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Seasonality of picophytoplankton chlorophyll *a* and biomass in the central Cantabrian Sea, southern Bay of Biscay

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Abstract

Seasonal changes in the abundance and biomass of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and picoeukaryotes were studied by flow cytometry in the upper layers of the central Cantabrian Sea continental shelf, from April 2002 to April 2006. The study area displayed the typical hydrographic conditions of temperate coastal zones. A marked seasonality of the relative contribution of prokaryotes and eukaryotes was found. While cyanobacteria were generally more abundant for most of the year (up to $2.4\ 10^5$ cells mL^{-1}), picoeukaryotes dominated the community (up to 10^4 cells mL^{-1}) from February to May. The disappearance of *Prochlorococcus* from spring through summer is likely related to shifts in the prevailing current regime. The maximum total abundance of picophytoplankton was consistently found in late summer–early autumn. Mean photic-layer picoplanktonic chlorophyll *a* ranged from 0.06 to 0.53 μg L^{-1} with a relatively high mean contribution to total values (33±2% SE), showing maxima around autumn and minima in spring. Biomass (range 0.58–40.16 mg C m^{-3}) was generally dominated by picoeukaryotes (mean±SE, 4.28±0.27 mg C m^{-3}) with an average contribution of cyanobacteria of 30±2%. Different seasonality of pigment and biomass values resulted in a clear temporal pattern of picophytoplanktonic carbon to chlorophyll *a* ratio, which ranged from 10 (winter) to 140 (summer). This study highlights the important contribution of picoplanktonic chlorophyll *a* and carbon biomass in this coastal ecosystem.

Keywords: Picoplankton; Phytoplankton; Chlorophyll; Biomass; Temporal variations; Coastal waters; Bay of Biscay; Cantabrian Sea

1. Introduction

The size structure of the phytoplankton community is an important factor in controlling the carbon cycle and food web dynamics in pelagic ecosystems (Azam et al., 1983; Legendre and Le Fèvre 1991). Chlorophyll, cell abundance and primary production in oceanic oligotrophic areas are dominated by picoplankton cells (<2 μm) (Zubkov et al., 1998, 2000; Poulton et al., 2006). By contrast, in coastal areas where factors influencing the composition and dynamics of the phytoplankton community (nutrient and light availability, turbulence and predation, among others) are more variable in time and space, nano- and microplankton usually dominate both biomass and production for considerable periods of time (Malone et al., 1991; Iriarte and Purdie 1994). However, there are transitional situations deeply tied to physical events, as downwelling intermittency in coastal areas, with occasional picoplankton dominance (Cermeño et al., 2006).

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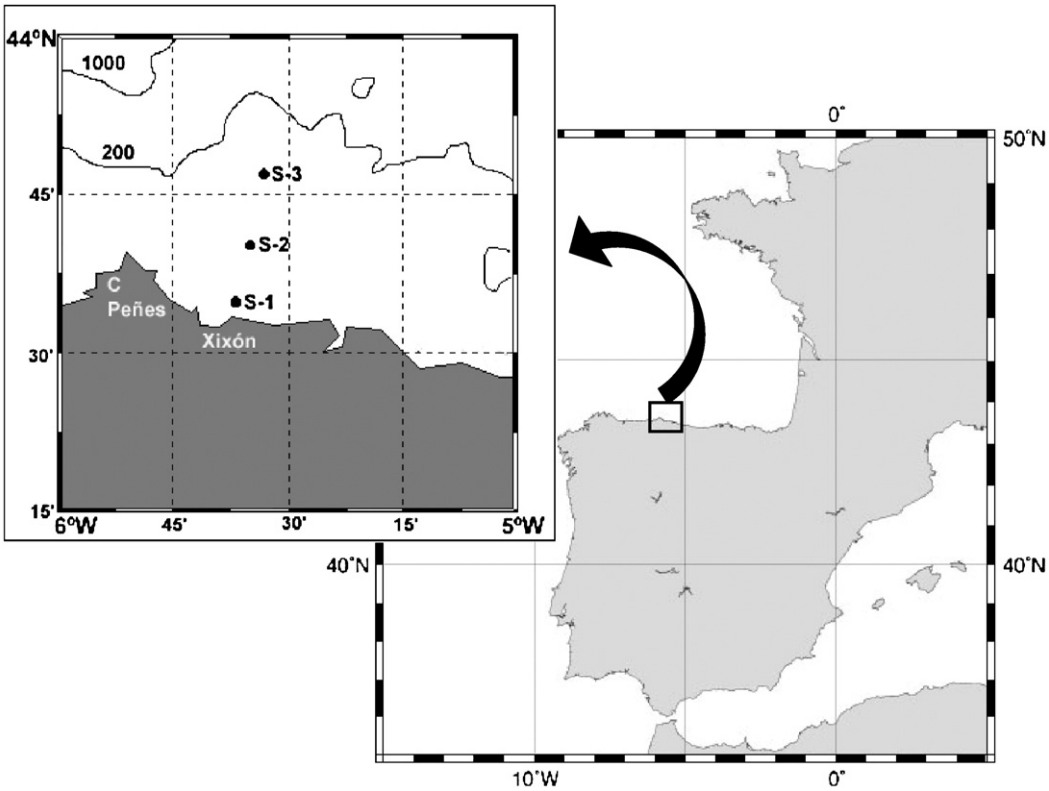

Fig. 1. Map of the Iberian peninsula showing the location of the sampling stations.

