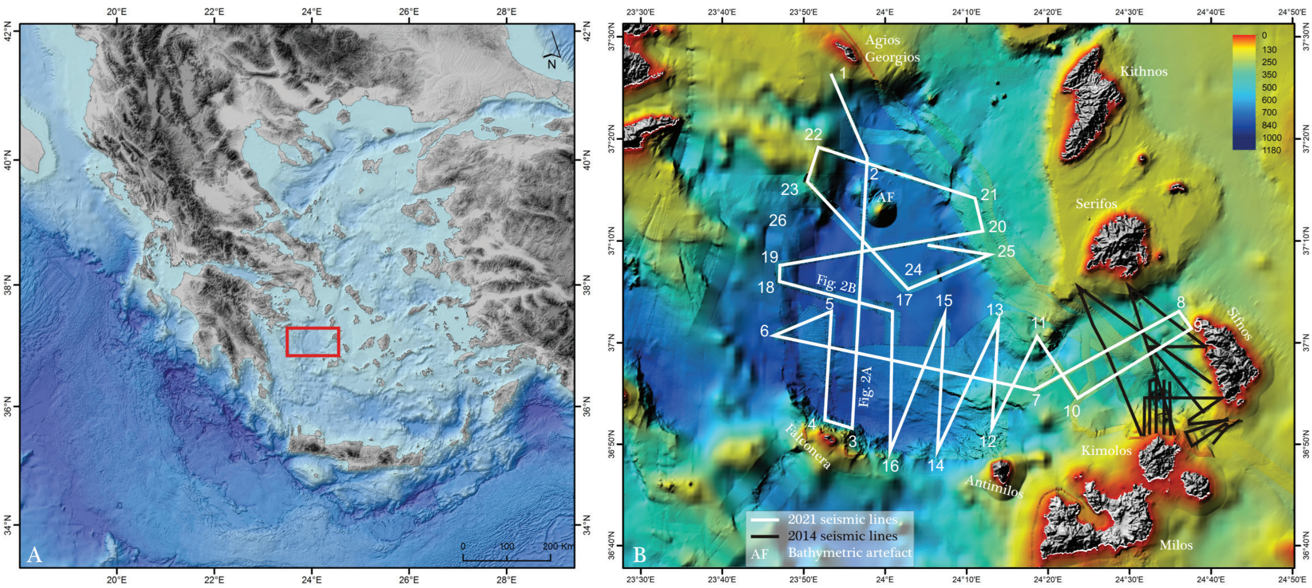

Fig. 1: (A) Aegean bathymetric ma p. The red square indicates the location of the study area. (B) Bathymetric map of Myrtoon Basin. Data from EMODnet Bathymetry Lot (release year 2021) and recently acquired swath bathymetric data. Location of seismic profiles is shown in white and black lines.

Previous studies in the area (Anastasakis & Piper, 2005; Anastasakis *et al.*, 2006) recognized 8 regional reflections (B-D and 1-5) above this surface. Piper and Perissoratis (2003) correlated the upper part of the sedimentary sequence with rhyolithic volcanism and provided some constraints on the prevailing tectonic movements. Piper & Perissoratis (2003) recognized an angular unconformity (UF1) below the basin margins near Milos islands. The seismic unit overlying this unconformity is cut by E-W faults which appear to have been inactive during the deposition of the upper-most units. The same authors also mapped mainly N-S trending faults and at least one major NE trending fault. The current study aims to present the preliminary results of a geological survey of Myrtoon Basin through the interpretation of the seismic stratigraphy, the geomorphology and the dominant fault network of the area.

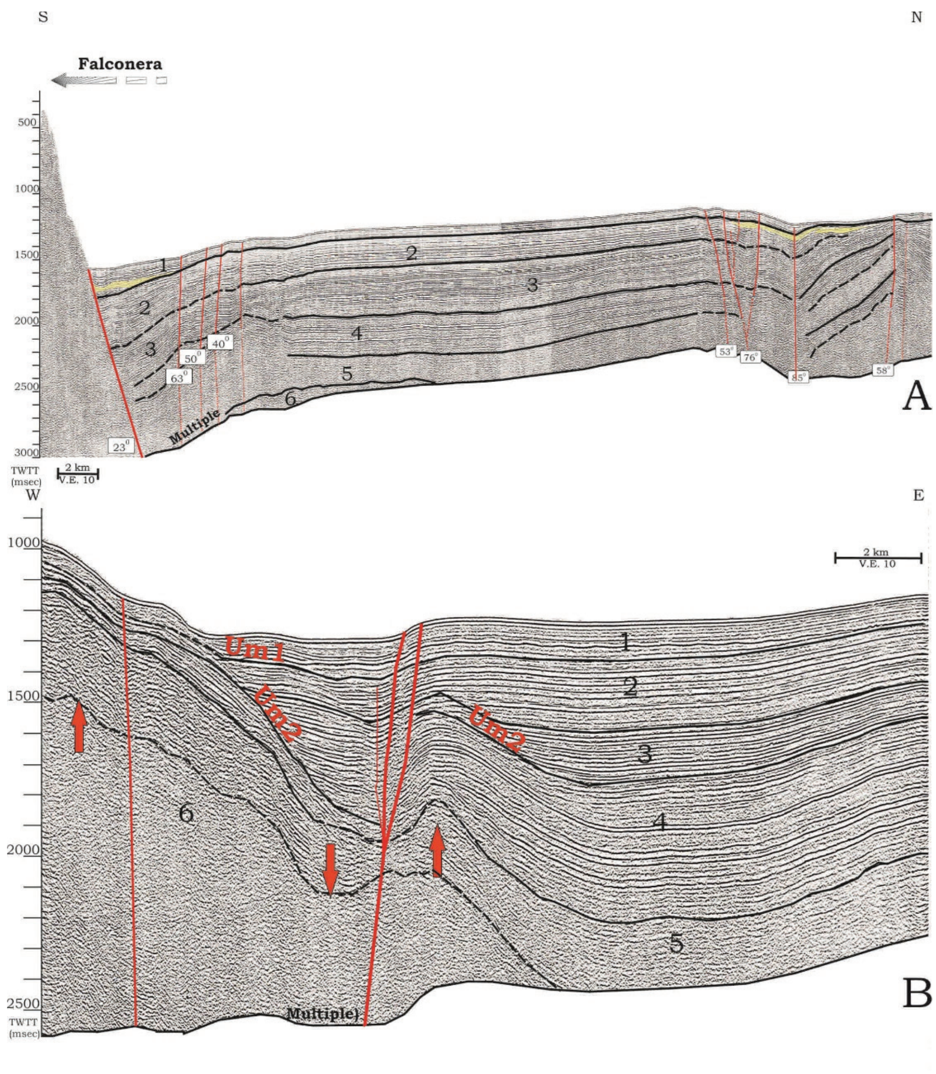

Fig. 2: (A) Air gun 40 ci seismic profiles (A) in S-N direction crossing the central Myrtoon Basin (Line 2-3 in fig.1) and (B) in W-E direction across the western margin of Myrtoon Basin (Line 17-18 in fig.1).

2. Material and Methods

Swath bathymetry and seismic data were acquired in the frame of the Eurofleets+ "Myrtoon" cruise in 2021 aboard the research vessel AEGAEO. For the acquisition of the bathymetric data, a Wärtsilä ELAC SeaBeam 3030 (30 kHz) multibeam system, was used. The single-channel seismic reflection profiles (Fig. 1) were acquired with a 40 cubic inches airgun sound source and a SIG seismic streamer (65 m active length, 48 hydrophones at 1 m spacing) with a CodaOctopus DA4G™ 2000 acquisition software and hardware. The shooting rate was set to 4 seconds. The total length of the recorded track lines was 523 km. Additional swath bathymetry and seismic profiles that were obtained during the YPOTHER project in 2014 in the area between Milos and Serifos were also used for this survey.

3. Preliminary results

The main, deep basin of Myrtoon displays an irregular shape with its longest dimension aligned in a N-S direction. The maximum depth of roughly 1100 m occurs in the southernmost part of the basin, north of the steep slopes off Falconera Islet. Moderate steep slopes mark the western and eastern margins of the deep basin. A channel-like feature, which has also been described and by Tripsanas *et al.*, (2016), runs along the southern slopes of Serifos Island and outflows in the southeastern part of Myrtoon deep basin. The seafloor between Serifos and Kimolos Islands is shallower and relative flat, with wide and smooth depressions and local highs. A spectacular, crater-like, 700 m deep depression occurs north of Kimolos Island. These observations, are in agreement with the results of the geological survey of Karageorgis *et al.*, (2016).

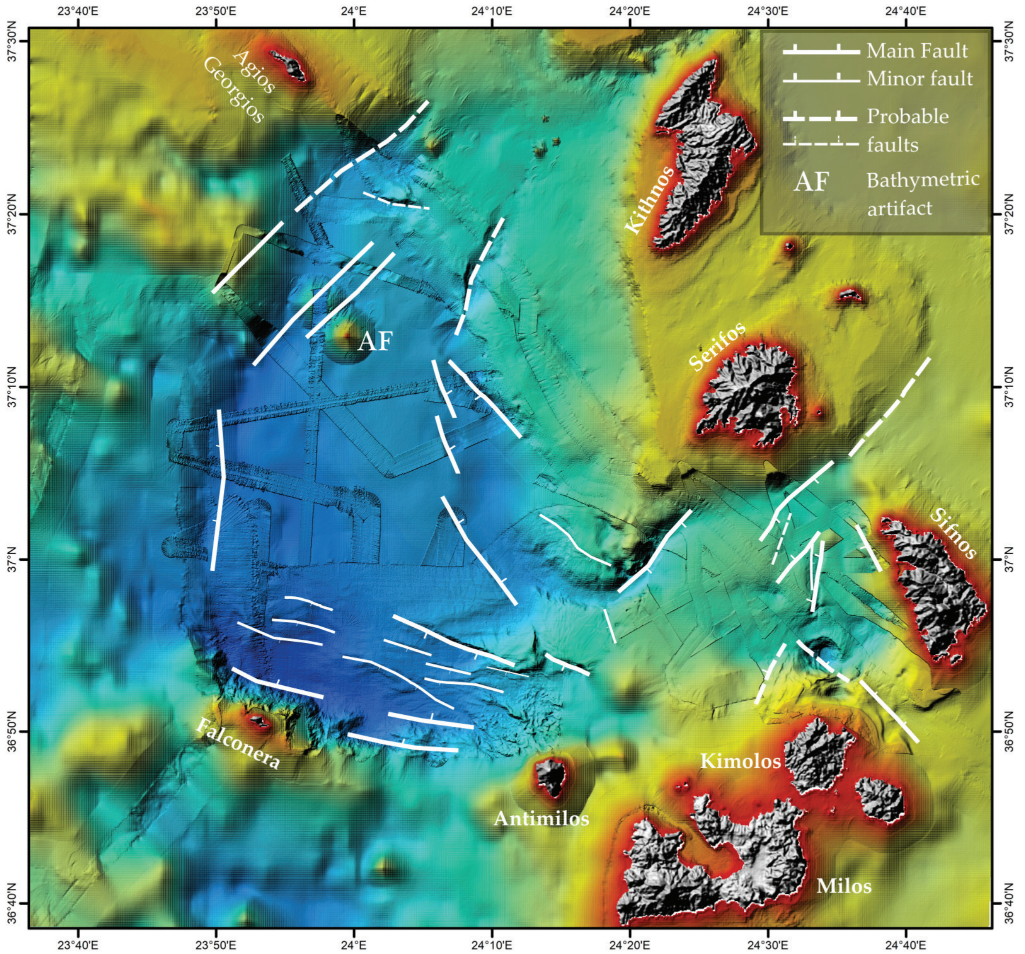

Fig. 3: Tectonic map of Myrtoon Basin.

The stratigraphic interpretation (Fig. 2) of the seismic profiles reveals the existence of at least six different seismic facies (1 to 6 from top to bottom). The thickness of seismic units 1 and 2 remains constant at the center of the basin, thins out towards the western margin, and increases towards the southern margin (Fig. 2A). The thickness of units 3 and 4 varies at the center of the basin (from 150 to 350 msec and 300 to >300 msec respectively) and remains constant towards the margins. The thickness of units 5 and 6 cannot be resolved accurately due to the attenuation of the seismic signal. However, the reflector that separates unit 4 from unit 5 has been mapped below the 2100 msec at the center of the basin.

At least two unconformities (Um1 and Um2) were mapped within the basin (Fig. 2B). Sediments of unit 1 are deposited unconformably on unit 2. Um1 seems to coincide with the base of seismic unit 1. A second unconformity (Um2) marks the interface between seismic units 3 and 4.

The structural interpretation of Myrtoon Basin (Fig. 3) shows a rather complicate tectonic pattern. The steep, southern margin of the basin, north of Falconera Islet is controlled by a major NNE-dipping, normal fault, that along with a series of SSW-facing faults control the subsidence of the southern part of the basin. The increase of the thickness of units 1 and 2, close to the south faulted margin, indicates rapid and recent subsidence. A series of major SW- and NE- dipping, NW-SE striking faults demarcate the basin's margins to the east, towards the shallower plateau west of Kythnos and Serifos Islands The western margin of the deep Myrtoon basins represents rather a flexure than a faulted margin. The N-S striking fault parallel to the western margin, downthrows its western block. NE-SW striking faults dominate in the northwestern edge of the basin. The deformation induced by them indicates a horizontal sense of movement along with a subordinate vertical throw. Similar kinematics is observed in the southeastern part of the survey area, between Serifos and Kimolos Islands. Some parts of the Myrtoon Basin are still not surveyed with seismic profiling methods, therefore understanding of the fault network requires additional seismic and swath bathymetry data.

4. Discussion/Conclusion

Six different acoustic facies characterize the seismic stratigraphy of Myrtoon Basin. The interface between seismic units 5 and 6 may be related to the Messinian erosional surface which has been observed by Anastasakis *et al.*, (2006). Two major unconformities (Um1 and Um2) have been mapped within the sedimentary sequence. Um1 coincides with the unconformity UF1 as has been described by Piper & Perissoratis (2003) in Folegandros basin and eastern Myrtoon Basin. The same authors have also observed a thin unit with incoherent reflections, below a seismic unit with high amplitude reflectors on the top of the sedimentary sequence and above the aforementioned unconformity. They dated this unit at 0.7 Ma. and they suggest that the unit with the high amplitude reflectors is the result of volcanic tephra derived from the late rhyolitic centers of Milos, dated from 0.49 Ma to 0.09 Ma. This is in agreement with our observations. We can reasonably assume that unit 1 includes volcanic deposits derived from the rhyolitic centers of Milos. The interpretation of the bathymetric data in combination with the seismic data revealed the existence of a channel-like feature running along the southern slope of Serifos island (see also Tripsanas *et al.*, 2016).

Preliminary interpretation of the structural data indicates that the recent evolution of the Myrtoon Basin has been controlled by almost W-E to WNW-ESE striking normal faults, NW-SE striking faults predominantly of extensional character and NE-SW striking faults with a possible horizontal sense of movement. Older faults that were active before the deposition of seismic unit 1 are also mapped within the basin. This is in agreement with the observations of Piper & Perissoratis (2003) on the presence of older E-W trending faults that affect the sedimentary sequence below unconformity UF1.

5. Acknowledgements

Recent seismic and swath bathymetry data in the Myrtoon Basin were acquired in October 2021 during the Eurofleets+ Myrtoon cruise aboard the R/V Aegaeo. The seismic and swath bathymetry data in the area between Serifos and Kimolos Islands were acquired aboard R/V Aegaeo in 2014 in the framework of the nationally funded “YPOTHER/Aegean Explorations” project.

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SEDIMENTATIOM PROCESSES IN THE SOUTH AEGEAN SEA (PRELIMINARY RESULTS)

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Abstract

Six sediment cores were analyzed in order to better understand the dominant factors controlling the sedimentation processes in the Cretan and Carpathian Sea and the Amorgos Basin. Preliminary results of the vertical distribution patterns of grain size, carbonate and organic carbon contents, and major and trace elements reveal the abundance of gravity driven deposits as well as hemipelagic sedimentation in the Cretan Sea and in Kamilonisi Basin. In Amorgos and Cretan Basins, mass movements deposits are dominant. Sapropels S1 (with an interruption), were found in three cores (SA-3, SA-7B, SA-8). In the Kamilonisi Basin (2290 m), a 150 cm thick unit was composed of a mixture of terrigenous and pelagic sediments.

Keywords: gravity driven deposits, sapropel S1, seismic events.

1. Introduction

South Aegean has undergone major subsidence as a result of the back-arc extension during the Neogene. The largest earthquake in the last century in the Aegean Sea and the tsunami that followed, occurred in July 1956 southwest of Amorgos Island, in the central part of the South Aegean (Perissoratis & Papadopoulos, 1999). Variably shaped basins, with depths from 1,000 to more than 2,000 m (Cretan Basin, Maleas Basin, and others), separated by shallow ridges characterize the morphology of the South Aegean. Several studies have been conducted focusing on the main sedimentary processes in those basins [i.e., Myrtoon (Geraga *et al*., 2000), Christiana (Tsampouraki-Kraounaki & Sakellariou, 2018), and Cretan (Anastasakis, 2007)]. Thick volcaniclastic flows have been recognized within Cretan Basin, with the absence of the S-1 sapropel (Anastasakis, 2007) and input of terrigenous material (Hsü *et al*. 1973). The Kamilonisi Basin and the steeply inclined lower slope are filled with multiple stacked mass transport deposits (MTDs) (Strozyk *et al*., 2010). It is evident that the South Aegean Sea is a rather complicated and scientifically interesting marine area, wherein hemipelagic sediments, mass transport deposits, turbidites, as well as volcanic and terrigenous material have been accumulated in the basins. This research aims to improve the existing knowledge of the sedimentary processes that control sedimentation in specific areas of the South Aegean (Fig. 1); to achieve it physical and geochemical properties have been studied and relationships between the units have been found.

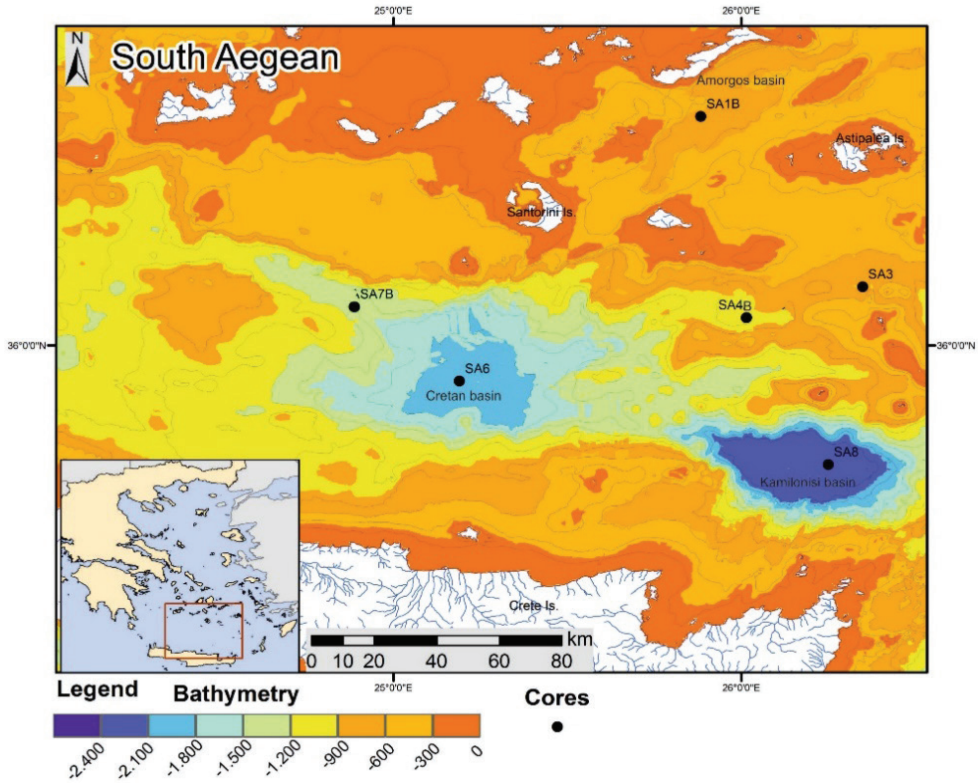

Fig. 1: Location and bathymetry (in meters; grid interval of 100 m) of the study area. Black dots show the location of the retrieved cores.

2. Material and Methods

The current study is based on the sedimentological and geochemical analysis of 6 sediment cores (table1) that were collected during 2017, through two oceanographic cruises aboard the R/V Aegaeo

Table 1. Cores with their coordinates, depths, lengths and locations.

Core	Longitude	Latitude	Depth (m)	Length (cm)	Location
SA-1B	25° 52.974 E	36°40.304 N	720	144	Amorgos Basin
SA-3	26°20.920 E	36°10.302 N	895	335	South Aegean Sea
SA-4B	26°00.835 E	36°04.873 N	1030	129	South Aegean Sea
SA-6	25°11.365 E	35°53.692 N	1870	326	Cretan Sea
SA-7B	24°53.235 E	36°06.798 N	1390	391	Cretan Sea
SA-8	26° 14.984 E	35°38.988 N	2290	373	Carpathion Sea

Initially, the cores were scanned with a multi-sensor core logger (Geotek), at a 1-cm sample interval, for the determination of fundamental attributes such as magnetic susceptibility, gamma-ray density, and P wave velocity. Then, the cores were longitudinally split into two halves. The one half has been photographed, visually described, and samples for grain size analysis, total organic carbon (TOC), total carbon (C), nitrogen (N), major and minor elements were carefully collected (83 samples from core SA-1B, 174 from core SA-3, 68 from core SA-4B, 238 from core SA-6, 211 from core SA-7B, and 245 from core SA-8). The second half of each of the sediment cores was divided into 30-cm-long sections and subjected to X-radiography, using a Faxitron apparatus (Cabinet X-ray). After the preparation of the samples, grain size analysis was carried out using a SediGraph (Micromeritics SediGraph III Plus). Textural sediment classification followed the subdivisions of Folk (1974). For C and TOC measurements the samples were placed in a CHN Elemental Analyzer (Fisons Elemental Analyzer EA1108). In addition, $CaCO_3$ values were calculated by the standard formula: $CaCO_3 = (C–TOC) × 8.333$. For the estimation of the terrestrial organic matter inputs, the C/N ratios were calculated (Prahl *et al*., 1980). Fuse beads were prepared and analyzed in the X-ray fluorescence spectrometer (Phillips PW–2400), for the determination of the major elements. Powder pellets were prepared and analyzed in the X-ray fluorescence spectrometer (Phillips PW–2400), for the determination of the trace elements contents. Finally, the collected foraminifera from core SA-8 were used for radiocarbon (^{14}C) dating, which was accomplished through accelerator mass spectrometry (AMS), undertaken by Beta Analytic Laboratory.

3. Results

Some preliminary results of the laboratory analysis are listed below:

1: SA-1B and SA-4B present similar results, having four distinct units of similar sedimentological characteristics. The second unit shows a thickness up to 100cm in SA-1B (Fig. 2) and 59cm in SA-4B. In this unit the sand fraction presents its highest values up to 80% and lowest in some trace elements (Co, Cr, I, Sr, V). This unit is observed between oxidized units. Also in core SA-8, the first three units present similar characteristics with the three units of the aforementioned cores.

2: A double sapropel S1 layer was found in cores SA-3 (54-72 cmbsf), SA-7B (38-57 cmbsf) and SA-8 (268-289 cmbsf). The onset of S1 deposition occurs at 9300-9070 B.P. in the Kamilonisi Basin (core SA-8) and highest TOC value is 2.52%. The characterization as Sapropel at cores SA-3 and SA-7B has been made through the macroscopic description (color change), magnetic susceptibility measurements (decrease) and higher Ba/Al ratios than the sediments above and below.

3: In core SA-8, from the Kamilonisi Basin, seven units are observed. In the fourth unit from 60 to 210 cmbsf, the $CaCO_3$ values rapidly decrease, C/N ratios reveal mixed and terrigenous origin, and silt and sand fractions present high values.

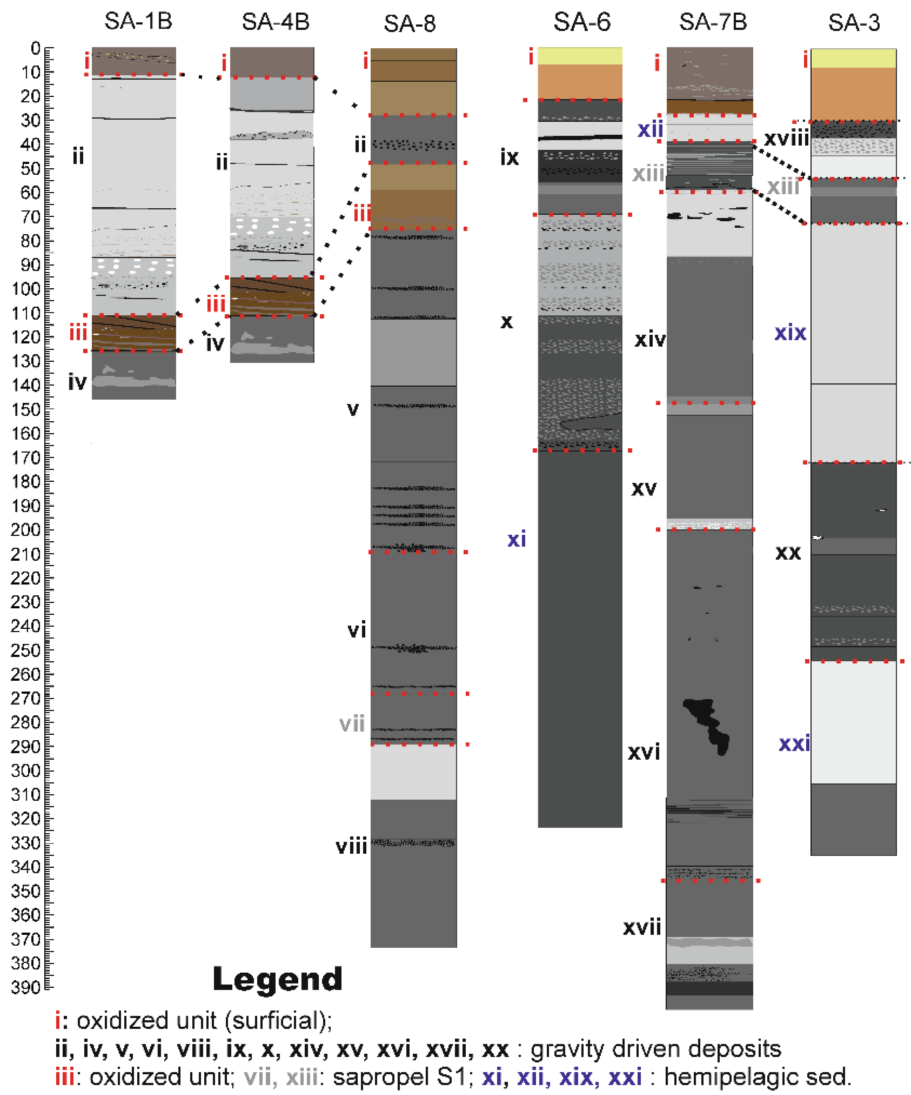

Fig. 2: Sedimentary units (I–XXI) identified in the gravity cores. The vertical scale is in centimeters. For core locations, see Figure 1.

4. Discussion/Conclusion

All cores are largely composed of gravity driven deposits.

Mass movements deposits have been found amongst other deposits in Amorgos, Cretan and Kamilonisi basins. The second unit in the Amorgos Basin (core SA-1B) and in SA-4B indicate MTDs. Similar MTDs have been found in several cores obtained from the area between Amorgos and Astypalea Islands that were associated with the 1956 earthquake and may have triggered the tsunami that followed, according to radioactive tracers Pb^{210} (Sakellariou *et al.*, 2021). Also, the second unit in core SA-8 (Kamilonisi Basin) present similar characteristics probably reveals the same seismic event, further investigation is needed.

In cores SA-3, SA-7B, SA-8 a double Sapropel S1 layer is observed (hemipelagic sedimentation). The onset of S1 deposition in the Kamilonisi basin occurs at 9300-9070 B.P.

The following tasks are still in progress, and will help us to recognize the triggering mechanism (seismic events, sea-level changes, bottom currents, and/or tectonic movements) of gravity flow deposits, their origin and their possible repeatability in periods of sediment failure, are the following

C, N measurements, to determine the origin of sediments (marine, terrigenous, or mix.)

Foraminifera picking, for radiocarbon (^{14}C) dating, to determine the age of the events of interest.

XRD analysis to indicate environmental changes and pathways of suspended sediments.

Examination of possible associations with seismic events, sea-level changes, etc.

5. Acknowledgements

The sediment cores presented in this study were acquired in the context of the South Aegean Geodynamics 2017.

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GEOMORPHOLOGY OF THE CURRENT-SWEPT SOUTH EVIA SHELF, GREECE

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Abstract

This study presents the results of a high resolution marine geophysical survey and especially the findings of the side scan sonar imagery in the south Evia shelf. The scope of this research was the identification of the geomorphological characteristics of the seabed that indicate the presence of bottom currents. The main geomorphological features that denote the occurrence of near seabed flows are: (i) bedforms in the form of ripples, small dunes and comet marks, (ii) biogenic formations, whose origin is partly attributed to low-medium speed currents, (iii) an extended area barren of sediments, with anomalous and rocky relief outcropping, and (iv) loose (sandy) sediments covering the seafloor. Man-made features also demonstrate indications of current activity. Different, probably overlapping, bedform generations are present, formed by diverse currents, flowing in general southwards.

Keywords: bottom-currents, transverse bedforms, biogenic formations, South Evia shelf.

1. Introduction

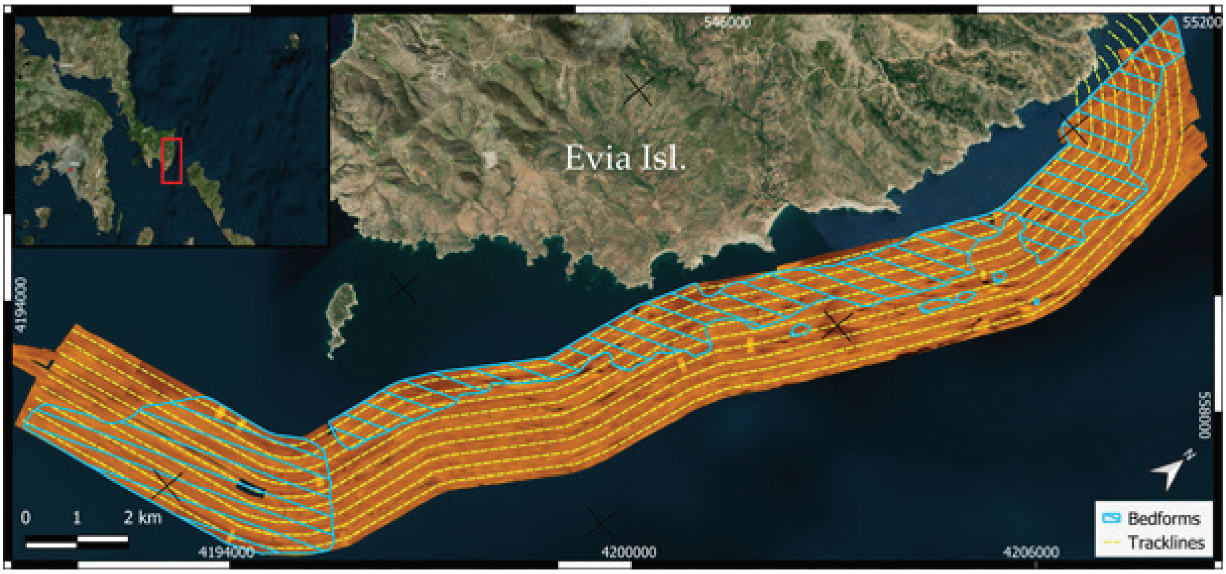
The south Evia shelf lies at the eastern part of Kafireas Strait (between Evia and Andros), an area well known for the very rough seas, the prevailing strong north winds and the intense surficial currents (Pechlivanoglou, 2001). Very few studies have documented a variety of sediment bedforms in the Strait suggesting bottom-current speeds up to 150 cm/s (i.e., Lykousis, 2001). The south Evia shelf has been less studied in relation to the other morphological units of the Kafireas Strait (Stefa *et al.*, 2015). This paper examines indirect evidence of bottom current activity in order to document morphological patterns and current directions in this part of the strait.

2. Material and Methods

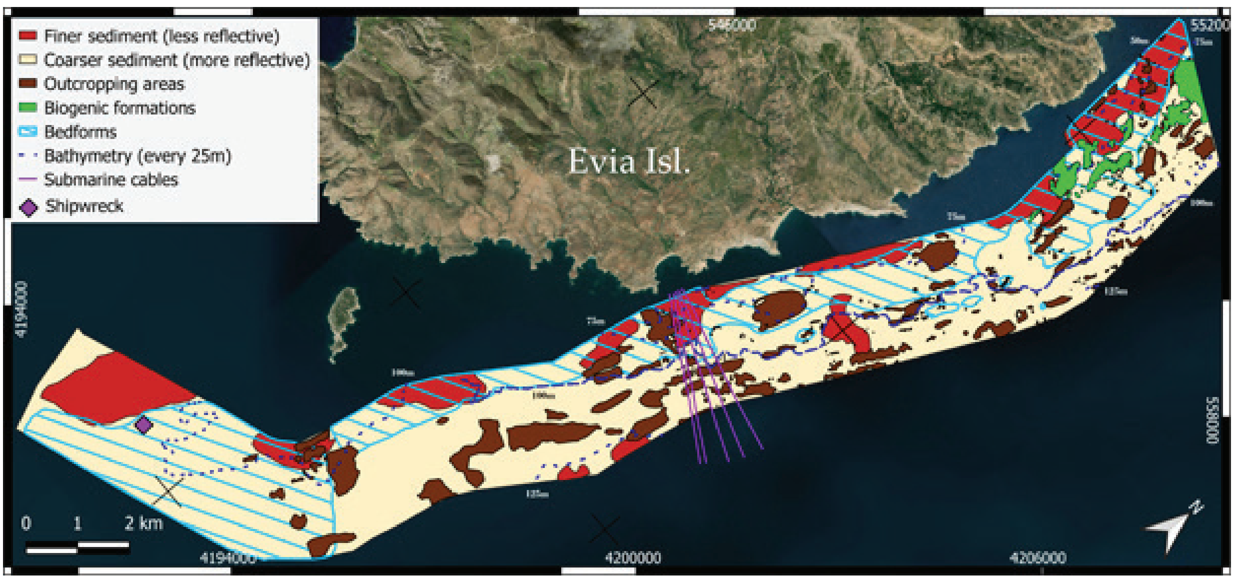
Geomorphological information was collected in 2021, during a marine survey for the submarine power interconnection between Lavrio (Attica) and Kafireas (Evia) with “Astrea”, a DP II multi-purpose support vessel (owned by Asso.subsea). The study comprised data from a Kongsberg EM2040 multibeam (MBES), a Geopulse 3.5 kHz subbottom profiler (SBP) and an EdgeTech 4205 side scan sonar (SSS) transmitting at 325 kHz. Navigation and positioning were supported by Hypack-Max, a Trimble RTK-GNSS and a Super Short Base Line Kongsberg HiPAP 500 for SSS tracking. SonarWiz, Autocad, QGIS and Excel were used for processing and mapping purposes. Here, data only from a certain stretch along the south Evia shelf are presented, covering an area of about 60 km^2 (Fig. 1a). Geophysical interpretation was based on changes of the backscatter intensity in the SSS images and variations in the acoustic character of the SBP profiles. No sediment samples were collected for ground-truthing. The study area was found to be occupied by bedforms. In order to study their distribution and orientation areas with distinct features were detected in the SSS images and then polygons were chosen depending on their homogeneity in the records, since it was noticed that they often appear different characteristics within the same region. Various transects were selected and the bedforms wavelength, direction and water depth were measured. Their height was estimated, wherever feasible, from the relevant SonarWiz tool.

3. Results

The combined MBES, SSS and SBP interpretation revealed a variety of morphological features, textural differences as-well as human related structures in the study area (Fig. 1b). The depths range from ~50 to 150 m, with a highly irregular seafloor developing mainly between 75 and 120 m, with inclinations exceeding 30° for short distances. Three main types of backscatter intensity were recognized: (a) high-energy backscatter with sparse tonal differences, attributed to coarse-grained sediments that occupy a large part of the study region, (b) lower-energy reflectivity returns corresponding to finer sediments, being either soft or loose material, and (c) very strong backscatter intensity in the form of extended areas or patches followed by acoustic shadows of various sizes implying to an intense and varying hard relief.



(a)



(b)

Fig. 1: (a) SSS mosaic with bedform distribution (inset showing the location of the study area). (b) Morphological features on the south Evia shelf.

The SBP profiles show, in general, a surficial semi-transparent unit of varying thickness underlain by a prolonged reflector suggesting a layer of almost homogeneous loose sediments overlying the harder basement. Areas of small sediment thickness appear more reflective in the sonar images indicating coarser material, whereas areas of thicker sediments are less reflecting representing finer material. Usually, the thickness of the surficial layer is very small and the hard basement outcrops either in the form of an almost planar and or slightly rugged seafloor or as patchy/scattered rocky outcrops returning isolated small hyperbolic echoes in the seismic profiles. Towards the northern part of the surveyed zone, between ~50 and 100 m depth and along areas covered by loose sediments, isolated or overlapping hyperbolic echoes with a relatively low-amplitude acoustic signal and a semi-transparent internal acoustic structure imply to the presence of biogenic formations (coralligene reefs – Georgiades *et al.*, 2009) that also create an uneven relief being very difficult to be distinguished solely from the MBES or the SSS images (Fig. 1b and 2a).

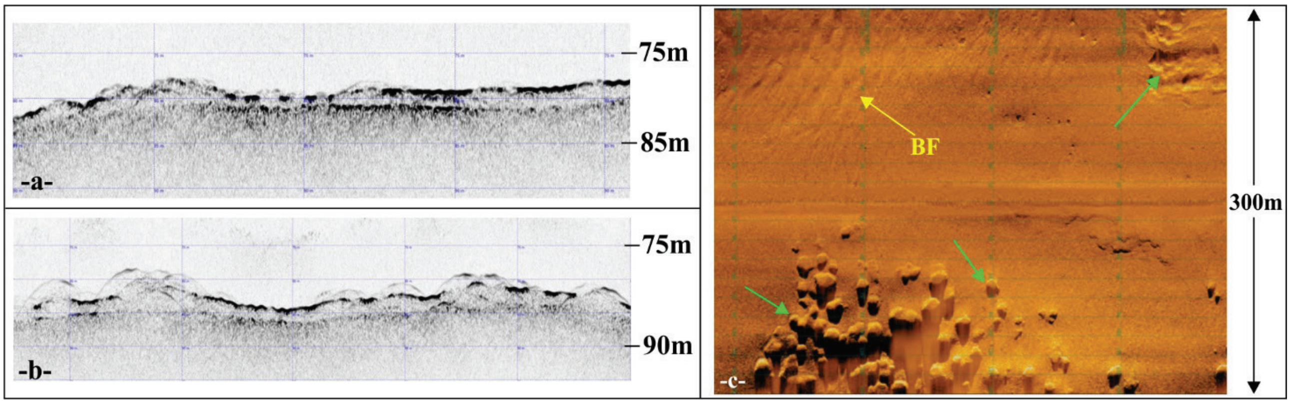


Fig. 2: Indicative SBP profiles (a,b) and SSS image (c) showing the acoustic character and the reflectivity pattern of the biogenic formations (arrows) respectively, next to seafloor bedforms (BF).

A major morphological characteristic is the presence of low-relief transverse bedforms as is indicated by the rhythmic alternations of relatively low-high reflectivity pattern in the SSS images (Fig. 3), suggestive of bottom-current activity. The bedforms develop in areas both of lower and higher backscatter implying that they both constitute of sandy or coarser material, probably with variations in textural sub-classes. They occupy ~28 km^2, they are less discernible in the MBES imagery and appear only randomly in the SBP profiles designating their small height (lower than the SBP resolution ~40-50 cm). Their wavelength varies from ~ 3 to 30 m and their height, which was rarely extracted from the SSS records, is usually less than 0.5 m. Bedforms with bigger wavelengths (15-30 m) develop between 60 and 85 m depth, whilst smaller wavelengths (3-15 m) appear in deeper waters, except from the southern part, where the extended field of bedforms has wavelengths bigger than 20 m. Their maximum height reaches 1.0 m and it was also confirmed in one SBP profile to the west over thicker finer sediments (Fig. 4).

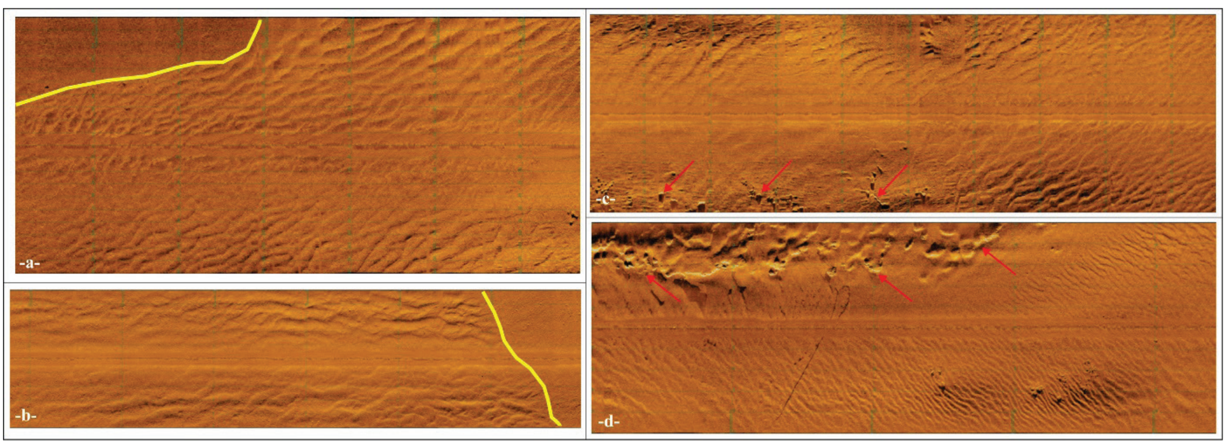


Fig. 3: SSS images showing bedforms of varying wavelength, next to areas of different texture (a,b yellow lines) and close to hard substrate outcrops (c,d red arrows).

According primary to their wavelength and secondly to their height, the small wavelength bedforms represent ripples, whilst the big wavelength features are small scale dunes (megaripples), which are formed by currents less than ~100 cm/s (Kuijpers *et al.,* 1993). According to their direction and their asymmetry in the SSS images the majority of the bedforms (47%) suggests bottom currents with a SSE direction; whereas the rest are equally formed by S and SSW (26%) bottom flows. Only in one case evidence of WNW current orientation was observed in the southern part of the study zone, where the coastline of Evia changes orientation probably influencing the flow direction. It is also interesting that (i) often neighboring bedforms have diverse dimensions and directions suggesting formation under different flow regimes, sometimes overlapping and (ii) transverse bedforms develop also between areas of hard substate outcrops, whose presence may influence/shift the near-bottom flows. The only longitudinal type of bedforms observed are randomly distributed comet marks, which are erosional areas observed at the downstream side of an obstacle (outcrop), yielding additional prove for the occurrence of bottom flow pulses of at least 70 cm/s (Kuijpers *et al.,* 1993). They are also directed, in general, towards the south, thus confirming the main direction of the bottom flows.

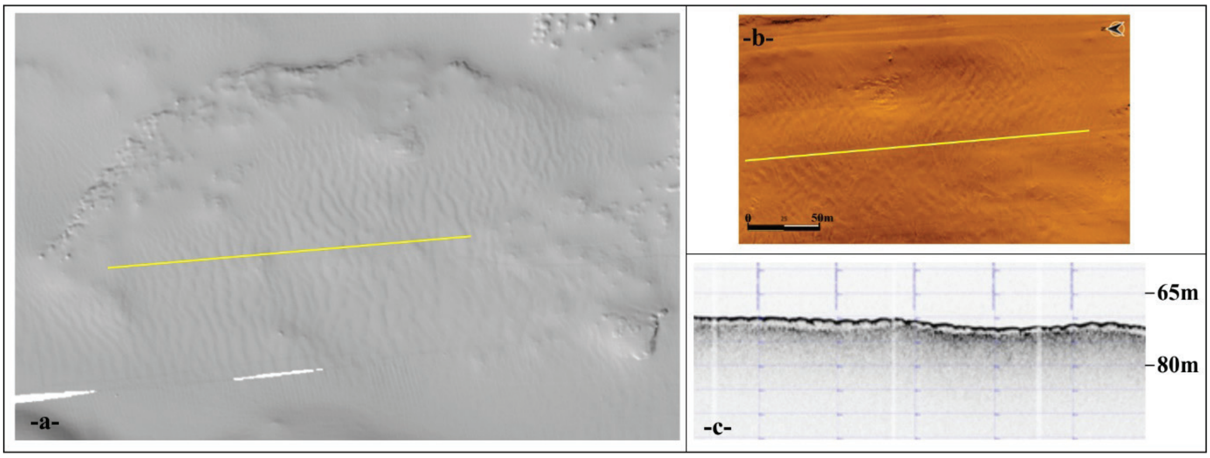


Fig. 4: (a) MBES shaded relief, (b) SSS image and (c) SBP profile, showing a stretch on the seafloor where the bigger bedforms were observed, and their acoustic appearance in the SBP record (yellow line shows the SBP profile location).

Man-made features providing indications of current-activity are (i) submarine cables (between Evia-Andros) found to be buried and/or unburied by loose sediments near areas occupied by bedforms, and (ii) a ~45 m wreck, located in the southwestern part of the studied region, followed by a distinct comet-mark feature pointing to main bottom current direction observed in the area.

4. Discussion/Conclusion

Since direct bottom-current measurements are lacking in the south Evia shelf, indirect geomorphological features offering indications of near bottom flow activity are (i) the presence of small-scale dunes, ripples and comet marks whose configuration is indicative of a southward flow direction; they are distributed mainly to the west, where sediment increases in thickness, (ii) the occurrence of biogenic formations, whose development require at least medium-intensity currents (Georgiades *et al.,* 2009), and (iii) the widespread anomalous hard substrate relief and the small thickness of the sedimentary loose deposits suggesting poor sediment supply (Stefa *et al.*, 2015) and also textural compatibility for modification under current speeds of up to 100 cm/sec. The latter also coincides with typical current-swept shelves (Green *et al.*, 2020) that consist of sandy/gravelly sediments blanketing a relatively even or low-relief hard substrate outcrop.

5. Acknowledgements

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MARINE AGGREGATES DEPOSITS IN THE INNER SHELF OF THE SE EVIA ISLAND, AEGEAN SEA, GREECE

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Abstract

The present study investigates the inner shelf of the SE Evia as a potential area hosting Marine Aggregate deposits. The quantitative estimation of the reserves was based on a geophysical survey, while the qualitative analyses, based on granulometric, mineralogic and geochemical analyses, revealed their suitability for various industrial uses. The results denote that the examined sites of SE Evia Island's inner shelf could be considered as promising MA deposits for exploitation.

Keywords: quartz sand, industrial minerals, nourishment.

1. Introduction

Sand and gravel are considered the largest in volume mined solids (e.g., UNEP, 2019). Marine aggregates (MA) are naturally occurring, non-metallic sediment deposits found in the inner continental shelf off the coast, consisting of sand, gravels and shells/shell debris (Velegrakis *et al.*, 2010) and are related to their terrestrial equivalents (e.g., Anastasatou *et al.*, 2019). However, MA extraction does not have a unique or similar legal framework worldwide, and in many countries, including Greece, the extraction depends on multi-level and non-coherent legislation (Velegrakis *et al.*, 2015). In Greece, since the 1960s, small-scale operations of MA extraction activities have been limited off to a few coastal areas such as Mykonos and Andros islands and more recently in west Crete Island, Trikeri area in Magnesia Prefecture, and SE Evia Island (Paramana *et al.*, 2021; Anastasatou *et al.*, 2019). Only during the last years a few integrated studies regarding the potential existence and exploitation of marine aggregate resources in Greece have been conducted. These areas are Kissamos Bay, NW Crete and Lesvos islands (Anastasatou *et al.*, 2019; Hasiotis *et al.*, 2020), along with additional reconnaissance studies in Afantou Bay, Rhodes Island (e,g., Kapsimalis *et al.*, 2013). The present study aims to investigate quantitatively and qualitatively the exploitation of an offshore potential marine aggregate deposit at the inner shelf of SE Evia Island.

2. Study Area

The present investigation covers a subaqueous area of 30 km^2 that lies along the SE coast of Evia Island, consisting mainly of steep cliffs and streambeds. The continental shelf is rather narrow, extending 800 m seaward to water depths of 200 m (Poulos, 2020). At the western study area, the lithology of the adjacent hinterland area includes mylonitized blueschist to greenschist facies assemblages/metamorphosed schists, including Na-amphibole, epidote group minerals, white micas, quartz, chlorite, stilpomelane, calcite, opaque minerals, and albite porphyroblasts with inclusions of glaucophane (Shaked *et al.*, 2000; Voudouris, 2011). The central-eastern part hosts marbles, quartzite, carbonate-rich schists, metapelites, metabauxite-bearing marble and serpentinite lenses, including coarse-grained calcite, detritus quartz, Si-rich micas and glaucophane relicts (Shaked *et al.*, 2000; Voudouris, 2011).

3. Material and Methods

In June 2014, a scientific cruise was carried out on the SE shelf of Evia Island with R/V Aegeon of the Hellenic Centre for Marine Research. The offshore survey included bathymetric and geophysical data acquisition and sampling of 14 sediment samples < 20 cm depth (Fig. 1) collected with a box corer.

The geophysical survey was conducted with a chirp GeoAcoustics sub-bottom profiler (SBP); during the survey, 110 km of seismic profiles were acquired and subsequently elaborated using Coda Octopus Survey Engine Seismic Plus. After seabed tracking, a 3db static gain and an envelope filter was applied to the seismic profiles to enhance seismic reflectors.

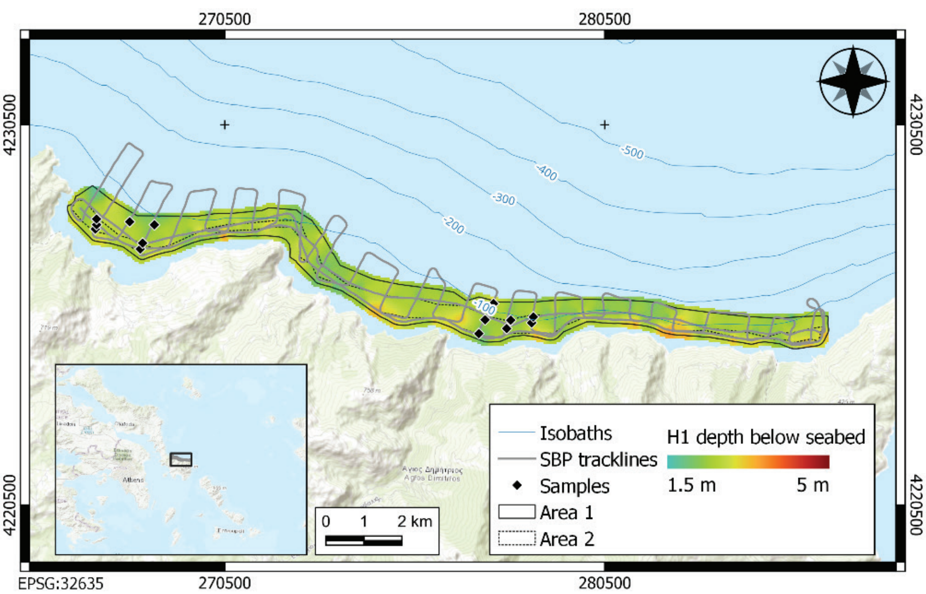

Fig. 1: Map showing the offshore study area in SE Evia Island including sampling points and seismic lines.

The samples were analyzed in terms of granulometry, sediment density, mineralogy and geochemistry. Grain size analysis was carried out by dry sieving (Folk, 1980) due to the coarse nature of the sediments (sands), whilst grain size parameters were further elaborated by Gradistat software. The mineralogical study was carried out with Powder X-Ray Diffraction (PXRD) on a Siemens Model 5005 XRD combined with the DIFFRACplus software package. The samples were chemically (major and trace elements) analyzed by the X-Ray Fluorescence (XRF) method in a BRUKER SRS 3400 sequential X-ray spectrometer of WDS (Wavelength Dispersive Spectrometer) type. The ArcGis 10.8 software, IDW interpolation method, was used to map and estimate the spatial heterogeneity of the qualitative characteristics of the mineral resources.

4. Results

In the study area, three main seismic horizons (H1, H2 and H3) have been recognized in seismic profiles delineating four seismic facies (A, B, C and D), as shown in Figure. 2. For the conversion of milliseconds to meters, an average sound velocity of 1600 m/s has been adopted. Seismic facies A is bounded by horizon H1, showing high reflectivity near the seabed that gradually decreases downwards, suggesting a seabed covered by coarse-grained sediment. In the deeper part (approx. >40 m), seismic facies A displays lower surficial reflectivity, indicating a lateral change to more fine-grain sediments. Seismic facies B possibly represents more fine-grained sediments, as is indicated by its medium to low reflectivity. Seismic facies C is characterized by multiple internal reflectors displaying layered structure interpreted as possible alterations of fine- and coarse-grained sediments. Seismic facies D displays high reflectivity at its upper part that gradually decreases to reflection-free areas and represents the acoustic basement that possibly correlates to an extended erosional surface related to a much lower stage of sea level (i.e., 20 ka BP).

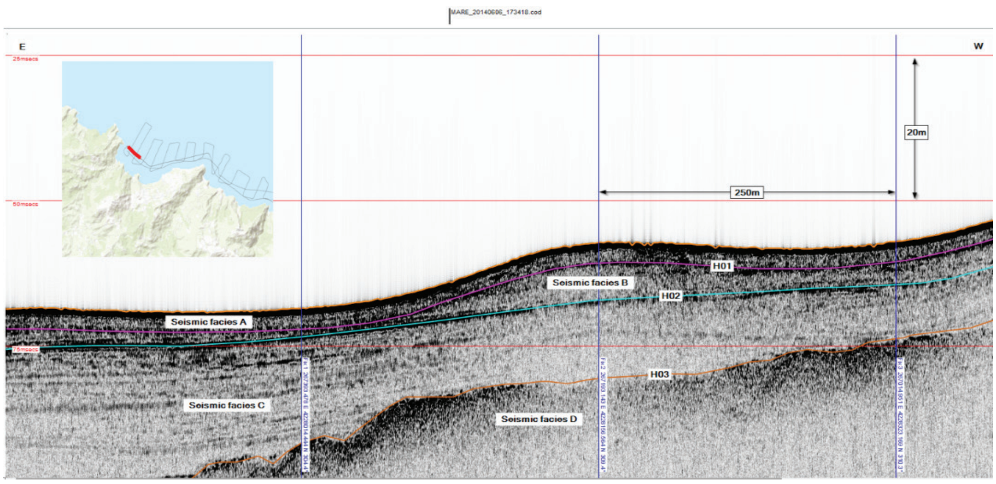

Fig.2: Typical acoustic profile in the area showing main seismic horizons H01, H02 and H03, and seismic facies A, B, C and D.

In the sampling sites, the surficial sediments are predominantly coarse-grained (sand with sand containing small percentages of gravel). According to Folk's classification, seven (7) sedimentary classes have been identified; these are: i) Gravels (G), ii) sandy Gravels (sG), iii) gravelly Sand (gS), iv) Sand (S), v) silty Sand (Zs), vi) gravelly sandy Mud (gsM) and v) sandy Silt (sZ), while at water depths 20-50 m sand content is >83% and at water depths 10-20 m sand content is >99%. An almost zonal distribution from shallow to deeper water depths is observed. Moreover, the surficial sediments of the eastern part of the sampling site (Fig. 1) are coarser (i.e., exhibit higher sand fraction) than those of the western part.

The mineralogical analysis of the surficial sediments revealed that quartz is the dominant mineral phase throughout the area, while albite is the second most significant phase. Calcite, chlorite group minerals, and clay minerals occur in trace amounts in all samples; talc and Mn-oxides often occur as trace phases and glaucophane and edenite as trace minerals in a few samples. Chemical analyses revealed that SiO_2 content varies from 60% to 80%, and CaO content, inversely distributed, ranges between 1.5% and 17%. Fe_2O_3 content is close to 2-3%, whilst SO_3 values are <1% throughout the study area.

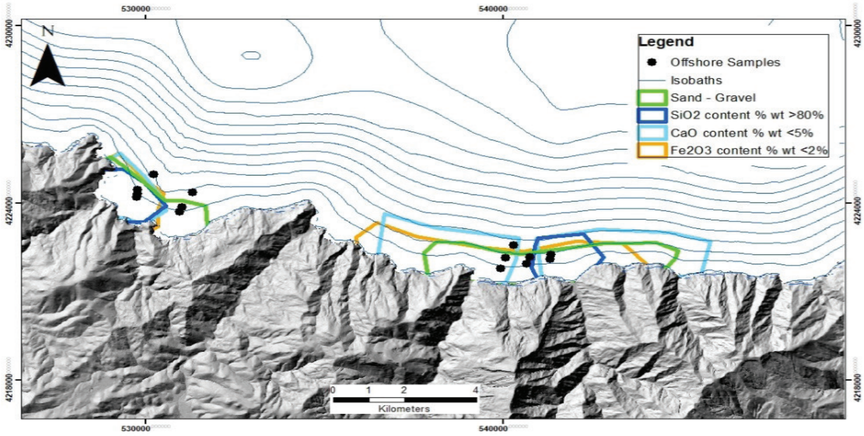

Fig. 3: Shaded onshore relief map with the restricted offshore sub-areas of SE Evia Island according to the suitable grain size, SiO_2, CaO and Fe_2O_3 content regarding various uses for the construction industry. The restricted areas emerged after IDW interpolation.

5. Discussion/Conclusion

The combination of sedimentological, mineralogical and geochemical results indicates that the offshore area of SE Evia Island hosts potential MA deposits that meet the quality requirements (i.e., geochemistry) regarding its industrial uses. Specifically, grain size, mineralogical and geochemical analysis revealed a high compositional maturity (e.g., relatively coarse-grained quartz-rich sand) of the depositional environment of the two sampling sites (Fig. 3). However, the sorting degree and quartz content decrease as depth increases. Overall, the mineralogical and chemical analysis didn't identify significant impurities according to various EU and national standards (e.g., EN 12620:2000 and GCCT, 2016) for aggregates use.

The estimated quantity of MA deposit in Area 1 (Fig.1), based on horizon H1, has been loosely outlined where horizon H1 has been picked along the seismic profiles; according to this distribution, the potential aggregate deposit volume has been estimated to be $43*10^6$ m^3 or $1.13*10^8$ tons. Area 2 (Fig. 1) has been outlined based on the coarse-grained surficial regions; this corresponds to a potential aggregate deposit volume of approximately $14*10^6$ m^3 or $3.68*10^7$ tons.

According to the quantitative and qualitative data from the surficial sampling, the investigated sites sound promising regarding MA exploitation. However, to increase the confidence level, an adequately detailed and reliable exploration, sampling, and testing should be obtained in terms of denser seismic lines and drill cores in sampling grid. Moreover, environmental characteristics (i.e., benthic analysis, Posidonia meadows) have to be further identified to eliminate potential adverse effects to the environment, whilst additional spatial constraints will be introduced when taking into consideration bathymetric and oceanographic characteristics (i.e., closure depth, maximum depth of exploitation) of the study area.

6. Acknowledgements

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COMPARISON BETWEEN CPT UNDRAINED SHEAR STRENGTH AND VANE TEST MEASUREMENTS IN SURFICIAL MARINE SEDIMENTS

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Abstract

Estimation of undrained shear strength (*Su*) of fine-grained sediments from cone penetration test (CPT) is based on empirical formulas where cone factors (*Nkt*) are used to estimate *Su*. Cone factors vary among studies due to differences in sampling and testing. For this reason, it is often necessary to validate CPT readings with lab tests in order to establish reliable *Nkt* values for specific deposits. In this study, CPT loggings are validated through Vane Shear Tests (VST) along with additional index properties measured in a set of vibro-cores in the lab. Index properties show that the fine-grained deposits exhibit geotechnical differences between relatively shallow- and deep-water samples. The former deposits are of mixed composition and low plasticity, while the latter contain finer material and display high plasticity. The VST distribution aligns better with the CPT remoulded *Su* values rather than with the initial CPT *Su* profiles. Simple linear regression analysis produces a moderately good correlation between cone resistance and VST, albeit with considerable variance, hence, prediction results are difficult to establish precise values for these deposits. Sample disturbance seems to be a crucial issue that must also be taken into account when considering *Nkt* validation.

Keywords: cone penetration test, vibro-cores, cone factor, Aegean Sea.

1. Introduction

Estimations of undrained shear strength (*Su*) using Cone Penetration Tests (CPT) or piezocone (CPTu) have become popular in the field due to the fast acquisition of accurate in-situ measurements and ability to obtain continuous strength profiles. For offshore integrated site investigations, *Su* is an important parameter since it indicates the accepted loads that the sediments can sustain for potential installations (e.g., platforms, pipelines etc.). Estimations of S_u from CPTu can be determined either with theoretical solutions or empirical formulas, the latter being the most common approach (Lunne *et al.* 1997). The following empirical formula uses total cone resistance to calculate *Su*:

$$S_u = \frac{q_{net}}{N_{kt}} = \frac{(q_t - \sigma_{vo})}{N_{kt}} \quad (kPa) \qquad (1)$$

where q_t is the corrected cone resistance for pore pressure, σ_{vo} is the total in-situ vertical stress and *Nkt* is the empirical cone factor. *Nkt* values vary among studies due to differences in sampling and testing equipment. For example, Lunne *et al.* (1985) has produced a range of 9-20, while other studies have expanded the range at 10-30 (Rémai, 2013). There is no current consensus on the exact range of *Nkt* (Li, *et al.*, 2019). For deposits where no laboratory measurements have been obtained, it is typical to use a range 15 to 20 (Lunne *et al.*, 1997). However, if samples are available, it is useful to produce representative *Nkt* values for the specific deposits. This study (i) briefly describes the physical properties of surficial marine fine-grained deposits along a transect between Evia and Skiathos Island in the Aegean Sea, and (ii) examines and compares vertical distributions of CPT *Su* with laboratory vane shear test (VST) measurements, in order to produce potential *Nkt* values for this specific data set.

2. Material and Methods

30 CPTs and 32 vibro-cores (VC) were obtained between Evia and Skiathos Islands in 2020 during a marine geophysical and geotechnical survey for the burial of a submarine power cable (Fig. 1). Both CPT and VC information was obtained in the same sampling location with penetration not exceeding the 3.5 m under the seafloor. CPT profiles were extracted using a Manta-heavy CPT system, with a 3.5 m penetration rod and a 1500mm^2 cone. The CPT gave a continuous measurement of the tip resistance (q_c), sleeve friction (f_s) and excess pore pressure. Penetration rate was implemented at 2 cm/sec. CPT logs were processed using the Geologismiki Software. *Su* profiles were extracted with an average *Nkt* of 14 (by default in the software). In the laboratory, undrained shear strength measurements were performed using a Torvane. Lab measurements also comprised of grain-size analysis, Atterberg limits, unit weight and water content. Sediment classification was performed according to the Unified Soil Classification System (USCS). In this paper, data from 11 CPT/VC locations are examined, consisting mainly of fine sediments that were distinguished into two groups: the first group corresponds to deeper water locations (95-205 m depth) between the 2 islands, whilst the second to shallow water small basins (54-80 m depth), restricted between the SE coastline of Skiathos and neighboring islets (Fig. 1).

3. Results

VC lab analysis shows discrepancies in terms of composition and geotechnical behavior between deep and shallow water deposits (Table 1). Deep-water sediments display high plastic behavior with liquid limit (LL) of ~ 60-50% and plasticity index (PI) of ~ 27-35%. This is also validated by the high presence of fine-grained (clay and silt) material, accounting for more than 80% of the sediment fraction, albeit with some small variations. Water content was also found to be higher (~60-80%) than the LL, indicating the very soft nature of the deposits that can become unstable upon disturbance. Shallow-water deposits show a coarser composition, with reduced values of the clay fraction (<20-30%) and varying amounts of silt (~10-40%); the presence of coarser-grained material (mainly sands with few small gravels and biogenic fragments) is also apparent, but it rarely reaches 50%, thus, it generally indicates a mixed composition. Moreover, the coarser texture of shallow water deposits is reflected by the lower LL (30-45%) and PI (12-18%) values, as well as the decreased water content (16-45%). The unit weight is less than 1.74 gr/cm^3 for the finer deep water sediments, whilst it ranges from 1.81 to 1.96 gr/cm^3 for the coarser deposits. According to the USCS classification scheme the deep water sediments are considered as inorganic clays of high plasticity, whereas the shallower sediments are inorganic clays, including sandy clays and silty clays, of low to medium plasticity.

Table 1. Index properties for deep-water and shallow-water sediments. Range of Liquid Limits (LL), Plasticity Index (PI), water content (w), and fine material (< 0.075 mm) measured in the corresponding number of samples.

	Number of samples	Water depth (m)	LL (%)	PI (%)	w (%)	Unit weight (gr/cm^3)	Fine material (%)	USCS Classification
Deep-water sediments	20	95-205	60-50	27-35	60-80	1.64-1.74	80-90	CH
Shallow-water sediments	24	54-80	30-45	12-18	20-40	1.81-1.96	50-60	CL

Interesting findings were observed from the comparison of undrained shear strength values logged by the CPT and VST measurements. Deep-water cores (Fig. 1A) show vertical distributions of *Su* values less than 20 kPa, while remoulded *Su* (expressed by the sleeve friction in the CPT log) display very low values (< 10 kPa). VST measurements were found to either align with the remoulded *Su* or exhibit relatively higher values that only occasionally aligned with CPT *Su*, specifically for the first meter of the softer deposits.

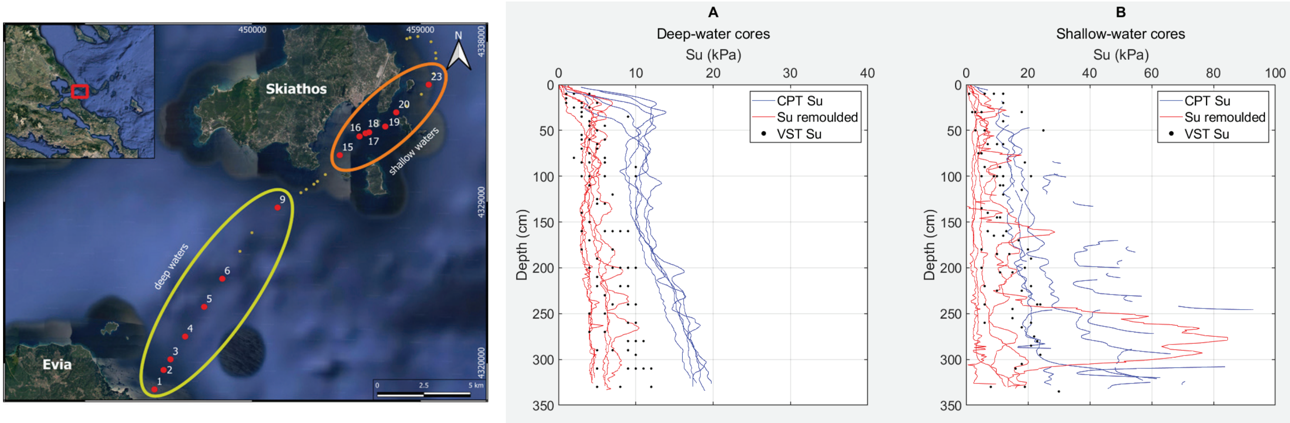

Fig. 1: (left) Location of the study area showing deep-water (yellow ellipse) and shallow-water (orange ellipse) cores. (right) CPT logs of *Su* and remoulded *Su* in comparison with VST measurements for (A) deep-water and (B) shallow-water cores.

Shallow-water cores display a similar pattern; however, there is considerable scatter regarding the CPT log values (Fig. 1B). It is evident that the relatively lower clay content and higher coarse fraction, consisting mainly of silts and fine sands, produces higher values of *Su* (> 40 kPa), especially with increasing depth under the seabed (> 1.5 m), where they become firm. Furthermore, the presence of coarse material is indicated by some intermittent *Su* profiles; the intermittent parts are represented by relative density loggings (not presented here) and are the typical readings for increasing sand content along the sedimentary column. No VST measurements were obtained from the relevant parts of the VCs. In general, few VST measurements align with CPT *Su* (corresponding to an *Nkt* factor of 14 assigned by default).

A clearer picture of the sediment behaviour stems from the comparison between deep-water and shallow-water cores. The slightly coarser sediments in shallow waters exhibit strengths considerably higher than the deeper softer deposits, exceeding 60 kPa, locally, deeper than 2.4 m under the seabed (Fig. 2A). The remoulded *Su* (Fig. 2B), even though it follows the same trend, appears to have comparable values for deep and shallow sediments, at least for the upper ~1.5 m where CPT *Su* is generally lower than 20 kPa. Deeper than 1.5 m, the differences increase slightly and only in one case was the remoulded *Su* very high (reaching 80 kPa) and in accordance with the increased values on the same CPT *Su* log. Torvane undrained strengths (Fig. 2C) exhibit higher values for shallower and slightly coarser sediments relatively to the finer deeper deposits.

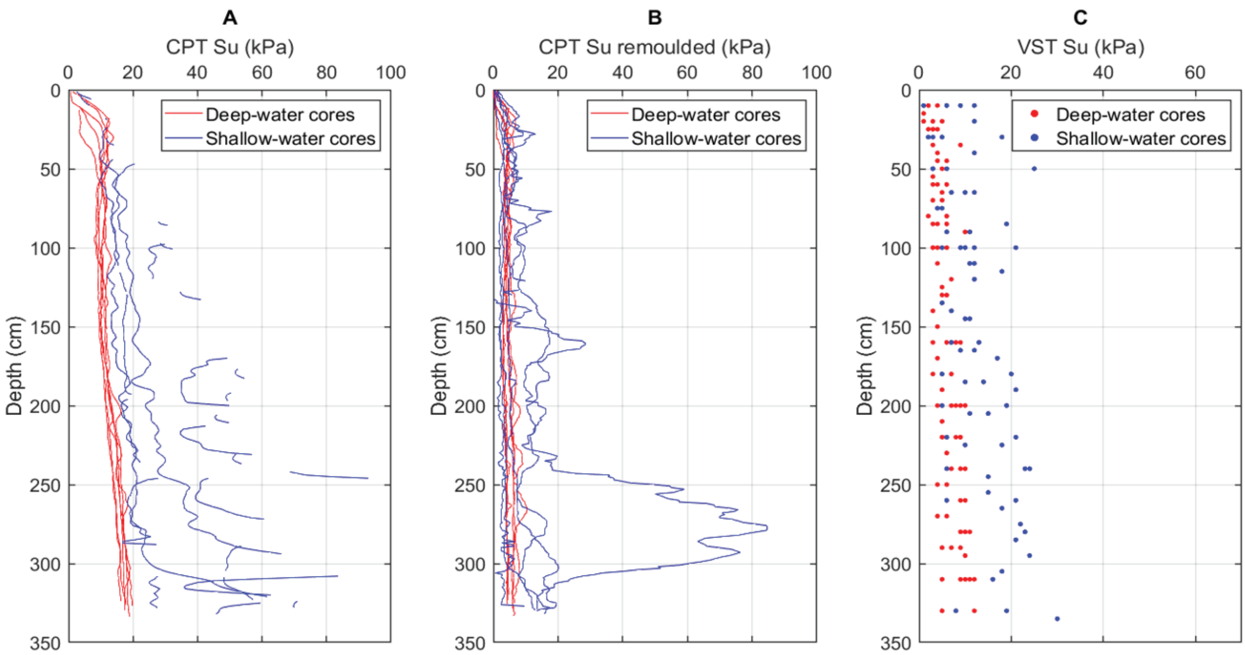

Fig. 2: Profiles of (A) *Su* measured from CPT, (B) CPT remoulded *Su*, and (C) VST *Su* from deep-water and shallow-water sediment cores.

According to Equation (1), a scatter plot was produced between the *Su* measured in the lab (VST) and the CPT data, in this case, the net cone resistance (q_{net}). The slope of this plot (Fig. 3) represents the *Nkt* cone factor; therefore, a simple linear regression suggests the best fit or rather the most suitable *Nkt* value for the measurements. From Fig.3A, the linear approximation of the fitting line indicates an Nkt of 28, with coefficient of determination R^2 of 0.63 showing moderately good correlation; however, the residual standard error (RSE) of the model is high and hence indicates considerable scatter in the data. Furthermore, residual plot of the tested variables (not presented here) shows that the data do not exhibit normal distribution. This is a perquisite, in order for a meaningful relationship to be established. Specifically, the evident scatter from shallow-water VST measurements (Fig.3B) shows that for these deposits, assigning cone factors could produce variable results.

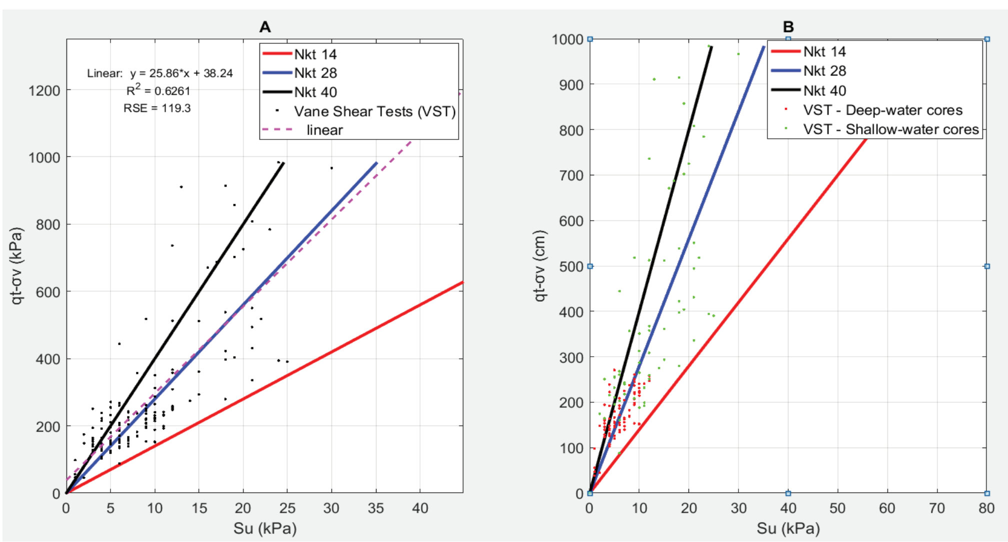

Fig. 3: Correlation of cone resistance (q_{net}) with vane shear strength showing (A) the best fit line for the data and (B) the variations of deep-water and shallow water measurements.

4. Discussion/Conclusion

CPT-derived *Su* constitutes probably the most effective in-situ measurement for the strength of fine-grained sediments. Yet, various studies report on the need and the difficulties to define the appropriate *Nkt* value for the appropriate definition of *Su* (i.e., Lunne *et al.*, 1985; Rémai, 2013). In this study, laboratory VST measurements were used in an effort to compare and discuss the *Nkt* for a specific CPT and VC data set between Evia and Skiathos. The results have shown that VST distribution do not reflect the initial CPT *Su* profiles and seem to align more with CPT *Su* remoulded values. From index properties, differences between shallow-water and deep-water environments could be established; these were also registered in the CPT *Su* profiles. For shallow-water sediments, closer to Skiathos Island, coarse content was further validated by the intermittent profiles of *Su* respective to relative density logs. The coarser texture reflects not only increased percentages of sand and small gravels, but also sediments rich in biogenic fragments and small shells, as observed in the VCs. Establishing *Nkt* values for these deposits was a difficult task due to the high scatter in the measurements (Fig. 3). Specifically, linear approximation between VST *Su* and net cone resistance from CPT produced a moderately good correlation and an *Nkt* factor of 28. Other studies have also produced high *Nkt* values for clay deposits (Rad and Lunne, 1988; Rémai, 2013). Sowers (1979) has suggested different cone factors for soil groups, which could reduce the margin of error; however, even when isolating the different data sets for shallow-water and deep-water environments (according to the index properties) credible results could not be easily produced, since variability in the data is still present. The findings indicate that a major source of the observed discrepancies could be sample disturbance resulting from the specific sampling technique, storage, transportation and handling issues in the lab that might have hindered the ability to establish accurate empirical relationships with CPT.

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MARINE GEOLOGY-GEOPHYSICS II: SEAFLOOR / HABITAT MAPPING - COASTAL DYNAMICS - OPTICS

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GUIDELINES AND BEST PRACTICES FOR MARINE HABITAT MAPPING IN THE HELLENIC SEAS

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Abstract

The "Guide for Marine Habitat Mapping" is the outcome of the Green Fund project entitled "Development of common methodology and techniques for marine habitat mapping". This Guide aims to provide a synopsis of the diverse array of concepts and methods used to undertake the marine habitat mapping adapted to the marine environment of the Hellenic Seas. The guide lists the intentional policy instruments that obligate the marine habitat mapping in protective sites, a selection of international guides for marine habitat mapping and also previous marine habitat mapping projects. The marine Habitat Types of the Greek Natura 2000 network are presented with their key ecological attributes. The geomorphology and bathymetry of the Hellenic seafloor is displayed with the bathymetric distribution of the Habitat Types. A review of the direct (*in situ* sampling and video/imaging techniques) and indirect (airborne/satellite/UAS and acoustic remote sensing) methodologies is given, along with ground truth methods. Best practices and key points are recommended for optimal marine habitat survey planning and different methodologies are suggested, based on each Habitat Type requirements. Additionally, issues of scale, spatial and temporal resolution, accuracy, errors, data confidence and habitat classification are discussed and selected case studies are reported.

Keywords: Natura 2000 marine habitat types, habitat mapping methods, Hellenic Seas.

1. Introduction

Mapping of marine habitats plays a key role in the management and conservation of natural systems and provides the spatial framework for ecosystem-based management (Deiana *et al*., 2019; Gerovasileiou *et al*., 2019). According to the EU Directive of Habitats (92/43/EU) it is mandatory to map the spatial distribution of habitat types and species of the protected sites in the NATURA 2000 network. Considering also that the benthic environment is prone to various types of anthropogenic impacts, there is an urgent need to develop tools for the rapid and accurate mapping of the seabed and to produce good quality maps of its seabed habitats.

However, marine ecosystems are poorly described compared to their terrestrial counterparts (Deiana *et al*., 2019). On land, the proportion of unknown habitats has been estimated at 17% whilst for the marine realm at 40% (EC, 2007). Furthermore, very few marine sites of the Natura 2000 network in Greece are efficiently mapped and after 20 years of its establishment, these maps require updating. This is a significant deficit that risks over time the ability of Greece to plan and apply effective conservation targets and measures for the management of protected areas so as to comply with its member state commitments.

Following this necessity, we have composed the "Guidelines and Best Practices for Marine Habitat Mapping" under the financial program of Green Fund "Natural Environment and Innovative actions 2020" within the Priority Axis "Actions to conserve Biodiversity" (Sakellariou *et al.*, 2021). The Guide aims to review and present the existing methodologies and techniques which are applied in Marine Habitat mapping worldwide. This Guide is based on the multidisciplinary experience of the collaborating scientists and summarizes the common practices reported in the current scientific literature, adapted to the marine environment of the Hellenic Seas.

2. Guidelines and Best practices for Marine Habitat mapping

Marine habitat mapping has been defined as "plotting the distribution and extent of habitats to create a map with complete coverage of the seafloor showing distinct boundaries separating adjacent habitats" (MESH, 2008). The process of producing seafloor habitat maps involves combining disparate data sets from various disciplines (i.e., marine biology, geology, hydrography, ecology, oceanography, remote sensing) to produce simplified spatial representations of the seafloor relating to the distribution of biological characteristics (Brown *et al*., 2011).

Thus, the necessity for creation of the "Guidelines and Best Practices for Marine Habitat Mapping" derives from the diversity of the different methodologies and techniques that are used for the mapping of the different bathymetric zones of the NATURA 2000 sites (i.e., shallow coastal, deeper coastal, deep sea), the different approaches for the description of the marine ecotypes, the different methods and techniques for the processing and interpretation of the raw data and the different means of ground truthing for the interpretation of marine acoustic and satellite data.

The objective of the present Guide is to provide a synopsis of the diverse array of concepts, methods and techniques used to map the Natura 2000 marine Habitat Types that require management and protection. The Guide is divided into nine chapters. A brief description of each chapter is given subsequently following the structure of the manuscript.

2.1 European and International policy instruments for the marine environment

Chapter 1 presents the main European and international policy instruments that promote and aim to ensure Marine Habitat protection, also identifies the necessary actions to be taken towards this direction. As the process of choosing Natura 2000 sites and their designation as Sites of Community Importance nears completion, member States need to adopt conservation measures involving appropriate management plans and other measures which correspond to the ecological requirements of the natural Habitat Types and Species of Community interest.

2.2 Guidelines for habitat mapping in the EU and worldwide and previous national mapping projects

Chapter 2 briefly reports on the marine habitat mapping projects that have taken place in Greece during the last 20 years after the initial establishment of the NATURA 2000 network. A current literature review showed that several studies were either involved with individual marine habitat ecotypes and their conservation or applied specific mapping and monitoring techniques. Also, it presents a selection of available guides and guidelines for marine habitat mapping that are currently applied at the national and international level.

2.3 Marine habitat types in the Natura 2000 network in Greece

Chapter 3 presents the marine habitat types and subtypes in the Greek Natura 2000 network. The habitat types are divided in two subgroups according to the Annex I of the Habitat Directive classification: a) Coastal and halophytic habitats and b) Rocky habitats and caves. They are coded and named according to Annex I of the Habitat Directive (as they are listed in the Standard Data Form (SDF), that contains an extensive description of the site and its ecology, Biogreece'95 and Natura databases) while the subtypes are based on the Barcelona Convention typology and modified for the Greek seas by Salomidi *et al*., (2014). The sign "*" indicates EU priority habitat types:

1110: Sandbanks which are slightly covered by seawater all the time

1110 A: Shallow subtype, Biocommunities of fine-grained sand in shallow waters

1110 B: Deep subtype, Rhodolith beds: "Tragana"/ "benches", Biocommunities of coarse-grained sand and pebbles of biogenic fragments

1120*: *Posidonia* beds (*Posidonia oceanica*)

1130: Estuaries

1140: Mudflats and sandflats not covered by seawater at low tide

1150*: Coastal lagoons

1160: Large shallow inlets and bays

1170: Reefs

1170 A: Shallow subtypes: Shallow reefs

1170 B: Deep subtype: Coralligenous habitats, Deep-sea corals or other biogenic reefs

1180: Submarine structures made by leaking gases

1180 A: Hot Seeps

1180 B: Cold Seeps

8330: Submerged or partially submerged sea caves.

A full description is given for each habitat type which includes: definition, ecological characteristics, flora composition and uniqueness, rarity and state of conservation and threats and also their association with other marine habitats and their geographic distribution (EU, 2013; Dafis *et al*., 2001; Salomidi *et al*., 2014).

2.4 Bathymetry of the Hellenic Seas and bathymetric distribution of habitat types

Chapter 4 summarizes the main geo-morphological characteristics of the seafloor of the Hellenic seas. Emphasis is given to the description of the shelf and the shallow terraces, the basins, the seafloor mounts and ridges. The bathymetric distribution of the marine Habitat Types of the Guide is also given.

2.5 Direct methods of marine habitat mapping (Data collection, processing and presentation)

Chapter 5 outlines the direct methods of marine habitat mapping. They include the direct sampling and imaging techniques using underwater cameras and remotely operated vehicles (ROV). They are usually used in conjunction with remote sensing or acoustic techniques and confirm the seafloor classification. The chapter presents the different methods with their advantages and limitations along with optimal practices for sample collection and processing. The detection and mapping methods of underwater caves are presented in a separate section.

2.6 Indirect methods of marine habitat mapping (Data collection, processing and presentation)

Chapter 6 reviews the indirect methods which include a) the remote sensing techniques and b) the acoustic/ geophysical techniques.

The remote sensing techniques include satellite images, aerial photography or orthomosaics from unoccupied aerial systems (UAS) in order to obtain frequent data on a synoptic scale about the state of the coastal areas and the surface of the oceans. In this section the applications of airborne and spaceborne data are discussed along with their advantages and limitations, methods of data processing, correction, analysis and accuracy estimation of the final product.

The section on the acoustic survey methods presents the basic systems that are usually applied to marine habitat mapping: Acoustic ground discrimination systems (through a single beam system), multibeam echosounders, multi-frequency side scan sonars and sub-bottom profilers. The operating principles of these systems are presented, along with their specifications, advantages and limitations of their application, the type and accuracy of the collecting data and the data processing and analysis techniques. Finally, the most common seafloor classification methods are presented.

2.7 Design of habitat mapping project and selection of optimal methodology

Chapter 7 indicates the importance of scale for the marine habitat mapping and its implications on the mapping products. Understanding the effects of scale is essential to the understanding of natural eco-systems, particularly in marine environments where sampling is more limited in respect to terrestrial environments (Lecours *et al*., 2015). According to Boyd *et al*., (2005) there is an inverse relationship between the information content (detail and resolution) of a map and the spatial area that it covers. In other words, the scope of the mapping project defines the scale of the survey.

During the planning of a new mapping project the following key points need to be considered: the available means and resources, the habitat type and the existing data. This process will determine the area to be mapped (extend), the scale of an operational map and resolution, the spatial precision required, the level of coverage required, how accurate the map should be, the level of detail needed in the habitat classification and the credibility and repeatability of the mapping methods. The selection of the suitable methodology and techniques for the mapping of marine habitat types is also defined by the geo-morphological characteristics and the bathymetric distribution of each habitat type. Thus, the best practices for the use of remote sensing (both satellite and drone) and acoustic systems are presented and an optimal combination of the different methods is suggested for each different marine habitat ecotype. Ground truthing methods are presented for each marine Habitat Type for the confirmation of the data collected by indirect methods of mapping (remote sensing and acoustic/geophysical systems).

2.8 Errors and quality of spatial data

Chapter 8 presents a review of the errors, both random and systematic that exist during the collection and analysis of the spatial data and also how these affect the accuracy and precision of the measurements. In conjunction the overall quality of the spatial data and the mapping products are discussed. Also, the main habitat classification systems are listed that cover the marine habitat types in Europe (and Greece) and the EU legislation INSPIRE (2007/2/EK) which defines the specifications and requirements of the produced spatial data. Finally, the production of confidence maps is suggested. They provide a quality estimation of the data reliability in relation with the purpose of the mapping products and their final use.

2.9 Case studies of marine habitat mapping in Greece

Chapter 9 presents selected case studies from actual marine habitat mapping projects that took place in Hellenic Seas. This chapter aims to demonstrate how the different marine habitat mapping methodologies can be applied to the study of different marine habitat types and produce successful habitat mapping products that satisfy the requirements of each project.

3. Discussion

The present Guide is based on the multidisciplinary experience of the involved scientists and sums up the scientific experience of the existing literature. The compilation and acceptance of the Guide by a number of different multidisciplinary scientists aims to establish a common practice for the mapping of marine habitat ecotypes which will be applied and followed by all involved research institutions on any occasion.

4. Acknowledgements

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HOLOCENE RECORDS OF OYSTER REEFS IN A SEMI-ENCLOSED EMBAYMENT, KALLONI GULF, GREECE

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Abstract

A high resolution geophysical survey, combined with ground-truthing methods, focused on the central part of the Kalloni Gulf (Lesvos, NE Aegean Sea), revealed the peculiar distribution of oyster reef structures with a relief up to 5.7 m. Their height reduces drastically towards shallower waters, but their distribution becomes denser. The subbottom structure revealed the presence of similar buried features. The buried reefs have been probably formed during the Holocene transgression and their development was interrupted at least twice probably from rapid environmental changes. Their formation has been undoubtedly influenced by the physical environmental conditions, although further research must be performed in order to discover the exact mode of their development.

Keywords: seabed morphology, sub-bottom structure, Lesvos Isl.

1. Introduction

Kalloni Gulf is a 20 km long semi-enclosed shallow embayment, with a maximum depth of ~19 m close to its entrance. It is located in the western part of Lesvos Island (North Aegean) and connects with the Aegean Sea to the southwest through a narrow strait (>25 m deep). Previous studies (Chronis *et al.*, 2014) revealed a peculiar seabed relief formed by numerous, randomly distributed reefs (consisting mainly of bivalves and other mollusks), which are directly linked with the high productivity spots of the gulf. Chronis *et al*. (2014) presented a preliminary distribution map of the reefs according to their reflectivity pattern and tried to make a link between the reefs and the presence of gas in the sediment pores. Widespread surficial sediment sampling (Manoutsoglou *et al*., 2021) showed that Kalloni Gulf is actually a sink for fine-grained (muddy) material, whereas the strait connecting the gulf with the open sea is covered by sandy sediments due to winnowing caused by seabed currents. In this study, geophysical data from Chronis *et al*., (2014) are re-analyzed in conjunction with ROV ground-truthing in order to update the distribution pattern of the reefs that have developed at the central part of the gulf, and also thoroughly investigate the shallow sub-bottom structure in order to discuss parameters that are involved in their formation, growth, and distribution during the Holocene.

2. Material and Methods

A 2-D seismic reflection survey in conjunction with side scan sonar mapping was carried out in 2013 using the parametric system of “Innomar” SES-2000 light plus comprising a subbottom profiler (SBP) at 6 Khz and a side scan sonar (SSS) operating simultaneously in 400 and 250 kHz. Both instruments were side-mounted onto R/V Amfitriti. Data acquisition was implemented by the SESWIN software and positioning was by a Hemisphere Differential GPS. In 2020, about 12 km of seismic data were collected with a Boomer (Applied Acoustics) SBP system in the shallow NNE part the Gulf, along with a StarFish 450F (Tritech) SSS at 450 kHz that covered a ~4 km grid over a selected area that emerged from the SBP data analysis. Spotted ROV (Teledyne Seabotix LBV200) dives in 8 sites were accomplished in 2021 for ground-truthing morphological data. Post processing, data analysis and mapping were performed using the softwares ISE (Innomar), SonarWiz and ArcGIS 10.1.

