'End to end' planktonic trophic web and its implications for the mussel farms in the Mar Piccolo of Taranto (Ionian Sea, Italy)

Ana Karuza, Carmela Caroppo, Marina Monti, Elisa Camatti, Elena Di Poi, Loredana Stabili, Rocco Auriemma, Marco Pansera, et al.

Environmental Science and Pollution Research

ISSN 0944-1344

Environ Sci Pollut Res
DOI 10.1007/s11356-015-5621-1

Springer

Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Author's personal copy

Environ Sci Pollut Res
DOI 10.1007/s11356-015-5621-1

'End to end' planktonic trophic web and its implications for the mussel farms in the Mar Piccolo of Taranto (Ionian Sea, Italy)

Ana Karuza[1] · Carmela Caroppo[2] · Marina Monti[1] · Elisa Camatti[3] · Elena Di Poi[1] · Loredana Stabili[2] · Rocco Auriemma[1] · Marco Pansera[3] · Tamara Cibic[1] · Paola Del Negro[1]

Received: 10 April 2015 / Accepted: 15 October 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract The Mar Piccolo is a semi-enclosed basin subject to different natural and anthropogenic stressors. In order to better understand plankton dynamics and preferential carbon pathways within the planktonic trophic web, an integrated approach was adopted for the first time by examining all trophic levels (virioplankton, the heterotrophic and phototrophic fractions of pico-, nano- and microplankton, as well as mesozooplankton). Plankton abundance and biomass were investigated during four surveys in the period 2013–2014. Beside unveiling the dynamics of different plankton groups in the Mar Piccolo, the study revealed that high portion of the plankton carbon (C) pool was constituted by small-sized (<2 μm) planktonic fractions. The prevalence of small-sized species within micro- and mesozooplankton communities was observed as well. The succession of planktonic communities was clearly driven by the seasonality, i.e. by the nutrient availability and physical features of the water column. Our hypothesis is that beside the 'bottom-up' control and the grazing pressure, inferred from the C pools of different plankton groups, the presence of mussel farms in the Mar Piccolo exerts a profound impact on plankton communities, not only due to the important sequestration of the plankton biomass but also by strongly influencing its structure.

Keywords Mar Piccolo · Plankton · Trophic web · Mussels · Carbon flux · Biodiversity · Taranto

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (doi:10.1007/s11356-015-5621-1) contains supplementary material, which is available to authorized users.

✉ Ana Karuza
akaruza@ogs.trieste.it

[1] Sezione Oceanografia, Istituto Nazionale di Oceanografia e Geofisica Sperimentale (OGS), Via A. Piccard 54, 34151 Trieste, Italy

[2] Consiglio Nazionale delle Ricerche (CNR), Istituto per l'Ambiente Marino Costiero (IAMC), 74123 Taranto, Italy

[3] Consiglio Nazionale delle Ricerche (CNR), Istituto di Scienze Marine (ISMAR), 30122 Venezia, Italy

Introduction

Trophic relationships determine the routes of energy flow and chemical cycling in aquatic systems. The strength of the match between the availability of resources (bottom-up) and the removal exerted by planktonic heterotrophs and viral lysis (Azam and Malfatti 2007) determines carbon (C) flow within aquatic food webs (Legendre and Rivkin 2002; Pugnetti et al. 2008) and the biomass distribution in the different planktonic compartments. In most marine environments, organic matter flows to heterotrophic prokaryotes that are able to use dissolved organic substrates incorporating them in new biomass (Amon and Benner 1996). In turn, prokaryotic biomass may be removed from the aquatic system by several mechanisms among which the grazing by heterotrophic flagellates is one of the most important (Fenchel 1982; Sherr et al. 1989). This step is essential for conveying the C from heterotrophic prokaryotes to the higher trophic levels through grazing by heterotrophic flagellates and then by larger predators. Also, the C incorporated by primary producers is channelled to the higher trophic levels through the microbial loop by the primary production of prokaryotic phytoplankton or through the classic food chain (for phytoplankton >2 μm). Viral infection is common to all plankton components and when the cell lysis occurs it acts as catalyser of nutrient transfer from the particulate (cellular) to the dissolved form. In biological systems C is used as a general tracer of energy flow because all

Published online: 24 October 2015

Author's personal copy

organisms store energy in the form of chemical bonds within C-based complexes (Wilhelm and Suttle 1999).

In the sea, plankton is patchily distributed, due to the heterogeneous distribution of environmental abiotic and biotic factors (Belmonte et al. 2001). This feature is more evident in coastal areas than in the open sea, and mainly in temperate latitudes, where seasons heavily affect plankton composition and abundance (e.g. Raymont 1983). In the Mediterranean waters, the changes in the biomass distribution of marine plankton communities among different size classes (Sieburth 1979) and preferential energy pathways (autotrophic vs heterotrophic) occur in relation to the trophic status of the environment (Duarte et al. 2000).

The Mar Piccolo (Ionian Sea, Italy) is an example of the Mediterranean coastal marine ecosystem whose biological balances have been modified as a result of the considerable anthropogenic activities. Besides the eutrophication processes, such as the discharge of urban wastewaters and the freshwater draining from the surrounding agriculture soil, this sea area has been subjected for decades to the impacts of the most important steelwork complex in Europe (ILVA), the major Italian Navy shipyard and arsenal as well as an oil refinery and shipbuilding activities (Caroppo et al. 2012). Moreover, due to its hydrological features such as the restricted circulation, the low water exchanges and shallow depth, this 'semi-enclosed' coastal ecosystem is particularly suitable for the intensive mussel rearing and represents the largest mussel farming site in Italy (Cardellicchio et al. 2015).

The quality of the farmed mussels (*Mytilus galloprovincialis* L., Parenzan 1984; Pastore 1993) in the Mar Piccolo has been historically known. In the 1990s, the mussel farming strategy has been improved. Recently, several modifications related to socio-economic and sanitary problems occurred in the area exerting an influence on the trophic relationships of the Mar Piccolo. In the years 2000–2006, six sewage outfalls were relocated to the Gulf of Taranto in order to reduce bacterial exposure and to allow mussel farmers to extend their activities. The production reached a maximum of ~60,000 tons year^{-1} in 2005–2006, but instead of the expected extension after the year 2006, the production progressively declined to 40,000 tons years^{-1} in 2010 and the quality of the mussels, measured as flesh-to-shell dry weight, dropped to 50 % compared to its level in 2004 (Caroppo et al. 2012).

Several studies carried out in that area were mostly aimed to larger planktonic fractions as mesozooplankton (De Angelis and Della Valle 1959; Crisafi and Crescenti 1975; Belmonte et al. 2001, 2013) and microphytoplankton (Caroppo and Cardellicchio 1995; Caroppo 1996; Caroppo et al. 2006, 2014). Given the rising interest for the microbial components, in the last decade, prokaryotic (Caroppo et al. 2006) and viral (Stabili et al. 2004) abundances have been investigated as well. However, with the exception of a model-based study that also included the plankton compartment of the Taranto Sea (Caroppo et al. 2012), there is no evidence on an integrated study on plankton communities in the Mar Piccolo.

In a view of the lacking knowledge on the Mar Piccolo ecosystem, the present study primarily aims to provide, as much as possible, the synoptical characterization of the whole planktonic compartment (from viruses to mesozooplankton) in terms of abundance, biomass partitioning and taxonomy (the latter is limited to the microplankton and mesozooplankton communities). In order to achieve representative scenarios of seasonal dynamics, four surveys were conducted in both inlets at surface and bottom depths. For this purpose, the stations were selected in order to depict the maximum variability of the main hydrographic features, trophic state, mussel farming and other anthropogenic pressures. Moreover, the relationship between plankton community structure and environmental variables (physical features and bottom-up control) was assessed as well. Finally, given the extent and the hypothetic C removal due to the mussel farming in the area, the influence of plankton community structure on the mussel filter feeding and vice versa has been approached by examining the life cycle and dietary preferences of the mussels. The implications of the recent changes in the trophic regime of the Mar Piccolo on the plankton structure and mussel harvest have been investigated as well.

Materials and methods

Study area

The Mar Piccolo is a shallow, nearly enclosed sea, situated in the centre of the Mediterranean region, at the northern end of the Ionian Sea, in south-eastern Italy (Fig. 1). It is divided by a promontory into two basins, i.e. first and second inlet, stretching in a NE–SW direction and with maximum depths of 13 and 10 m and a surface area of 8.28 km^2 and 12.43 km, respectively. As an inner sea, the circulation of the Mar Piccolo is restricted: water exchanges which occur with Mar Grande through two channels are mainly due to moderate sea tides with excursions <30–40 cm (Caroppo and Cardellicchio 1995). The scarce hydrodynamism and the reduced water exchange with the nearby Mar Grande (Umgiesser et al. 2007) is particularly evident in the second inlet and determines the water stratification, especially in summer. Moreover, the exchange with Mar Grande and the hydrodynamism, especially of the first inlet, has been modified in 1985 by the installation of a water-scooping machine (0.15 M m^3 day^{-1}) to provide cooling water for the iron and steel industry.

The Mar Piccolo is generally characterized by low-velocity currents (~5–10 cm s^{-1}). Two dominant currents at 1 and 6 m depth (De Pascalis 2013) have been identified. At subsurface (−1 m), the dominant current flows from the second to the first inlet towards the Mar Grande through the navigation and

Author's personal copy

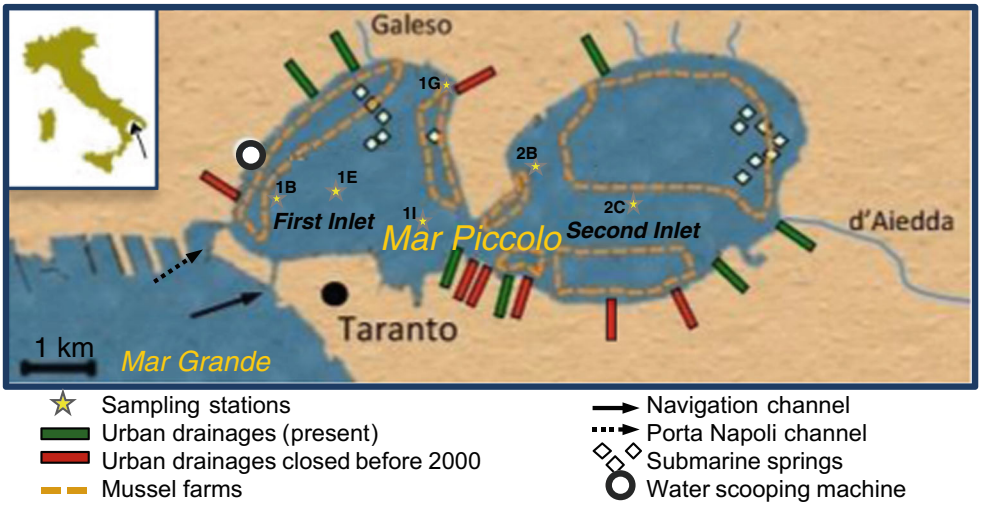

Fig. 1 Mar Piccolo of Taranto: location of six sampling stations in the first and the second inlet, mussel farm areas, urban drainage system, freshwater inputs (rivers and submarine spring) and water-scooping machine (modified from Caroppo et al. 2012)

Porta Napoli channels, generating the clockwise currents inside both inlets. At 6 m depth, the current flows from Mar Grande through the two channels into the first and then into the second inlet.

The confinement (Alabiso et al. 2006), defined as an expression of different degrees of connection to the open sea and of sea renewal time (Melaku Canu et al. 2012), is evident from the changes in physicochemical features moving from the first to the second inlet. Salinity is influenced by the input of freshwater deriving from small tributary rivers (the most important is Galeso, with a mean flow of 50,000 m^3 day^{-1}) and submarine springs, by water draining from the surrounding agricultural soils and by 34 freshwater springs (locally called 'Citri'). Moreover, seven sewage pipes (three in the first and four in the second inlet) are currently operating. The total flow of freshwater inputs is seasonal, reaching a maximum of 0.01 km^3 $week^{-1}$, allowing to consider the system similar to an estuary (Strusi and Pastore 1975). Mussel farms are widely distributed in both inlets of the Mar Piccolo covering the 61 % of the sea surface.