The central Cantabrian Sea is a temperate coastal ecosystem located in the southern Bay of Biscay (NE Atlantic) characterized by two marked hydrographic periods. Downwelling conditions prevail from October to March, while intermittent upwelling characterizes the period from April to September (Fernandez and Bode 1991). Annual maxima of phytoplanktonic biomass and production occur between late winter and spring while minimum values are found in summer stratified waters (Fernandez and Bode 1991, X.A.G. Morán pers. comm.). With regard to the picoplanktonic size-class of autotrophs, a distinct seasonality was found in a one year study by Calvo-Díaz and Morán (2006), with cyanobacteria dominating in abundance for most of the year. However, eukaryotes made up the bulk of picophytoplankton biomass (82±2% SE) except in surface nutrient-depleted waters in summer. The mentioned study was mostly focused on changes in abundance and cellular characteristics, with little information on pigment content and the contribution of this size-class to total phytoplankton chlorophyll and biomass. Variability of carbon to chlorophyll *a* ratios (C:Chl *a*) has long been documented (e.g. Buck et al., 1996; Pérez et al., 2006) but mostly for the entire phytoplankton community rather than for specific size-fractions (e.g. Arin et al., 2002; Pérez et al., 2006). With an adequate calibration, flow cytometry techniques provide a fast estimation of the size of picophytoplankton cells, which in turn permits reliable estimates of their biomass due to the relatively small variability of the carbon to biovolume conversion factors within this size-class (e.g. Li, 1986; Worden et al., 2004).

Given the reportedly high interannual variability of planktonic assemblages (e.g. Mura et al., 1996), by considering a complete 4-year sampling we can assess the consistency of the seasonal patterns previously described for the major groups found in our area, *Prochlorococcus* and *Synechococcus* cyanobacteria and two groups of picoeukaryotic cells of differing size. In this study we also focus on the seasonal variability of picoplanktonic C:Chl *a* ratios. We show that small phytoplankters contribute significantly to total phytoplankton in terms of both pigment and carbon biomass in this coastal temperate ecosystem.

2. Materials and methods

Sampling was carried out from 14 April 2002 to 11 April 2006 at approximately monthly intervals on board of R/V *José de Rioja* as part of the IEO time-series project RADIALES. Samples were taken from 4 to 9 depths between the surface and the near-bottom of three

stations located over the shelf off Xixón (Asturies, N Spain) (Fig. 1). More details can be found in Calvo-Díaz and Morán (2006).

Temperature, salinity and fluorescence data were acquired with a CTD *SeaBird 25* equipped with a *Sea-Point* fluorometer. Photosynthetically active radiation (PAR) in the water column was measured every month except in December 2005, January and February 2006, with a spherical quantum sensor (Biospherical QSP-2200). Values at 1-m depth intervals were used to calculate the vertical light attenuation coefficient (K_d), which was in turn used to calculate the depth of the photic layer, defined as the depth receiving 1% of surface irradiance.

Water samples were taken from 5 l Niskin bottles in a rosette sampler attached to the CTD. Fractionated chlorophyll *a* concentration (chl *a*) was obtained after sequential filtration of 100 mL samples through polycarbonate 47 mm (Millipore) filters of 20, 2 and 0.2 μm of pore-size. A very small fraction of cyanobacteria and Small picoeukaryotes (<10%) was retained by the 2 μm filters used for chl *a* fractionation. Although most of the Large picoeukaryotes were retained by these filters due to their larger size (mostly slightly higher than 2 μm, Table 1), their considerable lower abundance made the possible over-estimation of picophytoplankton biomass by avoiding the filtration step of minor importance. Glass fibre filters (Whatman GF/F, 25 mm diameter) for estimating total chl *a* were used before January 2003. Filters were frozen until analysis, generally within one week. Pigments were extracted in 90% acetone for 24 h in the dark at 4 °C and chl *a* was measured with a Perkin Elmer LB-50s spectrofluorometer (Neveux and Panouse, 1987) calibrated with pure chl *a*. Samples for nutrients (NO_3^-, NO_2^- and PO_4^-) were frozen and their concentrations determined with a Technicon autoanalyzer within 6 months. The nutricline depth was defined as that where the NO_3^- concentration equalled 1 μmol l^{-1}.

Table 1
Monthly means±SE of cell size (diameter, μm) of the different picophytoplanktonic groups at the surface of the central Cantabrian Sea continental shelf for the period 2002–2006

	Cell size (μm)			
	Synechococcus	*Prochlorococcus*	Small pEukar	Large pEukar
January	1.00±0.01	0.70±0.04	1.35±0.03	2.06±0.09
February	1.01±0.01		1.31±0.04	2.11±0.04
March	1.02±0.02		1.35±0.05	2.18±0.12
April	1.07±0.02		1.25±0.06	2.32±0.13
May	1.00±0.07		1.22±0.07	2.00±0.13
June	0.87±0.01		1.32±0.08	2.25±0.13
July	0.87±0.02		1.31±0.05	2.06±0.07
August	0.92±0.04	0.51	1.41±0.13	1.96±0.12
September	0.91±0.04	0.54±0.03	1.52±0.04	2.09±0.04
October	0.94±0.04	0.54±0.02	1.29±0.01	2.00±0.19
November	1.02±0.02	0.68±0.02	1.41±0.05	2.01±0.05
December	1.02±0.02	0.71±0.03	1.41±0.06	2.05±0.08

pEukar, picoeukaryotes.