3. Results

According to the observed reflectivity patterns in the SSS images four backscatter types (BT) were distinguished (Fig. 1) that coincide with different reef patterns in the study area. BT1 corresponds to a patchy acoustic pattern with small, dense, and strong backscatter patches referring to low relief reefs (average height of 1 m) in a moderate reflectivity background. BT2 contains short and long curved streaks of high backscatter. Those features represent coalescing reef structures that rise up to 5.7 m in height (average height of 3.5 m, according to bathymetric profiles), creating chains with maximum length of ~1.4 km and with a direction almost perpendicular to the gulf axis. BT3 occurs close to the deepest part of the study area and returns a low backscatter that corresponds to a flat seabed covered in mud, with the appearance of a few small reefs. BT4 consists of two relatively small areas of high backscatter at the two sides of the central part of the gulf, representing regions covered by coarser materials stemming from the neighboring small fan deltas. As it was revealed from SSS and SBP data, the relief diminishes gradually towards the inner and shallower part of the gulf.

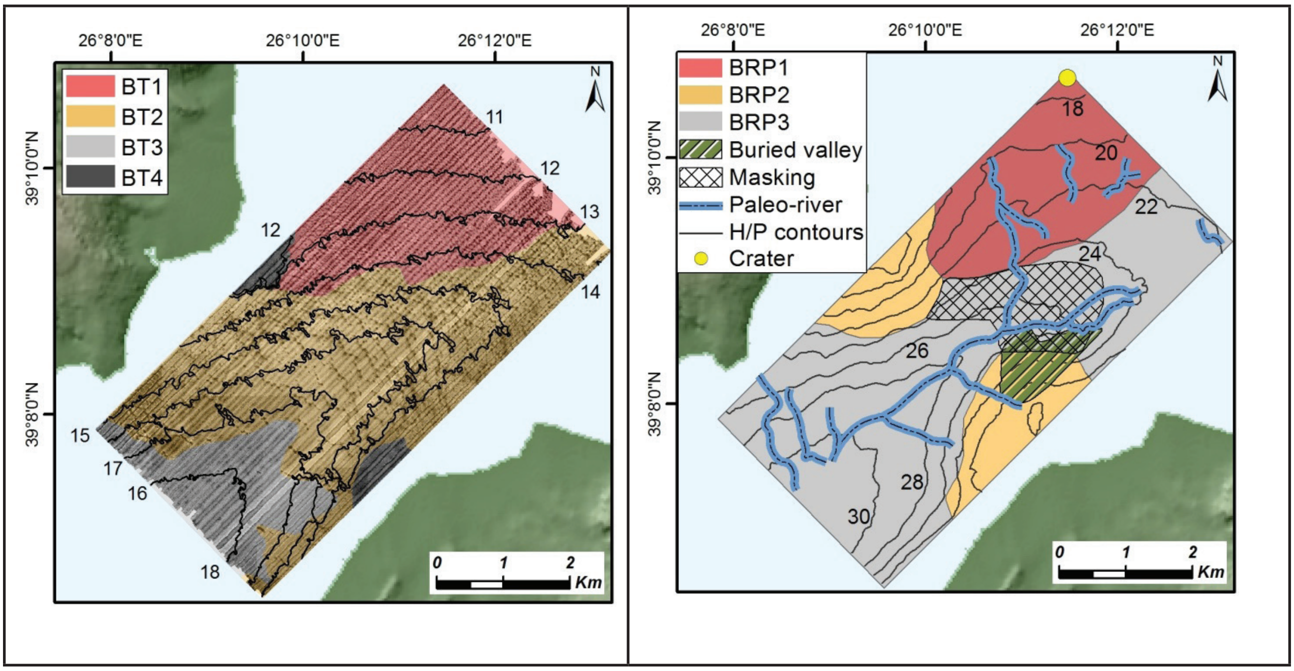

Fig. 1: (left) Backscatter types distributed over the SSS mosaic. (right) Buried reef patterns, valleys and paleo-rivers, as distinguished in the SBP profiles, over the H/P topography.

The general stratigraphy of the gulf is expressed by three main seismic units (SU) (Fig. 2). SU1 corresponds to an acoustically semi-transparent layer with few faint internal reflectors indicative of an almost homogeneous surficial layer with few slightly coarser internal layers. The surficial echo is mounded due to the numerous reefs that populate the seafloor, reaches a maximum thickness of ~4 m and most likely corresponds to the Holocene high stand system tract (HST). In the deeper areas of the gulf, a higher relief has developed with mostly asymmetrical larger bio-structures followed by shallow depressions around their base. The reefs produce a strong masking, locally, that covers the underlying reflectors and prevents the continuous record of the stratigraphy.

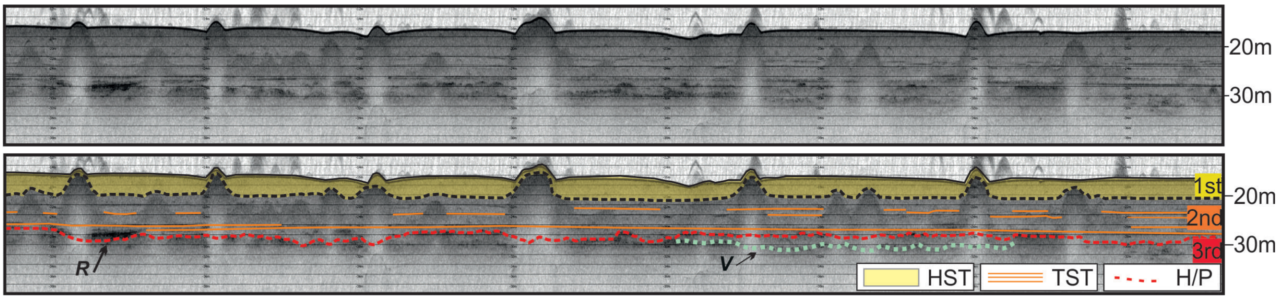

Fig. 2: Seismic units SU1 (HST), SU2 (TST) and SU3 (H/P) and the two distinct buried reef phases (1st - 2nd) in the Gulf of Kalloni (R: buried river channel, V: buried valley).

SU1 overlies unconformably the SU2 which also shows a mounded topography of low to medium intensity along with few semi-prolonged related probably to transgressive deposits (TST). The resemblance of the SU2 mounded reflectors with those of the surficial layer indicates probably the existence of buried reefs laying on various stratigraphic levels, implying that they have been buried in different phases during the TST. The boundary between SU2 and SU3 is a distinct basal unconformity that appears as a strong irregular reflector that most likely corresponds to an erosional surface. On this surface, numerous buried river channels were marked, connected, and formed the trace of an old drainage system, which is likely to be the sequel of the modern river system (Fig. 1). A buried valley was also observed at the south-eastern part of the studied area (Fig. 1). Although the SBP data showed evidence of various acoustic anomalies in SU2, which could be possibly related to the presence of fluids in the sediments pores, the general masking produced by the reefs in conjunction with the potential existence of coarser biogenic material stemming from the buried relief, make insufficient the source of those acoustic signatures in the seismic profiles. However, the presence of a small crater, detected in the SBP profile (Fig. 3a), reinforced the fluid's initial hypothesis. The crater is buried by recent sediments, but indications of fluids exist immediately under its base. A small SSS mosaic (Fig. 3b) revealed that the crater is 90x110 m and also hosts 3-4 smaller depressions randomly distributed along its base.

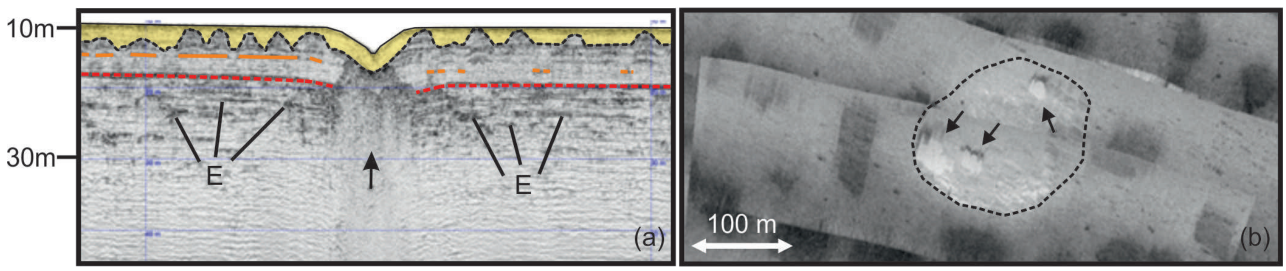

Fig. 3: (a) Seismic profile of the crater represented along with the seismic units and other stratigraphic features (Arrow: acoustic turbidity under the crater, E: enhanced reflectors), (b) Part of the SSS mosaic (Starfish) showing the surface of the crater (dashed line) with the smaller depressions along its base (arrows).

The most distinctive sub-bottom characteristic observed in the seismic profiles is the presence of buried reefs in the upper part of SU2. According to their topology (height, conjunction, and density) three types of buried reef patterns (BRP) were distinguished (Fig. 1). The BRP1 presents a pattern similar to the patchy acoustic pattern of BT1 on the SSS mosaic, with small, low relief (~4 m max height) and very dense reefs that create a strong masking. The BRP2 extends at the two sides of the central area, coinciding with BT4 but being more extended and reveals a very low relief (~2 m max height) of sparsely distributed reefs that stand either single or as an amalgamation of more reefs with a height increasing as they rich the central part of the zone. The BRP3 consists of sparsely distributed higher reefs (~8 m max height) that appear individual or locally coalescing creating 3D bioconstructions. Parallel SBP lines also show similar buried chain-shaped reefs, like the ones that appear in BT3 of the SSS mosaic. At the central part of the surveyed zone, due to the increased noise and density of the reefs it was impossible to make any discriminations.

ROV dives along the BT1 and BT2 verified the differences between the two areas (Fig. 4). Inspection of the BT1 area showed a highly turbid environment, semi-burial of the reef relief and a lot of bivalves, sponges and anthozoa. In contrast, the BT2 displayed a greater visibility due to the lower turbidity towards deeper waters. Remarkable bivalve assemblages (mainly oysters) along with other organisms created a higher relief. The variety and density of organisms was considerably greater than BT1. Sponges, anthozoa, various bivalves, ascidians, echinoderms, crustaceans were mostly observed in BT2. The most pronounced differences observed between the BT1 and BT2 types was the relief, that certainly appeared higher in BT2. The number of species and the size of individual organisms look better developed in the BT2 area. The presence of particular species, such as the coral *Cladocora caespitosa* and sessile organisms of the Vermetidae family (known to coexist and characterised as reef builders), have probably contributed to the development of oyster reefs. The last are known to create tube-shaped shells permanently attached to hard substrates that form dense, cemented, aggregations.

4. Discussion/Conclusion

A wide distribution of modern and buried reefs appears to populate the modern seabed but also to occupy deeper stratigraphic levels in the Kalloni Gulf. Previous data re-analysis ended with a different backscatter distribution in relation to Chronis *et al.*, (2014) results. The SBP data revealed various reef profiles and showed that they can create unique distribution patterns, resulting even in higher relief, with asymmetrical larger bio-structures followed by shallow depressions around their base, in the deeper parts of the gulf. Those features are probably related to the current circulation pattern, with higher velocity currents favoring the reef growth, and mining the base by removing the fine sediments. The ROV dives highlighted the distinction between BT1 and BT2 and moreover the differences along the same reef from the base to the top. The SBP demonstrated a buried relief, almost similar to the surficial, suggesting different burial phases (two at least). The majority of the buried reefs is found within SU2, indicating an initial growth during the early TST. Data re-analysis revealed poor evidence of gas presence in relation to Chronis *et al.*, (2014) observations, although the presence of a shallow water depression enhances the fluid-presence scenario.

Fig. 4: Camera stills from the ROV dives in the BT1 (a) and BT2 (b, c) verifying differences in the backscatter types.

A critical factor that can affect the oyster reef development, even deteriorate it, is a rapid salinity change (Parker, 1955). The salinity in the gulf has seasonal variations with the biggest changes occurring next to the entrance and the NE area due to increased freshwater inflow by the surrounding drainage system. This probably explains the lower and diminishing reef relief at the NE part and towards the shallower parts of the gulf. In addition, the absence of reefs close to the entrance could be a result of stronger currents, increased depth and other biological factors such as the presence of harmful species (oyster predators etc). Abrupt changes in the environmental parameters, due to the climate change, have taken place during Holocene (i.e., Triantaphyllou *et al.*, 2014). Possible evidence of these climatic alterations is imprinted in the stratigraphy of the Kalloni Gulf. The different burial phases of the reefs indicate environmental instabilities that slowed down their growth and even deteriorated them. A possible counterpart could be a period around Holocene Climatic Optimum (~10-6 ka) (Triantaphyllou *et al.*, 2014) or the 8.2 k event (Weninger *et al.*, 2006) that both affected the Aegean Sea's freshwater budget and sedimentation rates. Future interdisciplinary work (i.e., multi-beam morphology, radiocarbon dating, hydrodynamics) is required in order to precisely determine the parameters that affect the development and distribution patterns of the reefs, but also to identify the periods when the buried oyster reefs were thriving as well as the main causes of their earliest extinction-phases.

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AN UNUSUAL COASTAL DEPRESSION AT EASTERN LESVOS ISLAND - ASSUMING POSSIBLE FORMATION MECHANISMS

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Abstract

An odd depression, resembling to an elongated pockmark, was detected during a previous marine survey in the eastern coastal waters of Lesvos, and it was re-visited in order to study in detail its morphology and possible causes of its formation. The pockmark is about 400 m long and 5 m deep, it has a complex relief towards its SE edge and its base is covered by organic-rich fine-grained sediments. Neighboring small depressions are attributed to sand patches within the surrounding *P. oceanica* meadow. Seismic data in the wider area has not given concrete indications regarding the presence of fluids in the sediment pores (either gas or of hydrothermal origin). Also, no visual evidence of seepage in the water column exists. The depression could not have originated due to sediment instability, and it cannot be an artificial feature related to human intervention. It is profound that the depression's mode of formation is ambiguous and needs to be thoroughly investigated.

Keywords: multi-beam, ROV, pockmark, NE Aegean Sea.

1. Introduction

Observations of unusual seabed features in coastal areas such as mound clusters and depressions have been mounting in recent years (i.e., Manoutsoglou *et al.*, 2018). Regarding the latter, depressions (pockmarks) found on the surficial sediments, in shallow and deep waters, have been mainly attributed to fluid flow processes related to e.g., methane gas, freshwater or hydrothermal fluid venting/seepage (i.e., Hasiotis *et al.*, 1996; Judd and Hovland, 2007; Rousakis *et al.*, 2014). Pockmarks usually develop in clusters, whilst isolated depression features are rarely documented and harder to explain.

In October 2018, during a marine geophysical survey in the coastal area of eastern Lesvos Isl. (Fig. 1), seismic profiles at the edge of the survey grid revealed the presence of a depression, resembling to an instability or a crater-like feature. In February 2022, the area was re-visited in order to obtain detailed bathymetric data over this feature using a multi-beam echo-sounder (MBES); the results showed that the feature is an odd, elongated depression (Fig. 1b-A). Thus, the objective of this paper is to describe in detail the depression and discuss on the possible formation mechanisms.

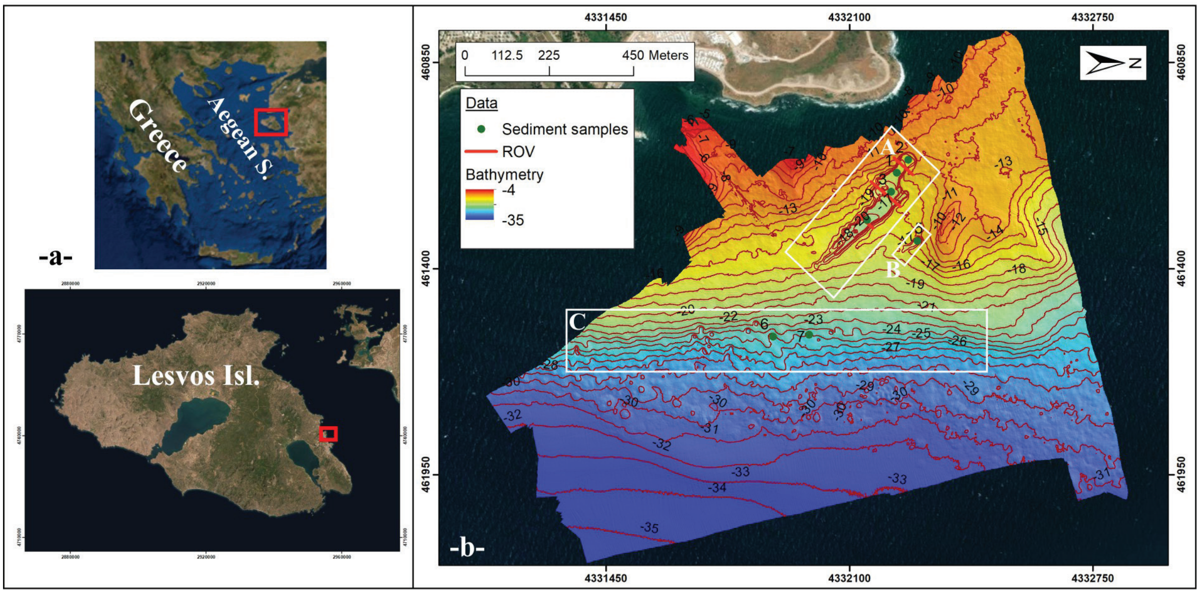

Fig. 1: (a) Location of Lesvos and of the study area. (b) MBES bathymetry of the study site, showing the location of the observed elongated depression (A), the neighboring (B) and the smaller circular depressions (C). The ROV dives and the surficial samples are also displayed in the map.

2. Material and Methods

A Teledyne Reson SeaBat T20-R MBES was used at a frequency of 420 kHz, recording simultaneously 512 beams over a swath angle of up to 140°. The system consists of a TC2181 projector transducer and an EM7219 receiver transducer and a rack-mounted sonar processor that is configured with a fully integrated inertial navigation system (INS Type-20). The INS uses RTK GNSS (Trimble) for position and time, and IMU (IP68) for measuring roll, heave, and acceleration. The INS is fully compliant with the Applanix POSMV Wavemaster II. Sound velocity profiles were obtained using a Valeport SWIFT SVP memory probe. The TDY-PDS (Teledyne RESON) software was used for hydrographic data acquisition and processing. The MBES data were gridded to a DTM of 1 m cell size. MBES data were acquired from the vessel “Amfitriti” over a dense grid of survey lines with a speed of about 4 knots. A small ROV (Teledyne Seabotix LBV200) was used for ground-truthing of the hydroacoustic data along 3 profiles (Fig. 1). Seven surficial samples were collected using a Van-veen grab (Fig. 1) and they were only macroscopically described. Unfortunately, subbottom profiling that could give information for the underlying structure and stratigraphy, was not available during the survey. ArcGIS 10.4 was used for mapping purposes.

3. Results

The study area is located at the east Lesvos, ~4 km north from the port of Mytilene. Geologically, the wider onshore area built on Pliocene lavas laying, locally, over Pliocene marly (mainly) freshwater limestones (Katsikatsos *et al.*, 1982). Holocene alluvial deposits occupy restricted low-lying coastal areas. No major or minor tectonic lineaments are reported in the wider area (Soulakellis *et al.*, 2006; Pavlides *et al.*, 2009).

The study site encompassed a 1.3 km^2 area, with depths ranging from 4 to 35 m (Fig. 1). The MBES morphology revealed a distinct depression developing between 12 and 17 m water depth (Fig. 1b-A and 2). It is 400 m long, up to 87 m wide, and up 5 m deep near its center, with slope gradients reaching 50º at its northern edge, covering an area of 17300 m^2. Its shape is elongated with an almost NW-SE orientation and present irregularities at its center and towards the SE edge, where it becomes more complex and seems to split into two very narrow segments separated by an internal small, elongated ridge reaching 2 m in height (Fig. 2). The base of the depression is almost flat; few modest mound-like features near its middle section are probably due to small-scale instabilities that have affected the steep slopes. A neighboring smaller depression to the east (Fig. 1b-B and 2), with the same orientation, is ~65 m and 20 m long and wide, respectively, about 3 m deep, with inclinations of its banks of up to 30º. It is also elongated in shape covering an area of 1150 m^2. Finally, some almost circular (up to 20x20 m) depressions were detected between 22 and 27 m (Fig. 1b-C), being about 2 m deep, and having gradients up to 30º.

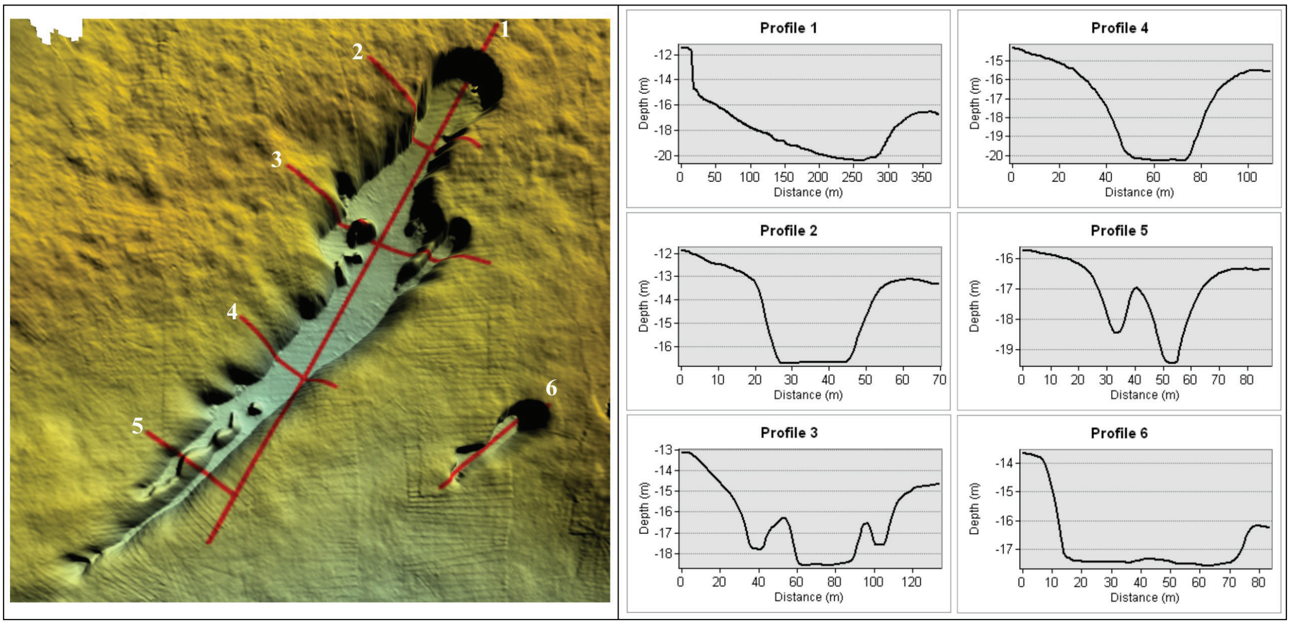

Fig. 2: 3-D image of the elongated and the smaller neighboring depressions and location of the seabed profiles. Profiles displaying the geometry/internal seabed features along and across the depressions.

The MBES backscatter mosaic presented, in general, high intensity levels all over the study area, attributed to the presence of a dense *P. oceanica* meadow covering the wider region, which was also verified by the ROV dives (Fig. 3). A small outcropping area also returns a restricted but higher backscatter. Lower backscatter intensities stem mainly from the base of the depressions suggesting that they are covered by soft/loose sediments as it was also validated by the ROV (Fig. 3) and the collected samples. The samples from the elongated depression appeared a quite different color, in relation to the other seabed depressions in the area, being dark grey/black muddy sand to sandy mud with small shells and biogenic fragments. The sediment characteristics may suggest potential anoxic conditions in the uppermost sedimentary column. The base of the smaller depressions is covered by brownish muddy sand with small biogenic fragments suggesting different environmental conditions. Hence, the smaller depressions most probably represent sand patches within the *P. oceanica* meadow. The ROV video also depicted dead Posidonia locally littering the elongated depression seabed (Fig. 3). However, it is intriguing that *P. oceanica* does not appear to grow in the depression.

The subbottom profiler records acquired to the N and NE of the observed depression (with an Applied Acoustics Boomer system) have not given indications of faulting or acoustic anomalies implying the presence of fluids in the sediment pores. No columnar disturbances or chimneys or other gas eruption features suggesting intense and disruptive for the underlying layering fluid escape has been observed, nor buried depressions were detected in the seismic records. The acoustic facies imply to a generally fine-grained texture for the surficial sediments, as is also indicated by the facilitation of the pockmark's steep slopes, although *P. oceanica* could be also a stabilizing factor. Finally, seepages have not been reported from this coastal area nor have been identified in the MBES records or the ROV during the survey.

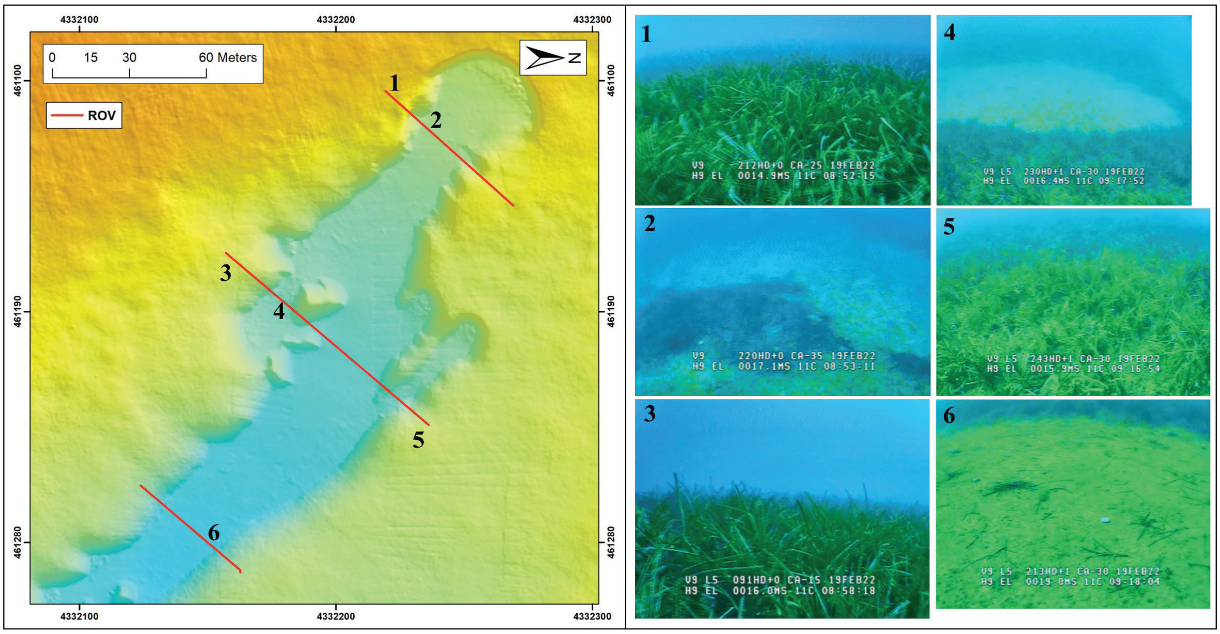

Fig. 3: ROV images showing *P. oceanica* surrounding the depression (1,3,5) but also covering its flanks (4) and soft/loose sediments and dead Posidonia blanketing the base of the depression (2,4,6).

4. Discussion/Conclusion

The source fluid that has possibly contributed to the formation of the unusual depression detected at eastern Lesvos is uncertain. Pockmark characteristics (e.g., location, dimensions, shapes) are controlled by the fluid type and fluxes, the thickness and nature of near bottom sediments and the underlying structure and lithology (Judd & Hovland, 2007). In the present case, however, seismic signatures, features and structures related to fluid migration through the overlying sedimentary layers (i.e., acoustic anomalies, chimneys or faults), were not detected in neighboring seismic records. Moreover, hydrothermal activity has not been reported from this area, although basalts appear onshore.

Alternatively, the depression might have developed due to hydrodynamic seabed scouring. Its elongation could have developed over time, in response to the prevailing current activity and particular coastal circulation patterns. Yet, currents must be sustained and strong in order to allow for such scouring in the direction of the prevailing flows. Concerning the present conditions, field information and model results from the wider area (Kolovoyiannis & Tsirtsis, 2013) have shown wind-driven alongshore circulation in the area, subsurface currents and rotational flows in the embayments; nevertheless, offshore coastal current speeds that could have formed the elongated depression have been found to be up to 0.1 m/s, which are hardly adequate to trigger and sustain the sediment transport required to form this elongated seabed scouring. It should be mentioned, however, that the depression could be a relict seabed feature developed during the transgressive Holocene phase, when coastal hydrodynamics could have been more energetic than those at the present conditions.

Also, the observed feature does not appear to be a collapse depression, since neither its shape resembles the typical bowl-shaped instability feature, nor the common triggering factors (storm waves, intense seismic activity) (Prior & Coleman, 1982) are intensive and/or frequent in the region.

Finally, an artificial origin (e.g., dredging activities), is not supported by the available historical records nor by the sediment texture, the shape/dimensions of the depression and the widespread presence of *P. oceanica*.

It becomes apparent, that on the basis of the available evidence the formation and maintenance mechanism of the detected elongated depression cannot be ascertained. Only a comprehensive oceanographic and geophysical survey (i.e., subbottom profiling) together with sediment coring could shed light to the formation time and mechanisms of this intriguing feature.

5. Acknowledgements

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HIGH FREQUENCY SHORELINE AND WAVE RUN-UP DETECTIONS THROUGH OPTICAL VIDEO IMAGERY. EXAMPLE FROM KAMARI BEACH, SANTORINI

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Abstract

Shoreline and wave run-up positions and their changes form fundamental morphological parameters of coastal zone dynamics. In this study, the winter 2-D shoreline and wave run-up/swash maxima positions are detected/monitored in geo-rectified coastal imagery of high spatio-temporal resolution obtained from a Beach Optical Monitoring System (BOMS) installed in a highly touristic beach (Kamari, Santorini). The variability of the shoreline and wave run-up positions is linked with concurrent high-frequency wind and wave records. During the 106-day monitoring period, it was found that shoreline displacement was ranging between 6-19 m and wave run-up position changes were ranging between 21-40 m. Correlation of beach morphodynamics with the wave forcing has shown that energetic wave events can trigger changes in the beach spatio-temporal dynamics. The study results suggest that the BOMS could provide a fast, powerful and efficient beach monitoring tool. It is noted that the shoreline and wave run-up maxima are also important for beach management, as the latter plays the most significant role in the definition of the landward boundary of the Public Maritime Domain (i.e. the “aigialos” line) according to the legislation in many European (and in Greece).

Keywords: beach morphodynamics, shoreline detection, wave run-up, coastal video monitoring, image processing.

1. Introduction

Beaches form one the most dynamic environments in earth. Their morphological changes can be very frequent, being dependent on beach exposure to the hydrodynamic action and the sediment supply. Beaches are also important coastal ecosystems, providing flood protection to other significant coastal ecosystems (e.g., wetlands and lagoons) and the very substantial and increasing infrastructure/assets (e.g., coastal roads, airports, industrial/urban development) they front/support/host. Moreover, they have a high aesthetic/hedonic and socio-economic value, being the focus of the 3S (Sun-Sea-Sand) tourism, a most significant sector of the touristic industry. Therefore, studying of beach morphodynamics is of paramount environmental and socio-economic significance, especially when concerning that most beaches worldwide are under an increasing erosion risk, which is projected to exacerbate in the future (Monioudi *et al.*, 2017).

Shoreline and wave run-up positions are fundamental parameters of the swash zone dynamics and are crucial factors for coastal planners, engineers, and local authorities, as they are typically used for effective coastal planning and the design of coastal protection works (Vousdoukas, 2014). In addition, they form important regulatory boundaries. Shoreline position defines the extent of the dry beach, and thus has impact on the carrying capacity (i.e., the number of beach visitors that can be hosted simultaneously), whereas the maximum position of the wave run-up forms a reference line (defined as the “aigialos line” in Greek) beyond which a ‘setback’ zone of no further development/constructions are allowed according to the national (Greek Law 2971/2001) and European legislation (e.g., the ICZM Protocol to the Barcelona Convention [Art. 8(2) and the EU Directive 2014/52/EU].

Estimation of wave run-up and shoreline positions is a complicated task, as nearshore hydro-morphological changes (or coastal morphodynamics) are based on complex processes-response mechanisms driving the swash zone, operating at various spatio-temporal scales (Suanez *et al.,* 2015). The traditional mapping techniques are not able to cope with the issue satisfactory, as they are not able to provide accurate records of high spatio-temporal coverage. High-resolution satellite images, which are commonly used for the extraction of such morphological parameters in large scale, are not only characterized by high cost, but also from low temporal coverage, while in many cases images cannot be recorded due to physical restrictions (e.g. cloudiness occurring during extreme storminess). On the other hand, repeated topographic records through classic leveling/positioning methods, require dedicated human efforts especially during extreme storm events. Over the recent years, emphasis has been given to the development of image processing algorithms/techniques, capable to record/monitor with high accuracy specific coastal features of interest on specialized optical datasets deriving from coastal video monitoring systems (e.g., Vousdoukas, 2014; Velegrakis *et al.*, 2016).

2. Material and Methods

An autonomous Beach Optical Monitoring System (BOMS) was installed at the southernmost part of Kamari beach at an elevation of 17 m, close to St. Nicholas church, set to monitor a beach stretch of about 680 m long at the southern part of Kamari beach (Fig. 1). The BOMS comprises of a station PC and 2 video cameras set to obtain beach imagery of high resolution (3gp videos, 1920 × 1080 pixels) with a sampling rate of 5 frames per second (fps) in burst mode (for 10 minutes at the beginning of each day-light hour). Images are pre-processed by the station PC. Initially, they are corrected for lens distortion, geo-rectified and projected on real-world (UTM) coordinates using standard photogrammetric methods and Ground Control Points (GCPs), collected with a Differential GPS (Topcon HiPer RTK-DGPS). The geo-rectified and UTM-projected images of each hourly 10-minute burst (3,000 snapshots/frames) are then furthermore processed in order to generate high resolution (always less than 0.25 m) time-stack images expressing the time-average (TIMEX images) and the time-maxima (IMMAX images) foaming positions, amongst other coastal optical products (see also Velegrakis *et al.*, 2016). Separate software tools have been developed/used in order to i) generate a single mosaic that combines the mosaics generated for each of the 2 cameras; and ii) rotate the generated mosaics by setting as reference point (x = 0, y = 0) the position of the optical system (Fig. 1). For the purpose of this study, TIMEX and IMMAX datasets for a highly energetic period (in terms of wave activity) have been extracted, covering 106 days (16/12/2016 - 29/03/2017). However, due to video system downtime, optical data were not available for 14 days (21-24/01/2017 and 06-15/02/2017).

In order to facilitate the shoreline and wave run-up detection monitoring procedure, two automated coastal feature detectors have been developed/used. The detectors are based on a localised kernel that progressively 'walks' along the feature of interest on the georectified TIMEX or IMMAX imagery, automatically following the high intensity zone along the shoreline. The site-specific configuration parameters of the detectors are: i) the preferable, in terms of shoreline/wave run-up following, general direction of kernel movement along the imagery (left to right in this case); and ii) a corresponding user-defined "root" cross-shore transect at the edge of the image which spans across the feature of interest (i.e. the shoreline or/and the wave run-up).

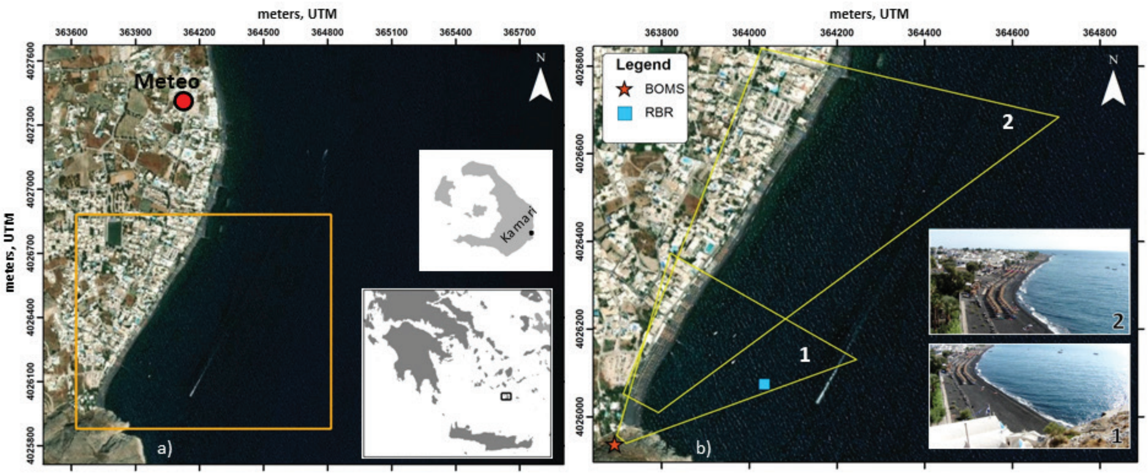

Fig. 1: a) Kamari beach, Santorini; the orange box shows the area studied, whereas the position of the deployed meteorological station is also shown. b) The study area (southern part of the beach), showing the field of vision of the 2 video cameras and 2 corresponding frames; the location of the RBR wave logger is also shown (*satellite image source: Google Earth*).

In addition to the BOMS, a wave logger (RBRvirtuoso D|) was deployed 220 m offshore the beach at 9.2 m water depth (Fig. 1b) and provided high frequency (6 Hz) wave data during 10-minute hourly bursts, concurrent to the optical records, during the period of 17/12/2016 - 29/03/2017. Using these data, hourly values of the wave climate (zero-moment wave height - Hm0 and peak wave period - Tp) were estimated. In addition, a meteorological station was installed at the roof of a hotel (*Alexandra beach*), located close to the deployment area at about 150 m from the BOMS deployment site - Fig. 1a) for a longer period (20/11/2016 - 26/06/2017) providing wind velocity and direction data. Furthermore, wind velocities over 6 Beaufort deriving from the sector where Kamari beach is exposed (between 35°-180°N) were selected and the criterion of Sanchez-Arcilla *et al.*, (2008) according to which: "*the minimum time to consider a storm event as a storm surge is 6 hours and the maximum gap between the observations 18 hours*" was used in order to isolate the storm events that affected the beach.

3. Results

During the 106-day monitoring period, cross-shore shoreline and wave run-up positions showed significant variability. At any shoreline section 0.25 m long, the differences between the minimum and maximum y-point was ranging between 6-19 m (Fig. 2a), whereas wave run-up changes along the shoreline were found to range between 21 - 40 m (Fig. 2b). Areas of increased shoreline variability are associated mainly with areas of the southern (at about x between 50 - 225 m) and central (at about x between 475 - 550 m) parts of the beach (Fig. 2c). By comparison, two sections of the beach showed standard deviations lower than 2 m and seem to be quite stabilized (at about x between 250 - 300 m and 575 - 650 m). The maximum recorded wave run-up variability is concentrated at the central sector of the monitored beach (at about x between 125 - 525 m) with wave run-up variability ranging between 30 - 40 m in most cases, whereas the edge parts of the monitored beach show lower values (Fig. 2d).

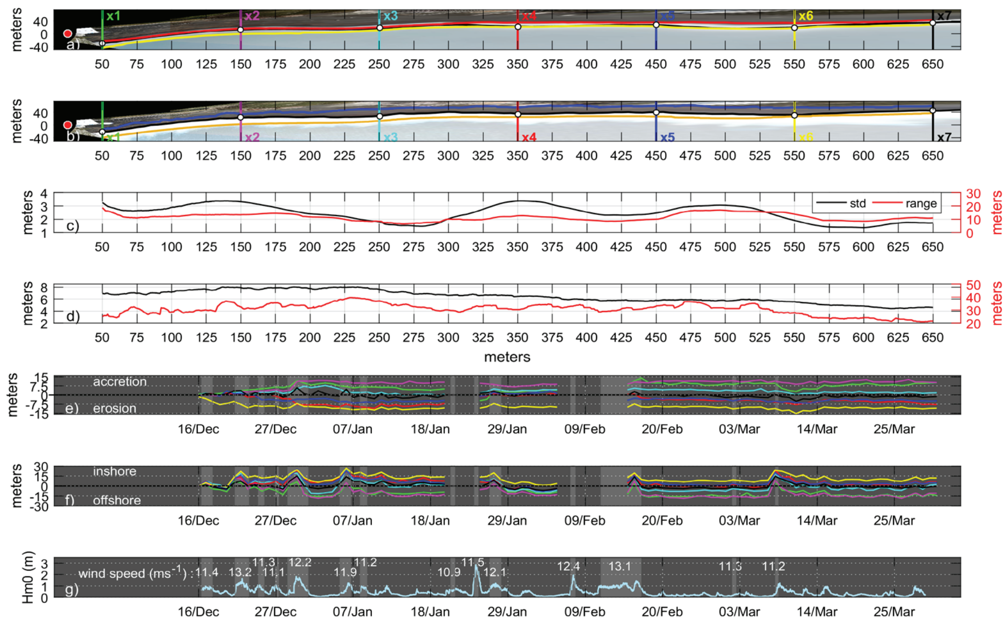

Fig. 2: a)-b) Georectified TIMEX and IMMAX mosaics at the beginning of the monitoring period from the southern part of Kamari beach showing also the locations of the 7 selected/representative profiles, the BOMS (red circle) and the range between the minimum and maximum recorded positions. c)-d) Spatial distributions of the standard deviation (std) of cross-shore shoreline position and range and wave run-up position and range, respectively, detected during the monitoring period. e)-f) Temporal changes in cross-shore beach accretion/erosion and wave run-up, respectively, at the 7 locations shown in panel a; changes are relative to the shoreline and wave run-up position recorded at 16/12/2016 09:00. g) Wave heights recorded from the RBR logger during the monitoring period; light gray stripes indicate the timing, duration and speeds of energetic wind events (winds from the southern sector with speeds > 10.8 ms^{-1} and duration > 6 hours).

Morphodynamics becomes clearer when examining the temporal changes of the 7 selected/representative equally distanced profiles (Fig. 2e and 2f). The cross-shore profiles located at the southern part of the monitored beach sector (x1 and x2) showed accretion behavior (of about 10 m) compared to the starting day of the monitored period. On the contrary, for the same period, cross-shore profiles x4, x5 and x6 showed beach erosion of similar magnitude (10 m), whereas cross-shore profile x7 located at the northernmost part of the monitored beach area didn't show significant changes. Beach response is found to be very energetic to the events detected/recorded during the monitored period (Fig. 2g). Shoreline positions in all of the examined cross-shore sections are found to respond to detected storm events with wave heights greater than 1 m approaching from directions where the beach is exposed (e.g. at the events of 27/01 14/02 and 09/03), whereas wave run-up changes follow a similar trend. It is evident that during events of increased wave energy approaching the beach, wave run-up responds in all cases by excursion to the inshore. In general, higher values of wave run-up are recorded for the central section of the beach, compared to the southern and the northernmost sectors, which may be attributed to milder slopes.

4. Discussion / Conclusions

Beach morphology of the southern part of Kamari beach was found to be highly variable during the monitoring period. Maximum changes in shoreline position are recorded for the southernmost sector of the beach, whereas wave run-up variability for the same sector was found to be close to the lowest recorded value (of about 25 m). In addition, the minimum changes in shoreline position are found at the same location, with the highest recorded wave run-up values (at x about 200 - 250 m). Areas of common high shoreline and wave run-up variability are found at the central part of the examined beach area. The shoreline was more variable (over time) at the southern and central sectors of the beach. Most of the recorded energetic wave events of the monitored period were found to approach the beach from the NE, E and SE sectors (Fig. 2g).

The automated approach developed to extract shoreline and wave run-up positions from TIMEX and IMMAX images showed to be an efficient tool in resolving beach variability in fine spatio-temporal scales. Both positions are of high importance for coastal planners and engineers, as they form fundamental parameters of the swash zone dynamics. At the same time, these morphological parameters form regulatory boundaries. The shoreline position defines the beach carrying capacity (i.e. the number of visitors/tourists that can be hosted simultaneously in a beach), whereas the swash maxima (i.e. the maximum recorded wave run-up) of a beach forms a reference line (defined as the “aigialos line” in Greek) beyond which a ‘set-back’ zone of no further development/constructions are allowed according to the national (Greek Law 2971/2001), European (e.g., the Floods Directive 2007/60/EEC and the amended EIA Directive 2014/52/EU) and international legislation [e.g., the ICZM Protocol to the Barcelona Convention (Art. 8(2)]. A significant result of the present work is the development of an objective and cost-effective methodology to define such coastal regulatory boundaries. It has to be noted that detections are focused at (proximal) beach sections for both beaches, due to the increased pixel footprint in these areas; thus, the results are characterized by extremely high accuracy. However, in the case of defining set-back zones (see above), detections of lower accuracy could be also used, and thus, longer beach sections can be monitored.

5. Acknowledgements

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MEASURING AND MODELLING THE OPTICALPROPERTIES IN THE CRETAN SEA

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Abstract

The main objectives of Hellenic Center for Marine Research (HCMR) optical work during the MARRE project were the collection of in situ optical data, radiative transfer modelling and thus the improvement of ocean colour algorithms and satellite validation as well. This was designed to contribute to one of MARRE ‘s general objectives of ensuring accurate monitoring of the marine environment using ocean colour satellite data. Therefore, the present work took a comparative approach to the calculation of a key apparent optical property in ocean colour validation, remote sensing reflectance, in three different ways: (a) in situ measurement (b) satellite data; and (c) Hydrolight modelling. The study area was the Cretan Sea during the MARRE project research cruise. The methodology followed during both the acquisition of the in situ measurements and the post - processing was in accordance with international protocols. Our results, in addition to their intrinsic value for an area of high interest to the ocean colour satellite validation and optical oceanography community, will alsocontribute to the improving of the individual assessment methods towards a more accurate calculation approach for the Cretan Sea and the wider area of the oligotrophic eastern Mediterranean.

Keywords: marine optics, apparent optical properties, remote sensing, hydrolight, satellite ocean colour validation, Eastern Mediterranean.

1. Introduction

The study of optical properties is a particularly critical factor in a number of studies concerning the marine environment. This is especially important in an ultra-oligotrophic environment such as that of the wider region of the eastern Mediterranean (Karageorgis *et al.*, 2008; Banks *et al.*, 2020b; Chaikalis *et al.*, 2021) and more specifically for the marine periphery of the island of Crete. From this point of view, the study of the optical properties of this area acquires its own special value as it adds valuable information to the understanding of satellite ocean colour products and the marine optics of oligotrophic areas in general.

The remote sensing reflectance [R_{rs}(λ)] parameter is a measure of how much of the downwelling light that is incident onto the water surface is eventually returned through the surface, so that it can be detected by a radiometer pointed in the opposite direction. R_{rs} (λ) is the fundamental measurement from which satellite ocean color (OC) products, such as phytoplankton chlorophyll concentration, are developed. In addition, the variation of the Inherent Optical Properties (IOP) estimates, as a description of the light absorption and scattering in the water column, are an important basis for understanding the levels of R_{rs}. These were also measured and will be presented in a relatively independent way.

The in situ measurements used for the MARRE project were carried out in September 2020. The optical measurements were made with the integrated HCMR optics suite system at specific stations, so that they coincided with the overpass of the Sentinel-3 OLCI satellite. Figure 1 shows the stations for the MARRE research cruise.

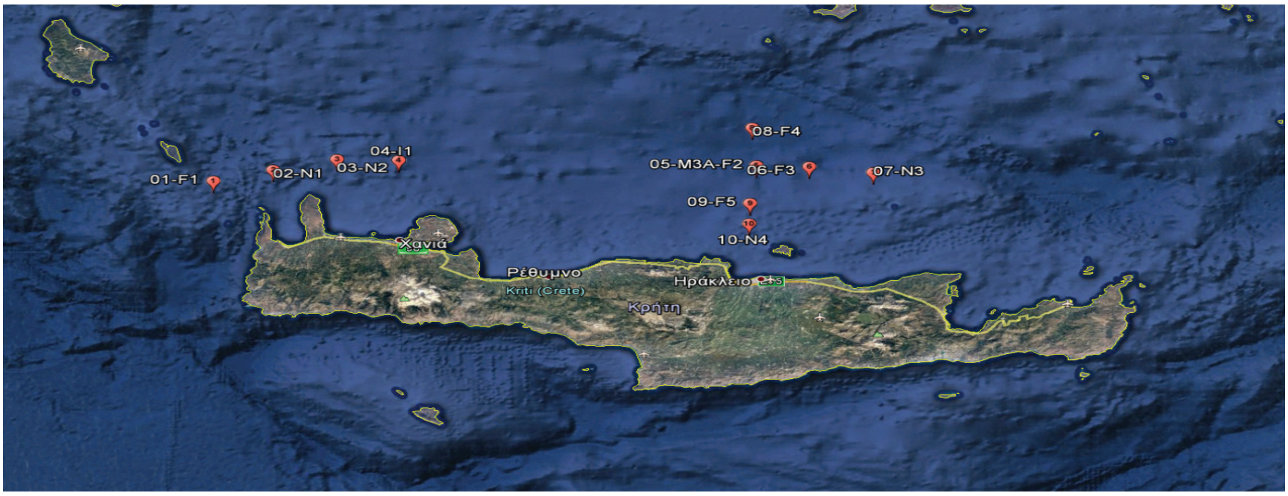

Fig. 1: Marine optics stations during the MARRE cruise.

2. Material and Methods

The in situ measurements used for the MARRE project were carried out in September 2020. The optical measurements were made with the integrated HCMR optics suite system at specific stations, so that they coincided with the overpass of the Sentinel-3 OLCI satellite.

The HCMR optics suite was deployed using one of the winches of the research vessel of the cruise (R/V Aegaeo) following the NASA and FRM4SOC protocols to retrieve in water measurements of upwelling radiance and downwelling irradiance (Mueller *et al.*, 2003; Ruddick *et al.*, 2019a, 2019b; Banks *et al.*, 2020a). For data processing the IOCCG protocols (Zibordi *et al.,* 2019) were applied.

In addition to the HCMR optics suite, for a few stations on the MARRE cruise, the portable above water radiometry system from the University of West Attica was used to take measurements for comparison with those from the in-water Trios radiometry. For the above water measurements, the Skylight Blocked Approach was followed (Drakopoulos *et al.,* 2015; Banks *et al.,* 2020a).

Hydrolight is a numerical model based on solving the radiative transfer equation; it calculates AOPs (apparent optical properties) through six different models of IOPs (inherent optical properties) (Mobley and Sundman, 2001). The use of the Hydrolight 5.0 numerical model was run using chlorophyll data as input, which were sampled at discrete depths during the oceanographic cruise.

3. Results

The satellite data were filtered using the L2 flags to remove any matchups where the satellite reflectance retrieval was not meeting minimum quality standards (e.g. pixels containing cloud/cloud shadow, suspect or erroneous atmospheric correction due to very high dust aerosol load in the atmosphere, etc). In total6 stations met all the necessary criteria, both in situ and satellite. The initial R_{rs} comparisons for two of these stations are shown in Figure 2.

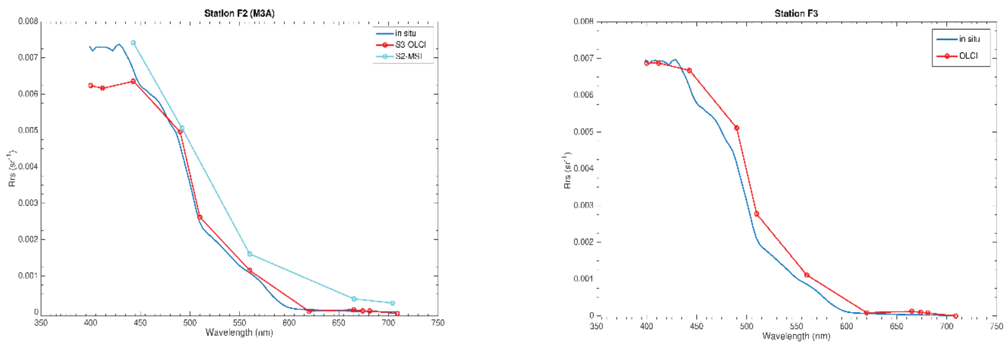

Fig. 2: Initial comparisons of R_{rs} spectra between in situ and satellite data.

As already mentioned, during the MARRE project, we also aimed to test the coherence between the in water and above water profiles. The results of an R_{rs} spectral comparison between the two systems are shown in Figure 3.

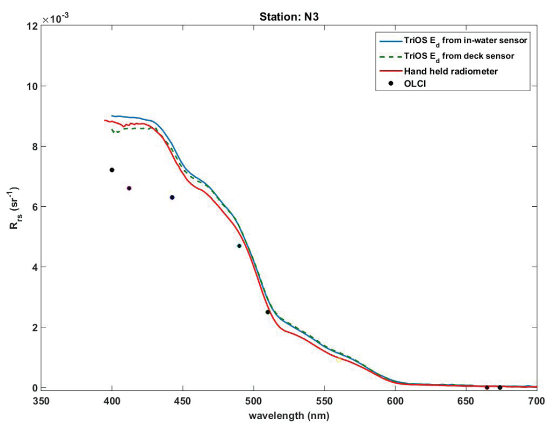

Fig. 3: Extracted R_{rs} at MARRE N3 station as measured by 3 different instrument configurations and approaches: (a) Use of in water TriOS radiance and irradiance profiles to calculate R_{rs}; b) Use of in water TriOS radiance and above water TriOS irradiance to calculate R_{rs}; c) using the handheld portable radiometer over the water.

Additionally, modelling the optical radiation transmission of oligotrophic waters can also help us better understand marine optics and thus to develop more accurate satellite retrieval algorithms. The results of the first comparison between in situ and satellite data, and the simulations based on the Hydrolight radiative transfer model for the two above - mentioned stations of the MARRE cruise are shown in Figure 3.

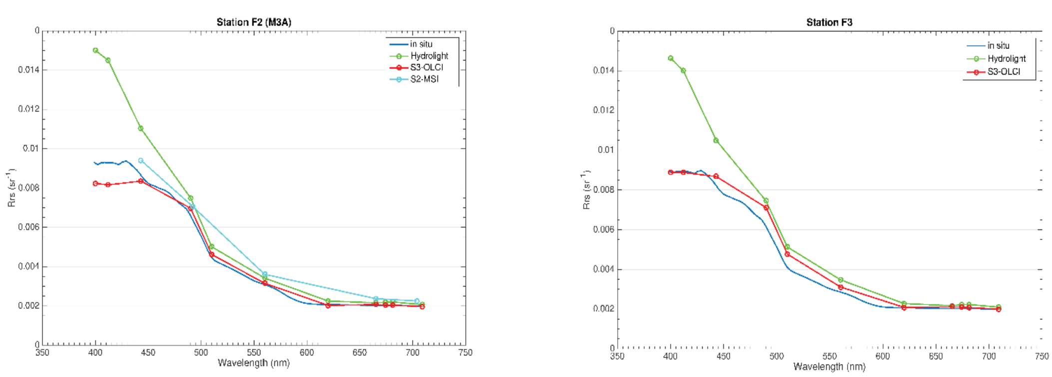

Fig. 4: Comparison of R_{rs} spectra between Hydrolight simulations and data from in situ and satellite data.

These simulated spectra show a reasonable fit for longer wavelengths (> 500nm), but much more work is needed to constrain the model for shorter wavelengths (<500nm). A further simulation error may be due to the inaccurate simulation of the atmospheric dust model dealing with Saharan dust, and this needs further matchup data to examine.

4. Discussion/Conclusion

As already indicated by previous marine optics studies in the Eastern Mediterranean region, the specific area is highly oligotrophic and also is an under-sampled region in terms of optics measurements. Thus there is a need for systematic measurements in the area.

The first attempt to calculate the apparent optical property of remote sensing reflectance in multiple ways gave encouraging results, especially at wavelengths larger than 500nm.

In addition, the observance of the fiducial reference measurement and processing protocols is estimated to help significantly for the better and fairer comparison of the results.

This study instigates the potential of using the inherent optical properties as input to the Hydrolight model towards more accurate results at lower wavelengths as well.

5. Acknowledgements

This work was supported by the "MARine monitoring system of the Hellenic Seas using REmote sensing satellite data and in situ measurements (MARRE)" project (T1EDK-02966) financed by the Operational Programme Competitiveness, Entrepreneurship and Innovation (EPAnEK, ESPA 2014–2020) of the Greek Ministry of Economy and Development, and co-funded by the EU (European Regional Development Fund) and by national funds. The authors also acknowledge ESA, EUMETSAT, and Copernicus for the provision of the satellite data.

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IMPACTS OF AQUACULTURE ON CHEMICAL COMPONENTS IN THE WATER COLUMN ON HORIZONTAL AND VERTICAL SCALES

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Abstract

Vertical variability of chemical components of the water column (including concentrations of inorganic nutrients, particulate organic phosphorus and carbon, Chl-a and phaeophytin) were investigated at three depths in 49 fish farms and 17 undisturbed locations in the Aegean and Ionian seas. Fish farm samples were collected at a distance of 50 m from cages, within Allowable Zone of Effects (AZE) limits. Ecological quality was assigned to each site according to the WFD and drivers of this assignment were investigated. The limited spatial extent of nutrient enrichment due to aquaculture seen in this study (undetectable at 50 m) is validated by previous efforts. Influence of depth in chemical parameters was reported only for fish farm stations and requires further validation.

Keywords: fish farming, nutrients, ecological quality, Mediterranean.

1. Introduction

The aquaculture industry has grown rapidly over the past decades, and will continue to do so in the years to come (Zhou, 2020). As such, there is growing scientific concern regarding the impact of aquaculture on the marine environment (Price *et al.*, 2014; Weitzman *et al.*, 2018)coastal stakeholders require tools to evaluate the risks that marine aquaculture poses and to craft science-based policies and practices which safeguard marine ecosystems. We summarized current knowledge regarding dissolved nutrient loading from marine fish farms around the world, direct impacts on water quality and secondary impacts on primary production, including formation of harmful algal blooms. We found that modern operating conditions have minimized impacts of individual fish farms on marine water quality. Effects on dissolved oxygen and turbidity are largely eliminated through better management. Nutrient enrichment of the near-field water column is not detectable beyond 100 m of a farm when formulated feeds are used, and feed waste is minimized. We highlight the role of siting fish farms in deep waters with sufficient current to disperse nutrients and prevent water quality impacts. We extensively discuss the potential for advances in integrated multi-trophic aquaculture (IMTA. In the pelagic environment, water quality is influenced by the continual release of dissolved organic matter and inorganic nutrients into the water column from aquaculture installations (Pitta *et al.*, 1998). The spatial extent of aquaculture effects on chemical and biological parameters in the water column has been addressed in multiple studies (Rosa *et al.*, 2002; Pitta *et al.*, 2005, 2006; Tsagaraki *et al.*, 2013;Jansen *et al.*, 2018;)two sub-areas were sampled: one with fish farming zones (within 2-3 nm. However, the challenging nature of discrete water column sampling has often led to discrete sampling being overlooked in favour of integrated water column sampling, at least where chemical parameters are concerned (Pitta *et al.*, 2006; Jansen *et al.*, 2016;) Italy and Greece. In this study, we aim to investigate the impact of aquaculture on chemical parameters of the water column in discrete depths. Furthermore, differences in chemical parameters among sites of varying ecological quality as defined by the European Water Framework Directive (WFD) were tested. We also compare ecological quality of aquaculture sites with the standards set by the WFD, aiming to test whether quality stays within acceptable levels. To this end, fish farms and undisturbed locations with distinct environmental properties and physicochemical characteristics were sampled during the peak production period for Greek aquaculture (June-September).

2. Materials and Methods

2.1. Study sites and sampling strategy

Seawater samples were collected from a total of 66 stations (Fig. 1), including 49 fish farms and 17 control sites in the Aegean and Ionian seas via Niskin bottle, between June and September 2020. Control sites were within the same geographic region of sampled fish farms. Sampling was carried out at three distinct depths, surface (2 m), middle and bottom, dependent on the maximum depth of the water column at each site, and at a single distance from the cages (50 m downstream of the main current) on fish farms, considered to be within the limit of the Allowable Zone of Effects (AZE). Samples were analysed for chemical parameters of the water column including the concentrations of inorganic nutrients (PO_4, NO_2, NO_3, NH_4, SiO_2), particulate organic phosphorus (POP), carbon (POC), chlorophyll-a (Chl-a) and phaeophytin.

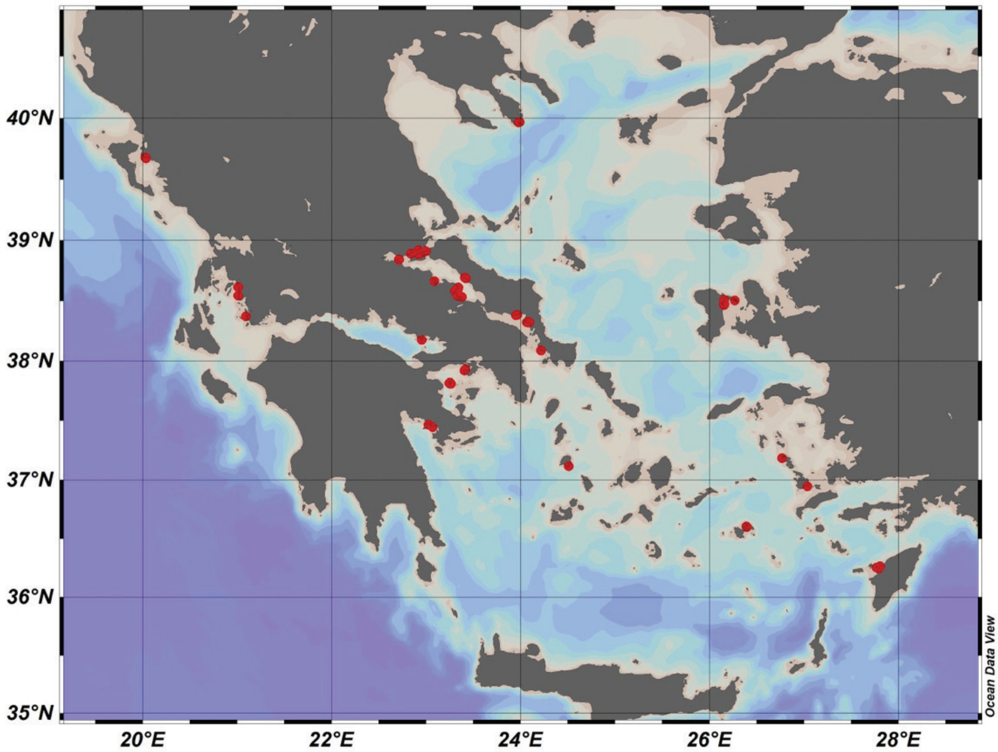

Fig. 1: Map of sampling stations in the Ionian and Aegean seas.

2.2 Laboratory analysis

In the laboratory, the concentration of phosphates, nitrates, nitrites and silicates was estimated according to Strickland & Parsons (1972). The concentration of dissolved ammonium was measured in accordance with Ivančič & Degobbis (1984). Particulate organic phosphorus (POP) and particulate organic carbon (POC) were quantified using methods by Pujopay and Raimbault (1994) and Hedges and Stern (1984) respectively. Chlorophyll-a and phaeophytin concentration were assessed through fluorometry as per Yentsch & Menzel (1963).

2.3 Assignment of ecological quality

Using a five-level scale based on the determined concentration of Chl-a (Simboura *et al.*, 2005), ecological quality was assigned to each station. The scale is adjusted for the Greek seas and ranges from High to Poor ecological quality in compliance with the Water Framework Directive (European Union, 2000) (Table 1).

Table 1. Eutrophication scale on the basis of Chl-a concentration (μg/l) ((Simboura et al., 2005).

Eutrophication scale	Chlorophyll-a (μg/l)	Ecological Quality Status
Oligotrophic	<0.1	High
Lower mesotrophic	0.1–0.4	Good
	0.4–0.6	Moderate
Higher mesotrophic	0.6–2.21	Poor
Eutrophic	>2.21	Bad

2.4. Statistical analysis

To test for the influence of depth on the concentration of the chemical and biological parameters mentioned above, firstly, all stations were considered as replicates. One-way ANOVA was used to test the effect of water depth on all examined parameters. Then, the analysis was repeated for fish farms only to check depth influence on parameters in an aquaculture setting. PERMANOVA was used to investigate the differences in all parameters based on the three sampling depths considering all stations. Furthermore, one-way ANOVA was used to check which of the tested parameters differentiate on the basis of ecological quality when this is assigned to each station with the scale given by Simboura *et al.*, (2005). Statistical analyses were run using IBM SPSS 23 and PRIMER version 6 (PRIMER-E Ltd., Plymouth Marine Laboratory, Natural Environmental Research Council, UK) with PERMANOVA+.

3. Results

3.1 Influence of depth

When all stations were considered as replicates, no significant differences between the three depths were observed among parameters. Repeating the analysis for fish farm stations only revealed significant influence of depth on chemical parameters (PERMANOVA, $p<0.05$). Specifically, bottom was the most distinct depth layer in fish farm stations (Tukey HSD, $p<0.05$). Furthermore, no significant differences in any parameter were noted for individual depths when all stations were grouped together (One-way ANOVA, $p<0.05$).

Altogether, although PERMANOVA results indicate a significant influence of depth on chemical parameters examined here, fish farm stations are indistinguishable from undisturbed locations in the three depths (surface-middle-bottom).

3.2 Influence of ecological quality

Differences were observed among stations based on ecological quality assigned using the scale of Simboura *et al.*, (2005), vastly attributable to chlorophyll a, POP, POC and NH_4 concentrations. Stations with Good ecological quality differ significantly from those with Poor with regard to PO_4, Chl-a, Phaeophytin, POC and POP concentrations (Tukey HSD, $p<0.05$). Significant differences in Chl-a, Phaeophytin and POP concentrations were observed between stations of Moderate and Poor ecological quality. The same was true between stations of Good and Moderate ecological quality (Tukey HSD, $p<0.05$).

4. Discussion/Conclusions

The release of nutrients in oligotrophic environments is promptly followed by their assimilation by the microbial food web and subsequent transfer to higher trophic levels (Pitta *et al.*, 2009). Indeed, our results indicate that aquaculture impacts with regard to nutrient enrichment of the water column are not detectable 50 m from cages, regardless of depth. Although establishing a set distance for assessing aquaculture impacts maybe questionable from an ecosystem perspective, we have demonstrated that a 50 m distance falls within an Allowable Zone of Effects (AZE) (Jerez &Karakassis, 2012; Weitzman *et al.*, 2018). According to Jerez & Karakassis (2012), an AZE in the Mediterranean is a zone within which fish farmers are allowed to exceed WFD Ecological Quality standards limits with ensuing environmental impacts.

Evidence suggests that localised nutrient enrichment from aquaculture does not exceed 100 m (Price *et al.*, 2014)coastal stakeholders require tools to evaluate the risks that marine aquaculture poses and to craft science-based policies and practices which safeguard marine ecosystems. We summarized current knowledge regarding dissolved nutrient loading from marine fish farms around the world, direct impacts on water quality and secondary impacts on primary production, including formation of harmful algal blooms. We found that modern operating conditions have minimized impacts of individual fish farms on marine water quality. Effects on dissolved oxygen and turbidity are largely eliminated through better management. Nutrient enrichment of the near-field water column is not detectable beyond 100 m of a farm when formulated feeds are used, and feed waste is minimized. We highlight the role of siting fish farms in deep waters with sufficient current to disperse nutrients and prevent water quality impacts. We extensively discuss the potential for advances in integrated multi-trophic aquaculture (IMTA or even 30 m in the case of Pitta *et al.* (2006) in the Mediterranean, confirming our findings. Nonetheless, although imperceptible, continuous release of nutrients from aquaculture has been shown to lead to structural changes in the food web (Tsagaraki *et al.*, 2013; Tsiaras *et al.*, 2022)Greece. The effect of depth on chemical parameters in fish farms seen in this study must be validated further as previous studies have suggested the influence of aquaculture in discrete depths is not consistent with regard to relevant parameters (Pitta *et al.*, 2006). If confirmed, our results could have major implications for management and monitoring aquaculture schemes.

In our study grouped stations in all depths range from High to Poor ecological quality according to the scale of Simboura *et al.*, (2005). Differences in chemical parameters driving ecological quality assignment using this scale can be explained by the sensitivity of chlorophyll-a levels to nutrient concentrations (Simboura *et al.*, 2016).

Both previous studies cited and the present one confirm the limited spatial scale of nutrient enrichment in the water column as a result of aquaculture in the Mediterranean. However, our understanding of the vertical distribution of nutrients released by aquaculture is limited. Given the ecological and management implications of this further research effort is required to validate the results of the present study and beyond.

5. Acknowledgements

The authors would like to thank fish farm staff for their assistance during sampling.

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FISH FARM CAGES ABOVE SEAGRASS MEADOWS: CAN WE REDUCE THEIR FOOTPRINT?

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Abstract

Marine aquaculture sector is rapidly growing especially in the Mediterranean Sea. As the sector develops, fish farm companies implement improvements that could reduce their ecological impacts. Here we assess the effectiveness of automatic feeders as an improvement to reduce fish farm impact on *P. oceanica* meadows. We sampled *P. oceanica* meadows close to fish farm cages where different feeding approaches are implemented (automatic vs conventional handfeeding). We then applied a reconstructive technique to assess productivity and trace element content and their trajectory of change for almost a decade. We also assessed differences between seagrass compartments. We compared our results with a pristine location. Meadows in proximity to fish farm had lower productivity than the pristine location (ANOVA, $P < 0.001$). Meadows close to fish cages in 'Improvement' had the lowest productivity (0.02 ± 0.01 g DW shoot^{-1} y^{-1}) and shoot density (307 ± 33 shoots m^{-2}), while meadows in 'non-Improvement' were in better condition with no difference in shoot density or productivity between cage and control stations. Our results assess the effectiveness of automatic feeders as a tool to reduce fish farm ecological impacts and draw attention to the necessity of more implementations, towards a more ecologically aware industry.

Keywords: lepidochronology, marine aquaculture, macrophytes, East Mediterranean.

1. Introduction

Seagrass meadows rank among the most productive ecosystems on Earth (Duarte *et al.*, 2010) providing numerous ecological services (Nordlund *et al.*, 2016). However, they are facing global decline (Waycott *et al.*, 2009) due to human-induced pressures that include habitat loss and degradation, pollution, eutrophication, introduction of alien species and climate change .

Marine aquaculture is a fast-growing sector in the Mediterranean basin, expanding approximately 5 % annually (Massa *et al.*, 2017). The development of fish farm activities in proximity to the Mediterranean coasts have both direct and indirect effects on its marine macrophytes, including its endemic seagrass *Posidonia oceanica* (L.) Delile. Seagrass decline and shoot mortality have been related to fish farm activities including organic enrichment that leads to anoxic conditions and sulfate reduction, nutrient overload that stimulates epiphytic and macroalgae overgrowth, reduction of light penetration under fish farm cages and increased herbivory (Boudouresque *et al.*, 2020).

Moving towards a more ecologically aware fish farm industry, it is essential to identify its impacts and implement appropriate ecological improvements. Here we examine the potential of automatic feeders in reducing the magnitude of fish farm impact on *P. oceanica* meadows. For this purpose, we sampled *P. oceanica* meadows in two fish farm locations (Chios and Oinouses Islands) that use different feeding approaches (automatic vs conventional handfeeding). We then applied a reconstructive technique to assess productivity, carbon, nitrogen and trace element concentrations and their trajectory of change in a decadal scale, using different *P. oceanica* compartments (leaves, rhizomes, sheaths, roots, and epiphytes). We compared our results with a pristine location, unimpacted by any human pressure.

2. Material and Methods

2.1 Sampling

During September 2021, we sampled *P. oceanic*a meadows in two sites in proximity to fish farm units. Both units are operating since 1992, with fish cages located in distance 50-100 m from the shore. The first site (hereafter called 'Improvement), located in Chios Island, operates using automatic feeders, in an effort to minimize feed waste. The second site (hereafter called 'non-Improvement') is in Oinousses Island and operates using conventional hand-feeding methods. In each site, one station was chosen in proximity (40-80 m,) to the fish cages (hereafter called 'Cage') while another station was chosen as reference in safe distance (~ 800 m) from the cages (hereafter called 'Control'). One more site was selected, located in south of Chios Island far from any anthropogenic pressures, to serve as baseline conditions for the area (hereafter called 'Pristine'). In each station, fifteen shoots were randomly collected by divers in depths 3 to 5 m. Shoot density (shoots m^{-2}) was estimated by divers in each station from five replicates of 40 x 40 cm quadrates.

2.2 Laboratory analysis

Shoots from each station were dated using the reconstructive technique of lepidochronolgy (Pergent, 1990). Starting from the rhizome apex and downwards, one lepidochronological year was identified between two consecutive minima in sheath thickness. The last two years of each shoot were excluded from the analysis, as they may have not been complete in the time of sampling (Peirano, 2002). All samples were lyophilized and separated into leaves, rhizomes, roots, sheaths, and epiphytes. Each rhizome segment was dried at 60^{o} C for 48 - 72 h to obtain dry weight and estimate rhizome productivity (g DW shoot^{-1} y^{-1}). Trace element concentrations in the different shoot compartments and epiphytes for each reconstructed year were measured using an Inductively Coupled Plasma Mass Spectometer (ICP-MS).

2.3 Statistical analysis

We compared *P. oceanica* shoot density (shoots m^{-2}) and annual productivity (g DW shoot^{-1} y^{-1}) in the 'Pristine' site with the ones in the 'Control 'sites by analysis of variance (ANOVA). Tukey post-hoc test was used to assess which sites differed. The effect of each fish farm unit ('Improvement' and 'Non-Improvement') on *P. oceanica* shoot density and annual productivity (g DW shoot^{-1} y^{-1}) was assessed by separate Student t-test's (Control vs Impacted for each unit). Prior to all analyses, data were checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). When these assumptions were not met, data were log10 or square root transformed. All analyses were performed in R v.4.0.5 (R Core Team, 2012).

3. Results

Mean shoot density did not differ between the 'Pristine' site (522 ± 78 shoots m^{-2}) and the Control sites (Improvement: 492 ± 166 shoots m^{-2}, non-Improvement: 574 ± 46 shoots m^{-2}) (ANOVA, df=2, MS=8518, F=0.72, $P > 0.05$). Mean shoot density in 'Improvement' was significantly lower in 'Cage' than 'Control' station (Cage: 307 ± 33 shoots m^{-2}, Control: 492 ± 166 shoots m^{-2}) (Student t-test, t= -2.45, df=8, $P < 0.05$), while it did not differ between stations in 'Non-Improvement' location (Cage: 690 ± 175 shoots m^{-2}, Control: 574 ± 46 shoots m^{-2}) (Student t-test, $P > 0.05$)

The lepidochronological dating resulted in the analysis of 386 rhizome segments, reconstructing the period between 2012 and 2021. The highest mean annual productivity was found in the 'Pristine' site (0.068 ± 0.05 g DW shoot^{-1} y^{-1}) significantly different than all the other stations (ANOVA, df=4, MS=0.43, F=8.01; Tukey post-hoc, $P < 0.001$). 'Improvement-Cage' (0.02 ± 0.01 g DW shoot^{-1} y^{-1}) had significantly lower mean annual productivity than 'Improvement-Control' (0.048 ± 0.019 g DW shoot^{-1} y^{-1}) (Student t-test, t=-3.98, df=26, P <0.001) while in 'non-Improvement' the two stations had no significant difference (Student t-test, t= -0.03, df=28, $P > 0.05$)(Fig. 1).

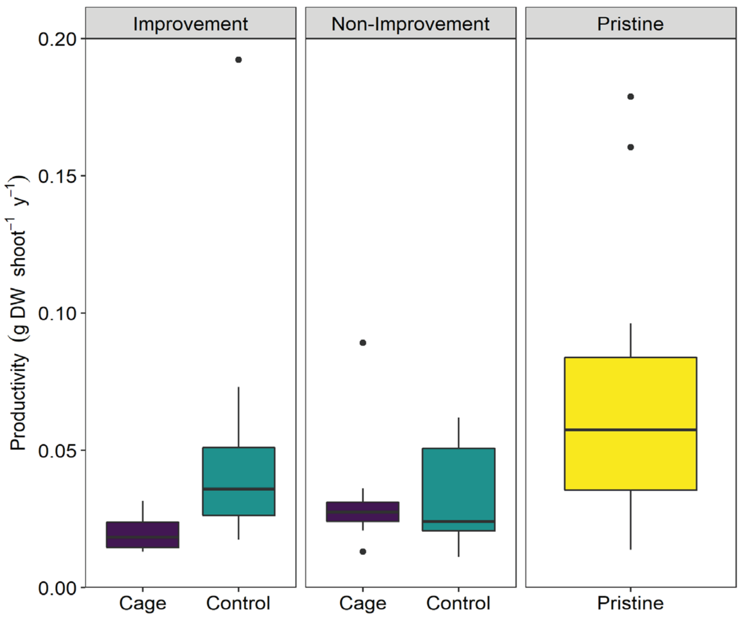

Fig. 1: Productivity (g DW shoot^{-1} y^{-1}) in 'Improvement' ('Cage' and 'Control), in 'non-Improvement' ('Cage' and 'Control') and in the 'Pristine' sites. The black lines represent the medians, the boxes encompass the 25 % and 75% quantiles, and the black circles show data points beyond the whiskers.

4. Discussion

Both 'Improvement' and 'Non-Improvement' locations had lower productivity when compared to a Pristine location, unimpacted by any human activity. Overall, meadows of 'non-Improvement' seem to be in better condition than the ones in 'Improvement', having the highest density, and similar productivity between 'Cage' and 'Control' stations. The location's nature of 'non-Improvement' site, in Oinousses Island, which is far from any other anthropogenic pressure, expect the fish farm, could explain the better condition of *P. oceanica* meadows which are not subjected to cumulative impacts. On the other hand, productivity in 'Improvement-Cage' was 2.4-fold lower than the one in 'Improvement- Control' and had shoot density similar to the ones previously reported in meadows impacted by fish farm activities (Apostolaki *et al.*, 2009; Kletou *et al.*, 2018). Our results, assess the effectiveness of automatic feeders to improve the ecology quality around fish cages by reducing the feed waste. Apart from the automatic feeders, other implementations should be considered by fish farmers and policy makers to minimize their ecological impacts.

5. Acknowledgments

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SEASONAL CHANGES OF ENERGY RESERVES AND METABOLIC PATTERNS IN *PAGRUS PAGRUS* (RED PORGY)

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Abstract

Recent studies have shown that aquaculture, a major sector of economic activity, is threatened by climate change. At the same time, the growth of global population requires the increase of food production which promotes the development of aquaculture. The present research focuses on the study of the seasonal changes on the energetic and metabolic patterns of red porgy (*Pagrus pagrus*) in order to evaluate their correlation with high seasonal temperatures. Sampling took place at a fish farm in Larymna. Heart, red muscle, white muscle, and liver all showed changes in total lipids, carbohydrates, and proteins. This research is expected to shed light on the possible relationship between seasonality and energy reserves, as well as changes in the quality of red porgy proteins, total lipids, and ultimately flesh quality, by examining how seasonal changes affect the oxidation of biological energy 'biofuels'. The latter contribute to the elucidation of the physiological mechanisms underlying farmed fish thermal tolerance.

Keywords: Proteins, Lipids, Carbohydrates, metabolism, fish.

1. Introduction

Energy possesses a key role regarding tolerance to environmental conditions, among which temperature is a main stressor, affecting physiological processes ranging from protein denaturation to cell membrane fluidity changes, that affect organ function (Hochachka & Somero, 2002). Fish growth involves the synthesis of new proteins which is dependent on the regular supply of amino acids resulting from the digestion and absorption of proteins through the diet (Counthno, 2017). Lipids can be stored as triglycerides and cholesterol in adipose tissue, muscle, and liver. Moreover, carbohydrates can be stored as glycogen in muscle and liver, while proteins cannot. Although recently, research has been carried out in this scientific field, marine ecosystems' responses to climate change are still little understood. One of the possible ways to sustain intensive Mediterranean aquaculture is to breed healthy fish with high nutritional value. The present study examines the seasonal changes in energy reserves in open field conditions of the economically important fish species *Pagrus pagrus* (red porgy).

2. Material and Methods

2.1 Tissue Sampling

The study was carried out in a fish farm located in the North Euboean Gulf in cooperation with Prometheus Ltd. After collection, individuals were immediately anesthetized and their weight (gr) and total length (cm) were recorded. Per sampling, ten (N = 10) fish were dissected, from which the white muscle, liver, heart and red muscle were removed. Immediately, tissues were stored at -80°C until further analysis.

2.2 Biochemical Analysis

Proteins were determined with the Kjeldahl method by AOAC (Association of Analytical Chemists, 1990). Total lipids were determined with the chloroform/methanol method (Bligh & Dyer, 1959) and the phenol-sulfuric acid method was used to determine total carbohydrates (AOAC,1990; Dubois,1956).

3. Results

Total lipids content in the heart showed significant increase in June, whereas total carbohydrates showed sharp increase from April to July (Fig. 1). Similarly, total proteins exhibited a significant increase from April to June. Regarding red muscle, the content of total lipids showed a decrease from December to April. On the other hand, total carbohydrates exhibited a significant increase in December and total proteins from December to April. In the white muscle, total lipids content increased from September to December, total carbohydrates and proteins from December to July. In the liver, decrease in lipid and carbohydrate content was observed intense from September to April, while total protein levels remained seasonally equal (Fig. 1).

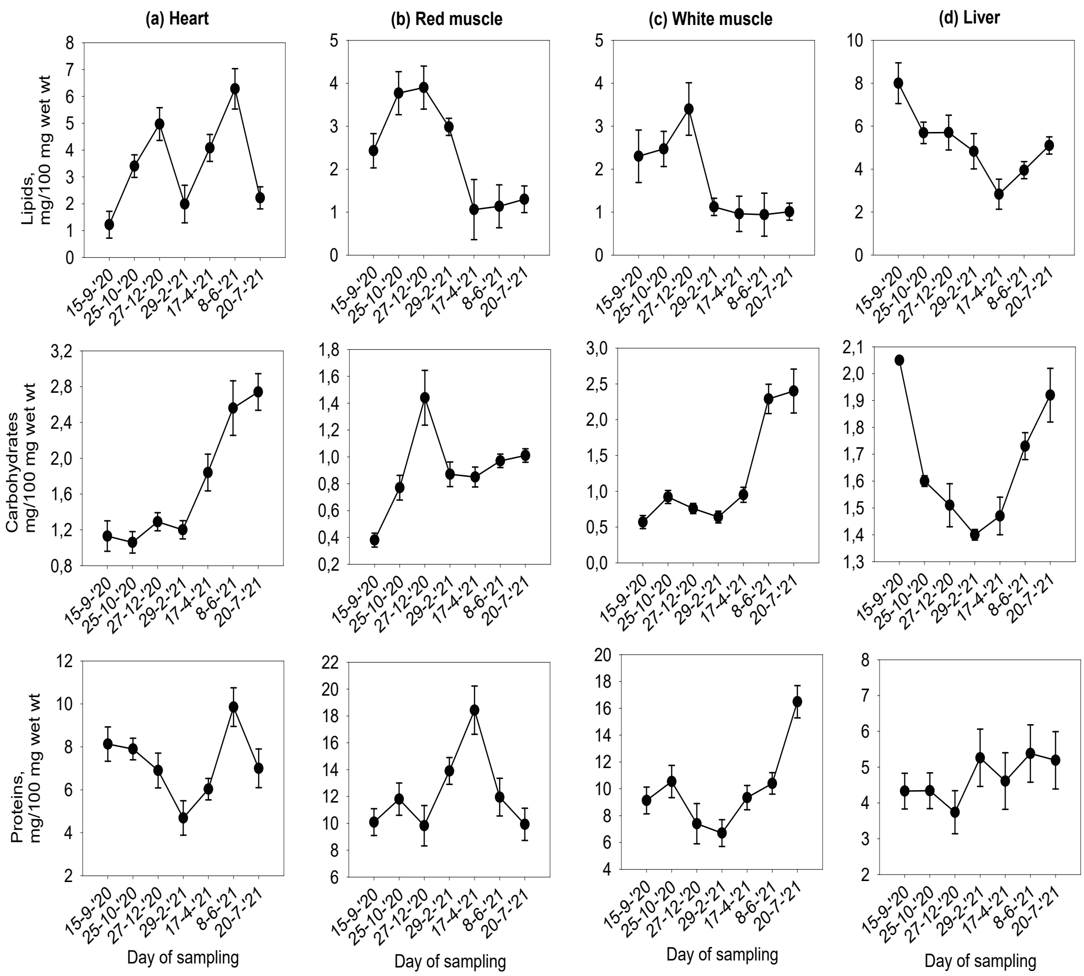

Fig. 1: Total lipids, total carbohydrates and total proteins in the heart (a), red muscle (b), white muscle (c) and liver (d) of *P. pagrus*.

4. Discussion/Conclusions

The present research focuses on the metabolic patterns in four tissues of the *P. pagrus* in order to investigate the seasonal changes of energy reserves in relation to the content of carbohydrates, lipids and proteins. The stability of protein levels during sexual maturation may be due to the continuous feeding of fish during the winter as well as to the lack of complete maturity (Grigorakis *et al.*, 2002). Also, it has been found that sexual maturation reduces the lipid reserves in salmon because this species ceases to feed during maturation and the lipid reserves are directed to the gonadal development or used for energy reserves (Bell *et al.*, 1998). Reduced feeding activity and maturation will help reduce lipid levels during the winter and a low rate of flesh lipid content from January to May after gonadal growth is ceased (Grigorakis *et al.*, 2002). At low temperatures, lipid absorption in the liver is relatively fast, reducing input activities (Ibarz et al., 2007). In physiological states of demanding energy supply, such as fasting or stress, stored triacylglycerols in perivascular fat, liver, and muscle are catabolized to glycerol and fatty acids (Sheridan, 1988).

In summary, in winter, fish reduce food intake due to low water temperatures and begin mobilizing fat deposits. On the other hand, in the summer, a stimulating eating behavior with high catabolic and active enzymatic properties is observed. The results of the present study demonstrate seasonal changes in the physiological and metabolic parameters of red porgy, which can be attributed to various environmental factors, the temperature of which appears to be the major factor.

5. Acknowledgements

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DIEL OSCILLATIONS IN MICROBIAL PLANKTON COMMUNITIES IN AQUACULTURE ENVIRONMENT

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Abstract

The diel variability in prokaryotic and eukaryotic community composition was studied under the influence of aquaculture activity using DNA metabarcoding analysis. Samples were collected at 3 time points during the day (morning, noon, evening), from a non-impacted site, a site with one fish cage and a site with many fish cages. Increased aquaculture activity led to changes in free and particle-attached prokaryotic community composition, with opportunistic strains “replacing” strains that are not adapted to high concentrations of nutrients near cages. Diurnal variability was found in particle-attached prokaryotes, possibly related to their direct dependence on particulate organic matter produced and released in different diels rhythms by phytoplankton and farming activity. Meso-zooplankton and pico- and nano-autotrophic and heterotrophic eukaryotes and fungi were affected by aquaculture activity. Several zooplankton phyla that perform diel vertical migration showed maximum contribution at surface waters at night. Small diatoms contributed less at aquaculture sites, while dinoflagellates contributed less at all stations at night. Further exploration of the observed spatiotemporal patterns will advance our understanding of nutrient cycling and aquaculture-environment function.

Keywords: fish farm, bacteria, eukaryotes, fungi, metabarcoding.

1. Introduction

The contribution of microbial plankton in oceanic function has been long recognized: it drives biogeochemical cycling, fuels higher trophic levels with energy, and may also modulate climate (Azam and Malfatti, 2007). Microbial communities are highly dynamic and change at various spatiotemporal scales. Indeed, several biological features oscillate over diurnal, monthly and annual cycles. Diel oscillations are mainly driven by changes in light intensity but also by changes in nutrient and chlorophyll concentration, temperature, day length and the presence of other organisms. Diel oscillations have been studied for phytoplankton and heterotrophs, including the well-studied diel vertical migration of mesozooplankton, a pattern of downwards movement with daylight and upwards movement with darkness (Davis *et al.,* 2019). Prokaryotic community diversity, metabolism and the quantity of cell-division transcripts were higher during the night (Vislova *et al.,* 2019)with diversity negatively correlated with day-length. Here we determine whether metagenomes and metatranscriptomes follow similar patterns. We generated 16S rRNA datasets, metagenomes (1.2 GB and similarly, particle-attached bacteria were found to be more active and abundant during the night in the Mediterranean (Ghiglione *et al.,* 2007; Ruiz-González *et al.,* 2012).. Various patterns have been observed for ciliates in the Mediterranean (Pérez et al., 2000), while a consistent peak of heterotrophic protist abundance was detected during the night and bacterial abundance during the second half of the day (Mary *et al.,* 2008; Tsai *et al.,* 2005)Synechococcus spp., and nanoflagellates at a coastal station at the southern edge of the East China Sea. Synechococcus spp. and nanoflagellates exhibited diel fluctuation at water temperatures above 25°C. Cell concentrations of Synechococcus spp. were significantly higher during the evening, whereas those of nanoflagellates were higher during the day. The day and night amounts of heterotrophic bacteria did not differ significantly, and we did not observe diel rhythms in these organisms below 25°C. The fractionation experiments we performed between August and October showed that growth rates of bacteria were high (0.73-1.00 μg C L-1 h -1.

