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Photoacclimation of picophytoplankton in the central Cantabrian Sea

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ABSTRACT: Photoacclimation of picophytoplankton was studied in the mixed layer of 3 stations in the central Cantabrian Sea (southern Bay of Biscay). Picophytoplankton chl *a*:carbon ratios (θ) presented minimum values during summer, when irradiance, temperature, and biomass of prokaryotes reached maximum values and inorganic nutrient concentrations were low. Conversely, the maximum θ were reached during winter, coincident with lowest annual irradiance but maximum concentration of inorganic nutrients and higher relative biomass of eukaryotes. Changes in θ were modeled using irradiance as an independent variable. Exponentially decreasing functions of θ with irradiance were significant only when the mean temperatures in the mixed layer were above 14°C. These functions presented light-saturated minimum ratios (θ_{min}) that decreased linearly with temperature and low-light maximum ratios (θ_{max}) that increased exponentially with temperature. Such relationships were used to establish an empirical model that reproduced the seasonality of picophytoplankton θ in the mixed layer, with minima in summer and maxima in winter. A maximum potential θ, $\theta_{N,T\text{-}max}$, was determined to estimate picophytoplankton growth rates in the central Cantabrian Sea. Combinations of picophytoplankton growth rates and biomass in the mixed layer were used to estimate areal picophytoplankton primary production rates in the euphotic zone that presented a bimodal seasonal cycle, with maxima in late winter (ca. 100 mg C m^{-2} d^{-1}) and in late autumn (>200 mg C m^{-2} d^{-1}) and mean annual values around 120 mg C m^{-2} d^{-1}.

KEY WORDS: Light · Temperature · Nutrients · Picophytoplankton · Chl *a*:carbon ratio · Modeling

INTRODUCTION

Phytoplankton photoacclimation processes have been assessed classically by changes in the carbon: chl *a* ratio in laboratory and field studies (i.e. Geider 1987, Geider et al. 1997), although recent studies preferentially report the chl *a*:carbon ratio (θ). Phytoplankton cells may change their θ in a physiological process called photoacclimation, which ensures that light harvesting and electron production match the cellular demands of ATP and NADPH (Behrenfeld et al. 2008). θ is highly variable and can depend on phytoplankton species composition (Geider 1987, Sathyendranath et al. 2009) and a diverse set of physicochemical variables such as irradiance (i.e. photon flux density) (Geider 1987, Arín et al. 2002), temperature (Geider 1987, Maxwell et al. 1994, Behrenfeld et al. 2005), and inorganic nutrient availability (Geider et al. 1998, Behrenfeld et al. 2005).

The decay of θ with irradiance can be modeled as an exponential function (Geider 1987, Behrenfeld et al. 2005). Such exponentially decreasing functions have low-light maximum chl *a*:carbon ratios (θ_{max}) that increase exponentially with temperature because of the positive effect of temperature on metabolism and light-saturated minimum chl *a*:carbon ratios (θ_{min}) that decrease with temperature because of the covariation between temperature and nutrient

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stress (Behrenfeld et al. 2005). Therefore, a third factor influencing photoacclimation is nutrient stress, with increasing nutrient availability having a positive effect on θ (Sakshaug et al. 1989, Cloern et al. 1995, Geider et al. 1998). All these photoacclimation changes have been studied and modeled under pure culture conditions (Geider 1987, Geider et al. 1997, 1998), and they are at the basis of photoacclimation models of phytoplankton growth rates and ocean productivity from satellite-based measurements (Behrenfeld et al. 2005). However, few photoacclimation studies have been conducted with *in situ* data (e.g. Behrenfeld et al. 2008).

Picophytoplankton are the most abundant primary producers within oceanic systems (Waterbury et al. 1979, Chisholm et al. 1988, Courties et al. 1994, Partensky et al. 1999). Their dominance is related to their advantage in terms of resource acquisition and subsequent growth and reproduction in oligotrophic conditions (Raven 1998). Picophytoplankton contribute to >20% of total primary production in the global ocean (Uitz et al. 2010), and higher percentages have been measured occasionally in coastal mesotrophic ecosystems (Cermeño et al. 2006, Morán 2007). Diverse prokaryotic and eukaryotic picophytoplankton groups can be easily resolved by flow cytometry (Gasol & Del Giorgio 2000), their size can be estimated from light scatter properties (e.g Calvo-Díaz & Morán 2006), and this size can be transformed into cellular carbon (Worden et al. 2004). Thus, θ of picophytoplankton can be precisely estimated as the quotient between chl *a* concentration in the <2 µm fraction and picophytoplankton biomass.

The central Cantabrian Sea is a temperate coastal ecosystem located in the southern Bay of Biscay (northeastern Atlantic). Picophytoplankton there comprise between 10 and 60% of total chl *a* (Calvo-Díaz & Morán 2006, Morán 2007, Calvo-Díaz et al. 2008), and its dominance shifts from prokaryotes in late summer and early autumn to eukaryotes in late winter and early spring (Calvo-Díaz & Morán 2006, Morán 2007, Calvo-Díaz et al. 2008). In surface waters, picophytoplankton θ presents a typical seasonal pattern, with low values in summer and high values in winter (Calvo-Díaz & Morán 2006, Morán 2007). These θ changes have been related to irradiance differences yielding higher chl *a* synthesis in winter and to changes in the relative contribution of prokaryotes and eukaryotes to total picophytoplankton biomass (Calvo-Díaz et al. 2008).

The mixed layer is a region in the upper ocean where there is little variation in temperature and density (Kara et al. 2000) and where frequently vigorous turbulent mixing and important biological processes take place (de Boyer Montégut et al. 2004). For a given space and time, the mixed layer of the central Cantabrian Sea is a rather homogeneous physicochemical environment. However, the mixed layer depth over the continental shelf varies annually between non-existent or just a few meters in summer (and occasionally in winter because of haline stratification) and the whole water column through the bottom in winter. Thus, picophytoplankton thriving in this mixed layer are subject to strongly different conditions of irradiance, temperature, and concentration of inorganic nutrients in different periods of the annual cycle. Here we analyze 8 yr of measurements of physicochemical and biological variables in the mixed layer at 3 stations located in the central Cantabrian Sea continental shelf with the aim to (1) understand the influence of temperature, irradiance, nutrient concentration, and community composition on the *in situ* θ of picophytoplankton; (2) study the applicability of a photoacclimation model (Behrenfeld et al. 2005) with *in situ* measurements to derive an empirical photoacclimation model of the variation of picophytoplankton θ; and (3) determine the growth and primary production rates of picophytoplankton based on θ values.

MATERIALS AND METHODS

Physicochemical and environmental variables

Sampling was carried out on board the RV 'José de Rioja' from April 2003 to September 2010. Samples were withdrawn between the surface and 150 m depth at 3 stations located in the central Cantabrian Sea: a coastal station (43.58° N, 5.61° W), a shelf station (43.67° N, 5.58° W), and an open sea station (43.78° N, 5.55° W).

Photosynthetic active radiation (PAR) at the surface of the 3 stations was assumed to be equal to the PAR obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) (mol photons m^{-2} d^{-1}) for the day of sampling (180 × 180 km^2 resolution) transformed to µmol photons m^{-2} h^{-1} with a factor of 1/DL, where DL is the daylength in hours during the day of sampling. Because of the lack of some data from MODIS, a recovered PAR data series (R-PAR, mol photons m^{-2} d^{-1}) was established with the PAR obtained from MODIS and by filling the gaps with the monthly PAR obtained from SeaWiFS for the same area.

