

Physiological and behavioral responses of phytoplankton communities to nutrient availability in a disturbed Mediterranean coastal lagoon

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ABSTRACT

Short-term bioassays were conducted in Biguglia lagoon (Corsica) to study the physiological and behavioral responses of phytoplankton to N- and P-availability. Natural communities were collected in two stations representative of the two sub-basins, at three periods with contrasting environmental characteristics to address the impact of seasonal variability. These samples were separately enriched with a full N and P enrichment, and with enrichments minus N or minus P. Phytoplankton size structuration, diversity, and growth of the total phytoplankton, the micro-, nano- and ultraphytoplankton were evaluated using spectrofluorimetry, and optical microscopy. Results showed that the communities were fueled by NO_3^- in the wet periods (autumn and spring) and NH_4^+ in summer. The phytoplankton communities displayed highest cell size in autumn, with high abundances of nanoflagellates, and smallest cell size in summer with a large dominance of phycocyanin-rich picocyanobacteria. Blooms of dinoflagellates also occurred during the wet periods, coinciding with high N:P ratios. The full enrichment has not stimulated phytoplankton growth in autumn, suggesting the importance of other controlling factors such as light, a possible NH_4^+ inhibition or the use of mixotrophic abilities. In spring, communities have displayed single P-limitation in the northern basin and different N and P co-limitations in the southern basin. In summer, the full enrichment consistently stimulated the growth of all cell sizes. The communities showed high N and P co-limitations, which is consistent with growing observations in aquatic ecosystems, and reflects the different functional responses of phytoplankton communities to the nutrient availability.

1. Introduction

Coastal lagoons are very productive ecosystems at the sea-land interface hosting a high degree of biodiversity and providing numerous ecosystem services for human wellbeing (Barbier et al., 2011). These services confer a high economical value for the coastal lagoons, e.g. as water and food supply (fishing, shellfish farming), tourism and recreation (Franco et al., 2010; Rochette et al., 2010). However, because of their location in densely populated littoral areas and the services they provide, coastal lagoons and their surroundings have been increasingly exploited for human uses. Overexploitation combined with the hydromorphology of these semi-enclosed systems and climate change have increased their vulnerability to anthropogenic impacts. Hence, coastal lagoons are among the most threatened ecosystems (Nixon, 2009). This threat is mainly associated with changes in their hydrology and with an increase of nutrients and pollutants inputs from

sewage effluents and watershed run-off (Mouillot et al., 2000; Cloern, 2001). Due to shallowness and confinement, these inputs result in high concentration of nutrients and pollutants in coastal lagoons, thereby exacerbating their degradation (Donald et al., 2013).

An excessive or unbalanced input of nutrients disturbs the community of primary producers, leading to an increase of the eutrophication processes and to drastic changes in the biodiversity of the autotrophic compartment. Thus, it may promote fast-growing opportunistic algae in the first place and finally phytoplankton at the expense of benthic organisms such as macrophytes, and may result in an increase of the frequency of harmful algal blooms (Schramm, 1999; Livingston, 2000; Le Fur et al., 2018). These changes in the composition of the autotrophic communities impact all the trophic levels, altering the ecosystem functioning (Cloern, 2001). High nutrient inputs can also, through an accumulation of macroalgal or phytoplankton biomass, lead to the development of dystrophic crisis and hypoxia and anoxia

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events, having dramatic impacts on all the living organisms (Cloern, 2001). Therefore, the management of a eutrophicated coastal lagoon needs to target the reduction of nutrient loadings. In addition, knowledge is required about the availability of nutrients within lagoons and the autotrophic communities responses (Duarte et al., 2000; Glibert et al., 2015). This allows to assess the resilience of the aquatic communities, and to estimate the critical nutrient loading for the ecosystem (Pasqualini et al., 2017; Le Fur et al., 2018).

Phytoplankton represents the basis of food webs and biochemical cycles, and is generally the first autotrophic compartment responding to a change of nutrient availability (Leruste et al., 2016). Understanding how the ecosystem functioning influences the composition of phytoplankton communities and their growth mechanisms is indispensable (Livingston, 2000; Paerl et al., 2003). Hence, it is particularly important to understand and predict how N and P inputs affect the growth and community assembly of phytoplankton. These responses are indicators of water quality, and are consequently informative for water management (Duarte et al., 2000; Reed et al., 2016). Morphological and physiological traits such as cell size and maximal growth rates shape the phytoplankton functional responses to nutrient availability. Small-size fast-growing algae are competitive at low nutrient concentrations and low light, while larger cells can generally thrive under a pulsed nutrient supply and higher light intensities (Litchman et al., 2007; Bec et al., 2011; Andersen et al., 2015). The community composition also reflects which nutrients are available. Diatoms are particularly competitive for nitrate, while green algae and picocyanobacteria are more competitive for ammonium. The latter often originates from regenerated internal sources. Dinoflagellates can develop even under dissolved inorganic nutrient limitation, because of their relatively low growth rates and potential mixotrophic abilities (Litchman et al., 2007; Glibert et al., 2015).

The general aim of this study was to explore the responses of the phytoplankton communities of a disturbed Mediterranean coastal lagoon to the nutrient availability. Therefore, we choose Biguglia lagoon, which is the largest lagoon on the island of Corsica, as the study site. This Mediterranean lagoon has been increasingly impacted since 1980 by a strong increase of the human population density, industrial and agricultural uses in its catchment and its surrounding areas. This lagoon receives particularly high amounts of nitrate from runoffs, tributaries, and from groundwater flows from a coastal aquifer connected with the lagoon (Erostate et al., 2018). Biguglia lagoon also displays a high confinement and restricted exchanges with the Tyrrhenian Sea that have induced higher levels of nutrient concentration than in most other French Mediterranean lagoons these last decades (Souchu et al., 2010; Pasqualini et al., 2017). Hence, the lagoon has experienced strong changes in its ecological state, *i.e.* increasing eutrophication and pollutant levels linked with changes in the composition of its autotrophic community (Mouillot et al., 2000; Pasqualini et al., 2017). Moreover, a dystrophic crisis associated with a bloom of toxic cyanobacteria in 2007 has raised the awareness of the water quality in the lagoon among the responsible management authorities. Therefore, they have taken remediation measures to reduce the confinement in order to decrease the concentration of nutrients. This included the modification of the lagoon hydrology since 2009 to increase dilution processes (Cecchi et al., 2016; Garrido et al., 2016). However, so far, no efficient direct management measures have been implemented to reduce the external nutrient loadings that remain important and uncontrolled. Moreover, although the measures taken in 2009 may have helped the development of aquatic angiosperms (Pasqualini et al., 2017), these may have also promoted the occurrence of blooms of potentially toxic and or harmful dinoflagellate species (Cecchi et al., 2016).

The specific objectives of this study were to describe in different seasons with contrasting environmental conditions including nutrient form and origin, (1) the structure of phytoplankton communities in Biguglia lagoon submitted to a strong anthropogenic impact in term of size classes and species diversity, and (2) their growth rate in response

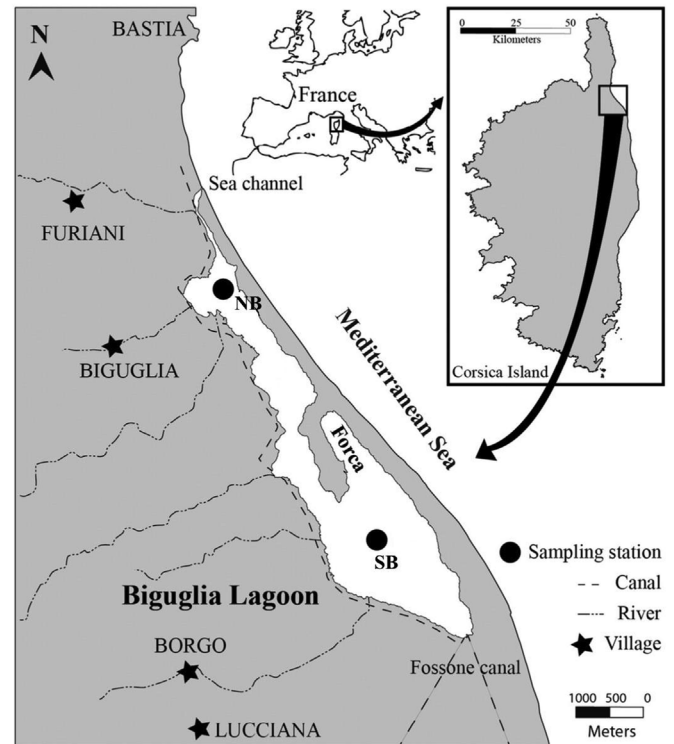


Fig. 1. Location of the two stations representatives of the Northern Basin (NB) and the Southern Basin (SB) of Biguglia lagoon.

to various forms of nutrients inputs. These two observations illustrate phytoplankton functional responses to contrasting nutrient availability, and the latter specially reflects the vulnerability of the whole ecosystem to a short-term nutrient pulse. We experimentally incubated phytoplankton communities under *in situ* light and temperature conditions with enrichments containing N (NO_3^- , NH_4^+) and P (PO_4^{3-}), either in combination or separately to selectively induce either N- or P-limitation. Exploring the responses of phytoplankton communities to a change in nutrient availability helped understanding the impact of these changes on the ecosystem functioning.

2. Material and methods

2.1. Characterization of the study site

Biguglia lagoon ($42^\circ36'N$; $9^\circ28'E$) is a shallow brackish coastal lagoon (average depth 1.2 m), located on the East coast of Corsica, and separated from the Tyrrhenian Sea by a sandy beach barrier (Fig. 1). This choked lagoon *sensu* Kjerfve (1994) covers 14.5 km^2 with a single inlet in the north to the sea that consists of a 1.5 km long, narrow and shallow natural channel. Biguglia lagoon is divided into two sub-basins by a peninsula in its middle. Each of these sub-basins displays a specific hydrological functioning resulting in differences in salinity, nutrient concentrations and phytoplankton biomasses (Garrido et al., 2016).

The microtidal conditions, and the morphology of this inlet and its natural inclination to silt up restrict the exchanges with the sea and thus limit marine water input in the lagoon (Mouillot et al., 2000). This lagoon is highly influenced by freshwater inputs from its watershed (182 km^2), and its salinity remains particularly low (Pasqualini et al., 2017). For example, this ranged from 2.0 to 18.5 between November 2013 and December 2014. Freshwater comes from rainfall, sewage plants, several rivers draining the watershed and flowing in the north-west of the lagoon, the Golu River connected to the lagoon by the Fossone channel in the south-western part, and from pumping stations draining the agricultural plain in the southern part of the watershed

(Erostate et al., 2018) (Fig. 1). Therefore, Biguglia lagoon displays a salinity gradient from the north to the south (Garrido et al., 2016).

Biguglia lagoon has been increasingly eutrophicated since the 1980s due to an intensification of human activities and urbanization on its watershed southward of city of Bastia, including four towns hosting approximately 35,000 inhabitants in winter (Lafabrie et al., 2013). During summer touristic period, the population doubles, which results in increasing anthropogenic pressure. Hence, this lagoon displays very high nutrients concentrations (NH_4^+ , NO_2^- , NO_3^- , DIN, Si, TP, TN) in the water column, enhanced by the reduced exchanges with the sea (Orsoni et al., 2001). The sediment compartment shows an important silting with high nutrient concentrations and organic matter content compared to other French Mediterranean coastal lagoons (Souchu et al., 2010). An eutrophication gradient is also observed from the south to the north because of substantial anthropogenic inputs in the southern part of the lagoon.

