

Planktonic Assemblages in a Coastal Mediterranean Area Subjected to Anthropogenic Pressure

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Abstract

In marine environment phytoplankton and picoplankton are responsible for a bulk of production and nutrient cycling and may give important information about the different seawater habitats. In the present paper, the temporal changes of phytoplankton as well as autotrophic and heterotrophic picoplankton abundance and biomass in two coastal areas in the Gulf of Taranto (Mediterranean Sea) subjected to different levels of anthropogenic pressure were studied and related to the main environmental variables. The two analysed areas were significantly different as regards the abiotic conditions which also varied temporally. Univariate analyses revealed that larger phytoplankton and heterotrophic picoplankton abundance and biomass varied as well. The multivariate analyses showed a complex distribution of the whole planktonic assemblages, which varied in time and space without a decipherable pattern, presumably due to the peculiar spatial-temporal dynamics of the sole autotrophic picoplankton abundance. Significant correlations between planktonic assemblages and environmental variables were discussed by taking into account also the potential role of the considered planktonic components as useful environmental monitoring parameters.

Keywords: Abiotic Factors; Nutrients; Picoplankton; Phytoplankton; Statistical Analysis; Mediterranean Sea

Introduction

More than 70% of the world's human population lives in the coastal environments where are the boundaries between a positive and negative effect in terms of biomass production and fuelling the whole trophic web [1]. Thus coastal man-caused nutrient over enrichment and subsequent eutrophication result in reduced water quality and habitat changes which are recognized as problems in most coastal European countries [2]. In the last fifty years, the growing human impact in the Mediterranean has strongly enhanced the fluxes of nutrients, mainly nitrogen while phosphorus, after an initial increase in 1980-1990s, rapidly dropped down as a consequence of the policy in combating phosphorus pollution in different countries [3]. Although Mediterranean Sea is considered oligotrophic and a phosphorus limited system, its coastal waters could be subjected to eutrophication phenomena, mainly due to the high density of population and the low intensity of the currents which reduces the dilution of sewages [4]. According to the European Water Framework Directive 2000/60 (WFD) [5] the environmental quality of marine ecosystems is defined using the Ecological Quality Ratio (EQR) for a number of biological and chemical quality elements. Up to now among the key biological elements specified for the assessment of the coastal waters quality, phytoplankton is the planktonic element mainly considered. Phytoplankton indeed respond quickly to many environmental stressors and provide useful information on water quality, hydrology or climate changes [6] and is considered a better indicator of nutrient loads changes than nutrient and chlorophyll *a* concentrations in water [7]. Also the more recent Marine Strategy Framework Directive 2008/56 (MSFD) [8], in the frame of the descriptor 5 (Eutrophication), takes in consideration phytoplankton biomass and composition to evaluate the effects of human pressure on coastal systems. In addition, recently, new protocols and set of tools to help guide the ecosystem management are developing. These consider the effects of environmental changes on living organisms and, more specifically, on the whole pelagic component including also picoplankton [9]. Picoplankton has been reported to include not only piko-sized, heterotrophic bacteria but

also, often to a considerable extent, < 2 μm pigmented organisms, i.e. cyanobacteria, and small pigmented eukaryotes [10]. Small autotrophs constitute a significant fraction of the total primary production in many systems where they are superimposed on the classical pathway based on the larger phytoplankton and thus are responsible for the bulk of productivity and nutrient cycling.

Usually, increasing nutrient inputs, as experienced in many coastal sites, are expected to lead to increased plankton biomass. Knowledge of the capacity of plankton communities to increase the growth rates as response to the input of nutrients is very important taking into account that this is one of the triggers of the problems associated to eutrophication [11]. The implications of an increase in primary production are expected at all the trophic levels of the ecosystems, particularly at the microbial level taking into account that algae liberate a variety of monomeric and polymeric organic compounds [12] which constitute a major resource for the heterotrophic bacteria. Bacteria indeed respond quickly to biotic and abiotic changes in their environment and data on the bacterial community may give important information about the different seawater habitats since the bacterial metabolic activity influences the water quality [13,14]. In this framework our aim was to evaluate the spatial and temporal dynamics of the phytoplankton and picoplankton communities in two coastal areas of the Taranto Gulf (Northern Ionian Sea, Mediterranean Sea) subjected to different levels of anthropogenic impact. We considered the two areas as "living laboratories" useful to evaluate whether

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| Code | Location | WWTPs | WWTPs CAPACITY (p. e.) | TOTAL DEMAND (p. e.) | TREATMENT LEVEL |
|------|---|----------------------|------------------------|----------------------|-----------------|
| a | Ginosa Marina | Ginosa 2 Marina | 51640 | 14948 | Secondary |
| b | Castellaneta Marina Castellaneta 2 Marina | | 60000 | 18606 | Secondary |
| c | Taranto | Taranto 1 Gennarini | 100000 | 252267 | Secondary |
| d | | Taranto 2 Bellavista | 116723 | 65561 | Tertiary |
| e | Porto Cesareo | Porto Cesareo | 31200 | 29429 | Secondary |
| f | Nardò | Nardò | 31627 | 58131 | Secondary |
| g | Gallipoli | Gallipoli | 80000 | 73887 | Secondary |

Table 1: Capacity of the WWTPs (Waste Water Treatment Plans) located in the study area (coast between Ginosa and Gallipoli). Capacity and Total Demand are expressed as p.e. – person equivalent. Data from Apulia Region, 2009. Individuazione e perimetrazione degli agglomerati urbani della Regione Puglia. Environmental Emergency Final Report. Regional Law n° 3184 of the Council of 22 March 2002. Apulia Region, Bari (Italy).

phytoplankton and picoplankton constitute useful and sensitive environmental descriptors and their abundances and biomass are related to the variations of the physico-chemical parameters.

