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Shifts in taxonomic composition and size structure of phytoplankton and their effects on light absorption properties and nutrient uptake rates in contrasting areas of the Alboran Sea

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Running page head: Phytoplankton size structure and metabolic rates

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ABSTRACT: Biomass size distribution, light absorption properties and carbon and nitrogen uptake rates were analysed in phytoplankton assemblages along coast-offshore gradients. During the samplings, the coastal stations were affected by upwelling. In contrast, the offshore stations were located at the western Alboran anticyclonic gyre core. Surface nitrate concentration was higher than 1 μM at the coastal stations and lower than the detection limit at the offshore stations. Furthermore, the thickness of the upper mixed layer was progressively deeper from coast (ca. 20-30 m depth) to offshore (80-100 m depth). In terms of biomass, the coastal communities were dominated by diatoms; dinoflagellates and pico-plankton contributed to phytoplankton biomass by less than 30% and 7%, respectively. At the offshore stations, diatom abundance decreased while dinoflagellate abundance did not change significantly in comparison with the coastal stations, becoming the dominant group in terms of biomass. The mean length of the microplankton cells increased from coast to offshore due to decrease in abundance of diatoms smaller than 50 μm in length. The coastal communities featured lower chlorophyll *a* specific absorption coefficient in the blue spectral band than the offshore communities. In contrast, the biomass specific uptake rates of inorganic carbon and nitrate estimated for the coastal communities (3.5 $\text{nmol C } \mu\text{g}^{-1} \text{ POC h}^{-1}$ and 0.2 $\text{nmol N } \mu\text{g}^{-1} \text{ POC h}^{-1}$, respectively) were higher than for the offshore communities (1.0 $\text{nmol C } \mu\text{g}^{-1} \text{ POC h}^{-1}$ and 0.03 $\text{nmol N } \mu\text{g}^{-1} \text{ POC h}^{-1}$, respectively). These differences in light absorption efficiency and nutrient uptake rates were significantly correlated with the change in the mean cell size of the communities from coast to offshore that were mainly due to the decrease in biomass of diatoms smaller than 50 μm and the increase in the dinoflagellate contribution to phytoplankton biomass that produced.

KEY WORDS: Anticyclonic gyre · Primary productivity · Diatoms · Upwelling · Carbon uptake · Nutrients

1 INTRODUCTION

2
3 Cell size modulates the light absorption and nutrient uptake by phytoplankton. Theoretically, the
4 probability of absorbing photons per chlorophyll unit under a given light field decreases with cell
5 size, consequently the efficiency of light absorption in large cells should reduce in comparison with
6 small cells (Geider et al. 1986, Raven & Kübler 2002). Laboratory data obtained from experiments
7 performed with isolated cultures corroborate this expected relationship between cell size and light
8 absorption properties (e.g. see Finkel 2001). Coincidentally, phytoplankton communities dominated
9 by small cells (cell size $< 2 \mu\text{m}$) feature higher values of light absorption coefficient per chlorophyll
10 a unit than communities dominated by larger phytoplankton (Ciotti et al. 2002, Ciotti & Bricaud
11 2006). Ciotti et al. (2002) concluded that about 80% of variability in the absorption properties of the
12 phytoplankton can be explained by changes in the mean size of the communities. On the other hand,
13 small cells theoretically require lower nutrient concentrations than large cells to keep an equivalent
14 influx per volume unit (Armbrust & Chisholm 1992). As a result, small cells should maintain high
15 growth rates at low nutrient concentrations while large cells have a higher capacity for nutrient
16 uptake at high nutrient concentrations. Recently, Edwards et al. (2012) reviewed the relationship
17 between nitrate and phosphate uptake kinetic parameters per cell and cell size in phytoplankton.
18 According to that study, both nutrient uptake maximum rate per cell and half-saturation constant
19 tend to increase with cell volume. Concordantly, field data indicate that nitrate uptake rates are
20 normally higher for large-sized phytoplankton than for small-sized cells (Harrison & Wood 1988,
21 Tremblay et al. 1997).

22 Based on these relationships between resource acquisition and cell size, size constraints have
23 been used to explain the main macro-ecological distribution patterns of phytoplankton. Thus, it has
24 been postulated that the predominance of communities dominated by large cells in upwelling or high
25 dynamic areas (i.e. under non-restrictive conditions of nutrients or light, at least temporally) is

1 related to their higher growth rates and carbon-specific photosynthesis in comparison with small
2 phytoplankton (Stolte & Riegman 1995, Raven & Kubler 2002, Lohrenz et al. 2003, Irwin et al.
3 2006, Litchman et al. 2009, Key et al. 2010). In contrast, dominance of picoplankton in poor
4 nutrient and strong stratified open ocean waters is normally attributed to higher affinity of this group
5 for nutrients (Agawin et al. 2002, Gutiérrez-Rodríguez et al. 2011, Silovic et al. 2011). However,
6 exceptions to this expected relationship between nutrient usage and community size structure have
7 been described (Rodríguez et al. 2001, Armstrong 2003, Nogueira et al. 2004, Morin & Fox 2004,
8 Irigoien et al. 2005). Normally, these deviations are attributed to biological interactions (e.g.
9 grazing) that could have a preponderant role in regulating the abundance of some size groups in
10 several ecosystems.

11 The aim of this study is to asses if shifts in taxonomic composition and biomass size
12 distribution of phytoplankton communities in the Alboran Sea are reflected in their light absorption
13 efficiencies and nutrient uptake rates according to the expected theoretical relationships between
14 size constraints and metabolism. This hypothesis is tested by analysing the results of an
15 oceanographic research survey conducted in contrasting areas (coastal upwelling vs. offshore
16 oligotrophic areas) of the western Alboran Sea. The jet of Atlantic water that penetrates into the
17 Alboran Sea through the Strait of Gibraltar feeds two nutrient depleted quasi-permanent anticyclonic
18 gyres which occupy the entire central part of the western and eastern sub-basins (Parrilla & Kinder
19 1987, Minas et al. 1991, Tintoré et al. 1991). At the northern edge of the western anticyclonic gyre
20 there are intense geostrophic fronts that promote upwelling of subsurface waters. Furthermore, the
21 upwellings are frequently intensified by favourable winds blowing along the northern coast (Parrilla
22 & Kinder 1987, Sarhan et al. 2000). Consequently, the presence of strong decreasing gradients of
23 nutrients and chlorophyll from coast to offshore is a typical feature of the northwestern Alboran Sea
24 (Rodríguez 1994, Ramírez et al. 2005, Mercado et al. 2007, 2012). The changes in the shape of the
25 phytoplankton size spectra in this Alboran sub-basin have been extensively studied (Rodríguez et al.

