Structural and functional response of phytoplankton to reduced river inputs and anomalous physical-chemical conditions in the Gulf of Trieste (northern Adriatic Sea)

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HIGHLIGHTS

- Phytoplankton structure and function were related to anomalous seawater conditions.
- Microphytoplankton and cyanobacteria densities decreased (up to 60% and 47%).
- The late spring diatom bloom was not reflected in high photosynthetic rates.
- An unusually high primary production in autumn was concomitant to a mucilage event.
- The typical seasonal succession of pelagic phototrophs was altered.

GRAPHICAL ABSTRACT
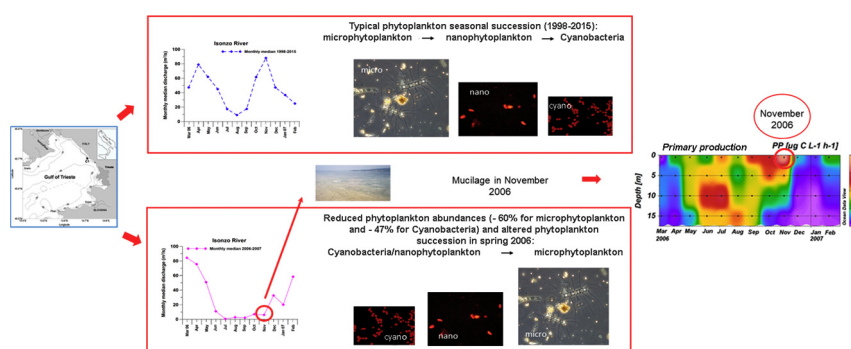
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ABSTRACT

We studied the influence of anomalous meteorological and hydrological conditions that occurred in the Gulf of Trieste from March 2006 to February 2007 on phytoplankton structure and function. We computed monthly mean (or median) air temperature, total precipitation, wind speed, river discharge, seawater temperature, salinity, photosynthetic available radiation (PAR), cyanobacteria, nano- and microphytoplankton abundances during the study year and compared them to climatological (1999–2014 for PAR; 1999–2007 for nanophytoplankton; 1998–2015 for the other variables) mean/median data. We then related the cyanobacteria (0.2–2 μm), nano- (2–20 μm) and microphytoplankton (20–200 μm) of the study year to inorganic nutrient concentrations. Median river inputs in October and November were 9- and 15-fold lower, respectively, than the time series medians, with consequent high salinity from May to November (up to +1.26 compared to the climatological data). Monthly mean seawater temperatures were lower than the climatological values (−2.95 °C at the surface) from March to August 2006 and higher (+2.15 °C at the surface) from September to February 2007. Reductions in freshwater input and nutrient depletion were likely responsible for a decrease in microphytoplankton (median annual abundance over 60% lower than the climatologic median) and cyanobacteria (up to 47% lower than the climatology). Significant seasonal differences in cyanobacteria and microphytoplankton abundances ($R_{ANOSIM} = 0.52$; $p < 0.05$), as well as in seawater temperature and salinity ($R_{ANOSIM} = 0.73$; $p < 0.05$) between the study period and the climatology were highlighted. The late spring diatom bloom was not reflected in high photosynthetic rates whereas an unusually high primary production was estimated in November (7.11 ± 1.01 μgC L^{-1} h^{-1}), when a mucilage event occurred due to very stable atmospheric

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and oceanographic conditions. The typical seasonal succession of pelagic phototrophs (micro-, nanophytoplankton and cyanobacteria) was altered since an exceptional cyanobacteria bloom first developed in April, followed by a delayed diatom bloom in May.

1. Introduction

Primary production measurement in marine waters is one of the most important tools to understand ecosystem functioning and the transport of inorganic/organic matter through the food web (Williams et al., 2002). Overall, shallow coastal photic systems are among the most productive on the planet (Odum, 1983). However, in oligotrophic systems like the Mediterranean Sea, coastal production largely depends on rivers and freshwater-borne nutrient inputs (Mozetič et al., 2012 and references therein), and internal recycling of nutrients particularly from sediments under seasonally elevated temperatures (Kennish et al., 2014). In smaller basins and gulfs, the timing and magnitude of the biological responses depend on a combination of different factors (Malej et al., 1995). Aside from nutrients, coastal processes are largely influenced by physical factors such as light, temperature, stratification, winds and local currents that are key parameters regulating pelagic processes. Variations in precipitation and evaporation, together with wind forcing and density gradients greatly affect the ecosystem primary production. Short-term, acute meteorological perturbations, including heat waves and droughts, cause extreme fluxes in hydrodynamics and biotic responses in coastal systems (Kennish et al., 2014). Because of their small size, rapid nutrient uptake and growth rates, phytoplankton are particularly responsive to variations in environmental conditions. Deviations from typical phytoplankton abundances and compositional patterns through time can thus be used to detect the occurrence of ecological changes in shallow coastal photic systems (Valdes-Weaver et al., 2006).

The Gulf of Trieste, located in the northern part of the Adriatic Sea, is a semi-enclosed basin with a maximum depth of 25 m, where the response to any kind of environmental variation can be rapid and thus easier to detect. In this area, the carbon cycle is strongly affected by freshwater input, mainly from Isonzo River (Cozzi et al., 2012), and atmospheric forcing, mostly on account of the strong ENE Bora wind (Celio et al., 2006). A major disturbance event in the northern Adriatic Sea is the appearance of mucous aggregates; this has been recorded often in the past centuries (Precali et al., 2005 and references therein) and severely affects ecosystem functioning. Phytoplankton development, in terms of microalgal blooms, community succession (Malej et al., 1995; Cabrini et al., 2012; Mozetič et al., 2012) and photosynthetic activity (Fonda Umani et al., 2004, 2007; Ingrosso et al., 2016) are highly dependent on nutrient availability originating from freshwater discharges, and therefore respond to seasonal and interannual variations of riverine fluxes. On a seasonal basis, the pelagic ecosystem of the gulf shifts from a more eutrophic condition, typical of the late winter-spring season, when sufficient inorganic nutrients are available to sustain the main diatom bloom of the year, to an oligotrophic condition in summer-autumn dominated by pico-sized photoautotrophs (Fonda Umani et al., 2012). Therefore, the typical phytoplankton seasonal succession displays the following pattern: a late winter-early spring diatom bloom, followed by a nanophytoplankton bloom in late spring-early summer, a cyanobacteria peak in late summer-early autumn and a second relative diatom maximum, usually in November. From a long term perspective, phytoplankton density in the Gulf of Trieste was quite high from 1986 until the end of 1992. Thereafter, phytoplankton abundance declined to reach minimum values in the mid-1990s as a result of both climatic changes and environmental protection measures that progressively lowered the trophic state of the system: from a eutrophic, nutrient-enriched system, to an oligotrophic, nutrient-depleted one

(Fonda Umani et al., 2004). In a recent study, Mozetič et al. (2012) analysing the phytoplankton trends and community changes in the period 1989–2009, have highlighted that the absolute minimum of chl *a* concentration and the second maximum of salinity were reached in 2006. In this particular year, the highest abundances of nanoflagellates were observed also, in their long time series.

The aim of this study was to investigate to what extent phytoplankton abundance, structure and photosynthetic activity were affected by the anomalous environmental conditions that occurred from March 2006 to February 2007. To highlight the possible effect of meteorological and hydrological variables on phytoplankton development, we computed monthly mean (or median) air temperature, total precipitation, wind speed, Isonzo River discharge, seawater temperature, salinity, photosynthetic available radiation (PAR), cyanobacteria, nano- and microphytoplankton during the study year and compared these data to climatological (1999–2014 for PAR; 1999–2007 for nanophytoplankton; 1998–2015 for the other variables) mean/median data. We further related cyanobacteria (0.2–2 μm), nano- (2–20 μm) and microphytoplankton (20–200 μm) abundances of the study year to inorganic nutrient concentrations. In order to investigate phytoplankton functional response to the observed anomalous physical-chemical conditions, we estimated the photosynthetic activity of total phytoplankton. In addition, from June to November 2006, we evaluated the contribution of the nanoplankton fraction (2–20 μm) to total primary production. Finally, we discussed the implications of very low phytoplankton abundances over the study year on the ecosystem functioning of the whole basin.

We hypothesized that because pelagic primary producers, in terms of standing stock and primary production, are largely controlled by nutrient availability in this shallow basin (as in many others), reductions in river flow and nutrient loads would lead to reduced phytoplankton abundances and primary production. We also hypothesized that the synergistic effect of anomalous physical-chemical conditions (nutrients, seawater temperature, wind forcing, *etc.*) could alter the typical seasonal succession of pelagic phototrophs and their primary productivity patterns. Our guiding questions were: i) How are phytoplankton abundance, structure and production affected by reductions in freshwater and nutrient inputs? ii) Could the synergistic effect of anomalous physical-chemical conditions alter the typical micro- → nanophytoplankton → cyanobacteria succession in this area and overall primary production? iii) What may be the consequences for upper trophic levels in terms of food availability?

2. Material and methods

2.1. Study area

The Gulf of Trieste is a small (~500 km^2) and shallow (maximum depth 25 m) semi-enclosed basin in the north-western part of the Adriatic Sea (Fig. 1). In this area, freshwater inputs and atmospheric forcing greatly influence seawater temperature, salinity and water column stratification (Malačič and Petelin, 2001). Seawater temperature displays seasonal oscillation from 8 °C (February) to 26 °C (August), whereas salinity in surface waters ranges between 24, in spring during high riverine discharge, and 38.3 (Celio et al., 2006). Typically, in winter, the water column is well-mixed, whereas during spring freshwater input and surface heating lead to thermohaline stratification. The period between May and September is characterised by strong density
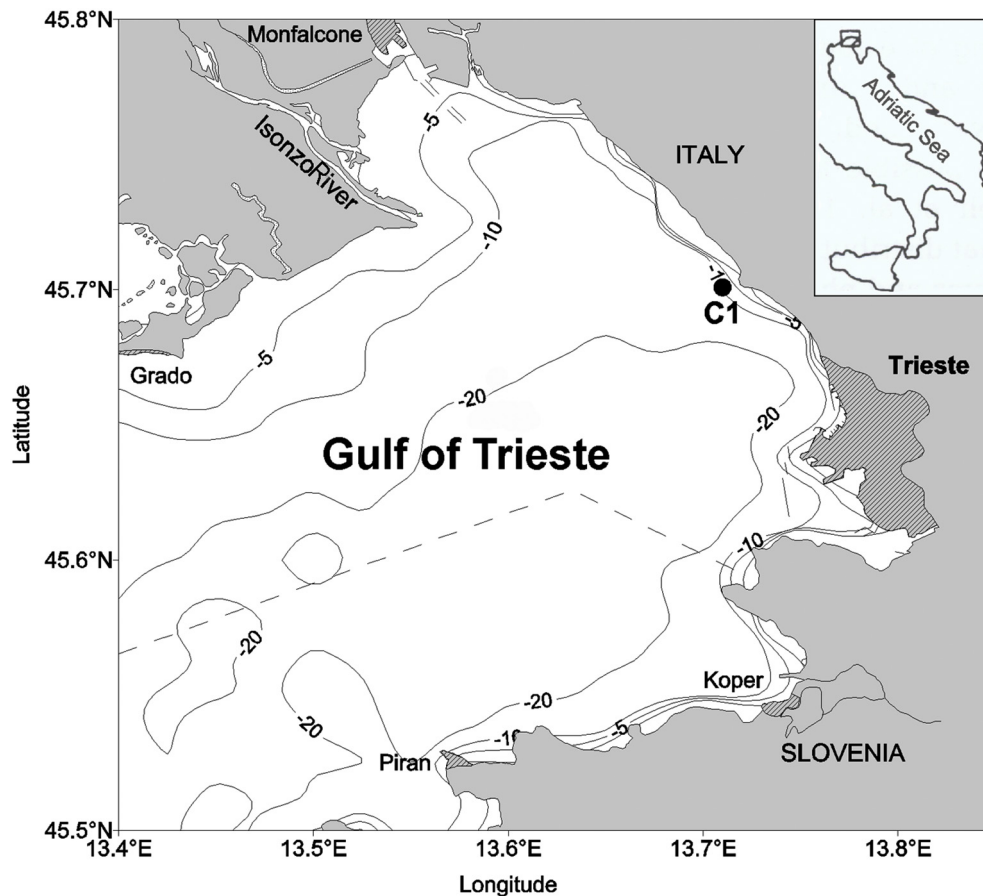
Fig. 1. Map of the study area showing the location of the sampling station (C1) in the northern Adriatic Sea.

gradients and the prevalence of respiration processes at the bottom layer, which determine low oxygen concentration and occasionally hypoxia events (Faganeli et al., 1985; Malej and Malačič, 1995). In autumn, convective and mechanical mixing, induced by water cooling and wind, disrupt vertical stratification, oxygenate the bottom water and distribute the re-generated nutrients to the entire water column.

