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Seasonal variability of picophytoplankton under contrasting environments in northern Tunisian coasts, southwestern Mediterranean Sea

N. Salhi^{a,b,g,1}, H. Zmerli Triki^{a,1}, J.C. Molinero^{c,f}, M. Laabir^d, E. Sehli^e, A. Bellaaj-Zouari^e,
N. Daly Yahia^{b,1}, O. Kefi-Daly Yahia^{a,*}

^a Institut National Agronomique de Tunisie, IRESA – Université de Carthage, 43 avenue Charles Nicolle, 1082 Tunis, Tunisia

^b Laboratoire de Biodiversité et Fonctionnement des Systèmes Aquatiques, Faculté des Sciences de Bizerte, Université de Carthage, 7021 Zarzouna, Tunisia

^c GEOMAR Helmholtz Center for Ocean Research, Kiel, Germany

^d Center for Marine Biodiversity, Exploitation and Conservation (MARBEC), Montpellier University, CNRS, IRD, Ifremer, Place Eugène, Bataillon, CC093, 34095 Montpellier Cedex 5, France

^e Institut National des Sciences et Technologies de la Mer, 28 rue 2 Mars 1934, 2025 Carthage Salammbô, Tunisia

^f Marine Ecology/Food Webs, Duesternbrooker Weg 20, D-24105 Kiel, Germany

^g Laboratory of Water, Membrane and Biotechnologies of Environnement, Centre of Research and Water Technologies (CERTe), Technopark of Borj-Cedria, Tunisia

We investigated at the single cell level during 16 months (June 2012 to September 2013) the temporal distribution of picophytoplankton (picoeukaryotes, *Synechococcus* and *Prochlorococcus*) communities in two contrasting ecosystems: the Bay of Bizerte characterised by an oligotrophic regime typical of the Mediterranean Sea and the Bizerte Lagoon that exhibits a mesotrophic/eutrophic state. We aimed at depicting seasonal variations and quantifying the relationships between the environmental factors and the structure and abundance of picophytoplankton communities. Results showed that picophytoplankton groups were able to grow under a wide range of environmental conditions varying seasonally, although their abundances and contributions to the total chlorophyll biomass significantly varied and showed importance in the Bay of Bizerte. *Synechococcus* was the most abundant group reaching $225 \times 10^3 \text{ cells}\cdot\text{cm}^{-3}$ in the Bay and $278 \times 10^3 \text{ cells}\cdot\text{cm}^{-3}$ in the lagoon. This group was present all over the year in both ecosystems. Structural equation model results pointed out a different configuration regarding the picophytoplankton environmental drivers. The complexity of the configuration, i.e. number of significant links within the system, decreased under enhanced eutrophication conditions. The less exposure to anthropogenic stress, i.e. in the Bay of Bizerte, highlight a larger role of nutrient and hydrological conditions on the seasonal variations of picophytoplankton, whereas a negative effect of eutrophication on picophytoplankton communities was unveiled in the Bizerte Lagoon. We stress that such influence may be exacerbated under expected scenarios of Mediterranean warming conditions and nutrient release in coastal eco-systems.

Picophytoplankton cells are autotrophic microorganisms, $< 2 \mu\text{m}$ in size, that substantially contribute to the phytoplankton biomass and carbon export in all aquatic environments (Richardson and Jackson, 2007; Stockner, 1988). In particular, these microorganisms dominate the total phytoplankton biomass and production in oligotrophic ecosystems (Li et al., 1993; Šilović et al., 2011). Such dominance is based on the efficient uptake of dissolved nutrients at low bulk concentrations, coupled with reduced sedimentation loss and subsequent grazing (Caroppo, 2000; Raven, 1986). In environments characterised by a marked seasonality, the abundance and structure of picophytoplankton change throughout the year along with the decrease of nutrient

concentration and increasing temperatures, which characterize the shift from new to regenerated production (Kuosa, 1991).

Picophytoplankton is represented by two genera of picocyanobacteria *Synechococcus*, *Prochlorococcus* and picoeukaryotes (Sieburth et al., 1978). The genus *Synechococcus* is ubiquitous in all marine environments, although it shows larger abundances in nutrient-rich environments than in oligotrophic ones (Partensky et al., 1999a, 1999b) and appears also favoured by low salinity and/or low temperature (Dvořák et al., 2014). Conversely, *Prochlorococcus* is well adapted to high light levels of stratified oligotrophic waters, although the genus has been also recorded in shallow, meso- and eutrophic systems

* Corresponding author.

E-mail address: onskefidaly@gmail.com (O. Kefi-Daly Yahia).

¹ U.R Biologie marine, UR13ES36 - Université El Manar I.

(Partensky et al., 1999a, 1999b). In turn, Picoeukaryotes (cells < 3 μm) form a diverse group widely distributed in oceanic, coastal, and freshwater ecosystems; their biomass and high productivity suggest that they play a much larger role in planktonic ecosystems (Diez et al., 2001; Worden et al., 2004).

The Mediterranean Sea is characterised by substantial proliferations of ultraphytoplankton (< 10 μm) and a dominance of picophytoplankton over large phytoplankton cells in offshore areas (Casotti et al., 2003; Conan et al., 1998; Denis et al., 2010; Minas et al., 1988; Siokou-Frangou et al., 2010). Research efforts to understand picoplankton dynamics however, have mainly covered northern Mediterranean coasts, whereas in the southern Mediterranean Sea similar studies are scarce. Former reports have shown that *Prochlorococcus* displays a conspicuous distribution in a variety of Mediterranean habitats, such as offshore stratified areas and mixed coastal waters, whereas *Synechococcus* has been reported mainly in stratified waters (Marty et al., 2002). This pattern of distribution probably results from the presence of ecotypes favoring different strains of *Prochlorococcus* and *Synechococcus*. Here we investigate picophytoplankton communities in the Bay and Lagoon of Bizerte, located in the northern Tunisian coast, south-western Mediterranean Sea.

Bizerte lagoon is a highly productive and eutrophicated lagoon, and an important economic area holding aquaculture and fishing activities. This ecosystem however is heavily affected by anthropogenic stress as it receives constant discharges of domestic and industrial runoffs that alter the nutrient dynamic balance and therefore autotroph communities at the basis of the food web. Conversely, the Bay of Bizerte is exposed to the influence of oligotrophic Mediterranean waters and is connected to the Lagoon of Bizerte.

In the present study, we focused on seasonal changes of picophytoplankton assessed at the single cell level in these ecosystems characterised by contrasted trophic states. We aim to depict the seasonal distribution and abundance of picophytoplankton groups, their contribution to the total chlorophyll biomass and to quantify potential relationships linking their temporal changes to environmental factors (nutrients, temperature and salinity) prevailing from June 2012 to September 2013.

