

Picophytoplankton intaglios in temperate waters of the southern Bay of Biscay

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ABSTRACT: The influence of irradiance, temperature, and nitrate (NO₃) concentration over the biomass, growth rate, and primary production rate of picophytoplankton was assessed in an inshore to offshore gradient in the temperate waters of the southern Bay of Biscay (NE Atlantic). Moreover, we analyzed the effect of these variables on the seasonal succession of a broad picophytoplankton community structure (i.e. *Synechococcus*, *Prochlorococcus*, small and large picoeukaryotes). Picophytoplankton showed higher biomasses, primary production rates, and growth rates inshore, which were associated with higher irradiances and NO₃ concentrations in these mixed layers as compared to offshore locations. Seasonally, picophytoplankton presented high biomasses in summer, growth rates in spring, and primary productions in autumn. Most picophytoplankton biomass was represented by picoeukaryotes inshore (70.8%), while cyanobacteria were more important offshore (42.3%). Seasonally, picoeukaryotes were the higher contributors to picophytoplankton biomass in spring and winter (nearly 75%). Moreover, small picoeukaryotes represented nearly 50% of the picophytoplankton biomass in winter, and the greater presence of large picoeukaryotes was found in spring (nearly 35%). Conversely, cyanobacteria peaked in summer (55.4%) with *Synechococcus* amounting to 52.4% of picophytoplankton biomass. Maximum contribution of *Prochlorococcus* was found in autumn (10.4%). A multiple regression model was used to analyze the association between the biomass of different groups of picophytoplankton in the mixed layer and its irradiance and temperature, and the interaction between irradiance and temperature. This shed light on the ecological conditions affecting the community structure of a planktonic size class traditionally regarded as homogeneous. Finally, an intaglio representation showed the seasonal succession of picophytoplankton.

KEY WORDS: Irradiance · Temperature · Picophytoplankton · Community structure · Intaglio

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INTRODUCTION

Phytoplankton contribute half of the global primary production (Field et al. 1998); in other planktonic functional groups, the smallest size class of phytoplankton (~3 µm) is also the most abundant in marine ecosystems, indeed comprising the most abundant autotrophic organisms on Earth (Waterbury et al. 1979, Chisholm et al. 1988, Partensky et al. 1999). Picophytoplankton are clearly the dominant autotrophs under oligotrophic conditions, in

which they can reach more than 50% of chl *a* (Agawin et al. 2000, Morán et al. 2010) and can be responsible for most primary production (i.e. Fernández et al. 2003, Uitz et al. 2010). The success of picophytoplankton under oligotrophic conditions is related to their small size, which provides a high surface to volume ratio and gives advantages in terms of resource acquisition and growth rate (Raven 1998). However, picophytoplankton may also thrive well in coastal ecosystems, where they can contribute up to 40% of total chl *a* and 50% of

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net primary production (Calvo-Díaz et al. 2004, Cermeno et al. 2006, Morán 2007).

Coastal marine ecosystems are collectively responsible for more than 20% of primary production in the ocean. These regions are highly variable in physico-chemical conditions (e.g. irradiance, temperature, and inorganic nutrient concentrations/fluxes), since they are usually influenced by complex physical processes such as mixing, tides, sedimentation, and river runoff (Wollast 1998). This variability in physico-chemical drivers has a strong influence on the structure and function of picophytoplankton in coastal ecosystems (Tamigneaux et al. 1995, Calvo-Díaz & Morán 2006, Cabello et al. 2016). In a model approach, it has been shown that the start of the stratification period could lead to a succession of phytoplankton groups and that this succession is influenced by changes in physico-chemical variables (Ruudij et al. 1997). Coastal marine ecosystems are prone to relevant successions of phytoplankton species because the physico-chemical constraints in these areas are highly variable, which makes them an interesting location to study the effect of these constraints on the seasonal succession of picophytoplankton.

The continental shelf in the southern Bay of Biscay (NE Atlantic) is a good example of a temperate coastal ecosystem, experiencing complex hydrodynamic processes including upwelling, continental water discharges, superficial currents, and intrusions of high salinity (Botas et al. 1989, Bode et al. 2002, García-Soto et al. 2002, González-Quirós et al. 2004). In spite of the sporadic upwellings that occur mostly in summer (Llope et al. 2007), the main input of nutrients in the southern Bay of Biscay takes place during winter mixing (Fernández & Bode 1991). As elsewhere, in these waters the main drivers of picophytoplankton growth rates are irradiance, temperature, and inorganic nutrient concentration (Morán 2007, Vázquez-Domínguez et al. 2013). Moreover, these changing physico-chemical conditions in the region's mixed layers have been related to seasonal changes in the biomass, growth rate, primary production (Vázquez-Domínguez et al. 2013), and community structure (Calvo-Díaz et al. 2004, Calvo-Díaz & Morán 2006) of picophytoplankton. All in all, these studies showed high growth rates in winter and inshore, with cyanobacteria peaking under oligotrophic conditions and picoeukaryotes being more abundant under eutrophic conditions. They paid less attention, however, to understanding which of these physico-chemical factors (i.e. irradiance, temperature, and inorganic nutrient concentration) were more important during the seasonal succession.

Studying aquatic ecosystems in the late 1970s, Margalef (1978) proposed an elegant solution to decipher both the seasonal succession of plankton communities and the so-called paradox of the plankton. This idea introduced by Hutchinson in the 1960s investigated 'how it is possible for a number of species to coexist in a relatively isotropic or unstructured environment all competing for the same sorts of materials' (Hutchinson 1961, p. 137). In his seminal work, Margalef proposed that the variability in physico-chemical conditions in the environment may lead to the coexistence and succession of a myriad of phytoplankton species. He thus established his conceptual mandala, where turbulence and nutrient availability were the main drivers of phytoplankton species competition, cohabitation, and succession (Margalef 1978, Margalef et al. 1979). Subsequently, the study of plankton seasonal succession evolved towards more mechanistic models. For instance, Reynolds' C-S-R intaglio (Smayda & Reynolds 2001, Reynolds 2003) considers a continuum of nutrient availability and irradiance intensity to disentangle the spatio-temporal succession of phytoplankton communities. Reynolds' C-S-R intaglio distributes phytoplankton communities depending on habitat and resource availability, functional and morphological traits, physiological attributes, and life history strategies. One example of this C-S-R intaglio is the distribution of harmful algal blooms in 3 functional communities (Smayda & Reynolds 2001): (1) small to intermediate-sized, colonizing, fast growing, r-selected competitor species (C), which bloom in chemically disturbed waters; (2) acquisitive, larger-celled, slow-growing, K-selected, nutrient stress-tolerant species (S), which thrive in oligotrophic waters; and (3) disturbance-tolerant ruderal species (R), which develop in physically disturbed water masses. 'Within each of these primary adaptive strategies, both r- and K-selected species occur' (Smayda & Reynolds 2001, p. 450).

Despite our increasing knowledge about the seasonality of picophytoplankton in marine ecosystems, there are very few studies placing picophytoplankton inside mandalas or intaglios (cf. Follows et al. 2007, Chisholm 2014, Mouriño-Carballido et al. 2016). Moreover, the latter studies focused their attention on cyanobacteria, mainly *Prochlorococcus* spp. (Follows et al. 2007, Chisholm 2014), or were restricted to 1 yr of sampling (Mouriño-Carballido et al. 2016). Our main objective here was to study the influence of irradiance and temperature, which was highly correlated with nitrate (NO₃) concentration (as a proxy of nutrient fluxes), on the seasonal succession of the biomass, growth rate, primary production, and com-

munity structure of picophytoplankton. This analysis was performed along an inshore–offshore gradient in the southern Bay of Biscay by taking into consideration a 7 yr time series. We established multiple regression models relating irradiance and temperature in the mixed layer with picophytoplankton biomass, primary production, growth rate, and community composition. Finally, we proposed the use of intaglios, representations of habitats against the axes of relative stress and disruption of growth opportunities (Reynolds 2003), summarizing the effect of major environmental drivers underlying seasonal variability on the structure and function of the smallest yet important size class of phytoplankton in the mixed layer of this temperate ecosystem.

MATERIALS AND METHODS

Physico-chemical variables

Sampling was carried out in the southern Bay of Biscay near the city of Xixón (Asturies, Spain) from April 2003 through September 2010 onboard the RV 'José de Rioja'. Seawater samples were collected between the surface and 150 m depth (at 5 or 10 m depth intervals up to 50 m and at 75, 100, and 150 m) at 3 stations (Fig. 1): an inshore station (43.58°N, 5.61°W) with a maximum depth of 20 m, a middle shelf station (43.67°N, 5.58°W) with a maximum depth of 100 m, and an offshore station (43.78°N, 5.55°W) with a maximum depth of 150 m. For this study, we exclusively considered samples that were collected simultaneously at the 3 stations during the same day of sampling.

Photosynthetically active radiation (PAR) at the surface was assumed to be equal to the PAR obtained from MODIS ($E \text{ m}^{-2} \text{ d}^{-1}$) for the day of sampling (180

$\times 180 \text{ km}^2$ resolution). This PAR was transformed into moles of photons per square meter per hour with a factor of $1/\text{DL}$, where DL is the day length in hours. In the water column, a vertical light attenuation coefficient (K_d, m^{-1}) was calculated by measuring PAR values at 1 m depth intervals with a spherical quantum sensor (Biospherical QSP-2200). Then, irradiance reaching each depth (E , mol photons $\text{m}^{-2} \text{ h}^{-1}$) was estimated as the product between PAR (mol photons $\text{m}^{-2} \text{ h}^{-1}$) and the exponential decaying Beer-Lambert law, $E = \text{PAR} e^{-K_d \times z}$, where $K_d (\text{m}^{-1})$ is the attenuation coefficient and $z (\text{m})$ is the depth of sampling. Moreover, a median irradiance was estimated for the mixed layer (E_g , mol photons $\text{m}^{-2} \text{ h}^{-1}$).

Temperature (T , °C) and salinity were profiled at each station with a CTD probe (SeaBird 25). For each date of sampling, the mixed layer was established with the criterion of a change in density of 0.05 kg m^{-3} within 5 m that has been previously used in this region (Morán 2007) and is within the usual range found in the literature (de Boyer Montégut et al. 2004). At different depths and with Niskin bottles (5 l), samples for nutrient analysis were collected and frozen (-20°C) until nutrient analysis. NO_3 concentration ($\mu\text{mol l}^{-1}$) was determined within 6 mo of sampling with an autoanalyzer following standard methods (Grashoff et al. 1999). Temperatures and NO_3 concentrations were averaged for each mixed layer.

Picophytoplankton variables

For each sampling depth, picophytoplankton chl a concentration (chl $a_{0.2}$) was obtained after sequential filtration of 100 ml of sample through polycarbonate filters of 2 and $0.2 \mu\text{m}$ pore size (47 mm, Millipore). Although the $2 \mu\text{m}$ filters retained a small fraction ($<10\%$) of *Prochlorococcus*, *Synechococcus*, and small and large picoeukaryotes, the effect of such retention was of minor importance in the concentration of chl $a_{0.2}$ (Calvo-Díaz & Morán 2006). Filters containing the chl $a_{0.2}$ were frozen (-20°C) until posterior analysis, which was performed within 1 wk after sampling. The chl $a_{0.2}$ was extracted in 90% acetone for 24 h in the dark at 4°C and measured with a spectrofluorometer calibrated with pure chl a (Neveux & Panouse 1987).