The aim of this work is to describe diel oscillations in all microbial groups for the first time in aquaculture environment with metabarcoding analysis. We studied prokaryotic and eukaryotic diversity at three time points within the day, and tried to correlate diversity shifts with dissolved and particulate nutrient pulses from the fish farm.

2. Material and Methods

2.1 Sampling

First sampling took place in Argolikos gulf in June-July 2017 on board the R/V *Philia* in the framework of the TAPAS project (http://tapas-h2020.eu/) and second sampling in Chios island in September 2021 within the LIFE+ AquaPEF project (https://lifeaquapef.eu/). Intensive aquaculture production of European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) is occurring in both areas. Samples were collected from 2 sites within the aquaculture area: from one site next to one fish cage (One Farm: OF) and one site next to multiple fish cages (Multiple Farms: MF) and from a non-impacted site (Control: C). At each site, samples were collected at 3 time points within the day, at 06:00 am (morning), 14:00 pm (noon) and 22:00 pm (evening). Sampling was repeated at 3 consecutive days for validity reasons. Surface seawater was collected with Niskin deployment for the study of pico-, nano- and micro-plankton communities. Following collection, water was filtered (10-20 liters) sequentially through membranes of 20 (micro-plankton), 2 (nano-plankton), and 0.2 μm (pico-plankton) pore sizes. Filtration was done using a peristaltic pump. Meso-plankton was collected via a vertical haul (Mean ± SD: Summer=4.8 ±0.3 m^3, Winter=6.3 ± 1.1 m^3) and the collected material of the net was further filtered. All membranes were stored at -20°C until return to the laboratory. In total, there were 27 samples for each size class.

2.2 DNA extraction, PCR amplification, library preparation and sequencing

DNA extraction from the membranes was done following a modified CTAB protocol and PCR amplifications following a two-step protocol, as described elsewhere (Tsiola et al., 2020). PCR amplification of the 16S rRNA gene was done in the 0.2-2 μm fraction (free prokaryotes) and >2 μm fraction (particle-attached prokaryotes). The 18S rRNA gene was amplified in all fractions: 0.2-20 μm (pico- and nano-eukaryotes), 20-200 μm (micro-eukaryotes) and >200 μm (meso-zooplankton). The region ITS1-ITS2 was amplified to affiliated fungi many ecological aspects of their counterparts in coastal ecosystems remain largely elusive. Using high-throughput sequencing, quantitative PCR, and environmental data analyses, we studied the spatiotemporal changes in the abundance and diversity of planktonic fungi and their abiotic and biotic interactions in the coastal waters of three transects along the Bohai Sea. A total of 4362 ITS OTUs were identified and more than 60% of which were unclassified Fungi. Of the classified OTUs three major fungal phyla, Ascomycota, Basidiomycota, and Chytridiomycota were predominant with episodic low dominance phyla Cryptomycota and Mucoromycota (Mortierellales. Library preparation was done using Illumina indices and sequencing was accomplished in the HCMR Illumina MiSeq platform.

2.3 Sequence processing and Statistics

Sequence processing was conducted via PEMA (Zafeiropoulos et al., 2020)providing support to an increasing number and diversity of users. Pairing with the impetus offered by high-throughput methods to key areas such as non-model organism studies, their operation continuously evolves to meet the corresponding computational challenges. Here, we present a Tier 2 (regional as offered by the IMBBC HCMR high-performance computing system (Zafeiropoulos *et al.*, 2020). Raw sequences were processed with trimmomatic (v.0.38) and the paired-end sequences were constructed with the algorithm PANDAseq (v.2.11) with minimum overlap of 5, 20 and 10 nucleotides for 16S, 18S and ITS1-ITS2, respectively. Only reads of >150 nucleotides were analyzed and organized into Operational Taxonomic Units (OTUs) with 98% similarity, using the VSEARCH algorithm for 16S and 18S rRNA and SWARM v2 algorithm for ITS region. Silva_132 database was used for 16S, PRS2 for 18S and PRS2 and MIDORI v2 for ITS region.

Principal coordinates analysis was applied to coordinate the data. Multivariate analysis of variance using the factors "aquaculture" (Farm, Control), "type of aquaculture" (C, OF, MF) and "time of the day" (morning, noon, evening) was applied to compare communities composition. Our null hypothesis was there are no differences. Bray–Curtis dissimilarity matrices on square-root transformed biological data were constructed and pairwise tests were done with significance level of 0.05 (PRIMER v6, PRIMER-E Ltd., Plymouth Marine Laboratory, NERC, UK). Distance-based redundancy analysis (dbRDA) was used to summarize the variation in the biological data that was explained by the abiotic data.

3. Results

The final OTU table for free prokaryotes consisted of 815.242 sequences, corresponding to 4.101 OTUs. Free prokaryotic community (species level) was significantly different between Farm and C (PERMANOVA, $p<0.05$, Fig. 1a) but not between time points. The dominant phyla were Proteobacteria, Bacteroidetes, Cyanobacteria and Verrucomicrobia, with Cyanobacteria contributing more in C than Farm and Verrucomicrobia contributing less in C than Farm sites. Flavobacteriales ranged between 50 and 80%, while Chitinophagales and Cytophagales contributed less but showed an interesting pattern: their contribution increased from C to OF and further to MF. *Rubritaleaceae* family within Verrucomicrobia also showed the same pattern. Cyanobacterial diversity was higher in Farm sites with members of *Synechococcales*, *Nostocales* and *Phormidesmiales* being present. The final OTU table for particle-attached prokaryotes consisted of 2.017.116 sequences. Prokaryotic community attached to particles (species level) was significantly different between Farm and C and between morning, noon and evening (PERMANOVA, $p<0.05$, Fig. 1b). The contribution of free SAR11 clade ranged between 19-41% in C, 5-19% in OF and 5-6% in MF, while the opposite was noticed for the *Rhodobacteraceae* family (21-48% in C, 61-82 in OF and 78-81% in MF). Approx. 24% of free prokaryote variability was explained by the concentrations of PON, NO_3, NO_2 and NH_4, while approx. 27% of particle-attached prokaryote variability by POC, PON and NH_4 (dbRDA, $p<0.05$, Fig. 1c).

The final OTU table for meso-zooplankton consisted of 351.211 sequences, corresponding to 1.177 OTUs. Meso-zooplankton community (species level) was significantly different between C, OF and MF stations (PERMANOVA, $p<0.05$)but not between time points . The dominant phylum was Arthropoda, mainly consisted of Crustaceae that contributed more in Farm sites than C, similarly to Mollusca and Brachiopoda. On the opposite, Cnidaria contributed more in C. Some zooplankton phyla showed diurnal pattern, namely Arthropoda were higher towards the evening in MF. The final OTU table for pico- and nano- eukaryotes consisted of 368.188 sequences, corresponding to 3.568 OTUs. Pico- and nano- eukaryotic community (species level) was significantly different between C, OF and MF stations (PERMANOVA, $p<0.05$), although the contribution of the dominant Dinoflagellata phyla decreased from morning to noon and further to evening. Chlorophyta were also abundant (5% in C noon up to 35% in Farm noon). Bacillariophyta and Radiolaria showed maximum contribution in C noon and evening. The final OTU table for fungi consisted of 559.802 sequences, corresponding to 5.285 OTUs. Fungal community (species level) was significantly different between C and Farm stations and between C, OF and MF (PERMANOVA, $p<0.05$, Fig. 1d). A large fraction (65-85%) of reads could not be assigned. The dominant phyla were Ascomycota and Basidiomycota. Approx. 14% of fungal community variation was explained by the combination of POC, PON, NH_4, NO_2 and PO_4 (dbRDA, $p<0.05$). The microplankton community is not yet presented since 18S rRNA gene amplification was not successful in the collected membranes.

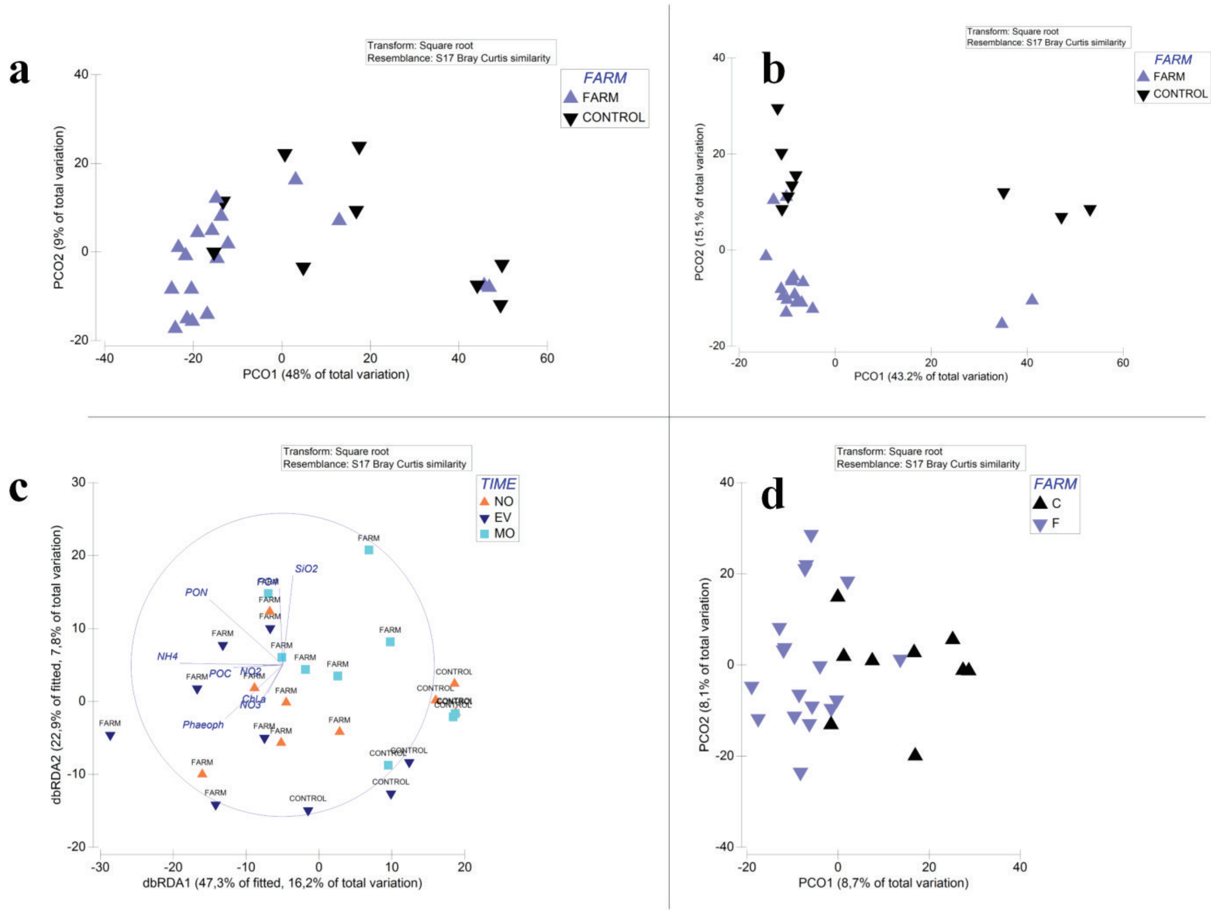

Fig. 1: Principal CoOrdinates plot of OTU composition of the free (a) and particle-attached (b) prokaryotic and fungal community (d). Colors represent the type of stations. Distance-based redundancy analysis plot of the linear model of the abiotic parameters fitted to the particle-attached prokaryotic community composition (c). Length and direction of the vectors indicate relative strength and direction of relationship in the ordination plot, respectively.

4. Conclusions

Knowing the synthesis of a microbial plankton community may help us understand the function and performance of a given environment. This is particularly important in aquaculture areas that have major sustainability issues nowadays due to the substantial amounts of nutrients that are released on a daily basis. Our study showed that prokaryotic community (in both free and particle-attached form) was significantly different between control and fish-farming sites. Taxa that are known for their opportunistic behavior (i.e., *Rhodobacteraceae*) prevailed near fish cages, while oligotrophic taxa (i.e., *Pelagibacter*) increased in contribution away from cages. Increased overall prokaryotic diversity was noticed near fish cages, with pronounced levels within Cyanobacteria phylum. The eukaryotic community also differed with respect to fish farming, with small diatom species contributing less near fish cages. The time of the day seemed to affect several zooplankton phyla (but not the whole community), as well as particle-attached prokaryotes that are frequently affected by organic matter produced by phytoplankton, and small dinoflagellate species that were less abundant in the evening. Different zooplankton community was found at the third sampling day, possibly related to environmental parameters that changed (e.g., water circulation and temperature). A large percentage of uncharacterized taxa were found within the fungal community. The primers used to amplify the viral genes may not have been suitable for the studied environment. An association with inorganic and organic forms of nitrogen prevailed in all datasets. Still, biological interactions between dominant taxa should be explored with statistical and bioinformatics approaches in order to picture ecosystem status and dynamics.

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PRELIMINARY RESULTS ON THE EFFECT ON GROWTH PERFORMANCE OF ZEBRA-FISH *DANIO RERIO* WHEN FED ON DIETS CONTAINING DIFFERENT BIOACTIVE COMPOUNDS

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Abstract

The use of plant derived compounds known to have various biological activities and potential health benefits, has an increase interest over the last decade in the aquaculture as functional feed additives. This study aimed to evaluate the effects of bioactive compounds, derived from Spirulina (*Arthrospira platensis*) and olive by-products on the growth parameters of adult zebrafish. In this study fish were fed with 3 different olive by-products and 1 olive oil, 1 Spirulina hydrolysate fraction (>30kDa), resulting in 6 different diets, plus a commercial diet as a control. Diets were produced at the installations of HCMR. Zebrafish 50 days -old were randomly divided into 7 groups, each group had three replicates. After 12 weeks of feeding trial, growth performance of the fish was assessed. Growth performance was improved compared to the commercial diet. In all diets where the bioactive compounds were added.

Keywords: Danio rerio, nutrition, growth performance, zebrafish, Spirulina hydrolysate, olive by-products.

1. Introduction

Bioactive compounds derived from plants contain a large number of phytochemicals. Many of their beneficial effects are connected to their antioxidant properties and their immunostimulatory functions. They are able to affect positively fish performance and resistance to diseases (Ahmadifar *et al.*, 2020). Spirulina is a small, blue-green algae with long history as a safe, non-toxic nutritional supplement rich in proteins, carbohydrates, lipids, carotenoids, vitamins and trace elements (Jung *et al.*, 2019). It holds great promise as a cheaper nutritional ingredient compared to those of animal origin for complete or partial replacement of protein in aquaculture (Taelman *et al.*, 2013). These ingredients, added as fish food additives, are expected to enhance the survival, and improve additional biotic indices (condition factor, growth rate, etc.) and biochemical/hematological parameters of the cultured fish. The current study presents preliminary results on the effects of bioactive compounds such as in zebrafish growth performance.

2. Materials and Methods

2.1. Preparation of olive by-products

The used olive-pomace oil -was enzymatically enriched with hydroxytyrosol through transesterification reaction catalyzed by immobilized lipase from *Thermomyces lanuginosus* in rotating bed-reactor at 40°C. All reaction aliquots were analyzed and quantified by high performance liquid chromatography

(HPLC), equipped with a μBondapack C18 reversed-phase column (particle size 10 μm, length 300 mm, diameter 3.9 mm) and a diode array UV detector. The column temperature was set at 35°C and the flow rate and injection volume was 1 mL min-1 and 20 μL, respectively. The mobile phase consisted of methanol (A) and water (B, with 0.1% acetic acid) with a gradient elution of 40-60% A at 0-4 min, 20-80% A at 4-8 min, 0-100% A at 8-25 min and reset at initial conditions. Hydroxytyrosol and hydroxytyrosyl-fatty acids esters were detected at 280 nm using a PDA detector. ^{1}H-^{13}C HSQC-HMBC NMR experiments were employed for the characterization of the hydroxytyrosol esters. The olive byproducts supplements Stymon feed 10+ and Stymon oliva with different phenolic content were provided by STYMON Natural Products IKE The Stymon feed 10+ treated was prepared through the enzymatic treatment of phenolic content for 24 h at 30°C by laccase from *Trametes vesrsicolor.*

2.2. Preparation of Spirulina hydrolysate fraction

Spirulina hydrolysate fraction (>30kDa) was prepared as follows. Spirulina powder was defatted overnight with hexane (ratio 1:20 w/v) under magnetic stirring. After drying, the defatted powder was subjected to protein extraction by suspension of dry mass in NH_4Cl (0,01 M, pH 4,39). Cell disruption was achieved by 3 sequential rounds of freeze-thaw and the lysed cells were cleared via centrifugation at 7200g at 4 °C for 30 min. The supernatant containing the total protein content of spirulina was collected, the pH was adjusted to pH 8 to accommodate trypsin digestion and trypsin was added at a 1:50 (w/w) E/S ratio. Enzymatic hydrolysis was performed at 37 °C for 3h under stirring. The digested material was subjected to filtration using a 30kDa cutoff filter and the recovered peptide fraction containing protein fragment >30kDa was collected and lyophilized (Aiello *et al.*, 2019).

2.3. Experimental diets

All diets were isonitrogenous and isoenergetic (53/15*)* containing 35% FM and other plant raw materials like soya protein concentrate, corn and wheat gluten, fish oil, mineral, and vitamin premix. The raw materials were fine grinded and mixed, while supplements were added as follow: Stymon feed 10+ at 0.05 and 0.2% (Diet 1 & 2), Stymon oliva at 0.2% (Diet 5), Stymon feed 10+ treated 0.05% (Diet 7), olive oil at 0.5% (Diet 10) and Spirulina hydrolysate fraction (>30kDa) at 0.1% (Diet 14). All supplements were added before cold pelleting. Pellets of 0.5mm were left to dry and conserved at 4***°C***. Before use in growth trial experiments, pellets were grinded until powdered for the better intake from zebrafish juveniles.

2.4. Zebrafish maintenance

AB wild type zebrafish were bred and maintained at the Laboratory of Zoology, University of Ioannina, Greece. Water supply to all tanks was continuous, while water temperature was kept constant at 28***°C***, pH at 7-8, and water conductivity at 500 ±50 μS. The photoperiod was set to 14:10 hours light: dark respectively. Fish were maintained at 3,5L tanks in groups of 27 individuals in total.

2.5. Growth trial

Wild-type zebrafish were fed with commercial feed for 50 approx. days post fertilization. After this period, fish with an average initial weight of 0.100 g each, were randomly distributed in 3.5 lt tanks. Individuals of all different diets were left to acclimatize themselves in the new tanks for one week and fed with commercial food during this time. After the acclimatization period, fish were fed for 12 weeks with the experimental diets. Food was provided twice a day and was estimated at 3% of the total body weight of the individuals in each tank. A group of the same number of fish, continued to be fed commercial food and this was used for comparisons. Fish were weighed at the end of every week in groups of nine in a petri dish and returned to the tanks they came from. Weight gain was calculated as the final fish weight (g) – the initial fish weight, while SGR was calculated as the weight fish increase percentage per day [SGR= (Ln (Wt)-Ln(W0) *100/t(d)].

3. Results

Regarding the growth parameters shown in Table 1, experimental diets expressed similar or higher growth performance compared to the commercial feed. Specifically, this was significantly highest in diet 2 (0.2% Stymon feed 10+) and 10 (0.5% Olive oil) ($P < 0.05$), while no statistical differences were verified between commercial, 5, 7 experimental diets as well as between 2, 10, 14 experimental diets.

Table 1. Growth performance parameters fed with different experimental diets.

	Diet Group						
	Commercial	**1**	**5**	**2**	**7**	**10**	**14**
IW (g)	0,11±0,02	0,10±0,02	0,10±0,02	0,12±0,02	0,11±0,02	0,11±0,02	0,11±0,02
FW (g)	0,49±0,02[a]	0,48±0,01[a]	0,48±0,04[a]	0,69±0,04[b]	0,47±0,02[a]	0,72±0,08[b]	0,65±0,14[ab]
WG (g)	0,38±0,01[ab]	0,38±0,01[ab]	0,37±0,04[ab]	0,57±0,06[c]	0,36±0,01[a]	0,61±0,07[c]	0,54±0,13[bc]
SGR (%)	1,83±0,16[ab]	1,84±0,03[ab]	1,83±0,09[ab]	2,07±0,27[ab]	1,77±0,06[a]	2,23±0,09[b]	2,12±0,20[ab]

Effects of different types of FM replacements on growth performance of zebrafish. Parameters assessed were initial individual weight (Wi, g); final individual weight (Wf, g); weight gain (WG, g), and specific growth rate (SGR; %) after 12 weeks of feeding trial. Values are expressed as mean ± SD of three replicates.

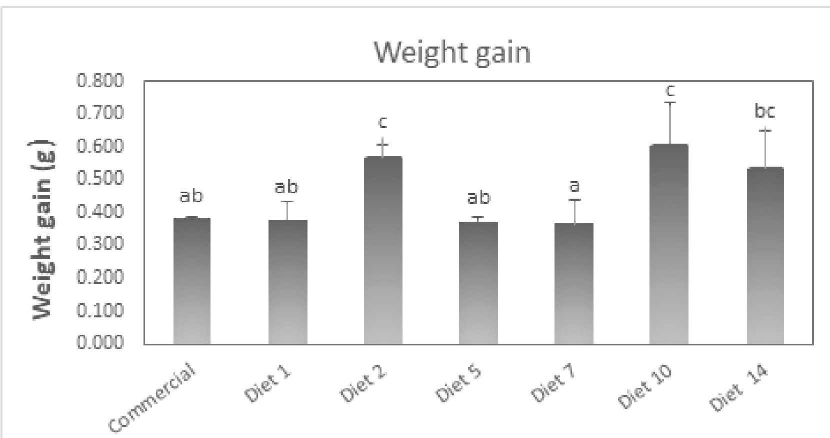

Fig. 1: Weight gain rate (%) at the end of the feeding trial. Values are expressed as means ± standard deviation.

4. Discussion

In the present study significant differences were revealed between growth performance of *Danio rerio* when fed with olive byproducts supplement, against a commercial type of diet. Diets 2, 10 and 14 showed the highest WGR and SGR showing that this by-product rich in bioactive compounds like polyphenols, Phycocynines, are very effective in ameliorate fish performance as in other species (Ahmadifar *et al.*, 2020; Mahmoud et al 2018). As for the lowest weight gain performances, they were recorded by fish fed with 5, 7 experimental feeds as well as the commercial diet. Experimental diets 2 and 10 showed significant differences between commercial, 1, 5 & 7 experimental diets. Overall, diet 10 showed the highest WGR and SGR in contrast to all remaining diets.

5. Acknowledgements

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CONSUMER ACCEPTANCE OF FRESH FISH PACKAGED WITH CO_2-EMITTING PADS

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Abstract

Aim: Active packaging with CO_2-emitters (pads) has been recently used for shelf-life extension of fresh fish. The aim of this study was to identify consumer attitudes towards fresh fish packaging, to examine whether Greek consumers prefer active packaging with pad over active packaging without pad, and to investigate any perceived differences in the sensory quality of the fish. Methods: 274 consumers participated in the study which included freshness sensory evaluation of gilthead sea bream and seabass, whole fish and fillets, at high quality and at the end of high-quality shelf-life. Samples were packaged under modified atmosphere either with a pad or without. Questions about frequency of fish consumption and attitudes towards fresh fish packaging were also included. Results: Preference for packages with pads, especially for the fillets at the end of high quality, was confirmed. Samples packed with a pad were generally perceived as fresher and closer to the ideal product, while more differences were perceived due to the storage time of the samples. Most consumers are positive towards fresh fish packaging although they usually buy unpacked fresh fish. Conclusion: Our results imply that active packaging with pads has a positive potential in the Greek market.

Keywords: consumer acceptance, pad, freshness, active packaging, sensory evaluation.

1. Introduction

Active packaging is widely used for fresh fish storage as a means to enhance shelf-life (Ashie *et al*., 1996). CO_2-emitters, i.e., pads, have been used in the recent years to maintain the CO_2 concentration in the packaging and to prevent spoilage in addition to the commonly used modified atmosphere (Hansen *et al*., 2007; Hansen *et al*., 2016; Tsironi *et al*., 2019). However, there is no research on the use of active packaging with CO_2-emitters in the acceptance of fish products by the consumers. Nevertheless, examining the attitudes of the consumers towards active packaging with pads would give an important insight to the utility and the potential of this method as a means to limit food waste by extending the shelf-life of the product (Tsironi *et al*., 2019). The aim of this research was to identify consumer attitudes towards fresh fish packaging and to examine whether Greek consumers prefer active packaging with pad over active packaging without pad. Furthermore, we aimed to investigate any perceived differences in the sensory quality of the fish, both whole and filleted, in different packaging (active packaging with and without pad) and for the same storage period.

2. Material and Methods

2.1 Samples

Gilthead sea bream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*) were provided by Avramar S.A. Both the gutted fish and their fillets, packed under modified atmosphere (40% CO_2, 40% N_2, 20% O_2), were tested in this study. Half of the packages additionally contained a CO_2-emitting pad (90 × 255 mm, 300 mL eluted CO_2) kindly offered by McAirlaid’s (Berlingerode, Germany). Samples were packed as soon as possible after harvesting and were immediately transported to HCMR under cold chain. Samples were kept in refrigeration at 2,5°C until used in the consumer test. Gas concentration (%CO_2, %O_2) in the packaging headspace and microbial growth i.e., total viable count (TVC), *Pseudomonas* spp., *Enterobacteriaceae* spp., and H_2S-producing bacteria, mainly *Shewanella putrefaciens*, were determined during storage.

2.2 Participants

In total 274 consumers took part in the study (44% male; 56% female). Participants were staff of the HCMR and were recruited on the basis of their interest and availability to participate in the study, through invitation e-mails sent to all personnel. The only prerequisite for participation was not to detest fish. Details on the distribution of the consumers over the test sessions and their age can be seen in Figure 1.

2.3 Questionnaire

The questionnaire consisted of questions regarding the freshness of the samples and consumers' preference. Odor, appearance and texture attributes were evaluated, as well as overall freshness perception, purchase intention and proximity to the ideal product. Participants were asked to respond on category scales or to select the appropriate answers for them from multiple choices. Slightly different attributes were used in the questionnaire for the whole fish and in that of the fish fillets reflecting the different nature of the samples. The last part of the questionnaire consisted of demographic questions and questions regarding attitudes towards fish consumption and packaging.

2.4 Consumer test

The test took place in the Sensory Lab of the Institute of Marine Biology, Biotechnology and Aquaculture at HCMR in Anavyssos. Four sessions took place between October and November 2021. Sessions were run from 10.30 am to 16.30 pm. Two freshness time points were chosen to test the samples; one at high quality shelf-life and one at the end of high-quality shelf-life. Four samples were evaluated in each session by each consumer; two sea bream packages, one with a pad and one without a pad, and two respective seabass packages. Participants were asked to first open the package and then start evaluating successively the odor, appearance and texture of the sample. A schematic overview of the test organization can be seen in Figure 1.

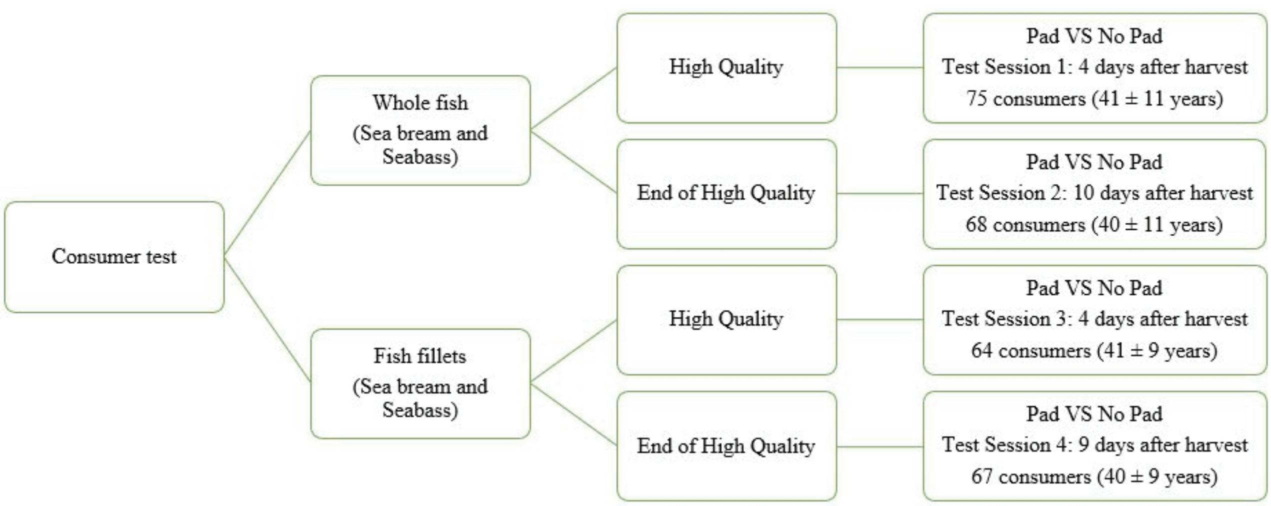

Fig. 1: Schematic overview of the consumer test organization.

2.5 Statistical analysis

Preference data were analyzed as frequencies and then were cross-checked with the corresponding statistical tables (Lawless & Heymann, 2010). Sensory freshness data were checked for normality and were found not to be normal (Shapiro-Wilk test, histograms and P-Plots). Thus, Mann-Whitney U test was run to test differences in the scores of the attributes between samples with and without a pad, at high quality and at the end of high-quality time points. Attributes were treated as dependent variables, while presence or absence of a pad was treated as an independent variable, and data were analyzed at high quality (H.Q.) and end of high quality (End) time points, separately. Chi-square test was used for categorical data, such as attitudes and frequency of consumption. All analyses were based on the 5 % significance level ($\alpha = 0,05$). IBM SPSS Statistics Version 28 (IBM Corporation, Armonk, New York, USA) was used for the analyses.

3. Results

3.1 Whole fish

54 out of 66 consumers preferred sea bream with a pad over packaging without pad at the end of high quality ($p < 0,05$). Differences in sensory attributes, purchase intention and proximity to the ideal product of whole fish appear in Table 1. CO_2 concentration in the packaging of gutted seabass and sea bream with a pad, at the end of high quality, was on average 24,4% and 30,4%, respectively, while the respective values for packaging without a pad were 21,9% and 19,2%. The initial TVC for gutted seabass and sea bream was 4,2 ± 0,2 log CFU g^{-1} and 5,0 ± 0,2 log CFU g^{-1}, respectively. Furthermore, for all samples at high quality, *Pseudomonas* spp., *Enterobacteriaceae* spp. and *Shewanella* counts were low (3,0 – 4,0 log CFU g^{-1}), reaching the value of 5,0 log CFU g^{-1} for *Pseudomonas* spp., and 6,0 log CFU $g^{-1}$for *Shewanella* at the end of high quality. *Enterobacteriaceae* spp.

Counts remained low i.e., 4,7 and 4,9 log CFU g^{-1} on average for seabass with and without a pad, respectively. The respective values for sea bream with and without a pad were 3,4 and 4,0 log CFU g^{-1}.

Table 1. Evaluation of attributes from consumer test, presented as means ± standard deviation, for whole fish samples of sea bream and seabass at high quality and end of high-quality shelf-life. Type of category scale for each attribute used in the questionnaire is also presented. Significant differences at a=0,05 are depicted with (*).

	Sea bream (whole fish)						Seabass (whole fish)						
	High Quality			End of High Quality			High Quality			End of High Quality			
	Pad	No Pad	*p-value*	Pad	No Pad	*p-value*	Pad	No Pad	*p-value*	Pad	No Pad	*p-value*	Type of Scale
Fresh Odor	3,57 ± 0,95	3,35 ± 1,12	0,219	3,35 ± 0,94	2,84 ± 1,14	0,004*	3,85 ± 0,87	3,71 ± 0,85	0,385	3,45 ± 0,88	3,26 ± 1,10	0,479	5-point
Bright Skin	2,08 ± 0,61	2,04 ± 0,69	0,731	2,03 ± 0,62	1,94 ± 0,64	0,414	2,15 ± 0,63	2,20 ± 0,55	0,632	2,07 ± 0,58	2,10 ± 0,58	0,757	3-point
Convex-Shiny Eyes	2,14 ± 0,64	2,21 ± 0,66	0,459	2,31 ± 0,61	2,16 ± 0,59	0,143	2,31 ± 0,64	2,21 ± 0,66	0,373	2,25 ± 0,61	2,27 ± 0,64	0,812	3-point
Coherent Flesh	1,96 ± 0,71	1,86 ± 0,70	0,411	2,04 ± 0,68	1,85 ± 0,74	0,112	2,19 ± 0,71	2,04 ± 0,65	0,166	2,21 ± 0,62	2,18 ± 0,58	0,730	3-point
Overall Freshness	3,25 ± 1,02	3,09 ± 1,14	0,369	3,28 ± 0,90	2,79 ± 1,15	0,011*	3,56 ± 0,96	3,51 ± 0,97	0,702	3,25 ± 0,87	3,18 ± 1,02	0,996	5-point
Purchase Intention	3,53 ± 1,25	3,32 ± 1,29	0,343	3,68 ± 1,04	3,18 ± 1,26	0,018*	3,73 ± 1,19	3,90 ± 1,10	0,396	3,68 ± 1,11	3,56 ± 1,32	0,753	5-point
Close to Ideal	3,22 ± 1,04	3,11 ± 1,20	0,614	3,28 ± 0,93	2,78 ± 1,13	0,009*	3,55 ± 1,18	3,60 ± 1,11	0,824	3,38 ± 1,07	3,25 ± 1,06	0,646	5-point

3.2 Fish fillets

Table 2. Evaluation of attributes from consumer test, presented as means ± standard deviation, for fillets of sea bream and seabass at high quality and end of high-quality shelf-life. Type of category scale for each attribute used in the questionnaire is also presented. Significant differences at a=0,05 are depicted with (*).

	Sea bream (fillets)						Seabass (fillets)						
	High Quality			End of High Quality			High Quality			End of High Quality			
	Pad	No Pad	p-value	Pad	No Pad	p-value	Pad	No Pad	p-value	Pad	No Pad	p-value	Type of Scale
Fresh Odor	3,66 ± 0,93	3,70 ± 0,87	0,793	3,16 ± 0,86	3,06 ± 0,97	0,544	3,77 ± 0,79	3,63 ± 0,81	0,402	3,40 ± 0,80	3,04 ± 0,91	0,030*	5-point
Flesh	2,34 ± 0,67	2,30 ± 0,71	0,740	2,19 ± 0,74	1,99 ± 0,71	0,091	2,53 ± 0,62	2,48 ± 0,64	0,677	2,55 ± 0,56	2,38 ± 0,58	0,071	3-point
Bright Skin	2,08 ± 0,64	2,10 ± 0,59	0,916	2,00 ± 0,53	2,00 ± 0,50	1,000	2,20 ± 0,60	2,12 ± 0,56	0,454	2,03 ± 0,55	2,00 ± 0,54	0,735	3-point
Overall Freshness	3,52 ± 1,13	3,48 ± 1,07	0,671	3,15 ± 1,03	3,00 ± 1,03	0,382	3,73 ± 0,91	3,59 ± 0,83	0,284	3,51 ± 0,86	3,18 ± 0,92	0,028*	5-point
Purchase Intention	3,78 ± 1,27	3,75 ± 1,17	0,719	3,45 ± 1,03	3,19 ± 1,05	0,205	3,97 ± 1,02	3,94 ± 0,97	0,764	3,79 ± 0,93	3,42 ± 1,08	0,045*	5-point
Close to Ideal	3,52 ±1,23	3,34 ± 1,12	0,336	3,03 ± 1,08	2,98 ± 1,05	0,846	3,77 ± 1,00	3,57 ± 1,03	0,249	3,53 ± 0,95	3,08 ± 0,99	0,010*	5-point

44 out of 64 and 48 out of 62 consumers preferred packaging with a pad at the end of high quality, for sea bream and seabass, respectively ($p < 0,05$). Regarding, high quality shelf-life, a significant preference for seabass packed with pad was also shown ($p < 0,05$). Differences in sensory attributes, purchase intention and proximity to the ideal product of fish fillets appear in Table 2. CO_2 concentration in the packaging, at the end of high quality, was on average 36% and 34% CO_2, while for samples without a pad it was below 30%. The initial TVC, *Pseudomonas* spp., *Enterobacteriaceae* spp., and *Shewanella* counts for the seabass fillets were 5,0 ± 0,5, 4,2 ± 0,2, 3,4 ± 0,6 and 4,7 ± 0,5 log CFU g^{-1}, respectively. The corresponding values for sea bream fillets were 4,5 ± 0,9, 4,1 ± 0,6, 3,5 ± 0,6 and 4,6 ± 0,9 log CFU g^{-1}. The inhibitory effect of CO_2-emitters was observed at the end of high quality for *Pseudomonas* spp., and *Shewanella*, as counts were approximately 4,0 log CFU g^{-1}, for both seabass and sea bream fillets. Also, the *Enterobacteriaceae* spp. counts were approximately 3,0 log CFU g^{-1}, for both seabass and sea bream fillets.

3.3 Consumer attitudes

The second part of the questionnaire included questions regarding consumption frequency of fish and attitudes towards fish packaging. Results showed that all participants were regular consumers of fish, especially fresh fish that is not packaged. Regarding their attitudes and beliefs, 38% of them believe that packaged fresh fish is safer, 59% find it more convenient, while 50% believe that it is less fresh than unpacked fresh fish. Finally, they were asked if they would prefer to buy a packaging with a pad and 55% of the consumers replied positively, while 31% remained indifferent.

4. Discussion/Conclusion

This study was the first one to examine consumer attitudes regarding active packaging with CO_2-emitters in Mediterranean farmed fish. As stated in previous research, CO_2-emitters may improve preservation and extend the shelf-life of fresh fish (Hansen *et al.*, 2007; Hansen *et al.*, 2016; Tsironi *et al.*, 2019). However, their impact on consumer's perception remained unknown until now. Our research showed that consumers preferred active packaging with pads over packaging without pads, especially during the end of high quality of the samples. This preference can be attributed to the increased CO_2 concentration in the packaging with a pad, which led to a delay in the aerobic and Gram-negative bacteria growth and, consequently, to an extension of the microbial and sensory shelf-life. Sensory evaluation by consumers showed that whole sea bream packaged with a pad and seabass fillets with a pad at the end of high-quality shelf life had a significantly higher score in terms of fresh odor, overall freshness, purchase intention, and were closer to consumer's ideal product. All participants were regular consumers of fish, especially fresh fish that is not packaged. However, results indicated that they have a positive attitude towards fresh fish packaging even when it contains a pad. To conclude, our results imply that active packaging with CO_2-emitters has a positive potential in the Greek market and further research should focus on the purchase intention of the consumers taking into consideration factors such as price and labelling of the products, as well as the investigation of the sensory perception and potential sensory superiority of the cooked product.

5. Acknowledgements

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THE EFFECT OF SALINITY AND LIGHT INTENSITY ON THE BATCH CULTURED CYANOBACTERIA *ANABAENA* SP. AND *CYANOTHECE* SP.

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Abstract

A culture of the local cyanobacterial strains *Anabaena* sp. and *Cyanothece* sp. at 20-21.5 °C in 6 combinations of 3 salinities (20, 40 and 60 ppt) and 2 light intensities (2000 and 8000 lux) resulted in: a) *Anabaena* grew best at 20 and 40 ppt at high light of 8000 lux. b) *Cyanothece* grew best at 40 and 60 ppt at high light. c) Low light of 2000 lux resulted in much reduced growth in all treatments. d) Maximal biomass yield was 1.27 and 1.77 g d.w./L for *Anabaena* and *Cyanothece*, respectively.

Keywords: cyanobacterial culture, *Anabaena*, *Cyanothece*, growth, salinity, light.

1. Introduction

Microalgae are sources of various products, e.g., aquaculture feedstuff, bio-fuels, healthy food pills, antioxidants, pharmaceuticals, etc, (Priyadarshani & Rath, 2012; Sun *et al*., 2018) and the quest for novel species suitable for culture with characteristics facilitating the implementation of cost-effective techniques is always welcomed. Cyanobacteria occupy a prominent part of interest for mass cultivation and among them *Spirulina* (*Arthrospira*) species were the starting point for exploitation (Sill *et al*., 2012). Many other species have been also the target of research, expanding thus the spectrum of cyanobacteria with the capability to grow fast and massively at various environmental conditions (Morais *et al*., 2015). To be commercially exploitable a candidate species must be easily cultured. Light is the most essential and critical factor affecting directly the photosynthesis from which biosynthesis of biomass ensues (Sun *et al*., 2018). Low illumination has a limiting effect and increasing the light intensity is a common practice to enhance growth but care should be taken as too much of it can cause photoinhibition (Raqiba & Sibi, 2019). Salinity affects the growth of microalgae acting directly on the osmoregulatory mechanism of the cell. Cyanobacteria can endure several ranges of salinity but the existing information in the literature is rather complicated on conclusions about both the range of tolerance and the optimum value. There are studies suggesting that the elevated salinities affect negatively the growth of microalgae acting directly on their photosynthetic apparatus (Vonshak & Torzillo, 2004; Bilanovic *et al*., 2009). In the present work the filamentous diazotroph *Anabaena* sp. and the coccoid diazotroph *Cyanothece* sp., two local strains isolated from lagoonal and hypersaline waters, respectively, were cultured on the assumption that are hardy species as lagoons and hypersaline ponds are among the harshest water environments due to intense seasonal fluctuations of salinity, light, temperature and nutrient input. So, it is logical to assume that species encountered there are sturdy because of their adaptability to such constantly changing water bodies. Furthermore, these local strains can enrich the collections of algae preserved worldwide in order to create an expanding deposit of strains of sibling or novel species.

2. Material and Methods

Anabaena sp. and *Cyanothece* sp. (Fig. 1 A & B, respectively) originated from a screening survey of the adjacent saline waters of Messolonghi lagoon and the hypersaline ponds of Messolonghi saltworks, respectively in W. Greece. Water samples were cultured and renovated and finally through continuous serial dilutions monospecific cultures were stabilized and kept at a salinity of 40 ppt. They were batch-cultured (Fig. 1C) in 2-L glass conical Erlenmeyer flasks in 2 replicates for each combination of salinity and light intensity. Water of salinities 20, 40 and 60 ppt, and light intensities of 2000 and 8000 lux from 20 watt 1600 lm LED lamps, measured at the middle of the outer surface of the vessel (Lux meter BIOBLOCK LX-101), were combined so as to create 6 treatments (3 salinities x 2 light intensities). Temperature was maintained at 20–21.5 °C by a 18,000 BTU air condition. A 16 hL:8 hD light duration was maintained by an electric timer controller. The water (enriched by Walne's medium) used to prepare the various salinities originated from the nearby seacoast. It was first autoclaved at 121 °C for 20 minutes and then adjusted to 20 ppt by sterilized distilled water or 60 ppt by the dilution of the proper quality of sterilized artificial salt (Instant Ocean®, Blacksburg, Virginia, USA). Suspension of cells and supply of CO_2 were accomplished using coarse air bubbling through 2-mL glass pipettes of half culture volume/min fed by a blower. The density of *Anabaena*'s in g dry biomass/L and *Cyanothece* as cells/mL were monitored daily by the recording of their optical density at 750 nm in a visible-UV spectrophotometer (Shimadzu UVmini-1240 UV-visible) and transformed to mass density by a proper calibration curve (Hotos *et al.*, 2020). The specific growth rate (SGR as doublings day^{-1}) was estimated during the early exponential (log) phase of the culture's growth curve using the equation: SGR = $(\ln C_2 - \ln C_1)/(t_2 - t_1)$ where C_1 and C_2 stand for g d.w./L or cells/mL at days t_1 and t_2, respectively ($t_2 > t_1$). Next the generation time Tg (in days) was calculated as days required for duplication using the formula: Tg = 0.6931/SGR. The calculation of the dry weight (in triplicate for each treatment) was made by filtering a known amount of culture through 0.45 μm GF/C filters (Fig. 1D) in a vacuum pump (Heto-SUE-3Q), washing the filter with ammonium carbonate and drying the filter in an oven to 100 °C for 2 h before weighing. The pH was daily measured by a digital pH-meter (HACH-HQ30d-flexi). Statistical treatment of the different variables was done with ANOVA and pair-wise Tukey's test for comparison of the means at a 0.05 level of significance using the free PAST3 software.

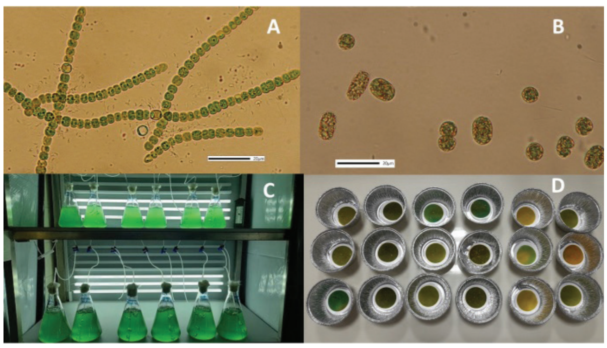

Fig. 1: Photos of the treated material. A: filaments of *Anabaena* sp. B: cells of *Cyanothece* sp. Scale bars in both photos are 20 μm. C: Culture vessels of *Anabaena* on the 4th day. D: Dried GF/C filters with dry material of *Cyanothece* masses during dry weight measurements.

3. Results

The culture of *Anabaena* sp. lasted 24 days (Fig. 2 A1-A3) and presented a very short lag phase with the highest value of 1.24 g d.w./L at the salinity of 20 ppt on the 22nd day (Fig. 2 A1). The specific growth rate (SGR) was above 0.13 doubl./d at all treatments presenting its highest value (0.213) at the salinity of 40 ppt-XL and the lowest (0.131) at 60 ppt-XL (Fig. 3 A1, Table 1). In every salinity there were considerably higher values each day at the high light intensity (XL-8000 lux) compared to its counterpart at low intensity (L-2000 lux). At the cultures with salinity of 20 and 40 ppt, pH started from values slightly lower than 8.0 and then grew steadily to values exceeding 8.5 at high light remaining always higher than its counterparts of low light which kept under 8.5. Total biomass yield calculated on the 22nd day was much higher ($p<0.05$) at 20 and 40 ppt salinities (1.27 and 0.97 g d.w./L at XL, respectively) compared to 60 ppt (0.78 g/L, XL). The same was observed also at low light (L) with ~0.6 g/L at 20 and 40 ppt compared to 0.37 g/L at 60 ppt (Fig. 3 A2).

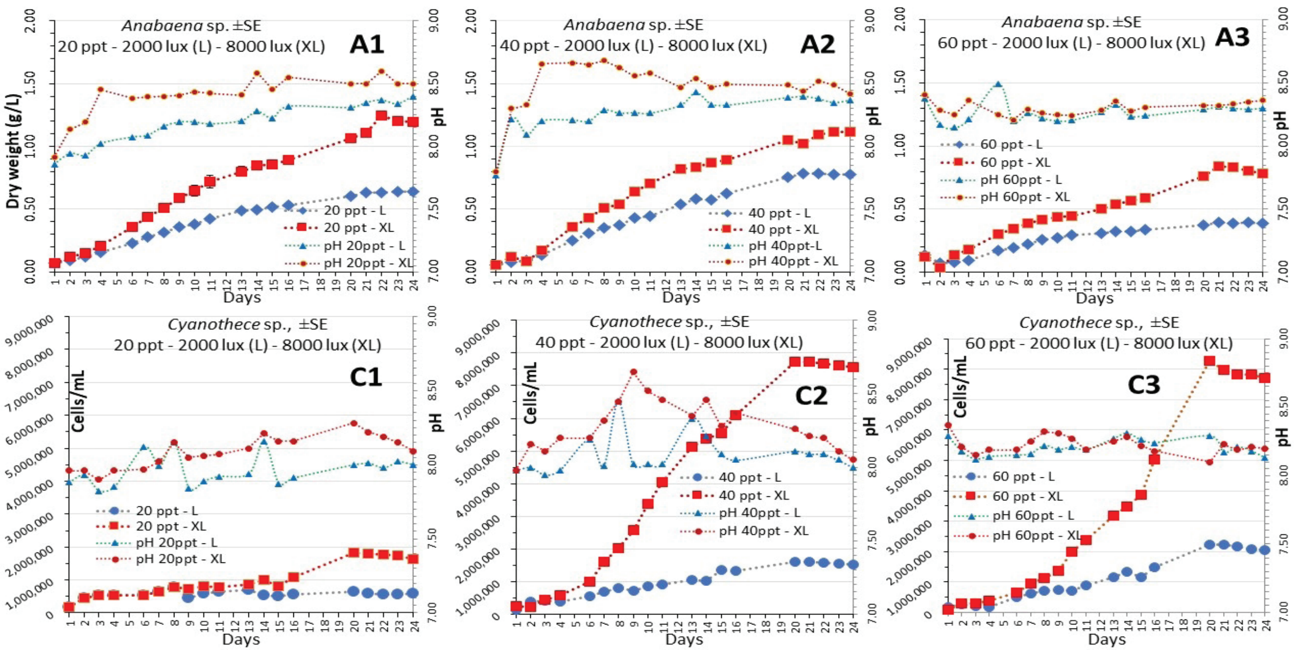

Fig. 2: Growth curves of the cultures of *Anabaena* sp. (A1-A3 in g dry weight/L) and *Cyanothece* sp. (C1-C3 in cells/mL) at the salinities of 20 ppt (A1 & C1), 40 ppt (A2 & C2) and 60 ppt (A3 & C3) and at each light intensity (L: 2000 lux, XL: 8000 lux). Also depicted are the pH daily values in each condition. Values are means of 3 measurements ± standard error (SE).

The culture of *Cyanothece* sp. (Fig. 2 C1-C3) presented the highest value of 7.5-8.0 x 10^6 cells/mL at the salinities of 40 and 60 ppt at high light (8000 lux) on the 20^{th} day (Fig. 2 C2 & C3). Contrary to these salinities, at the salinity of 20 ppt growth was much lower reaching 2.0 x 10^6 cells/mL (Fig. 2 C1). The specific growth rate (SGR) was maximal at the salinities of 40 ppt-XL and 60 ppt XL (0.281±0.003 and 0.260 ±0.005, respectively) and minimal (0.041±0.004 - 0.084±0.002) at the salinity of 20 ppt in both light regimes (Fig. 3 C1, Table 1). At the higher salinities (40 and 60 ppt) there were considerably higher values each day at the high light intensity (XL-8000 lux) compared to their counterparts at low intensity (L-2000 lux). In the cultures with salinities of 20 and 40 ppt, pH started from values slightly lower than 8.0 and then grew steadily (with much fluctuation) to values between 8.0 and 8.5 at high light. At the salinity of 60 ppt both light treatments had similar pH around 8.3 during the whole period. Total biomass yield calculated on the 20^{th} day reached its highest values at 40 and 60 ppt salinities (1.60 and 1.77 g d.w./L at XL, respectively) compared ($p<0.05$) to 20 ppt (0.30 g/L, XL). At all low light regimes yield was much lower ($p<0.05$) in all salinities (0.16 and 0.19 g/L at 20 and 40 ppt respectively) but the treatment 60 ppt-L gave considerably higher yield of 0.56 g/L (Fig. 3 C2).

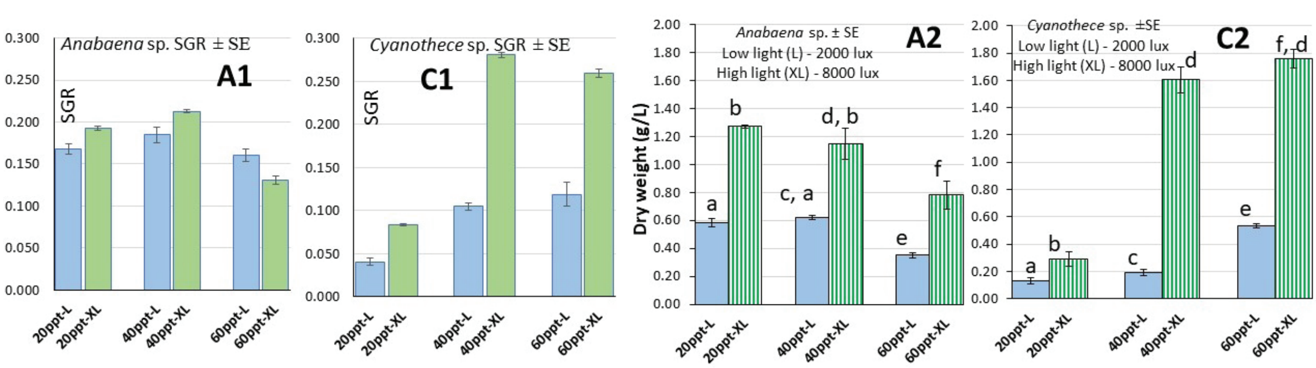

Fig. 3: Data (± SE) of cultures on the SGR (doubl./d) and biomass yield (g d.w./L) of (A1 & A2) for *Anabaena*, respectively and (C1 & C2) for *Cyanothece*, respectively at salinities of 20, 40 and 60 ppt and at each light intensity (L: 2000 lux, XL: 8000 lux). Different superscripts stand for difference at the 0.05 level (ANOVA and then pair-wise comparison with Tukey's test). A second superscript means statistical equality.

Table 1. Data (± SE) on specific growth rate (SGR) and generation or doubling time (Tg) of *Anabaena* and *Cyanothece* cultures at salinities of 20, 40 and 60 ppt and at each light intensity (L: 2000 lux, XL: 8000 lux). Different superscripts indicate a statistically significant difference at the 0.05 level of confidence (ANOVA and then pair-wise comparison with Tukey's test). A second superscript means statistically equal to the value of the condition of the corresponding letter.

Conditions	20ppt-L	20ppt-XL	40ppt-L	40ppt-XL	60ppt-L	60ppt-X
			Anabaena sp.			
SGR± SE	0.168^{a} **±0.006**	0.192^{b} **±0.003**	$0.185^{c.b.}$ **±0.007**	$0.213^{d.}$ **±0.009**	$0.160^{e.a}$ **±0.002**	0.131^{f} **±0.001**
Day interval	4^{th}-11^{th}	4-11	4-11	4-11	4-11	4-11
Tg ± SE (days)	4.212**±0.143**	3.371**±0.031**	3.416**±0.065**	3.052**±0.065**	3.534 **±0.071**	3.316 **±0.031**
			Cyanothece sp.			
SGR± SE	0.041^{a} **±0.004**	0.084^{b} **±0.002**	0.105^{c} **±0.004**	0.281^{d} **±0.003**	$0.119^{e.c}$ **±0.014**	0.260^{f} **±0.005**
Day interval	6^{th} – 11^{th}	6-11	6-11	6-11	6-11	6-11
Tg ± SE (days)	16.99 **±1.395**	8.29 **±0.162**	6.61 **±0.253**	2.471 **±0.023**	5.84 **±0.643**	2.67 **± 0.051**

4. Discussion/Conclusion

The effect of light on growth of the local strains *Anabaena* sp. and *Cyanothece* sp. (Hotos, 2021a) was examined using low (2000 lux) and high (8000 lux) illumination, combined with several salinities because on the one hand, these are marine species and on the other, future mass cultivation of cyanobacteria for various products is desirable to be conducted in seawater due to the preservation of limited freshwater resources which are destined for drinking and crop irrigation (Chisti, 2013). The two species exhibited totally different response to the various salinities. *Anabaena* clearly grew more efficiently at the lower salinity of 20 ppt while *Cyanothece* at the higher of 40 and 60 ppt. Cyanobacteria can adapt to the variations of salinities (Joset *et al.*, 1996) and optimum growth occurs at a specific range of salinity (Rippka *et al.*, 1979). Apart from salinity, it seems that the above physiological responses are also influenced by the intensity of light as, in *Anabaena* the influence of both light intensities exhibited a lesser degree of difference between low and high illumination on both growth rate and biomass yield compared to *Cyanothece*, where the differences were much higher at all salinities. The influence of light is catalytic and we are faced with the need to adjust its intensity to a compromising level between opposite directions. On the one hand, cyanobacteria grow faster in continuous high light intensities (Klepacz-Smółka *et al.*, 2020; Hotos, 2021c) and this was corroborated in the present study in both *Anabaena* with 1.26 g dw/L at high light of 8000 lux as compared to 0.60 g/L in low light of 2000 lux and *Cyanothece* with 1.75 and 0.55 g/L, respectively. On the other hand, cyanobacteria can grow effectively in low light because they have low energy requirement for maintenance and a unique flexible photo-capture apparatus that maximizes the spectrum of available low light (Kumar *et al.*, 2011). In the present work it was proven beyond any doubt that both cyanobacteria responded more efficiently at the higher level of illumination (8000 lux) than in the lower of 2000 lux and this was more pronounced in *Cyanothece*. Our results of yields of *Anabaena* and *Cyanothece* rank among the highest level of those reported in the literature concerning e.g., *Phormidium* (1.20 g/L, Hotos, 2021b), *Arthrospira* from 0.2 g/L (Walter *et al.*, 2011), to 1.3 g/L (Raqiba & Sibi, 2019), to 1.7 g/L (Pandey *et al.*, 2010), *Spirulina* 101 mg/L/d (Ho *et al.*, 2018) and *Pseudoanabaena* 0.55 g/L (Khatoon *et al.*, 2018). Considering that the optimum temperature range for culturing microalgae in general is 20–30 °C (Renaud *et al.*, 2002) and for cyanobacteria 25-35 °C (Robarts & Zohary, 1987), it is logical to assume that these particular strains of *Anabaena* and *Cyanothece* could attain higher both growth rate and yield if cultured at temperatures of 25-30 °C. In conclusion, these local strains of

Anabaena sp. and *Cyanothece* sp. have potential for mass culture presenting a long exponential growth phase even at a moderate temperature of ~ 20 °C. They are growing best at 8000 lux yielding from ~ 1.2 to 1.7 g dry weight per Liter and while they present a wide salinity tolerance, nevertheless *Anabaena* grows best at 20 ppt and *Cyanothece* at 40 – 60 ppt enabling thus the proper use of various salinities available in particular locations.

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DETERMINATION OF THE CAROTENOID PROFILE OF *TETRASELMIS STRIATA* GROWN UNDER DIFFERENT CULTIVATION CONDITIONS USING UPLC-QTOF

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Abstract

Recently an ever-increasing trend towards sustainable ingredients has driven the aquaculture industry in search of alternatives bioactive compounds. For this purpose, molecules derived from cultivated microalgal strains have been a point of particular interest, as they have already found uses in the production of high-added value products. The aim of this study was to evaluate the carotenoids profile of the microalgae *Tetraselmis striata* cultivated in laboratory or pilot scale production, under different conditions, in correlation with its biomass productivity and crude protein. For this reason, a new method for the determination and quantification of the targeted carotenoids (astaxanthin, lutein, zeaxanthin, canthaxanthin, b-cryptoxanthin, echinenone, lycopene and b-carotene) was developed using a UPLC-q-TOF-MS. The results of the different trials showed, that the pilot scale *T. striata* which was grown in uncontrolled temperatures (24-27), pH= 8 and under constant illumination, presented to be the best possible conditions for the formulation of a favorable carotenoid profile in terms of b-carotene and lutein concentrations. However, more research is necessary, to be able to clarify the potential correlations and understand the interactions between the aforementioned parameters and the carotenoid levels.

Keywords: microalgae, pigments, UPLC-QTOF, extraction.

1. Introduction

Microalgae are unicellular microscopic photosynthetic microorganisms, which are commonly found in marine and freshwater ecosystems (Milledge, 2011). They are characterized as the most productive plant-like organisms in the world due to their superior growth rate (Marshall, 2007) and have been a point of interest due to their production of high-added value molecules which are accumulated in their biomass (Barsanti & Gualtieri 2018). The perspectives and benefits of microalgae as food additives lie in the diversity of their biomass composition in wide ranges of pH, light intensity, temperature. Amongst the several microalgal derived high-value added products; mainly two products have made a real impact on global economy, namely, polyunsaturated fatty acids and pigments such as carotenoids (Rahman, 2020). Carotenoids are natural occurring lipophilic isoprenoid pigments, composed of a 40-carbon backbone and can be divided into two classes, carotenes and xanthophylls (Varela *et al.*, 2015). In the industry they can find applications as food colorants, fish natural pigments and cosmetic additives. Among the identified carotenoids in microalgae, b-carotene, astaxanthin, and lutein have the most significant market potential (Novoveská *et al*. 2019), although violaxanthin, antheraxanthin, zeaxanthin, and neoxanthin are also commonly found (Egeland, 2016). The objective of this study was to evaluate the carotenoid profile of the microalgae *Tetraselmis striata* cultivated in laboratory or pilot scale production, under different pH, temperature and photoperiod conditions in correlation with its biomass productivity and crude protein content, to determine the conditions under which can promote the optimum carotenoid profile.

The specific strain was selected as presented high salinity tolerance with high biomass productivities.

2. Material and Methods

2.1. Tetraselmis striata cultivation

A pure culture of *T. striata* was purchased from the Culture Collection of Algae at Göttingen University in Germany (SAG). The strain was cultivated in drilling waters obtained from the commercial fishery Plagton S.A.. In Total, eight different cultivations *T. striata* were employed in laboratory-scale under different pH, temperature and photoperiod conditions, followed by two repetitions of an up-scale cultivation under the conditions (pH 8, under constant illumination and uncontrolled temperatures 24-27°C) based on the results of the samples that presented the optimum growth parameters (such as biomass productivity, crude protein content etc.). In detail, the initial two acquired *T. striata* originated from an experimentation which was examining the growth optimization of microalgae cultivated under the same conditions (temperature 25 °C and constant illumination) but with different pH values, 7 and 8. The *T. striata* cultivated with pH 8 presented higher biomass productivity (79.8 mg L^{-1} d^{-1}) compared to pH 7 (60.1 mg L^{-1} d^{-1}) and consequently it was used in the next experiment which compared to the *T. striata* that were cultivated under controlled temperatures (19 ± 1 °C, 25 ± 1 °C and 28 ± 1 °C). Lower and higher temperatures reduced significantly its biomass productivity (69.3 and 61.5 mg $L^{-1}d^{-1}$) compared to cultivation at 25 ± 1 °C (93.7 mg $L^{-1}d^{-1}$). Thus, in the final lab-scale experiment, three different photoperiodic conditions were tested, 18:06 h, 12:12 h and 20:04 h (light: dark), at 25 ± 1 °C and pH= 8. Based on the results of all the experimentations, as well as biomass productivity and the crude protein content of each sample, cultivation under uncontrolled temperatures (24-27 °C) with pH 8 and constant illumination where the conditions that were selected to conduct an upscale experimentation.

2.2. Chromatographic and mass spectrometry conditions

An Acquity UPLC H-Class system coupled with a quadrapole-orthogonal acceleration Time of Flight Premier mass spectrometer by Waters (Q-TOF Premier, MassLynx 4.1 software). The chromatographic separation on the UPLC system was carried out with a C18 BEH column (50 mm × 2.1 mm, 1.7 m, Waters). Mobile phases consisting of 0.1% aqueous FA (solvent A) and acetonitrile with 0.1% FA (solvent B). A gradient flow rate and elution program were selected for carotenoids separation as follows: 85-79% A from 0 to 2 min; 79-75% from 2 to 5 min; 75-15% from 5 to 7 min; 15–85 from 7 to 8 min; equilibrate at 85% from 8 to 10 min. Column temperature was maintained at 32◦C and the sample's temperature was 15◦C. The injection volume was 5 μL. The qTOF-MS detector was operated using an orthogonal-V electrospray ionization interface (ESI) in positive mode.

2.4. Sample preparation

All the *T. striata* biomass samples were acquired at the last days of the experimentations and they were transferred to HCMR facilities in Athens. Afterwards, the wet microalgae biomass was centrifuged, while a clean-up protocol was applied to remove seawater salts (Mohamed *et al*., 2014). The samples where then lyophilized and homogenized with a mortar and pestle. Standard stock solutions of all carotenoids and internal standard (trans- 8'- apo- β- caroten- 8'- al) were prepared in dichloromethane.

2.5. Carorenoid extraction

A carotenoid solid-liquid extraction (SLE) followed by a liquid-liquid extraction method was developed based on already published literature (do Nascimento *et al*., 2020; Vendruscolo *et al*., 2021) and chloroform was selected as the final extraction solvent. Following the extraction of the analytes, a saponification protocol was applied (Larsen & Christensen, 2005). However, this method had a notable deterioration effect on the remaining targeted carotenoids. Thus, a simultaneous extraction protocol without saponification was followed to quantify the rest of the carotenoids. In both extraction protocols, with or without the saponification, 15 mg of freeze-dried microalgae biomass were weighted in 2 ml Eppendorf tubes, then 38.5 μL of trans-β-apo-carotenal were added as an internal standard and the samples remained in the dark for 15 minutes. Afterwards, 450 μL of MeOH/H_2O (1:1) were added in each Eppendorf tube and the samples were vortexed for an hour at room temperature (25 °C). After an hour, 450 μL of chloroform were added, vortexed for 30 seconds and centrifuged using at 7000 rpm for 5 minutes at 5 °C. A volume of 400 μL was carefully collected from the bottom chloroform layer and transferred to an evaporating flask. Then another 450 μL of chloroform were added and the same extraction steps were repeated until the samples in each Eppendorf tube appeared colorless. The contents in the flasks were then evaporated. The contents of the flasks that did not follow the saponification protocol, were reconstituted in 1 ml of MeOH:$CHCl_3$ (9:1) and transferred in glass vials through a syringe polyvinylidene difluoride (PVDF) filter with pore size of 0.22 μm, while 4 ml Petroleum ether : Diethyl ether (1:1) were added in samples that would be saponified and transferred to a 20 ml liquid scintillation vials with screw cap. An equivalent volume of 10 % KOH in MeOH was added and the samples were subjected to an overnight magnetic stirring. In continuation the samples were transferred into 50 mL round-bottom glass centrifuge tubes and 4 ml of deionized water was added. Then the samples were vortexed for 30 seconds and centrifuged at 3000 rpm for 10 minutes at 5 °C. The upper layer was collected in an evaporating flask and the samples were subjected to the same extraction steps two additional times. Afterwards, the extracts were evaporated, reconstituted in 1 ml of MeOH:$CHCl_3$ (9:1) and transferred in glass vials through a syringe polyvinylidene difluoride (PVDF) filter with pore size of 0.22 μm for samples.

3. Results

The results from the pH experimentations indicated that even though *T. striata* at pH 8 was chosen as the optimum choice for further experiments due to its higher biomass productivity (79.8 mg $L^{-1}d^{-1}$), pH 7 provided a richer carotenoid profile since it presented, higher concentration of astaxanthin (324.15 mg/kg dry microalgae biomass), lutein & zeaxanthin (1,454.92 mg/kg) and echinenone (497.32 mg/kg) compared to *T. striata* pH 8 with contents of 130.03, 916.37 and 238.98 mg/kg, respectively. When the temperature was controlled at 25 ± 1 °C, where the optimum biomass productivity was observed (93.7 mg $L^{-1}d^{-1}$), there was a major increase in lutein & zeaxanthin concentration (2,026.54 mg/kg dry microalgae biomass), conversely to b-carotene which had a reduced concentration (3,355.85 mg/kg), compared to the strain cultivated at uncontrolled temperatures 24-27 °C and pH 8. Other carotenoids such as astaxanthin (284.42 mg/kg), canthaxanthin (43.54 mg/kg) and echinenone (403.64 mg/kg) presented a mild increase. On the other hand, when the temperature was set at 28 ± 1 °C, b-carotene concentration was measured at 8,151.77 mg/kg, while at the same time lutein & zeaxanthin was reduced to 401.68 mg/kg. Higher temperature also aided in the increase of both astaxanthin (428.69 mg/kg) and echinenone (403.64 mg/kg) as well. When the strain was cultivated at 19 ± 2 °C, b-carotene (4,905.19 mg/kg) and lutein & zeaxanthin (270.42 mg/kg) concentration were lower compared to the samples cultivated with uncontrolled temperatures. Astaxanthin content (336.81 mg/kg) presented a mild increase, unlike canthaxanthin, b-cryptoxanthin and echinenone, where no important difference was observed. Photoperiod experimentations offered the lowest biomass productivity compared to the previous cultivation experiments (30-46.3 mg $L^{-1}d^{-1}$). In all three different photoperiodic conditions a high concentration of b-carotene was observed, where *T. striata* cultivated at 20h/04h presented the highest value (12,395.39 mg/kg dry microalgae biomass), followed by 12h/12h (8,639.20 mg/kg) and 18h/06h (7,644.58 mg/kg). Most of the carotenoids, had lower concentration compared to the strain cultivated under constant illumination, except from echinenone which presented increased concentration at illumination 18h/06h (395.96 mg/kg) and 20h/4h (281.75 mg/kg). *T. striata* cultivated in pH 8, constant illumination and uncontrolled temperature was selected as the best candidate for an up-scale cultivation, as a result of its relatively high biomass productivity (79.8 mg $L^{-1}d^{-1}$), crude protein (51.26 g/100g dry microalgae biomass), as well as its enriched carotenoid profile consisting mainly of lutein & zeaxanthin (916.37 mg/kg dry microalgae biomass), echinenone (238.98 mg/kg) and b-carotene (5359.9 mg/kg). The results of *T. striata* cultivated at uncontrolled temperature, pH 8 and constant illumination showed increased content of lutein & zeaxanthin (1214.60–1692.78 mg/kg dry microalgae biomass) and b-carotene (7063.36–9835.11 mg/kg), while at the same time astaxanthin (33.49-46.46 mg/kg), canthaxanthin (1.96–2.12 mg/kg), b-cryptoxanthin (33.73–40.72 mg/kg) and echinenone (190.94–205.68 mg/kg) were quantified at lower concentrations.

4. Discussion/Conclusion

To be successfully incorporated in an industrial set up, an important bottleneck that we still need to overcome, is the cost, with astaxanthin price ranging from 1200 to 1500 USD per kg wholesale of 10% Astaxanthin oil in the nutraceutical market (Acién *et al.,* 2017). The main carotenoids extracted from all *T. striata* biomass samples were b-carotene, lutein and zeaxanthin. This is in agreement with other studies where b-carotene and lutein were contained in different *Tetraselmis* species (Schüler *et al*., 2020). The optimum carotenoid production of strains *Chlorococcum citriforme* and *Neospongiococcus gelatinosum*, was observed when they were cultivated at pH 7 (Del Campo *et al*., 2000), similarly to this study results. In alternates experimentation, an increase in the cultivation temperature of the microalgae *Phaeodactylum tricornutum* (from 22-24 to 27-28 °C), led to an increase in b-carotene content. Furthermore, when the temperature shifted from 25 °C and 37 °C in the cultivation of *Chlamydomonas reinhardtii*, an increase in β-carotene and lutein concentrations was observed (Napaumpaiporn P. & Sirikhachornkit A., 2016). This comes in agreement with this study results where a temperature increase (25-28 °C) led to higher b-carotene and lutein & zeaxanthin content. The results from the photoperiod trials appear to be in line with the published literature as the increased light exposure displayed significantly higher amounts of b-carotene. Similarly, *D. salina* cultivated under different photoperiod conditions presented higher carotenoid and b-carotene content when it was grown at 12:12h (Reshma *et al*., 2021). Based on the results of the performed trials, we were able to scrutinize to which extend changes in the cultivation conditions (pH, temperature and photoperiod) may affect the carotenoid content of *T. striata*. However, more research is necessary, to be able to clearly target the potential correlation and understand the interactions between the aforementioned parameters and the carotenoid levels.

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TEMPORAL DYNAMICS OF THE MICROBIAL FOOD WEB IN A COASTAL STATION OF THE EASTERN MEDITERRANEAN SEA: A FOCUS ON CILIATES

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Abstract

The present study, conducted at a coastal station of the Eastern Mediterranean Sea, analyzed the temporal and vertical changes of all components of the microbial food web (MFW), with a specific focus on heterotrophic and mixotrophic ciliates, and their correlation with potential prey. Abundance, biomass, size classes, mixotrophy vs. heterotrophy, and species composition of MFW components were investigated using two series of samples fixed with Lugol and formaldehyde. The MFW was dominated by heterotrophic picoplankton in all months and depths analyzed, whereas autotrophic nanoplankton took advantage in cold months with higher nutrient availability. On the other hand, mixotrophic microplankton biomass was higher in summer.
Total ciliate abundance showed the highest abundance and biomass in summer with a peak in September. Small heterotrophs dominated the ciliate community at all depths and months. Mixotrophic ciliates were correlated with their "potential" prey at the surface and deep chlorophyll maximum

Keywords: Mixotrophy, Oligotrophic Marine Environment, Planktonic food web, Temporal variability.

1. Introduction

Marine pelagic microbial food web comprises different organisms belonging to size classes from fempto- to microplankton (Azam *et al.*, 1983; Pomeroy, 1974; Sherr & Sherr, 1988). All components of the MFW are characterized by different trophic modes such as photo-autotrophy, phago-heterotrophy and mixotrophy, and are connected with complex trophic relationships that potentially depend on size. Ciliates are an important component of the pelagic ecosystem, and they represent the link between the picoplankton and nanoplankton on one hand, and mesozooplankton and the higher trophic levels on the other. Especially in oligotrophic environments (Atlantic Ocean and Mediterranean Sea), ciliates are even more important because they are the main grazers due to the small size of primary producers (Burkill *et al.*, 1994; Pitta *et al.*, 2001; Sherr & Sherr, 1988). Because of the high range of sizes, pelagic planktonic ciliates play different roles in the marine MFW; mixotrophic and heterotrophic ciliates, on one hand, and nano- and microciliates on the other, have different temporal and vertical distributions in the oligotrophic Mediterranean Sea (Bojanić *et al.*, 2012; Dolan *et al.*, 2019; El-Shabrawy *et al.*, 2018; Heneash *et al.*, 2015; Pitta & Giannakourou, 2000; Romano *et al.*, 2021). To our knowledge, very little is known on the vertical and temporal variability of different size classes of mixotrophic and heterotrophic ciliates. Moreover, studies combining ciliates with the other components of pico and nanoplankton are fragmented.

The present study assesses the temporal and vertical variability of microbial food web in the ultra-oligotrophic Eastern Mediterranean Sea, with a focus on ciliate groups, trophic strategies and size classes and their correlation with abiotic factors and the numerical abundance of the other groups. This approach, applied to a complete series of vertical and temporal samples resulted in a unique and complex dataset.

2. Materials and Methods

A coastal station (POSEIDON-HCB, 35.426°N - 25.072°E) was sampled using 5L Niskin bottles during 2019, on a monthly basis at seven different depths in the euphotic zone (2, 10, 20, 50, 75, 100 and 120 m). Abiotic factors (temperature, salinity) and chlorophyll a (Chla) were measured with a Seabird CTD profiler equipped with a fluorescent sensor.

Picoplankton and nanoplankton abundance was calculated following Romano et *al*. 2021 Picoplankton abundance was measured by flow cytometry according to Marie *et al.* 1999, and nanoplankton abundance by means of epifluorescence microscopy (Porter & Feig, 1980). or microplankton enumeration, two parallel series of samples were examined. One series was fixed with 2% acid Lugol's solution; the second series with 2% buffered formaldehyde, which allows the capture and identification of mixotrophic species. Ciliates were further divided in four different groups according to size classes: very small (<18 μm), small (18-30 μm), medium (30-50 μm) and large (> 50 μm).

Pearson correlation analysis was used to estimate relationships between biological and environmental variables. Two-way ANOVA (water column structure by depth) was used in order to reveal significant differences among samples grouped according to certain criteria (mixing/intermediate/stratification period and depth layers 2–120 m). This analysis was performed on four mixotrophic ciliate size classes (very small, small, medium, and large). The post hoc Tukey test was performed for multiple comparisons among groups of samples assembled according to the significant factors. Methods in details are found in Romano et *al*. (2021) and Romano & Pitta (2021).

3. Results

3.1 Picoplankton, nanoplankton and microplankton

Heterotrophic bacteria dominated picoplankton in all samples, while picoeukaryotes were more abundant during June.

Total nanoflagellate abundance was higher from January until May, and then it dropped significantly during summer. For microplankton components, on average, diatoms were detected only during the mixing period and were not present or were present at very low densities during the stratification period, whereas dinoflagellates and ciliates were more abundant during the stratification period. All integrated abundances based on Lugol samples are shown in Table 1.

3.2 Mixotrophic vs. heterotrophic ciliates

Throughout the water column, heterotrophic ciliates numerically dominated the aloricate community by 75 ± 8.6%, leaving the remaining 24 ± 8.6% to mixotrophic ciliates. Differences between heterotrophic and mixotrophic ciliates were found in both vertical and temporal distributions. Integrated abundance of heterotrophic ciliates presented no obvious pattern, while mixotrophic one was higher during summer. In terms of size classes and trophic modes, small heterotrophic ciliates dominated the total ciliate community, while large (>50 μm) mixotrophic cells were observed only above the DCM (at 50 m), and the deeper layers were populated by very small (<18 μm) and small (18-30 μm) mixotrophic ciliates (Fig. 1). According to the two-way ANOVA, abundances of the very small (<18 μm) mixotrophs were significantly different ($p < 0.05$, $p < 0.01$, respectively) between the stratification and mixing and between the mixing and intermediate periods, respectively. On the other hand, the medium and large (30– 50 μm and >50 μm) mixotrophic ciliates significantly differed numerically among depths ($p < 0.01$). Pearson correlation showed that large mixotrophic species were correlated to a specific potential prey, while small species were correlated to different prey according to depth.

4. Discussion/Conclusion

Our results are at first a significant contribution to the knowledge of the MFW and pelagic ciliate dynamics with a focus on mixotrophic species. Few studies on planktonic ciliates have been conducted in oligotrophic marine environments, and most of them do not report on mixotrophs. The present study demonstrated the importance of mixotrophic ciliates in summer for the Mediterranean Sea, where mixotrophic ciliates contributed 37% to the total integrated abundance during May, June, July, and September, while heterotrophs did not show any specific seasonal pattern. Mitra et *al*. (2014) hypothesized that autotrophic and heterotrophic organisms dominate during the developmental phase of the ecosystems, while mixotrophs dominate in mature systems, benefiting from a flexible nutrition. Our study showed that ciliate mixotrophy was increasingly important at the decline of the spring bloom and during summer, when the system shifts from net autotrophy to net heterotrophy. Since it is known that oligotrophic conditions favor nanoplankton feeding on pico-bacterioplankton in tropical and temperate areas (Zubkov & Tarran, 2008), it is possible that nanociliates are more benefited compared to bigger species, since they can feed on picoplankton. This could explain the differences in the vertical distribution of the four size classes of mixotrophic ciliates. Based on this difference, we may conclude that large and small mixotrophs could be considered as two different groups following different distribution patterns and driven by different factors.

Table 1. Integrated abundance (cells m^{-2}) of heterotrophic bacteria (Bact), Cyanobacteria (Cyano), picoeukaryotes (pEuk), pigmented nanoflagellates (PnFlag), non-pigmented nanoflagellates (NPnFlag), diatoms (Diat), dinoflagellates (Dino) and ciliates (Cil) for all months sampled.

Month	Bact	Cyano	pEuk	PnFlag	NPnFlag	Diat	Dino	Cil
	10^{10}	10^{9}	10^{8}	10^{9}	10^{9}	10^{8}	10^{6}	10^{6}
January	2958	2100	1205	2449	2415	187	4214	348
March	1968	2377	3953	623	722	8	8267	285
April	2044	1518	1834	1012	653	536	4094	348
May	7270	2143	637	705	526	0	9158	305
June	1152	1118	9781	352	675	0	9141	445
July	2323	2007	494	185	176	0	16968	399
September	6210	1431	290	147	172	0	6185	523
October	3944	1777	420	97	200	0	6511	364
November	3367	789	259	112	206	169	4477	198
December	2576	941	271	127	139	97	3475	256

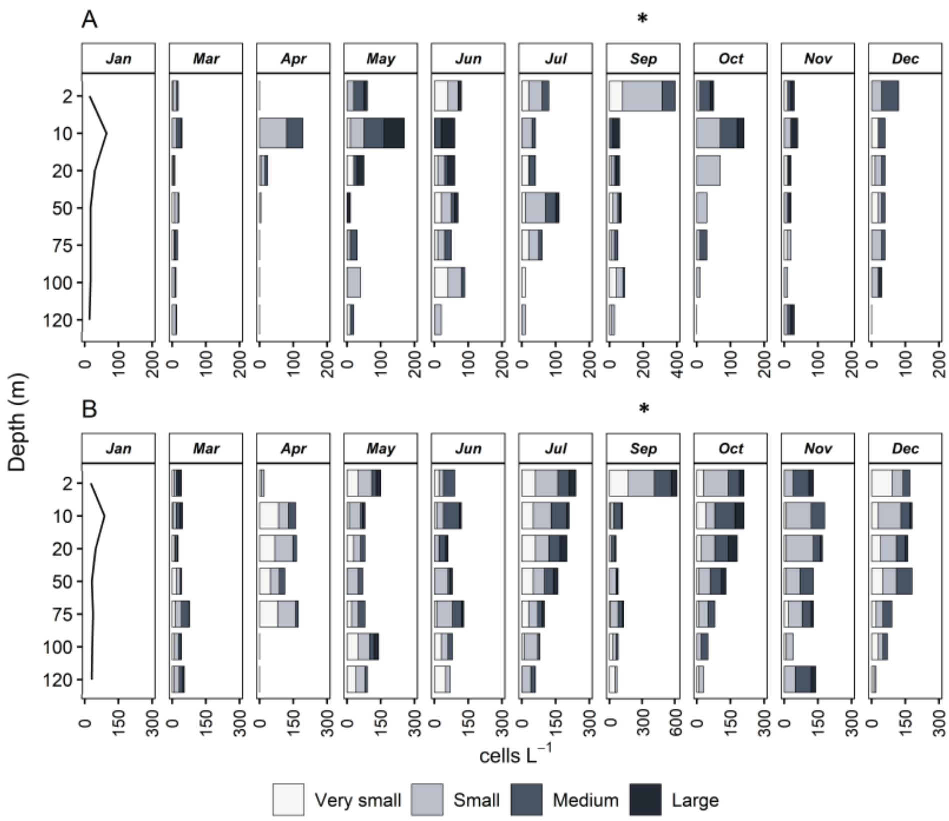

Fig. 1: Vertical distribution of abundance (cells L^{-1}) of four size classes of mixotrophic **(A)** and heterotrophic ciliates **(B)** at all months sampled. Very small: <18 μm, Small: 18-30 μm, Medium: 30-50 μm, Large: >50 μm. Note that in September, the x axis has a different scale compared to all other months.

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AQUACOSM-PLUS: AN EU-FUNDED RI-PROJECT PROVIDING ACCESS TO EXPERIMENTAL ECOSYSTEMS

Pitta P.[1], Magiopoulos I.[1,2], Tsiola A.[1] and Tsapakis M.[1] on behalf of the AQUACOSM-plus consortium

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Abstract

AQUACOSM-plus aims to efficiently address the dramatic challenges aquatic ecosystems are facing by providing, to a broad multidisciplinary international research community, an open-access, high-quality and innovative European-driven research infrastructure. In this way, AQUACOSM-plus supports excellent research and fast-track to innovation, and brings together a research community specialised in experimental mesocosm-based research, covering freshwater and marine ecosystems from the Arctic to the Mediterranean.

Keywords: Research Infrastructures, mesocosms, TransNational Access, marine, freshwater.

Human impact on the environment manifests in rapidly changing status of marine and freshwater environments around the globe. Ecosystem-scale experiments yield more comprehensive data allowing to understand how aquatic ecosystems respond to anthropogenic (and natural) stressors (Riebesell & Gattuso, 2015, Davidson *et al.*, 2018) and assisting to predict the ecosystem response to future scenarios of climate change. However, while knowledge on biological and other processes prevailing in the marine and freshwater environments increases, the need for active mitigation of natural and anthropogenic challenges these environments face increases faster; thus, testing nature-based solutions is urgent. Large-scale mesocosms are an excellent tool for this. Mesocosms are experimental bags or tanks of large volume thatallow experimentation at the ecosystem level (water column alone or with benthos) under controlled conditions. The mesocosm-based science combines advantages of laboratory experiments (hypothesis testing with replicates, controls, factorial experimental design and replication possibility) and of work in the field (complexity of natural ecosystems). Mesocosms may be used in a range of research contexts: 1) to advance understanding of the food web interactions and ecosystem functioning, 2) to examine food web dynamics, diversity, community composition and resource partitioning, nutrient pathways and carbon flow, 3) to study effects of perturbations/hazards at the ecosystem level, 4) to investigate phenomena including climate change, and 5) to investigate nature-based solutions to mitigate negative impacts. However, the access to such tools is still limited for most systems. One reason is the need for substantial technical investments and know-how to experimentally study ecosystems on adequate scales.

We therefore present open opportunities in the EU-funded RI-project **AQUACOSM-plus** (**www.aquacosm.eu**, 2020-2024) that aims to mitigate this problem. **AQUACOSM-plus** offers >13.000 days of access to >60 research facilities across the EU and is linked to world-wide cooperation through the **MESOCOSM.EU** portal. These networks comprise mesocosm facilities in rivers, ponds, lakes, estuaries and marine systems – offering unique opportunities to conduct ecosystem-scale experimental studies of relevance to a range of environmental forcing.

As a RI project, AQUACOSM-plus offers 3 types of activities.

First, Networking activities comprise interaction and collaboration with industry, European RIs to RIs, and policy makers; identify grand challenges that can best benefit from AQUACOSM facilities; build-up and train a new generation of scientists and engineers prepared to maintain and further develop mesocosm-based research and services; develop and implement approaches towards open science compliancy, and ease the application of principles of open data, open workflows and open access results.

Second, Joint Research activities among partners will develop affordable, light-weight, and easily transportable mesocosms allowing for standardized experimentation over wide geographical and environmental gradients; automated, high-resolution imaging techniques, coupled to Artificial Intelligence developments, as wells as high frequency sensing of greenhouse gas fluxes that will be effectively shared among the consortia, associated partners, and beyond.

Third, AQUACOSM-plus has increased focus on user needs for easy, wide and efficient TransNational Access (TA) to standardised and harmonised use of the RIs. To reach these goals emphasis is given to Pre-TA training of participants to ensure that TA users receive essential skills.

The AQUACOSM-plus project is significantly extending the initial network of 6 marine mesocosm facilities in the FP7 MESOAQUA that was expanded to 21 partners spanning rivers, ponds, lakes estuaries and marine systems in the H2020 AQUACOSM (Fig. 1). AQUACOSM-plus is effectively expanding the consortium, by strategically adding more partners reaching 30.

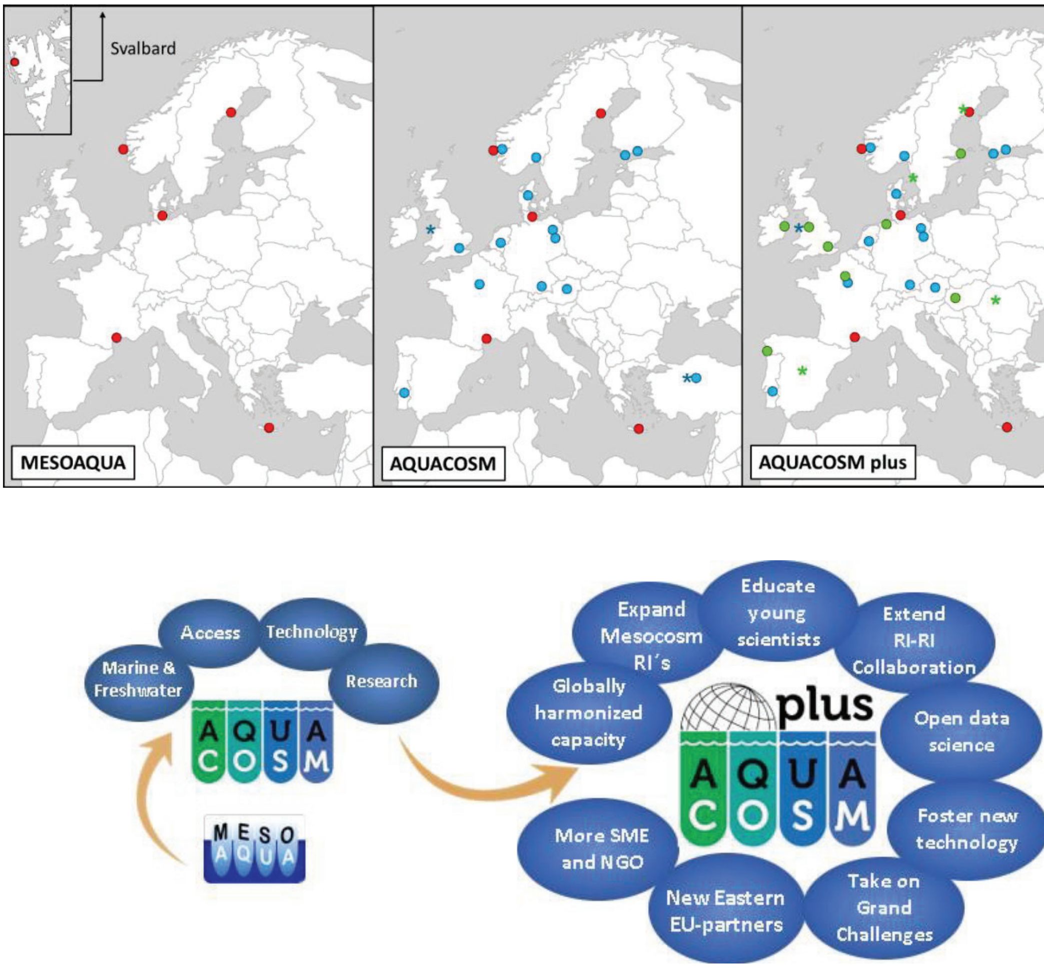

Fig. 1: Expansion of mesocosm facilities from FP7 MESOAQUA via H2020 AQUACOSM to AQUACOSM-plus, in terms of partners (upper panels, red: partners MESOAQUA, blue: new partners AQUACOSM, green: new partners AQUACOSM-plus), and increase in activities (lower panel).