2001, Arín et al. 2002, Reul et al. 2005). There is also available information about light absorption properties and nutrient uptake kinetics (Arin et al. 2002, Mercado et al. 2008a). However, the studies that analyse the relationship between size structure and metabolism are scarce.

MATERIAL AND METHODS

The sampling was carried out in the northwest Alboran Sea (latitude 35° 54' 36" N and longitude 4° 11' 4° 54' W) between 9-14 May 2008, on board R/V García del Cid in the framework of the Project NITROALBORAN. Ten stations distributed into two transects perpendicular to the coast were sampled (Fig. 1). A vertical profile of temperature, salinity and chlorophyll *a* fluorescence was obtained at each station with a CTD Seabird 25 equipped with fluorescence probe (Seapoint 6000) and PAR radiometer (LICOR). The euphotic layer depth in each station was estimated from the ascending profiles of PAR as the depth at which 1% of surface irradiance was reached. The mixed-layer (ML) depth was estimated according to the method proposed by Kara et al. (2000) based on the use of a finite difference criterion where the ML depth is the depth where potential density has changed by a fixed amount from the surface reference value. Specifically, the ML depth was estimated as that at which the density difference from the surface was 0.5. This threshold was chosen as it gave the highest success rate for finding the proper pycnocline in previous studies performed at the northern Alboran Sea (Mercado et al. 2007, 2008a,b). Additionally, vertical variation of the Brunt-Väisälä frequency (N^2) was estimated at each station from the potential density profiles according to Millard et al. (1990).

Samples of seawater were collected with a rosette equipped with Niskin bottles at 7 fixed depths (0, 10, 20, 30, 50, 75 and 100 m). An additional sample was collected at the depth where the

sub-surface chlorophyll *a* fluorescence maximum (SFM) was detected (usually at 20-50 m depth). For nutrient analysis, two replicates of seawater from each depth were taken and immediately frozen at -20°C. At the laboratory, nutrients (nitrate plus nitrite, nitrite, ammonium, phosphate and silicate) were analysed by means of segmented flow analysis using a Bran-Luebbe AA3 autoanalyser, following the methods described in Ramírez et al. (2005). The detection limits were: 0.05 µM for nitrate, 0.01 µM for nitrite, 0.04 µM for phosphate and ammonium and 0.10 µM for silicate. For the determination of chlorophyll *a* (chl *a*) a volume from 0.5 to 1 L of seawater was filtered through Whatman GF/F filters. The filters were subsequently frozen at -20 °C until their analysis at the laboratory. The analysis of chl *a* was conducted by spectrophotometry, after extraction in 90% acetone overnight at 4-5°C.

Structure of the phytoplankton communities

Abundance of *Prochlorococcus*, *Synechococcus*, eukaryotic picoplankton and nanoplankton were determined in samples collected at each depth. The samples were fixed with glutaraldehyde (1% f.c.) and immediately frozen in liquid nitrogen (Vaulot et al. 1989). The samples were analysed with a Becton Dickinson FACScan flow cytometer. Counting of cells was performed based on the forward-light scatter and the orange and red fluorescence signals. BD TrueCOUNT Tubes were used to determine absolute counts. Abundance and taxonomy of phytoplankton >5 µm were determined in water samples collected at surface and SFM depth. The water samples were fixed in dark glass bottles with Lugol's solution (2% f.c.). At the laboratory, 100 mL of each fixed sample were sedimented in a composite chamber for 24 h, following the technique developed by Utermöhl (1958). Cells were counted at 200x and 400x with a Leica DMIL inverted microscope connected to a Leica DFC video-camera. The species nomenclature was validated following Tomas (1997).

Cell bio-volume of the most abundant species and genus of diatoms and dinoflagellates was calculated by using the most appropriate formula according to their geometric shape (Sun and Liu

2003, Olenina et al. 2006, Vadrucchi et al. 2007). Bio-volume of unidentified autotrophic flagellates was also calculated. The figures of main cell axis length measured by image analysis with Leica Application Suite software were used to perform these calculations. Equivalent spherical diameter (ESD) of each cell was estimated from the length of the longer axis. Bio-volume of forty-two taxonomic groups (most of them genera or species of diatoms) were characterised. Biovolumes of *Synechococcus*, *Prochlorococcus*, nanoeukaryotes and picoeukaryotes were calculated using the values given in Ribes et al. (1999) for samples collected in the northwestern Mediterranean Sea. Bio-volume values were converted into biomass using the formulae proposed by Morel et al. (1993) for *Prochlorococcus*, Kana & Glibert (1987) for *Synechococcus*, Verity et al. (1992) for phytoplankton smaller than 15 µm in length and Menden-Deuder & Lessard (2000) for dinoflagellates and diatoms higher than 15 µm in length.

Light absorption properties

Absorption spectra were obtained on Whatman GF/F filters with a Shimadzu UV10100 spectrophotometer following the modified method by Trüper & Yentsch (1967). The absorbance of the filters (OD_{filt}) before and after the extraction of the chl *a* with acetone was measured as described in Mercado et al. (2008b). The equation proposed by Cleveland and Weidemann (1993) was used to calculate the absorbance spectrum of the suspension [OD_{susp}(λ)] from OD_{filt}(λ).

Absorption coefficient [a(λ)] was calculated according to:

$$a(\lambda) = 2.3 \text{ OD}_{\text{susp}}(\lambda) / (V/A)$$

where V is the filtered volume (m³) and A the filtered area (m²).

The absorption coefficient of the phytoplankton (a_{phy}(λ); m⁻¹) was calculated by subtracting the absorption of particulate matter [a_p(λ); unit m⁻¹] from detritus [a_d(λ); unit m⁻¹] which were obtained

before and after extraction, respectively. Specific absorption coefficient spectra [$a^*(\lambda)$; $\text{m}^2 \text{mg}^{-1} \text{chl } a$] were calculated by dividing $a_{\text{phy}}(\lambda)$ by chl a concentration.