The main riverine input in the Gulf of Trieste derives from Isonzo/Soča River on the north-western coast (Cozzi et al., 2012), which controls the salinity and nutrient concentration of the system with a highly variable outflow. On a seasonal time scale, however, spring and autumn are generally characterised by the highest river discharges (due to snowmelt and rain, respectively), while drought periods occur during winter and summer (Comici and Bussani, 2007).

The trophic status of the gulf also depends on the prevailing circulation patterns and not only on the intensity of the Isonzo River discharge rate. Circulation in the gulf is mainly cyclonic at the transitional and lower layer (10 m – bottom), while the surface layer (0–4 m) is affected by wind conditions (Stravisi, 1983). There are mainly two dominant winds: the SE Scirocco and the NE Bora (Stravisi, 1977, 1983). Bora-induced circulation is more frequent in autumn and in winter and it generates a cyclonic gyre at the surface layer, which causes a fast outflow of riverine waters from the gulf. When the Scirocco wind blows, instead, anticyclonic surface circulation favours eastward spreading of nutrient-rich freshwater, which increases primary production (Cantoni et al., 2003; Querin et al., 2006).

The Gulf of Trieste is also influenced by the Eastern Adriatic Current (EAC), a current flowing northwards along the Croatian coast and advecting warmer, saltier, and more oligotrophic waters coming from the Ionian Sea (Poulain et al., 2001). The ingression of EAC is more frequent in the cold seasons, when a cyclonic circulation is present, which can lead to oligotrophic conditions of the gulf.

2.2. Sampling and environmental data collection

Sampling was performed monthly at the Long-Term Ecological Research (LTER) station C1 (45°42′2″N and 13°42′36″E, maximum depth 17.5 m) located in the Gulf of Trieste (Fig. 1). From March 2006 to February 2007, seawater temperature and salinity were recorded by a CTD probe model Sea-Bird Electronics SBE 19plus SeaCAT profiler. These data were compared to the monthly means (1998–2015) that were recorded with the following probes: from March 1998 to October 1999 with an Idronaut mod. 401 probe; from November 1999 to September 2003 with an Idronaut mod. 316 probe. These CTD probes were calibrated with an interval of 6–12 months. From October 2003 onwards, a Sea Bird Electronics SBE 19 plus SeaCAT was used that was calibrated every year.

Air temperature, wind speed and total precipitation data (1998–2015), provided by ISMAR-CNR Trieste, were recorded at the Molo Fratelli Bandiera station (45°38′59.96″N; 13°45′8.07″E). The Isonzo River discharge data (1998–2015), provided by Regione Autonoma Friuli Venezia Giulia, was calculated by a rating curve from the hydrometric level registered at Turriaco station (13 km from the Isonzo River mouth). Photosynthetic available radiation (PAR) (1999–2014) was recorded by a PNF-300 Profiling Natural Fluorometer (Biospherical Instruments Inc., San Diego, USA).

From March 2006 to February 2007, discrete seawater samples were collected monthly with 5-L Niskin bottles at four depths (0.5, 5, 10, 15 m) for nutrient, phytoplankton and primary production analyses. Samples for the determination of dissolved inorganic nutrient concentrations (nitrite, $N-NO_2$; nitrate, $N-NO_3$; ammonium, $N-NH_4$; phosphate, $P-PO_4$; and silicate, $Si-Si(OH)_4$) were pre-filtered through glass-fibre filters, pore size 0.7 μm (Whatmann GF/F), stored at −20 °C and analysed by a flow injection spectrophotometric method on a five-channel Bran-

Luebbe Autoanalyzer 3 (Norderstedt, Germany), using standard procedures (Grasshoff et al., 1983).

2.3. Phytoplankton abundance

In the shallow waters of the Gulf of Trieste, pico-sized (0.2–2 μm) prokaryote assemblages are generally largely dominated by the coccoid cyanobacterium *Synechococcus* (Paoli et al., 2008), while *Prochlorochoccus* is nearly absent. Therefore, we only present the abundance of *Synechococcus*, but we use the term cyanobacteria in order to avoid classification up to genus level, as for the other phytoplankton groups.

Cyanobacteria abundance was estimated from 50 mL-samples, preserved in 0.2 μm pre-filtered formaldehyde (2% v/v final concentration) in the dark at 4 °C and processed within 48 h. Samples were filtered in triplicate (3–15 mL per subsample) through black-stained polycarbonate membranes, pore size 0.2 μm (Ø 25 mm, Nuclepore). Filters were mounted on microscope slides using non-fluorescent oil and stored at −20 °C. Enumeration was carried out using an Olympus BX51 epifluorescence microscope equipped with a 100 W high-pressure mercury burner (HPO 100 W/2) at 1000× final magnification. Cells were counted in randomly selected fields under a green (BP 480–550, BA 590 nm) filter set. A minimum of 200 cells was counted for each sample. Cyanobacteria cell numbers were converted into carbon biomass using a factor of 200 fg C cell^{-1} (Caron et al., 1991). This conversion factor was preferred to 250 fgC cell^{-1} to avoid an overestimation of the C biomass attributable to cyanobacteria since their abundance is considerably higher than that of larger phototrophic plankton.

For nanophytoplankton (2–20 μm) analysis, water samples were collected in 100 mL-dark bottles, fixed with pre-filtered glutaraldehyde (1% final concentration) and stored in the dark at 4 °C until the analyses. Subsamples (30 mL) were filtered at low pressure (max 100 mmHg) through black polycarbonate membranes, pore size 0.8 mm (Ø 25 mm, Nuclepore). Filters were stained with DAPI (4′6′-diamidino-2-phenylindole) and mounted on glass slides in three replicates for each sample (Porter and Feig, 1980). A minimum of 200 nanophytoplankton cells per filter were counted in randomly selected fields at 1000× final magnification using an Olympus BX51 microscope equipped with a mercury burner light. The set of filters for chlorophyll fluorescence (BP450–490/FT 510/LP520) was used. Cells were distinguished according to five different shapes (sphere, cone, ellipse, cylinder and as pennate diatoms) and six standard dimensional sizes (2–5 μm, 5–8 μm, 8–11 μm, 11–14 μm, 14–17 μm, 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).

For microphytoplankton (20–200 μm) analysis, samples were collected in 500 mL-dark bottles and preserved with pre-filtered and neutralized 1.6% formaldehyde (Throndsen, 1978). Cell counts were performed following the Utermöhl method (Utermöhl, 1958). A variable volume of seawater (25–50 mL) was settled depending on cell concentrations. Counting was performed in random fields (20–40) using an inverted microscope (Leitz Fluovert FS) equipped with phase contrast, at a final magnification of 320×. In addition, one half of the Utermöhl chamber was also examined at a magnification of 200×, to obtain a more correct evaluation of less abundant microphytoplankton taxa. The biovolume of microphytoplankton cells was calculated according to Edler (1979) and Hillebrand et al. (1999). Cell volumes were converted to carbon content using the formula introduced by Menden-Deuer and Lessard (2000).

Phytoplankton community loss factors (grazing pressure, viral infection) were not considered in this study.

Cyanobacteria, nano- and microphytoplankton abundance were plotted using ODV (Schlitzer, 2015) and weighted-average gridding (Ocean Data View User's Guide, Version 4.7.7, 2016).

2.4. Primary production

From March 2006 to February 2007, total primary production (PP) was estimated *in situ* by the ^{14}C technique (Steeman Nielsen, 1952). Water samples were poured into three light and one dark 70 mL polycarbonate carboy (Nalgene) per depth. The samples were kept in the dark for 30 min to stop residual photosynthetic activity. Subsequently, 0.22 MBq (6 μCi) of NaH^{14}CO$_3$ (DHI, Denmark) was added to each carboy. The samples were then fixed on a rosette, lowered to the corresponding sampling depth, and incubated for 2 h around noon. At the end of the incubation, the samples were stored in dark and cold conditions, and immediately transferred to the laboratory. From each sample, an aliquot of 25 mL was filtered through 0.2 μm polycarbonate filters (Nuclepore) applying low vacuum pressure (100 mmHg) in order to avoid cell damage. The filters were placed in 6 mL plastic scintillation vials (Perkin Elmer), acidified with 200 μL of HCl 0.5 M (Cibic and Virgilio, 2011) to remove the residual ^{14}C-bicarbonate not assimilated by the phytoplankton, and an aliquot of 5 mL of scintillation cocktail (Filter Count; Perkin-Elmer) was added to each vial. Disintegrations per minute (DPM) were measured by a QuantaSmart TRI-CARB 2900 TR Liquid Scintillation Analyzer (Packard BioScience, USA) including quenching correction, obtained using internal standards. Assimilation of carbon was calculated as described by Gargas (1975), assuming 5% isotope discrimination. Activities of the added NaH^{14}CO$_3$ and inorganic carbon concentration (tCO$_2$) were calculated on the basis of total alkalinity measured in the same samples. Standard deviation (SD) of three replicate values was below 25% except for rates close to zero for which SD was over 50%.

Nanoplankton Primary Production (nPP) was estimated from June to November 2006 following the same methodology described above for PP. In this case, to select the nanoplankton fraction, water samples were first pre-filtered through 20 μm mesh to remove larger organisms and then filtered through 2 μm polycarbonate filters (Nuclepore).

PP and nPP plots were drawn using ODV (Schlitzer, 2015) and applying the same gridding used for the three phytoplankton communities.

Volumetric PP and nPP data were converted to areal data using standard trapezoidal integration over the top 15 m of the water column.

Global solar radiation data were used to convert primary production hourly rates to daily rates. Data were recorded at the Molo Fratelli Bandiera station in Trieste and downloaded from the website of Regione Autonoma Friuli Venezia Giulia, ARPA FVG- Agenzia Regionale per la Protezione dell'Ambiente, OSMER – Osservatorio Meteorologico Regionale (www.osmer.fvg.it).