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BROWNIFICATION IN AN OLIGOTROPHIC SEA: HOW A SINGLE ADDITION OF DISSOLVED ORGANIC CARBON (DOC) AFFECTS THE EASTERN MEDITERRANEAN PLANKTON COMMUNITY, PRELIMINARY DATA

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Abstract

The inflow of dissolved organic carbon (DOC) from terrestrial to coastal ecosystems colors the water brown. This "brownification" phenomenon causes shading, affecting primary production. Moreover, increasing the concentration of DOC can lead to increased biomass and production of heterotrophic organisms, especially heterotrophic bacteria. The present study focused on the effect of this phenomenon on different plankton communities. Brownification was simulated in a mesocosm experiment with the addition of HuminFeed to seawater. The mesocosm experiment took place in June 2021 at CretaCosmos facility of HCMR and had 2 treatments: Control (C) and HuminFeed (HF); each treatment had 3 replicates. HuminFeed was added at a concentration of 2 mg L^{-1} in the HF mesocosms while no manipulation was performed in the C treatment. The experiment lasted in total 15 days during which it was observed that a single addition of HuminFeed caused brownification and reduced light penetration which resulted in a decrease in the abundance of cyanobacteria *Synechococcus*. The abundance of heterotrophic bacteria was higher, mainly during the first 5 days of the experiment in HF, while after day 5, similar abundances were observed in both treatments. The abundance of 1-3 μm Heterotrophic NanoFlagellates (HNF) showed no significant differences in the two treatments.

Keywords: plankton, microbial ecology, brownification, mesocosms, oligotrophic Eastern Mediterranean.

1. Introduction

In the last twenty years, it has been noticed that surface waters in large parts of the northern Hemisphere receive increased concentrations of dissolved organic carbon (DOC) from terrestrial origins (Nydahl *et al.,* 2019). Increased soil surface runoff, which is intensified by increased rainfall, might be the main cause of DOC inflows from land to rivers, coastal and freshwaters ecosystems (Fonseca *et al.,* 2021). Other causes are changes in land use, global warming, acid rain and a combination of all the above (Graneli, 2012; Lebret *et al.,* 2018). This increase in the amounts of DOC, along with the humic organic substances it contains, colors the water brown; this phenomenon is called "brownification".

Brownification primarily causes shading, which means that the light that enters the water column is reduced. Shading is mainly caused by the chromatic aromatic structures of the humic organic substances present in DOC; these substances absorb photosynthetically active radiation (PAR) (Nydahl *et al.,* 2019). Thus, this phenomenon affects the primary productivity that is photo-dependent through the reduction of PAR that is available to photosynthetic organisms (Lebret *et al.,* 2018).

In addition, the increase in the concentration of available carbon in the water can lead to an increase in bacterial abundance, production and biomass (Ask *et al.,* 2009). Under high DOC availability, heterotrophic bacteria may be more efficient in nutrient uptake than phytoplankton. This may lead to an increase in secondary production and indirectly to a decrease in primary production (Ask *et al.,* 2009; Nydahl *et al.,* 2019).

Brownification has received a lot of attention by the scientific community; it has been mainly studied in freshwaters through mesocosm experiments (Lebret *et al.,* 2018; Nydahl *et al.,* 2019; Fonseca et al., 2021; Caldero-Pascual *et al.,* 2021) while few studies have been done in brackish waters (Spilling, personal communication) and in marine environments. The goals of this study were to investigate the effect of water coloring and the addition of DOC to the planktonic groups of the microbial food web in the oligotrophic environment of the Eastern Mediterranean Sea.

2. Materials and Methods

2.1 Mesocosm Experimental Design

The mesocosm experiment took place at the CretaCosmos facility of HCMR (Crete, Greece) in June 2021, in the framework of the project AQUACOSM. This experiment was part of a study about brownification performed along a salinity and latitudinal gradient. All experiments had a common design where a single addition of DOC was the experimental manipulation. The experiment had 2 treatments: Control (C) and HuminFeed (HF); each treatment had 3 replicates. No manipulation was performed in C while in the HF treatment, HuminFeed was added at a concentration of 2 mg L^{-1}. HuminFeed® is an alkaline extract of leonardite, used as live-stock feed. HuminFeed has been commonly used in other aquatic experiments (Nydahl *et al.*, 2019), in this way all experiments had the same DOC and color source.

The coastal water was collected in 1m^3 tanks, from 1,5m depth at the north side of the port located in front of the HCMR premises, in Gournes, Heraklion, Crete. The full tanks were then transported by truck to the mesocosms. The water from each one of the tanks was evenly distributed among the mesocosms to ensure homogeneity of the initial conditions. The mesocosms were made from polyethylene bags with a capacity of 3 m^3 each. A HOBO sensor was installed in each mesocosm to record temperature and average light intensity.

2.2 Plankton counting

Picoplankton samples were collected on a daily basis from the mesocosms and the abundances of cyanobacteria *Synechococcus* spp. and heterotrophic bacteria were counted on a FACSCalibur™ flow cytometer (Becton Dickinson) according to Marie *et al.* (2000). The software used was CellQuest Pro. *Synechococcus* samples were counted without any treatment. Heterotrophic bacterial samples were fixed with glutaraldehyde (25%) at a final concentration of 0.5%, deep frozen in liquid nitrogen, and then transferred to -80°C until analysis. Prior to analysis, the samples were thawed at room temperature, then stained with SYBR Green I nucleic dye at a final dilution 4×10^{-4} and incubated in the dark for 10 min prior analysis.

Samples for counting Heterotrophic NanoFlagellates (HNF) were collected every other day in 50 mL falcons and fixed with formalin at a final concentration of 5%, then they were stored in the refrigerator (4°C). Samples were prepared for enumeration according to Porter & Feig (1980) using DAPI staining. The filters were placed on microscope slides and stored in the freezer (–20 °C). Enumeration was done using an Olympus BX60 epifluorescence microscope. All HNF cells were sized and divided into 3 categories (1-3, 3–5, >5 μm) using the ocular micrometer.

3. Results

The average light intensity decreased sharply on day 1 in both experimental treatments, then increased and fluctuated until the end of the experiment (Fig. 1-A). The average light intensity was lower in the HF treatment compared to control C on all days of the experiment.

Cyanobacteria *Synechococcus* were more abundant in C replicates (C_1, C_2, C_3), during the first days of the experiment (Figure 1-B). Subsequently, *Synechococcus* abundance did not show significant differences in the two treatments until the end of the experiment. In the control C, it increased in the first days of the experiment and the maximum value recorded was 48049 cells mL^{-1}, in C_1. Then, it showed a gradual decrease until day 6 and remained low until the end of the experiment. In the HF treatment, immediately after the addition of HuminFeed to the seawater, a gradual decrease was recorded until day 6 and remained low until the end of the experiment.

The abundance of heterotrophic bacteria was higher during the first days of the experiment in the HF replicates (Figure 1-C), followed by similar abundances in the two treatments. Similar fluctuations in the abundance of heterotrophic bacteria were observed in all replicates, initially their abundance gradually decreased in both treatments by day 5, then increased by day 8 and finally decreased slightly by the end of the experiment.

The abundance of 1-3 μm HNF (Heterotrophic NanoFlagellates) showed no significant differences in the two treatments (Figure 1-D). In all replicates, their abundance increased until day 3 (the maximum value recorded was 7500 cells mL^{-1} in HF_2). After that their abundance decreased until day 9, followed by a small increase until day 15.

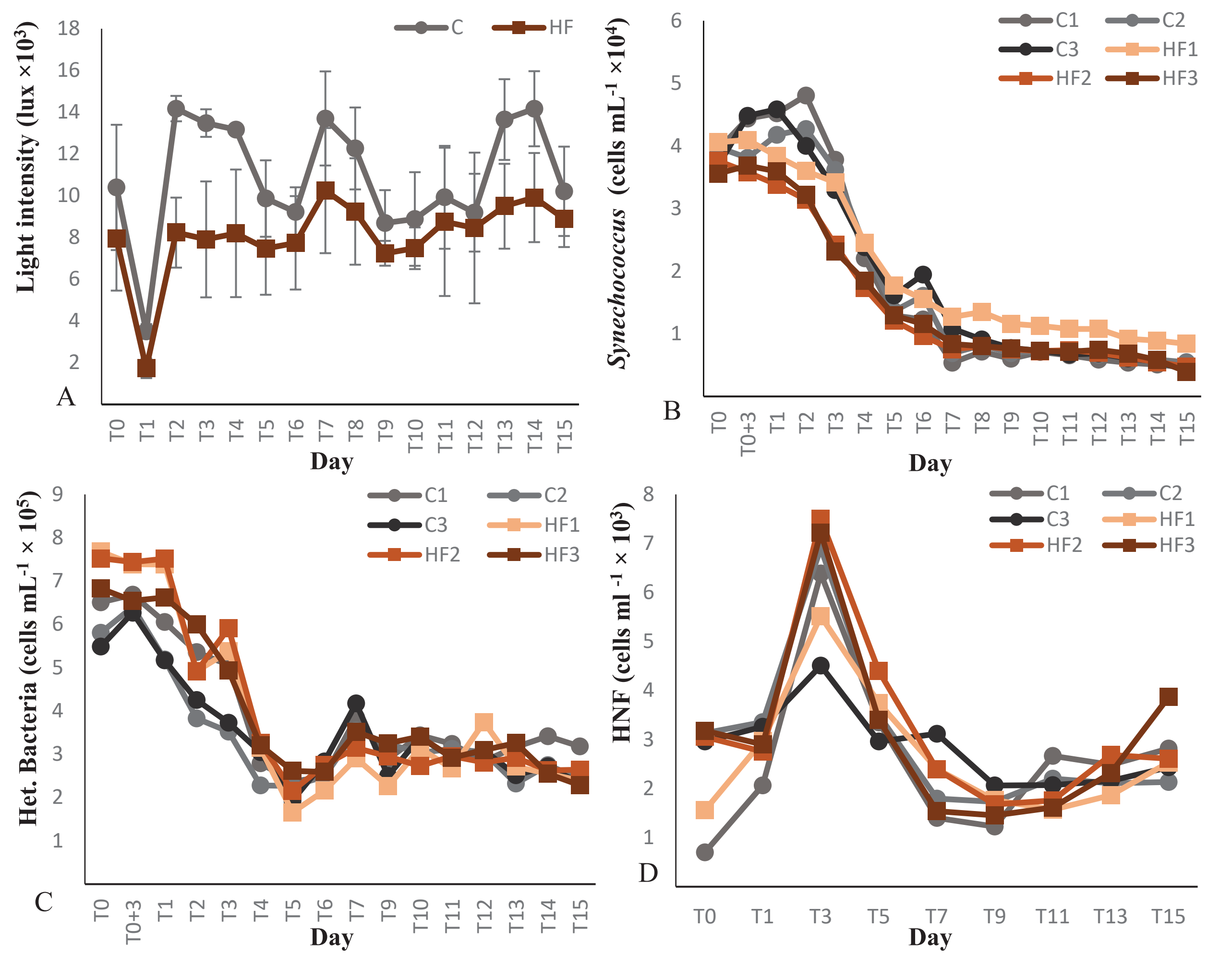

Fig. 1: Average light intensity during the experiment (Data are mean ± SD) (A), *Synechococcus* spp. abundance (B), heterotrophic bacteria abundance (C) and HNF (1-3 μm) abundance (D). Each mesocosm replicate is represented individually. C: Control, no addition. HF: mesocosms in which HuminFeed was added.

4. Discussion/Conclusions

The addition of HuminFeed in the water resulted in a decrease in light intensity. Decrease of light intensity was observed in other experiments in which HuminFeed was added at a concentration of 2 mg L^{-1} (Fonsesca *et al.*, 2021; Spilling personal communication), as well as at higher concentrations (Lebret *et al.*, 2018; Nydahl *et al.*, 2019). Also, the abundance of cyanobacteria *Synechococcus* which are strictly photo-autotrophic organisms was slightly lower in HF treatment ($HF_{1\text{-}3}$) probably due to shading caused by HuminFeed. On the other hand, the abundance of heterotrophic bacteria was higher in the HF treatment (HF 1-3), during the first days of the experiment. It is therefore reasonable to assume that at least a part of HuminFeed was available as a carbon source for bacteria. The decrease observed in picoplankton (cyanobacteria *Synechococcus* and heterotrophic bacteria) abundances in both treatments was probably due not to the decrease in nutrient concentration but to predation by HNFs whose maximum abundance was observed in day 3 in all replicates.

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ASSESSING THE MARINE SCRUBBERS' IMPACT ON THE SARONIKOS GULF MARINE ECOSYSTEM THROUGH THE PROJECT EMERGE

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Abstract

In the framework of the European Project "Evaluation, control and mitigation of the environmental impacts of shipping emissions (EMERGE)", a wide spectrum of Greek scientists from several disciplines ranging from atmospheric sciences to marine biology have joined forces in order to assess the impact of the widespread adoption of marine scrubbers by the shipping industry on the marine environment. To that aim, a comprehensive simulation strategy, comprised of a set of atmospheric, marine circulation, surface waves and biogeochemical / pollutant dispersion models has been designed, aiming not only to simulate the current biogeochemical and marine pollution "state", but also to assess the impact of the adoption of various strategies by the shipping industry in the Gulf of Saronikos through different future scenarios. Intensive effort has been given in collecting and processing information on pollution sources and (when possible) fluxes as well as oceanographic biochemical variables. Furthermore, dedicated field sampling missions have been performed in order to record the potential impact of shipping lanes on the marine environment of the Saronikos Gulf. Finally, ecotoxicological experiments have been designed and conducted to assess the impact of pollutants on coastal phytoplankton communities.

Keywords: Aquatic Pollution, Shipping, Coastal Environment.

1. Introduction

Marine shipping is considered a significant contributor to atmospheric pollution, both on planetary (Al Baroudi *et al.*, 2021) and local/regional (Radonja *et al.*, 2020; Wan *et al.*, 2020) scale. While regulation of air emissions from shipping becomes progressively stricter, introducing Emission Control Areas (ECAs) within regional seas such as the Baltic and North seas, the English Channel, the coasts of North America, technological solutions are progressively being adopted by the shipping industry to allow the continuation of use of "heavy" fuels while significantly reducing air emissions. The most widely used solution are marine scrubbers (Komissarova & Krasova, 2020), equipment which sprays with water the exhaust funnels of vessels, thus removing the pollutants (mostly SO_x and particulate matter (PM)) from the atmospheric emission plume and diluting them in water. In the case of "open loop" devices, the scrubber water, highly acidic and often enriched in PM, heavy metals and aromatic hydrocarbons is released in the sea, thus transferring the pollution from the atmospheric to the marine environment.

The impact of the scrubber water release in the sea has not been fully assessed yet. Toxicological experiments have shown adverse effects both on phytoplankton (Ytreberg *et al.*, 2021) and zooplankton (Thor *et al.*, 2021) communities, while simulation studies have shown significant potential impacts on basin-scale marine ecosystems (Stips *et al.*, 2016). In order to comprehensively assess the potential impact of the widespread use of scrubbers on the marine environment at European and regional scales, the European Union has funded the project "Evaluation, control and mitigation of the environmental impacts of shipping emissions (EMERGE)" through the Horizon Europe programme. In the following sections we provide an overall summary of the project EMERGE, as well as a description of its implementation in the Gulf of Saronikos.

2. Material and Methods

2.1 The EMERGE project: consortium, experimental design and case studies

The project EMERGE is the E.U.'s response to the urgent need to assess the potential impact of the widespread adoption of the marine scrubbers' technology by the shipping industry. The consortium consists of 18 partners, of which 8 are Universities, 7 Research Institutes, 2 private Companies and 1 international organization. Four partners are from Greece, while the others are based in Finland, Sweden, Norway, U.K., Portugal, Italy and Cyprus. The project's design is based on the following structure:

- identifying the most significant pollutants from shipping in the marine environment through extended bibliographic research,
- assessing the atmospheric and marine emissions at European scale through the combination of an updated version of the STEAM model (Jalkanen *et al*., 2009) and best estimates of pollutant concentrations in the scrubbers' liquid effluents,
- estimating pollutant dispersion and concentrations at European scale using regional – scale atmospheric models for the atmosphere and an enhanced version of the Lagrangian OpenDrift model (Dagestad *et al*., 2018) applied on Copernicus fields of surface circulation for the marine environment.
- In addition to the European-scale modeling effort, there will be more detailed studies of the impact on the marine environment at the following case studies:
  - ◇ Piraeus harbor and Saronikos Gulf, Greece
  - ◇ Venice harbor and northern Adriatic, Italy
  - ◇ Lagoon of Aveiro, Portugal
  - ◇ Southampton and Portsmouth harbors, Solent Strait, U.K. and
  - ◇ Öresund Strait, between Sweden and Denmark
- Additional field data have been collected along the European coasts on a route from the North to the Black Seas aboard a container ship.

While the atmospheric modeling tools used throughout the various case study regions were the same (described in 2.2), the approach regarding the pollution pathways and impacts within the marine ecosystem vary significantly among the Case Studies.

In order to assess the potential impact on the marine environment, the year 2018 will be used as a reference period, with which all different scenarios for future marine traffic and emissions will be compared. The different scenarios will be temporally located in years 2030 and 2050. There has been no decision to vary atmospheric and hydrographic conditions based on climatological projections.

In addition to the modeling effort, field campaigns of varying extents and ambitions have been designed for each Case Study, in order to complement the available information regarding the parameters considered as the most critical in each. Furthermore, in some cases, dedicated ecotoxicological experiments have been planned.

2.2 The Piraeus / Saronikos Gulf case study. modeling approach.

In the case of Piraeus / Saronikos Gulf, the dispersion of the shipping pollutants in the atmosphere, deposition at the sea-surface, as well as the atmospheric and radiative forcing for the sea circulation, are simulated using the following model chain (Fig. 1):

- MEMO (Moussiopoulos et al., 1993), covered a 120x120 km2 broad region over Central Greece and the Peloponnese, as well as the western part of the Aegean Sea, at a 2 km resolution.
- Nested in this, a combination of the physical / chemical MEMO/MARSaero models, covering the Athens metropolitan area, including the port and coastal region of Piraeus, at a 500 m resolution.

The impact on the marine environment is simulated using the following models:

- Sea circulation is simulated by the Delft3D-FLOW model, (Deltares, 2022a) forced by the models described above, covering the Saronikos Gulf, at a variable grid of a horizontal resolution ranging from about 50 to 800 m and 15 σ layers in the vertical (Kolovoyiannis et al, 2021).
- Surface waves will be simulated using the Swan model (Delft, 2022)
- The impact on the marine ecosystem is assessed using the Delft3D Water Quality model (Deltares, 2022b), based on the same grid as the circulation model.

Boundary conditions for the oceanographic models are provided by the Copernicus Marine Service (Copernicus, 2022). Shipping-pollutant boundary conditions are provided by the OpenDrift model as described in 2.1.

2.3 The Piraeus / Saronikos Gulf case study. Field experiments.

Two field oceanographic campaigns have been held in the Saronikos Gulf to record pollutant dispersion and provide additional reference measurements for the model validation. The expeditions were held in November 2021 and June 2022, in order to record conditions during low and high vertical stratification respectively. Five hydrographic stations were positioned along the northwest to southeast-oriented shipping lane of the approach to the port of Piraeus, and two more across this axis. Temperature salinity, dissolved oxygen, chlorophyll-a concentration, turbidity, nutrients, heavy metals, PAHs and suspended matter samples have been collected and are being analyzed. In addition, water samples are collected in both expeditions to study the spatial distribution of the phytoplankton communities. Furthermore, water samples have been collected in February 2022 to act as the initial natural plankton communities in ecotoxicological experiments.

Additional experiments are planned in the wake of vessels in the more pristine environment of Lesvos island, in order to isolate the impact of the passage of single vessels.

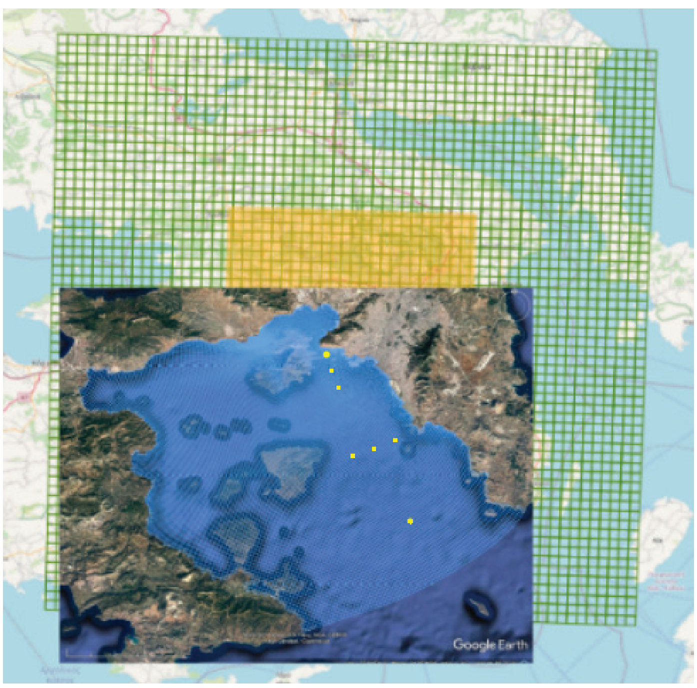

Fig. 1: The three model grids overlaid on a map of the region of interest. The models MEMO (green), MEMO/Mars (orange) and Delft3D-FLOW (white shade) are shown. The seven yellow bullets identify the two hydrographic transects.

2.4 The Piraeus / Saronikos Gulf case study. Ecotoxicological experiments.

During these experiments we examine the effects of scrubber wash-water (chemically characterized) on two natural plankton communities originated from vulnerable marine areas of Saronikos Gulf (Floisvos and Vouliagmeni). For this, mesocosms were set-up indoors in 24 glass containers under temperature-controlled environments (the *in situ* temperature) with natural light:dark cycles. Each treatment was replicated three times. Samples were taken every 24 hours for 96 hours and every second day until the termination of the experiment. The water samples (controls and treatments) are examined microscopically and with modern eDNA sequencing technologies to characterize the planktonic microbial communities at multiple taxonomic and functional levels and explore the impacts of scrubber wash-water on the pico-, nano- and micro- plankton communities focusing on phytoplankton, towards multiple ecological endpoints (from gene to community level). Overall, the response of the multi-domain planktonic microbial community will be used to address whether the scrubber wash water discharge can be disruptive at the ecosystem level via assessing potential impacts on Good Environmental Status.

3. Conclusions/Discussion

The investigation of the impact of a potential extended use of marine scrubbers on the marine environment is a very demanding exercise, requiring the involvement of scientists from a wide range of disciplines. The task of isolating this impact in a coastal environment such as the Saronikos Gulf, as several polluting activities operate along the coast (such as industrial and ship-building industries), in addition to intense traffic from ships not using scrubbers, urban pollution and sewage treatment facilities. Thus, the isolation and assessment of the scrubbers' impact requires first the quantification and simulation of all significant polluting sources, the production of marine ecosystem simulations without any polluters, with the presence of current polluting activities without scrubbers, and then the application of current scrubber use estimates and future scenarios. The outcome of this concentrated effort is expected to extend far beyond the assessment of the impact of scrubbers, but to lead to a comprehensive understanding of the relative impacts of different polluting sources in the congested marine environment of the Saronikos Gulf and provide a very useful tool for the management of this very important coastal region in the future.

4. Acknowledgements

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FIRST RECORD OF *SALMO PELAGONICUS* KARAMAN, 1938 MOUNTAINOUS POPULATIONS IN ALIAKMONAS AND AXIOS RIVER BASINS OF NORTH GREECE AND COMPARISON TO LOWER ALTITUDES

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Abstract

This study presents preliminary monitoring results of *Salmo pelagonicus* populations, recorded for the first time, in two high altitude sites (> 1000m); Floros stream of Axios River system, and Trypimeni stream of Aliakmonas River system. Physicochemical parameters were also measured and QBR index values were also calculated for each site. The pristine conditions of the sites and the rarity of their populations are highlighted by comparison to lower altitude ones (< 600m). Impacts of climate change and anthropogenic activities (e.g., creation of dams) are also discussed. Additional monitoring of the sites, collaboration with local inhabitants and authorities, and dissemination of scientific knowledge are necessary, in order to establish conservation and management measures to alter species' unfavourable conservation status and enhance its protection.

Keywords: Salmonidae, monitoring, endemic, conservation, dam.

1. Introduction

Fig. 1: Sampling sites of the study.

Pelagonian trout (*Salmo pelagonicus* Karaman, 1938) is an endemic species found in mountainous streams and rivers in Axios (Vardar) and Aliakmon River basins (Kottelat & Freyhof, 2007), listed as "vulnerable" in the IUCN Red List and included in Annex II of the 92/43/EEC Directive with unfavourable – bad (U2) conservation status. Due to its limited range, small population sizes, anthropogenic degradation of its habitats (Cheimonopoulou *et al.*, 2011; 2019), and pressures arising from climate change, there is an urgent need for conservation actions to ensure its protection. Since 2016, the Hydrobiological Station of Pella (HSP), an agency of the Greek Ministry of Rural Development and Agriculture, in collaboration with the Fisheries Departments of Imathia, Grevena, and Pella Prefectures, and the Management Unit of Northern Pindos National Park, have been investigating Pelagonian trout populations in Northern Greece. The objectives of this study are to: a) present preliminary monitoring results of two populations of the Pelagonian trout, recorded for the first time, in high altitude sites (> 1000m altitude) in Axios and Aliakmonas River basins; b) compare the aforementioned findings with those of lower altitude populations (< 600m altitude, Aliakmonas River system), and c) highlight the urgent need for the establishment of conservation measures. Environmental Literacy and dissemination of scientific knowledge among local communities, could provide substantial support towards this direction.

2. Material and Methods

Fish were sampled at two high altitude sites (> 1000m, Fig. 1), Floros stream (site 1) of Axios (Vardar) River system (summer 2018, winter 2019, fall 2020, summer 2021), and Trypimeni stream (site 2) of Venetikos-Aliakmonas River system (summer 2019, winter 2019, fall 2020) and two lower altitude sites (< 600m, Fig. 1), Arapitsa (site 3) and Tripotamos (site 4) Rivers of Aliakmonas River system (Winter 2019), located in the trout zone (Table 1). According to the 5 gradient classes of the Greek Soil Map (2019), site 1 has a slight and steep ground slope (18 - 40%), site 2, a slight and medium ground slope (6 - 18%), and sites 3 and 4, a slight ground slope (1 - 6%). Geology of the upstream drainage basin of site 1 is of igneous origin (gneiss), site 2, of siliceous and calcareous origin, while sites 3 and 4, mainly of calcareous origin (see https://www.europe-geology.eu). Sites 1 and 2 are located far from human settlements, while sites 3 and 4 are close to them. Riparian vegetation is dominated by: conifers, the Scots and black pine in site 1; King Boris fir, black pine, and European yew in site 2; and deciduous trees, plane trees in site 3; and, willow trees in site 4. A hydropower dam is under construction, 450 meters upstream of site 2. Site 1 is situated higher than the Skopos area, where *Salmo pelagonicus* was previously recorded (Kottelat & Freyhof, 2007).

Dissolved oxygen (DO in mg/l and %), pH, water temperature (^oC), conductivity (μS/cm), and turbidity (NTU), were measured *in situ*, using a WTW Oxi 315i oximeter; a WTW pH 315i pH meter, a WTW LF 196 conductivity meter and an EXTECH TB400 turbidimeter, accordingly, while wet river width (m) was also recorded and the substrate composition was assessed (Cheimonopoulou *et al*., 2011). The quality of the riparian habitat of each sampling site was evaluated by the QBR index (Munne *et al.,* 2003). Fish data were obtained by electrofishing (wading, gft model 550D & Hans Grassl IG200 devices), according to CEN standards (CEN 2003). Measurements of Total Length (TL, cm), Fork Length (FL, cm), and Weight (W, g) were recorded *in situ*, after individuals had been anesthetized with 2-phenoxyethanol. Revival and return of fish to the river followed. Captures were estimated based on the number of individuals (NPUE, individuals/100m^2) and their biomass (BPUE, g/100m^2). The Mann-Whitney U-test was applied to identify any statistically significant differences for BPUE, NPUE, TL, FL, W, among sites and sampling periods, where available, by using Statistica, version 7 (StatSoft, Inc., Tulsa, OK, USA). For sites 1 and 2, in addition, the Length-Weight Relationships (LWRs) were estimated from the formula, $W = a\ TL^b$ (a, b, regressions' coefficients between W and TL; Le Cren, 1951) and fish were grouped into size groups of 5 cm intervals.

3. Results

Physicochemical parameters and QBR index values are presented in Table 1. Mean DO values were higher than the lower limit (7 mg/l, Directive 2006/44/EC) for salmonid survival, while pH values were within the limits of the same Directive (6 - 9). The highest mean pH value was recorded in site 2. Sites 1 and 2 exhibited much lower mean conductivity values than sites 3 and 4, while site 1 exhibited extremely low values, along with limited wetted width. The substrate was characterized as coarse (> 70% boulders, cobbles, and pebbles) in all sites. Sites 1 and 2 were dominated by boulders (> 40%), while sites 3 and 4 by cobbles (> 40%). The quality of the riparian habitat, according to the QBR index, was in a natural state for sites 1 and 2 (high quality), while it was satisfactory for sites 3 and 4, characterized by significant anthropogenic intervention (moderate quality).

Table 1: Range of physicochemical parameters and width at the sampling sites during the study period (mean ± SE, min: minimum value, max: maximum value), altitude, coordinates, and QBR values.

Site/ Alt.(m)	Settlements nearby sites	Width (m)	Water Temperature (°C)	DO %	DO (mg/l)	pH	Conductivity μS/cm	QBR 2019
1/ 1800	Voras Snow Resort, Pella	2.60 ± 0.28 1.80 - 3.00	12.05 ± 1.15 9.50 - 14.00	91.42 ± 2.35 88.10 - 98.10	8.20 ± 0.34 7.61 - 8.89	7.56 ± 0.08 7.36 - 7.70	49.93 ± 15.03 34.90 - 80.00	100
2/ 1008	Mikrolivado, Grevena	6.00 ± 0.52 5.20 - 07.00	9.46 ± 2.71 4.30 - 13.50	113,83 ± 4.47 105,00 - 119.50	10.88 ± 0.56 9.80 - 11.70	8.20 ± 0.10 8.00 - 8.34	211.26 ± 19.86 179.80 - 248.00	100
3/ 513	Naousa, Imathia	15.20 ± 1.31 12.00 - 20.00	10.51 ± 0.24 9.90 - 11.40	96.33 ± 4.39 88.20 - 116.90	10.19 ± 0.45 9.17 - 12.21	7.85 ± 0.10 7.56 - 8.27	383.40 ± 26.20 322.00 - 454.00	70
4/ 404	Veria, Imathia	3.65 ± 0.23 3.00 - 4.00	13.01 ± 0.27 12.10 - 13.90	97.35 ± 3.86 81.70 - 110.90	9.84 ± 0.50 8.06 - 11.90	7.77 ± 0.15 7.15 - 8.01	399.20 ± 4.16 391.00 - 412.00	55

In total, 161 fish individuals were captured in high altitude sites (site 1: n = 24, site 2: n = 137) and 16 in the lower altitude ones (site 3: n = 5, site 4: n = 11) (Table 2). In addition, in sites 3 and 4, 20 individuals of the alien trout species *Oncorhynchus mykiss* Walbaum, 1792 (site 3: n = 19, site 4: n = 1) were captured in winter 2019 (Table 2). Regarding W, TL, and FL, no statistically significant difference was evident among the four sampling periods in site 1 (U-test, $p > 0.05$). This was also the case for site 2, except for summer 2019 and fall 2020 samples, where a statistically significant difference was evident for the same parameters (U-test, $p < 0.001$). No statistically significant difference was also evident for W, TL and FL values among the four sites in winter 2019 (U-test, $p > 0.05$). In site 2, NPUE, in fall 2020, was about two times higher compared to summer values of 2019, which was not the case for the BPUE values (Table 2). No statistically significant difference was evident for NPUE and BPUE values between sites 1 and 2 during all sampling periods (U-test, $p > 0.05$). Length groups of 0 - 5 cm and 20 - 25 cm of TL were absent in both sites 1 and 2 (Fig. 2a, c), except for site 2 (Fig. 2c), in fall 2020 (low flow season), where only 3 individuals with a TL > 20 cm (Table 2) were captured. For LWRs, all regressions were statistically highly significant, ($R^2 = 0.98$, $p < 0.001$, Bayesian length-weight: a = 0.007 (0.005 - 0.010), b = 3.122 (2.997 - 3.248) for site 1 (Fig. 2b); a = 0.009 (0.008 - 0.011), b = 3.042 (2.983 - 3.102) for site 2 (Fig. 2d)).

Table 2: NPUE (individuals/100 m^2), BPUE (g/100m^2), Total Length (TL, cm), Fork Length (FL, cm), Weight (W, g), n: number of individuals, mean: mean value, SE: standard error, min: minimum value, max: maximum value.

Site /Seasons	Species	n	NPUE (individuals/ 100 m^2)	BPUE (g/100 m^2)	TL mean ± SE (min - max) (cm)	FL mean ± SE (min - max) (cm)	W mean ± SE (min - max) (g)
1/Summer 2018	*S. pelagonicus*	12	3.07	62.82	11.90 ± 0.83 (9.10 - 18.00)	11.45 ± 0.80 (8.80 -17.20)	20.41 ± 5.08 (7.00 - 65.00)
1/Winter 2019	*S. pelagonicus*	4	1.33	21.00	10.60 ± 2.15 (6.90 - 16.40)	10.15 ± 2.05 (6.60 - 15.70)	15.75 ± 8.84 (3.00 - 41.00)
1/Fall 2020	*S. pelagonicus*	4	1.48	35.25	11.80 ± 2.37 (7.30 - 18.50)	11.30 ± 2.35 (7.00 - 18.00)	23.80 ± 14.33 (4.20 - 66.40)
1/Summer 2021	*S. pelagonicus*	4	0.88	36.31	14.80 ± 1.97 (9.20 - 18.40)	14.15 ± 1.95 (8.60 - 17.60)	40.85 ± 12.14 (9.00 - 65.20)
2/Summer 2019	*S. pelagonicus*	37	4.25	147.35	14.45 ± 0,29 (11.20 - 17.80)	13.73 ± 0.28 (10.60 - 17.00)	34.64 ± 2.10 (15.00 - 61.00)
2/Winter 2019	*S. pelagonicus*	23	2.19	34.09	10.49 ± 0.69 (7.50 - 18.70)	10.02 ± 0.65 (7.20 - 17.80)	15.56 ± 4.00 (4.00 - 68.00)
2/Fall 2020	*S. pelagonicus*	77	9.87	216.12	11.08 ± 0.52 (5.60 - 20.50)	10.47 ± 0.50 (5.40 - 19.80)	21.89 ± 2.82 (1.80 - 95.80)
3/Winter 2019	*S. pelagonicus*	5	0.22	17.41	17.62 ± 3.22 (7.20 - 24.80)	16.98 ± 3.16 (6.90 - 24.10)	78.36 ± 34.76 (3.80 - 165.00)
	O. mykiss	19	0.84	21.46	12.63 ± 0.93 (7.40 - 25.90)	11.93 ± 0.88 (6.90 - 24.50)	25.42 ± 7.18 (3.60 - 147.20)
4/Winter 2019	*S. pelagonicus*	11	3.05	36.94	10.60 ± 0.49 (8.60 - 13.70)	10.09 ± 0.47 (8.10 - 13.10)	12.09 ± 2.03 (5.00 - 26.00)
	O. mykiss	1	0.27	23.61	19.9	19.00	85

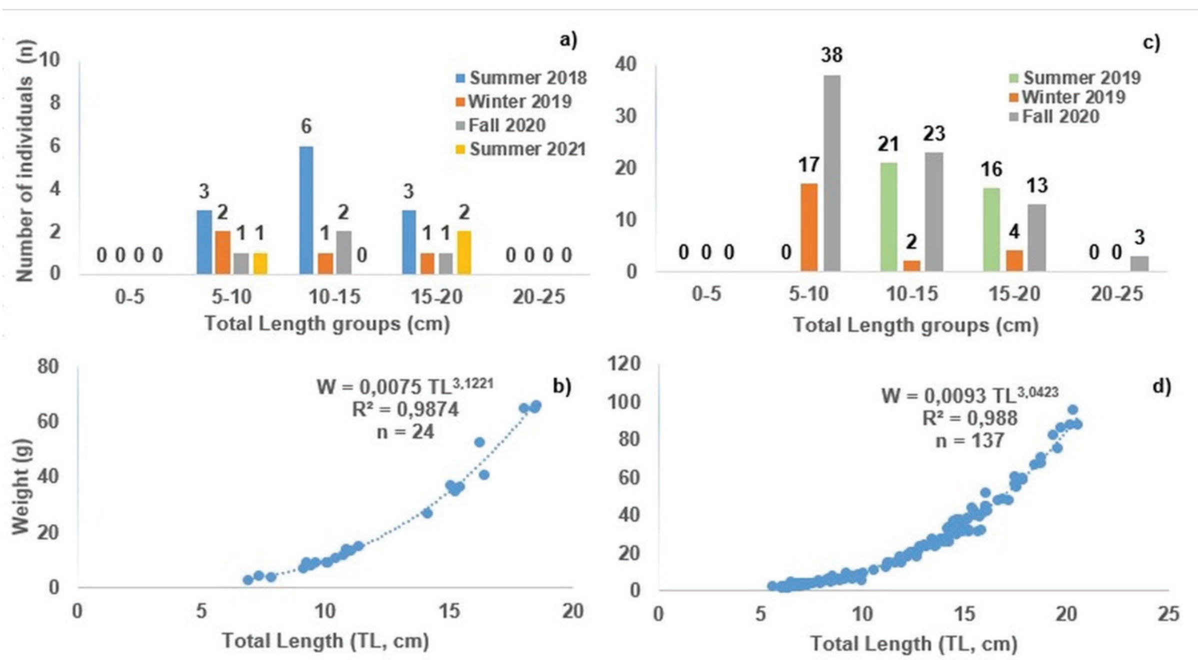

Fig. 2: Length - frequency distribution and Length (TL) - Weight (W) equations for site 1 (a, b) and site 2 (c, d).

4. Discussion/Conclusion

Pelagonian trout is a species with a limited range (Kottelat & Freyhof, 2007) and unfavourable conservation status (see https://nature-art17.eionet.europa.eu). The decline of its population in site 4, over time, has already been demonstrated (Cheimonopoulou *et al.*, 2019), while it co-exists with a larger population of *O. mykiss* in site 3 (HSP unpublished data since 2016). Therefore, newly discovered populations, as in Floros and Trypimeni streams (sites 1, 2), are important for the conservation of the species. The pristine conditions of these sites are pointed out by low conductivity values, maximum QBR index values, high altitude, relatively steep ground slope, lack of anthropogenic activities and absence of alien species, in contrast to lower altitude sites (3, 4). The NPUE value in site 2, in fall 2020 (low flow season), is considered relatively high and unlikely to be found, compared to previous data from site 4 (Cheimonopoulou *et al.*, 2019) and site 3 (HSP, unpublished data), indicating a robust population. The lack of larger individuals (TL > 20cm) may be related to lower growth rates, evident in streams with low conductivity values and low prey availability, arising from gneiss and siliceous watersheds upstream the higher altitudes sites 1 and 2 (> 1000m), as demonstrated elsewhere (Nicola & Almodovar, 2004). In addition, previous research (Cheimonopoulou, 2005; Cheimonopoulou *et al.*, 2019; HSP, unpublished data) showed the opposite for sites 3 and 4, where conductivity is higher and therefore conditions may be more favourable for growth (Nicola & Almodovar, 2004). In site 2, differences in NPUE and BPUE values and fish size in high and low flow season (early summer 2019 and fall 2020, respectively) indicate a seasonal fluctuation of population and individuals' size due to interannual variability. The *b* values demonstrated an isometric growth (b = 3), of *S. pelagonicus*, in both high altitude sites.

Pelagonian trout populations in Floros (site 1) and Trypimeni streams (site 2) are vulnerable to new water regime conditions induced by climate change (OECD, 2013), and anthropogenic activities, such as the construction of hydropower dams (Hocking *et al.,* 2021). Additional monitoring of sites and comparison on intraspecific/stream-specific level, are necessary to support the conclusions of this study. The collaboration with local inhabitants and authorities is of paramount significance for the establishment of management and conservation measures, in order to alter species' unfavourable conservation status and enhance its protection. Environmental Literacy and dissemination of scientific knowledge among local communities, could also provide substantial support towards this direction (Chepesiuk, 2007).

5. Acknowledgements

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VARIATIONS IN *POSIDONIA OCEANICA* MEADOWS IN THERMAIKOS GULF (NORTH AEGEAN SEA, GREECE)

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Abstract

Variations of *Posidonia oceanica* features were studied in Thermaikos Gulf (North Aegean Sea, Greece), in Epanomi region, which constitutes a Site of Community Importance (GR1220012) and a Special Protection Area (GR1220011), characterized by the presence of marine and terrestrial habitat types of community interest (Directive 92/43/EEC). Epanomi region and particularly Mytikas Cape, is the border between the inner and outer Thermaikos Gulf. Two sites were selected: Site 1, situated in the inner Thermaikos Gulf and Site 2, in the outer part of the Gulf. Although, the selected sites are very close to each other, the anthropogenic pressures' impacts, and the environmental factors vary. The aim of this study was to compare meadows' descriptors, between the neighbor sites. The study was conducted via the combination of nondestructive (in situ measurements) and destructive (sampling and laboratory analysis) methods. According to our results, the two meadows differ regarding specific metrics, with the meadow in Site 1 being degraded, comparing with the meadow in Site 2. This is believed to be related to its proximity to the urban center of Thessaloniki, the limited water renewal, the increased turbidity and the sediments' features.

Keywords: *Posidonia oceanica,* Thermaikos Gulf, meadows' features.

1. Introduction

Seagrass communities have an essential role in the coastal environments and are referred to, as "ecosystem engineers" (Boudouresque *et al.*, 2012). Given their increased light requirements levels, they are very sensitive to environmental changes that alter water clarity (Orth *et al.*, 2006). The decline of seagrass communities is a worldwide phenomenon, caused by increased exposure to natural and human-induced pressures (Hemminga & Duarte, 2000).

In the Mediterranean Sea, the endemic *Posidonia oceanica* (L.) Delile is the predominant seagrass species, covering 20%-50% of the seabed between 0 and 50 meters depth (Boudouresque *et al.*, 2012). Its meadows are considered to be one of the most important providers of ecological goods and services in the Mediterranean basin. At the same time, *P. oceanica* meadows are experiencing regression during the last century, especially near urban areas (Montefalcone *et al.*, 2007; Boudouresque *et al.*, 2012). The growing human activities on the coastline are the major cause of meadows' regression, either by causing mechanical damage or environmental alterations (e.g., coastal development, fish farming, trawling, anchoring, nutrient inputs) (Boudouresque *et al.*, 2012).

The sensitivity of *P. oceanica,* in combination to its response to various anthropogenic pressures, as well its wide distribution across the Mediterranean Sea, makes this ecosystem an effective bioindicator (Boudouresque *et al.*, 2012). The European Union, in order to maintain and recover the ecological quality of the marine environment, has established the Water Framework Directive (EU, 2000) and the Marine Strategy Framework Directive (EU, 2008). To this end, both directives recognize the use of *P. oceanica* as a suitable bioindicator for the ecological status assessment and therefore various indices have been developed and surveys have been conducted on the use of *P. oceanica* as a tool for assessing the ecological status of coastal waters (Gobert *et al.*, 2009; Lopez y Royo *et al.*, 2010; Gerakaris *et al.*, 2017).

This study aims to investigate the variations on specific *P. oceanica* features in the North Aegean, specifically at Thermaikos Gulf at two neighboring sites, located at both sides of Mytikas Cape, which is the border between inner and outer parts of Thermaikos Gulf. Thus, the selected sites are characterized by differentiation in the effect of natural and human pressures. Various descriptors were applied taking into account structural and functional features of *P. oceanica* meadows, across three biological levels: individual (plant descriptors), population (meadow descriptors) and community (associated flora/fauna descriptors) (Gerakaris *et al.*, 2021).

2. Material and Methods

2.1 Study area

Epanomi region (Thermaikos Gulf, North Aegean Sea) was selected as the study site. Thermaikos Gulf is a relatively closed and shallow Gulf, where numerous of economic – productive activities take place (shellfish farming, fisheries, navigation, industrial production, etc.). Moreover, rivers in the western coastal zone (Axios, Gallikos, Loudias, Aliakmonas) supply the Gulf with nutrients, resulting in its eutrophication. In addition, the city of Thessaloniki, with a population exceeding 1.5 million, is built in the coastal zone of Thermaikos Gulf.

Epanomi region, approximately 25 km from the city of Thessaloniki, is a very significant site, as for its ecological role, and hosts important fauna and flora species, protected by EC, International and National legislation. A part of the selected area constitutes a Site of Community Importance - GR1220012 and a Special Protection Area - GR1220011, being characterized by the presence of marine and terrestrial habitat types of community interest, including priority ones, such as *Posidonia* meadows, lagoons, dunes, etc (EU, 1992). In addition, the wider area of Epanomi is a summer touristic/leisure destination for the inhabitants of Thessaloniki and unfortunately numerous of uncontrolled touristic activities, that affect the conservation and/or restoration of coastal habitats, take place in the area.

For the needs of this research, two stations in the studied area were selected. One in the northern part of Mytikas cape, inner Thermaikos Gulf (Site 1) and the other located in the southern part of Mytikas cape, outer Thermaikos Gulf (Site 2) (Fig. 1). The two stations, although being very close to each other, exhibit different characteristics, in terms of natural and anthropogenic environmental pressures.

2.2 Methodology

Data were collected during 2020 from the two sites, in Epanomi area. At each site, the survey was conducted with the combination of nondestructive and destructive methods. Nondestructive methods included in situ meadows' observation and measurements (distribution, lower limit depth and type, shoot density). Destructive methods included random sampling (20 shoots/station), via SCUBA diving. In laboratory, the phenological analysis of the collected samples took place. Leaves were divided into three categories (adult, intermediate and juvenile), as defined by Pergent *et al.* (1995). The following phenological measurements were measured: leaf number, brown (dead) part of leaves, leaves' length and width. Based on the length and width of leaves, photosynthetic leaf surface per shoot has been calculated (cm^2/shoot) and taking into consideration the meadows' density, Leaf Area Index (m^2/m^2) was also calculated (Pergent *et al.*, 1995). In addition, Coefficient A (Leaf Apex Erosion) for adult and intermediate leaves was calculated (Giraud, 1977, Pergent *et al.*, 1995). Furthermore, the epiphytes were removed, and both leaves and epiphytes were weighted, after dried at 70°C for 48 h.

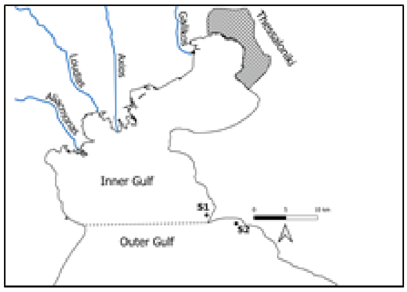

Fig. 1: Geographic location of the studied areas – Site 1 (S1) and Site 2 (S2) (Thermaikos Gulf, Greece).

3. Results

Based on in situ observations, via SCUBA diving, soft substrate dominates in both sites, either without vegetation or with the presence of angiosperms' meadows. In Site 1, the dominant community is that of *Cymodocea nodosa*, whereas in Site 2, particularly at the southern part of the Site, an extended meadow of *Posidonia oceanica* is present.

Site 1, which is located within the boundaries of the protected area, is characterized by the presence of a sparse, non-typical *Posidonia oceanica* meadow, while at Site 2, which is located outside the boundaries of the protected area, a relatively dense and typical form of *Posidonia oceanica* meadow develops.

The meadow in Site 1 is heterogeneous, extends from 4m to 14m depth and its lower limit is regressive, based on Meinesz & Laurent (1978). During the dives, visibility was very limited, due to turbidity. The meadow in Site 2 is continuous, forms an extensive zone, from 3m to over 23m depth and its lower limit type is stable.

In Table 1, the mean values of the measured and/or calculated *P. oceanica* features are given.

Table 1. *Posidonia oceanica* meadows' features at the two sampling sites (mean value ± standard deviation).

Feature	Site 1	Site 2
Lower limit depth (m)	14	23
Lower limit type (based on Meinesz & Laurent, 1978)	regressive	stable
Meadow cover (%)	25.12±6.84	72.35±3.62
Shoot density (shoots/m^2)	188.5±42.5	371.8 ± 64.3
Number of leaves per shoot	5.4±0.9	5.6±1.1
Adult leaves' length (cm)	21.4±10	25.1±9.1
Intermediate leaves' length (cm)	19.3±10.1	21.6±10.5
Juvenile leaves' length (cm)	3±1.4	4.1±0.7
Adult leaves' width (cm)	0.76±0.08	0.72±0.08
Intermediate leaves' width (cm)	0.74±0.07	0.68±0.05
Juvenile leaves' width (cm)	0.70±0.06	0.65±0.05
Coefficient A (%/shoot)	42.3±19.8	33.4±26.9
Adults	73.4±25.6	49.5±31.1
Intermediates	22.1±31.1	2.5±11.2
Leaf necrosis (%/shoot)	7.4±11.6	9.9±14.7
Adults	16.3±23.9	15.2±21.4
Photosynthetic Leaf surface (cm^2/shoot)	77.5±28.9	90.6±29.2
Leaf Area Index (m^2/m^2)	1.5±0.5	3.4±1.1
Epiphytic biomass (dry mg/shoot)	118±12.2	67.5±6.5
Leaf biomass (dry mg/shoot)	543.7±90.5	711.6±59.4
Epiphytic/Leaf Biomass	0.22±0.02	0.09 ± 0.01

4. Discussion/Conclusion

According to the results, the studied features of *P. oceanica* meadows evidence two different trends, when compared with previous surveys (Lazaridou *et al.*, 2011), as well as unpublished personal observations. Meadow in Site 1 showed signs of regression, in contrast to meadow in Site 2 which is in a stable status. This is believed to be related to the first's placement in the inner part of Thermaikos Gulf.

Eutrophication is a serious problem in coastal areas of the Mediterranean Sea, especially in closed gulfs such as Thermaikos. River inputs, in addition to urban and industrial wastewater, are the main factors contributing to eutrophication phenomena in the surrounding area. The ecological conditions in closed gulfs are gradually improving while moving from inner to outer regions (Simboura *et al.*, 1995). As the Cape of Mytikas is the geographical border between inner and outer Thermaikos Gulf, the current state of the meadows reflects the environmental conditions of these two sub-ecoregions. Similar observations, regarding differences in *P. oceanica* meadows along a distance from big urban centers and harbors have been reported from other Mediterranean areas (Montefalcone *et al.*, 2007).

Light is one of the most important parameters that determine the lower depth limit of the meadow, as well as the plants' growth. In detail, specific environmental factors associated with light (turbidity, nutrients concentration, primary production, temperature, etc.), are strongly related to *P. oceanica* meadows' differentiation among sub-ecoregions (Gerakaris *et al.* 2021). According to Pergent *et al.* (1995), lower depth limit is used as an indicator of water clarity. Based on this, Site 1 can be characterized as «Waters of increased turbidity», while Site 2 as «Waters of moderate turbidity». This characterization is in agreement with in situ observations, as visibility was restricted in Site 1, due to the suspended materials, resulting from the fine sediment as well. Nevertheless, we must be very careful in such characterizations, as there are other parameters that prevent the growth of a species at a given depth, such as the lack of a suitable substrate, etc.

Regarding the meadows' density, according to the classification of Giraud (1977), the meadow on Site 1 is 'very sparse bed undergoing regression' and the meadow on Site 2 is 'sparse beds possibly tending toward regression'. Additionally, the meadows' densities measured for both locations at 10 m depth are characterized as abnormal for Site 1 and normal for Site 2, based on Pergent *et al.* (1995).

The number of leaves, leaves' length and width are similar at both sampling sites. The coefficient A (%/shoot) describes the percentage of the leaves having lost their apex, due to water movement and/or grazing. The high value in adult leaves of Site 1 is probably due to grazing. As for the metric of leaves with necrosis marks/per shoot, adult leaves necrosis is 15.2% for Site 2 and 16.3 for Site 1, while intermediate leaves necrosis is 0% for both sides. The difference of LAI (in m^2/m^2) between the two sites is significant and occurs, due to the difference of meadows' density in two sites. As for the higher value in epiphytic biomass in Site 1, this is most likely related to the proximity of this region to the inner Thermaikos Gulf, where there is a high input of nutrients.

According to Gerakaris *et al.* (2021), when comparing spatial patterns of *P. oceanica* features in Aegean and Ionian Seas, meadows developing near mouth/estuaries and small rivers basins exhibit the lowest mean values in most of the meadows' population metrics and the highest of community level metrics. This is the case in our survey as well, as metrics such as shoot density; meadow cover, etc. are higher in Site 2, whereas the epiphytic biomass is higher in Site 1.

Based on: i. The significance of Epanomi region as a protected area, hosting numerous of habitats and species of European Community interest, ii. Its location and proximity with Thessaloniki city, and iii. The degradation of *Posidonia* meadows, during the last decade, the importance of monitoring as well as management of the marine habitat types arises.

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SHORELINE DISPLACEMENTS BASED ON REMOTE SENSING DATA OVER A PERIOD OF 73 YEARS AT SCHINIAS -MARATHON NATIONAL PARK, GREECE

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Abstract

A shoreline detection through remote sensing data covering a period of 73 years was realized at the coastal front of Schinias-Marathon National Park, in order to identify current trends of the coastline and the reasons for the intense erosion that has been caused. Comparison of the digitized historical coastlines, with the use of the Digital Shoreline Analysis System software, showed that the entire study area has undergone retreat in the last 73 years with changes ranging from 5 to 36 meters and setback rates from 0.07 to 0.5 m / yr. The setback rate seems to have variably changed through space and time, with the construction of the Marathon Dam severely affecting the sedimentary equilibrium of the beach until the 80's, while presenting gradual signs of restoration from then onwards. As compared to the western, the eastern part of Schinias beach shows high values and rates of setback, despite it being more sheltered from wave action. This may be due to various geomorphological and biotic features, namely the western part presenting higher beach foreshore slopes, slightly coarser sediments, as well scattered 'Posidonia reefs' in the sublittoral zone, probably acting as underwater barriers which further enhance protection at the beach front.

Keywords: Schinias-Marathon National Park, Shoreline displacement rate, Shoreline evolution, DSAS.

1. Introduction

Coastal areas are highly dynamic systems of particular socio-economic and environmental interest. They are sensitive environments affected by the interaction of multiple physical factors such as tidal inundation, sea level rise, land subsidence, erosion or deposition, etc. Environmental deterioration and beach erosion are major global problems already (Eurosion, 2004;), and are only expected to worsen with climate change. Monitoring of coastal changes allows the spatial allocation of erosion dangers and prediction of their evolution (Nassar *et al*., 2018). This study focuses on the coastal front of Schinias, to investigate the trends of shoreline displacement, as well as possible causes of the erosion phenomena that have been taking place in the area for several decades.

The beach of Schinias-Marathon National Park covers 3,165 m of coastline, which is part of the wider coastal area of Marathon Bay, located at northeast Attica, Greece. The broader area has great historical and social significance. The Marathon coastal plain has been continuously inhabited since the Neolithic and according to archaeologists, has remained geomorphologically stable since Classical times (Kraft, 1972). The extensive wetland of Marathon, also known as the Great Marsh, is separated from the sea by a barrier beach with low sand dunes. This area was a lagoon 3,500 yrs BP (Pavlopoulos *et al*., 2006). It is characterized by low inclinations and smooth slopes and it is typical of coastal plains of Greece. In the wider onshore area of Marathon Bay, military bases and archeological sites are present. In the area of the now drained Great Marsh, an artificial canal was constructed for the 2004 Olympic Games in Athens to serve as the Olympic Rowing Center. The plain is widely cultivated, being one of the main vegetable producer areas in Attica. Most of it is now characterized by intense coastal tourism development.

In 2000, Schinias was declared a National Park (Government Gazette 395 D2000). The area is also part of the Natura 2000 European Network of protected areas (GR3000016 - Schinias Wetland and GR 3000003 – Schinias-Marathon National Park).

The beach of Schinias has an almost linear shoreline with an almost WSW-ENE direction and it is characterized as an extended low-lying sandy beach (Dimou *et al.,* 2014) comprising important ecological features, such as the protected pine forest (i.e., *Pinus pinea* and *Pinus halepensis*). No significant changes have been observed on the coastline limit between 1889 and 1938. A significant retreat of the shoreline near the estuary of Kenourgio Rema took place in the 1950s and 1960s with a rate of about 2 m / y, slowing down to 1 m / y within the next two decades (Maroukian, *et al.,* 1993). This is mainly caused due to the reduction of riverine sediment supply by the Marathon dam built in 1929 to meet Athen's water supply demands, together with sand extraction activities taking place on the lower course of the riverbed (Dimou *et al.,* 2014).

Shoreline change detection with remote sensing techniques is a valuable means in monitoring coastal areas through time (Nassar *et al.,* 2018). Collection and analysis of these data is essential in coastal management and planning, to help prevent impacts on shores on both anthropogenic and natural assets (Ciavola *et al.,* 2011).

2. Materials and Methods

The quantification of long-term shoreline displacements was performed using remote sensing data covering a period of 73 years (1945 - 2018). In particular, a comparison of digitized coastlines, which were extracted from the respective orthophotomosaics and images created (one for each time period), was conducted (Tsokos *et al.,* 2018). Coastlines were digitized in ArcGIS (version 10.2) using WGS '84 World Mercator as the geographic reference system.

Historical high-resolution analog aerial photographs and recent high-resolution digital satellite imagery were collected, for the detailed extraction of the exact location of the shoreline for each time period. The aerial photographs were taken during the years 1945, 1960, 1969, 1988, 1996, 2001 and 2010. The satellite images were taken in 2012, 2014, 2018

The quantification of long-term shoreline displacements was made using the Digital Shoreline Analysis System (DSAS) software, which is a sub-routine of ArcGIS 10.2 software (Himmelstoss *et al.,* 2018). This was accomplished by assessing 322 transects, placed perpendicular relatively to the historical shorelines from a stable baseline (on the land side) parallel to the shorelines (Tsokos *et al.,* 2018). The distance between the transects was set at 10 m while the length of each one was 200 m. Statistical values of the measurement have been denoted as negative for erosion rate and positive for accretion rate. The shoreline uncertainty variable for each coastline was defined as the spatial resolution (cell size) of the corresponding mosaic or image whose coastline was digitized.

At each transect the Net Shoreline Movement (NSM), the Shoreline Change Envelope (SCE) and the End Point Rate (EPR) was calculated. Selectively for some transects, the linear correlation of the points (Linear Regression Rate - LRR), which intersects a specific transect of the coastlines, was calculated and analyzed to check for systematic shifts.

3. Results

The results of NSM show that the coastline in the entire study area (all 322 transects) has undergone retreat in the last 73 years. The largest changes (25 - 30 m) are observed at the eastern part, with the maximum value (-36 m) at transect 3 at the eastern end of the beach. From east to west, values gradually decrease (minimum value -5.4 m at transect 320). The average change of the coastline across the study site is –15.9 m. The maximum observed distance (SCE) values in the study area range from 39.4 m (transect 6) to 5.9 m (transect 304). Also, the zone of total range displacement gradually decreases westward.

The average displacement rate in the study area shows only negative values (regression) for the period 1945 – 2018 (Fig. 1a). The maximum setback value is found in transect 3 (-0.5 m / yr) and the minimum in transect 320 (-0.07 m / yr). As for the above parameters, the values of the erosion rate in the eastern part are higher and decrease to the west. The overall average coastline retreat rate for the study site is 0.21 m / yr. From the study of the Linear Regression Rate (LRR) at transects 3 (Fig. 1b), 120 (Fig. 1c), and 240 (Fig. 1d), the regression rate has been estimated not to have been constant: changes from the period

1945 – 1969, where the coastline is constantly eroding, to the period 1988 – 2018, where erosion phenomena alter with periods of advance, are also evident from the linear correlation – R^2 of the points.

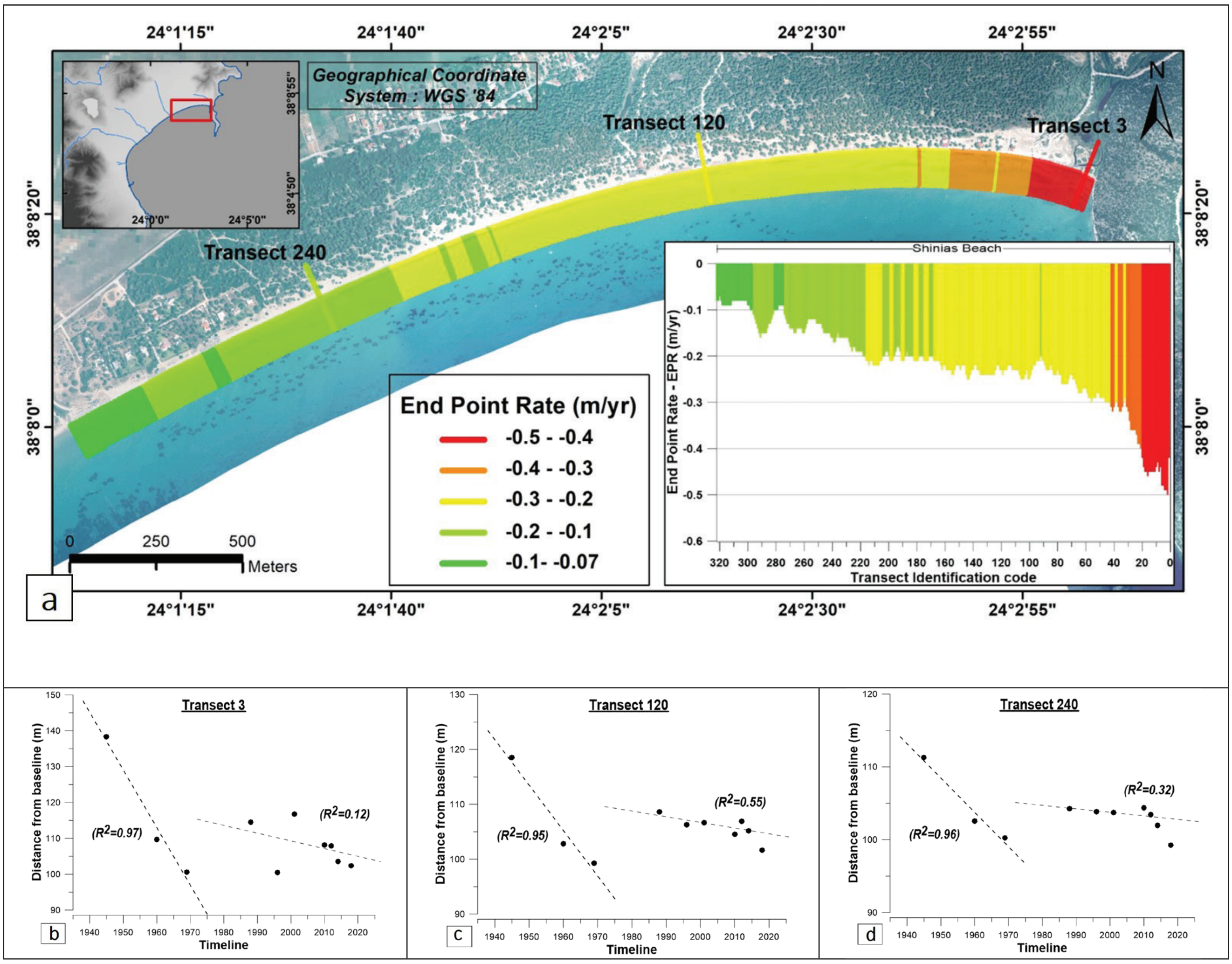

Fig. 1: Representation of the shoreline displacement rate at each transect (in meters per year) across the coastal front of Schinias-Marathon National Park, for the period 1945 – 2018 (a), and the positions where the representative transects 3 (b), 120 (c) and 240 (d) intersect the coastline as well as the linear correlation of the points at each transect.

4. Discussion/Conclusion

Historical change analysis of Schinias presents an ongoing coastline setback from 1945 until recent years, resulting to a loss of land surface of the order of 91,217 m^2. The operation of the Marathon Dam caused high rates of coastline regression for about 25 years and severely disturbed the sedimentological balance of the wider coast, indicating that torrents flowing into the Gulf played a decisive role in the sediment supply of the beach. The regression rate seems not to be constant but changes over large time periods, i.e., 1945 - 1969 and 1988 – 2018. It seems that part of the coastal sedimentological balance disrupted by the operation of the Marathon dam was rather restored from the 80's onwards, when regression rates decrease, and periods of shoreline advance appear. The western part of Schinias beach shows smaller changes and regression rates, although it is directly exposed to predominant south waves, as opposed to the eastern part which appears more sheltered due to Kynosoura cape. This may be explained by wave diffraction due again to Kynosoura cape: the direction of south waves that affect the coast create a coastal current alongside the coast with east direction at Schinias beach which changes direction to the south; being bounded by Kynosoura cape, this results in more intense beach erosion and deposition of sediments at shallow sublittoral depths (Poulos *et al.*, 2004; Kourliaftis, 2019). At the same time, the lower setback values at the western part may further be explained by higher slopes of the beach foreshore, coarser sediments (as compared to finer ones prevailing at the eastern side where massive *Posidonia* banquettes occur), as well as the presence of '*Posidonia* reefs' probably acting as underwater barriers which further enhance protection at the beach front (Kourliaftis, 2019). Also noteworthy for the period 2014 – 2018, milder regression and lower setback values are generally observed along the beach of Schinias, particularly pronounced at the easternmost part of beach where the greatest setbacks had been previously detected. This finding interestingly coincides with regulations set forth in 2018 by the Management Board of Schinias - Marathon National Park, forbidding removal of Posidonia banquettes from this specific area, and may further corroborate the importance of undisturbed banquettes in maintaining the beach sediment budget (De Falco *et al.,* 2003). According to literature, other factors that have been suggested as further affecting this coastline's regression are the human intervention in riverbeds (i.e., extensive sand extraction) (Kouli *et al.*, 2009), drying out of parts of the Marathon Wetland, subsidence of the alluvial riverplain due to the inactivation of torrents, and over-pumping of groundwater resources (Aucelli *et al*., 2017). More extensive monitoring, considering both long-term and seasonal changes is required. In this way, coastal trends and spatiotemporal sedimentological changes in direct relation with key environmental factors will be better comprehended, providing valuable input to management planning and decision-making processes for this area of high ecological interest.

5. Acknowledgements

This research was funded by the Hellenic Society for the Protection of Nature (HSPN) and carried out in the scope of the international InterregMED project "POSBEMED2: Governance and Management of Posidonia Beach-Dune Systems Across The Mediterranean".

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INTERGRATED APPROACH FOR HABITAT MAPPING USING UAV TECHNOLOGY AND ITS CONTRIBUTION TO FURTHER IN SITU INVESTIGATION

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Abstract

This study focuses on the use of UAV technology for coastal habitat mapping prior to dredging operations in a case study area (Vouliagmeni bay, Attica). Scientific flights with UAV equipped with RTK technology were planned in order to acquire the proper data. Also, GCPs were placed in order to augment the accuracy of the georeferenced information thus, securing the high-quality of the final product (i.e., orthophotomosaic). Prior to the flight, GCPs were surveyed with RTK GPS. The results from the RTK GPS survey along with the images collected from the drone flights, were processed via the software Pix4D react. The ArcMap software. v 10.7.1 was used in order to georeference the orthophotomosaic to its correct position (according to RTK-GPS). The production of the orthophotomosaic helps towards an accurate detection and estimation of habitats' extent as well as for the planning of detailed fieldwork to evaluate our results.

Keywords: Vouliagmeni, Habitat mapping, Posidonia oceanica, UAV, GCPs.

1. Introduction

Sensitive coastal ecosystems such as seagrass (marine angiosperm) meadows are highly affected by several human activities (i.e., dredging, beach stabilization and construction of permanent structures, e.g., harbors). This fact contribute mainly to changes of the sedimentary dynamics and thus, to their loss (Ruiz and Romero, 2003; Badalamenti *et al.*, 2006).

When such human activities are present in an area, where the presence of the strictly protected *P. oceanica* meadows (Habitat Type 1120* sensu the Habitats Directive 92/43/EEC) is recorded, detailed mapping of the meadows is considered as a critical monitoring tool. This study focuses on the use of UAV technology for high resolution coastal habitat mapping prior to dredging operations and the construction of the new marina in a case study area (Vouliagmeni bay, Attica).

2. Materials and Methods

The coasts of this area generally present smoother slopes than those of the southeastern part of Attica, except for the coastal areas consisted by alpine formations.

In the wider area of Vouliagmeni (Fig. 1) a scientific flight by using UAV was held on 12 May 2021 by to monitor-map with significant accuracy and analysis, the current extension of *P. oceanica* meadows, in order to be studied extensively and the final results to be evaluated.

The UAV, enterprise grade, DJI Matrice 210 RTK v.1 used for the data collection, it is equipped with RTK technology which makes it ideal for coastal environments, since it can withstand strong winds. The RTK system of the drone corresponds to its own geo-fence created on the air and not on the ground, giving a substantial accuracy and stability during flight only.

Due to the smoothness of the ground (low inclination beach and the surface of the sea), the flights were horizontal parallel to the ground at a certain altitude AGL (above ground level) (Trajkovski *et al.*, 2020). The UAV sensor was headed to 80 degrees (nadir). The images were taken in a specific overlay (75% side, 75% front), in order to achieve the most accurate 2D representation of the study area. The software used to create the flight plans was Pix4D capture (v.4.12.1).

The use of ground control points (GCPs) in such research is fundamental because they increase the accuracy of UAV imagery at the cm level (Turner *et al.,* 2016; Kabiri *et al.,* 2020; Fallati *et al.,* 2020; Lu *et al.,* 2020). Ten (10) targets-GCPs, three (3) terrestrial targets-GCPs and seven (7) marine targets-GCPs (buoys) were used for data collection. The terrestrial targets were black and white in color with a defined center, and exact dimensions of 25 x 25 cm. The marine targets were buoys, either inflatable or plastic-fixed (of the construction site), which were scattered throughout the study area. Prior to the flight, GCPs were surveyed with RTK GPS. The buoys were anchored to the bottom and they were stable at the sea surface. Since the weather conditions of the survey were great the buoys didn't move more than few centimeters from their current position. Nevertheless, the sea surface is not stable but for the purpose of the study (monitor subsurface habitats) the use of such technique is ideal. Especially in enclosed bays such as the area of Vouliagmeni.

The RTK GPS survey results and the images collected from the drone flights were processed via the software Pix4D react. This software was chosen over the "conventional" ones because it can make high-precision orthophotos from images taken over the sea. "Conventional" software does not have the ability to build such orthophotomosaic, or it has significant inaccuracies due to the lack of fixed points since the sea surface does not remain stationary. The ArcMap software v10.7.1 was used in order to georeference the targets to their correct position (according to RTK-GPS), a process that dramatically increased the accuracy of the results. They also improved the results from Structure from Motion (SfM) and the overall photogrammetry process in general (Windle *et al.,* 2019).

3. Results

For the marine area, all the necessary photos were taken from an altitude of 120 m AGL (Above Ground Level), while for the coastal area, the flight altitude was fixed at 100 m (AGL). The GSD (Ground Sampling Distance) was calculated at 2.87 cm/pixel. Ten (10) GCPs were generated and calculated during the construction of the orthophotomosaic giving an RMSE (Root Mean Square Error) of approximately 1.48 m for the XY of the survey area. This result is due to the presence of the buoys as GCPs. However, employing buoys like GCPs improved the absolute horizontal accuracy to ~2 m, which is not much at first glance, nevertheless, it is much better than the ±10m accuracy given by the automatic georeferencing of the drone (without GCPs). The area represented was 623,824 $m^{2,}$ and the coordinate system was chosen to be WGS 84.

As observed, the higher GSD of the drone photos could produce a precise and accurate thematic map including the different habitat types before the evaluation comprised by in situ investigations.

The orthophotomosaic of the study area that was constructed from the interpretation of the images through the Pix4d react v. 1.3.0. software is given in Figure 1. In total, three different marine habitats were identified: *Posidonia* meadows, shallow rocky reefs, and sandy areas.

The field survey (i.e., GCPs-buoys placement and drone flight) was conducted within 2 h, while lab processing (i.e., photo mosaicking, on-screen digitizing, and topological mapping) took less than 40 h.

4. Discussion/Conclusion

This study has demonstrated the potential of UAV data coupled with SfM analysis as a tool for mapping and monitoring sensitive coastal habitats over time. Although with certain limitations, the resulted accuracy (1.48 m) achieved with the combined use of GCPs and RTK, as well as, the time and cost efficiency of the proposed protocol, indicate that high-resolution imagery collected with UAVs can be adequately analyzed for habitat mapping and monitoring purposes.

This technique is beneficial since it is effective, time-saving, accurate (in flight-geolocation), and it has great applicability, especially in studies like the abovementioned one and after events that could be harmful to the coastal marine ecosystems. The place of the buoys in such type of research seems to have good results and augments the accuracy of the products derived from the current survey.

From the production of the orthophotomosaic a rough estimation of the current condition can be provided, and a detailed fieldwork plan can be made. In addition, the maps and the DTMs generated in this work prove that quantitative data are successfully produced for a long-term and cost-effective monitoring program to check the dynamics of shallow-water habitats.

In addition to all the above, the proposed technique can highly assist in well-organized fieldwork activities to monitor and evaluate marine habitats' state and extent and, thus, provide important scientific information for coastal management and conservation.

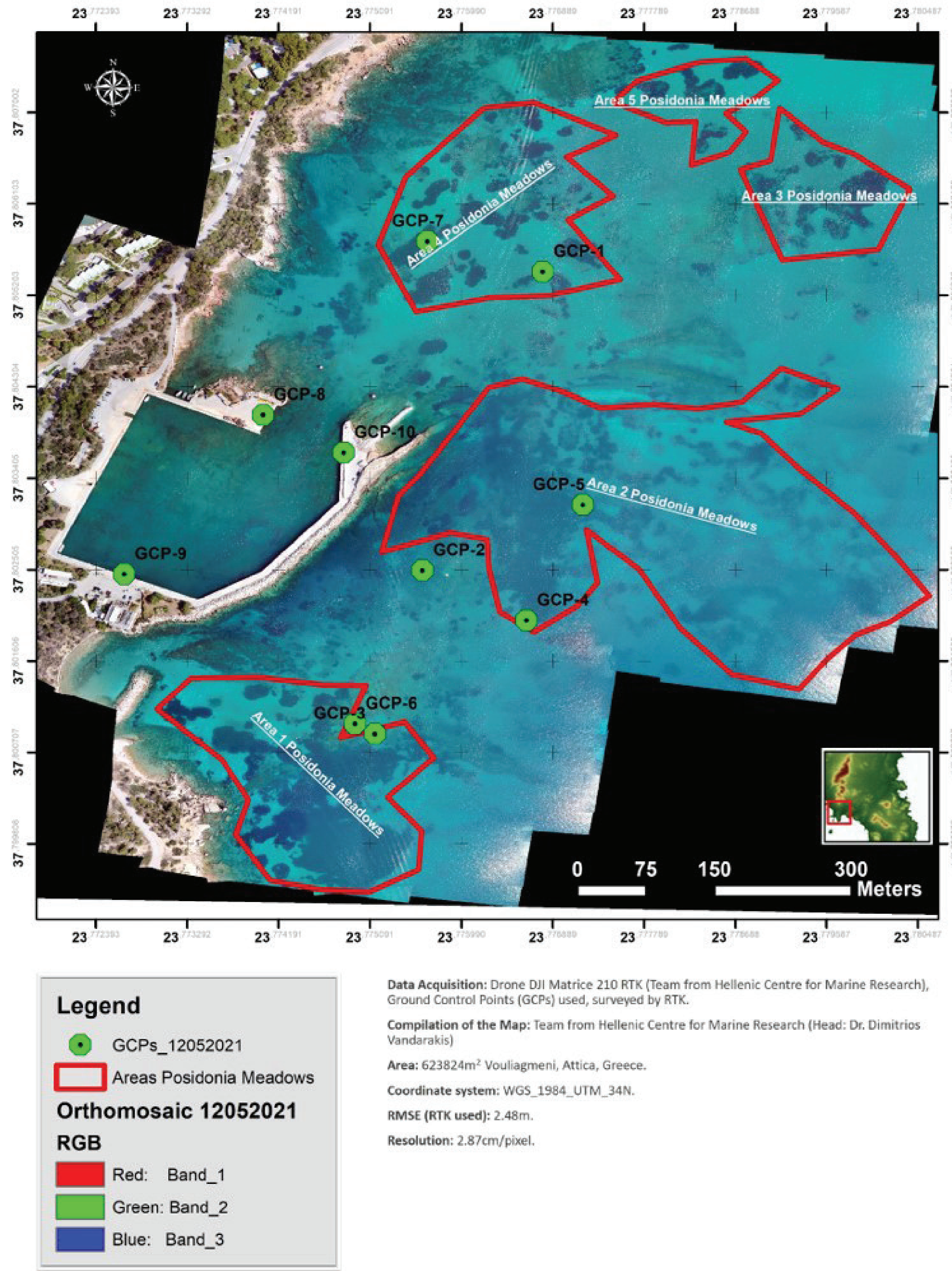

Fig. 1: Orthophotomosaic of the study area. Ground control points (GCPs) are presented.

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IN VIVO & *IN VITRO* EVALUATION OF PRAZIQUANTEL TOXICITY

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Abstract

This study assessed the toxicity of praziquantel (PZQ), a promising fish anthelmintic on cell culture and selected target organisms. The evaluation of PZQ toxicity on cell culture exhibited mild cytotoxicity. Likewise, measurements of PZQ toxicity on *Pseudomonas* sp. and *Daphnia magna* revealed similar findings. In agreement with existed limited knowledge, this trial suggests that PZQ use in aquaculture is relatively safe for the treated animal and the environment.

Keywords: Praziquantel, antiparasitic, cytotoxicity, bacteria, invertebrates.

1. Introduction

Praziquantel (PZQ) is a synthetic broad-spectrum anthelmintic, widely used in veterinary and human helminthiasis. In farmed fish, the drug has proven to be effective against monogeneans infecting the gills, skin or branchial cavities, as well as against intestinal cestodes. PZQ has proven to be an essential anthelmintic for Asian fish farming where it is used in a dietary premix as well as for more than 2 decades in the Norwegian salmon farming industry based on "off-label" application. Apart from bath-administered formalin, there are currently no anthelmintic compounds as authorized dietary premixes in farmed fish in the EU. Thus, PZQ constitutes a good candidate substance based on its proven efficacy against parasitic flatworms of fish elsewhere (Bader *et al.,* 2019) or in Mediterranean area (Rigos *et al.,* 2021). PZQ has a wide margin of safety, and is generally considered safe in treated animals, with very low toxicity in several tested species (Norbury *et al*., 2022); however, no PZQ studies have been carried out in Mediterranean farmed fish species. Since little work has been done examining the effects of PZQ in the aquatic environment or against target organisms, the aim of this study was therefore to evaluate the toxicity of PZQ on selected target organisms and cell culture.

2. Material and Methods

2.1 Cell culture

The cellular toxicity of PZQ was evaluated in epithelial cell line cultures. RTgill-W1 (rainbow trout gill cell line) monolayers were grown in 75 cm^2 polystyrene tissue culture flasks at 19ºC in the basal medium, L-15, supplemented with 10% fetal bovine serum (FBS). The cells were dispersed with trypsin-EDTA, plated in flat bottom 96-well plates for cell cytotoxicity assays. The cell lines were used between 3 and 5 passages.

2.2 Cytotoxicity assays

The cytotoxicity of PZQ was evaluated in 96-well plate cultures applying the MTT assay for energy metabolism and mitochondrial function. In a flat bottom 96-well plate, 15×10^4 cells per well were placed to a final volume of 0.2 mL and incubated at 19ºC for 24 h until adherence. Next, the medium was replaced and PZQ was diluted in DMSO (final concentration 2% v/v) and added in culture in different concentrations. Cells were measured using MTT after 24 h. For this purpose, 0.02 mL MTT was added into the cell culture and subsequently cells were incubated for 4 h at 19ºC. At the end of incubation, MTT was discarded and replaced by DMSO. Optical density was measured on a Microplate Spectrophotometer at 550 nm.

2.3 Daphnia magna toxicity assay

The acute *D. magna* toxicity test was used to determine the toxicity of PZQ. Hatching of *D. magna* ephippia was carried out in standard FW (ISO medium, formula according to ISO 6341) at a temperature of 20±1°C and lateral illumination (6000 Lux). Toxicity assays were carried out in two independent experiments in 24well plates with four replicates for each concentration. After 72 h, twenty neonates were transferred with a Pasteur pipette into the 24-well plates that were filled with 10 mL of dilution water (control well, 2.5 % DMSO) or 10 mL of the respective concentrations of PZQ in 2.5% DMSO. Plates were incubated in the dark at 20°C for 48 h. Mortality was assessed by recording the number of individuals with no movement of their appendages within 10 s and is expressed as the percentage of the total number of assessed individuals for each concentration.

2.4 Marine bacteria growth assay

The ability of PZQ to inhibit the growth of bacteria was assessed using marine bacteria such as *Pseudomonas* sp. Sterile filter paper discs (4 mm) were loaded with 40 μL samples of serial dilutions of PZQ, allowed to dry at room temperature and then were placed on agar plates (TSA 2%), which were seeded with the strain of bacteria. Plates were incubated for 24 h at 35°C. Marine bacteria growth inhibition was determined by measuring the zone of inhibition in mm around the filter paper disc. As a positive and negative control, standard discs were loaded with 40 μL of 0.5 M penicillin G or solvent (40 μL DMSO), respectively.

2.5 Statistical analysis

For statistical comparisons between groups, one-way ANOVA followed by Dunnett's multiple comparisons test at P<0.05 was used where appropriate using GraphPad Prism v. 8.4.2. For LC_{50} (median lethal concentration) all data were fitted to a three-parameter logistic curve according to the following model: Y=Bottom + (Top – Bottom)/(1+10^((X – LogIC50)) depending on the trend of the curve.

3. Results

3.1 Cytotoxicity assay

The results showed that very low and low concentrations (0.1 ng/L-1 mg/L) of PZQ did not have an effect on mitochondrial activity of the rainbow trout gill cell line utilized since no statistical difference was observed between PZQ-treated and control cells at these PZQ dosages (Fig. 1). Overall, the results of one-way ANOVA with the Dunnett's multiple comparisons test between control and the various concentrations of PZQ revealed statistical significance between the 10-200 mg/L concentrations of PZQ and the control group (P<0.05) indicating cytotoxic effects of PZQ on RTgill-W1 cells only at high concentrations.

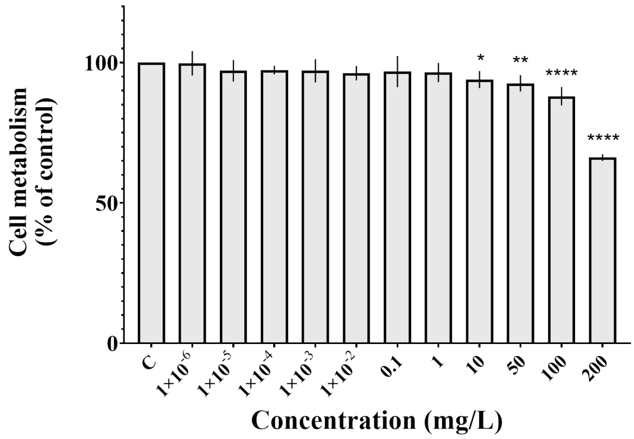

Fig. 1: Results of MTT assays on RTgill-W1 cells in the presence of varying concentrations of PZQ for 24 h. The cumulative results from five independent experiments with three replicates each are shown and are expressed as percentage (mean ± SD) of PZQ-treated cells compared to untreated (control, C) cells. * indicates a statistically significant difference. *: P < 0.05; **: P < 0.01; ****: P < 0.0001.

3.2 Evaluation of toxicity against Daphnia magna

The selected concentrations were subsequently tested in *D. magna* toxicity assays at 24 and 48 h of exposure. These toxicity assays were conducted to identify if PZQ exhibits toxicity towards another invertebrate organism. PZQ showed low toxicity against *D. magna* and it was lethal only at high concentrations (>50 mg/L). Results of one-way ANOVA with the Dunnett's multiple comparisons test between control and the various concentrations of PZQ at 24 h revealed statistical significance between PZQ concentrations of 10 mg/L to 250 mg/L and the control group (P<0.0001). Furthermore, at 48 h the multiple comparisons test revealed statistical significance between 1 mg/L to 250 mg/L of PZQ and the control group (P<0.0001). The results are schematically shown in Figure 2.

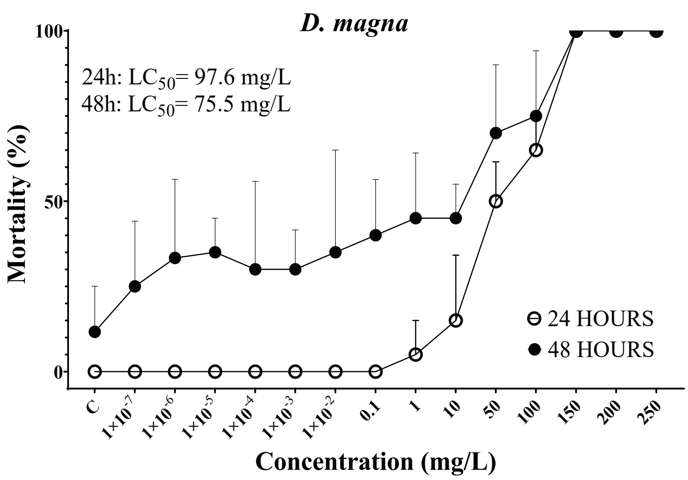

Fig. 2: Results of *D. magna* toxicity assays in the presence of varying concentrations of PZQ. The cumulative results from two independent experiments with four replicates each is shown.

3.3 Evaluation of growth of marine bacteria

The activity of PZQ was evaluated against marine bacteria known to exist in marine fish and coastal marine areas. The results showed that that PZQ induced no effect on the growth of the tested bacterial strain (Fig. 3).

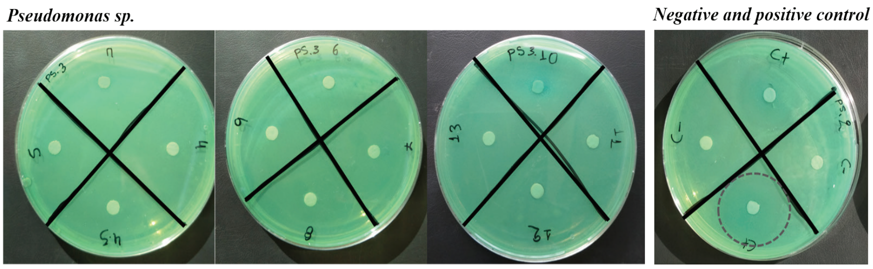

Fig. 3: Results of marine bacteria growth assay in the presence of PZQ. TSA agar plates were split into four sections (each corresponding to a serial dilution (1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}, 1×10^{-3}, 1×10^{-2}, 0.1, 1, 10, 50, 100 and 1×10^{3} mg/L). Indicative plates from the experiment with three replicates each are shown.

4. Discussion/Conclusion

The findings of this study indicated that low concentrations (< 10 mg/L) of PZQ induced no effect on RTgill-W1 cells mitochondrial function after 24 h of exposure, while medium and high concentrations of PZQ exerted cytotoxic effects by inhibiting the mitochondrial function of the cells; however, no more than 12% for the 100 mg/L concentration and 34% for the 200 mg/L concentration compared to the control cells (Fig. 1). Based on ecotoxicity tests against *D. magna* (24 h: LC_{50}= 97.6 mg/L, Fig. 2) which were also confirmed by marine bacteria growth assays where PZQ induced no effect on the growth of the tested bacterial strain, the toxicity of PZQ was found to be low (Figs. 2, 3). This conclusion is in agreement with previous findings in acute and long-term experiments (Frohberg, 1984; Cioli *et al*., 2003).

While the environmental concentrations are likely dependent upon dose and stocking density, there is no evidence that high concentrations of PZQ are being discharged into the environment after a treatment regime. Data from a previous pilot study have shown that PZQ was detected in very small concentrations (0.003 mg/L) in water collected from the surface water of the net pen during PZQ administration and was not detected in the sediment (Ido *et al*., 2019). Similarly, another study demonstrated that 2 mg/L PZQ was degraded below the detectable limit in a recirculating system within 2-3 days following administration (Thomas *et al*., 2016).

Despite the expected release of PZQ into the surrounding environment following its use in aquaculture, a limited number of trials have been performed to observe the effects of PZQ on arthropods and plants. Going forward, there is a need to investigate the fate of this anthelminthic in aquaculture systems in order to more accurately assess concerns about environmental contamination and ecological impacts.

5. Acknowledgements

The project is co-funded by Greece and the European Union under the Fisheries and Maritime Operational Program 2014-2020 (75% EMFF contribution, 25% National Contribution).

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THE DEVELOPMENT AND APPLICATION OF AN UPGRADED SAMPLING PLATFORM FOR THE STUDY OF BENTHIC BOUNDARY LAYER HABITAT

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Abstract

The marine sediment-water interface is the most extensive habitat occupying 75% of the Earth's surface. Fluxes of materials across this interface and the mechanisms that mediate and constrain those fluxes are likely to be of global importance. Since 2001, IMBBC has developed and applied in the field a novel sampling platform for the study of Benthic Boundary Layer (BBL) habitat providing findings that contribute towards a better understanding of the role of biodiversity related to the sediment-water interface with respect to nutrient regeneration, carbon cycling and energy transfer to higher trophic levels. The upgrading of this bottom sampling system was currently funded aiming to simulate the natural resuspension of the sediment surface for the study of marine biodiversity at the sediment-water interface while ensuring reliability and safety during operation. The upgraded sampling platform is described. Preliminary data derived from its application are presented as well as future challenges.