Flow cytometry was used to differentiate picophytoplankton groups (Gasol & Del Giorgio 2000). Picophytoplankton samples (1.8 ml) were preserved with 1% paraformaldehyde plus 0.05% glutaraldehyde (final concentration) and frozen at -80°C until they were analyzed in the laboratory. Analysis was car-



Fig. 1. Stations located in the southern Bay of Biscay: inshore (43.58°N, 5.61°W), middle shelf (43.67°N, 5.58°W), and offshore (43.78°N, 5.55°W). Caption from Googlemaps

ried out with a flow cytometer (FACSCalibur, Becton Dickinson) equipped with an argon laser emitting at 488 nm. Based on fluorescence and light scatter, autotrophic cells were divided into *Synechococcus*, *Prochlorococcus*, and small or large picoeukaryotes (Calvo-Díaz & Morán 2006). Flow rate of the flow cytometer was volumetrically calibrated daily. In addition, picoplankton cellular biovolume was estimated with an empirical calibration between side scatter and cell diameter (Calvo-Díaz & Morán 2006), and picoplankton cellular biomass was calculated by using $230 \text{ fg C } \mu\text{m}^{-3}$ for *Synechococcus*, $240 \text{ fg C } \mu\text{m}^{-3}$ for *Prochlorococcus*, and $237 \text{ fg C } \mu\text{m}^{-3}$ for picoeukaryotes (Worden & Nolan 2004). The biomass (mg C m^{-3}) of each group of picophytoplankton was determined, and picophytoplankton biomass (P_{BM} , mg C m^{-3}) was estimated by adding the biomass of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and picoeukaryotes (small and large). Thereafter, we determined the percentage of cyanobacteria biomass (Cyano, %) with respect to P_{BM} as well as the percentages of picoeukaryote biomass (Picoeuk, %), *Synechococcus* biomass (Syne, %), *Prochlorococcus* biomass (Proch, %), small picoeukaryotic biomass (Small, %), and large picoeukaryotic biomass (Large, %). All picophytoplankton variables were averaged for the mixed layer depth.

Picophytoplankton growth rates (μ , d^{-1}) in the mixed layer were estimated according to the following model (Behrenfeld et al. 2005):

$$\mu = \mu_{\text{max}} (\theta \theta_{\text{N}, T-\text{max}}) (1 - e^{-3E_g})$$

where μ_{max} was assumed to be 2 d^{-1} , θ was the chl *a* to carbon ratio measured in the mixed layer ($\text{chl } a_{0.2} / P_{\text{BM}}$), and $\theta_{\text{N}, T-\text{max}}$ was defined as the 85 % envelope of the θ ratio in the region: $\theta_{\text{N}, T-\text{max}} = 0.049 + 0.021 e^{-3E_g}$ (Vázquez-Domínguez et al. 2013). This model has been validated in the southern Bay of Biscay with *in situ* ^{14}C estimations of picophytoplankton growth rates.

Volumetric picophytoplankton primary production rates (P_p , $\text{mg C m}^{-3} \text{ d}^{-1}$) were estimated as:

$$P_p = \mu \times P_{\text{BM}}$$

Multiple regression model

During this study, several physico-chemical variables were measured: mixed layer depth, irradiance, temperature, and NO_3 concentration. We did not measure turbulence, inorganic nutrient flow, or consumption rate. Irradiance (E_g , $\text{mol photons m}^{-2} \text{ h}^{-1}$), temperature (T , $^{\circ}\text{C}$), and the factor station were

included in the multiple regression models as independent variables. Irradiance, temperature, and NO_3 concentration may influence the community composition of picophytoplankton (i.e. Morán et al. 2010, Flombaum et al. 2013, Cabello et al. 2016); however, a significant relationship was found between temperature and NO_3 concentration (Table A1 in the Appendix), therefore we decided not to include the latter variable in the model. Each picophytoplankton dependent variable was thus related to the model $Y \sim E_g \times T \times \text{Station}$. Adjusted determination coefficients were estimated for each model. All statistical analyses were performed with R (R Development Core Team 2011).

Seasonality and intaglio representations of picophytoplankton

To determine the seasonality of picophytoplankton in the mixed layer of the southern Bay of Biscay, all picophytoplankton variables were summarized by considering 4 different seasons: winter from December 21 to March 20, spring from March 21 to June 20, summer from June 21 to September 20, and autumn from September 21 to December 20. Picophytoplankton intaglios were drawn by plotting the value of each functional and structural variable (i.e. yellow to red scale) against the corresponding irradiance and temperature values in the mixed layer. Originally, Reynolds' intaglio representations were described with mixing and nutrient availability as the main descriptors (Reynolds 2006). However, here we relied on irradiance in the mixed layer as a proxy of turbulence and temperature in the mixed layer as a variable related to both nutrient concentration (e.g. this study) and community structure of picophytoplankton (Morán et al. 2010).

Four domains of succession were defined in these intaglios (Wyatt 2014): domain I, low irradiance and low temperature, related to high turbulence and high nutrients (i.e. mainly winter season); domain II, high irradiance and low temperature, related to low turbulence and high nutrients (i.e. mainly spring season); domain III, high irradiance and high temperature, related to low turbulence and low nutrients (i.e. mainly summer season); and domain IV, low irradiance and high temperature, related to high turbulence and low nutrients (i.e. mainly autumn season). These 4 domains were divided by (1) the average of the mean of the minima temperatures found at the 3 stations in autumn and the mean of the maxima temperatures found at the 3 stations in winter, and the

mean of the maxima temperatures found at the 3 stations in spring and the mean of the minima temperatures found at the 3 stations in summer; and (2) the average of the mean of the maxima irradiances found at the 3 stations in winter and the mean of the minima irradiances found at the 3 stations in spring, and the mean of the minima irradiances found at the 3 stations in summer and the mean of the maxima irradiances found at the 3 stations in autumn. This is an approximation of temperatures, distinguishing autumn from winter and spring from summer, and irradiances, distinguishing winter from spring and summer from autumn.

RESULTS

Physico-chemical conditions

Mixed layer depths (Table 1, Fig. 2A) varied inshore between 8.2 m in summer and 16.0 m in autumn; in the middle shelf, they varied from 16.0 m in

summer to 58.7 m in winter, and offshore they ranged between 16.4 m in summer and 131.2 m in winter. Average mixed layer depth was 5 times shallower inshore than offshore, 11.8 and 57.1 m, respectively.

Median irradiances in the mixed layer ranged inshore between 0.8 mol photons $\text{m}^{-2} \text{h}^{-1}$ in autumn and 2.1 mol photons $\text{m}^{-2} \text{h}^{-1}$ in spring or summer (Table 1, Fig. 2B); in the middle shelf, they were between 0.6 mol photons $\text{m}^{-2} \text{h}^{-1}$ in autumn and 1.8 mol photons $\text{m}^{-2} \text{h}^{-1}$ in summer, and offshore they varied from 0.3 mol photons $\text{m}^{-2} \text{h}^{-1}$ in winter to 1.9 mol photons $\text{m}^{-2} \text{h}^{-1}$ in summer. On average, median irradiance inshore reached a 30 % higher level, 1.6 mol photons $\text{m}^{-2} \text{h}^{-1}$, than offshore, 1.1 mol photons $\text{m}^{-2} \text{h}^{-1}$, which was due to shallower mixed layer depths.

Temperatures in the mixed layer varied inshore between 12.6°C in winter and 19.2°C in summer, and in the middle shelf and offshore, they ranged from 12.8 to nearly 20.0°C for the same seasons (Table 1, Fig. 2C). Temperature in the mixed layer was on average 0.6°C lower inshore as compared to offshore, but this difference was not significant.

Table 1. Seasonal (winter, W; spring, Sp; summer, S; autumn, A) and annual averages (\pm SE) of physico-chemical variables measured in inshore, middle shelf, and offshore mixed layers of the southern Bay of Biscay (see Fig. 1 for coordinates). Number of profiles (n), mixed layer depth (MLD, m), median irradiance (E_g , mol photons $\text{m}^{-2} \text{h}^{-1}$), temperature (T , °C), nitrate concentration (NO_3 , $\mu\text{mol l}^{-1}$) and picophytoplankton chl *a* (chl $a_{0.2}$, mg chl *a* m^{-3}). Different superscript letters indicate significant pairwise differences among stations; significant differences among seasons are indicated by inequalities (ANOVA and Tukey-Kramer honestly significant difference; $p < 0.05$)

Season	n	MLD	E_g	T	NO_3	Chl $a_{0.2}$
Inshore						
W	13	13.7 \pm 1.9	1.2 \pm 0.2	12.6 \pm 0.3	4.7 \pm 0.6	0.26 \pm 0.03
Sp	12	8.8 \pm 2.0	2.1 \pm 0.2	14.5 \pm 0.5	1.9 \pm 0.5	0.20 \pm 0.04
S	16	8.2 \pm 1.5	2.1 \pm 0.2	19.2 \pm 0.4	1.1 \pm 0.2	0.24 \pm 0.03
A	16	16.0 \pm 1.2	0.8 \pm 0.1	15.3 \pm 0.5	3.3 \pm 0.7	0.46 \pm 0.07
Annual	57	11.8 \pm 0.9 ^a	1.6 \pm 0.1 ^a	15.6 \pm 0.4 ^a	2.7 \pm 0.3 ^a	0.29 \pm 0.03 ^a
		A > Sp,S	Sp,S > W; Sp,S > A	Sp,S,A > W; S > A,Sp	W > Sp,S; A > S	A > W,Sp,S
Middle shelf						
W	13	58.7 \pm 12.9	1.0 \pm 0.3	12.8 \pm 0.2	4.2 \pm 0.5	0.20 \pm 0.02
Sp	12	19.7 \pm 4.2	1.7 \pm 0.3	14.6 \pm 0.5	0.7 \pm 0.1	0.26 \pm 0.13
S	16	15.2 \pm 2.1	1.8 \pm 0.2	20.2 \pm 0.4	0.6 \pm 0.1	0.14 \pm 0.03
A	16	39.6 \pm 7.1	0.6 \pm 0.1	15.8 \pm 0.5	1.8 \pm 0.4	0.36 \pm 0.08
Annual	57	32.9 \pm 4.3 ^b	1.3 \pm 0.1 ^{a,b}	16.1 \pm 0.4 ^a	1.8 \pm 0.2 ^b	0.24 \pm 0.04 ^{a,b}
		W > Sp,S	S > W; Sp,S > A	Sp,S,A > W; S > A,Sp	W > S,A,Sp; A > S	A > S,Sp,W
Offshore						
W	13	131.2 \pm 13.2	0.3 \pm 0.1	12.8 \pm 0.2	3.9 \pm 0.4	0.19 \pm 0.02
Sp	12	30.2 \pm 11.4	1.5 \pm 0.3	14.7 \pm 0.5	0.9 \pm 0.2	0.13 \pm 0.03
S	16	16.4 \pm 2.6	1.9 \pm 0.2	20.4 \pm 0.39	0.8 \pm 0.1	0.09 \pm 0.01
A	16	57.9 \pm 8.9	0.5 \pm 0.1	16.1 \pm 0.5	1.9 \pm 0.5	0.28 \pm 0.03
Annual	57	57.1 \pm 7.3 ^c	1.1 \pm 0.1 ^b	16.3 \pm 0.4 ^a	1.8 \pm 0.2 ^b	0.18 \pm 0.02 ^b
		W > Sp,S,A; A > S	Sp,S > W; Sp,S > A	Sp,S,A > W; S > A,Sp	W > Sp,S,A	A > Sp,S; W > S
Average						
W	39	67.9 \pm 4.7	1.2 \pm 0.2	12.7 \pm 0.1	4.3 \pm 0.1	0.22 \pm 0.01
Sp	36	19.5 \pm 2.0	2.1 \pm 0.2	14.6 \pm 0.1	1.1 \pm 0.5	0.19 \pm 0.02
S	48	13.2 \pm 0.7	2.1 \pm 0.2	19.9 \pm 0.1	0.8 \pm 0.2	0.15 \pm 0.01
A	48	37.8 \pm 2.4	0.8 \pm 0.1	15.7 \pm 0.1	2.3 \pm 0.2	0.36 \pm 0.02
		W > A,Sp,S; A > S	Sp,S > W; Sp,S > A	Sp,S,A > W; S > A,Sp,W; A > Sp	W > Sp,S,A; A > S,Sp	A > W,Sp,S; A > W