The lagoon has a Mediterranean climate with a succession of a dry and warm summer and wet autumn, winter and spring seasons with sudden and plentiful rainfalls causing flash floods (Pasqualini et al., 2017). The salinity and nutrients inputs are strongly influenced by theseasonal variability of the climate (Collos et al., 2003). This generally determines three hydrological seasonal periods characterized by differences in their nutrient origin and availability, in phytoplankton biomass and size class structure, and in photosynthetic performance (Cecchi et al., 2016; Garrido et al., 2016).

2.2. Sampling procedures

Water samples were collected in two stations representatives of the northern (NB) and the southern (SB) basins (Figs. 1 and 42°38'12"N, 9°27'15"E and 42°35'00"N, 9°29'18"E, respectively).

Experiments were carried out in autumn 2013 (26 November to 5 December), spring 2014 (2–8 April), and summer 2014 (9–12 September). In each of the two NB and SB stations, 70 l of pre-filtered water through 1000 μm mesh to remove larger debris without removing zooplankton or larger phytoplankton cells (Collos et al., 2005) were sampled in sub-surface (20 cm depth) with a pump from a small boat between 7.00 a.m. and 8.00 a.m., and kept in the dark. Sub-surface salinity, temperature, percentage of dissolved oxygen (DO) and turbidity were measured *in situ* with a multi parameter Water Quality Probe (YSI[®] 6600 V2-2). Upon return to the laboratory, water samples were homogenized by gentle shaking, aliquoted and immediately stored at -20°C for the characterization of phytoplankton communities. Measures of NH_4^+ , PO_4^{3-} , NO_3^- , NO_2^- , TN, and TP concentrations (μM) were performed on duplicates of 80 ml, filtered on 20 μm meshes for the dissolved inorganic nutrients (Aminot and Chaussepied, 1983).

2.3. Characterization of phytoplankton biomass

Chlorophyll *a* (Chl *a*) concentrations ($\mu\text{g l}^{-1}$) were used as a proxy for phytoplankton biomass. Chl *a* measurements were performed on the total and the fractionated water samples. This allowed assessing the contribution of the ultraphytoplankton $< 5 \mu\text{m}$, the nanophytoplankton between 5 and 20 μm and the microphytoplankton $> 20 \mu\text{m}$ to the total biomass. Upon return to the laboratory, triplicates of water samples (maximum 100 ml, depending on phytoplankton abundance) and of samples pre-filtered on 20 μm and on 5 μm meshes were analyzed by spectrofluorimetry (Neveux and Lantoiné, 1993). The biomasses of the three size classes were estimated by subtracting the concentrations obtained for the fraction $< 20 \mu\text{m}$ to the total water (microphytoplankton), and the fraction $< 5 \mu\text{m}$ to the fraction $< 20 \mu\text{m}$ (nanophytoplankton). The Chl *a* concentration of the fraction $< 5 \mu\text{m}$ estimated the ultraphytoplankton biomass.

2.4. Assessment of phytoplankton community composition

Composition and abundance of the ultraphytoplankton $< 5 \mu\text{m}$ were studied using flow cytometry. Triplicates of 1 ml of sample fixed with 2% formaldehyde (filtered on 0.2 μm , final concentration) were submitted to a flash freeze in liquid nitrogen and then stored at -80°C until analysis. Cells counts were performed with a FACSCalibur flow cytometer (Becton Dickinson), using beads for size calibration. Populations of coccoid phycoerythrin-rich picocyanobacteria (PE-cyanos) were identified by light diffraction (FSC) and their orange fluorescence emissions, while phycocyanin-rich picocyanobacteria (PC-cyanos) were distinguished by their low red fluorescence and their absence of orange fluorescence. Photosynthetic picoeukaryotes $< 3 \mu\text{m}$ were identified by their red fluorescence emissions (Chl *a*, wavelength $> 650 \text{ nm}$).

Optical microscopy was used to describe nano- and micro-phytoplankton ($> 5 \mu\text{m}$) composition and abundance. Triplicates of 1 l of sample fixed with formaldehyde (5% final concentration) were stored at obscurity prior to analysis. A modified method of the Utermöhl protocol was used (Leruste et al., 2018). Samples were examined with an optic microscope Olympus AX10. At least 400 cells per sample were counted to obtain a relevant assessment of the assemblage. Taxonomic resolution was realized at species level whenever possible, and identification was verified according to several books (Bellinger and Sigeo, 2015; Bérard-Therriault et al., 1999; Loir, 2004; Tomas, 1997) and database such as the World Register of Marine Species (<http://www.marinespecies.org/>, databases available online). Taxonomic diversity was explored using species richness and Shannon index (Leruste et al., 2018).

2.5. Experimental procedure of bioassay experiments

To measure maximal growth rate, five dilutions of water sample (9, 17, 43, 74 and 100%) in 0.2 μm filtered sample in duplicate were incubated with an enrichment containing vitamins, silica, metals trace, nitrogen and phosphorus (NaH_2PO_4 , final concentration 0.8 μM) (Landry and Hassett, 1982). Nitrogen was supplied as nitrate and/or ammonium (f/2 and h/2 media, respectively, Guillard and Ryther, 1962) depending on season. In April 2014, N was supplied as nitrate (20 μM final concentration), assuming that nitrate inputs from the watershed were the main nitrogen source. In September 2014, N was supplied as ammonium (20 μM final concentration), assuming that this may be the predominant form provided from remineralization processes in the sediment (Collos et al., 2003). In November–December 2013, N was supplied as a mix of nitrate and ammonium (10 μM final concentration each), assuming that sufficiently high temperatures allow the regeneration of ammonium from the sediments, associated with flash floods bringing nitrate from the watershed. This nitrogen concentration has been chosen to avoid phytoplankton growth limitation during the incubation, which has been occasionally observed at 10 μM in another Mediterranean lagoon (Bec et al., 2005). To highlight the potential growth limitation of phytoplankton by N and/or P, two other dilution series were enriched either without nitrogen or phosphorus (Andersen et al., 1991). Two bottles of sample without dilution and enrichment were incubated as control, and the all bottles were simultaneously incubated for 24 h in Biguglia lagoon under *in situ* temperature and light conditions at 30 cm depth.

2.6. Estimation of maximal growth rate and N-/P-limitation

After 24 h incubation, the temporal changes of the total and size-fractionated Chlorophyll *a* in each bottle were used to calculate their apparent specific growth rate $k(x)$ for each dilution x . The relationship between the apparent growth rates $k(x)$ in the fully-enriched bottles and dilution factor x were fitted to the linear equation (1):

Table 1

Mean values of environmental parameters in the two NB and SB stations of Biguglia lagoon in November–December 2013, April and September 2014. DO: dissolved oxygen. Dissolved inorganic nitrogen is expressed as $\text{DIN} = \text{NH}_4 + \text{NO}_3 + \text{NO}_2$. Dissolved inorganic phosphorus corresponds to PO_4^{3-} concentration.

Sampling periods	Stations	Date	T(°C)	Salinity	DO (%)	Turbidity (NTU)	TN	NH_4^+	NO_3^-	NO_2^-	DIN	TP	PO_4^{3-}	TN:TP	DIN:DIP
Nov–Dec 2013	NB	26/11/13	8.3	4.7	100.8	2.87	84.37	7.52	70.01	0.49	78.03	1.37	0.73	61.4	107.3
	SB	04/12/13	8.2	6.1	101.5	16.83	61.87	0.69	40.72	0.29	41.70	1.77	0.64	34.9	65.5
April 2014	NB	07/04/14	15.0	2.0	113.0	0.80	34.55	2.18	17.47	0.16	19.80	0.82	0.16	41.9	124.5
	SB	02/04/14	15.5	6.0	93.2	4.63	106.47	1.85	93.22	0.30	95.38	1.50	0.00	71.0	–
September 2014	NB	11/09/14	24.8	10.9	97.0	1.83	38.51	0.37	0.03	0.00	0.40	0.89	0.03	43.3	13.3
	SB	09/09/14	25.9	6.0	86.2	10.50	29.95	1.24	0.11	0.09	1.44	1.17	0.11	25.6	13.1

$$k(x) = \mu_{\max} - gx \quad (1)$$

Where $k(x)$ is the phytoplankton apparent growth rate in the bottles at dilution x , μ_{\max} is the maximal growth rate under nutrient replete conditions, and g is the grazing rate. All rates were expressed on a per day basis (d^{-1}). The maximal growth rate μ_{\max} was obtained from the Y axis intercept, and the phytoplankton mortality rates g as the slope of the linear regressions assuming that mortality was due to grazing (Landry and Hassett, 1982). Equations were fitted with the 'lmer' function in the 'lme4' library (version 1.1–10, (Bates et al., 2015)) to evaluate if the linear model best fitted the trend of the relationship between the apparent growth rate and the dilution factor. To check whether the linear equation (1) was acceptable, we also considered a quadratic function by adding the term ax^2 . The quadratic function has a parabolic pattern and could reflect the contribution of regenerated resources or a saturation of the grazing rate in the less diluted bottles (Andersen et al., 1991). All combinations of the parameters of the quadratic function were considered, and model selection was based on parsimony using the small-sample corrected Akaike's information criterion (AIC_c) (Burnham and Anderson, 2004). Therefore, we calculated the difference between the AIC_c of the linear model and the model having the lowest AIC_c , i.e. the most parsimonious model, to obtain a ΔAIC_c value. The models with a $\Delta\text{AIC}_c < 2$ were considered as having equivalent levels of support (Burnham and Anderson, 2004), using the 'dredge' function of the MuMIn package (Bartón, 2013). Hence for $\Delta\text{AIC}_c < 2$ we selected the linear model as it allows calculating μ_{\max} and g according to equation (1) (Landry and Hassett, 1982), while the quadratic relationship did not allow calculating μ_{\max} and g .

Phytoplankton net growth rate in controls (μ_0) was calculated by adding g to the apparent growth rate (k_0) i.e. $\mu_0 = g + k_0$. Hence, it was assumed that grazing rate was not impacted by nutrient enrichments. The $\mu_0:\mu_{\max}$ ratio assessed the impact of inorganic nutrient enrichment on growth, and estimated the nutrient sufficiency for phytoplankton growth (Landry and Hassett, 1982; Landry et al., 1998).

In the selectively enriched bioassays, we took account that apparent growth rate is not necessarily a linear function of the dilution factor, because the instantaneous growth rate may decline as internal and external nutrient pools become depleted. μ_N and μ_P were calculated as followed: $\mu_N = g + k_N$ and $\mu_P = g + k_P$. To estimate how much phytoplankton growth was N- and/or P-limited, the ratio between growth rate with the enrichments minus N or minus P (μ_N or μ_P) and maximal growth rate in theoretically non-limiting nutrient condition (μ_{\max}) was calculated. Differences between growth rates with full enrichment, enrichment minus N and enrichment minus P helped distinguish different types of co-limitation (Harpole et al., 2011; Burson et al., 2016).

2.7. Statistical analyses

Data analysis was performed on R (R Core Team, 2013).

