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# Vertical structure of picophytoplankton at different trophic sites of the tropical northeastern Atlantic Ocean

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**Abstract**—The vertical structure and chlorophyllous pigments of picophytoplanktonic populations of the northeastern Atlantic Ocean were studied by flow cytometry and spectrofluorometry. Three sites (EU, MESO and OLIGO) on a hydrological gradient from near coastal, eutrophic waters to offshore, oligotrophic waters were occupied in October 1991 (except EU), June 1992 and December 1992. The population structure of the EU site (20°32′N 18°34′W) was greatly influenced by a permanent, wind-induced upwelling. The latter was well developed in June, and an important nutrient enrichment of surface waters ensued (> 10 mM $NO_2 + NO_3$). The latter favored the blooming of diatoms, but picophytoplanktonic populations remained low. In December the upwelling was less developed, and there was a dramatic increase of the cell abundances of both prokaryotic (*Prochlorococcus* and *Synechococcus*) and picoeukaryotic populations at the EU site. Cells of all groups were concentrated and homogeneously distributed in the upper, 30–35 m thick, mixed layer. Similar population structures were observed in June and December at the MESO site (18°29′N 21°05′W). In these cases, both *Synechococcus* cyanobacteria (with concentrations of up to $5 \times 10^5$ cells $ml^{-1}$ during winter) and picoeukaryotes (typically $1–2 \times 10^4$ cells $ml^{-1}$) made significant contributions to the integrated picophytoplanktonic biomass in terms of carbon (166–333 μg C $cm^{-2}$ and 92–155 μg C $cm^{-2}$, respectively). *Prochlorococcus* made a smaller contribution (6–48 μg C $cm^{-2}$), as also indicated by a low ratio of divinyl-chlorophyll *a* to total chlorophyll *a* (≤22%). The population structure observed in October at the MESO site was much more variable, even at the time-scale of hours. At the OLIGO site (21°02′N 31°08′W), the relative contribution of *Prochlorococcus* to picophytoplankton carbon and total chlorophyll standing stocks increased dramatically (> 50%), mainly as a result of a sharp decrease of both *Synechococcus* and picoeukaryotes cell concentrations down to a few thousands cells per ml. There was little seasonal change in the vertical structure of any of the three populations at this site. From analyses of cell cycle distributions during a 31 h time-series, growth rates were estimated for *Prochlorococcus* as 0.41 $day^{-1}$ at mid-depth (80 m) and 0.39 $day^{-1}$ in the deep chlorophyll maximum. 

## INTRODUCTION

The picophytoplankton, operationally defined as the fraction of autotrophic cells passing through 2 μm filters (Stockner, 1988), includes both eukaryotes, which belong to a variety of algal families but are poorly characterized at the specific or even generic level (Takahashi and Hori, 1984;Murphy and Haugen, 1985; Simon *et al.*, 1994), and prokaryotes. The latter

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are represented by two phylogenetically closely related genera (Palenik and Haselkorn, 1994; Urbach *et al.*, 1992), *Synechococcus* (Waterbury *et al.*, 1979) and *Prochlorococcus* (Chisholm *et al.*, 1988, 1992), which can be readily identified and counted by flow cytometry (Olson *et al.*, 1988, 1990a,b; Neveux *et al.*, 1989; Veldhuis and Kraay, 1990). From a pigmentary viewpoint, *Prochlorococcus* is fundamentally different from *Synechococcus*, since it contains divinyl derivatives of chlorophylls (DV-Chl) *a* and *b* and no phycobilins (Goericke and Repeta, 1992; Morel *et al.*, 1993; Partensky *et al.*, 1993). Recent studies in which both photosynthetic prokaryotes were discriminated (see e.g. Olson *et al.*, 1990a; Li *et al.*, 1992; Campbell and Vaulot, 1993; Veldhuis and Kraay, 1993) show that although these two ubiquitous groups generally co-occur, their depth distributions differ both in absolute concentrations and vertical extension, except when the water column is homogeneous, e.g. during winter in the Mediterranean Sea (Vaulot *et al.*, 1990). Moreover, in areas undergoing seasonal variations of hydrological parameters, the profiles of cell abundances of these two picoplankters exhibit different patterns. *Synechococcus* concentrations generally peak in late winter and are low in summer; the converse is true for *Prochlorococcus* (Olson *et al.*, 1990a). This suggests that the distribution and dynamics of these two prokaryotic groups are subject to different environmental controls. In the Atlantic Ocean, the latitudinal distribution of *Prochlorococcus* seems limited by seawater temperature below 17°C in the northwestern part, i.e. north of the Gulf Stream (40°N, Olson *et al.*, 1990a), and 14–22°C (range of temperature between 90 m and the surface) in the northeastern part (43°N). In contrast, *Synechococcus* can stand temperatures down to 12°C and extend much further north, up to 63°N (Veldhuis *et al.*, 1993). *Prochlorococcus* cells have also been detected at temperatures as low as 12.5°C, but only in the Mediterranean Sea in winter (Vaulot and Partensky, 1992). At low latitudes, where temperature is not expected to be a limiting factor for *Prochlorococcus* growth, the relative contributions of *Prochlorococcus* and *Synechococcus* must depend upon other physico-chemical parameters. *Synechococcus* is found at low concentrations in the oligotrophic, subtropical oceans (Campbell and Vaulot, 1993), whereas it is very dense (*ca* $10^5$ cells $ml^{-1}$) in coastal areas (Olson *et al.*, 1988; Vaulot and Ning Xiuren, 1988). This prokaryote reaches some intermediate abundances in temperate open sea areas (Veldhuis *et al.*, 1993) and equatorial areas (Blanchot *et al.*, 1992) that transiently or permanently exhibit some nutrient enrichment of surface waters. This suggests that *Synechococcus* and possibly the picoeukaryotic community as a whole, which follows the same patterns (Olson *et al.*, 1990a), might be limited by low concentrations of inorganic nutrients. However, factors controlling the growth and abundance of *Prochlorococcus*, which has been reported at high abundances in many oligotrophic areas (Campbell and Vaulot, 1993; Shimada *et al.*, 1993; McManus and Dawson, 1994), are very poorly understood.

The present paper compares different sites of the tropical northeastern Atlantic Ocean on a gradient between eutrophic and oligotrophic situations at different seasons (winter, late spring and fall). A large set of flow cytometric (cell concentrations) and spectrofluorometric (pigment) data were obtained from three cruises off Mauritania, part of the French EUMELI (EUtrophe, MEsotrophe, oLIgotrophe) Programme (JGOFS–France). The large contribution of *Prochlorococcus* to the picophytoplanktonic biomass at the OLIGO site allowed us to study, in great detail, vertical profiles of its specific chlorophyllous pigments and associated red fluorescence. We also applied a new method, developed by Carpenter and Chang (1988) and Vaulot *et al.* (1995), to estimate its species-specific growth rate at the base of the euphotic layer.

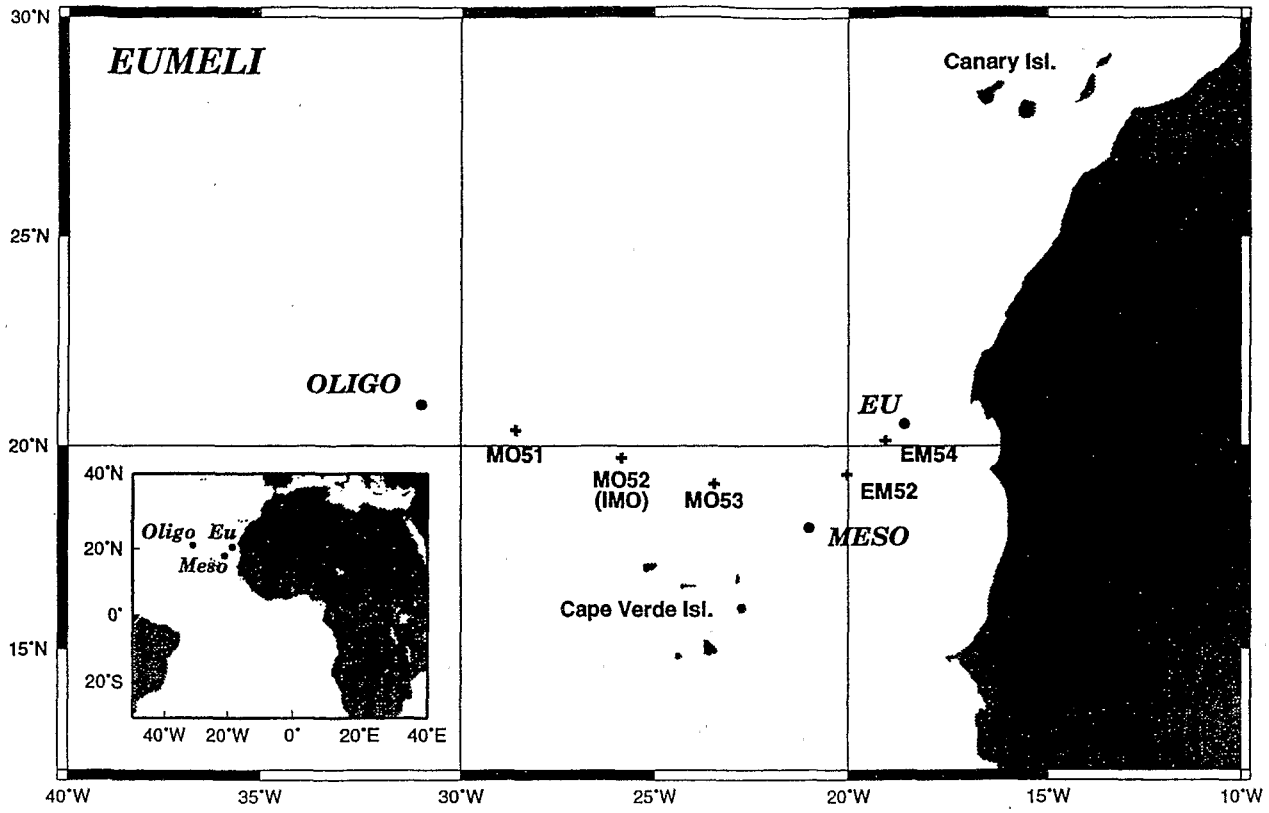


Fig. 1. Map showing the sites sampled during the EUMELI 5 cruise (December 1992). MESO, OLIGO and IMO (equivalent to MO 52) sites were also sampled at EUMELI 3 (October 1991), and EU, MESO and OLIGO sites were occupied during EUMELI 4 (June 1992).