The study was carried out in the Bay and the Lagoon of Bizerte, northern coast of Tunisia (Fig. 1). The Bay of Bizerte is an open oligotrophic/mesotrophic ecosystem characterised by a salinity ranging

between 36 and 38 (Addad et al., 2008), water depth ranging from 16 to 20 m, chlorophyll *a* ranging between 0.5 and 2.5 $\text{mg}\cdot\text{m}^{-3}$ and moderate nutrient concentrations: total nitrogen (1–4 μM), orthophosphate (0.01–0.71 μM) and silicate (0.4–2.2 μM) (Sahraoui et al., 2012). The Lagoon of Bizerte is a semi-enclosed area covering 128 km^2 with a mean depth of 8 m.

This Lagoon communicates with the Mediterranean Sea via the Bizerte channel (7 km long, 300 m width and 12 m depth) and receives important freshwater inputs from several tributaries (Béjaoui et al., 2008). This ecosystem is exposed to heavy anthropogenic stress, i.e. aquaculture activities, industrial discharges, and intensive agriculture in the southeast watershed. It shows eutrophic conditions with (1–24 μM) total nitrogen; (0.14–0.48 μM) orthophosphate, (1.87–13 μM) silicate, and is characterised by high chlorophyll *a* concentrations (3–8 $\text{mg}\cdot\text{m}^{-3}$) (Grami et al., 2008; Sakka Hlaili et al., 2008, 2007).

The seawater samples were collected using a pipe line on a fortnightly basis from June 2012 to September 2013 at the Bay (20 m deep; 37°17'09.2"N, 09°53'91.1"E) and the Lagoon (37°12'46.0"N, 09°50'48.0"E) 8 m deep stations. At each sampling site, 500 cm^3 of seawater were filtered for determining chlorophyll *a* concentration and the filtrate was used to assess nutrient concentrations (NO_2^- , NO_3^- , PO_4^{3-} and SiO_4^{4-}). For NH_4^+ analysis, 250 cm^3 were collected and stored at -20°C until their analysis in the laboratory. For flow cytometry analysis, subsamples (2 cm^3) of collected seawater were placed into cryogenic tubes, fixed with paraformaldehyde (2% final concentration) and frozen in liquid nitrogen on the boat to be further stored at -80°C in the laboratory until analysis (Vaulot et al., 1989). Temperature and salinity were measured using the multiprobe WTW, Cond 3110/SET model.

For chlorophyll *a* analysis, 500 cm^3 of seawater were filtered using low vacuum pressure firstly on 20 μm pore-size polycarbonate membranes then on Whatman GF/D filters (2.7 μm) and finally through Whatman GF/F polycarbonate filters (0.7 μm). Microphytoplankton cells were retained by the 20 μm filters, whereas nanophytoplankton and picophytoplankton were retained by the 2.7 μm and 0.7 μm pore-size filters respectively. These successive filtrations allowed the estimation of contribution percentages of the different fractions to total chlorophyll *a* concentrations that were computed as the sum of the concentrations related to the three fractions. Each single filter was

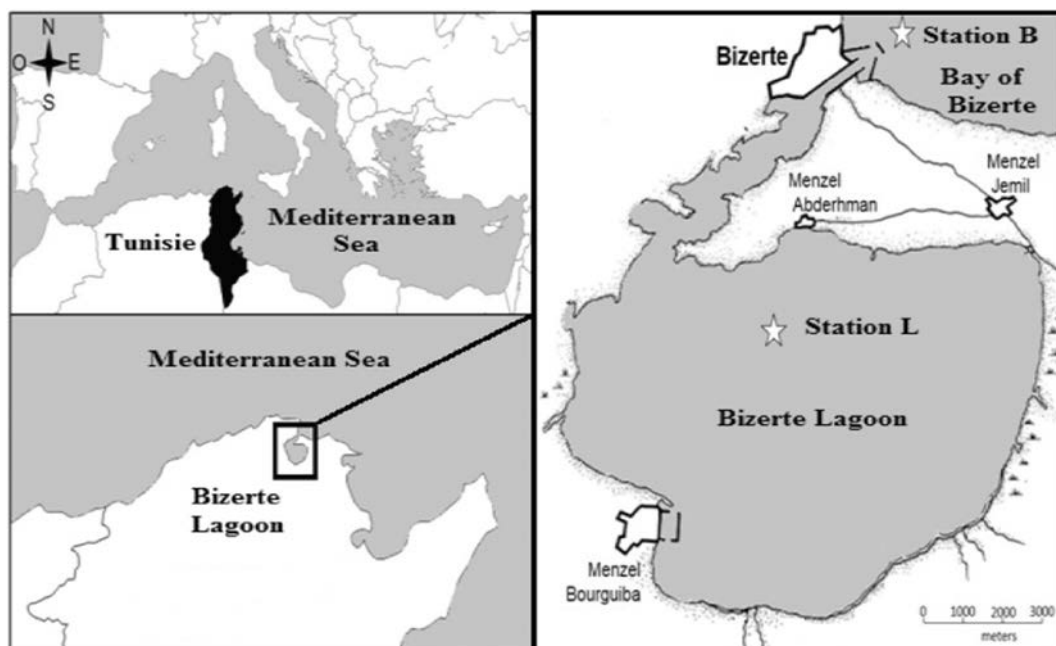


Fig. 1. Location of the study area and sampling stations in the Bay (A) and Lagoon (B) of Bizerte.

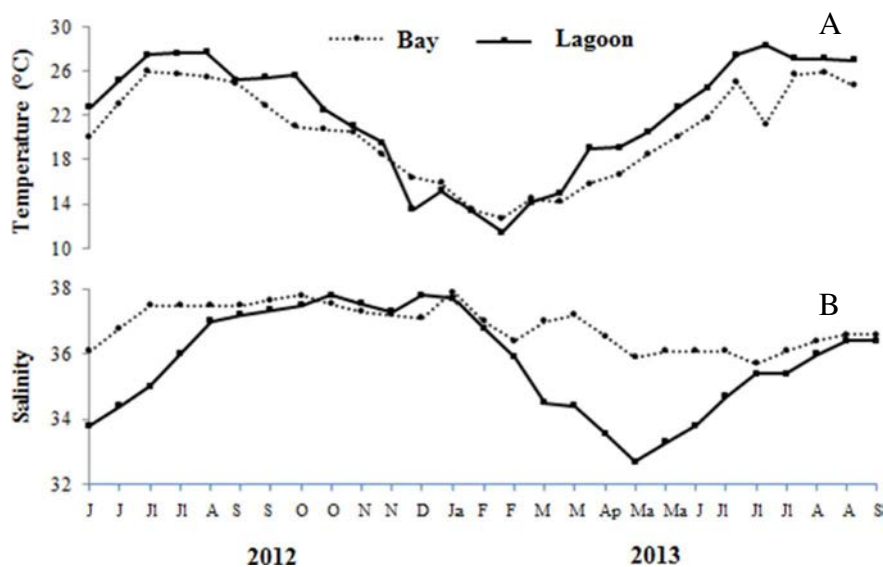


Fig. 2. Seasonal variation of temperature (°C) (A) and salinity (B) in the Bay and Lagoon of Bizerte.