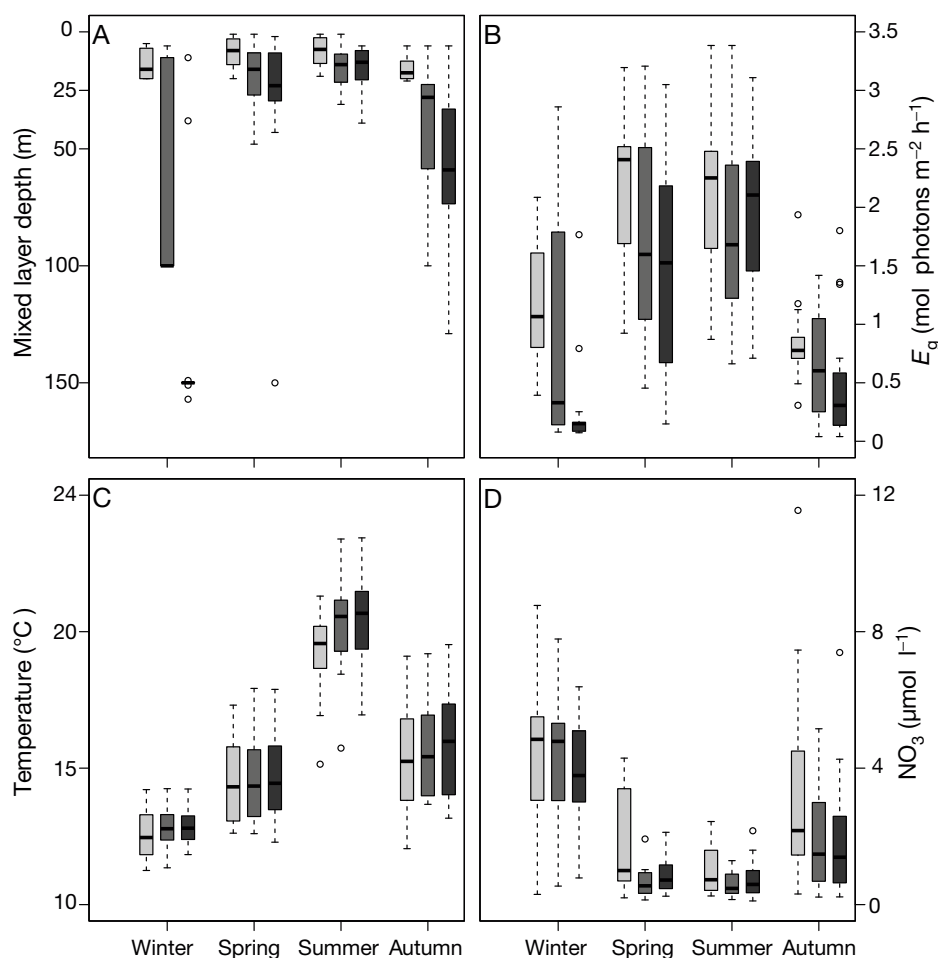


Fig. 2. Seasonality of physico-chemical variables measured in the southern Bay of Biscay: (A) mixed layer depth (m), (B) irradiance (E_g , mol photons $m^{-2} h^{-1}$), (C) temperature ($^{\circ}C$), and (D) nitrate concentration (NO_3 , $\mu mol l^{-1}$). Inshore (pale gray), middle shelf (gray), and offshore (dark gray). Line: median; box: 25th–75th percentiles; whiskers: 10th–90th percentiles; dots: outliers

The concentration of NO_3 in the mixed layer varied by a factor of 5 between summer and winter (Table 1, Fig. 2D). Inshore concentration ranged between 1.1 and $4.7 \mu mol l^{-1}$, middle shelf concentration ranged from 0.6 to $4.2 \mu mol l^{-1}$, and offshore concentration ranged between 0.8 and $3.9 \mu mol l^{-1}$. The concentration of NO_3 was on average 50% higher inshore as compared to the middle shelf and offshore. In addition, negative relationships were found between NO_3 concentration and temperature, and though they showed similar slopes at the 3 stations, they had higher intercepts as we moved inshore (Table A1).

In addition, picophytoplankton chl *a* (chl $a_{0.2}$) in the mixed layer increased seasonally (Table 1) more than 2-fold inshore, from $0.2 mg m^{-3}$ in spring to $0.5 mg m^{-3}$ in autumn, and nearly tripled in the middle shelf and offshore, from 0.14 and $0.09 mg m^{-3}$ in summer to 0.36 and $0.28 mg m^{-3}$ in autumn, respectively. Finally, chl $a_{0.2}$ values in the mixed layer were 50% higher inshore than those found in the middle shelf and offshore.

Spatio-temporal variability of picophytoplankton

There was a generalized decrease in most picophytoplankton variables assessed along the inshore–offshore gradient (Table 2, Fig. 3): biomasses dropped from 11.0 to $6.5 mg C m^{-3}$, primary production rates declined from 12.3 to $5.3 mg C m^{-3} d^{-1}$, and growth rates decreased from 1.2 to $0.8 d^{-1}$. Seasonally, the higher biomasses of picophytoplankton were reached during autumn, $10.7 mg C m^{-3}$, while the lower biomasses were found during winter, $5.4 mg C m^{-3}$. Moreover, picophytoplankton primary productions were higher in autumn, $11.3 mg C m^{-3} d^{-1}$, as compared to winter, $6.1 mg C m^{-3} d^{-1}$. However, growth rates peaked in winter, $1.3 d^{-1}$, and they dropped to nearly half this value in summer, $0.7 d^{-1}$. On average ($\pm SE$), picophytoplankton biomass was $8.4 \pm 0.5 mg C m^{-3}$, primary production was $8.5 \pm 0.7 mg C m^{-3} d^{-1}$, and growth rate was $1.0 \pm 0.1 d^{-1}$.

Usually, there was an inshore–offshore gradient in the biomass contributed by each picophytoplankton

Table 2. Seasonal (winter, W; spring, Sp; summer, S; autumn, A) and annual averages (\pm SE) of variables measured in inshore, middle shelf, and offshore mixed layers of the southern Bay of Biscay (see Fig. 1 for coordinates). Picophytoplankton biomass (P_{BM} , mg C m^{-3}), primary production of picophytoplankton (Pp, $\text{mg C m}^{-3} \text{d}^{-1}$), and growth rate of picophytoplankton (μ , d^{-1}). Different superscript letters indicate significant pairwise differences among stations; significant differences among seasons are indicated by inequalities (ANOVA and Tukey-Kramer honestly significant difference; $p < 0.05$)

Season	n	P_{BM}	Pp	μ
Inshore				
W	13	6.3 ± 0.8	9.7 ± 1.3	1.5 ± 0.1
Sp	12	8.4 ± 2.4	11.5 ± 3.6	1.4 ± 0.1
S	16	13.5 ± 1.7	10.2 ± 1.8	0.7 ± 0.1
A	16	14.4 ± 3.3	17.2 ± 3.8	1.2 ± 0.1
Annual	57	11.0 ± 1.2^a	12.3 ± 1.5^a	1.2 ± 0.1^a
W, Sp, A > S; W > A				
Middle shelf				
W	13	4.6 ± 0.5	5.5 ± 1.1	1.1 ± 0.2
Sp	12	7.6 ± 2.6	10.3 ± 3.6	1.3 ± 0.1
S	16	8.7 ± 1.4	6.2 ± 1.8	0.7 ± 0.1
A	16	9.6 ± 1.7	9.7 ± 2.2	1.0 ± 0.1
Annual	57	$7.8 \pm 0.8^{a,b}$	7.9 ± 1.1^b	1.0 ± 0.1^b
W, Sp > S				
Offshore				
W	13	5.1 ± 0.8	3.3 ± 0.7	0.7 ± 0.1
Sp	12	6.3 ± 1.1	7.2 ± 1.3	1.2 ± 0.1
S	16	6.1 ± 0.5	3.7 ± 0.5	0.6 ± 0.1
A	16	8.0 ± 1.0	7.0 ± 1.1	0.8 ± 0.1
Annual	57	6.5 ± 0.4^b	5.3 ± 0.5^c	0.8 ± 0.1^c
Sp > W; Sp > W, S, A				
Average				
W	39	5.4 ± 0.2	6.1 ± 0.3	1.1 ± 0.1
Sp	36	7.4 ± 0.5	9.6 ± 0.8	1.3 ± 0.1
S	48	9.4 ± 0.4	6.7 ± 0.5	0.7 ± 0.1
A	48	10.7 ± 0.7	11.3 ± 0.8	1.0 ± 0.1
A > W; S > W; A > S, W; A, Sp, W > S; Sp > A				

group (Table 3, Fig. 4). Cyanobacteria presented a slight decrease in biomass, going from 3.9 mg C m^{-3} inshore to 2.9 mg C m^{-3} offshore, and the same happened with *Synechococcus*, going from 3.6 mg C m^{-3} inshore to 2.5 mg C m^{-3} offshore. Conversely, *Prochlorococcus* increased from 0.3 mg C m^{-3} inshore to 0.4 mg C m^{-3} offshore. Seasonally, maximum cyanobacterial and *Synechococcus* biomasses were reached in summer, and minima were observed in winter or spring. However, *Prochlorococcus* were undetectable in springtime, and maxima were attained in autumn. On average, biomass of cyanobacteria was $3.2 \pm 0.3 \text{ mg C m}^{-3}$, with *Synechococcus* being $2.9 \pm 0.2 \text{ mg C m}^{-3}$ and *Prochlorococcus* $0.3 \pm 0.1 \text{ mg C m}^{-3}$. Conversely, the biomass of picoeukaryotes declined as we moved offshore, going from 7.2 mg C m^{-3} inshore to 3.6 mg C m^{-3} offshore. The same happened with small picoeukaryotes, which declined from 5.0 mg C m^{-3} inshore to 2.3 mg C m^{-3} offshore, and large picoeukaryotes, which declined from 2.2 mg C m^{-3} inshore to 1.3 mg C m^{-3} offshore. Seasonally, picoeukaryotes and small picoeukaryotes presented the highest biomasses in autumn, ca. 6.4 and 4.6 mg C m^{-3} , respectively, while the highest bio-

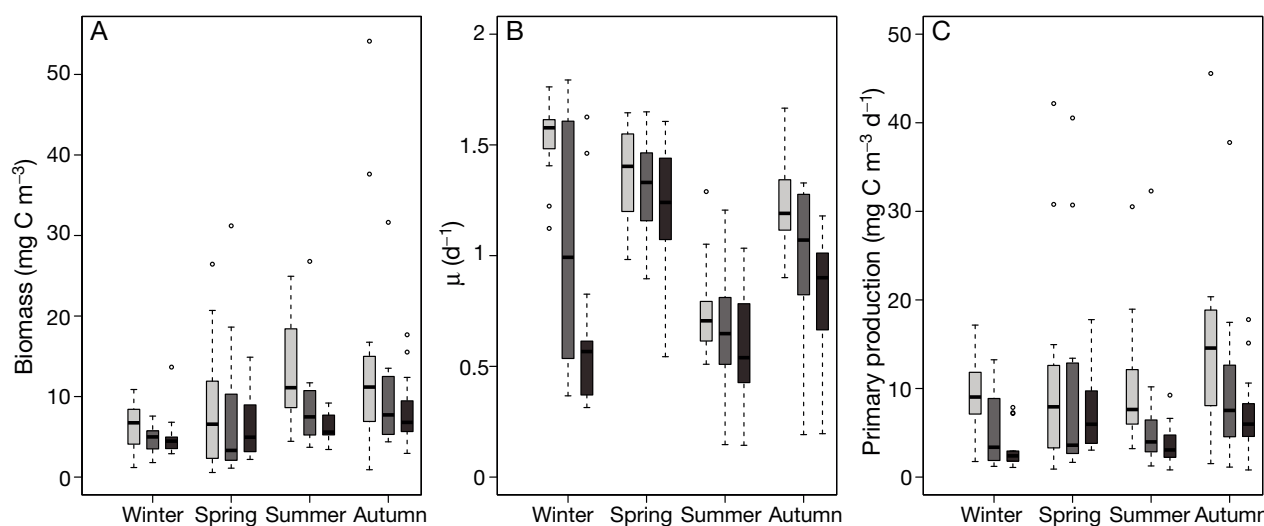


Fig. 3. Seasonality of picophytoplankton variables measured in the southern Bay of Biscay: (A) biomass (P_{BM} , mg C m^{-3}), (B) growth rate (μ , d^{-1}), and (C) production rates (Pp, $\text{mg C m}^{-3} \text{d}^{-1}$). Inshore (pale gray), middle shelf (gray), and offshore (dark gray). Boxplot features as in Fig. 2