## MATERIALS AND METHODS

### *Sampling*

Two sites of the subtropical northeastern Atlantic, MESO (18°29′N 21°05′W) and OLIGO (21°02′N 31°08′W), were occupied with the R.V. *l'Atalante* for 5 and 7 days, respectively, in October 1991 (EUMELI 3). In June 1992 (EUMELI 4), measurements were made at the latter two sites, plus a more coastal site called EU (20°32′ 18°34′W). Finally, in December 1992 (EUMELI 5), eight stations were occupied along a transect from the EU site to the OLIGO site (Fig. 1). Water sampling was performed using a rosette equipped with 12 Niskin bottles. Conductivity, temperature, barometric pressure and chlorophyll *a* fluorescence (referred to in the text and figures as *in situ* fluorescence) were measured on-line. Inorganic nutrients (data kindly provided by P. Raimbault) were measured with a Technicon® Autoanalyzer according to Raimbault *et al.* (1990) for nitrates and Tréguer and LeCorre (1975) for phosphates. The vertical profile of photosynthetically available radiation (PAR) was measured with a custom-designed immersible quantum-meter (data kindly provided by A. Morel). A time series of casts was performed at the OLIGO site during EUMELI 3 to study the short-term variability of physical and biological parameters and to assess the species-specific growth rate of the *Prochlorococcus* population (see below). Two depths were sampled at 1 h intervals during a 31 h time-series using Niskin bottles: a fixed one (80 m) and a variable one that tracked the chlorophyll *a* fluorescence maximum.

*Flow cytometric cell counting*

During the EUMELI 3 cruise, a FACScan™ flow cytometer (Becton Dickinson) was used aboard the ship in a thermostated room ($23 \pm 2°C$) to count live picophytoplanktonic populations. This system provides two light scatter signals at forward (FALS) and right (RALS) angles and three fluorescence signals, which will be referred to as "green" ($530 \pm 15$ nm), "orange" ($585 \pm 21$ nm) and "red" (> 650 nm) fluorescences. Seawater filtered through 0.2 µm filters was used as sheath fluid. Data were collected in list mode, then analyzed on a PC computer using custom-designed software, called CytoPC (Vaulot, 1989). The parameters measured for the different populations were normalized to the mean values calculated for 1.98 µm fluorescent beads (Polysciences) systematically added to samples. Cell concentrations were estimated from the cell number obtained for a fixed time (3 min) and the calibrated flow rate. Daily calibration of the flow rate was done using a suspension of beads, the concentration of which was determined in triplicate with an epifluorescence microscope. An average flow rate of $49 \pm 8$ µl $min^{-1}$ ($n = 15$) was used for calculations of cell concentrations for all samples.

A few samples analyzed onboard during EUMELI 3 were fixed, using a modification of the Vaulot *et al.* (1989) method, with 0.5% paraformaldehyde for 10–15 min then frozen in liquid nitrogen. These samples were analyzed in the laboratory with both the FACScan™ and an EPICS® 541 flow cytometer (Coultronics, Hialeah, FL). The latter was equipped with a 6 W argon laser (Coherent, Palo Alto, FL), delivering 1.3 W at 488 nm, and a Micro-Sample Delivery System (MSDS). Optical configuration and filters were as described by Vaulot *et al.* (1990). Re-analysis of samples aimed at checking the quality of the fixation and at intercalibrating these two flow cytometers, since all samples from the EUMELI 4 and 5 cruises were fixed and analyzed with the EPICS®. With both systems, *Prochlorococcus* cells were too dimly red-fluorescing in near-surface waters (0–60 m) of the OLIGO site in October 1991 and June 1992 to be completely resolved. Thus, approximate *Prochlorococcus* concentrations were estimated by multiplying by two the cell number in the right part of the red-fluorescence (RF) distributions, assumed to be log normal. Table 1 shows the results of the analysis of seawater samples from EUMELI 3 with the FACScan™ before and after paraformaldehyde fixation. For *Prochlorococcus* below 60 m and *Synechococcus* at all depths, most cells were well preserved by the method (loss = 3–4%), while picoeukaryotes were preserved poorly (loss = 23%). The estimated *Prochlorococcus* cell concentrations above 60 m at the OLIGO site were more variable, with an average 11% apparent loss. Intercalibration between the EPICS® and the FACScan™ shows that the former underestimated deep *Prochlorococcus*, *Synechococcus* and picoeukaryotes concentrations by 20, 21 and 24%, respectively, compared to FACScan™ counts on live cells (Table 1). Since correlation coefficients between cell counts made with the two systems were high ($r^2 > 0.98$), cell concentrations obtained with the EPICS® have been corrected in subsequent figures and tables to allow better intercomparison of the data obtained from the two systems. The other flow cytometric parameters (fluorescence and scatter signals) were differently affected by the fixation, depending on the population type, as already reported by Vaulot *et al.* (1989) and Landry and Kirshtein (1993). Moreover, the fluorescence or scatter values obtained for a given population with the two systems were often poorly correlated (Table 1).

*Table 1. Effect of the preservation method on the parameters measured with the FACScan flow cytometer on natural picoplanktonic populations at the OLIGO site of EUMELI 3 and intercalibration between FACScan and EPICS*

| Population type | Number of samples | Cell concentration | FALS | RALS | Red fluorescence | Orange fluorescence | Green fluorescence |
|---|---|---|---|---|---|---|---|
| FACScan | | | | | | | |
| *Prochlorococcus* | 16 | 0.97±0.01 | 3.08±0.18 | 0.83±0.02 | 0.99±0.01 | na | na |
| *Synechococcus* | 21 | 0.96±0.01 | 1.98±0.07 | 0.91±0.04 | 1.51±0.03 | 2.10±0.06 | 0.75±0.04 |
| Picoeukaryotes | 21 | 0.77±0.01 | 1.07±0.03 | lcc | 0.79±0.02 | na | na |
| EPICS | | | | | | | |
| *Prochlorococcus* | 24 | 0.80±0.01 | na | 1.06±0.02 | 0.71±0.01 | na | na |
| *Synechococcus* | 27 | 0.79±0.01 | 2.43±0.08 | lcc | 0.21±0.01 | 0.44±0.02 | na |
| Picoeukaryotes | 33 | 0.76±0.01 | lcc | lcc | lcc | na | na |

For each parameter, values correspond to the slope of the relationship between measurements made on live cells with the FACscan vs that made on fixed cells with either the FACScan or the EPICS (intercept = 0). For *Prochlorococcus*, only the populations below 60 m were considered, as near-surface populations were not completely resolved. For *Synechococcus*, computation on green fluorescence also takes into account only the deepest populations.

na = Not applicable.

lcc = Low correlation coefficient ($r^2 < 0.75$).

### *DNA analyses and growth-rate computation*

Samples from the time-series of EUMELI 3 were analyzed for DNA after staining for 1 h with 1 μg ml$^{-1}$ Hoechst 33342 (Sigma). Measurements were made with the EPICS® using UV light for excitation (353–357 nm, 400 mW). DNA distributions of *Prochlorococcus* cells were analyzed as described by Vaulot and Partensky (1992), except that we used the M-cycle software (Phoenix Flow Systems) to calculate the percentage of cells within the different phases of the cell cycle: $G_1$, S and $G_2$. A slight skewness on the right of the $G_1$ peak caused a systematic overestimation of the S%, so all values were corrected by subtracting the minimum S% value obtained during the period without division (see below). Growth rate of *Prochlorococcus* cells was computed using Carpenter and Chang's model (1988). The model requires knowledge of the duration of the division period ($t_{S+G2}$), estimated as twice the distance between the peak of cells in S and the peak of cells in $G_2$. Daily gross production rates were estimated at both depths according to Vaulot *et al.* (1995), as $P = C_{cell}\, N_{20:00}\, (\exp[\mu(z)] - 1)$, where $C_{cell}$ is the intracellular carbon content of *Prochlorococcus* estimated as 53 fg per cell (Campbell *et al.*, 1994) and $N_{20:00}$ is the cell concentration at 20:00 h, i.e. just prior to division (see Results).

### *Pigment analyses*

Half-liter samples were filtered through 47 mm Whatman GF/F filters. After extraction in 90% acetone, chlorophyllous pigments, including DV-Chl *a* and *b*, were analyzed by spectrofluorometry, as described by Neveux and Lantoine (1993).

## RESULTS

### *Hydrological conditions*

The main physico-chemical and biological characteristics of the stations occupied during the different EUMELI cruises are given in Table 2. For the EU and MESO sites, the nutrient enrichment (inorganic nitrogen and phosphorus) of the upper layer exhibited large seasonal variations. These changes were due mainly to the variable influence of a permanent upwelling, caused by strong trade-winds along the African coast. This phenomenon occurs between 20°N and 24°N and typically reaches its maximum activity in spring and summer (Mittelstaedt, 1991; Nykjaer and Van Camp, 1994). In our data set, the upwelling most strongly influenced the hydrological structure of the EU site in June 1992, as shown by very high values of nitrates and phosphorus, accompanied by large Chl *a* concentrations. The mixed layer of the MESO site also showed some nutrient enrichment in June, allowing phytoplankton growth near the surface. In contrast, in October 1991, the Chl *a* maximum was generally found below the thermocline at the MESO site, the upper mixed layer being nutrient-poor (0–50 nM $NO_2+NO_3$; Table 2). Inorganic nutrients were always undetectable in the mixed layer of all stations of the MESO site, whatever the season. At the OLIGO site, the deep chlorophyll *a* maximum (DCM), which exhibited large diel oscillations (see below), was observed at 90–140 m, i.e. well below the thermocline, but also slightly below the euphotic layer (Table 2).

Given the high seasonal variability in the hydrological characteristics of the EU and MESO sites, these are not necessarily representative of typical eu- or mesotrophic conditions (e.g. the MESO site in June is actually richer in nutrients than the EU site in December). Thus, the terms EU, MESO and OLIGO must rather be considered as representing different enrichment stages of a peculiar seasonal gradient.