Experimental Procedures

Study area

The study area is the coastal Taranto Gulf, which is affected by the general circulation dynamics of the Ionian Sea and represents the transition zone of different water masses: one from the Northern Adriatic Sea (Adriatic Surface Water, ASW) and the other from the Ionian Sea (Ionian Surface Water, ISW). The surface circulation is generally cyclonic, with a high variability and a vertical structure strongly dependent on a seasonal cycle. During winter-spring, the ISW, characterised by low temperature and high salinity [15], invades both the Taranto Gulf and the Southern Adriatic Sea [16]. During summer-autumn, the low-salinity ASW moves southward and occupies both the Southern Adriatic Sea and the Taranto Gulf [16]. Levantine Intermediate Water (LIW) may be also present in this area, when vertical mixing occurs; as it is particularly rich in nutrients, LIW may enhance the productivity of the basin [15]. Eight coastal sampling points were selected randomly monthly in the Gulf of Taranto (Figure 1) located at a distance of 0.5 km from the coastline and with an average depth of 20 m (range between 5 and 40 m) in two areas subjected to different anthropogenic impact [17].

All the sampling points were chosen at random being distant each other from 10 to 15 km (Figure 1). Four sampling points were located in the western-side of the study area (comprised between Castellaneta and L. Silvana) being characterized by the presence of industrial wastes (stainless-steel and oil refinery), urban waste-water treatment plants and rivers discharge outflow (Lenne, Lato and Galaso) collecting runoff from the surroundings fertilized fields (Figure 1). In this area, the urban waste-water Gennarini has a depuration capacity corresponding to approximately 100,000 person equivalents (p.e.) which is widely under the real influent load of about 250,000 p.e. [18]. Four sampling points were also selected in the eastern-side (comprised between Campomarino and Santa Maria al Bagno) subjected to less pressure in terms of river inflow and human population. In fact, the only sewage treatment plant of Nardò is slightly under-sized respect to the real total demand of about 58,000 p.e.; furthermore, in 1997 at Porto Cesareo (in the center of the eastern area) a Marine Protected Area has been established to preserve the present high water quality.

Sample collection and data analyses

Surface seawater samples were collected monthly from January to December using a 5 L Niskin bottle. Water temperature and salinity were recorded by an Idronaut Ocean Seven 501 multiprobe. Nutrient

(NH₄⁺ -N, NO₂⁻ -N, NO₃⁻ -N, PO₄³⁻ -P and Ptot) concentrations

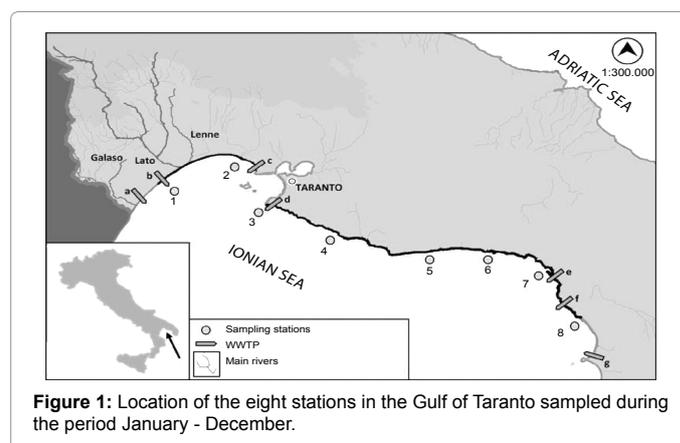


Figure 1: Location of the eight stations in the Gulf of Taranto sampled during the period January - December.

were measured by the spectrophotometric method according to Strickland & Parsons [19].

Larger phytoplankton abundance: Phytoplankton samples, freshly collected, were fixed with Lugol's iodine solution and examined under inverted microscope (Labovert FS Leitz equipped with phase contrast) at a magnification of 400× and 630×. Depending on phytoplankton densities, sub-samples varying from 50 to 100 ml were allowed to settle for 24-48 hours and examined following the Utermöhl method [20, 21]. Phytoplankton larger than 2-3 μm was mainly represented by diatoms, dinoflagellates, coccolithophorids, and phytoflagellates (forms of uncertain taxonomic classification <10 μm, prasinophyceans, chrysophyceans, cryptophyceans, euglenophyceans and silicoflagellates). Total chamber bottom was scanned for taxa larger than 30 μm, while abundant microphytoplankton (>20 μm) were counted at two transects. Nanophytoplankton cells (2–20 μm) were counted in 15 randomly selected fields with a magnifications of 630×.

Picoplankton abundance: In order to estimate the picoplankton abundance, water samples were preserved with formaldehyde (2%) and kept at 4 °C until they could be counted (within four weeks). The cell counts were made using a Zeiss Standard Axioplan microscope equipped with a halogen (Hg 100) light. For picophytoplankton (APP) duplicate slides were prepared from each sample by filtering 10 ml of seawater onto 0.2 μm (pore size) Millipore black membranes. Under blue light excitation, cyanobacterial cells fluoresced yellow-orange whereas eukaryotic algae fluoresced deep red. A BP 485/20 exciter filter, a FT 510 chromatic beam splitter and a LP 520 barrier filter were used. For heterotrophic picoplankton (HPP) duplicate slides were prepared from each sample by filtering 1 ml of seawater onto a 0.2 μm (pore size) Millipore filter, using DAPI (4,6 - diamidino-2 phenylindole) as fluorochrome [22]. A G 365 exciter filter, a FT 395 chromatic beam

splitter and a LP 420 barrier filter were used. At least 40 microscopic fields at $\times 1000$ magnification were counted for each preparation.

