PROCHLOROCOCCUS AND SYNECHOCOCCUS CELLS IN THE CENTRAL ATLANTIC OCEAN: DISTRIBUTION, GROWTH AND MORTALITY (GRAZING) RATES

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PICOCYANOBACTERIA
GROWTH AND GRAZING RATES
SYNECHOCOCCUS
PROCHLOROCOCCUS
TROPICAL ATLANTIC

ABSTRACT. – The distribution, primary production, growth and grazing rates of *Prochlorococcus* and *Synechococcus* were investigated across the tropical Atlantic ocean during boreal autumn and spring. The picophytoplankton fraction contributed significantly to total phytoplankton community biomass and production (>70% and > 50%, respectively). *Prochlorococcus* reaching up to $2.5 \bullet 10^5$ cells ml^{-1} numerically dominated the picophytoplankton community in the central oligotrophic Atlantic waters while *Synechococcus* was abundant at the ends of the transects (i.e. in nutrient rich coastal waters). High resolution surface samples showed the meridional patterns of *Prochlorococcus* and *Synechococcus* abundance to be highly variable within a few degrees latitude and correlated well with intermediate scale variability (< 500 km) in water mass structure along the transects. Growth rates of *Prochlorococcus* and *Synechococcus* in surface waters range from 0.40 to 1.47 d^{-1}, and 0.22 to 1.58 d^{-1}, respectively. There was a positive relationship ($r=0.77$, $p<0.05$) between growth and grazing rates, and the average differences between growth and grazing rates were -0.17 ± 0.25 d^{-1}, and 0.15 ± 0.11 d^{-1}, for *Prochlorococcus* and *Synechococcus*, respectively. High grazing rates on *Prochlorococcus* and *Synechococcus* indicate that top down control of picophytoplankton abundance is most likely important in Central Atlantic waters.

PICOCYANOBACTERIES
CROISSANCE ET GRAZING
SYNECHOCOCCUS
PROCHLOROCOCCUS
ATLANTIQUE TROPICAL

RÉSUMÉ. – La distribution, la production, et la croissance de *Prochlorococcus* et de *Synechococcus*, ainsi que les taux de prédation ont été étudiés dans l'Océan Atlantique tropical au cours de l'automne et du printemps boréaux. La fraction picophytoplanctonique contribue significativement à la biomasse et à la production totale de la communauté phytoplanctonique (respectivement > 70 % et > 50 %). *Prochlorococcus* atteignant $2{,}5.10^5$ cellules.ml^{-1} domine numériquement la communauté picophytoplanctonique dans les eaux centrales atlantiques oligotrophes alors que *Synechococcus* a été abondant en fin de transects (i.e. dans les eaux côtières riches [« en sels nutritifs »]). Les prélèvements de hautes fréquences effectués en surface ont révélé tout d'abord que les modèles « méridionaux » d'abondance de *Prochlorococcus* et *Synechococcus* présentaient une grande variabilité aux basses latitudes. Ces modèles ont présenté une bonne corrélation à une échelle intermédiaire (< 500 Km) le long des transects dans les structures de masses d'eaux. Les gammes de variation du taux de croissance ont été respectivement de 0,40 à 1,47 j^{-1} pour *Prochlorococcus* et de 0,22 à 1,58 j^{-1} pour *Synechococcus*. Il a été observé une relation positive ($r=0{,}77$, $p<0{,}05$) entre la croissance et les taux de prédation; les différences moyennes calculées entre la croissance et les taux de prédation étaient respectivement de $-0{,}17 \pm 0{,}25$ j^{-1} pour *Prochlorococcus* et de $0{,}15 \pm 0{,}11$ j^{-1} pour *Synechococcus*. Les valeurs importantes des taux de prédation exercés sur *Prochlorococcus* et *Synechococcus* révèlent la prédominance du top down control sur l'abondance du picophytoplancton dans l'Atlantique central.

INTRODUCTION

Picoplankton are important components of the subtropical and tropical Atlantic ocean (Partensky *et al.* 1996, Zubkov *et al.* 2000a, Agustí *et al.* 2001). *Prochlorococcus* are particularly abundant in the tropical gyres in the Atlantic (Zubkov *et al.* 2000a), where nutrients are extremely depleted and temperature conditions are optimum for their growth (~24°C, Partensky *et al.* 1999). *Synechococcus*, however, can be encountered at lower temperature (Shapiro & Haugen 1988), and are more abundant in oligotrophic to mesotrophic waters of the subtropical and temperate Atlantic (Zubkov *et al.* 2000a).

Although the community structure of the picophytoplankton in the subtropical Atlantic ocean has been investigated quite extensively (Li 1995, Zubkov *et al.* 2000a), measurements of their growth and loss rates (i.e. grazing rates) are lacking. The growth rate of *Prochlorococcus* was estimated, using cell cycle analysis based on flow cytometry, during the boreal autumn (austral spring) to average 0.15 d^{-1}, in subtropical gyres and equatorial waters (Zubkov *et al*. 2000b), well below the maximal rates possible for these organisms (Shalapyonok *et al*. 1998). Partensky *et al*. (1996) reported growth rates of 0.39 – 0.41 d^{-1} in deeper waters of the tropical northeastern Atlantic ocean. Recently, Agustí (2004) reported a high proportion of dead cells of *Synechococcus* and *Prochlorococcus* in the Central Atlantic Ocean, and suggested cell death as an important loss process structuring the picophytoplankton communities in the region. The grazing mortality loss rates however, were not yet reported, but are essential to understand the maintenance of the planktonic populations (Lehman 1991). Moreover, both growth and loss rates are expected to vary over time, as suggested by the reported shifts of picophytoplankton populations (Zubkov *et al.* 2000a) and the temporal variability of photosynthetic parameters of phytoplankton (Marañon & Holligan 1999), between boreal autumn (austral spring) and boreal spring (austral autumn) in the region. The trophic degree of the different regions in the central Atlantic may also influence both growth and loss rates of *Prochlorococcus* and *Synechococcus*. Hence, present knowledge is insufficient to evaluate the importance of loss processes (i.e. grazing mortality) in controlling pico-cyanobacteria communities in the central Atlantic.

Here, we present the distribution and dynamics of pico-cyanobacteria along the subtropical and tropical Atlantic Ocean. We do so on the basis of two extensive meridional cruises conducted during boreal autumn (austral spring) and boreal spring (austral autumn). We first present the distribution of *Prochlorococcus* and *Synechococcus* in both seasons in an extensive continuous sampling of surface waters and also along depths. Secondly, we provide estimates of gross growth and mortality grazing rates estimated using cell cycle analysis and a refined dilution method (Landry *et al*. 1995), respectively. Size fractionated biomass and primary production determinations were done to assess the contribution of picophytoplankton to community biomass and production.