### *Seasonal variations of the vertical distributions of picoplankton at the different sites*

Dramatic changes in the vertical structure of picophytoplanktonic populations were observed at the EU site between June and December 1992 (Fig. 2). In late springtime, this site was characterized by homogeneously cool (17.5°C), nutrient-rich waters down to 100 m, although the occurrence of a pycnocline indicated a weak stratification (Fig. 2(B)). All picoplanktonic populations exhibited strikingly low cell concentrations (Fig. 2(A)). Altogether, the contribution of picoplankton represented only a small fraction (6–9%) of the total chlorophyll standing stock (averaging 7.2 $\mu g\, l^{-1}$ over the first 40 m). The latter was largely dominated by a diatom bloom, as shown by the high concentration of Chl *c* (see Table 3) and the abundance of carotenoids (fucoxanthins) specific to this algal group (H. Claustre, personal communication, 1992). In winter, the stratification was well marked at this site. The cell abundances of all three picophytoplanktonic groups were much higher than in June (Fig. 2(C)), although the total phytoplankton biomass was *ca* 6–7 times lower (Fig. 2(D) and Table 2). Cells were restricted and homogeneously distributed over the mixed layer. *Synechococcus* cells were numerically dominant, but their contribution to the picoplankton biomass in terms of carbon only slightly exceeded that of picoeukaryotes (Table 4).

The picophytoplankton population structures observed in June and December 1992 at the MESO site (Fig. 3(E), (G)) were globally similar to that observed at the EU site in December, except that the layer of maximum abundance was thicker, as a result of a deeper

*Table 2. Main characteristics of the sites sampled during the EUMELI cruises*

| Sampling date | EUMELI cruise no. | Station | Position | tChl *a* ($\mu g\,l^{-1}$) | Inorganic nutrient concentrations ($nmol\,l^{-1}$) in the mixed layer | | Average depth (m) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $NO_2+NO_3$ | $PO_4$ | Mixed layer | Euphotic zone | DCM |
| October 1991 | 3 | MESO | 18°29'N 21°05W | 1.42±0.61 | 25±24 | 131±11 | 30 | 40 | 40±5 |
| | 3 | IMO | 19°42'N 25°52W | 0.39 | 3 | <20 | 35 | nd* | 100 |
| | 3 | OLIGO | 21°02'N 31°08W | 0.38±0.04 | <3 | <20 | 40 | 89±5 | 100±11 |
| June 1992 | 4 | EU | 20°32'N 18°34W | 7.38±0.92 | 10 450±1 040 | 710±60 | 48±8 | 20±3 | none† |
| | 4 | MESO | 18°29'N 21°05W | 1.14±0.20 | 590±290 | 230±30 | 45±8 | 24±4 | none |
| | 4 | OLIGO | 21°02'N 31°08W | 0.41±0.04 | <3 | <20 | 50–70‡ | 102±2 | 123±15 |
| December 92 | 5 | EU | 20°32'N 18°34W | 1.15±0.01 | 255±80 | 210±20 | 31±3 | 33 | none |
| | 5 | EM 54 | 20°08'N 19°03W | 1.02 | nd | nd | 35 | nd | none |
| | 5 | EM 52 | 19°17'N 20°01W | 0.76 | nd | nd | 47 | nd | none |
| | 5 | MESO | 18°29'N 21°05W | 0.68±0.05 | 22±8 | 170±20 | 36±6 | 36 | none |
| | 5 | MO 53 | 19°06'N 23°30W | 0.52 | nd | nd | 62 | nd | 75 |
| | 5 | MO 52 | 19°45'N 25°51W | 0.66 | nd | nd | 75 | nd | 75 |
| | 5 | MO 51 | 20°23'N 28°37W | 0.39 | nd | nd | 75 | nd | 75 |
| | 5 | OLIGO | 21°02'N 31°08W | 0.42±0.04 | <3 | <20 | 85±10 | 95 | 103±10 |

Nutrient values were averaged over the mixed layer. Values for total chlorophyll *a* (tChl *a*) correspond either to the average concentration in the DCM or in the mixed layer when there was no DCM. Variations of the width of the mixed layer, depth of the euphotic zone (i.e. the depth reached by 1% of the PAR in surface) and the depth of the DCM are also indicated.

*nd = Not determined.

†none = No Chl *a* maximum occurred.

‡Average depths at the beginning (21–22 June 1992) and end (25 June 1992) of the sampling period.

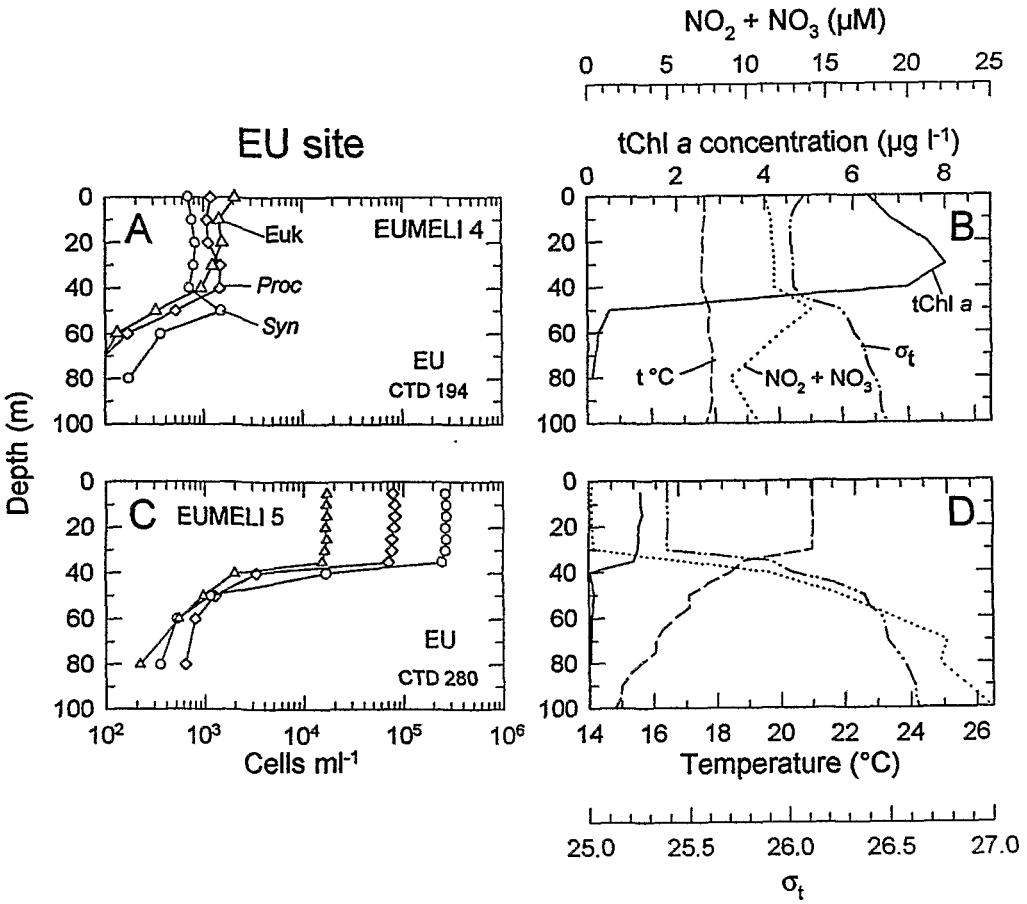


Fig. 2. Vertical profiles of *Prochlorococcus* (*Proc*), *Synechococcus* (*Syn*) and picoeukaryotes (Euk) cell concentrations and corresponding total Chl *a* concentration (tChl *a*), temperature ($T$°C), density ($\sigma_t$) and $NO_2 + NO_3$ concentration at the EU site in June (A)–(B) and December 1992 (C)–(D).

mixed layer (Fig. 3(F), (H)). Very dense populations of *Synechococcus* were observed at the MESO site in June, reaching exceptional densities of up to $5.2 \times 10^5$ cells ml$^{-1}$ at 15 m and integrated concentrations of up to $2 \times 10^9$ cells cm$^{-2}$. Thus, cyanobacteria accounted for almost half of the POC (data not shown) and 22–32% of the chlorophyll standing stock (estimated contribution from fractionated filtrations: 0.3 µg l$^{-1}$). None of the vertical distributions observed at the MESO site in October 1991 (Fig. 3(A), (C)) showed any point in common with those observed in the EU-MESO area at other seasons. Moreover, the variability of cell concentrations of picoplankton was considerable, even at short time-scales. This variability is illustrated in Fig. 3(A) and 3(C), which correspond to casts done less than 24 h apart. Figure 3(A) displays well-developed abundance maxima of all three populations at 35–45 m, coincident with the chlorophyll maximum depth. These maxima occurred at the base of a very sharp thermocline, in waters at 18–20°C (Fig. 3(B)). In contrast, in Fig. 3(C), picoeukaryotes show no abundance maximum, consistent with a sharp reduction of the tChl *a* maximum (Fig. 3(D)). Concomitantly, a sharp depression of both *Prochlorococcus* and *Synechococcus* cell numbers occurred at 30 m, i.e. right at the level of the thermocline ($\Delta T = 7$°C between 25 and 35 m; Fig. 3(C)). This biological variability can apparently not be accounted for by changes in the physico-chemical characteristics of the water column, which were stable during the sampling period (Fig. 3(B), (D)). At both

*Table 3. Integrated concentrations of chlorophyllous pigments measured by spectrofluorimetry during the different EUMELI cruises*

| EUMELI cruise no. | Station | Integration depth (m) | $n$ | Pigment concentrations ($\mu g\ cm^{-2}$) Chl *a* | Chl *b* | Chl *c* | DV-Chl *a* | DV-Chl *b* | DV-Chl *a*/ tChl *a* (%) |
|---|---|---|---|---|---|---|---|---|---|
| 3 | MESO | 100 | 8 | 3.66±0.76 | 1.05±0.26 | 1.11±0.26 | 0.82±0.42 | 0.42±0.16 | 18.3±6.0 |
| 3 | IMO | 100 | 1 | 1.62 | 0.56 | 0.65 | 1.99 | 0.77 | 55.1 |
| 3 | OLIGO | 200 | 13 | 1.45±0.15 | 0.43±0.06 | 0.56±0.06 | 1.82±0.18 | 0.83±0.12 | 55.6±3.2 |
| 4 | EU | 100 | 6 | 32.67±7.46 | 1.40±0.52 | 9.36±2.39 | nd* | nd | 0.5±0.2 |
| 4 | MESO | 100 | 7 | 5.38±0.81 | 1.15±0.19 | 1.29±0.37 | 0.20±0.12 | 0.03±0.05 | 3.5±1.8 |
| 4 | OLIGO | 200 | 10 | 1.79±0.25 | 0.49±0.08 | 0.70±0.10 | 1.67±0.17 | 0.80±0.17 | 48.3±2.0 |
| 5 | EU | 100 | 3 | 3.85±0.43 | 0.99±0.12 | 0.78±0.14 | 0.48±0.02 | 0.06±0.02 | 11.2±0.1 |
| 5 | EM 54 | 100 | 1 | 3.93 | 1.21 | 0.81 | 0.71 | 0.05 | 15.3 |
| 5 | EM 52 | 100 | 1 | 3.50 | 0.80 | 0.75 | 0.23 | 0.06 | 6.1 |
| 5 | MESO | 100 | 3 | 2.56±0.22 | 0.64±0.04 | 0.57±0.06 | 0.73±0.08 | 0.10±0.01 | 22.2±0.0 |
| 5 | MO 53 | 100 | 1 | 1.63 | 0.52 | 0.55 | 1.54 | 0.61 | 48.7 |
| 5 | MO 52 | 100 | 1 | 1.62 | 0.53 | 0.56 | 1.72 | 0.45 | 51.6 |
| 5 | MO 51 | 100 | 1 | 1.11 | 0.31 | 0.45 | 1.55 | 0.33 | 58.2 |
| 5 | OLIGO | 200 | 4 | 1.69±0.24 | 0.41±0.06 | 0.61±0.05 | 1.73±0.11 | 0.74±0.09 | 50.8±2.2 |