For each sampling date and station, a vertical light attenuation coefficient (k_d, m^{-1}) was calculated by

measuring PAR values at 1 m depth intervals in the water column with a spherical quantum sensor (Biospherical QSP-2200). Irradiance reaching each sampling depth (E, mol photons m^{-2} h^{-1}) was estimated as the product between the R-PAR (mol photons m^{-2} h^{-1}) and the exponential decaying Beer-Lambert law, $E = \text{R-PAR} \exp^{-k_d z}$, where z (m) is the sampling depth and k_d (m^{-1}) is the attenuation coefficient. The euphotic depth was the depth where irradiance reached 1% of surface irradiance.

Temperature and salinity were acquired *in situ* with a CTD probe (SeaBird 25), and seawater samples were withdrawn with 5 l Niskin bottles in a rosette sampler attached to a CTD. Fractionated chl *a* concentrations were obtained after sequential filtration of 100 ml samples through polycarbonate filters with 20, 2, and 0.2 µm pore-sizes (47 mm, Millipore). Small fractions of *Prochlorococcus* sp., *Synechococcus* sp., and small picoeukaryotes (<10%) were retained by the 2 µm filters, while most large picoeukaryotes were retained by the 2 µm filters because of their size (slightly larger than 2 µm). However, because of the low abundance of large picoeukaryotes at the stations, the overestimation of picophytoplankton chl *a* (i.e. chl *a* passing through 2 µm and retained by 0.2 µm) was of minor importance (Calvo-Díaz & Morán 2006). Filters containing picophytoplankton chl *a* were frozen until analysis, which was performed within 1 wk after sampling. Pigments were extracted in 90% acetone for 24 h in the dark at 4°C, and chl *a* was measured with a Perkin Elmer spectrofluorometer calibrated with pure chl *a* (Neveux & Panouse 1987). Samples for nutrient analysis were frozen (–20°C), and inorganic nutrient concentration was determined within 6 mo of sampling with a Technicon autoanalyzer by following standard methods (Grasshoff et al. 1999).

Picophytoplankton samples (1.8 ml) were preserved with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration). Samples were frozen at –80°C until they were analyzed in the laboratory. The analysis was carried out with a flow cytometer (FACSCalibur, Becton Dickinson) equipped with an argon laser emitting at 488 nm. Autotrophic cells were separated into prokaryotes and eukaryotes based in their fluorescence and light scatter signals (Calvo-Díaz & Morán 2006). The abundance of the different picophytoplankton groups was determined after a volumetric calibration of the flow rate that was performed daily. A solution of 1 µm fluorescent latex beads (F-13081, Molecular Probes) was added to the samples as an internal standard of fluorescence and side scatter (Calvo-Díaz & Morán 2006), and all cellular variables were related to bead values. 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 the following conversion factors: 230 fg C μm^{-3} for *Synechococcus* sp., 240 fg C μm^{-3} for *Prochlorococcus* sp., and 237 fg C μm^{-3} for picoeukaryotes (Worden et al. 2004). Furthermore, θ of picophytoplankton were estimated in each sample as the concentration of chl *a* in the picophytoplankton fraction (<2 µm) divided by the biomass of picophytoplankton.

The mixed layer was established for each date of sampling at each station with a criterion of a change in density of 0.05 kg m^{-3} within 5 m (Mitchell & Holm-Hansen 1991), and the different variables measured at each station and depth were averaged for this mixed layer.

Photoacclimation modeling

The θ of picophytoplankton in the mixed layer was modeled within 3 ranges of temperature (<14°C, 14 to 19°C, and >19°C) as an exponential decaying function of irradiance (Cloern et al. 1995, Behrenfeld et al. 2002, 2005), such as:

$$\theta = \theta_{min} + (\theta_{max} - \theta_{min}) \exp^{-3E_g} \quad (1)$$

where θ is the chl *a*:carbon ratio of picophytoplankton in the mixed layer, θ_{max} is a low-light maximum ratio, θ_{min} is a light-saturated minimum ratio, and E_g was the median irradiance in the mixed layer. In this sense, θ_{min} and θ_{max} are constants estimated by linearization (model I regression) with the function:

$$\theta = a + b \exp^{-3E_g} \quad (2)$$

where $a = \theta_{min}$, and $b = \theta_{max} - \theta_{min}$.

In addition, θ_{min} was related to the mean temperature in the mixed layer for each of the 3 temperature ranges with a linear model, such as:

$$\theta_{min} = c + dT \quad (3)$$

where T was the mean temperature in the mixed layer for each of the 3 temperature ranges, and c and d are constants estimated by linear regression (model I regression).

Similarly, θ_{max} was related to the mean temperature in the mixed layer for each of the 3 temperature ranges with an exponential model, such as:

$$\theta_{max} = f \exp^{hT} \quad (4)$$

where T was the mean temperature in the mixed layer for each of the 3 temperature ranges, and f

and h are constants estimated by linearization (i.e. $\ln \theta_{max} = \ln f + hT$, model I regression).

The linear model between θ_{min} and temperature in the mixed layer was related to the positive linear relationship between temperature and nutrient stress in the mixed layer and the negative linear relationship between θ_{min} and increasing nutrient stress (Behrenfeld et al. 2005). However, the exponential model between θ_{max} and temperature in the mixed layer was related to temperature-independent biophysical reactions involved in light harvesting and the exponential temperature dependence of biochemical reactions (Geider 1987).

According to the photoacclimation model described above (Eq. 1), an empirical photoacclimation model of the variation of θ of picophytoplankton in the mixed layer of the central Cantabrian Sea was established by taking into account, on the one hand, the irradiance and temperature in the mixed layer during the period of sampling and, on the other hand, the relationship between θ_{min} and θ_{max} and the mean temperatures in this mixed layer. Model performance was evaluated in terms of the root mean square deviation [$RMSD = (1/(N - 1)\ \Sigma_{i=1}^{N}\ (\theta_{mod(i)} - \theta_{Is(i)})^2)^{0.5}$], where θ_{mod} corresponded to the modeled values and θ_{Is} corresponded to the *in situ* values (Piñeiro et al. 2008). RMSD describes the adjustment of the observed values to the model, is expected to be small with reference to the measurements, and is used to compare different data modeled with the same units.

Picophytoplankton growth and production rates

To estimate the picophytoplankton growth rates from *in situ* θ, it was assumed that increases in nutrient and temperature stress cause decreases in the picophytoplankton growth rates that are paralleled by decreases in θ (Geider 1987, Cloern et al. 1995, Behrenfeld et al. 2005). This physiological response was quantified by dividing the *in situ* θ of picophytoplankton in the mixed layer by a maximum potential ratio ($\theta_{N,T\text{-max}}$) for a given irradiance in the same mixed layer, and growth rates were estimated as (Behrenfeld et al. 2005):

$$\mu = \mu_{max}\ [\theta{:}\ \theta_{N,T\text{-max}}]\ (1 - \exp^{-3E_g}) \quad (5)$$

where μ_{max} was assumed to be 2 d^{-1} (Behrenfeld et al. 2005), θ was the chl *a*:carbon ratio in the mixed layer, $\theta_{N,T\text{-max}}$ was defined as the 85% envelope of the different θ measured *in situ* in the mixed layer of the 3 stations in the central Cantabrian Sea estimated by quartile regression (tau = 0.85), and $1 - \exp^{-3E_g}$ is from the decreasing relationship between growth rate and light that is related to the fact that physiological adjustments in pigmentation are insufficient to maintain constant levels of light absorption (Behrenfeld et al. 2005).