Phytoplankton and picoplankton biomass estimation: Phytoplankton cell volumes were calculated for 45 photosynthetic taxa which on the average comprised 98% of total cell numbers. Cell volumes were calculated by assigning cells to one geometrical body or, in some cases, to a combination of more geometrical bodies, and applying standard formulae [23]. Phytoplankton biomass was calculated by multiplying the cell abundances for the Carbon Content calculated using the relationship reported by Menden-Deuer & Lessard [24]. Cell size of APP and HPP was estimated by epifluorescence microscopy using microphotographs. Each cell size was determined after projection on a screen and at least 60 cells per filter were measured manually. For APP cell volume was calculated assuming that the shape of picoplankton was spherical or cylindrical with hemispheric ends [25] and using the Bratbak formulas [26]. The biomass was calculated by multiplying the cell abundances for a Carbon Content of $254 \text{ fg C } \mu\text{m}^{-3}$ [27] for cyanobacteria, $1500 \text{ fg C } \mu\text{m}^{-3}$ for picoeukaryotes [28]. HPP cells were subdivided into three size classes: small, medium and large (<0.065 , $0.065\text{--}0.320$ and $0.320\text{--}0.780 \text{ } \mu\text{m}^3$) [29]. HPP biovolume was converted into biomass assuming a carbon content of $310 \text{ fg C } \mu\text{m}^{-3}$ [30].

Statistical analyses

Univariate analyses: Spatial-temporal differences in the univariate patterns of temperature, salinity, nutrient concentration, N:P ratio, phytoplankton, autotrophic and heterotrophic picoplankton abundances were analysed using 2-way ANOVA performed by PERMANOVA [31] based on Euclidean distance measure [32], in order to avoid any assumption about the distribution of the variables [31,33]. In this analysis the F statistics were calculated but the p-values were obtained by permutation.

Multivariate analyses: Spatial-temporal differences in the multivariate pattern of abundance of the planktonic assemblages were analysed using 2-way PERMANOVA [30] based on Bray-Curtis similarity measure. In order to detect possible correlations between the planktonic assemblages and the environmental variables (temperature, salinity, nutrient concentration, and N:P ratio) the standard BEST-BVSTEP routine was performed. The BEST-BVSTEP routine was used to month-by-month test for correlations between the distribution pattern of the planktonic assemblages and the normalized matrix of the environmental predictor variables. This routine is a stepwise procedure searching the best combination of environmental variables correlated with the plankton assemblage distribution by Spearman rank correlation. All the analyses were performed using the computer program PRIMER v6 (PRIMER-E ltd), including the add-on package PERMANOVA+ [34].

Results

Environmental parameters

Results concerning the hydrological parameters are reported in Figure 2. In particular the annual trend of water temperature was sinusoidal. Average values ranged from $12.66 \pm 0.23 \text{ } ^\circ\text{C}$ (winter) to $27.39 \pm 0.27 \text{ } ^\circ\text{C}$ (summer) (Figure 2A). Salinity ranged between 37.49 ± 0.37 and $38.54 \pm 0.09 \text{ PSU}$ (Figure 2B).

As regards the distribution and concentration of N and P, high values of these nutrients were generally observed in winter and in the

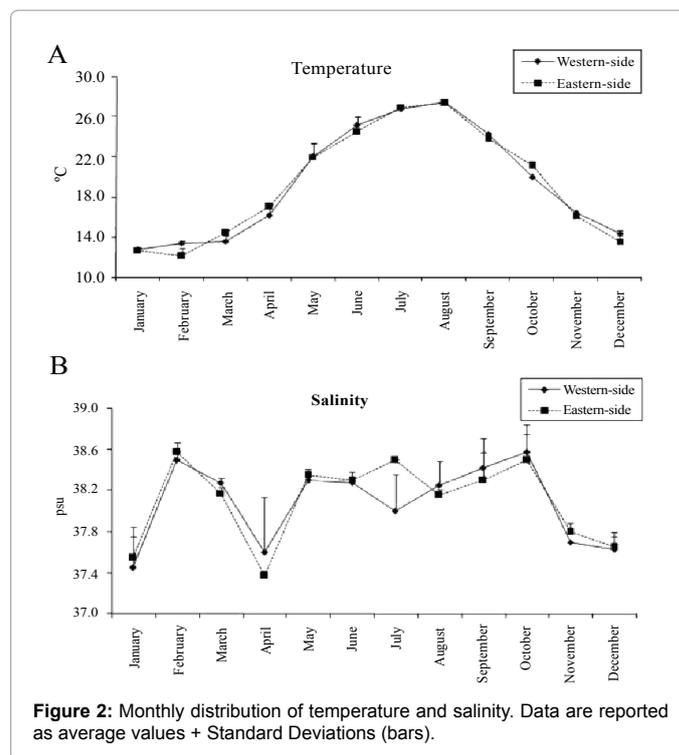


Figure 2: Monthly distribution of temperature and salinity. Data are reported as average values + Standard Deviations (bars).

| Variable | | position F11,72 | position F1,11 | time F11,72 |
|-----------------|------------------|--------------------|-------------------|----------------|
| Temperature | | 3.3718** | 0.10204 | 819.49** |
| Salinity | | 1.5558 | 0.15652 | 28.16** |
| NH ₄ | ⁻ -N | 1.2568 | 17.219** | 1.0665 |
| NO ₂ | ⁻ -N | 2.7159** | 23.513*** | 1.5734 |
| NO ₃ | ⁻ -N | 4.7301*** | 1.7072 | 19.332*** |
| PO ₄ | ³⁻ -P | 1.2248 | 26.61** | 4.6113** |
| Ptot | | 1.2857 | 56.192** | 1.0955 |
| N:P | | 0.080787 | 61.664** | 1.473 |

Table 2. F-ratios and associated level of significance (** = $p < 0.01$, *** = $p < 0.001$) resulting from the 2-way ANOVAs testing spatial-temporal differences in the nutrients concentrations, N:P ratio, temperature and salinity distribution.

