Picophytoplankton abundance and biomass in the western tropical Pacific Ocean during the 1992 El Niño year: results from flow cytometry

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Abstract—Natural populations of phytoplankton from the western tropical Pacific Ocean were analyzed by flow cytometry from a transect along 165°E between 20°S and 7°N. The abnormal hydrological situation corresponded to a weak El Niño event, with no equatorial upwelling and a marked nutrient ridge centered on 10°S. Prochlorophytes dominated numerically everywhere along the vertical, whatever the depth, in the 0–160-m layer (96% of cell abundance). Paradoxically, the highest concentrations, up to 4.4×10^5 cells ml^{-1}, were found in oligotrophic waters ($<0.1\,\mu$M NO$_3$). In contrast, the highest concentrations of orange cyanobacteria and red-fluorescing picoeukaryotes were observed when nitrate was present in the photic layer, i.e. around 10°S (up to 6.4×10^4 cells ml^{-1} and 1.3×10^4 cells ml^{-1}), and, to a lesser extent in the vicinity of the deep nitracline north of 8°S. Along the transect we encountered two hydrological situations, characterized by different community structures. The first one, found from 15°S to 7°N, except at 10°S, was a two-layer structure (Typical Tropical Structure, TTS) defined by a strong pycnocline in the upper 180 m and a well-marked nitracline. In this region, *Prochlorococcus* and picoeukaryotes co-dominated the 180-m integrated fluorescence and carbon biomass, but *Prochlorococcus* were the major component in the upper nitrate-depleted layer, while picoeukaryotes dominated the underlying rich layer. Inversely, *Synechococcus* were a relatively minor contributor to fluorescence ($\sim$4%) and phytoplankton biomass ($<$1%) in comparison to the other cell types. The second structure observed in the southernmost part of the transect (20°S–16°S) was defined by the absence of a density gradient, and therefore by deep vertical mixing. In this case, the concentration of *Prochlorococcus* in the upper nitrate-depleted layer was reduced, whereas *Synechococcus* percentage contribution in the upper 180 m was significantly higher than in the TTS ($>$30% of total fluorescence and $\sim$4% of carbon biomass). According to our results, we discuss the expected role of each phytoplankton group in the regenerated and new production. Finally, we discuss the importance of cell size as a factor in the expected roles of the different phytoplankton groups in the carbon sink. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In subtropical and tropical open oceans, the picoplankton, as defined by Sieburth *et al.* (1978), dominates in the upper nitrate-depleted layers in terms of numerical abundance (Campbell and Vaulot, 1993) and pigment biomass (Le Bouteiller *et al.*, 1992). This result is due mainly to the discovery in recent years of two minute photoprokaryotes referred to as *Synechococcus* and *Prochlorococcus*. In the 1980s, observations using epifluorescent microscopy evidenced the abundance and the ubiquitous distribution of the first group of

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cyanobacteria assigned to the genus *Synechococcus* (Johnson and Sieburth, 1979; Waterbury *et al.*, 1979). Ten years later, the introduction of flow-cytometry in sea-going marine research led to the discovery of a new type of cyanobacteria, *Prochlorococcus* sp. (Chisholm *et al.*, 1988). In 1992, Chisholm *et al.* (1992) gave a formal description of this new cell type, referred to as *Prochlorococcus marinus*. More recently, it was demonstrated that the phylogenic positions of the two types of cyanobacterial picoplankter were close (Palenik and Haselkorn, 1992; Urbach *et al.*, 1992). The cell-cycle distribution of *Prochlorococcus* was described by Vaulot and Partensky (1992) using the DNA-specific fluorochrome DAPI.