A more exhaustive description of the study area is given by Cardellicchio et al. (2015).

Sampling strategy

Samplings were carried out in four different periods in order to depict the interseasonal variability in the Mar Piccolo of Taranto (during 10–17 June 2013, 24 September–2 October 2013, 3–5 February 2014 and 31 March–8 April 2014). Samples were collected in six selected stations (Fig. 1), four in the first inlet (1G, 1E, 1B and 1I) and two in the second inlet (2B and 2C): station (st.) 1G (13.0 m deep) was located near the Galeso River inflow and the former shipyard (40° 29′ 48″ N, 17° 15′ 53″ E), st. 1E (11.2 m deep) in the middle of the first inlet (40° 29′ 01″ N, 17° 14′ 46″ E), st. 1B (10 m deep) near the connection channels with Mar Grande (40° 28′ 59″ N, 17° 14′ 10″ E), st. 1I (11.0 m deep) near the connection with the second inlet, between the navy arsenal and Italian Military Navy (40° 28′ 35″ N, 17° 15′ 37″ E), st. 2B (7.0 m deep) near the Punta Penna Bridge and the former Polveriera Buffoluto (40° 28′ 57″ N, 17° 16′ 42″ E) and st. 2C (8.0 m deep) in the middle of the second inlet in the correspondence of a long-term monitoring site (40° 28′ 57″ N, 17° 17′ 41″ E). In order to simplify the further description in the text and figures, the surveys conducted in September/October will be labelled as October whereas the samplings conducted in March/April will be considered as April survey hereinafter.

Hydrological parameters were obtained using a Seabird 19 Plus Seacat multiparametric probe in June and April surveys, whereas in October survey, the CTD acquisition was done using an Idromar IPO5OD multiparametric probe. In the February survey, the seawater temperature was measured using a PNF-300 Profiling Natural Fluorometer whereas salinity was measured using a CDM83 conductivity meter (Radiometer Copenhagen).

Surface and bottom water samples for chemical and biological characterization were collected using an acid-rinsed 5-L Niskin bottle, equipped with silicon elastic and red silicon O-ring. Only mesozooplankton samples were collected by vertical hauls, from the bottom to the surface, using a WP2 net (0.57 m Ø, 200-μm mesh).

Sample analyses

Nutrients and pigments

Samples for dissolved inorganic nutrients [nitrite ($N\text{-}NO_2$), nitrate ($N\text{-}NO_3$), ammonium ($N\text{-}NH_4$), phosphate ($P\text{-}PO_4$) and silicate ($Si\text{-}Si(OH)_4$)] were filtered on board on pre-combusted Whatman GF/F filters and stored at −20 °C until laboratory analysis which was performed with a segmented flow Bran+Luebbe AutoAnalyzer 3 following standard

Author's personal copy

colorimetric methods (Hansen and Koroleff 1999). The detection limits of nutrient concentrations reported by the analytical methods were set to 0.02, 0.02, 0.04, 0.02 and 0.02 μM, respectively, for nitrite, nitrate, ammonium, phosphate and silicate.

Samples for the estimate of chlorophyll *a* (Chl-*a*) concentration were filtered onto 47-mm Whatman GF/F filters that were stored at −20 °C until laboratory analysis. Chl-*a* was extracted overnight (4 °C) with 90 % acetone from the homogenate filter and determined spectrofluorometrically according to Lorenzen and Jeffrey (1980) using Jasco FP 6500 spectrofluorometer. The coefficient of variation for three replicate samples was lower than 5 %, and the detection limit, defined as twice the standard deviation of three blank filters, was 0.002 μg L^{-1}.

Particulate organic carbon (POC) was measured using an elemental analyzer CHNO-S Costech mod. ECS 4010 according to the methods performed by Pella and Colombo (1973) and Sharp (1974). Two subsamples of about 0.5 L each were filtered on 25-mm Whatman GF/F pre-combusted filters and stored at −20 °C. Before analysis, filters were treated with 200 μL of HCl 1 N to remove the carbonate and then dried in oven at 60 °C for about 1 h with the similar method of Lorrain et al. (2003). The detection, defined as twice the standard deviation of the blank, was 0.001 μmol C L^{-1}, and the relative standard deviation for three replicates was <10 %.

More exhaustive description and quality check of analytical procedures are described in Kralj et al. (2015).

Plankton

Viruses

Viral abundance was estimated according to Noble and Fuhrman (1998). Formalin-fixed samples (1 % final concentration in 10 mL) were processed within few days from the collection. Samples were diluted 1:10 in Milli-Q-ultrafiltered sterile water and filtered in triplicate (100 μL) onto 0.02-μm pore-sized Al_2O_3 inorganic membrane filters (Anodisc, Whatman) using a vacuum flask and maintaining the filtration pressure <0.1 atm. The membrane was filtered to dryness and stained with 20 μL of SYBR Green I (Molecular Probes stock solution diluted 1:20). After 15 min in darkness, the filters were mounted on a glass slide between 2 drops (25 μL each) of antifade solution [50 % glycerol, 49 % PBS (6.7 mM, pH 7.8) and 1 % ascorbic acid]. Viral counts were obtained by epifluorescence microscopy (Zeiss Axioplan; magnification ×1000) under a blue filter set [bandpass (BP) 450–490 nm, barrier filter (BA) 515–565 nm]. A minimum of 200 viruses were counted for each filter within at least 20 randomly selected fields or more to ensure ±10 % confidence levels.

Viruses were expressed in biomass by converting the abundance of particles into carbon content using a factor of 0.2 fg C (Suttle 2005). Virus-to-prokaryote ratio (VPR), generally considered a proxy of viral infection on prokaryotes, was calculated by dividing the viral abundance with the abundance of heterotrophic prokaryotes.

Prokaryotes

Samples (50 mL) for the determination of the abundance of heterotrophic prokaryotes were preserved in pre-filtered (through a 0.2-μm Acrodisc syringe filter) buffered formalin (2 % *v*/*v* final concentration) at 4 °C and processed within 48 h. After staining for 15' with 4′,6-diamidino-2-phenylindole (DAPI; Sigma) at 1 μg mL^{-1} final concentration (Porter and Feig 1980), samples were filtered in triplicate (3 mL) onto 0.2-μm pore-sized black-stained polycarbonate filters (Ø 25 mm, Nuclepore; Whatman) that were positioned on 0.45-μm nitrocellulose backing filters (Millipore). Filters were mounted on microscope slides using non-fluorescent oil and stored at −20 °C. The abundance of picophytoplankton was estimated from the same microscope slides without any further staining procedure. The enumeration was carried out using a Leica DM 2500 epifluorescence microscope equipped with a 100-W high-pressure mercury burner (HPO 100 W/2) at ×1000 magnification. Heterotrophic prokaryotes and picophytoplankton were accounted respectively under UV (BP 340–380 nm, BA 430 nm) and green (BP 515–560 nm, BA 590 nm) filter sets. A minimum of 200 cells were counted for each filter within at least 20 randomly selected fields or more to ensure ±10 % confidence levels. Cell number was converted into carbon biomass using a factor of 200 fg C $cell^{-1}$ (Caron et al. 1991) for picophytoplankton and 20 fg C $cell^{-1}$ for heterotrophic prokaryotes (Lee and Fuhrman 1987).

Nanoplankton

Samples (125 mL) for the determination of the abundance of nanoplankton (heterotrophic flagellates and nanophytoplankton) were preserved in glutaraldehyde (1 % final concentration) according to Bloem et al. (1986). Samples (30 mL) were filtered in triplicate onto 0.8-μm black-stained polycarbonate filters (Ø 25 mm, Nuclepore; Whatman) that were positioned on 1.2-μm nitrocellulose backing filters (Millipore). The cells were stained with DAPI (Sigma) at a final concentration of 1 μg mL^{-1} (Porter and Feig 1980) and stored at −20 °C. The enumeration was carried out using a Leica DM 2500 epifluorescence microscope equipped with a 100-W high-pressure mercury burner (HPO 100 W/2) at ×1000 magnification. The heterotrophic flagellates and nanophytoplankton were enumerated respectively under a UV (BP 340–380 nm, BA 430 nm) and blue (BP 450–490 nm, BA 515 nm) filter sets. Cells were counted in at least 20 randomly selected fields to give ±15 % confidence levels (Lund et al. 1958). The accounted cells were distinguished according to

Author's personal copy

five different shapes (circle, cone, ellipse, cylinder and as pinnate diatoms) and six standard dimensional sizes (2–5, 5–8, 8–11, 11–14, 14–17 and 17–20 μm); for each of the 30 resulting classes, the biovolume was estimated and converted to carbon content using a conversion factor of 0.14 pg C μm^{-3} (Lessard 1991).

Microphytoplankton

Samples (500 mL) were fixed with Lugol's iodine solution, stored at 4 °C and processed within 4 weeks. The preliminary examination was carried out under an inverted microscope (Leitz Labovert FS equipped with phase contrast) at a magnification of ×400 and ×630. According to the observed microphytoplankton cell densities, a variable volume of sample (50–100 mL) was settled in an Utermöhl chamber (Utermöhl 1958). Microphytoplankton was enumerated in 30–60 randomly selected fields or along 1–4 transects; in addition, the half of the Utermöhl chamber was examined at ×200 magnification in order to obtain better evaluation of less abundant taxa. The minimum value of counted cells has been 200 cells per sample, by accepting a confidence limit of 14 % (Andersen and Throndsen 2004). This is a generally accepted limit (Zingone et al. 2010), especially when samples of oligotrophic waters are considered. Biovolume was calculated by assigning to each cell one geometrical body or, in some cases, to a combination of more geometrical bodies and by applying standard formulae according to Hillebrand et al. (1999). The obtained biovolumes were converted to carbon content using the conversion factors introduced by Menden-Deuer and Lessard (2000).

Microzooplankton

Samples (5 L) were concentrated with a 10-μm mesh, reduced to 250 mL and immediately fixed with buffered formalin (4 % final concentration). Subsamples (10–25 cm^3) were examined in a settling chamber using a Leica DMI 3000B inverted microscope equipped with phase contrast and bright-field illumination at ×200 magnification, according to the method of Utermöhl (1958). The entire surface of the chamber was examined.

Among the microzooplankton communities, five main groups were considered and distinguished as ciliates (naked and tintinnids), heterotrophic dinoflagellates, other protozoa and micrometazoans. The phototrophic ciliate *Mesodinium rubrum* was treated together with the aloricate heterotrophic ciliates. Empty loricae of tintinnids were not differentiated from filled ones because tintinnid protoplasts are attached to the lorica by a fragile strand, which detaches with ease during collection and fixation of the samples. For each taxon, biovolumes were estimated by measuring the linear dimension of each organism with eyepiece scale and equating shapes to standard geometric figures. The obtained biovolumes were converted to carbon content by applying the following conversion factors and formulae according to Verity and Langdon (1984) for tintinnids, Putt and Stoecker (1989) for naked ciliates, Edler (1979) for athecate and thecate dinoflagellates and Beers and Stewart (1970) for other protozoa.

Mesozooplankton

After collection, all the samples were dried of excess seawater (using a sieve with mesh of the same size) and 95 % ethyl alcohol was added for organism preservation. Taxonomic and quantitative zooplankton determinations were performed using a Zeiss stereomicroscope (Stemi 2000CS model, ×50 magnification) at the lowest possible taxonomic level (up to species level for copepods and cladocerans); larval or juvenile stages were classified at genus, family or class level. Each sample was poured into a beaker to allow thorough mixing in order to obtain the random distribution of the organisms: representative sub-sample, or the total sample when abundances were scarce, was analyzed. For the estimation of rare species, total sample volume was analyzed according to Harris et al. (2000).