Inorganic carbon and nitrogen uptake

Experiments of inorganic carbon, nitrate and ammonium uptake were performed with samples collected at the stations A1, A5, B1 and B5. Samples obtained at the surface and SFM depth were incubated for 4-5 h in 1 L polycarbonate transparent bottles in light-attenuated deck boxes, cooled by flowing surface seawater. Before the incubations, the samples were pre-screened through a 200 μm mesh and additions of sodium bicarbonate- ^{13}C (99 atom % ^{13}C ; 150 μM final concentration), potassium nitrate- N^{15} (99 at.% ^{15}N ; 0.05 μM final concentration) or ammonium- N^{15} sulphate (98 at.% ^{15}N ; 0.05 μM final concentration) were performed. The final enrichment of dissolved inorganic carbon was 7%. The nitrate final enrichment calculated for the samples with nitrate concentrations above the detection limit varied from 1% to 55%. The final ammonium enrichment was 40% on average. Controls (i.e. samples without addition of ^{13}C or ^{15}N) were also incubated. The isotope enrichment and the concentration of particulate organic carbon (POC) and nitrogen (PON) was estimated at the beginning of the experiments by filtering 1 L of initial non-incubated seawater (i.e. without ^{13}C or ^{15}N added) through precombusted (450°C for 2 h) Whatman GF/F filters. After incubation, the samples were filtered through precombusted Whatman GF/F filters. The filters were frozen until they were exposed to HCl fumes overnight and then dried and pelletized for isotopic analysis. Carbon and nitrogen stable isotope natural abundance measurements were made by continuous flow isotope-ratio mass spectrometry with a DELTA PLUS Finnigan MAT mass spectrometer. This technique also provided data of POC and PON.

The calculations of inorganic carbon uptake rates were performed following Dugdale & Wilkerson (1986):

$$\text{Carbon uptake } (\mu\text{g m}^{-3} \text{ h}^{-1}) = [^{13}\text{C}_{s(t)} - ^{13}\text{C}_{s(0)}] \times \text{POC} / (^{13}\text{C}_{\text{enr}} - ^{13}\text{C}_{\text{nat}}) \times t^{-1}$$

where $^{13}\text{C}_{s(0)}$ and $^{13}\text{C}_{s(t)}$ are the atom % ^{13}C excess at the beginning of (0) and after (t) the incubation period, $^{13}\text{C}_{\text{enr}}$ is the initially labelled fraction (7%), $^{13}\text{C}_{\text{nat}}$ is the percentage of dissolved ^{13}C occurring naturally and t is the incubation time in hours.

The calculations of nitrogen uptake were performed following Knap et al. (1996). The initial PON concentration was used for the calculations, as it varied on average <10% after incubation. The main problem in calculating *in situ* uptake rates in our experiments was that the addition of ^{15}N increased the nitrogen concentration >10% in some samples. This could lead to an overestimation of the nitrogen uptake rates (Dugdale & Goering 1967). For these samples, the uptake rates were corrected following the procedure proposed by MacIsaac & Dugdale (1972). Another problem inherent to the ^{15}N uptake experiments is the possible change of the isotope enrichment of ammonium during incubation due to N regeneration (dilution effect). The potential under-estimation of the NH_4^+ uptake rates was evaluated following the numerical procedure proposed by Kanda et al. (1987). It was assumed that uptake and regeneration rates were tightly coupled (i.e. a value of 1 for parameter a in Kanda et al.'s 1987 Eq. [8] was assumed). According to the results of these calculations, the under-estimation of the uptake rates was <5%. The relative nitrate uptake was estimated as the ratio between NO_3^- uptake and $(\text{NO}_3^- + \text{NH}_4^+)$ uptake.

Statistical analysis

A principal component analysis (PCA) was performed in order to identify the main variation patterns among the physical and chemical variables (Savenkoff et al. 1995). The variables included in the analysis were temperature, salinity, nutrient concentrations (nitrate, ammonium, silicate and phosphate), nutrient molar ratios (nitrate:phosphate, N:P; nitrate:silicate, N:Si; silicate:phosphate, Si:P) and chl a concentration. Each variable was normalized to 0 mean by first subtracting the mean

value for the whole data set and then dividing it by the standard deviation. PCA was performed with the data obtained in the 10 stations at each sampling depth (excluding the data obtained at depth higher than the lower limit of the mixed layer). The scores of the two first components (PC1 and PC2) extracted from the PCA for each sample were used to research the relationships among the main hydrological patterns and the changes in composition and cell size of the phytoplankton communities. For this objective, the correlations among the biological variables (biomass of picoplankton, diatoms, dinoflagellates and flagellates, ESD of microplankton and diatoms) and the scores for PC1 and PC2 were analysed. The correlations were studied by Pearson's correlation analysis. *p* values of the coefficient correlations were calculated in order to determine their statistical significance.

One-way ANOVA was performed in order to determine the statistical significance of the differences in diatom cell size, ratio of chlorophyll *a* to particulate organic carbon, light specific absorption coefficient and uptake rates of inorganic carbon, nitrate and ammonium between the coastal stations (A1 and B1) and offshore stations (B5 and B5). Additionally, Pearson's correlation analyses among metabolic variables, biomass and ESD were performed in order to research the possible relationships among composition, size and metabolism of the communities.

Prior to perform the statistical tests, normality and homogeneity of variances of the biological variables were assessed. Normality was evaluated from the normal probability plot of the residuals of each variable. Homogeneity of variances was tested with a Levene's test. The assumptions of the parametric correlations and ANOVA were satisfied by all the variables. The software package Statistica 7.1 (Statsoft, Inc. 1984-2005) was used for the statistical analysis.

RESULTS

1 Surface temperature increased sharply from the coastal stations to the centre of the basin (Fig. 2a).
2 In contrast, the highest surface salinity (> 37.5) was registered in the coastal stations while salinity
3 was < 36.5 at the offshore stations (Fig. 2b). Furthermore, the vertical profiles of salinity in the
4 coastal stations (A1, A2, B1 and B2) indicated the presence of a strong vertical gradient of salinity
5 located at 30-40 m depth. The gradient was deeper at the offshore stations (80-100 m depth; stations
6 A4, A5, B4, B5). Consequently, the vertical maximum of Brunt Väisälä frequency (that indicates the
7 pycnocline position) in the offshore stations was located at higher depth in comparison with the
8 coastal stations (Fig. 3). Concordantly, the thickness of the upper mixed layer (ML) estimated as the
9 depth where potential density changed by 0.5 from surface, was shallower in the coastal stations
10 (Fig. 3). These differences in the hydrological features suggest that stations A1, A2, B1 and B2 were
11 affected by upwelling of deep water during the survey whereas stations A4, B4, A5 and B5 were
12 located within the core of the western Alboran anticyclonic gyre. Coincidentally, wind records at the
13 study area indicate that westerly winds (that favour the coastal upwelling) started to blow two days
14 prior to the samplings and were predominant during the survey (data not shown).