Volumetric net picophytoplankton primary production (NPP) in the mixed layer of the 3 stations was estimated as picophytoplankton growth rates times biomass (B), NPP (mg C m^{-3} d^{-1}) = μ (d^{-1}) B (mg C m^{-3}), and areal primary production rate, PP (mg C m^{-2} d^{-1}), was obtained as the product between the volumetric NPP and the minimum depth between the mixed and euphotic layer depth (m). To ascertain the accuracy of the picophytoplankton growth and areal primary production rates, the picophytoplankton growth rates modeled in 2003 in the euphotic layer of the shelf station were compared with those estimated in the same samples with the ^{14}C method (Morán 2007). All data processing and statistical analysis were performed with R (R Development Core Team 2011).

RESULTS

Environmental conditions in the mixed layer of the central Cantabrian Sea

Monthly PAR at the sea surface (daily averages throughout the day) varied between 0.7 mol photons m^{-2} h^{-1} in winter and 3.4 mol photons m^{-2} h^{-1} in summer. The mixed layer depths frequently extended to the bottom of the 3 stations in winter (Table 1), while stratification started at the surface in summer (i.e. apparent mixed layer depths <5 m). Irradiance (E_g) was close to 2.2 mol photons m^{-2} h^{-1} in summer and varied between 0.2 and 0.5 mol photons m^{-2} h^{-1} in winter (Fig. 1 A–C). Temperatures were close to 11°C in winter, while in summer they were slightly lower near the coast, ca. 20°C, than at the other stations, ca. 21°C (Fig. 1D–F).

Chl *a* presented a seasonal cycle with 2 maxima, one in springtime and the other in autumn (Fig. 1G–I). The mean chl *a* concentration was higher at the coastal station than at the other 2 stations (Table 1). NO_3 varied between low concentrations in summer, ca. 0.1 µmol l^{-1}, and high concentrations in winter, >4 µmol l^{-1} (Fig. 2A–C). The mean NO_3 concentration was significantly higher at the coastal station than at the shelf and open sea stations (Table 1), and there was a negative relation between temperature and NO_3 concentration at the 3 stations ($r^2 > 0.44$, $n > 140$, $p < 0.01$). The community of picophyto-

Table 1. Mean and range of different environmental and biological variables measured at the 3 stations in the central Cantabrian Sea. MLD: mixed layer depth, k_d: light/irradiance attenuation coefficient, z_{eu}: euphotic layer depth, E_g: median irradiance, *T*: temperature, $Chl_{0.2}$ *a*: picophytoplankton chl *a*, Biomass: picophytoplankton biomass, PProk: percentage of prokaryotic biomass of picophytoplankton, θ: chl *a*:carbon ratio of picophytoplankton, μ: picophytoplankton growth rate, PP: areal picophytoplankton primary production. Significant differences (Tukey's HSD: $p < 0.05$): *between Stns 1 and 2, **between coastal and open sea stations, ***between shelf and open sea stations

Variable	Coastal station	Shelf station	Open sea station
MLD (m)	11 (1–20)*,**	30 (1–100)***	56 (1–150)
k_d (m^{-1})	0.129 (0.044–0.404)*,**	0.092 (0.055–0.171)***	0.086 (0.057–0.144)
z_{eu} (m)	42.1 (11.4–102)*,**	53.0 (26.8–75.5)***	55.5 (31.8–80.9)
E_g (mol photons m^{-2} h^{-1})	1.50 (0.15–3.38)*	1.34 (0.02–3.38)	1.06 (0.04–3.10)
T (°C)	15.6 (11.2–21.4)	16.2 (11.3–23.4)	16.3 (11.8–23.4)
NO_3 (μmol l^{-1})	2.76 (0.16–11.55)*,**	1.85 (0.14–7.78)	1.83 (0.11–7.39)
chl *a* (mg chl *a* m^{-3})	1.07 (0.08–6.13)*,**	0.69 (0.08–3.42)	0.53 (0.09–2.32)
$chl_{0.2}$ *a* (mg chl *a* m^{-3})	0.27 (0.02–1.22)*	0.23 (0.04–1.68)	0.18 (0.03–0.66)
Biomass (mg C m^{-3})	10.77 (0.57–54.13)*,**	7.59 (1.11–31.64)	6.77 (1.50–22.81)
PProk (% picophytoplankton biomass)	30 (1.2–75)*	40 (1–88)	43 (3–88)
θ (mg chl *a:* mg C)	0.034 (0.006–0.118)	0.034 (0.006–0.103)	0.030 (0.006–0.102)
μ (d^{-1})	1.23 (0.25–4.02)*,**	1.03 (0.06–4.16)***	0.72 (0.14–2.72)
PP (mg C m^{-2} d^{-1})	113.4 (2.33–457.42)	125.15 (1.95–114.67)	118.0 (0.01–523.63)