Mesozooplankton biomass was obtained through measurements of ash-free dry mass (Harris et al. 2000): sub-samples previously cleaned of debris were filtered onto pre-combusted GF C glass and then dried and weighed. Then, the same filters were combusted in a muffle furnace at 500 °C for 12 h in order to eliminate the organic matter and then weighed again. The estimates were obtained by the difference between dried and ash samples.

Data analyses

Multivariate statistical analysis was used to assess the differences in planktonic community structure between the sampling depths/inlets/surveys. First of all, a one-way analysis of similarities (ANOSIM) was performed on plankton abundance data set at surface and bottom depths (except for the mesozooplankton abundance that was sampled in the whole water column, i.e. providing only one data for each sampling station). This was done in order to assess, in case of further analysis, whether the variability between the sampling depths is statistically acceptable to consent data processing of a single variable as a mean value for each sampling station.

To test for differences in plankton community structure between inlets and among surveys, ANOSIM was performed on mean plankton abundances in the water column (calculated on surface and bottom values) for each sampling station (except for the mesozooplankton abundance that was obtained for the whole water column and thus was used as such). Log-transformed abundances of the plankton groups were used to estimate the Bray-Curtis similarity. The test output (R) can

Author's personal copy

range from −1 to 1 with R close to 1 when groups are very dissimilar and approach to 0 as they become more similar.

A non-metric multidimensional scaling (nMDS) ordination (Kruskal and Wish 1978) was used to assess the differences in plankton community structure among different surveys. The analysis was performed on the abundances of main plankton groups, from viruses to mesozooplankton. The environmental variables were fitted as supplementary variables (vectors) onto ordination spaces to investigate their effects on the plankton community structure. When the nMDS ordination analysis was carried out for environmental data, the ordination was based on a Euclidean distance matrix derived from normalized (z standardization) physical-chemical data.

In order to investigate the relationship between viruses and heterotrophic prokaryotes as their most probable hosts (thus possibly representing an important C pathway), linear correlation analysis was applied. The Shapiro-Wilk test was used to verify the normal data distribution. Because data within single variables did not result as normally distributed, a non-parametric Spearman rank correlation analysis was used to assess the association between viruses and heterotrophic prokaryotes. A non-parametric analysis of variance Mann-Whitney U test was used to test the differences in the abundances of viruses and heterotrophic prokaryotes between the two inlets.

The analyses were performed using Primer-E Software package v7.0 (Plymouth Marine Laboratory, UK) according to Clarke and Warwick (2001) for ANOSIM and nMDS ordination. Statistica software package (StatSoft) was run for the analysis of variance and the correlation analysis.

Results

Physical and chemical features of the water column

Physical and chemical characterization of the water column, presented by means of a range of values for every single parameter, is listed in Table 1. In all surveys, salinity was lower at the bottom depth, outlining the influence of the surface freshwater inputs. Only in June, the time-shift between the samplings in different stations (8 days) considerably affected the water column properties. In that survey, the difference in the thermocline properties between the first and the second inlet was due to the complete water column mixing at the beginning of the survey (first inlet) to the progressive stratification towards the sampling end (second inlet). Thus, slightly higher temperature was detected in the second inlet (st. 2B) whereas minimum temperature characterized stations 1G and 1I. This event also affected salinity that was more variable in the first inlet which is also the deeper one, where higher values characterized the bottom depth. POC concentrations widely varied displaying the marked difference between surface and bottom samples. Phosphates dropped to the minimum concentrations in June, whereas ammonium, with 91 %, largely prevailed over other dissolved inorganic nitrogen (DIN) compounds. In October, the water column profile was rather homogeneous, especially in the shallowest station of the second inlet (st. 2B) where complete mixing was observed. As a consequence, also nutrient concentrations and Chl-*a* did not display considerable differences between surface and bottom depths. Phosphate concentrations ranged between the lower detection limit and 0.14 μM.

In February, seawater temperature was comprised between 12.0 and 13.2 °C and was characterized by progressive increasing surface-to-bottom gradient. The stratification of the water masses was evident also from salinity profiles that displayed pronounced variability, ranging between 34.3 and 38.3. Lower salinity was detected in the offshore stations at the bottom, with the minimum value that was detected at the deepest station (st. 1I). Nutrients reached the maximum concentrations among the surveys with higher values that were measured at the surface, likely due to the precipitations that occurred in the days before the samplings. Chl-*a* concentrations displayed annual minima.

In April, the thermohaline profile evidenced the beginning of the water stratification. Seawater temperature ranged between 15.5 and 17.0 °C (surface of st. 1I), whereas salinity varied between 35.1 (surface of st. 1B) and 37.5 (bottom of st. 1I), outlining the presence of freshwater due to the precipitations that occurred in the days before. The low concentration of phosphates highlighted the depletion, whereas within DIN pool, nitrates largely prevailed as the most oxidized form (86–99 %). Chlorophyll-*a* reached the maximum concentrations for the study period. More exhaustive characterization of the physical and chemical features is reported in Kralj et al. (2015).

Plankton abundance and biomass

Viruses and prokaryotes

The abundance of viruses ranged between $4.7 \pm 0.5 \times 10^9$ particles L^{-1} and $1.0 \pm 0.02 \times 10^{11}$ L^{-1} (Fig. 2(a)). The abundances remained rather stable across the different surveys with the exception on October 2013 when higher abundances were detected in the second inlet ($3.7 \pm 0.2 \times 10^{10}$ and $7.3 \pm 0.43 \times 10^{10}$ L^{-1} at the surface and $0.6 \pm 0.04 \times 10^{11}$ and $1.0 \pm 0.02 \times 10^{11}$ at the bottom, for stations 2B and 2C, respectively).

The abundance of picophytoplankton (Fig. 2(b)) varied in the range of 2 orders of magnitude (from $5.5 \pm 0.3 \times 10^6$ to $1.0 \pm 0.1 \times 10^9$ cells L^{-1}). Considerably higher abundances were detected on October 2013 at all stations and especially in the second inlet where, at the surface of st. 2B, the abundance reached the maximum value for the entire study period.

Author's personal copy

Table 1 Physical and chemical features at surface (S) and bottom (B) depths at six stations (mean values) in the Mar Piccolo of Taranto over different surveys

Survey	Depth (m)	Range	Temp (°C)	Sal	POC (μg C L^{-1})	P-PO_4 (μM)	N-NH_4 (μM)	N-NO_2 (μM)	N-NO_3 (μM)	Si-$Si(OH)_4$ (μM)	Chl-*a* (μg L^{-1})
June	S	Min	21.44	35.18	263.6	0.02	0.50	0.03	0.04	6.44	0.95
		Max	24.78	36.75	644.6	0.03	0.85	0.13	7.41	17.84	1.84
	B	Min	20.79	37.34	191.6	0.02	0.48	0.02	0.05	3.01	0.69
		Max	22.29	38.41	292.6	0.03	0.83	0.06	0.74	11.88	1.63
October	S	Min	23.12	37.24	409.2	0.06	0.50	0.10	1.40	15.09	1.18
		Max	23.42	37.60	599.5	0.14	1.58	0.22	7.41	19.61	2.55
	B	Min	23.35	37.49	297.1	0.02	0.79	0.08	0.98	8.77	0.44
		Max	24.19	39.04	639.8	0.13	2.27	0.22	4.00	27.64	2.34
February	S	Min	11.97	34.32	90.2	0.15	1.06	0.28	10.15	16.69	0.56
		Max	12.24	36.29	287.6	0.64	3.14	0.54	19.92	22.23	1.35
	B	Min	12.80	35.71	79.6	0.11	0.84	0.27	4.29	7.43	0.36
		Max	13.20	38.28	117.1	0.28	1.57	0.33	7.80	13.20	0.78
April	S	Min	16.04	35.10	191.9	0.02	0.00	0.13	3.17	7.99	1.74
		Max	16.98	35.91	288.9	0.05	0.67	0.23	36.92	32.53	3.25
	B	Min	15.46	36.73	149.8	0.02	0.02	0.12	2.01	3.71	1.23
		Max	16.78	37.47	284.1	0.05	0.38	0.15	6.26	10.23	4.59

Data were kindly provided by M. Kralj

Temp temperature, *Sal* salinity, *POC* particulate organic matter, *P-PO_4* phosphate, *N-NH_4* ammonium, *N-NO_2* nitrite, *N-NO_3* nitrate, *Si-$Si(OH)_4$* silicate, *Chl-a* chlorophyll *a*

The abundance of heterotrophic prokaryotes ranged between $7.1\pm0.4\times10^8$ and $4.2\pm2.1\times10^9$ cells L^{-1} (Fig. 2(c)). The abundance of both viruses and heterotrophic prokaryotes was significantly higher in the second inlet relative to the first inlet (U=24, p<0.01, and U=28, p<0.02, for viruses and heterotrophic prokaryotes, respectively). The viral biomass ranged between 0.9±0.2 and 20.2±0.4 μg C L^{-1}. The biomass of heterotrophic prokaryotes was comprised between 14.1±0.1 and 85.7±4.7 μg C L^{-1}, whilst the picophytoplankton biomass widely varied between 1.1±0.06 and 203.3±23.2 μg C L^{-1}.

Heterotrophic nanoflagellates and nanophytoplankton

Nanoflagellates displayed high abundance variability in the investigated area, ranging from $2.4\pm0.1\times10^5$ to $6.5\pm0.05\times10^6$ for the heterotrophs and from $3.6\pm0.5\times10^5$ to $1.3\pm0.06\times10^7$ cells L^{-1} for the phototrophs (Fig. 2(d)). The minimum values were found on February 2014 at all stations: in that period, the abundances were on average $1.4\pm0.5\times10^6$ and $4.5\pm0.7\times10^6$ cells L^{-1} for the heterotrophs and phototrophs respectively. Even if the similar trend was observed in the distribution of heterotrophs (Fig. 2(e)) and phototrophs, the latter significantly increased their abundances on June 2013 when they reached their absolute maximum for the entire study. The distinction of nano-sized plankton into different shape classes enabled us to detect the presence of *Chaetoceros* spp. assuming that the cylinder shape organisms belong exclusively to this genus. In June, in fact, relatively high abundance of *Chaetoceros* spp. was found, especially at the surface layer, where at st. 1I, their abundance reached 3×10^6 cells L^{-1} (28 % of total nanophytoplankton). In April, small-sized *Chaetoceros* spp. (<8 μm) contributed with >50 % to the nanophytoplankton at st. 1B, but the highest abundance characterized st. 2B (3.1×10^6 cells L^{-1}).

Nanoplankton biomass displayed minimum values in February, when the values ranged between 0.9±0.02 and 3.5±0.41 μg C L^{-1} for the autotrophs and between 0.5±0.06 and 2.1±0.07 μg C L^{-1} for the heterotrophs. The highest biomass of nanophytoplankton (39.0±4.2 and 34.8±5.1 for the surface and bottom, respectively) was encountered in June in the innermost station (st. 2C) when small-sized *Chaetoceros* spp. contributed with 16.9±6.5 μg C L^{-1}. The high biomass in April at st. 2B (31.8±4.5 μg C L^{-1}) was limited to the bottom depth only. Heterotrophic nanoflagellates reached the maximum biomass of 13.0±1.3 μg C L^{-1} in April at the surface of st. 1I, prevalently due to small-sized cells (2–5 μm).