Picoplankton samples (1.8 ml) were preserved with 1% paraformaldehyde+0.05% glutaraldehyde (final concentration). Samples were frozen at −80 °C until analysis in the laboratory with a FACSCalibur flow cytometer (Becton-Dickinson) equipped with a laser emitting at 488 nm. Autotrophic cells were separated into two groups of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and two groups of picoeukaryotes based in their fluorescence and light scatter signals, as explained in Calvo-Díaz & Morán (2006). For estimating the abundance of the different groups, calibration of the cytometer flow rate was performed daily and a solution of 1 μm fluorescent latex beads (ref. F-13081, Molecular Probes) was added as an internal standard (Calvo-Díaz and Morán, 2006). All cellular variables were related to fluorescent beads values.

An empirical calibration between side scatter (SSC) and cell diameter (Calvo-Díaz and Morán, 2006) was used to estimate biovolume. Picoplankton biomass was calculated by using the following volume-to-carbon conversion factors: 230 fg C μm^{-3} for *Synechococcus*, 240 fg C μm^{-3} for *Prochlorococcus* and 237 fg C μm^{-3} for picoeukaryotes (Worden et al., 2004).

All variables were log-transformed to attain normality and homogeneity of variances. Mean values provided were either integrated or averaged for the photic layer at each station, except where otherwise indicated.

3. Results and discussion

3.1. Hydrography

The sampled stations showed typical hydrographic conditions of temperate coastal zones for the whole study period (from April 2002 to April 2006). As shown in Fig. 2 in Calvo-Díaz & Morán (2006) for the 2002–2003 period, a marked stratification with small upwelling pulses on occasions was consistently found in mid summer every year whereas a well-mixed water column was detected from November to April. The surface water temperature gradually increased from January–February (minimum 11.6 °C, January 2006) to August, when the highest temperatures were usually reached (maximum 23.4 °C, August 2003). Surface haline stratification was frequently observed in winter months. High salinities (>35.7) were found in April 2002 and 2006, suggesting an influence of the Iberian Poleward Current (IPC) in this zone, as described in detail by González-Nuevo & Nogueira (2005).

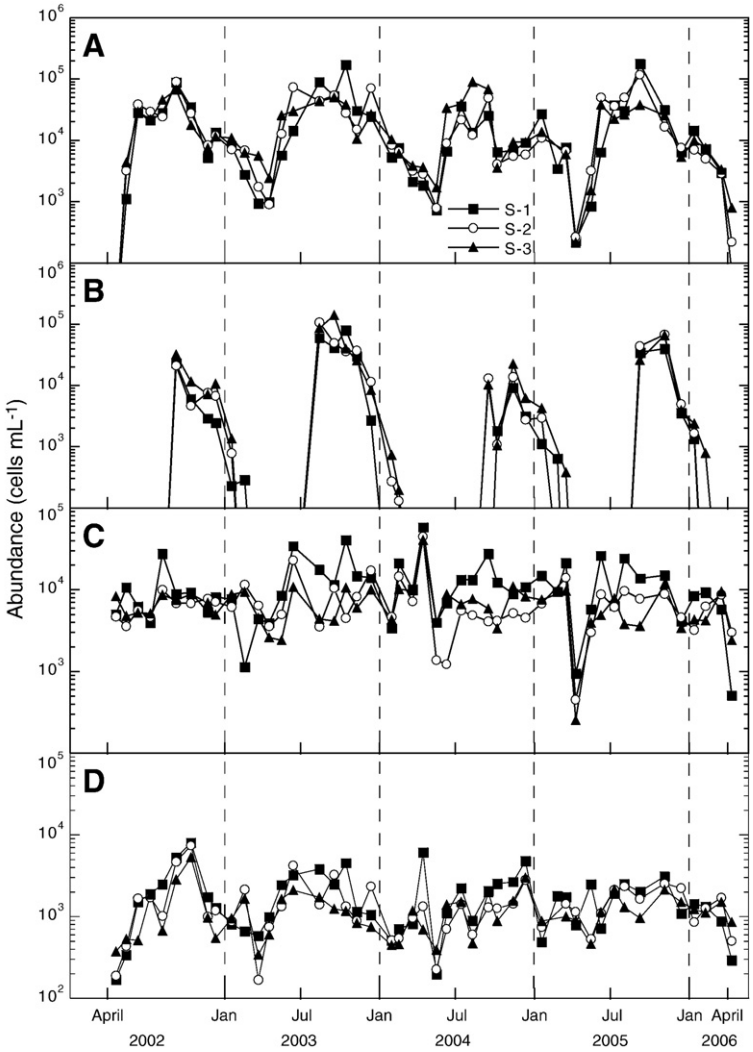

Fig. 2. Interannual variability of the photic-layer abundance at the three stations (S-1, S-2, S-3) of the four groups of picophytoplankton considered, (A) *Synechococcus*, (B) *Prochlorococcus*, (C) Small and (D) Large picoeukaryotes.