Keywords: sampling methods, bottom sampling, hyperbenthos, marine crustaceans, continental shelf.

1. Introduction

The marine sediment-water interface is one of the most extensive habitat on the planet occupying 75% of the Earth's surface. Because of differing ecosystem structure above and below the sediment-water interface, the ecologists who study these domains use different techniques and ask different research questions leading often to scientific isolation of the two domains. Consequently, the role of biodiversity related to the interface between the sediment and the overlying water (benthic boundary layer, BBL) is underestimated, especially in studies of benthic-pelagic coupling related to energy fluxes and also in holistic approaches of the marine ecosystem (Gili *et al*., 2020).The BBL, characterized by strong gradients of flow and high concentrations of dissolved and particulate matter supports both benthic and pelagic, mostly macrofaunal hyperbenthic, species classified in different groups according to their different mobility and bottom dependence (Dauvin & Vallet, 2006). For the study of BBL macrofauna, specially designed samplers, generally known as hyperbenthic or suprabenthic sledges, have been used over the last 50 years. Nevertheless, there are still practical technical difficulties in sampling efficiently the lowermost layer of the water column above the seabed where most of these species are concentrated (Koulouri *et al*., 2013).

Since 2001, a novel sampling platform (a modified hyperbenthic sledge equipped either with planktonic nets or water bottles) for the study of BBL habitat has been developed and applied in the field providing information and new insights towards a better understanding of the role of biodiversity related to the sediment-water interface with respect to nutrient regeneration, carbon cycling and energy transfer to higher trophic levels (Koulouri *et al*., 2003; 2005; 2009; 2013; 2015; Dounas, 2006; Dounas *et al*., 2007). The upgrading of this bottom sampling platform was funded by a project entitled "MOdern UNifying Trends in marine biology-MOUNT" aiming to simulate the natural resuspension of the sediment surface for the study of marine biodiversity at the sediment-water interface while ensuring reliability and safety during operation. The objectives of the present study are: a) the description of the upgraded sampling platform; b) the presentation of preliminary data derived from its application and, c) future challenges.

2. Material and Methods

2.1 Development and implementation of the sampling platform

Upgrading the system with individual components and materials had benefits in several areas, such as full manufacturing quality control of all parts used in the upgrade; full control of all materials and parts manufactured by contractors on our behalf with our own technical design and specifications; increased capabilities provided to the end user of the system (e.g. ability to connect more instruments and more complex ones, such as releasers, sonar, etc.); better video quality (with the ability to upgrade the video resolution to HD for scientific observations, etc.); use of fiber optics for data transmission, resulting in the elimination of any electronic noise during the transmission of data and video; low maintenance costs and high reliability of the existing system; by increasing the reliability ratio.

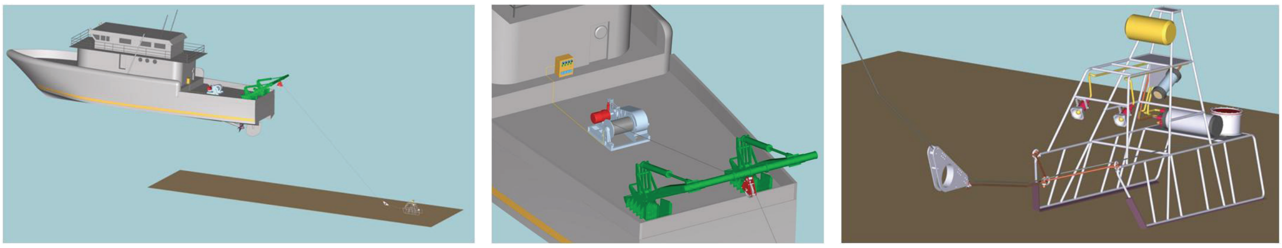

Fig. 1: The upgrading of the sampling platform.

The system as it worked up to that time could not allow us adding new devices and/or sensors due to the lack of more twisted copper lines for control and data signals. The use of a single towing cable for both power supply and signal transmission, resulted the use of a winch with a corresponding copper and fiber optic slipring, as well as the use of electrical to optical signal converters and vice versa, both on the surface section and on the sledge (Fig. 1). The installation of electrical to optical signal transducers in the sledge section gave us the possibility to have additional instruments connected to the control vessel by adding many connection ports. In this way, we are able to control whether to put them into operation, without having to activate switches on the surface, but to give commands with corresponding software. Because of the short time available for the upgrade, and trying to prevent any manufacturing failure, we chose to work on all mechanical and manufacturing drawings in a three-dimensional environment. This choice allowed us to study both the usability of the structures and the minimization of corrections during construction. Particular effort was made to fit the winch and slipring with the new towing cable. A new winch was built to use the specific electro-optical slipring and cable, but for cost reasons we did not add its own hydraulic PSU but used the one of the ship's own winches.

2.2 Field experiments design and data analysis

One field survey (June 2019) was conducted on the continental shelf of Heraklion Bay, an area of 110 km^2 located on the northern coast of Crete. Sampling was carried out in one transect of three stations (St. 1, St. 2, St. 3) at depths of 50, 100 and 200 m. The upgraded hyperbenthic sledge, equipped with three nets (0.5-mm mesh size), was used in order to collect macrofaunal samples during daylight hours (Fig. 2). Deployment of the sledge was made with (one reference sample-tow) and without resuspension (three replicate samples-tows) of the sediment surface (caused by a tickler chain). Samples were fixed in 90% alcohol on board immediately after collection and sorted under a dissecting microscope upon return to the laboratory. The hyperbenthic macrofaunal organisms (peracarids and decapods) were then identified to the species level, when possible, and counted. Densities estimated in the samples collected in the three nets of the hyperbenthic sledge were standardized to the number of individuals per m^3 by multiplying 0.45 m^2 surface area of the opening of the three nets and 50 m length of each tow of the sledge (estimated by using GPS). Water samples were also collected by using the sledge equipped with six bottles (1l, Fig. 2) for the determination of nutrients (PO4, NO2, NO3, NH4, SiO2), chloroplastic pigments (chlorophyll *a*, phaeopigments) and organic carbon concentrations. Samples were filtered through Whatman GF/F glass fibre filters on board, frozen immediately and stored at -20°C and analysed according to the protocols of the laboratory (Strickland & Parsons, 1972; Yentsch & Menzel, 1963). By using the electro-mechanical switch, which is installed to the right of the camera, or on the bar, we can choose when to open the inlet of the nets or when one of the six water samplers closes. All instruments are protected inside the cage of the sledge from the small obstacles of the sea bottom.

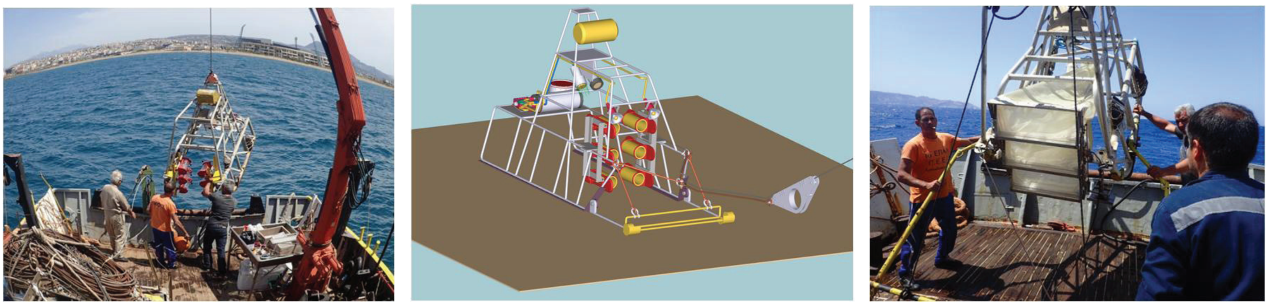

Fig. 2: The upgraded sampling paltform equipped either with water bottles or with planktonic nets depending on the parametres sampled.

3. Results

As far as nutrients concentrations in the study area is concerned, phosphates ranged from 0.09 to 0.14μM, nitrites from 0.02 to 0.16μM NO2, nitrates from 0.68 to 1.62μM, ammonium from 0.45 to 0.94μM, silicates from 0.8 to 1.69. Concentrations of chlorophyll *a* ranged from 0.12 to 0.19μg/l, phaeopigments from 0.04 to 0.05 μg/l and particulate organic carbon from 576.9 to 1008.5μg/l. In total, 7,036 individuals were collected and identified to 23 major taxonomic groups. The most abundant major taxonomic groups were: larvae of Crustacea, Copepoda, Chaetognatha, Cnidaria, Thaliacea, Amphipoda and Mysidacea. The most important hyperbenthic groups (peracarids and decapods) were analysed and 46 taxa were identified. The most abundant ones were: the decapods *Parapenaeus longirostris* (Lucas, 1846), *Philocheras bispinosus* (Hailstone, 1835), *Philocheras monacanthus* (Holthuis, 1961), the mysids *Leptomysis megalops* Zimmer, 1915, *Anchialina* sp., *Erythrops* sp., *Diastyloides serratus* (Sars, 1865), *Diastylis rugosa* Sars, 1865, *Iphinoe rhodaniensis* Ledoyer, 1965, the amphipods *Perioculodes longimanus longimanus* (Bate &Westwood, 1868), *Microdeutopus versiculatus* (Bate, 1856), *Synchelidium haplocheles* (Grube, 1864), *Westwoodila rectirostris* (Della Valle, 1893). The BBL densities estimated in the non-resuspended samples of the sledge were lower than the resuspended ones. More specifically, densities of the non-resuspended samples ranged from 38.9 ind/m^3 at 50m to 63.7 ind/m^3 at 100m and 36.83 ind/m^3 at 200m, while densities of the resuspended samples ranged from 40.26 (±12.45) ind/m^3 at 50m, 80.95 (±22.33) ind/m^3 at 100m and 49.9 (24.08) ind/m^3 at 200m.

4. Discussion/Conclusion

The conclusions that can be drawn from the upgrading of the sledge system are the following: a) significant economic benefit and experience from the upgrade using our own engineers for designing, manufacturing and maintenance; c) an open system easily upgradable in the future; d) responsible for our entire system since we have designed it, with a few exceptions; e) acquirement or even improvement of our techniques in both mechanical constructions and electronic design; f) integration of many communication and electrical connection protocols in our system, so that they can be used in the future, for the connection of more devices, such as sonars (sub botton profiler & scanning DTS sonar) as shown in Figure 3.

The old version of the modified hyperbenthic sledge aimed at identifying the direct impact of otter trawling on the BBL macrofaunal communities and the resulting ecosystem response (Koulouri *et al.*, 2003; 2005; Dounas 2006; Dounas *et al.*, 2005; 2007). The current version -either equipped with bottles or nets- was developed in order to simulate the natural resuspension of the sediment surface for the study of BBL habitat. However, comparison of results seems to efficiently sample BBL macrofauna (Koulouri *et al.*, 2013). According to Eleftheriou (2013) benthic research has been accompanied by significant technological research in acoustic techniques, deep-sea technology, diving techniques, while there is a scarcity of ideas in technical and methodological issues on macrofauna techniques. One can hardly conclude that the existing methodology concerning sampling of this extremely complex environment has reached any degree of perfection.

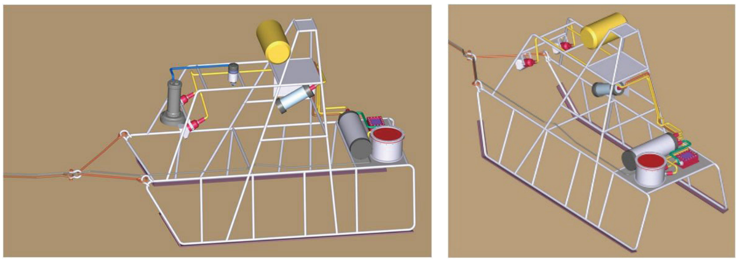

Fig. 3: Future uses of the upgraded sampling platform.

5. Acknowledgements

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EVALUATION OF FERMENTED SOYA (ME-PRO®) AS PROTEIN SOURCE/FISH MEAL REPLACER IN EUROPEAN SEA BASS DIETS. EFFECTS ON GROWTH AND FEED UTILIZATION

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Abstract

Several studies on the development of practical diets for fish using microbial enhanced proteins through fermentation have shown to be a promising solution to produce eco-friendly aquafeeds. Results have demonstrated that these products can sustain fish health, high-performance growth, and feed efficiency with high inclusion levels in the diet. The aim of this study is to evaluate fermented soya as fish meal replacer in European sea bass diets. To achieve this goal, the effects of dietary incorporation of ME-PRO® on growth, and feed utilization of juvenile sea bass were determined. The results showed that within the specific formulation, this ingredient can effectively replace 8% of fishmeal without compromising key performance indices.

Keywords: Aquaculture nutrition, feed ingredients, sea bass, fish meal replacer.

1. Introduction

Ensuring food supply to a population that is expected to exceed 9 billion until the middle of the century remains one of the biggest challenges according to the World Food Organization. Farming aquatic organisms is one of the world's fastest growing food sectors, providing the planet with about half of all the globally consumed fish. However, the expansion of aquaculture production has been accompanied by the need of rapid growth of aquafeed production. The challenge that the aquaculture industry is facing is to identify economically viable and environmentally friendly alternatives to fish meal, on which many present aquafeeds are largely based. The reasons why fish meal has been the protein source of choice in aquafeeds are its high protein content, excellent amino acid profile, high nutrient digestibility, and general lack of antinutrients. The decrease, however, of wild fish population and the increase of demand for seafood put enormous pressure on fish meal availability and price. Hence, while the supply of fish meal and oil is not sustainable, the anticipated growth in demand internationally for use in aquafeeds is expected to exceed the supply in the next decade. Thus, the aquafeeds industry has recognized for many years that viable utilization of plant feedstuffs for the production of aquatic species is an essential requirement for future development of aquaculture. Such plant feedstuffs must provide nutritious diets that will electively grow aquatic species with minimal environmental impact and produce high-quality fish flesh to confer human health benefits in a cost-effective manner (Gatlin *et al.*, 2017). Although several plant feed ingredients contain a moderately high amount of protein, calories, certain minerals and vitamins, their use in food and feed is still limited due to the presence of several endogenous antinutritional factors (ANFs) that adversely affect enzyme activity or the absorption of minerals and other nutrients. Some of the antinutritional factors can be partially or totally inactivated by heat processing such as roasting, autoclaving, extrusion or cooking prior to inclusion in fish feeds (Francis *et al.*, 2001). However, high heat compromises the nutritional quality of these ingredients due to partial destruction of heat sensitive nutrients and in not effective on some of these antinutritional factors. One way to enable the extensive use of plant ingredients is increasing their nutritional value and minimize the antinutritional factors by processing them. In animal nutrition, biotechnology can play a significant role in improving the nutritional value of feed ingredients of plant origin using modern processes. These include fermentation using various microorganisms.

Fermentation is a dynamic process involving microorganisms, substrates and environmental conditions to convert complex substrates into simple compounds. Fermentation outcomes depend on the nature and characteristic of the type of microorganisms and substrates being used as well as conditions; temperature, pH, the nature and composition of the medium, dissolved O_2 and CO_2, operational systems, addition of precursors, mixing and the length of the fermentation process may all influence the rate of fermentation and quality of the fermented products (Renge *et al.*, 2012).

Fermentation may enhance the nutritional quality of feedstuffs by lowering the fibre content, increasing crude protein and lipid content, improving vitamin availability, protein solubility and amino acid patterns (Borresen *et al.*, 2012). It has also been shown to increase the digestibility of various nutrients such as organic matter, nitrogen, amino acids, fibre and calcium, and to increase feedstuff palatability (Shahowna *et al.*, 2013). Also, it has been reported to reduce antinutritional factors content in feed ingredients, e.g., lectins and trypsin inhibitors in soybean meal, glucosinolates in rapeseed meal, tannins, haemagglutinins and prosopine in prosopis seed meal and phytate in maize (Olukomaiya *et al.*, 2019). In addition, fermentation has been shown to decrease mycotoxins in feedstuffs (Okeke *et al.*, 2015).

Fermentation, in particular SSF, has been recently documented targeting the improvement of the nutritional value mainly of plant ingredients for aquafeeds for shrimp *Penaeus vannamei* (Jannathulla *et al.*, 2018), rainbow trout, *Oncorhynchus mykiss* (Yamamoto *et al.*, 2010), barramundi, *Lates calcarifer*, (Ilham, I., *et al.*, 2017), Red sea bream, *Pagrus major* (Dossou *et al.*, 2019), Yellowtail amberjack, *Seriola lalandi* and Atlantic salmon, *Salmo salar* (Refstie *et al.*, 2005).

Several studies on the development of practical diets for fish using a microbial enhanced protein, ME-PRO® (Prairie Aquatech, South Dakota, USA), have shown to be a promising solution to produce eco-friendly aquafeeds. The protein is processed at a state-of-the-art plant using non-GMO (non-genetically modified) soybean meal and a natural occurring, non-toxigenic, fungi, *Aureobasidium pullulans*.

2. Material and Methods

2.1 Diets

Four diets were evaluated through *in vivo* tests. Specifically, four commercial type diets; one control and three diets containing different levels of the tested ingredient (Table 1), directly substituting fish meal, were fed in juvenile sea bass (23g initial size). The experimental diets were formulated to be isonitrogenous (46% crude protein), isolipidic (17% crude fat) and isoenergetic. Micronutrients (essential amino acids, phosphorus, essential vitamins and minerals) were balanced among the experimental diets. The experimental diets were produced by extrusion at HCMR.

2.2 Rearing Trial setup

- Sea bass individuals of 23g initial mean weight were used.
- Fish were distributed in 12 tanks. Four diets were tested in triplicate groups of fish.
- Fish were fed for 16 weeks in an open flow system with controlled and monitored water parameters.
- Temperature was held at 20°C and O_2 at >90% saturation.
- Fish were fed at *libitum* two times per day.
- Uneaten feed was collected, and feed consumed (g) was recorded daily.

2.3 Sampling

Fish were weighted individually at the beginning, intermediately and at the end of the experimental trial. In total 4 weightings were performed.

2.4 Statistics

All data were subjected to ANOVA. When significant differences among groups are identified, multiple comparisons among means were made using the Duncan's and/or Tukey tests. Treatment effects were considered at a significance level of P<0.05.

Table 1. Formulation and Composition of tested diets (%).

	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
Fish meal 67	20	15	12	8
MEPRO		5	8	12
Wheat Gluten	11	11	10	10
Corn gluten	15.5	15	15	16
Soy Bean meal 47	8	7	8	7
Soy bean Concentrate	15	15	15	15
Wheat meal	11.6	11.95	11.4	10.4
Fish Oil	13.7	14.2	14.5	15
Vitamin premix	0.1	0.1	0.1	0.1
Monocalcium Phosphate	4	4.5	4.6	5
Taurine	0.5	0.5	0.5	0.5
Lysine	0.3	0.4	0.4	0.45
Methionine	0.1	0.15	0.2	0.25
Mineral pack	0.2	0.2	0.3	0.3
Nutrients				
Protein %	46.00	45.87	46.04	45.96
Fat %	16.98	16.99	16.97	17.07
Ash %	5.11	4.33	3.94	3.30
Fiber %	1.51	1.72	1.88	2.04
NFE %	20.14	20.18	20.23	19.97
Starch %	12.75	12.41	11.78	11.27
DHA %	1.82	1.82	1.82	1.83
EPA %	2.39	2.39	2.39	2.40

3. Results

At the end of the 16-week feeding fish were individually sampled and the Key Production Indices were calculated as described in Table 2.

No statistically significant differences (P value>0.05) were observed for SGR, FCR and SFR. However, there was a trend for decreased KPIs between diet 1 and diet 4. A slight tendency for decreased feed consumption with the incorporation of ME-PRO® was measured, which might have resulted in decreased growth and consequently increase of FCR for diets 4. Up to 8% Me-Pro inclusion (diet 2 and diet 3) brought no difference in KPIs compared with diet 1.

4. Discussion/Conclusion

Fermented soya ME-PRO®, proved, within the specific formulation, to effectively replace fishmeal up to 8% inclusion rate without compromising the key performance indices measured.

As observed during the trial period, the inclusion of ME-PRO® was negatively related to feed consumption at the highest level, possibly due to some palatability issues. However, at 8% inclusion rate no such drawback was determined. Reformulation of the feeds at higher inclusion level with the addition of feed attractants could possibly support higher ME-PRO® inclusion and subsequent high performance overall.

Table 2. Growth Key Performance Indices.

	Diet 1	Diet 2	Diet 3	Diet 4
Initial weight (g) 19/11/2020	23.3	23.48	23.47	23.21
STDEV	0.29	0.41	0.43	0.44
Fish weight (g) 23/03/2021	80.38	79.19	77.71	73.26
STDEV	0.17	1.45	3.05	4.74
Weight increase	57.08	55.71	54.25	50.04
STDEV	0.21	1.04	2.63	5.18
Feed Conversion Ratio	1.29	1.29	1.27	1.31
STDEV	0.02	0.03	0.02	0.03
Specific Growth Rate	1	0.98	0.97	0.92
STDEV	0.02	0.01	0.03	0.06
Specific Feeding Rate	1.14	1.13	1.10	1.08
STDEV	0.03	0.03	0.01	0.04
Viscera Somatic Index	10.92	10.28	10.36	11.07
STDEV	1.55	1.56	1.73	1.52
Hepato Somatic Index	1.83	1.69	1.82	1.67
STDEV	0.39	0.27	0.23	0.25

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OTOLITHS MORPHOMETRICS IN *DENTEX MAROCCANUS* (VALENCIENNES, 1830) FROM THE AEGEAN SEA

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Abstract

400 otoliths were extracted from *Dentex maroccanus* specimens from the Aegean Sea to investigate their morphologies using morphometrics and shape analysis indices for the first time. Images of right otoliths were obtained using the Image - ProPlus software to examine ten otolith morphometric parameters. Using linear regressions, the relationship of each otolith parameter with fish total length was examined. A strong correlation exists between five of the otolith parameters (Diameter, Width, Radius, Area, Perimeter) and fish total length. This information is particularly useful for further otolith-size predictions and other otolith related studies.

Keywords: Otolith, Morphometry, Morocco dentex.

1. Introduction

The Morocco dentex (*Dentex maroccanus* Valenciennes, 1830) is a commercial species of the genus *Dentex* (family Sparidae), which occurs at depths ranging between 20 and 500 m. Its distribution extends into the Eastern Atlantic, from the Bay of Biscay to the Gulf of Guinea, and into the southern and eastern Mediterranean Sea, but the species is absent in the Adriatic and Black Sea (Bauchot & Hureau, 1986; Golani *et al.*, 2006). Thanks to their allometric growth fish otoliths have a species-specific morphology and are frequently used in fish stock assessment studies. Extrinsic and intrinsic factors can potentially affect fish growth and thus, affect otolith size and shape (e.g., Monteiro *et al.*, 2005; Lombarte & Cruz, 2007). Tuset *et al.* (2008) have presented two otolith shape indices for the Morocco dentex in the western Mediterranean Sea and the central Atlantic, but no such information is available for the species in the eastern part of the Mediterranean Sea. Here, ten otolith morphometric parameters were studied for the first time for the Morocco dentex in the South Aegean Sea (Eastern Mediterranean Sea). These results will be particularly useful for studies examining fish populations based on otolith characteristics, such as stock identification studies (Legua *et al.*, 2013).

2. Material and Methods

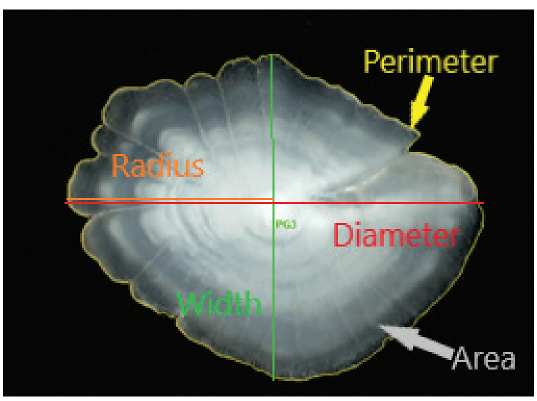

Fig. 1: Image of otolith parameters of *Dentex maroccanus.*

We used 400 specimens sampled from the Aegean Sea during 2014 and 2015. Their size ranged from 92 to 192 mm total length (TL). Sagittal otoliths from each fish were extracted, rinsed with fresh water and stored dry in a plastic tube. Calibrated digital images of the right otolith distal surface (Fig.1) were obtained under reflected light through a stereoscope connected with a digital image analysis system. The morphometric measurements were carried out using the Image-ProPlus software. We measured the otolith *Diameter* (D, mm), *Width* (W, mm), *Radius* (R, mm), *Area* (A, mm^2), *Perimeter* (P, mm), and *Roundness* (Rn), using the measurement tools of Image-ProPlus software. *Diameter* is the maximum distance of the outer point of rostrum and antirostrum which passes through the nucleus of the otolith, *Width* is the otolith height passing through the nucleus and *Radius* is the longest distance from the nucleus to the outer part of rostrum. Based on these parameters, we additionally calculated *Circularity* (C), *Form factor* (F), *Rectangularity* (Rc) and *Ellipticity* (E) for each specimen according to Tuset *et al.* (2003). For the description of the relationship between fish length and otolith characteristics, a simple linear function was used, following the methodology presented in other studies (Zorica *et al.*, 2010; Battaglia *et al.*, 2015). Linear regression was fitted to estimate the relationship between each otolith parameter and fish total length (y = a + b*x, where y is otolith morphometric parameter, and x is TL).

3. Results

The relationships between TL and each otolith parameter were statistically significant, except for Rectangularity Rc ($P > 0.05$). Area (A), Diameter (D), Width (W), Radius (R) and Perimeter (P) of the otolith showed a strong positive relationship with TL ($R^2 \geq 0.88$). The values of the shape indices Roundness, Circularity, Form Factor and Ellipticity showed a significant but weak ($R^2 \leq 0.1$) correlation with TL.

Table 1. Intercept values (a), regression slope (b), coefficient of determination (R^2), correlation coefficient (r) and significant relation (P-value < 0.05) for linear relationship between fish total length (TL) and otolith morphometric parameters (D, Diameter; W, Width; R, Radius; A, Area; P, Perimeter; Rn, Roundness; C, Circularity; Rc, Rectangularity; FF, Form Factor; E, Ellipticity).

Relation	a	b	R^2	r	P-value
D-TL	0.94	0.04	**0.88**	0.94	< 0.05
W-TL	0.35	0.03	**0.89**	0.95	< 0.05
R-TL	0.43	0.02	**0.90**	0.95	< 0.05
A-TL	-17.91	0.31	**0.91**	0.95	< 0.05
P-TL	2.98	0.12	**0.89**	0.94	< 0.05
Rn-TL	1.29	-0.0008	0.10	-0.32	< 0.05
C-TL	16.16	-0.01	0.10	-0.31	< 0.05
Rc-TL	0.79	-0.00001	0.0002	-0.01	**> 0.05**
FF-TL	0.78	0.0005	0.09	0.30	< 0.05
E-TL	0.20	-0.0002	0.08	-0.28	< 0.05

4. Discussion/Conclusion

We present otolith morphometry and shape indices of otoliths from the species *Dentex maroccanus* from the Eastern Mediterranean Sea for the first time. The high correlation coefficient (r = 0.94-0.95) for the otolith variables D, W, R, A and P indicate strong relationships with fish total length (P-value < 0.05). These equations can be particularly useful for further otolith-size predictions (e.g., dietary studies) and paleontological studies related to back-calculations of fish size based on otolith size and shape. Estimated length of fish from its fossil otoliths can occur when the relationship between fish length and otolith length is given as was the case of *Apogon townsendi* (Stringer *et al.*, 2017). The correlation of shape indices with fish length is very low, showing that shape of otolith does not change evidently with the change of the fish total length. When changes in otolith shape are less prominent, shape indices may remain constant, information useful for ontogenetic studies (Tuset *et al.*, 2021). Ontogeny has been found to affect the formation of otolith shape (Vignon, 2012), therefore studies about ontogenetic variations in otolith shape can offer information about the ecology of a species (Carvalho *et al.*, 2020).

5. Acknowledgements

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INVESTIGATING PRECIPITATION VARIABILITIES IN GREECE DURING 1950-2020 USING ERA5 DATA

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Abstract

This study aims at exploring the inter-annual variabilities of precipitation over Greece from 1950 to 2020. To this aim, ERA5 monthly precipitation reanalysis data covering Greece and some adjacent areas for this period were used to estimate inter-annual Theil–Sen linear trends and Mann–Kendall significance. Furthermore, generalized additive models (GAMs) were used to analyze and model nonlinear variabilities of the precipitation data. The results showed significant decreasing inter-annual trends of precipitation, more pronounced over western and eastern Greece. GAMs highlighted that the precipitation over the study area during the 71-year period presented high nonlinear, inter-decadal variabilities. Summarizing, this study concluded that precipitation over Greece and surrounding areas during the past 7 decades initially increased from 1950 to the late 1960s, then decreased until the beginning of 1990s and afterwards increased until 2020 with a smaller trend than the decades of 1950 and 1960.

Keywords: climate change, precipitation reanalysis, Theil–Sen trend analysis, Mann-Kendall significance, GAM.

1. Introduction

Mediterranean countries such as Greece are vulnerable to the climate change having effects on many atmospheric parameters like precipitation. Several studies have been conducted to investigate inter-annual variabilities of precipitation in Greece during the previous decades (e.g., Feidas *et al.*, 2006; Hatzianastassiou *et al.*, 2008; Markonis *et al.*, 2017). Nevertheless, the meteorological station networks are usually short-term, discontinuous and sparse. Therefore, spatially distributed long-term reanalysis datasets that consistently cover space and time can facilitate multi-year analyses of precipitation variabilities including also ungauged areas. Furthermore, the last two decades should be included in current climatic studies, because previous studies exploring precipitation temporal variabilities mainly focused on the 20th century. In this context, this study aims to unravel statistically significant inter-annual precipitation trends in Greece and surrounding areas for seven decades from 1950 to 2020, considering land and sea regions. We used monthly precipitation data from the ERA5 reanalysis dataset, which is one of the most modern and reliable sources. Robust statistical methods and tools such as the generalized additive models (GAMs) were used to investigate precipitation trends and to estimate nonlinear variabilities that occurred during the period 1950-2020. Hence, our study indicates regions susceptible to climate change effects on precipitation, which is critical for today and the future.

2. Material and Methods

The study area includes Greece and some surrounding areas between 18.75° W – 29.75° E and 34.5° N – 42° N (Fig. 1, more information can be found in Varlas *et al.,* 2022). The climatic precipitation data from 1950 to 2020 used in this study were retrieved from the ERA5 climate reanalysis dataset (Hersbach *et al.,* 2020). ERA5 data were created by the European Centre for Medium-Range Weather Forecasts (ECMWF). The data can be online obtained from the Copernicus Climate Change Service (C3S, 2021). ERA5 data include sea and land regions with a grid spacing of 0.25° × 0.25°. After retrieving and editing the data as described in Varlas *et al.,* (2022), first, we made a map illustrating the spatial pattern of average annual precipitation across the study area. Consequently, we estimated Theil–Sen linear trends (Theil 1950; Sen, 1968) from 1950 to 2020 for each ERA5 grid point in the study area. We also applied Mann–Kendall (M–K) nonparametric tests for monotonic trends (Mann, 1945; Kendall, 1975; Gilbert, 1987) to check the significance of trends for each grid point. Moreover, we estimated Theil–Sen linear trends and M–K significance considering time series of areal precipitation (i.e., annual precipitation spatially averaged over the study area). Afterwards, we used GAMs to analyze and model the areal precipitation time series aiming at exploring nonlinear trends and variabilities. We selected GAMs as they can model nonlinear relationships between the response variable and the predictors (Varlas *et al.,* 2022 and references therein).

3. Results

Regarding the spatial distribution of average annual precipitation from 1950 to 2020, the highest values are demonstrated in western parts of Greece with maxima exceeding 1250-1350 mm (Fig. 1a). High values are also observed in the eastern parts of Aegean Sea as well as in parts of western Peloponnese and western Crete. The lowest values are depicted in parts of southeastern Aegean Sea, as well as in Athens and eastern Peloponnese (Fig. 1a) with average annual precipitation amounts even lower than 350 mm. Fig. 1b presents regions with increasing, stable and decreasing Theil–Sen linear trends, as well as the Mann–Kendall significance. Decreasing trends of annual precipitation are generally depicted. The trends are statistically significant mainly in parts of the western Greece and the eastern Aegean Sea, as well as in limited regions of the northern Aegean Sea and in the west of Crete.

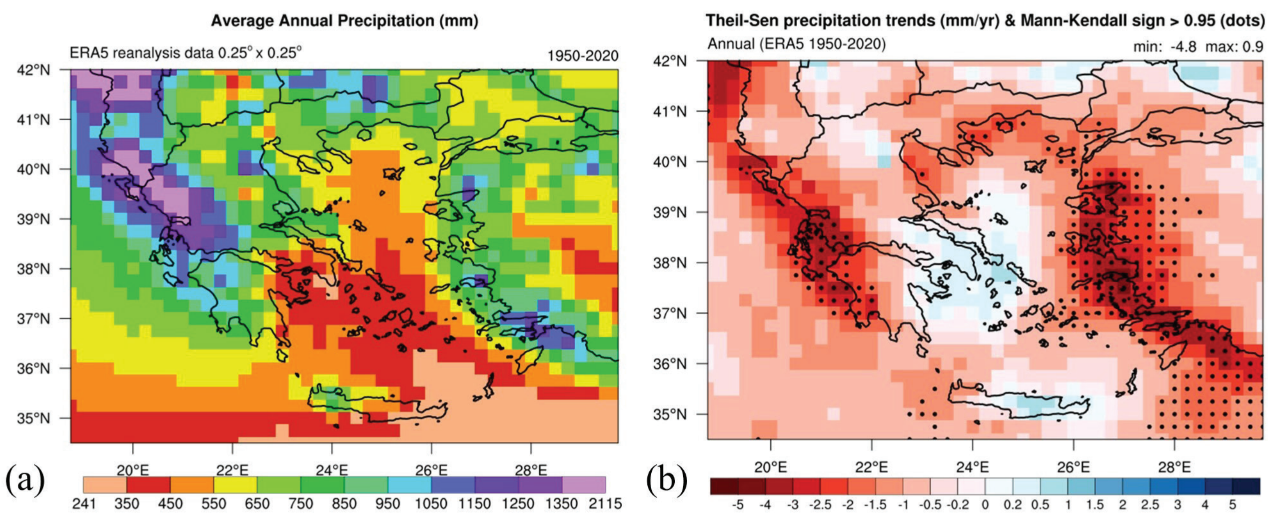

Fig. 1: Spatial pattern of **(a)** average annual precipitation (mm) and **(b)** of Theil–Sen trends for annual precipitation (mm/yr) during the period 1950–2020. The dots on maps represent statistically significant trends at the 95% significance level.

Figure 2a depicts time series of annual areal precipitation from 1950 to 2020 and the respective trend line. The annual areal precipitation was characterized by a decreasing Theil–Sen trend line during the 71-year period with a trend of −1.02 mm/yr. Nevertheless, Mann–Kendall significance was 93% and, thus, inter-annual trend is not significant at the 95% level. It is noteworthy that the areal precipitation did not linearly change over time (Fig. 2a). It is better depicted by the time series of areal precipitation anomalies that were created by subtracting the 71-year average precipitation from annual precipitation values for each year, as well as by the results of GAM modeling nonlinear variabilities (Fig. 2c). Hence, areal precipitation was firstly characterized by an increase from the 1950s to the late 1960s, then by a decrease until the early 1990s and afterwards by a small increase until 2020.

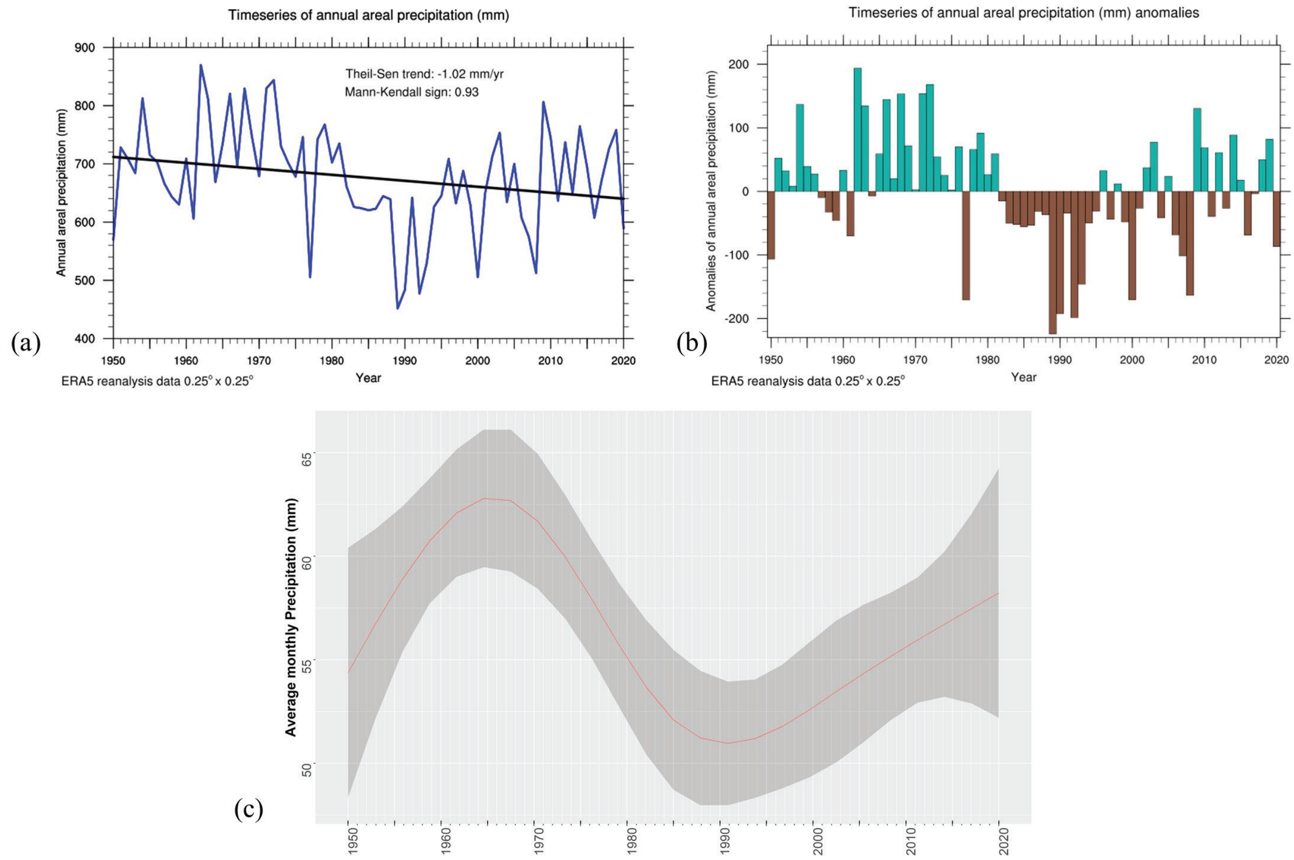

Fig. 2: Time series from 1950 to 2020 of annual areal precipitation across the study area. Theil–Sen trend, trend line, and Mann–Kendall significance are also demonstrated; (**b**) Same as (**a**) but for precipitation anomalies with 1950–2020 average precipitation used as reference; **(c)** Fitted GAM nonlinear inter-annual trend of areal precipitation (mm) over the study area. The shaded area represents the 95% confidence intervals.

4. Discussion/Conclusion

Our results indicated a spatially inhomogeneous pattern of variabilities over time. Significant Theil–Sen decreasing linear trends were identified mostly in parts of western Greece and the eastern Aegean Sea. Overall precipitation across the study area first increased until the late 1960s, then decreased until the early 1990s and afterwards increased until 2020 with a small rate. Our results generally confirm previous studies that presented inter-annual trends of precipitation in our study area (e.g., Feidas *et al.*, 2006; Hatzianastassiou *et al.*, 2008; Markonis *et al.*, 2017). The use of ERA5 reanalysis precipitation data, which are consistent in space and time, is one innovative feature of our study. ERA5 can facilitate the investigation of inter-annual precipitation variabilities even in ungauged areas during seven decades, including the past two decades from 2000 to 2020 which are critical in terms of climate change intensity. The results of this study can be a basis of other climate studies while they can be exploited by decision-makers and civil protection for designing management plans, regarding the mitigation and adaptation of climate change impacts on precipitation. The findings of this study have significant implications for freshwater availability, focusing on the inland freshwater ecosystems and their delivered services. A general decreasing trend in water availability would affect the anthropogenic water uses and the ecological integrity. It is also noteworthy that the present study could contribute to applications of the Indicators of Hydrologic Alteration (IHA) methodology in river ecosystems to investigate their ecological integrity and functioning.

5. Acknowledgements

We gratefully acknowledge Copernicus Climate Change Service for the provision of ERA5 climate reanalysis data that were used in this study. The ERA5 climate reanalysis dataset was generated using Copernicus Climate Change Service Information [2021]. ERA5 data used in this study for the period from 1979 to 2020 are available at: https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels-monthly-means?tab=overview (Accessed 3 March 2022), and respectively, for the period from 1950 to 1978 at: https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels-monthly-means-preliminary-back-extension?tab=overview (Accessed 3 March 2022).

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THE MODERN TOMBOLOS OF GREECE: MORPHOMETRIC CHARACTERISTICS AND MULTIDECADAL EVOLUTION

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Abstract

Tombolos (or isthmuses) are natural formations, not so common among the coastal landforms, due to the rather strict wave and sediment yield regime needed at a coastal environment, to allow their formation and evolution. The present study focuses on the largest 20 tombolos of Greece, which were identified, measured and studied, on the basis of their evolution since 1945, their morphometric characteristics and the stability of their sedimentary body. Most of the studied tombolos remain active, whereas around 2/3 of them are under erosion. Those that appear to gain space do so mostly due to artificial constructions at the body of the tombolos. The tombolos with sedimentary body lengths < 200 m present an L/D ratio < 2, whereas longer tombolos have an L/D ratio of > 2.

Keywords: Coastal erosion, Spatial variations, Coastal landforms, Tombolo formation.

1. Introduction

A tombolo (or isthmus) is a sandbar, barrier or spit that joins an island (called tied island) with the mainland or another, larger island, resulting from longshore drift or the migration of an offshore bar toward the coast (Ward, 2006). Tombolos are constructive coastal landforms, in which the main sediment supply derives predominantly from the adjacent coastal area, through wave mechanisms, which accumulate and build the sandbar between the mainland and the tied island. In Greece, tombolo formations are rather rare. In a total coastline length of about 16,000 km, only 20 natural tombolos of significant size (tombolo length >50 m) have been observed, scattered all around Greece, 3/4 of them being formed on island coasts, and the rest on mainland coasts (Fig. 1, Table 1).

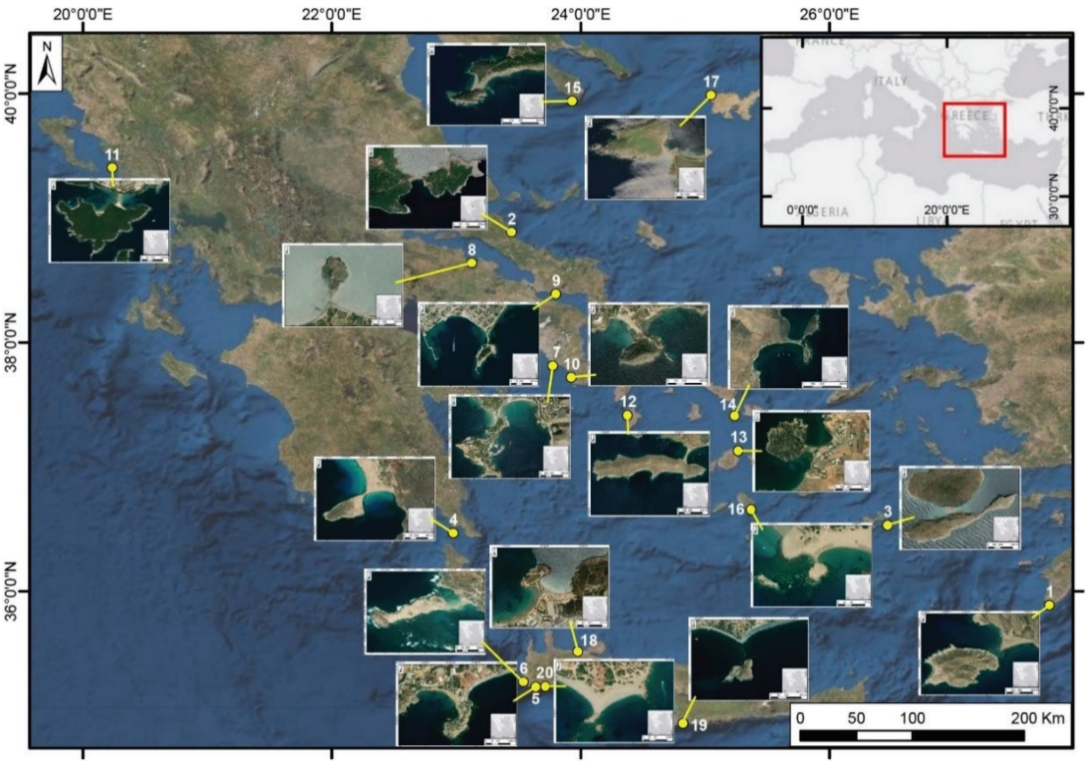

Fig. 1: Map of the studied tombolos' locations in Greece.

Table 1: List of the studied tombolos.

No.	Tombolo
1	Prasonisi, Rodos isl.
2	Agia Anna, Evoia isl.
3	Kounoupoi, Astypalea isl.
4	Elafonisos, Crete isl.
5	Gyalos, Crete isl.
6	Elafonisi, Crete isl.
7	Laimos, Attika
8	Mikrovivos, Fthiotida
9	Eretria, Evoia isl.
10	Agios Nikolaos, Fokea
11	Sivota, Thesprotia
12	Apokrisi, Kithnos isl.
13	Lagkeri, Paros isl.
14	Agia Kiriaki, Rinia isl.
15	Akrotiri, Halkidiki
16	Magkanari, Ios isl.
17	Mourtzeflos, Limnos isl.
18	Agioi Apostoloi, Crete isl.
19	Kali Limenes, Crete isl.
20	Gialiskari, Crete isl.

2. Material and Methods

The tombolo formations of Greece were located with the use of satellite images from Google Earth, while aerial photos from 1945, provided by the Hellenic Military Geographical Service (HMGS), were georeferenced and the tombolo shoreline was digitized, to study the evolution of the tombolos during the last 75 years. Through this collection of images, the evolution of each tombolo was studied, regarding the shifting of the sandbar, the stability of the connection between the mainland and the tied island, the existence of artificial constructions on the tombolo's sandbar and its touristic exploitation. Finally, the tombolo beach area gained or lost in the last 75 years was measured, using a GIS application.

As for the morphometry, the main features measured in each tombolo were: the tied island length (*L*), the tombolo length from the initial shoreline to the island (*D*) the smallest tombolo width (*b*) and the average tombolo width (b_{av}) (Fig. 2a). The surrounding bathymetry of each tombolo site, usually at the depth of 5-10 m, was digitized, using the detailed bathymetric basemaps provided by the Navionics webapp (Fig. 2b). For each tombolo site, a vertical transect to the deployment of the tombolo was created, depicting the bathymetry on both sides of the tombolo (Fig. 2c), in order to derive the local wave and sedimentological regime, responsible for its formation.

A statistical analysis followed next, focused mainly on the ratio between the tied island length (L) and the tombolo length (D).

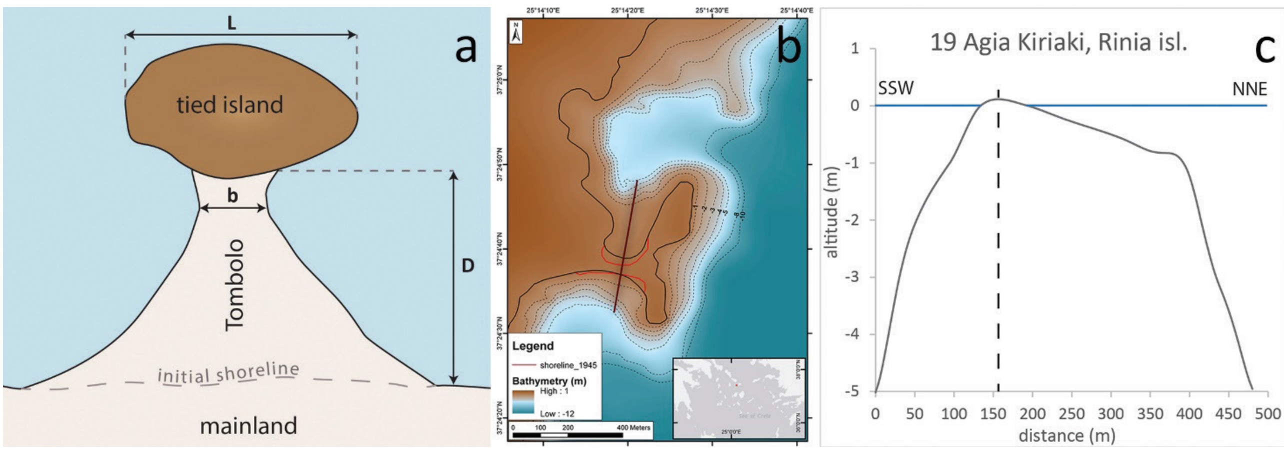

Fig. 2: (a) main morphometric characteristics of the studied tombolos. (b) bathymetry of the tombolo surrounding area. (c) transect vertical to the tombolo development.

3. Results

The majority of the studied tombolos present morphometric characteristics that can be easily categorized in distinct groups, based on the morphology due to which they have been formed and evolved, and the area on which they are present in terms of anthropogenic activities.

Thus, 13 of the 20 studied tombolos remain active until nowadays, as they constantly shift their sedimentary body width, whereas the other 7 seem to remain inactive during the last 75 years.

13 tombolos have been constantly connected to the tied island, whereas the other 7, at some point during the last 75 years, have been detached from it.

4 of the studied tombolos of Greece have permanent artificial constructions on their sedimentary body, usually touristic facilities, while on the aspect of touristic development, 1/4 of them are intensively exploited, 1/2 are moderately exploited and 1/4 have not been exploited touristically.

Referring to the tombolos' shoreline displacement since 1945, 13 of the tombolos seem to erode their sediment body through time, whereas only 7 of them seem to present accretion of the beach area. The present average erosion has been measured at - 15.3% of the initial area in 1945, ranging from - 0.5% (in Kali Limenes, Crete isl.) to - 30.8% (in Magkanari, Ios isl.). On the contrary, the corresponding average accretion has been measured to be + 26.2%, ranging from + 0.8% (in Mourtzeflos, Limnos isl.) to + 45.3%

(in Agia Kiriaki, Rinia isl).

The outcome of the morphometric analysis of all 20 tombolos, according to their L/D ratio, revealed a distinct characteristic, applicable for the majority (> 90%) of the studied cases. The tombolos whose lengths (D) are rather short (< 200 m) present a L/D ratio of < 2, ranging from 1.2 to 1.9, whereas tombolos of longer sandbars (> 200 m) present a L/D ratio of > 2, ranging from 2.7 to 7.6.

Finally, in most of the studied tombolos, the bathymetry is generally symmetrical in both sides of the tombolos, as expected.

4. Discussion/Conclusion

Tombolos, due to their distinct morphology and their most often easy access from the land, since they are formed of – and connected to – sandy beach environments, are common touristic destinations. As mentioned earlier, most Greek tombolos are highly or moderately touristically exploited.

Such formations are generally fragile, as they are in a constant shifting shoreline condition. 1/3 of the studied examples are characterized as instable, due to the constant detachment between the mainland and the island seawards. Concerning the continuous sandbar shifting, about 2/3 of the studied tombolos are under erosion, reaching to the loss of up to 1/3 of their initial area of 1945. On the other hand, though about 1/3 of the studied tombolos seem to have increased their sandy area during the last 75 years, it must be mentioned that for most of the cases where accretion is observed, it is due to artificial constructions along the tombolo, aiming either to its protection from erosion, or to the construction of touristic or port infrastructures.

Morphometrically, tombolos with lengths of < 200 m have L/D ratio of < 2, whereas those with longer sedimentary bodies have L/D ratio of > 2. Those ratios are in accordance with the relevant international literature (Nir, 1982; Sunamura and Mizuno, 1987; Ming and Chiew, 2000; Black and Andrews, 2001; Gonzales and Medina, 2001; Bricio *et al.*, 2008; Van Rijn, 2013;), where L/D ratios of usually < 1 lead to the formation of salients (or "uncompleted tombolos, according to Gulliver, 1899"). It has also been observed that the length (L) of the tied island responsible for the formation of the tombolo is not crucial for its sandbar length, as for both cases the islands lengths vary from about 60 to 1500 m, though smaller islands (< 100 m) tend to form thinner tombolos.

The usually similar, almost symmetrical bathymetry of the near-shore zone (usually to the depth of -10) on both sides of the studied tombolos indicates a rather normal and continuous wave/sediment regime in most of the studied cases. This could be attributed to the fact that, in those cases, tombolos are formed mainly by wave refraction and diffraction around the tied island (Easterbrook, 1999), whereas in the other cases their formation is usually as a result of a unidirectional longshore drift due to oblique wave incidence in one side of the tombolo.

5. Acknowledgements

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PROXIMATE COMPOSITION AND FILLETING YIELD OF WILD AND EXTENSIVELY CULTIVATED *CHELON LABROSUS* (RISSO, 1827)

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Abstract

The thicklip grey mullet *Chelon labrosus*, is found throughout Greece's marine and brackish waters. It is a promising new species for stocking freshwater ecosystems and aquaculture because it is capable of thriving in inland freshwater aquatic ecosystems. Consumers of fish place a premium on filleting yield and proximate composition. The purpose of the present work was to compare the filleting yield, and proximate composition of extensively cultivated and wild thicklip grey mullet. Extensively cultivated samples were acquired from Lugaru Lagoon, while wild fish were obtained from Amvrakikos Gulf's nearby brackish waters (N.W. Greece).The results indicate that compared to wild thicklip grey mullet, extensively cultivated fish fillets had a higher fat but lower protein and moisture content. However, there was no significant difference in the filleting yield between farmed and wild fish.

Keywords: thicklip grey mullet, aquaculture, proximate composition, filleting yield.

1. Introduction

Fish quality is defined by several parameters including organoleptic and nutritional properties. Fish biochemical composition is influenced by sexual maturity, size, age, and diet. Environmental parameters such as temperature and salinity also have an effect on lipid metabolism, thereby affecting the nutritional composition and value of fish. As a result, the proximate composition and nutritional value of wild and farmed fish species may differ (Kandyliari *et al.*, 2020; Oztekin & Oztekinal, 2020; García-Márquez *et al.*, 2021).

The thicklip grey mullet (*Chelon labrosus*) is found throughout Greece's marine and brackish waters. It is capable of thriving in inland freshwater ecosystems and is a promising new species for stocking aquatic ecosystems and aquaculture (Besbes *et al.*, 2020). As is the case in a number of other countries, grey mullet fry is extensively or semi-extensively cultivated in lagoons in Greece. The Lugaru lagoon's principal fish species include grey mullets, seabass, gilthead seabream, and eels. This lagoon covers an area of around 4000 hectares and is one of several lagoons formed by the drainage of Louros and Arachthos rivers in Amvrakikos Gulf in N.W. Greece. Fisherman cooperatives run the lagoon's fisheries and fish rearing, with main production based on extensively cultivated fish in enclosures.

Consumers appear to place a premium on filleting yield and proximate composition of both farmed and wild fish (Lazo *et al.*, 2017; Bland *et al.*, 2021; Tarricone *et al.*, 2022), two parameters which can vary according to several parameters. The present work was conducted in order to determine differences between extensively cultivated and wild thicklip grey mullet *Chelon labrosus* in terms of filleting yield and proximate composition.

2. Materials and Methods

Samples of extensively reared thicklip grey mullet were obtained from Lugaru Lagoon (Amvrakikos Gulf, N.W. Greece). Wild fish were obtained from the nearby brackish waters of Amvrakikos Gulf. The sampling took place in spring 2019. The fish were freshly caught and obtained by local fishermen a few hours after capture. Thereafter, samples were transported to the laboratory on ice and subjected to somatometric and filleting measurements. Prior to filleting, fish were gutted, skinned, and beheaded manually. Total body and fillet weight were used to calculate the filleting yield. After that, the fillets were frozen to facilitate further analysis.

The Association of Official Analytical Chemist (AOAC, 1990) method of drying fish samples (5 g) at 105°C was used to determine the moisture content. The results were expressed as percentage (%) of total weight when wet. The Kjeldahl method was used to determine the total protein content (5 g) (AOAC, 1990). Total lipids were extracted using the chloroform/methanol (2/1) method (Folch *et al.,* 1957). Aliquots of the chloroform layer extract were evaporated to dryness under nitrogen and gravimetrically quantified for lipids. Samples from 5 specimens (in triplicates) from each group (wild, farmed fish) were used for each type of analysis. Prior to statistical analysis, data were arc–sin transformed to follow normality (Nathanailides *et al.,* 2011). Students' t-test was used to test for significant differences between wild and farmed fish groups.

3. Results

The proximate composition of fish used is shown in Table 1. The lipid, protein, and moisture content varied significantly between wild and farmed fish ($p<0.05$). Specifically, the wild fish exhibited higher protein and moisture but lower fat content than farmed fish. Moreover, no significant difference was shown in filleting yield of wild and farmed fish ($p>0.05$).

Table 1. Mean (±sd) proximate carcass composition (% wet weight) and filleting yield (%) of wild and farmed thicklip grey mullet (n=5 fish for each group) and filleting yield (FY) (n=25 fish for each group).

Carcass chemical composition	**Farmed**	**Wild**	**p value**
Protein (%)	19.67 (0.43)	21.68 (0.66)	0,012
Fat (%)	1.88 (0.07)	1.42 (0.11)	0,004
Moisture (%)	75.61 (0.83)	76.82 (0.88)	0,023
Filleting yield (%)	58.91 (6.82)	57.73 (6.08)	NS

4. Discussion

In comparison to wild thicklip grey mullet, intensively farmed fish had a higher fat content but a lower protein and water content. However, there was no significant difference between farmed and wild fish filleting yield. Different factors may have contributed to the observed differences between the two groups. Grey mullet species choose their habitat and modify their feeding behavior in response to ontogenetic and environmental factors (Gisbert *et al.,* 1995; Cardona *et al.,* 2006). Clearly, the cultivated fish in the present study lacked the spatial migration options available to the wild fish group. Several environmental factors can affect fish growth, filleting yield, and proximate composition (Grigorakis, 2017; Garca-Márquez *et al.*, 2021). In lagoon ecosystems, temperature and salinity are two parameters that exhibit spatial variability. The differences in the proximate composition of the two groups can be partially attributed to spatial variation in the salinity and temperature gradient of their habitat (Christia & Papastergiadou, 2006). Likewise, feeding, digestion, and proximate composition of thicklip grey mullet are affected by temperature and salinity (Rabeh *et al.,* 2015; Pujante *et al.,* 2018). The obtained results are comparable to those reported by other studies that examined the proximate composition of grey mullet with a thicklip in Greece (Parlapani *et al.,* 2021).

Filleting yield is a good indicator of how much of a farmed fish's total body weight is edible. The filleting yield of farmed and wild thicklip grey mullet rerorted in this study is within the previously reported range for other euryhaline fish in N.W. Greece (Grigorakis *et al.,* 2011; Nathanailides *et al.,* 2019). Given, the high potential contribution of *C. labrosus* to improving the dietary intake of highly polyunsaturated n-3 fatty acids, with a benefit to human health (García-Márque *et al.*, 2021), further research is required to investigate in depth the fatty acids profile and any potential differences between locally distributed wild and farmed populationsof this species.

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ZINC SPECIATION IN THE MARINE COASTAL SURFACE MICROLAYER

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Abstract

Trace metals speciation in seawater is principally controlled by the formation of complexes, through their interaction with organic ligands and particles. In the case of the sea surface microlayer (SML), which constitutes the boundary layer between the ocean and the atmosphere, trace metals are enriched in both the dissolved and particulate phases, with enrichment mechanisms characterized by high complexity.

A limited number of works have been published so far providing data on zinc complexation in seawater, without however any reference to SML. In the present study Zn complexing capacity, L_T, was determined employing anodic stripping voltammetry, during 3 seasonal samplings, at two coastal sites within the Aegean Sea, Eastern Mediterranean, at 3 different depths including the surface microlayer and subsurface water at 20 cm ($SSW_{0.2}$) and 2 m depth (SSW_2). The nature of the organic matter in marine SML samples was additionally investigated by Attenuated Total Reflectance Fourier Transform Infrared (ATR FT-IR) spectroscopy. Statistically significant differences were recorded among different water depths.

Keywords: marine surface microlayer; zinc complexation; ATR FT-IR spectra.

1. Introduction

The biological availability of trace metals in seawater is regulated by their chemical composition (speciation) and interactions with organic matter, interfaces, biota, chemical transformations and solubility, as well as uptake kinetics. The sea surface microlayer (SML) constitutes the boundary layer between the ocean and the atmosphere, representing a critical interface for physical, chemical and biological processes. Complexing agents of metals present in SML derive both from allochthonous (e.g. atmospheric particles deposition) and autochthonous sources, with abundant microorganisms, called neuston having a significant contribution. The SML consists generally of adsorbed surface active substances (SASs), which are amphiphilic molecules reducing the surface tension of the water–air interface. They include a large variety of substances such as polysaccharides, transparent exopolymer particles (TEPs), polypeptides, lipid-like material, carbohydrates, amino acids, bacterioneuston and phytoneuston (Karavoltsos *et al*., 2015 and references therein).

Zinc participates in numerous enzyme systems involved in a variety of metabolic processes (Vallee & Auld, 1990). In the ocean, total dissolved Zn concentration (C_{Zn}) presents a nutrient–like vertical profile (Bruland, 1980). At high concentrations, Zn could be toxic to phytoplankton and bacteria (Sunda & Huntsman, 1996). Moreover, low Zn concentrations in surface seawater could limit the growth of some kinds of phytoplankton (Brand *et al*., 1983; Sunda & Huntsman, 2000). In most surface waters, natural organic ligands strongly bind Zn, hence dominating the speciation of the total Zn pool. Studies referring to Zn complexing capacity in the marine environment are limited (Kim *et al*., 2015), whereas no pertinent data have been published so far regarding SML.

Spectroscopic techniques such as Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) can be effectively used for quantitative functional group analysis of bulk particulate organic matter (POM) collected on filters (Tremblay *et al.*, 2011). The specific technique, which offers valuable information on the nature of organic matter related to trace elements complexation, is non destructive, permitting the reuse of filters.

Zinc speciation in seasonal samples of surface microlayer at 2 coastal sites within the Saronicos gulf was examined herewith, employing Differential Pulse Anodic Stripping Voltammetry (DPASV), in order to characterize organic Zn complexing ligands and their influence on its chemical speciation. Data regarding the nature of organic matter were obtained by ATR FT-IR scans in the suspended particulate matter.

2. Material and Methods

2.1 Study area, sampling

Three seasonal samplings were performed in winter, spring and early autumn (January, May and September 2018), at two coastal areas, namely Loutropyrgos, within the Elefsis gulf and Pahi of Megara, in contact with open Saronicos gulf. Samplings were performed under calm weather conditions (wind speed <5 m s^{-1}) with a small rubber boat equipped with an electric engine. For SML sampling, a glass plate sampler was employed.

2.2 Methodology

Electrochemical measurements were carried out using an Autolab III (Eco-Chemie, The Netherlands) voltammetric instrument connected to a three electrode cell (VA 663, Metrohm stand, Switzerland) with a static mercury drop electrode (SMDE) as the working electrode. The reference electrode was an Ag/AgCl (3 M KCl). A carbon rod electrode served as the auxiliary electrode. Differential Pulse Anodic Stripping Voltammetry (DPASV) was used for complexing capacity determinations. Zn complexing capacity is the concentration of Zn ions which could be added to the seawater sample before the Zn ions could be measured as “free” Zn ions (including hydrated Zn ions plus inorganic complexes, being labile for the applied electrochemical method). Zn complexing capacity could be regarded as “Zn ions buffering capacity”. The strength of the formed organic complexes could be compared by their calculated apparent stability constant (K_{app}). The values of the complexing capacity as well as of the corresponding stability constant were calculated by applying the linear transformation plot (Ružić, 1982).

Total dissolved zinc concentrations were determined using the rapid solvent extraction technique described by Danielsson *et al.* (1982). Determination of trace elements in the preconcentrated samples was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a Thermo Scientific ICAP Qc instrument (Waltham, MA USA).

The potential enrichment of the SML relatively to subsurface water, in terms of Zn complexing capacity and total dissolved Zn, was estimated through the calculation of the enrichment factor ($EF = C_{SML}/C_{subsurface\ water}$), with EF values >1 termed as “enrichments” and EF values <1 designated as “depletions” (Chen *et al.*, 2013).

The attenuated total reflection (ATR)–Fourier transform infrared (FTIR) spectra of samples were recorded by a Spectrum Two FTIR spectrometer equipped with a Diamond ATR compartment (Perkin Elmer, USA) using the provided Spectrum 10 spectroscopy software. For each sample, 32 scans of the infrared region between 4000 and 450 cm^{-1} at a resolution of 4 cm^{-1} were recorded in duplicate and averaged. The recorded spectra were afterwards ATR-corrected with a refractive index of 1.5 for all samples, in order to be comparable to spectral libraries available and peak tables published.

3. Results

The complexing capacity of Zn ions was found ranging from 24 to 149 nM (Fig. 1), while the corresponding logarithmic values of the apparent stability constant from 7.4 to 8.8. These results are in accordance to the concentrations of Zn complexing capacity (46 – 104 nM) reported for Narragansett Bay (Muller & Kester, 1991).

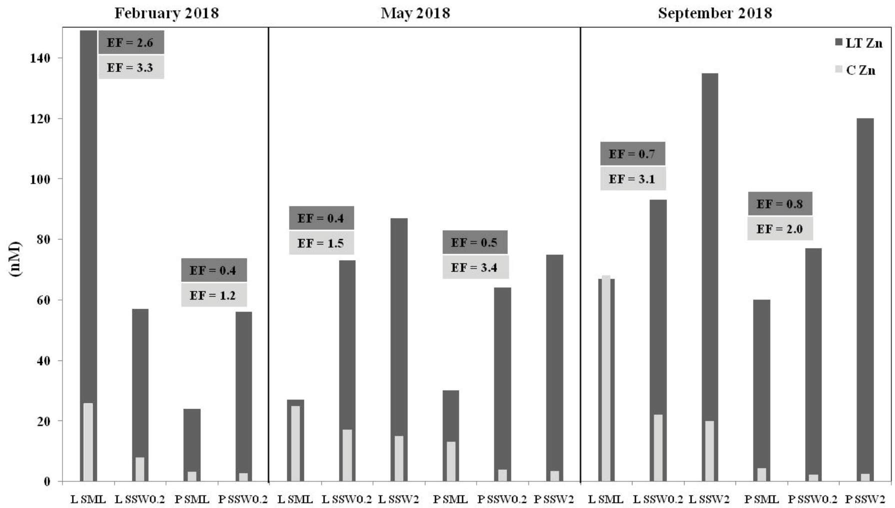

Fig. 1: Zinc-binding ligands ($L_{T\ Zn}$), dissolved Zn concentrations (C_{Zn}) and their enrichment factors (EF), in surface microlayer (SML) and sub-surface ($SSW_{0.2}$, SSW_2) marine coastal waters of Loutropyrgos (L) and Pahi of Megara (P) sampling sites.

4. Discussion/Conclusion

In several samples, a depletion of Zn ligands in the surface microlayer (EF<1) is calculated, resulting in the saturation of Zn complexing sites (Fig. 1). Since the amount of ligands available in SML is not adequate for a complete Zn complexation, a significant amount of Zn is expected to be present in either ionic form or labile complexes.

In order to chemically characterize the organic matter occurring at various sampling depths, a Principal Component Analysis (PCA) was performed on the recorded ATR-FTIR spectra, for the identification of any grouping of samples on the basis of their sampling depth (surface microlayer and subsurface waters at 0.2 m depth) (Fig. 2). Projection of PC1 and PC2 allowed for separation (across PC2 axis) of the recorded spectra of surface microlayer samples from those originating from the subsurface layer.

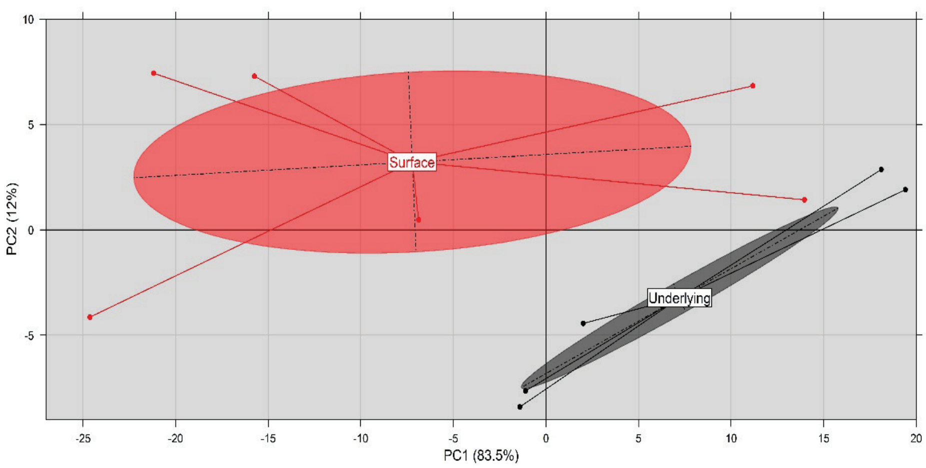

Fig. 2: Principal component analysis of the recorded ATR-FTIR spectra and separation on the basis of sampling depth (red: surface microlayer; grey: sampling depth 0.2 m).

The complexing capacity of Zn ions was determined, for the first time herewith, in marine surface microlayer samples. The observed Zn speciation is characterized as particularly interesting, since in several cases a depletion of Zn ligands is determined. ATR-FTIR spectra analysis of particulate matter revealed a differentiation of organic matter between SML and subsurface waters.

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IMPLEMENTATION OF MARITIME SPATIAL PLANNING AT REGIONAL-LOCAL SCALES: LESSONS LEARNED FROM RESEARCH EFFORTS IN THE IONIAN SEA

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Abstract

The study presents key outcomes derived using the PORTODIMARE project geoportal (GAIR) modules and tools with the aim to address challenges related to analyzing existing and future conditions in the Ionian Sea study area, as an effort to contribute towards paving the way for robust planning at the sub-regional, regional and interregional scale, according to the provisions of the Greek MSP law. Recommendations and key actions included in the final deliverable of PORTODIMARE, the so called 'Action Plan', are also included. The web portal of PORTODIMARE can be found at the URL address: https://www.portodimare.eu/.

Keywords: conflicts, ecosystem, uses, stakeholders, recommendations.

1. Introduction

Greece is a maritime nation by tradition, and shipping and maritime trade have been key elements of the Greek economy since the antiquity. Furthermore, fisheries have been also a sector of vital importance for the communities of the island and for the coastal regions of Greece for thousands of years. In the last decades tourism, and particularly maritime/coastal tourism, has been one of the main contributors to the country's economy, while marine aquaculture is also considered as a success story in Greece's Blue Growth agenda. What is more, Greece has to comply with the EU Biodiversity Strategy for 2030, according to which protected areas should be established for at least 30% of the country's land, sea and freshwater ecosystems by 2030, considering also the adoption of nature-based solutions related to climate change resilience. Consequently, an increasing demand for coastal and marine space, particularly in specific areas, is expected in the next years. In this vein, Greece is in the process of preparing maritime plans according to the provisions of the Maritime Spatial Planning Directive (2014/89/EU) aiming to balance ecological, economic and social interests and following an ecosystem-based approach linked with Green Deal goals and aspirations; the MSPD was transposed to the Greek legal system in 2018 (Law 4546) and was amended in 2020 with Law 4759.

As MSP should pave the way for practical tailor-made planning, that takes into account the characteristics of the different marine/coastal social-ecological systems (Papageorgiou & Kyvelou, 2018), promoting concepts of 'maritime cohesion' and regional smart specialization strategy (RIS3) (Kyvelou & Ierapetritis, 2019), the objective of this study is to share experience gained from a recent effort aiming to apply MSP approaches and tools in the Greek case study of the Interreg ADRION project PORTODIMARE. In the frame of this project the Geoportal for the Adriatic Ionian Region (GAIR) has been developed including spatial databases and a set of MSP tools (modules), with the aim to contribute to analyzing existing and future conditions in the study area, the latter being a key step of the MSP process (UNESCO/IOC, 2021). Indeed, the latter is imperative not only for the implementation of the planning process, but it should also be considered in relevant consultations by all stakeholders to determine and evaluate planning scenarios and their implications (Coccossis *et al.* 2020). Hence, the main aim of the study is to present key outcomes from the project and share the proposed recommendations and suggested key actions of the project's Action Plan that may potentially be useful towards paving the way for inclusive ecosystem-based MSP at regional/local scales.

2. Material and Methods

Existing information from deliverables and publications of MSP-research projects that took place in the Ionian Sea were considered for the organization of the PORTODIMARE Greek case study. MSP should consider the characteristics of the social-ecological system (SES) of the area under study, considering the spatial challenges linked with both the natural and the socio-economic capital, to promote sustainable Blue Economy goals. Hence, spatial data on maritime activities/uses in the central Ionian Sea, with special focus on aquaculture (as the area hosts the 18% of the units in Greece), small scale fisheries (being an important socio-economic activity with more than 1500 vessels registered there), and tourism (sites of massive and milder development exist within the borderlines of the case study) were compiled along with spatial data on priority conservation ecosystem components ((e.g., Neptune's seagrass (*Posidonia oceanica*), monk seals (*Monachus monachus*), common dolphin (*Delphinus delphis*).

Data were stored in the GAIR, and the modules that were applied in the Ionian Sea case study were: the Medium Scale Fishery (MSF) and the Small-Scale Fishery (SSF), both developed by the HCMR team, the Allocation of Zones for Aquaculture (AZA), developed by CORILA, and the Cumulative Effects Assessment (CEA), developed by CNR-ISMAR. The above modules were selected to be applied following consultations with local stakeholders, that took place in 2018 back-to-back with the Interreg ADRION ARIEL, according to which spatial conflicts in the study area seemed to exist between aquaculture and SSF, and then aquaculture and coastal tourism (Anon., 2018). Hence, the focus of the Greek case study of PORTODIMARE has been on addressing spatial challenges that may be stemming from interactions between the aforementioned maritime activities, considering also their impacts on the selected ecosystem components. Project outcomes were then discussed with key actors, and following stakeholder interactions, proposed recommendations were integrated in the final deliverable of the project, that aimed to provide a so-called 'Action Plan' proposing key actions for addressing challenges identified in the frame of PORTODIMARE (Vassilopoulou *et al*., 2021); recommendations and key actions referring to the MSP Step on 'analyzing existing and future conditions' in the study area are presented in the following section.

3. Results

Outcomes of the CEA module applied to the Greek case study are shown in Figure 1a, in which sites with higher conflict scores, considering cumulative impacts on the selected ecosystem components, are visualized. However, a crucial point that has emerged from the study, but also from previous ones in the Ionian Sea, refers to the important data deficiency regarding ecosystem components. Hence, CEA outcomes should be considered as preliminary and indicative. Indeed, the application of CEA should include comprehensive data and assessment of environmental impacts with the lowest possible uncertainty, which underlines the need to couple MSPD efforts with monitoring activities under relevant environmental and sectoral policies (e.g., the MSFD, the WFD, the CFP).

The spatial distribution of aquaculture units and tourism-related facilities in the study area were combined with the outcomes of the SSF module, in order to visualize potential existing spatial conflicts. In Figure 1b, the officially designated zones for aquaculture development are indicated, along with the spatial distribution of SSF operations. Results corroborate stakeholder views on existing spatial conflicts between SSF traditional fishing grounds and existing aquaculture units, as well as on their anticipations that these conflicts may further increase due to the potential future expansion of units, following the provisions of the Special Spatial Framework for Aquaculture in Greece (SSFA) (CMD 31722/2011). Another point that may be also important to note is that small scale fishers have mentioned that they were never consulted by State authorities while they were developing the SSFA (Anon., 2018).

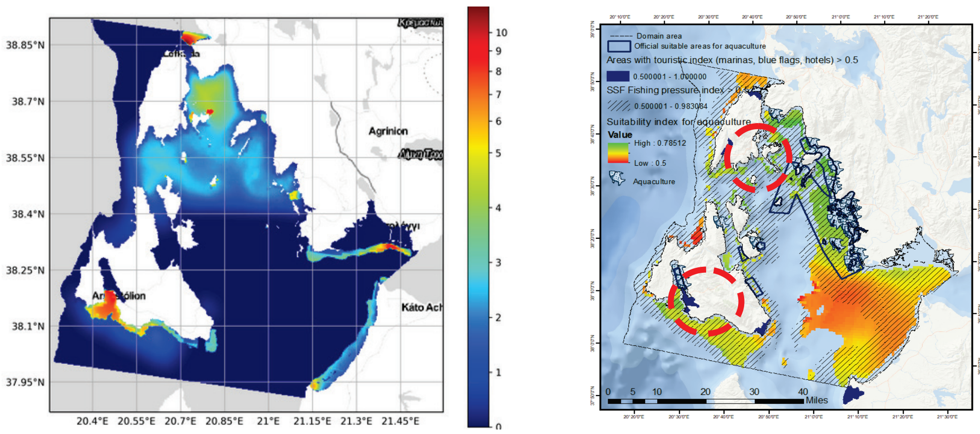

Fig. 1: *a/left*: Geospatial distribution of CEA scores in the Ionian Sea case study. *b/right*: Map highlighting conflicting hotspots with coastal tourism and SSF in areas identified as suitable by the AZA module; the officially designated zones for aquaculture development (SSFA) are indicated, highlighting potential future spatial conflicts mainly with the SSF sector.

The synthesis of PORTODIMARE outcomes, integrating also experience gained from other research projects (MESMA, MSP-Med, ADRIPLAN, ECOAST, ARIEL) conducted in the case study area, were then summarized in proposed recommendations and suggested key actions. The respective ones referring to the MSP Step on 'Defining and analyzing existing and future conditions' are presented in Table 1.

Table 1. Recommendations linked to the MSP steps on 'Defining and analysing existing and future conditions' stemming from the planning exercise in western Greek waters, including suggested actions and relevant actors.

MSP steps	GAIR support	Recommendations
Defining and analyzing existing and future conditions	Module engines -fisheries module - AZA module - CEA module	- Operational objectives integrating national strategies with regional/local needs reflecting practical socio-economic and environmental existing and future demands, the latter emerging during stakeholder consultations, need to be identified. - Spatial information on ecosystem components and human activities linked to the objectives should be collected. - Data format, resolution, timeframe, attributes, as the quality of data upon which the analysis/application of tools will be based is crucial for restricting uncertainty and improving the robustness of results.

Suggested Actions	Relevant Actors
• MSP plans should consider environmental impact assessments based on optimal data aggregations and sound scientific interpretations, including confidence intervals /approaches to evaluate uncertainty of the model outputs. • Monitoring efforts are needed to fill the important data gaps particularly in areas that seem to constitute hot spots of conflicts with conservation priority species as well as between uses competing for the limited marine space. • Further research should be streamlined towards improved understanding of the links between ecosystem pressures, impacts, status and the capacity of the ecosystem to deliver ecosystem services, and such efforts should be linked particularly with WFD and MSFD ones. • Joint monitoring activities should be pursued adapting existing monitoring (WFD, MSFD, CFP) to provide relevant and cost-effective input to MSPD requirements	✓ Research institutions ✓ Research institutions ✓ Research institutions ✓ Competent authorities & res. institutions

4. Discussion/Conclusion

An effort to synthesize the key actions and recommendations, highlighting also potential ways forward, was made with the aim to contribute towards adopting an integrated approach for ‘analyzing existing and future conditions’ towards improving MSP implementation. A crucial take out from the study is that joint monitoring efforts are needed to fill the important data gaps and further research should be streamlined towards improved understanding of the links between pressures, impacts and environmental status, considering also climate change effects under the implementation of climate-smart MSP (Vassilopoulou, 2021); such monitoring and research efforts should be linked in a truly coordinated way with those already taking place under the implementation of other policies (e.g., HD, WFD, MSFD, CFP) identifying synergies with MSP data and knowledge needs. The latter underlines the need for MSP to be based on integrated governance approaches, as there are different public authorities dealing with the management of coastal and marine space, which are responsible for the implementation of the aforementioned policies; the MSP competent authority should ensure horizontal and vertical governance integration, promoting also cooperation with maritime sectors as well as research centers/academia.

5. Acknowledgements

This work has been supported by the Interreg ADRION program. The contribution of the CNR-ISMAR and the CORILA teams that developed the GAIR CEA and AZA modules is greatly appreciated by the authors.

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HEAVY METALS IN SEDIMENT CORES FROM WEST SARONIKOS GULF

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Abstract

Four sediment cores from the coastal marine area of the West Saronikos Gulf have been analyzed for their grain size and elemental geochemistry. The concentrations of 3 metals (Al, Ni, Zn), along with total organic carbon (TOC) and carbonate content were measured. The cores are fairly homogeneous, in terms of carbonates, while the down-core variability of TOC is characterized by high surficial values that decrease with depth. The vertical distributions of Zn present a constant decrease over depth along the cores, indicating the anthropogenic effects to the upper parts of the sediment cores, while Ni is increased with depth. The concentrations of Zn are increased from the northeast to the southwest part of the studied area, in contrast to Ni which is increased in the northern stations. The concentrations of Ni at the surface sediments are higher than the ERM value (51.6 mg Kg^{-1}) and those of Zn, below the ERL value (150 mg Kg^{-1}). Finally, the levels of Zn at most stations remain fairly constant, compared to a previous study of 2007.

Keywords: nickel, zinc, spatial distribution, anthropogenic contamination.

1. Introduction

Saronikos Gulf is situated at the central Aegean Sea (northeastern Mediterranean) near Athens, the capital of Greece. Its western sector (West Saronikos Gulf) incorporates the deep basins of Megara (max depth 234 m) and Epidavros (max depth 440 m). Saronikos is subjected to a strong seasonal cycle of heating and cooling, with air temperatures between 0-40°C, which causes the formation of a seasonal pycnocline from May to November. In winter, the water column is homogenized down to 120m. In the western Saronikos, vertical mixing never reached the sea bottom (440m) in the years after 1992 and dissolved oxygen concentration has approached nearly anoxic conditions (D.O. < 1mL L^{-1}) (Paraskevopoulou *et al*., 2014).

The Susaki area, which is near to the northern coast of West Saronikos, is known for its volcanic activity which took place during Pliocene-Quaternary. Most of the volcanic materials were transported by fluvial processes and deposited in the alluvial plains and coastal regions. The formations that are observed are peridotites and serpentinites, neogene deposits and Quaternary deposits (Kelepertsis *et al. 2001).*

The coastal marine area of the northwest part of Saronikos Gulf is affected by a few types of industries, with an oil refinery unit among them, touristic facilities in the nearby coastal villages and small towns and settlements.

The main aim of this work is to assess the levels and the distribution of Al, Ni and Zn in sediment cores of West Saronikos, in order to discern between geological and anthropogenic origin of heavy metals and to identify the major sources of metal pollution. The second aim is to determine the evolution of metal pollution in the area by comparing the results with those of a similar study ten years ago.

2. Materials and Methods

Four sediment cores (core length: 24-32 cm) were obtained at corresponding stations of varying depths (100-420m) in Western Saronikos Gulf (Fig. 1) using a box corer in October 2017 with R/V AEGAEO. Stations MOT16A, UN5, UN6, near the Susaki area, are affected by the coastal industrial zone, urbanization, touristic activities and the local geological background. Station UN11, located at the deepest area of West Saronikos, Epidavros basin, is probably less affected by the coastal anthropogenic activities.

The cores were cut in layers of 1cm for the top 10cm and of 2cm below 10cm, stored and were frozen until analysis. The sediment layers were freeze-dried and sieved by Retsch stainless steel 'Test Sieves' for the separation of the silt and clay fraction (<63μm) from the sand fraction (> 63μm).

The total organic carbon (TOC) content was measured using the method of the oxidation of sediments with potassium dichromate ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4), followed by back-titration with ferrous ammonium sulfate ($FeSO_4$) and ferroine indicator, and the carbonate content by calculating the weight difference of the sample before and after the strong effervescence by adding HCl (Loring & Rantala, 1992). The total metal contents were extracted via complete dissolution of sediment samples with an acid mixture of HNO_3-$HClO_4$-HF (ISO-14869-1:2000) (Peña-Icart *et al.*, 2011). The total metal concentrations were determined by Flame Atomic Absorption Spectroscopy (FAAS-Varian SpectrAA-200) (Skoog *et al.*, 1997). The results were evaluated by using statistical methods, environmental indicators and comparison with previous data.

Fig. 1: Map of Saronikos Gulf and sampling stations.

3. Results

3.1 Geochemical results

Table 1 presents the main findings from the determination of geochemical parameters. The percentage of TOC and carbonates and the heavy metal concentrations were determined in the fine sediment fraction (f<63μm). The percentage of sand fraction (63μm<f<1mm) was negligible (lower than 10%), which can be attributed to the depth of these stations which is above 100m. The percentages of carbonates at surface sediments are similar to those at deeper sediment layers of the collected cores. Regarding TOC, high surficial values are observed that decrease with depth.

Table 1. TOC (%), carbonates (%), and concentrations of metals (in mg Kg^{-1}), and ratios to Al (10^{-4}) in the surface and the maximum depths of cores.

Station	Depth(m)	Layer(cm)	TOC	CO_3^{2-}	Al	Ni	Zn	Ni Al^{-1}	Zn Al^{-1}
MOT 16A	100	0-1	0.67	22	27009	375	48.3	139	17.9
		30-32	0.38	23	30070	382	44.1	127	14.7
UN 5	140	0-1	0.98	21	32705	305	74.5	93.1	22.8
		30-32	0.54	22	37885	320	57.5	84.5	15.2
UN6	193	0-1	1.12	22	43264	253	92.1	58.4	21.3
		24-26	0.85	22	44547	274	62.4	61.6	14.0
UN11	420	0-1	2.35	17	54626	230	110	42.0	20.2
		30-32	1.10	16	48186	217	71.4	45.1	14.8

The higher content of Al at core UN11, in relation to the other cores, can be explained by the maximum depth of 400m at station UN11 and the association of Al with aluminosilicate minerals (Karageorgis *et al.*, 2005). The concentrations of Al and Zn at the surface sediments of the studied area are increased from the northeast to the southwest, from the shallow to the deeper part. On the other hand, the concentrations of Ni at the north area are higher than at the southern, which can be explained by the existence of the ultrabasic rocks in the soils of Susaki area and the natural weathering and transport to the coastal marine environment (Kelepertsis *et al.,* 2001).

Figure 2 presents selected vertical distributions of Ni and Zn in collected cores. Al presents fairly constant profiles in all stations. The Ni concentrations are increased with depth along cores MOT16A, UN5, UN6 and are decreased with depth in core UN11. The Zn levels show a constant decrease with depth of the collected cores, because the surface sediments are much more affected by recent anthropogenic activities than the deeper sediments at the time of their deposition (Karageorgis *et al.,* 2005).

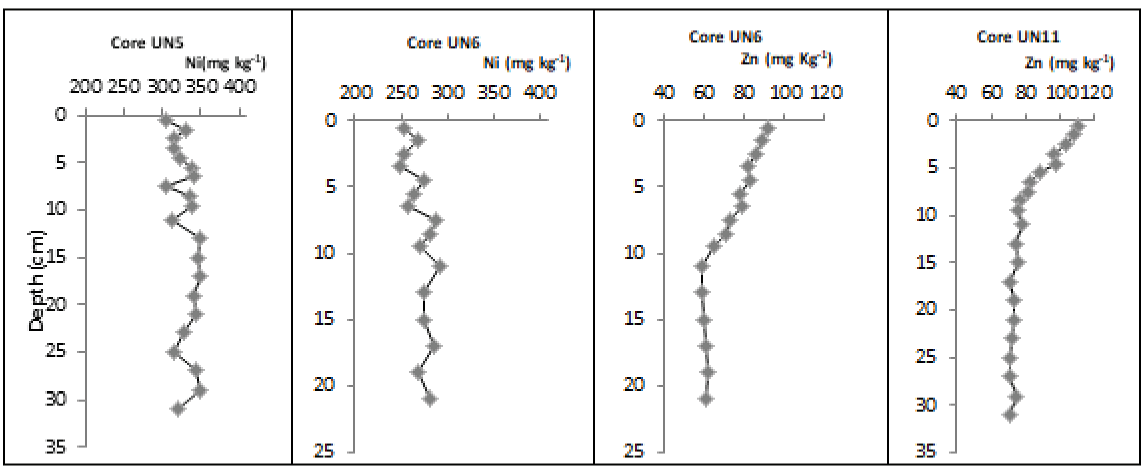

Fig. 2: Selected vertical distributions of heavy metals at the collected cores.

4. Discussion/Conclusion

The distribution of metals in sediment cores of West Saronikos Gulf, indicates enrichment from both geological and anthropogenic origins. Sediment Quality Guidelines (SQG) of effect range low (ERL) and effect range median (ERM) were used to assess the level of toxicity of metals in the surface sediments. The concentrations of Ni in the surface sediments are higher than the ERM value (51.6 mg Kg^{-1}) and as a result, they may frequently affect the biota, while the Zn levels are below the ERL (150 mg Kg^{-1}) and ERM (410 mg Kg^{-1}) values, which indicates that effects on biota may rarely be observed (Long *et al.*, 1995).