METHODS

Picophytoplankton abundance and biomass: Seawater samples were collected daily on two cruises aboard BIO Hesperidés along the Central Atlantic waters (Fig. 1). The first cruise (LATITUD-III) started on November 1st, 1999 in Las Palmas de Gran Canaria (Spain) and ended on November 20th, 1999 (boreal autumn, austral spring) in Montevideo, sampling 15 stations in between. In the second cruise (LATITUD-IV), starting in Rio de Janeiro on the March 16th 2000 and ending April 4th 2000 (boreal spring, austral autumn), 14 stations were visited. The stations were located at intervals of 3° to 5° latitude from each other. The transect stations in both cruises were similar in the Central Atlantic but during the LATITUD-III cruise, the transect crossed near the NW African upwelling area, and included a coastal station near Montevideo (Fig. 1). The cruise track of both cruises included 3 main biogeochemical provinces as defined by Longhurst *et al.* (1995): (1) the North Atlantic Tropical Gyre (NATR, 25° to 19° N); (2) the Western Tropical Atlantic (WTRA, 10° N to 5° S); and (3) the South Atlantic Tropical Gyre (SATL, 5° to 40° S). During the two cruises, seawater samples were collected in Niskin bottles on a Rosette system fitted with a CTD at an average of 10 depths from the surface down to 200 to 300 m. These samples were used for various biological analyses and experiments, and for chemical (dissolved nutrient concentrations) analyses.

The abundance and cell size (diameter) of pico-cyanobacteria cells (*Prochlorococcus* and *Synechococcus*) in each depth and station were determined on board by

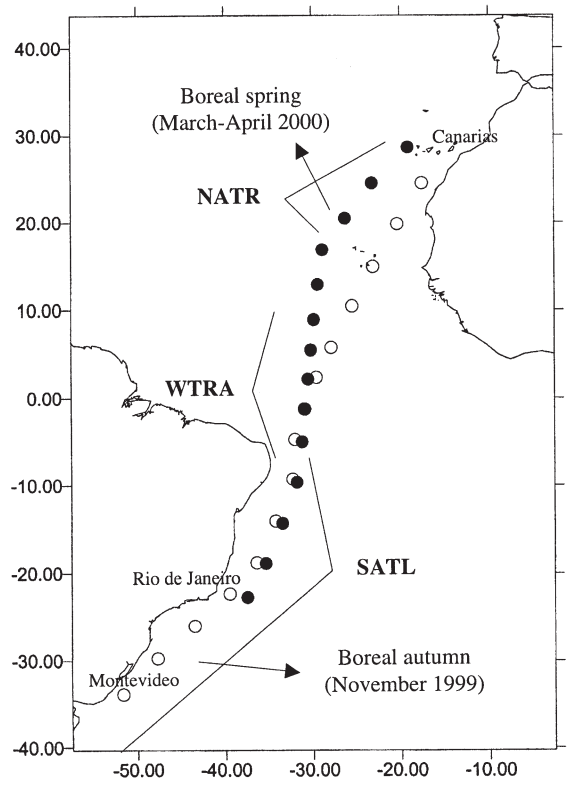


Fig. 1. – Cruise tracks of the BIO Hesperidés across the Atlantic ocean during the LATITUDE-III (open circles, boreal autumn, austral spring) and LATITUDE-IV (filled circles, boreal spring, austral autumn) cruises. The cruise track included the North Atlantic Tropical Gyre (NATR), Western Tropical Atlantic (WTRA) and South Atlantic Tropical Gyre (SATL) regions.

flow cytometric analysis of duplicate fresh samples with a FACSCALIBUR (Becton-Dickinson) flow cytometer (FCM), fitted with a 488 nm laser and a photomultiplier for detection of forward scattered light according to population fluorescence and light scatter characteristics following Vaulot *et al*. (1990). Running time of samples on FCM was about 5 to 10 minutes (flow mode and time depending on the abundance of the cells analyzed). An aliquot of a calibrated solution of 1 µm diameter high-green fluorescent beads (Polysciences) was added to the samples as an internal Standard for the quantification of cell concentration. Cell size (diameter) was calculated from the FSC (Forward Scatter) signal of the picophytoplankton cells and calibrated using fluorescent beads of various sizes (0.2, 0.5, 1.0, 2.0 µm, Fluospheres Size kit # 2, Molecular probes) mixed with algal cultures of known sizes, determined through epifluorescent microscope measurements (*Chlorella* sp., 2.7 µm; *Dunaliella* sp., 3.8 µm). Average cell volume was calculated from the equivalent spherical diameter derived from the FSC signals. To convert cell volumes to biomass, a specific carbon content of 200 fg C μm^{-3} was used (Christian & Karl 1994, Zubkov *et al.* 2000b). Picophytoplankton biomass values were vertically integrated over the euphotic zone (~ 200 m) in each station.

High resolution estimates of pico-cyanobacteria abundance were also obtained in surface waters (~ 5 m) along the transects in both cruises. An automated sampling device, attached to the outlet of continuously sampled surface (~ 5 m) waters, was programmed to take samples every 2 hrs (approximately every 0.36° latitude) along the entire cruise paths. Two ml samples were fixed with 1% (final concentration) glutaraldehyde, frozen in liquid nitrogen and stored at –80°C until analysis in the flow cytometer as described above. Concurrent physical (temperature, salinity and sigma-t) and meteorological data were also recorded. The resulting spatial variability in temperature, salinity and picophytoplankton abundance reflects differences among the main water masses crossed along the transect, as well as smaller-scale variability derived from instabilities and mesoscale features. To explore the relationship between these small to intermediate-scale features and picophytoplankton distribution, we removed the large-scale water mass signals using a running average of approximately 5° latitude, which was subtracted from the observed data to obtain residuals highlighting the small to intermediate-scale variability. These residuals were then correlated to the high resolution picophytoplankton abundance data, to examine their coherence.

Primary production: Primary production by the < 2 µm fraction and the entire community were determined for surface waters during the LATITUD-IV cruise. Size fractionation was done as described for chlorophyll determination. Three (two clear and one dark) 125-ml PC Nalgene bottles each received 120 ml of the < 2 µm fraction, while another set of three bottles were filled with whole seawater. One milliliter of ^{14}C solution (20 µCi concentration) was added in each bottle. The bottles were immediately incubated on deck in a tank with flow-through surface seawater for three hours. Neutral density screens were used to maintain the photosynthetically active radiation (PAR) in the water bath similar to surface (~ 5 m) waters. After the incubation, samples were filtered through 0.45 µm Millipore filters, and filters were fumed over concentrated HCl to remove traces of inorganic C. Radioactivity on the filters was measured with a liquid scintillation counter, with correction for quenching, and converted to C incorporation rates as described by Strickland & Parsons (1972). All materials were acid-cleaned prior to use.