Microphytoplankton

Microphytoplankton abundance widely ranged between 6.0×10^3 and 2.0×10^6 cells L^{-1} (Fig. 2(f)). Considerably higher abundances were related to the campaign conducted on April 2014 (mean=9.4×10^5 cells L^{-1}) with respect to the

Author's personal copy

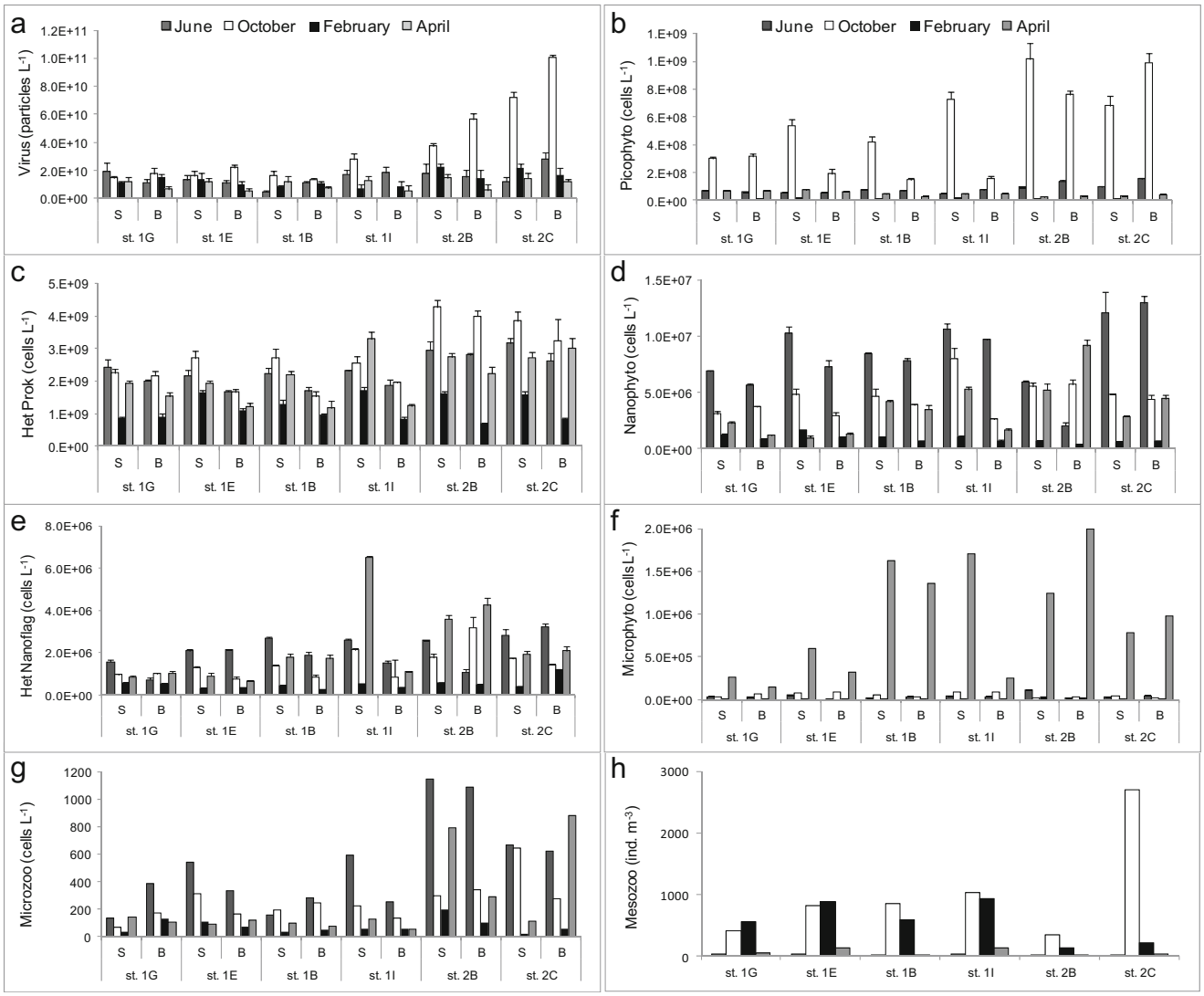

Fig. 2 The abundance of plankton communities in the Mar Piccolo of Taranto examined in four different surveys at surface (*S*) and bottom (*B*) sampling depths: *a* viruses, *b* picophytoplankton, *c* heterotrophic prokaryotes, *d* nanophytoplankton, *e* heterotrophic nanoflagellates, *f* microphytoplankton, *g* microzooplankton and *h* mesozooplankton

other surveys (mean=3.4×10^4 L^{-1}), especially by approaching the second inlet (st. 1B, st. 1I, st. 2B and st. 2C). A wide range of microphytoplankton biomass values was detected at the surface in February: the minimum of 2.9 μg C L^{-1} was detected in the first inlet (st. 1E), whereas the maximum of 56.6 μg C L^{-1} was observed in the second inlet (st. 2B).

The microphytoplankton community was generally dominated by diatoms and dinoflagellates. In particular, diatoms represented, on average, about 57.5±21.9 % of total abundance and 44.8±25.6 % of total biomass. Dinoflagellates accounted for 30.3±22.6 % of total abundance and 53.1± 26.5 % of total carbon. Micro-sized phytoflagellates never dominated the algal community (11.9±14.7 % of total abundance and 1.8±2.9 % of total biomass), and coccolithophorids were present at background levels. In the first inlet, diatoms and phytoflagellates reached the highest abundance and biomass values with respect to those detected in the second inlet. On the contrary, dinoflagellates in the second inlet reached maximum percentages in terms of cell counts and C content.

As concerning the species composition, a total of 117 species have been identified: 54 diatoms, 54 dinoflagellates, 6 coccolithophorids and 3 phytoflagellates. On June 2013, diatoms dominated the community with the species *Chaetoceros* spp., *Ceratoneis closterium*, *Proboscia alata*, *Pseudo-nitzschia delicatissima* group, *Navicula* spp. and *Nitzschia* spp. Also on April 2014, diatoms represented the most important components of the microphytoplankton assemblage, when the potentially toxic species *Pseudo-nitzschia* cf. *galaxiae* was responsible for a bloom in both inlets. Dinoflagellates reached their highest abundances and biomass on October 2013 with the

Author's personal copy

genera *Alexandrium*, *Gymnodinium*, *Gonyaulax*, *Prorocentrum* and *Scrippsiella*. Throughout the entire sampling period, among typically phototrophic dinoflagellates, mixotrophic species were also detected: *Akashiwo sanguinea*, *Alexandrium minutum*, *Prorocentrum micans*, *Prorocentrum triestinum*, *Ceratium furca* and *Lingulodinium polyedrum*.

On February 2014, coccolithophorids and phytoflagellates reached their highest abundance and biomass percentages with the coccolithophorids *Anoplosolenia brasiliensis*, *Calciopappus caudatus*, *Calciopappus murrayi* and *Syracosphaera* spp. and the phytoflagellates *Dictyocha fibula*, *Dictyocha octonaria* and *Hermesium adriaticum*.

Microzooplankton

The abundance of microzooplankton varied between 20 and 1150 organisms L^{-1} (Fig. 2(g)). In general, maximum abundances were found on June 2013 at both sampling depths. An increasing gradient in both the abundance and number of microzooplankton taxa was observed from the external to the more internal stations sampled (st. 2B and 2C) in June and April (Fig. 2(g)). During February 2014, this trend was not evident as microzooplankton abundance remained always <200 individuals L^{-1}. The more internal stations were characterized by a higher presence of tintinnids in June and October and aloricate ciliates in April.

Microzooplankton biomass ranged from 0.33 μg C L^{-1} (st. 1B at the surface in February) to 6.6 μg C L^{-1} (st. 2B at the bottom in June). The highest biomass was mostly due to tintinnids and micrometazoans.

Tintinnids, constituted by 16 genera and 36 species, dominated microzooplankton in June and October. They showed the highest abundance in June with the genera *Eutintinnus*, *Steenstrupiella* and *Tintinnopsis*. In particular, *Eutintinnus* cfr. *tubulosus* that reached high values at both depths in the second inlet presented smaller size than typically observed for the species (length of 60–90 μm and diameter of 18–24 μm). In October, when tintinnid abundance lowered, the most frequent genera were *Tintinnopsis*, *Salpingella* and *Helicostomella*. In February and April, the abundance of tintinnids drastically decreased and the most represented species were *Favella serrata*, *Stenosemella nivalis* and *Tintinnopsis campanula*. Aloricate ciliates, mostly Strombidiidae and Strobilidiidae, represented the second major group. Among the identified species, *Tiarina fusus* was present in June whilst *M. rubrum* dominated in April. Heterotrophic dinoflagellates, especially constituted by genera *Gyrodinium* and *Protoperidinium*, showed high values in June and October. Micrometazoans comprised mainly copepod nauplia, bivalve larvae and rotifera. In particular, rotifera *Trichocerca marina* was identified in June and April.

Mesozooplankton

The abundance of mesozooplankton widely ranged between 8 and 2705 individuals m^{-3} (Fig. 2(h)). Maximum abundances were observed in October and February, whereas minimum abundances characterized the surveys conducted in June (8 organisms m^{-3}) and April. The absolute maximum was detected at st. 2C in October survey with 80 % of the mesozooplankton assemblage that was constituted by *Oithona brevicornis*. Copepods were the most abundant group within the mesozooplankton assemblage in all surveys reaching up to 90 % of the abundance in February and followed by 80 % in October, relative to both inlets. Cladocerans contributed with the higher percentage in June (14 % in the first inlet and 1 % in the second inlet) and April (3 % in the first inlet and 2 % in the second inlet).

Generally, all surveys evidenced rather low species and taxa diversity: copepods were characterized by *Acartia*, *Oithona*, *Paracalanus* and *Centropages* genera. The rest of the assemblage was prevalently characterized by meroplanktonic forms as larvae of decapods, gasteropods and Ascidiacea in June and April, larvae of echinoderms and Cirripedia in September and, finally, idrozoans and appendicolaria in February.

Two new copepod species were recorded for the first time in the Taranto Sea: the Asian egg-carrying calanoida *Pseudodiaptomus marinus* Sato (1913) and the cyclopoida *O. brevicornis* Giesbrecht (1891). *P. marinus* was detected in June, October and April whilst, *O. brevicornis* was found only in October at all stations and with significant abundances.

Low values of mesozooplankton biomass are due to the low abundances that were registered over different surveys. The highest biomass that was found in February was mostly due to the presence of larger copepods, contrary to September when the maximum abundance of mesozooplankton organisms was detected when prevailing groups were characterized by smaller biovolume (bloom of the small species *O. brevicornis*).

The differences in the abundance and structure of planktonic community in the Mar Piccolo were investigated with respect to the water depth and station locations. The structure of the planktonic community did not show statistically significant differences between surface and bottom depths (R=0.017; p=0.67). This made possible to run all the tests on mean values in order to include also the mesozooplankton fraction which was sampled in the whole water column and thus was represented by one value only. Although the plankton community in the Mar Piccolo did not display statistically significant differences between the inlets either in terms of abundances (R=0.094; p=0.11) or biomass (0.089; p=0.13), slightly higher biomass, characterized by the major variability among the sampling surveys, was found in the second inlet (Fig. 3).