Two-way ANOVAs assessed if the total phytoplankton biomass significantly changed among the three samplings and between NB and SB. Posteriori pairwise mean comparisons between stations and samplings

were made using Tukey-Kramer's post-hoc analysis (alpha level = 0.05). Permanovas assessed if the size class structure varied among stations and samplings, according to differences of biomass, temperature, salinity, dissolved inorganic nutrients, TN and TP concentrations, turbidity and DO (Anderson, 2001). Taxonomic diversity indices i.e. the Shannon index on the relative abundance of taxa and morphotypes (H), and the species richness (S) were estimated using Vegan package (Oksanen et al., 2018). ANOVAs assessed if S and H significantly changed among stations and samplings. Spearman's rank correlations between S, H, total Chl *a* biomass and environmental variables (temperature, salinity, inorganic nutrient concentrations, TN and TP concentrations, turbidity and DO) were assessed for the two stations and the three samplings.

Regression analyses determined which mixed models (i.e. combinations of explanatory factors) best fitted the biomass of total phytoplankton and the three size classes. Among all the possible fixed effects (stations, samplings, salinity, temperature, nutrients concentrations, dissolved oxygen, and turbidity), the most relevant were chosen according to a correlation matrix. Values of fixed effects were scaled prior to analysis. Because of pseudo-replication (each triplicate was taken from the same bottle), a random effect was added as a bottle effect. Models were fitted with the 'lmer' function and model selection was assessed using the AIC_c (see below). To estimate the relative importance of each fixed effect, the AIC_c -w of every model was summed (Patiño et al., 2013), and to estimate the variable force effect, we used the mean of the relative coefficient weighted by the AIC_c -w.

3. Results

3.1. Environmental variables and phytoplankton biomass

Salinities were low during the three dilution experiments, ranging from 2.0 in NB in April, to 10.9 in September 2014 (Table 1). The percentage of DO was the lowest in September 2014 for both stations, and was negatively correlated with the temperature for the three sampling periods (Table 2, Spearman's rank correlations, p -value < 0.05). Turbidity was highest in the autumn sampling (Table 1).

Concentrations of dissolved inorganic nutrients (NH_4^+ , NO_3^- , NO_2^- , DIN, PO_4^{3-}) and of TN and TP were extremely variable. In the sampling in November–December 2013, both stations presented the highest PO_4^{3-} concentrations, and NB displayed the highest concentration of dissolved inorganic nutrients, DIN being mainly composed of NO_3^- (Table 1). In April 2014, N was mainly represented by NO_3^- in the two stations, and N levels were especially high in SB, with 106.5 μM of TN and 95.4 μM of DIN. Inversely, in September 2014, both stations displayed low NO_3^- concentrations (< 0.15 μM) and NH_4^+ was the main nitrogen form. TP was higher in SB than in NB in all three samplings (Table 1). All dissolved inorganic nutrient concentrations and TN and TP concentrations were negatively correlated with the temperature. Moreover, NH_4^+ concentrations were also negatively correlated with the salinity. DIN forms were positively correlated between them, and NO_3^- and NO_2^- concentrations were also positively correlated with TN

Table 2

Spearman's rank correlation between species richness (S), Shannon index (H), Chl *a* concentrations and environmental variables at the 95% confidence interval for the three sampling periods in both NB and SB stations of Biguglia lagoon. Significant correlations are in bold. DO: dissolved oxygen. ****p*-value < 0.001, ***p*-value < 0.01, **p*-value < 0.05.

	S	H	Chl <i>a</i>	Temperature	Salinity	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	PO ₄ ³⁻	TN	TP	Turbidity
H	-0.314											
Chl <i>a</i>	-0.312	0.626**										
Temperature	0.469*	-0.661**	-0.900***									
Salinity	-0.051	-0.693**	-0.242	0.203								
NH ₄ ⁺	-0.066	0.680**	0.260	-0.314	-0.928***							
NO ₂ ⁻	-0.513*	0.536*	0.530*	-0.657**	-0.464	0.714***						
NO ₃ ⁻	-0.494*	0.335	0.492*	-0.543*	-0.377	0.600**	0.943***					
PO ₄ ³⁻	-0.405	0.836***	0.709***	-0.880***	-0.400	0.516*	0.637**	0.395				
TN	-0.280	-0.009	0.360	-0.486*	0.058	0.257	0.771***	0.829***	0.273			
TP	-0.809***	0.047	0.398	-0.486*	0.319	-0.086	0.600**	0.657*	0.273	0.657**		
Turbidity	-0.828***	-0.041	0.122	0.143	0.406	-0.314	0.200	0.257	0.030	0.143	0.829***	
DO	0.041	0.624**	0.774***	-0.771***	-0.348	0.257	0.200	0.086	0.698**	0.029	-0.143	-0.371

and TP concentrations. PO₄³⁻ concentrations were positively correlated with the percentage of DO (Table 2). The TN:TP and DIN:DIP ratios displayed highest values in April 2014, respectively 71.0 in SB and 124.5 in NB.

The biomass of the total phytoplankton significantly differed among stations and samplings, and the differences among samplings also changed between the two stations (Fig. 2, ANOVA, *p*-value < 0.05). Chlorophyll *a* (Chl *a*) concentration generally ranged between 3.5 and 6.0 µg l⁻¹, with the exception in SB in December 2013 when it peaked at 20.6 µg l⁻¹ (Fig. 2A). Taking all samplings collectively, we found that Chl *a* concentration showed a negative relationship with the salinity and NH₄⁺ concentrations, the latter effect changing among stations (Regression analyses, force of the salinity effect: 4.5, and of the NH₄⁺ concentration: 8.1). Total biomass was significantly higher (i) in SB than in NB, (ii) in the autumn sampling than in April and in September 2014, as well as (iii) in April than in September samplings (Fig. 2, pairwise Tukey multiple comparisons, *p* < 0.05). The total biomass was negatively correlated with temperature, and positively correlated with NO₂⁻, NO₃⁻, PO₄³⁻ concentrations and with the percentage of

DO, reflecting the peak of biomass observed in December in SB (Table 2, Spearman's rank correlations, *p*-value < 0.05).

3.1.1. Phytoplankton community composition

The size class structuration of phytoplankton communities was significantly different between the NB and SB stations among the three samplings (Fig. 2B), and the differences between samplings changed between stations (Permanova, *p*-value < 0.05). Taking all samplings collectively, the ultra- and the nanophytoplankton biomasses showed a positive relationship with the salinity and the phosphorus concentrations. In contrast, the microphytoplankton biomasses showed a positive relationship with NO₃⁻ concentrations and a negative relationship with NH₄⁺ concentrations (Regression analyses, delta-AICc < 2). Ultra-phytoplankton was the major group in the phytoplankton communities (up to 87% of Chl *a* in September 2014) (Fig. 2B), except for NB in November 2013, when a higher proportion of nanophytoplankton (40%) than ultraphytoplankton (34%) was observed. For both stations, the nanophytoplankton biomass was highest in November 2013.

The taxonomic diversity indices showed different patterns at the two stations and among the three samplings. Both the species richness (S) and the Shannon index (H) significantly changed among stations and samplings (ANOVAs, both *p*-values < 0.05). S was the lowest in the autumn sampling and highest in the sampling in September for the two stations, while H showed the inverse trend. Hence, S was positively correlated with temperature, and negatively correlated with NO₂⁻, NO₃⁻, TP concentrations and turbidity (Table 2). H was positively correlated with total biomass, NH₄⁺, NO₂⁻ and PO₄³⁻ concentrations and percentage of DO, while it was negatively correlated with temperature and salinity (Table 2).

Fig. 3 gives the contributions of four size classes (< 3 µm, between 3 and 5 µm, between 5 and 20 µm and > 20 µm) to the total abundances of cell counts in the two stations at the three sampling periods, and Fig. 4 specifies the main taxonomic classes represented in each of the size classes. For the three samplings, small cells dominated the phytoplankton communities (Fig. 3). The NB station showed higher Bacillariophyceae abundances than SB, while the SB station showed higher Chlorophyta and Cryptophyceae abundances (Fig. 4). NB has also displayed higher dinoflagellates abundances than SB, except in November-December 2013 (Fig. 4).

In the sampling in November 2013, the phytoplankton community in NB showed a bloom of the dictyochophycean *Apedinella radians* (Lohmann) P.H. Campbell, 1973 (1.3 × 10⁶ cells.l⁻¹, i.e. 41.6% of total abundances, Figs. 3 and 4). This community also comprised high abundances of eukaryotes with flagella between 3 and 5 µm (6.9 × 10⁵ cells.l⁻¹, Figs. 3 and 4), and of *Prorocentrum cordatum* (Ostenfeld) J.D.Dodge, 1975 *alias* *Prorocentrum minimum* (Pavillard, 1916) J.Schiller, 1933 (2.9 × 10⁵ cells.l⁻¹, Fig. 4). Dinoflagellates dominated the community in SB in December, mainly represented by *Heterocapsa*

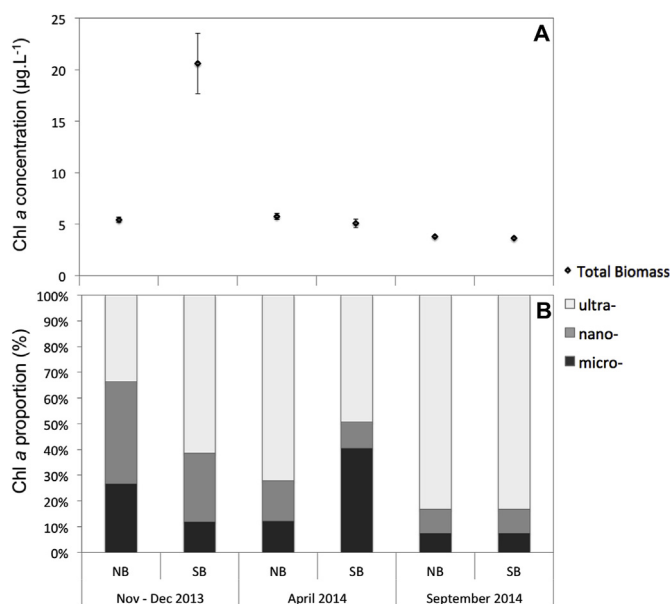


Fig. 2. Mean chlorophyll *a* (Chl *a*) concentration (µg l⁻¹) (A) in the three samplings at the NB and SB stations in Biguglia lagoon. Error bar indicates standard deviations. Proportions of the different size classes of phytoplankton contributing to phytoplankton biomass (B), i.e. microphytoplankton > 20 µm (dark grey), nanophytoplankton between 5 and 20 µm (grey) and ultraphytoplankton < 5 µm (light grey).

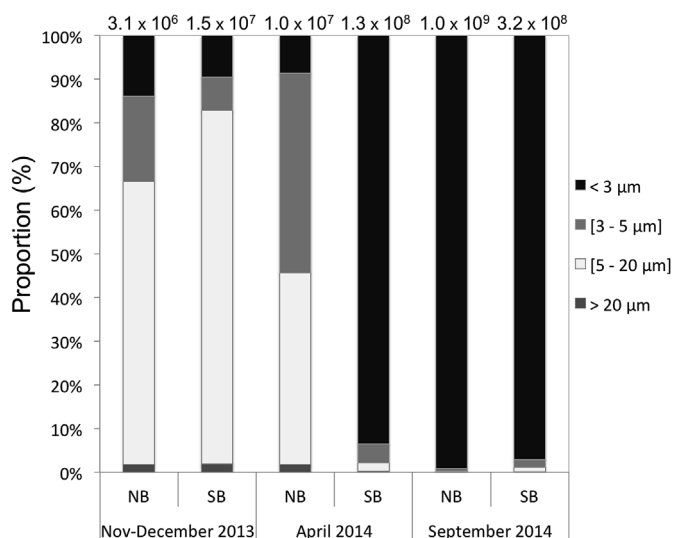


Fig. 3. Contribution of the size classes < 3 μm, [3–5 μm], [5–20 μm] and > 20 μm to the total abundances of cell counts in the two NB and SB of Biguglia lagoon at the three sampling periods. Total abundances are specified on the top of the bars.

minima Pomroy, 1989 (5.1×10^6 , i.e. 34.2% of total abundances Fig. 3), associated with *A. radicans* (4.8×10^6 cells.l⁻¹, Fig. 4). Microphytoplanktonic cells were scarce (e.g. *Kryptoperidinium foliaceum* Lindemann, 1924: 3.7×10^3 cells.l⁻¹; *Cocconeis* sp.: 3.7×10^3 cells.l⁻¹; *Prorocentrum micans* Ehrenberg 1834: 1.5×10^3 cells.l⁻¹). Conversely, we observed a bloom of *Mesodinium rubrum* Lohmann, 1908 (2.3×10^5 cells.l⁻¹, i.e. 78% of the microplanktonic cells, Fig. 4).