15 Surface concentrations of nitrate and phosphate decreased from coast to offshore following
16 the vertical pattern of salinity and temperature (Fig. 4a, b). The highest concentrations of both
17 nutrients were obtained at the stations where upwelling was more intense (i.e. stations A1, A2, B1
18 and B2). In contrast, the surface concentrations were lower than the detection limit in the offshore
19 stations (A4, B4, A5 and B5).

20 Chlorophyll *a* concentration in the surface layer decreased from coast to offshore in the two
21 transects (Fig. 5). A sub-superficial maximum of chl *a* fluorescence was detected in all stations
22 although it became progressively deeper and less intense as the distance to the coast increased. Chl *a*
23 concentration at SFM depth varied from $2.6 \mu\text{g L}^{-1}$ in the coastal stations to $0.3 \mu\text{g L}^{-1}$ in the
24 offshore stations. The SFM was located within ML at the ten sampling stations. However, the
25 irradiance at that depth decreased from coast to offshore. Thus, SFM was located at 10%- I_0 depth in

stations A1, A2, A3, B1 and B2 and close to the euphotic layer lower limit (i.e. 1%-I₀ depth) in stations A4, A5, B4 and B5 (Fig. 3).

The results of the PCA performed with the hydrological and chemical variables are shown in Fig. 6. The two first principal components (PC1 and PC2) explained 67% of the variability. Temperature and salinity were correlated with PC1 although with opposite signs (Fig. 6a). Consequently, PC1 represented the horizontal hydrological gradient from coastal to offshore. As expected, the nutrient concentrations and their molar ratios (except silicate:phosphate molar ratio) were negatively correlated with PC1 corroborating that the upwelling of subsurface Mediterranean water produced enrichment of the ML at the coastal stations. In concordance, stations A1 and B1 had the most negative scores for PC1 (Fig. 6b). Stations A5 and B5 had positive (or slightly negative) scores for this variance component (with the exception of B5 at 100 m depth). The variability explained by PC2 was 12.5%. Ammonium and chl *a* concentrations were negatively correlated with PC2 (note that chl *a* concentration was weakly correlated with PC1). On the contrary, optical depth was positively correlated with PC2. Superficial samples collected at stations A1, A2, B1 and B2 had the most negative scores for PC2 while the samples collected below 20 m depth had positive scores. Furthermore, most of the samples collected at stations A3, A4, A5, B3, B4 and B5 had positive scores for PC2 (with the exception of deeper samples collected at station B5). Based on these results, it can be assessed that PC1 and PC2 gathered the most superficial samples of the stations affected by upwelling.

Distribution of the phytoplankton abundance

The three groups of picoplankton presented distinguishing horizontal and vertical distribution patterns (Fig. 7). The maximal abundances of *Prochlorococcus* were obtained at the central stations of the two transects although the vertical distribution range was wider at stations A3 and A4 (between 20-80 m depth) than at station B3 (where its abundance decreased sharply below 40 m

depth; Fig. 7a). The highest abundances of *Synechococcus* were obtained at 20-30 m depth at the offshore stations (A4, A5, B4 and B5; Fig. 7b). The abundance of picoeukaryotes (and nanoeukaryotes) followed horizontal and vertical distribution patterns similar to those described for chl *a* (Fig. 7c-d) as the highest abundances of both groups were obtained in the surface layer of the coastal stations (A1, A2, B1 and B2).

The highest abundances of microplankton and diatoms were obtained at station B2 although both cell groups decreased from coast to offshore in the two transects (Fig. 8). Flagellates abundance tended to be also higher at the coastal stations in comparison with the offshore stations, especially in the transect B. The highest abundance of dinoflagellates was obtained at SFM depth at station B2, though a clear horizontal variability pattern from coast to offshore was not obtained for this group. Diatom cells were the most abundant in all the samples analysed with the exception of the surface samples of stations A4, B4 and B5 and the SFM samples of stations A5, B3 which were dominated by flagellates. There was not any sample dominated (in terms of abundance) by dinoflagellates.

The results of the correlation analyses among abundances of the phytoplankton groups and scores for PC1 and PC2 support the differences among the horizontal and vertical distribution patterns commented above. Abundance of *Synechococcus* was positively correlated with the scores for PC1 (Table 1). This result corroborates that *Synechococcus* was more abundant in the warmer and less salty waters of the offshore stations. The correlations between scores for PC2 and abundances of pico- and nanoeukaryotes, diatoms and flagellates were statistically significant, probably because higher abundances of these four groups co-occurred in the surface layer of the coastal stations. Furthermore, abundance of <50 μm diatoms and ratio of diatoms to dinoflagellates was negatively correlated with both scores for PC1 and PC2. In contrast, abundances of *Prochlorococcus* and dinoflagellates were not significantly correlated with the scores for PC1 or PC2 indicating that their distribution patterns were not clearly related with the hydrological patterns identified by means of the PCA.

Size structure of the phytoplankton communities

In most of the samples analysed, the diatoms covered a wide range of cell length, from 14 μm to 140 μm of equivalent spherical diameter (ESD). However, the mean length of the dominant species differed between the coastal and offshore stations. The diatom community in the coastal stations was dominated by *Chaetoceros*, *Asterionellopsis* and *Leptocylindrus minimum* with ESD lower than 50 μm . At the offshore stations, the diatom community was dominated by large diatoms (>50 μm ESD) of the genus *Leptocylindrus* and other species of the genera *Nitzschia* and *Pseudonitzschia*. Consequently, the mean of ESD for the diatom community was about 40% lower in stations A1, A2, B1 and B2 than in stations A4, A5, B4 and B5 (Fig. 9; Table 2). In contrast, ESD averaged for flagellates and dinoflagellates did not change significantly from coast to offshore (data not shown). ESD averaged for the whole micro-plankton community (i.e. including diatoms, flagellates and dinoflagellates) followed the same variability pattern as the one obtained for diatoms although the differences between the coastal and offshore stations were not significant statistically (Table 2). ESD averaged for both microplankton and diatoms were positively correlated with the scores for PC1 (Table 1). Therefore, the changes in cell length of the microplankton communities were mainly related to changes in size structure of the diatom community following the coast-offshore hydrological gradient.