Author's personal copy

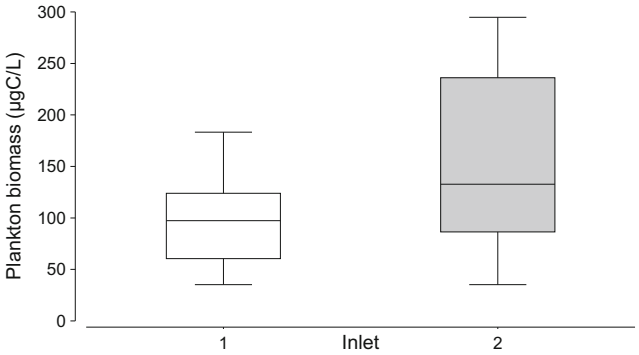

Fig. 3 Box plot of whole plankton biomass (µg C L^{-1}) in the water column (averaged between surface and bottom depth) distinguished between first (*1*) and second (*2*) inlets

The strong survey-based grouping in the resulting non-metric multidimensional scaling ordination (Fig. 4) evidenced that the plankton community structure is mostly and strongly influenced by seasonality (stress=0.08). That is confirmed by the superimposed ordination of the environmental variables on the nMDS ordination grouping reported in the same figure. The vectors of nutrients were associated to February, clearly indicating the maximum concentrations of all nutrients in that survey. On the contrary, planktonic community was associated to the minimum nutrient (in decreasing order phosphates, ammonia, nitrites, silicates and nitrates) and maximum Chl-*a* concentrations in the April survey. Among the environmental variables examined, water temperature and low nutrient concentrations (in decreasing order nitrates, nitrites and phosphates) determined the plankton community structure in June. Conversely, the plankton distribution in October was mainly influenced by physical features of the water column, when temperature and salinity reached their maximum values. Highly significant differences among the surveys were revealed not only globally (R=0.946; p<0.001), i.e. by taking account all the surveys, but also by the pairwise comparison as follows: February strongly differed from June and October (R=1; p<0.001), February differed from April (R=0.887; p<0.001), October differed from June and April (R=0.828; p<0.001), whereas April only partly, but still highly significantly, differed from June (R=0.487; p<0.001).

Carbon partitioning in the planktonic trophic web

The whole plankton C pool (mean±SD), calculated as a mean value for all stations, was the lowest in February (44.6±13.6 µg C L^{-1}) followed by June and April (109.4±22.1 and 111.3±36.1 µg C L^{-1}, respectively) and reached the maximum value in October (197.1±77.3 µg C L^{-1}) when, in the second inlet, it peaked up to 293.8 (±1.4) µg C L^{-1}.

The C partitioning among the planktonic groups was generally rather similar in different stations (Fig. 5). The major discrepancies were referred to st. 2B where the prokaryotic fraction in June increased to 50 and 20 % of total biomass for heterotrophs and picophytoplankton, respectively. Also in October, the picophytoplankton biomass at that station contributed with a higher percentage (61 %) compared to the other stations. At st. 2B, high microphytoplankton biomass that was detected in February (71.3 µg C L^{-1}) and April (179.1 µg C L^{-1}) largely exceeded the mean whole plankton biomass in the other stations in the same months (39.0±4.0 in February and 97.6±15.5 µg C L^{-1} in April), with considerably higher biomass that was detected at surface with respect to

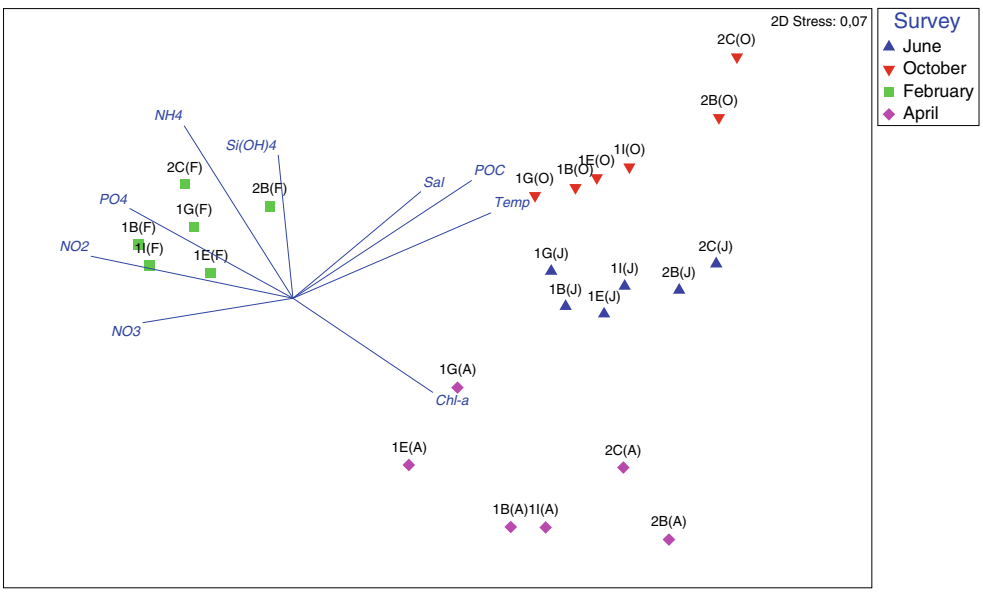

Fig. 4 Non-metric multidimensional scaling (nMDS) ordination plot of plankton abundances in four seasonal surveys (June, October, February and April) in the Mar Piccolo of Taranto. The correlation analysis of environmental variables (*Temp* temperature, *Sal* salinity, *Chl-a* chlorophyll *a*, NH_4 ammonium, NO_2 nitrite, NO_3 nitrate, PO_4 phosphate, $Si(OH)_4$ silicate) was superimposed to the nMDS ordination plot. The *length of arrows* indicates the correlations between the environmental variables and the ordination axes

Author's personal copy

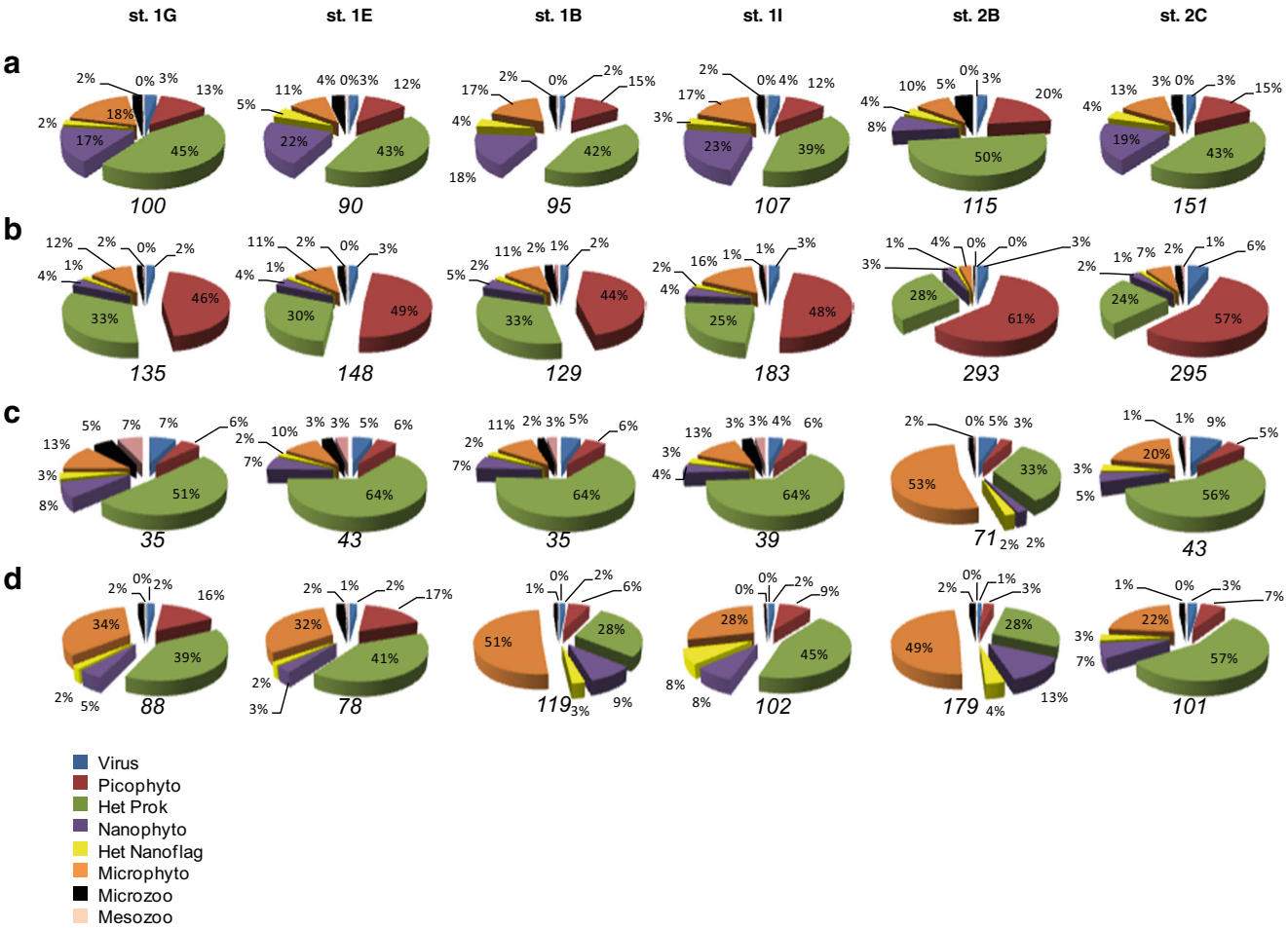

Fig. 5 Total plankton biomass (*value in italics below each plot*, expressed in μg C L^{-1}) and its partitioning in the Mar Piccolo of Taranto in six sampling stations (averaged for surface and bottom depths) in *a* June, *b* October, *c* February and *d* April

bottom depth (56.6 and 18.6 μg C L^{-1} in February and 107.1 and 69.1 μg C L^{-1} in April).

Prokaryotic biomass in the water column generally largely prevailed over other planktonic fractions (averaged between surface and bottom except for the mesozooplankton) in all surveys accounting between the minimum of 49 % in April and the maximum of 81 % in October (Fig. 5). By taking into account different stations, the exceptions where the biomass of micro-sized cells prevailed over prokaryotes were st. 1B in April (52 %) and st. 2B in February and April (54 % in the former and 51 % in the latter). The October sampling was characterized by particularly high biomass of small-sized fractions at all stations. In that month, the maximum was reached at stations 2B and 2C with up to 90 % of biomass belonging to <2 μm (pico-sized organisms, i.e. prokaryotes) and only 6 % of biomass belonging to >20 μm organisms (micro- and meso-sized).

Heterotrophic prokaryotes dominated within the whole C pool in February (53 %) and June (43 %), whereas in April their biomass equalled to those of microphytoplankton. In October, when picophytoplankton accounted for 53 % of the total plankton biomass, heterotrophs contributed with 28 % only.

The abundance of heterotrophic prokaryotes positively highly correlated with the abundance of viruses (R=0.48; p<0.01).

From the partitioning of whole plankton C biomass between phototrophs and heterotrophs (Fig. 6), the results show that the phototrophs, largely constituted by cyanobacteria, prevailed in October whereas the heterotrophs dominated in February, with the only exception of st. 2B where the phototrophic biomass in February accounted for 60 %. The situation in June was rather balanced between phototrophy and heterotrophy, whereas in April, the repartition between two compartments was more variable: the phototrophic biomass (>66 %) dominated at stations 1B and 2B, whilst at stations 1I and 2C, heterotrophs (>53 % of total biomass) slightly prevailed.