In the sampling in April 2014, dinoflagellates dominated the NB community mainly due to a bloom of *H. minima* (3.7×10^6 cells.l⁻¹, i.e. 35.8% of total abundances, Figs. 3 and 4). Bacillariophyceae were also abundant and distributed among the three size classes > 3 μm (3.1×10^6 cells.l⁻¹, Fig. 4) with e.g. *Thalassiosira* sp., *Diatoma* sp., and *Chaetoceros* spp. The SB community was dominated by picocyanobacteria (PC-cyanos, 6.4×10^7 cells.l⁻¹, i.e. 49.6% of total abundances, Figs. 3 and 4), Picoeukaryotes (Peuk) without flagella (5.6×10^7 cells.l⁻¹) and eukaryotes with flagella between 3 and 5 μm (5.9×10^6 cells.l⁻¹, Fig. 4). The two latest morphotypes probably belonged to the green algae according to the pigment composition (data not shown). *M. rubrum* also bloomed as in December 2013 (1.6×10^5 cells.l⁻¹, Fig. 4).

In the sampling in September 2014 the communities of the two stations were dominated by PC-cyanos (9.4×10^8 cells.l⁻¹, i.e. 92.7% of total abundances in NB, 3.0×10^8 cells.l⁻¹ i.e. 91.9% of total abundances in SB, Figs. 3 and 4) and Peuk without flagella (6.5×10^7 cells.l⁻¹ in NB, 1.7×10^7 cells.l⁻¹ in SB, Figs. 3 and 4), that most likely belonged to the green algae according to the pigment composition (data not shown). The nanophytoplankton in NB displayed high abundances of *Tetraselmis* sp. (2.0×10^5 cells.l⁻¹), *P. cordatum* (8.2×10^4 cells.l⁻¹) and *H. minima* (3.2×10^4 cells.l⁻¹). Dinoflagellates dominated the microphytoplankton with *Prorocentrum* sp. cf. *mexicanum* Osorio-Tafall, 1942, and *Gonyaulax* sp. (10^4 cells.l⁻¹). We also observed high abundances of *Chaetoceros* spp., and *Ceratoneis closterium* Ehrenberg, 1839 (10^4 cells.l⁻¹). The ultra- and the nanophytoplankton in SB also contained high abundances of *A. radicans* (1.6×10^6 cells.l⁻¹), *Pyramimonas* sp. (8.9×10^5 cells.l⁻¹), *Plagioselmis* sp., *H. minima*, *P. minimum* and nanoplanktonic centric diatoms (10^4 cells.l⁻¹). *C. closterium*, and *Gonyaulax* sp. cf. *spinifera* (10^4 cells.l⁻¹) were the most abundant microplanktonic cells in SB (Fig. 4).

3.1.2. Phytoplankton net growth and mortality rates without enrichment

Phytoplankton net growth rates (μ_0) without enrichment ranged from negative values for the microphytoplankton of NB in November 2013 and April 2014, to 1.91 d^{-1} for the nanophytoplankton of SB in April 2014 (Table 3). In November-December 2013, the total phytoplankton displayed low net growth rates (0.45 d^{-1} in NB and 0.48 d^{-1} in SB), and similar mortality rates (0.62 d^{-1} in NB to 0.69 d^{-1} in SB). In NB the ultraphytoplankton dominated by picoeukaryotes (Peuk) displayed a two-fold higher growth rate, while its mortality rate was three times higher than those of the total phytoplankton (Table 3). The nanophytoplankton, mainly comprising *A. radicans* and *P. cordatum*, and particularly the microphytoplankton comprising diatoms and dinoflagellates species, showed a loss of biomass after 24 h incubation (Table 3). In SB, only the net growth rate (μ_0) of ultraphytoplankton could have been estimated, but this size class dominated by Peuk has shown a loss of biomass after the 24 h incubation (Table 3, Fig. S2A).

In April, we were able to calculate the μ_0 only for the nanophytoplankton (Table 3, Fig. S2B). In NB, this size class dominated by *H. minima* displayed a high net growth rate ($\mu_0 = 1.13 \text{ d}^{-1}$) and a relatively low mortality rate (0.28 d^{-1}), while in SB, it was dominated by Chlorophyta and Cryptophyta cells, which displayed the highest estimated μ_0 (1.91 d^{-1}) and a high mortality rate (0.83 d^{-1}).

In September, the net growth rates in NB ranged between 0.50 d^{-1} for the ultraphytoplankton dominated by PC-cyanos and 1.84 d^{-1} for the microphytoplankton dominated by dinoflagellates and Bacillariophyceae. The nano-, dominated by *Plagioselmis* sp., *Tetraselmis* sp., and *P. cordatum*, and the microphytoplankton showed the highest growth rates ($\mu_0 = 1.6$ and $\mu_0 = 1.8 \text{ d}^{-1}$, respectively) with similar mortality rates ($g = 0.57 \text{ d}^{-1}$). In SB, the total phytoplankton and the ultraphytoplankton also dominated by PC-cyanos experienced a loss of biomass after 24 h incubation (Table 3). The nanophytoplankton dominated by *A. radicans* and *Plagioselmis* sp. displayed high growth and mortality rates ($\mu_0 = 1.4 \text{ d}^{-1}$; $g = 1.1 \text{ d}^{-1}$). The biomass of microphytoplankton at T0 was too low to estimate their apparent growth rate, we were not able to truly calculate its specific growth and mortality rates.

3.2. Bioassay experiments

In November and December 2013, the full enrichment including NO_3^- , NH_4^+ and PO_4^{3-} did not enhance phytoplankton growth rates in the two stations. The total phytoplankton, the micro- and ultraphytoplankton in NB experienced a loss of biomass after 24 h incubation compared with the controls. Only the growth rate of the ultraphytoplankton of SB was higher with the enrichment, and showed a linear relationship between the apparent growth rate and the dilution factor. This indicates a nutrient limitation for this class size (Table 3). In NB, the enrichment minus N has only resulted in a higher growth rate of the nanophytoplankton. The non-linear relationship between the apparent growth rate and the dilution factor for the ultraphytoplankton (Fig. S2A) suggests the use of other resources than the externally supplied nutrients, or a saturation of predation in the less diluted samples. The enrichment minus P partly released the total phytoplankton growth from N-limitation, allowing a positive growth rate. Ratios between μ_{-N} or μ_{-P} and μ_{max} for the nanophytoplankton indicate an independent co-limitation by N and P. The negative growth rates of the micro- and ultraphytoplankton, and the non-linear relationship between their apparent growth rate and the dilution factor with the enrichments minus N and minus P more likely suggest a co-limitation by N and P (Table 3, Fig. S2A). In SB, ratios between μ_{-N} or μ_{-P} and μ_{max} of the total phytoplankton and of the nanophytoplankton, as well as their non-linear relationship between apparent growth rates and dilution factors suggest a single N-limitation (Table 3, Fig. S2B). The higher growth rate of microphytoplankton with the enrichments minus N and minus P compared with μ_{max} (Table 3) suggests a co-limitation by N and P. Ratios between μ_{-N} or μ_{-P} and μ_{max} for the ultraphytoplankton suggest an

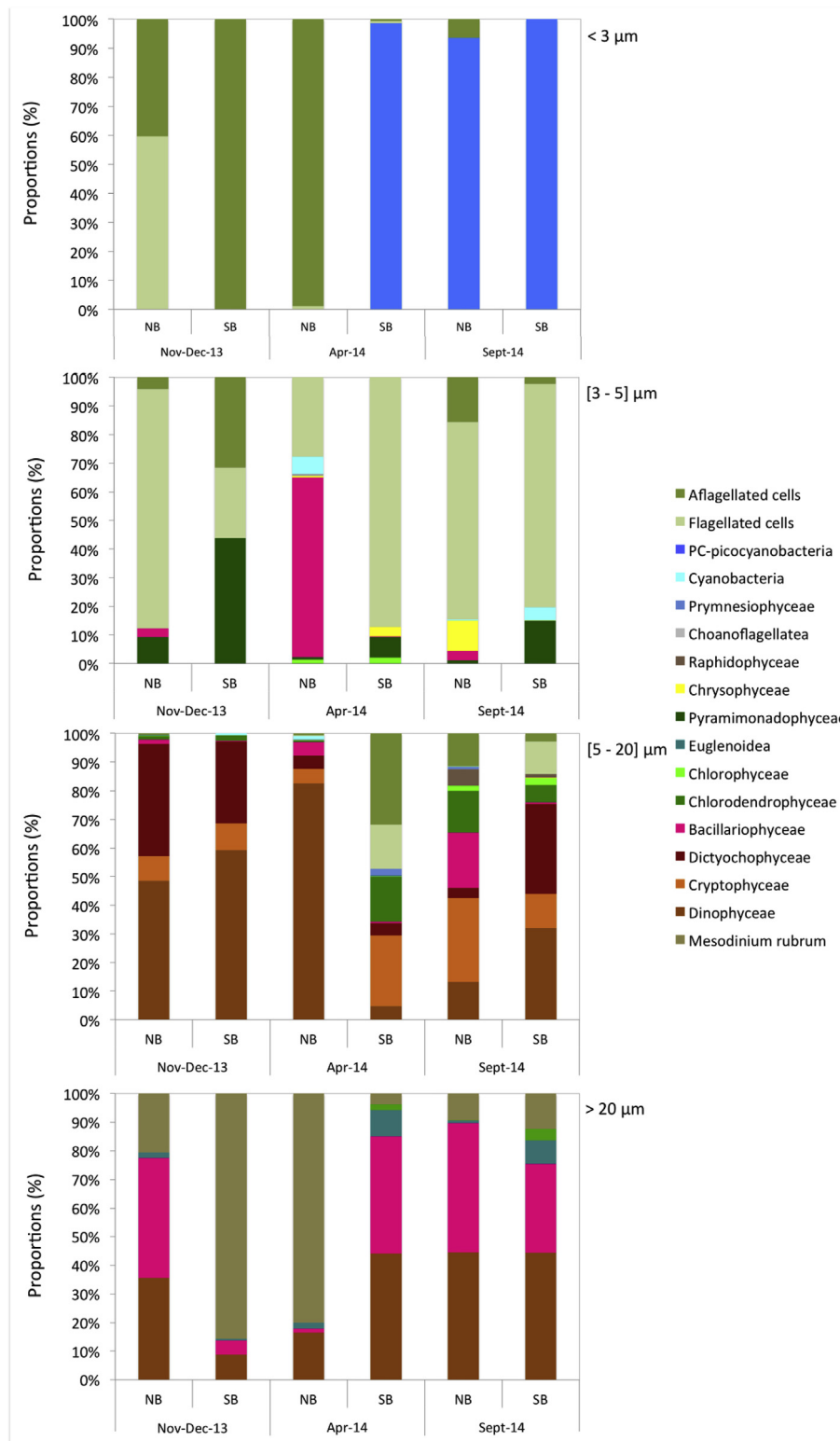


Fig. 4. Contribution of the main taxonomic classes to the abundances of the four size classes < 3 μm, [3–5 μm], [5–20 μm] and > 20 μm in the two stations of Biguglia lagoon at the three sampling periods.

independent co-limitation by N and P (Table 3).