For every depth profile, total Chlorophyll a was measured for each depth, while fractionated chlorophyll measurements were done only at the surface and at the Deep Chlorophyll Maximum (DCM) layer. Water (500 ml) for fractionated chlorophyll was filtered through 2 µm (pore size) polycarbonate filters (Poretics). The filtration efficiency of this method was shown to be 93% (percentage of picocyanobacteria, e.g. *Synechococcus*, passing through the 2 µm polycarbonate filters, Agawin *et al*. 2000). Phytoplankton Chl a concentration in whole and fractionated samples was determined fluorometrically using the method of Parsons *et al*. (1984). Duplicate subsamples of 200 ml water were filtered through Whatman GF/F filters, which later were homogenized. The pigments were then extracted in 90% acetone for 6 h and refrigerated in the dark. Following extraction, fluorescence was measured in a Turner Designs fluorometer.

Grazing experiments: Grazing experiments were carried out in four stations during the LATITUD-III cruise and in three stations during the LATITUD-IV cruise, following a modified dilution method of Landry *et al*. (1995). Eleven L of surface water were collected for each experiment, of which at least 4 L were filtered through Whatman and 0.2 µm PC filters. Each experiment was set-up in eleven 2.7 L polycarbonate Nalgene bottles, eight of which were used for nutrient-enriched dilution series. Prior to filling the bottles with the filtered seawater (to achieve picophytoplankton abundance of approximately 25, 50, 75 and 100% ambient levels, in duplicate bottles), 0.5 µM N-ammonium and 0.03 µM phosphate (final concentrations) were added in each bottle. One bottle was also filled with only the filtered seawater to account for picophytoplankton cells that passed through the filter. Additional duplicate whole water samples were dispensed in two bottles without nutrient-enrichment. The bottles were incubated for 24 hrs on deck in a tank with a flow-through of surface seawater. Duplicate subsamples of 2 ml were collected from each bottle just before incubation and after 24 hrs, fixed with glutaraldehyde (1% final concentration), quickly frozen in liquid nitrogen and stored at –80 °C until flow cytometric analysis for picophytoplankton counts as described above. Grazing rate was calculated as the slope of the regression between apparent growth rates of the picophytoplankton cells (in the nutrient-enriched dilution series) and the dilution factor. The dilution factor was determined by flow cytometric analyses of the picophytoplankton cells at the start of each experiment relative to undiluted samples (Landry *et al.* 1995). Gross growth rate without nutrient amendments was calculated as the sum of the grazing or mortality estimate provided by the regression analysis and the mean observed net rate of picophytoplankton growth in bottles without nutrient amendment.

Cell cycle analysis of Prochlorococcus: Cell cycle analyses to estimate the specific growth rate of *Prochlorococcus* in surface waters were carried out in four stations during the LATITUD-III cruise and in three stations during the LATITUD-IV cruise. In each experi-

ment, duplicate 2-L surface seawater samples were dispensed in acid-cleaned 2-L PC Nalgene bottles. These bottles were incubated on deck in a tank with a flow-through of surface seawater. Every two hours for 24 hrs, subsamples of 2 ml were taken from each bottle either manually or though an automatic sampler, fixed with glutaraldehyde (1% final concentration), quickly frozen in liquid nitrogen and stored at –80 °C until flow cytometric analysis. Prior to staining with a DNA-specific dye, SYBR-GREEN I, samples were quickly thawed for 5 min at 37 °C and incubated for 30 min in the presence of 0.1 g mixture of RNAse A and B (Sigma R-4875 and R-5750, 1:1 wt/wt l^{-1}). Potassium citrate (final concentration 25 mM) and a latex bead solution were also added before incubation. After the 30 min incubation, the SYBR-GREEN dye was added (at a final concentration of 10^{-4} of the commercial solution), and the mixture was allowed to incubate for 15 minutes in the dark at room temperature prior to flow cytometric analyses (Marie *et al*. 1999). All flow cytometric parameters (forward and side scatter characteristics, green, red and orange fluorescence) were first collected in logarithmic scales. Second, data acquisitions were done with linear green fluorescence signals for cell cycle analyses. All data were recorded in listmode files and cell cycle analyses were performed with the ModFit LT cell cycle analyses software (Verity Software House, Inc. 1995) where the percentages of cells in G1, G2-M, S phases were estimated. The average CV of the G1 peak was 11.9%, with a standard deviation of 2.6%. Average daily specific growth rates of *Prochlorococcus* were calculated following Liu *et al*. (1997):

$$\mu = \frac{1}{(T_s + T_{G2M})} \int_0^{24} \ln\left[1 + f_s(t) + f_{G2M}(t)\right] dt$$

where μ is the daily mean specific growth rate, T_s + T_{G2M} is the duration of the terminal event estimated graphically from the time lag between an S phase fraction maximum and the following maximum in G2+M phase, multiplying this time by 2 (Carpenter & Chang 1988). $f_s(t_i)$ and $f_{G2M}(t_i)$ are the fractions of cells in the S and G2M phases respectively at each sampling occasion. Using this approach, the T_s + T_{G2M} was 4 h in most of the stations, similar to those found in Mann & Chisholm (2000), Shalapyonok *et al*. (1998) and Partensky *et al.* 1996. In one station however (9.28 ºS, 32.27 ºW, boreal autumn cruise) where specific growth rate was found the lowest, T_s + T_{G2M} was longer (8 h). The terms G_1, S and G_2+M are not really appropriate for prokaryotes, but can be used as a convenience because *Prochlorococcus* seems to have a discrete DNA synthesis phase, similar to the eukaryotic cell cycle, even when dividing at more than one division per day (Shalapyonok *et al*. 1998). Cell cycle analyses for *Synechococcus* were not done because of its highly variable cell cycle patterns.

RESULTS

Surface layer properties

Figure 2 shows sigma-t, temperature and salinity properties of the surface layer in the two cruises. The surface layer temperature reached up to 29 °C in equatorial waters and decreased down to 19 °C to 20 °C in subtropical waters in both seasons. There was however, a clear variation of surface layer temperature in the two cruises, with a general increase in temperature differences farther away from the equator. Surface temperatures during the boreal autumn were up to 4 °C warmer than during the boreal spring while surface temperature during the austral autumn were up to 4 °C warmer than during the austral spring (Fig. 2).