2.5. Statistical analyses

Meteorological, physical and biological data of the study year were compared to the monthly mean climatology. The latter was computed on seawater temperature, salinity, Isonzo River discharge and meteorological data recorded between March 1998 and February 2015, and on PAR data measured from March 1999 to December 2014. For cyanobacteria and microphytoplankton, climatology was computed on data from March 1998 to February 2015, while for nanophytoplankton on data from March 1999 to February 2007. The monthly mean climatology was computed for each parameter at four sampling depths (0.5, 5, 10 and 15 m), except for surface PAR measurements that were carried out at 1 m instead of 0.5 m in order to reduce light scattering in the coastal waters. Whenever CTD and PAR measurements were performed with different sampling frequency (weekly and biweekly), data were averaged to a monthly value. The Isonzo River climatology was displayed as monthly median values (rather than monthly means) in order to exclude the extremely high values that were occasionally observed. The monthly climatologies were compared to the data of the study year (March 2006–February 2007).

To analyse the temporal pattern of the phytoplankton community structure and primary production in the study year, a non-metric

Multi-Dimensional Scaling (*n*MDS) (Kruskal and Wish, 1978) ordination method was applied using a data matrix of cyanobacteria, nano- and microphytoplankton monthly abundances and primary production values at each sampled depth (0.5, 5, 10 and 15 m), and Bray-Curtis similarity, after log $(X + 1)$ transformation of the data. On the same Bray-Curtis similarity matrix, cluster analysis (complete linkage) was performed in conjunction with the similarity profile (SIMPROF) routine to determine significant differences among clusters. This technique is a permutation test of the null hypothesis that specified sets of samples, which are not *a priori* divided into groups, do not differ from each other in multivariate structure (at a 5% significance level) (Clarke et al., 2008). The groups of samples significantly gathered by the SIMPROF test were overlaid on the nMDS ordination plot. The normalized (z-standardization) environmental variables [temperature, salinity and dissolved inorganic nutrients: N-NO$_2$, N-NO$_3$, N-NH$_4$, P-PO$_4$ and Si-Si(OH)$_4$] were fitted as supplementary variables (vectors) onto ordination spaces to investigate their effects on community structure, using a Euclidean distance matrix for physical-chemical data. An analysis of similarity (ANOSIM) was used to assess differences in phytoplankton assemblages and photosynthetic rates among *a priori*-fixed seasons (winter: January, February, March; spring: April, May, June; summer: July, August, September; autumn: October, November, December). The resulting pair-wise R-values give an absolute measure of separation among groups with zero (0) indicating no difference among groups, while one (1) indicating that all the samples within groups are more similar to one another than any samples from different groups.

Furthermore, to assess significant differences in the microphytoplankton and cyanobacteria abundances of the four sampling depths, between seasons and sampling periods (study year: March 2006–February 2007 *vs* climatology: March 1998–February 2015), two-way nested analysis of similarity (ANOSIM) was used, where 'season' and 'period' were fixed factors/groups. Nanophytoplankton was not included in the latter analysis because its time series was not comparable to the other biotic series. ANOSIM was also performed on abiotic variables (*i.e.* seawater temperature and salinity at the four sampling depths), using the same fixed factors. Prior to analysis, the two matrices (*i.e.* with biotic and abiotic variables) were modified as follows: log $(X + 1)$ and Bray-Curtis similarity transformation on the biotic matrix, whereas square root and Euclidean distance on the abiotic one. Multivariate analyses were performed using PRIMER 7 (PRIMER-E Ltd., Plymouth, UK) software (Clarke et al., 2014). The Spearman rank correlation test (R) was used to investigate relationships between variables (STATISTICA 7, StatSoft, Inc., USA).

3. Results

3.1. Meteorological variables and Isonzo River discharge

From July 2006 to February 2007, air temperature (Fig. 2a) was much higher (up to 2.76 °C in February) compared to the climatological mean data (1998–2015), except for August when monthly mean temperature was 3.49 °C lower than the mean value. Lower wind intensity compared to the monthly means (1998–2015) was recorded from October to February (Fig. 2b) when mean wind speed reached the annual minimum (1.63 m s^{-1}). Low temperatures in August were due to particularly intense and persistent rain in that month, when total precipitation reached 243 mm (Fig. 2c), strongly exceeding values generally recorded in that period, in the time span from 1998 to 2015 (mean = 78 mm; median = 46 mm; third interquartile = 112 mm). In contrast, the months before (June and July) and after (September and October) that rainy August were particularly dry, and characterised by total monthly precipitation below 31 mm. Except for March 2006 and February 2007, during the rest of the study year monthly median Isonzo River discharge in the Gulf of Trieste was much lower than the time series medians (1998–2015) (Fig. 2d). The highest discrepancy between the values of the time series and those of the study year was observed in
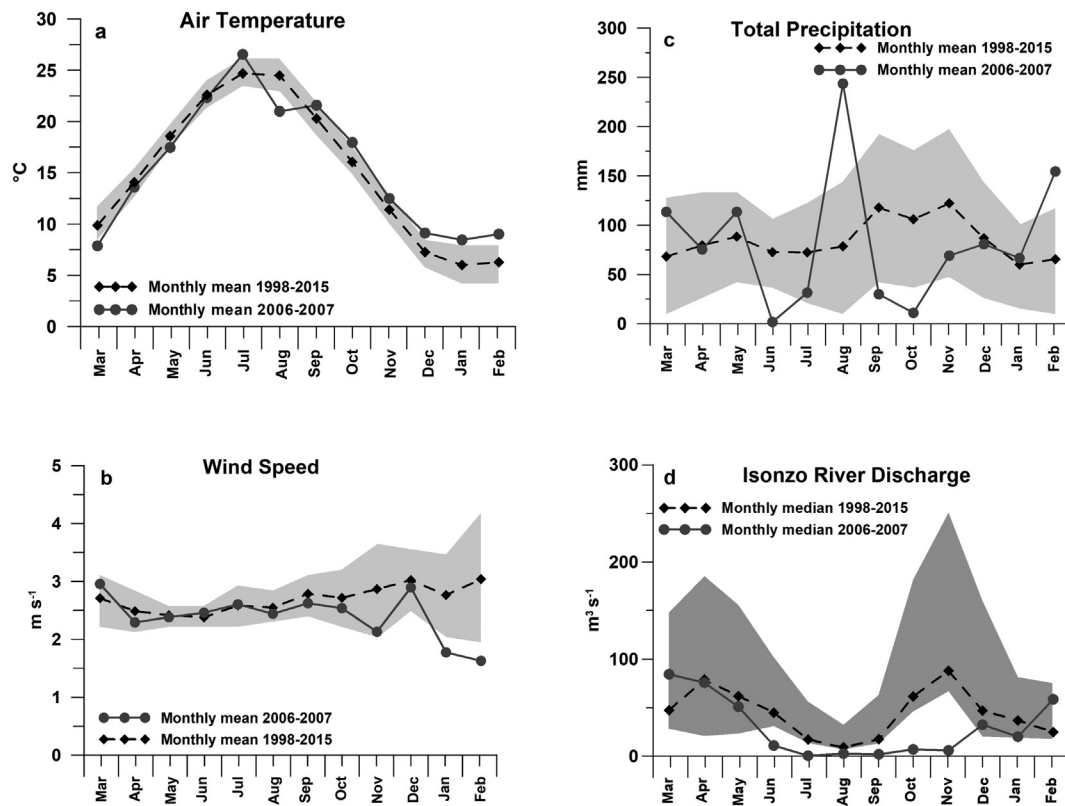

Fig. 2. Monthly means of air temperature a), wind speed b), total precipitation c), and monthly medians of Isonzo River discharge d) in the time series (March 1998–February 2015) and in the study year (March 2006 – February 2007). Shaded area enveloping monthly means in a), b) and c) represents the standard deviation of data computed over the period 1998–2015; darker shaded area enveloping monthly median in d) represents river discharge data (1998–2015) falling between the 1st and the 3rd quartile.

October and November when the flows were respectively 9- and 15-fold lower than the time series medians.

3.2. Physical-chemical properties of the water column

From March to August 2006, seawater temperature at the surface was much lower (up to −4.42 °C in June) than the monthly means of the considered time series (1998–2015) (Fig. 3a). In contrast, from September 2006 to the end of the study period, seawater temperature increased and, particularly from December 2006 to February 2007, it was >2 °C higher compared to the monthly means. The same dynamics were observed at 5 m depth although the differences were not so marked (up to −3.50 °C in June and +2.32 °C in December 2006) (Fig. 3b). At 10 m depth, the greatest differences between climatological data and those of the 2006–2007 period were observed in July (−3.21

°C) and December (+2.40 °C), whereas at the bottom major differences were recorded in May (−2.65 °C) and December (+2.37 °C) (Fig. 3c, d).

An evident drop in salinity was recorded in April 2006 at the surface layer (−0.78), whereas from May to October 2006 data were quite higher (up to +2.19 in May) than the monthly means for the 1998–2015 period (Fig. 3e). From November 2006 to the end of the study period, salinity data were comparable to the monthly means of the time series. At 5 m depth, the differences between climatological data and those of the study period were more muted, both the relative minimum (−0.30) in April and the higher values over the May–August 2006 period (Fig. 3f), and disappeared completely at 10 and 15 m depths (Fig. 3g, h).

At the surface, PAR was lower in April 2006 compared to the monthly mean for the 1999–2014 period, and constantly higher from June to October 2006, and decreased in the last three months of the study period (Fig. 3i). The same pattern was observed at the other
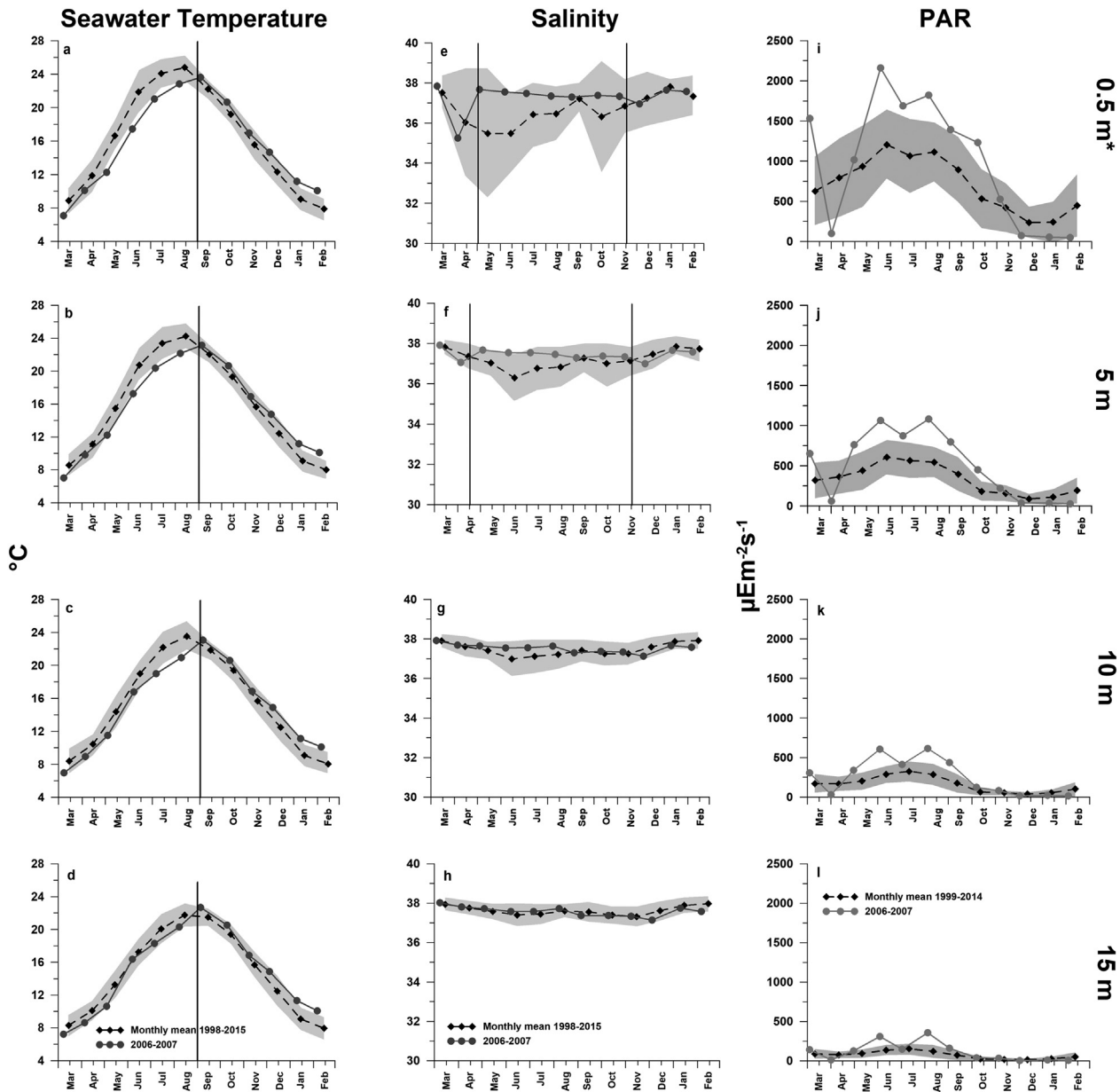