In April 2014, the full enrichment with NO_3^- and PO_4^{3-} resulted in an increase of the growth rate of the nanophytoplankton by 62% and 5% in NB and SB respectively. The non-linear relationship between the apparent growth rate and the dilution factor indicates that other fractions in the two stations may have been limited despite the full nutrient

enrichment (Table 3, Fig. S3). In NB, ratios between μ_N or μ_P and μ_{max} of the total phytoplankton and of the nanophytoplankton suggest a single P-limitation. The microphytoplankton displayed an independent co-limitation of N and P, which was more pronounced for P. The negative μ_N and μ_P of the ultraphytoplankton, and the relationship between their apparent growth rate and the dilution factor in the

Table 3

Net growth rate without enrichment (μ_0) and maximal growth rates, with (i) full enrichment (μ_{max}), (ii) enrichment minus N (μ_N), (iii) enrichment minus P (μ_P) for the NB and SB stations of Biguglia lagoon in November–December 2013, April and September 2014. g represents the mortality rate. Bold values specify when the relationship between apparent growth rate as a function of the dilution factor can be explained as a linear model, although for the minus N and minus P enrichments other models can also fit this relationship; the $\mu_0:\mu_{max}$ ratio illustrates the impact of the full enrichment on phytoplankton growth; the ratios between growth rates with selectively N- or P-enrichment (μ_N or μ_P) and μ_{max} highlight N- or P-limitations ($\mu_X:\mu_{max} = 1$ no limitation, $\mu_X:\mu_{max} < 1$ limitation of the lacking nutrient X, $\mu_X:\mu_{max} > 1$ limitation of the added nutrient).

Stations	Samplings	Fraction	μ_0	g	μ_{max}	$\mu_0:\mu_{max}$	μ_N	$\mu_N:\mu_{max}$	μ_P	$\mu_P:\mu_{max}$
				(d ⁻¹)			(d ⁻¹)		(d ⁻¹)	
NB	Nov–Dec 2013	Total	0.45	0.62	< 0	–	< 0	–	0.30	< 0
		Micro-	< 0	1.45	< 0	–	< 0	–	< 0	–
		Nano-	0.37	0.26	0.46	0.79	0.62	1.34	1.13	2.44
		Ultra-	0.96	1.82	< 0	–	< 0	–	< 0	–
	April 2014	Total	< 0	< 0	0.25	–	0.51	1.99	0.07	0.29
		Micro-	< 0	1.01	0.24	–	1.59	6.70	1.01	4.24
		Nano-	1.13	0.28	1.83	0.62	1.95	1.06	1.54	0.84
		Ultra-	< 0	0.43	< 0	–	< 0	–	< 0	–
	September 2014	Total	0.64	0.30	1.78	0.36	0.86	0.48	1.03	0.58
		Micro-	1.84	0.57	3.40	0.54	1.78	0.52	2.24	0.66
		Nano-	1.60	0.57	2.70	0.59	1.77	0.65	2.13	0.79
		Ultra-	0.51	0.45	1.12	0.45	0.51	0.46	0.52	0.46
SB	Nov–Dec 2013	Total	0.48	0.69	0.41	1.19	0.26	0.64	0.53	1.31
		Micro-	1.12	1.12	0.45	2.48	1.09	2.41	1.05	2.32
		Nano-	1.17	0.50	0.90	1.30	0.78	0.87	1.07	1.19
		Ultra-	< 0	1.10	0.14	< 0	1.85	13.68	2.02	14.94
	April 2014	Total	0.17	< 0	0.19	0.92	0.06	0.31	0.26	1.35
		Micro-	–	–	–	–	–	–	–	–
		Nano-	1.91	0.83	2.00	0.95	1.64	0.82	1.64	0.82
		Ultra-	< 0	< 0	0.01	< 0	0.10	8.73	0.34	27.96
	September 2014	Total	< 0	0.16	1.79	< 0	0.28	0.16	1.34	0.75
		Micro-	–	–	–	–	–	–	–	–
		Nano-	1.42	1.14	3.23	0.44	1.58	0.48	< 0	< 0
		Ultra-	< 0	< 0	0.87	< 0	< 0	< 0	< 0	< 0

enrichments minus N and minus P also suggest a co-limitation by N and P (Table 3, Fig. S3A). In SB, ratios between μ_N or μ_P and μ_{max} of the total phytoplankton indicate a single N-limitation, while those of the nano- and of the ultraphytoplankton indicate a simultaneous and an independent co-limitation by N and P respectively (Table 3, Fig. S3). This is also supported by the non-linear relationship between their apparent growth rate and the dilution factor that may highlight the use of other N and P resources to cope with the nutrient limitation (Table 3, Fig. S3B).

In September 2014, the enrichment with NH_4^+ and PO_4^{3-} led to more than the doubling of the growth rate of most of phytoplankton size classes in NB (Table 3), indicating a strong nutrient limitation. The microphytoplankton of NB displayed the highest μ_{max} of the two communities for the three bioassays. In SB, the full enrichment has allowed a release from nutrient limitation of the entire community, especially for the ultraphytoplankton and the nanophytoplankton (Table 3). In NB, ratios between μ_N or μ_P and μ_{max} of the total phytoplankton and the three size classes suggest a N and P co-limitation, that was serial for the micro- and for the ultraphytoplankton, and independent for the nanophytoplankton (Table 3). In SB, ratios between μ_N or μ_P and μ_{max} of the three size classes indicate an independent co-limitation by N and P for the total phytoplankton, a serial co-limitation by N and P for the nanophytoplankton and a simultaneous co-limitation by N and P for the ultraphytoplankton (Table 3, Fig. S4B).

4. Discussion

In this study, we aimed to describe functional traits of phytoplankton communities in Biguglia lagoon submitted to a strong anthropogenic impact in different seasons with contrasting environmental conditions, and the response of these communities to various forms of nutrients inputs simulating nutrient pulses to predict their impact on ecosystem functioning.

4.1. Functional traits of phytoplankton communities in response to environmental conditions

Biguglia lagoon was globally strongly influenced by freshwater inputs from the watershed and from the connection with one of the main river in Corsica in the south of the lagoon. These freshwater inputs have contributed to maintain a low salinity that prevailed in the two sub-basins during the year 2014, despite their distinct hydrological dynamics. This freshwater influence has led to the dominance of species associated with brackish or fresh waters in phytoplankton assemblages. Phycocyanin-rich picocyanobacteria (PC-cyanos) and flagellates from the Dictyochophyceae, dinoflagellates, Chlorophytes, and Cryptophytes were the main representative groups in the two stations at the three samplings. Their size structure, community composition and productivity illustrate their functional responses to lagoon variability.

Biguglia lagoon was principally enriched by high nitrogen inputs, especially during the wet periods. Indeed, high nitrate inputs from a huge historical accumulation are carried to the lagoon by groundwater flows during strong episodic rainfall events (Erostate et al., 2018). The negative correlations, both between temperature and nutrient concentrations, and between salinity and ammonium concentration also support that freshwater discharge was the main source for nutrient enrichment in the lagoon at the three sampling periods (Garrido et al., 2016; Pasqualini et al., 2017). The important precipitations in November 2013 and March 2014 (150.4 mm and 92.6 mm respectively, <https://www.infoclimat.fr/>, Station of Bastia Poretta) explain the high NO_3^- concentrations and high TN:TP and DIN:DIP values in the autumn and spring samplings. In contrast, low dissolved inorganic nitrogen and phosphorus concentrations were observed in September, although the summer period corresponds to the most important period for the recycling of ammonium and phosphate in the lagoon (Collos et al., 2003).

Phytoplankton cell size decreased with the decrease of DIN availability, which may be explained by higher uptake affinities of small

cells under nutrient-limiting conditions (Litchman et al., 2007). High nanophytoplankton biomasses in Biguglia lagoon have already been related to the availability of oxidized forms of nitrogen (Cecchi et al., 2016). In this study, ultra- and nanophytoplankton biomasses were rather positively related to PO_4^{3-} availability. Dominance of fast-growing pico- and nanoflagellates can be frequent in shallow lagoons exposed to high nutrient loads and submitted to continuous inputs of freshwater or discontinuous floods. These nutrient loads allow the release of the limitation of cells $> 3 \mu\text{m}$ that can coexist with fast growing and highly competitive picoeukaryotes (Bec et al., 2011; Coelho et al., 2015).

Nanoplanktonic dinoflagellates were particularly abundant during the samplings in the wet seasons (autumn and spring). We particularly observed blooms ($> 10^5 \text{ cells.l}^{-1}$) of *P. cordatum* in NB in November 2013, and of *H. minima* in SB in December 2013 and in NB in April 2014. Blooms of *P. cordatum* are frequently related to high N:P ratios in coastal transitional ecosystems characterized by brackish waters (Johnson, 2015), and to freshwater inflows that cause low salinities and high nitrate concentrations (Heil et al., 2005; Coelho et al., 2007; Cecchi et al., 2016). Moreover, *P. cordatum* and some *Heterocapsa* species have demonstrated potential mixotrophic abilities, especially under N- or P-starvation (Legrand et al., 1998; Jeong et al., 2005; Johnson, 2015). The presence of potential preys such as picocyanobacteria, Cryptophytes and small diatoms may thus have stimulated their development despite the potential nutrient limitation.

Biomass of the microphytoplankton, mainly composed of Bacillariophyceae and dinoflagellates in the three samplings, has been positively related to the high NO_3^- concentrations. Indeed, pulses of NO_3^- especially favor fast-growing diatoms during spring blooms in river-dominated coastal ecosystems (Paerl et al., 2010; Donald et al., 2011; Glibert et al., 2015). They were generally most abundant in the northern basin, probably due to their resistance to turbulent environments, their high affinity for nitrate, and the high freshwater inputs (Garrido et al., 2016).

Picophytoplankton (picocyanobacteria and picoeukaryotes) often represents an important component of phytoplankton communities in brackish lagoons. Particularly, phycocyanin-rich picocyanobacteria have shown blooms after floods when salinities achieved their minimal values (Bec et al., 2011; Lafabrie et al., 2013). Small cells have physiological advantages that confer them a high competitiveness to acquire and use nutrients under limiting conditions (Raven, 1998). They are especially competitive in warmer waters and under low N-availability as observed during the summer period (Bec et al., 2005; Leruste et al., 2016; Domingues et al., 2017). Moreover, they are able to use nitrate, ammonium as well as organic N-sources in a highly efficient way (Domingues et al., 2011), and are hardly inhibited by occasionally occurring high ammonium concentrations (Collos and Harrison, 2014). Bloom of picophytoplankton in summer coincides with high regeneration process, and a dominance of ammonium that picophytoplankton can preferentially use, while diatoms and dinoflagellates preferentially use NO_3^- , and are able to show high growth rate in nitrate-rich waters (Domingues et al., 2011; Glibert et al., 2015; Reed et al., 2016).