Table 3. Seasonal (winter, W; spring, Sp; summer, S; autumn, A) and annual averages (\pm SE) of variables measured in inshore, middle shelf, and offshore mixed layers of the southern Bay of Biscay (see Fig. 1 for coordinates). Picophytoplankton biomass (mg C m^{-3}) due to cyanobacteria (Cyano), *Synechococcus* (Syne), *Prochlorococcus* (Proch), picoeukaryotes (Picoeuk), small picoeukaryotes (Small), and large picoeukaryotes (Large). Different superscript letters indicate significant pairwise differences among stations; significant differences among seasons are indicated by inequalities (ANOVA and Tukey-Kramer honestly significant difference; $p < 0.05$). nd: not detectable

Season	n	Cyano	Syne	Proch	Picoeuk	Small	Large
Inshore							
W	13	1.6 ± 0.4	1.6 ± 0.4	<0.1 ± <0.1	4.7 ± 0.6	3.7 ± 0.5	1.1 ± 0.2
Sp	12	1.4 ± 0.6	1.4 ± 0.6	nd	7.0 ± 2.2	4.1 ± 1.3	2.9 ± 1.0
S	16	6.1 ± 1.2	5.9 ± 1.1	0.2 ± 0.1	7.3 ± 1.0	5.0 ± 0.8	2.4 ± 0.3
A	16	5.3 ± 1.9	4.5 ± 1.7	0.8 ± 0.3	9.1 ± 1.8	6.7 ± 1.7	2.4 ± 0.4
Annual	57	3.9 ± 0.7 ^a	3.6 ± 0.6 ^a	0.3 ± 0.1 ^a	7.2 ± 0.8 ^a	5.0 ± 0.6 ^a	2.2 ± 0.3 ^a
				A > S,Sp,W			
Middle shelf							
W	13	1.0 ± 0.2	1.0 ± 0.2	<0.1 ± <0.1	3.6 ± 0.5	2.5 ± 0.4	1.1 ± 0.2
Sp	12	1.8 ± 0.7	1.8 ± 0.7	nd	5.8 ± 2.2	3.6 ± 1.5	2.2 ± 1.0
S	16	4.5 ± 0.6	4.2 ± 0.5	0.3 ± 0.1	4.2 ± 1.5	2.9 ± 1.5	1.3 ± 0.2
A	16	3.7 ± 0.6	2.8 ± 0.6	0.9 ± 0.2	5.9 ± 1.3	4.1 ± 1.3	1.8 ± 0.3
Annual	57	2.9 ± 0.3 ^a	2.6 ± 0.3 ^b	0.3 ± 1.2 ^a	4.9 ± 0.7 ^b	3.3 ± 0.6 ^{a,b}	1.6 ± 0.2 ^{a,b}
		S > W,Sp; A > W	S > W,Sp	A > S,Sp,W			
Offshore							
W	13	1.3 ± 0.2	1.2 ± 0.1	0.1 ± <0.1	3.8 ± 3.8	2.7 ± 0.5	1.2 ± 0.2
Sp	12	2.0 ± 0.6	2.0 ± 0.6	nd	4.2 ± 6.4	2.2 ± 0.5	2.0 ± 0.5
S	16	3.8 ± 0.5	3.6 ± 0.5	0.3 ± 0.1	2.3 ± 5.2	1.5 ± 0.4	0.8 ± 0.1
A	16	3.8 ± 0.8	2.7 ± 0.7	1.1 ± 0.2	4.3 ± 4.2	2.9 ± 0.4	1.4 ± 0.2
Annual	57	2.9 ± 0.3 ^a	2.5 ± 0.3 ^b	0.4 ± 0.1 ^a	3.6 ± 0.3 ^c	2.3 ± 0.2 ^b	1.3 ± 0.1 ^b
		A,S > W	S > W	A > S,Sp,W		Sp > S	
Average							
W	39	1.3 ± 0.1	1.2 ± 0.1	0.1 ± <0.1	4.1 ± 0.3	2.9 ± 0.7	1.1 ± 0.1
Sp	36	1.7 ± 0.4	1.7 ± 0.4	nd	5.7 ± 1.1	3.1 ± 0.6	2.4 ± 0.5
S	48	4.8 ± 0.5	4.6 ± 0.5	0.3 ± 0.1	4.6 ± 0.7	3.3 ± 0.7	1.5 ± 0.2
A	48	4.3 ± 0.7	3.4 ± 0.6	0.9 ± 0.1	6.4 ± 0.8	4.6 ± 0.7	1.9 ± 0.2
		S > W,Sp; A > W,Sp	S > W,Sp; A > W	A > S,Sp,W		Sp > W	

masses of large picoeukaryotes were found in spring, ca. 2.4 mg C m^{-3} . On average, picoeukaryotic biomass was $5.2 \pm 0.4 \text{ mg C m}^{-3}$, with small picoeukaryotic biomass being $3.5 \pm 0.3 \text{ mg C m}^{-3}$ and large picoeukaryotic biomass $1.7 \pm 0.1 \text{ mg C m}^{-3} \text{ d}^{-1}$.

The percentage of picophytoplankton biomass represented by the different groups of picophytoplankton also presented inshore–offshore gradients (Table 4, Fig. 5). Cyanobacteria represented 29.2% of picophytoplankton biomass inshore and 42.3% offshore, and seasonality was found with maxima in summer, ca. 50%, and minima in spring, ca. 23%. Similarly, *Synechococcus* were 27.2% of picophytoplankton biomass inshore and 36.7% offshore, and seasonality was found with maxima in summer, ca. 50%, and minima in winter, ca. 23%. Moreover, *Prochlorococcus* were 2.0% of picophytoplankton biomass inshore and 5.6% offshore, and they presented a maximum in autumn, ca. 10%, while they were undetectable in spring. On average, cyanobacteria represented $36.9 \pm 1.7\%$ of picophytoplankton biomass, with *Synechococcus* being $32.8 \pm$

1.6% and *Prochlorococcus* $4.0 \pm 0.6\%$. Conversely, picoeukaryotes represented 70.8% of picophytoplankton biomass inshore and 57.6% offshore, and they presented a seasonality with maxima in spring inshore, ca. 85%, and in winter in the middle shelf, ca. 77%, and offshore, ca. 72%. Moreover, picoeukaryotes presented minimum biomasses in summer, varying between 56% inshore and 37.3% offshore. Similarly, small picoeukaryotes represented 44.8% of picophytoplankton biomass inshore and 36.1% offshore and presented a seasonality with maxima in winter, ca. 50%, and minima in summer, varying between 37.1% inshore and 23.3% offshore. Finally, large picoeukaryotes were close to 20% of picophytoplankton biomass at the 3 stations; maxima were reached in spring, varying between 43.4% inshore and 33.5% offshore, while minima were found in summer, ca. 17%. On average, picoeukaryotes represented $63.1 \pm 1.7\%$ of picophytoplankton biomass, with small picoeukaryotes being $39.5 \pm 1.5\%$ and large picoeukaryotes $23.6 \pm 1.2\%$.

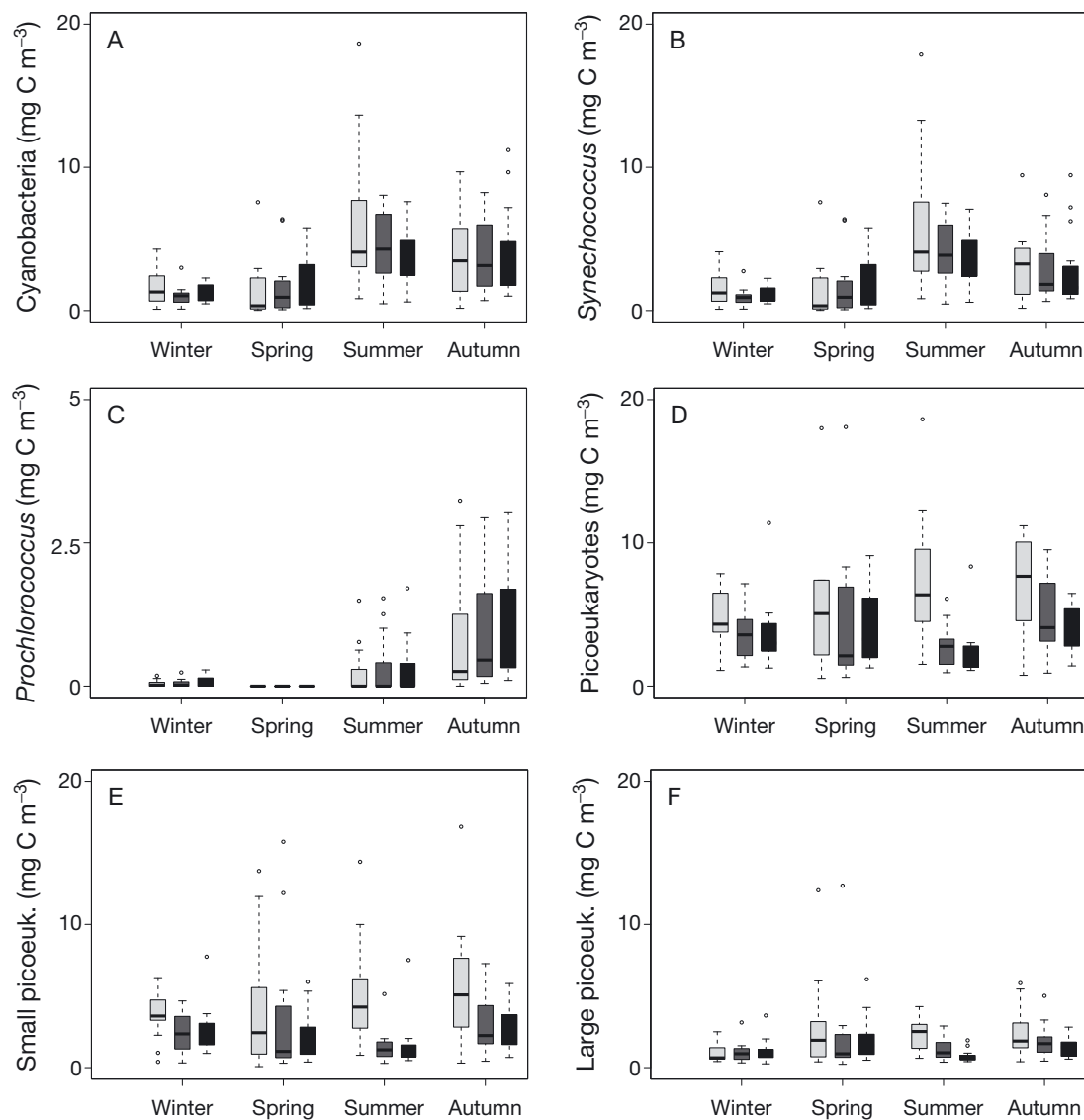


Fig. 4. Seasonality of picophytoplankton biomass (mg C m⁻³) due to (A) cyanobacteria, (B) *Synechococcus*, (C) *Prochlorococcus*, (D) picoeukaryotes, (E) small picoeukaryotes, and (F) large picoeukaryotes. Inshore (pale gray), middle shelf (gray), and offshore (dark gray). Boxplot features as in Fig. 2

Picophytoplankton biomass, growth rate, primary production, and community structure

Multiple regression showed that picophytoplankton biomass was significantly related to temperature (Table 5, $r^2 = 0.09$), and there was an effect of the station on these relationships. Intercepts were higher in the middle shelf and offshore as compared to inshore and, moreover, the coefficients influencing temperature decreased as we moved offshore.