Fig. 3. Monthly means of seawater temperature (a–d), salinity (e–h) and PAR – photosynthetically available radiation (i–l) at sampling depths (0.5 m, 5 m, 10 m and 15 m) in the time series (March 1998–February 2015; except for PAR: March 1999–February 2014) and in the study year (March 2006 – February 2007). Shaded area enveloping monthly means represents the standard deviation of data computed over the period 1998–2015 (for a–h) and over the period 1999–2014 for (i–l). Vertical line between Aug-Sep (a–d) denotes the transition from warmer to colder than average seawater conditions; vertical lines in e) and f) delimit the period (Apr–Nov) with higher than average salinity. 0.5 m*: depth of surface PAR (i) is 1 m instead of 0.5 m

sampling depths, where higher PAR values than the monthly means were recorded, especially in June and August 2006 (Fig. 3j, k, l).

During the study year, N-NH$_4$ displayed the highest values (3.41 and 3.14 μM, respectively) in January at the surface and 5 m depth, whereas at 10 m depth and at the bottom the maxima were recorded in December (3.09 and 3.15 μM, respectively) (Fig. 4). In contrast, the minima were recorded in February, at the surface and bottom layers, and in May at the intermediate layers (<0.53 μM). At all four depths, N-NO$_2$ displayed the highest values, up to 0.93 μM in January, and a second relative maximum was also consistently recorded over the water column in February. Minima of N-NO$_2$ mostly occurred in June and July. N-NO$_3$ peaked in April both at the surface (8.75 μM) and at 5 m depth (2.28 μM) whereas at 10 m depth and at the bottom the maxima were measured in December. Again, minima were recorded over the summer period, from July to September. The highest Si-Si(OH)$_4$ values were recorded at the surface layer in April (6.80 μM) with high concentrations towards the bottom also (4.03 μM). High Si-Si(OH)$_4$ values were also recorded from October to December over the water column while minima were observed mostly in July. P-PO$_4$ was consistently low at all sampling depths, never exceeding 0.12 μM over the study period (Fig. 4).

3.3. Phytoplankton community structure

Temporal variations of cyanobacteria, nano- and microphytoplankton from March 2006 to February 2007 are shown in Fig. 5. In terms of biomass, microphytoplankton was the most represented fraction, accounting on average for 46.3% of the total phytoplankton biomass, followed by nanophytoplankton (40.4% of the total biomass) and cyanobacteria (13.3%) (Cibic et al., 2018, Table 1).

3.3.1. Cyanobacteria

The abundance of cyanobacteria in the March 2006–February 2007 period (Fig. 5a) ranged between 2.4·10^6 (May 2006 at the bottom) and 209.6·10^6 (September 2006 at 5 m) cells L^{-1}. Rather homogeneous cyanobacteria distributions were found along the water column profile, characterised by a slight decreasing surface to bottom gradient (surface median = 26.3·10^6 cells L^{-1}; bottom median = 18.4·10^6 cells L^{-1}). The highest monthly water column average of 193.1·10^6 cells L^{-1} was found in September followed by 99.7·10^6 cells L^{-1} in October, whereas
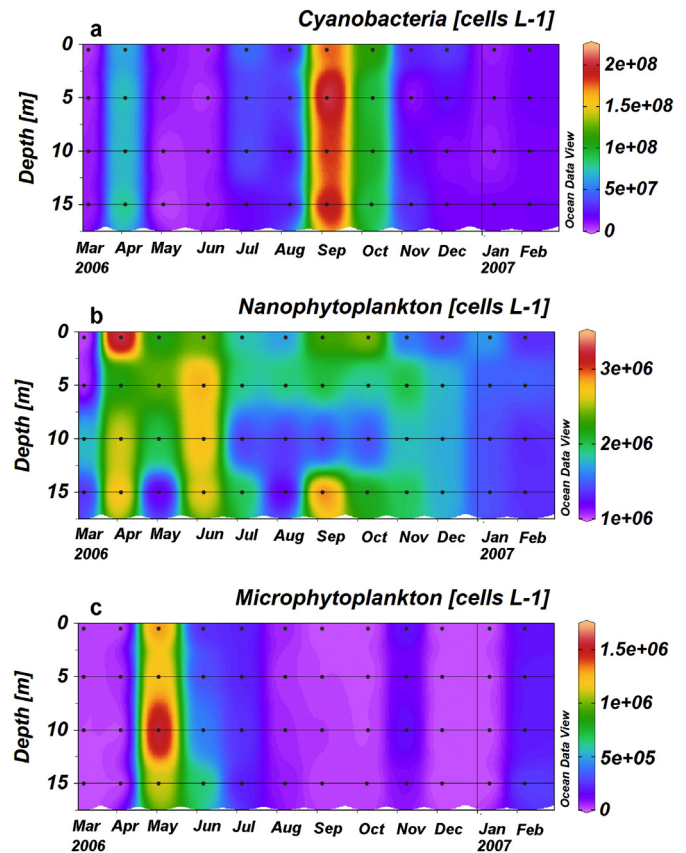

Fig. 5. Temporal variations (March 2006–February 2007) of cyanobacteria (a), nano- (b), and microphytoplankton (c) abundances. Colour bar scales are different for the three panels.

a spring peak of 73.4·10^6 cells L^{-1} was recorded in April. For the rest of the year, average column abundances remained <38·10^6 cells L^{-1}.

The temporal distribution of cyanobacteria during the study year (March 2006 – February 2007) followed the typical progressive increase of cyanobacteria abundance towards summer, usually reaching the

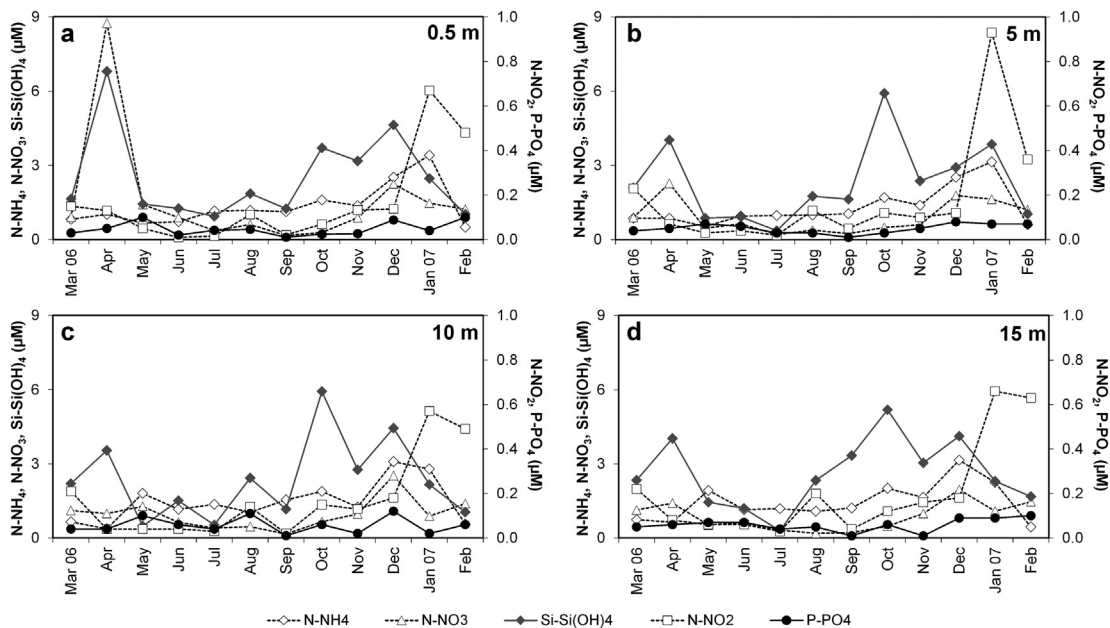**Fig. 4.** Temporal variations (March 2006–February 2007) of dissolved inorganic nutrient (ammonium N-NH$_4$, nitrite N-NO$_2$, nitrate N-NO$_3$, phosphate P-PO$_4$, and silicate Si-Si(OH)$_4$) concentrations at 0.5 m (a), 5 m (b), 10 m (c) and 15 m (d).

Table 1
Mean (± standard deviation) and median abundance values of the three phytoplankton communities, expressed as cells L^{-1}, over the study year (March 2006–February 2007) and over the climatology (March 1998–February 2015 for microphytoplankton and cyanobacteria; March 1999–February 2007 for nanophytoplankton). Micro: microphytoplankton; Nano: nanophytoplankton; Cyano: cyanobacteria. The number 0.5, 5, 10 and 15 indicates the sampling depth.

	Study year			Climatology		
	Mean	±SD	Median	Mean	±SD	Median
Micro0.5	1.91E + 05	3.71E + 05	3.68E + 04	3.76E + 05	9.22E + 05	7.59E + 04
Micro5	1.85E + 05	3.47E + 05	3.08E + 04	3.04E + 05	6.03E + 05	7.96E + 04
Micro10	2.32E + 05	4.49E + 05	4.29E + 04	3.03E + 05	4.68E + 05	1.09E + 05
Micro15	2.08E + 05	3.44E + 05	4.86E + 04	2.71E + 05	5.30E + 05	8.12E + 04
Nano0.5	1.94E + 06	6.20E + 05	1.76E + 06	2.19E + 06	1.42E + 06	1.84E + 06
Nano5	1.93E + 06	4.62E + 05	1.92E + 06	2.02E + 06	1.27E + 06	1.73E + 06
Nano10	1.75E + 06	5.00E + 05	1.60E + 06	2.00E + 06	1.50E + 06	1.60E + 06
Nano15	1.89E + 06	6.27E + 05	1.87E + 06	1.87E + 06	1.57E + 06	1.47E + 06
Cyano0.5	4.53E + 07	5.25E + 07	2.63E + 07	5.85E + 07	8.42E + 07	3.30E + 07
Cyano5	4.43E + 07	5.92E + 07	2.08E + 07	5.64E + 07	7.98E + 07	3.08E + 07
Cyano10	4.14E + 07	5.43E + 07	1.76E + 07	4.85E + 07	5.25E + 07	3.32E + 07
Cyano15	4.26E + 07	5.63E + 07	1.84E + 07	4.46E + 07	5.72E + 07	2.44E + 07

peak in September–October, as depicted by the seasonal cycle of the 1998–2015 time series (Fig. 6a–d). Moreover, an intense cyanobacterial bloom was recorded in September, when the maximum abundance was observed at 5 m, but of minor extent (proximal or above 3rd interquartile) when compared to several other years within the 1998–2015 period. During the study year, the occurrence of an exceptional (about 3-fold higher compared to the time-series average) spring bloom was recorded in April, when cyanobacteria abundance reached a maximum of 79.8·10^6 cells L^{-1} at the bottom depth. Contrary to the autumn bloom, an increasing surface to bottom gradient was observed for the spring bloom.