These competitive trade-offs may thus explain the competitive exclusion of the larger cells by the PC-cyanos in September. Shift between diatoms and dinoflagellates to picophytoplankton from spring to late summer frequently occur in coastal aquatic systems with the shift of available N forms, from NO_3^- ($> 88\%$ of the DIN in the two stations in November-December 2013 and April 2014) to NH_4^+ ($> 85\%$ of the DIN in the two stations in September 2014) (Glibert et al., 2015; Domingues et al., 2017). However, ambient nutrient concentrations are measures of the residual nutrients after biogeochemical activity, and may not reflect the real available nutrient concentrations for phytoplankton growth. The bioassay experiments gave further insights about the relationship between their functional responses to the nutrient availability. This especially specified which nutrient was potentially limiting for phytoplankton growth at the three sampling periods.

4.2. Phytoplankton behavior under induced nutrient limitation

Temperate coastal ecosystems generally display seasonal changes of nutrient availability and limitation, characterized by shifts from a P-limitation during the wet seasons after high nitrate-rich freshwater inputs, to N-limitation during the dry season (Lomas and Glibert, 2000; Kemp et al., 2005). In contrast, we did not observe this pattern in Biguglia lagoon, despite the high DIN inputs from the watershed during the wet seasons, reflected by high TN:TP and DIN:DIP⁻ values. The results of the bioassay experiments more often show a co-limitation by N and P for the phytoplankton communities. Nevertheless, some size fractions were limited by one of these elements alone, e.g. the nanophytoplankton of the NB station limited by P in April 2014, and the microphytoplankton of the SB station limited by N in December 2013. A review showed that co-limitation by N and P in freshwater and marine ecosystems is more common than previously thought (Elser et al., 2007). Interestingly, the single limitation for some size fractions within a co-limited community, such as observed in SB in September 2014 may indicate niche differentiation. We also observed that the phytoplankton in SB was more often N-limited than in NB despite high DIN:DIP ratios. Conclusions from the dilution experiment should be drawn with caution, because incubations have been conducted on small spatial and temporal scales. The responses of phytoplankton communities at these scales can differ from the overall ecology of the entire ecosystem (Pehler et al., 2004; Reed et al., 2016). However, these results support that ambient nutrient ratios do not necessarily reflect the availability of nutrients for autotrophic communities, particularly because of the known variability of nutrient stoichiometry of phytoplankton. The communities of the two stations showed the strongest simultaneous or serial co-limitation by N and P in summer while in the wet seasons they showed single N- or P-limitations or independent co-limitation depending on fractions. Fig. 5 conceptualizes our observations for the three samplings, and links the environmental specificities, the differences in community structure and composition, with the calculated growth rates and the observed N- and P-limitation.

Picocyanobacteria, dinoflagellates and other phytoplankton groups have demonstrated numerous ways to acclimate to temporally and varying N- and P-limitation (Lin et al., 2015). For example, luxury PO_4^{3-} uptake prior to sampling for the bioassays could have provided the necessary intracellular P for phytoplankton growth during the 24 h incubation. Picocyanobacteria may have used high P-storage abilities that could have allowed them to maintain their bloom despite low P-availability (Sorokin and Dallochio, 2008). Moreover, they have shown high inorganic P-uptake efficiency at low levels of available soluble reactive phosphorus (Collos et al., 2009). Alternatively, the high dissolved and particulate organic N and P stocks in the sediment of Biguglia lagoon may have been used by the phytoplankton to thrive despite low dissolved inorganic N- and P-availability. Indeed, the very low dissolved inorganic phosphorus concentrations at the three samplings may have stimulated the production of alkaline phosphatase, allowing the use of dissolved organic phosphorus (Pehler et al., 2004; Lin et al., 2015). Moreover, high abundances of potentially mixotrophic species among *Prorocentrum*, *Heterocapsa*, *Gonyaulax* and other genera, and of *Mesodinium rubrum* (Collos et al., 2014; Fischer et al., 2016; Johnson, 2015) have been observed during these experiments. Mixotrophy is an advantageous behavioral response under nutrient limiting conditions, allowing cells to reduce direct competition for inorganic nutrients (Jeong et al., 2005; Mitra et al., 2014; Johnson, 2015). Hence, the high abundances of picocyanobacteria, physiologically able to cope with P-limitation, and of potentially mixotrophic dinoflagellates and nanoflagellates, could explain the N and P co-limitation of the communities despite high DIN:DIP ratios (Burson et al., 2016). Indeed, these physiological and behavioral capacities may have offset an inorganic P-limitation, either by the ingestion of starving preys that may benefit from the added inorganic nutrients, or of dissolved organic substances. This may have led to a N and P co-limitation rather than a

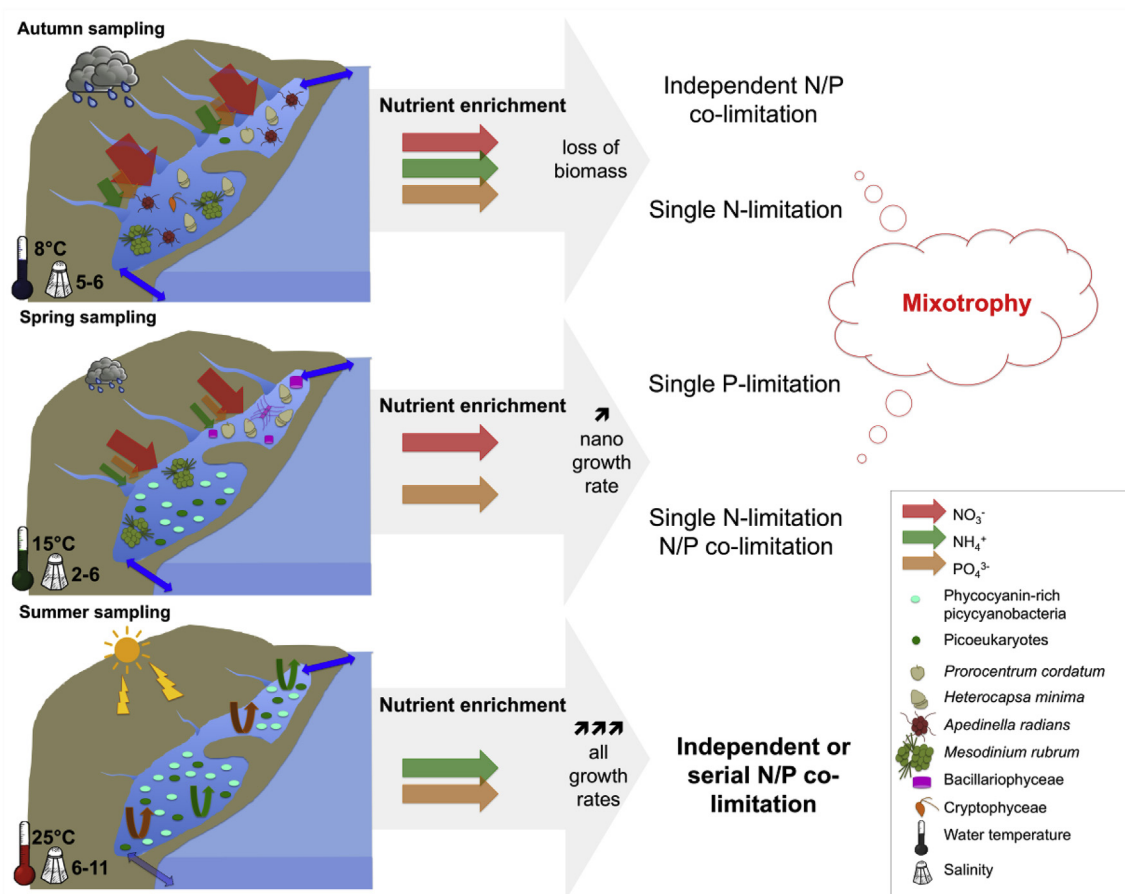


Fig. 5. Schematic representation of the environmental characteristics, phytoplankton community composition and their relationship with nutrient availability during the three bioassays periods in the two stations of Biguglia lagoon. For each sampling, the schematic representation specifies the water temperature, the salinity, the freshwater influence, and the nutrients inputs whether they come from the watershed or from the sediments. Main phytoplankters are specified as pictograms. Phytoplankton co-limitation by N and P is inferred according to ratios between growth rate under experimentally induced N- or P-limitation and maximal growth rate (see Results).

single P-limitation (Burson et al., 2016; Fischer et al., 2016). Mixotrophy is also stimulated by nutrient limitation, and would have been particularly competitive in Biguglia lagoon during the three sampling periods. This may explain the bloom of the mixotrophic *Mesodinium rubrum* (Tong et al., 2015; Seong et al., 2017) in the southern basin in the autumn and spring samplings (Fig. 5).

4.3. Assumptions about responses of phytoplankton communities to a nutrient pulse

Bioassay experiments can provide valuable insights about relationship between nutrient dynamics and phytoplankton physiological responses. This can simulate a nutrient pulse, helping to predict algal-based expression of eutrophication (Xu et al., 2012).

The composition of nutrient enrichments used at the three sampling periods was based on previous studies and assumptions about ecosystem functioning, and may have not always reflected the real environmental conditions or the potential nutrient pulses threatening Biguglia lagoon due to its high variability. These enrichments may thus have not always been the most suitable to involve an optimum response of the phytoplankton communities. Hence, the addition of the full enrichment has not always allowed the estimation of maximal growth and mortality rates probably because the underlying assumptions of this method were not always fulfilled. For almost the half of the fractions (11 out of 24) the relationship between the apparent growth rates and the dilution factor was non-linear. Non-linear trends may indicate microzooplankton feeding threshold or saturation depending on the curve

pattern (Chen et al., 2014). Indeed, the shape of some of these plots (Fig. S1, red lines) may indicate a strong nutrient limitation and suggests the use of other nutrient resources such as those regenerated through cell lyses, grazing and mineralization that increase with the proportion of undiluted sample (Andersen et al., 1991).

The responses of the phytoplankton communities to the nutrient enrichment in the three bioassays have reflected contrasting environmental vulnerability of Biguglia lagoon to short-term nutrient enrichment. Nutrient limitation can have many indirect effects on phytoplankton productivity, e.g. a modification of the internal cell stoichiometry that changes its nutritional quality and modify predation rates (Harpole et al., 2011). In November-December 2013, the addition of the full enrichment comprising a mixture of nitrate and ammonium (10 μM each) seems to have had a negative impact. In certain cases, ammonium can negatively affect nitrate uptake and assimilation, especially in nitrate-dominated systems (Domingues et al., 2011, 2017), and particularly for dinoflagellates, the least ammonium-tolerant group (Collos and Harrison, 2014). Hence, the addition of ammonium when nitrogen was mainly available as nitrate, and dinoflagellates were the most abundant, may have inhibited its uptake and led to a decrease of growth rate (Dodds and Whiles, 2010; Glibert et al., 2015). As nutrient addition is expected to increase limitation by other resources, a negative response may also reflect strongly constrained N:P stoichiometry. This is suggested by high TN:TP and DIN:DIP values. Rather than re-equilibrating these ratios, nutrient addition may have led to an exacerbated internal stoichiometric imbalance of N and P nutrients with negative impact on phytoplankton growth (Harpole et al., 2011).