western-side. In particular, ammonium (Figure 3A) showed the highest values from November to March (up to $22.90 \text{ } \mu\text{M}$ in December). Nitrite showed an increase of the concentrations in winter (up to $1.46 \text{ } \mu\text{M}$ in February) (Figure 3B). A decrease of nitrite levels (around $0.3 \text{ } \mu\text{M}$) was generally detected in spring. As regards nitrate distribution (Figure 3C) the highest concentrations (from 9.0 to $11.0 \text{ } \mu\text{M}$ during the January-May period) were detected at the western-side. In summer, nitrate concentration leveled off at around $1 \text{ } \mu\text{M}$ in the whole study area. Finally, phosphorus, as orthophosphate (Figure 3D) accounted for the least abundant element among the nutrients studied with extremely low values ranging from 0.001 to $0.55 \text{ } \mu\text{M}$ for the period under examination and with significantly higher values in December ($0.55 \text{ } \mu\text{M}$) in the western side. As regards the total phosphorus distribution (Figure 3E), it did not show significant fluctuations throughout the year with values ranging between 0.14 and $1.25 \text{ } \mu\text{M}$. The horizontal distribution of the N:P ratio evidenced values usually higher in the western-side (Figure 3F). As regards the seasonal distribution, N:P ratio appeared, on average, higher than the theoretical value of 16 for all the sampling period, except for the summer period (in the whole area) and the fall period (only at the eastern-side).

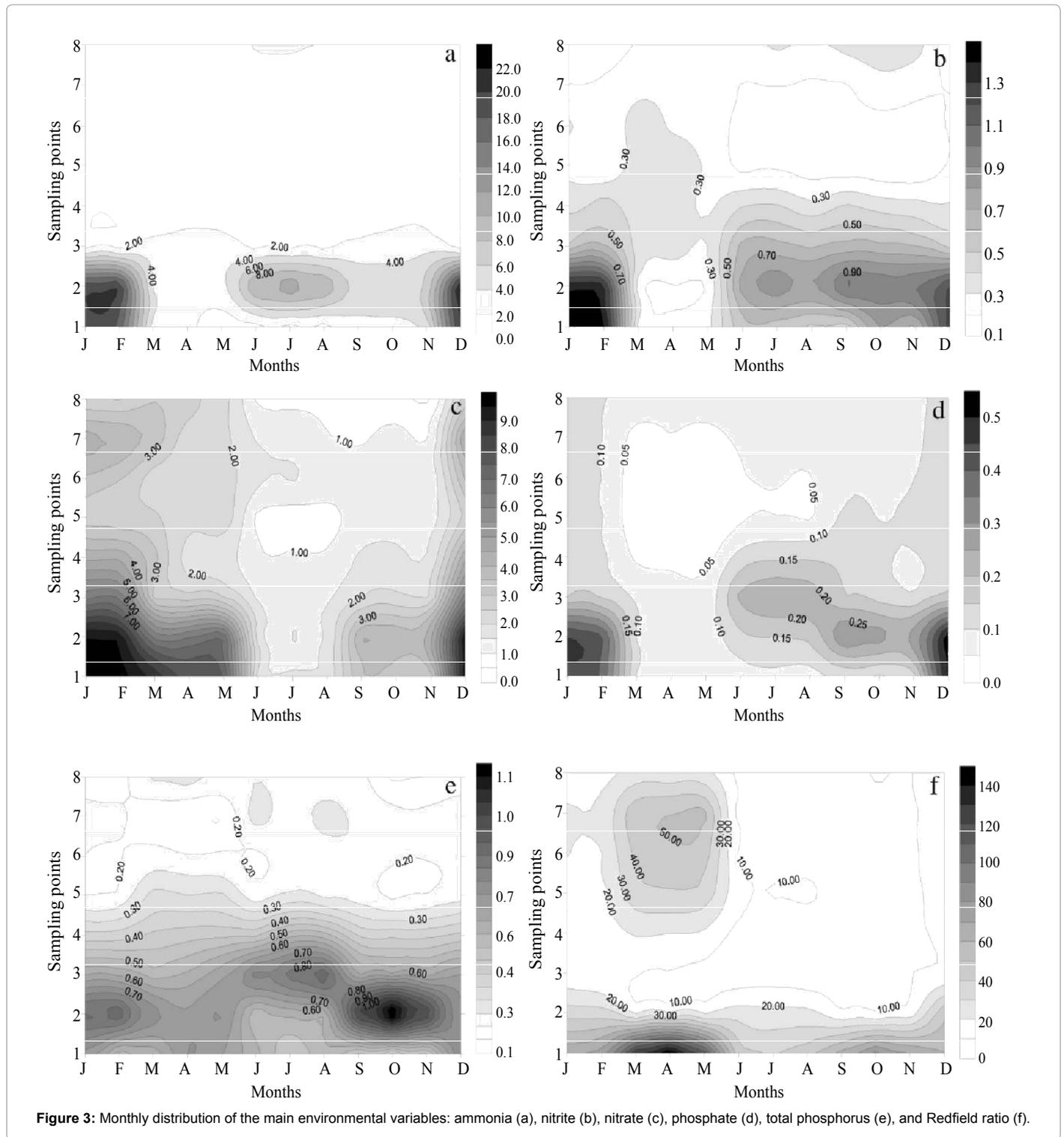


Table 2 reports the outcomes of the 2-way ANOVAs testing spatial-temporal differences in the univariate pattern of temperature, salinity, nutrient concentration, and N:P ratio distribution. Ammonium, phosphate concentrations and N:P ratio showed significant differences between the two analyzed areas, as well as phosphate concentration that, in addition, showed significant differences also among sampling times. The significance of the position \times time interaction term indicates

that temperature, nitrite, nitrate concentrations varied among times depending on the considered sector without a clear pattern. In particular, the pair-wise tests (data not showed) indicate that nitrite concentration was significantly different between the two areas from June to February but not in March, April and May. As far as nitrate concentration, it was significantly different between the two areas from September to February but not during the rest of the year. Temperature