*nd = Not detected.

stations, the upper layer had characteristics of oligotrophic waters with nutrient-depletion (<20 nM $NO_2+NO_3$), relatively low Chl biomass (0.42 $\mu g\ l^{-1}$ at 20 m) and high temperature (>25°C). The nutrient-depletion may have resulted from the biological activity of phytoplankters, which then sank to track the nitracline. Alternatively, the sharp temperature gradient observed at the thermocline suggests that the hydrological structure may have resulted from the sinking of cool water masses, originating from the already mentioned permanent upwelling, below more oceanic waters.

The vertical structure of picophytoplankton at the OLIGO site showed little variation between seasons (Fig. 4). This site was always characterized by a large numerical predominance of *Prochlorococcus*. Although there was *ca* 100-fold less *Synechococcus* than *Prochlorococcus*, they both reached their abundance maxima at least 30 m above the DCM (Fig. 4). At greater depth, cyanobacteria cell concentrations decreased rapidly, so that there were almost no cells left at the depth of the DCM. In contrast, *Prochlorococcus* concentrations showed a more progressive decrease at depth, and cells could still be found down to 160–200 m. The most striking seasonal change in the vertical structure of picoplankton was a variation in the average depth of the picoeukaryote maximum, which was always tightly associated with the DCM. The latter oscillated between 90 and 120 m in October 1991 (average = 100 m), 100 and 140 m (average = 123 m) in June 1992 and 80 and 110 m (average = 103 m) in December 1992. Concentrations of both *Synechococcus* and picoeukaryotes varied only slightly. The apparently larger seasonal variations of *Prochlorococcus* cell concentrations in near surface waters may be related to seasonal changes in the average incident irradiance. Cells were less abundant in June, when the irradiance reaching the surface was the highest (*ca* 51.7 mol quanta $m^{-2}\ day^{-1}$), than during the two other seasons (*ca* 36.5 mol quanta $m^{-2}\ day^{-1}$ in October and 31.0 mol quanta $m^{-2}\ day^{-1}$ in December). Alternatively, these changes may be artifactual, since *Prochlorococcus* were dimly fluorescent in June and more cells may have

*Table 4. Integrated cell concentrations of the three populations of picoplankton during the different EUMELI cruises and corresponding carbon biomass*

| EUMELI cruise no. | Station | *n* | Million cells per cm$^2$ | | | µg C per cm$^2$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Prochlorococcus* | *Synechococcus* | Picoeuk.* | *Prochlorococcus* | *Synechococcus* | Picoeuk.* |
| 3 | MESO | 7 | 613±509 | 404±259 | 54±30 | 32±27 (13) | 101±65 (41) | 113±63 (46) |
| 3 | IMO | 1 | 2 224 | 23 | 19 | 118 (63) | 5.9 (3) | 63 (34) |
| 3 | OLIGO | 7 | 2 508±156 | 25±2 | 17±2 | 133±8 (76) | 6.4±0.5 (4) | 36±4 (20) |
| 4 | EU | 1 | 37 | 10 | 15 | 2.0 (5) | 2.5 (7) | 32 (88) |
| 4 | MESO | 5 | 122±47 | 1 332±550 | 61±22 | 6.4±2.5 (1) | 333±138 (71) | 128±47 (28) |
| 4 | OLIGO | 4 | 2 311±151 | 38±1 | 33±6 | 122±8 (61) | 9.5±0.1 (5) | 69±12 (34) |
| 5 | EU | 2 | 297±6 | 800±313 | 62±5 | 16±1 (5) | 200±78 (57) | 131±11 (38) |
| 5 | EM 54 | 1 | 553 | 664 | 64 | 29 (9) | 166 (50) | 136 (41) |
| 5 | EM 52 | 1 | 368 | 1 334 | 54 | 20 (4) | 333 (71) | 114 (25) |
| 5 | MESO | 2 | 901±80 | 690±60 | 38±2 | 48±4 (16) | 172±15 (57) | 81±4 (27) |
| 5 | MO 53 | 1 | 1 750 | 141 | 40 | 93 (44) | 35 (17) | 83 (39) |
| 5 | MO 52 | 1 | 1 908 | 87 | 33 | 101 (53) | 22 (11) | 69 (36) |
| 5 | MO 51 | 1 | 1 730 | 40 | 26 | 92 (59) | 10 (6) | 55 (35) |
| 5 | OLIGO | 2 | 1 513±38 | 21±1 | 25±1 | 80±2 (58) | 5.2±0.1 (4) | 53±4 (38) |

The following conversion coefficients were used: *Prochlorococcus*: 53 fg C cell$^{-1}$, *Synechococcus*: 250 fg C cell$^{-1}$; picoeukaryotes: 2108 fg C cell$^{-1}$ (Campbell *et al.*, 1994). Numbers between parentheses correspond to the average percentage of total picophytoplankton carbon.

*Values do not include large eukaryotic cells, such as diatoms, which were not detectable with the flow cytometer setup used.

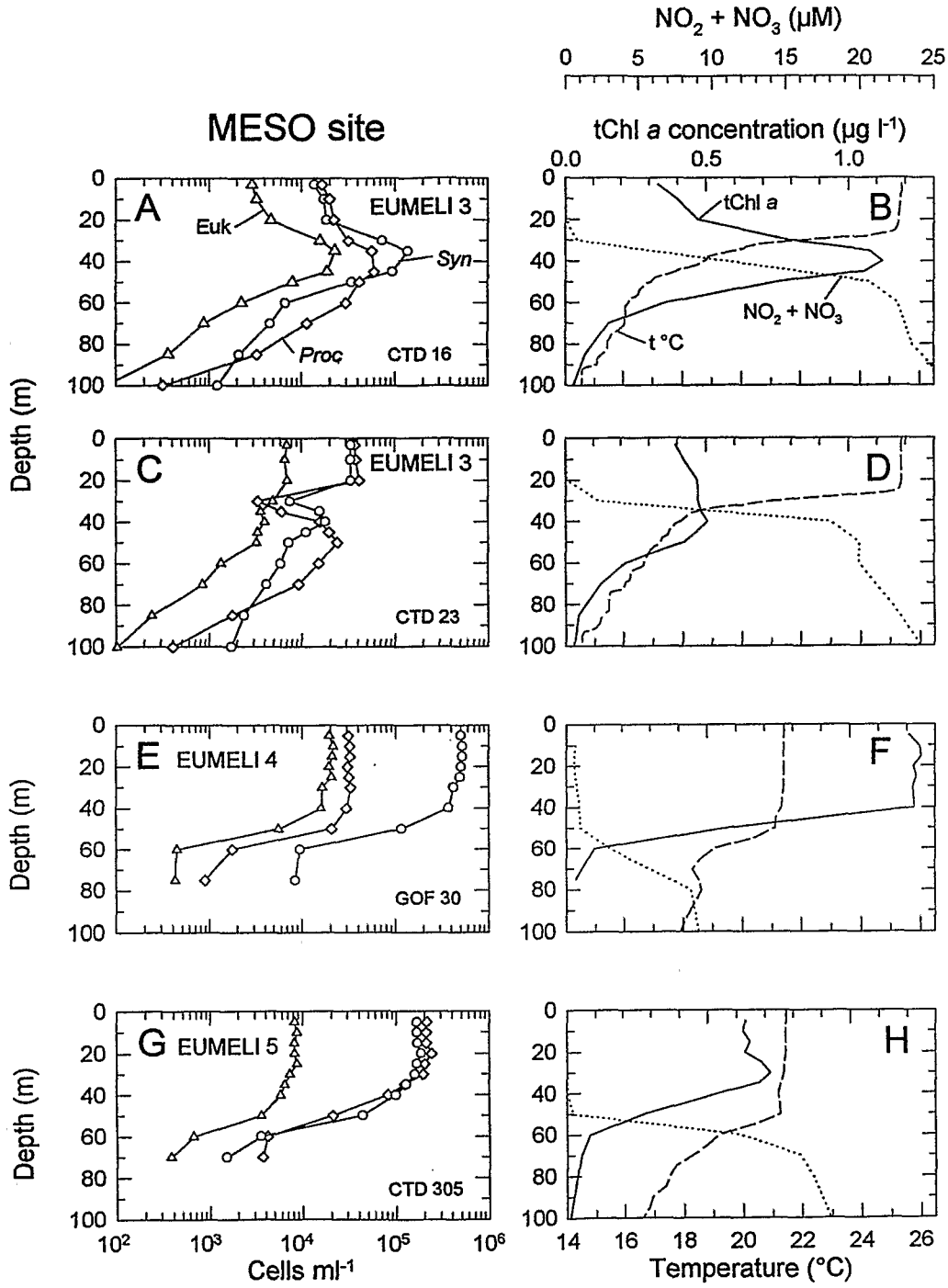


Fig. 3. Vertical profiles of *Prochlorococcus* (*Proc*), *Synechococcus* (*Syn*) and picoeukaryotes (Euk) cell concentrations and corresponding total Chl *a* concentration (tChl *a*), temperature ($T$°C) and $NO_2+NO_3$ concentration at the MESO site in October 1991 (A)–(D), June 1992 (E)–(F) and December 1992 (G)–(H). Data for inorganic nitrogen were taken from different casts (done at ±1–3 h) and therefore are only indicative. Density profiles, which mirrored temperature profiles, have been omitted for readibility.

escaped detection than at other seasons. The highest integrated concentrations of *Prochlorococcus* were observed in the fall ($2.5 \times 10^9$ cells per $cm^2$) with maximum concentrations of up to $4 \times 10^5$ cells $ml^{-1}$, whereas *Synechococcus* and picoeukaryotes peaked in late spring (3.8 and $3.3 \times 10^7$ cells per $cm^2$, respectively; Table 4).