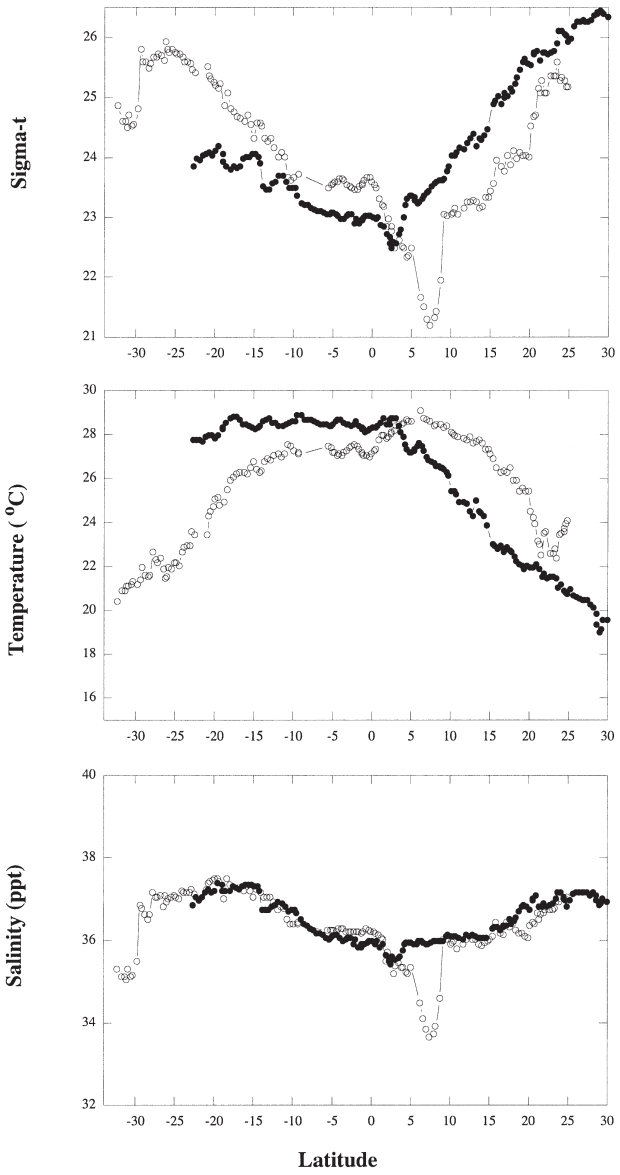


Fig. 2. – Latitudinal distribution of surface water density, temperature and salinity during the LATITUDE-III (open circles, boreal autumn, austral spring) and LATITUDE-IV (filled circles, boreal spring, austral autumn) cruises.

Picophytoplankton abundance and biomass

Prochlorococcus, reaching up to 2.5 • 10^5 cells ml^{-1} were the most abundant among the picophytoplankton groups particularly in deep waters. They were found still at significant numbers at deep waters (>150m). The highest abundance of *Prochlorococcus* shifts from the surface to deeper water from the NATR waters to SATL waters. *Synechococcus* was most abundant in the NATR waters and their numbers decreased southwards to SATL waters. *Synechococcus* was generally abundant in deeper waters. High resolution surface samples showed small-scale variability in pico-cyanobacteria abundance (Fig. 3), which was correlated well with variability (< 500 km) in water mass properties along the transects. During boreal autumn, the residuals of temperature and sigma-t describing their variability at scales < 500 km, were significantly correlated with the abundance of *Prochlorococcus* (pearson r = –0.31 and r = 0.23, $p < 0.05$, respectively) and, particularly, *Synechococcus* (pearson r = –0.76 and 0.69, $p < 0.05$ respectively). During boreal spring, the residuals of temperature, sigma-t and salinity were significantly correlated with the high resolution abundance of *Prochlorococcus* (pearson r = 0.45, r = –0.58, and r = –0.54, $p < 0.05$, respectively) and *Synechococcus* (pearson r = 0.36, r = –0.47, and r = –0.43, $p < 0.05$, respectively). *Prochlorococcus* was generally more abundant in surface waters of WTRA and NATL regions than in the SATL region during boreal spring and autumn. *Synechococcus* was also generally more abundant in WTRA and NATL waters than in SATL waters and particularly during the boreal autumn (Fig. 3) when the cruise path crossed near the NW African upwelling area (Fig. 1). The abundance of both *Synechococcus* and *Prochlorococcus* varied somewhat (about 2 fold on average) between cruises. Small eukaryotes, with average size (diameter) of 3.9 (± 0.24) µm, were also detected during the cruises, but with less abundance (0.07 to 9.5 cells • 10^3 cells ml^{-1}) than *Synechococcus* and *Prochlorococcus*, whose size (diameter) averaged 1.01(± 0.09) and µm 0.47 (± 0.04) µm, respectively.

The depth-integrated *Prochlorococcus* C biomass was higher in the SATL waters than in the NATR waters during austral spring (boreal autumn) (Fig. 5). This may be due to the presence of *Prochlorococcus* at greater depths in the SATL

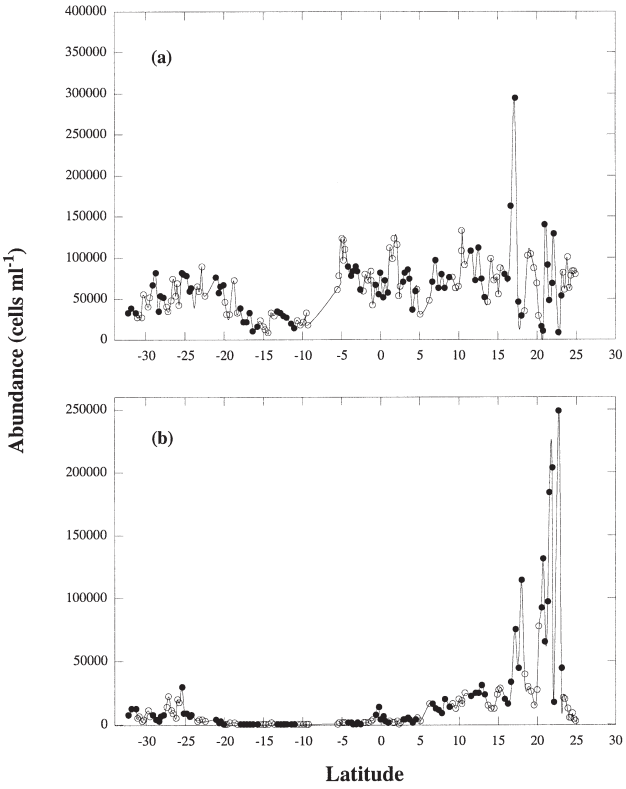


Fig. 3. – Latitudinal distribution of surface abundances of (a) *Prochlorococcus*, and (b) *Synechococcus* across the tropical Atlantic transect during the LATITUDE-III cruise (boreal autumn, austral spring). Open and filled circles denote samples taken during day and night respectively.