recovered with 90% acetone and grounded manually into glass centrifuge tube filled with 90% acetone. The mixture was kept in the dark at -4°C for 24 h to allow total extraction of chlorophyll *a* whose fluorescence was measured using the standard spectrometric Lorenzen method (Aminot and Chaussepied, 1983). Nutrient concentrations were determined by using conventional colorimetric methods on automated channel Technicon auto analyzer, according to the protocols of Tréguer and Le Corre (1975). Seven independent analyses were performed for each sample. Ammonium concentration analyses were carried out using a spectrometric method as described in Aminot and Chaussepied (1983). The detection limits for ammonium, nitrate, silicates and orthophosphates were $0.04\ \mu\text{M}$, $0.02\ \mu\text{M}$, $0.05\ \mu\text{M}$ and $0.002\ \mu\text{M}$, respectively.

In order to identify picophytoplankton groups, the fixed seawater samples ($2\ \text{cm}^3$) were analysed according to Marie et al. (2000) protocol using a FacsCalibur flow cytometer (BD Biosciences) and a Cyflow space (Partec) equipped with vertically polarised blue laser (488 nm) and a 638 nm red laser. Cell Quest Pro and Flow Max softwares were used for data treatments. In flow cytometry, allometric (size versus pigment fluorescence) and taxonomic (two-natural pigment fluorescences: Chlorophyll *a* and phycoerythrin autofluorescences) data were used to resolve phytoplankton groups. Orange autofluorescence corresponds to the presence of phycoerythrin and red autofluorescence corresponds to the presence of chlorophyll *a*. Before analysis, samples were prefiltered on $3\ \mu\text{m}$ mesh size and fluorescent beads of $0.2\ \mu\text{m}$ and $2\ \mu\text{m}$ were added in order to normalise the flow cytometer settings and enumeration of picophytoplankton cells (Olson et al., 1993). Bead counts provided a reference to determine the analysed volumes and derive cell concentrations for each cell group. For Cyflow space (Partec) machine, the software directly calculated cell concentrations ($\text{cells}\cdot\text{cm}^{-3}$) by using the sample volume injected through the micro-syringe.

For statistical analysis, data were normalised, while the abundance of each picophytoplanktonic group was arcsine square-root transformed (Legendre and Legendre, 1998). To assess the environmental influence on picophytoplankton we used Structural Equation Models (SEM). The structure of the path model is based on the potential influence of hydrological factors and nutrient loading on picoplankton communities, their structure and functioning; the strength of such links however may vary under contrasting trophic states (mesotrophy vs eutrophy) and anthropogenic stress. For each location, specific effects of hydrography and nutrient loading and their co-variations on picoplankton biomass were assessed using variance partitioning and explored through SEM

(Neto et al., 2006). The strength of the links and the quantification of the overall model were determined by simple and partial multivariate regression and Monte Carlo permutation tests (1000 replicates), while Chi-square values were used to assess the robustness of models (Alsterberg et al., 2013). Path coefficients indicate the strength of the connection between causal and response variables. Path analysis was performed using AMOS version 22.

The measured physico-chemical water-sample parameters were temperature, salinity and nutrients. Seasonal changes of temperature showed a close covariation between the two sites (Fig. 2A). In the Lagoon, temperature reached the highest value (28.3°C) in July 2013 whereas the lowest value (11.4°C) was registered in February 2013. In the Bay, temperature ranged from 12.7°C in February 2013 to 25.9°C in August 2013. Marked differences however, were shown in the pattern of salinity. In the Lagoon, salinity displayed a marked seasonality, ranging from 32.7 to 37.8 with a maximum value observed in winter (December 2012), while the minimum was registered in spring (May 2013). In clear contrast, seasonal changes in salinity were less pronounced in the Bay, which also showed higher values ranging from 35.7 to 37.9 (Fig. 2B). Similar changes were noticed in the nutrient concentration. It was higher in the Lagoon than in the Bay over the entire sampling period (Fig. 3). Nitrate concentrations ranged from 0.02 to $4.94\ \mu\text{M}$ in the Bay, and from 0.02 to $5.03\ \mu\text{M}$ in the Lagoon (Fig. 3A). In the Bay, nitrite was characterised by relatively lower values with concentrations below $0.7\ \mu\text{M}$, in contrast with the Lagoon where nitrite concentrations ranged between 0.02 and $2.84\ \mu\text{M}$ (Fig. 3B). Ammonium concentrations showed strong temporal variations during the survey in both ecosystems with the highest values being registered in the Lagoon of Bizerte (Fig. 3C). The observed concentration ranged from 0.04 to $8.7\ \mu\text{M}$ in the Bay, while in the Lagoon it ranged 0.04 to $11.53\ \mu\text{M}$ (Fig. 3C). Orthophosphate reached its highest concentrations in summer (August 2012) with $4.76\ \mu\text{M}$ in the Bay, and $8.8\ \mu\text{M}$ in the Lagoon (Fig. 3D). Silicate concentrations were high in both ecosystems, ranging from 0.05 to $6.82\ \mu\text{M}$ in the Bay and from 0.05 to $6.7\ \mu\text{M}$ in the Lagoon (Fig. 3E). Moreover, it is worth noting that water transparency was significantly higher in the Bay than in the Lagoon (Mann-Whitney *U* test, $p < 0.05$), with average values of 11.7 m and 3.5 m, respectively.