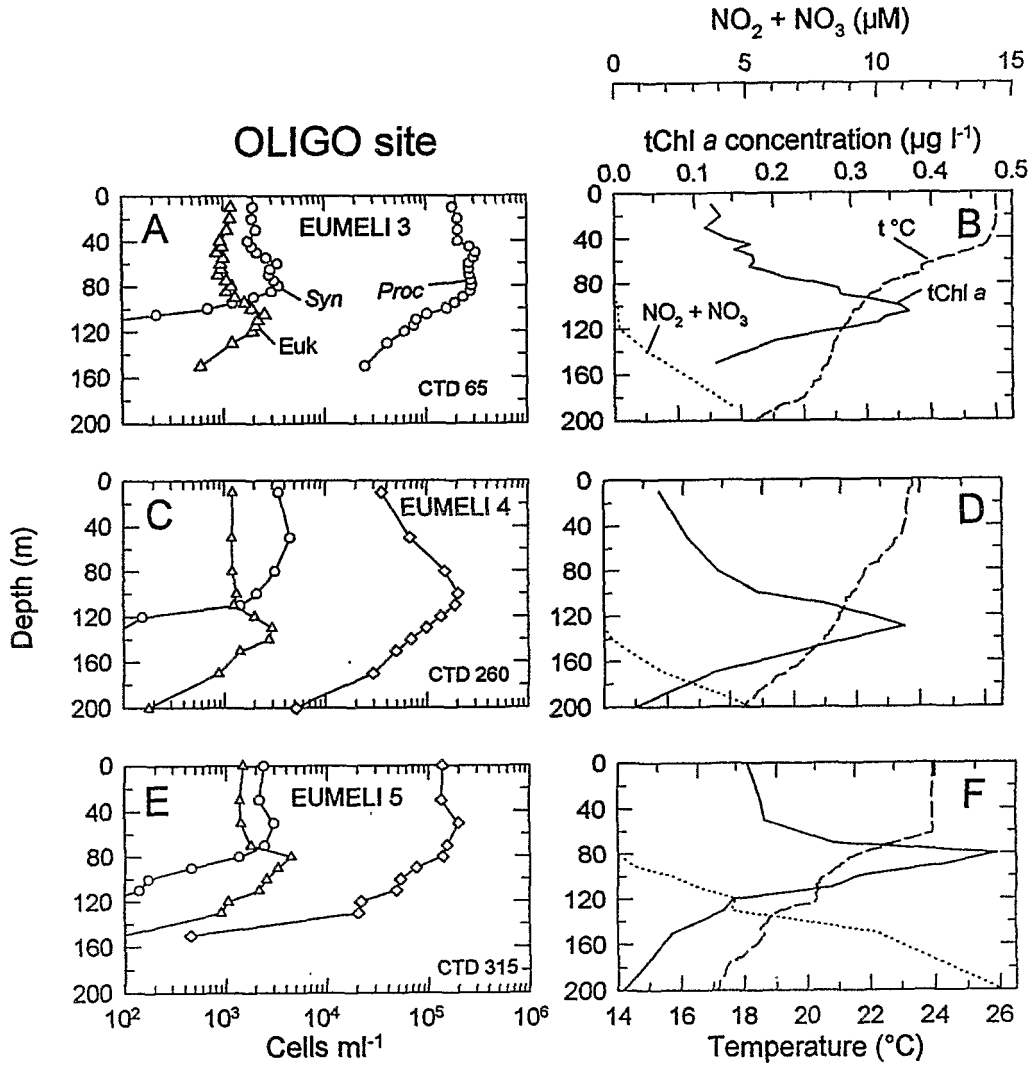


Fig. 4. Vertical profiles of *Prochlorococcus* (*Proc*), *Synechococcus* (*Syn*) and picoeukaryotes (Euk) cell concentrations and corresponding total Chl *a* concentration (tChl *a*), temperature ($T$°C) and $NO_2 + NO_3$ concentration at the OLIGO site in October 1991 (A)–(B), June 1992 (C)–(D) and December 1992 (E)–(F). Same comments as Fig. 3.

*Spatial variations of the vertical distributions of picoplankton along the trophic gradient*

Spatial changes in the vertical distribution of picophytoplankton along the gradient were studied in most detail during the winter cruise (EUMELI 5), which included several intermediary stations between the main sites (Fig. 1). At this season, the trophic gradient from the EU to the OLIGO site was clearly much weaker than in June (Table 2). Figure 5 shows that in all the EU-MESO area, the three picoplanktonic populations were abundant in the upper 30–40 m layer. The decrease of cell concentrations below this layer was, however, sharper at the EU site than at more offshore stations. From the EU to the MESO site, integrated concentrations of both *Synechococcus* and picoeukaryotes had a tendency to decrease (*ca* 2-fold), while those of *Prochlorococcus* increased (Table 4). There was a slight reversal of this general tendency at station EM 52 for the prokaryotes. At this station, a *Synechococcus* bloom, similar to that described at the MESO site in June 1992 (Fig. 3(E) and Table 2), was observed. More significant variations occurred off the MESO site. Between the MESO and OLIGO sites, the maximum cell concentrations decreased dramatically for *Synechococcus* from $1.8 \times 10^5$ cells $ml^{-1}$ to $3 \times 10^3$ cells $ml^{-1}$ and decreased only 2-fold for

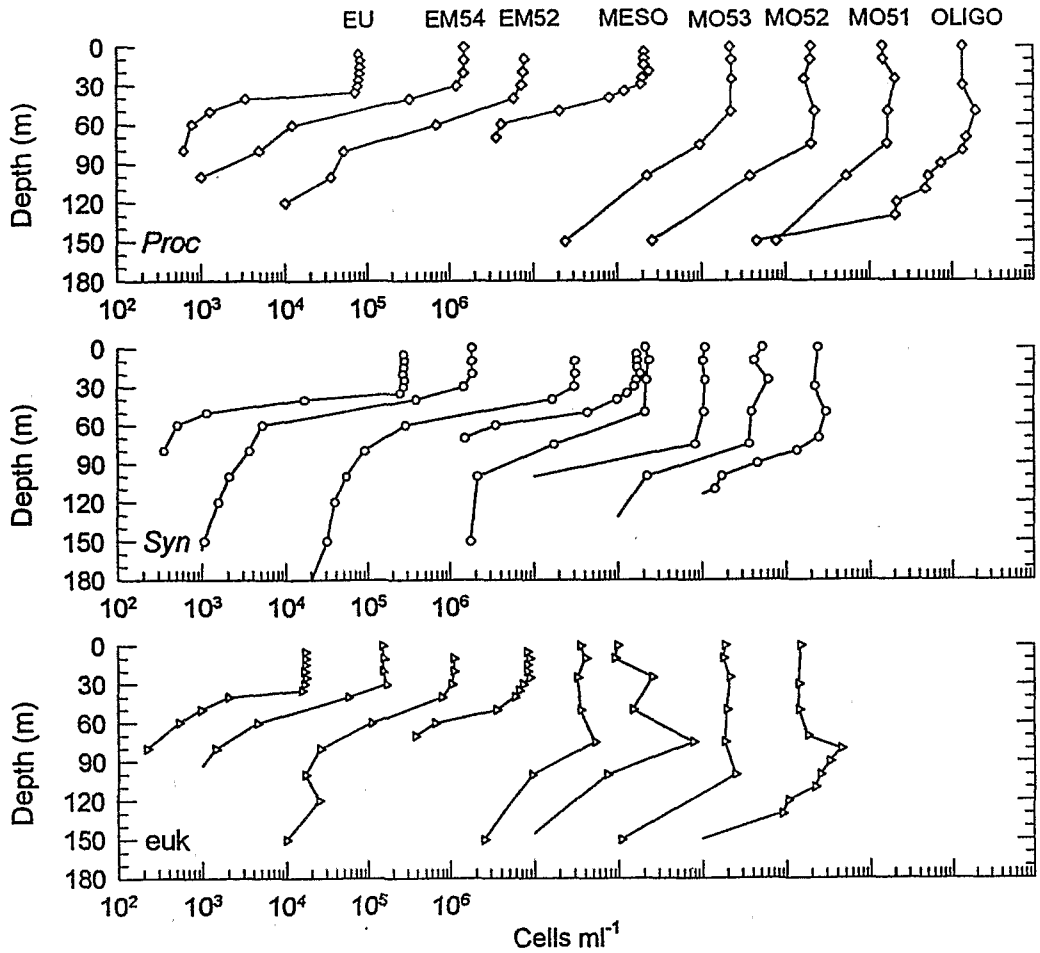


Fig. 5. Vertical profiles of *Prochlorococcus* (*Proc*), *Synechococcus* (*Syn*) and picoeucaryotes (Euk) cell concentrations along the transect from the EU to OLIGO site during December 1992 (EUMELI 5). For each profile, the abscissa ranges from $10^2$ to $10^6$ cells ml$^{-1}$, but consecutive profiles are shifted by one decade. Details on stations are given in Table 2.

the microalgae from $8.7 \times 10^3$ cells ml$^{-1}$ to $4.4 \times 10^3$ cells ml$^{-1}$. In the upper layer, the picoeukaryote concentration, however, decreased more than 6-fold. In contrast, *Prochlorococcus* concentrations varied little (range = 2.0–2.4 $\times 10^5$ cells ml$^{-1}$) between the MESO site and the more offshore stations. Corresponding changes in integrated cell concentrations were 1.7-fold, 33-fold and 1.5-fold for *Prochlorococcus*, *Synechococcus* and picoeukaryotes, respectively (Table 4). The relative contribution of *Prochlorococcus* to both picoplanktonic carbon (Table 4) and to total Chl *a* (as estimated from the ratio of DV-Chl *a* to tChl *a*, Table 3) doubled from EU to MESO and again from MESO to MO 53 but did not significantly increase further offshore. It is noteworthy that the layer of high abundances of these three populations (Fig. 5) was significantly thicker at the OLIGO site (80 m for *Synechococcus*, 120–130 m for the others) than in the EU-MESO area (33–36 m for all populations), consistent with an increase of the depth of the euphotic layer (Table 2). While there was no subsurface maximum at the EU, EM 52, EM 54 or MESO sites for any of the picophytoplankton populations, both *Prochlorococcus* and *Synechococcus* showed a slight maximum at 50 m at the OLIGO site, and the picoeukaryotes showed a marked maximum 30–40 m below. The formation of a cell density maximum of microalgae first occurred at MO 53.