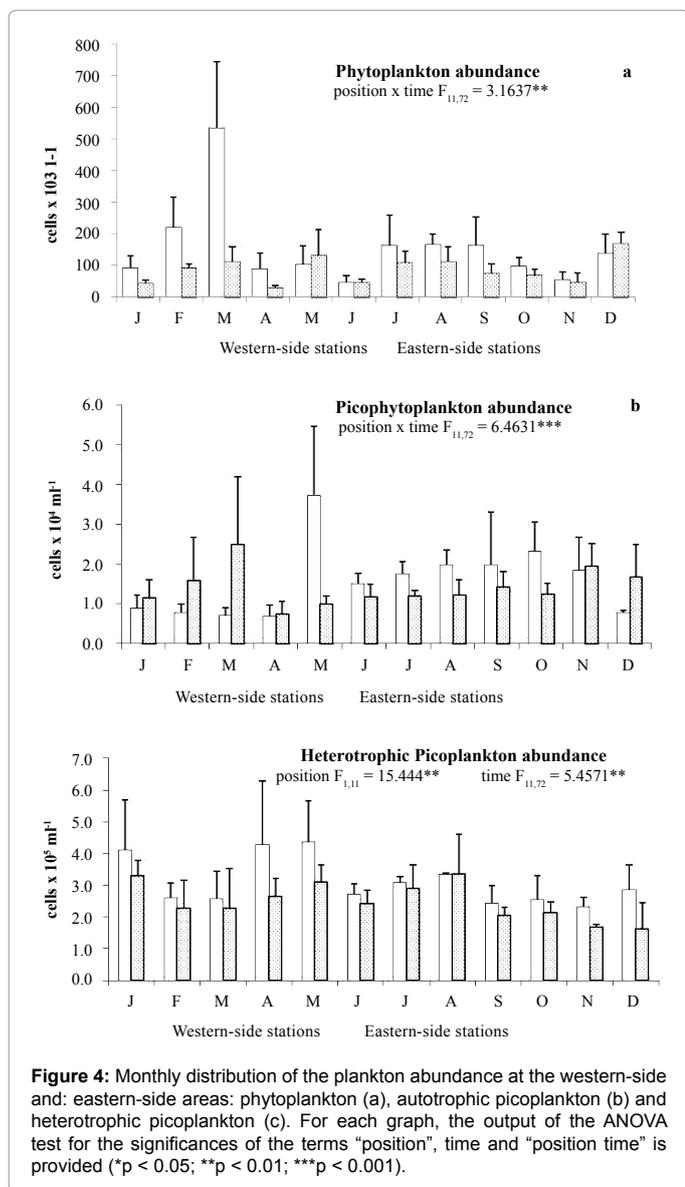


Figure 4: Monthly distribution of the plankton abundance at the western-side and eastern-side areas: phytoplankton (a), autotrophic picoplankton (b) and heterotrophic picoplankton (c). For each graph, the output of the ANOVA test for the significances of the terms "position", time and "position time" is provided (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

was significantly different between the two areas in February, March, October, November and December.

Planktonic abundance and biomass

Larger phytoplankton: Average phytoplankton abundance generally showed low concentrations, usually below $200 \times 10^3 \text{ cells l}^{-1}$ (Figure 4A), except for February and March. The western-side was generally characterized by higher values ($155.3 \pm 145.4 \times 10^3 \text{ cells l}^{-1}$) than the eastern-side ($87.5 \pm 50.7 \times 10^3 \text{ cells l}^{-1}$), especially in winter. At the eastern-side phytoplankton abundance value trend was almost stable with sporadic increases of values only in December.

Like abundance, also biomass was usually higher at the western-side ($62.6 \pm 70.9 \mu\text{g C l}^{-1}$) than at the eastern-side ($20.8 \pm 10.4 \mu\text{g C l}^{-1}$). In particular, higher values (up to $261.3 \mu\text{g C l}^{-1}$) were detected in winter at the western-side, but an increase of biomass was observed in May and December (up to 35.7 g C l^{-1}) at the eastern-side (Figure 5a, 5b). Phytoplankton biomass was mainly represented by diatoms and dinoflagellates which formed the 69% (western-side) and 58% (eastern-

side) of the total biomass, respectively. Nano-sized phytoflagellates contributed to the biomass mainly in the fall-winter period at the eastern-side. The linear regression analysis revealed that phytoplankton biomass and abundance were significantly related ($r^2 = 0.60$, $p < 0.05$). The significance of the position \times time interaction term in the ANOVA test indicates that the larger phytoplankton abundance varied among times depending on the considered sector without a clear pattern (Figure 4A). The pairwise test (data not showed) revealed significant differences between the two areas in January, February, March, and April.

Autotrophic Picoplankton (APP): Autotrophic picoplankton abundance showed similar average values in the western-side ($1.6 \pm 0.9 \times 10^4 \text{ cells ml}^{-1}$) and in the eastern-side ($1.4 \pm 0.5 \times 10^4 \text{ cells ml}^{-1}$) throughout the year (Figure 4B). At the western-side the seasonal trend showed low concentrations from January to April with a peak in May (up to $3.8 \pm 1.7 \times 10^4 \text{ cells ml}^{-1}$). Relatively higher abundances were detected from July to November with the maximum concentrations in October ($2.3 \pm 0.7 \times 10^4 \text{ cells ml}^{-1}$). In the easternside APP abundance was almost stable throughout the year, except for an increase during the February-March period. APP biomass was slightly higher in the western area ($2.1 \pm 1.1 \mu\text{g C l}^{-1}$) than in the eastern-side ($1.7 \pm 0.5 \mu\text{g C l}^{-1}$) (Figure 5C-D). At the western-side the higher biomass values were detected from May ($4.6 \pm 1.2 \text{ g C l}^{-1}$) to November while at the eastern-side in the late winter ($2.5 \pm 1.1 \mu\text{g C l}^{-1}$, March) and fall periods. As regards the composition, the population was predominantly composed by yellow-fluorescing cyanobacteria $0.5\text{-}1.5 \mu\text{m}$ in diameter of the *Synechococcus* type. The eukaryotic APP component represented on