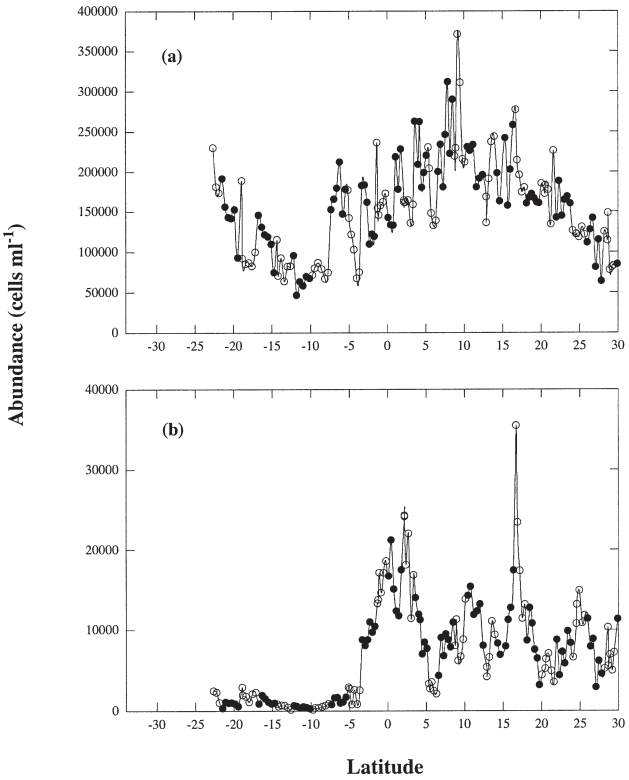


Fig. 4. – Latitudinal distribution of surface abundances of (a) *Prochlorococcus* and (b) *Synechococcus* across the tropical Atlantic transect during the LATITUDE-IV cruise (boreal spring, austral autumn). Open and filled circles denote samples taken during day and night respectively.

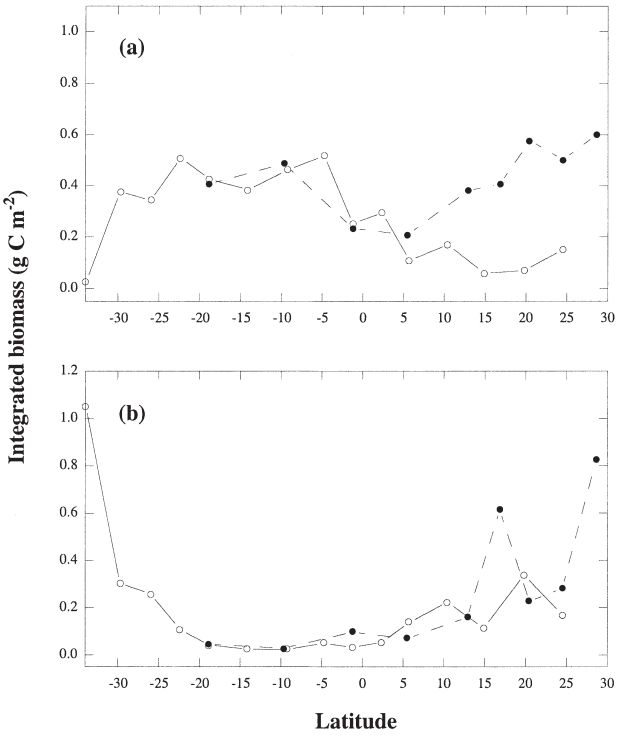


Fig. 5. – Latitudinal distribution of the integrated C biomass of (a) *Prochlorococcus* and (b) *Synechococcus* across the tropical Atlantic during the LATITUDE-III (open circles, boreal autumn, austral spring) and LATITUDE-IV (filled circles, boreal spring, austral autumn) cruises.

than in the NATR profile during austral spring. Their C biomass was also high in both SATL and NATR waters during austral autumn (boreal spring) (Fig. 5). Depth integrated *Synechococcus* C biomass was higher in the coastal station near Montevideo during austral spring (boreal autumn) and at the northernmost (29 °N) station (already in the northern subtropical region) during austral autumn (boreal spring) (Fig. 5). A significant negative correlation was found between the integrated C biomass of *Prochlorococcus* and *Synechococcus* particularly during boreal autumn (austral spring) ($r = -0.67$, $p < 0.05$, both parameters were log-transformed). The depth-integrated biomass of *Prochlorococcus* exceeded that of *Synechococcus* in the south subtropical gyre and the equatorial area (Fig. 5). Size fractionated (< 2 µm) chlorophyll concentration measurements showed picophytoplankton biomass to be generally higher at the northern tropical waters, particularly at the deep chlorophyll maximum (Fig. 6). The < 2 µm fraction contributed on average, 72% and 75% of the total chlorophyll biomass at the surface and DCM waters, respectively during boreal autumn (austral spring), and 77% and 62% of the total chlorophyll biomass at the surface and DCM waters, respectively during boreal spring (austral autumn).