The vertical distribution (not shown) and integrated abundances (Table 4) observed at

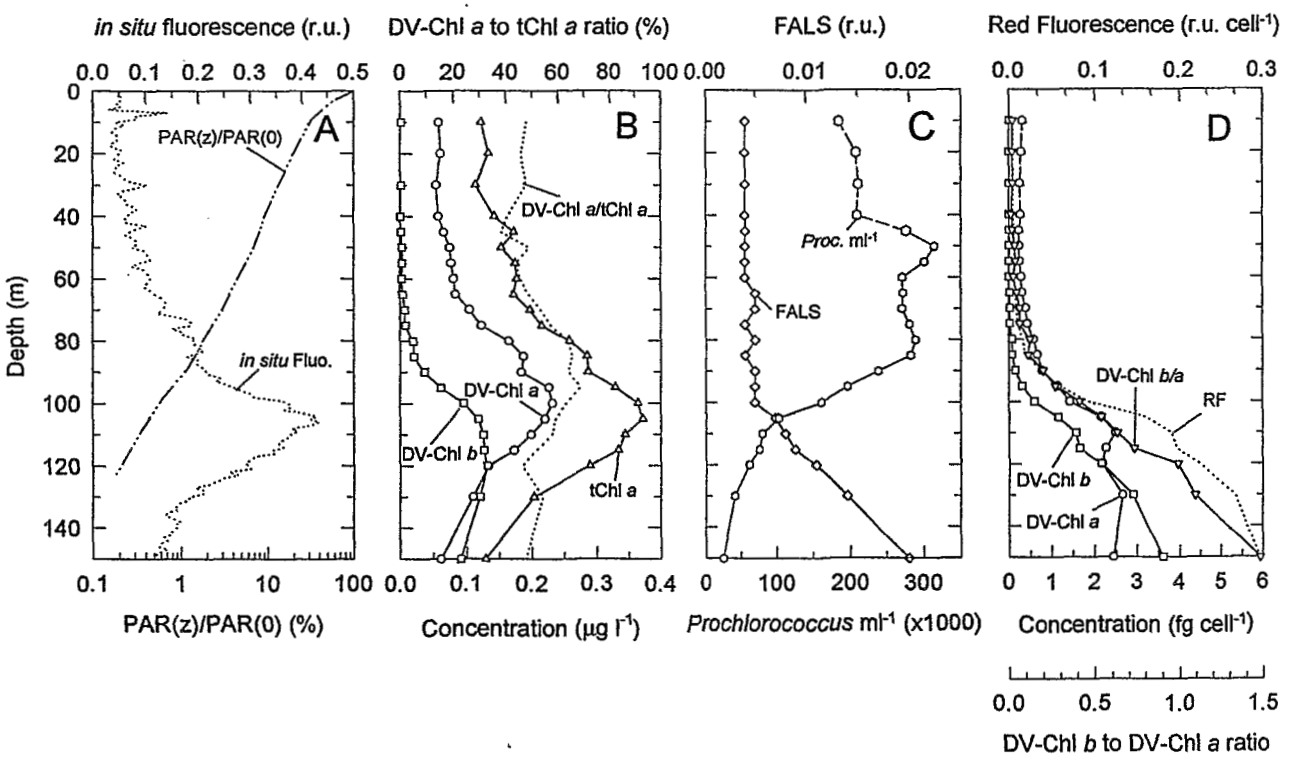


Fig. 6. Vertical profiles of various parameters measured at the OLIGO site (CTD 65) during October 1991 (EUMELI 3): (A) photosynthetically available radiation [PAR(*z*)/PAR(0)] and *in situ* fluorescence; (B) DV-Chl *a*, DV-Chl *b* and tChl *a* concentrations in seawater and DV-Chl *a* to tChl *a* ratio; (C) *Prochlorococcus* cell concentrations and forward angle light scatter (FALS); (D) *Prochlorococcus* RF, cell content in DV-Chl *a*, DV-Chl *b* and ratio of DV-Chl *b* to *a*.

station IMO during EUMELI 3 were similar to those observed at OLIGO and not intermediate between mesotrophic and oligotrophic structures, as was the equivalent station MO 52 during winter.

### *Vertical profiles of* Prochlorococcus *pigments and fluorescence at the OLIGO site*

In all the seasons examined, *Prochlorococcus* not only was the most abundant photosynthetic organism at the OLIGO site (see above), but also accounted for half or more of the chlorophyll standing stock (Table 3). In October 1991, the ratio of DV-Chl *a* to tChl *a* averaged 53% from the surface down to 150 m, but the relative contribution of *Prochlorococcus* to total chlorophyll increased with depth: the DV-Chl *a*/tChl *a* ratio averaged 44% in the mixed (0–45 m) layer, 52% in the layer of maximum *Prochlorococcus* abundance (45–85 m) and 58% in the deep layer (85–150 m), including the DCM (Fig. 6(B)). That *Prochlorococcus* contributes most to the chlorophyll below its layer of maximum cell abundance might seem paradoxical. In fact, changes of DV-Chl *a* concentration in seawater (DCS) with depth (Fig. 6(B)) result from the combined variations of *Prochlorococcus* cell concentration (Fig. 6(C)) and DV-Chl *a* concentration per cell (Fig. 6(D)). Thus, the DCS did increase between 85 m and 100 m, because the sharp decrease in cell density was more than compensated by the increase in DV-Chl *a* concentration per cell. The DCS began to decrease only below 100 m (Fig. 6(B)), because the DV-Chl *a* content per cell ceased increasing (Fig. 6(D)). In contrast to the latter, the DV-Chl *b* content per cell went on

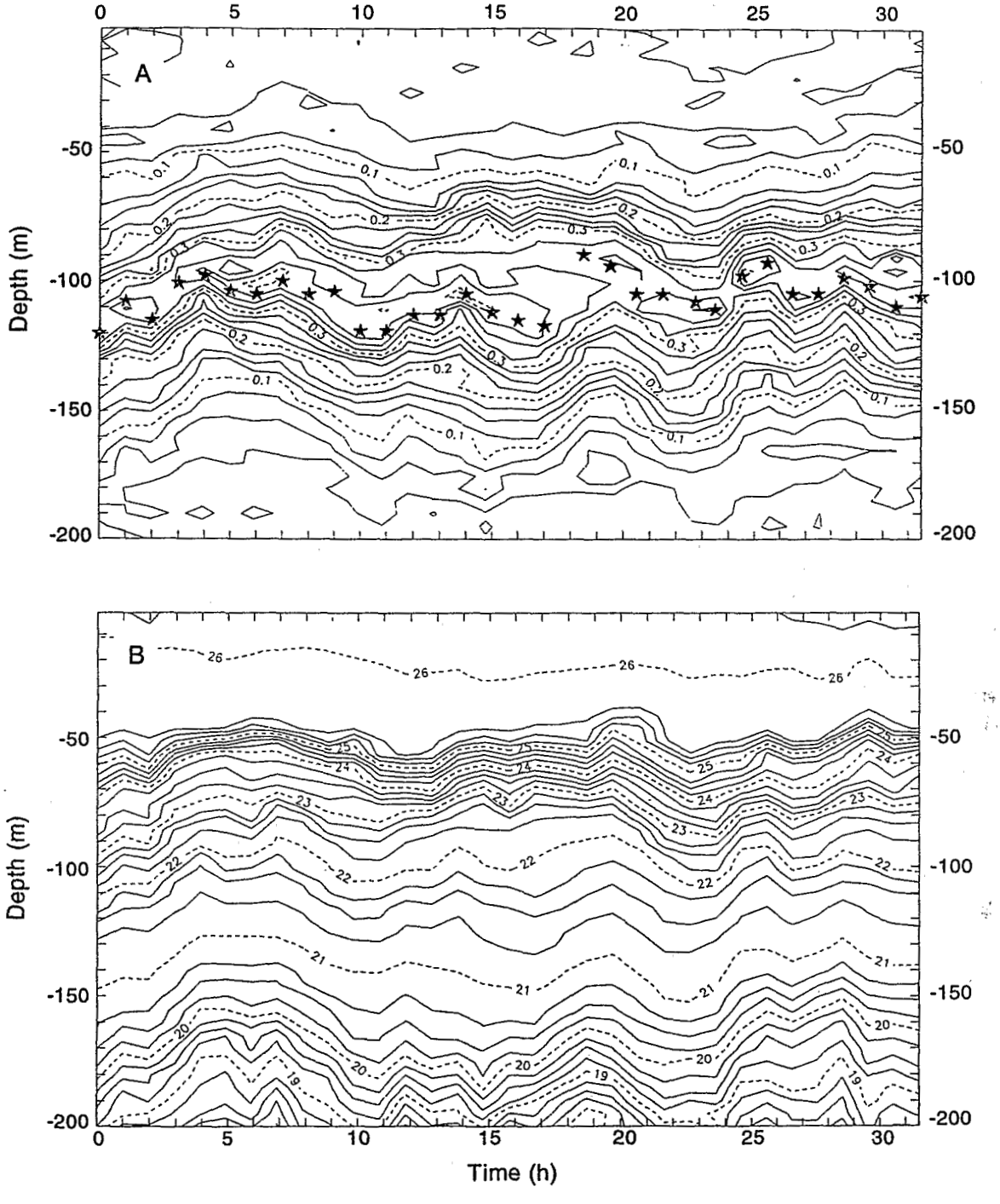


Fig. 7. Time series of continuous fluorescence (A) and temperature (B) measurements at the OLIGO site during October 1991 (EUMELI 3). Stars indicate the depths at which bottles were closed to sample the CFM level.

increasing down to *ca* 150 m, suggesting that *Prochlorococcus* cells were still able to photoacclimate at very low ambient light levels (0.5–0.6% of the photosynthetically available radiation incident on the surface reached 100 m and less than 0.1% reached 150 m, Fig. 6(A)). The good ability of *Prochlorococcus* to acclimate its pigment content in response to decreased light levels is also shown by changes in the flow cytometric red fluorescence (RF) signal. The latter correlated well with the DV-Chl *b* to *a* ratio [RF = 0.257 DV-Chl *b*/*a* −0.003 ($r^2 = 0.93$, $n = 72$)], a good index of the photoacclimation state of

*Prochlorococcus* cells (Partensky *et al.*, 1993). Beside photoacclimation, another phenomenon also strongly contributed to the RF increase with depth. At the base of the euphotic zone (i.e. at *ca* 90 m), the frequent bimodality of the RF signal suggested the co-occurrence of two subpopulations of *Prochlorococcus*. The dimmest subpopulation dominated in the upper layer and the brightest in the deep layer (not shown; for illustration, see Campbell and Vaulot (1993) who observed a similar phenomenon in the tropical Pacific Ocean).