Total chlorophyll *a* displayed marked seasonal changes ranging between 0.4 and $15.75\ \text{mg}\cdot\text{m}^{-3}$ in the Lagoon of Bizerte, with highest values observed in July and October 2012 (15.75 and $10.94\ \text{mg}\cdot\text{m}^{-3}$, respectively). In the Bay, the total chlorophyll *a* concentration ranged between 0.04 and $8.54\ \text{mg}\cdot\text{m}^{-3}$ with the highest values observed in

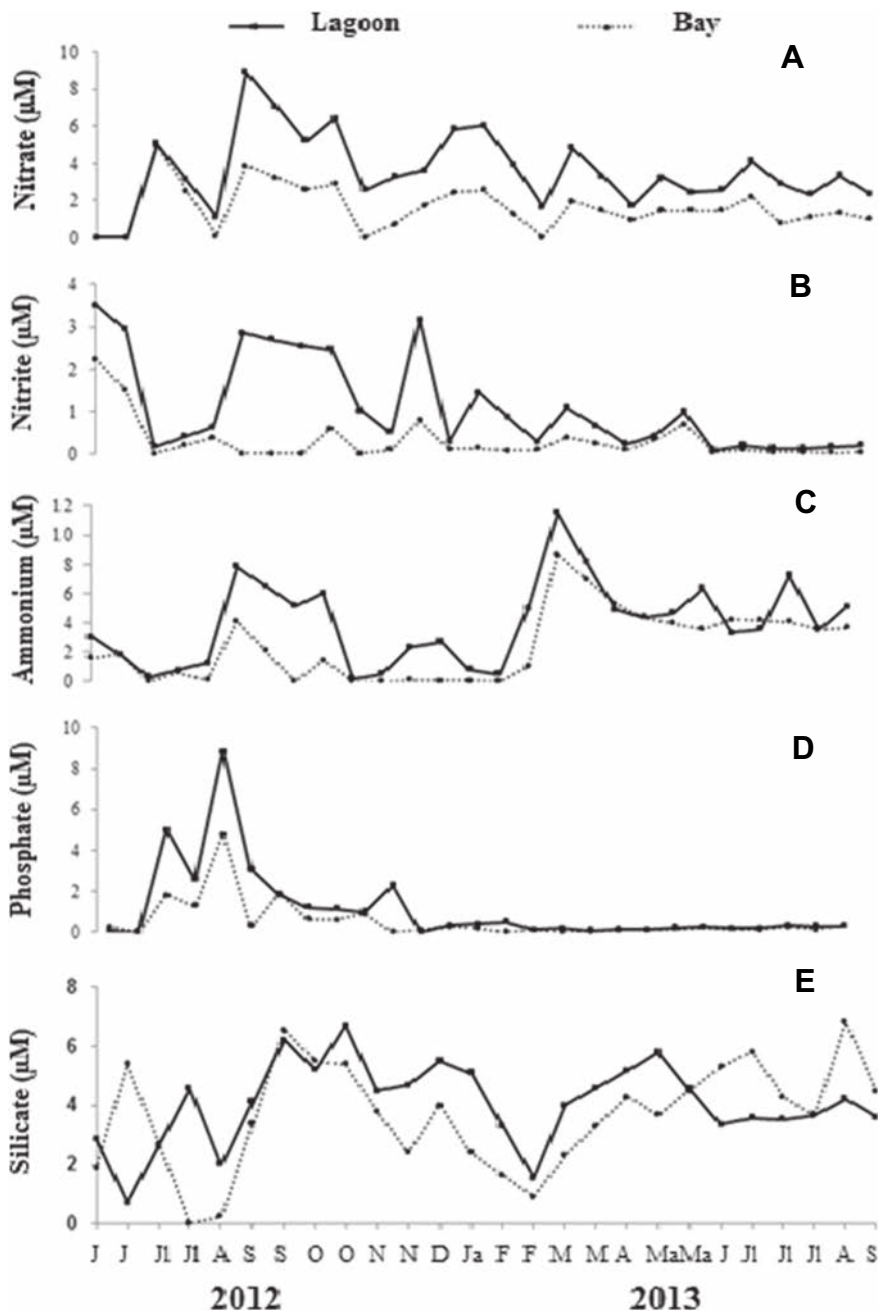


Fig. 3. Temporal variation of nutrient concentrations in the Bay and Lagoon of Bizerte.

June and October 2012, 3.47 and $8.54 \text{ mg}\cdot\text{m}^{-3}$ respectively (Fig. 4A). Total chlorophyll *a* concentration resulted from summing the chlorophyll *a* concentrations in the three fractions (picophytoplankton, nanophytoplankton and microphytoplankton). The Bay of Bizerte, characterised by low nutrient availability and relatively low chlorophyll concentration, was mainly represented by the phytoplankton group with 39.56% of total chlorophyll *a*, followed by the nanophytoplankton and microphytoplankton fractions with 35.7% and 24.7% of total chlorophyll *a*, respectively. Regarding the biomass of autotrophs, picophytoplankton was also dominant, reaching up to 60 to 80% of the total phytoplankton biomass. These field observations are in line with previous studies in the Mediterranean Sea, an overall oligotrophic ecosystem (Berman et al., 1984; Siokou-Frangou et al., 2010). Under low nutrient supply, picophytoplankton develops better than microphytoplankton, which explains their dominance in oligotrophic waters where the primary production is based on regenerated nutrients and rapid recycling of organic matter (Dugdale and Goering, 1967; Eppley

and Peterson, 1979). In the Lagoon of Bizerte, the nutrient concentration was substantially higher as a consequence of the continuous nutrient supply by water discharge from urban area and river outflow (Harzallah, 2003). In this ecosystem, the phytoplankton community was dominated by microphytoplankton in summer with a relative abundance of 41.69% of total chlorophyll *a*, whereas in autumn picophytoplankton was the main group representing 41.9% of total chlorophyll *a* (Fig. 4B, C). Similarly, previous studies pointed out a dominance of microphytoplankton in nutrient-rich waters (Casotti et al., 2003; Denis et al., 2010; Li et al., 1993; Siokou-Frangou et al., 2010), including previous studies in the Lagoon of Bizerte (Grami et al., 2008; Sahraoui et al., 2012; Sakka Hlaili et al., 2008, 2007; Salhi et al., 2016).