Concerning picophytoplankton community structure, the percentage of picophytoplankton biomass

contributed by cyanobacteria was significantly related to irradiance, temperature, and the interaction between irradiance and temperature (Table 5, $r^2 = 0.59$). There was also an effect of the station on the percentage of picophytoplankton biomass contributed by cyanobacteria, with lower intercepts as we moved offshore. Conversely, the higher coefficients related to temperature that influenced the percentage of biomass of cyanobacteria were found as we moved offshore. In addition, the percentage of *Synechococcus* biomass was significantly related to irradiance, temperature, and the interaction between irradiance and temperature (Table 5, $r^2 = 0.50$). Similarly, the per-

Table 4. Seasonal (winter, W; spring, Sp; summer, S; autumn, A) and annual average (\pm SE variables measured in inshore, middle shelf, and offshore mixed layers of the southern Bay of Biscay (see Fig. 1 for coordinates). Percentage of picophytoplankton biomass (%) due to cyanobacteria (Cyano), *Synechococcus* (Syne), *Prochlorococcus* spp. (Proch), picoeukaryotes (Picoeuk), small picoeukaryotes (Small), and large picoeukaryotes (Large). Different superscript letters indicate significant pairwise differences among stations; significant differences among seasons are indicated by inequalities (ANOVA and Tukey-Kramer honestly significant difference; $p < 0.05$). nd: not detectable

Season	n	Cyano	Syne	Proch	Picoeuk	Small	Large
Inshore							
W	13	22.7 \pm 3.6	22.2 \pm 3.5	0.6 \pm 0.2	77.3 \pm 3.6	56.5 \pm 3.6	20.8 \pm 4.10
Sp	12	14.5 \pm 4.7	14.5 \pm 4.7	nd	85.5 \pm 4.7	42.1 \pm 5.6	43.4 \pm 6.4
S	16	44.0 \pm 4.6	42.7 \pm 4.5	1.3 \pm 0.6	56.0 \pm 4.6	37.1 \pm 3.8	18.9 \pm 2.1
A	16	30.8 \pm 3.5	25.5 \pm 2.5	5.3 \pm 4.2	69.2 \pm 3.5	44.9 \pm 3.1	24.4 \pm 3.8
Annual	57	29.2 \pm 2.5 ^a	27.2 \pm 2.3 ^a	2.0 \pm 0.6 ^a	70.8 \pm 2.5 ^a	44.8 \pm 2.2 ^a	26.0 \pm 2.3 ^a
		S > W,Sp; A > Sp	S > Sp,W,A	A > W,Sp,S	W,Sp > S; Sp > A	W > S	Sp > A,W,Sp
Middle shelf							
W	13	23.3 \pm 4.1	22.1 \pm 3.7	1.2 \pm 0.5	76.7 \pm 4.1	50.8 \pm 4.4	25.9 \pm 5.0
Sp	12	28.1 \pm 7.3	28.1 \pm 7.3	nd	71.9 \pm 7.3	43.0 \pm 6.5	28.9 \pm 3.3
S	16	59.4 \pm 5.3	56.1 \pm 4.9	3.2 \pm 1.3	40.6 \pm 5.3	23.8 \pm 5.4	16.8 \pm 2.4
A	16	40.1 \pm 5.1	28.1 \pm 3.1	11.9 \pm 1.7	59.9 \pm 5.1	36.3 \pm 4.1	23.6 \pm 3.6
Annual	57	39.1 \pm 3.3 ^b	34.6 \pm 3.0 ^{a,b}	4.5 \pm 1.2 ^{a,b}	60.9 \pm 3.3 ^b	37.5 \pm 2.8 ^{a,b}	23.3 \pm 1.8 ^a
		S > W,Sp,A	S > Sp,W,A	A > W,Sp,S	W, Sp,A > S	W > S	
Offshore							
W	13	28.2 \pm 3.8	26.1 \pm 3.3	2.0 \pm 0.7	71.8 \pm 3.8	49.9 \pm 3.2	21.9 \pm 2.1
Sp	12	39.3 \pm 6.4	29.3 \pm 6.4	nd	70.7 \pm 6.4	37.2 \pm 6.7	33.5 \pm 4.5
S	16	62.7 \pm 5.2	58.2 \pm 4.9	4.5 \pm 2.0	37.3 \pm 5.2	23.3 \pm 4.5	14.0 \pm 2.2
A	16	43.3 \pm 4.2	29.3 \pm 3.0	13.9 \pm 4.5	56.7 \pm 4.2	36.9 \pm 2.7	19.9 \pm 2.5
Annual	57	42.3 \pm 3.1 ^b	36.7 \pm 2.8 ^b	5.6 \pm 1.3 ^b	57.6 \pm 3.1 ^c	36.1 \pm 2.5 ^b	21.6 \pm 1.7 ^a
		S > W,Sp,A	S > Sp,W,A	A > W,Sp,S	W,Sp,A > S	W > S	Sp > A,W,Sp
Average							
W	39	24.7 \pm 1.1	23.5 \pm 0.9	1.3 \pm 0.1	75.3 \pm 1.1	52.4 \pm 1.0	22.8 \pm 1.1
Sp	36	24.0 \pm 1.7	24.0 \pm 1.7	nd	76.0 \pm 1.7	40.8 \pm 1.6	35.3 \pm 1.3
S	48	55.4 \pm 1.6	52.4 \pm 1.5	3.0 \pm 0.4	44.6 \pm 1.6	28.1 \pm 1.5	16.6 \pm 0.7
A	48	38.0 \pm 1.4	27.6 \pm 0.9	10.4 \pm 0.9	62.0 \pm 1.4	39.4 \pm 1.0	22.6 \pm 1.0
		S > A,Sp,W; A > Sp,W	A > S,Sp,W	A > S,Sp,W	A > S,Sp,W; Sp,W > S	Sp,W,A > S; W > Sp,S,A	Sp > W,A,S

centage of *Prochlorococcus* biomass was also significantly related to irradiance, temperature, and the interaction between irradiance and temperature (Table 5, $r^2 = 0.29$).

Percentages of picophytoplankton biomass contributed by picoeukaryotes were significantly related to irradiance, temperature, and the interaction between irradiance and temperature (Table 5, $r^2 = 0.59$). As we moved offshore, these relationships presented increasing intercepts and decreasing coefficients related to temperature. However, the percentage of small picoeukaryotes was significantly related to irradiance and temperature (Table 5, $r^2 = 0.36$). Similarly, large picoeukaryotes were significantly related to irradiance and temperature (Table 5, $r^2 = 0.17$).

Picophytoplankton primary production was significantly related to irradiance, temperature, and the interaction between irradiance and temperature (Table 5, $r^2 = 0.15$), and there was also an influence of

the station on the intercepts that decreased as we went offshore.

Finally, growth rates were significantly related to irradiance, temperature, and the interaction between irradiance and temperature (Table 5, $r^2 = 0.81$). Intercepts and coefficients related to the interaction between irradiance and temperature decreased as we moved offshore, while coefficients affecting irradiance and temperature increased as we went offshore.

Picophytoplankton intaglios

Intaglios of picophytoplankton biomass showed minima (Fig. 6A) at low irradiances and low temperatures (domain I), while maxima were attained at low irradiances and high temperatures (domain IV). Among picophytoplankton, cyanobacteria were more than 38% of picophytoplankton biomass (Fig. 6B)

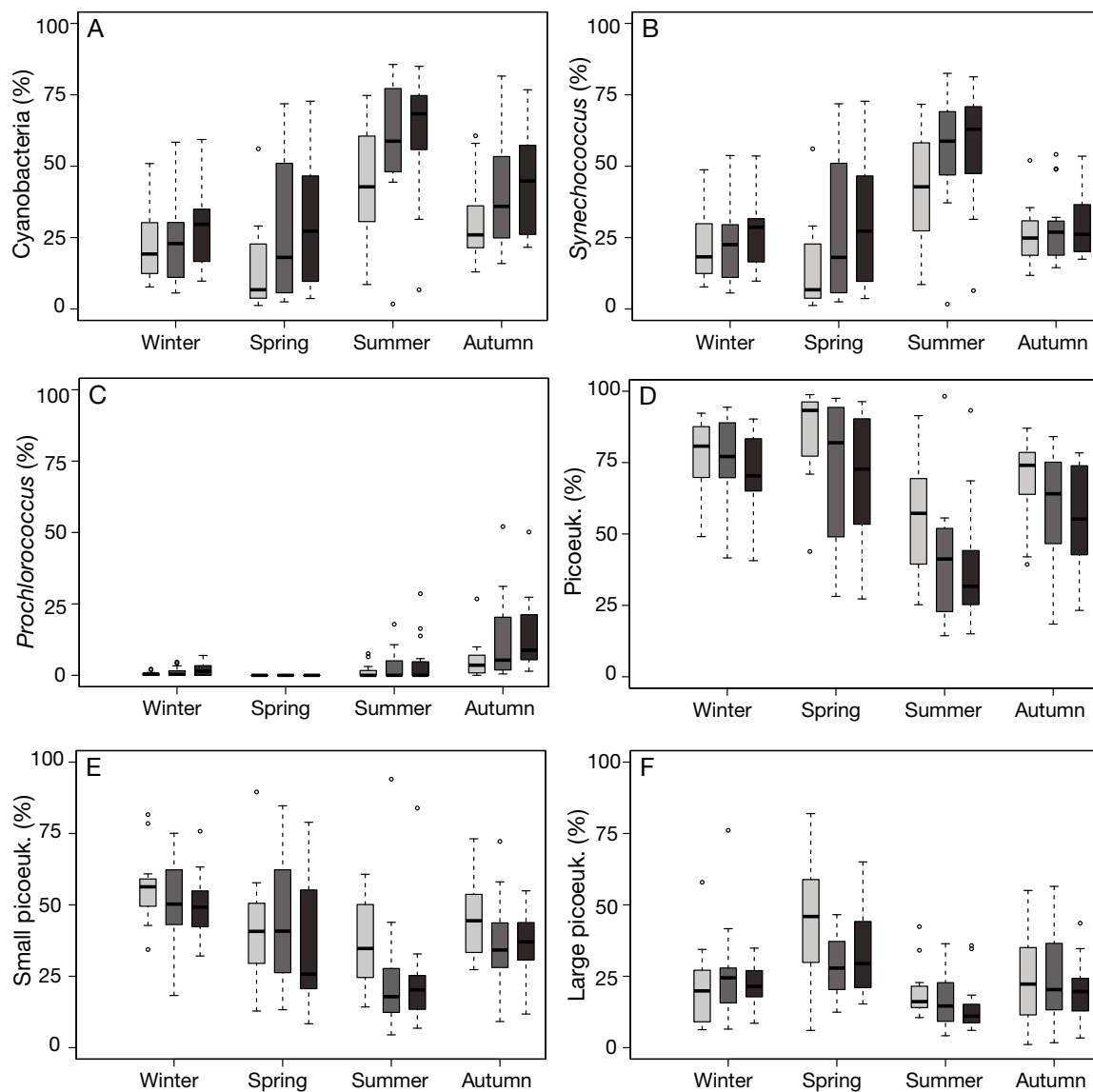


Fig. 5. Seasonality of the percentage of picophytoplankton biomass (%) due to (A) cyanobacteria, (B) *Synechococcus*, (C) *Prochlorococcus*, (D) picoeukaryotes, (E) small picoeukaryotes, and (F) large picoeukaryotes. Inshore (pale gray), middle shelf (gray), and offshore (dark gray). Boxplot features as in Fig. 2

during high temperatures (domains III and IV), whereas picoeukaryotes were particularly important for picophytoplankton biomass (Fig. 6C) during low temperatures (domains I and II).

Within cyanobacteria, *Synechococcus* (Fig. 7A) amounted to nearly 50% of picophytoplankton biomass during high irradiances and high temperatures (domain III), while *Prochlorococcus* (Fig. 7B) contributed to nearly 10% of picophytoplankton biomass during low irradiances and high temperatures (domain IV). Conversely, within picoeukaryotes, small picoeukaryotes (Fig. 7C) amounted to nearly 50% of picophytoplankton biomass in low

irradiances and low temperatures (domain I), while large picoeukaryotes (Fig. 7D) were 35% of picophytoplankton biomass in high irradiances and low temperatures (domain II). Moreover, minimum picophytoplankton primary production rates (Fig. 7E) were found during low irradiances and low temperatures (domain I), and maxima were found in low irradiances and high temperatures (domain IV). Finally, minimum picophytoplankton growth rates (Fig. 7F) were found during high irradiances and high temperatures (domain III), whereas maxima were found during high irradiances and low temperatures (domain II).