3.2. Picophytoplankton abundance and photosynthetic pigments

Variability of the photic-layer abundances of the four groups is shown in Fig. 2 for the studied period. Very similar patterns were found for the three stations. Numbers of *Synechococcus* and *Prochlorococcus* were much more variable [coefficients of variation (CV) of 131% and 136%, respectively] and predictable than those of the two groups of picoeukaryotes (CV of 93% for small and 86% for large). A very coherent pattern emerged from extending the study of Calvo-Díaz and Morán (2006) to three more years. Cyanobacterial abundance (Fig. 2A, B) varied approximately two orders of magnitude between maxima in summer (August–September) and minima in late winter–early spring (February–April), with abundances similar to those found in NE Atlantic waters (e.g. Tarran et al., 2001). As in other coastal zones (e.g. Worden et al., 2004), *Prochlorococcus* cyanobacteria were only present in the study area from September to February every year, except in 2003 when they re-appeared in mid August. Preliminary results from samplings along two transects perpendicular to the N Iberian coast at 8°W and 4°W carried out in January and August–September 2005, and February 2006 (X.A.G. Morán & L.A. Suárez, unpublished data) showed the continuous presence of *Prochlorococcus* from shore to deep (>2000–3000 m depth) waters well within the Bay of Biscay offshore waters. However, its persistent and sudden appearance in shelf waters in September cannot be readily associated

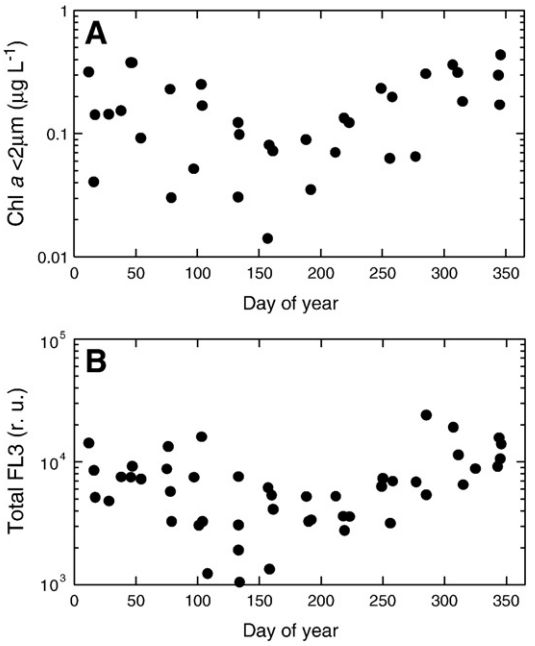

Fig. 3. Average seasonal distribution of (A) picoplanktonic chl *a* and (B) total red fluorescence (∑FL3) as a proxy of chl *a* content at the surface of station 2.

with any reported changes in the current regime or upwelling pulses. In Suruga and Uchiumi Bays, Japan (Shimada et al., 1995; Katano et al., 2005), the presence of *Prochlorococcus* was closely associated to the intermittent "*Kyucho*" phenomenon, an intrusion of warm surface water from the Pacific Ocean, throughout spring and summer. Although we had previously proposed *Prochlorococcus* as a tracer of the IPC, especially in the Galician area (Calvo-Díaz et al., 2004, X.A.G. Morán & L.A. Suárez, unpublished results) there is no strong singular hydrographic signal in September in the Central Cantabrian Sea (C. González-Pola, pers. comm.) that could possibly explain the seasonality of this organism. The possibility of a higher exchange of water between the offshore Bay of Biscay area and the Cantabrian shelf during this month cannot be discarded, but further studies are obviously necessary to shed light on the possible mechanism of advection of *Prochlorococcus* into these coastal waters.

Slightly different seasonal patterns were observed for the two groups of picoeukaryotes (Fig. 2C, D). Small picoeukaryotes were approximately one order of magnitude more abundant than large picoeukaryotes during the first half of the year, whereas an increase in the abundance of the latter group in summer and autumn mainly at the most coastal stations (S-1 and S-2) made them show more similar numbers (Fig. 2). These differences in seasonality could be partially explained by a different taxonomic composition (see below).

Interannual variability was of the minor importance compared with the seasonal signal (Fig. 2) with the exception of *Prochlorococcus*. This cyanobacteria showed 2.2-times higher abundances in 2003 compared with the mean value for the 4-year period (1.9 10^4 cells mL^{-1}, calculated only for presence months), while *Synecochoccus* and picoeukaryotes abundances were apparently not affected. This result suggests a different impact of local versus larger scale oceanographic conditions on the ecology of the different groups, with *Prochlorococcus* likely being more dependent on advection processes as discussed above.