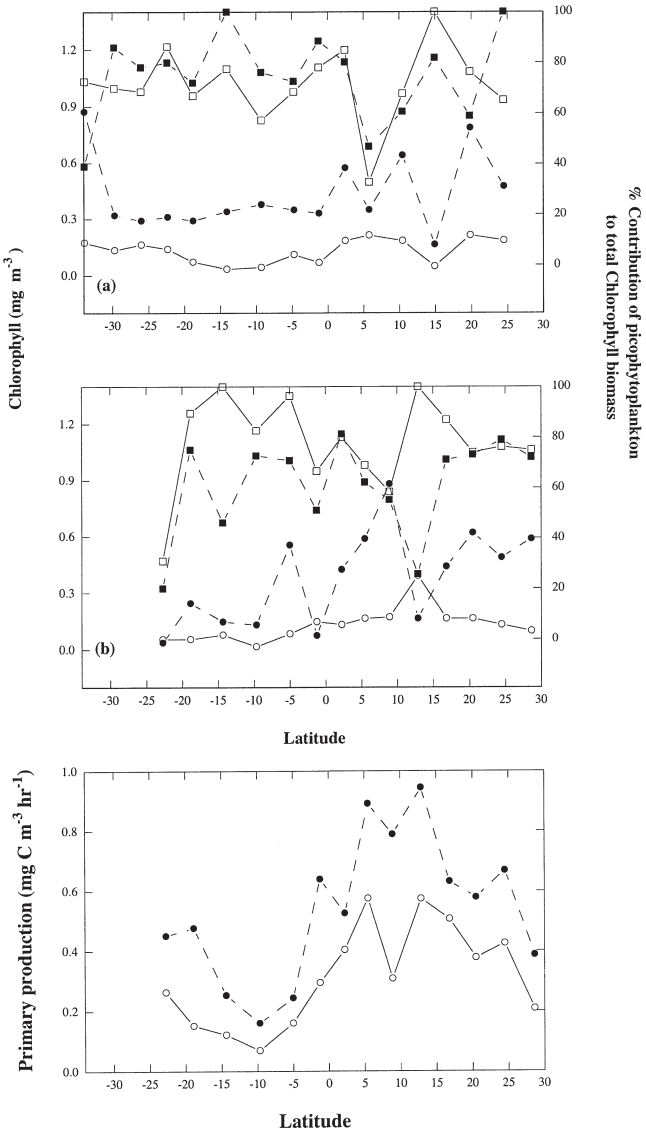
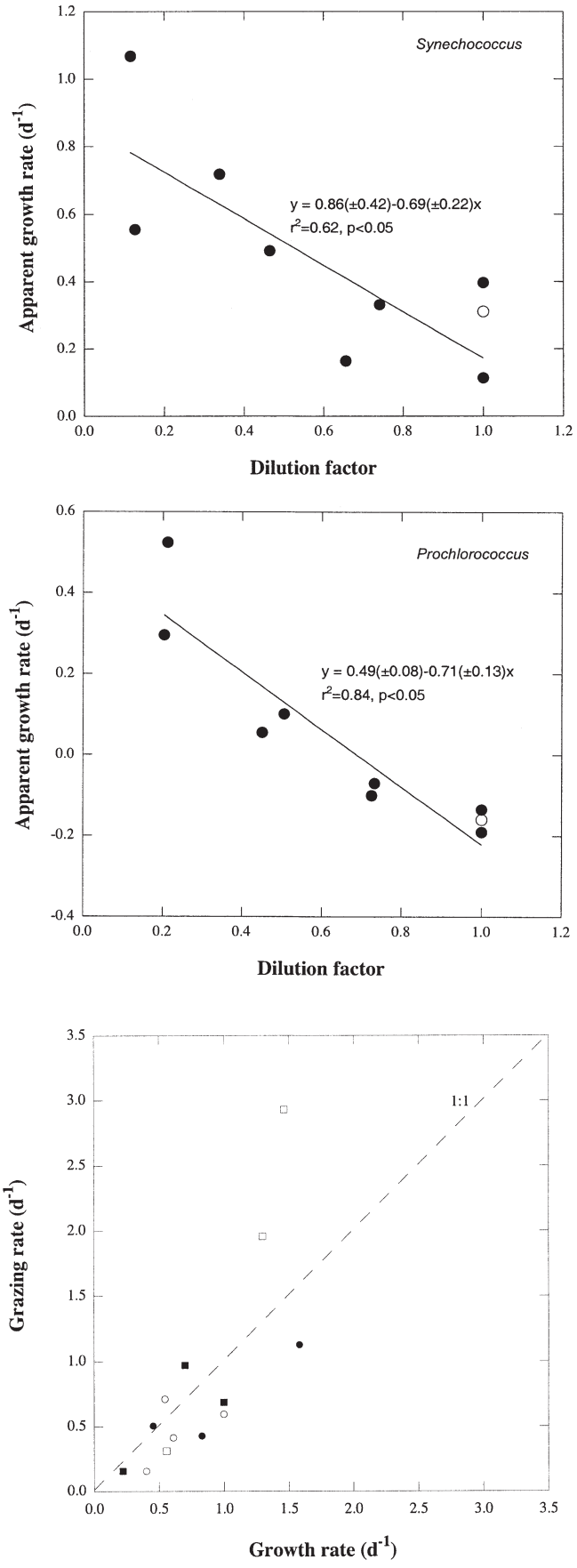


Fig. 6. – Top and middle, latitudinal distribution of chlorophyll concentrations of the < 2 µm fraction in surface (○) and in Deep Chlorophyll Maximum layers, DCM (●), and their contribution to the bulk phytoplankton chlorophyll biomass in the surface (□) and DCM layers (■), respectively, during (a) LATITUDE-III (boreal autumn, austral spring) and (b) LATITUDE-IV(boreal spring, austral autumn) cruises. Bottom, latitudinal distribution of the bulk phytoplankton primary production (●) and that of the < 2 µm fraction (○) in surface waters during the LATITUDE-IV (boreal spring, austral autumn) cruise.

Primary production, growth and grazing rates

The meridional pattern of primary production by the picophytoplankton fraction (< 2 µm) during the boreal spring (austral autumn) paralleled that of the whole community ($r = 0.88$, $p < 0.05$), being higher at the northern tropical waters (Fig. 6). Picophytoplankton contributed, on average, 57% of



total phytoplankton primary production in the surface waters. The primary production and chlorophyll biomass of picophytoplankton were significantly correlated ($r = 0.77$, $p < 0.05$).

The results from the grazing experiment, showed that gross growth rates of *Synechococcus* ranged from 0.46 to 1.58 d^{-1} during the boreal autumn (austral spring), and 0.22 to 1 d^{-1} during the boreal spring (austral autumn) (Table I). The gross growth rates of *Prochlorococcus* ranged from 0.40 to 1 d^{-1} during the boreal autumn (austral spring), and from 0.56 to 1.47 d^{-1} during boreal spring (austral autumn, Table I). Grazing rates on *Prochlorococcus* were also higher during austral autumn than during austral spring (Table I). Figure 7 shows representative plots used to generate the results of the grazing experiments. Regression analyses between apparent growth rates of picophytoplankton in the nutrient enriched dilution series and the dilution factor showed fairly linear plots with an average r^2 of 0.63 ($p < 0.05$). There was a positive significant relationship ($r = 0.77$, $p < 0.05$) between growth and grazing rates (Fig. 7), and the average differences between growth and grazing rates (μ-g) were 0.15 ± 0.11 d^{-1} and –0.17 ± 0.25 d^{-1}, for *Synechococcus* and *Prochlorococcus*, respectively.

The diel patterns of cell cycle of *Prochlorococcus* were generally similar in all stations in both seasons with peak division (highest G2-M percentage of cells) occurring around midnight (2300 h, Fig. 8). Specific growth rates of *Prochlorococcus* based on cell cycle analyses were similar as those estimated from the grazing dilution experiments, and showed the same trend of increased growth rates during boreal spring compared during boreal autumn (Table II). *Prochlorococcus* cell division and mortality rates were higher during the boreal spring cruise only south of the equator, where the surface temperatures were generally higher.