Table 5. Multiple regression model, $Y \sim E_g \times T \times \text{Station}$ (i.e. $Y = a + \beta_1 \times E_g + \beta_2 \times T + \beta_3 \times E_g \times T$), for the mixed layer at 3 stations in the southern Bay of Biscay. Independent variables: median irradiance (E_g , mol photons $\text{m}^{-2} \text{h}^{-1}$) and temperature (T , °C). Factor station: inshore, middle shelf, and offshore. Dependent variables: picophytoplankton biomass (P_{BM} , mg C m^{-3}); percentage of biomass (%) due to cyanobacteria (Cyano), *Synechococcus* (Syne), *Prochlorococcus* (Proch), picoeukaryotes (Picoeuk), small picoeukaryotes (Small), and large picoeukaryotes (Large); picophytoplankton primary production (Pp, mg C $\text{m}^{-3} \text{d}^{-1}$); and growth rate (μ , d^{-1}). A step function, taking into account Akaike's information criterion, was used to minimize the number of variables and factors ($\pm \text{SE}$) of each multiple regression. In the case of *Synechococcus*, *Prochlorococcus*, and the small and large picoeukaryotes, there were no significant differences between stations (ANOVA, $p < 0.05$)

Station	Y	a	β_1	β_2	β_3	df	Adjusted r^2	p
Inshore	P_{BM}	-2.76 ± 4.89		0.88 ± 0.31		165	0.09	0.01
Middle shelf		5.81 ± 11.59		0.12 ± 0.72				
Offshore		5.08 ± 11.58		0.09 ± 0.72				
Inshore	Cyano	-11.78 ± 16.4	-19.1 ± 8.1	3.34 ± 1.07	0.75 ± 0.50	163	0.59	0.01
Middle shelf		-37.06 ± 31.2	-19.1 ± 8.1	5.26 ± 1.99	0.75 ± 0.50			
Offshore		-39.39 ± 31.5	-19.1 ± 8.1	5.40 ± 1.99	0.75 ± 0.50			
	Syne	-4.64 ± 12.3	-29.63 ± 7.8	2.52 ± 0.82	1.64 ± 0.49	167	0.50	0.01
	Proch	-30.1 ± 5.8	11.9 ± 3.7	2.54 ± 0.38	-1.01 ± 0.23	167	0.29	0.01
Inshore	Picoeuk	111.8 ± 16.4	19.1 ± 8.1	-3.34 ± 1.07	-0.75 ± 0.50	163	0.59	0.01
Middle shelf		137.1 ± 31.2	19.1 ± 8.1	-5.26 ± 1.99	-0.75 ± 0.50			
Offshore		139.4 ± 31.5	19.1 ± 8.1	-5.40 ± 1.99	-0.75 ± 0.50			
	Small	98.21 ± 6.16	3.21 ± 1.38	-3.93 ± 0.40		168	0.36	0.01
	Large	50.17 ± 5.5	4.75 ± 1.23	-2.05 ± 0.36		168	0.17	0.01
Inshore	Pp	-1.91 ± 6.97	14.34 ± 4.43	0.82 ± 0.46	-0.83 ± 0.28	165	0.15	0.01
Middle shelf		-5.94 ± 8.52	14.34 ± 4.43	0.82 ± 0.46	-0.83 ± 0.28			
Offshore		-7.48 ± 8.57	14.34 ± 4.43	0.82 ± 0.46	-0.83 ± 0.28			
Inshore	μ	1.96 ± 0.34	0.70 ± 0.20	-0.05 ± 0.02	-0.04 ± 0.01	159	0.81	0.01
Middle shelf		0.13 ± 0.77	1.64 ± 0.46	0.05 ± 0.05	-0.09 ± 0.03			
Offshore		-0.30 ± 0.76	1.96 ± 0.47	0.07 ± 0.05	-0.11 ± 0.03			

DISCUSSION

Picophytoplankton in oceanic ecosystems can account for nearly 24 % of global phytoplankton primary production (Uitz et al. 2010), as they are the most abundant phytoplankton (Waterbury et al. 1979, Chisholm et al. 1988, Partensky et al. 1999). However, picophytoplankton can also play an important role in coastal ecosystems (Waterbury et al. 1986, Tamigneaux et al. 1995). In the southern Bay of Biscay, previous studies have shown that picophytoplankton are a keystone group within phytoplankton, contributing up to 40 % of total chl *a* and nearly 50 % of primary production (Calvo-Díaz et al. 2004, Cermeno et al. 2006, Morán 2007). In this regard, between 2002 and 2006, a succession of different phylogenetic groups of picophytoplankton has been described for the southern Bay of Biscay with small picoeukaryotes as the most abundant group in springtime, large picoeukaryotes thriving in autumn, *Synechococcus* attaining high numbers in summer, and *Prochlorococcus* being conspicuous in autumn (Calvo-Díaz & Morán 2006). Here, we expanded the period of time studied from 2003 to 2010, concentrat-

ing on main physico-chemical factors driving picophytoplankton seasonal succession and trying to understand which of those drivers affected picophytoplankton biomass, primary production, growth rate, and community composition.

Picophytoplankton biomass, community structure, primary production, and growth rates

From 2003 to 2010, there was a positive relationship between picophytoplankton biomass and temperature in mixed layers of the southern Bay of Biscay. This positive effect has been previously described for Atlantic waters (Morán et al. 2010) and is related to a combination of 2 patterns affecting the relationship between body size and cellular abundance: (1) the temperature–size rule, which relates higher temperatures with smaller planktonic sizes (Atkinson et al. 2003, Chen & Liu 2010, Morán et al. 2010), and (2) the cross-community scaling relationship, which inversely relates organism size to its abundance in the community (White et al. 2007). In this regard, the increase in picophytoplankton biomass with increasing

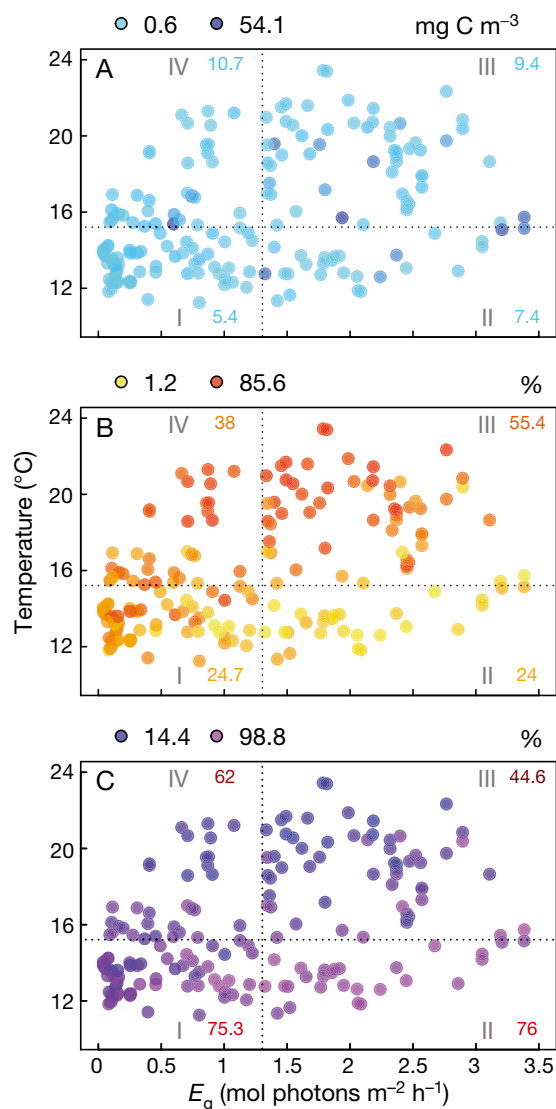


Fig. 6. Picophytoplankton intaglio representations containing (A) biomass (P_{BM} , mg C m^{-3}) and (B,C) percentage of picophytoplankton biomass (%) due to (B) cyanobacteria and (C) picoeukaryotes. All stations are plotted together. Domains: I, low irradiance and low temperature; II, high irradiance and low temperature; III, high irradiance and high temperature; and IV, low irradiance and high temperature. Color intensity within each symbol is related to the range of the variable reported in the label. Delimitation of each domain was established through slashed horizontal and vertical lines (see 'Materials and methods')

temperatures in the southern Bay of Biscay has been mainly related to an increase in the cellular abundance of picophytoplankton (Calvo-Díaz & Morán 2006). However, we cannot rule out that a fraction of the positive relationship found between biomass and temperature could also be due to changes in the community structure of picophytoplankton. Thus, the higher coefficients in the relationships between tem-

perature and biomass as we moved towards the coast could be influenced by seasonal changes in the relative abundance of small and large picoeukaryotes. The fact that there was nearly half the amount of picophytoplankton biomass in the mixed layer during winter as compared to autumn points towards a dilution and export to deeper waters of picophytoplankton due to increasing mixed layers (Richardson & Jackson 2007).

The factor station was significant in the relationship between temperature and picophytoplankton biomass (ANOVA, $p < 0.05$), which points towards an influence of inorganic nutrients on the biomass of picophytoplankton, as has been observed in other marine communities (Morán et al. 2010, Flombaum et al. 2013, Stawiarski et al. 2018). On that point, a fraction of the relationship between picophytoplankton biomass and temperature could have been influenced by NO_3 concentration, as there was co-linearity between both variables. However, a recent study has shown significant relationships between picophytoplankton biomass and seawater temperature by using generalized additive models, thus avoiding cross-correlation between temperature and NO_3 concentration (Otero-Ferrer et al. 2018), though we have to consider that general additive models are not exempt from problems such as overfitting, which may lead in some cases to poorer results than those obtained with multiple linear regression models (Bishop & McBratney 2001). In addition, a good relationship between picophytoplankton abundance, irradiance, and temperature has been found in oceanic ecosystems with a low influence of inorganic nutrients (Flombaum et al. 2013). Had we included NO_3 concentration in our multiple regression models, the slope between picophytoplankton biomass and temperature would have remained positive and the adjusted r^2 would have increased from 0.09 to 0.11, yet there would have been no significant differences in the adjusted r^2 for the remaining dependent variables (data not shown). In any case, the differences in NO_3 concentration between the 3 stations might help to explain why inshore waters presented a steeper increase in picophytoplankton biomass for the same increase in temperature as compared to middle shelf and offshore waters, which could reflect a differential effect of temperature on the carrying capacity of the ecosystem, meaning the maximum population density that could sustain a set of environmental conditions (Sarker & Whiltshire 2017).

Irradiance was the other independent variable used in the multiple regression models. In this regard, the estimation of irradiance in the southern