Overall, total chl *a* concentration ranged from 0.03 to 7.56 µg L^{-1} with an exceptionally high value (12 µg L^{-1}) in March 2005. Maximum values were usually found in late winter or early spring, the same as for the >20 µm size-fraction. Picoplanktonic chl *a* ranged from 0.002 to 1.14 µg L^{-1} and it showed a distinct temporal pattern in surface waters (Fig. 3A) with maxima in late autumn–early winter and minima in late spring in contrast to the more homogeneous values found year-round by Cermeño et al. (2006) in the Ria of Vigo (NW Iberian coast). Fig. 4 represents the average monthly relative contribution of each phytoplankton size-class to photic-layer values at station 2. Picoplankton contribution was maxima in early winter and autumn (50%) while minima in spring (10%) were coincident with the highest total chl *a* values of the year (Fig. 4), similarly to previous reports on the contribution of small cells in temperate nearshore waters (e.g. Tamigneaux et al., 1999). Yet, absolute picoplankton chl *a* values increased with trophic status (estimated as total chl *a*). As previously reported by Agawin et al. (2000) and Bell & Kalff (2001) the slope of the log–log Model I linear regression of picoplanktonic chl *a* versus the total (log pico chl $a = -0.65 + 0.72$ log total chl *a*, $r^2 = 0.48$, $p < 0.001$, $n = 662$) was significantly <1 ($b = 0.72$, *t*-test, $p < 0.05$). However, the slope was not significantly different from 1 when we used Model II regression analysis ($b = 1.04$, 95% confidence limits: 0.98 to1.09), thus indicating that the frequently reported decrease in the relative importance of picoplankton with increasing trophic status would not be fully applicable to our dataset. The contribution of small, non-microplanktonic (i.e. <20 µm) phytoplankton to total chl *a* was always higher than 30%, attaining a maximum of 90% in late autumn–early winter (Fig. 4) whereas large phytoplankton dominated the phytoplankton community during late winter and early spring.

If relative red fluorescence (FL3) can be used as a proxy of chl *a* content per cell, total FL3 (∑ relative FL3 × group abundance, relative units) should then reflect the variation of picoplanktonic chl *a*. Indeed, similar seasonal patterns

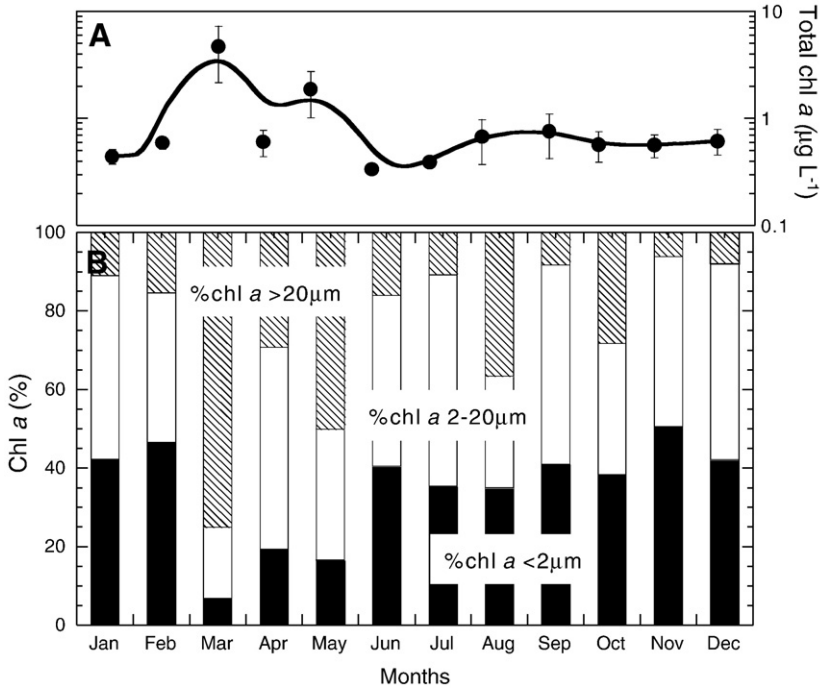

Fig. 4. (A) Photic-layer average of total chl *a* and (B) contribution (%) of each phytoplankton size-fraction (<2, 2–20, >20 µm) to total chl *a* at station 2 for the period 2003–2006.

were observed for picoplanktonic contribution to total chl *a* (chl *a* <2 µm) and total FL3 at the surface (Fig. 3A, B), which were significantly correlated ($r=0.61$, $p<0.001$, $n=104$). Seasonal variability of FL3 for each group followed the same pattern as the total values, showing relative minima in summer (Fig. 5A), less marked for