In general, the phytoplankton maximal growth rates followed a predictable seasonal trend, with minima in autumn and maxima in summer. These changes are correlated with water temperatures and photoperiods length, providing more favorable growth conditions in summer. Lower temperatures and light availability in autumn may have altered the uptake rate of phytoplankton (Dodds and Whiles, 2010; Domingues et al., 2011; Reed et al., 2016). This is also consistent with the community composition, the picophytoplankton dominating the community in September potentially having higher growth rate than the nanophytoplankton dominating the autumn and spring samplings (Bec et al., 2011). Phytoplankton was thus more reactive to a nutrient addition during the summer season when the nutrient demand is the highest. This suggests that Biguglia lagoon is the most vulnerable to nutrient inputs during summer.

5. Conclusion

Experimental enrichments bioassays are particularly appropriate to explore cause-effect relationships between nutrient availability and phytoplankton dynamics, which can reflect the vulnerability of the whole ecosystem. This is an important issue for managers to draw up appropriate nutrient loading budgets, and to help evaluating the efficiency of subsequent nutrient reduction strategies. In our study, N inputs, particularly NO_3^- during the wet periods and NH_4^+ in summer, stimulated phytoplankton growth in Biguglia lagoon emphasizing the need to mitigate these inputs. The phytoplankton community was strongly influenced by freshwater and nutrients inputs that have led to different physiological and behavioral responses over seasons. During autumn and spring, high DIN:DIP ratios have been associated with blooms of potentially harmful dinoflagellates, which are increasingly observed in Biguglia lagoon.

The absence of a significant positive effect of the enrichment could be explained through several hypotheses. The first is the presence of sufficient amounts of dissolved inorganic nutrients in the water column to sustain the phytoplankton growth during the incubation time. The second could be a more intensive use of other resources than the external pool, such as internal or regenerated inorganic nutrients. The third could be a strong competition for available nutrient with the bacterial communities. Of increasing concern is thus the potential for organic nutrients and high abundances of small preys to promote mixotrophic species, some of which are potentially harmful. It would be important to quantify the availability of dissolved organic nutrients and its effect on phytoplankton growth and trophic modes. This should be linked with a study about the interaction between phytoplankton and bacteria, such as competition for organic and inorganic available nutrients. The impact of bacterial activity on the available nutrients stocks has also to be explored. Indeed, bacterial communities can play an essential role in nutrient cycling, e.g. through the remobilization of organic matter, leading to a tight phytoplankton-bacterioplankton coupling (Caroppo, 2002). The possible use of external, internal and regenerated N and P pools, as well as the mixotrophic abilities of the phytoplankton communities also need to be studied.

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Appendix A. Supplementary data

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References

- Aminot, A., Chaussepied, M.C., 1983. *Manuel des analyses chimiques en milieu marin*. CNEXO, Brest.
- Andersen, K.H., Aksnes, D.L., Berge, T., Fiksen, O., Visser, A., 2015. Modelling emergent trophic strategies in plankton. *J. Plankton Res.* 37, 862–868. <https://doi.org/10.1093/plankt/fbv054>.
- Andersen, T., Schartau, A., Paasche, E., 1991. Quantifying External and Internal Nitrogen and Phosphorus Pools, as Well. *Mar. Ecol.: Prog. Ser.* 69, 67–80. <https://doi.org/10.3354/meps069067>.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>.
- Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. *Ecol. Monogr.* 81, 169–193. <https://doi.org/10.1890/10-1510.1>.
- Bartón, K., 2013. MuMIn: Multi-modal inference. Model selection and model averaging based on information criteria (AICc and alike). <http://cran.r-project.org/web/packages/MuMIn/index.html>.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Software* 67. <https://doi.org/10.18637/jss.v067.i01>.
- Bec, B., Collos, Y., Souchu, P., Vaquer, A., Lautier, J., Fiandrino, A., Benau, L., Orsoni, V., Laugier, T., 2011. Distribution of picophytoplankton and nanophytoplankton along an anthropogenic eutrophication gradient in French Mediterranean coastal lagoons. *Aquat. Microb. Ecol.* 63, 29–45. <https://doi.org/10.3354/ame01480>.
- Bec, B., Hussein-Ratrema, J., Collos, Y., Souchu, P., Vaquer, A., 2005. Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: emphasis on the picoeukaryote community. *J. Plankton Res.* 27, 881–894. <https://doi.org/10.1093/plankt/fbi061>.
- Bellingier, E.G., Sigee, D.C. (Eds.), 2015. *Freshwater Algae: Identification, Enumeration and Use as Bioindicators*. John Wiley & Sons, Inc., Hoboken, NJ, USA. <https://doi.org/10.1002/9781118917152>.
- Bérard-Therriault, L., Poulin, M., Bossé, L., 1999. *Guide d'identification du phytoplancton marin de l'estuaire et du golfe du Saint-Laurent incluant également certains protozoaires* Canadian Special Publication of Fisheries and Aquatic Sciences No. 128. NRC Research Press.
- Burnham, K.P., Anderson, D.R. (Eds.), 2004. *Model Selection and Multimodel Inference*. Springer New York, New York, NY.
- Burson, A., Stomp, M., Akil, L., Brussaard, C.P.D., Huisman, J., 2016. Unbalanced reduction of nutrient loads has created an offshore gradient from phosphorus to nitrogen limitation in the North Sea. *Limnol. Oceanogr.* 61, 869–888. <https://doi.org/10.1002/lno.10257>.
- Caroppo, C., 2002. Variability and interactions of phytoplankton and bacterioplankton in Varano lagoon (Adriatic Sea). *J. Plankton Res.* 24, 267–273. <https://doi.org/10.1093/plankt/24.3.267>.
- Cecchi, P., Garrido, M., Collos, Y., Pasqualini, V., 2016. Water flux management and phytoplankton communities in a Mediterranean coastal lagoon. Part II: Mixotrophy of dinoflagellates as an adaptive strategy? *Mar. Pollut. Bull.* <https://doi.org/10.1016/j.marpolbul.2016.04.041>.
- Chen, B., Laws, E.A., Liu, H., Huang, B., 2014. Estimating microzooplankton grazing half-saturation constants from dilution experiments with nonlinear feeding kinetics. pp. 639–644. <https://doi.org/10.4319/lo.2014.59.3.0639>.
- Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. *Mar. Ecol.: Prog. Ser.* 210, 223–253. <https://doi.org/10.3354/meps210223>.
- Coelho, S., Gamito, S., Perez-Ruzafa, A., 2007. Trophic state of Foz de Almagem coastal lagoon (Algarve, South Portugal) based on the water quality and the phytoplankton community. *Estuar. Coast Shelf Sci.* 71, 218–231. <https://doi.org/10.1016/j.ecss.2006.07.017>.
- Coelho, S., Perez-Ruzafa, A., Gamito, S., 2015. Phytoplankton community dynamics in an intermittently open hypereutrophic coastal lagoon in Southern Portugal. *Estuar. Coast Shelf Sci.* 167, 102–112. <https://doi.org/10.1016/j.ecss.2015.07.022>.
- Collos, Y., Vaquer, A., Bibent, B., Souchu, P., Slawky, G., Garcia, N., 2003. Response of coastal phytoplankton to ammonium and nitrate pulses: seasonal variations of nitrogen uptake and regeneration. *Aquat. Ecol.* 37, 227–236. <https://doi.org/10.1023/A:1025881323812>.
- Collos, Y., Hussein-Ratrema, J., Bec, B., Vaquer, A., Hoai, T.L., Rougier, C., Pons, V., Souchu, P., 2005. Pheopigment dynamics, zooplankton grazing rates and the autumnal ammonium peak in a Mediterranean lagoon. *Hydrobiologia* 550, 83–93. <https://doi.org/10.1007/s10750-005-4365-1>.
- Collos, Y., Bec, B., Jauzein, C., Abadie, E., Laugier, T., Lautier, J., Pastoureaud, A., Souchu, P., Vaquer, A., 2009. Oligotrophication and emergence of picocyanobacteria and a toxic dinoflagellate in Thau lagoon, southern France. *J. Sea Res.* 61, 68–75.