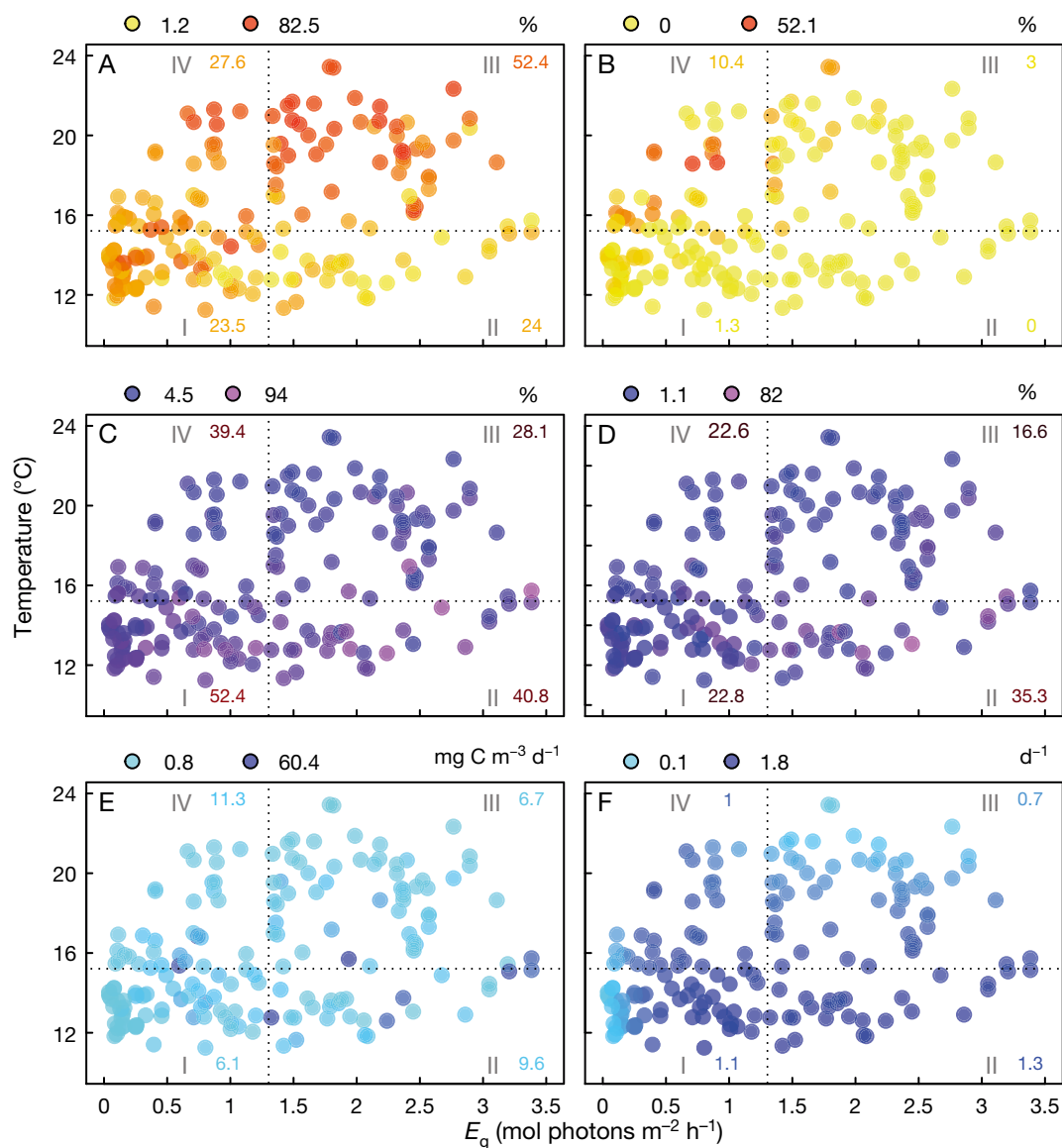


Fig. 7. Picophytoplankton intaglio representations containing (A–D) percentage of picophytoplankton biomass (%) due to (A) *Synechococcus*, (B) *Prochlorococcus*, (C) small picoeukaryotes, (D) large picoeukaryotes, (E) production rate (P_p , $mg\ C\ m^{-3}\ d^{-1}$), and (F) growth rate (μ , d^{-1}). All stations are plotted together. Domains and lines as in Fig. 6

Bay of Biscay is influenced by the mixed layer depth, with larger mixed layer depths and lower irradiances found in winter. Conversely, increases in temperature of the upper ocean may lead to the development of a seasonal thermocline and stable water column with high irradiances and shallow mixed layer depths found in summer (Kara et al. 2000). Therefore, using irradiance as the best available measure of turbulent mixing, we expected to have high mixing coincident with low irradiances and, in contrast, low mixing when irradiances were high. However, there must be exceptions with increased turbulence and nutrient supply in stratified waters due to internal

wave formation or propagation of internal tides that may lead to high turbulence and high nutrient supply towards the base of the mixed layer (Baines 1982, Sharples et al. 2001) or a decrease in turbulence and nutrient supply through stratification of storm-homogenized mixed layers by dynamical adjustment (Boccaletti et al. 2007). In addition, we cannot disregard that under some circumstances, the cells thriving in the shallower mixed layers could have suffered lower irradiances than those presented here or, conversely, some of the cells thriving near the surface in the deeper mixed layers possibly were not well mixed and could have had higher irradiances than those

presented here. In any case, if highly contrasting hydrographic regimes are compared, as presented in this study, stratification can be a good proxy for mixing and nutrient supply (Mouriño-Carballido et al. 2016).

Picophytoplankton communities can be structured through changes in the proportion of different groups of picophytoplankton that were adapted to different irradiances (Veldhuis et al. 2005, Johnson et al. 2006, Huang et al. 2012), as maximum growth rates occur over a wide spectrum of irradiances in both cyanobacteria (Moore et al. 1995, West & Scanlan 1999, Johnson et al. 2006, Chen et al. 2014) and picoeukaryotes (Rodríguez et al. 2005, Veldhuis et al. 2005). Here, the biomass of small picoeukaryotes increased in low irradiances and low temperatures, while large picoeukaryotes were more abundant in high irradiance and low temperatures. This could be related to a good adaptation/response of small picoeukaryotes to low irradiances due to the low package effect of their photosynthetic systems (Cermeno et al. 2005, Falkowski & Raven 2007, Álvarez et al. 2016) and, conversely, higher nutrient uptake and assimilation rates by larger phytoplankton (Marañón et al. 2013), as in this region high irradiances and nutrient concentrations were concurrent during springtime (Calvo-Díaz & Morán 2006, Morán 2007, Vázquez-Domínguez et al. 2013). Strikingly, but confirming previous studies at the site (Calvo-Díaz et al. 2004, Calvo-Díaz & Morán 2006), *Synechococcus* were particularly abundant in mixed layers with high irradiances and high temperatures, while *Prochlorococcus* were abundant in mixed layers with low irradiances and high temperatures. This could be related to the fact that irradiance may affect the growth of picophytoplankton depending on the genotype; for example, low-light *Prochlorococcus* can grow particularly well at low irradiances but may suffer photoinhibition or even death at high irradiances (Agustí 2004, Llabrés & Agustí 2006, Stawiariski et al. 2018), whereas *Synechococcus* may grow at high irradiances with higher growth rates than *Prochlorococcus* and picoeukaryotes (Moore et al. 1995, Agustí 2004, Llabrés & Agustí 2006) but may also suffer photoinhibition and cell death (Agustí 2004, Llabrés & Agustí 2006, Stawiariski et al. 2018). In addition, cyanobacteria are better adapted to oligotrophic conditions than picoeukaryotes, as they are smaller than the latter and may be more efficient at taking up nutrients that are present at very low concentrations (Raven 1998, Zubkov et al. 2003, Mackey et al. 2009, Mouriño-Carballido et al. 2016). All these circumstances may help to explain why cyanobacteria were

so abundant in summer and autumn, when there was a broad range of irradiances and nutrients varied between low and medium concentrations, but also why picoeukaryotes thrived in winter and springtime, when there was also a broad range of irradiances and nutrients were between high and medium concentrations.

Temperature may also influence the community structure of picophytoplankton in the southern Bay of Biscay. Temperature has been observed and modeled in different marine ecosystems, and results showed an increase in cyanobacteria with increasing temperatures (Agawin et al. 2000, Follows et al. 2007, Vázquez-Domínguez et al. 2008, Chen et al. 2014) and an increase in picoeukaryotes with decreasing temperatures (Morán 2007, Morán et al. 2010). Our multiple regression models show a positive effect of temperature on cyanobacteria, that is *Synechococcus* and *Prochlorococcus*. It has been shown that phytoplankton is adapted to local temperatures, reaching maximum growth rates with increasing temperatures (Thomas et al. 2012). Thus, one may expect higher growth rates and abundances of *Synechococcus* in summer, as these cyanobacteria are well adapted to oligotrophy and they present optimal growth rates within a range from 18 to 28°C (Chen et al. 2014). Conversely, *Prochlorococcus* reached higher levels in autumn, which could be related to both their slightly higher thermal niche as compared to *Synechococcus*, between 24 and 28°C (Chen et al. 2014), and their lower tolerance to high irradiance conditions (Agustí 2004, Llabrés & Agustí 2006, Stawiariski et al. 2018). Moreover, the absence of *Prochlorococcus* in springtime could be related to the combined effect of both winter dilution, due to higher mixed layer depths along this season, and winter temperatures, as they are absent at low temperatures in marine ecosystems (Johnson et al. 2006, Flombaum et al. 2013). Conversely, picoeukaryotes usually peaked at low temperatures, which could be related to optimal growth temperatures slightly lower than cyanobacteria, between 20 and 25°C (Chen et al. 2014). Among picoeukaryotes, small picoeukaryotes were more prone to be found in winter as compared to large picoeukaryotes, in seeming contradiction with the temperature–size rule. Nevertheless, this may be related to the smaller package effect of small cells (Cermeno et al. 2005, Falkowski & Raven 2007, Álvarez et al. 2016). Therefore, under light-limiting conditions, as for example in winter or when mixed layers are large, small picoeukaryotes may use light more efficiently than large picoeukaryotes. Finally, different studies about picophytoplankton communi-

ties have shown higher growth rates related to larger sizes (Bec et al. 2008, Marañón et al. 2013, Stawiarski et al. 2018), which may help to explain the presence of large picoeukaryotes and high growth rates in springtime.

Picophytoplankton primary production rates were positively related to irradiance, as with growth rates, and temperature, as with biomass. Therefore, the variability of picophytoplankton primary production was not only related to picophytoplankton biomass but also to the growth rates of these tiny microorganisms. Furthermore, there was a significant decrease in picophytoplankton primary production as we moved offshore (ANOVA, $p < 0.05$).

Maximum growth rates were found during winter inshore, while minima were reached during summer offshore. Such differences were related to high NO_3 concentrations inshore and low NO_3 concentrations offshore. Moreover, the variation in growth rates is coincident with the dominance of cyanobacteria offshore and picoeukaryotes inshore. Picophytoplankton growth rates in the southern Bay of Biscay have been positively related to the presence of picoeukaryotes (Morán 2007), which in this region increase with decreasing temperatures (Calvo-Díaz & Morán 2006, Vázquez-Domínguez et al. 2013). The multiple regression model showed a significant effect of station (ANOVA, $p < 0.05$) and a significant effect of irradiance, temperature, and the interaction between irradiance and temperature. The determination coefficient of this multiple regression was very high ($r^2 = 0.81$), as expected, since growth rate was modeled from the picophytoplankton carbon to chl *a* ratio that depends on an ecosystem's irradiance and temperature (Vázquez-Domínguez et al. 2013).

Succession of picophytoplankton

Picophytoplankton intaglios showed higher picophytoplankton biomass under oligotrophic warm conditions, when cyanobacteria were more abundant (i.e. domains III and IV). This may be related to, on the one hand, the lower nutrient requirements of cyanobacteria as compared to picoeukaryotes (Raven 1998, Zubkov et al. 2003, Mackey et al. 2009, Mouriño-Carballido et al. 2016) and, on the other hand, the lower grazing rates by protists that often support cyanobacteria as compared to picoeukaryotes (Christaki et al. 2005). The higher picophytoplankton biomass as we moved inshore might be related to a higher carrying capacity of the inshore station due to its higher NO_3 concentrations, as both

Prochlorococcus and *Synechococcus* may use inorganic forms of nitrogen to grow (Martiny et al. 2009, Berube et al. 2015). Growth rate intaglios showed higher rates when picoeukaryotes were abundant (i.e. domains I and II), which is concordant with positive relationships found between picophytoplankton growth rate and abundance of picoeukaryotes (Morán 2007) and between picophytoplankton growth rate and size (Bec et al. 2008, Marañón et al. 2013, Stawiarski et al. 2018), as in the southern Bay of Biscay, cyanobacteria are smaller than picoeukaryotes (Calvo-Díaz & Morán 2006). Consequently, primary production intaglios showed high productions when large picoeukaryotes were abundant but also when cyanobacteria dominate the ecosystem (i.e. domains II and IV).

In this study, we did not consider the composition of picoeukaryotes during the period of study, but a recent work in the Avilés Canyon has shown a highly diverse community of picoeukaryotes (Cabello et al. 2016) containing prymnesophytes that can be found in similar concentrations in surface waters and the deep chlorophyll maximum (DCM); chlorophytes that present 2 peaks, one above and one below the DCM; and pelagophytes that dominate in deep waters. Though we do not have any information about the genetic composition of picoeukaryotes, chlorophytes and pelagophytes are often smaller than prymnesophytes (Cabello et al. 2016), which could suggest that the small picoeukaryotes described here could be composed of chlorophytes and pelagophytes, while the large picoeukaryotes could be prymnesophytes. In this regard, the presence of the chlorophyte *Micromonas* has been associated in regions with eutrophic waters (Not et al. 2004, 2007, Cabello et al. 2016).