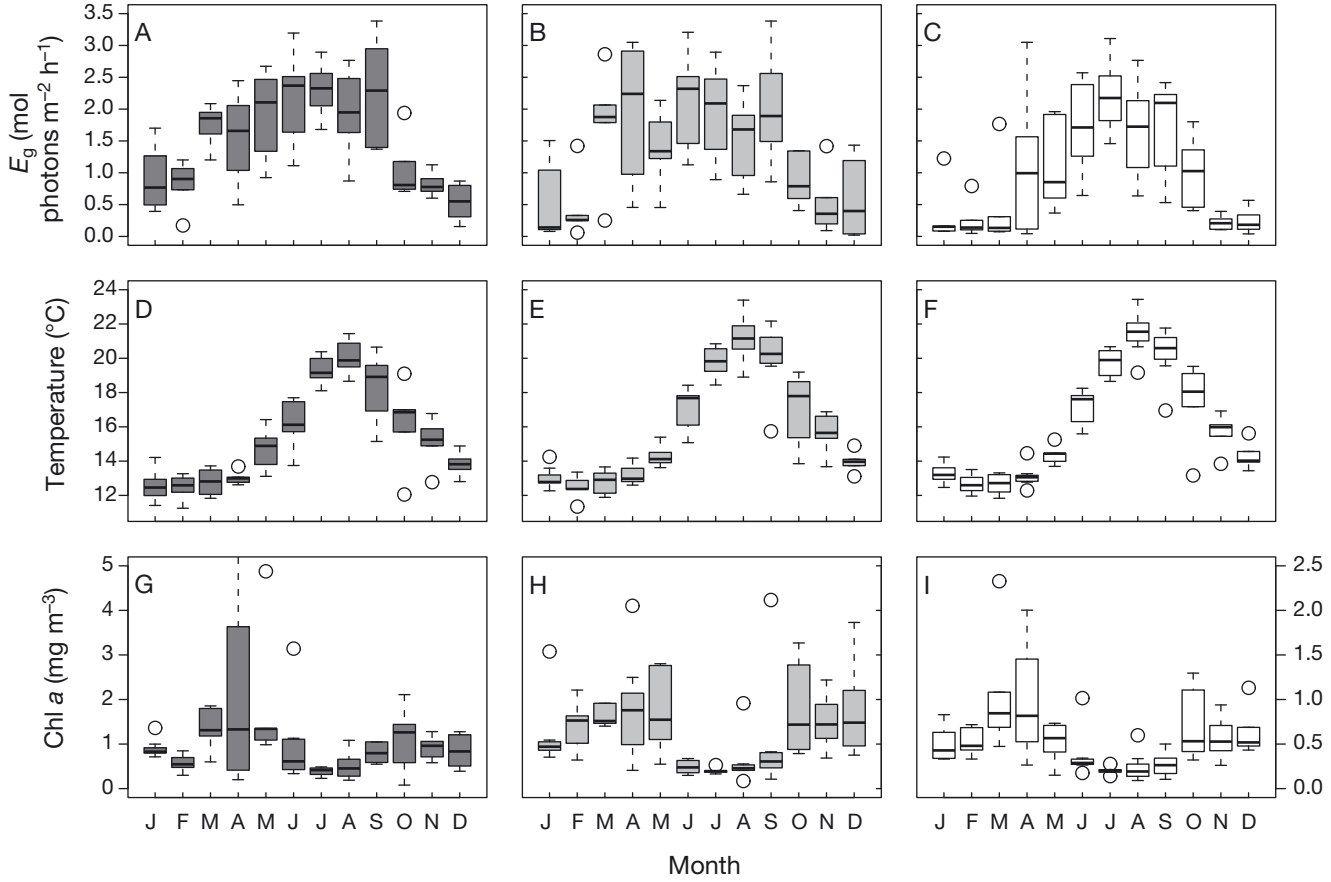

Fig. 1. Monthly variability of (A–C) irradiance, (D–F) temperature, and (G–I) chl *a* concentration in the mixed layer of the coastal (dark grey bars), shelf (light grey bars), and open sea (white bars) stations. Note change of scale in panels H and I. Box plots show the median values (line), 25 and 75% quantiles (box), 5 and 95% quantiles (whiskers), and outliers (>1.5 interquartile range; circles)

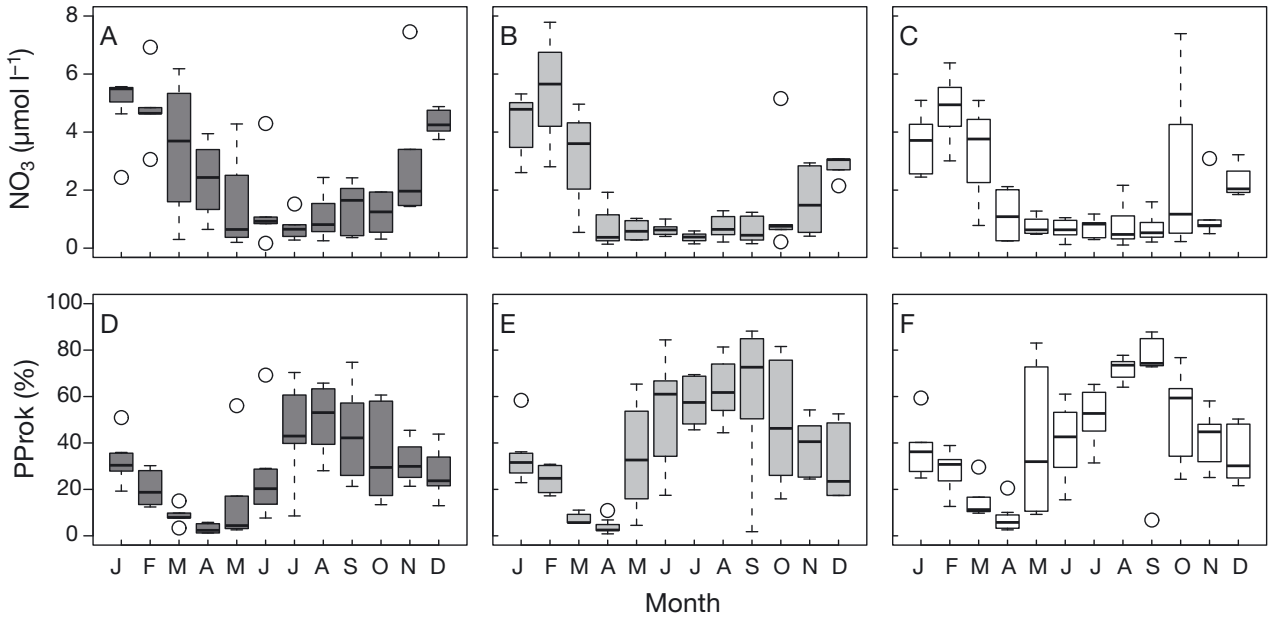

Fig. 2. (A–C) NO_3 concentration and (D–F) biomass of prokaryotes as percentage of picophytoplankton biomass (PProk) in the mixed layer of the coastal (dark grey bars), shelf (light grey bars), and open sea (white bars) stations. Box plots as in Fig. 1

plankton was mainly composed of eukaryotes in spring, >90% of biomass, and by prokaryotes in autumn, when *Synechococcus* sp. and *Prochlorococcus* sp. accounted for >40% of total picophytoplankton biomass at the coastal station and >60% at the shelf and open sea stations (Fig. 2D–F).

Picophytoplankton chl *a* reached minimum values in summer, ca. 0.1 mg chl *a* m^{-3}, and maximum values in late autumn, 0.3 mg chl *a* m^{-3} (Fig. 3A–C). The mean picophytoplankton chl *a* was significantly higher at the coastal station than at the other stations (Table 1). Picophytoplankton biomass varied between minimum values in late winter, near 4 mg C m^{-3}, and maximum values in early autumn, >9 mg C m^{-3} (Fig. 3D–F). The mean picophytoplankton biomass presented an open sea to coastal gradient and was significantly lower at the open sea station (Table 1). The θ of picophytoplankton reached minimum values in summer, ca. 0.01 mg chl *a* mg C^{-1}, and maximum values in winter, ca. 0.05 mg chl *a* mg C^{-1} (Fig. 3G–I). The mean θ were not significantly different between stations (Table 1), even if we consider the different months of sampling (ANCOVA, $p > 0.1$).

Influence of irradiance and temperature on θ of picophytoplankton

As predicted by photoacclimation modeling, θ of picophytoplankton decreased exponentially with increasing irradiance in the mixed layer, and such negative relationships were significant only when the mean temperatures were above 14°C (Fig. 4).

Once the effect of irradiance is taken into account with the photoacclimation model, there were no significant differences in θ_{min} and θ_{max} between stations (ANOVA, $p > 0.2$). However, we further explored the dependence of θ_{min} and θ_{max} on temperature. Light-saturated chl *a*:carbon ratios (θ_{min}) reached minimum values in summer, near 0.014 mg chl *a* mg C^{-1}, when the mean temperature in the mixed layer of the 3 stations was above 19°C. However, θ_{min} reached maximum values in winter, near 0.04 mg chl *a* mg C^{-1}, when the mean temperature in the mixed layer of the stations was below 14°C. Thus, θ_{min} was negatively related to the mean temperature (Fig. 5A), with a slope of –0.0035 $°C^{-1}$ (Table 2, CI –0.0044 to –0.0026), and no significant differences in intercepts or slopes were found between stations (ANCOVA, $p > 0.85$). Low-light maximum chl *a*:carbon ratios (θ_{max}) varied in winter between 0.054 and 0.078 mg chl *a* mg C^{-1} at the shelf and coastal stations, respectively, and varied in summer between 0.084 and 0.235 mg chl *a* mg C^{-1} at the open sea and coastal stations, respectively. Thus, θ_{max} increased with the mean temperature in the mixed layer (Fig. 5B), with an exponent of 0.114 $°C^{-1}$ (Table 2, 95% CI 0.009 to 0.218), and no significant differences in intercepts or exponents were found between stations (ANCOVA, $p > 0.09$).