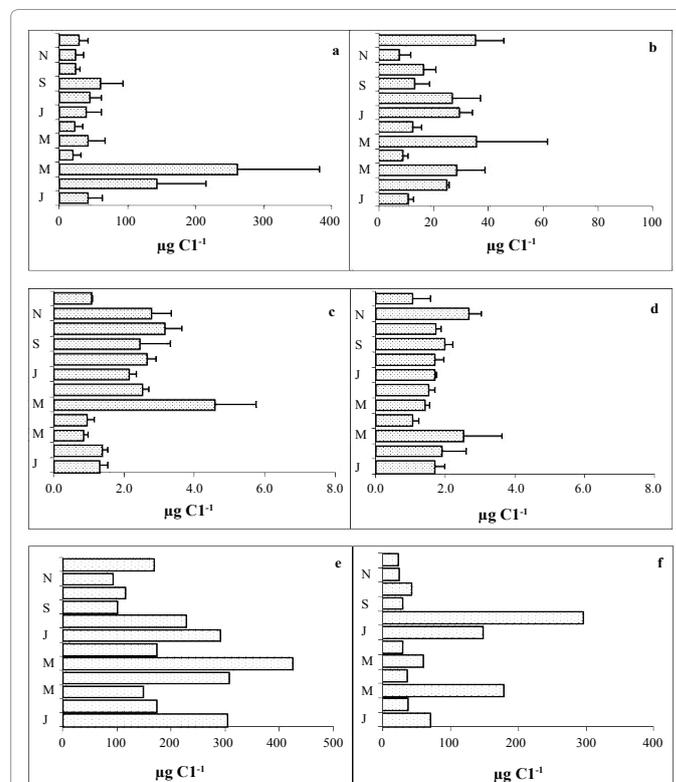


Figure 5: Monthly distribution of the plankton biomass. Western-side area: phytoplankton (a), autotrophic picoplankton (c) and heterotrophic picoplankton (e). Eastern-side area: phytoplankton (b), autotrophic picoplankton (d) and heterotrophic picoplankton (f). Data are reported as average values + Standard Deviations (bars).

average only the 3.0% of the total APP biomass, with maximum values detected in winter (January- February) in the whole study area. The linear regression analysis revealed that APP biomass and abundance were significantly related ($r^2 = 0.79$, $p < 0.05$). The significance of the position \times time interaction term in the ANOVA test indicates that the APP abundance varied among times depending on the considered sector without a clear pattern (Figure 4B). The pairwise test (data not showed) revealed significant differences between the two areas in March, May, July, August and October.

Heterotrophic picoplankton (HPP): Heterotrophic picoplankton abundance showed higher values in the western-side than in the eastern side (Figure 4c) throughout the year with a mean density of 3.0×10^5 cellsml⁻¹ in the former and 2.4×10^5 cellsml⁻¹ in the latter. The trend of HPP was characterized by two major peaks (January, spring) in the western-side and by two major peaks (January, and August) in the eastern-side. In particular the highest HPP abundances were recorded in April (up to 7.1×10^5 cellsml⁻¹) and in May (up to 6.2×10^5 cellsml⁻¹). By contrast, as mentioned above, in the eastern-side the maximum values were observed in August (5.0×10^5 cells ml⁻¹) and in January (3.7×10^5 cellsml⁻¹). In the whole area the lowest values were recorded from September to December. Biomass averaged values were usually higher at the western-side ($210.6 \pm 9.8 \mu\text{g Cl}^{-1}$) than at the eastern side ($81 \pm 7.2 \mu\text{g Cl}^{-1}$). In particular, the highest values were observed in May ($420 \mu\text{g Cl}^{-1}$) at the western-side and in August ($296 \mu\text{g Cl}^{-1}$) at the eastern side (Figure 5E, 5F). The linear regression analysis revealed that heterotrophic picoplankton biomass and density were significantly related ($r^2 = 0.65$, $p < 0.05$). The ANOVA showed significant temporal

variation in the HPP abundance as well as significant differences between the two areas (Figure 4C).

Multivariate statistical analyses

The PERMANOVA test analysing the multivariate pattern of spatial-temporal distribution of the whole examined planktonic assemblages revealed that they varied among times in different ways depending on the sampled area without a decipherable pattern (position \times time: $MS = 11.73$; Pseudo- $F_{11,72} = 3.9462$; $p < 0.001$). PERMANOVA pair-wise test showed significant differences in the planktonic assemblages between the two analysed areas in February, March, April, May, October and December.

Relationships of planktonic assemblages with environmental factors: The BEST-BVSTEP tests (Table 3) indicated that the multivariate pattern of distribution of the planktonic assemblages was correlated with the set of selected variables in January, March, May, September and October. The strong levels of correlation (especially for January, June, September and October) suggest the direct influence of nutrients and physical-chemical parameters on the assemblages. Temperature appears in the variable selection in March, May and September while salinity in March and May. The analyses indicate general high level of correlation among the nutrients and nitrogen salts (especially ammonium) playing a major role, while the phosphorous appears to have important influence during spring (March, May, June) and autumn (October). The N:P ratio appears among the predictor variables only in June when it is the only strongly correlated variable.