In order to estimate the contribution of each phytoplankton group to the whole community, cell abundances were transformed into equivalent biomass and afterwards the biomass percentage in relation to phytoplankton total biomass was quantified (biomass percentage; Fig. 10). *Prochlorococcus* counted for less than 1% of the phytoplankton biomass (note that the x-axis scale in Figure 10 hampers the visualization of the *Prochlorococcus* biomass percentage). *Synechococcus* biomass percentage was maximal in the surface sample of station A4 and minimal in the surface sample of station B1. Concordantly, *Synechococcus* biomass percentage and scores for PC1 were

positively correlated (Table 1). Picoeukaryote biomass percentage was comparatively lower in transect B although it tended to decrease in the offshore stations of the two transects. As described for cell abundance, the diatoms were the dominant group in terms of biomass at the coastal stations (A1, A2, B1 and B2; Fig. 10). In contrast, the community was dominated by dinoflagellates at the offshore stations. Consequently, diatom biomass percentage was negatively correlated with the scores for PC1 while dinoflagellate biomass percentage was positively correlated (Table 1). Interestingly, small diatom biomass percentage (ESD <50 μm) was significantly correlated with the scores for PC1, being the correlation between large diatom biomass and scores for PC1 not statistically significant. Therefore, the differences in diatom biomass following the coast-offshore gradient were mainly due to changes in the abundance of the smaller diatoms. Biomass percentages of *Prochlorococcus*, nanoplankton and dinoflagellates were positively correlated with the scores for PC2, whereas the correlation between scores PC2 and diatom biomass was negative.

In order to visualize how the changes in the community composition affected the size structure, the biomass percentages by different cell size classes were calculated (Fig. 11 a-b, only data of stations A1, A5, B1 and B5 are shown.). For these comparisons, size classes relative to cell bio-volume were used instead of ESD. The main difference in the distribution of biomass percentages by size classes between coastal and offshore stations was a drastic reduction of the biomass of the 100-1000 μm^3 size class in included most of <50 μm diatoms. Furthermore, in the samples collected at offshore stations, the 10^3 - 10^4 μm^3 size class counted for more than 70% of the phytoplankton biomass.

Physiological features of the phytoplankton communities

In our study, cell content of chl *a* was indirectly estimated from the ratio of chl *a* to particulate organic carbon (Westberry et al. 2008). Chl *a*/POC (Fig. 11a) ranged from 5 to 41 $\mu\text{g chl } a \text{ mg}^{-1}$ POC obtained in the surface samples of stations B5 and B1, respectively. Furthermore, chl *a*/POC

was significantly higher at the coastal stations in comparison with the off-shore stations (Table 2). The highest $a^*(440)$ value ($0.08 \text{ m}^2 \text{ mg}^{-1} \text{ chl } a$) was also obtained in the surface sample of station B5 although the differences between coastal and offshore stations were not statistically significant (Fig. 12b). In order to research which phytoplankton group contributed to these differences in chl a /POC and $a^*(440)$, correlation analyses among these two variables and biomass percentages for each group were performed (Table 3). Chl a /POC was negatively correlated with *Synechococcus* biomass percentage and positively with $<50 \text{ }\mu\text{m}$ diatom biomass percentage. The correlation between $<50 \text{ }\mu\text{m}$ diatom biomass percentage and $a^*(440)$ was also significant although negative. In contrast, $a^*(440)$ was positively correlated with the biomass percentages of *Synechococcus*, nanoplankton and dinoflagellates.

The biomass specific rates of carbon uptake (VDIC) were about four-fold higher in the coastal samples in comparison with the offshore samples (Fig. 12c, Table 2). The variability in the biomass-specific nitrate uptake rates (VNO_3^-) was substantial among the eight samples analysed although on average VNO_3^- was also significantly higher at the coastal stations (Fig. 12d, Table 2). In contrast, the biomass-specific ammonium uptake rates (VNH_4^+ ; Fig. 12e) were higher in the offshore samples even though the differences between coast and offshore were not statistically significant (Table 2). VNH_4^+ at the coastal stations was lower than VNO_3^- by 5-20 times. On the contrary, VNH_4^+ was higher than VNO_3^- at the offshore stations. The coefficients for the correlations among uptake rates and biomass percentages of the phytoplankton groups are showed in Table 3. Biomass percentage of $<50 \text{ }\mu\text{m}$ diatoms was positively correlated with VDIC and VNO_3^- and negatively with VNH_4^+ . Furthermore, pico-eukaryote biomass percentage was correlated with VDIC and VNH_4^+ and biomass percentages of *Synechococcus* and dinoflagellates were negatively correlated with VDIC.

DISCUSSION

Hydrological variability, nutrients and chlorophyll *a*

The surface layer at the western sector of the Alboran Sea is usually occupied by Modified Atlantic Water (MAW) with 36.5 salinity and low nutrient concentration (Minas et al. 1991, Rodríguez et al. 1997). However, saltier and colder waters are frequently detected along the northern coast of the Alboran Sea due to subsurface Mediterranean water upwelling driven by westerlies (Minas et al. 1991, LaFuente et al. 1998, Rodríguez et al. 1998, Sarhan et al. 2000, Ramírez et al., 2005, Mercado et al. 2007). Westerly winds started to blow 2 days prior the samplings presented in this report. Therefore, it is probable that the nutrient enrichment and the decrease of the upper mixed layer thickness obtained in the coastal stations were due to upwelling of Mediterranean water. In contrast, salinity values in the surface layer of the offshore stations were similar to those described in the literature for the MAW. The thickness of the MAW layer was almost 100 m at stations A4, A5, B4 and B5, indicating that these stations were located within the western Alboran anticyclonic gyre core (Parrilla & Kinder 1987).

Our data show that the upwelling modified the availability of resources for phytoplankton growth (nutrients and irradiance) at the coastal stations in comparison with the offshore stations. On average, nitrate concentration in the ML of the coastal stations was 6-folds higher than in the offshore stations, while silicate and phosphate concentrations were higher by 2.5 times. Consequently, the upwelling produced an increase of nitrate relative to silicate and phosphate concentration that is attributable to the different nutrient composition of the upwelled Mediterranean water (Dafner et al. 2003, Ramírez et al. 2005, Reul et al. 2005). In addition, the light conditions in the water column were modified by the upwelling because the phytoplankton cell displacement underneath the first optical depth layer (20-30 m depth) must be hampered by the reduction of the ML thickness. In contrast, the ML thickness (70-80 m) at the offshore stations in combination with

the vertical surface water downwelling caused by the circulation pattern in the anticyclonic gyre core would permit phytoplankton cell displacement below the first optical depth layer.