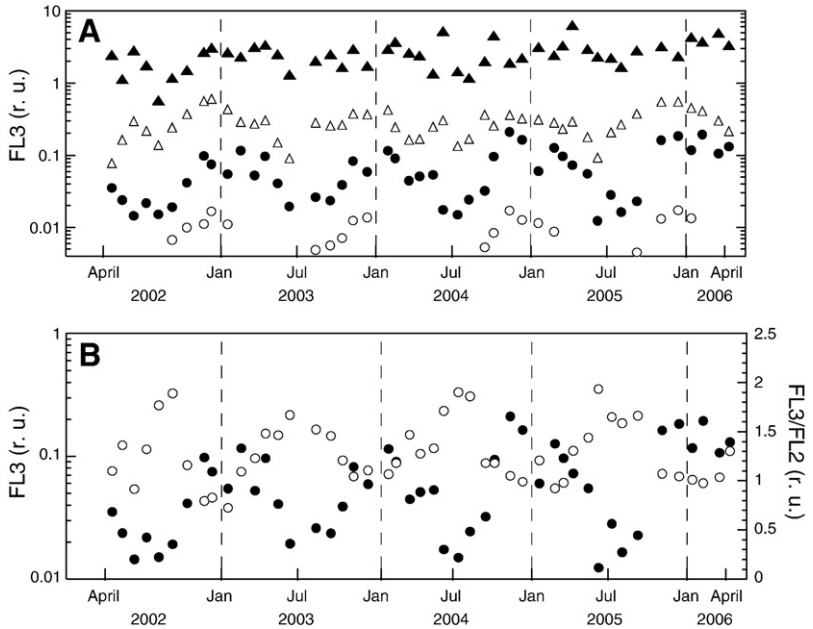

Fig. 5. Interannual variability of (A) relative red fluorescence (FL3) of *Synechococcus* (filled circles), *Prochlorococcus* (open circles), Small (open triangles) and Large picoeukaryotes (filled triangles), and (B) FL3 (filled symbols) and the ratio of red to orange fluorescence (FL3/FL2) (open symbols) of *Synechococcus* at the surface of station 2 for the period 2002–2006.

Large eukaryotes. This finding can be explained by the lower photosynthetic pigment content under higher irradiances and longer daylight periods. Phycoerythrin content of *Synechococcus*, estimated from the relative orange fluorescence (FL2), showed a temporal pattern similar to FL3 (data not shown). However, high irradiances in summer were associated with a more pronounced decrease in FL2 than in FL3, causing a two-fold increase in the FL3/FL2 ratio during those months (Fig. 5B, open symbols). These temporal variations in FL3/FL2 ratio confirm our previous findings (Calvo-Díaz and Morán, 2006). The gradual change observed led us to hypothesize that variability is related with chromatic adaptation (Palenik, 2001) within the same "species" rather than the presence of different *Synechococcus* strains along the annual cycle.

3.3. Cell size and biomass

Changes in picophytoplankton cell size (Table 1) strongly support previous accounts (Calvo-Díaz and Morán, 2006). Vertical profiles also displayed a marked seasonality, with maxima at depth during stratification and more homogeneous profiles during the mixing period (data not shown). Cyanobacteria and Small picoeukaryotes showed minimum sizes in the upper layers during summer whereas larger *Synechococcus* cells with low size variation were usually detected from November to April. The relationship between cell size and nutrient conditions reported in Calvo-Díaz & Morán (2006) held for this extended sampling, with a significant difference in cyanobacteria and Small picoeukaryotes cell size above and below the nutricline (*t*-test, $p<0.001$, $df=444$ and 124 for *Prochlorococcus*). These results are in agreement with those reported by DuRand et al. (2001). When the nitracline is shallow nutrient concentrations in the upper layers are usually also higher, cells are larger and show greater fluorescence, whereas in summer nutrient-depleted surface waters, cells are smaller and less fluorescent. Concurrent variations in cell size and fluorescence were very consistent (Fig. 6). Yet, differences in the magnitude of change of each variable over the year drive a significant seasonality of the carbon to chl *a* (C:Chl *a*) ratio (see below). The absence of any clear seasonal pattern for the size of Large picoeukaryotes can be a consequence of their different taxonomic composition with shifts in dominant species with season or depth. In this regard, a recent study at a Western English Channel coastal site found that *Micromonas pusilla* dominated the eukaryotic community (75% of picoeukaryotic cells on average) for most of the year (Not et al., 2004). It seems that this species is usually detected in the Small rather than Large picoeukaryotes cluster in cytograms (F. Not, personal communication). Were the Small picoeukaryotes group mostly monospecific like the two clusters of cyanobacteria identified here, this would help explain its higher predictability compared with the Large group (Fig. 2). However, taxonomical studies are clearly needed to confirm this hypothesis.

Strong vertical and seasonal variations in cell size (Table 1 and Calvo-Díaz and Morán, 2006) stress the need to use information on biovolume to estimate biomass from abundance data rather than relying on constant cell carbon values (e.g. Zubkov et al., 1998). Although variations in the specific carbon to biovolume relationship for phytoplankton have also been reported (Taylor et al., 1997) it seems that they are more constant in the case of picoplankton cells (Li, 1986; Worden et al., 2004). Notwithstanding these possible limitations, our observations

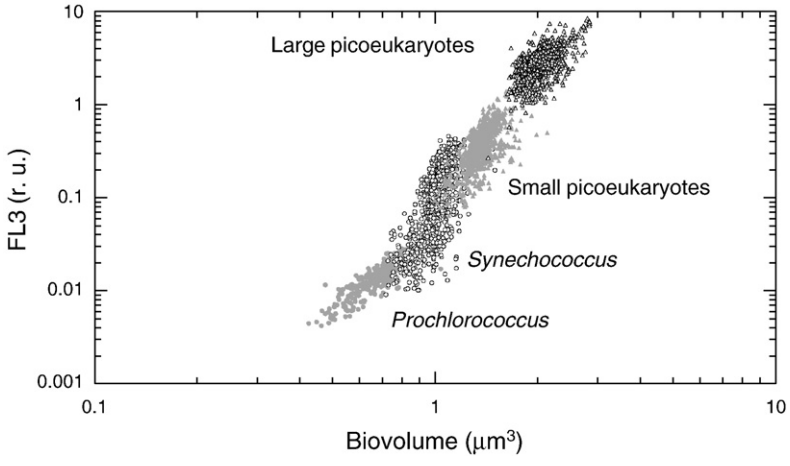

Fig. 6. Scatter plot of cell size vs. relative red fluorescence (FL3) for the four groups.