| | Rho | p | var. sel. | corr. var. |
|------|-------|----|----------------------------------|---|
| Jan | 0.727 | * | Ptot | NH ₄ ⁺ -N (0.84), NO ₂ ⁻ -N (0.91); NO ₃ ⁻ -N (0.95); PO ₄ ³⁻ -P (0.88) |
| Feb | 0.436 | ns | T | |
| - | | | Ptot | |
| Mar | 0.799 | ** | T | |
| | | | S | T (-0.66), NH ₄ ⁺ -N (0.66), Ptot (0.70) |
| | | | NH ₄ ⁺ -N | T (-0.70), S (0.66), PO ₄ ³⁻ -P (0.77), Ptot (0.96) |
| Apr | 0.655 | ns | NH ₄ ⁺ -N | |
| | | | NO ₂ ⁻ -N | |
| | | | NO ₃ ⁻ -N | |
| | | | Ptot | |
| | | | N:P | |
| May | 0.681 | * | T | nc |
| | | | S | NO ₂ ⁻ -N (0.74) |
| | | | NH ₄ ⁺ -N | Ptot (0.73) |
| | | | NO ₂ ⁻ -N | S (0.74) |
| | | | Ptot | NH ₄ ⁺ -N (0.73) |
| June | 0.394 | ns | PO ₄ ³⁻ -P | |
| July | 0.274 | ns | NO ₃ ⁻ -N | |
| | | | N:P | |
| Aug | 0.488 | ns | T | |
| | | | NO ₃ ⁻ -N | |
| Sep | 0.714 | * | T | NO ₃ (0.68) |
| | | | NH ₄ ⁺ -N | NO ₂ ⁻ -N (0.94), NO ₃ ⁻ -N (0.95), PO ₄ ³⁻ -P (0.85), Ptot (0.94) |
| | | | NO ₃ ⁻ -N | T (0.68), NH ₄ ⁺ -N (0.95), NO ₂ ⁻ -N (0.79), PO ₄ ³⁻ -P (0.72), Ptot (0.84) |
| Oct | 0.815 | ** | NO ₃ ⁻ -N | T (-0.79), NH ₄ ⁺ -N (0.95), NO ₂ ⁻ -N (0.90), PO ₄ ³⁻ -P (0.76), Ptot (0.86) |
| | | | PO ₄ ³⁻ -P | NH ₄ ⁺ -N (0.84), NO ₂ ⁻ -N (0.71), NO ₃ ⁻ -N (0.76), Ptot (0.96) |
| Nov | 0.05 | ns | Ptot | |
| Dec | 0.173 | ns | T | |
| | | | Ptot | |

Table 3: Results of the BEST-BVSTEP routine reporting the correlation among the abiotic variables and the planktonic assemblages. Rho = level of correlation; p = significance (** = $p < 0.01$; * = $0.05 > p > 0.01$; ns = not significant); var. sel. = selected variables. When the test is significant, the column "corr. var." reports the level of correlation between the selected variables and the other abiotic variables.

Discussion

In this study we examined and compared autotrophic and heterotrophic planktonic components as well as physical-chemical variables in two coastal areas of the Gulf of Taranto (Northern Ionian Sea) subjected to different anthropogenic pollution. Although the monthly sampling scale and the single year examined could have affected the detection of the actual seasonal cycle in the study area, our data represent a contribute to the global observations of microbes and their reactions to environmental factors variability and several interesting issues can be gathered.

Inorganic nutrient concentration

As indicated by the univariate analyses all inorganic nutrient concentrations, except nitrate, were significantly higher in the western-side area than those observed in the eastern-side one thus confirming that the two examined areas have different features. The differences between the two investigated areas were due to the presence of industrial wastes (stainless-steel and oil refinery), rivers discharge outflow (Lenne, Lato and Galaso) collecting runoff from the surroundings fertilized fields and urban waste-water treatment plants including the large sewage treatment plant of Gennarini which has a depuration capacity widely under the real urban influent load. As concerning the nutrient concentrations, in the former area they were similar to those recorded in other mesotrophic areas in the Ionian Sea [35], Cretan Sea [36], and other Mediterranean coastal sites [4]. By contrast in the eastern-side area inorganic nutrients showed values comparable to those observed in the Mediterranean open waters [37] and higher than those observed in open oligotrophic waters [38-40].

Phytoplankton and picoplankton abundance and biomass

The PERMANOVA test analysing the spatial-temporal pattern of distribution of the whole examined planktonic assemblages revealed that they varied both in space and time without a decipherable pattern. However, the univariate tests separately considering the phytoplankton, autotrophic and heterotrophic picoplankton abundances depicted different spatial-temporal distribution patterns of these three components in the area. Concerning the phytoplankton abundance and biomass, we recorded values lower than those observed in other coastal Mediterranean sites [41]. By contrast the herein observed values were higher than those reported for the open waters [38-42]. Usually higher phytoplankton abundances and biomass were detected in the polluted western-side area confirming phytoplankton as a sensitive and important indicator of eutrophication, on account of the fast growth rates (i.e. doubling times of a day or less) and rapid response to a wide range of environmental perturbations including nutrient enrichment [43]. Our data are also in agreement with laboratory experiments, demonstrating the impact of a domestic sewage effluent on the dynamics of phytoplankton assemblages [44]. Picophytoplankton abundances and biomass were of the same order of magnitude as those reported in other coastal Mediterranean environments [45]. Moreover, the picophytoplankton abundance and biomass showed a peculiar spatial-temporal distribution being characterized by higher values in the summer-autumn period at the western side stations and in the winter-spring period at the eastern-side stations, respectively. The high values observed in summer autumn in the western side stations, are in agreement with data available in literature from oceanic and coastal areas where picophytoplankton is reported to play an important role in the production and eutrophication potential [46, 47]. The heterotrophic picoplankton densities were also comparable to those reported in other coastal Mediterranean environments [48]. Moreover, the ANOVA

test revealed that heterotrophic picoplankton abundances varied significantly throughout the year. In all the examined sampling points the lowest bacterial density values were evidenced from September until December (autumn period). It is well known that bacterial growth may be limited by several factors such as temperature, DOM, labile organic carbon, inorganic nutrients or micronutrients [49] as well as by biotic factors (e.g. grazing, competition). At the moment explanations can be only hypothetical and further studies are needed to explain the bacterial distribution observed in this study. The same ANOVA analysis showed significant differences between the two analysed areas indicating that they experienced different environmental conditions. We can hypothesize that "nutrient-fertilisation" associated with sewage and industrial pollution in the western-side area stimulated heterotrophic picoplankton at least in two ways: (i) by modifying the quantity and availability of organic nutrients to heterotrophic bacteria and/or (ii) by supplying inorganic nutrients, which can be used by heterotrophic bacteria as nutritional supplements. The pattern of heterotrophic picoplankton biomass followed that of abundance, with bacterial biomass values about two order of magnitude higher in the western-side area throughout the year. Thus, heterotrophic picoplankton, as already reported for other studies [50], seems a sensitive indicator of the environmental conditions. The increase of heterotrophic picoplankton in the polluted area is of particular interest since the microbial community mediates, by the specific metabolic activities, several important ecological processes related to pollution of aquatic systems. They indeed clean up contaminated environment by degradation, transformation or accumulation of a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals [51,52].