In the field, knowledge of the distribution of *Prochlorococcus* progressed rapidly. Reported for the first time at the bottom of the euphotic zone in stratified pelagic waters (Chisholm *et al.*, 1988), they were detected throughout the entire euphotic zone in coastal (Vaulot *et al.*, 1990) and offshore waters (Veldhuis and Kraay, 1990; Olson *et al.*, 1990a). In these studies, nitrate was generally present at the surface, suggesting that *Prochlorococcus* was sensitive to nitrate. However, recent observations in the subtropical Pacific have established that it is indeed present and abundant in the nitrate-depleted mixed layer (Campbell and Vaulot, 1993). Actually, one of the main problems is to determine the contribution of this very small organism to carbon biomass, fluorescence and light scatter (Li *et al.*, 1992, 1993; Shimada *et al.*, 1993; Campbell *et al.*, 1994; Ishizaka *et al.*, 1994) and to primary production (Goericke and Welschmeyer, 1993; Li, 1994).

As part of the PROPPAC program, we examined in detail the phytoplankton biomass and composition in the western tropical Pacific along 165°E. The shipboard use of a flow cytometer gave us the opportunity to collect new data on phytoplankton community structure along 165°E, which improved our former description made with an epifluorescent microscope (Blanchot *et al.*, 1992). A set of contrasted hydrological situations encountered along the transect (20°S–7°N) allowed us to study the role of physico-chemical parameters on the phytoplankton structure and function. We considered more precisely the effect of nitrate concentrations and light intensity on the fluorescence and scatter signatures of *Prochlorococcus*. The results are also presented in terms of carbon, even if the factors available to convert cell numbers to carbon are far from perfect. Our results show that, paradoxically, *Prochlorococcus* reach their maximum abundance within nitrate-depleted layers. The ecological and biochemical implications of this new type of distribution in the oligotrophic ecosystems are discussed. We hypothesize on the expected role of prochlorophytes in regenerated production, the carbon sink and the microbial food web.

MATERIALS AND METHODS

Observations were made during the SURTROPAC 17 cruise, aboard R.V. *Le Noroit*, 6 August–2 September 1992, and extending from 20°S to 8°N along 165°E (Fig. 1). The cruise was organized by the ORSTOM-SURTROPAC group (Nouméa, New Caledonia), with the participation of the JGOFS-Pacific ORSTOM-FLUPAC group.

Hydrocasts to 1000 m were made every degree and every 0.5 degree in the equatorial zone with a Seabird CTDO2 (Conductivity–Temperature–Depth–Oxygen sensors, SBE 9). Water samples from 12 discrete depths were collected with 5-l Niskin bottles (General Oceanics) attached to a rosette sampler. The standard depths are shown in the typical depth profiles at the selected stations.
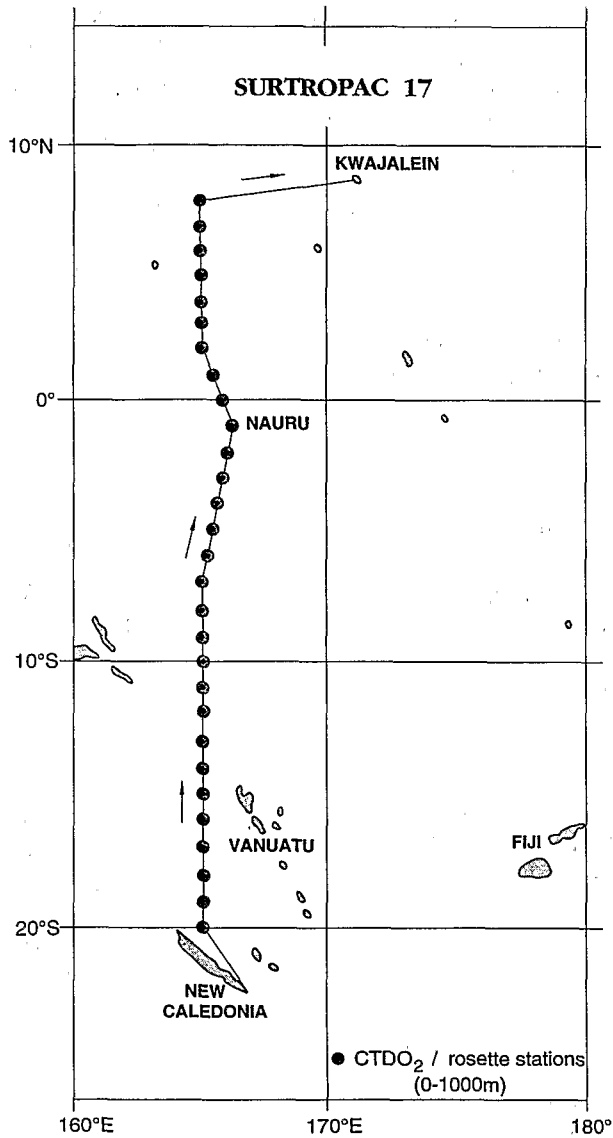
Fig. 1. Cruise track of the SURTROPAC cruise aboard R.V. *Le Noroit* and locations of sampling stations.