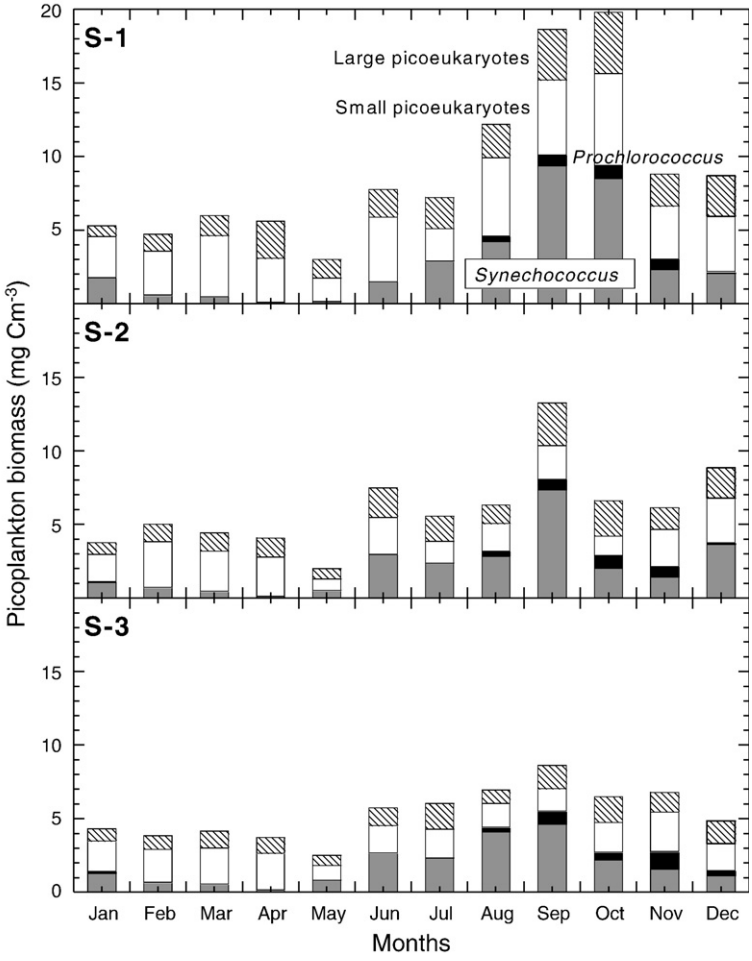

Fig. 7. Monthly variation of photic-layer average biomass (mg C m^{-3}) of the four picoplanktonic groups at the three sampled stations for the period 2002–2006.

showed a clear and consistent seasonality in the biomass contributed by the different groups at the three stations (Fig. 7). Consistent with variations in abundance (Fig. 2), the lowest biomass of cyanobacteria was found in late winter and early spring, in April for *Synechococcus* (0.12 ± 0.07 mg C m^{-3}) and in January for *Prochlorococcus* (0.06 ± 0.04 mg C m^{-3}). Picoeukaryotes biomass was generally more homogeneous, although it varied in one order of magnitude (0.8–3.1 for Small and 0.7–2.9 mg C m^{-3} for Large picoeukaryotes). Small picoeukaryotes dominated over the rest of the groups except in summer, when *Synechococcus* and *Prochlorococcus* reached their highest biomass (7.3 ± 1.5 and 0.7 ± 0.2 mg C m^{-3}, respectively). Fig. 8 shows that the prokaryotes:eukaryotes biomass ratio was generally <1 although their abundance ratio was consistently >1 except in spring. The tight match displayed by both ratios indicates that picoplankton biomass was largely determined by changes in abundance rather than changes in individual carbon content derived from changes in cell size (Worden et al., 2004; Calvo-Díaz and Morán, 2006). Although cell size varied noticeably for most groups (Table 1), changes in abundance were of one to several

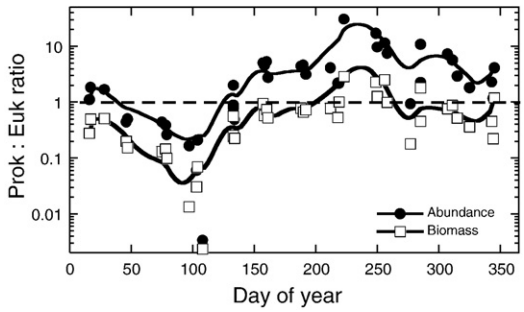

Fig. 8. Ratio of prokaryotes:eukaryotes abundance and biomass for all sampled months within the 2002–2006 period at station 2. A smooth curve was fitted for each ratio.

orders of magnitude over the year (Fig. 2). The conspicuous, constant pattern displayed by the prokaryotes: eukaryotes ratio regardless of the sampling year suggest that picoplankton dominant cell type tracks changes in the hydrographic regime of the southern Bay of Biscay continental shelf. The ratio consistently attains minimum values at the beginning of the stratification period and maxima are found when stratification erodes due to the passage of autumn storms. We suggest that these ratios could be successfully used to characterize hydrographic conditions of the Cantabrian Sea.