Fig. 7. – Top and middle, representative plots between apparent growth rates of *Synechococcus* and *Prochlorococcus* in the nutrient enriched dilution series (●) and the dilution factor. Gross growth rates of picophytoplankton were calculated as sum of grazing estimate provided by the regression analysis and mean observed net rate of picophytoplankton in bottles without nutrient amendment (○). Bottom, the relationship between gross growth and grazing rates of *Prochlorococcus* (empty symbols) and *Synechococcus* (filled symbols) estimated from the refined dilution experiments during the LATITUDE III (square) and LATITUDE IV (circle) cruises. Dashed line indicates the 1:1 correspondence ratio.

Table I. – Gross growth (μ, d^{-1}) and grazing (g, d^{-1}) rates of *Synechococcus* and *Prochlorococcus* in surface central Atlantic waters, from the refined dilution experiments during LATITUDE-III (November 1 to 20, 1999) and LATITUDE-IV (March 16 to April 4, 2000) cruises.

	November 1999 (Boreal autumn)					March 2000 (Boreal spring)			
	Synechococcus		*Prochlorococcus*			*Synechococcus*		*Prochlorococcus*	
	μ (d^{-1})	g (d^{-1})	μ (d^{-1})	g (d^{-1})		μ (d^{-1})	g (d^{-1})	μ (d^{-1})	g (d^{-1})
14.89 °N 23.07 °W	0.83	0.43	1.0	0.59	12.87 °N 29.37 °W	1.0	0.69	0.56	0.31
1.24 °S 30.96 °W			0.40	0.16	1.25 °S 30.84 °W	0.22	0.15	1.3	1.96
18.79 °S 36.4 °W	1.58	1.13	0.55	0.71	18.87 °S 35.38 °W	0.70	0.97	1.47	2.93
29.75 °S 47.7 °W	0.46	0.50	0.61	0.42					
Mean (± SE)	0.96 (± 0.33)	0.69 (± 0.22)	0.64 (± 0.13)	0.47 (± 0.12)		0.64 (± 0.23)	0.60 (± 0.24)	1.11 (± 0.28)	1.73 (± 0.76)

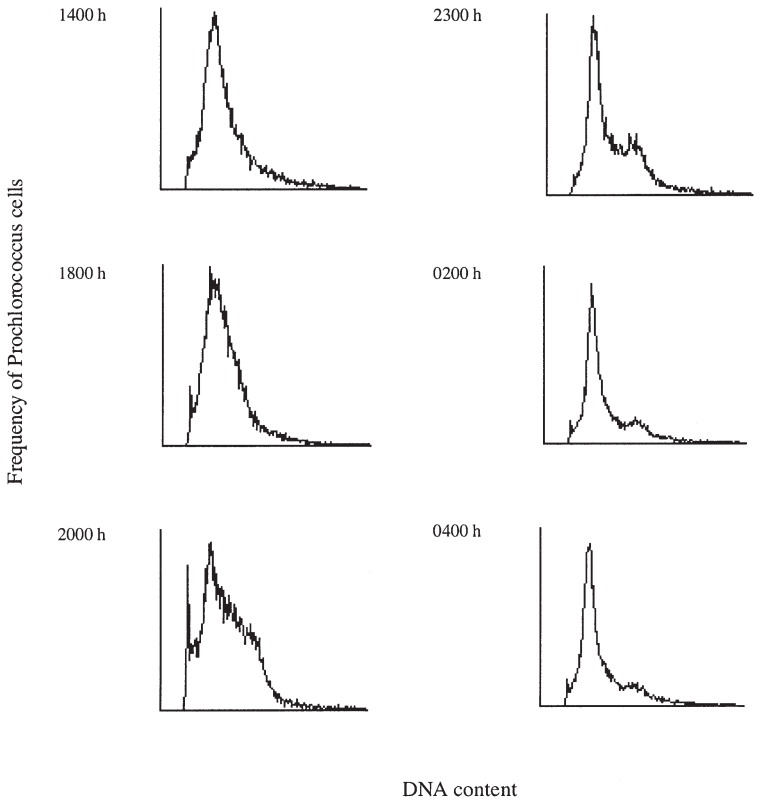


Fig. 8. – Representative flow cytometry histograms of the relative DNA content (as measured by the green fluorescence) of SYBR-stained *Prochlorococcus* cells at selected times.

DISCUSSION

The low chlorophyll concentrations (< 0.3 mg m^{-3}) found in the upper mixed layer in the north and south central gyres were consistent with those observed in previous studies in the region (Agustí & Duarte 1999, Marañon & Holligan 1999). The abundance of *Prochlorococcus*, the numerically most abundant picophytoplankton, was comparable to those reported in previous studies in the Atlantic ocean (Zubkov *et al*. 2000a) and in the Pacific equatorial regions (Vaulot & Marie 1999, André *et al*. 1999). The picophytoplankton community dominated the biomass (>70%) and primary production (on average, 57%) in the North Atlantic Tropical

Table II. – Mean (± se) specific growth rates (μ, d^{-1}) of *Prochlorococcus* in surface central Atlantic waters, from cell cycle analyses during the LATITUDE-III (boreal autumn, austral spring) and LATITUDE-IV (boreal spring, austral autumn) cruises.

November 1999 (Boreal autumn)	μ (d^{-1})	**March 2000** (Boreal spring)	μ (d^{-1})
24.49 °N 17.53 °W	0.84 (± 0.19)	20.48 °N 26.24 °W	1.04 (± 0.03)
2.38 °N 29.55 °W	1.18 (± 0.06)	5.46 °N 30.17 °W	1.01 (± 0.23)
4.74 °S 32.01 °W	0.72 (± 0.01)	9.69 °S 31.80 °W	1.58 (± 0.07)
9.28 °S 32.27 °W	0.36 (± 0.04)		
Mean (± SE)	0.77 (± 0.11)		1.21 (± 0.13)

Gyre, Western Tropical Atlantic and South Atlantic Tropical Gyre waters, consistent with previous studies (Marañon & Holligan 1999). The contribution of picoplankton to the total primary production reported by Marañon & Holligan (1999) was somewhat greater (> 60%) than that reported here.

Prochlorococcus and *Synechococcus* were generally more abundant and had higher chlorophyll biomasses in deeper waters than in the surface waters, consistent with previous studies in the Atlantic ocean (Zubkov *et al.* 2000a) and other oceanic sites (Goericke *et al.* 2000). This is also consistent with the observation that both groups showed the highest fraction of dead cells at depths receiving the highest irradiance in the Central Atlantic Ocean (Agustí 2004). *Prochlorococcus* generally was found still at significant numbers at deep (> 150 m) waters, which supports the suggestion that *Prochlorococcus* has a greater capacity to survive at low irradiance than *Synechococcus* (Agustí 2004). *Synechococcus* was found in other studies, to be more abundant in the surface layers (Ishizaka *et al.* 1994, Bustillos-Guzmán *et al.* 1995) while *Prochlorococcus* can be equally abundant in surface and in deeper waters (Goericke *et al.* 2000). The deepening of the *Prochlorococcus* layer with increasing oligotrophy from NATR to SATL waters, similar to observations in the Pacific ocean (Partensky *et al.* 1999) may be due to the decrease in light penetration in the North Atlantic region (associated with plankton response to the upwelling). Recently, *Prochlorococcus* has been found to be more sensitive to UV-stress than *Synechococcus* in the Atlantic Ocean (Llabrés & Agustí, in press).