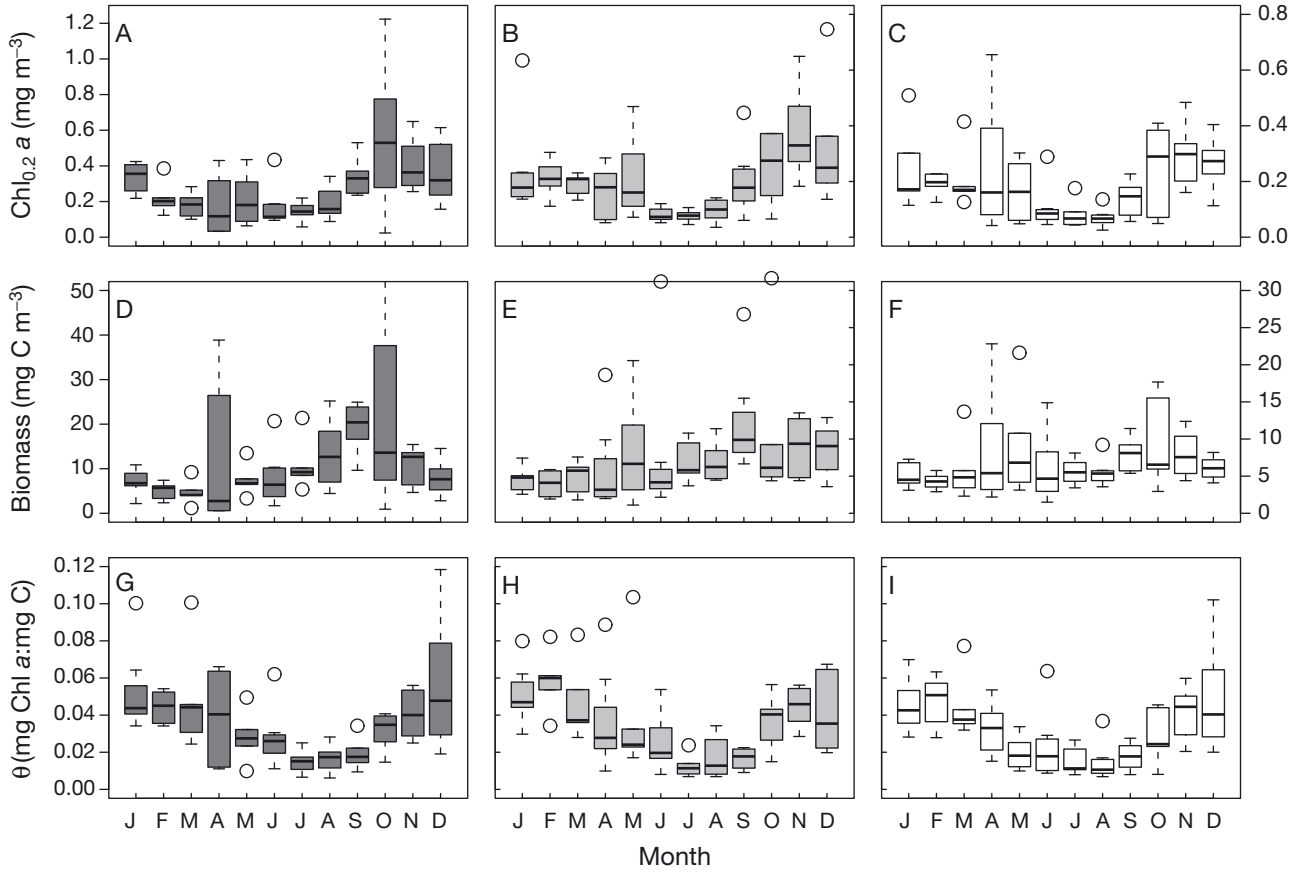

Fig. 3. Monthly variability of (A–C) picophytoplankton chl *a* (chl$_{0.2}$ *a*), (D–F) biomass, and (G–I) chl *a*:carbon ratios (θ) in the mixed layer of the coastal (dark grey bars), shelf (light grey bars), and open sea (white bars) stations. Note change of scale in panels B, C, E, and F. Box plots as in Fig. 1

Considering the irradiance and temperature in the mixed layer of the central Cantabrian Sea and the relationship between θ_{min} and θ_{max} and the mean temperature in this mixed layer, the empirical photoacclimation model explained between 33 and 38% of the variability of θ of picophytoplankton (Table 3). This empirical photoacclimation model significantly reproduced the *in situ* θ (Fig. 6A), as the relationships between *in situ* and modeled values presented slopes not significantly different from 1 and intercepts not significantly different from 0 (Table 3). In addition, the model depicted a seasonality of θ of picophytoplankton, with maximum values in winter and minimum values in summer that matched the seasonality of the *in situ* observations (Fig. 6B). Finally, the RMSD was 0.019 (bias −4.1%, precision 11.9%) in the coastal station, 0.018 (bias −21%, precision 8.4%) in the shelf station, and 0.016 (bias −17.6%, precision 10.7%) in the open sea station; thus, model performance was similar for all the stations.

Picophytoplankton growth and primary production rates

Picophytoplankton growth rates were estimated with a maximum potential $\theta_{N,T\text{-max}} = 0.049 + 0.021 \exp^{-3E_g}$ (thau = 0.85). The comparison between modeled and measured picophytoplankton growth rates in the mixed layer of the shelf station gives a relationship that was marginally significant, $\mu = 0.38 + 0.87\ ^{14}C\ \mu$ (n = 8, $r^2 = 0.32$, p = 0.08). Picophytoplankton growth rates in the mixed layer reached minimum values in summer, ca. 0.5 d^{-1}, and maximum values in early spring, ca. 1 d^{-1} (Fig. 7A–C), and the mean picophytoplankton growth rate was significantly lower at the open sea station, ca. 0.7 d^{-1}, than at the shelf and coastal stations, >0.8 d^{-1} (Table 1).

Picophytoplankton primary production rates in the mixed layer reached minimum values in summer, varying between 2.75 and 5.85 mg C m^{-3} d^{-1} at the open sea and coastal stations, respectively, whereas