The analysis of the abundance and structure of picophytoplankton communities highlights the presence of five distinct subgroups in the study area: *Prochlorococcus*, exhibiting only red fluorescence (divinyl chlorophyll *a*), two types of *Synechococcus* ($< 1.5 \mu\text{m}$) labelled

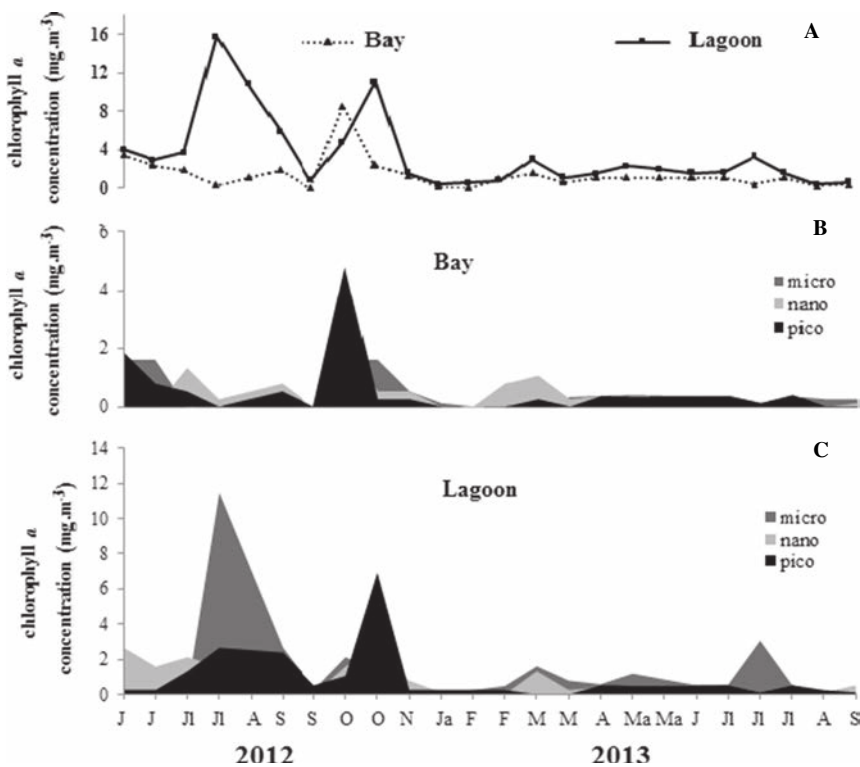


Fig. 4. Seasonal variation of total chlorophyll *a* concentration ($\text{mg}\cdot\text{m}^{-3}$) and evolution of chlorophyll *a* concentration ($\text{mg}\cdot\text{m}^{-3}$) in size fractions of phytoplankton sampled from the Bay and Lagoon of Bizerte.

Synechococcus type (I) and *Synechococcus* type (II), which displayed red (chlorophyll *a*) and orange (phycoerythrin) fluorescences. In addition, two types of picoeukaryotes ($< 3 \mu\text{m}$) were identified; these were labelled picoeukaryotes type (I) and picoeukaryotes type (II) mainly emitting red (chlorophyll *a*) fluorescence. The picophytoplankton structure was thus based on 3 major groups, two of them split into two subgroups (Fig. 5).

The total cell abundance of picophytoplankton was significantly different in the two sites (Mann-Whitney *U* test, $p < 0.05$) and exhibited higher values in the Bay from August 2012 to February 2013 and June–July 2013. In the Bay, picophytoplankton abundance ranged between 1365 and $236 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$, whereas in the Lagoon it ranged between 2939 and $291 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ (Fig. 6A). *Synechococcus* was numerically dominant in both ecosystems with its abundance ranged from 628 to $225 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ in the Bay and from 1575 to $278 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ in the Lagoon (Fig. 6B). The *Synechococcus* abundance maximum was observed in July 2012 in the Bay and in June 2012 in the Lagoon. These abundances were similar to previously reported field records in the Gulf of Gabès, southern Tunisia, where *Synechococcus* abundance ranged from 0.30 to $210.51 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ in June 2008 (Hamdi et al., 2015). In the Bay of Marseille (France), northwestern Mediterranean Sea, maximum abundance registered for *Synechococcus* was $7 \cdot 10^4 \text{ cells}\cdot\text{cm}^{-3}$ (Grégori et al., 2001). In the Ionian Sea, eastern Mediterranean Sea, the highest *Synechococcus* concentration was relatively high with $3 \cdot 10^4 \text{ cells}\cdot\text{cm}^{-3}$ (Casotti et al., 2003).

For *Prochlorococcus* group, it was more abundant in the Bay showing abundance values ranging from 0 to $123 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ (maximum in December 2012), while in the Lagoon the abundances ranged from 0 to $17 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ (maximum in September 2012) (Fig. 6C). These values were relatively higher than those recorded in other Mediterranean coastal and open waters where *Prochlorococcus* reached a maximum of $10^4 \text{ cells}\cdot\text{cm}^{-3}$ (Garczarek et al., 2007; Šantić et al., 2011; Sommaruga et al., 2005). *Prochlorococcus* abundance reached $26.25 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ in the Gulf of Gabès (Hamdi et al., 2015) and $7 \cdot 10^4 \text{ cells}\cdot\text{cm}^{-3}$ in the Bay of Marseille (Grégori et al., 2001).

In contrast, picoeukaryote was less abundant in the Bay where

values ranged from 20 to $161 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$, and in the Lagoon abundances ranged from 603 to $82 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ (Fig. 6D). The highest picoeukaryotes abundances were recorded in February 2013 in the Bay and in July 2012 in the Lagoon. These values were similar to those observed in other ecosystems (Table 1). For instance, in the Gulf of Gabès, picoeukaryote cells were less abundant than other groups with maximal values of $48.19 \cdot 10^3 \text{ cells}\cdot\text{cm}^{-3}$ (Hamdi et al., 2015). In the Bay of Marseilles, the abundance maximum reported for this group was $3 \cdot 10^4 \text{ cells}\cdot\text{cm}^{-3}$ (Grégori et al., 2001).

Studies conducted on Mediterranean picophytoplankton communities focused mainly on the Mediterranean Sea northern coast (Caroppo, 2000; Casotti et al., 2003; Denis et al., 2010; Grégori et al., 2001; Sommaruga et al., 2005) and little is known on seasonal changes of autotroph communities in southern Mediterranean coasts, although these ecosystems may be more vulnerable to global anthropogenic changes due to water scarcity, concentration of anthropogenic activities and population density in coastal areas (Pachauri et al., 2014). The present study provides the first data on the seasonal distribution and contribution of picophytoplankton communities to the total autotrophic biomass in the study area.

Multivariate drivers of picophytoplankton were depicted by SEM (Fig. 7). In the Bay of Bizerte, model results showed that picophytoplankton was modulated by the combination of temperature, salinity, and nutrient loading relative to nitrates and ammonium. A large amount of the variability of picoeukaryotes and *Synechococcus* was positively linked to nitrates + nitrites, while salinity and ammonium showed a negative effect. In turn, *Prochlorococcus* abundance variations were mainly linked to temperature and ammonium. SEM results showed noticeable differences regarding the main drivers of picophytoplankton in the eutrophic site, the Lagoon. Results showed an enhanced effect of orthophosphate on the picoeukaryote abundance, *Synechococcus* appeared mainly driven by temperature, whereas *Prochlorococcus* was driven by nitrates + nitrites, temperature and salinity.