At the surface layer, cyanobacteria mean abundance over the study period (March 2006 – February 2007) was up to 22% lower than that calculated over the time series (March 1998 – February 2015) whereas the median value of the study year was up to 47% lower than the median climatological data at 10 m depth (Table 1).

3.3.2. Nanophytoplankton

During the March 2006–February 2007 period, nanophytoplankton abundances underwent major oscillations at the surface, varying from 1.0·10^6 cells L^{-1} in March to 3.3·10^6 cells L^{-1} in April, with a mean value of 1.9 ± 0.5·10^6 cells L^{-1} (Fig. 5b). The highest abundances were recorded in spring along the water column (on average 2.4 ± 0.5·10^6 cells L^{-1} from April to June). A less pronounced increase in abundance was also observed in late summer-early autumn (on average 2.1 ± 0.5·10^6 cells L^{-1} in September–October), with a peak at 15 m in September (about 3.0·10^6 cells L^{-1}), while during the rest of the year nanophytoplankton displayed lower abundance values (1.6 ± 0.3·10^6 cells L^{-1}, on average).

Compared to the seasonal cycle of the 1999–2007 time series (Fig. 6e–h), the nanophytoplankton generally showed higher abundance values than the median in April and even higher than the third interquartile in autumn at the surface and bottom (Fig. 6e and h). On the contrary, in March 2006 and February 2007, values comparable to the first interquartile were recorded at the surface and 5 m depth (Fig. 6e–f). Finally, an extremely low value compared to the time series was recorded in May 2006 at the bottom.

Overall, the mean and median annual nanophytoplankton abundances of the study period were comparable to those of the climatological data (Table 1).

3.3.3. Microphytoplankton

Compared to the smaller phytoplankton fractions, microphytoplankton was less abundant, with densities ranging from 8.4·10^2 cells L^{-1} in December to 1.6·10^6 cells L^{-1} in May, and a mean value of 1.9 ± 3.6·10^5 cells L^{-1} (Fig. 5c). Maximum abundances were recorded in spring-early summer along the water column (6.5·10^5 cells L^{-1} on average from May to July). Another two slight increases

were observed in November (1.5·10^5 cells L^{-1}) and February (2.2·10^5 cells L^{-1}), while during the rest of the year abundance was on average 1.6 ± 0.2·10^4 cells L^{-1}.

The microphytoplankton abundances recorded in March and April of the study year were lower compared to the climatology (Fig. 6i–l). On the contrary, very high values were recorded in May and June along the whole water column, often well over the third interquartile of the climatology (Fig. 6i–l).

Mean annual abundance of microphytoplankton (March 2006–February 2007) was up to 49% lower than the climatologic mean data at the surface layer whereas the median annual abundances were over 60% lower than the climatological ones at 5 and 10 m depth (Table 1).

3.3.4. Microphytoplankton composition

Considering that the size class division (micro-, nanophytoplankton and cyanobacteria) does not allow highlighting of the phytoplankton community structure in terms of the main taxa and group composition, we presented the results concerning only the composition of the microphytoplankton assemblages in terms of taxa identified by light microscopy using the Utermöhl method. Diatoms characterised the main microphytoplankton peaks and, therefore, dominated total microphytoplankton abundance (study year average 95.2%). Minima occurred in December at the surface and 5 m depth (36.9 and 51.3%, respectively) when dinoflagellates (study year average 3.8%) were more abundant (54.8 and 44.1%). In particular, *Gonyaulax polygramma* reached 53.5% and 42.3% of total microphytoplankton abundance at the surface and 5 m depth, respectively. Coccolithophores and other flagellates accounted for very low percentages (0.6 and 0.4%, respectively) of the microphytoplankton assemblages. Focusing on diatoms, 44 diatom taxa were identified during the study year. Species of the genera *Chaetoceros* and *Pseudo-nitzschia* dominated during the spring bloom, reaching 72.6% and 26.8%, on average, of the total microphytoplankton abundance in May, and 7.9% and 88.7% in June, respectively (Table 2). On the contrary, *Proboscia alata* dominated (96.2%) the microphytoplankton assemblage in July. In November 2006 and February 2007, the microphytoplankton assemblage was more biodiverse and composed of: *Bacteriastrum delicatulum*, *Cerataulina pelagica*, *Chaetoceros curvisetus*, *Chaetoceros* spp., *Leptocylindrus* spp., *Pseudo-nitzschia* spp., *Skeletonema* sp. and *Thalassiosira* sp. (Table 2).

3.4. Primary production

Total primary production (PP) varied from values close to zero, particularly at the bottom layers in the winter months, to 7.11 ± 1.01 μgC L^{-1} h^{-1} in November 2006 at the surface layer (Fig. 7a). Furthermore, two relative maxima were observed in June and July 2006 both at 10 m depth, reaching 5.28 ± 0.43 and 5.80 ± 0.64 μgC L^{-1} h^{-1},
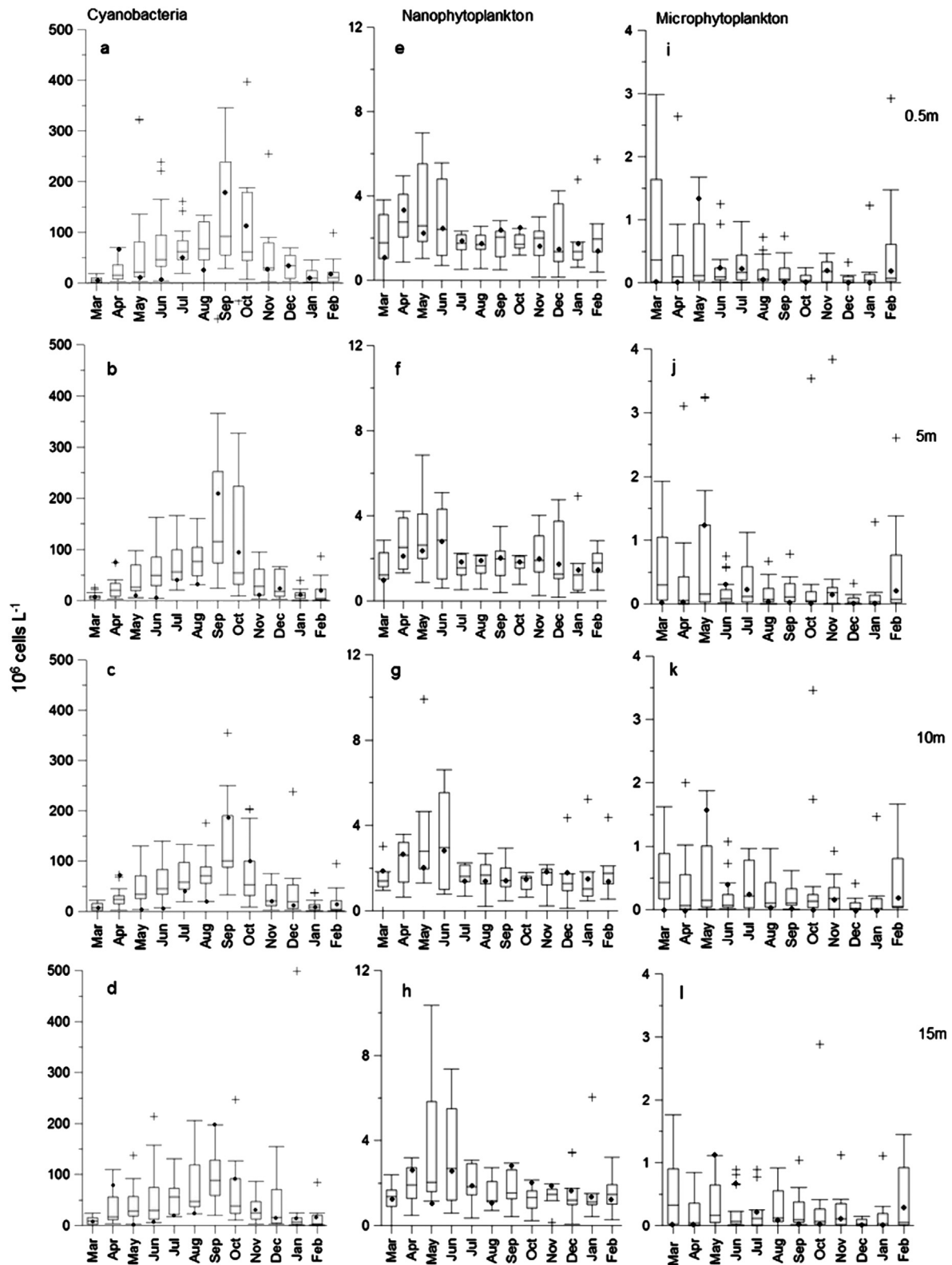
Fig. 6. Temporal variations of cyanobacteria (a–d), nano- (e–h) and microphytoplankton (i–l) abundances at four sampling depths (0.5 m, 5 m, 10 m and 15 m). Box plots report the time series distribution (March 1998–February 2015 for cyanobacteria and microphytoplankton; March 1999–February 2007 for nanophytoplankton). Outliers (data points that fall above the third quartile and below the first quartile) are depicted as crosses. Full circles represent monthly abundance in the study period (March 2006 – February 2007). Y-scales are different for the three communities.

respectively. With a few exceptions, the highest rates were generally estimated at the surface layer.

The nanophytoplankton primary production (nPP), estimated from June to November 2006, varied from values close to zero, measured in October and November at the bottom layers, to 1.98 ± 0.17 $\mu gC\,L^{-1}\,h^{-1}$ obtained in October at the surface layer (Fig. 7b). Moreover, for this fraction, a relative maximum of $1.46 \pm 0.07\,\mu gC\,L^{-1}\,h^{-1}$ was observed in July at 10 m depth.

Table 2
Abundance values of diatoms, over the water column, expressed as percentage of total microphytoplankton from March 2006 to February 2007. Only diatoms with a mean relative abundance >1% are listed.