Phytoplankton biomass is still frequently estimated from the application of C:Chl *a* ratios to chl *a* measurements, although the use of constant values is not appropriate for ecological studies (Geider, 1987) since its variability in response to irradiance, nutrient availability and temperature has long been documented (e.g. Geider, 1987; Geider et al., 1997). The problem aggravates when a unique literature value is applied over a complete seasonal cycle in temperate ecosystems, characterized by strong changes of the above-mentioned variables. Previous studies were mostly focused on the spatial variation of C:Chl *a* ratios (Chang et al., 2003), although different models have been put forward to account for seasonal, latitudinal and vertical variations (Cloern et al., 1995; Taylor et al., 1997). Yet relatively little is known about size-fractionated C:Chl *a* ratios (e.g. Arin et al., 2002; Pérez et al., 2006) especially in shelf waters. We calculated what we believe are the first reported C:Chl *a* ratios for picophytoplankton along an annual cycle using the sum of individual carbon contents and picoplanktonic contribution to total chl *a* values. Similarly to larger or bulk phytoplankton studies, we found a clear temporal variation of picophytoplankton C:Chl *a* ratios (Fig. 9). Overall, they ranged from 0.07 to 282 (except for a exceptionally high value of 1086 measured at S-2 in June 2005). Maximum values were found in the summer as a consequence of low cell-specific chl *a* values (Fig. 5A) rather than an increase in carbon content: FL3 had CVs which ranged between 40 and 71% whereas variability in size was always much lower (9–10%). Higher values were systematically found at the surface during the stratification period also as a consequence of a higher chl *a* concentration per cell with depth due to acclimation to lower irradiances (Arin et al., 2002; Pérez et al., 2006). The highest ratios found during summer stratification are in agreement with the results of Buck et al. (1996), who reported the highest values in the most oligotrophic and stratified region in a latitudinal transect across the north Atlantic. The ratios obtained were in the range reported by Buck et al. (1996) and Pérez et al. (2006) for subtropical and subarctic Atlantic regions, and slightly higher than those reported by Arin et al. (2002) in May for the SW

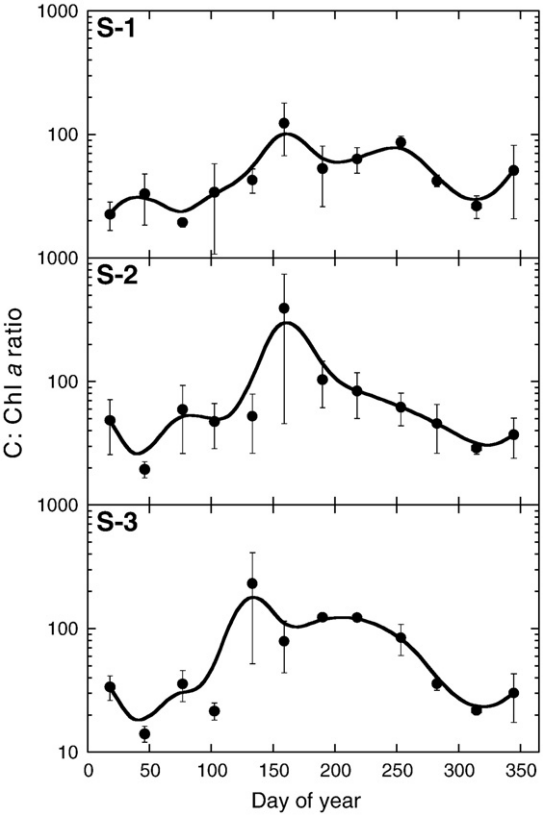

Fig. 9. Average seasonal variation (±SE) of the picophytoplankton carbon to chl *a* ratio at the surface of the three stations for the period 2003–2006.

Mediterranean. Picoplankton C:Chl *a* ratios in summer showed a significant coastal-offshore variability with higher values in the outer shelf station (Fig. 9) (ANOVA, $p<0.05$, SNK-test, S-1=S-2<S-2=S-3, $n=15$, July and August data). These results can be explained by the concurrent spatial gradient displayed by Small picoeukaryotes, with significantly higher abundances towards the coast (ANOVA, $p<0.001$, SNK-test, S-1>S-2=S-3, $n=114$), since C:Chl *a* of cyanobacteria is known to be higher than that of eukaryotes (Geider, 1987; Furuya, 1990). In summary, the large temporal variations of the C:Chl *a* ratio observed here (Fig. 9) strongly discourage the use of constant ratios for biomass estimations based on chl *a* values regardless of the sampling month.

In coastal areas, phytoplankton biomass and production is still usually assumed to be dominated by nano- and microplankton size-classes (Malone et al., 1991; Iriarte and Purdie, 1994, Tarran et al., 2006) and only sporadically by picoplankton [e.g., with short-lived downwelling events as reported by Cermeño et al. (2006)]. The overwhelming dominance of picophytoplankton cells in terms of absolute numbers (Li et al., 2006) is not necessarily reflected in

terms of biomass, because of their small size compared with bloom forming phytoplankton cells. However, with the exception of the spring bloom, our results show a minimum relative contribution to total chl *a* of 35% with maxima of ca. 50% in autumn. This study demonstrates a strongly coherent seasonal trend in the relative importance of picoplankton in terms of both pigment and carbon biomass in a temperate coastal site.

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