Variations of *Prochlorococcus* FALS (Fig. 6(C)), a flow cytometric signal correlated with cell size (Sieracki *et al.*, 1995), seem to indicate that the average *Prochlorococcus* size was constant or only slightly increasing over the top 100 m, but increased more sharply below this depth.

## Prochlorococcus *dynamics at the OLIGO site*

Figure 7 shows the variations of fluorescence and temperature measured in the euphotic zone (0–200 m) during a 31 h time series at the OLIGO site in October 1991. Both measurements show the large, vertical oscillations of the water column due to internal waves. The chlorophyll fluorescence maximum (CFM) sometimes became thick but on average followed closely the isotherms 21.4–21.8°C. Two depths, that of the CFM (stars in Fig. 7(A)) and a fixed depth (80 m) were sampled every hour, and the percentage of *Prochlorococcus* cells in the active phases of the cell cycle, S (DNA synthesis phase) and $G_2$ (cells prior to division), was then computed (Fig. 8). Results suggest that *Prochlorococcus* cells exhibit a highly synchronized division, closely coupled to the natural diel light cycle. The first cells of the cohort entered the S phase at 12:30–13:00 (solar time) at 80 m and about 1 h before at the CFM. Cells entered the $G_2$ phase 4 to 5 h later. Division was completed for all the cohort within 12–14 h, then daughter cells remained in the $G_1$ phase until the next division period. From these time series of DNA distributions we computed the growth rate, according to Carpenter and Chang (1988). The duration of the division period ($t_{S+G2}$) was estimated as 4.5 h for both depths. Computation led to growth rates of 0.41 $day^{-1}$ and 0.39 $day^{-1}$ at 80 m and the CFM, respectively. Parallel samples incubated onboard in 50-ml culture flasks in a light- and temperature-controlled room (23 ± 2°C) displayed clear signs of artifact (see inserts in Fig. 8(B), (C)). Incubated populations showed a pattern similar to that observed in the field for the first division period, but probably as a result of either the "bottle" effect or poisoning due to sampling with Niskin bottles, fewer cells entered the S-phase. After 24 h, almost no cells were cycling.

Carbon assimilation rate of *Prochlorococcus* cells was derived to be 4.77 and 1.32 mg C $m^{-3}$ $day^{-1}$ at 80 m and at the CFM, respectively. These values can be compared to the average $^{14}C$-derived total gross production (Y. Dandonneau, personal communication, 1992) at corresponding depths, divided by 0.4 to account for the difference between gross and net production, as described by Vaulot *et al.* (1995). Thus *Prochlorococcus* contributed an estimated 95% of the total production at 80 m (5.00 ± 1.98 mg C $m^{-3}$ $day^{-1}$) and 44.7% at the CFM (2.95 ± 1.31 mg C $m^{-3}$ $day^{-1}$ for the 90–120 m layer).

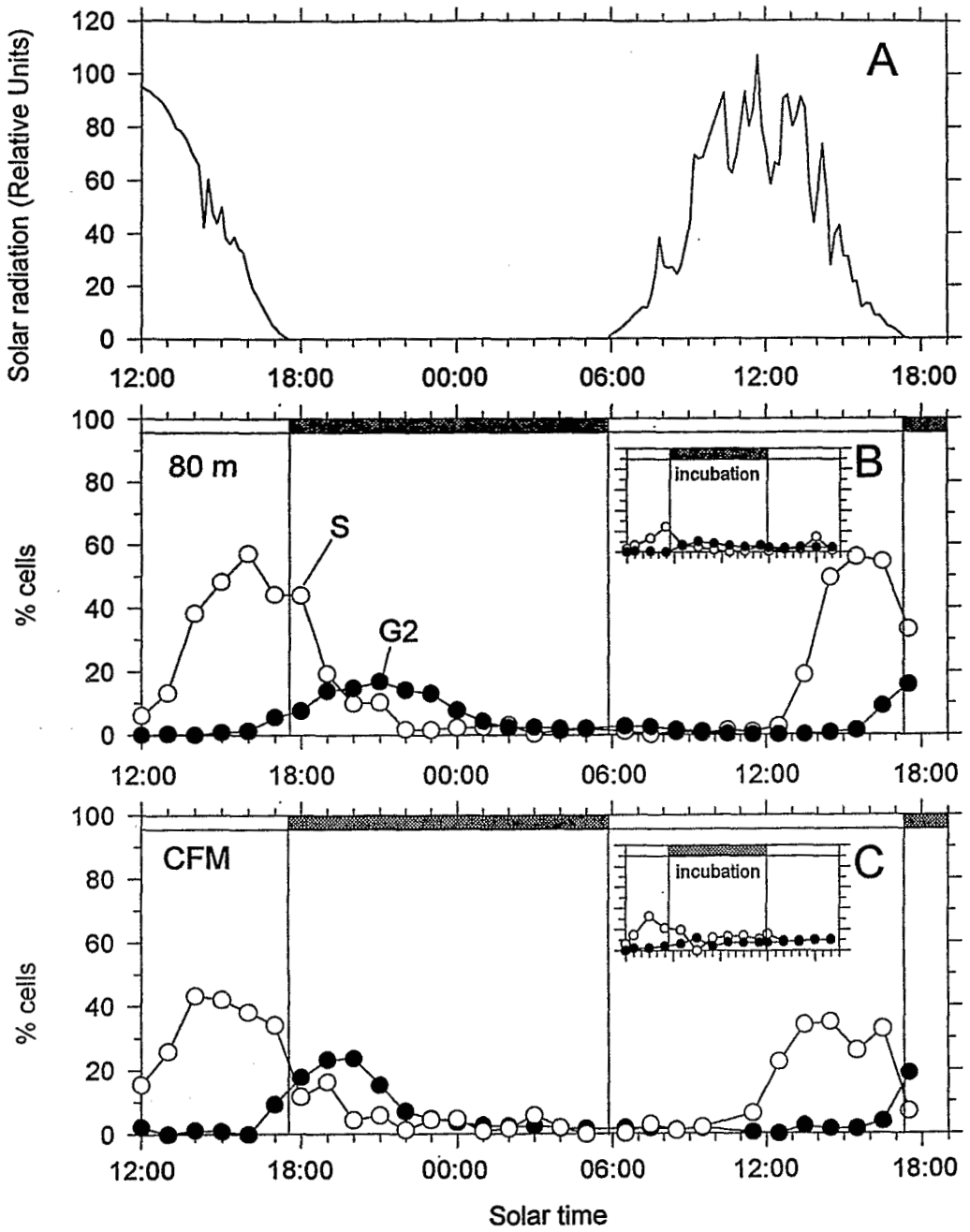


Fig. 8. Incident solar irradiance at the surface and percentage of *Prochlorococcus* cells in S and $G_2$ phases of the cell cycle at 80 m and the CFM as a function of time. Inserts show the results of incubations of seawater samples taken at T0 from 80 m (*ca* 1% incident PAR in surface) and the CFM (*ca* 0.1–0.2% incident PAR in surface) then kept for 26 h under a 12 h: 12 h L:D cycle and 14.5 and 1.2 $\mu E\, m^{-2}\, s^{-1}$ blue light, respectively.

## DISCUSSION

### *Characteristics of the vertical structures of picophytoplankton*

The different hydrological situations that we observed during the three EUMELI cruises may be roughly classified into four distinct cases, each one associated with a typical picophytoplankton structure. The first case (case 1), observed in June at the EU station (Fig. 2(B)), was characterized by a quasi-absence of vertical stratification, a high nutrient enrichment and a low temperature. This situation appeared deleterious to the growth of all

picophytoplanktonic populations, which occurred at very low concentrations. Most of the other hydrological situations observed in the EU-MESO area belonged to a second case.

Case 2 was characterized by a well developed stratification and the superimposition of the thermocline, pycnocline and nitracline at the base of a thick (30–40 m) upper mixed layer, generally containing detectable amounts of inorganic nutrients (range 22–590 nM $NO_2 + NO_3$). In that case, cells of the three picoplanktonic populations were concentrated and homogeneously distributed in the upper layer. In terms of carbon, the picophytoplanktonic biomass was mainly shared between picoeukaryotes and cyanobacteria (e.g. their average contributions in the EU-MESO area in winter were 33 and 59%, respectively), whereas the contribution of *Prochlorococcus* was always weak (4–16%). The relative contribution of these groups seemed to depend strongly on the seawater concentration of inorganic nutrients. When the latter was high (e.g. 590 nM $NO_2 + NO_3$ in June 1992 at the MESO site), it apparently favored, selectively, the blooming of *Synechococcus*, the contribution of which to the picophytoplanktonic carbon could rise to 71%. Such conditions appeared to a small extent favorable to picoeukaryotes but were clearly unfavorable to *Prochlorococcus*. In contrast, when the nutrient enrichment in the upper layer tended towards zero (e.g. at the MESO site in December 1992), both *Synechococcus* and picoeukaryote cell concentrations significantly decreased (2–4-fold), whereas *Prochlorococcus* cells became more abundant (*ca* $2 \times 10^5$ cells per ml).

The hydrological situation observed at the MESO site in October (CTD 16, Fig. 4(A)) constituted the third case. Case 3 differed from case 2 by stronger temperature and density gradients and was characterized by very low to undetectable inorganic nutrient concentrations in the upper layer. In four out of the eight stations sampled during our occupation of the MESO site in fall 1991, cells of all three populations peaked in the chlorophyll maximum located at the depth of the nitracline and just below the thermocline. In this hydrological situation, which corresponds to the typical tropical structure (TTS) defined by Herbland and Voituriez (1979), there was a numerical co-dominance of *Synechococcus* and *Prochlorococcus*. However, because of their larger size, the picoeukaryotes were actually the main contributors to the picoplanktonic carbon (46%). Although the physical characteristics of the water column were stable, large variations of both the total chlorophyll biomass and the relative concentrations of picoplankton populations were observed at the other four stations sampled. A specific impoverishment of the prokaryotes, especially *Prochlorococcus*, occurred to various degrees (see Fig. 3(C) for illustration). The latter phenomenon may have resulted either from a growth stress due to the sharpness of the thermocline or an unbalanced grazing pressure over the smallest size fractions.