Diatoms	Study year											
	Mar 06	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan 07	Feb
Bacteriastrum delicatulum Cleve	0.00	0.00	0.01	2.34	0.00	1.15	0.00	0.00	4.85	2.03	6.90	0.00
Cerataulina pelagica (Cleve) Hendey	0.00	0.40	0.04	0.36	0.19	11.71	2.72	3.82	18.62	3.33	2.10	6.48
Chaetoceros spp.	27.19	43.54	72.62	7.93	2.12	48.55	12.67	5.71	45.02	8.29	0.29	57.71
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin	3.21	9.81	0.05	0.04	0.00	0.18	0.15	0.93	0.10	0.45	0.29	0.10
Leptocylindrus spp.	0.00	0.63	0.00	0.05	0.03	16.44	65.47	11.63	20.37	0.00	0.00	5.26
Lauderia annulata Cleve	1.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.21	22.14	0.28
Nitzschia spp.	0.16	6.23	0.07	0.02	0.29	1.08	0.39	37.06	0.28	11.20	0.57	0.01
Proboscia alata (Brightwell) Sundström	0.00	0.00	0.01	0.09	96.20	8.69	0.20	3.93	0.27	0.68	0.00	0.01
Pseudo-nitzschia spp. "delicatissima complex"	0.00	0.00	26.84	88.70	0.00	0.00	0.00	1.49	0.00	0.00	0.00	0.00
Pseudo-nitzschia spp. "seriata complex"	0.16	0.00	0.04	0.00	0.01	1.83	1.32	12.91	1.63	0.00	21.26	18.56
Skeletonema spp.	63.26	14.19	0.07	0.10	0.00	0.00	0.00	0.00	0.88	0.00	15.89	9.07
Thalassiosira spp.	0.10	0.13	0.00	0.01	0.03	0.01	2.14	0.07	0.54	2.31	17.30	1.87

The maxima of primary production depth-integrated rates (PPi) were recorded in July and June and the minima in January and December (Table 3). Over the six months (June–November 2006), the nPP depth-integrated rates (nPPi) ranged from 15.7% of total PPi in November to 33.6% in October. In the period June–November 2006, the nanophytoplankton fraction contributed, on average, 24.9 ± 5.8% to total PPi.

3.5. Influence of abiotic factors on phytoplankton structure and function

The nMDS analysis based on the cyanobacteria, nano- and microphytoplankton abundances, and PP rates of the study year revealed temporal differences among samplings (Fig. 8). The superimposed abiotic variables indicated the main discriminating factors responsible for the separation of samplings. Six significantly different groups were identified using the SIMPROF test: the first one gathered all September and two October samples, positioned in the upper part of the plot, characterised by high temperature. The second group gathered the four August samples and the 15-m depth sample of November. In the third cluster, April data were grouped with two October and December samples. In the lower part of the plot, in correspondence to the highest nutrient concentrations, another two clusters were identified: one gathering March data and one gathering all January and
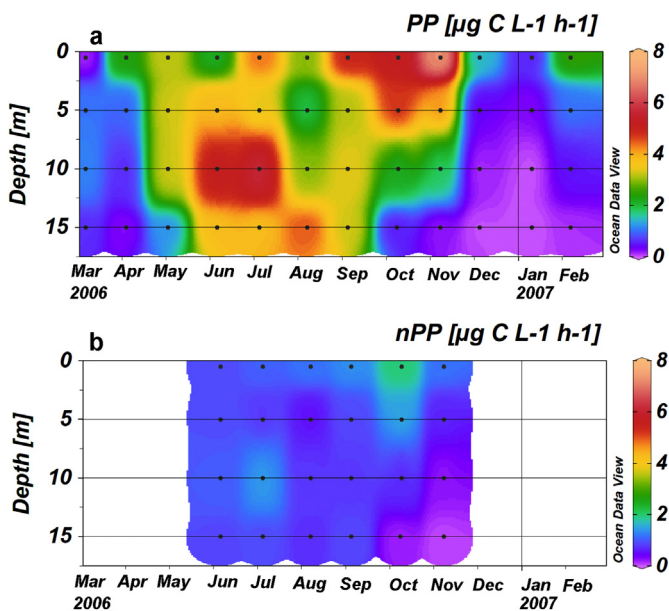

Fig. 7. Temporal variations of total Primary Production (PP) from March 2006 to February 2007 (a) and nanophytoplankton Primary Production (nPP) from June to November 2006 (b).
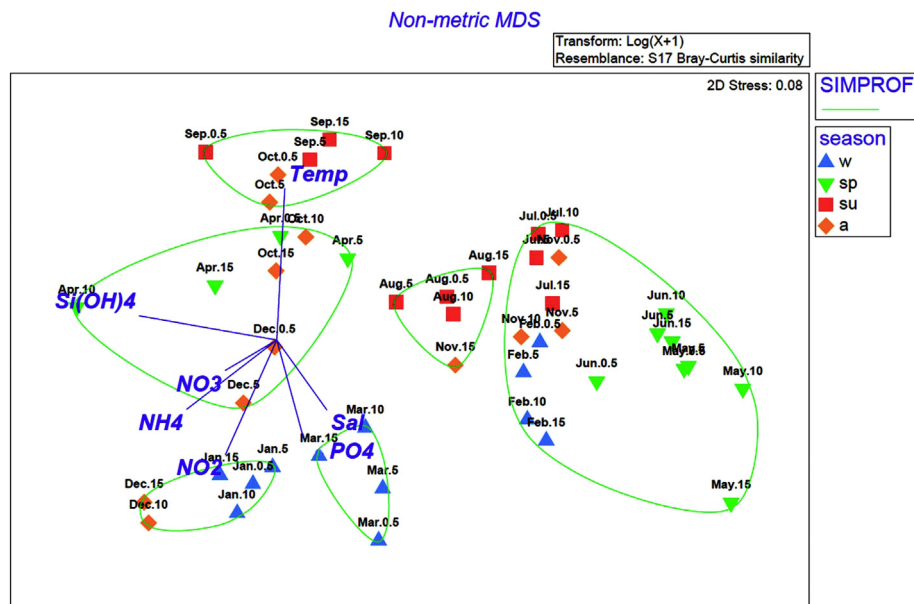
Table 3
Total primary production measured at the four sampling depths during the study period. PPi = primary production depth-integrated rates; PPd = primary production daily rates; nPP = nanophytoplankton primary production rates; nPPi = nanophytoplankton primary production depth-integrated rates. N.A = not available.

Sampling dd/mm/yyyy	depth m	PP µgC L^{-1} h^{-1}	PPi mgC m^{-2} h^{-1}	PPd mgC m^{-2}d^{-1}	nPP µgC L^{-1} h^{-1}	nPPi mgC m^{-2} h^{-1}
08/03/2006	0.5	0.17	12.83	102.16	N.A.	
	5	1.04				
	10	1.13				
	15	0.62				
05/04/2006	0.5	2.34	14.30	96.05	N.A.	
	5	0.96				
	10	0.60				
	15	0.27				
04/05/2006	0.5	3.23	44.08	381.16	N.A.	
	5	3.30				
	10	3.30				
	15	1.21				
07/06/2006	0.5	2.13	60.43	580.78	0.91	14.17
	5	3.83			0.90	
	10	5.28			1.04	
	15	3.83			0.87	
06/07/2006	0.5	4.41	66.44	620.82	1.05	16.06
	5	3.37			0.77	
	10	5.80			1.46	
	15	3.83			0.91	
08/08/2006	0.5	2.94	43.05	379.34	1.18	11.34
	5	1.87			0.52	
	10	2.99			0.80	
	15	4.56			0.72	
05/09/2006	0.5	4.97	53.64	499.57	1.35	14.02
	5	3.17			0.90	
	10	3.43			0.78	
	15	3.28			0.90	
10/10/2006	0.5	5.23	49.28	456.24	1.98	16.55
	5	4.89			1.54	
	10	2.09			0.67	
	15	0.52			0.21	
08/11/2006	0.5	7.11	48.39	300.93	1.17	7.59
	5	4.24			0.66	
	10	1.78			0.24	
	15	0.21			0.07	
05/12/2006	0.5	1.41	6.01	64.99	N.A.	
	5	0.36				
	10	0.12				
	15	0.03				
10/01/2007	0.5	0.56	2.88	21.59	N.A.	
	5	0.29				
	10	0.00				
	15	0.00				
06/02/2007	0.5	2.50	14.11	168.76	N.A.	
	5	1.06				
	10	0.46				
	15	0.12				

Fig. 8. Non-metric multidimensional scaling (nMDS) ordination plots based on cyanobacteria, nano- and microphytoplankton abundances, and total primary production rates during the study year. On the nMDS ordination plot, significantly (5% significance level) distinct groups identified by the SIMPROF test are overlaid and indicated by green full circles. The superimposed abiotic variables indicate which are the main discriminating factors responsible for the separation of samples. Temp: temperature; Sal: salinity; NO_2: nitrite; NO_3: nitrate; NH_4: ammonium; PO_4: phosphate; $Si(OH)_4$: silicate. W: winter; sp.: spring; su: summer; a: autumn. Labels of individual datapoints indicate the month and depth. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

two December samples. Interestingly, the largest group clustered May, June and July samples together with February and November ones.

Overall, the phytoplankton assemblages and photosynthetic rates significantly differed among seasons ($R_{ANOSIM} = 0.29$; p = 0.1%). All pairwise combinations of seasons significantly differed: (w, sp) $R = 0.363$, p = 0.4%; (w, su) $R = 0.541$, p = 0.1%; (w, a) $R = 0.18$, p = 1.9%; (sp, su) $R = 0.313$, p = 0.1%; (sp, a) $R = 0.243$, p = 1%; however, the R value was lower for (su, a) $R = 0.115$, p = 5%.

The two-way nested ('period' within 'season') ANOSIM highlighted seasonal differences in the cyanobacteria and microphytoplankton abundances between the study period and the climatological one ($R_{ANOSIM} = 0.52$; p < 0.05). Similarly, seasonal differences between the two periods were also obtained for seawater temperature and salinity ($R_{ANOSIM} = 0.73$; p < 0.05).

4. Discussion

4.1. Phytoplankton community development in relation to physical-chemical constrains

From a meteorological point of view, the study year could be roughly divided into two distinct parts: a first part characterised by monthly mean air temperatures lower than the climatological mean data, and a second part with much higher monthly means, especially towards the end of the study period. August 2006 represented a quite anomalous month, when the highest precipitation of the study period was recorded, accompanied by very low air temperatures compared to the climatological data. Despite the persistent rain that occurred in that month, total precipitation did not alter the discharge of the Isonzo River, likely because the previous months were particularly dry. In contrast, from September to November, total precipitation was very low, and consequently the maximum freshwater inflow typical of October–November did not occur. Moreover, the strong Bora wind events that usually persist in winter months were not as intense in January and February 2007. These meteorological events were clearly mirrored in the thermohaline properties of the water column in the shallow Gulf of Trieste that, in turn, influenced phytoplankton abundance and structure.