- <https://doi.org/10.1016/j.seares.2008.05.008>.
- Collos, Y., Harrison, P.J., 2014. Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Mar. Pollut. Bull.* 80, 8–23. <https://doi.org/10.1016/j.marpolbul.2014.01.006>.
- Collos, Y., Jauzein, C., Ratmaya, W., Souchu, P., Abadie, E., Vaguer, A., 2014. Comparing diatom and *Alexandrium catenella*/tamarensis blooms in Thau lagoon: Importance of dissolved organic nitrogen in seasonally N-limited systems. *Harmful Algae* 37, 84–91. <https://doi.org/10.1016/j.hal.2014.05.008>.
- Dodds, W., Whiles, M., 2010. Chapter 16: Nutrient Use and Remineralization. *Freshwater Ecology - Concepts and Environmental Applications*. pp. 437–467.
- Domingues, R.B., Barbosa, A.B., Sommer, U., Galvao, H.M., 2011. Ammonium, nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: potential effects of cultural eutrophication. *Aquat. Sci.* 73, 331–343. <https://doi.org/10.1007/s00027-011-0180-0>.
- Domingues, R.B., Guerra, C.C., Barbosa, A.B., Galvão, H.M., 2017. Will nutrient and light limitation prevent eutrophication in an anthropogenically-impacted coastal lagoon? *Cont. Shelf Res.* 141, 11–25.
- Donald, D.B., Bogard, M.J., Finlay, K., Bunting, L., Leavitt, P.R., 2013. Phytoplankton-Specific Response to Enrichment of Phosphorus-Rich Surface Waters with Ammonium, Nitrate, and Urea. *PLoS One* 8. <https://doi.org/10.1371/journal.pone.0053277>.
- Donald, D.B., Bogard, M.J., Finlay, K., Leavitt, P.R., 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnol. Oceanogr.* 56, 2161–2175. <https://doi.org/10.4319/lno.2011.56.6.2161>.
- Duarte, C.M., Agust, S., Gasol, J.M., Vaqu, D., VazquezDominguez, E., 2000. Effect of nutrient supply on the biomass structure of planktonic communities: an experimental test on a Mediterranean coastal community. *Mar. Ecol. Prog. Ser.* 206, 87–95. <https://doi.org/10.3354/meps206087>.
- Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>.
- Erostate, M., Huneau, F., Garel, E., Lehmann, M.F., Kuhn, T., Aquilina, L., Vergnaud-Ayraud, V., Labasque, T., Santoni, S., Robert, S., Provitolo, D., Pasqualini, V., 2018. Delayed nitrate dispersion within a coastal aquifer provides constraints on land-use evolution and nitrate contamination in the past. *Sci. Total Environ.* 644, 928–940. <https://doi.org/10.1016/j.scitotenv.2018.06.375>.
- Fischer, R., Giebel, H., Ptacnik, R., 2016. The identity of the limiting nutrient (N vs. P) affects the competitive success of mixotrophs. *Mar. Ecol. Prog. Ser.* <https://doi.org/10.3354/meps11968>.
- Franco, A., Fiorini, R., Zucchetto, M., Torricelli, P., Franzoi, P., 2010. Flounder growth and production as indicators of the nursery value of marsh habitats in a Mediterranean lagoon. *J. Sea Res.* 64, 457–464. <https://doi.org/10.1016/j.seares.2010.01.006>.
- Garrido, M., Cecchi, P., Collos, Y., Agostini, S., Pasqualini, V., 2016. Water flux management and phytoplankton communities in a Mediterranean coastal lagoon. Part I: How to promote dinoflagellate dominance? *Mar. Pollut. Bull.* 104, 139–152. <https://doi.org/10.1016/j.marpolbul.2016.01.049>.
- Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2015. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnol. Oceanogr.* <https://doi.org/10.1002/lno.10203>.
- Guillard, R.R.L., Ryther, J.H., 1962. Studies of Marine Planktonic Diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Grun. *Can. J. Microbiol.* 8, 229–239. <https://doi.org/10.1139/m62-029>.
- Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S., Elser, J.J., Gruner, D.S., Hillebrand, H., Shurin, J.B., Smith, J.E., 2011. Nutrient co-limitation of primary producer communities. *Ecol. Lett.* 14, 852–862. <https://doi.org/10.1111/j.1461-0248.2011.01651.x>.
- Heil, C.A., Glibert, P.M., Fan, C.L., 2005. *Prorocentrum minimum* (Pavillard) Schiller - A review of a harmful algal bloom species of growing worldwide importance. *Harmful Algae* 4, 449–470. <https://doi.org/10.1016/j.hal.2004.08.003>.
- Jeong, H.J., Park, J.Y., Nho, J.H., Park, M.O., Ha, J.H., Seong, K.A., Jeng, C., Seong, C.N., Lee, K.Y., Yih, W.H., 2005. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. *Aquat. Microb. Ecol.* 41, 131–143. <https://doi.org/10.3354/ame041131>.
- Johnson, M.D., 2015. Inducible Mixotrophy in the Dinoflagellate *Prorocentrum minimum*. *J. Eukaryot. Microbiol.* 62, 431–443.
- Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller, W.D., Newell, R.E., Roman, M.R., Smith, E.M., Stevenson, J.C., 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Mar. Ecol. Prog. Ser.* 303, 1–29.
- Kjerfve, B., 1994. *Coastal Lagoon Processes*. Elsevier.
- Lafabrie, C., Garrido, M., Leboulanger, C., Cecchi, P., Grégori, G., Pasqualini, V., Pringault, O., 2013. Impact of contaminated-sediment resuspension on phytoplankton in the Biguglia lagoon (Corsica, Mediterranean Sea). *Estuar. Coast Shelf Sci.* 130, 70–80. Pressures, Stresses and Trends in Estuarine Ecosystems. <https://doi.org/10.1016/j.eccs.2013.06.025>.
- Landry, M., Hassett, R., 1982. Estimating the Grazing Impact of Marine Micro-Zooplankton. *Mar. Biol.* 67, 283–288. <https://doi.org/10.1007/BF00397668>.
- Landry, M.R., Brown, S.L., Campbell, L., Constantinou, J., Liu, H., 1998. Spatial patterns in phytoplankton growth and microzooplankton grazing in the Arabian Sea during monsoon forcing. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* 45, 2353–2368. [https://doi.org/10.1016/S0967-0645\(98\)00074-5](https://doi.org/10.1016/S0967-0645(98)00074-5).
- Le Fur, I., De Wit, R., Plus, M., Oheix, J., Simier, M., Ouisse, V., 2018. Submerged benthic macrophytes in Mediterranean lagoons: distribution patterns in relation to water chemistry and depth. *Hydrobiologia* 175–200. <https://doi.org/10.1007/s10750-017-3421-y>.
- Legrand, C., Graneli, E., Carlsson, P., 1998. Induced phagotrophy in the photosynthetic dinoflagellate *Heterocapsa triquetra*. *Aquat. Microb. Ecol.* 15, 65–75. <https://doi.org/10.3354/ame015065>.
- Leruste, A., Malet, N., Munaron, D., Derolez, V., Hately, E., Collos, Y., De Wit, R., Bec, B., 2016. First steps of ecological restoration in Mediterranean lagoons: Shifts in phytoplankton communities. *Estuar. Coast Shelf Sci.* 180, 190–203. <https://doi.org/10.1016/j.eccs.2016.06.029>.
- Leruste, A., Villéger, S., Malet, N., Wit, R.D., Bec, B., 2018. Complementarity of the multidimensional functional and the taxonomic approaches to study phytoplankton communities in three Mediterranean coastal lagoons of different trophic status. *Hydrobiologia* 815. <https://doi.org/10.1007/s10750-018-3565-4>.
- Lin, S., Litaker, R.W., Sunda, W.G., 2015. Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. *J. Phycol.* 52, 10–36.
- Litchman, E., Klausmeier, C.A., Schofield, O.M., Falkowski, P.G., 2007. The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. *Ecol. Lett.* 10, 1170–1181. <https://doi.org/10.1111/j.1461-0248.2007.01117.x>.
- Livingston, R.J., 2000. *Eutrophication Processes in Coastal Systems: Origin and Succession of Plankton Blooms and Effects on Secondary Production in Gulf Coast Estuaries*. CRC Press.
- Loir, M., 2004. *Guide des diatomées: Plus de 200 micro-algues silicieuses photographiées*. Delachaux & Niestlé, Paris.
- Lomas, M.W., Glibert, P.M., 2000. Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. *J. Phycol.* 36, 903–913. <https://doi.org/10.1046/j.1529-8817.2000.9029.x>.
- Mitra, A., Flynn, K.J., Berge, T., Calbet, A., Raven, J.A., Graneli, E., Glibert, P.M., Hansen, P., Stoecker, D.K., Thingstad, T.F., Tillmann, U., Våge, S., Wilken, S., Zubkov, M.V., 2014. The role of mixotrophic protists in the biological carbon pump. *Biogeosciences* 11, 995–1005.
- Mouillot, D., Titeux, A., Migon, C., Sandroni, V., Frodello, J.-P., Viale, D., 2000. Anthropogenic influences on a mediterranean Nature Reserve: modelling and forecasting. *Environ. Model. Assess.* 5, 185–192. <https://doi.org/10.1023/A:1011533811237>.
- Neveux, J., Lantoine, F., 1993. Spectrofluorometric assay of chlorophylls and phaeopigments using the least squares approximation technique. *Deep Sea Res. Oceanogr. Res. Pap.* 40, 1747–1765. [https://doi.org/10.1016/0967-0637\(93\)90030-7](https://doi.org/10.1016/0967-0637(93)90030-7).
- Nixon, S.W., 2009. Eutrophication and the microscope. *Hydrobiologia* 629, 5–19. <https://doi.org/10.1007/s10750-009-9759-z>.
- Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P., O'Hara, R., Simpson, G., Stevens, M., Wagner, H., 2018. *Vegan: Community Ecology Package*.
- Orsoni, V., Souchu, P., Sauzade, D., 2001. Caractérisation de l'état d'eutrophication des trois principaux étangs corses (Biguglia, Diana et Urbino), et proposition de renforcement de leur surveillance. *Rapport final*.
- Paerl, H.W., Dymbly, J., Moisaner, P.H., Noble, R.T., Piehler, M.F., Pinckney, J.L., Steppe, T.F., Twomey, L., Valdes, L.M., 2003. Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. *FEMS Microbiol. Ecol.* 46, 233–246. [https://doi.org/10.1016/S0168-6496\(03\)00200-9](https://doi.org/10.1016/S0168-6496(03)00200-9).
- Paerl, H.W., Rossignol, K.L., Hall, N.S., Peierls, B.L., Wetz, M.S., 2010. Phytoplankton Community Indicators of Short- and Long-term Ecological Change in the Anthropogenically and Climatically Impacted Neuse River Estuary, North Carolina, USA. *Estuar. Coasts* 33, 485–497. <https://doi.org/10.1007/s12237-009-9137-0>.
- Pasqualini, V., Derolez, V., Garrido, M., Orsoni, V., Baldi, Y., Etourneau, S., Leoni, V., Rébillout, P., Laugier, T., Souchu, P., Malet, N., 2017. Spatiotemporal dynamics of submerged macrophyte status and watershed exploitation in a Mediterranean coastal lagoon: Understanding critical factors in ecosystem degradation and restoration. *Ecol. Eng.* 102, 1–14. <https://doi.org/10.1016/j.ecoleng.2017.01.027>.
- Patiño, J., Guilhaumon, F., Whittaker, R.J., Triantis, K.A., Gradstein, S.R., Hedenäs, L., González-Mancebo, J.M., Vanderpoorten, A., 2013. Accounting for data heterogeneity in patterns of biodiversity: an application of linear mixed effect models to the oceanic island biogeography of spore-producing plants. *Ecography* 36, 904–913. <https://doi.org/10.1111/j.1600-0587.2012.00020.x>.
- Piehl, M.F., Twomey, L.J., Hall, N.S., Paerl, H.W., 2004. Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA. *Estuar. Coast Shelf Sci.* 61, 197–209. <https://doi.org/10.1016/j.eccs.2004.05.001>.
- R Core Team, 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Raven, J.A., 1998. The twelfth Tansley Lecture. Small is beautiful: the picophytoplankton. *Funct. Ecol.* 12, 503–513. <https://doi.org/10.1046/j.1365-2435.1998.00233.x>.
- Reed, M., Pinckney, J.L., Keppler, C., Brock, L., Hogan, S., Greenfield, D., 2016. The influence of nitrogen and phosphorus on phytoplankton growth and assemblage composition in four coastal, southeastern USA systems. *Estuar. Coast Shelf Sci.* 177, 71–82.
- Rochette, S., Rivot, E., Morin, J., Mackinson, S., Riou, P., Le Pape, O., 2010. Effect of nursery habitat degradation on flatfish population: Application to Solea solea in the Eastern Channel (Western Europe). *J. Sea Res.* 64, 34–44. <https://doi.org/10.1016/j.seares.2009.08.003>.
- Schramm, W., 1999. Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. *J. Appl. Phycol.* 11, 69–78. <https://doi.org/10.1023/A:1008076026792>.

- Seong, K.A., Myung, G., Jeong, H.J., Yih, W., Kim, H.S., Park, J.Y., Yoo, Y.D., 2017. Ingestion rate and grazing impact by the mixotrophic ciliate *Mesodinium rubrum* on natural populations of marine heterotrophic bacteria in the coastal waters of Korea. *ALGAE* 32, 47–55.
- Sorokin, Y., Dallochio, F., 2008. Dynamics of phosphorus in the Venice lagoon during a picocyanobacteria bloom. *J. Plankton Res.* 30, 1019–1026.
- Souchu, P., Bec, B., Smith, V.H., Laugier, T., Fiandrino, A., Benau, L., Orsoni, V., Collos, Y., Vaquer, A., 2010. Patterns in nutrient limitation and chlorophyll a along an anthropogenic eutrophication gradient in French Mediterranean coastal lagoons. *Can. J. Fish. Aquat. Sci.* 67, 743–753. <https://doi.org/10.1139/F10-018>.
- Tomas, C.R., 1997. *Identifying Marine Phytoplankton*. Academic Press.
- Tong, M., Smith, J., Anderson, D.M., 2015. Role of dissolved nitrate and phosphate in isolates of *Mesodinium rubrum* and toxin-producing *Dinophysis acuminata*. *Aquat. Microb. Ecol.* 75, 169–185.
- Xu, J., Yin, K., Lee, J.H.W., Anderson, D.M., Jiang, Y., Yuan, X., Ho, A.Y.T., Harrison, P.J., 2012. Resistance of Hong Kong waters to nutrient enrichment: assessment of the role of physical processes in reducing eutrophication. *J. Oceanogr.* 68, 545–560. <https://doi.org/10.1007/s10872-012-0118-8>.