The spatial structure of picocyanobacterial abundance in surface waters was associated with the variability in the water mass structure at intermediate (< 500 km) scales. This coupling suggests that instabilities and mesoscale processes affect picophytoplankton abundance. Between cruise variability in picophytoplankton abundance can be due to seasonal variability. Seasonal variability has also been suggested to be related to the two-fold variations in bacterial abundance and production rates between two past cruises in the Equatorial Atlantic (Zubkov *et al.* 2000, 2000a). Variability in space (the cruise tracks of the two transects in this study were not exactly the same) may also account for between cruise variability in picocyanobacterial abundance. *Prochlorococcus* and *Synechococcus* showed segregated spatial distributions along central Atlantic, particularly during the boreal autumn (austral spring) when *Prochlorococcus* dominated in the ultra-oligotrophic tropical gyres (NATR, WTRA and SATL), and *Synechococcus* was more abundant in coastal stations and the northern subtropical region. This segregation is consistent with past observations in the same region (Zubkov *et al.* 2000a), and supports the contention that the abundance of these groups are negatively correlated in the ocean (Campbell *et al.* 1994). The mechanisms behind this segregation, which suggests, competitive interactions and/or differing growth requirements, are however, unclear. The growth rates of the two groups were somewhat inversely related. The growth rates of *Prochlorococcus* based on cell cycle analyses was inversely correlated (pearson $r = -0.77$, $p < 0.05$, $n = 7$) with nitrate concentrations (< 0.1 μM in surface waters, Agustí 2004), giving evidence to the recent observation that *Prochlorococcus* can not utilize nitrate for growth (Ripka *et al.* 2000). This, along with (1) the idea that their small size gives *Prochlorococcus* a growth advantage at low nutrient concentrations through reduction of their threshold for diffusion limitation (Chisholm 1992) and (2) the suggestion that *Prochlorococcus* can utilize organic forms of N and P (Cavender-Bares *et al.* 2001) which may be in higher concentration than nitrate in oligotrophic oceans, may explain the dominance of *Prochlorococcus* in the ultraoligotrophic tropical gyres (Zubkov *et al.* 2003). With increased concentration of inorganic nutrients (e.g. nitrate), *Prochlorococcus*, which can not utilize nitrate, is outcompeted with *Synechococcus* whose maximum growth rate is faster (Cavender-Bares *et al.* 2001). The dominance of *Synechococcus* in relatively colder waters in the subtropical Atlantic maybe also explained by the evidence that low temperatures are lethal to *Prochlorococcus* (Moore *et al.* 1995), explaining their absence at temperatures < 10 °C, while *Synechococcus* still thrives in even colder waters (Shapiro & Haugen 1988).

The contrasting abundance of *Prochlorococcus* during spring and autumn could be associated to differences in growth rates. The growth rates of *Prochlorococcus* during boreal and austral autumn

tended to be higher than those during austral and boreal spring, both when examining growth rates derived from dilution and cell cycle analyses. This may be due to higher temperature during autumn. This pattern was, however not so clearly identified for *Synechococcus*. The gross growth rates of *Prochlorococcus* derived from cell cycles analysis were similar to the maximal rates of 1.4 d^{-1} found in the eastern equatorial Pacific (Mann & Chisholm 2000). *Prochlorococcus* showed highly synchronized cell division in surface waters, with a maximum fraction of cells undergoing DNA replication in the S or G2 phases at midnight, consistent with the previous study in the Atlantic (Zubkov *et al*. 2000b). In the Pacific, *Prochlorococcus* division is also tightly synchronized (Vaulot *et al*. 1995, Liu *et al.* 1997). The high gross growth rates of *Synechococcus* and *Prochlorococcus* in the tropical central Atlantic estimated from the dilution technique and from cell cycle analyses are consistent with the high bulk picophytoplankton growth rate (an average of 0.76 d^{-1} in surface waters), estimated from the combination of size fractionated primary production and C biomass measurements. The high growth rates of picophytoplankton, particularly, *Prochlorococcus* whose growth rates reached up to 1.58 d^{-1} in the oligotrophic tropical central Atlantic may explain their ubiquitous presence and dominance in the area.

The observed distributions of *Synechococcus* and *Prochlorococcus* in the Central Atlantic Ocean should not only be attributed to different growth capacities, but also to probable differences in mortality rates, as abundance is the net result of growth and mortality. Recent evidences indicated that the differing optimal niches the two groups occupy in the Central Atlantic were well identified when analyzing the cell death distribution of the two picocyanobacteria which reflects their different sensitivity to stress conditions (Agustí 2004, Llabrés & Agustí in press). There may also be differences in predation rates on *Synechococcus* and *Prochlorococcus*, but here, we found no consistent differences of grazing rates between *Synechococcus* and *Prochlorococcus*, probably because the two groups may have an assemblage of flagellates as the main grazers and there might be differences in predator assemblages in the different areas in the Central Atlantic. On both groups, there were generally higher grazing rates experiments conducted in sites in the SATL, which may have resulted to a reduction of surface abundances of the picophytoplankton groups in these areas than areas in the WTRA and NATR regions. The increased *Prochlorococcus* growth rates found in boreal and austral autumn were however overcome by high grazing mortality in autumn explaining the small differences in abundances found between seasons.

The upper range of grazing rates on *Synechococcus* and *Prochlorococcus* are found here to be high compared to those reported in Christaki *et al*. (1999, Table V), indicating that top down control of picophytoplankton abundance is most likely important in the region. There was an overall balance between growth and loss (grazing) rates of *Synechococcus* (mean net population growth rate = 0.15 ± 0.11 d^{-1}) and *Prochlorococcus* (population growth rate = –0.17 ± 0.25 d^{-1}) as reported in previous studies (Agawin *et al.* 1998, André *et al*. 1999). In the equatorial Pacific region, a tight coupling between picoplankton cell division rate and mortality (microzooplankton grazing) has been also reported (Liu *et al*. 1997, Mann & Chisholm 2000). The high growth and turnover rate of picophytoplankton indicates that their biomass underestimates their role in carbon cycling, and that recycling processes must be very fast in the oligotrophic waters of the central Atlantic.

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