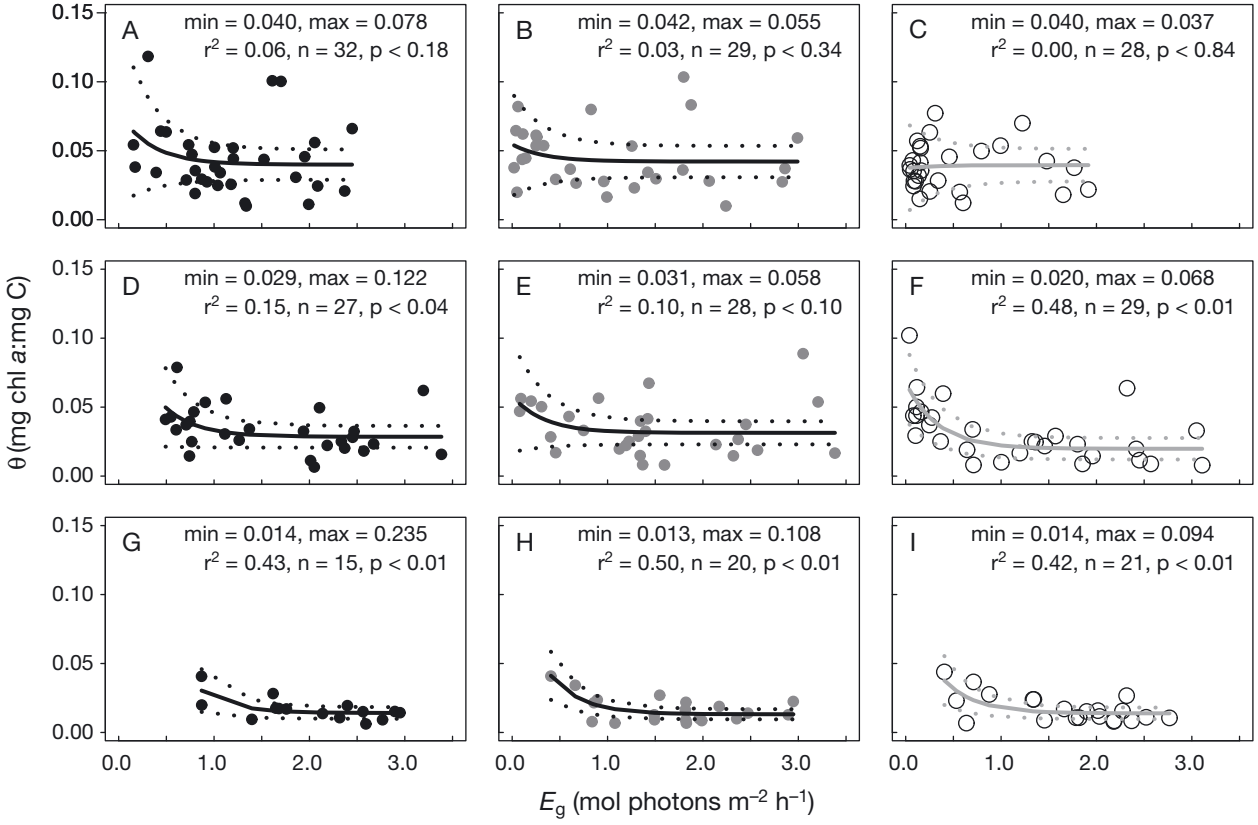

Fig. 4. Fits of the photoacclimation model between chl *a*:carbon ratios (θ) of picophytoplankton and the irradiance in the mixed layer of the coastal (●), shelf (●), and open sea (○) stations. The fits were established for 3 ranges of temperature in the mixed layer: <14°C (A–C), 14 to 19°C (D–F), and >19°C (G–I). min: light-saturated minimum ratios, max: low-light maximum ratios. Dotted line corresponds to 1 standard error of the fitted curves

maximum production rates were found in autumn and spring, varying between 8.8 and 20.9 mg C m^{-3} d^{-1} at the open sea and coastal stations, respectively. The mean picophytoplankton primary production rate was significantly lower at the open sea station, 4.3 mg C m^{-3} d^{-1}, than at the shelf and coastal stations, >7 mg C m^{-3} d^{-1} (Table 1). Areal picophytoplankton primary production rates reached minimum values in late spring or summer, near 40 mg C m^{-2} d^{-1} (Fig. 7D–F), and reached maximum values in late summer or autumn, varying between 250 and 360 mg C m^{-2} d^{-1}, at the open sea and shelf stations, respectively. Because of the higher mixed layer integration depths, areal picophytoplankton primary production rates were not different between stations, and the annual means varied between 113.4 and 125.15 mg C m^{-2} d^{-1} at the coastal and shelf stations, respectively (Table 1).

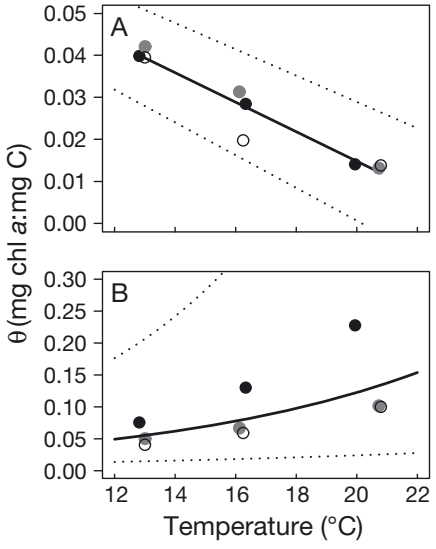

Fig. 5. Relationships between θ_{min} and θ_{max} ratios (A: light-saturated minimum ratios, black line; B: low-light maximum ratios, black line) and the mean temperatures in the mixed layer of the central Cantabrian Sea for the 3 ranges of temperature considered (<14°C, 14 to 19°C, >19°C) in the coastal (●), shelf (●), and open sea (○) stations. Dotted lines correspond to 1 standard error of the fitted curves

Table 2. Relationships between the light-saturated minimum chl *a*:carbon ratios (θ_{min}) and low-light maximum chl *a*:carbon ratios (θ_{max}) (SE in parentheses) and the mean temperature (T) in the mixed layer of the sampling stations

Ratio	Relationship	r^2	n	p
θ_{min}	θ_{min} = 0.085 (0.007) – 0.0035 (0.0004) T	0.91	9	<0.01
θ_{max}	θ_{max} = 0.012 (0.02) exp [0.114 (0.04) T]	0.42	9	<0.04

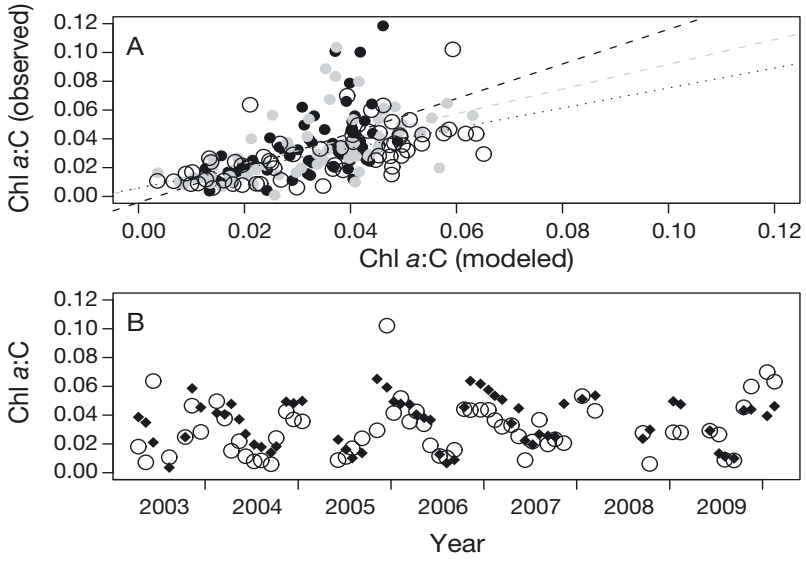

Fig. 6. Relationships (A) between modeled and *in situ* chl *a*:carbon ratios (θ) of picophytoplankton in the mixed layer of the coastal (black circles and slashed line), shelf (grey circles and slashed line), and open sea (open circles and dotted line) stations. Open sea time series (B) of *in situ* (open circles) and modeled (diamonds) θ of picophytoplankton determined with the empirical photoacclimation model

DISCUSSION

Influence of environmental conditions on θ of picophytoplankton

Picophytoplankton θ in the mixed layer of the central Cantabrian Sea presented a seasonality, with minimum values in summer and maximum values in winter likely reflecting physiological changes aimed at minimizing the influence of light availability on growth (Geider 1987, MacIntyre et al. 2002, Behrenfeld et al. 2005). However, the winter values of ca. 0.1 mg chl *a* mg C^{-1} are unrealistically high and should be considered as outliers, possibly because of an underestimation of picophytoplankton biomass from a higher presence of large picoeukaryotes that are less concentrated and more difficult to determine with accuracy by flow cytometry. Alternatively, there could be an overestimation of chl *a* if some of the nano- and microphytoplankton have lost a fraction of their chloroplasts during filtration. Because of the lower median irradiance in the mixed layer, θ was higher than that found in surface waters of the same system that varied between 0.003 mg chl *a* mg C^{-1} in summer and 0.06 mg chl *a* mg C^{-1} in winter (Calvo-Díaz et al. 2008) and that found in the upper 20 m of the Ría de Vigo that varied between 0.006 mg chl *a* mg C^{-1} in summer and 0.025 mg chl *a* mg C^{-1} in winter (Cermeño et al. 2005).