As expected, the highest chl *a* concentrations of the ML were obtained at the coastal stations where the nutrient availability was also relatively high. However, the correlation between chl *a* and salinity, temperature and nutrient concentration was weak as revealed by the structure of the first principal component extracted from the PCA (Fig. 6). The lack of correlation between chl *a* and nutrients could be partially due to consumption by phytoplankton. This was particularly noticeable in station B2 where the highest concentration of chl *a* was obtained although the nitrate concentration was relatively low. However, the chl *a* concentration was low in the surface layer of the station B3 despite nutrient concentrations were high. These findings suggest that nutrient availability was not the only pre-requisite for stimulating the phytoplankton growth. In fact, the results of the PCA hint that the phytoplankton bloom was jointly triggered by the nutrient enrichment and ML thickness decrease. This conclusion is in agreement with previous observations performed at the Alboran Sea (Claustre et al. 1994, Morán et al. 2001, Ramírez et al. 2005) and broadly supports the Sverdrup's hypothesis (1953) that bloom initiation is produced when the surface mixed layer becomes shallower.

Effects of hydrological variability on abundance and composition of the phytoplankton communities

The three groups of pico-plankton analysed presented different horizontal distribution patterns. Higher abundances of *Synechococcus* were obtained at the stations occupied by MAW whereas *Prochlorococcus* was more abundant at intermediate depths in the stations with surface salinity values and nutrient concentrations midway between those featuring the MAW and the upwelled Mediterranean water. Pico-eukaryotes were more abundant in the recently upwelled surface waters of the coastal stations, indicating that they responded more rapidly than *Prochlorococcus* and

Synechococcus to the changes induced by upwelling. These distribution patterns fit only partially the ones described by Reul et al. (2005) for the same study area as these authors obtained that both *Prochlorococcus* and *Synechococcus* increased in the western Alboran oligotrophic gyre in comparison to the northern coastal area. In the eastern Alboran Sea, Jacquet et al. (2010) also found that the picoplankton community was dominated by *Synechococcus* in the anticyclonic gyre while *Prochlorococcus* did at intermediate depths (ca. from 30 to 100 m depth) in the edge of this gyre. Jacquet et al. (2010) attributed the distribution pattern of *Synechococcus* at the eastern Alboran Sea to the horizontal gradient of nutrients, which is in agreement with the paradigm widely accepted that *Synechococcus* grows optimally in enriched-nutrient and mixed waters (Li 1994). However, Reul et al. (2005) demonstrated that *Synechococcus* abundance in the western sector of the Alboran Sea was negatively correlated with nutrient concentrations, which is corroborated by the results of the present study.

Contribution of picoplankton to phytoplankton biomass at the offshore stations varied from 11% to 26%. These figures are similar to those reported by Reul et al. (2005) for the western Alboran Sea in spring. Picoplankton biomass percentage was slightly lower at the coastal stations mainly due to lower abundance of *Synechococcus*. These changes were reflected in a higher biomass percentage of cells smaller than $1\ \mu\text{m}^3$ in bio-volume at offshore stations in comparison with coastal stations. However, the most conspicuous horizontal variability pattern in the size structure of the communities from coast to offshore was obtained for the size classes higher than $10^3\ \mu\text{m}^3$ in bio-volume (that roughly included cells higher than $10\ \mu\text{m}$ in length). Particularly, the biomass percentage of 10^3 - $10^4\ \mu\text{m}^3$ bio-volume cells was drastically reduced following the coast-offshore gradient. These changes went in parallel to an increase in ESD of the microplankton community. There were not differences in ESD of flagellates or dinoflagellates attributable to the hydrological gradient. However, ESD of diatoms was positively correlated with the first factor extracted from the PCA which can be attributed to high abundance of $<50\ \mu\text{m}$ diatoms in comparison with $>50\ \mu\text{m}$

1 diatoms at the coastal stations. Consequently, the showed data demonstrate that changes in cell size
2 following the coast-offshore gradient were due to changes in the abundance of diatoms relative to
3 dinoflagellates.

4 The samplings started just two days after westerly winds began to blow and this was the
5 wind regime predominating during the survey. Consequently it can be hypothesized that the
6 communities sampled at the coastal stations represented a relatively early phase of the
7 phytoplankton bloom induced by upwelling. According to Mercado et al. (2005, 2011), diatom
8 communities in the northwestern Alboran Sea during this bloom phase are dominated by
9 *Chaetoceros* which is consistent with the results of the present study. Moreover, our results support
10 the paradigm that diatoms dominate the phytoplankton community in areas affected by upwelling,
11 although the outcomes of this study indicate that <50 μm diatoms responded more rapidly than the
12 other size classes to the upwelling-induced changes. In contrast, the diatom communities in the
13 offshore stations were dominated by *Leptocylindrus*, *Asterionellopsis* and *Rhizosolenia* with
14 relatively large ESD. Chlorophyll *a* satellite images for the sampling days indicate that the
15 chlorophyll produced at the upwelling area was displaced towards the east by the current induced by
16 the Atlantic jet, away from the offshore stations. Consequently, it is improbable that these large cells
17 reached the offshore stations by advection from the coastal areas. This predominance (in terms of
18 biomass) of large cells at the offshore stations apparently contradicts the widely accepted paradigm
19 that small cells dominate the phytoplankton community in oligotrophic areas (Azam et al. 1983,
20 Goldman 1993).

22 **Relationship between phytoplankton composition and metabolic rates**

23 In our study, the changes in community composition were correlated with variations in the
24 efficiency of light absorption, which was higher in the offshore communities (dominated by
25 dinoflagellates) in comparison with the coastal communities (dominated by <50 μm diatoms).