Considering the main adaptive strategies described for plants (Grime 1977), the Reynolds' intaglio (Smayda & Reynolds 2001, Reynolds 2003), and the 4 domains of the mandala proposed by Wyatt (2014), a conceptual intaglio is proposed for picophytoplankton living in the southern Bay of Biscay (Fig. 8). The succession is as follows: During winter mixing, small picoeukaryotes flourish under low irradiance and low temperature, and they may be included in the disturbance-tolerant ruderal R strategists group (Grime 1977, Smayda & Reynolds 2001, Reynolds 2003), as they are light-harvesting picophytoplankton that may use low irradiance levels efficiently because of the small package effect of their pigments (Raven 1998). Moreover, they live in physically disturbed water masses with low stress (i.e. high nutrients). Simultaneously, they can be also included in domain I, characterized by high nutrients and high turbu-

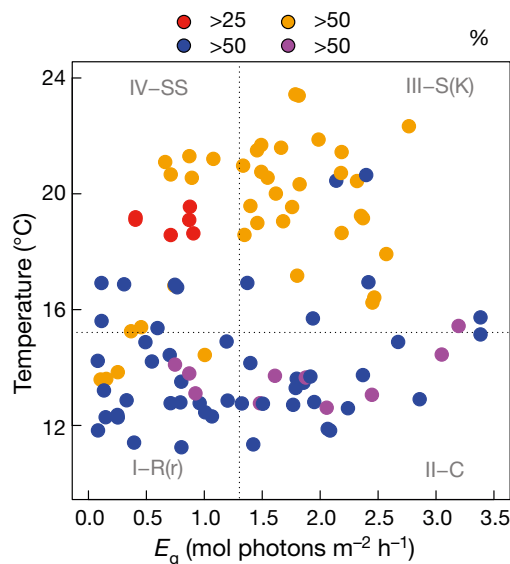


Fig. 8. Conceptual intaglio showing biomass seasonality of 4 different groups of picophytoplankton in the southern Bay of Biscay. The presence of these groups is shown when they share more than 25 or 50% of total picophytoplankton biomass. Domains: I, low irradiance and low temperature (usually coincident with high turbulence and high nutrients), where the most successful picophytoplankton are small picoeukaryotes (blue) and may be qualified as ruderal R strategists; II, high irradiance and low temperature (commonly concurrent with low turbulence and high nutrients), where large picoeukaryotes (magenta) are conspicuous and may be qualified as competitive C strategists; III, high irradiance and high temperature (frequently coincident with low turbulence and low nutrients), where the most successful picophytoplankton are *Synechococcus* (orange) and may be qualified as stress-tolerant S strategists; and IV, low irradiance and high temperature (mostly concurrent with high turbulence and low nutrients), where *Prochlorococcus* (red) are noticeable and may be qualified as chronic stress-tolerant SS strategists

lence (Wyatt 2014, this study). From the point of view of growth rate, they are r strategists (Grime 1977) and are in the high nutrients, high turbulence extreme of Margalef's mandala (Margalef 1978), as has been shown for the whole community of picoeukaryotes in the Mediterranean Sea (Mouriño-Carballido et al. 2016). When springtime arrives, large picoeukaryotes prosper under high irradiance and low temperatures, as larger phytoplankton possess a larger ability to take up nitrogen (Marañón et al. 2013), and they have higher growth rates than the smaller picoeukaryotes (Bec et al. 2008). Large picoeukaryotes may be considered competitor C strategists, as they thrive under low nutrient stress and low physical disturbance (Grime 1977); in addition, they are of intermediate size among picophytoplankton and are also fast growing (Smayda & Reynolds 2001, Reynolds 2003). Competitive strategists can be considered between r

and K strategists (Grime 1977) but actually can also be r strategists because within each functional category, there can be r and K strategists (Smayda & Reynolds 2001). In fact, the higher growth rates for the whole community of picophytoplankton in the southern Bay of Biscay were reached when large picoeukaryotes were present; thus, they can be considered r strategists. In this sense, large picoeukaryotes have also been included in the r strategy in Mediterranean waters (Mouriño-Carballido et al. 2016), as they have not been split within the picoeukaryote community. Considering the approximation of domains, they occupy domain II, with low turbulence and high nutrients (Wyatt 2014, this study). During summer, the dominant condition in the southern Bay of Biscay was high temperature with shallow mixed layer depths, which coincides with low nutrients and low turbulence, thus conditions of domain III (Wyatt 2014, this study). Under such circumstances, *Synechococcus* grew well, as they are tolerant to nutrient stress (Duarte et al. 2000, Vázquez-Domínguez et al. 2008, Suggett et al. 2009) and high irradiances (Agustí 2004, Llabrés & Agustí 2006, Stawiariski et al. 2018). Thus, they may be included in the group called stress-tolerant S strategists (Grime 1977, Smayda & Reynolds 2001, Reynolds 2003). However, they can be mainly considered K strategists from the point of view of growth rate (Grime 1977), though they have also been considered halfway between r and K strategists in Mediterranean waters (Mouriño-Carballido et al. 2016). During autumn, *Prochlorococcus* flourish at the low irradiance and high temperature of domain IV (Wyatt 2014, this study), mostly with high turbulence and often low to medium nutrients, a domain generally void of phytoplankton (Margalef 1978, Smayda & Reynolds 2001, Reynolds 2003); thus, they can be included in an adaptive strategy called chronic stress-tolerant SS or void strategists (Reynolds 2006, Chisholm 2014). From the point of view of growth rate, they can also be included in the K strategy, with lower rates than picoeukaryotes and a good adaptation to low light conditions, and they have also been included in this strategy in Mediterranean waters (Mouriño-Carballido et al. 2016). In this case, however, they have been included in the low turbulence regime; thus, strictly speaking, they are a K strategist within a Margalef mandala (Margalef 1978).

Our conceptual intaglio is slightly different from the picophytoplankton mandala described for the Mediterranean Sea, where the main succession is as follows (Mouriño-Carballido et al. 2016): picoeukaryotes thriving under high turbulence and high nutri-

ent fluxes, *r* strategists; then, *Synechococcus* growing at medium NO_3 fluxes and mixing rates; and, finally, *Prochlorococcus* being abundant at low turbulence and low nutrient flux, *K* strategists. The small differences between the Mediterranean Sea and the southern Bay of Biscay, where *Synechococcus* can be a better *K* strategist than *Prochlorococcus*, as the lower NO_3 concentrations were reached in summer, could be related to different factors. (1) In the Mediterranean Sea study, the dissipation rates of turbulent kinetic energy and estimates of vertical diffusivity were available, and the diffusive fluxes of NO_3 could then be estimated, while in this study, NO_3 concentration was used as a proxy of nutrient fluxes; thus, we could be underestimating nutrient flux in the southern Bay of Biscay during summer. (2) Our proxy of turbulence is median irradiance in the mixed layer depth, and it has been noted that the mixed layer depth is not necessarily related to turbulence (Baines 1982, Mouriño-Carballido et al. 2016), though in highly contrasting hydrographic regimes, as those studied here, stratification could be a valid proxy for mixing (Kara et al. 2000, Mouriño-Carballido et al. 2016). (3) There are different genotypes of *Prochlorococcus* (Moore et al. 1995, Chisholm 2014 and references therein), and it has been shown that some Atlantic *Prochlorococcus* (i.e. eNATL) may be better adapted to low light conditions than their Mediterranean counterparts (i.e. eMED4). In fact, *Synechococcus* were also positively related to irradiance in Mediterranean waters, while *Prochlorococcus* were not (Mouriño-Carballido et al. 2016). (4) Under high irradiance, cell death may affect more pronounced *Prochlorococcus* than *Synechococcus* (Agustí 2004, Llabrés & Agustí 2006), which may lead to a dominance of the former when irradiance is high in the ecosystem. (5) Once a certain level of NO_3 concentration is reached, its concentration is probably less important than irradiance for the growth of cyanobacteria, as *Prochlorococcus* and *Synechococcus* may use both inorganic and recycled forms of nitrogen (Moore et al. 2007, Martiny et al. 2009, Wawrik et al. 2009, Berube et al. 2015). (6) There are differences between growth and grazing rates of protists growing on cyanobacteria or picoeukaryotes (Christaki et al. 1999, 2005, Gereia et al. 2018), with higher grazing rates occurring often when protists are feeding on *Synechococcus*. As grazing is a process that depends on temperature (Sarmiento et al. 2010), it can have an effect on the community composition of picophytoplankton. (7) The effect of viruses may also shape the community of cyanobacteria and picoeukaryotes (Baudoux et al. 2007)

Though molecular tools were not used in this study, they would have been very useful to study the seasonal succession of genotypes and groups of picophytoplankton in the southern Bay of Biscay. In this regard, different studies dealing with the composition of picophytoplankton in marine ecosystems have shown a diversified community of cyanobacteria (Martiny et al. 2009, Chisholm 2014, Berube et al. 2015) and picoeukaryotes (Biegala et al. 2003, Not et al. 2004, 2007), but some groups are more abundant than others in the southern Bay of Biscay (Cabello et al. 2016): prymnesophytes can be found in similar concentrations in surface waters and the DCM; chlorophytes can present 2 peaks, one above and one below the DCM; and pelagophytes can dominate in deep waters. Then, the use of these tools may help to disentangle the composition and succession of picophytoplankton genotypes in the southern Bay of Biscay during the year. They may also help to open the black box of these tiny microbes and explain their position within the intaglio suggested here, showing their different life strategies in the ecosystem.

Outlook

There may be several mechanisms driving the functional and structural succession of picophytoplankton in the southern Bay of Biscay. This study is focused on bottom-up factors such as irradiance, temperature, and NO_3 concentration. In winter, disturbance (i.e. high turbulence) brings nutrients to the system, whereas irradiance and temperature are the limiting factors. Under such circumstances, small picoeukaryotes are the best competitors because of the lower package effect of their pigments as compared to large picoeukaryotes, while their mitochondria might produce maintenance energy when the temperature is low as compared to cyanobacteria. In spring, large picoeukaryotes are abundant, and they could be boosted by well-lit conditions and medium nutrient concentrations. In summer, however, *Synechococcus* are dominant picophytoplankton, as they are well adapted to both well-lit and oligotrophic conditions. *Prochlorococcus* become conspicuous in autumn, probably because of their low irradiance requirements as compared to *Synechococcus*. A conceptual intaglio is proposed with a seasonal succession of ruderal *R* small picoeukaryotes growing in winter, competitive *C* large picoeukaryotes thriving well in spring, nutrient-stressed *S* *Synechococcus* abundant in summer, and stress-tolerant *SS* *Prochlorococcus* thriving in autumn.

Acknowledgements. This study was held during the stay of E.V.D. at the Centro Oceanográfico de Xixón IEO (Spanish Institute of Oceanography). E.V.D. especially acknowledges the helpful comments from Ángel López-Urrutia; the friendly treatment provided by the personnel at COX-IEO, particularly the help provided by Rafael González-Quirós, Eva Santos, and Itziar Munuera-Fernandez; and the BCN Nautic Cluster Association. Funding was supported by the projects BLUES, BLUE growth connects European Seas (UE, 2017-1-EL01-KA202-036307); MEECE, Marine Ecosystem Evolution in a Changing Environment (UE, No. 212085); Radiales (IEO); and METOCA, Predicción del balance metabólico de los océanos (METOCA, CTM2009-13882-MAR).

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Appendix

Table A1. Linear relationships ($y = a + \beta \times x$) between nitrate concentration (NO_3 , in $\mu\text{mol l}^{-1}$) and temperature (T , °C) (\pm SE) in the mixed layer of inshore, middle shelf, and offshore waters of the southern Bay of Biscay. Different superscript letters indicate significant differences between intercepts (linear mixed effects model, with random effects = station; ANOVA, $p < 0.05$)

Station	y	x	a	β	n	Adjusted r^2	p
Inshore	NO_3	T	4.34 ± 0.56^a	-0.24 ± 0.03	57	0.45	0.01
Middle shelf	NO_3	T	3.27 ± 0.60^b	-0.20 ± 0.04	57	0.34	0.01
Offshore	NO_3	T	3.10 ± 0.56^b	-0.18 ± 0.03	57	0.33	0.01

Editorial responsibility: Josep Gasol,
Barcelona, Spain

Submitted: August 15, 2017; Accepted: July 17, 2018
Proofs received from author(s): November 12, 2018