SEM results pointed out a different configuration regarding the picophytoplankton environmental drivers. The complexity of the configuration, i.e. number of significant links within the system, decreases

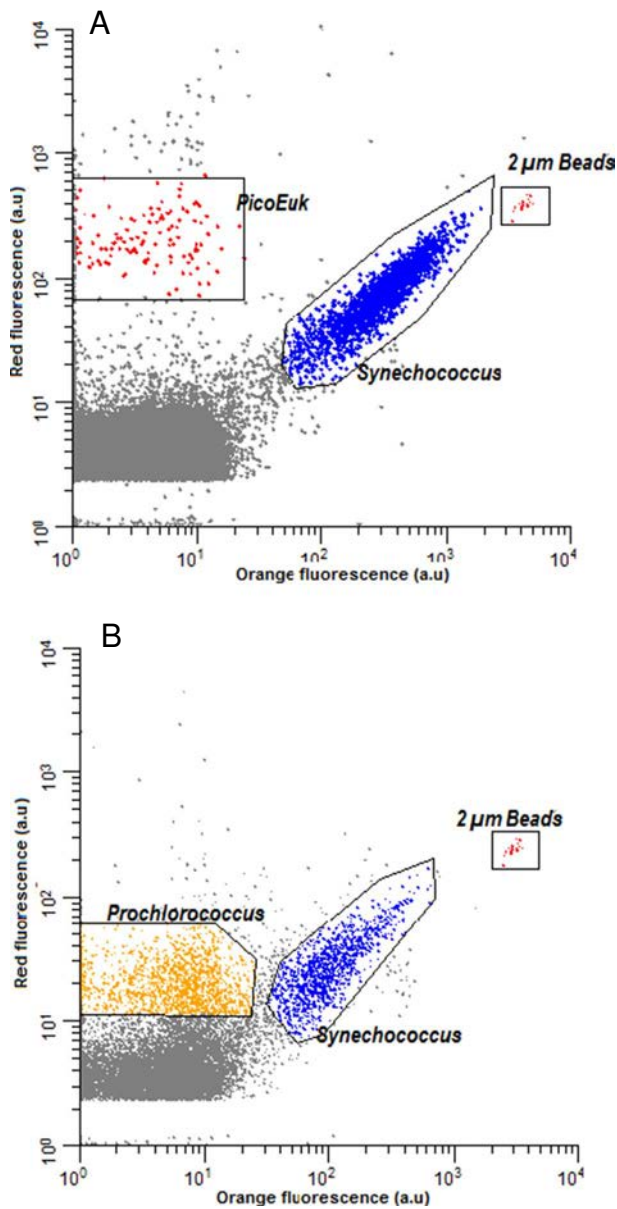


Fig. 5. Resolution of picophytoplankton cell groups by flow cytometry. Typical red vs orange fluorescence cytograms exhibiting the resolution of *Synechococcus* and picoeukaryotes in [A, 19 February 2013], and *Prochlorococcus* and *Synechococcus* in [B, 25 May 2013]. Samples were collected in the Bay of Bizerte and were analysed with a Cyflow space (Partec) by using the same instrument settings which also illustrates seasonal changes in the picophytoplankton composition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

along with the increase of eutrophication. Indeed, under less exposure to anthropogenic stress, i.e. in the Bay of Bizerte, a larger role of nutrient dynamics and hydrological conditions on the picophytoplankton distribution was outlined, whereas in the eutrophic system, the Lagoon of Bizerte, the picophytoplankton seasonal abundance variation and contribution to the phytoplankton bulk biomass is curved by the excess of nutrient loading yielded by the anthropogenic stress. Indeed, the model highlighted a significant negative effect of eutrophication on picophytoplankton communities. Moreover, linked to the excess of nutrient loading we noticed that water transparency was significantly lower in the Lagoon, which might also impair picophytoplankton communities. These results are in line with previous reports on the relationship between picophytoplankton and nutrients. For instance, *Prochlorococcus* optimally grows at low nutrient concentration as it cannot use nitrate, and thus depends on the high surface area to volume

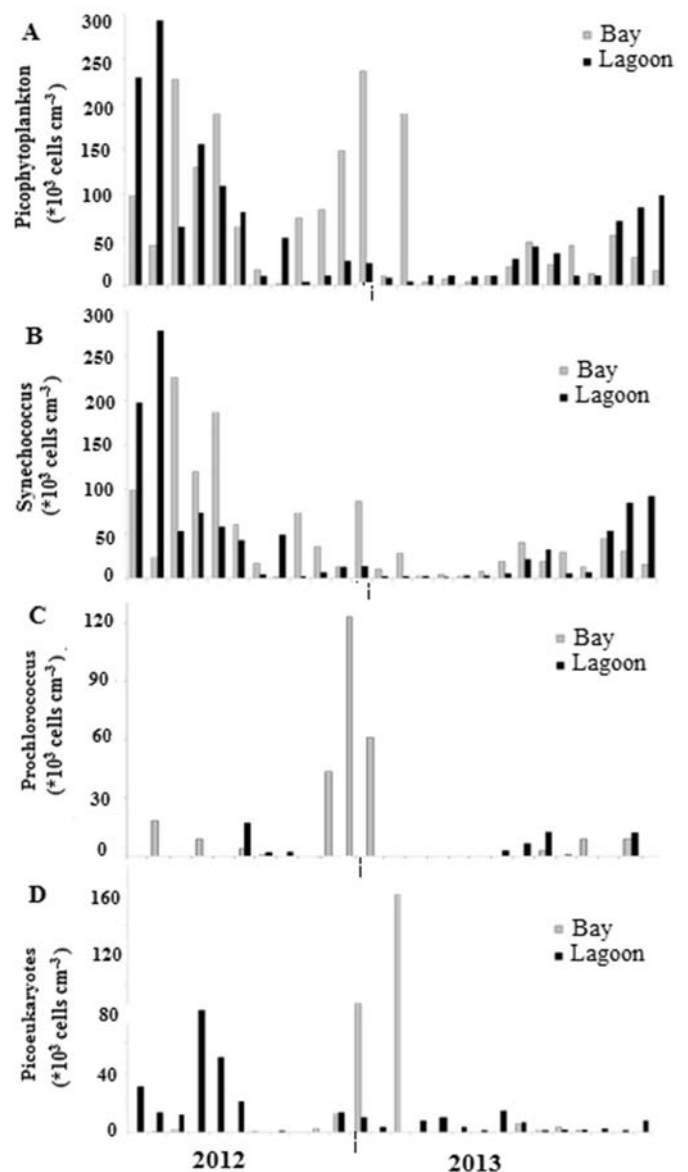


Fig. 6. Temporal variability of picophytoplankton (A), picoeukaryotes (B), *Synechococcus* (C), *Prochlorococcus* (D) cell abundances (cells cm^{-3}) in the surface water of the Bay and Lagoon of Bizerte.