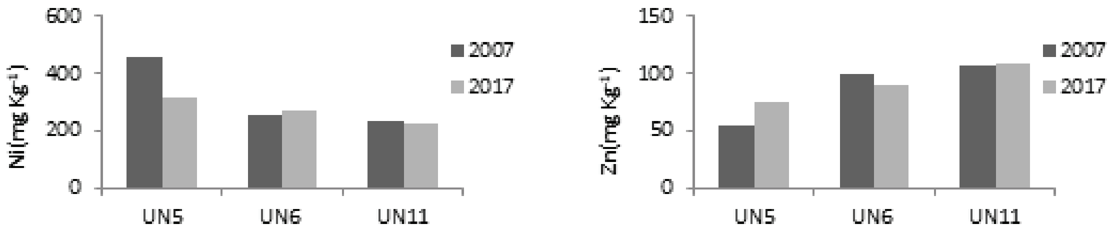

Fig. 3: Levels of heavy metals in surface sediments of 2017 and 2007.

Moreover, the metal concentrations in the surface sediments were compared with those of a similar study ten years ago and the results are depicted in Fig.3 (Paraskevopoulou, 2009). In most stations Zn and Ni concentrations remain fairly constant. In station UN5 there is a slight increase of Zn and a more prominent decrease of Ni.

The Ni and Zn concentrations at surface sediments range between 230-375 mg Kg^{-1} and 48.3-110 mg Kg^{-1}, respectively. Coarser sediments from adjacent locations collected during the same 2017 sampling present slightly higher concentrations of Ni (377-411 mg Kg-1) and somewhat lower concentrations of Zn (49.6-53.7 mg Kg-1) (Filippi, 2022). The increased Ni concentrations of the coarser West Saronikos sediments are attributed to the closer proximity to the Susaki shore line. The Zn differentiation between the coarse and finer sediments of West Saronikos is attributed to the known increased affinity of fine-grained materials for metals (Karageorgis *et al.*, 2005).

Ni concentrations throughout the northwest area of Saronikos are higher than the mean background Ni content (117 mg Kg^{-1}) estimated from sediments from various areas of Greece and similar to concentrations reported for Aliveri Bay and the Asopos river basin, due to the presence of ultrabasic rocks (Kanellopoulos *et al.*, 2022).

Compared to sediments from East Saronikos and Elefsis Gulf where Ni is found at varying concentrations below 160 mg Kg^{-1} (Karageorgis *et al.*, 2020), the Ni enrichment of West Saronikos sediments can be attributed to the geological contribution from the Susaki ophiolite complexes.

Zn levels of the surface sediments of West Saronikos, presented here, are mostly higher than the mean Zn background value of various sediments of Greece (62.8 mg Kg^{-1}) (Kanellopoulos *et al.*, 2022) indicating the anthropogenic impact for this metal. Furthermore, Zn concentrations at the center of Megara and Epidavros basins are similar to those at surface sediments of Malliakos and Pagassitikos Gulf (Kanellopoulos *et al.*, 2022) and the concentrations found in most regions of Inner and Outer Saronikos (Karageorgis *et al.*, 2020) but higher than less affected island areas such as Chios Port, Milos and Andros (Kanellopoulos *et al.*, 2022). However the West Saronikos sediments, affected by a relatively small industrial zone, are much less contaminated by Zn compared to the concentrations well above 150 mg Kg^{-1} found in specific locations of Elefsis, Inner Saronikos, Thessaloniki Bay, Ierissos and Lavrio (Karageorgis *et al.*, 2020, Kanellopoulos *et al.*, 2022) with extensive pollution sources such as the Elefsis industrial zone, Piraeus Port, the Athens Waste Water Treatment Plant outfall, major rivers of Northern Greece and current or historical mining operations.

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DIVERSITY-ECOSYSTEM FUNCTIONING RELATIONSHIP IN BENTHIC DIATOM ASSEMBLAGES IN RIVERS

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Abstract

Biodiversity-Ecosystem Functioning (BEF) relationships are important indicators of ecosystem health and species interactions. Despite their extensive study in terrestrial plants, the BEF relationship in benthic diatoms in rivers has been considerably understudied. In the present study, benthic diatom samples from nine rivers across Greece were obtained and species richness and abundance, diatoms functional traits and biomass production (as chlorophyll-a concentration) were measured. The observed relationship was not universal and differed among rivers, presenting also a strong response on environmental factors like flow and geology. This is the first extensive study of BEF in benthic river diatoms and gives important insights on possible mechanisms that require further research.

Keywords: biomass production, chlorophyll-a, functional diversity, Greece.

1. Introduction

Ecosystem functioning comprises of ecosystem processes that account for ecosystem health and sustain ecosystem services. Diversity plays a pivotal role in driving ecosystem functioning and the form of the Biodiversity-Ecosystem Functioning (BEF) relationship is indicative of species interactions and their contribution to ecosystems. BEF studies, especially early ones, mainly focus on positive relationships (i.e., increase of ecosystem functions with increased diversity). More recent research and meta-analysis, suggest different relationships (e.g., negative or hump-shaped relationships), depending on the type and duration of the study (i.e., observational field or experiment), the ecosystem type and the taxa studied (Daam *et al*., 2019). Most research occurred in terrestrial plants, whereas aquatic environments and especially freshwater remain understudied (Daam *et al*., 2019). River benthic biofilm studies are nearly missing (but see Smeti *et al*., 2019), making the importance of field observations in these systems imperative for our understanding of BEF relationships in phytobenthos.

A major component of phytobenthos in rivers are diatoms, unicellular algae with cell wall of silica, responsible for 50% of O_2 production and important indicators of water quality. Their growth depends on nutrient concentrations, and they contribute immensely to biofilm primary productivity, an important ecosystem function. A surrogate of primary productivity in algae is the concentration of chlorophyll-a (a measure of biomass accumulation) which can also be associated to the total volume of the cells in the community (i.e., biovolume). Thus, the important contribution of benthic diatoms in a fundamental ecosystem function together with the fact that they are the most diverse protists, constitute them an ideal group of organisms to study the BEF relationship.

The use of community biovolume as a measure of ecosystem function highlights the importance of cell size as functional trait. Other important traits are related to adherence to substrates and life forms and are increasingly used in describing benthic diatom assemblages. Growing evidence suggests that functional richness could be more important in driving ecosystem functions than taxonomic richness (Abonyi *et al.*, 2018). Despite their importance, functional diversity metrics are not widely used to BEF studies, denoting another gap in the research of the BEF shape.

The aim of this study was to identify the shape of the BEF relationship in benthic diatom assemblages in rivers using both taxonomic and functional diversity metrics. We further investigate possible environmental explanatory factors that underpin it. This is the first extensive field survey in rivers towards this aim and it provides useful insights in the function and diversity of these overlooked systems.

2. Material and Methods

2.1 Field sampling

Nine rivers across Greece (Nestos, Lisos, Fonias, Spercheios, Mornos, Alfeios, Arkadikos, Neda, Evrotas) were sampled in summer 2020. These rivers were selected based on available access and sampling substrate (stones) as well as due to their differences in terms of size, geology and environmental conditions. Pollution levels slightly differed even between sites of the same river, based on a biological quality diatom index, but quality classes didn't seem to play an important role in the BEF relationship (results not shown). In each river, five sampling sites were sampled across their linear flow from upstream to downstream, apart from Arkadikos and Lisos, where only four samples were taken. To ensure replication, in each site three spots were sampled, comprising of three stones each. From each stone, two surfaces of defined area were scraped, one to be used for chlorophyll analysis (immediately put in a dark bag and freezed) and the other for species identification and counting (preserved with 70% ethanol). This ensured the direct comparison between species diversity and productivity. At each site, physico-chemical parameters (Temperature, DO, pH, Conductivity, Turbidity) were also measured in-situ using a Portable multiparameter Aquaprobe and water was collected for the determination of main nutrients (NO2, NO3, NH4, TN, PO4, TP, SiO4).

2.2 Analysis of samples

In the laboratory, after filtration through 0.45 μm pore size membrane filters, nutrients were determined by a Skalar San++ Continuous Flow Analyzer (APHA, 1980). For the determination of chlorophyll, the trichromatic equations were applied (Jeffrey and Humphrey 1975), where all three main chlorophylls were measured and corrected for (Chl-a, Chl-b, Chl-c). Chl-a is a measure of the whole phytobenthos biomass production, whereas Chl-c is more indicative of the biomass produced by benthic diatoms.

Diatom species samples were treated with hot hydrogen peroxide to remove organic matter and obtain clean frustules, used for diatom species identification (Battarbee, 1986). Clean frustules were mounted with Naphrax, identified to species level with a light microscope at 1000X magnification and counted until no more new species were detected in each sample. For the taxonomy, the work of Cantonati *et al.* (2017) was mainly used. Functional traits used were linked to cell size (L/W ratio, biovolume), substrate adherence (high profile, low profile, motile and planktonic guilds), life forms (colonial, singular) and nitrogen fixation (Rimet & Bouchez, 2012).

2.3 Data analysis

Taxonomic diversity (Species Richness (S) and Evenness (J)) and functional diversity (Functional Richness based on functional traits assigned to species) indices were defined. The form of the relationship between the different diversity metrics and Chl-a and Chl-c was determined, both within each river and with the aggregated dataset. For the aggregated dataset, to define more clearly linear trends, Chl-a concentrations were log-transformed. Data analyses and illustrations were performed in R (v.4.0.3), using packages vegan v.2.5-7 (Oksanen *et al.*, 2020), BAT v.2.7.1 (Cardoso *et al.*, 2021) and ggplot2 (Wickham, 2016).

3. Results

Overall, the sampled rivers presented different environmental conditions, as depicted in the physico-chemical parameters and nutrient concentrations measured (Fig. 1). This had also an effect on diversity indices and biomass production (Fig. 1).

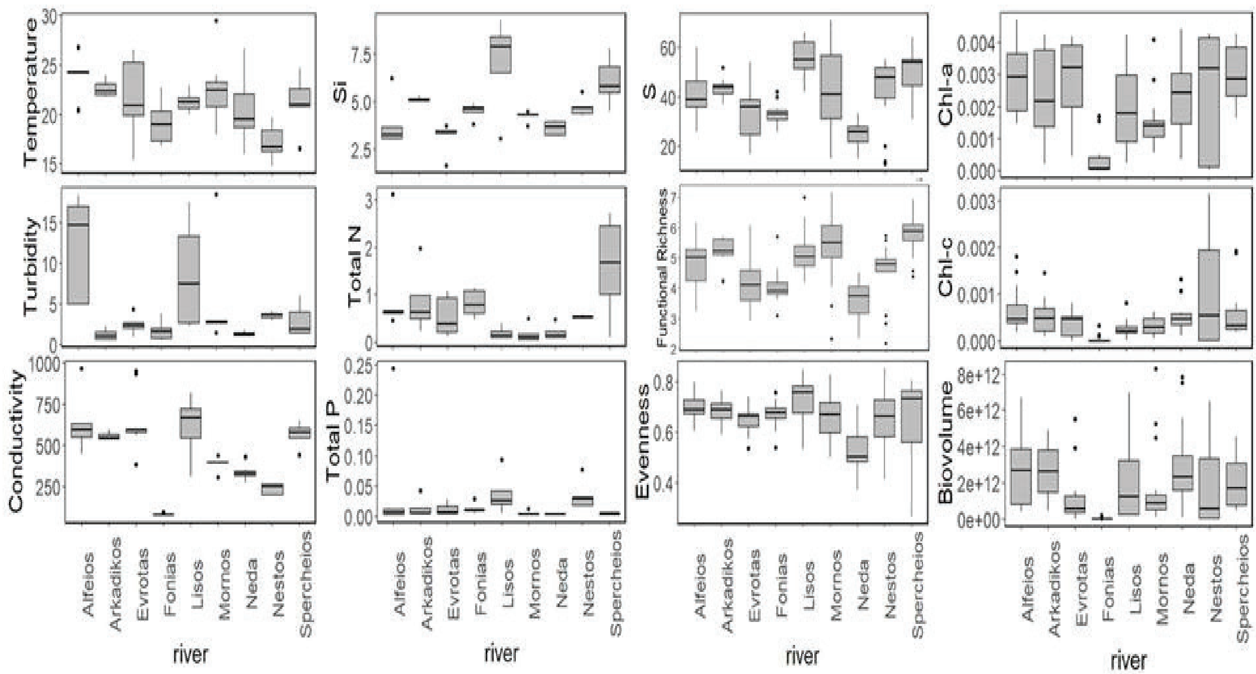

Fig. 1: Physico-chemical (Temperature, Turbidity, Conductivity), Nutrient concentration (Si, TN, TP), Diversity (Species richness –S, Functional Richness, Evenness) and Biomass (Chl-a, Chla-c, Total biovolume) variation between the nine rivers of the study.

Alfeios and Lisos presented the highest turbidity, Spercheios presented the highest TN concentration, Lisos and Nestos presented the highest TP concentration, Lisos and Spercheios presented the highest Si concentration. Lisos, Nestos and Spercheios were the rivers with the highest species richness. Functional richness presented the same trend with species richness, with the exception of Mornos that presented an increased functional richness. Fonias was the river with the lowest biomass production. All the above presented results were significant following Tukey post-hoc test. Chl-c and Total biovolume - biomass production metrics only based on benthic diatoms- presented the same trend as Chl-a – the metric of total biomass production- thus ensuring its use interchangeably.

The relationship between species richness and ecosystem functioning (i.e., biomass production measured as Chl-a concentration) is variable between the different rivers sampled (Fig. 2a). Indeed, Chl-a was best explained when the interaction between species richness and river was also considered (adjusted R^2=0.55, p<0.001). This variation is also apparent when considering other diversity metrics (Evenness J and Functional Richness, Fig. 2b,c). However, the overall relationship (when all samples were pooled together) is positive, albeit rather weak (adjusted R^2 =0.047, p<0.01, Fig. 2d). When considering the flow intensity as an additional explanatory variable, the relationship stays positive, whereas sites with faster flow present increased biomass production (adjusted R^2=0.15, p<0.001, Fig. 2e). A strong interaction effect is apparent when testing for the substrate geology. When testing for siliceous substrate, the BEF relationship is positive, whereas in non-siliceous substrate, there is no relationship (Fig.2f). Results are similar when testing for relationships between species richness and Chl-c (not presented).

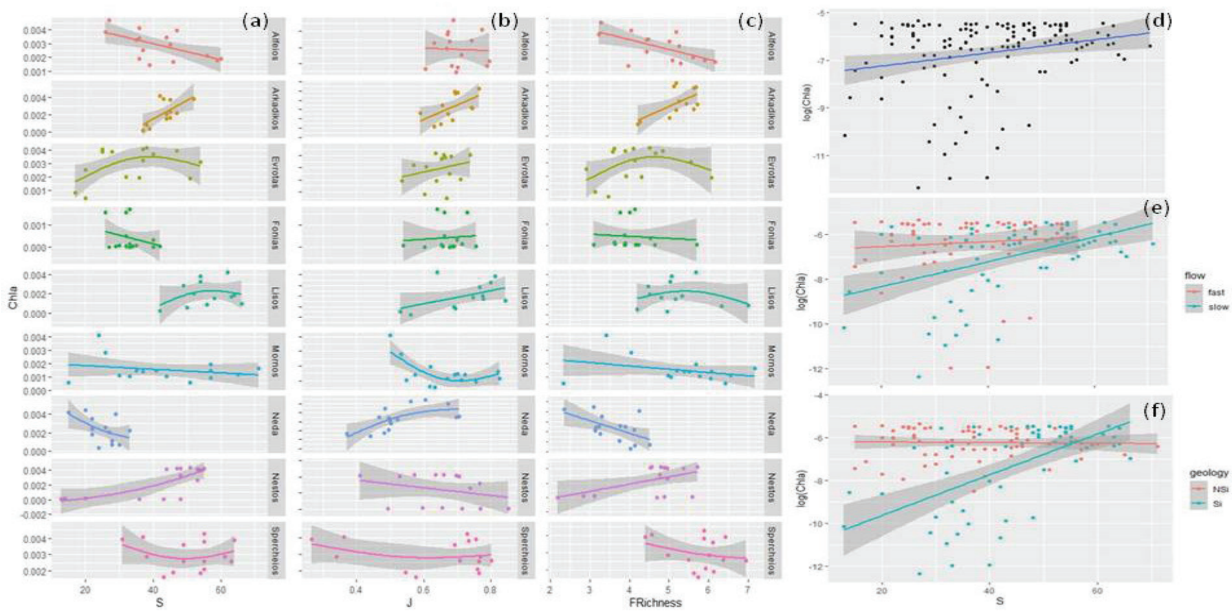

Fig. 2: Diversity (presented as species richness –S on x-axis in panels a,d,e,f) and ecosystem functioning (presented as Chl-a on y-axis) relationships in each river (a-S, b-Evenness J, c-Functional Richness), (d) the whole dataset, (e) at different flow levels (fast vs slow) and (f) in siliceous (Si) or non-siliceous (NSi) substrate. In panels d,e,f the y axis is log-transformed, to better express the linear trends.

4. Discussion/Conclusion

The shape of BEF relationship in benthic river diatoms is not universal. Different species interactions occur at different rivers, indicating a strong environmental effect on the form of the BEF relationship. Indeed, the response of biomass production to species richness depended on multiple environmental factors (i.e., geology, flow). The most pronounced effect is the dependence of the positive BEF relationship on river geology: the increase of species number results in an increase in biomass production only in rivers with siliceous substrate. Geology is linked to phosphorus concentrations, as calcareous soils can retain phosphorous, contrary to siliceous soils (Skoulikids, 2018), thus, changing nutrient stoichiometry. Therefore, it is possible that the effect of geology indicates an effect of differing nutrient limitation and species competition on the BEF relationship (i.e., on species richness and biomass production). Competition for available nutrients shapes microalgae communities and affects biomass production (Tilman, 1982), whereas the shape of the BEF relationship depends on species life-history traits (i.e., growth rate and competitive ability for nutrients) (Smeti *et al.*, 2018).

Functional traits important for river ecosystems and life-history traits of diatoms could provide a mechanistic understanding of the shape of the BEF relationship and consequently give us important insights on ecosystem health and protection. These aspects could not be controlled in this first extensive field survey, as in all field surveys. However, we gained important insights on the complexity of the drivers and factors emerged in this study (i.e., geology, nutrient concentrations, flow), that will be used for further exploration using experimental set-ups or numerical modeling.

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A MACROALGAE-BASED ECOLOGICAL STATUS ASSESSMENT OF SELECTED AREAS ADJACENT TO FISH-FARMS IN GREECE.

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Abstract

Constant anthropogenic pressures to coastal ecosystems oblige the need for their continuous monitoring and ecological quality evaluation. In this framework, macroalgal assemblages have often been used for the assessment of coastal ecosystems status. The aim of the present study is to evaluate the pressure imposed by aquaculture on littoral rocky-shore ecosystems with the aid of benthic macroalgal communities. In different regions of Greece, areas adjacent to fish farms were selected and divided into two sites, “far” and “near”. Both *in situ* samples and representative photos (photoquadrats) were obtained from each site, during the period July-September 2021. Afterwards the main algae genera covering the rocky bottoms were identified and categorized into Ecological Status Groups (ESGs) based on EEI index. Results showed that “far” sites hosted more genera which belonged to the I EGS (late-successional), while in the “near” sites diversity was lower and the number of II ESG (opportunistic) genera appeared increased. The above results are indicative of the impact of fish farms on hard substrate macroalgal communities. The obtained data are discussed in the context of the Ecological Evaluation Index continuous formula (EEI-c) application.

Keywords: monitoring, aquaculture, seaweeds, indices, hard substrate.

1. Introduction

Marine coastal areas are among the most subjugated habitats and therefore predominantly prone to human pressures. Thus, governmental legislation was developed for sustainably managing marine coastal resources, waters and habitats (Borja *et al.*, 2010), requiring assessment of coastal system ecological quality and capacity. Ideally, early warnings of change should be evaluated. In this context, long-term monitoring project is an ideal tool for identifying changes in biodiversity and ecosystem functioning at a given space and time, to recognize any response to anthropogenic pressures and - if necessary- propose the appropriate measures (Birk *et al.*, 2012).

Macroalgae are being used in ecological assessments (Stevenson, 2014) since they are sensitive to stress and are important components of the coastal systems structure (Piazzi & Ceccherelli, 2020). Monitoring programs using macroalgae have utilized a variety of methods of mapping and sampling, and different ecological descriptors have been employed (Krumhansl *et al.*, 2016; Duffy *et al.*, 2019), while a number of different ecological indices based on macroalgal assemblages have been developed (Orfanidis *et al.*, 2003; Cecchi *et al.*, 2014).

In macroalgae-based environment-health assessments, the use of photographic techniques along with identification of the main macroalgae taxa coverage is considered as a reliable and cost-effective method to study changes in the macroalgal community structure, especially in habitats with high spatial variability (Cecchi *et al.*, 2014). Furthermore, photographic sampling allows the collection of a large number of samples and keeps the time spent underwater at a minimum (Benedetti-Cecchi *et al.*, 2001).

The aim of this study was to use macroalgal assemblages in order to provide a preliminary assessment of the impact of aquaculture units on benthic hard-substrate macroalgal communities in two different proximities, using a combination of photoquadrads and laboratory identification of genera. Data assembled are discussed in the context of applying an ecological index.

2. Material and Methods

During the period of July – September 2021 locations adjacent to fish farms in a number of different regions of Greece (depicted on Fig. 1) were visited. The main hypothesis was that the closer the distance to the cages was, the more intense the impact fish farms would pose to the macroalgae population. Consequently, each sampling location was divided in two regions. Sites referred as“near” were the ones considered to be more affected and were closer to the fish farms, while those named as “far”, were considered less affected, and they were more distant to the aquaculture cages (Table 1). In some cases, only “far” samples were collected, due to the potential installation of fish farms in the area (new position). *In situ* qualitative samples of benthic macroalgal species were collected from rocky substrates in depths between 0 to 1 m by snorkeling. The samples were preserved in a 4% formalin solution. Additionally, representative photographs using a 25 x 25cm quadrat were taken from the selected sites following a specific protocol (transect). Subsequently, taxa were categorized into ecological groups following the EEI-c index of Orfanidis *et al.*, 2011, with the future aim of calculating the ecological status of the areas.

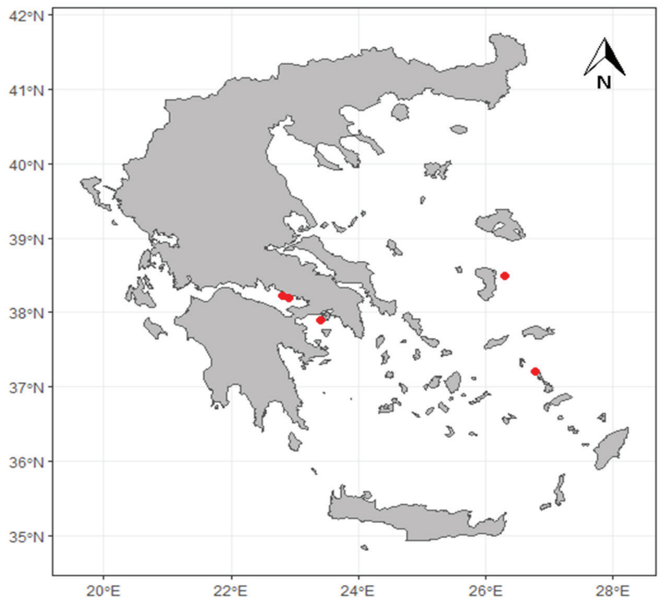

Fig. 1: The study sites (red dots on the map). In some locations two sites were selected, one considered as “near” and one as “far”.

Table 1. Description of the study sites. N=Sites referred to as “near”, F=Sites referred to as “far”.

Location Name	Track Code	Track length (m)	Geographic Area	Status	Latitude	Longitude	Closest distance to farm (m)	Years of Farms' operation
Tsoumas	TSOU_F	364	Gulf of Corinth	F	38,19429	22,90282	No farms	
Biotia	BIO_F	226	Gulf of Corinth	F	38,22940	22,81185	140	NA
Salamina	SAL_N	231	Saronic Gulf	N	37,89314	23,41102	60	32
Salamina	SAL_F	181	Saronic Gulf	F	37,89267	23,40770	275	
Leros	LER_F	301	SE Aegean	F	37,20121	26,77600	180	
Leros	LER_N	127	SE Aegean	N	37,20310	26,77686	70	30
Batos	BAT_N	155	N. Aegean	N	38,49128	26,28759	80	27

3. Results

The analysis of the *in situ* collected macroalgae samples revealed taxa belonging to 13 genera some of which remained unidentified, but were assigned to the 3 following categories: filamentous and encrusting rhodophyta and filamentous chlorophyta. The following native macroalgae genera - classified in the appropriate Ecological Status Groups (ESG) according to the EEI-c index (Orfanidis *et al.*, 2011) respectively - were found at the study sites: *Amphiroa* (IC), *Bryopsis* (IIB), *Ceramium* (IIB), *Corallina* (IC), *Dictyota* (IIA), *Halimeda* (IC), *Halopteris* (IIA), *Jania* (IC), *Laurencia* (IIB), *Padina* (IB), *Sargassum* (IB), *Valonia* (IIB). In LER_N and BAT_N sites the invasive chlorophyte *Caulerpa* (IIB) was also present, while the invasive seagrass *Halophila* (No classification) was also found in LER_N site.

Table 2. Ecological status groups of macroalgae found at the studying sites, according to *in situ* samples' analysis. All organisms have been classified at the genus level or in a larger taxonomic category when this was not possible, so that they can be classified in the appropriate Ecological Status Group (ESG) according to the EEI-c index (Orfanidis *et al.*, 2011).

Area	IB	IC	IIA	IIB	*Halophila*	Sum
BAT_N		1		2		**3**
LER_N	1	1	2	1	1	**6**
SAL_N	1	3	1	1		**6**
BIO_F		6	2	1		**9**
LER_F	2	3	1	4		**10**
SAL_F	1	4	2	1		**8**
TSOU_F	1	6	2	2		**11**
Sum	**6**	**24**	**10**	**12**	**1**	**53**

ESG I=late-successional; ESG II=opportunistic; ESG IA=thick perennial; ESG IB=thick plastic; ESG IC=shade-adapted plastic; ESG IIA=fleshy opportunistic; ESG IIB=filamentous sheet-like, opportunistic.

The most abundant ESG which was present in all examined sites, according to the *in situ* samples' analysis, was IC (Table 2). Genera categorized as late successionals were present 30 (59%) times, while opportunistic ones 22 (41%) times in total. The late successional ESG IA was totally missing from all the examined sites. The site with the highest diversity of genera was TSOU_F. In all "far" sites except from Leros, as well as the "near" site Salamina, macroalgae classified as late successional (ESG I) were dominant, whereas in the "near" sites of Batos and Leros, genera of ESG II (opportunistic) were dominant.

Regarding the photographic sampling most of the genera found *in situ* could also be discerned in the photos, with a representative example shown in Figure 2. Strictly considering the presence/absence of certain ecological groups, the genera found at SAL_F belong mainly to ESG IC (*Amphiroa*, *Corallina*, *Jania*) and one genus of ESG IIA was present (*Dictyota*). In SAL_N there were 2 genera of ESG IC (*Amphiroa*, *Jania*) and 1 genus of ESG IIA (*Dictyota*). In LER_F there were 2 genera of ESG IC (*Amphiroa*, *Jania*) 2 genera of ESG IIA (*Dictyota*, *Laurencia*) and 1 genus of ESG IB (*Padina*) while in LER_N there was 1 genus of each ESG IC (*Jania*), IB (*Padina*) and IIA (*Dictyota*), respectively, and the invasive seagrass *Halophila*. However, in Figure 2, a clear difference in macroalgal prevalence between "far" and "near" sites can be observed, as the different genera show a very different coverage, a factor which has not yet been taken into account in the present work.

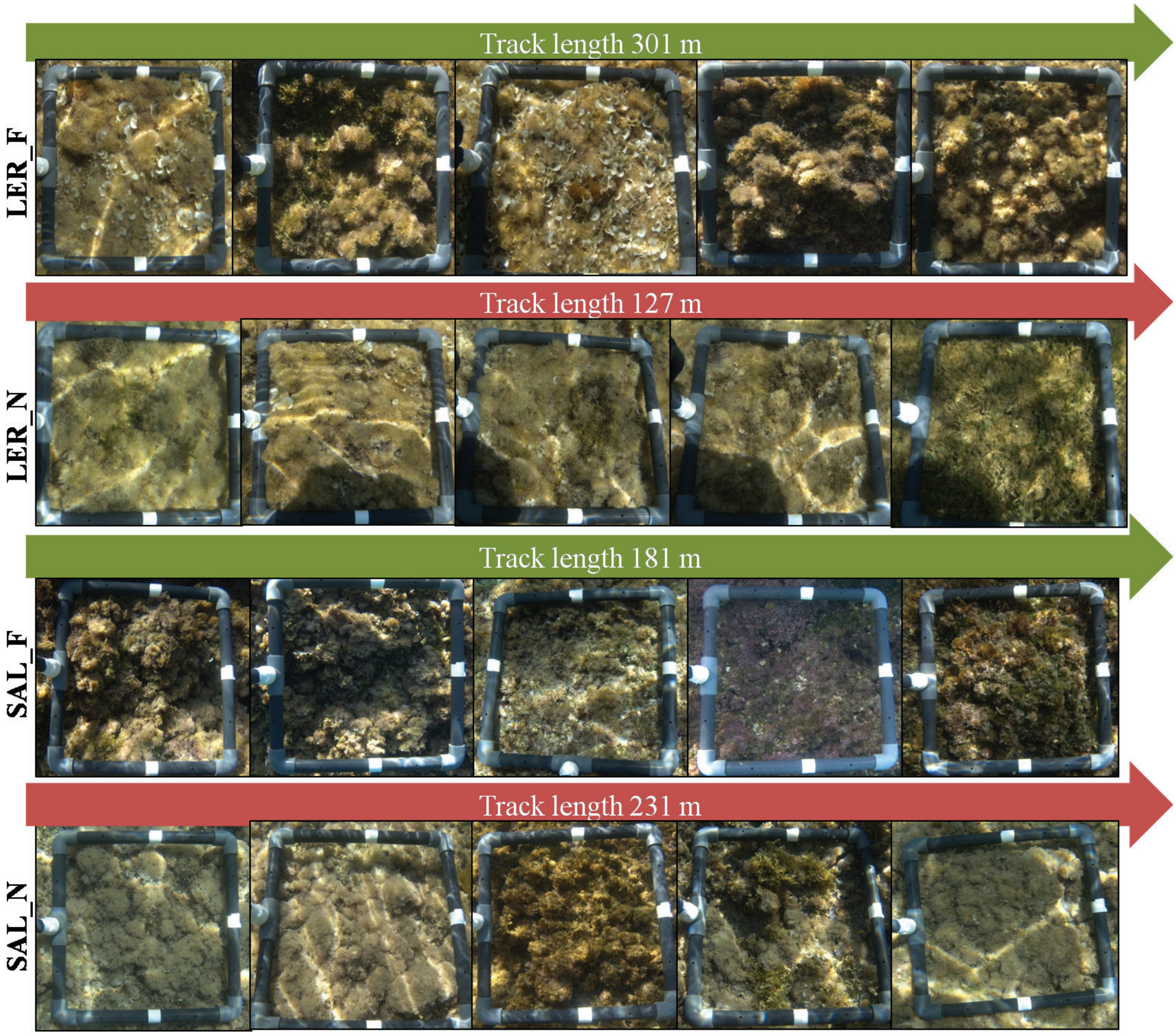

Fig. 2: Comparison of "far" (LER_F, SAL_F) and "near" (LER_N, SAL_N) photographic samples along a transect (indicated by the arrows) from Salamina and Leros.

4. Discussion/Conclusion

As a general trend, "far" and "near" sites exhibited different composition of macroalgae genera, also detected by observation of the photographic samples (Fig. 2). The number of genera present at the "near" sites was reduced compared with the "far" ones. In addition, "far" sites showed a higher percentage of genera belonging to ESG I than "near" sites. The effluent discharge from open cage fish farms includes, among other things organic matter, nutrients, suspended solids, is usually high. However, the level of impact depends on a variety of factors such as depth, sediment type, strength and direction of currents and farming intensity (Ruiz *et al*. 2001). Several studies have been focused on direct and indirect effects of nutrient enrichment on macroalgal assemblages (Martínez *et al*. 2012; Bulleri *et al*. 2020). Generally, opportunistic and fast growing algae (ESG II) can be rapidly boosted by nutrient enrichment (Pinedo *et al*. 2015; D'Archino *et al*., 2021). On the contrary, late successional and slow-growing macroalgae (ESG I), especially the habitat-forming ones, as well as their early life stages, can be impaired (Bergström *et al*. 2003). As a result, and in accordance to previous studies, macroalgae are specifically suitable for monitoring fish farm impacts (Howarth *et al*., 2019). Thus, both methologiues (in situ and photographic samples), showed a clear response of the macroalgal assemblage's structure (Table 2; Fig. 2).

However, these results are preliminary and coverage (and thus potential dominance of taxa) has not yet been taken into account. Consequently, the next step is to include the calculation of an ecological index, including both identity and dominance of taxa, which will lead to more robust conclusions. An indicative index that could be applied is the Ecological Evaluation Index continuous formula (EEI-c) which was designed to evaluate habitat- based ecological status of rocky coastal and sedimentary transitional waters using shallow benthic macrophyte assemblages as bioindicators (Orfanidis *et al.,* 2011).

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COMPARATIVE STUDY OF OTOLITH BILATERAL ASYMMETRY BETWEEN TWO FISH SPECIES OF THE FAMILY MULLIDAE: THE NATIVE *MULLUS BARBATUS* AND THE LESSEPSIAN MIGRANT *UPENEUS PORI*

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Abstract

Otolith bilateral asymmetry may reflect the environmental stress experienced by fish during their life. The present study compared the otolith asymmetry between two species of the family Mullidae, the native *Mullus barbatus* (red mullet) and the alien *Upeneus pori*, in order to evaluate the degree of adaptation of *U. pori* to its new environment. The analysis of 10 otolith traits (size and shape descriptors) showed that otolith asymmetry was not higher in the alien species which suggests that *U. pori* is comparatively well adapted to its new habitat. Future research is warranted in order to further assess the utility of otolith bilateral asymmetry as a bioindicator for evaluating the degree of adaptation of Lessepsian migrants.

Keywords: otolith morphology, alien species, adaptation.

1. Introduction

Otolith morphology is determined by genetic and environmental interactions and presents a sensitive indicator of developmental errors (Geladakis *et al*., 2021, 2022). In particular, otolith bilateral asymmetry is a popular tool for examining the effects of environmental stress on the health status of wild populations (Gagliano *et al*., 2008). The aim of the present study was to explore the use of otolith asymmetry as a bioindicator for evaluating the adaptation of invasive species to their new (foreign) habitats. For this purpose, a comparatively study was performed on two species of the family Mullidae, namely, the native *Mullus barbatus* (red mullet) and the alien *Upeneus pori*, caught in the Gulf of Heraklion Crete.

U. pori is a subtropical species, distributed along the western Indian Ocean (Ben-Tuvia & Golani, 1989). It entered the Mediterranean Sea via the Suez Canal (Lessepsian migrant) and was first recorded in 1950 (Deidun *et al*., 2018). Since its initial detection, *U. pori* has expanded its distribution in the Mediterranean Sea and established successful populations (Deidun *et al*., 2018).

2. Material and Methods

A total of 105 and 117 specimens of the native (*M. barbatus*) and alien (*U. pori*) species were used for this study. All individuals were caught with an experimental bottom trawl (with 40-mm stretched, diamond-mesh net in the cod-end) on board the R/V PHILIA during the "*LeFish*" research action (supported by "*RePhil*") in the Gulf of Heraklion, Crete, on January 20-21, 2022. Trawling was carried out on a shallow (30-35 m) sandy area covered with the seaweed *Caulerpa prolifera*.

In the laboratory, each fish was measured for standard length (SL, tip of snout to base of the central caudal lepidotrichia, mm) and weighed (TW, g). The length-weight relationship ($TW = a \times SL^b$) was fitted in both species by linear regression analysis after logarithmic transformation of TW and SL. From each specimen, the largest pair of otoliths (*sagittae*) was removed and individually photographed. All otolith images were analyzed using the "ShapeR" package (Libungan & Palsson, 2015), open software in R (version 4.0.03, R Core Team, 2020). After the extraction of otolith contours, otolith bilateral asymmetry was assessed for ten traits; four univariate morphometric descriptors (maximum length, O_L; maximum depth, O_D; surface, O_S; perimeter, O_P) and six high-amplitude harmonics (H_2-H_7), produced by a normalized elliptic Fourier technique (Geladakis *et al.*, 2021, 2022).

All traits were tested for the type of bilateral asymmetry, i.e., fluctuating asymmetry, directional asymmetry or antisymmetry (Somarakis *et al.*, 1997). Specifically, one-sample t-tests were carried out to evaluate the presence of directional asymmetry (skew, g1≠0) and antisymmetry (kurtosis, g2≠0) in the R-L (right minus left) distribution for each otolith trait. The variance of the bilateral difference [index FA_1=var(R-L)] was estimated for each species and otolith trait, as an index of fluctuating asymmetry (Palmer & Strobeck, 1986). The significance of differences in FA_1 between the two species was tested using F-tests (Somarakis *et al.*, 1997).

3. Results

The standard length (SL) of the native fish (*M. barbatus*) ranged from 69 to 104 mm, whereas of the alien fish (*U. pori*), from 76 to 127 mm. The growth in weight of *M. barbatus* presented a uniform allometric pattern (Fig. 1A). Instead, in the case of *U. pori*, the TW-SL relationship presented an inflection point at 85.9 mm SL (logSL=1.935), above which the rate of TW increase was lower (blue points in Fig. 1B).

For all otolith traits examined, antisymmetry was absent, with the kurtosis (g2) estimates being either non-statistically different from zero or significantly positive (leptokurtic). Directional asymmetry was detected for the shape descriptors H_4 and H_7 (skew significantly positive, R > L) and the size descriptors O_D and O_P (skew significantly negative, R < L). However, fluctuating asymmetry indices based on signed R–L values (e.g., FA_1) are not biased by skewed distributions (Palmer & Strobeck, 1986). Interestingly, for four shape morphometric descriptors (H_2, H_3, H_4, H_6), FA_1 was significantly higher in the native *M. barbatus* compared to the alien *U. pori* (Fig. 2).

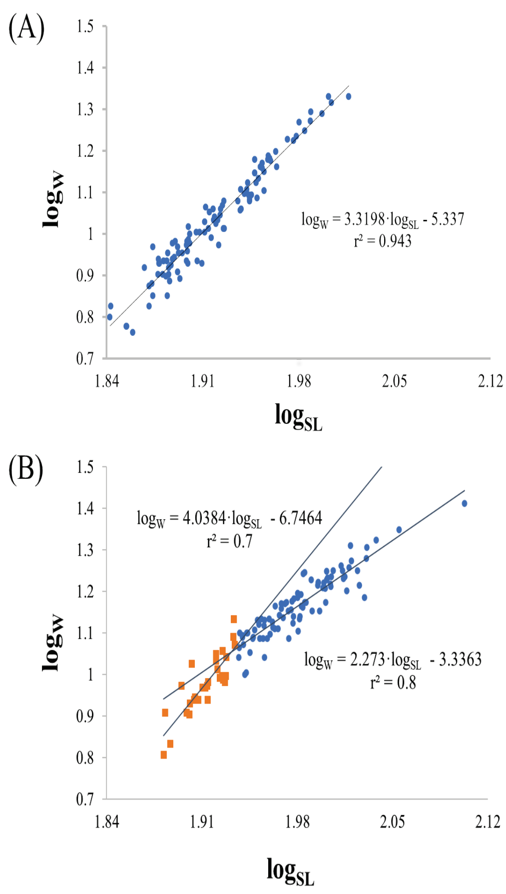

Fig. 1: Allometric relationship between total weight (TW, g) and standard length (SL, mm). (A) *Mullus barbatus* and (B) *Upeneus pori*. Different colors in *U. porri* indicate the change of the TW-SL relationship.

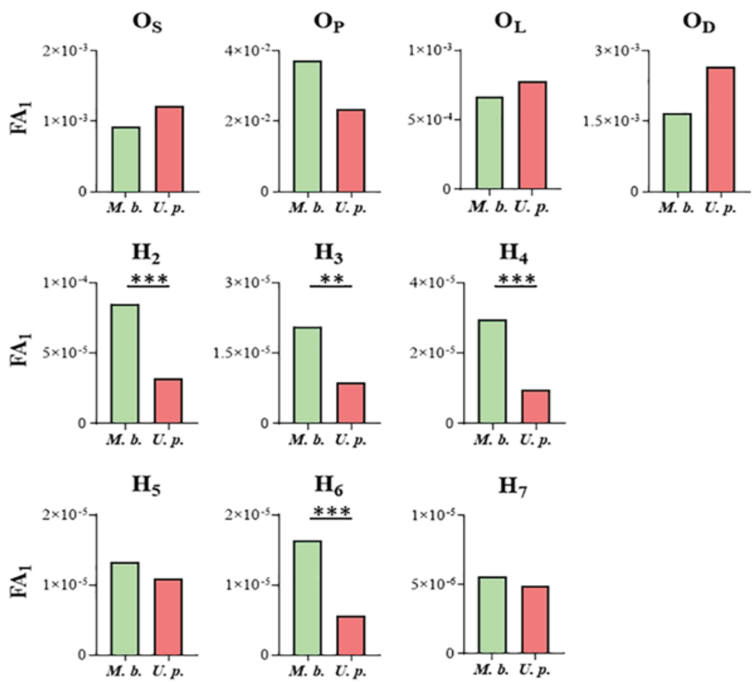

Fig. 2: Comparison of the index FA_1 between the native fish species *Mullus barbatus* (*M.b.*; green) and the alien species *Upeneus pori* (*U.p.*; red). O_L, maximum otolith length; O_D, maximum otolith depth; O_S, otolith surface area; O_P, otolith perimeter; H_2-H_7, harmonics two to seven. Significant differences between the two species are shown with **p < 0.01, ***p < 0.001.

4. Discussion/Conclusion

Existing literature indicates that deviations from perfect bilateral symmetry in otolith morphology may result from different environmental stressors during fish development (expressed as fluctuating asymmetry, FA). For all of the examined otolith traits, the alien species *U. pori* did not exhibit higher bilateral asymmetry in comparison to the native *M. barbatus*. Instead, *M. barbatus* presented significantly higher otolith asymmetry in several shape descriptors. Given that the level of fluctuating asymmetry is a measure of environmental stress (Palmer & Strobeck, 1986), these findings suggest that the examined alien species is comparatively well adapted to its new habitat. In other cases, departures from bilateral asymmetry may arise as a phenotypically plastic response, induced by environmental and/or genetic drivers (expressed as directional asymmetry, DA) (reviewed in Geladakis *et al*. 2021, 2022). The comparatively lower otolith asymmetry of *U. pori* may reflect a strong selection against individuals with high asymmetry and/or other developmental disadvantages (e.g., deformities) within the current (new) environmental conditions, probably during early developmental stages (e.g., larval stages). On the other hand, the significantly higher otolith asymmetry of *M. barbatus* may imply a stressful situation for native species in the Mediterranean Sea under the current trend of increasing sea temperature.

More research is warranted (including the study of alien fish samples at different stages of development and geographical sites), in order to further evaluate the utility of otolith bilateral asymmetry as a tool to assess the adaptation of alien species in their new environments and the chances of establishing abundant populations in the Mediterranean Sea.

5. Acknowledgements

The action *''LeFish''* - Study on the degree of adaptation of Lessepsian migrants to Cretan coastal ecosystems" was part of the project "Hellenic Research Fleet - Reconstruction of the Research Vessel PHILIA, funded by the Operational Program (OP) "Competitiveness Entrepreneurship and Innovation" (NSRF) for the period 2014-2020 and co-financed by the European Regional Development Fund (ERDF).

We would like to thank Dr. Fragkoulis Stefanos (member of "*LeFish*" research team) and Dr. Nota Peristeraki for their help during the experimental bottom trawling onboard the R/V PHILIA.

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PHYTOPLANKTON SAMPLING IN GREEK TRANSIENT WATERS: PRELIMINARY RESULTS

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Abstract

Fifteen transient water systems including mostly coastal lagoons in both the Aegean Sea and the Ionian Sea were sampled for physicochemical parameters and key phytoplankton species composition. According to the results of chlorophyll a, all the ecosystems examined could be classified as eutrophic. Less eutrophic, close to mesotrophic were the coastal lagoons Tsoukalio and Logarou in the close embayment of Amvrakikos Bay whereas the Rhodia Lagoon within the Tsoukalio Lagoon in the same area, showed the most eutrophic character. Mavrolimni a coastal lagoon in the Corinthian Gulf and the closed embayment of Kalloni in Lesvos Island in the Aegean showed high eutrophic character as well. Cyanobacteria and diatoms species were always among the most abundant phytoplankters with at times representatives from chrysophytes and cryptophytes. *Tetraselmis* and other greens were abundant as well.

Keywords: Greek coastal lagoons, phytoplankton, trophic level.

1. Introduction

Transient water systems such as coastal lagoons, estuaries etc., are very interesting systems as they host numerous opportunistic phytoplankton species with dominance continuously changing as the environmental conditions are changing frequently showing high variation at times. In this study fifteen transient water systems were sampled during early autumn for physicochemical parameters, photosynthetic pigments and phytoplankton species composition.

2. Material and Methods

All transient systems were sampled once during autumn 2020 (except for the Lesvos Island's ones, sampled during summer). A most representative spot was chosen for sampling from each system, far from the inlet of the open sea. Sampling depth was set at least to one meter from the surface and one meter from the bottom, to get the most phytoplankters avoiding benthic species and the photoinhibiting parts close to surface. However, most systems were very shallow thus in most cases sampling depth was set to 0.5 m from surface. Sampling was done by manual pumping to avoid disturbing the sentiments. Salinity (translated from conductivity compensated for temperature), dissolved oxygen and temperature presented in this report were determined using a multi-sensor instrument with long cable (Lovibond). Water samples were filtered on GF/C filters for photosynthetic pigment analysis based on Parsons *et al.*, (1984) using the equations of Mitchell & Kiefer, (1984). Water for community and abundance analyses was collected and fixed with Lugol's solution. Species identification was carried out using common keys while phytoplankton abundance was assessed by inverted microscope (Zeiss) using the method described by Utermöhl (Edler, & Elbrächter, 2010). As most of the phytoplankters were very small (micro- and nannoplankton) precise identification was not possible; therefore, the semi-quantitative method was used as described by Hällfors, (2013).

3. Results

In Table 1 a gross description of the status of each transient ecosystem assessed is presented as well as the gross species composition and semi-quantitative abundance.

Table 1. Gross description of certain transient marine Ecosystems status in Greece (BG: blue-green algae species, the more the * the more abundant the species).

Location coordinates	type	time	Sampling depth (m)	Temperature °C	Salinity (ppt)	DO (mg/L)	Chl a (μg/L)	Dominant phytoplankters
Corinthian Gulf, Ionian Sea								
Mavrolimni 38.059378, 23.106576	Coastal lagoon	OCT 2020 2:00 pm	2	26.2	43	7.45	8.038	BG sp.1*** Oedogoniales sp.1* Chrysophyte sp.1**
Louros -Arachthos Delta, Amvrakikos Embayment, Ionian Sea								
Mazoma 39.012917, 20.753685	Coastal lagoon	OCT 2020 10:00 am	2	20	39	5.60	4.01	BG sp.1*** Pennate diatom sp.1* Red cryptophyte sp.1* Coccoid green sp.1* Chrysophyte sp.1*
Rhodia 39.100646, 20.831652	Coastal Lagoon inner	OCT 2020 3:00 pm	2	23.4	33	7.61	11.22	BG sp.1*** Pennate diatom sp.1* *Tetraselmis* sp.*
Tsoukalio 39.044947, 20.859865	Coastal lagoon	OCT 2020 4:00 pm	2	24.7	31	8.94	1.19	BG sp.1* Pennate sp.1 ** Pennate sp.2 * *Cymbella* sp.1** *Entomoneis* sp. ** Cryptophyte sp.1 * *Tetraselmis* sp. *
Logarou 39.046258, 20.861544	Coastal lagoon	OCT 2020 3:00 pm	0.5	23.7	46	6.90	1.28	Helical BG sp.1* Filamentous BG sp.1*** Pennate diatom sp.1* Brown cryptophyte sp.1* Red cryptophyte sp.1* *Tetraselmis* sp. *
Agrilos 39.054287, 21.090420	Coastal lagoon	OCT 2020 3:00 pm	0.5	22.1	34	7.10	4.25	BG sp.1 ** BG sp.2 ** Coccoid green sp.1**

Location coordinates	type	time	Sampling depth (m)	Temperature °C	Salinity (ppt)	DO (mg/L)	Chl a (μg/L)	Dominant phytoplankters
Kalamas River Delta, Ioanian Sea								
Richo 39.521091, 20.190589	Coastal lagoon	SEP 2020 12:00 pm	0.5	22.2	24	5.50	4.57	BG sp.1** Pennate sp.1*** *Nitzchia* sp. ** Red Cryptophyte sp.1* Coccoid green sp.1*
Loutsa-Papadia 39.521697, 20.161451	Coastal lagoon	SEP 2020 10:00 am	0.5	20.7	38	6.93	1.49	BG sp.1* Pennate diatom sp.1* Chrysophyte sp.1* *Tetraselmis* sp. *
Vatatsa 39.521091, 20.190589	Coastal lagoon	SEP 2020 11:00 am	0.5	22.2	27	8.00	3.22	Oscillatoriales sp.1* Brown cryptophyte sp.1* Coccoid green sp.1*
Calanga 39.529492, 20.149835	Coastal lagoon	SEP 2020 2:00 pm	0.5	21.8	15	7.70	5.44	BG sp.1 ** Pennate diatom sp.1** Brown cryptophyte sp.1* green coccoid sp.1 *** coccoid green sp.2 **
Vondas 39.552491, 20.160810	Coastal lagoon	SEP 2020 12:00 pm	0.5	23.0	43	7.36	5.48	Helical BG sp.1* Nostocales sp.1 ** Amphidinium sp.* Red cryptophyte sp.1** Coccoid green ***
Bastia 39.563491, 20.164525	Coastal lagoon	SEP 2020 5:00 pm	0.5	24.9	35	8.94	4.22	Nostocales sp.1*** sp. Diatom sp.1* Brown cryptophyte sp.1* Haptophyte sp.1* *Tetraselmis* sp. **
Argolic Gulf Aegean Sea								

Location coordinates	type	time	Sampling depth (m)	Temperature °C	Salinity (ppt)	DO (mg/L)	Chl a (μg/L)	Dominant phytoplankters
Vivari 37.534066, 22.905895	Coastal lagoon	OCT 2020 11:00 am	1.5	24.2	45	3.90	3.64	BG sp.1* *Chaetoceros* sp. *** Brown cryptophyte sp.1* Red cryptophyte sp.1** *Tetraselmis* sp. * Coccoid green sp.1*
Lesvos Island Aegean Sea								
Gera Bay 39.087975, 26.520430	Closed embayment	JUN 2020 1:00 pm	2	23.0	45	7.20	2.05	BG sp.1*** Haptophyte sp.1*
Kalloni Bay 39.168961, 26.155181	Closed embayment	JUN 2020 11:00 am	2	24	45	6.70	7.4	BG sp.1*** Helical BG sp.2 * *Entomoneis* sp.* Brown cryptophyte sp.1* Chrysophyte sp.1*

4. Discussion/Conclusion

All aquatic ecosystems sampled were characterised as eutrophic according to classification based on the Chl a content per litre (Beiras, 2018). Of those, close to mesotrophic were the coastal lagoons Tsoukalio and Logarou in the close embayment of Amvrakikos Bay, whereas the Rhodia Lagoon within the Tsoukalio Lagoon in the same area, showed the most eutrophic character. Mavrolimni a coastal lagoon in the Corinthian Gulf and the closed embayment of Kalloni in Lesvos Island in the Aegean showed high eutrophic character as well. Cyanobacteria and diatoms species were always among the most abundant phytoplankters with at times representatives from chrysophytes, cryptophytes, *Tetraselmis* and other greens were abundant as well.

5. Acknowledgements

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ISOLATION AND GROWTH IN CULTURES OF THE MICROALGA *PHAEOCYSTIS*, A MUCILAGE BLOOMER FROM THE AEGEAN SEA

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Abstract

Harmful Algal Blooms (HABs) play a central role as traits for ecological/environmental status assessment. Phytoplankton species that produce HABs are therefore considered as key elements for marine monitoring. Some coastal areas in the Mediterranean Sea are listed as "hot spots" due to the formation of blooms with toxic species or high biomass producers. During summer 2021, a persistent outbreak of dense foamy mucilage phenomena was reported from the Sea of Marmara, where the microalga *Phaeocystis pouchetii* was dominating. Mucilage phenomena of lower intensity were reported from the North Aegean coasts as well. A short-lived bloom of *Phaeocystis* sp. with foamy mucilage aggregates was also observed on the coast of Mount Pelion in North Aegean. In this study, we present the isolation of the microalga *Phaeocystis globosa* from Saronikos Gulf waters in the Aegean Sea. We also present a culture experiment and discuss about the characteristics and growth of *Phaeocystis* in culture conditions.

Keywords: *Phaeocystis,* Harmful Algal Bloom HAB, mucilage foam, microalgae cultures, phytoplankton.

1. Introduction

Some phytoplankton species are considered as key elements to monitor the marine environment in certain cases, for example when they produce harmful algal blooms (HABs). Phytoplankton blooms play a central role as ecological/environmental status assessment traits of high policy importance for Water Framework Directive WFD (2000/60/EC) and Marine Strategy Framework Directive MSFD (2008/56/EC amended by 2017/845/EU) (Ferreira *et al.,* 2011). However, one of the main challenges in their practical application is the need of data with frequency, corresponding to the spatial and temporal scales of phytoplankton variability (Varkitzi *et al.*, 2018a).

The risk for important HAB events is rather low in the open waters of the Mediterranean Sea due to their oligotrophic character (Varkitzi et al., 2020), but potentially toxic algae may occasionally occur during the seasonal phytoplankton peaks (Garcés & Camp, 2012; Zingone *et al.,* 2021). In the Mediterranean coastal waters, some regions are listed as "hot spots" because they frequently generate the formation of blooms with toxic species or high biomass producers, namely the Alboran, Ligurian, Adriatic and Aegean Seas.

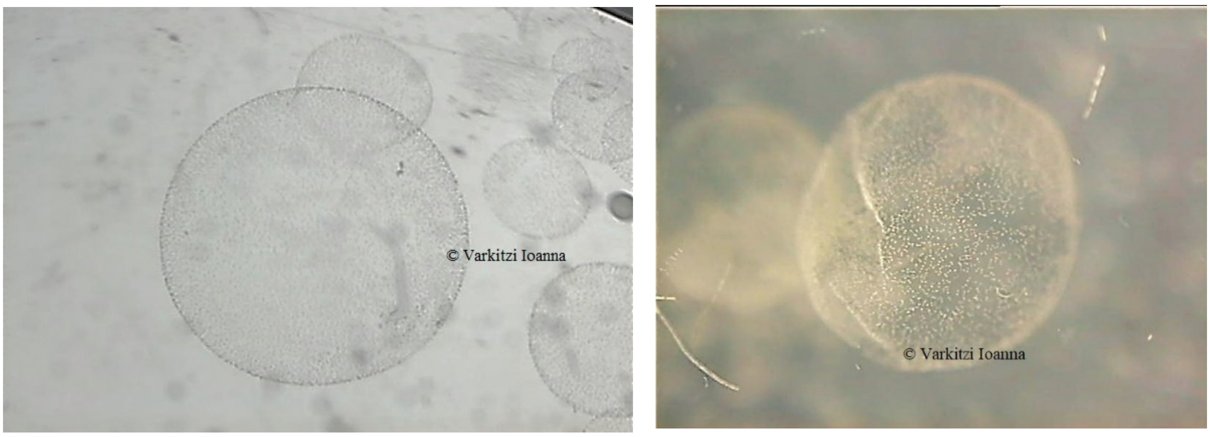
During summer 2021, a persistent HAB outbreak was reported from the Sea of Marmara, with the formation of dense foamy mucilage phenomena and the dominance of the microalga *Phaeocystis pouchetii* (Balkis-Ozdelice *et al.,* 2021). At the same time, mucilage phenomena of lower intensity and extent were reported from the North Aegean coasts, namely from Alexandroupolis to Chalkidiki and Limnos Island (Moustaka *et al.,* 2021). Furthermore, short-lived foamy mucilage aggregates were observed on the coast of Mount Pelion in North Aegean (I. Varkitzi personal observation). In this study, we present the isolation of the microalga *Phaeocystis globosa* from Saronikos Gulf waters in the Aegean Sea. We also present a culture experiment during which the characteristics and growth of *Phaeocystis globosa* were studied.

2. Materials and Methods

Seawater samples were collected from the Gulf of Saronikos in the Aegean Sea, with oceanographic bottles according to standard sampling procedures (see Varkitzi *et al.*, 2018b). Experiments with isolation protocols for microalgae were performed, while after isolation, *Phaeocystis* was established in mono-specific cultures according to standard culturing methodologies (Andersen & Kawachi, 2005). A growth experiment was performed in batch cultures under nitrogen and phosphorus replete conditions (for details see Varkitzi *et al.* 2010, 2017).

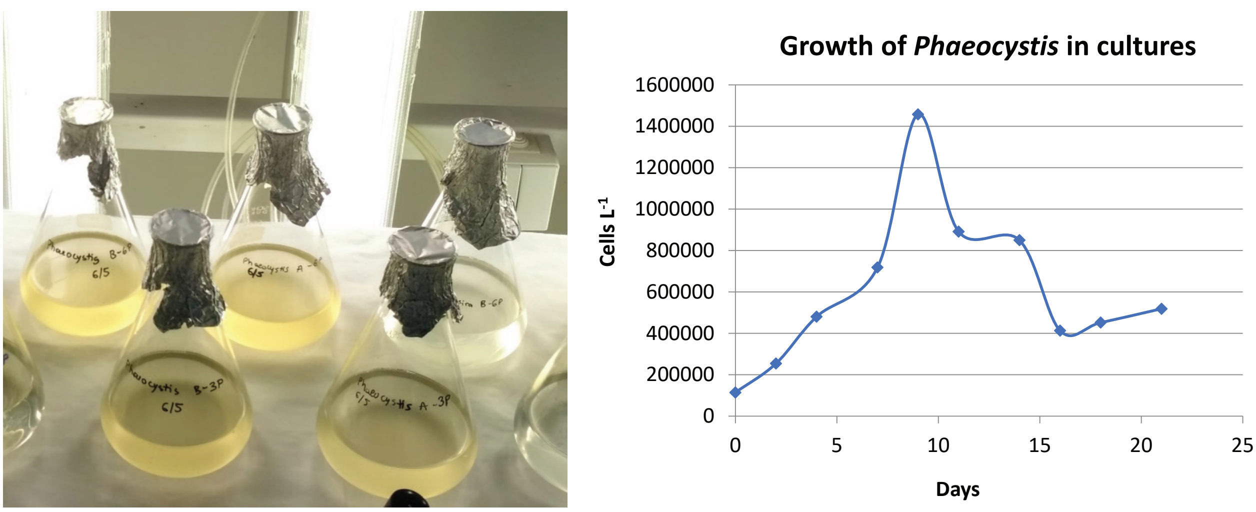
3. Results

The isolated strain of *Phaeocystis* (Coccolithophyceae, Prymnesiophysidae, Phaeocystales) is presented in Figure 1. The species *Phaeocystis globosa* Scherffel 1899 was identified according to Throndsen (1997), and the nomenclature was updated from *AlgaeBase* (Guiry & Guiry, 2018). *P. globosa* is distributed in oceanic and coastal waters of temperate latitudes. The motile cells of the isolated *P. globosa* strain (ca. 5 μm cell length) had a short haptonema and two equal flagella greater than the cell length. The non-motile *P. globosa* cells (5-7 μm cell length) lacked the haptonema or flagella, and they formed spherical smooth colonies without lobes, up to 2 mm in diameter. Non-motile cells were evenly distributed along the surface of the colony. All cells had two equal yellow-brown chloroplasts.



(a) **(b)**

Fig. 1: Colonies of *Phaeocystis globosa* under bright field (a) and phase contrast microscopy during isolation experiments in the laboratory.



(a) **(b)**

Fig. 2: Batch cultures of the isolated *Phaeocystis globosa* strain (a), and growth experiment of *Phaeocystis globosa* in culture conditions (b).

During the isolation experiments in the laboratory, *P. globosa* colonies and cells were separated under the microscope from the rest of the living material in seawater samples, freshly collected from Saronikos Gulf, Aegean Sea. Eventually and after consequent isolation experiments in the laboratory, the cells were established in monospecific cultures. *P. globosa* cultures were grown in natural aged seawater, 0.2 μm filtered and autoclaved, with the addition of f/2 medium (Guillard 1975), in a cool room with 20 °C and 12:12 light period (Fig. 2a).

As a next step, an experiment was performed in order to examine the growth of the isolated *P. globosa* strain in culture conditions with sufficient nitrogen and phosphorus supply (Fig. 2b). *P. globosa* cells were growing exponentially since the beginning of the incubation. Densities reached 1.46 10^6 cells L^{-1} after 9 days of growth. Cell densities started to decrease since day 10. The incubation lasted for three weeks.

4. Discussion/Conclusion

Species of the prymnesiophyte genus *Phaeocystis,* such as *P. globosa* and *P. pouchetii,* are well known agents for the production of high biomass blooms in cold and temperate waters (Verity et al., 2007). The lysis and degradation of *Phaeocystis* cells produce foam that causes low oxygen and increased viscosity conditions, with negative impacts for aquaculture and bathing beaches (Schoemann et al. 2005). *Phaeocystis* spp. are also known to produce high concentrations of the exometabolite dimethylsulfoniopropionate (DMSP) during blooms. Anthropogenic nutrient enrichment is considered as the main trigger factor for *Phaeocystis* spp. blooms.

During the massive mucilage phenomena that stroke the Adriatic Sea in the past, diatoms together with *Phaeocystis* spp. were reported to dominate (Turk *et al.,* 2010). Recently in the Sea of Marmara (spring-summer 2021), *P. pouchetii* together with diatoms, dinoflagellates and chrysophytes dominated the intense and persistent foamy mucilage aggregates (Balkis-Ozdelice *et al.,* 2021). In summer 2021, mucilage phenomena of lower intensity in the North Aegean coasts were dominated by diatoms and the dinoflagellate genus *Gonyaulax* mostly and less by *Phaeocystis* (Moustaka *et al.,* 2021), whereas foamy mucilage aggregates of a short-lived *Phaeocystis* sp. bloom were observed on the coast of Mount Pelion in North Aegean (I. Varkitzi personal observation). High densities of mucilage producing diatoms and dinoflagellates co-existing with high densities of *Phaeocystis* sp. (max 25 × 10^6 cells L–1) have been previously reported from Thermaikos Gulf (Genitsaris et al. 2019). In the past decades, water discolorations and mucilage problems in Saronikos Gulf (March 1989, August 1993) and Evoikos Gulf (September 1999) were attributed to high densities of *P. pouchetii* (max 35 × 10^6 cells L–1) (Metaxatos *et al.,* 2003; Ignatiades & Gotsis-Skretas, 2010).

In the present study, a *Phaeocystis* strain was collected from Saronikos Gulf and it was identified as *P. globosa* (Throndsen 1993). The strain was isolated in laboratory conditions and it was grown in monospecific cultures. The growth of the *P. globosa* strain was studied during an experiment for three weeks, in culture conditions with sufficient nitrogen and phosphorus supply. *P. globosa* was a fast growing species. Cells did not demonstrate a lag phase at the beginning of the incubation, and started to grow exponentially almost right after the inoculation. Cell densities maximized after 9 days, reaching 1.46 10^6 cells L^{-1}. Since day 10, cell densities started to decrease, reaching almost 1/3 of the maximum density during the following 11 days. In a recent study about the allelopathic effect of exometabolites from *Phaeocystis* sp., *Heterosigma akashiwo*, *Tetraselmis* sp. and *Thalassiosira* sp. in culture conditions, *Phaeocystis* produced the exometabolite DMSP in high levels that enhanced cell yield and photosynthetic biomass of the ochrophyte *Heterosigma akashiwo*, whereas *Phaeocystis* sp. growth was suggested to be inhibited by the presence of the exometabolite L-histidinal, produced by the chlorophyte *Tetraselmis* sp. (Apostopoloulou *et al.,* 2022).

In conclusion, the genus *Phaeocystis* is not an unknown producer of blooms in coastal waters of the Aegean Sea. The strain of *P. globosa* isolated from Saronikos Gulf can grow in culture conditions and be used in culture experiments. Future studies on this strain could throw light in active research about the algal exometabolome and its biotechnological implications.

5. Acknowledgements

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PHYTOPLANKTON STUDIES IN GREEK COASTAL LAGOONS

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Abstract

Nineteen transitional aquatic systems (including coastal lagoons and estuaries) located in several areas of Greece (North, Central and Western Greece) were sampled for phytoplankton species, while physical-chemical parameters of the sampling stations were also measured. Chlorophyll a results indicate that all coastal lagoons sampled were eutrophic at the time. Some of them exhibited very high eutrophication (Tourlida, Kotychi) while others like Kokkala Lagoon and Strymon River estuary enclosure were less eutrophic. Salinity was very variant with Strymon estuary enclosure and Vouliagmeni Lake coastal lagoon exhibiting very high figures. In other lagoons like Prokopos and Louros salinity was very low. Consequently, phytoplankton species composition was varying as well, with Cyanobacteria and diatoms always among the most abundant species. Certain chrysophytes and cryptophytes were also abundant as well as *Tetraselmis* and other green microalgae.

Keywords: coastal lagoons, phytoplankton, trophic level, Ionian Sea, Aegean Sea, transitional waters.

1. Introduction

Transitional aquatic systems, including coastal lagoons, estuaries, salt marshes etc., are among the most interesting and diverse ecosystems, as they exhibit a plethora of interchangeable conditions both in space and time. As a result, their biotic component and especially the phytoplankton community, is directly influenced by those conditions, involving several opportunistic species which can take advantage of the brief nutrient enrichment incidents under fluctuating conditions. In the present study, a series of samplings were performed during autumn (September or October 2020), in nineteen transitional aquatic systems, aiming to investigate the phytoplankton community photosynthetic pigments and abiotic parameters of those environments and the preliminary results are presented herein.

2. Material and Methods

All transient systems were sampled once during autumn 2020. A most representative spot was chosen for sampling from each system, far from the inlet of the open sea. Sampling depth was set at least to one meter from the surface and one meter from the bottom, to get the most phytoplankters avoiding benthic species and the photoinhibiting parts close to surface. However, most systems were very shallow thus in most cases sampling depth was set to 0.5 m from surface. Sampling was done by manual pumping to avoid disturbing the sentiments. Salinity (translated from conductivity compensated for temperature), dissolved oxygen and temperature presented in this report were determined using a multi-sensor instrument with long cable (Lovibond). Water samples were filtered on GF/C filters for photosynthetic pigment analysis based on Parsons et al., (1984) using the equations of Mitchell & Kiefer, (1984). Water for community and abundance analyses was collected and fixed with Lugol's solution. Species identification was carried out using common keys while phytoplankton abundance was assessed by inverted microscope (Zeiss) using the method described by Utermöhl (Edler, & Elbrächter, 2010). As most of the phytoplankters were very small (micro- and nannoplankton) precise identification was not possible; therefore, the semi-quantitative method was used as described by Hällfors, (2013).

3. Results

A map showing all the sites included in this study is presented in Figure 1. All sampling stations were selected within coastal lagoons or estuarine systems of great rivers of Greece, located all over the country (North, Central and Western Greece). In Table 1 a preliminary description of the status of each transitional aquatic system investigated is presented, along with the gross species composition and semi-quantitative abundance.

Fig. 1: Map of Greece, showing the location of all sampled sites included in this study. (For sampling station codes see Table 1).

Table 1. Preliminary results of sampled transitional aquatic ecosystems in Greece. (BG: blue-green algae species, the number of * corresponds semi-quantitatively to the abundance of the taxon in each sample).

Location coordinates	type	time	Sampling depth (m)	Temperature °C	Salinity ppt	DO mg/L	Chl a μg/L	Dominant phytoplankters
Northern Greece (Macedonia-Thrace, Aegean Sea)								
Vistonida 41.001751 25.161311	Lagoon inner	3:20 pm	1	24.0	25.0	6.30	10.08	BG sp.*** Diatom sp.** Coccoid green sp1** Coccoid green sp2**
Porto Lagos 40.980144 25.150225	Coastal lagoon	2:45 pm	1	23.7	49.0	7.90	8.07	Helical BG sp.** Filamentous BG sp.* Diatom sp1** Diatom sp2** *Tetraselmis* sp.** Coccoid green sp1* Coccoid green sp2* Cryptophyte*

Location coordinates	**type**	**time**	**Sampling depth (m)**	**Temperature °C**	**Salinity ppt**	**DO mg/L**	**Chl a μg/L**	**Dominant phytoplankters**
Ksirolimni 40.954368 25.152906	Coastal lagoon	2:10 pm	1	23.9	47.0	6.20	8.25	BG sp1*** BG sp2** BG sp3 (helical)** Diatom sp1*** Diatom sp2* Diatom sp3* Filamentous chrolophyte sp** *Tetraselmis* sp.** Chrysophyte***
Karatzia 40.957316 25.182462	Coastal lagoon	1:30 pm	1	24.6	55.0	8.50	10.21	Filamentous BG sp**** Helical BG sp* Cryptophyte* *Tetraselmis* sp.** Coccoid green sp* Coccoid brown*
Mesi (Alyki) 40.948374 25.203697	Coastal Lagoon/ Salt marsh	12:04 pm	1	22.8	55.0	6.70	12.21	Filamentous BG sp** Helical BG sp* BG sp* Diatom sp1* Diatom sp2* Cryptophyte* Filamentous green** *Tetraselmis* sp** Chrysophyte*
Elos 40.935299 25.275975	Coastal Lagoon	10:15 am	1	22.0	50.0	5.60	9.21	Filamentous BG sp**** Helical BG sp** Cryptophyte* *Tetraselmis* sp1** *Tetraselmis* sp2**
Strymonas 40.781828 23.877924	Estuary	12:15 pm	0.5	17.4	80	6.80	5.35	BG sp1** BG sp2* Diatom sp1* Diatom sp2* Coccoid green sp* Chrysophyte*
Kalochori Thessaloniki 40.631731 22.838038	Coastal lagoon	2:30 pm	1	19.8	35	5.70	9.37	BG sp** BG sp2* Pennate diatom sp* Filamentous green sp* Cryptophyte**
Central Greece (Spercheios River, Aegean Sea and Korinth, Korinthiakos Gulf)								
Agia Marina 38.888394 22.572386	Coastal lagoon	1:10 pm	1	22.0	42.0	5.20	10.32	BG sp.*** *Amphidinium* sp** Filamentous green sp*
Agia Triada-Spercheios Estuary 38.821383 22.603709	Estuary	2:30 pm	1	18.8	45.0	3.5	10.09	BG sp. *** Diatom sp*** *Amphidinium* sp** Cryptophyte * Coccoid green sp** Chrysophyte**** Haptophyte**

Location coordinates	**type**	**time**	**Sampling depth (m)**	**Temperature °C**	**Salinity ppt**	**DO mg/L**	**Chl a μg/L**	**Dominant phytoplankters**
Vouliagmeni 38.026188, 22.874110	Coastal lagoon	12:30 pm	1	25.0	61.1	12.8	5.10	BG sp.***
Western Greece (Ionian Sea)								
Aetoliko 38.436912 21.347736	Lagoon inner	3:45 pm	1	26.3	17.0	7.2	10.25	BG sp.* Pennate diatom sp1**** Pennate diatom sp2** Diatom sp*** Red cryptophyte sp* Brown cryptophyte sp2* Coccoid green sp1 * Coccoid green sp2 * Chrysophyte**
Tourlida 38.339815, 21.423702	Coastal lagoon	3:45 pm	1	27.2	40.0	7.7	30.82	BG sp.* Diatom * *Amphidinium* sp** Brown cryptophyte* *Tetraselmis* sp.* Chrysophyte **** Haptophyte***
Kleisova 38.347182 21.428595	Coastal lagoon	13:30 pm	1	28.1	43.0	5.7	9.82	BG sp.* Pennate diatom * *Amphidinium* sp* Brown cryptophyte* Red cryptophyte* *Tetraselmis* sp.*** Coccoid green** Chrysophyte **
Louros 38.317934 21.216322	Coastal lagoon	12:00 pm	1	25.8	17.0	4.7	11.43	BG sp*** Diatom *** Coccoid green**
Porto 38.314051 21.203079	Coastal lagoon	11:00 am	0.5	25.3	66.0	6.1	10.66	BG sp** Diatom sp1** Diatom sp2 ** Diatom sp3** Diatom sp4* *Tetraselmis* sp.* Chrysophyte****
Kokkala-Oiniades 38.339109 21.119181	Coastal lagoon	1:30 pm	1	26.0	20.0	8.2	4.47	BG sp*** Diatom sp1*** Diatom sp2 ** Brown cryptophyte** Red cryptophyte* Coccoid green sp1* Coccoid green sp2* Chrysophyte***
Kotychi 38.001249 21.279476	Coastal lagoon	1:30 pm	0.5	26.0	22.0	7.5	27.57	Filamentous brown BG* Filamentous green BG*** Coccoid green*

Location coordinates	type	time	Sampling depth (m)	Temperature °C	Salinity ppt	DO mg/L	Chl a μg/L	Dominant phytoplankters
Prokopos 38.156779 21.399787	Lagoon inner	10:00 am	1	24.3	7.0	3.6	7.81	Filamentous BG sp1*** Filamentous BG sp2** Pennate diatom *** Chlorphyte ** Coccoid green*

4. Discussion/Conclusion

All aquatic ecosystems sampled were characterised as eutrophic according to classification based on the Chl a content per litre (Beiras, 2018). Some lagoons exhibit very high eutrophication (Tourlida, Kotychi) while Kokkala Lagoon and Strymon River estuary enclosure had the less eutrophic character at the time of sampling. Salinity was very variant also with Strymon estuary enclosure and Vouliagmeni Lake coastal lagoon exhibiting very high figures and some lagoons like Prokopos and Louros very low. Dissolved oxygen was almost in every ecosystem of adequate saturation except for Prokopos lagoon where it was quite low. In contrast DO in Vouliagmeni Lake lagoon it was exceptionally high. Consequently, phytoplankton species composition was varying lot between these transient ecosystems with Cyanobacteria and diatoms always among the most abundant species while Certain chrysophytes and cryptophytes were also abundant as well as *Tetraselmis* and other green microalgae.

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EFFECT OF DIMETHOATE AND ITS COMMERCIAL FORMULATION, PERFEKTHION, ON PHOTOSYNTHESIS OF SELECTED PHYTOPLANKTON SPECIES

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Abstract

Dimethoate is an organophosphate insecticide. It was used as technical grade and compared to the commercial formulation Perfekthion. Perfekthion contains 37% dimethoate as active ingredient and is used in agriculture to kill insects. The effects of both dimethoate variants on photosynthesis of seven selected phytoplankton species was compared to each other and to DCMU, a PSII inhibitor. Oxygen evolution, chlorophyll fluorescence (OJIP, Fv/Fm and NPQ) and xanthophyll pigment experiments were performed. Perfekthion acted stronger than dimethoate on oxygen rate inhibition, OJIP fluorescence and Fv/Fm ratio in the used species. OJIP measurements showed that the two insecticides inhibited PSII like DCMU. Decreasing Fv/Fm with increasing Perfekthion concentrations indicated that its solvents probably dissociated the light harvesting complex from PSII. Non-photochemical quenching (NPQ) in the light of the Perfekthion treated samples was increasing with time and higher than NPQ of dimethoate treated *Chlorophyceae* and *Bacillariophyceae* species except *Dunaliella tertiolecta*. Perfekthion induced a higher NPQ in the dark phase compared to dimethoate applied on *Chlamydomonas reinhardtii* and the two *Bacillariophyceae* species. Specific xanthophyll pigments decreased compared to control at 3000 nmol/μg chla dimethoate and 500 nmol/μg chla Perfekthion in most tested phytoplankton species.

Keywords: insecticide, OJIP fluorescence, NPQ, xathophylls.

1. Introduction

The use of dimethoate was permitted in EU till June 2020 but is still not prohibited in other countries. It is an acetyl cholinesterase inhibitor that was used in the Greek agriculture primary against the fruit fly (*Bactrocera oleae*, *Dacus oleae*) which lays its eggs into the olive fruit. According to different studies on phytoplankton species the main target of dimethoate (Mohapatra & Schiewer, 1996) or Rogor (Jena *et al*., 2012) is photosynthesis. Rogor is the older name of Perfekthion, the most common commercial dimethoate product that contains 37% dimethoate as active ingredient. The rest are solvents.

When a Pulse Amplitude Modulated (PAM) fluorometer is used to measure photosynthesis, the fluorescence signal reduction is characterised as quenching. Photochemical quenching (qP) is caused when photochemical energy is utilised by charge separation at the reaction centers of PSII. Non-photochemical quenching (NPQ) is a mechanism employed by plants and algae to protect themselves from the adverse effects of high light intensity, where light energy absorption exceeds the capacity for light utilization in photosynthesis (Horton *et al*., 2005) dissipating the excessive energy as heat. NPQ, fluorescence and photochemistry (qP) are in concurrence to each other. OJIP fluorescence reflects the time course of photosynthesis from PSII to PSI where O, J, I, and P represents the reduced parts on the electron transport chain (ETC). The relation of variable to maximum fluorescence (Fv/Fm), called maximum photochemical efficiency, describes the yield of the excitation energy that get transferred from the LHC to the reaction centers of PSII (Schreiber *et al*., 1994).

Xanthophylls are involved in NPQ of excitation energy in light harvesting antenna proteins (Li Z.R. *et al*., 2009). Two xanthophyll cycles are known. Under high light conditions violaxanthin (Vx) is converted via antheraxanthin (Ax) to zeaxanthin (Zx) (Sapozhnikov *et al*., 1957) in the Vx cycle of the *Chlorophyceae* species (Hager 1980) and Dd to diatoxanthin (Dt) in the Dd cycle of the *Bacillariophyceae*. The new formed pigments of the xanthophyll cycles dissipate excessive energy in thermal energy (NPQ increase).

Primary goal of the study was to identify the acting place of dimethoate in the photosynthesis mechanism of the phytoplankton cells and to compare the technical grade dimethoate with the commercial formulation Perfekthion.

2. Material and Methods

C. reinhardtii, *D. tertiolecta*, *Tetraselmis sp*. (three *Chlorophyceae* species), *Phaeodactylum tricornutum* and *Thalassiosira pseudonana (*two *Bacillariophyceae* species), *Anabaena sp*. (*Cyanophyceae*) and *Porphyrridium sp*. (*Rhodophyceae*) were cultivated in 250 ml Erlenmeyer flasks under 15-45 µE white-light, 16/8 h light/dark cycle, 20°C, on shakers at 120-130 rpm. *Anabaena sp*. and *C. reinhardtii* were cultivated in bidistilled water, the other species in 33,6-35 %o artificial seawater. *f2* medium was used for *D. tertiolecta*, *Tetraselmis sp.* and *P. tricornutum* cells (Guillard and Ryther, 1962; Guillard, 1975). $f2_{Si/Se}$ medium was used for *T. pseudonana* (Guillard and Ryther, 1962; Guillard, 1975). The recipe for *C. reinhardtii* and *Porphyrridium sp.* culture medium originates from Pringsheim and Koch (1964) and for *Anabaena sp.* medium from Rippka *et al.,* 1979.

Perfekthion contains 37,2% (w/w) dimethoate as active ingredient, 43,5-48% (w/w) cyclohexanone, 4,2-5,2% (w/w) solvent naphtha and 4,2-5,2% (w/w) acetic anhydride and was purchased from BASF company, Ludwigshafen, Germany. Dimethoate (*O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] dithiophosphate) PESTANAL, analytical standard was used as technical grade from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. DCMU (3-(3,4-Dichlorophenyl)-1,1-dimethylurea), a PSII inhibitor, was ordered from Sigma-Aldrich Chemie GmbH. The concentrations of dimethoate, Perfekthion and DCMU used in the Clark electrode, fluorescence and HPLC measurements were listed in Table 1.

Oxygen production of the phytoplankton species was measured by Clark electrode (Hansatech Instruments, Oxy-Lab, Helmut Saur, Reutlingen, Germany) in order to study the effect of the insecticides and DCMU on the phytoplankton photosynthesis (at 150 µE light intensity). 2 ml of the phytoplankton sample with a normalised concentration of 2,0 µg chla/ml were placed into the chamber of the Clark electrode and the oxygen rate (net photosynthetic rate) was measured after insecticide application. The light intensity of the Clark electrode experiments intended for HPLC analysis was 500 µE in order to induce a pigment deepoxidation.

The fluorescence measurements of the phytoplankton cells were performed by Aqua Pen instrument (Aqua Pen-C, AP-C 100, Czech Republic, www.psi.cz). Blue excitation light (450 nm) was used to measure chl fluorescence in the tested *Chlorophyceae* and *Bacillariophyceae* samples and red excitation light (620 nm) in *Anabaena sp.* and *Porphyrridium sp.*. 1 ml of the phytoplankton sample with a normalised chl a concentration of 1,0 µg/ml was placed into a cuvette and dimethoate, Perfekthion or DCMU was added. OJIP fluorescence, NPQ in the light and the dark and Fv/Fm was measured.

The frozen filters with the pesticide treated phytoplankton samples of the Clark electrode experiments were used for HPLC analysis in order to measure the convertion of the xanthophyll pigments. 700 µl extractant containing at 90% a mixture of methanol (90%) and 0,2 M ammonium acetate (10%) and at 10 % ethyl acetate and about 40µg glass beads were put into each small tube with the frozen filter. Each tube was vortexed for 30 sec at the highest level and immediately afterwards centrifugated (Eppendorf Centrifuge 5415 D) at 13.200 rpm for 2,5 min. 400 µl of the yellow supernatant (opganic phase) from each sample tube were put into a HPLC vial and the extracts were measured by HPLC. Pigment concentrations were determined by reversed phase HPLC (VWR Elite LaChrome, Germany) according to Jacob *et al.* (1998). The HPLC consisted of two high pressure pumps and a mixing chamber, an autosampler (model L-2200) regulated to work at 10° C, from which the samples are injected on the column (mainly 60 µL) and a photodiode array (model L-2455). The samples were detected at 440 nm and integrated. The separation took place at 20° C using a EC 125/4 Nucleosil 120-5 C18 reversed phase column (Macherey-Nagel, Düren, Germany).

The Clark electrode, the fluorescence and HPLC experiments were carried out in the University of Konstanz in Germany, Department of Plant Physiology, supervised under Prof. Peter Kroth.

Table 1. Used dimethoate, Perfekthion and DCMU concentrations in the different experiments.

experiments	dimethoate	Perfekthion	DCMU
Clark electrode	125-18000	12,5-2000	4,5-4500
Fluorescence measurements	83-18000	8-2000	1,5-4500
HPLC	125-3000	25-500	4,5-4500

3. Results

3.1 Oxygen evolution measurements by Clark electrode

Oxygen rate inhibition increased with increasing dimethoate, Perfekthion and DCMU concentrations in all seven tested phytoplankton species. 500 nmol Perfekthion /μg chla caused an oxygen rate inhibition of about 90% while 3000 nmol dimethoate /μg chla of 25-60% (*C. reinhardtii* 25%, *Porphyrridium sp*. 40% at 4000 nmol/μg chla, the rest at least 50%). A similar trend to dimethoate was observed when 45 nmol DCMU /μg chla were added to the phytoplankton species although DCMU concentration was about 60.000 times higher than that of dimethoate.

3.2 Chlorophyll fluorescence measurements by PAM instrument

The OJIP fluorescence measurements showed that 3000 nmol dimethoate /μg chla, 500 nmol Perfekthion /μg chla and 45 pmol DCMU /μg chla had a similar effect on the three *Chlorophyceae* and the two *Bacillariophyceae* species. Dimethoate and Perfekthion inhibited PSII beyond Qa like DCMU in the seven used phytoplankton species. PSI was not inhibited.

Increasing Perfekthion concentrations decreased the maximum photochemical efficiency of the three *Chlorophyceae* and the two *Bacillariophyceae* species stronger compared to the same concentrations of dimethoate and DCMU (fig. 1 for *D. tertiolecta*). 500 nmol Perfekthion /μg chla, 3000 nmol dimethoate / μg chla and 45 pmol DCMU /μg chla presented a similar effect on Fv/Fm of *P. tricornutum* that was close to the control value.

3000 nmol dimethoate /μg chla and 45 pmol DCMU /μg chla decreased NPQ in the light with increasing time to similar values and significant lower than the control in *C. reinhardtii*, *Tetraselmis sp*. and the 2 *Bacillariophyceae* species. 3000 nmol dimethoate /μg chla increased NPQ development in *D. tertiolecta* but still under the control values. 500 nmol Perfekthion /μg chla increased NPQ with time and did not significant differ to the control, except in *D. tertiolecta* and *T. pseudonana* where it was *lower* than control.

NPQ after 230 seconds in the dark (NPQ_{D230}) of the 500 nmol Perfekthion /μg chla treated *C. reinhardtii* and the two *Bacillariophyceae* species was significant higher than the respective 3000 nmol/μg chla dimethoate treated samples. In *C. reinhardtii* and *P. tricornutum* NPQ_{D230} was about five times higher in the 500 nmol/μg chla Perfekthion treated cells compared to the control. The NPQ_{D230} values of the 3000 nmol dimethoate /μg chla and 45 pmol DCMU/μg chla treated cells of *C. reinhardtii*, *Tetraselmis sp*. and the two *Bacillariophyceae* species were not significantly different to each other (no DCMU data for *D. tertiolecta*).

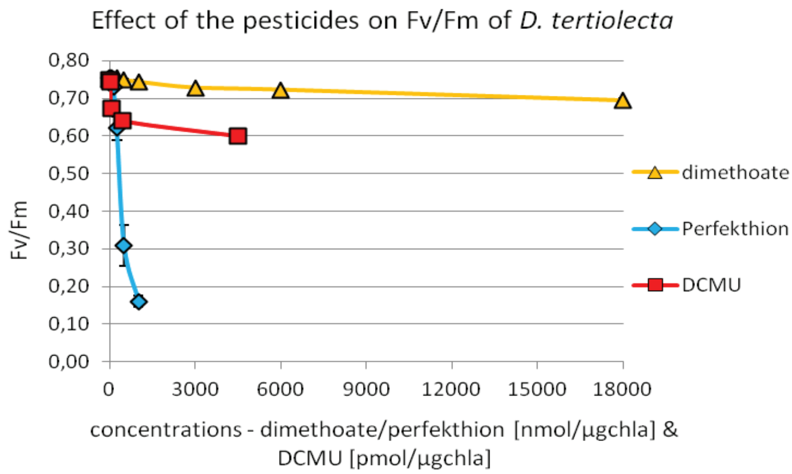

Fig. 1: Effect of dimethoate, Perfekthion and DCMU on maximum photochemical efficiency (Fv/Fm) of *D. tertiolecta*. A pesticide comparison.

3.3 Xanthophyll pigment measurements by HPLC

Under high light Ax/Vx was not significant different in the 3000 nmol dimethoate /µg chla treated cells of *C. reinhardtii* and *D. tertiolecta* compared to the 500 nmol Perfekthion /µg chla treated cells and the values lower than control. In *Tetraselmis sp*. 500 nmol Perfekthion /µg chla induced a four times higher Ax/Vx compared to 3000 nmol dimethoate /µg chla (still under control). Dt/(Dd+Dt) of the 500 nmol/µg chla Perfekthion treated *T. pseudonana* cells was two times higher than the 3000 nmol dimethoate /µg chla treated ones and similar to the control value. In *P. tricornutum* Dt/(Dd+Dt) of the 3000 nmol dimethoate /µg chla and the 500 nmol Perfekthion /µg chla treated sample was 5-20 times lower than the control.

4. Discussion/Conclusion

Perfekthion inhibited oxygen evolution of the tested phytoplankton species about 10 times stronger than dimethoate, probably because of the solvents contained in the commercial formulation. Dimethoate and Perfekthion inhibited PSII beyond Qa, showing the same effect on the ETC like DCMU. 500 nmol Perfekthion /µg chla significant decreased Fv/Fm of the 3 *Chlorophyceae* and the 2 *Bacillariophyceae* species up to 80% compared to 3000 nmol dimethoate /µg chla. Probably the solvents of Perfekthion dissociated the light harvesting complex from PSII after application of Perfekthion concentrations higher than 500 or 1000 nmol/µg chla increasing the ground fluoerescence, drecreasing Fv.

3000 nmol dimethoate /µg chla decreased NPQ in the light with increasing time while 500 nmol Perfekthion /µg chla increased NPQ. The first is explained as dimethoate inhibited the electron passing from PSII towards PSI resulting in a decreased proton gradient and NPQ (as NPQ depends on the proton gradient). Perfekthion induced probably a pigment removal and destruction as the exposed pigments got less protection. Less intact chlorophylls caused a fluorescence decrease and NPQ increase.

500 nmol Perfekthion /µg chla significantly increased NPQ of the relaxation phase in *C. reinhardtii* and *P. tricornutum* compared to 3000 nmol dimethoate /µg chla as the commercial formulation may cause a photoinhibition.

500 nmol Perfekthion /µg chla and 3000 nmol dimethoate /µg chla affected Ax/Vx in the three *Chlorophyceae* and Dt/(Dd+Dt) in the two *Bacillariophyceae* species and the rates were lower than control (exception Perfekthion in. *T. pseudonana*). The insecticide in both forms disrupted the transfer of the electrons on the ETC after PSII, leading in decreased proton gradient and xanthophyll ratios under high light conditions.

In general the effect of dimethoate on the photosynthesis of the selected phytoplankton species was similar to the effect of DCMU while Perfekthion showed additional effects in all main experiments probably because of the solvents contained.