Relationships of planktonic components with environmental factors

The multivariate correlation analysis indicated that the abiotic parameters are significantly correlated with the spatial-temporal distribution of the planktonic assemblages in January, March, May, September and October. On the other hand, the PERMANOVA pairwise test revealed significant differences in the planktonic assemblages between the two analysed areas in February, March, April, May, October and December. Comparing the two analyses, it is possible to identify the "critical" months during which the considered abiotic variables might presumably be responsible for the observed differences between the two areas, namely March, May and October. Particularly, in March temperature, salinity, and ammonia were the variables mostly correlated to the planktonic assemblages distribution and they were also collinear with the temperature, phosphate and ammonium. In fact, during the winter-spring period, the Ionian Surface Waters (ISW), characterized by low temperature and high salinity, invades both the Gulf of Taranto and the Southern Adriatic Sea. These waters are especially rich in nutrients and presumably contribute to the primary production increase. In particular, in the oligotrophic eastern side area, the input of nutrients seems to favour the pico-sized autotrophs, which in this area reached their annual peak in March and gave their highest contribution to the total phytoplankton biomass. Our data confirm that, on account of their small size and high surface-to-volume ratios, picophytoplankton are more competitive than larger phytoplankton in acquiring nutrients in oligotrophic systems [53]. In the mesotrophic western side the further nutrient input due to the ISW together with the availability of ammonia, significantly different between the two areas, were presumably responsible for the abundance and biomass increase of larger phytoplankton dominating over the pico-sized

autotrophs. In May, temperature, salinity, ammonia, nitrite, and Ptot were significantly correlated with the planktonic assemblages. The ANOVA showed that, among the three considered components, picophytoplankton reached the highest abundance values in the impacted area. The increase of rain events detected in April (C.C. personal observation) could be considered as responsible for the low level of salinity particularly evident in the western-side where some freshwaters tributaries flow (small rivers and wastewaters). The subsequent increase in seawater turbidity could be responsible for the development of picophytoplankton in the western-side. In fact, picophytoplankton, and particularly phycoerythrin cyanobacteria, like *Synechococcus* observed in May, are well adapted to freshwater and coastal marine systems where the spectral light quality is altered (from green to red) by turbid waters rich in dissolved and particulate organic matter [54]. It may be hypothesized that in May the high nutrient levels usually advantaging the larger phytoplankton, in combination with low salinity and particular light condition, altered the competition in favour of picophytoplankton. Moreover, the evident increase of the heterotrophic component in April and May especially in the western-side, seems to be consistent with the increase of both wastewater influx and exudates derived from the primary pico-producers peak. In October nitrate and phosphate concentrations were higher in the western-side but they also increased in the eastern-side compared to the rest of the year. Advective transportation processes associated to the disruption of the water column vertical stability and presumably represent the responsible of turbulent diffusion supplying nutrients as already observed in the coastal Southern Adriatic Sea in the same period of the year [55]. This condition, however, possibly favoured the autotrophic picoplankton component which appeared significantly more abundant in the western-side area. It could be suggested that the surplus of nutrients, coupled with the reduced intensity of the light due to the incoming autumn, determined the shift of competition between the larger and the smaller phytoplankton components, as already observed in May. Relationships between phytoplankton structure and light absorption was already observed in the Mediterranean Sea [56]. It is possible to hypothesize that in the competition between the two autotrophic components, change in light quality and intensity, whatever its cause (turbidity or natural cycle), represents a key factor. The PERMANOVA pair-wise test revealed significant differences in the planktonic assemblages between the two analysed areas also in February, April and December which are not related to the abiotic parameters herein considered. Thus, other factors, for example, light limitation due high turbidity in the impacted areas or intensive grazing/filtration activity could be involved in modulating the autotrophic and heterotrophic distribution.

General remarks

Considering that the status of the ecological quality of ecosystems must be monitored by taking into account biological, hydro-morphological and physical-chemical criteria, and under the premise of accurate simplicity, rapidity, and sensitivity, we confirm to consider the coastal planktonic assemblages herein simultaneously examined as potential useful and sensitive environmental descriptors. This would provide a more holistic approach to the analysis of coastal marine systems environmental quality. Statistical analyses indeed evidenced that apart phytoplankton, also picophytoplankton variability contributes to make the difference between the polluted and the unpolluted areas. In fact, picophytoplankton showed the capability to react rapidly to each "evident" change surplus of nutrients if coupled with a change in light quality, whatever its cause (turbidity or natural cycle). As a consequence shift of competition between the larger and

the smaller phytoplankton components have to be considered in analysis of the planktonic producers dynamics. In addition, from the analysis of the responses of the examined planktonic assemblages we provide some evidences that the bacterial community responds rapidly to even the most subtle changes in the monitored areas with important consequences for organic matter cycling and overall ecosystem functioning. On account of these features heterotrophic picoplankton is confirmed as a parameter for monitoring which can be used to derive useful indicators, as already suggested by other authors [57, 58]. More data are necessary to confirm the hypothesis of the employment of the suggested planktonic assemblages as useful environmental monitoring parameters to be used, in addition to those already existing, for the elaboration of new indicators of the state of coastal systems as the first step to their sustainable management. Because the implementation of the Urban Wastewater Treatment Directive 91/271/EC [59] and the more recent WFD [5] and MSFD [8] are a reality in all the coastal European sites, included the herein studied Taranto Gulf, this study may serve as a starting point for long term studies. These studies would facilitate and add to the compliance monitoring conducted in relation to the mentioned Directive in coastal Mediterranean waters and would be aimed to evaluate the effects of environmental changes due to the future reconstruction of the wastewater plants on the considered planktonic assemblages [57,58].

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