Author's personal copy

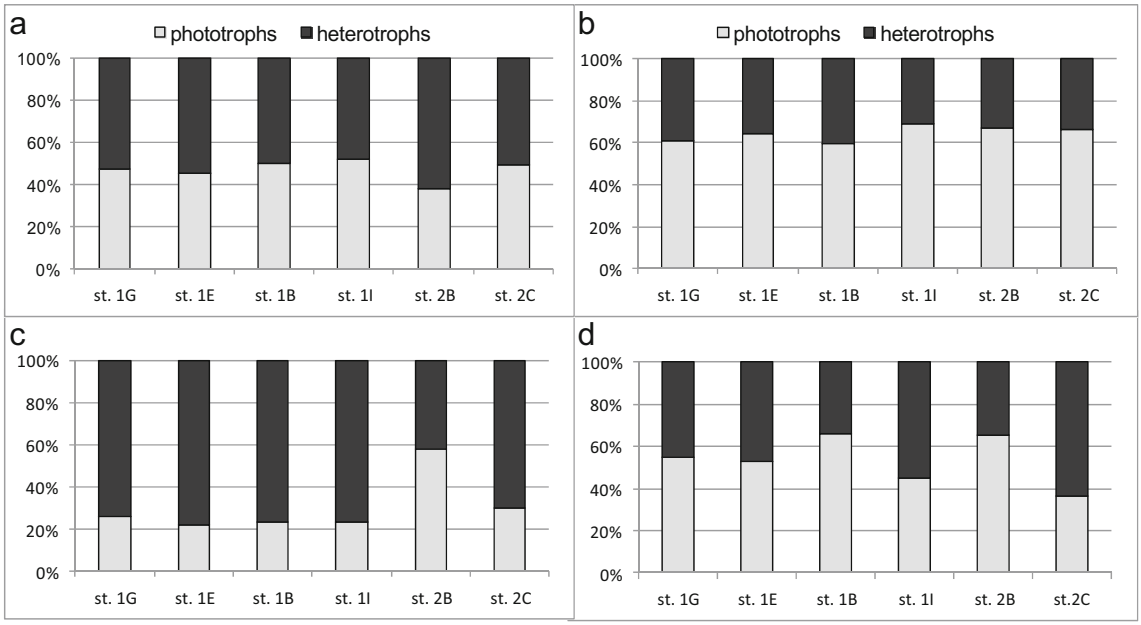

Fig. 6 Phototrophs vs phototrophs (μg C L^{-1}) in the Mar Piccolo of Taranto (averaged for surface and bottom depths) in *a* June, *b* October, *c* February and *d* April

Discussion

Plankton web structure

Planktonic community displayed a particularly wide range of variability over different surveys, especially with respect to the microbial fraction. Prokaryotic abundances were markedly higher, approximately by 1 order of magnitude, compared to the values previously observed out of the Mar Piccolo, at the eastern coast of the Mar Grande in the Gulf of Taranto, where the average annual prokaryotic abundance was $1.6\pm0.3\times10^{8}$ and $2.1\pm0.9\times10^{9}$ L^{-1} for picophytoplankton and heterotrophic prokaryotes, respectively (Caroppo et al. 2014). The abundance of the latter, which was higher also with respect to the Adriatic (Fonda Umani et al. 2012) and other Mediterranean areas (Jacquet et al. 1998), is likely associated to the estuarine feature of the Mar Piccolo. Similar values of abundance in heterotrophic prokaryotes were recorded in estuarine environments such as the coastal Mediterranean Thau lagoon (Bouvy et al. 2011), Chesapeake Bay (Kan et al. 2006) and Long Island estuary (Boissonneault-Cellineri et al. 2011). The higher mean abundance of picophytoplankton that emerged from our study is due to the bloom observed in October (up to $1.21\pm0.01\times10^{9}$ cells L^{-1} at st. 2B). This is in accordance with previous findings of consistently low picophytoplankton abundances in the Gulf of Taranto (Caroppo et al. 2006), with the exception of late summer when the peak of 4.5×10^{8} L^{-1} was detected for the Mar Piccolo. It is noteworthy to outline that the high picophytoplankton abundance observed in October matches with high viral abundances, supposing that virus-mediated mortality could have contributed to the end of the bloom (Weinbauer 2004), in accordance with the typical seasonal cycle reported for the Gulf of Trieste (Karuza et al. 2012). The coupling between the abundance of viruses and that of heterotrophic prokaryotes (as their most probable hosts) suggests the importance of virus-mediated mortality on a huge C pool of heterotrophic prokaryotes in the Mar Piccolo, especially in the second inlet where their abundances were significantly higher than those in the first one. In fact, compared to the other coastal ecosystems (Weinbauer 2004; Karuza et al. 2012) and in accordance with the previous findings for the Mar Piccolo (Stabili et al. 2004), our study outlined that, on average, viral abundance in the Mar Piccolo was generally high.

The distinction of plankton community into different size classes outlined the C partitioning towards the small-sized groups with respect to the eutrophic conditions. In fact, in the study conducted along a trophic gradient in the Adriatic Sea, pico-sized organisms did never exceed 48 % of planktonic C (Cabrini et al. 2002), in contrast to the ecosystem shift towards a lower trophic level that was previously observed by Caroppo et al. (2010) for the Mar Piccolo. High viral abundances, together with the positive relationship between viruses and heterotrophic prokaryotes, further strengthen our hypothesis that the C flow in the Mar Piccolo, and especially in its second inlet, is mainly conveyed to the microbial loop, thus reducing the transfer towards the higher trophic levels.

To the best of our knowledge, no integrated studies are available for this area; therefore, our data were compared with the exhaustive data set for the Adriatic Sea (Online Resource 1),

Author's personal copy

characterized by the pulses of freshwater input in the presence of mussel farm in a shallow system. From the comparison with the euryhaline waters of the Gulf of Venice (Pugnetti et al. 2008), the plankton biomass in the Mar Piccolo was higher, but mainly on the account of the small-sized planktonic fractions. Here, heterotrophic prokaryotes dominated over the large-sized plankton biomass with the evidence of the noticeably lower presence of grazers, especially mesozooplankton, if compared to the seasonal dynamics of other estuarine environments such as Chesapeake Bay (Glibert et al. 1991). The total plankton C pool of the Mar Piccolo was comparable to that reported in the Gulf of Trieste (Fonda Umani et al. 2012), characterized by more pronounced heterotrophy, also in this case mostly on the account of the heterotrophic prokaryotes but with low proportion of grazers in the trophic web. The major discrepancy, however, regarded mesozooplankton that displayed an overall lower abundance in the Mar Piccolo, up to 1 or 2 orders of magnitude with respect to the coastal environments of the Gulf of Trieste (Fonda Umani et al. 2012) and the Gulf of Venice (Acri et al. 2004) where the mussel farming was present and with respect to an offshore stations (Holligan et al. 1984), as reported in the Online Resource 1.

Among the investigated stations in the Mar Piccolo, only coastal st. 2B displayed a marked difference in community structure and was characterized also by higher plankton biomass, as has been inferred from Fig. 5. Even if none of the parameters analyzed in this study clearly evidenced the reason of different plankton structures of st. 2B with respect to the other stations, it is plausible that an external source played a key role in shaping the planktonic structure by favouring the following processes: more intense microphytoplankton proliferation in February and April (limited to the surface level), more pronounced picophytoplankton bloom in October and the more abundant presence of heterotrophic prokaryotes in June. This peculiarity of coastal st. 2B could be attributable to the different compositions of organic and inorganic matrixes since the presence of the terrigenous input, detected by means of stable isotopes analysis, was slightly higher at that station (Cibic et al. 2015).

Plankton seasonal dynamics

The ecological succession observed in the present study which was mainly driven by the seasonal variability of the environmental features, as inferred from the nMDS ordination analysis (Fig. 4), fits with previous findings from studies conducted in the Mar Piccolo (Caroppo and Cardellicchio 1995; Caroppo et al. 2006) and other coastal areas of the Mediterranean such as the Adriatic Sea (Fonda Umani et al. 1992), the Gulf of Naples (Carrada et al. 1992) and the Ligurian Sea (Innamorati et al. 1990).

In April, the nutrient availability favoured the bloom of large-sized phytoplankton (microphytoplankton), taking them into account for a third of the total planktonic C. The silicates were progressively consumed due to the diatom proliferation leading to the depletion of phosphates that was observed also in June (Kralj et al. 2015). In April, also heterotrophic prokaryotes were largely present, especially at the surface of st. 1I. Their high abundance, which also represented the maximum for the study period, matched with the peak of the abundance in heterotrophic nanoflagellates, suggesting the efficient proliferation of heterotrophic flagellates sustained by the availability of the prokaryotic prey. Also, the mesozooplankton abundance at st. 1I was the highest together with st. 1E, even if their overall abundance resulted low in that period (<140 individuals m^{-3}). The abundance of microzooplankton in April considerably differed between the two basins, with higher abundances encountered in the inner basin (especially at st. 2B) probably sustained by the rising availability of the phytoplankton prey.

In June, the amount of the whole plankton biomass was comparable to that observed in April, but with a heterotrophic prokaryotic biomass that raised to 45 % of the whole plankton C. In that period, nanoflagellates displayed their maxima, especially relative to their autotrophic fraction at the surface layer. These, in turn, probably constituted the prey for large microzooplankton organisms, such as those belonging to *Tintinnopsis* and *Eutintinnus* genera.

In October, the plankton C pool reached its maximum among the four surveys. The bloom of picophytoplankton, i.e. cyanobacteria belonging to *Synechococcus* spp. (Waterbury et al. 1979), was likely favoured by seawater temperature (Collos et al. 2009). Slightly increased availability of phosphates and lower inorganic nitrogen concentrations in the second inlet favoured the cyanobacterial proliferation. It is interesting to note the increasing gradient of viral abundances towards the inner inlet. As already reported above, the match between this peak of viruses and the maximum abundance of cyanobacteria suggests that viruses in that period were mostly constituted by cyanophages that successfully encounter, infect and proliferate on cyanobacterial hosts, likely contributing to terminate the picophytoplankton bloom.

In February, the biomass in the water column dropped to 261 μg C L^{-1} and was characterized by a high heterotroph-to-phototroph ratio. The heterotrophic prokaryotes were the major constituent of the planktonic C pool (54 %). Generally, all planktonic fractions displayed their minima with the exception of mesozooplankton that, especially in the first inlet, showed relatively high abundances. Moreover, high values of virus-to-prokaryote ratio (from 14 to 20) suggest the occurrence of intense lytic processes in the second inlet in February and, therefore, an intense C flow from the pool of heterotrophic prokaryotes into the dissolved organic one.

Author's personal copy

The implication of mussel cultures for the plankton web structuring

In the C flux investigation of the Mar Piccolo, the influence of intensive mussel culture must be unavoidably considered. Mussels, being filter feeders, retain a major portion of suspended particles. Considering the C content of flesh dry weight, equal to 1.6 % of the mussel dry weight (Caroppo et al. 2012), and the current estimates of the mussel harvest of 40,000 tons (Caroppo, personal communication), this would equal to 640 tons of C sequestered from the two basins of the Mar Piccolo. The presence of mussel farming does not only subtract a large amount of planktonic C but likely shapes the planktonic food web in the Mar Piccolo, in accordance with a study on *M. galloprovincialis* diet conducted in the northern Adriatic (Del Negro et al. 2014). In particular, recent study showed that microzooplankton plays an important role for the diet of *M. galloprovincialis* in the Gulf of Trieste, accounting for ~50 % of mussels' stomach content (Solidoro et al. 2010). Also harpaticoids, bivalves (larvae), cirripeds (nauplii), copepods (copepodites), gastropods (larvae) and undetermined eggs represented the highly desirable diet being characterized by the maximum selection index of ~+1 (Jacobs 1974), whereas only the copepods nauplii were partly positively selected (~0.25), hence constituting less desirable diet for the mussels. Thus, the particularly low biomass of mesozooplankton that was detected in the Mar Piccolo could be explained by high removal of mesozooplankton larval stages, thus further inhibiting the possibility of maintaining their standing stock. Unfortunately, no previous data on microzooplankton are available for the area and we cannot compare our results with micrometazoans previously recorded in the Mar Piccolo. According to Solidoro et al. (2010), planktonic microalgae represented a less desirable diet for *M. galloprovincialis*, never reaching the maximum selection index. Indeed, the high abundance of *Chaetoceros* spp. observed in our study could be due to the rejection (i.e. negative selection, index ~−1) by *M. galloprovincialis* as referred by Solidoro et al. (2010) for the Gulf of Trieste.

The mesozooplankton biomass between the two inlets did not statistically differ, but generally higher mesozooplankton C was found in the first inlet in all seasons with the only exception of the October survey. Based on the fact that the mussel farms are mainly present in the second inlet, we suppose that the presence of scarce mesozooplankton in the second inlet is likely due to the mussel filter feeding as mussels and zooplankton compete for the same food resources (Rodhouse and Roden 1987) or being larval stages rapidly removed by the mussels (Cranford et al. 2006). By contrast, virio- and picoplankton apparently dominated in the coastal areas devoted to the mussel farming, likely because they are too small to be captured by mussels. Mussels retain particles >3 μm, and in the case of heterotrophic prokaryotes, they are able to eject them as pseudofaeces (Dame 2012). Moreover, the proliferation of heterotrophic prokaryotes together with the evidence of their increased metabolic activity (Cibic et al. 2015) could be attributed to their role in organic matter decomposition originating from mussel's faeces and pseudofaeces.