The last case (case 4) corresponded to the purely oligotrophic situations observed, e.g. at the OLIGO site (Fig. 4). It was characterized by the complete absence of detectable nutrients over a layer much thicker than the upper mixed layer (e.g. the nitracline and the thermocline were on average at 100 and 40 m, respectively, during EUMELI 3). In this oligotrophic extreme of the TTS, the DCM was always located at the depth of the nitracline, as outlined by Herbland and Voituriez (1979). The remarkable stability of this hydrological structure (apart from the oscillations due to internal waves), led to less variability in the vertical structure of picophytoplankton than in the EU-MESO area. *Prochlorococcus* was by far the numerically dominant group at all depths. It was also the dominant component in terms of integrated picophytoplankton carbon (65% on average), but the contribution of picoeukaryotes was also significant (31% on average). The

contribution of *Synechococcus* cells to carbon was very reduced (≤5%), both because of their low concentration and because of their vertical confinement to the first two orders of magnitude of the light gradient. In contrast, both *Prochlorococcus* and picoeukaryotes were found down to 200 m, a depth reached by less than 0.1% of light irradiance incident at the surface. This population structure was very similar to that previously described at station ALOHA in the subtropical Pacific (Campbell and Vaulot, 1993). Moreover, the relative contributions of the three populations to the total integrated picophytoplanktonic carbon estimated at this station (with the same conversion factors that we used) were also quite comparable to our data (*Prochlorococcus*: 68%, picoeukaryotes: 28%, *Synechococcus*: 4%; Campbell *et al.*, 1994).

The apparent ability of picoeukaryotes and *Prochlorococcus* to grow over a very wide light gradient may in fact result from the genetic and ecophysiological heterogeneity of the components of these populations. The vertical extent of most, if not all, individual picoeukaryote species is probably restricted to a limited part of the water column. This is indicated on pigment profiles made by HPLC at the OLIGO site in October 1991 (Claustre and Marty, 1995), by the increase in the ratio of 19′-butanoyloxyfucoxanthin (19′-BF) to 19′-hexanoyloxyfucoxanthin (19′-HF) below 80 m, suggesting that Prymnesiophytes, which possess a low ratio of 19′-BF to 19′-HF, dominate in the upper layer, while Chrysophytes or Pelagophytes (*sensu* Andersen *et al.*, 1993), which have a high ratio of 19′-BF to 19′-HF, are mainly located at depth. Another indication is provided by the systematic observation, during EUMELI 3, of a very small population of picoeukaryotes displaying characteristic flow cytometric RF and RALS signals and being restricted to the 90 to 130 m layer (F. Partensky, unpublished data). The heterogeneity of *Prochlorococcus* populations is suggested by the frequent occurrence along the vertical light gradient of two *Prochlorococcus* subpopulations with distinct RFs (i.e. DV-Chl *b* to DV-Chl *a* ratios, see above), overlapping at intermediate depths. A similar observation was made in several other oligotrophic areas (Olson *et al.*, 1991; Campbell and Vaulot, 1993; McManus and Dawson, 1994; Blanchot and Rodier, 1996). In the subtropical Pacific, Campbell and Vaulot (1993) showed that these populations possessed different DNA contents, i.e. were genetically distinct. The study of two cultured isolates of *Prochlorococcus* also demonstrated that a large ecophysiological variability existed within this genus (Moore *et al.*, 1995).

### Prochlorococcus *dynamics at the OLIGO site*

Most methods currently used to estimate *in situ* growth rates provide information only on total phytoplankton communities and require incubations, which are the source of artifacts known as the "bottle effect" (see Furnas, 1990 for a review). In contrast, the "mitotic index" method, first applied to the analysis of cell cycle distributions by Carpenter and Chang (1988), has the advantages of allowing the estimation of the growth rate of individual species or populations and being applied on seawater samples without incubation (Vaulot, 1995). Nevertheless, the accuracy of the method depends on a correct estimation of the duration of terminal events of the cell cycle (i.e. $S+G_2$ phases in the case of prokaryotes). During EUMELI 3, this method was applied to *Prochlorococcus* at two depths of the OLIGO site with a high sampling frequency (1 h). This allowed an accurate determination of the time at which cells in S and $G_2$ peaked and therefore a good estimation of $t_{S+G2}$. Only a slight difference was observed between the estimated growth rates at 80 m (0.41 $day^{-1}$), i.e. in the layer of maximal *Prochlorococcus* abundance, and the CFM (0.39 $day^{-1}$), where it was

almost three times less abundant. Because of the internal waves, the sampling at fixed depth probably led to less reliable data, since the populations sampled at different times of the diel cycle did not all have exactly the same light history. However, these results suggest that *Prochlorococcus* concentrations at the CFM were low not because the growth rate was depressed, but probably because of a higher grazing pressure and (or) vertical diffusion at this depth than farther up in the water column. That *Prochlorococcus* growth rate only slightly decreased as a result of decreased incident light might be explained by the already mentioned occurrence, at different depths of the water column, of two or more *Prochlorococcus* sub-populations having different physiological requirements for light. The population dominating the upper part of the water column might be adapted to grow under high light–low inorganic nutrient conditions and the bottom one under low light–high inorganic nutrient conditions, so that they could sustain similar growth rates under very different environmental conditions. Whether the estimated growth rates of *ca* 0.4 $day^{-1}$ were optimal for these populations may be questioned. Using the incorporation of $^{14}C$ into pigments to estimate *Prochlorococcus* growth rate at a station off Bermuda (OFP: 31°50′N, 64°10′W), Goericke and Welschmeyer (1993) found rates ranging between 0.1 and 0.3 $day^{-1}$. In contrast, the same method as we used applied to *Prochlorococcus* populations at one station and many depths in the equatorial Pacific (Vaulot *et al.*, 1995) led to estimated rates as high as 0.69 $day^{-1}$ (i.e. one division per day) at the level of the DCM, which in this area occurs at *ca* 30 m. Below this depth, the growth rate progressively decreased, due to light limitation. These rates must be compared to the light-saturated, maximum growth rate of *Prochlorococcus* in culture ($0.53 \pm 0.06$ $day^{-1}$ and $0.63 \pm 0.06$ $day^{-1}$ for SS120 and MED4 clones, respectively; Moore *et al.*, 1995). Our data set for natural populations of the subtropical Atlantic is difficult to compare with that from the equatorial Pacific, because we have no data for near surface populations. If we assume that the growth rate of *Prochlorococcus* in the mixed layer is similar to that estimated at 80 m, it can be concluded that the different hydrological conditions prevailing in the equatorial and subtropical areas induce significantly different growth kinetics for *Prochlorococcus* populations. Rates could therefore be optimal in the former area and suboptimal in the latter.

Both the Vaulot *et al.* (1995) study and the present one demonstrate the tight coupling between cell cycle and the daily light cycle. In our data set, entry in S occurred around 13:00 at 80 m and noon at the CFM. Although the time span between consecutive samplings was larger in the data set from the equatorial Pacific (3 h), a similar shift of the time of entry in S between the surface and the deep layers was also obvious (surface cells replicated their DNA *ca* 3 h later than at 100 m), and was interpreted by Vaulot *et al.* (1995) as a possible protective mechanism to prevent cells from replicating their DNA at the time of highest irradiance exposure during the day.

The contribution of *Prochlorococcus* to the total gross production, estimated from its growth rate and conversion factors between cell and carbon content derived from cultures (Campbell *et al.*, 1994), was much more important at 80 m (95%) than at the depth of the CFM (45%). The high value found at 80 m was probably overestimated and might have been lower if we had applied conversion factors taking into account the true average size of *Prochlorococcus* cells, which in the upper layer is probably smaller than in culture (*ca* 0.4–0.5 μm instead of 0.6 μm). It is noteworthy, that the *Prochlorococcus* contribution to the chlorophyll standing stock was similar at these depths (e.g. at Station 65 of the OLIGO site in October 1991, the ratio of DV-Chl *a* to tChl *a* was 0.64 at 80 m and 0.59 at 105 m). This

points out that, in the oligotrophic area, the CFM (as well as the DCM) does not correspond to a maximum of biomass and production for *Prochlorococcus*. In contrast, some "shade species" of picoeukaryotes (chryso- or pelagophytes) might be adapted to grow at low light level (Claustre and Marty, 1995) and therefore have their peak of production at or near the depth of the DCM. Thus, in an oligotrophic situation, the different populations of picoplankton may exhibit very different vertical profiles of production.

## CONCLUSION

The current view of the specific composition and size structure of picophytoplankton has completely changed since *Prochlorococcus* was discovered (Chisholm *et al.*, 1988, 1992). In oligotrophic areas, the ecological importance of the other, very ubiquitous genus *Synechococcus* (Waterbury *et al.*, 1979) has probably been largely overestimated, since its contribution was often determined by fractionation through small pore-size filters or counting by epifluorescence microscopy, the latter method overlooking the tiny and rapidly fading *Prochlorococcus* cells (e.g. Glover *et al.*, 1986; Ituriaga and Mitchell, 1986). A clearer view now emerges.

The contribution of *Synechococcus* to the autotrophic standing stock is highest in mesotrophic situations, where it can form local blooms, whereas *Prochlorococcus* contributes the most to the biomass and production in oligotrophic areas (see e.g. Olson *et al.*, 1990a; Goericke and Welschmeyer, 1993; Campbell *et al.*, 1994).

Picoeukaryotic species are also present everywhere, and their contribution to the picophytoplanktonic biomass is always significant. The taxonomical value of flow cytometry fluorescence and scatter signals is, however, too low to draw any firm conclusion about species composition or even to check whether some species can photoacclimate over the wide light gradient found at the OLIGO site. It is clear, however, that some picoeukaryotes must be restricted to a limited part of the light gradient. Due to the ecological importance of these poorly known components of the picoplanktonic community, future studies should combine flow cytometry, HPLC pigment analyses and the use of species-specific probes (oligonucleotides or antibodies) to gain a better level of resolution and help identify the major species. Use of molecular biology techniques (e.g. see Palenik, 1994) may also help in studying the genotypic variability of picophytoplanktonic species, especially *Prochlorococcus* and *Synechococcus*, and understanding the way they adapt to a variety of hydrological conditions.

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