Nutrients and chlorophyll determination

$NO_3^- + NO_2^-$ analyses were performed immediately onboard with a Technicon Autoanalyzer II, using slight modifications of standard colorimetric methods (Strickland and Parsons, 1972). Additionally, a low level (10–1500 nM) module was used, according to the high sensitivity method defined by Oudot and Montel (1988). In this paper, for convenience, $NO_3^- + NO_2^-$ will be referred to as nitrate (NO_3).

Samples for chlorophyll a (Chl a) were stored frozen after filtration onto Whatman GF/F filters, and returned to the Nouméa ORSTOM Center for analysis. Chl a was determined fluorometrically on a methanol (95%) extract using a Turner model 112 fluorimeter calibrated with commercial pure Chl a (Sigma). Size fractionations were performed using Nuclepore polycarbonate filters (0.6 μm and 1 μm), and Chl a in the fractions was estimated in the filtrate after a second filtration onto a GF/F filter. Details of the fluorometric method and size fractionations are given in Le Bouteiller et al. (1992).

Euphotic depth

The 1% light level was computed as a function of chlorophyllous pigment concentrations, using the spectral model of Morel (1988). The bio-optical model prediction and actual 1% depths would agree within a few meters.

Flow-cytometry

Samples for flow cytometer (FCM) analysis were run at sea, and typically 0.1 ml was analyzed. FCM data acquisition was performed within 2 h after sampling. A FACScan flow cytometer (Becton-Dickinson), equipped with argon laser (power = 15 mW, at 488 nm), was installed in a dark and temperature controlled laboratory. Seawater collected at 1000 m and filtered through GF/F filters was used as sheath fluid. For each cell, five signals were recorded on 4-decade logarithmic scales: two light scatter (size side scatter, SSC, and forward light scatter, FLS), and three fluorescences. The photomultipliers were set up to quantify: the red fluorescence (RF) from Chl (wavelength > 650 nm), the orange fluorescence (OF) from phycoerythrin PE (564–606 nm), and the green fluorescence (GF) from phycourobilin PUB (515–545 nm), following Wood et al. (1985) and Olson et al. (1988). Olson et al. (1988) clearly demonstrated (Fig. 4) that different strains of Synechococcus had different ratios of orange/green fluorescences. In this paper the same technique is used to distinguish different field populations at 488 nm excitation. Each individual signal was stored in "list-mode" and analyzed with Lysis II software. All the signals of the samples were calibrated against the same internal standard beads (2-μm Polysciences Fluoresbrite beads, cat. 18604). Cellular fluorescence was always expressed relative to the fluorescence of the beads (in arbitrary units, a.u.), by dividing the mean cell fluorescence by the mean bead fluorescence. Before and after running each sample set, flow rate was determined using a known concentration of beads (counted by microscopy) according to Olson et al. (1988).