By analyzing the life cycle of mussels, they exhibit, as other marine bivalves in temperate latitudes, cyclic changes in reproductive stages in relation to the seasonal oscillations of environmental conditions. For *M. galloprovincialis* in the Mar Piccolo, Matarrese et al. (1993) reported one phase of reproductive activity (autumn-winter), one in which a decrease in gametogenic activity begins (spring), and one of quiescence (summer), when the water temperature rises to >20 °C. The highest values of plankton biomass have been detected in October when the energy of mussels is conveyed towards the reproductive activity rather than to the biomass increase. This is also in accordance with the out-of-phase fluctuations between mussel yield and zooplankton that was observed for the intensive cultivation of *Mytilus edulis* in Killary Harbour (Rodhouse and Roden 1987). Interestingly, high values of the C biomass match with summer survey when a high biomass of mussels is removed for the sale.

Finally, the different features of the two inlets of the Mar Piccolo with respect to the presence of mussels farms, trophic state and anthropogenic pollutants could be responsible for the signal of a slightly higher plankton pool that was observed in the second inlet. In this sub-basin, also a higher productivity was observed, as supported by the in situ production estimates (Cibic et al. 2015). According to these findings, both primary and secondary C production resulted lower in the first inlet that is considered also the more contaminated one (Cardellicchio et al. 2007) and where the shellfish farming for commercial use is forbidden and, in case, limited to the juvenile stages only (G. Portacci, personal communication). Despite the higher C sequestration due to the extensive mussel farms in the second inlet, there are at least two factors that could have contributed to the major variability among different plankton groups and higher plankton C pool and productivity (Cibic et al. 2015) in the second inlet: (1) more lagoonal features and (2) lower contamination levels (Cibic et al. 2015) and, therefore, a less detrimental effect on the pelagic system as already observed on the benthic domain (Franzo et al. 2015; Rubino et al. 2015). On the other hand, it is plausible that the factors that concurred in determining the decreased mussel production in the Mar Piccolo in the recent years are (1) the enlargement of the mussel farms in 2006 that exceeded the carrying capacity of the system (Caroppo et al. 2012) and (2) the removal of a half of urban waste outflows in 2000 that reduced the nutrient load (Kralj et al. 2015) and, in turn, diminished the phytoplankton biomass (Caroppo et al. 2015).

Author's personal copy

Plankton structure tendency towards small-sized fractions

Although the overall C pool does not seem to be reduced compared to the past (Caroppo et al. 2012) but mostly repartitioned among the pico-sized organisms, the C transfer along the meso-micro-nano-picoplankton is longer and, therefore, more dispersive compared to the grazing on the microphytoplanktonic primary producers. Thus, the low standing stock of mesozooplankton revealed in our study and supported by the previous observation by Belmonte et al. (2013) could be almost partly related to the general decrease of the prey availability due to the bottom-up controlled phytoplankton standing stocks. Comparing the phytoplankton abundance of the Mar Piccolo in the first half of 1990 and in the last decade, Caroppo et al. (2015) evidenced the decrease in phytoplankton abundance in the first inlet only, likely related to the closure of the most important urban wastewater pipes, as confirmed by Kralj et al. (2015) through the study of the changes in physical and chemical conditions.

Beside the tendency towards small-sized fractions, also the tendency towards small-sized species was observed, probably associated with increased confinement (Riccardi 2010) as in the case of small copepods (Turner 2004). It cannot be excluded that a mesh size of 200 μm used in the present study for the mesozooplankton samplings probably has not retained significantly smaller species (Riccardi 2010). The small-sized *Chaetoceros* spp., belonging to the nanoplanktonic fraction that was widely observed, probably undermined the large-sized species according to the historical records in the Mar Piccolo (Caroppo et al. 2015). Also within the microzooplankton population the presence of particularly small tintinnid *Eutintinnus* cfr. *tubulosus* was encountered, with especially high abundance in the second inlet in June, species normally present in the Ionian and Adriatic seas.

Moreover, the tendency towards small-sized planktonic fractions could further reduce the C transfer along the food web due to the viral shunt (Suttle 2005). Taking into account that the viral infection is a density-dependent process (Wiggins and Alexander 1985) and that a major abundance of prokaryotes is needed to obtain the same amount of C pool of the large-sized plankton, the intense viral lysis of these communities would enhance the C transfer towards the dissolved organic pool, being repeatedly recycled between the dissolved and prokaryotic C pool.

Conclusions

The present study represents the first integrated investigation on the structure of the planktonic trophic web in the Mar Piccolo of Taranto, the area subject to different natural and anthropogenic stress factors. The study unveiled plankton community composition in terms of abundance and biomass and its annual succession. Moreover, it evidenced that high portion of the C pool was constituted by small-sized planktonic fractions, mostly prokaryotes and especially in the second inlet. The tendency towards the copious presence of small-sized species among phyto-, micro- and mesozooplankton communities (such as *Oithona* spp., *Eutintinnus* cfr. *tubulosus* and *Chaetoceros* spp.) was observed as well.

The dynamics of the plankton community were likely influenced by the mussel cultures throughout different processes: (1) due to the C sequestration into the mussel harvest without accounting for the energy dispersed in growth processes, (2) by influencing the C pathways within the plankton trophic web and (3) by shaping the structure of plankton communities operated by selective grazing. Our hypothesis is that the lower mussel harvest in terms of flesh dry weight that was observed in the Mar Piccolo in the last decade could be attributable to the following processes tightly coupled with the plankton dynamics: (1) to the reduced C transfer towards the upper trophic levels due to the impoverishment of the mussels diet, i.e. phytoplankton prey, grazers and the larval stages of the latter, and (2) the tendency towards the more intense recycle of C within the microbial loop due to the enhanced viral lysis. All these dynamics are unavoidably related to the environmental changes that resulted in the enhanced bottom-up control of phytoplankton communities as a consequence of the reduced nutrient load (Kralj et al. 2015).

Generally, higher plankton biomass in the second inlet, which also represents the main mussel farming area in the Mar Piccolo, represented probably a positive feedback effect to overwhelm high C sequestration. However, there is a need of further studies to investigate the importance of heterotrophic prokaryotes and suspended particles in the diet of *M. galloprovincialis* as well as the further investigations on microzooplankton to assess the role of micrometazoans as a food source in the mussel diet. In order to obtain direct estimates on C transfer with respect to different seasons, grazing experiments could be taken into account; moreover, the transfer of pollutants within the planktonic trophic web up to higher trophic levels should also be considered. To deepen the knowledge on plankton dynamics, the higher sampling frequency is needed, also along the gradient towards the open sea in order to depict the variability due to the confinement effect.

Acknowledgments We would like to thank to M. Kralj for providing the chemical data and F. Cerino for her stimulating discussions. We are truly grateful to the anonymous reviewers for their valuable suggestions for improving the manuscript. The activities described in this publication were funded by the Project Bandiera RITMARE (Ricerca Italiana per il Mare), coordinated by the National Research Council and funded by the Ministry for Education, University and Research within the National Research Programme 2011–2013.

Author's personal copy

References

Acri F, Benardi Aubry F, Berton A, Bianchi F, Boldrin A, Camatti E, Comaschi A, Rabitti S, Socal G (2004) Plankton communities and nutrients in the Venice lagoon. Comparison between current and old data. J Mar Syst 51:321–329

Alabiso G, Giacomini M, Milillo M, Ricci P (2006) Chemical-physical conditions in the Taranto sea system from 2002 to 2004. Biol Mar Medit 13(1):1055–1058

Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr 41(1):41–51

Andersen P, Throndsen J (2004) Estimating cell numbers. In: Hallegraeff GM, Anderson DM, Cembella A (eds) Manual on harmful marine microalgae. Monographs on oceanographic methodology n. 11. UNESCO, Paris, pp 99–130

Azam F, Malfatti F (2007) Microbial structuring of marine ecosystems. Nature 5:782–789

Beers JR, Stewart GL (1970) Numerical abundance and estimated biomass of microzooplankton. In: Strickland JDH (ed) The ecology of the plankton off La Jolla, California, in the period April through September 1967. University of California Press, Berkeley, pp 67–87

Belmonte G, Fanelli G, Gravili C, Rubino F (2001) Composition, distribution and seasonality of zooplankton in Taranto seas (Ionian Sea, Italy). Biol Mar Medit 8(1):352–362

Belmonte G, Vaglio I, Rubino F, Alabiso G (2013) Zooplankton composition along the confinement gradient of the Taranto Sea System (Ionian Sea, south-eastern Italy). J Mar Syst 128:222–238

Bloem J, Bar-Gilissen MJ, Cappenberg TE (1986) Fixation, counting, and manipulation of heterotrophic nanoflagellates. Appl Environ Microbiol 52:1266–1986

Boissonneault-Cellineri KR, Mehta M, Lonsdale DJ, Caron DA (2011) Microbial food web interactions in two long island embayments. Aquat Microb Ecol 26:139–155

Bouvy M, Bettarel Y, Bouvier C, Domaizon I, Jacquet S, Le Floc'h E, Montanié H, Mostajir B, Sime-Ngando T, Torréton JP, Vidussi F, Bouvier T (2011) Trophic interactions between viruses, bacteria and nanoflagellates under various nutrient conditions and simulated climate change. Environ Microbiol 13(7):1842–1857

Cabrini M, Andri M, Cok S, Pecchiar I, Fonda Umani S (2002) Carbon partitioning among the first trophic levels in the north western Adriatic basin. Chem Ecol 18(1–2):95–105

Cardellicchio N, Buccolieri A, Giandomenico S, Lopez L, Pizzuli F, Spada L (2007) Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, southern Italy). Mar Pollut Bull 55(10–12):451–458

Cardellicchio N, Annicchiarico C, Di Leo A, Giandomenico S, Spada L (2015) The Mar Piccolo of Taranto: an interesting ecosystem for the study of the environmental problems. Spec Issue Ritmare. doi:10.1007/s11356-015-4924-6

Caron DA, Lim EL, Miceli G, Waterbury JB, Valois FW (1991) Grazing and utilization of chrococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. Mar Ecol Prog Ser 76:205–217

Caroppo C (1996) Phytoplankton successions and biodiversity in Mar Piccolo of Taranto. SItE Atti 17:355–358

Caroppo C, Cardellicchio N (1995) Preliminary study on phytoplankton communities of Mar Piccolo in Taranto (Ionian Sea). Oebalia 21:61–76

Caroppo C, Turicchia S, Margheri MC (2006) Phytoplankton assemblages in coastal waters of the northern Ionian Sea (eastern Mediterranean), with special reference to cyanobacteria. J Mar Biol Assoc UK 86:927–937

Caroppo C, Giordano L, Rubino F, Bisci AP, Hopkins TS (2010) Phytoplankton communities as indicators of ecological change in the anthropogenically impacted Mar Piccolo of Taranto (Ionian Sea). Biol Mar Medit 17(1):102–105

Caroppo C, Giordano L, Palmieri N, Bellio G, Bisci AP, Portacci G, Sclafani P, Hopkins TS (2012) Progress towards sustainable mussel aquaculture in Mar Piccolo, Italy. Ecol Soc 17(3):10

Caroppo C, Musco L, Stabili L (2014) Planktonic assemblages in a coastal Mediterranean Sea subjected to anthropogenic pressure. J Geogr Nat Disaster 4(1):121

Caroppo C, Cerino F, Auriemma R, Cibic T (2015) Phytoplankton dynamics with a special emphasis on harmful algal blooms in the Mar Piccolo of Taranto (Ionian Sea, Italy). Environ Sci Pollut Res. doi:10.1007/s11356-015-5000-y