ratio to uptake ammonium more efficiently or to take up organic nitrogen compounds at high rates (Worden et al., 2004). However, Berube et al. (2015) showed that some *Prochlorococcus* strains are indeed able to grow by using nitrate as the sole nitrogen source. In the case of *Synechococcus*, a positive correlation with nutrients in both mesotrophic and eutrophic ecosystems has been observed in the North Western Mediterranean Sea (Uysal and Köksalan, 2006; Vaulot et al., 1990). In oligotrophic Mediterranean waters, *Synechococcus* appeared better adapted to nutrient depleted waters because of the small cell size (Martin, 1997). These results may explain higher *Synechococcus* abundances in the bay of Bizerte. Other factors however are not discarded, such a potential larger top-down control in the Lagoon than in the Bay, i.e. microzooplankton grazing on bacteria and picoeukaryotes. Indeed, microzooplankton communities, which are mainly composed of dinoflagellates (*Torodinium*, *Protoperidinium* and *Dinophysis*) and aloricate (*Lohmaniella* and *Strombidium*) and tintinnid (*Tintinnopsis*, *Tintinnus*, *Favella* and *Eutintinnus*) ciliates have been shown to seasonally control ultraplankton abundances in the Lagoon of Bizerte (Dhib et al., 2016). Moreover, microzooplankton biomass can reach up to 359 mg C m^{-3} ,

Table 1
Abundance of different picophytoplankton groups recorded within the Mediterranean Sea.

Location	Ecosystem	Period investigated	Taxa	Cells densities	Reference
A					
North Western Mediterranean Sea	Alboran Sea	2008–2012 (11 cruises)	<i>Prochlorococcus</i> <i>Synechococcus</i> Picoeukaryotes	Max: $12.7 * 10^4$ cells cm^{-3} Max: $13.9 * 10^4$ cells cm^{-3} Max: $8.6 * 10^4$ cells cm^{-3}	Amorim et al., 2016
	Bay of Marseille Bay of Villefranche	1997–1999 July 1996	<i>Picoeukaryotes</i> <i>Synechococcus</i> Picoeukaryotes	Max: $3 * 10^4$ cells cm^{-3} Mean: $43 * 10^3$ cells cm^{-3} Mean: $2.5 * 10^3$ cells cm^{-3}	Grégori et al., 2001 Jacquet et al., 1998
	Bay of Blanes (Spain)	May–November 2003	<i>Synechococcus</i> <i>Prochlorococcus</i> Picophytoplankton	Max: $1.06 * 10^5$ cm^{-3} Max: $3.64 * 10^4$ cm^{-3} $0.7\text{--}448.6 * 10^9$ cells cm^{-3}	Sommaruga et al., 2005 Caroppo, 2000
North Eastern Mediterranean Sea	Varano lagoon	March 1997–February 1998			
North Eastern Mediterranean Sea (Ionian Sea)	Italian Side	Spring 1999	<i>Synechococcus</i> <i>Prochlorococcus</i> Picoeukaryote	Mean: $1.62 * 10^4$ cells cm^{-3} Mean: $7.37 * 10^3$ cells cm^{-3} Mean: $3.10 * 10^3$ cells cm^{-3}	Casotti et al., 2003
	Greek Side	Spring 1999	<i>Synechococcus</i> <i>Prochlorococcus</i> Picoeukaryotes	Mean: $1.29 * 10^4$ cells cm^{-3} Mean: $2.92 * 10^3$ cells cm^{-3} Mean: $2.16 * 10^3$ cells cm^{-3}	
North Eastern Mediterranean Sea	Greek coast Levantine basin Rhodes gyre Ionian basin	Dec. 1994–Feb. 1995	<i>Prochlorococcus</i> <i>Prochlorococcus</i> <i>Prochlorococcus</i>	Max: $117.6 * 10^{10}$ cells m^{-2} Max: $60.8 * 10^{10}$ cells m^{-2} Max: $30 * 10^{10}$ cells m^{-2}	Denis et al., 2010
			<i>Prochlorococcus</i> <i>Synechococcus</i>	$14.1\text{--}24.7 * 10^{10}$ cells m^{-2} Max: $120 * 10^{10}$ cells m^{-2}	
			<i>Synechococcus</i> <i>Prochlorococcus</i> <i>Synechococcus</i>	Max: $275 * 10^{10}$ cells m^{-2} Max: $23.3 * 10^{10}$ cells m^{-2} Max: $33.8 * 10^{10}$ cells m^{-2}	
	South of Crete Southern Adriatic basin				
	B				
South of Mediterranean Sea (Tunisia)	Bay of Bizerte	Jun. 2012–Sep. 2013	<i>Synechococcus</i> <i>Prochlorococcus</i> Picoeukaryotes	$628\text{--}225 * 10^3$ cells cm^{-3} $0\text{--}123 * 10^3$ cells cm^{-3} $20\text{--}161 * 10^3$ cells cm^{-3}	Present study
	Lagoon of Bizerte		<i>Synechococcus</i> <i>Prochlorococcus</i> Picoeukaryotes	$1575\text{--}278 * 10^3$ cells cm^{-3} $0\text{--}17 * 10^3$ cells cm^{-3} $603\text{--}82 * 10^3$ cells cm^{-3}	
	Ghar el Melh Lagoon	Autumn, winter 2012; spring, summer 2013	<i>Synechococcus</i> <i>Prochlorococcus</i> Picoeukaryotes	$0\text{--}8.26 * 10^4$ cells cm^{-3} $0\text{--}1.69 * 10^3$ cells cm^{-3} $0\text{--}1.7 * 10^4$ cells cm^{-3}	
South Eastern Mediterranean Sea (Tunisia)	Gulf of Gabes	June 2008	<i>Synechococcus</i> <i>Prochlorococcus</i> Picoeukaryotes	$0.30\text{--}210.51 * 10^3$ cell cm^{-3} $0.16\text{--}26.25 * 10^3$ cell cm^{-3} Max: $48.19 * 10^3$ cell cm^{-3}	Hamdi et al., 2015
	Coasts of Sfax	May 2010	<i>Synechococcus</i> <i>Prochlorococcus</i> Picoeukaryotes	Max: $16.65 * 10^4$ cells cm^{-3} Max: $40.41 * 10^4$ cells cm^{-3} Max: $64.19 * 10^4$ cells cm^{-3}	Rekik et al., 2013

whereas their grazing controlled 67–90% of pico- and nanophytoplankton (Sakka Hlaili et al., 2008).