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THE LEVELS AND HISTORICAL EVOLUTION OF MERCURY IN SEDIMENTS OF SARONIKOS GULF

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Abstract

Mercury (Hg) is a highly toxic element with various sources to the marine environment and implications to ecosystem and human health. It is recognized as a priority pollutant in European Environmental Legislation. The present paper is a preliminary presentation of Hg levels in sediments from Saronikos Gulf. The range of Hg in Saronikos surface sediments is 0.037-0.888mg/kg, while the range in deeper layers from short sediment cores is 0.019-0.041mg/kg. The levels of Hg in most stations were below the Effects Range Low Limit (ERL-0.150mg/kg) for which adverse effects to biota are rare. However, two of the stations near the most pronounced sources of pollution, i.e., the port of Piraeus and the Waste Water Treatment Plant outfall of the metropolitan Athens area, were found significantly polluted with Hg. The background levels for Hg in the Hellenic marine environment are currently being established and there is need for more research in order to connect the concentrations in water and sediments to Hg content in marine biota along with biomarker studies to identify possible physiological effects.

Keywords: industrial pollution, urban pollution, background levels, enrichment factors, quality guidelines.

1. Introduction

Mercury (Hg) presents complex environmental chemistry and biogeochemical cycling with enhanced lability and re-emission of existing Hg forms in water, soil and sediments to the atmosphere by conversion into volatile elemental Hg. The methylation of inorganic Hg to organic forms via natural bacterial processes increases bioaccumulation and toxicity. Hg, is released to the environment from several sources both natural, such as erosion, volcanoes and hydrothermia and human induced, i.e., fossil fuel combustion, mining, industrial waste. The study of Hg is of great importance for the Mediterranean due to increased geological background abundance. Sediments are considered to trap particulate Hg but bioturbation, bacterial methylation, dredging, and changes in physicochemical parameters may cause remobilization to the overlying water column (Horvat, *et al.* 1999; Scoullos, *et al.* 2000; Rajar, *et al.*, 2007; UNEP, 2013; Kehrig, *et al.*; 2010, Manta, *et al.*, 2016).

Mercury is known to accumulate in marine organisms and biomagnify along trophic webs. It has been reported that fish of the Mediterranean contain higher levels of Hg than populations of other seas. Mercury can subsequently be transferred to humans via fish and seafood consumption. Quite often Hg levels in food exceed safety limits and long term consumption of Hg contaminated fish can lead to potential health hazards (Kousteni, *et al.* 2006; Damiano *et al.* 2011; Renieri, *et al.* 2014). Therefore, Hg constitutes a priority element in Mediterranean environmental monitoring of biotic and abiotic components, including sediments.

At European level, Hg is a priority pollutant as designated in environmental legislation [Marine Strategy Framework Directive (MSFD) 2008/56/EC and Water Framework Directive (WFD) 2000/60/EC]. The protection of human health and the environment from Hg is also recognized in the Mediterranean Action Plan of the Barcelona Convention and the global treaty “Minamata Convention on Mercury”. There is no European legislative limit for Hg in sediments. In order to evaluate sediment quality regarding Hg the limits ERL (Effects Range Low-150μg/kg) and ERM (Effects Range Median-710μg/kg) are widely used (McDonald *et al.*, 1996).

This paper presents a preliminary attempt to evaluate Hg levels, spatial patterns, background values and Enrichment Factors in sediments from Saronikos Gulf.

2. Material and Methods

2.1 Study area - Sampling

Saronikos Gulf is the marine area bordering the largest cities of Greece, Athens and Piraeus, with ~4 million inhabitants and extensive industrial areas. The main sources of trace metals, including Hg, to the seawater and sediments of Saronikos are: (1) the industrial zones of Elefis Bay and Thriasio on the north coast hosting oil refineries, shipyards, food processing, iron steelworks, cement factories, cable manufacturing, waste recycling plants, landfills, military installations; (2) Piraeus port, one of the largest in the Mediterranean, with increased marine traffic; (3) the Athens wastewater treatment plant (WWPT) on Psitalia island and (4) the atmospheric emissions by vehicle traffic and the heating systems of the urban area. Furthermore, a smaller industrial zone, with a major oil refinery and smaller food processing and cable manufacturing installations, is situated on the north-western coast of Saronikos (Valavanidis *et al*. 2006, Paraskevopoulou *et al*. 2014).

The sediment samples (5 surface and 13 short cores) from Saronikos gulf were collected in September and October of 2017 by the R/V AEGAEO of HCMR. The study area with station locations and indicative pollution sources of Saronikos Gulf are presented in Figure 1.

2.2 Analytical procedure

The surface sediments and cores were stored frozen until analysis. The cores were cut in layers of 1-2cm for the top 10cm and of 2cm below 10 cm. The sediment samples were freeze-dried and dry sieved for the separation of the silt/clay (< 63 μm) from the sand fraction (> 63μm), using stainless steel sieves. The percentages of both fractions were calculated and analytical procedures were carried out when the mass of the fractions was adequate (>10% of the total sediment).

For the extraction of Hg from the sediment samples approximately 0.5 g of dry sediment were weighed in Teflon beakers and 8 mL of concentrated nitric acid p.a. were added. The beakers were placed on a hot plate for 4-5 hours at 50-60 °C. After cooling, the digestates were taken up to 50mL in class A volumetric flasks with ultrapure Milli-Q water and filtered (Millipore 0.45μm nitrocellulose filters) directly into pre-cleaned plastic bottles. All labware used were previously soaked in 10% nitric acid for 24-48 h and rinsed with ultrapure Milli-Q water. The Teflon beakers were digested open with concentrated nitric acid on the hotplate at a temperature above 70 °C for cleaning between sample digestions. Each digestion batch included a blank sample (in alternating beakers) and two secondary reference materials, which are sourced by an interlaboratory comparison. The target values of the reference materials are: ISE 848 - 0.073mg/kg and ISE 860 - 0.412 mg/kg, which are averaged by the consensus values given for three extraction techniques with different acids (nitric acid, aqua regia or other acid mixtures). Most samples were digested once and only a few randomly selected (approximately one layer from each core) were digested in duplicate. The Hg concentration of the digestates was measured by CVAAS (Cold Vapour Atomic Absorption Spectrometry) using a Varian SpectrAA 200 model equipped with a VGA77 peristaltic pump. The recoveries of the reference materials ranged between 85 and 112%.

2.3 Evaluation of results

Sediment cores are commonly used to evaluate metal background levels and the historical evolution of metal enrichment in coastal marine areas (Birch 2017). One of the most commonly used pollution indices is the Enrichment Factor (EF) using aluminium for normalization. Subsequently, EF is applied to classify sediments into five (5) contamination classes (after Sutherland, 2000), which are: (1) EF<2 (Depletion to minimal enrichment / no or minimal pollution), (2) EF 2-5 (Moderate enrichment / pollution), (3) EF 5-20 (Significant enrichment / pollution signal), (4) EF 20-40 (Very highly enriched / very strong pollution signal, (5) EF>40 (Extremely enriched / an extreme pollution signal).

The calculation formula is:

$$EF = \frac{(\frac{Me}{Al})_{sample}}{(\frac{Me}{Al})_{background}} \text{ (1) (Salomons \& Förstner, 1984)}$$

3. Results

The concentrations of Hg in the surface sediments and the deepest layers of the cores in every sub-area are given in Table 1 along with granulometry, aluminium content and the calculated Enrichment Factor (EF) for each of the surface sediments. Hg concentrations from the intermediate layers of sediments cores are not provided here. In the case of the surface sediments OS2-OS7 and S7 the background values used for EF calculations are the averages of the deeper layer concentrations from cores S13 and S16, while for stations OS5 and OS6 the deeper layer concentrations of core S11. The choice of background values, when no deep layer sediment was available at the exact position, was based on proximity and granulometry.

The minimum Hg concentrations were found in the coarse-grained sediments (i.e., MOT13A, MOT16, S16 and UN22) and the stations farther from polluting activities (S21, UN12A). The maximum Hg value is found at the outfall of the Psitalia WWTP (S7).

Table 1. Mercury, aluminium concentrations and calculated Enrichment Factors in Saronikos stations [(s1): core surface layer (0-1 cm), (s2): surface sediment (0-2cm) from box corer and (d): deeper layers of sediment cores, (*) granulometry at the deeper layers resembles the corresponding surface sediment at each station, values in bold are above ERM (0.710mg/kg), values in bold/italics are between ERL (0.150 mg/kg) and ERM].

Area / Station (Bottom Depth) / Core depth	Sample Type / % sand - %silt & clay / Al (mg/kg)	Hg (mg/kg)	EF
West Saronikos			
UN4 (95m) / (22cm)	(s1) / (51 - 49) / 22702	0.073	2.2 – Moderate Enr.
MOT13A (50m) / (12cm)	(s1) / (60 - 40) / 5697	0.037	1.9 – Minimal Enr.
MOT16 (87m) / (20cm)	(s1) / (58 - 42) / 16050	0.042	1.5– Minimal Enr.
UN5 (150m) / (32cm)	(s1) / (<10 - >90) / 32705	0.076	2.1– Moderate Enr
UN6 (200m) / (26cm)	(s1) / (<10 - >90) / 43264	0.082	2.2– Moderate Enr
UN6A (167m) / (24cm)	(s1) / (<10 - >90) / 39314	0.074	2.4– Moderate Enr
S25 (400m) / (32cm)	(s1)/(<10 - >90) / 54626	0.076	1.8– Minimal Enr.
West background levels	(d) / (*)/ 9342 - 48186	0.030 - 0.041	
Inner Saronikos			
OS2 (56m)	(s2) / (<10 - >90) / 12118	***0.432***	11.4 – Significant Enr.
S7 (70m)	(s2) / (39 - 61) / 13385	**0.888**	21.1 - Significant Enr.
OS5 (70m)	(s2) / (78 - 22) / 4559	0.103	5.1 - Significant Enr.
OS6 (35m)	(s2) / (78 - 22) / 2850	0.054	4.3– Moderate Enr
OS7 (92m)	(s2) / (68 - 32) / 10168	0.103	3.2 – Moderate Enr
S11 (77m) / (17cm)	(s1) / (82 - 18) / 6948	***0.168***	5.1– Significant Enr
S13 (88m) / (20cm)	(s1) / (>90 - <10) / 9922	0.106	3.0– Moderate Enr
S16 (85m) / (9cm)	(s1) / (82 - 18) / 4424	0.048	3.8– Moderate Enr
Inner background levels	(d) /(*) / 8271 - 11951	0.034 - 0.040	
Outer Saronikos			
S21 (220m) / (39cm)	(s1) / (<10 - >90) / 44902	0.057	1.7– Minimal Enr.
UN12A (216m) / (28cm)	(s1) / (<10 - >90) / 45891	0.058	2.2– Moderate Enr
UN22 (193m) / (13cm)	(s1) / (>90 - <10) / 11451	0.026	1.9– Minimal Enr.
Outer background levels	(d) / (*) / 5835 - 52562	0.019 – 0.039	

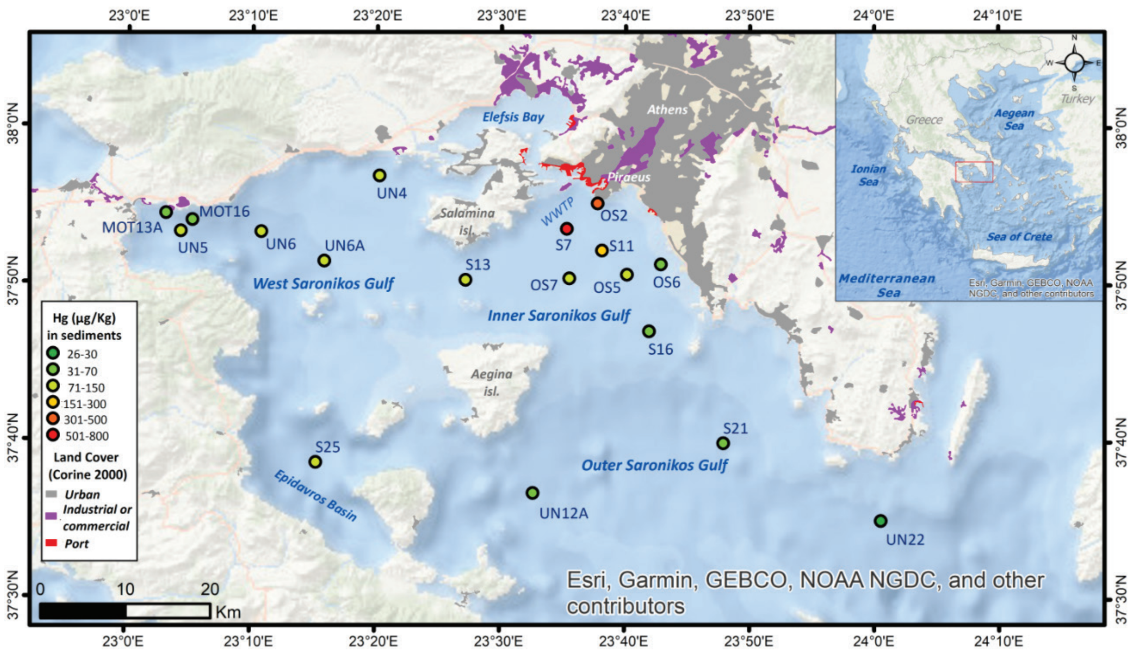

Fig. 1: Study area, sampling stations and the spatial pattern of Hg concentrations(μg/kg) in Saronikos Gulf sediments.

In terms of the Enrichment Factors most stations are characterized as minimally or moderately enriched except for stations S7, OS2, S11 and OS5 in Inner Saronikos where the Hg pollution signal compared to background values is significant.

4. Discussion/Conclusion

The background values of Hg in Saronikos Gulf sediments are similar to the average crust level of 0.040mg/kg (Wedepohl, 1995). The Saronikos sediments seem affected by recent anthropogenic Hg pollution, since the surface content in most stations is 2 to 20-fold higher than the average background value.

The surface sediment Hg content in most stations is below the ERL value of 0.150mg/kg therefore adverse effects to benthic biota are unlikely. That is not the case for the most affected stations (OS7 close to Piraeus port and S7 at the Athens WWTP outfall) where the ERL (0.150 mg/kg) and ERM (0.710mg/kg) limits are surpassed, respectively. In station S7 adverse effects to benthic biota are definite. The presence of Hg in wastewater, affecting the sediments at station S7, may be attributed to various uses in commercial / industrial facilities connected to the main sewage system and household waste and accidental spillage of food and other products. Another possible explanation could be Hg use in dental amalgams which is still occurring despite trends for replacement with other materials. (Fricke *et al.*, 2015; UNEP, 2013).

There is need for an integrated study of Hg levels in Saronikos Gulf also incorporating biotic elements (benthic and pelagic species) as well as biomarker studies in order to fully grasp the effect of Hg in this coastal marine environment.

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SUSPENDED PARTICLE-ASSOCIATED ALIPHATIC AND POLYCYCLIC AROMATIC HYDROCARBONS IN OPEN/DEEP AREAS OF THE EASTERN MEDITERRANEAN SEA: OCCURRENCE, SOURCES AND DISTRIBUTION PATTERNS

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Abstract

In this study, we focus on providing a wide overview on the pollution levels, major sources and mechanisms driving the distribution and cycling of suspended particle-associated aliphatic (TAHC) and polycyclic aromatic hydrocarbons (PAHs; sum of 25 compounds/ groups) collected along the water column from 38 offshore/deep sea locations across the open eastern Mediterranean Sea. Total concentrations of the determined compounds ranged from 10.1 to 1080 ng L^{-1} for TAHC and from 59.3 to 1290 pg L^{-1} for PAHs, respectively. Their molecular profile and diagnostic indices reveal mixed contributions from both natural and anthropogenic sources across the study area. Hydrocarbon mixtures characteristics varied significantly within the studied sub-regions, highlighting the importance of inputs from various point sources, the presence of dispersed pollutants and dispersion pathways.

Keywords: Aliphatic hydrocarbons, PAH, Suspended particles, Eastern Mediterranean Sea.

1. Introduction

Hydrocarbons are ubiquitous components of the organic material introduced into coastal and open sites of the world's oceans. Although they may derive from both marine and terrestrial sources, a major amount of their contribution is related to various anthropogenic activities which result in the formation and release of hazardous organic contaminants. The eastern Mediterranean Sea (EMS) is a maritime environment under intense anthropogenic pressure which has been receiving major concern during the last decades regarding the assessment of its chemical pollution status (e.g., Hatzianestis *et al.,* 2020). The study of suspended particle-associated hydrocarbons in the marine environment can reveal information about their sources, standing stocks and inventories, but also the processes affecting their occurrence and vertical/advective transport pathways following the circulation of water masses. Herein, we aim to provide an overview on the pollution levels, major sources and mechanisms driving the distribution and cycling of suspended particle-associated TAHC and PAHs collected along the water column in offshore/deep sea locations across the open EMS.

2. Material and Methods

Suspended particle-associated TAHC and PAHs were collected from various depths across the open EMS (Aegean, Ionian and northwestern Levantine Seas; 38 stations, 67– 4400 m water column depth), during various oceanographic cruises conducted between 2013 and 2021 (Fig. 1). Samples were taken close to the sea surface, within the maximum of chlorophyll fluorescence, the base of the euphotic zone, mesopelagic and bathypelagic layers. All samples were collected with a rosette sampler and were filtered through GF/F filters. The analysis of aliphatic hydrocarbons and PAHs in freeze-dried filters was carried out by GC/MS after Parinos *et al.,* (2013). *n*-C_{10} to *n*-C_{40} aliphatic compounds along with the UCM of aliphatic hydrocarbons, and 25 PAH compounds/groups (ΣPAH_{25}) including 15 parent (unsubstitued) priority PAHs along with the alkylated homologues of dibenzothiophene, phenanthrene, pyrene and chrysene where determined/quantified. In order to assess the main processes driving the distribution of the determined hydrocarbons, correlation analysis was performed for particle-associated aliphatic and polycyclic aromatic hydrocarbons concentrations, particulate organic carbon (POC) content, water column depth as well as concentrations of lipid biomarkers having a marine/algal origin (TPhyto; i.e. brassicasterol, dinosterol and C_{30} diols&keto-ols) and cholesterol associated with the existence of marine consumers such as zooplankton.

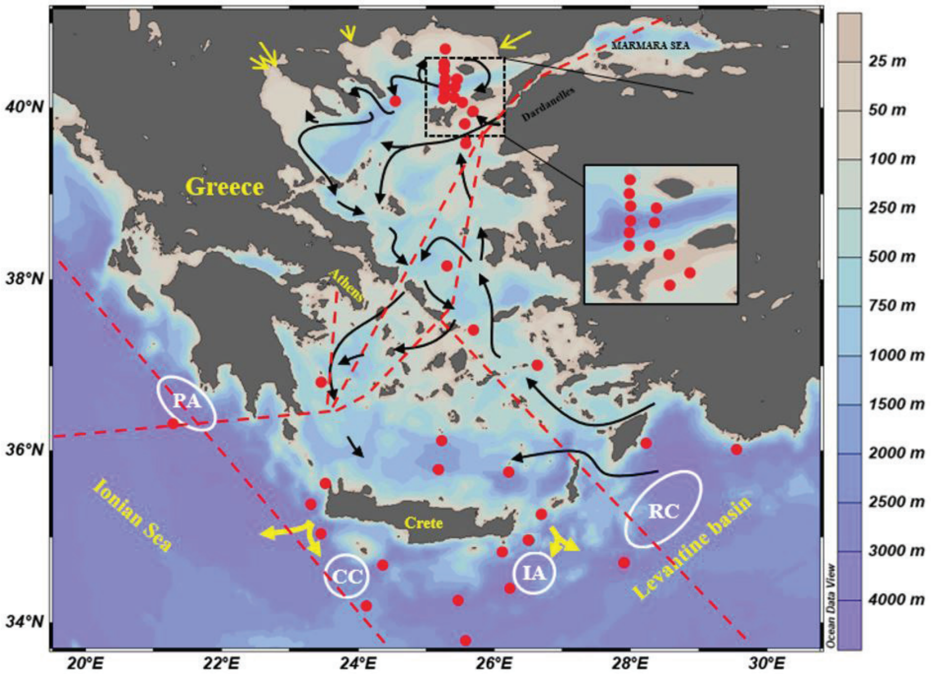

Fig. 1: Location of sampling sites in the study area along with the main tanker/crude oil shipping routes (red dotted lines), main surface circulation patterns (black lines) and Cretan intermediate/deep waters outflow (yellow lines). Yellow arrows indicate the main rivers outflowing into the north Aegean Sea area.

3. Results

TAHC concentrations ranged from 10.1 to 1080 ng L^{-1}, averaging 192 ng L^{-1}. The highest TAHC levels were recorded in the north Aegean Sea, averaging 223 ng L^{-1} followed by the Cretan Sea stations where TAHC averaged 151 ng L^{-1}. ΣPAH_{25} concentrations ranged from 59.3 to 1290 pg L^{-1}, 283 pg L^{-1} on average. The highest ΣPAH_{25} levels were recorded in the north Aegean Sea stations, averaging 439 pg L^{-1}, while lower concentrations were recorded at the Cretan Sea, averaging 167 pg L^{-1}. Concerning TAHC and ΣPAH_{25} levels with depth, higher concentrations were observed within the top meters of the water column, during fall and spring. No significant seasonal variations were observed within the sampling periods. Suspended particle-associated aliphatic hydrocarbons in all samples were dominated by long-chain *n*-alkanes ($C_n \geq 24$) maximizing at *n*-C_{29} and a UCM present as a unimodal hump centered around *n*-C_{30} (Fig. 2A). UCM was the major component of aliphatic compounds accounting for >70% of their total sum in all cases. Concerning PAHs, phenanthrene and its alkylated homologues dominated amongst 3-ring compounds, while amongst the ≥ 4-ring parent compounds of predominantly pyrolytic origin, fluoranthene and pyrene dominated amongst 4-ring compounds, while benzofluoranthenes and benzo[*ghi*]perylene dominated amongst 5-ring and 6-ring compounds, respectively (Fig. 2B). Concerning, PAH source-specific diagnostic indices, which could reveal information regarding specific PAH sources in the marine environment, the Phe/ (Phe + C_1-Phe) ratio exhibited values between 0.29 and 0.75 (0.55 ± 0.09 on average; Fig. 3A) and the BaA/ (BaA + Chry) ratio ranged between 0.42 and 0.96 (average 0.72 ± 0.15; Fig. 3B). TAHC and ΣPAH_{25} concentrations are significantly correlated with the corresponding POC content in the considered samples ($r = 0.360$, $p = 0.001$; 2-tailed, $n = 181$ and $r = 0.600$, $p = 0.001$; 2-tailed, $n = 135$, respectively), while low-MW phenanthrenes concentrations also show positive correlation with the concentration of cholesterol and TPhyto (r = 0.317, p = 0.001; 2-tailed, n = 189 and r = 0.430, p = 0.001; 2-tailed, n = 189, respectively).

4. Discussion/Conclusion

Preliminary results highlight the fact that the reported levels of the determined particle-associated TAHC and PAHs fall within a range comparable to that previously reported for non-polluted coastal and offshore/deep sea locations in the EMS and worldwide (reviewd by Parinos & Gogou, 2016). Overall, the molecular profile and source-specific diagnostic ratios of the determined organic compounds indicate an admixture of pyrolytic/combustion sources, oil derived compounds and natural inputs associated to higher plant waxes. High molecular weight *n*-alkanes are major components of epicuticular higher plant waxes and the presence of UCM in marine samples is mostly being attributed to products of petroleum origin and/or to oil residues which have undergone extensive degradation. The molecular profile and source-specific diagnostic ratios of low and high-MW PAHs in the study area (Figs. 2 and 3) reflects an admixture of both unburned fossil sources, the combustion/pyrolysis of fossil fuels and the combustion of grass, wood and coal.

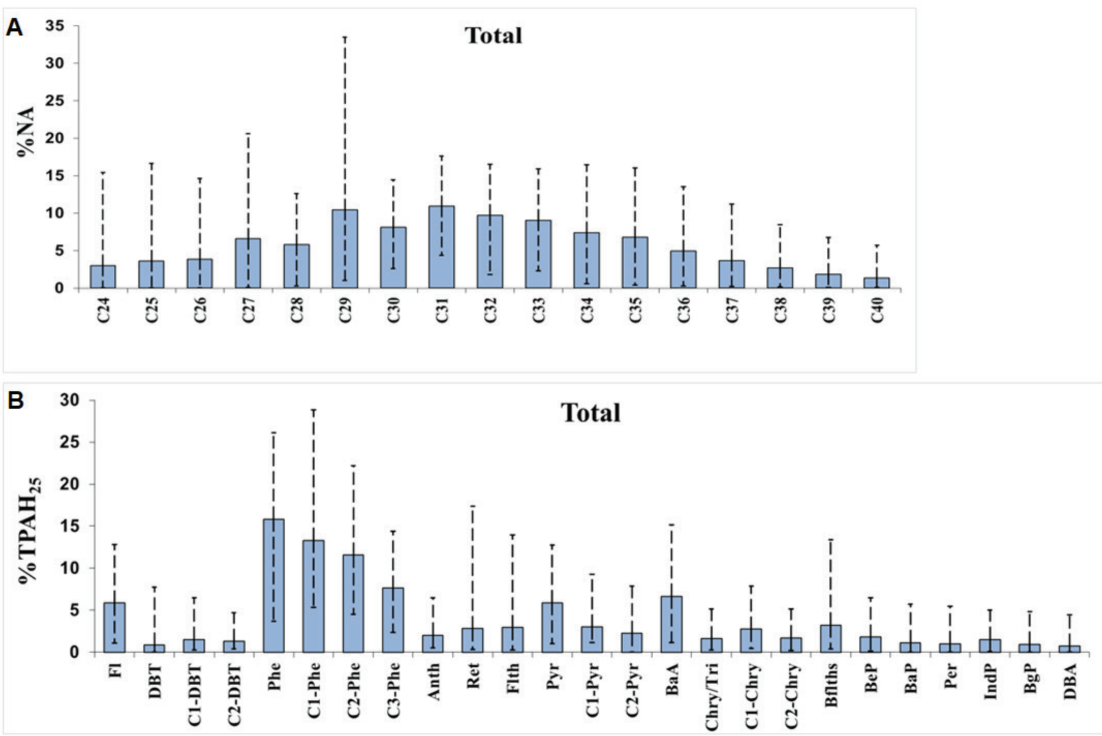

Fig. 2: Averaged molecular profile of *n*-alkanes - assigned with their number of carbon atoms **(A)** and the determined PAH compounds **(B)** in suspended particles across the study area. Individual compound abundances are normalized as percentage of the total sum of *n*-alkanes (NA) or PAHs (ΣPAH_{25}) and presented along with min–max deviation lines.

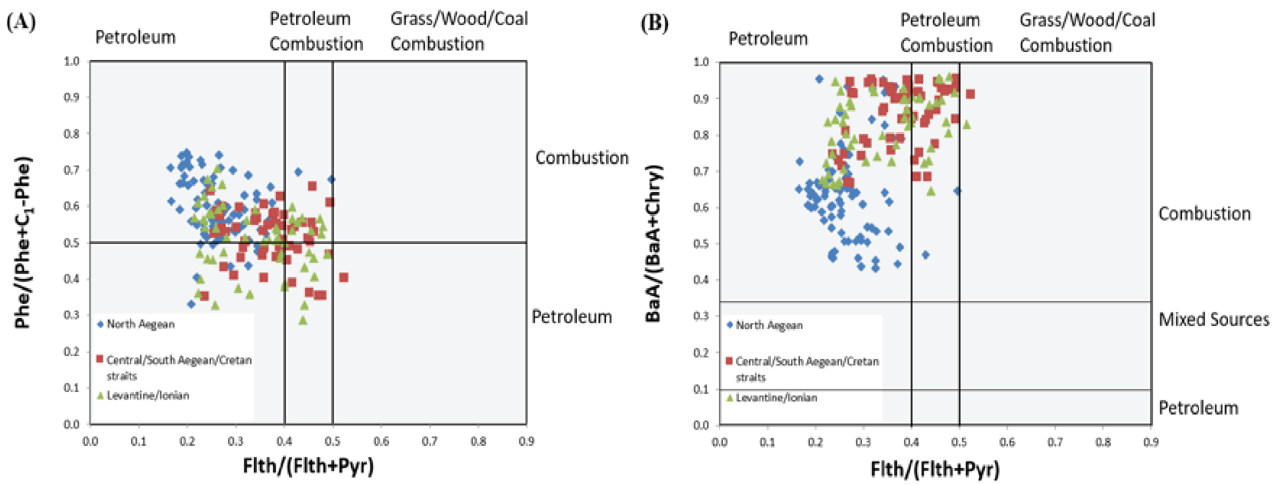

Fig. 3: The Phe/ (Phe + C_1-Phe) vs. Flth/ (Flth + Pyr) **(A)** and BaA/ (BaA + Chry) vs. Flth/ (Flth + Pyr) **(B)** diagnostic ratios cross plots for the study area, along with values corresponding to specific sources (after Yunker *et al.*, 2002).

Within the studied sub-regions, the north Aegean Sea stations display the highest levels, on average, of both anthropogenic and natural/terrestrial inputs, probably due to the intense merchant shipping/ oil transportation in the area as well as inputs through the inflow of the Black Sea water and from a number of rivers, which likely constitute the major transport pathways of both plant waxes and pyrolytic compounds, the latter being associated with fine combustion particles (char and soot-black carbon) as indicated by the source-specific diagnostic ratios of both low and high-MW PAH compounds (Fig. 3). In the central Aegean and the south Aegean/Cretan Sea, a mixed contribution from both pyrolytic and petrogenic sources is evident, however, PAH source-specific ratios values (Fig. 3) and the molecular profile of aliphatic hydrocarbons are indicative of enhanced fossil inputs typifying chronic oil pollution. The NW Levantine and Ionian regions are being characterized by elevated fossil inputs along the water column typifying chronic oil pollution. Deep water layers of the Ionian Sea present elevated natural inputs (plant wax *n*-alkanes) likely associated to the main water mass circulation features in the area. Deep-water layers of the area originate mainly in the Adriatic Sea and are exported to the abyssal layers of the Ionian Basin by bottom-arrested currents, flowing eastward all the way to the eastern Levantine Basin. Since the Ionian bottom waters have a renewal period of 58 years deep basins could function as a long-term repository of hydrocarbons exported from the Adriatic Sea.

TAHC and ΣPAH_{25} concentrations show significant correlation with POC, indicating that organic carbon exerts an important control on the distribution and accumulation of both natural and anthropogenic hydrocarbons in the study area, likely as a result of the well documented high affinity of the hydrophobic aliphatic and polycyclic aromatic compounds to organic matter. Furthermore, the positive correlation of low-MW phenanthrenes concentrations with the concentration of cholesterol and TPhyto in suspended particles of the study area, is attributed to the fact that low-MW phenanthrenes, related to the dissolved and colloidal phases in the water column, are more efficiently scavenged by organic-rich particles, which constitute the major vehicles for particulate organic carbon sinking and overall cycling in the marine environment.

Concluding, continental runoffs/riverine inputs, direct discharges, water masses' circulation patterns and biogeochemical features seem to influence the regional characteristics and distribution patterns of the studied suspended particle-associated aliphatic and polycyclic aromatic hydrocarbons in the study area. In all cases, an enhanced fossil input typifying chronic oil pollution is evident along the water column, with varying contributions depending on proximity to point sources, the presence of dispersed pollutants and vertical/advective transport pathways following the circulation of water masses.

5. Acknowledgements

The samples considered in this study have been collected within the framework of various national and EU research projects spanning the last decade. The synthesis of results presented herein has been supported by the project "ENIRISST – Intelligent Research Infrastructure for Shipping, Supply Chain, Transport and Logistics" (MIS 5027930) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020), co-financed by Greece and EU (European Regional Development Fund).

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BENTHIC RESPONSE TO THERMAL DISTURBANCE: PROJECT CONCEPT AND PRELIMINARY RESULTS

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Abstract

One of the most important outcomes of climate change with potentially significant ecological and economic consequences is the increase in seawater temperature. In this context, areas in the coast where warm discharges (i.e. industrial cooling water) are discharged in the sea inducing a mild and temporal change in water temperature, may be considered as an experimental simulation of climatic change. This study aims at assessing the effect of water temperature increase in macrofaunal community structure, ecological status as well as sediment environmental variables at four different locations where warm water emerges in the sea from industrial pipelines. Four locations were chosen, with three sampling stations each (Near the emerging point, Far 50 meters away and Control 1km upstream). The results indicate that there is one-degree °C water and sediment temperature increase in the Near stations. Based on the preliminary results presented here, there is an observable effect on sediment environmental variables but the Ecological Status based on the BQI_Family index does not differ between Near and Control stations.

Keywords: Benthos, Marine Ecology, Climate Change.

1. Introduction

The rapid response of benthic macroinvertebrates to environmental changes, such as raise of water temperature, has led to several studies examining the response of community structure and diversity patterns. Survey data over the past century suggest that there have been significant spatial changes in the distribution of intertidal organisms when sea temperatures have risen by as much as 1º C patterns (Xu *et al.,* 2020). Climate warming, over decades, has been suggested to modify the composition of resident organisms by promoting the spread of species from warm temperature areas. Change in temperature could affect mortality, reproduction, spawning, embryotic and gonad development of benthic species, modifying the community structure of benthic species. Furthermore, this change may also alter population dynamics at temporal and spatial scale and switch the geographical distribution of benthic communities which may induce species extinction as well as loss of diversity and ecosystem functionality (Birchenough *et al.,* 2011). Raise in water temperature caused by climate change could potentially increase the pressure on soft bottom benthic communities by reducing dissolved oxygen -increasing hypoxia, reducing the ability of benthic fauna to cope with large quantities of organic waste (Tett *et al.,* 2013).

The findings of this study will be used to assess the effects of thermal wastes on benthic communities. Furthermore, they will provide helpful information for monitoring marine ecosystems in view of the upcoming changes due to global warming. They will also result in a better understanding of the effects of climate change in coastal ecosystems, especially considering the oligotrophic conditions of the eastern Mediterranean, as well as the establishment of alien species in new areas.

2. Material and Methods

As a method of simulating scenarios of climate change, four areas were chosen, in which warm water discharge points are located. The warm water is used as a means of cooling heat exchangers of thermoelectric factories or industries and in the end emerges from pipelines and disperses to the sea. The temperature of the emerging water is reasonably higher than that of the sea and as it disperses it creates a zone of increased temperature which gradually decreases around the discharge point or pipeline.

In total, four locations were sampled in March 2022 two thermoelectric power plants at Ammoudara Beach and Atherinolakkos in Crete island and two oil. In Crete at, and two refineries oil companies, Oil Company 1 (OC1) and Oil company 2 (OC2) in Saronikos gulf. First of all, an accurate mapping of the exact location of the pipelines was created using satellite images extracted from Google Earth© and a set of three sampling stations at each location was determined. The sampling stations were codenamed: Station Near as close as possible to the water exit point, Far 50m away from the exit point and Control at least 1000m away upstream in a location with similar depth and sediment type. The sampling took place with the research vessel "Philia" in the context of the BENTHERM program funded by the RePhil project. At first, a video recording from the sediment at each location was captured with an underwater ROV, which possesses a real-time temperature sensor which was used to record sea bottom water temperature. Then, from each sampling station three replicated sediment samples were collected by using a Smith-McIntyre grab sampler ($0.1m^2$). Temperature (T) was measured in the 1 cm of the sediment, while redox potential (Eh) and hydrogen sulfide (H_2S), were measured in every 1 cm of the first 5 cm of the by means of a UNISENSE© Field-Microprofiler. Furthermore, samples were taken for granulometry from the upper 5cm and organic matter content from three layers (0-1, 1-3, 3-5 cm) and measured using the Loss of Ignition protocol (Loh et al., 2008). Macrofauna samples were sieved consecutively over a 1mm and a 0.5mm sieve, the retained materials were, fixed with Formalin solution (4%) and stored for sorting and taxonomic identification. The biotic index BQI_Family (Dimitriou *et al.,* 2012) was used to assess the Ecological Status (ES) of Near and Control stations.

3. Results

Habitat description based on CTD data, ROV videos and depth measurement (Table 1) showed that all locations had different environmental characteristics. However, based on the real-time ROV temperature sensor it was found that the water temperature at the sites close to the warm water effluent points was 1-2 ºC higher than that at the respective control sites. This was also confirmed from the temperature measurements of the Field Micro-profiler measured at the sediment corers (Table 1).

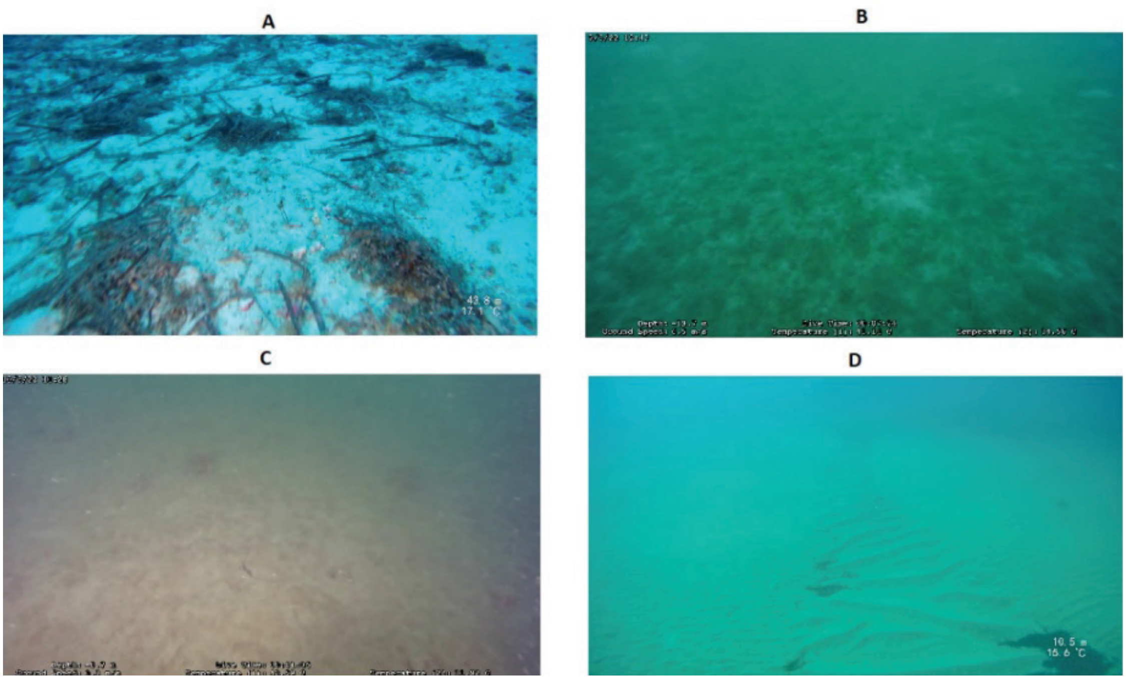

Fig. 1: Photographic images of the sediments taken using a camera on the underwater ROV near the warm effluents discharge points at locations: A) Atherinolakkos, B) Oil Company 1 (OC1), C) Oil Company 2 (OC2) and D) Ammoudara.

Table 1. Values of environmental variables and Ecological Status at each sampling station.

Station	Average Depth (m)	Habitat description	Average Water Temperature (C)	Average Sediment Temperature (C)	Ecological Status BQI_ Family
Ammoudara_near	5	Sand with no vegetation	16.6	16.3 ± 0.3	Moderate
Ammoudara_far	10	Sand with no vegetation	15.5	15.7 ± 0.3	-
Ammoudara_control	7.5	Sand with no vegetation	15.5	15.1 ± 0.2	Moderate
Atherinolakkos_ near	28.7	Sand with decaying vegetation	18	16.7 ± 0.2	Good
Atherinolakkos_far	37.4	Sand with decaying vegetation	17.1	16.3 ± 0.3	-
Atherinolakkos_ control	34.9	Sand with decaying vegetation	17.1	14.6 ± 0.2	Good
OC1_near	19.4	Sand covered with green algae	15.7	14.8 ± 0.3	Moderate
OC1_far	17.2	Sand covered with green algae	14.6	14 ± 0	-
OC1_control	24.2	Sand covered with green algae	14.6	13.8 ± 0.3	Good
OC2_near	7.4	Mud mixed with biogenic detritus	13	11.3 ± 0.6	Good
OC2_far	11.8	Mud mixed with biogenic detritus	11.6	10.7 ± 0.6	-
OC2_control	8.9	Mud mixed with biogenic detritus	11.6	9.7 ± 0.6	Good

In all cases the water and sediment temperature was around 1° C higher in the Near sampling locations compared to the control. The ES classification was Good in most cases. Sediment vertical redox profiles are presented in Figure 2 for all locations. No H_2S was detected in all sampling stations.

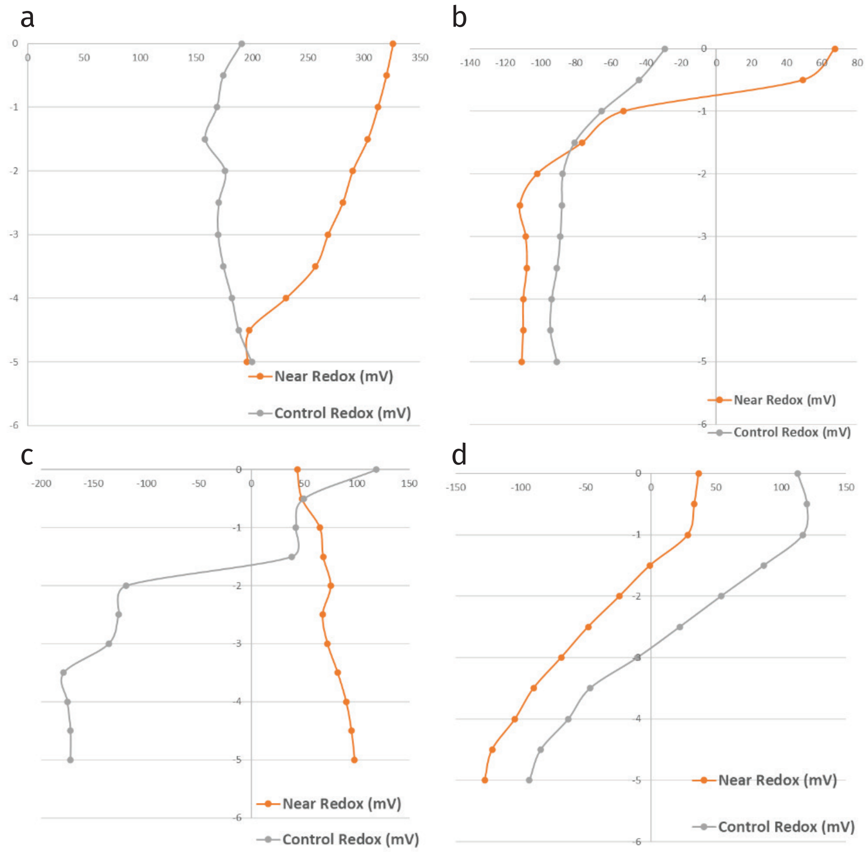

Fig. 2: Ventral profiles of sediment redox-potential at locations: a) Ammoudara, b) Oil Company 2, c) Atherinolakkos and d) Oil Company 1.

From the Redox vertical profiles it can be concluded that the locations with electric power plants have similar patterns with the Near station having higher Eh values. In the OC stations the opposite pattern is observed with the Near stations having lower Eh values after the 1 cm of the sediment.

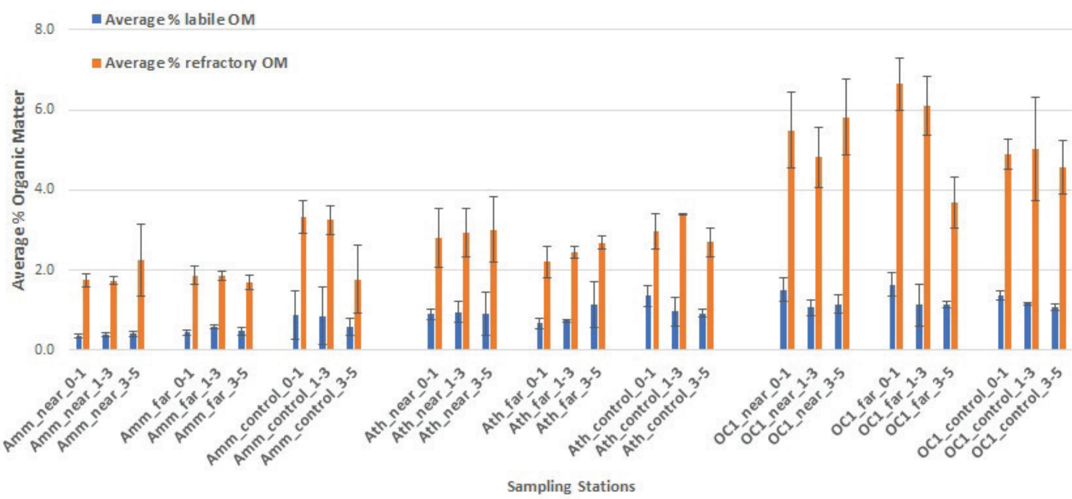

Fig. 3: Average (±SD) of labile organic matter (%) and refractory organic matter (%) in each sampling station of Ammoudara, Atherinolakkos and Oil Company (OC1).

The sampling stations in Crete had lower labile and refractory OM content compared to OC1 in Saronikos. Additionally, both in Ammoudara and Atherinolakkos the Control stations had higher OM content in the 0-1 layer of the sediment (ANOVA $p<0.05$), as opposed to the lower layers where no differences were observed (ANOVA $p>0.05$). In OC1 no significant differences were observed ($p>0.05$). OC2 OM data are not presented since the analysis of the samples is still ongoing, as is the case with macrofaunal community data (Fig 4).

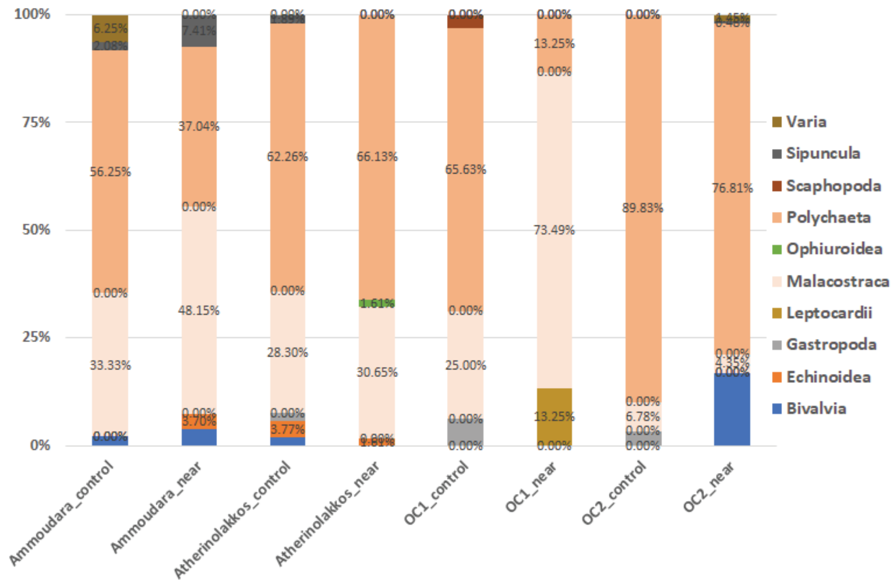

Fig. 4: Abundance percentage of macrofaunal classes in each sampling station.

4. Discussion/Conclusion

Based on the preliminary results presented here it can be concluded that:

the geochemical variables determined so far indicate that there is more variability between sites than between control and impact stations within each site (Fig. 2, Fig. 3)

There are some observable differences between environmental variables of the near and control stations but the effects are statistical significant only in a few cases, mainly in the stations in Crete. With the data available so far this cannot be attributed to the increased temperature.

The collection of macrofaunal specimens for the sediments in the close vicinity of the warm effluents discharge points has shown that the benthic communities are comprised of a large number of individuals, belonging to various families. Furthermore, with the exception of OC1, the ES classification of the Control and Near station was identical.

5. Acknowledgements

We would like to thank the captain and the crew of the RV Philia for assisting with the sampling. This project is part of the Hellenic Research Fleet - Reconstruction of the Research Vessel PHILIA, which is funded by the Operational Program (OP) "Competitiveness Entrepreneurship and Innovation" (NSRF) for the period 2014-2020 and co-financed by the European Regional Development Fund (ERDF)

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BIOCLIMATIC PROJECTIONS UP TO 2070 REVEAL ONE THIRD LOSS OF SUITABLE ENVIRONMENTAL CONDITIONS OF A VULNERABLE SALMONID

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Abstract

Climate change effect on species distribution and extinction risk constitutes a controversial topic among ecological researchers. To this end, habitat suitability assessments have been applied as a novel methodological approach to examine species' present and potential future distribution shifts. The present case-study proposes the application of the modern methodological approach of MaxEnt to estimate the suitable environmental conditions (SEC) for the vulnerable salmonid (*Salmo pelagonicus* Karaman, 1938) and predict the spatial and temporal shift of the species SEC under projections of the Global Circulation Model (GCM) MIROC5 for two climate change scenarios for the periods up to 2050 and 2070. The ratio of diurnal variation to annual variation in temperature, the coldest quarter of the year and the precipitation seasonality were the three most important bioclimatic variables for SEC. According to the results of the most ominous scenario (RCP 8.5), the SEC mean loss per habitat grid resulted at 26.3% and 32.9% for 2050 and 2070, respectively. Given the conservation status of the species and the projected SEC loss, proactive conservation practices and climatic mitigation practices are urgently needed.

Keywords: climate change, freshwater fish, General circulation models, MaxEnt, *Salmo pelagonicus*.

1. Introduction

Freshwater fish comprise the group of organisms that is most vulnerable to climatic changes (Markovic *et al*., 2017), with salmonids already experiencing important shifts and changes in their distributions (Comte & Grenouillet, 2013). To project species' distribution changes under a climate change scenario, niche modelling is often applied to inform about the suitable distribution area, assess the conservation needs and extinction risk of species and to plan conservation policy in order to assure the persistence of the species (Araújo *et al*., 2006). In recent years, several statistical and computer-based methods have been utilized to map ecological data and to spatially interpolate species' distributions. Among the ecological niche models, MaxEnt has shown accurate prediction capabilities in simulations and evaluations with presence-only data, outperforming classical modelling approaches (Su *et al*., 2021).

This is a case study proposing the application of a modern methodological approach, which could be used to assess the future impact of climate change on threatened Greek freshwater species. The main objectives of this study were to: (i) estimate the suitable habitat for the vulnerable Pelagonian trout (*Salmo pelagonicus* Karaman, 1938), since anticipated, climate-driven hydrological changes in mountain streams have clear consequences on salmonid survival (Jager *et al*., 1999) and (ii) proceed in a comparison analysis of the spatial and temporal shift of the habitat suitability values obtained through the simulated projections of the Global circulation model (GCM) MIROC5 under two representative concentration pathway (RCP 4.5 and 8.5) scenarios for the periods up to 2050 and 2070.

2. Material and Methods

Bioclimatic variables are derived from the monthly temperature and rainfall values, representing annual trends, seasonality and extreme or limiting environmental factors, used in species' distribution modelling and related ecological modelling techniques. To project the potential future distribution of the species, 15 bioclimatic variables (Table 1) were downloaded from WorldClim v. 2.1 database (https://www.worldclim.org/data/bioclim.html) , at a resolution of 5 arc-min (Hijmans *et al*., 2005), under two RCP scenarios: RCP 4.5 (intermediate) and RCP 8.5 (extreme), based on the corresponding trajectories of greenhouse gas (GHGs) concentrations adopted by the Intergovernmental Panel on Climate Change (IPCC), including projected changes of air pollutant emissions and land uses (Moss *et al*., 2008). MIROC5, which is considered one of the best performing models (Ayugi *et al*., 2020), was selected to acquire projections in two time periods: up to 2050 (average for 2041–2060, near future period) and up to 2070 (average for 2061–2080, distant future period) (Watanabe *et al*., 2010).

Salmo pelagonicus is endemic to the Aliakmon and Axios (Vardar) river basins (Barbieri *et al*., 2015), being found in the upper tributaries exhibiting a stationary distribution (extent of occurrence less than 20,000 km^2) (IUCN, 2008). Occurrence data were obtained from selected online biodiversity databases (GBIF - Global Biodiversity Information Facility, https://www.gbif.org/ and HCMR-https://watermonitoring.hcmr.gr/). Occurrences were partitioned (k-fold cross-validation) into those used to (i) make the model (i.e. “training”) and (ii) evaluate it (i.e. “testing”) (Peterson *et al*. 2011). MaxEnt machine learning algorithm applies a machine learning technique called maximum entropy modelling, being among the highest-performing niche/distributional modelling techniques for a wide range of environments and species (Elith *et al*., 2006), including for narrow or limited occurrence data (Hernandez *et al*., 2006). Model selection was based on the Akaike Information Criterion (AIC), with models with the lowest AIC being identified as optimal among candidate models. No threshold rule of training presence was applied. The SDM models were evaluated by measuring the Area Under the Curve (AUC) of the models, a widely used procedure for comparing species’ distribution model performance. The AUC value ranges from 0 to 1, the closer the value of the AUC to 1, the better the fit of the model. All analyses were implemented in Wallace (Kass *et al*., 2018).

Table 1. 15 bioclimatic variables of WorldClim v. 2.1 database included into the analysis.

bio1	Annual Mean Temperature	bio11	Mean Temperature of Coldest Quarter
bio2	Mean Diurnal Range	bio12	Annual Precipitation
bio3	Isothermality	bio13	Precipitation of Wettest Month
bio4	Temperature Seasonality	bio14	Precipitation of Driest Month
bio5	Max Temperature of Warmest Month	bio15	Precipitation Seasonality
bio6	Min Temperature of Coldest Month	bio16	Precipitation of Driest Quarter
bio7	Temperature Annual Range	bio17	Precipitation of Driest Quarter
bio10	Mean Temperature of Warmest Quarter		

Moreover, the present analysis explored spatial and temporal patterns of the Suitable Environmental Conditions (SEC). However, several problems occur during the comparative analysis of the produced patterns, since the combination of numerous geolocation data and the given SEC values demand increased computational power. In an attempt to simplify this multidimensional system, we calculated the delta difference [Δ_{SEC}] of the SEC values (y) that were obtained through bioclimatic scenarios on different time shifts (c). Hence, the Δ_{SEC} was applied on individual-based temporal shifts of each grid (i) regarding a timestep before (t) and after ($t+c$) as follows (Eq. [1]):

$$\Delta_{SECi} = y_{SECi\,[t+c]} - y_{SECi\,[t]} \; [1]$$

$$\Delta_{SEC\mu} = \frac{\sum_{i}^{n_{SEC}} \Delta_{SECi}}{n_{SEC}} \; [2]$$

$\Delta_{SEC\mu}$ (Eq. [2]) expresses the mean (μ) value of the temporal SEC shift and *n* denotes the total number of the geolocation grids. As a result, in case of $\Delta_{SEC\mu} < 0$, there is a decrease in the mean SEC compared to *c* years before, while if $\Delta_{SEC\mu} > 0$ the opposite occurs. The potential minimum value of the mean temporal shift of SEC is observed when $\Delta_{SEC\mu}$=-1 (i.e., 100% mean loss in SEC per habitat grid) and the potential maximum gain is when $\Delta_{SEC\mu}$=+1, with 0 referring to a stable temporal shift.

3. Results

Based on the results of the k-fold test in MaxEnt, only three out of the 15 variables were considered important. More specifically, the variables of the ratio of diurnal variation to annual variation in temperatures (°C), the coldest quarter of the year (°C) and the precipitation seasonality contributed as significant predictors of the distribution of *S. pelagonicus* and for the estimation of suitable habitat for the species in the study area. In the MaxEnt model, the average test AUC was 0.59 with a standard deviation of 0.18. Figure 1 depicts the SEC map generated values from MaxEnt (Fig. 1 left panel). By 2050 and 2070, the species is predicted to expand its range westerly, in tributaries of Aliakmon and Crna rivers (Axios/Vardar basin), under both the most conservative scenario in 2050 (RCP 4.5) (Fig. 1a, c) and the most extreme scenario, in 2070 (RCP 8.5) (Fig. 1b, d).

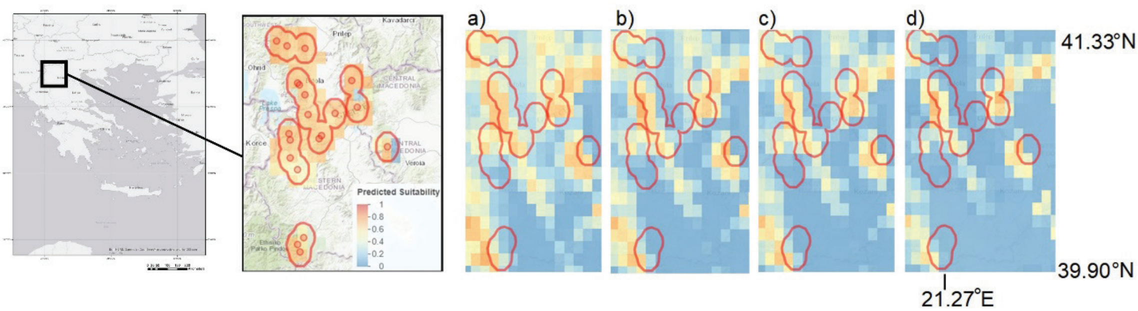

Fig. 1: Predicted suitable environmental conditions (SEC) of *Salmo pelagonicus* from MaxEnt. Presence points of the species in the study area are depicted with red circles (left panel). Distributions of *Salmo pelagonicus* under 4 scenarios of climate change: a) 2050 (RCP 4.5), b) 2050 (RCP 8.5), c) 2070 (RCP 4.5) and d) 2070 (RCP 8.5).

Overall, the calculated statistics derived through the bioclimatic projections demonstrated a constant decline of the mean and the minimum SEC values under both model scenarios (Table 2). On the contrary, there was a minor increase in the maximum value from 2020 to the 2050/2070 simulated projections, exhibiting a stable range of values across scenarios. According to the results of the delta temporal shifts based on the most ominous scenario (i.e., RCP 8.5 | MIROC 5), it appears that during the period of 2020 to 2050 there was a 26.3% mean loss of the SEC per grid of occurrence (2020-2050: $\Delta_{SEC\mu}$= -0.263). Correspondingly, the 2020 to 2070 projection for the same scenario exhibited a further increase in the mean loss of the SEC at 32.9% (2020-2070: $\Delta_{SEC\mu}$= -0.329). In the case of the 4.5 MIROC-5 scenario, the SEC mean loss per habitat grid resulted at 19.5% and 28.1%, respectively (2020-2050: $\Delta_{SEC\mu}$=-0.195; 2020-2070: $\Delta_{SEC\mu}$=-0.281).

Table 2. Descriptive statistics of the suitable environmental conditions (SEC) values based on present and projected data of two different time periods (2050; 2070) and scenarios (RCP 4.5; RCP 8.5), respectively.

Year	Scenario	SEC		
		Mean	Minimum	Maximum
2020	Present	0.607	0.160	0.728
2050	RCP 4.5	0.352	0.137	0.746
2050	RCP 8.5	0.300	0.138	0.747
2070	RCP 4.5	0.280	0.138	0.746
2070	RCP 8.5	0.245	0.136	0.740

4. Discussion/Conclusion

In response to the global biodiversity crisis, the 10th meeting of the CBD (CoP10) of the United Nations Convention approved a ten-year framework for action [Global Strategic Plan for Biodiversity (2011-2020); (CBD, 2010)], which specifies 20 ambitious targets, collectively known as the Aichi Targets. More specifically, Objectives 12 and 15 emphasize the protection of endangered species and the need for species to adapt to climate change. The European Member States are also called upon to draw up national plans to tackle climate change and reduce its impact on biodiversity. To this end, Greece, in 2014, issued the National Strategy for Biodiversity, which focuses on the development strategy for the adaptation to climate change and encourages research to meet the country's international obligations under the CBD (MoEE, 2014).

This study presents the first application of MaxEnt to map the suitable environmental conditions (SEC) of the threatened *Salmo pelagonicus* and estimate the temporal variability (gain or loss) of the mean SEC per grid. Similar studies on freshwaters of Greece are completely lacking, while they are very limited in terrestrial ecosystems (e.g., Kougioumoutzis *et al*., 2021), highlighting that climate change is still a marginal issue in the country. The catchments identified in the current study as most vulnerable for the survival of the species (Moglenitsas and Crna river) provide preliminary targets for development of climate change conservation management and mitigation strategies. Priority should be placed on enhancing stakeholder cooperation at the basin scale towards preventing further degradation of freshwater ecosystems and maintaining connectivity among catchments. Furthermore, river management plans could include the addition of riparian vegetation as a measure for mitigating the increased water temperatures, particularly during the summer heatwaves (EA, 2011; Dugdale *et al*., 2018). With the goal of contributing to clarity and replicability in climate change research in mind, we emphasize that projections and scenarios do not constitute statistical distributions of the future climate.

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COMPARING BIOLOGICAL QUALITY ELEMENTS IN GREEK RIVERS

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Abstract

Following the implementation of the WFD in Greece, three biological quality elements (BQEs) (diatoms, macroinvertebrates, fish), which are used to assess river biological quality for the Hellenic National Water Monitoring Programme, were compared among different river types. Furthermore, the environmental and geographic parameters that affect the biological quality indices of each BQE were assessed. Overall, the three BQEs agreed on the two extremes: Very Large rivers failed the good-moderate boundary, whereas the majority of sites in mountainous areas presented good quality. Diatoms proved to be more sensitive in detecting environmental alterations than the other BQEs, whereas biological quality based on fish was mostly affected by geographic parameters.

Keywords: WFD, biological quality, diatoms, macroinvertebrates, fish.

1. Introduction

The Water Framework Directive (WFD; 2000/60/EC) represents a milestone for the EU Member States in the field of water policy, since it aims at restoring or maintaining a good ecological status of European waters. The Directive provides very detailed guidance for improving water quality and thus enhancing the protection of aquatic ecosystems, by implementing ecological monitoring programmes within water bodies along with management and restoration measures at the catchment scale. The “good ecological status” is defined, in terms of quality of the community, using a multiple-taxa approach, based on five biological quality elements (BQEs) (benthic macroinvertebrates, macrophytes, diatoms, phytoplankton and fish) and supporting physico-chemical and hydromorphological quality elements. The biological quality is estimated by comparing the community in a site to the respective community that would be expected in environmental conditions of minimal anthropogenic impact. Bioassessments are expressed as an Ecological Quality Ratio (EQR) at a five-class system between one (High) and zero (Bad). The good-moderate boundary is essential in the WFD, because it defines if programmes of measures (POMs) are required. Thus, a two-class grouping may be applied, where all cases of high and good biological quality are considered as one “PASS” group, and the remaining cases of moderate, poor and bad biological quality are listed as “FAIL”.

Diatoms, benthic invertebrates and fish have long been used to assess anthropogenic pressures in rivers, due to their sensitivity to different degrees of stress and at different time scales (Birk *et al*., 2012). Diatoms can detect eutrophication gradients and have rapid responses to environmental alterations, due to their small generation times (e.g. Morin *et al*., 2016). Benthic invertebrates are more sensitive to organic pollution and general degradation, as well as to past stress events whereas fish detect habitat and morphological changes, connectivity, acidification and eutrophication (e.g. Hering *et al*., 2006). Apart from better detecting different pressures, BQEs can vary in their performance assessing different stream types (e.g. Karaouzas *et al*., 2019).

This study, using riverine data from the Hellenic National Water Monitoring Programme, aims at identifying patterns of biological quality, by addressing the following questions: (1) How is the biological quality, as measured by three BQEs, namely diatoms, macroinvertebrates and fish, distributed among the six Mediterranean intercalibration river types? (2) In cases of concordance of the three BQEs, how are the two groups of PASS/FAIL distributed among the six river types? (3) Is the value of the different biological quality indices explained by similar environmental and geographic factors?

2. Material and Methods

The WFD monitoring network includes 490 sites from 14 river basin districts, covering the entire national territory. In this study, a total of 669 samples, collected during a 3-year survey from 244 stream sites throughout Greece, were analyzed. The samples cover the six Mediterranean intercalibration river types: (1) small rivers (RM1) (38 sites, 93 samples), (2) medium rivers (RM2) (76 sites, 240 samples), (3) large rivers (RM3) (38 sites, 134 samples), (4) small/medium mountainous rivers (RM4) (32 sites, 84 samples), (5) temporary rivers (RM5) (37 sites, 42 samples) and (6) very large rivers (VL) (23 sites, 76 samples).

Environmental parameters [water temperature, conductivity, turbidity, dissolved oxygen (DO) and discharge] were measured in-situ, using multiparameter water quality probes. Water samples were collected for the determination of nutrient concentrations [total nitrogen (TN) and total phosphorus (TP)]. Geographic parameters (altitude and catchment area), and habitat characteristics were also recorded for each site. Sampling of biological groups followed standard EU protocols (EN 13946, EN 27828:1994). Biological quality, based on the assemblages of benthic diatoms, benthic macroinvertebrates and fish, was assessed using IPS (Specific Pollution Sensitivity index- Cemagref, 1982), HESY2 (Hellenic Evaluation System 2- Lazaridou *et al.,* 2018) and HeFI (Hellenic Fish Index- Zogaris *et al*., 2018), respectively. The above indices have been used for the quality assessment of rivers during the Hellenic National Water Monitoring Programme and quality boundaries have been adapted to national standards, based on the intercalibration exercise of the WFD (Lazaridou *et al*., 2016; Smeti & Karaouzas, 2016, Tachos *et al*., 2016) that resulted in a 5-class quality classification (Bad, Poor, Moderate, Good and High).

To identify potential relationships between biological (HESY2, IPS, HeFI) and physicochemical (Ph) indices, Spearman's correlation coefficient was used. Contingency tables were formed for the joint distribution of each pair of the categorical variables (biological quality, river typology) and were visualized through balloon plots, where each cell contains a dot, whose size reflects the relative magnitude of the corresponding component. To test for seasonal (two-level factor, spring/summer), environmental and geographic parameters that could affect the value of the biological quality index of each group, linear mixed-effects models, with the site as a random factor, were applied All quantitative variables were log-transformed before the analysis. Model selection was based on the Akaike Information Criterion (AIC), whereby the model with the lowest AIC is retained (Burnham & Anderson, 1998). All analyses were performed in the R statistical and programming environment (R. 4.1.2., R Development Core Team 2021), using lme4, MuMIn, corrplot, rcompanion and gplots packages.

3. Results

All indices were positively intercorrelated, with the IPS exhibiting the highest correlation with the Ph index (0.57), followed by IPS with HESY2 (0.53) and Ph with HESY2 (0.42). HeFI was moderately correlated with HESY2 (0.46), whereas poor correlation was observed between HeFI and Ph (0.22) and HeFI and IPS (0.36).

Diatom and macroinvertebrate indices presented more cases of good and high quality (56% of the cases), compared to fish that showed mostly poor and bad quality (48% of the cases). Very large rivers never presented high quality, irrespective of the investigated BQE (Fig. 1). This is also apparent when combining the three BQEs, where all the cases of VL rivers fail the good-moderate boundary for all BQEs. On the other hand, 90% of the samples of RM4 and RM5 rivers pass this boundary for all BQEs (Fig.1).

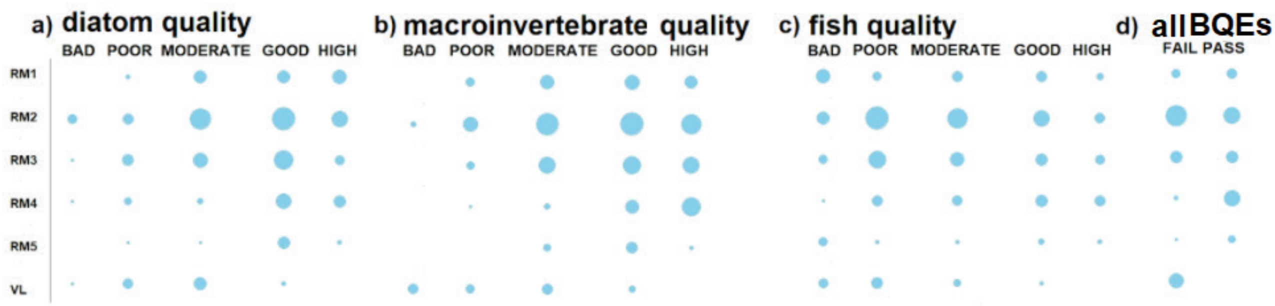

Fig. 1: Balloon plots showing the distribution of quality indices of diatom, macroinvertebrate and fish communities for all sites (a-c) and in cases where all three BQEs passed or failed the good-moderate boundary across the different Mediterranean River types (d). The size of the balloon corresponds to the number of occurrences in each category.

The biological quality based on diatoms was affected by season and environmental parameters, i.e. TP, D.O. and, followed by conductivity, water discharge and temperature. Environmental (TP and temperature) and geographic (altitude) parameters were important in explaining the variation of the macroinvertebrate biological quality. Concerning the biological quality based on fish, only the geographic parameters (altitude, followed by catchment area) were important (Table 1).

Table 1. Summary of linear mixed-effects models results. The percentages represent the frequency of appearance of each explanatory variable in all possible models explaining the for the percentages of geographic and environmental variables selection on the variation of biological quality indices. The higher the percentage, the more important the variable in explaining the quality indices of diatom, macroinvertebrate and fish communities in Greek rivers. Significant variables present in the selected model (with the lowest AIC) are shown in bold.

	Season	Altitude	Catchment area	Temperature	Conductivity	Turbidity	DO	Total N	Total P	Discharge
Diatoms (IPS)	**98%**	11%	13%	48%	**68%**	28%	**99%**	14%	**100%**	**60%**
Macro-invertebrates (HESY2)	3%	**90%**	<1%	**79%**	19%	2%	1%	8%	**98%**	<1%
Fish (HeFI)	3%	**69%**	29%	15%	6%	<1%	6%	3%	1%	13%

4. Discussion/Conclusion

Biological quality of different river types, based on the three BQEs, does agree on the two extremes , namely in very large rivers that fail the good-moderate boundary, as well as in the majority of sites located in mountainous areas that are of good ecological quality (Larsen *et al.*, 2012). Discrepancies between the three BQEs arise probably from the different variables that shape the communities of each biological group and thus, determine the respective quality indices. The three BQEs represent different groups of organisms, with different life cycles and different needs for habitat and growth, presenting thus specific responses to various pressures (Johnson & Hering, 2009).

Diatoms, that are unicellular, fast growing, primary producers, are largely affected by variables that are not characteristic of a site, but could be altered at a given time, after a pollution event (point source pressures). They are thus more indicative of fast and sudden environmental alterations. This is also in agreement with previous studies comparing diatom and macroinvertebrate responses to anthropogenic pressures (Karaouzas *et al.*, 2019). On the contrary, fish may be affected mainly by geographic (altitude, catchment area) parameters, highlighting the role of macroecological patterns of fish assemblages in broad scale studies (Oikonomou, 2021). Indeed, altitude could generate direct barrier effects on the distribution and life history traits of species and large catchment areas could support more coexisting fish species, verifying the species area relationship observed (Oikonomou & Stefanidis, 2020). Interestingly, HeFI increases with increasing altitude and may fail to capture anthropogenic pressures at narrow scales, already highlighted by Tachos *et al*. (2022), where water pollution was not recognized as a key stressor in most running waters. Macroinvertebrates seem to be affected by both environmental (TP, temperature) and geographical parameters (altitude).

This study highlights the need for implementing an ecological assessment of Greek rivers, using an approach of combining multiple biological assessment methods, as evidence from European case studies show that co-acting pressures may impact differently each BQE in a synergistic or antagonistic way. Thus, our findings can be used to further explore whether the integration of multiple BQEs into one biological classification (One-out, All-out rule) is affected by the biological index with the highest uncertainty and/or the highest sensitivity to environmental change, with the risk of false downgrading a water body (Carvalho *et al.,* 2019). By identifying patterns of quality assessments among river types, and cases where assessments result in different classifications, this study may offer water managers and policy makers insights on the classification capability of the current assessment scheme.

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MASS MORTALITY EVENT OF THE TOOTH-CORAL *BALANOPHYLLIA EUROPAEA* IN NATURA 2000 SITES OF CHALKIDIKI PENINSULA (NORTH AEGEAN SEA, EASTERN MEDITERRANEAN)

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Abstract

Coral mortality is a widespread global phenomenon of increasing magnitude, correlated with climate change. Prolonged marine heatwaves have affected the Aegean Sea in summer 2021, especially its northern part, threatening the populations of shallow water corals, such as *Balanophyllia europaea*. The present work aims to assess the population status of *B. europaea* in Natura 2000 sites along Chalkidiki peninsula (north Aegean Sea) after the "hot" summer of 2021. Sampling took place in September - October 2021 at 10 coastal, rocky-bottom stations spread over Kassandra and Sithonia by diving up to 20 m depth and applying non-destructive techniques. The abundance of *B. europaea* was estimated *in situ* by counting the number of corals within randomly placed 50 x 50 cm frames at the depth zone of the species' maximum density (3-6 m). The number of bleached or dead polyps was also recorded. *B. europaea* was found in 80% of stations, with a mean density of 9.5 N/m^2. In total, 58.17% of *B. europaea* individuals were affected by necrosis. Agios Nikolaos Bay in Kassandra gulf was the most affected site. These results highlight the need to establish monitoring programs on vulnerable coral populations along the Aegean Sea to assess climate change impacts.

Keywords: scleractinian corals, bleaching, necrosis, sea warming, climate change.

1. Introduction

The Mediterranean Sea is becoming warmer during the last decades and temperature increases much faster than global mean. This phenomenon is especially relevant along the eastern basin, which is suffering from "tropicalization" (Bianchi, 2007; WWF, 2021). Increased seawater temperature severely affects marine biota, mainly long-lived sessile epibenthic invertebrates, such as sponges, corals, bivalves and ascidians. Corals are among the most impacted species (Kružić *et al.*, 2016). Bleaching, i.e., the loss of endosymbiotic zooxanthellae from the host coral is the first signal of thermal distress, which if prolonged leads to mass mortality events. The frequency and severity of such events affecting various anthozoan species are steadily increasing over the Mediterranean Sea (Kružić & Popijač, 2014; Gómez-Gras *et al.*, 2021).

Focusing on the Aegean Sea, the case of the "hot" summer 2021 was rather unique due to the prevalence of very high sea surface temperature associated with a number of intense and prolonged (over 20 days) marine heatwaves that had especially affected its northern part (Androulidakis & Krestenitis, 2022). This prolonged warming may have strongly affected sessile marine biota.

Balanophyllia europaea (Risso, 1826) is an endemic scleractinian coral, widely distributed in the Mediterranean Sea. The species thrives on shallow rocks, usually between 2 and 8 meters, although it can be found much deeper, up to 40 m depth (Zibrowius, 1980). It is a zooxanthellate ahermatypic solitary coral, sensitive to climate change (Goffredo *et al.*, 2007, 2008; Kružić & Popijač, 2015; Otero *et al.*, 2017). Mortality events of *B. europaea* have been frequently reported from the Tyrrhenian and the Adriatic Sea during the last three decades (Kružić & Popijač, 2015; Kružić *et al.*, 2016). However, no relevant events have been reported, so far, from the Aegean Sea. In the latter area, the coral is among the most common invertebrates living on shallow rocky shores (Vafidis *et al.*, 1997). However, its biology has not been studied at all, except of some publications reporting its presence (Vafidis *et al.*, 1997; Morri *et al.*, 2000; Antoniadou *et al.*, 2006).

Considering all the above the present study aims to assess the population status of *B. europaea* in Special Protected Marine Areas of the Natura 2000 network in Chalkidiki peninsula (north Aegean Sea) after the particularly "hot" summer of 2021.

2. Material and Methods

2.1 Study area

The study was carried out at five Special Marine Protected Areas of the Natura 2000 network (Kassandra: GR1270010, GR1270008; Sithonia: GR1270007, GR1270002, GR1270009) in Chalkidiki peninsula, north Aegean Sea (Fig. 1). One up to four sampling stations were randomly set within each area on the rocky coastline. The sea bottom consisted of moderately to strongly inclined rocky cliffs (S3, S5, S7, S8, S9) or of large boulder reefs (S1, S2, S4, S6, S10) surrounded by dense *Posidonia oceanica* meadows.

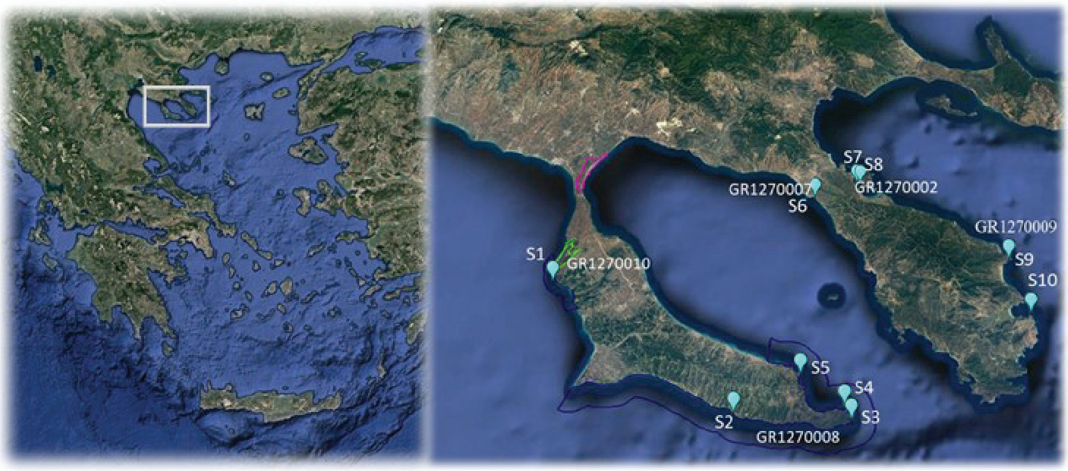

Fig. 1: Map of the Aegean Sea (left) and of the study area (right) indicating the location of sampling stations (from Google Earth).

2.2 Samplings

Samplings were carried out in September (Kassandra, 10-13) and October (Sithonia, 20-22) 2021 by scientific diving up to 20 m depth. The surveyed species, *B. europaea*, was found between two and 10 m depth, being particularly abundant around 3-6 m; therefore, population was surveyed at this depth level. Population density was *in situ*, visually estimated by counting all individuals present in 10 randomly placed quadrate frames (50 x 50 cm) at each station. The condition of individuals, *i.e.* alive (normally colored polyps), bleached (transparent polyps) or dead (bare white skeleton) was also recorded on an underwater slate. Primary data were used to assess mortality rate per sampling station, per protected site, and over the surveyed area.

3. Results

Balanophyllia europaea was found at eight out of the ten surveyed stations (80%), as the species was not recorded at S2 and S5. There is no previous information on the presence of *B. europaea* at S2, in contrast with S5 where the species was abundant (authors' personal data).

The species density, considering both alive and affected polyps, ranged from 2.8 N/m^2 (S6) to 23.2 N/m^2 (S7) with an overall mean 9.5 ± 8.3 N/m^2. No bleached polyps were observed. Mortality rate ranged from 45.45% (S10) to 82.14% (S4) with an overall mean at 58.17%. The highest necrosis was observed at Kassandra GR1270008 (Table 1), mostly due to the very dense and highly affected *B. europaea* population at Agios Nikolaos Bay (S4).

Table 1. Mortality rate of *Balanophyllia europaea* per sampling station at the surveyed Special Marine Protected Areas of the Natura 2000 network in Chalkidiki peninsula in autumn 2021.

Natura 2000 sites / Stations	**Kassandra**						**Sithonia**						
	GR1270010	**GR1270008**					**GR1270007**	**GR1270002**			**GR1270009**		
	S1	S2	S3	S4	S5	S_{3-4}	S6	S7	S8	S_{7-8}	S9	S10	S_{9-10}
N	12	0	17	56	0		7	58	16		13	11	
$N_{affected}$	8	-	9	46	-		4	34	9		6	5	
Mortality (%)	66.67	-	52.94	82.14	-	67.54	57.14	58.62	56.25	57.43	46.15	45.45	45.80

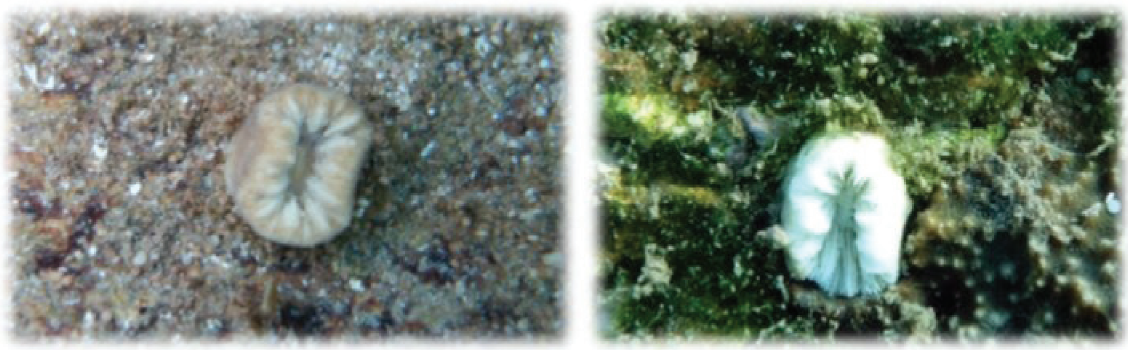

Fig. 2: Healthy (left) and dead (right) specimens of *B. europaea* in Chalkidiki during autumn 2021samplings.

4. Discussion/Conclusion

The tooth coral *B. europaea* has been evaluated as Least Concern by IUCN, as the species –despite being sensitive to marine warming– is widely distributed in the Mediterranean Sea and has dense populations in some marine protected areas (Otero *et al.*, 2017). However, since 1997 successive mortality events of growing magnitude have affected the species in the northwest, the central, and the eastern part of the basin (Metalpa *et al.*, 2000; Kružić & Popijač, 2014; Jimenez *et al.*, 2016) causing localized declines and reducing fitness (Otero *et al.*, 2017).

According to presented results, a mass mortality event has severely affected the population of *B. europaea* in Chalkidiki peninsula, as 58% of coral specimens suffered from necrosis. Persisting marine heatwaves that have affected the north Aegean Sea during summer 2021 (Androulidakis & Krestenitis, 2022) have probably caused the reported event. Coral mortality was especially high in Kassandra Bay, where necrosis affected, locally, over 80% of the surveyed population. Coastal topography (shelter bays and depth) combined with seawater circulation patterns and mixing may explain the observed differences in mortality rates between the sampling Natura 2000 sites, as healthier populations were recorded at the most exposed sites.

To our knowledge, this is the first report of *B. europaea* mass mortality event from the Aegean Sea. Despite the limited sampling effort, the endemic Mediterranean tooth-coral seems to be under serious threat in the study area, especially considering the predicted warming trend. Due to its sensitivity to warming, the species may serve as a climate-change descriptor over the Mediterranean Sea. Moreover, seeing that other benthic species, such as sponges (*Sarcotragus* spp., *Ircinia* spp.), corals (*Cladocora caespitosa*), bivalves (*Spondylus gaederopus*) and ascidians (*Microcosmus sabatieri*) suffered as well (authors' personal data), recursive monitoring using standard protocols is needed to detect future changes and impacts in larger spatial scales under climate crisis.

5. Acknowledgements

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AUTOTROPHIC VS. MIXOTROPHIC AND HETEROTROPHIC GROWTH IN CULTURES OF MICROALGAE ISOLATED FROM EASTERN MEDITERRANEAN SEA, A PRELIMINARY COMPARISON

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Abstract

In this study, we examine the growth of four microalgae strains of *Phaeocystis* sp., *Pleurochrysis* sp., *Chlorella* sp. and *Nannochloropsis* sp. with Mediterranean origin, which have been poorly addressed in laboratory studies under autotrophic against mixotrophic and heterotrophic conditions. Our results demonstrate that in autotrophic conditions, there were apparent differences in preference and ability to consume different nutrient sources among the isolated strains, opposing to biomass growth which was ubiquitous for all the strains tested. Different growth responses of the isolated strains tested under heterotrophic and mixotrophic conditions are discussed.

Keywords: microalgae growth, biomass production, nutrient consumption, ammonium, Volatile Fatty Acids (VFAs).

1. Introduction

There are several microalgae species which have attracted high scientific interest as model organisms in many applications, such as *Chlorella* and *Nannochloropsis* (Safi *et al.*, 2014; Liu *et al.*, 2017). The marine raphidophyte *Phaeocystis* has been mentioned mainly for high biomass blooms' production in northern latitudes (Hamm & Rousseau 2003). While *Phaeocystis antarctica* has been studied mostly for fatty acid composition under UV radiation effect (reviewed in Paliwal *et al.*, 2017), other *Phaeocystis* spp. remain rather unexploited. *Pleurochrysis* sp. is a coccolithophore, very scarcely mentioned in literature to our knowledge (Varkitzi *et al.*, 2017a). Coccolithophores are also well-known high biomass bloomers especially in the open ocean.

The aim of this research was to identify potential differences in biomass production and nutrient consumption under autotrophic, mixotrophic and heterotrophic conditions for three microalgae strains isolated from eastern Mediterranean, which have not been widely studied yet. Different nitrogen sources and stress conditions (nitrogen deplete cultures) were tested for their impact on the growth of *Chlorella* sp., *Nannochloropsis* sp., *Pleurochrysis* sp., isolated by the Department of Biology of National and Kapodistrian University of Athens from Koumoundourou lagoon in western Saronikos Gulf (Aegean Sea, Eastern Mediterranean) (Varkitzi *et al.*, 2017a; Savvides *et al.*, 2019), and *Phaeocystis* sp. which was isolated from the southeast coast of Saronikos Gulf (I. Varkitzi).

2. Material and Methods

2.1 Heterotrophic and mixotrophic growth conditions in preliminary experiments

Strains of *Chlorella*, *Phaeocystis* and *Nannochloropsis* sp. were tested under heterotrophic conditions in total darkness, and under mixotrophic conditions with a 12:12h light cycle, in a cool room at 25°C. Volatile Fatty Acids (VFAs) from Vegetable Garden Food (VGF) waste were used as a carbon source (for details see Chalima *et al.*, 2019). Three concentrations of VFAs were added in natural seawater (Table 1).

2.2 Autotrophic conditions

Strains of *Chlorella* sp., *Phaeocystis* sp. and *Pleurochrysis* sp. were chosen to be cultivated autotrophically in natural seawater and Walne's medium in order to increase biomass production potential (see Table 1). Nitrogen replete (full Walne) and N deplete conditions (half Walne) were used, with two combined nitrogen sources (50% NH4-N and 50% NO_3-N). All culture treatments were grown in triplicate 1L bottles, in a final upscale volume of 700ml and placed on a constant shaker at 100 rpm, in a cool room at 25°C. A 12h:12h light cycle was obtained with white fluorescent lamps providing an average light intensity of 60 μE m^{-2} s^{-1}.

2.3 Upscale, filtrations, biomass & nutrient sampling

The experiment included two phases: phase I with available N and P, and phase II with exhausted N and available P. Deplete and replete cultures were spiked only with P when exhausted. Samples for biomass production and nutrient analyses were taken every two or three days, while pH and cell vitality was monitored on regular intervals (for details of analyses see Varkitzi *et al.*, 2017a) The necessary volume for interim sampling was estimated beforehand in order to not affect the cultures' incubation.

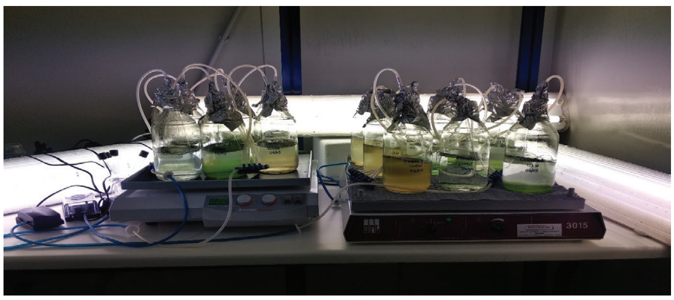

Fig. 1: Experimental cultures in borosilicate bottles with air supply, on a rotary shaker, in a cool room.

3. Results

In the preliminary experiments under heterotrophic and/or mixotrophic conditions, there was an overall poor growth for most strains under different concentrations of VFAs as a carbon source (Table 1). Particularly *Phaeocystis*' growth was totally inhibited in mixotrophy in any concentration of VFAs after less than 7 days of incubation, and therefore we did not proceed to the testing of heterotrophic conditions (Table 1). *Chlorella* and *Nannochloropsis* growth remained better in mixotrophic and heterotrophic conditions, even though they were rather inhibited in general.

Patterns of growth and biomass production under autotrophy are depicted in Figure 1(a-c). All strains increased their biomass significantly in both N-deplete and N-replete conditions, especially after one month of incubation. *Chlorella* sp. increased its biomass nearly 5-fold, reaching the highest average biomass among the tested strains in both replete and deplete conditions, with 7.802 and 5.670 g/L respectively on day 39. *Pleurochrysis* sp. increased its average biomass steadily, reaching 5.064 in deplete conditions and 4.325 g/L in control cultures. *Phaeocystis* sp. seemed to grow more slowly, but ultimately biomass reached similar levels with *Pleurochrysis* sp. and practically without significant differences in both treatments (4.686 g/L in control and 4.696 g/L in deplete conditions). pH was monitored regularly in the experimental cultures and it ranged at 8-8.5.