1 Differences in light absorption efficiency per chl *a* unit among marine phytoplankton assemblages
2 have been attributed to the distinguishing pigment composition of the taxonomic groups that shape
3 the community (Bidigare et al. 1990, Hoepffner and Sathyendranath 1991) and/or to changes in the
4 effect of packaging of the pigments (that diminishes their light absorption efficiency). The pigment
5 packaging effect is modulated by the cell size and shape traits and by the pigment intracellular
6 content. In particular, the packaging effect increases with cell size (Ciotti et al. 2002, Ciotti &
7 Bricaud 2006) and lessens with decreasing pigment internal content (Thompson et al. 1992,
8 Reynolds et al. 1997, Stramski et al. 2002). In our study, changes in taxonomic composition, cell
9 ESD (and bio-volume) and chl *a* per biomass (that is a proxy for the average chl *a* cell content for
10 the community; Westberry et al. 2008) co-varied following the coast-offshore gradient, therefore it
11 is difficult to isolate the factor which causes the differences in the light absorption efficiency
12 estimated from $a^*(440)$. However, the relationship between cell ESD and $a^*(440)$ was positive (i.e.
13 the opposite of the expected relationship between cell size and light absorption efficiency).
14 Therefore, it appears that cell size did not contribute to the observed variability in $a^*(440)$. In
15 contrast, chl *a*/POC and $a^*(440)$ were negatively correlated ($r=-0.87$, $p<0.01$) as expected if lower
16 chl *a* cell content lessened the packaging effect. The increase in internal chl *a* content and the
17 consequent reduction of $a^*(\lambda)$ in response to increasing nutrient concentration has been widely
18 described in laboratory experiments performed with both diatoms and dinoflagellates (Reynolds et
19 al. 1997, Stramski et al. 2002, Stehr & Cullen 2003). Similarly, Mercado et al. (2008b) reported
20 higher $a^*(440)$ values in natural communities of the Alboran Sea growing at nitrate limitation in
21 comparison with non-limited communities. Consequently, the higher light absorption efficiency in
22 the offshore communities fits the expected acclimation response to low nutrients. However, it cannot
23 be discarded that the distinguishable taxonomic composition of the offshore communities
24 contributed to their higher $a^*(440)$ since the chl *a* content per carbon unit in dinoflagellates is *per se*
25 low in comparison with diatoms and other phytoplankton groups (Tang 1996). Furthermore, the

larger cells has higher ability to build vacuoles (Raven 1995) that decreases the chl *a* content per cell volume (Raven 1995).

It should be expected that these changes in light absorption efficiency do not affect the photosynthetic capacity by communities growing under non-limiting irradiance conditions ($P_{\max}$). Consequently, the differences in biomass-specific carbon uptake rates (VDIC) among the surface communities of the coastal and off-shore stations (which photosynthesize under non-limiting light) should be attributed to differences in Calvin cycle activity rather than to photoacclimation. Our data show that the coastal surface communities dominated by <50 μm diatoms had higher carbon uptake rates than the offshore communities dominated by dinoflagellates. Changes in $P_{\max}$ coinciding with increases in contribution of dinoflagellates to phytoplankton biomass have been previously described in natural communities by different authors (Côté & Platt 1983, Shaw and Purdie 2001). Published data indicate that dinoflagellates have lower photosynthesis rates per carbon unit (Chan 1980) and higher metabolic costs in comparison with diatoms (Smayda 1997) which has been attributed to their lower chl *a* per carbon unit (Tang 1996). Therefore, the differences between coastal and offshore communities fit adequately the distinctive physiological performances of both groups. Dominance of dinoflagellates on diatoms at offshore stations could be explained by its capacity for adopting an alternative strategy to autotrophy for nutrient acquisition as have been reported that some of the species found in our samples have mixotrophic nutrition (e.g. *Prorocentrum minimum* and *Karenia brevis*; Li et al. 1996, Stoecker et al. 1997, Glibert et al. 2009). If that is the case, the inorganic nutrient uptake rates obtained in our study for the off-shore communities could not reflect their actual nutrient acquisition rates. Li et al. (2012) found that mixotrophy in dinoflagellates could be a competition strategy during nutrient limitation.

In overall, the results demonstrate that acclimation strategies and physiological features of the dominant groups modulate the usage of light and nutrients by the community. These changes appear to depend weakly on the size-constraints (at least at community level). Our results do not

1 fully support the expected relationship between cell size and photosynthetic capacity (Stolte &
2 Riegman 1995, Lohrenz et al. 2003, Irwin et al. 2006, Litchman et al. 2009, Key et al. 2010), at least
3 when the features of the community as a whole are examined. Similarly, Huete-Ortega (2011)
4 reported that phytoplankton carbon fixation rates and cell size are often un-correlated. Previously,
5 several authors demonstrated that the slope of the size-scaling function of the phytoplankton
6 metabolic rate is far from the expected value (the so called 3/4 rule; Dodds et al. 2001, Isaac &
7 Carbone 2010) and Finkel et al. (2010) obtained a slope much closer to 1 than 3/4 for the
8 relationship between the specific growth rate as a function of cell size. Huete-Ortega (2011)
9 attributed the deviation of metabolic rates, predicted from their cell size and those actually
10 measured, to the acquisition of taxa-specific physiological strategies by species belonging to certain
11 size class. Our data support this hypothesis and emphasize the necessity of considering the
12 acclimation capacity of the communities to predict their role in the carbon budgets.

13
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Table 1. Pearson's correlation coefficients of the correlations among the scores for the two first principal components extracted from the PCA (PC1 and PC2) and the cell abundances of each group (microplankton is the sum of diatoms, dinoflagellates and flagellates), their biomass percentages (i.e. the biomass of each group normalized by the total phytoplankton biomass) and the equivalent spherical diameter (ESD) averaged for the microplankton and diatoms. Coefficients statistically significant are in bold (*, $p < 0.05$; **, $p < 0.01$). N indicates the number of samples used in each comparison.

	PC1	PC2	N
<i>Cell abundances</i>			
<i>Prochlorococcus</i>	-0.08	0.23	57
<i>Synechococcus</i>	0.79**	-0.08	57
Picoeukaryotes	-0.08	-0.52**	57
Nano-eukaryotes	-0.06	-0.57**	57
Microplankton	0.01	-0.72**	20
Diatoms	-0.24	-0.71**	20
<50 μm diatoms	-0.57**	-0.73**	
>50 μm diatoms	0.02	-0.46*	
Flagellates	0.29	-0.74**	20
Dinoflagellates	0.16	-0.28	20
Diat/Dino	-0.40*	-0.63**	20
<i>Biomass percentage</i>			
<i>Prochlorococcus</i>	-0.23	0.48*	20
<i>Synechococcus</i>	0.56**	0.33	20
Picoeukaryotes	0.28	0.41	20
Nanoplankton	-0.23	0.50*	20
Microplankton	0.11	-0.58**	20
Total diatoms	-0.48*	-0.75**	20
<50 μm diatoms	-0.57**	-0.73**	20
>50 μm diatoms	0.02	-0.47*	20
Flagellates	-0.25	0.28	20
Dinoflagellates	0.53**	0.72**	20
<i>Cell size (ESD)</i>			
Microplankton	0.45*	0.07	20
Diatoms	0.66**	-0.11	20

Table 2. Results of the one-way ANOVA performed to determine the statistical significance of the differences in cell length and physiological features between the coastal communities (data obtained at the surface and SFM depth of stations A1 and B1) and the offshore communities (surface and SFM samples of stations A5 and B5). ESD: equivalent spherical diameter; $a^*(440)$, chl a specific absorption coefficient at 440 nm; VDIC, VNO_3^- and VNH_4^+ , biomass specific uptake rates of dissolved inorganic carbon, nitrate and ammonium, respectively; p, probability value associated to F.