Mean and median microphytoplankton and cyanobacteria abundances computed over the study period (March 2006–February 2007) were lower than the climatological mean and median data (1998–2015) at the same station (Table 1). Conversely, nanophytoplankton abundances were comparable to the climatological ones. Differences in cyanobacteria and microphytoplankton abundances between the study period and the climatology were corroborated by the analysis of similarity. This result was confirmed by Mozetič et al. (2012), who found the absolute minimum of chl *a* concentration in the Gulf of Trieste in 2006 over the 1989–2009 period. Additionally, the analysis of a four-decadal dataset from the eastern part of the northern Adriatic also revealed a significant change in phytoplankton abundances, with lower values in the 2000–2009 period compared to the 1972–1999 period (Marić et al., 2012). Both papers ascribed this phytoplankton decrease to lower nutrient loads resulting from the reduced outflows of Rivers Isonzo and Po, respectively, which is in accordance with our results since the Isonzo River discharge in our study year was much lower than the climatological monthly means. The influence of the river outflows on the trophic state (Socal et al., 2008), primary productivity (Socal et al., 2002) and phytoplankton seasonality (Malej et al., 1995; Mozetič et al., 1998, 2002; Viličić et al., 2007, 2009) in the northern Adriatic basin as well as in other areas (*e.g.* Watanabe et al., 2017) has been well-documented. Indeed, considering the whole study year, our microphytoplankton data showed significant negative relations with silicates, nitrites and ammonium ($R = -0.700$, p < 0.001; $R = -0.416$ and -0.388, p < 0.01, respectively), suggesting that the microphytoplankton scarce abundances were strongly influenced by reduced nutrient availability. Furthermore, nanophytoplankton showed a highly significant negative relation with nitrites ($R = -0.567$, p < 0.001) while cyanobacteria were negatively correlated with phosphates and nitrates ($R = -0.393$, p < 0.01 and $R = -0.327$, p < 0.05, respectively) (Table 4).

Nutrient uptake peculiarities among different phytoplankton size-groups likely played a crucial role in determining the distribution of different groups of autotrophs and their seasonal pattern during the study year, thus altering the typical ecological succession of micro-nano-picophytoplankton reported by Vanucci et al. (1994) for this study area. In March 2006, both the nano- and microphytoplankton

Table 4
Spearman rank correlation test (R) between the considered variables. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

	Temp	Sal	N-NH4	N-NO2	N-NO3	P-PO4	Si-Si(OH)4	Chl *a*	Micro	Nano	Cyano	PP
Temp	1											
Sal	**−0.514*****	1										
N-NH$_4$	**0.322***	**−0.367***	1									
N-NO$_2$	**−0.510*****	0.225	0.137	1								
N-NO$_3$	**−0.728*****	−0.017	0.020	**0.547*****	1							
P-PO$_4$	**−0.377****	0.156	0.004	**0.304***	**0.531*****	1						
Si-Si(OH)$_4$	−0.074	**−0.335***	**0.394****	**0.397****	**0.381****	0.021	1					
Chl *a*	**−0.287***	0.187	−0.281	0.135	0.055	−0.075	−0.149	1				
Micro	0.084	0.172	**−0.388****	**−0.416****	−0.261	0.122	**−0.700*****	**0.304***	1			
Nano	0.231	−0.272	−0.123	**−0.567*****	−0.096	−0.188	0.087	−0.007	0.058	1		
Cyano	**0.533*****	**−0.542*****	0.062	−0.134	**−0.327***	**−0.393****	**0.358***	−0.052	−0.276	0.142	1	
PP	**0.595*****	−0.180	−0.082	**−0.641*****	**−0.656*****	−0.269	**−0.361***	0.110	**0.478*****	**0.292***	−0.013	1

Values with a significance level <5% are marked in bold. Temp = temperature; Sal = salinity; Micro = microphytoplankton; Nano = nanophytoplankton; Cyano = cyanobacteria; PP = total primary production.

abundances at the uppermost 5 m were much lower than the climatological monthly means due to nutrient-depletion. In April, nutrient availability, particularly of nitrate, triggered an exceptional cyanobacteria bloom, which seldom occurs in this month (Karuza et al., 2012), and an increase in nanophytoplankton. Nanophytoplankton dominance in spring phytoplankton assemblages is a feature of the northern Adriatic (Marić et al., 2012; Godrijan et al., 2013; Talaber et al., 2014). Interestingly, Mozetič et al. (2012) also found significant differences in the nanophytoplankton abundances that were consistently higher in the 2003–2009 period, which includes our study year, in contrast to the 1989–2002 period. Due to these high cyanobacteria and nanophytoplankton abundances, the April samplings differed from the other spring samplings, as evidenced by their position on the nMDS plot.

In 2006, the late winter-early spring diatom bloom typical of this area (Cabrini et al., 2012) was much delayed since it occurred in May, following the high silicate and nitrate concentrations present in the water column in April. This is in accordance with the findings of Mozetič et al. (2010) who reported a shift in the late winter-early spring bloom from January–February to April–May over the last decade of the 1970–2007 period in the northern Adriatic Sea. In May 2006, the upper 7 m layer of the water column was thoroughly mixed (Cibic et al., 2018, Figs. 1 and 2) and the only diatom bloom of the year developed, mostly on account of *Chaetoceros* and *Pseudo-nitzschia* species that reached their highest density at 10 m depth. According to Viličić et al. (2009), these species are indicators of nutrient influx in the Adriatic Sea. The increase in microphytoplankton abundance in May occurred simultaneously with a drop in silicate and nitrate concentrations. By applying the Redfield-Brzezinski nutrient ratio C:Si:N:P = 106:15:16:1 proposed for diatoms by Brzezinski (1985) to our 2006–07 dataset (Cibic et al., 2018, Table 2), Si limitation in the upper 10 m of the water column (Si:P = 4.8) was evidenced in May 2006. Due to lower Si availability, a shift from the predominance of large-cell diatoms to phytoflagellates and small diatoms was observed in June. The presence of small cells in stratified and oligotrophic warm water conditions is typically due to their capability to exploit low nutrient levels (Agawin et al., 2000). Indeed, the epifluorescence microscopic observations revealed that very small *Chaetoceros* cells (2–8 μm) reached up to 16.9% of total nanophytoplankton at 15 m depth whereas pennate diatoms (5–8 μm) accounted for about 15.3% of this community at 10 m depth. Apart from these diatoms, the nanophytoplankton community was mostly dominated by phytoflagellates. In contrast to the findings of Mozetič et al. (2012) who reported an overall decline in microphytoplankton but an increase in phytoflagellates after the regime shift (2002/2003), we did not observe an increase in nanophytoflagellate numbers during the study year. However, our nanophytoplankton climatology encompassed only 8 years prior to the study period that may not be sufficient to highlight a shift in their pattern. Si limitation conditions were again present in July 2006 (Si:P

< 13.3 below 5 m depth) when large-sized *Proboscia alata* proliferated in the water column with over $2 \cdot 10^5$ cells L^{-1}. This species has often been observed in summer phytoplankton assemblages in the northern Adriatic (Bernardi Aubry et al., 2006, 2012; Godrijan et al., 2013) and it has been suggested that it is a thermophilic species (Bernardi Aubry et al., 2004).

In August, cyanobacteria displayed much lower abundances than the climatological mean values at all depths. Since cyanobacteria prefer high temperature seawater conditions, as indicated by a highly significant correlation with temperature ($R = 0.533$, p < 0.001), it is likely that the bad weather conditions that persisted in August did not favour the development of these phototrophs. However, the typical late summer cyanobacteria bloom developed in September, with up to $2 \cdot 10^8$ cells L^{-1} (and up to 64% of the total biomass) through the entire water column. Considering the 15 m layer, such an abundance value was exceptionally high compared to the climatological mean data. The high abundance of *Synechococcus* in oligotrophic systems, such as the Gulf of Trieste (Mozetič et al., 2010) indicates that, due to their small size, they are more efficient in nutrient uptake in oligotrophic environments than larger cells (Raven, 1986).

In October–November, the typical autumn maximum inflow of the Isonzo River did not occur. The silicates, regenerated in summer at the water-sediment interface (Cossarini et al., 2012), were highly available throughout the water column and diatom development was likely not Si-limited. A secondary peak dominated by diatoms is typical of the northern Adriatic in autumn (Mozetič et al., 1998, 2002, 2010; Viličić et al., 2009; Šupraha et al., 2011; Bernardi Aubry et al., 2012; Marić et al., 2012; Godrijan et al., 2013), although Mozetič et al. (2012) highlighted a substantial reduction in diatom abundances in November during the 2003–2009 period compared to the 1989–2002 period. Our microphytoplankton abundances in November, but especially in October 2006, were much lower than the climatological mean values, particularly considering the surface layer. In contrast, nanophytoplankton abundances were high and nano-sized diatoms were present, as revealed by epifluorescence microscopic observations of small *Chaetoceros* species.

In December, the first noticeable input of the Isonzo River occurred but it was still reduced compared to the monthly median climatological value. Phytoplankton was quite scarce in the water column due to light limitation, and the observed pennate diatoms (*Nitzschia*, *Navicula*, *Pleurosigma*, *Diploneis*) were temporarily present in the phytoplankton assemblage following the strong wind events that resuspended them from the bottom. In these high hydrodynamic conditions, the phototrophic dinoflagellate *Gonyaulax polygramma* developed, reaching up to 53.5% of the microphytoplankton assemblage. According to Smayda and Reynolds (2001), *Gonyaulax* possesses biophysical tolerance to elevated shear/stress and appears in physically disturbed water masses with pronounced vertical mixing, such as those of December 2006.

January and February 2007 were very mild and rainy, resulting in exceptional discharge of the Isonzo River, particularly in February, when the second maximum river discharge was observed. The anomalous conditions were also confirmed by the significant clustering of February samplings together with the summer ones on the nMDS plot. The CTD casts (Cibic et al., 2018, Figs. 1 and 2) revealed that the water column was thoroughly mixed in that month and therefore the nitrite peaks that were detected at all depths were likely freshwater-borne. However, the upper 5 m layer of the water column was Si-depleted (Si:P = 10.3 at the surface) and the late winter diatom bloom just began, mainly on account of *Chaetoceros* and *Pseudo-nitzschia* species.

4.2. Primary production in relation to different phytoplankton communities and anomalous meteorological and physical conditions

While for the meteorological, physical and phytoplankton data presented in this study we computed the climatological mean data over a long time series to compare them with our study period, it was not possible to follow the same approach for primary production (PP) since these data were not available. Therefore, we compared our PP rates with those available in the literature for the Gulf of Trieste. Our depth-integrated PP values were much lower than the spring (March to June) rates estimated in 1992 by Malej et al. (1995), but higher than their autumn data. Fonda Umani et al. (2007) reported integrated daily PP values obtained from January 1999 to December 2001 at the same station (C1). Considering the average value of our study period, our depth-integrated PP rate (PPd = 284 mgCm^{-2}d^{-1}) was roughly half of that estimated by Fonda Umani et al. (2007) (PPd > 500 mgC m^{-2}d^{-1}). According to Mozetič et al. (2012), a regime shift occurred in the Gulf of Trieste in 2002/2003 with a subsequent decline in phytoplankton biomass. In fact, our study period was subsequent to the regime shift and lower photosynthetic activities were therefore expected. Indeed, our depth-integrated PP rate of 284 mgC m^{-2}d^{-1} was highly comparable to the mean value (PPd = 250 mgC m^{-2}d^{-1}) estimated by Ingrosso et al. (2016) during a two-year study (March 2011–February 2013). In each of the three investigated years, Fonda Umani et al. (2007) regularly recorded a late winter-early spring PP (relative or absolute) maximum that was also observed by Ingrosso et al. (2016). In contrast, during our study period we did not detect the spring PP annual maximum. Also, in presence of a major microphytoplankton abundance (>1·10^6 cells L^{-1} along the water column) in May, the photosynthetic rate was not particularly high (PPd = 381 mgC m^{-2}d^{-1}). In terms of biomass, diatoms were the major constituent of the phototrophic community in that month, representing >87.2% of total phytoplankton (Cibic et al., 2018, Table 1). Interestingly, the microphytoplankton assemblage was not in a senescent phase yet, as indicated by the phaeopigment/chl *a* ratio ranging from 0.51 to 0.70 over the water column (Cibic et al., 2018, Table 3), nor was it light-depleted since there was no cloud coverage during *in situ* incubation. Cloud coverage is known to significantly affect summer primary production by reducing light availability for high-light adapted phytoplankton (Strom et al., 2010). The uncoupling between microphytoplankton abundance and primary production could not be ascribed to nutrient depletion either, since phosphates and DIN were not limiting in this month (Fig. 4 and Cibic et al., 2018, Table 2). Besides, nutrient depletion limits biomass production but not photosynthesis: under N and P limitation, when protein synthesis is reduced, a large proportion of the photosynthetically fixed carbon is channelled to the extrusion of extracellular polymeric substances as a result of cellular carbon overflow (Corzo et al., 2000; Engel, 2002). *Chaetoceros* species, in particular, that were dominant at the study site in May 2006, are known to produce large amounts of extracellular polysaccharides and, under P-deficiency, this production would have likely constituted the main photosynthetic activity (Myklestad, 1977; Corzo et al., 2000). However, in May, the ^{14}C uptake rate in the filtrated fraction, *i.e.* released from the cells as dissolved organic C (measured for control),

was comparable to that obtained in the other months of the study year and about 10% of the ^{14}C uptake measured in the filter, *i.e.* retained in the cells (Cibic and Virgilio, 2011). Therefore, other environmental factors were probably responsible for this uncoupling between the major abundance and not particularly high photosynthetic rates. Indeed, phytoplankton constantly adjust their photosynthetic output to match environmental constrains and optimize their growth (Talaber et al., 2014).