Consumption patterns of nutrients demonstrated rapid consumption of NH_4-N for most strains, especially in deplete cultures (*Phaeocystis* sp. on day 2 and *Chlorella* sp. on day 4), whereas *Pleurochrysis* was consuming NH_4-N more gradually (Fig. 1d-f). For *Chlorella*, NO_3-N (data not shown) was available until day 7 in deplete cultures, and until day 16 in replete cultures. NO_3-N remained available in *Phaeocystis* replete cultures for the whole incubation period, whereas in deplete cultures NO_3-N was exhausted on day 11. *Pleurochrysis* consumed NO_3-N after 29 days in all conditions. PO_4-P (data not shown) was exhausted soon in most cultures. Consequently, PO_4-P was spiked on day 22 in those cultures that P had been consumed, namely *Phaeocystis* deplete and *Chlorella* replete and deplete cultures, in order to avoid any impact of P limitation stress on the microalgae cells during the experiment.

4. Discussion/Conclusion

In general, there are several microalgae species which have attracted high scientific interest as model organisms, such as *Chlorella* spp. and *Nannochloropsis* spp. which were used in the preliminary experiments as reference strains for comparison with other less known species, such as *Pleurochrysis*. In the preliminary experiments under heterotrophic and mixotrophic conditions, there was an overall poor growth of *Chlorella* and *Nannochloropsis* under different concentrations of VFAs as a carbon source, whereas *Phaeocystis* growth was totally inhibited. *Chlorella* and *Nannochloropsis* have been studied before under heterotrophic or mixotrophic conditions and their survival and growth is considered very strain specific (Chiu *et al.*, 2015).

Table 1. Survival time and growth for *Chlorella* sp., *Phaeocystis* sp., *Nannochloropsis* sp. and *Pleurochrysis* sp. under autotrophic vs. heterotrophic and mixotrophic conditions, with Volatile Fatty Acids (VFAs) as a carbon source.

		Carbon and nutrient substrate	Survival time (days)	Growth
***Chlorella* sp.**	Autotrophy	Walne medium	39	Normal growth
	Mixotrophy	2 g/L VFAs plus f/2	7	Mildly inhibited growth
	Mixotrophy	1 g/L VFAs plus f/2	28	Mildly inhibited growth
	Mixotrophy	0.5 g/L VFAs plus f/2	28	Mildly inhibited growth
	Heterotrophy	1 g/L VFAs plus f/2	<29	Totally inhibited growth
***Phaeocystis* sp.**	Autotrophy	Walne medium	39	Normal growth
	Mixotrophy	2 g/L VFAs plus f/2	<7	Totally inhibited growth
	Mixotrophy	1 g/L VFAs plus f/2	<7	Totally inhibited growth
	Mixotrophy	0.5 g/L VFAs plus f/2	<7	Totally inhibited growth
	Heterotrophy	-	-	-
***Nannochloropsis* sp.**	Autotrophy	-	-	-
	Heterotrophy	1 g/L VFAs plus f/2	29	Mildly inhibited growth, heavy contamination (cyanobacteria) on day 7
	Mixotrophy	1 g/L VFAs plus f/2	<28	Heavy contamination (cyanobacteria) on day 13
	Mixotrophy	0.5 g/L VFAs plus f/2	<28	Heavy contamination (cyanobacteria) on day 13
***Pleurochrysis* sp.**	Autotrophy	Walne medium	39	Normal growth
	Heterotrophy	-	-	-
	Mixotrophy	-	-	-

In our experiments under autotrophic conditions, *Chlorella* sp., *Pleurochrysis* sp. and *Phaeocystis* sp. all increased their average dry biomass significantly in both replete and deplete conditions, with *Chlorella* sp. being the most successful biomass producer. Day 32 appeared to be a turning point in increasing average biomass. However, this observation was not strain-specific as was cohesive in all three strains. Strains' rapid consumption of NH_4-N states that NH_4-N was quite preferable, whereas some NO_3-N remain unutilized in some treatments till the end of incubation. *Phaeocystis* sp. and *Chlorella* sp. remarkably consumed NH_4-N since day 2 and 4 respectively in N-deplete cultures. *Chlorella* sp. is considered a faster N consumer than other species (Varkitzi *et al.*, 2017a), while in the present study *Pleurochrysis* was found to be the slowest N consumer. Many microalgae species have been found to prefer NH_4-N over other N substrates in cultures and natural populations (Varkitzi *et al.*, 2010; Fan *et al.*, 2003).

Some *Chlorella* and *Phaeocystis* deplete cultures consumed PO_4-P soon and they were spiked with PO_4-P during the incubation in order to avoid any impact of P limitation on the microalgae cells. pH was regularly monitored in the experimental cultures in order to ensure that the cultures were not deprived of CO_2 while their biomass was increasing, as it has been suggested in other studies (Varkitzi *et al.*, 2010; 2017a, b). A pH increase in culture incubation water is an indication of high CO_2 consumption by increasing algal biomass in laboratory cultures or natural algal blooms.

Our results demonstrate that there were some clear differences in preference and ability to consume nutrients by all the strains tested, opposing to biomass growth which was ubiquitous for all the different strains. The tested strains did not show any prominent preference for heterotrophic or mixotrophic growth conditions, with some survival of *Chlorella*, however. It is our expectation that these findings could contribute to the vivid research field on the production of metabolites and other added-value compounds from microalgae, such as food additives, pharmaceuticals, biofuels etc.

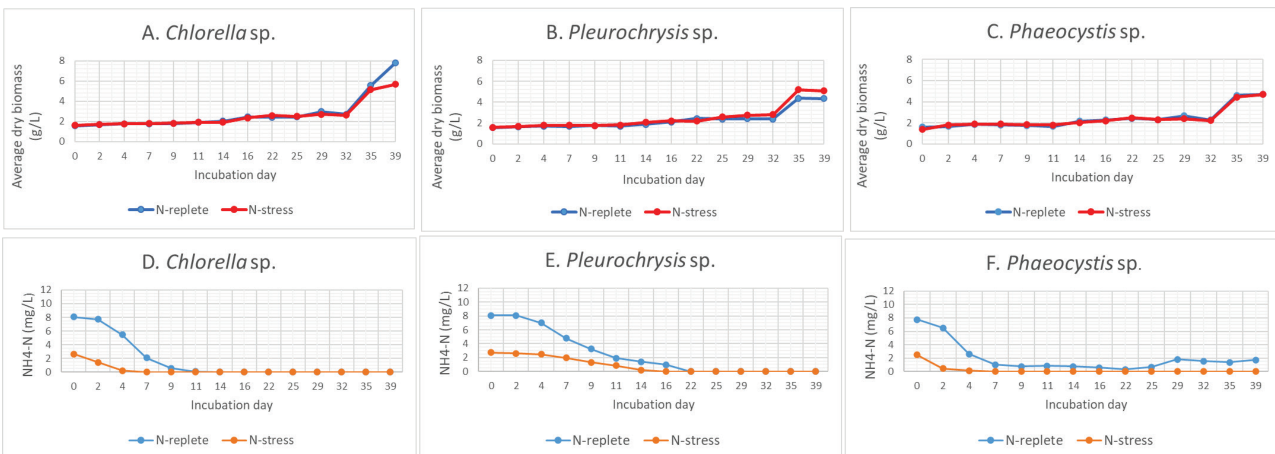

Fig. 1: Average dry biomass production (g/L in a, b, c) and temporal change of NH_4-N concentrations (d, e, f) in *Chlorella* sp., *Pleurochrysis* sp. and *Phaeocystis* sp. experimental cultures under autotrophic conditions, subjected to nitrogen-replete and nitrogen-stress conditions during an incubation period of 39 days.

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PRELIMINARY RESULTS OF TETRODOTOXIN THERMAL DEACTIVATION

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Abstract

The genus *Lagocephalus*, a member of the *Tetraodontidae* family, was introduced in the Mediterranean and the Aegean Sea in 2003 and since then has become successfully established. *Lagocephalus* sp. is highly adaptive and presents a rapid growth rate while it lacks predators resulting in a significant increase in its population. Their settlement in the area is causing considerable ecological disruption as well as economic losses. Tetrodotoxin (TTX), a strong neurotoxin, is detected in all *Lagocephalus* tissues and has been shown to cause multiple health issues or even be fatal when consumed. All the above factors in combination with its excessive fishing as bycatch have created the imperative need to research and plan techniques so that *Lagocephalus* can be rendered into an exploitable fish species. The current study scrutinizes new methods to inactivate the TTX so that the large quantities of bycatch can be used for fishmeal production and thus acquire a market value. Specifically, thermal treatments were performed for TTX, and TTX inactivation was analysed by ELISA TTX kit. So far, dry heating in high temperatures (160-210°C) has shown encouraging results in deactivating TTX.

Keywords: *Lagocephalus* sp., neurotoxin, fishmeal, inactivation, ELISA.

1. Introduction

In recent years, a massive invasion of non-native marine species has been observed in the Mediterranean and the Greek seas. In particular, among 17,000 existent species in the Mediterranean, more than 1,000 are non-native species, of which more than 214 have been recorded in Greece (Zenetos *et al.*, 2018). A large number of those non-native species are considered invasive and cause significant ecological problems, such as the displacement of native species, genetic drift, structural change of native communities, food web alterations and others. In addition, their presence damage local economies, as they affect fishery and tourism. The E.U. has estimated that the financial footprint of non-native species is approximately 12 billion euros per year.

A characteristic example of a Lessepsian migrant is the genus *Lagocephalus*, firstly reported in the Mediterranean Sea, as well as in the Aegean Sea, in 2003 (Akyol *et al.*, 2005; Kasapides *et al.*, 2007). *Lagocephalus* belongs to the family of *Tetraodontidae*. The name *Tetraodontidae* is associated with the characteristic denture of family's species, in which the teeth of each fish jaw are concave, but separated by a central suture, resulting in the appearance of 4 teeth. In the Greek Seas, 4 species have been identified, *L. lagocephalus*, *L. sceleratus*, *L. suezensis* and *L. guentheri* with *L.sceleratus* being the most commonly fished. The increase of *Lagocephalus* population in the Mediterranean is probably due to its rapid growth rate, the fact that it matures only at the age of two years and shows great adaptability, both in its eating habits and sea temperature. Furthermore, *Lagocephalus* sp. is not a target for inshore fishing, due to its toxicity and related marketing restrictions (Masuda *et al.*, 1984; Katikou *et al.*, 2009). It is very likely that *Lagocephelus* sp. will not compete or be hunted by other species. All the above factors, as well as the fact that it can be caught by almost every fishing gear, lead to its excessive fishing as a bycatch (Nader *et al.*, 2012).

A potent exogenous neurotoxin, TTX, is found in the *Lagocephalus* sp. tissues. TTX is produced by marine bacteria, such as *Vibrio alfinolyticus*. The concentration of this neurotoxin, depending on the development stage of the fish, increases gradually before the breeding season, mainly during summer, and decreases sharply at the end of the breeding season. Gonads, especially in female species, display the highest toxicity compared to other organs (Hassoun *et al.*, 2022). Some human case reports relate serious effects at a dose of 0.2 mg, corresponding to 4 μg/kg body weight (EFSA, 2017).

Over the last decade, the European Union countries have made the reduction of bycatches a priority. In particular, the European Commission, in the context of the new Common Fisheries Policy (EU Reg. No 1380/2013), has decided to impose the mandatory landing of unwanted bycatches. Discarded catches are used as food in aquaculture for the production of the fundamental ingredients of fish food, fish meal and fish oils.

Fishery products consist one of the most important sources of animal protein and the aquaculture's contribution to meeting animal protein global demand is becoming increasingly important. Mediterranean aquaculture is one of the most efficient sectors of high nutritional value protein production and serves a catalytic role in the attempt to meet the growing food demand.

Lagocephalus sp. has already adapted to the Mediterranean habits and is causing both long-term damage, by disrupting the ecology of its living areas, and short-term damage, by destroying fishing gear and especially those of small scale coastal fishery. Therefore, it is imperative to find ways to exploit this new fish species, which will augment the revenue of the fishery sector as well as the aquaculture sector.

Guided by the sustainable management of the aquatic biological resources and the protection of the environment, the aim of the present study is the use of *Lagocephalus* sp. for fish meal production, the fundamental ingredient of fish food. Towards this aim, the deactivation of TTX from fish tissues is essential.

2. Material and Methods

In total 39 individuals of *Lagocephalus sceleratus* were collected from the Aegean Sea during the spring and summer periods. They were transferred fresh, somatometric analysis took place, and subsequently, they were grounded. Also, their sex was determined macroscopically by visual inspection of gonads. Fishmeal was obtained after cooking, press drying of fresh grounded fish and milling, successively. In the stage of press drying, most of the water and some or all of the oil were removed (stick water, SW). The SW was collected and frozen at -20°C until analysis. After press drying, the wet meal (WM) was dried overnight in air ovens and the dry meal was obtained (DM). Lastly, fishmeal (FM) was treated at different temperatures (160°C and 210°C) and exposure times (10- 20- 40 min). TTX heat inactivation was analyzed by TTX ELISA (EuroProxima, 5191TTX). The TTX indirect competitive ELISA is an immunoassay for the quantitative and sensitive detection of TTX in fish samples (LOD: 7 ng/g). This test is specific for TTX and does not show cross-reactivity with the other marine biotoxins. The statistical analysis was done by the GraphPad Prism v. 8.4.2. software.

3. Results

Their total length (TL) ranged from 21.5 to 72 cm and their total weight from 100 to 4300 g (Fig. 1). Sex was determined in 39 *L. sceleratus* (19 males and 13 females). Males outnumbered females and the sex ratio was estimated at 1.46 (male: female).

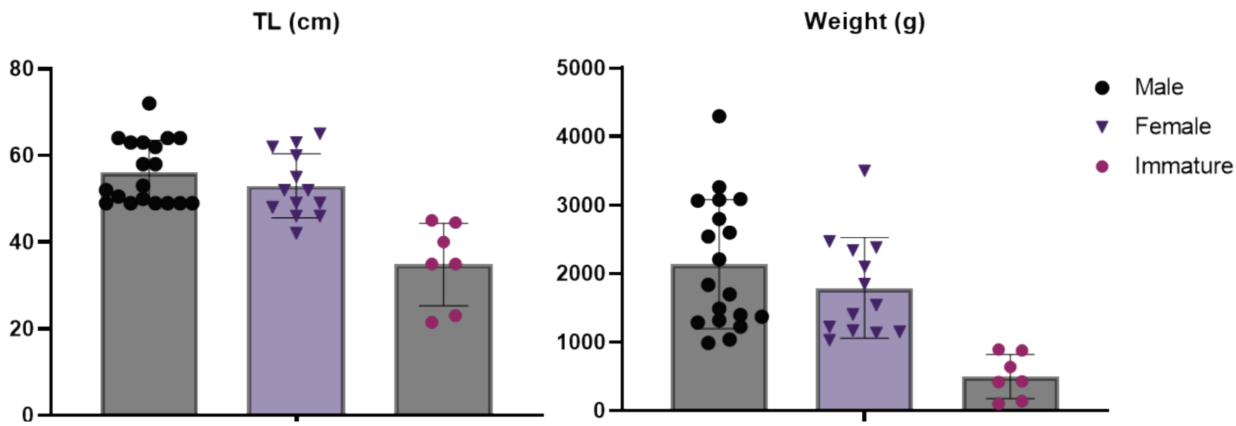

Fig. 1: Somatometry of 39 specimens sampled in the eastern Mediterranean Sea during the period 2021-2022.

The results showed (Fig. 2) that 210°C for 10 min exhibited the optimal capability for TTX inactivation, followed by 160°C for 40 min and 160°C for 20 min. In detail, TTX concentration at 210°C (10 min) was under the limit of detection and decreased from 3.4 μg/g to 0.5 μg/g by a 20-min thermal treatment and to 0.2 μg/g by a 40-min thermal treatment at 160°C, respectively.

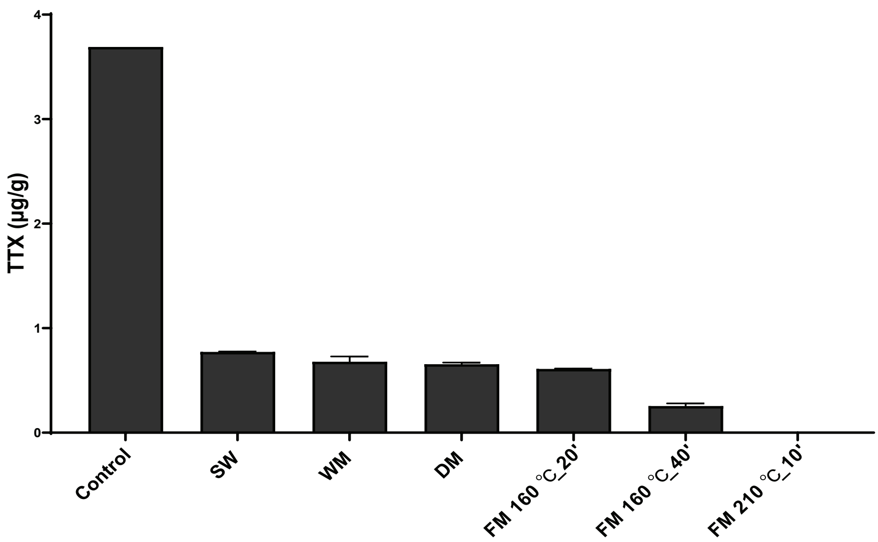

Fig. 2: Inactivation of TTX (μg/g) by time exposure to various temperatures of dry heat. SW: Stick water, WM: Wet meal, DM: Dry meal, FM: Fish meal.

4. Discussion/Conclusion

TTX and its analogues belong to a group of neurotoxins that are produced by various marine bacteria. On the cellular level TTX causes blockage of voltage-gated sodium channels that leads to alteration of neuronal functions and muscle paralysis (EFSA, 2017). Due to the worldwide increase in water temperature TTX has appeared also in the European waters. As for now there are no maximum limits for TTX in the European Union. According to the recent European Food Safety Authority Scientific Opinion the concentration of TTX and its analogues of 44 μg/kg of shellfish meat should not result in adverse effects in humans. TTX is a powerful neurotoxin, which is tolerance to salt, heat and cooking. According to literature TTX activity does not decrease after its exposure to steam autoclaving, after its 10 min exposure to 93.3°C (dry heat) and through freeze thaw (BMBL, 2009). In contrast, there are some reports that suggest that this toxin is inactivated after exposure to a 2.5% sodium hypochlotite (NaOCl) solution with or without sodium hydroxide's (NaOH) presence for 30 min, by using dry heating at 260°C for 10 min and by multi-gas plasma jet technology application (Takamatsu *et al.*, 2014). In addition, partial inactivation is observed by using hydrogen peroxide (H_2O_2), but long exposure (16 hours) and the presence of ultraviolet radiation are required. The effects of ozone and gamma radiation remain unknown (BMBL, 2009).

The aim of this study is to develop a protocol that will ensure the deactivation of TTX from all the tissues of fish of the genus *Lagocephalus* and at the same time, the high nutritional value of fishmeal in fish feed for the Mediterranean farmed fish species. Conclusively, our preliminary results showed that thermal treatment above 160°C is a very promising method for TTX inactivation, while it is easy to apply in practice allowing the fishmeal and fish oil industry to produce an innovative and cost-effective raw material.

5. Acknowledgements

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STRANDING EVENTS PROVIDING A FIRST INSIGHT ON THE INCIDENTAL CATCH OF VULNERABLE SPECIES BY THE SMALL-SCALE FISHERY

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Abstract

Stranding events of marine mammals and sea turtles with signs of gear entanglement can provide useful information on the incidental catch rate of vulnerable species in the Greek Seas. Here, stranding data on sea turtles and marine mammals (cetaceans and monk seals) was analyzed in relation to the presence of fishing gears, classified as longlines or nets as obtained from port authorities' reports during the period 2010-2021. The Kernel Density analysis was subsequently applied for sea turtles and marine mammals, separately for longlines and nets aiming to identify sites with high frequency of reported evidence of entanglement. Analysis showed that sea turtles are much more susceptible to incidental catch compared to marine mammals, independent of the fishing gear. Longlines were the fishing gear presenting the higher number of sea turtle entanglements, with the loggerhead turtle (*Caretta caretta*) being the main species caught. The number of entangled stranded Mediterranean monk seals (*Monachus monachus*), bottlenose dolphins (*Tursiops truncatus*) and striped dolphins (*Stenella coeruleoalba*) was generally low. Kernel density maps highlighted the occurrence of sites with recurrent entanglement for nets and longlines. This phenomenon should be further explored in terms of local conditions, such as local fisheries and current species population dynamics, to extract useful inferences with regards to mitigation measures in Greece.

Keywords: incidental catch, vulnerable species, stranding events, small-scale fishery, Greek waters.

1. Introduction

The incidental catch of vulnerable species (i.e., marine mammals, marine reptiles, and seabirds) is considered one of the main threats to marine biodiversity. As a result, the issue of incidental catch attracts high attention of most regional fisheries management organizations. Effective reporting and monitoring of vulnerable species incidental catches, allow scientists and managers to obtain a more thorough overview of the impacts of the interaction of fisheries and vulnerable species, and on this basis, to set priority areas for management actions. Even though incidental catch is often referred as one of the main threats for marine mammals and sea turtles in the Mediterranean Sea (Carpentieri *et al.*, 2021; Casale & Margaritoulis, 2010; Karamanlidis *et al.*, 2008), large knowledge gaps still exist on the actual estimates per species and especially, in the eastern part of the basin (Carpenteri *et al.*, 2021). As animals are generally either released alive (with unknown post-release survival) or discarded dead by fishers at sea, control and surveillance at landing sites become ineffective in recording incidental catches. This type of information in the Greek Seas is largely lacking. Stranding events datasets can be one of the primary sources of evidence of how anthropogenic activities may affect marine megafauna (Crosti *et al.*, 2017). Spatial analysis of stranding events can be a useful tool for identifying correlations between stranding patterns and human influences such as fishing effort (Crosti *et al.*, 2017). In an effort to estimate the magnitude of incidental catch in Greek waters, here we analyzed stranding events of marine mammals and sea turtles with signs of gear entanglement for a total period of twelve years (2010-2021).

2. Material and Methods

Stranding data on sea turtles, cetaceans and monk seals as recorded by port authorities reports (photos and filled protocol addressed to the National Network for Monitoring Stranding Events and compiled by the Hellenic Center of Marine Research) during the period 2010-2021 were analyzed in relation to the apparent evidence of fishing gear entanglement (remnants of the fishing gear were apparent in photos or reported), classified as longlines or nets. All stranding records were georeferenced based on toponym information and a GIS .shp file (ESRI, 2015) was produced. Subsequently, the Kernel Density analysis tool in GIS (v.10.4, ESRI 2015) was applied for sea turtles and marine mammals, separately for longlines and nets aiming to identify sites with high frequency of reported evidence of entanglement. The Kernel Density analysis tool calculates the density of features in a neighborhood around those features, based on the quartic Kernel function described in Silverman (1986).

3. Results

Fishing gear entanglement was observed in 368 stranding events for loggerhead turtle *Caretta caretta* (193 longlines, 141 nets, 10 longlines and nets, 1 bottom trawl, respectively) and 23 for green turtle *Chelonia mydas* (6 longlines, 17 nets) from 2010 to 2021 (Fig. 1). This means 16.1 (± 6.63) records per year on average loggerhead turtles entangled in longlines and 11.8 (± 4.83) records per year entangled in nets. For the green turtle, this equals 1.20 (± 0.45) records on average entanglements in longlines and 2.13 (±1.25) records entanglement in nets. All green turtles stranding events were referring to sub-adults (<80 cm straight carapace length). The spatial distribution of entangled sea turtle strandings underlined the presence of a single recurrent area in Zakynthos Island for longlines and several areas for nets (i.e., Zakynthos Island, Amvrakikos gulf, Saronikos gulf, Thermaikos gulf, Fig. 2).

Results on marine mammals showed that 53 records of entangled animals were encountered in the same period. Specifically, 46 dolphins [23 bottlenose dolphins (*Tursiops truncatus*) or 2.09 events per year on average, 15 striped dolphins (*Stenella coeruleoalba*) or 1.36 events per year on average, 3 common dolphins (*Delphinus delphis*), 1 harbour porpoise (*Phocoena phocoena*), 4 unrecognized dolphins], and 7 Mediterranean monk seals *(Monachus monachus)* or 0.63 per year on average, were encountered from 2010 to 2021 (Fig. 1). Spatial analysis showed recurrent sites of net entanglement mainly in the north part of Aegean Sea, such as Alexandroupoli, Kavala gulf in Thracian Sea, the west part of Thermaikos gulf and the north part of North Evoikos gulf. Additional areas were identified regarding longlines entanglement in the Ionian Sea (i.e., Zakynthos, Leukas, and Corfu islands; Fig. 2) and in the eastern Aegean Sea (i.e., Chios, Ikaria, and Kos islands; Fig. 2).

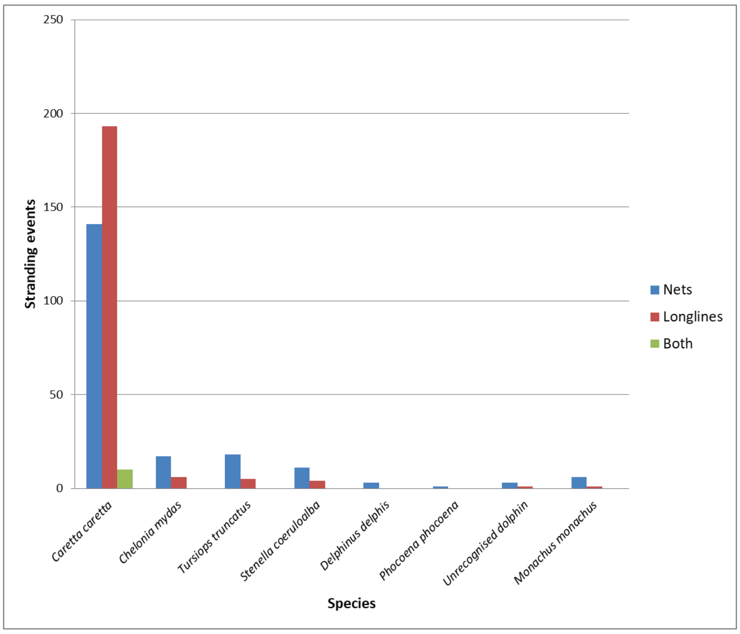

Fig. 1: Stranding events per species showing fishing gear entanglement.

4. Discussion

Depredation on fishing gears is known for both marine mammals (Jog *et al.*, 2022) and sea turtles (Casale & Margaritoulis, 2010). It is inevitably associated to gear damage and catch loss and often results to incidental catches or detrimental injuries and stranded animals (Carpentieri *et al.,* 2021). However, obtaining solid estimates on the incidental catch of vulnerable species is often difficult, since the available information is subjected to a number of limitations such as the lack of extensive onboard observer programs, difficulties in species identification, inadequate spatial and temporal coverage. Stranding records might provide an indication on incidental catch, especially regarding marine mammals and sea turtles. A first attempt to examine stranding records in the Greek seas was made by Kapiris *et al.* (2015) for 2010-2013. Here, stranding records from 2010 to 2021 showed that marine turtles are much more susceptible to incidental catch compared to marine mammals (368 vs 53 records). Although Carpentieri *et al.* (2021) mention set nets, and especially trammel nets, as being responsible for the highest direct mortality rate of sea turtles, here longlines was the fishing gear presenting the highest number of sea turtle entanglements, with the main species caught being predominantly the loggerhead turtle, especially in the Ionian Sea, an area with key foraging grounds and nesting sites of the species (Casale & Margaritoulis, 2010). Still the number of entangled sea turtles in the stranding incidents is well below the 2,500 sea turtle entanglement records on annual basis in set nets at Greek waters (Carpentieri *et al.,* 2021 and references therein) but not far from the 98 animals mentioned as incidental catch by set longlines in 2008 in the Aegean Sea (Carpentieri *et al.*, 2021 and references therein). Especially regarding longlines, the strandings information might reflect more the direct mortality caused by the gear, which is generally low, rather than the actual entanglement rate, as the hooked loggerhead turtles have the capacity and strength to rise to the surface, even when entangled, and breathe (Carpentieri *et al.*, 2021). In addition, delayed mortality due to injuries and necrosis after entanglement and/or release cannot be ignored although largely unknown. Spatial analysis underlined the importance of certain areas as recurrent sites of sea turtle entanglement. Zakynthos Island was the single area highlighted for longlines, whereas for nets the recurrent areas were Zakynthos Island and Amvrakikos gulf in the Ionian Sea, and Saronikos, Thermaikos and Lakonikos gulfs in the Aegean Sea. Taking into account the number of stranding events along with the presence of recurrent sites of entanglement, further investigation should be done to explore the potential of applying mitigation measures focusing on such specific areas.

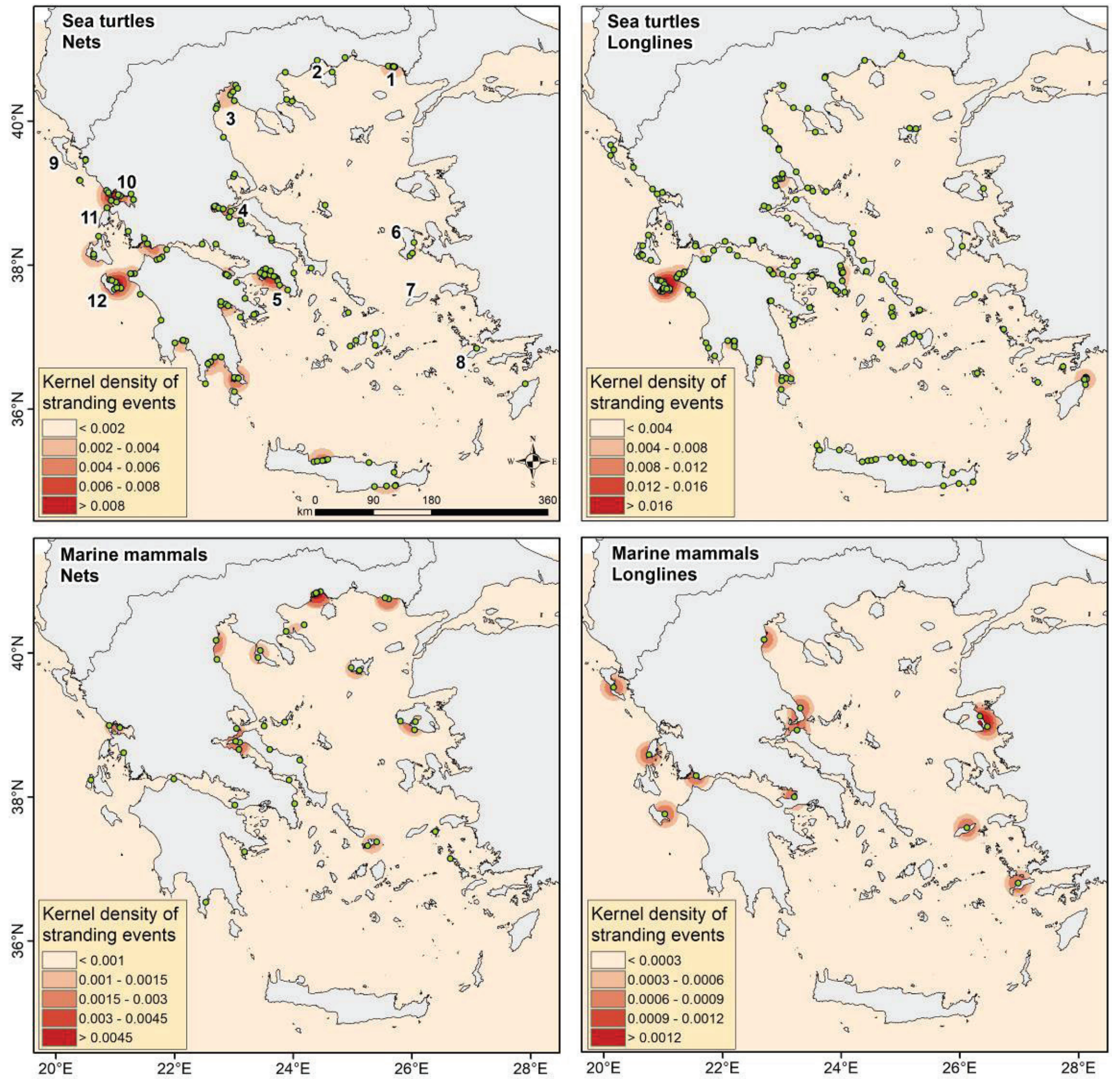

Fig. 2: Density maps based on stranding events with fishing gear entanglement (nets and longlines) for the period 2010-2021 in Greek seas for (a) sea turtles and (b) marine mammals. 1 Alexandroupoli; 2 Kavala gulf; 3 Thermaikos gulf; 4 North Evoikos gulf; 5 Saronikos gulf; 6 Chios Island; 7 Ikaria Island; 8 Kos Island; 9 Corfu Island; 10 Amvrakikos gulf; 11 Leukas Island; 12 Zakynthos Island. Green dots represent the positions of the recorded stranding events.

Though no published data based on fishery surveys exist in the eastern Mediterranean, incidental captures of cetacean in bottom-set nets have been reported (Carpentieri *et al.*, 2021). Although our results most probably underestimate the actual situation at sea either due to non-reported strandings, long decay or lack of vet report and stomach content analysis, they support the hypothesis that cetaceans and monk seals incidental catch is likely to be very low in Greek waters, and concern only sporadically medium and small-sized cetacean species, such as the bottlenose dolphins and the striped dolphins and or young/immature seals. Here, a small number of stranded bottlenose dolphins (less than 2 events per year on average) was found with signs of net entanglement, which can be related to the species common behavior of gillnet depredation (Frantzis & Alexiadou, 2003). Bottom-set gillnets and trammel nets targeting several demersal species, such as stripped red mullet *Mullus surmuletus*, and common cuttlefish *Sepia officinalis*, are among the fishing gears responsible for the most interactions with marine mammals (Carpentieri *et al.*, 2021). Striped dolphin stranding events in both nets and longlines entanglement were found at a low rate (less than 1.5 events per year on average). As striped dolphins are known to prefer open waters beyond the continental shelf, entanglement events observed could be accidental (Frantzis & Alexiadou, 2003). Seven stranded Mediterranean monk seals exhibiting signs of gear entanglement were also recorded within the study period. This number is close to the number mentioned by Karamanlidis *et al.* (2008) who have reported the accidental entanglement of 7 monk seals (mainly subadults) in the Greek Seas from 1991 to 2007. Spatial analysis showed high frequency sites of net entanglement mainly in Alexandroupolis and Kavala gulf in the Thracian Sea, and the west part of Thermaikos gulf and the north part of North Evoikos gulf in the Aegean Sea. Additional areas were identified regarding longlines in Zakynthos Island, Leukas and Corfu islands in the Ionian Sea, and Chios, Ikaria and Kos islands in the eastern Aegean Sea. Thus, despite the low rate of entanglement estimated, based on stranding events for marine mammals, results might imply the need to further investigate the local conditions in certain areas, such as fishing effort and species population dynamics and encourage the local application of mitigation measures.

5. Acknowledgements

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PRELIMINARY RESULTS ON THE REPRODUCTIVE BIOLOGY OF THE ALIEN RED SEA GOATFISH (*PARUPENEUS FORSSKALI*) IN THE HELLENIC AEGEAN SEA

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Abstract

The Red Sea goatfish is an alien species for the Mediterranean Sea introduced most likely through the Suez Canal. It was first reported in 2000 in the southern coasts of Turkey. Although there are some published reports regarding the species biology, data on its reproductive biology are rather limited. In this preliminary work, we provide information from the analysis of 116 Red Sea goatfish individuals caught in the southeastern Aegean Sea, on the gonadal maturity of the species along with data on the length and age structure of its population in the study area. Using non-linear models to estimate length and age at maturity, we show that 42% of the studied population was immature. Further research is required on this economically important alien species towards the effective management of its stock and the conservation of Mediterranean native biota.

Keywords: Biological invasions, fisheries biology, *Parupeneus forsskali,* length-age at maturity, Mediterranean Sea.

1. Introduction

The Red Sea goatfish *Parupeneus forsskali* (Fourmanoir & Guézé, 1976) belongs to the family Mullidae and has entered into the Mediterranean Sea most likely through the Suez Canal (Bariche *et al.,* 2013). The first occurrence of the species was recorded in 2000 in the southern Turkish coasts in Mersin, near Adana (Cinar *et al.,* 2006). Since then, the species has successively expanded its distribution within the eastern Mediterranean in other coasts of Turkey, Lebanon, Israel, Cyprus, Syria, Egypt, Tunisia and lastly in Greece (Evagelopoulos *et al.,* 2020 and references therein). The species is caught by the small-scale fisheries and is considered as economically important due to its high market value, in both its native and invaded range (Sabrah, 2015; Evagelopoulos *et al.,* 2020). Hence, there is a need for administrative and research initiatives towards the monitoring of its population and the investigation of the species biological features. However, there are limited data on the species biology in the literature, especially regarding its reproduction (Turan *et al.*, 2021).

The aim of this work was to provide preliminary information on the species gonadal maturity along with data on population size and age structures. The results of this study will contribute to the understanding of the species invasive character, as well as to the design of sustainable exploitation and management strategies.

2. Material and Methods

2.1 Sampling and study area

In total, 116 Red Sea goatfish were caught in coastal waters of Rhodes and Karpathos in the southeastern (SE) Aegean Sea (GSA 22). Collection involved the use of static trammel nets (mess sizes 32 to 160 mm) deployed from commercial small-scale fishing vessels following a seasonal sampling schedule, from September 2019 to January 2021. Individuals were placed in ice on board the vessel and then transferred to the laboratory for further analyses. The sampling depth ranged between 20 and 30 m while the type of substrate was classified as rocky, sandy while the majority of the individuals were collected from *Posidonia oceanica* meadows.

2.2 Laboratory and Data Analyses

After thawing, each individual was measured for total length (TL) and weighed (body weight, W) to the nearest 0.1 cm and 0.1 g, respectively. The age (years) was estimated from otolith readings (sagittae). Gonads of both sexes were weighed (W_g) to the nearest 0.001 g and the reproductive stage was determined according to the six–stage scale (I-VI) of Nikolskii (1963). Goatfish with gonads at the reproductive stage IV to VI were considered as mature individuals (Tsikliras & Antonopoulou, 2006). Additionally, the gonadosomatic index (GSI) was calculated, expressed as the % ratio between gonad weight the total body weight (Jakobsen *et al.*, 2009).

Length (L_m) and age (t_m) at maturity was defined as the length and age at which 50% of the population reaches first maturity (Tsikliras *et al.*, 2013; Tsikliras & Stergiou, 2014). In order to estimate L_m and t_m, the Logistic and Gompertz nonlinear regressions were applied. All analyses were performed using R 4.2.0. software for statistical computing and graphics (R Core Team, 2022).

3. Results

Overall, the TL of the goatfish population ranged from 6 to 26 cm (mean: 17.6; standard error: ± 0.53) while the age ranged from 1 to 4 years (mean: 2.16; standard error: ± 0.1). With respect to sex, 72 males and 44 females were identified. The GSI exhibited a minimum value of 0.019% for both males and females (maturity stage I) while the maximum GSI was 5.62% for females and 1.14% for males at maturity stage V (Figure 1). The GSI increased substantially from immature to mature stages in the examined specimens.

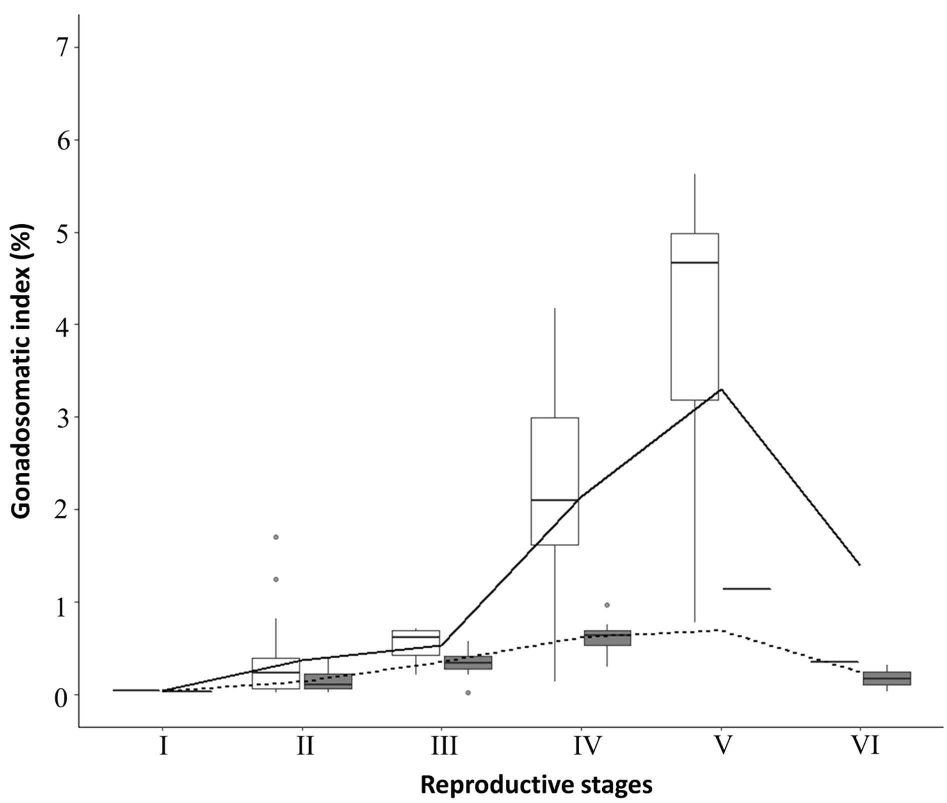

Fig. 1: Box and whisker plot for the gonadosomatic index (GSI; %) for different maturity stages of the Red Sea goatfish population in the Aegean Sea (male: black; female: white).

Both the Logistic and Gompertz functions had a good fit to the size- or age-specific maturity data ($R^2 \geq 0.96$) with statistically significant model parameters (t–tests; P<0.001). Model statistics revealed that the

Gompertz function behaved slightly better for the Red Sea goatfish in the examined population (Table 1). For pooled-sex data, length at maturity (L_m) was calculated at 19.6 cm and age at maturity (t_m) at 2.8 years (Figure 2). According to the length measurements, 42% of the individuals caught in the SE Aegean had sizes smaller than the estimated length at maturity (L_{50}), i.e., they were immature.

Table 1. Regression statistics for length/age at maturity analysis of the Red Sea goatfish in the Aegean Sea. The selected models are highlighted with bold (R^2: coefficient of determination; RSE: residual standard error; N: sample size; DF: degrees of freedom; AIC/BIC: Akaike and Bayesian Information Criterion).

Explanatory Variable	Model	Parameters			R^2	RSE	AIC	BIC
		a	b	c				
Length (cm)	Logistic	176.04	124.04	0.20	0.96	6.053	745.45	756.45
	Gompertz	**521.20**	**7.17**	**0.05**	**0.96**	**5.97**	**742.60**	**753.58**
Age (years)	Logistic	109.29	34.39	1.18	0.96	6.12	748.13	759.11
	Gompertz	**139.95**	**4.92**	**0.55**	**0.96**	**6.00**	**743.49**	**754.47**

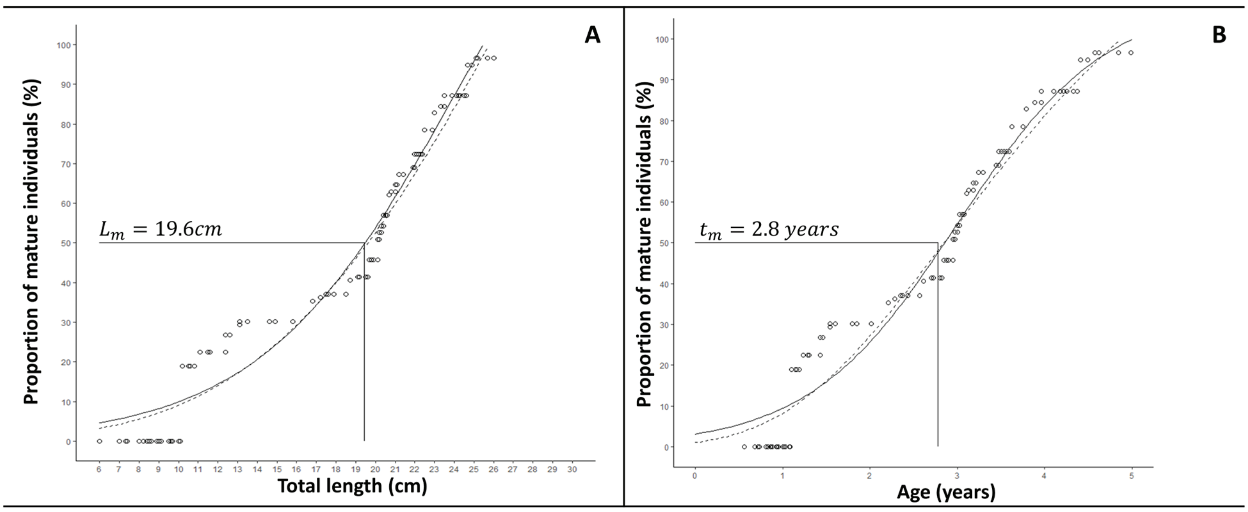

Fig. 2: Scatterplots of % mature individuals against Total length (cm; top) and age (years; bottom) along with the fitted Logistic (solid) and Gompertz (dashed) curves for the Red Sea goatfish population in the Aegean Sea (L_m: size at maturity; t_m: age at maturity).

4. Discussion

The Red Sea goatfish is an Indian Ocean fish species, native to the Gulf of Aden and the Red Sea, which migrated through the Suez Canal and currently is considered as an established Lessepsian species in the eastern Mediterranean Sea (Bariche *et al.*, 2013). Published studies on its reproduction (i.e., gonadal maturity) are limited in the literature. Specifically, in the recent work of Turan *et al.*, (2021) in Iskenderun Bay (SE Turkey), the L_{50} was estimated at 18.9 cm, for pooled sex data, a finding which is in agreement with the present study (L_{50}=19.6 cm). However, Sabrah (2015) found a considerably lower L_{50} in the native region of the Red Sea goatfish (15.38 cm for pooled sexes).

The population length distribution in the present study was similar to those in the studies of Sabrah (2015) and Turan *et al.*, (2021). However, differences in sample sizes, year of collection and region–specific physicochemical regimes do not allow drawing general conclusions regarding the invasive character of the species. It is important to mention that, during the last decade, the Red Sea goatfish has demonstrated a rapid expansion of its invaded range, as well as increased biomass in the eastern Mediterranean (Evagelopoulos *et al.,* 2020). Thus, it can potentially affect negatively the native, or even other introduced species with similar ecological role, due to interspecific competition.

In conclusion, the results of the present study show that 42% of the Red Sea goatfish caught in the SE Aegean with small-scale fishing gears are immature. A number of research initiatives in the Mediterranean have contributed to the elucidation of some basic biological traits of the species, yet many gaps in knowledge remain (e.g., diet and age-growth parameters), representing an impediment to the successful monitoring and effective management of the species.

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TEMPERATURE CURRENT TRENDS FOR ARAXOS AND IERAPETRA COASTAL REGIONS, AS INDICATORS OF FUTURE SEA LEVEL RISE

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Abstract

Sea-level rise -as a result of climate change- influences coasts all around the world. There is scientific consensus on the extent of sea-level rise to 2050 and a range of projections to 2100, which differ depending on the approach used and the carbon-reduction measures followed. The scope of this article is to compare the CMIP6 climate model temperature projections with the observational and reanalysis data for the Greek coastal areas of Araxos and Ierapetra and relate them to current and anticipated changes in sea level. The data for the Greek regions were obtained from the Hellenic National Meteorological Service (HNMS) of Greece and the ECMWF ERA5, for which the annual mean, maximum and minimum land surface air, sea surface and marine air temperature, for the period 1960–2010, were calculated. The results show an increase in air and sea surface temperature, which, given the geographic location of the studied areas, can be related to the probable thermal expansion that is considered as one of the key parameters for sea level rise in the Mediterranean Sea.

Keywords: Greece, coastal, sea level rise, climate change.

1. Introduction

Although climate is influenced by a variety of factors, there is general agreement on the catalytic role of human activities in shaping the climate system over the past 200 years (Hardy, 2003). Anthropogenic climate change lies in the increase of greenhouse gases concentration in the atmosphere, as well as airborne particles (aerosols), and changes in land cover and use.

Significant spatial heterogeneity of the effects of climate change has been widely studied (Thornton *et al.,* 2014). The average global temperature increase differs significantly between land and sea, as well as between high and low latitudes. Increased air temperatures, among other environmental impacts of climate change, affect the water cycle by increasing or changing water temperatures, causing changes in the adjacent ecosystems, water quality, and local weather (Gossiaux *et al.,* 2019). Rising global temperatures is the main contributor to the sea level rise either due to the melting ice or the thermal expansion of water; the latter accounts for roughly half of the observed global sea level rise on Earth, which is accelerating and will continue to do so throughout the twenty-first century and beyond. Reliable projections of future sea temperature increase and sea level change are critical for coastal management planning at the local level (Mimura, 2013; Allison *et al.,* 2022).

The scope of this article is to compare the CMIP6 climate model temperature projections with the observational and reanalysis data for the Greek coastal areas of Araxos and Ierapetra and to relate them to the current expected trends of sea level change.

2. Material and Methods

For the analysis, raw 3-hourly air-temperature (LSAT) measurements (0, 3, 6, 9, 12, 15, 18, 21) from the Hellenic National Meteorological Service (HNMS) stations at ‘Araxos’ and ‘Ierapetra’ between 1969 and 2010 were employed. At the same time, the sea surface temperature (SST) and marine air temperature (MAT) were retrieved from ECMWF ERA5 Global Reanalysis (C3S, 2017). Then, the annual mean, maximum and minimum values of the LSAT, SST and MAT were calculated for further analysis.

Finally, the NASA Sea Level Projection Tool (Garner *et al.*, 2021) was used to determine the expected regional Total Sea Level Rise (SLR) at the examined areas. This tool is based on the CMIP6 climate model median projections of global and regional sea level rise. Herein, we discuss the future projections, relative to a 1995–2014 baseline, under the SSP3-7.0 scenario, according to which emissions and temperatures rise steadily and, by the end of the century, average temperatures will have risen by 3.6°C.

3. Results

Figures 1 and 2 show the time series of the annual mean, maximum and minimum Land Surface Air Temperature (LSAT), Sea Surface Temperature (SST) and Marine Air Temperature (MAT) for Araxos and Ierapetra, respectively, according to the HNMS and ERA5 datasets. The future projections of SLR Rate (mm/yr), and Total SLR (m) for the two examined areas, along with the Mediterranean averages of SST and Total SLR, are shown in Figure 3.

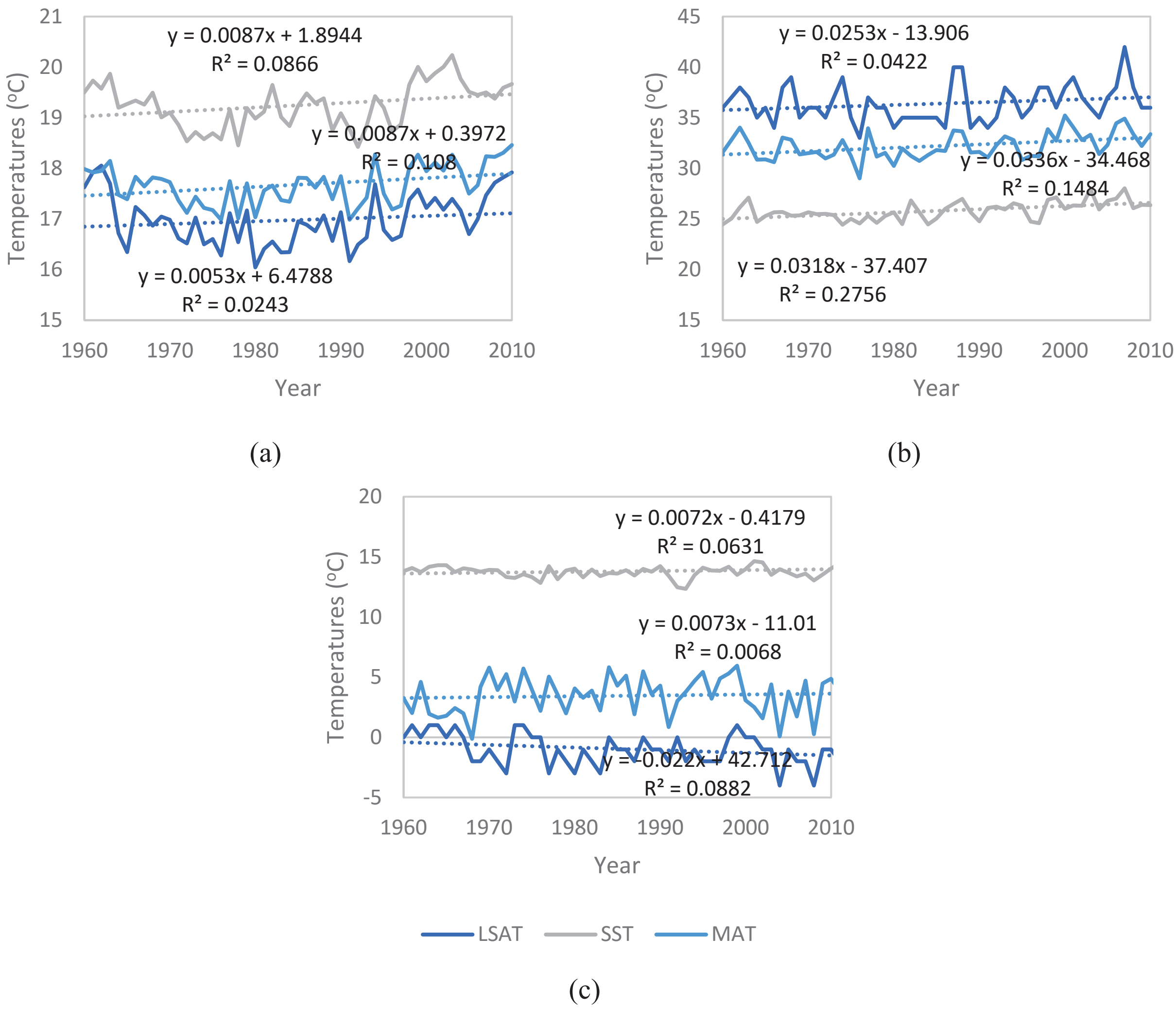
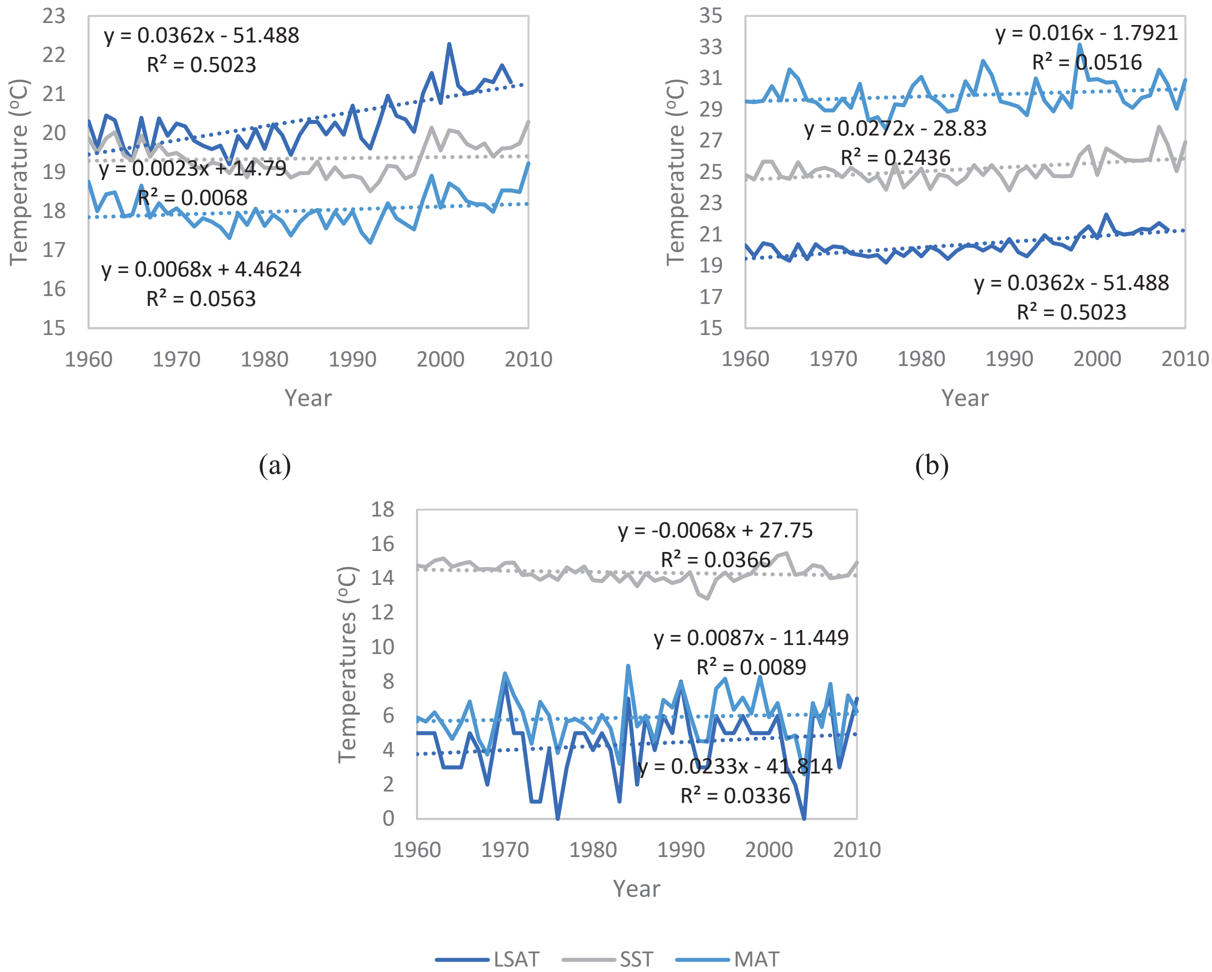


Fig. 1: Annual mean (a), max (b) and min (c) values of LSAT, SST and MAT for the period 1960-2010 for Araxos.



(c)

Fig. 2: Annual mean (a), max (b) and min (c) values of LSAT, SST and MAT for the period 1960-2010 for Ierapetra.

4. Discussion/Conclusion

According to the IPCC 6th Assessment Report (AR6; IPCC, 2021), the global mean surface temperature has increased by 0.85 (0.69 to 0.95) °C from 1850–1900 to 1995–2014, while the last decade is considered the warmest, compared to any multi-centennial period since the Last Interglacial (reference??). At the same time, the AR6 states that over the last century, the global ocean has warmed faster than it has since the end of the last deglacial transition (around 11,000 years ago). The warming of the climate system has already resulted in global mean sea level rise due to ice melt on land and ocean thermal expansion; the later has contributed by 50% to sea level rise between 1971 and 2018, while glacier ice loss, ice sheets loss and changes in land-water storage have contributed by 22%, 20% and 8%, respectively (IPCC, 2021). Based on the SSP3-7.0 scenario for the Mediterranean, the medium term (2041–2060) mean temperature is expected to increase by 1.7°C, while a3.5°C increase is expected by the end of the century. In addition, the NASA Sea Level Projection tool showed an increase in both the Total Regional Sea Level, as well as the corresponding rate.

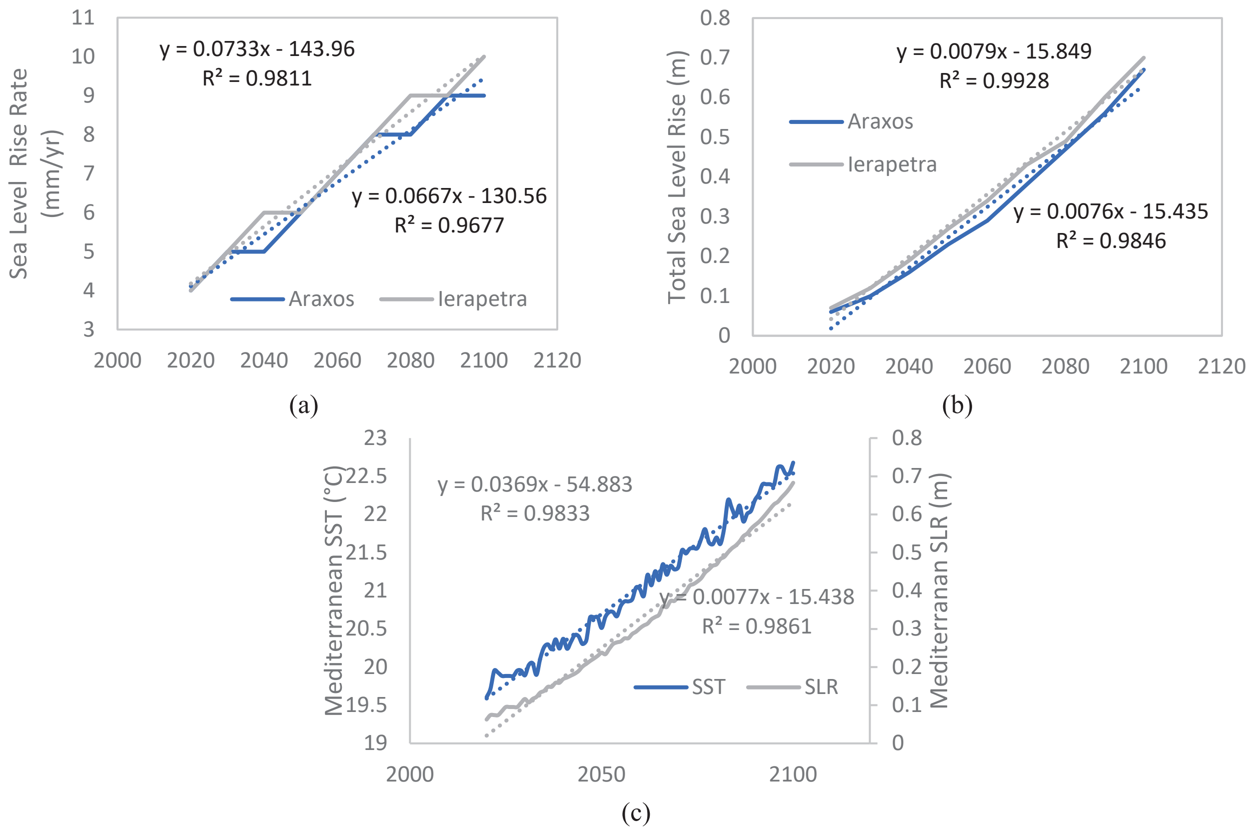


Fig. 3: Median projections from 2020 to the end of the century according to SSP3-7.0 of (a) SLR rate (mm/yr) and (b) total SLR (m), compared to 1995-2014 baseline, for Araxos and Ierapetra (NASA sea level projection tool), and (c) Mediterranean SST (°C) and Total SLR (m) (adapted from the IPCC WGI Interactive Atlas; https://interactive-atlas.ipcc.ch/).

The examination of the HNMS and ERA5 datasets long-term air temperature changes for the current climate, showed a decreasing trend in the annual mean air, sea surface and marine air temperatures between 1960 and 1977 and an increasing trend between 1978 and 2010 at both stations (Araxos and Ierapetra); which is particularly profound after 1990 (Fig.1). Moreover, Ierapetra station showed a weaker decreasing trend between 1960 and 1977 and a stronger warming trend in the annual mean air, sea surface and marine air temperatures between 1978 and 2010 (Fig. 2). The mean and maximum SST timeseries for both locations show a positive trend, which is in accordance with an expectancy of thermal expansion and a consequent sea level rise.

Considering that the studied areas belong to the semi-enclosed Mediterranean Sea, the anticipated rise in sea level (Fig. 3a,b) can be attributed primarily to the ocean's thermal expansion and the exchange of water masses between the Mediterranean and the Atlantic Ocean, rather than ice melting. This is also evident from Fig. 3c, exhibiting the dependence of SLR from the SST. On the other hand, the positive relationship between air temperature, sea surface temperature and sea level rise may interfere with several eustatic parameters (e.g., circulation, wind field) acting within the Mediterranean Sea.

5. Acknowledgements

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MICROPLASTIC INGESTION AND TOXICOLOGICAL BIOMARKERS IN SEA URCHINS AND STRIPED RED MULLETS FROM ZAKYNTHOS ISLAND (IONIAN SEA)

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Abstract

Marine plastic debris is increasing and consequently the microplastic (MP) ingestion by marine organisms. Detection of MPs in marine organisms is ongoing, however, there is lack of consensus regarding the effects of MP ingestion in marine organisms. Our aim is to investigate whether biomarker variations in two Mediterranean species can be related to the occurrence of MPs in their gastro-intestinal (GI) tract. Striped red mullets *Mullus surmuletus* and sea urchins *Paracentrotus lividus* were sampled along the highly touristic east coast of Zakynthos Island and inside the National Marine Park of Zakynthos (NMPZ). Blood and tissue samples were collected from alive *M. surmuletus* and *P. lividus* for biomarker measurements. The organisms' GI tracts were digested and then filtered to detect ingested MPs. Fewer ingested MPs were found in *M. surmuletus* sampled in NMPZ compared to those sampled along the east coast of the island. No differences in MP ingestion were observed between NMPZ and the east island coast for *P. lividus*. Principal Component Analysis showed limited and non-consistent relations of biomarkers with ingested MPs in the two species.

Keywords: ecotoxicology, microplastics, fish, Paracentrotus, Mediterranean Sea.

1. Introduction

The Mediterranean Sea is a semi-enclosed basin that is continuously exposed to high densities of marine debris. The increase of marine plastic debris is leading to increased microplastic (MP) occurrence in the marine environment and therefore increased potential of MP ingestion by marine organisms. Ingestion of MPs can increase the exposure of the organisms to chemical substances that are either among the plastic components (e.g., phthalates and flame retardants) or are adsorbed on the surface of the plastic particles (e.g., polychlorinated biphenyls and polycyclic aromatic hydrocarbons) (Rochman *et al.*, 2014). The ingestion of MPs increases the bioavailability of the associated chemical compounds which can alter the levels of ecotoxicological biomarkers in the organism's tissues (Wang *et al.*, 2019; Iheanacho & Odo, 2020).

The striped red mullet, *Mullus surmuletus*, is a native Mediterranean fish species of high commercial value proposed as bioindicator for MP monitoring in benthic habitats (Bray *et al.*, 2019). Sea urchins are key benthic species of rocky ecosystems both as predators and as prey and they have also high commercial value. Detection of MP impacts on biota in the marine environment is a challenging task and the physical and ecotoxicological effects that can be strictly attributed to MP ingestion in the environment have been addressed in very few studies (Alomar *et al.*, 2017). The aim of the present study was to evaluate the impact of MPs on commercially harvested species (invertebrates and fish) in a Mediterranean environment at coastal sites under different human pressure intensity (inside and outside of a marine protected area) in the Ionian Sea. To achieve this, we investigated the MP ingestion in two marine organisms of the seafloor habitat (the red striped mullet *Mullus surmuletus* and the sea urchin *Paracentrotus lividus*) in combination with a range of ecotoxicological biomarkers commonly used to assess toxicity of xenobiotics and investigated whether any changes in the biomarker levels can be attributed to the presence of MPs in the GI tract of the selected organisms.

2. Material and Methods

2.1 Sampling

The study area included the National Marine Park of Zakynthos (NMPZ), a marine protected area (MPA), and the coastal area of Zakynthos island, eastern Ionian Sea, Greece. Two sampling surveys were carried out in June and September 2019.

Alive samples of *M. surmuletus* were collected by trammel bottom net at one site inside the NMPZ and two sites outside the NMPZ and anaesthetised using clove oil as soon as they were collected on board. A total of 121 fish were sampled. Blood smears were prepared for application of the micronucleus test. Liver and muscle tissue were sampled, frozen in liquid nitrogen and stored at -80 °C. The GI tract was stored at -20 °C for microplastics analysis. Dissection was conducted on aluminum foil covered boards and procedural blanks were also placed near the dissection site.

Sea urchins were sampled by hand at one site within the NMPZ and two sites outside the NMPZ (5 - 6 individuals per site). Gonads were dissected, frozen in liquid nitrogen and stored at -80 °C (5 individuals per site) for biochemical biomarker measurements (GST, CAT), coelomic fluid was sampled and smears (5 individuals per site) were prepared for application of the MN test and the digestive system was stored at –20 °C for microplastics analysis (5 individuals per site).

2.2 Biomarker Analyses

The biochemical biomarkers evaluated included acetylcholinesterase (AChE) activity measured by the method of Ellman *et al.* (1961) adapted to microplate reading by Bocquené *et al.*, (1993), glutathione S transferase (GST) activity measured according to Habig *et al.*, (1974) with some modifications, lipid peroxidation (LPO) measured following the procedure of Ohkawa *et al.*, (1979) and Bird and Draper (1984) with some modifications and catalase (CAT) activity measured by the method of Cohen *et al.*, (1996). The MN test was performed using the method of Galloway *et al.* (2010).

2.3 Microplastic Analyses

Microplastic analyses were performed according to Tsangaris *et al.* (2021). Briefly, microplastic analysis was performed following extraction with 15% H_2O_2 digestion for the GI tract of *M. surmuletus* and with 10% KOH digestion for the *P. lividus* digestive system, stereomicroscope observation and Fourier Transform Infrared (FTIR) analysis performed on an FTIR Agilent Cary 630 spectroscope equipped with a Survey IRTM (Czitek) microspectroscopy accessory. To control airborne contamination, samples of all materials present on board that could potentially contaminate the GI tract samples were obtained and were later analysed by FTIR. Blanks were assessed in all steps of the microplastic analysis (i.e., digestion, filtration and stereoscopic observation).

2.4 Statistical Analyses

Microplastic data are presented as mean ± standard error of the mean. The Mann-Whitney U test was conducted to evaluate differences in the number of ingested MPs per individual between sampling sites ($p<0.05$). Principal component analysis (PCA) was applied to evaluate the influence of MPs and sampling site on the biomarker levels using the R software (R Development Core Team, 2018) and the packages FactoMineR and factoextra.

3. Results

The striped red mullets ingested significantly fewer MPs in the NMPZ than the those sampled along the east coast of the island in June (Mann-Whitney U test, $p= 0.0015$). Similarly, fewer MPs were ingested by red mullets in September at NMPZ, however the difference was not significant (Mann-Whitney U test, p= 0.07). In particular, the striped red mullets sampled in June ingested MPs with an average number of items per individual found at 0.16 ± 0.08 (n = 31) inside the NMPZ and at 0.69 ± 0.1 (n = 62) outside the protected area. In September, the striped red mullets ingested on average 0.27 ± 0.1 (n = 15) MPs per individual inside the NMPZ and 0.71 ± 0.2 (n = 14) MPs per individual outside the protected area. No differences were observed for sea urchins in either of the samplings with 0.4 ± 0.2 (n = 5) MPs per individual inside the NMPZ and 0.4 ± 0.4 (n = 5) MPs per individual outside the protected area in June; and 0.2 MPs ± 0.2, (n = 5) MPs per individual inside the NMPZ and 0.16 ± 0.2, (n = 6) MPs per individual outside the protected area in the September sampling.

PCA was conducted on June and September data separately because differences were observed in the level of biomarkers between the two samplings that can be attributed to natural seasonal variation. For the striped red mullet, (Fig. 1) for both samplings, the relative contribution of ingested MPs to the plotted PC axes is low and only in September parallel vectors of number of ingested MPs and MN indicate these variables are strongly correlated in terms of the displayed PCs. Regarding the sea urchin data, (Fig. 1) the variable vectors plots indicate a positive correlation of GST and a negative correlation of CAT with ingested MPs in terms of the displayed PCs in June and September, respectively. For both species and samplings, the PCA score plots show no differences between the animals sampled in the NMPZ and those sampled along the touristic east coast of the island.

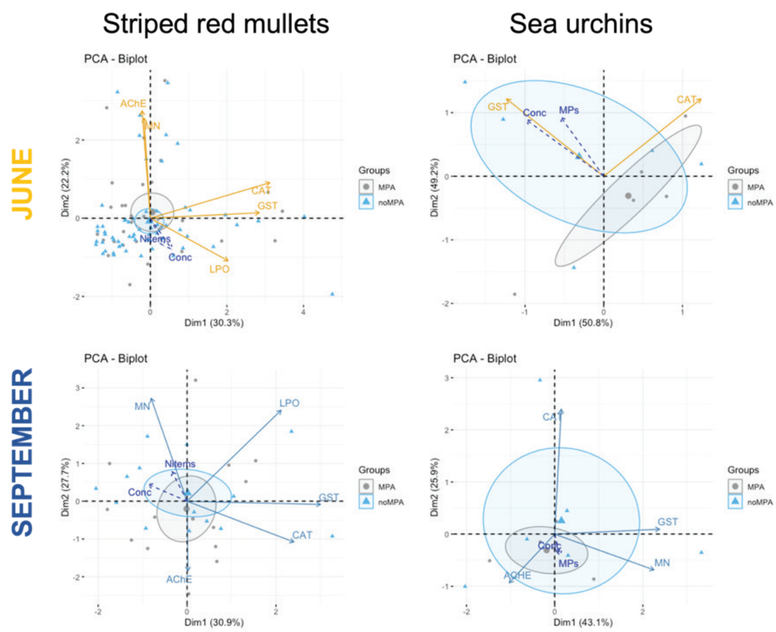

Fig. 1: PCA biplots of the responses of the selected biomarkers (AChE, CAT, GST, LPO and MN) for striped red mullets *Mullus surmuletus* (left) and sea urchins *Paracentrotus lividus* (right) with the ingested MPs of either species in total number of items (Nitems) and in number of items per gram of digested tissue (Conc). The data show the biomarker responses of the organisms sampled in the NMPZ (MPA, grey circles) and along the east coast of Zakynthos island (noMPA, blue triangles).

4. Discussion/Conclusion

Microplastic ingestion in striped red mullets has been reported to reach up to 1.53 MPs per individual in NW Iberian shelf (Filgueiras *et al.*, 2020) and the values observed in the present study lay in the mid and low range of the literature values for this species. Number of ingested microplastics in sea urchins at both sites was found lower than those reported in other studies in eastern Aegean Sea, Greece and in the gulf of Naples, Italy (Hennicke *et al.*, 2021; Murano *et al.*, 2022).

The PCA showed limited and non-consistent relations of biomarkers with ingested MPs in the two species. The MN frequency in *M. surmuletus* showed a positive correlation with the number of MPs in the GI tract, only in September. Genotoxicity induced by exposure to MPs at environmentally relevant concentrations has been reported in aquatic organisms (Sun *et al.*, 2021). Microplastic exposure can also induce antioxidant responses in fish and invertebrates (Wang *et al.*, 2019, Murano *et al.*, 2021). Nevertheless, our results lack of consistency in the two seasonal samplings and thus should be interpreted with caution.

Overall, no differences between sampling areas were observed regarding the toxicological response of the organisms assessed by the applied biomarkers, even though the striped red mullets ingested fewer MPs in the NMPZ compared to the east coast of Zakynthos island. The limited responses of biomarkers to MP ingestion in *P. lividus* and *M. surmulettus* from Zakynthos island, may be attributed to the low numbers of MPs ingested by the animals nad the low number of animals (i.e., 5 individuals of sea urchin per site) analysed in the current study. Further research is needed including sampling areas where higher levels of MP ingestion would be expected to obtain a clear indication of the MP ecotoxicological effects in the marine environment.

5. Acknowledgements

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CHEMICAL TRACE OF AN ANCIENT AQUEDUCT UNDER ATHENS RESILIENCE

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Abstract

In recent years, the need for high quality water mostly for irrigation purposes in the region of Attica has increased significantly. To meet Attica's irrigation demands, an alternative water resource for non-potable water is proposed which its origin dates back to the Roman period (140 AC). The Hadrian's Aqueduct, which after 1900 years continues to be operational with unutilized water, extends below eight municipalities of Athens and its aquifer derives from Parnitha Mountain. This study took place on the whole length of the Hadrian's Aqueduct, from April 2021 up to March 2022 and focused on determining the quantitative and qualitative characteristics of aqueduct water resource, as well as the interactions between the neighboring basins. The annually results provided us with better understandings of the quality and quantity of the non-potable water use along the Hadrian's Aqueduct. Indicatively, the data collected from the 14 water shafts display significant variation in the concentration of Ca^{2+} and NO_3^- throughout the season. The conclusions of this study will feed into designing a system of sustainable, coordinated pumping in 20 wells of Hardian's Aqueduct, contributing to the irrigation water needs of the eight Municipalities along its length.

Keywords: Hadrian aqueduct, groundwater, urban irrigation, chemical analysis.

1. Introduction

Nowadays, about 2 billion people live with less water than the ancient Athenians do, when they used the water supplied from the Hadrian's Aqueduct in the second century A.D. (Sargentis *et al.*, 2014). The Hadrian Aqueduct managed by the Athens Water Supply and Sewerage Company (EYDAP S.A.) is an underground tunnel that spreads out 23.7 km, mostly in lacustrine geological formations (Shawna L., 1998), under eight municipalities of Athens, from 10 – 42m depth (Fig. 2). It consists of four hydraulically independent aqueduct segments and is managed by EYDAP S.A. Its technology was inherited and further developed by Romans, whose Empire flourished after the Hellenic (Angelakis *et al.*, 2014). Specifically, it was constructed in 140 A. D. (Roman period) and it is maintained and utilized from 1870 to 1945. Its aquifer derives from the mountains of Parnitha and the gravitational water flows to the southern regions of Athens.

Aiming to utilize the untapped potential of the aqueduct, it is essential to have knowledge over the current situation, regarding parameters relevant with the water flow and its chemical constitution. Thus, we conducted pumping tests, as well as monthly chemical analysis to supplement them, carried out at multiple shafts along the aqueduct. Those tests have provided us with a significant amount of data regarding the quantity and quality of the water.

2. Material and Methods

2.1 Pumping tests

Seven pumping and water sampling tests performed throughout the year 2021 in seven different shafts (Fig. 2) along the way of Hadrian's Aqueduct. These tests gave us better insights of the water flow, the quality and quantity of the underground water, as well as the hydraulically independent segments and the interactions between them.

1. The pumping tests took place chronologically:
2. On April 14th at shaft #103
3. On April 22th between the shafts #85 and #86
4. On June 30th there were simultaneously three pumping tests at shafts #169, #180A and #192
5. On July 7th at shaft #18A
6. On July 14th between the shafts #123 and #124
7. On July 21st between the shafts #255 and #257
8. On October 6th at shaft #189

The above pumping tests affected the water level of the neighboring shafts, as well. After the pumping, there was immediate response of the aquifer and restoration of the water level inside the aqueduct.

2.2. Monitoring of Hadrian's Aqueduct

The monitoring of all the physicochemical parameters along Hadrian's Aqueduct groundwater focused on seven different basins on both wet (from October 2021 to March 2022) and dry (from May 2021 to September 2021) seasons. Specifically, there was a monthly sampling net in 14 water shafts, of which the name number increases from south to north, along Hadrian's Aqueduct, monitoring nine different physicochemical parameters: Ca^{2+}, NO_3^-, Cl^-, Mn^{2+}, Mg^{2+}, Al^{3+}, electrical conductivity (EC), pH and Turbidity.

3. Results

3.1 Outcome of pumping tests

Among other physicochemical parameters, we selected the electrical conductivity, nitrates, calcium, chloride and pH, as they comprise the main "Chemical Identity" of water. The above terminology assisted us, as we could have distinguished whether there is any inflow or any difference in the quality of the water, between the samples of the measured shafts, along Hadrian's aqueduct, regarding the above chemical parameters. Figure 1 presents the mean deviation of the parameters along the aqueduct.

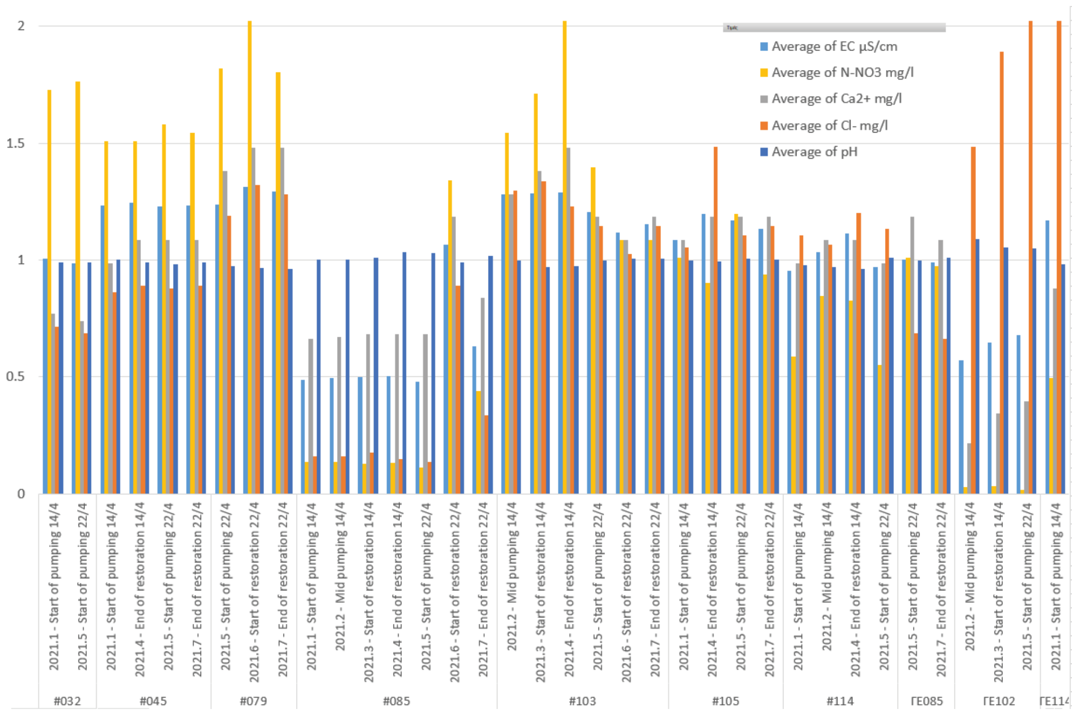

Fig. 1: Diagram with the mean deviation of the parameters that consists the "chemical identity" of water at basins of Cha-

landri region.

The study and the differentiation of the deviation of the chemical parameters on the pivot diagrams gave us better insight of the Hadrian's aqueduct, as well as the interactions between neighboring basins. We concluded that the aqueduct consists of four hydraulically independent segments (Fig. 2), with independent groundwater drainage inflow capacities. There is a fifth segment (#250 - #240) though, that the water level is extremely low.

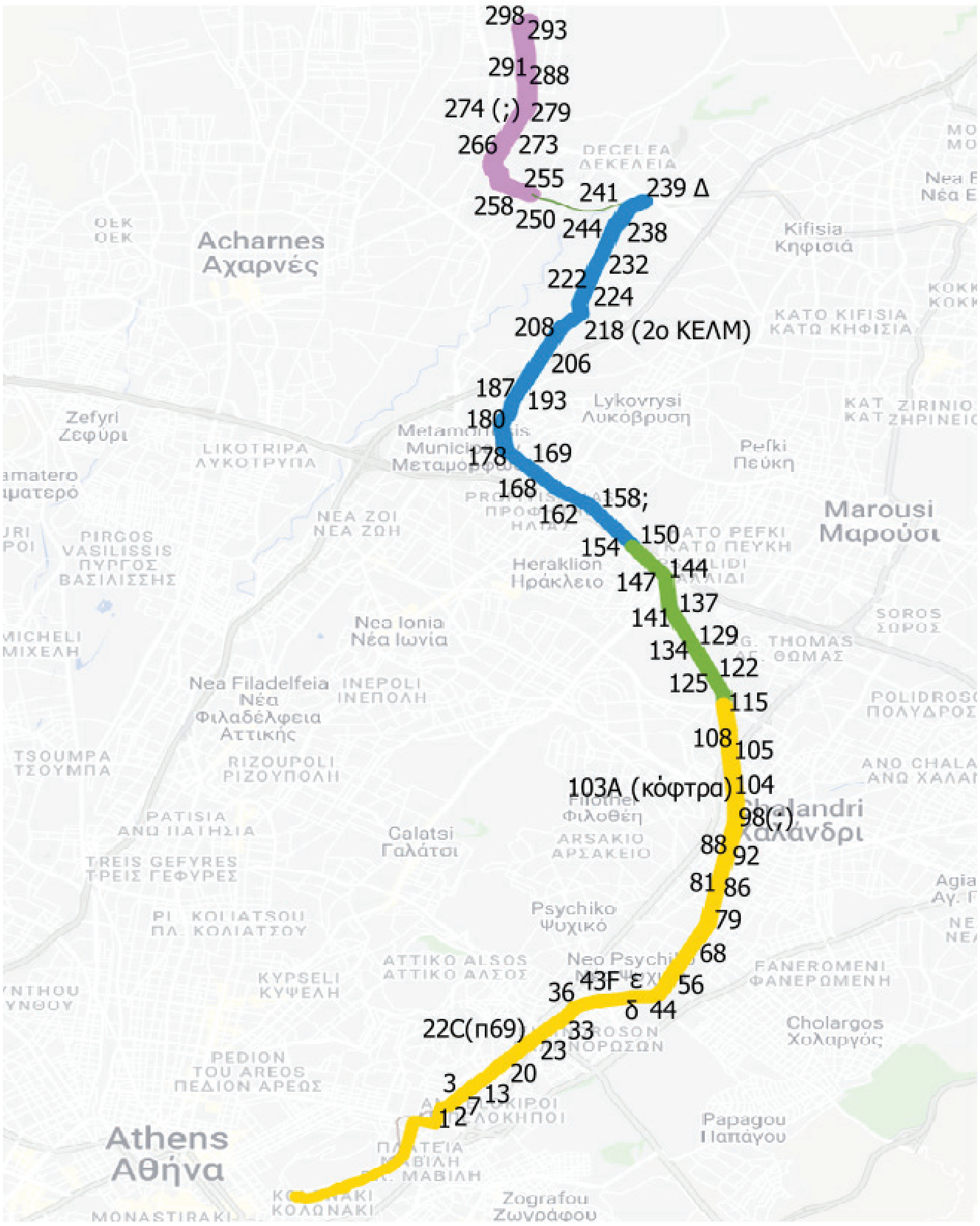

Fig 2: Trace of Hadrian's Aqueduct, with the 4 hydraulically independent segments.

In addition, we detected that along the way of Hadrian's aqueduct there are some blockages. The type of blockage differs accordingly the chemical identity of the water of neighboring shafts. In some cases, there is absolute blockage, in same partial and in some; there is a dam overflow that is bypassed when the lake, that was created, reaches a certain height. At shafts #253, #154, #150 and #118 - #119 there are absolute blockages, at shafts #199 and #43ε there are partial blockages and at shaft #18A there is a dam overflow that has to be bypassed.

3.2 Outcome of annually monitoring

After the sampling tests, diagrams (Fig. 3) were constructed accordingly and depicted the seasonal progress of nine different chemical parameters. Bellow, we illustrate the analysis and the results of two major, out of the above-mentioned nine, chemical parameters. The concentrations of calcium ranged from 60 to 150 mg/l (Fig. 3). Nitrates (NO3-) levels were always below 100 mg/l and differed from water shaft to water shaft, as well as slightly from the wet to the dry season. The presence of NO^{3-} could possibly be a source of fertilizer for the irrigation purposes of Hadrian's aqueduct.

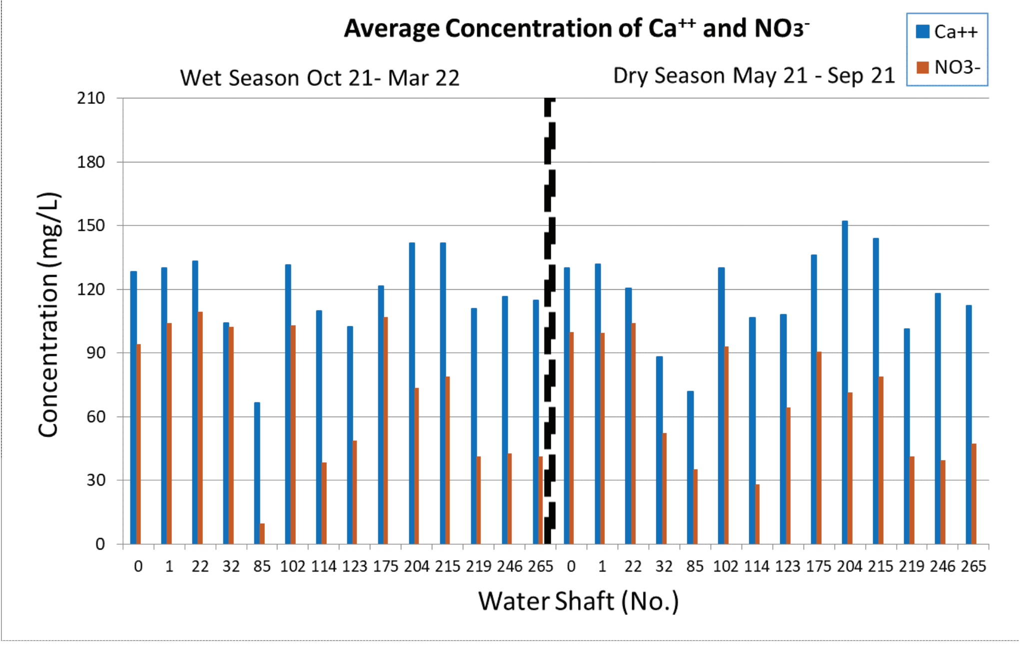

Fig. 3: Average Concentration of Ca^{2+}, NO_3^- during wet and dry season.

4. Discussion/Conclusion

Nowadays, there is an increasing water need and the utilization of the ancient aqueduct can contribute in relieving it. Hadrian's Aqueduct could provide the City of Athens with a high quality water that could be used for irrigation purposes, as well as for cleaning and other non-potable uses. In addition, its water use could reverse the negative impact on the aquifer and counter urban heat island effect. The study proved that the chemical identity for all parameters is characteristic of high quality underground water considering Hadrian's Aqueduct. In addition, we were able to define possible interactions between the neighboring basins and investigate chemically the possible water sources, in order to track down the water inflows. The general and future vision is to restart Hadrian Aqueduct and contribute to Athens water needs.

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