	F	p
Microplankton ESD	2.4	0.14
Diatom ESD	21.1	<0.001
$a^*(440)$	7.6	0.05
chl a /POC	12.2	0.01
VDIC	144.9	<0.001
VNO_3^-	7.2	0.004
VNH_4^+	5.4	0.06

Table 3. Pearson's correlation coefficients obtained from comparisons between the physiological variables and the biomass percentages of the different phytoplankton groups [chl *a*/POC, ratio of chlorophyll *a* to particulate organic carbon; *a**(440), chlorophyll *a* specific absorption coefficient at 440 nm; VDIC, VNO₃⁻ and VNH₄⁺, biomass specific uptake rates of dissolved inorganic carbon, nitrate and ammonium, respectively]. The coefficients of the correlations between mean cell length of microplankton and diatoms and the physiological variables are also showed [ESD, equivalent spherical diameter]. Coefficients statistically significant are in bold (*, p<0.05; **, p<0.01). N was 8 for all comparisons.

	chl <i>a</i> /POC	<i>a</i> *(440)	VDIC	VNO ₃ ⁻	VNH ₄ ⁺
<i>Biomass</i>					
<i>Prochlorococcus</i>	0.003	0.20	-0.28	-0.34	-0.30
<i>Synechococcus</i>	-0.75*	0.87*	-0.74*	-0.61	0.41
Picoeukaryotes	0.57	-0.72	0.73*	0.36	-0.81*
Nanoplankton	-0.34	0.81*	0.10	0.04	-0.66
<50 µm diatoms	0.88**	-0.97**	0.94**	0.77*	-0.71*
>50 µm diatoms	0.20	0.20	0.01	-0.06	0.22
Dinoflagellates	-0.70	0.90*	-0.84**	-0.63	0.59
Flagellates	0.50	0.09	0.06	0.1	0.11
<i>Cell size (ESD)</i>					
Microplankton	-0.54	0.12	-0.34	-0.29	0.14
Diatoms	-0.85**	0.98**	-0.79*	-0.63	0.85**

FIG. LEGENDS

Fig. 1. Position of the sampling stations. The grey arrows indicate the surface circulation pattern characteristic of the Alboran Sea. It is representative of the hydrological dynamics that occurred during the sampling cruise (WAG, western anticyclonic gyre; EAG, eastern anticyclonic gyre)

Fig. 2. Spatial variability of temperature (a) and salinity (b) along the two transects perpendicular to the coast sampled during the cruise.

Fig. 3. Spatial variability of Brunt-Väisälä frequency (N^2) estimated from the potential density profiles for the two transects sampled during the cruise. The position of the lower limit of the mixed layer calculated as the depth at which the density difference from the surface was 0.5 is also shown (dashed line). The depths at which 10% and 1% of surface incident light (I_0) was reached are indicated by the continuous line. Black points show the position of the sub-superficial chl *a* fluorescence maximum (SFM) where samples for analysis of taxonomic and physiological features of the phytoplankton were collected.

Fig. 4. Spatial variability of nitrate (a) and phosphate (b) along the two transects perpendicular to the coast sampled during the cruise.

Fig. 5. Spatial variability of chl *a* concentration along the two transects perpendicular to the coast sampled during the cruise.

Fig. 6. Structure of the two first principal components (a) extracted from the PCA performed with temperature (T^a), salinity (Sal.), nitrate (Nitra.), nitrite (Nitri.), ammonium (Ammo.), silicate (Sil.), phosphate (Phosp.), nitrate to phosphate molar ratio (N:P), nitrate to silicate molar ratio (N:Si), silicate to phosphate molar ratio (Si:P), chlorophyll *a* (chl *a*) and optical depth (Opt. depth). Panel b shows the scores for PC1 and PC2 of each sample that was included in the analysis. The numbers indicate the station (black points, transect A; white points: transect b).

Fig. 7. Spatial variability of the abundance of *Prochlorococcus* (a), *Synechococcus* (b), picoeukaryotes (c) and nanoeukaryotes (d) quantified by means of flow cytometry along the two transects sampled during the cruise.

Fig. 8. Abundance of microplankton cells (total cells estimated as the sum of diatoms, flagellates and dinoflagellates), diatoms, flagellates and dinoflagellates in the samples collected at the surface (white background) and at sub-superficial chl *a* fluorescence maximum (SFM) depth (grey background) of the ten sampling stations.

Fig. 9. Average values of the equivalent spherical diameter (ESD) of the diatom community in the samples collected at the surface (white columns) and at sub-superficial chl *a* fluorescence maximum depth (SFM; grey columns) of the ten sampling stations. Error bars indicate +1 standard error.

Fig. 10. Biomass percentages of the main groups of phytoplankton in the samples collected at the surface (SUP) and at the sub-superficial chl *a* fluorescence maximum depth (SFM) of the ten sampling stations.

Fig. 11. Biomass percentages of the different size classes of phytoplankton in the samples collected at the surface and the sub-superficial chlorophyll *a* fluorescence maximum depth (SFM) of the coastal (A1 and B1) and offshore (A5 and B5) stations. The x-axis labels indicate the higher limits of each bio-volume size class.

Fig. 12. Physiological performances of the phytoplankton communities collected at the coastal (A1 and B1) and offshore (A5 and B5) stations. White and dark columns indicate the results for samples collected at the surface and sub-surface chlorophyll *a* fluorescence maximum depth (SFM), respectively. (a) Chl/POC: ratio of chlorophyll *a* to particulate organic carbon concentration; (b) $a^*(440)$ and $a^*(675)$: chlorophyll *a* specific absorption coefficient at 440 and 675 nm, respectively; (c) VDIC, biomass specific dissolved inorganic carbon uptake rate ; (d) VNO_3^- , biomass specific nitrate uptake rate; (e) VNH_4^+ , biomass specific ammonium uptake rate;

- 1 (f) RNO_3^- , nitrate relative to (nitrate + ammonium) uptake rate. Error bars indicate +1SD. Note
- 2 that there are not available data of $a^*(440)$ or $a^*(675)$ for station A5.
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Fig. 1. Map

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