Over a broader context, mean θ of picophytoplankton in the central Cantabrian Sea (0.03 mg chl *a* mg C^{-1}) was 3-fold larger than in laboratory experiments (Behrenfeld et al. 2005). If θ are to be used in the estimation of primary production from carbon-based models (i.e. Behrenfeld et al. 2005, Westberry et al. 2008), we suggest accounting for these differences in studies of primary productivity of picophytoplankton (i.e. Uitz et al. 2008, 2009, 2010). However, a fraction of these differences can be related to the volume-to-carbon conversion factors, as there are studies suggesting both higher (350 fg C μm^{-3}) and lower (220 fg C μm^{-3}) conversion factors for *Synechococcus* sp. and picoeukaryotes, respectively (Dall'Olmo et al. 2011,

Table 3. Comparison between *in situ* chl *a*:carbon ratios (θ_{Is}) at the coastal, shelf, and open sea stations and modeled chl *a*:carbon ratios (θ_{Pred}) according to the empirical model established for the 3 stations (SE in parentheses). None of the slopes or intercepts were significantly different from 1 and 0, respectively (p > 0.05)

Ratio	Relationship	r^2	n	p
Coastal with empirical model	θ_{Is} = –0.004 (0.006) + 1.07 (0.17) θ_{Pred}	0.33	77	<0.01
Shelf with empirical model	θ_{Is} = –0.002 (0.006) + 0.99 (0.15) θ_{Pred}	0.35	77	<0.01
Open sea with empirical model	θ_{Is} = 0.001 (0.005) + 1.01 (0.14) θ_{Pred}	0.38	77	<0.01

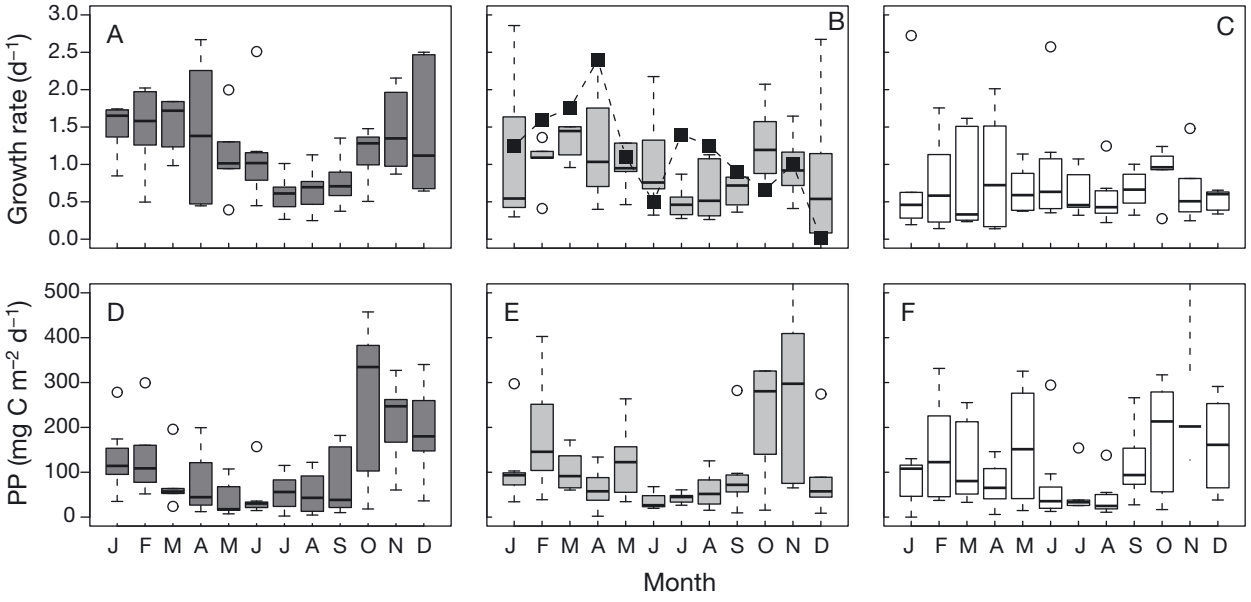

Fig. 7. Monthly variability of (A–C) picophytoplankton growth rate and (D–F) areal primary production rate (PP) measured in the mixed layer of the coastal (dark grey bars), shelf (light grey bars), and open sea (white bars) stations located in the central Cantabrian Sea. The squares joined by dashed lines in panel B correspond to growth rates determined by ^{14}C at the shelf station in 2003 (Morán 2007). Box plots as in Fig. 1

Martinez-Vicente et al. 2013). Had we used a slightly lower volume-to-carbon conversion factor for picoeukaryotes (ca. 10%), winter θ would still be higher than that reported for satellite-based measurements, while a higher conversion factor for *Synechococcus* would have resulted in lower (ca. 20%) summer θ values, much closer to the satellite-based measurements. However, there could still be large differences between measured and satellite-estimated picophytoplankton θ caused by uncertainties about the role of different seawater constituents in light backscattering, as for example small inorganic particles (Stramski et al. 2004), and the relationships between backscattering and carbon used in the satellite-based studies (Martinez-Vicente et al. 2013). Thus, more studies are necessary to constrain the spatio-temporal variability in volume-to-carbon conversion factors and the environmental drivers of cellular carbon content.

Modeling the photoacclimation of picophytoplankton in the mixed layer

In this study, θ of picophytoplankton was related to the median irradiance in the mixed layer depth, as has been proposed in other studies (Behrenfeld et al. 2005, Westberry et al. 2008). This is related to the light-dependent signal for chlorophyll synthesis that is intermediated by the plastoquinone pool acting as a switch; once phytoplankton reaches saturating light levels, the reduced plastoquinone pool stops the signal for chlorophyll synthesis (Escoubas et al. 1995). Overall, we found better adjustments with median than with other irradiances (data not shown), which is coincident with good relationships between photoacclimation and the light level at the bottom of the mixed layer (Behrenfeld et al. 2002) but is in contrast to high light acclimation of phytoplankton in well-mixed waters (Moore et al. 2006). However, the slight differences between stations in the fits between θ_{max} and temperature could suggest variations in the photoacclimation between different groups of picophytoplankton (Moore et al. 2006).

Our study shows the applicability of an exponentially decreasing function of θ with irradiance (Behrenfeld et al. 2005) to *in situ* picophytoplankton θ. The decrease in picophytoplankton θ is because of the lower requirements of cellular chl *a* for light harvesting when the irradiance reaching the cell is high (Geider 1987, Behrenfeld et al. 2005, Westberry et al. 2008). Determination coefficients of our linear regression models increased with mean temperature because of the interaction between irradiance, temperature, and nutrient availability in the mixed layer. In winter, temperatures in the mixed layer were generally <14°C, NO_3 concentration was generally >4 µmol l^{-1}, and the mixed layer was usually deeper

than the euphotic layer. Consequently, picophytoplankton spent an important fraction of the growing period under light-limiting conditions and must have high θ to prioritize light harvesting. In contrast, in summer, the temperature in the mixed layer was >14°C, NO_3 concentration was <1 µmol l^{-1}, and the euphotic layer was larger than the mixed layers. Then, picophytoplankton were under nutrient-depleted conditions and must have low θ to prioritize catabolic and anabolic metabolisms. Therefore, picophytoplankton adapted their θ during the year to match light harvesting with ATP and NADPH demands (Behrenfeld et al. 2008). To the best of our knowledge, this is the first report of the applicability of the photoacclimation model under *in situ* conditions, which gives further confidence to satellite-derived estimates of ocean primary productivity of phytoplankton (i.e. Behrenfeld et al. 2005).