In June and July 2006 the highest integrated daily rates of the study period were obtained. In fact, considering the whole study period, PP was highly related to seawater temperature (R = 0.595, p < 0.001). In these two months, the community at the 10 m layer was more productive than that at the surface, reaching 5.8 µgC L^{-1} h^{-1} in July. However, community composition differed between the two periods. In June, phytoplankton biomass was approximately equidistributed between the nano- and microphytoplankton fraction whereas in July it was dominated by diatoms, by large-sized *Proboscia alata* specifically. In June, averaging the data over the water column, nPP accounted for 27.18 ± 10.51%, while at the 10 m layer it accounted for about 20% of PP. In July, despite the dominance of large diatoms, the nanoplankton fraction appeared to display high photosynthetic activity, reaching 23.89 ± 0.97% of PP.

In August, the nanophytoplankton at the surface was quite photosynthetically active, accounting for 40.23% of the PP. Yet, the bottom layer was the most productive, with a PP rate of 4.56 µgC L^{-1} h^{-1}, mainly on account of the microphytoplankton community (*Chaetoceros* spp., *Leptocylindrus* spp., *Cerataulina pelagica*). In September, the cyanobacteria biomass accounted for over 61% of total phytoplankton in the upper 15 m-layer of the water column. The highest PP rate estimated at the surface (PP = 4.97 µgC L^{-1} h^{-1}) was likely ascribable to cyanobacteria since the micro-sized fraction was very scarce and nPP accounted for only 27.09% of the PP.

With 35.46% of PP, the highest nPP rate of the study period was estimated at the surface layer in October, where 34.18% of the phytoplankton biomass pool consisted of nano-sized cells. High photosynthetic rates were maintained at the 5 m-layer in which the biomass was evenly distributed among the three phototroph fractions. In fact, although microphytoplankton was quite scarce in this month, it was represented by quite large-sized cells.

In November, the highest PP rate of the study period was estimated at the surface layer. This value was twice higher than that reported by Ingrosso et al. (2016) and at least five times higher than that obtained by Fonda Umani et al. (2007) for the same month. In November 2006, the atmospheric conditions were very stable; in particular, the monthly mean wind speed was much lower than the climatological data and, together with the higher temperature, it likely represented favourable conditions for the persistence of an autumn diatom peak with high photosynthetic rates. However, we detected an uncoupling between the highest PP and the phytoplankton density responsible for this major PP rate. Interestingly, although microphytoplankton abundance at the upper 5 m-layer reached only 1.9·10^5 cells L^{-1}, at the same time the highest chl *a* concentration (3.69 µg L^{-1}) of the study period was recorded at this depth (Cibic et al., 2018, Table 3). Microphytoplankton, accounting for 74.9% of the total phototrophic biomass, was dominated by large-sized diatom cells, such as *Chaetoceros* spp., *Leptocylindrus* spp. and *Cerataulina pelagica*. The microscopic observations confirmed that these cells, though not very abundant, formed long chains and were very viable (D. Fornasaro, personal comment), and therefore most likely highly photosynthetically active. The nPP in this month accounted for only 20% of PP. This percentage roughly represented the nanophytoplankton fraction of the total phototrophic biomass at the surface layer, whereas the cyanobacteria biomass was negligible and therefore the high PP could not be attributed to the picophototrophs. The occasional high primary production rates that occur in autumn in favourable conditions are generally short-lasting due to both the strong Bora wind that disrupts water stratification and photolimitation.

According to the SIMPROF test, the phytoplankton assemblages and photosynthetic rates in summer and November were similar. This was likely due to the unusual warm temperature and stable water column conditions in November that triggered the highest PP of the study period.

Considering the entire study period, our PP was not correlated with total chl *a*, in contrast to the findings of Malej et al. (1995) and Fonda Umani et al. (2007). Chlorophyll concentration explains only 30–40% of the variance in primary production at the scale of a single station (Hyde et al., 2008). However, our PP rates were highly correlated with the microphytoplankton ($R = 0.478$, p < 0.001) and nanophytoplankton ($R = 0.292$, p < 0.05) abundances. Overall, in these oligotrophic conditions, PP rates seemed to be directly driven by nitrate, nitrite and silicate inputs ($R = -0.656$, p < 0.001; $R = -0.641$, p < 0.001; $R = -0.361$, p < 0.05, respectively).

Our areal daily rates were much lower than those estimated in 1995–96 by Pugnetti et al. (2004), using the ^{14}C uptake technique and *in situ* incubations, at a coastal site strongly affected by the Po River nutrient discharge, and even lower than their rates obtained at an offshore coastal site in the northern Adriatic basin. Furthermore, our areal daily rates were lower (particularly the spring data) than those obtained by Pugnetti et al. (2005) applying the same methodology, from June 1999 to June 2002, along two transects crossing the northern Adriatic basin from the Italian to the Croatian coast. This could be due to the fact that phosphate concentration at our site was lower than the average value reported by the authors, although we measured overall higher DIN concentrations. In the southern Tyrrhenian Sea, Decembrini et al. (2009) used the ^{14}C technique and "on deck" incubations in July and December 2005 and reported areal daily rates lower than or comparable to ours. Their lower rates were likely linked to very low chl *a* concentrations. Moreover, our PP rates were much lower than those estimated in the semi-enclosed nutrient-enriched (Caroppo et al., 2016) Mar Piccolo of Taranto (Ionian Sea) in June 2013, and February and April 2014, using the ^{14}C uptake technique and *in situ* incubations (Cibic et al., 2016).

The nanoplankton primary production was previously estimated at the same site from January to June 2005 (Guardiani et al., 2006). The authors estimated the highest nPP in April at the surface (3.73 µgC L^{-1} h^{-1}) when nPP accounted for 59% of total PP, whereas nPP accounted for up to 84% of PP at the bottom in May. Their values were higher than ours in June 2005, ranging from 1.13 to 1.78 µgC L^{-1} h^{-1} along the water column. Previously, Malej et al. (1995) also reported a noticeable percentage of production due to >10 µm cells in the surface layer during April and June 1992.

4.3. Implications of reduced phytoplankton biomass on the ecosystem functioning of the basin

Overall, the annual (March 2006 – February 2007) mean and median abundances of two out of three considered photoautotrophic communities were lower than the climatological mean and median data (Table 1). The reduced availability of phytoplankton biomass may have several repercussions on both the pelagic and benthic trophic webs. As already pointed out by Mozetič et al. (2012), the extremely low chl *a* concentration caused the disappearance of the typical November peak of mesozooplankton that occurred after the regime shift. In our study period, particularly in April 2006, mesozooplankton abundance was extremely low, 30 times lower than the monthly mean climatology over the 2000–2015 period (212 ind m^{-3} *vs* 6401 ± 2620 ind m^{-3} (as mean ± SD)) (De Olazabal, pers. comm.), when an increase in this community is generally observed. This could have led to a trophic cascading effect on planktivorous fish.

The Gulf of Trieste is historically devoted to extensive mussel farming that currently covers a 15 km long × 100 m wide area along the coastline, where the blue mussel *Mytilus galloprovincialis* is cultivated using the long-line system (Franzo et al., 2014). This economic activity represents a very important economic asset for the local fishing

industry. Farmed bivalves remove a large amount of phytoplankton (Karuza et al., 2016), primarily diatoms, phototrophic dinoflagellates and coccolithophores, and preferably 20–100 µm size class ones (Del Negro et al., 2014). During a spring diatom bloom, the stomach content analysis revealed that their diet was composed of up to 99% diatoms (Solidoro et al., 2010). When plankton is not available, they filter out suspended particulate matter from the water column, which has lower energy content with a resulting decline in mussel quality. A mussel diet based mainly on detritus leads to lower flesh weight and, consequently, significant economic losses (Caroppo et al., 2012).

The reduction in phytoplankton biomass is also expected to have a direct negative effect on the benthic trophic web through pelagic-benthic coupling (Griffiths et al., 2017). Sinking phytodetritus from surface waters represents a prime source of high-quality food supply for marine benthic organisms (Quijón et al., 2008). In the Gulf of Trieste, exceptionally low primary sedimentation rates were already observed in 2003, as a consequence of the absolute minimum phytoplankton abundance of the previous eighteen years (Cibic et al., 2007). Indeed, considering the 2002–2005 period, lower particulate organic C contents were measured in the water column in 2003, thus affecting macrofaunal community structure and feeding guilds (Nasi et al., 2017). Although we do not have macrobenthos data for the March 2006–February 2007 period to corroborate this assumption, it is likely that a similar negative effect on the benthic trophic web occurred following the reduced phytoplankton biomass observed during the study period, which may have affected the essential ecosystem services provided by benthic organisms.

5. Conclusions

The reductions in freshwater and nutrient inputs observed during the study period were very likely responsible for a decrease in pelagic phototroph abundances, particularly of microphytoplankton and cyanobacteria. In contrast, nanophytoplankton did not seem to be as severely affected, since comparable values to the climatological ones were observed. However, our nanophytoplankton time series was shorter than the other two biological climatologies, thus limiting the likelihood of highlighting deviations from the typical values for the area. The anomalous physical-chemical features that occurred in the study year altered the succession of the pelagic phototrophs typical of this area, since an exceptional cyanobacteria bloom, together with high nanophytoplankton abundances first occurred in April, followed by a delayed diatom bloom. We did not observe differences in the microphytoplankton community structure that was dominated by the usual keystone species. However, the late-winter/early-spring diatom bloom developed only in May and was not reflected in high photosynthetic rates. Extremely high primary production was estimated in autumn due to very stable atmospheric and oceanographic conditions. The reduced availability of phytoplankton biomass may have several repercussions on both the pelagic and benthic trophic webs as well as on the quality of the blue mussels extensively farmed in the area.

The Gulf of Trieste may be considered a natural megacosm due to its peculiar geomorphologic characteristics and we believe that the structural and functional response of phytoplankton to anomalous physical-chemical conditions observed in this area may have broader implications and could be extended beyond the geographical limits of this particular ecosystem.

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