Carrada GC, Ribera d'Alcalà M, Saggiomo V (1992) The pelagic system of the Southern Tyrrhenian Sea. Some comments and working hypotheses. Proc of IX AIOL Congress: 151–166

Cibic T, Bongiorni L, Borfecchia F, Di Leo A, Franzo A, Giandomenico S, Karuza A, Micheli C, Rogelja M, Spada L, Del Negro P (2015) Ecosystem functioning approach applied to a large contaminated coastal site: the study case of the Mar Piccolo of Taranto (Ionian Sea). Environ Sci Pollut Res. doi:10.1007/s11356-015-4997-2

Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation. Primer-E- Ltd, Plymouth

Collos Y, Bec B, Jauzein C, Abadie E, Laugier T, Lautier J, Astoureaud A, Souchu P, Vaquer A (2009) Oligotrophication and emergence of picocyanobacteria and a toxic dinoflagellate in Thau Lagoon, southern France. J Sea Res 61:68–75

Cranford PJ, Anderson R, Archambault P, Balch T, Bates SS, Bugden G, Callier MD, Carver C, Comeau L, Hargrave B, Harrison WG, Horne E, Kepkay PE, Li WKW, Mallet A, Ouellette M, Strain P (2006) Indicators and thresholds for use in assessing shellfish aquaculture impacts on fish habitat. Can Dept Fisheries and Oceans, Ottawa, 125 p. DFO Can Sci Advis Sec Res Doc 2006/034

Crisafi P, Crescenti M (1975) Conseguenze delle attività umane sullo zooplancton del Mare di Taranto. Boll Pesca Piscicolt Idrobiol 30(2):207–216

Dame RF (2012) Ecology of marine bivalves: an ecosystem approach. Francis & Taylor, Boca Raton

De Angelis CM, Della Valle R (1959) Il ciclo stagionale del plancton in rapporto alle condizioni fisico-chimiche del Mar Piccolo e del Mar Grande di Taranto. Boll Pesca Piscicolt Idrobiol 14:21–43 (in Italian)

De Pascalis F (2013) Correnti e T/S nei mari di Taranto. CNR Report 09/2013 SP3_WP4_AZ5_UO01_D01 RITMARE La Ricerca Italiana per il MARE (in Italian)

Del Negro P, Monti M, Beran A, De Vittor C, Celussi M, Libralato S, Solidoro C (2014) Plankton as inexhaustible resource for the mussel farms of the Gulf of Trieste. Biol Mar Medit 21(1):14–16

Duarte CM, Agusti S, Gasol JM, Vaquè D, Vazquez-Dominguez E (2000) Effect of nutrient supply on the biomass structure of plankton communities: an experimental test on a Mediterranean coastal community. Mar Ecol Prog Ser 206:87–95

Edler L (1979) Recommendations for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. Balt Mar Biol Public 5:1–37

Fenchel T (1982) Ecology of heterotrophic microflaggelates. IV. Quantitative occurrence and importance as bacterial consumers. Mar Ecol Prog Ser 9:35–42

Fonda Umani S, Franco P, Ghirardelli E, Malej A (1992) Outline of oceanography and the plankton of the Adriatic Sea. In: Colombo G, Ferrari I, Ceccherelli VU, Rossi R (eds) Marine eutrophication and population dynamics. Olsen & Olsen, Fredensborg, pp 347–365

Fonda Umani S, Malfatti F, Del Negro P (2012) Carbon fluxes in the pelagic ecosystem of the Gulf of Trieste. Estuar Coast Shelf Sci 115:170–185

Author's personal copy

Franzo A, Auriemma R, Nasi F, Vojvoda J, Pallavicini A, Cibic T, Del Negro P (2015) Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy): focus on heterotrophic pathways. Environ Sci Pollut Res. doi:10.1007/s11356-015-5339-0

Giesbrecht W (1891) Elenco dei copepodi pescati dalla R. Corvetta 'Vettor Pisani' secondo la loro distribuzione geografica. Atti della Accademia Nazionale dei Lincei, Classe di Scienze Fisiche Matematiche e Naturali Rendiconti (4)7 sem. 2:63–68, 276–282 (in Italian)

Glibert PM, Garside C, Fuhrman JA, Roman MR (1991) Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs. Limnol Oceanogr 36(5):895–909

Hansen HP, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (eds) Methods of seawater analysis, 3rd edn. Wiley, Weinheim, pp 159–228

Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley M (2000) ICES zooplankton methodology

Hillebrand H, Durselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403–424

Holligan PM, Harris RP, Newell RC, Harbour DS, Head RN, Linley EAS, Lucas MI, Tranter PRG, Weekley CM (1984) Vertical distribution and partitioning of organic carbon in mixed, frontal and stratified waters of the English Channel. Mar Ecol Prog Ser 14:111–127

Innamorati M, Lazzara L, Nuccio C, Senesi P, Buracchi G (1990) Variazioni stagionali e spaziali delle cenosi fitoplanctoniche nel Mar Ligure (1979–1982). Oebalia 16(1):93–102 (in Italian)

Jacobs J (1974) Quantitative measurement of food selection: a modification of the forage ratio and Ivlev's selectivity index. Oecologia 14: 413–417

Jacquet S, Lennon JF, Marie D, Vaulot D (1998) Picoplankton population dynamics in coastal waters of the northwestern Mediterranean Sea. Limnol Oceanogr 43:1916–1921

Kan J, Wang K, Chen F (2006) Bacterioplankton community in Chesapeake Bay: predictable or random assemblages. Limnol Oceanogr 51(5):2157–2169

Karuza A, Fonda Umani S, Del Negro P (2012) The (un)coupling between viruses and prokaryotes in the Gulf of Trieste. Estuar Coast Shelf Sci 115:87–97

Kralj M, Comici C, DeVittor C, Alabiso G (2015) Recent evolution of the physical-chemical characteristics of a site of national interest—the Mar Piccolo of Taranto (Ionian Sea)—and changes over the last 20 years. Environ Sci Pollut Res. doi:10.1007/s11356-015-5198-8

Kruskal JB, Wish M (1978) Multidimensional scaling. Sage, Beverly Hills, 96 pp

Lee S, Fuhrman JA (1987) Relationship between biovolume and biomass of naturally derived marine bacterio-plankton. Appl Environ Microbiol 54(6):1426–1429

Legendre L, Rivkin RB (2002) Pelagic food webs: responses to environmental processes and effects on the environment. Ecol Res 17:143–149

Lessard E (1991) The trophic role of the heterotrophic dinoflagellates in diverse marine environments. Mar Microb Food Webs 2:49–58

Lorenzen CJ, Jeffrey SW (1980) Determination of chlorophyll in seawater. UNESCO Tech Pap Mar Sci 35:1–20

Lorrain A, Savoye N, Chauvaud L, Paulet YM, Naulet N (2003) Decarbonation and preservation method for the analysis of organic C and N contents and stable isotope ratio of low-carbonated suspended particulate material. Anal Chim Acta 491:125–133

Lund JWG, Kilpling C, Le Cren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimation by counting. Hydrobiology 11:143–170

Matarrese A, Tursi A, Costantino G, Pollicoro R (1993) The reproductive cycle of *Mytilus galloprovincialis* in the Mar Piccolo and Mar Grande of Taranto (Ionian Sea). Oebalia 19:1–11

Melaku Canu D, Solidoro C, Umgiesser G, Cucco A, Ferrarin C (2012) Assessing confinement in coastal lagoons. Mar Poll Bull 64:2391–2398

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms and other protest plankton. Limnol Oceanogr 45(3):569–579

Noble RT, Fuhrman JA (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat Microb Ecol 14:113–118

Parenzan P (1984) Il Mar Piccolo di Taranto. Collana documenti della CCIAA, Taranto 25:319 (in Italian)

Pastore M (1993) Mar Piccolo. Nuova Apulia, Martina: 166 (in Italian)

Pella E, Colombo B (1973) Study of carbon, hydrogen and nitrogen determination by combustion-gas chromatography. Mikrochim Acta 5:697–719

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948

Pugnetti A, Bazzoni AM, Beran A, Bernardi Aubry F, Camatti E, Celussi M, Coppola J, Crevatin E, Del Negro P, Paoli A (2008) Changes in biomass structure and trophic status of the plankton communities in a highly dynamic ecosystem (Gulf of Venice, northern Adriatic Sea). Mar Ecol 29:367–374

Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34:1097–1107

Raymont JEG (1983) Plankton and productivity in the oceans: vol 2 zooplankton. Pergamon, Oxford, 824 pp

Riccardi N (2010) Selectivity of plankton nets over mesozooplankton taxa: implications for abundance, biomass and diversity estimation. J Limnol 69(2):287–296

Rodhouse PG, Roden CM (1987) Carbon budget for the coastal inlet in relation to intensive cultivation of suspension-feeding bivalve mollusks. Mar Ecol Prog Ser 36:225–236

Rubino F, Cibic T, Belmonte M, Rogelja M (2015) Microbenthic community structure and trophic status of sediments in Mar Piccolo of Taranto (Mediterranean, Ionian Sea). Environ Sci Pollut Res. doi:10.1007/s11356-015-5526-z

Sato T (1913) Pelagic copepods (n. 1). Scientific reports, Hokkaido Fisheries Experimental Station 1: 1–79

Sharp JH (1974) Improved analysis for 'particulate' organic carbon and nitrogen from seawater. Limnol Oceanogr 19(6):984–989

Sherr BF, Sherr EB, Pedros-Alio C (1989) Simultaneous measurement of bacterioplankton production and protozoan bacterivory in estuarine water. Mar Ecol Prog Ser 54:209–219

Sieburth JM (1979) Sea microbes. Oxford University Press, New York, 491 pp

Solidoro C, Del Negro P, Libralato S, Melaku Canu D (2010) Sostenibilità della mitilicoltura triestina Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS, 88

Stabili L, Caroppo C, Danovaro R (2004) Virioplankton abundance in relationship with the pico- and phytoplankton dynamics along gradients of anthropogenic impact (Ionian Sea, Mediterranean Sea). Biol Mar Medit 11(3):116

Strusi A, Pastore M (1975) Osservazioni idrografiche nel Mar Grande e nel Mar Piccolo di Taranto. Campagna 1970–71. Oebalia 1:1–64

Suttle CA (2005) Viruses in the sea. Nature 437:356–361

Turner JT (2004) The importance of small planktonic copepods and their roles in pelagic marine food webs. Zool Stud 43:255–266

Umgiesser G, Scroccaro I, Alabiso G (2007) Mass exchange mechanisms in the Taranto Sea. Transit Water Bull 2:59–71

Utermöhl H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitt Int Ver Theor Angew Limnol 9:1–38

Author's personal copy

Verity P, Langdon C (1984) Relationship between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. J Plankton Res 6(5):859–868

Waterbury JB, Watson SW, Guillard RRZ, Brand LE (1979) Widespread occurrence of a unicellular marine, planktonic cyanobacterium. Nature 277:293–294

Weinbauer MG (2004) Ecology of prokaryotic viruses. FEMS Microbiol Rev 28:127–181

Wiggins BA, Alexander M (1985) Minimum bacterial density for bacteriophage replication: implications for significance of bacteriophages in natural ecosystems. Appl Environ Microbiol 49:19–23

Wilhelm SW, Suttle CA (1999) Viruses and nutrient cycles in the sea. Bioscience 49(19):781–788

Zingone A, Totti C, Sarno D, Cabrini M, Caroppo C, Giacobbe MG, Luglie A, Nuccio C, Socal G (2010) Fitoplancton: metodiche di analisi quali-quantitativa. In: Socal G, Buttino I, Cabrini M, Mangoni O, Penna A, Totti C (eds), Metodologie di studio del plancton marino. ISPRA, Manuali e Linee Guida 56: 213–237 (in Italian)