Fits of the photoacclimation model at the coastal, shelf, and open sea station presented light-saturated minimum chl *a*:carbon ratios (θ_{min}) that were negatively related with temperature in the mixed layer. This negative covariation can be explained by the negative relation between temperature and inorganic nutrient concentration at the 3 stations and the negative effect of nutrient stress on θ of phytoplankton (Laws & Bannister 1980, Geider et al. 1998, Behrenfeld et al. 2005). However, the intercept of the relationship between θ_{min} and temperature was nearly 4-fold higher and the negative slope nearly 10-fold larger than satellite-based measurements (Behrenfeld et al. 2005). Such disparity could be related to differences between bulk phytoplankton properties measured on surface waters from satellites and the same properties measured from picophytoplankton at different depths and integrated for the mixed layer. Similar variations have been reported between satellite-based and laboratory-based θ of phytoplankton, which have been related to the rare replicability of laboratory conditions (i.e. nutrient-saturated, exponentially growing cultures) for all members of any natural phytoplankton community (Behrenfeld et al. 2005).

As has been observed in laboratory cultures (Geider 1987, Cloern et al. 1995), fits of the photoacclimation model at the coastal, shelf, and open sea stations presented low-light maximum chl *a*:carbon ratios (θ_{max}) that increased exponentially with temperature. In this regard, the positive effect of temperature on the θ_{max} could be related to the exponential enhancement of metabolic reactions with temperature (Brown 2004) and the concept of 'resource allocation' (Behrenfeld et al. 2008), which implies that the fraction of enzymatic machinery devoted to metabolic reactions of maintenance must increase when temperature in the mixed layer was low, and this would be reflected in an exponential increase of the cellular carbon content. However, the exponent found in the central Cantabrian Sea, 0.114 $°C^{-1}$, was lower than that described for satellite-based measurements, 0.215 $°C^{-1}$ (Behrenfeld et al. 2005). This could be related to differences in θ of the bulk phytoplankton pool as compared to the picophytoplankton community.

Overall, >30% of the variability in θ of picophytoplankton in the mixed layer of the central Cantabrian Sea was captured by taking into account, on the one hand, the exponential decrease of θ with irradiance and, on the other hand, the linear decrease of θ_{min} and the exponential increase of θ_{max} with temperature. However, the clear onshore-offshore gradient with a higher irradiance, NO_3 concentration, and percentage of eukaryotes onshore did not translate into significant differences in θ of picophytoplankton. Then, the unexplained variability of θ should be related to alternative factors, such as the different ecotypes of picophytoplankton in the ecosystem and the effect of alternative environmental variables on θ. In this sense, the larger differences between the *in situ* measurements and the empirical model were observed in winter, when picoeukaryotes were the dominant picophytoplankton. Thus, more studies are needed to better understand which environmental factors can determine the variability of θ among the different ecotypes of picoeukaryotes. In addition, it could be interesting to compare the picophytoplankton θ measured in the central Cantabrian Sea with that derived from satellites in the same region to ascertain the reasons for the differences between both estimates.

Picophytoplankton primary production in the coastal ecosystem

Several studies have shown that increases in nutrient and temperature stress cause decreases in phytoplankton growth rates paralleled by decreases in θ (Geider 1987, MacIntyre et al. 2002, Behrenfeld et al. 2005). Such a physiological response can be quantified by the quotient between the *in situ* θ and the potential $\theta_{N,T\text{-}max}$ (Behrenfeld et al. 2005). In the central Cantabrian Sea, the quotient between θ of picophytoplankton and $\theta_{N,T\text{-}max}$ was higher toward the coast than toward the open sea, thus suggesting a clear offshore-inshore increasing gradient of picophytoplankton growth rates. This gradient was paral-

leled by a change in the picophytoplankton community composition toward increasing dominance by picoeukaryotes as we moved onshore. In this connection, the higher growth rates at the coastal station were reflected in higher volumetric picophytoplankton primary production rates that were consistent with a positive relationship between the growth rates of picophytoplankton and the percentage of picoeukaryotes in the euphotic layer (Morán 2007). Unfortunately, the validation of the model was based on 8 data points available for 2003, and the relationship between modeled and *in situ* data was only marginally significant, making it necessary to increase the database of *in situ* measurements.

Areal picophytoplankton primary production rates presented a seasonal cycle, with the lowest productions, <50 mg C m^{-2} d^{-1}, found in summer and 2 peaks of production, one in late winter, >100 mg C m^{-2} d^{-1}, and the other in autumn, >200 mg C m^{-2} d^{-1}. This annual cycle was similar to that described with the ^{14}C method in the shelf station during 2003 (Morán 2007); however, the mean areal picophytoplankton production rate was half that measured during 2003 because of different integration depths (mixed layer versus euphotic layer). These areal picophytoplankton production rates were comparable to observations in the nearby Ría de Vigo, where they varied between 20 and 480 mg C m^{-2} d^{-1} during winter mixing and summer upwelling, respectively (Cermeño et al. 2006), and lower than rates measured in the Southampton estuary that varied between 10 and 1000 mg C m^{-2} d^{-1} in autumn and summer, respectively (Iriarte & Purdie 1994). However, they were slightly higher than rates estimated for the open (>200 m) temperate North Atlantic that varied between 40 and 120 mg C m^{-2} d^{-1} in January and July, respectively (Uitz et al. 2010). The small differences between studies may also be related to differences in taxonomic composition, as modeled areal primary production in the central Cantabrian Sea was estimated by using a maximum growth rate of 2 d^{-1} that is close to the growth rates observed for *Synechococcus* sp. and picoeukaryotes but is higher than the 1 d^{-1} described for *Prochlorococcus* sp. (Veldhuis et al. 2005) that are more abundant under open ocean conditions.

Picophytoplankton can share between 10 and 60% of the total primary production estimated in the open Atlantic Ocean (Glover et al. 1986, Fernandez et al. 2003, Uitz et al. 2010). Closer to the coast, this percentage can vary between 20% in the Southampton estuary (Iriarte & Purdie 1994) and up to 50% in the central Cantabrian Sea and in Carolina's Neuse River Estuary (Morán 2007, Gaulke et al. 2010). If we consider that the surface of the ocean is 353 × 10^6 km^2, with 22% of this surface covered by the Atlantic Ocean and nearly 3% below 200 m depth (Uitz et al. 2010), the coastal Atlantic Ocean could cover nearly 2.38 × 10^6 km^2. Taking into account the mean and maximum areal production rates obtained in this study, 118 and 1114 mg C m^{-2} d^{-1}, respectively, picophytoplankton could be producing between 0.10 and 0.94 Gt C yr^{-1}. Thus, picophytoplankton in the coastal Atlantic Ocean should be producing between 4 and 38% of the 2.5 Gt C yr^{-1} estimated for the open Atlantic Ocean (Uitz et al. 2010). These values should be increased by nearly 2-fold if the euphotic layer is considered instead of the mixed layer (Morán 2007), which gives an idea of the importance of picophytoplankton for the global carbon cycle.

CONCLUSIONS

More than 30% of picophytoplankton θ variability can be related to the irradiance and temperature in the mixed layer of the central Cantabrian Sea. This empirical photoacclimation model captured the seasonal cycle of θ of picophytoplankton and may serve to estimate the growth rates of picophytoplankton and the effect of different environmental conditions (i.e. light and temperature) on picophytoplankton primary production.

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