In the Bizerte Bay, *Prochlorococcus* showed maximum abundances in both summer and winter seasons. Although this group has been reported dominant in warm (> 20 °C) oligotrophic oceanic waters (Partensky et al., 1999a, 1999b), the South East Atlantic (Jameson et al., 2010; Zwirgmaier et al., 2008) and the North West Atlantic (Zinser et al., 2007), high abundances (higher than 10^4 cells cm^{-3}) have been also reported in relatively cold environments (< 15 °C) (Šantić et al., 2011). Moreover, in the north-western Mediterranean Sea (Vaulot et al., 1990), as well as in the central Adriatic Sea (Šantić et al., 2011) field studies have shown that *Prochlorococcus* high abundances are not restricted to the warm seasons (Zwirgmaier et al., 2008). According to the literature, *Prochlorococcus* is able to develop in a wide range of environmental conditions across marine ecosystems (Buck et al., 1996; Partensky et al., 1999a, 1999b; Worden et al., 2004), except in polar waters (Zwirgmaier et al., 2008). *Synechococcus* was recorded throughout the study period in both ecosystems, with high densities under both low and high temperature. Previous works highlighted the eurytherm character of this group, which allows it inhabiting within a wide range of temperatures (Goericke et al., 2000; Li, 1998; Ning et al., 2000; Tsai et al., 2005, 2008; Zwirgmaier et al., 2008). In the coastal ecosystems of the Adriatic Sea, highest cell abundances of *Synechococcus* have been reported in summer in Sibenik, Kastela Bay, Split and Ploce, although in the open sea, higher abundances have been observed in winter (Šantić et al., 2011). In our study,

the relationship of *Synechococcus* abundance with environmental factors (temperature and nutrients) suggests different populations having distinct environmental windows. Indeed, a number of studies demonstrated that *Synechococcus* is genetically complex with several clades widely distributed in the Mediterranean Sea (Ahlgren and Rocap, 2012; Mella-Flores et al., 2011; Scanlan et al., 2009). Clade II was observed in stable, low-nutrient conditions typical of summer season (Alonso-Sáez et al., 2008; Dufresne et al., 2008; Scanlan et al., 2009; Sohm et al., 2015; Zwirgmaier et al., 2008) whereas clades I and IV were adapted to high nutrient/low temperature waters of the Mediterranean Sea (Mella-Flores et al., 2011). The seasonal variation of physico-chemical conditions together with the trophic status of each marine ecosystem control the development of the specific clades adapted to the prevailing conditions, as has been described for some coastal sites of the Pacific Ocean (Tai and Palenik, 2009).

In this study, we have shown seasonal abundance changes of picophytoplankton communities and their relationship with environmental conditions. This group predominated in the Bay of Bizerte, coping with the local ecological niche of primary producers and substantially contributing to the total chlorophyll *a* biomass. Moreover, the close relationship of the picophytoplankton community with hydrographic conditions and anthropogenic stress, portrayed by nutrient concentration, warns on the negative effects anthropogenic related eutrophication may have at the base of planktonic food webs. This is important as such influence may be exacerbated under expected scenarios of Mediterranean warming conditions and nutrient release in coastal

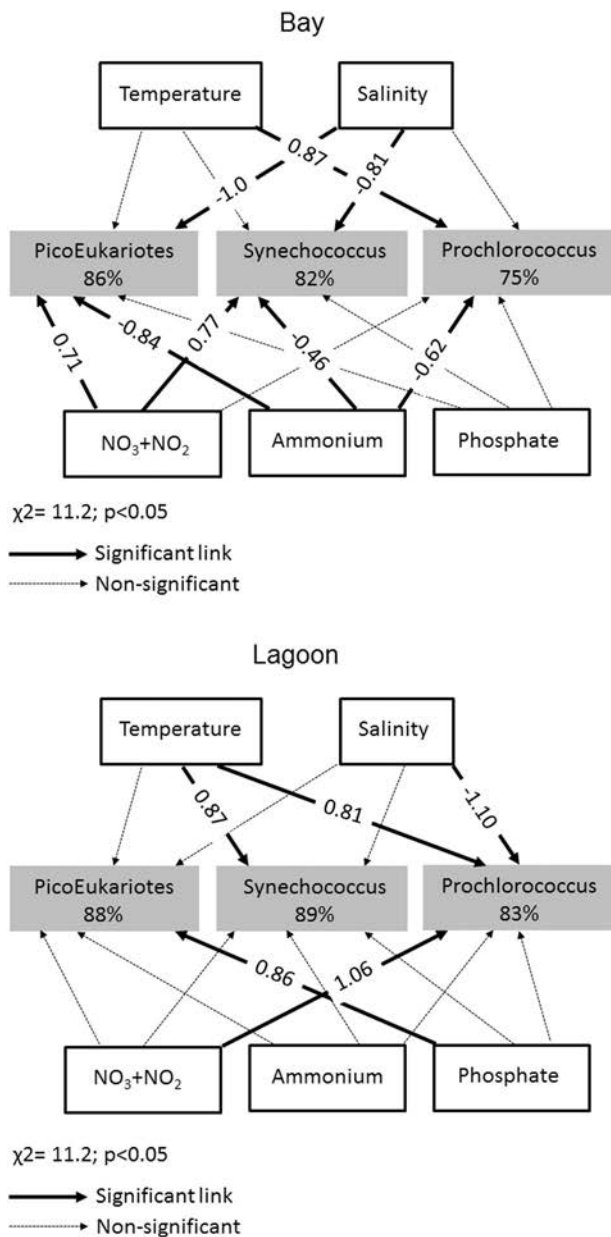


Fig. 7. Path diagramme showing environmental forcing on picophytoplankton communities under oligotrophic (top panel) and eutrophic (bottom panel) conditions. Solid paths are statistically significant ($p < 0.05$), whereas dashed lines are not. At each significant path the standard coefficients are displayed.

ecosystems. The seasonal patterns pointed out by our investigation might serve as a baseline for future studies addressing picophytoplankton dynamics that should also consider potential top-down controls in order to gain a thorough understanding of the role these groups play in the functioning of the southern coastal Mediterranean ecosystems.

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