Cell groups (prochlorophytes, orange cyanobacteria, picoeukaryotes) were determined, using gates which were combinations of regions defined in different two-dimensional dot-plots, mainly RF vs SSC, RF vs OF, OF vs SSC and GF vs FLS. Prochlorophytes, referred to as Prochlorococcus marinus, were easily discriminated from the picoeukaryotes by their much smaller red fluorescence and scatter. Orange cyanobacteria, most likely Synechococcus spp., have intermediate RF and SSC signals between those of Prochlorococcus and picoeukaryotes and were distinguished clearly by their OF. Picoeukaryotes always have the largest RF and SSC. In order to estimate numerical abundance, we used the histograms provided by the FACScan analysis system (number vs red fluorescence intensity; Lysis II software). In the near-surface nitrate-depleted samples, Prochlorococcus populations were too dim to be completely resolved by the FACScan

system, as already mentioned in the literature (Olson *et al.*, 1990a; Dusenberry and Frankel, 1994). Since then, we have checked with bright populations: that the distribution of prochlorophytes is a normal distribution (99%) and that the observations are contained in the interval mean value of fluorescence ± 2.57 standard deviation, and that the mean ($n = 10$) of half of the whole population and the mean ($n = 10$) of the right part of the distribution are equal to the 1% level. We have calculated indirectly the approximate concentration of the dim prochlorophytes by extrapolation of the missing part, by multiplying the right part of the distribution by two.

RESULTS AND DISCUSSION

Environmental setting

Our study took place during a temporary weakening of the 1991–1994 El Niño (Climates Diagnostics Bulletin, 1995 June). The main hydrographic features encountered along 165°E are described in Du Penhoat *et al.* (1993). The zonal flow was eastward down to 250 m, between 4°S and 6°N, with a subsurface maximum associated with the equatorial undercurrent EUC. The well-known warm pool ($T > 29°C$) extended from 6°S to 8°N down to 50–80 m. A low salinity surface layer (< 34.5) was observed in the equatorial region down to the bottom of the isothermal layer. The isolines were depressed around 14°S in relation to a convergence observed at this latitude. Inversely, an uplifting of the isolines was evident in a restricted area around 10°S, which was probably due to the positive Ekman pumping (Delcroix and Hénin, 1989) and, additionally, to the divergence between the South Equatorial Counter Current (SECC) and the southern branch of the South Equatorial Current (SEC) (Merle *et al.*, 1969; Oudot and Wauthy, 1976).

Nitrate remained undetectable at the surface through the whole transect (Fig. 2). The nitrate-depleted layer was > 70-m thick except at 10°S, where the top of the nitracline (defined as the first level where $NO_3 > 0.1 \ \mu M$) was only 5 m deep, as a result of a shoaling of the deep nutrient reservoir. The temporary weakening of El Niño seems not to induce equatorial change of NO_3 concentrations, which remained typical for an El Niño year.

Cell abundance distribution of phytoplankton groups

The meridional distributions of the abundances for the three groups at 165°E are shown in Fig. 2. Such a presentation improves our earlier description, made with an epifluorescent microscope (Blanchot *et al.*, 1992), by adding the *Prochlorococcus* distribution. In order to show the vertical structure of cell abundance in greater detail, we also present four typical vertical profiles at selected latitudes (Fig. 3).

Prochlorococcus dominated everywhere along the transect, at all depths, in the upper 180 m ($10–440 \times 10^3$ cells ml^{-1}): they were 1–2 orders of magnitude more abundant than the other two cell types. Paradoxically, the highest *Prochlorococcus* concentrations ($> 200 \times 10^3$ cells ml^{-1}) were observed in the nitrate-depleted layers ($< 0.1 \ \mu M$) at 28 out of 29 stations, whatever the depth of the nitracline. Thus, in the convergence at 14°S, when nitrate was depleted down to 120–140 m, the population of *Prochlorococcus* was present at high concentration down to 140 m (Figs 2 and 3). Inversely, in the rising zone at 10°S where the nitracline was shallow, *Prochlorococcus* were less abundant except at the surface ($< 200 \times 10^3$ cells ml^{-1}, Figs 2 and 3). The distribution of *Synechococcus* and

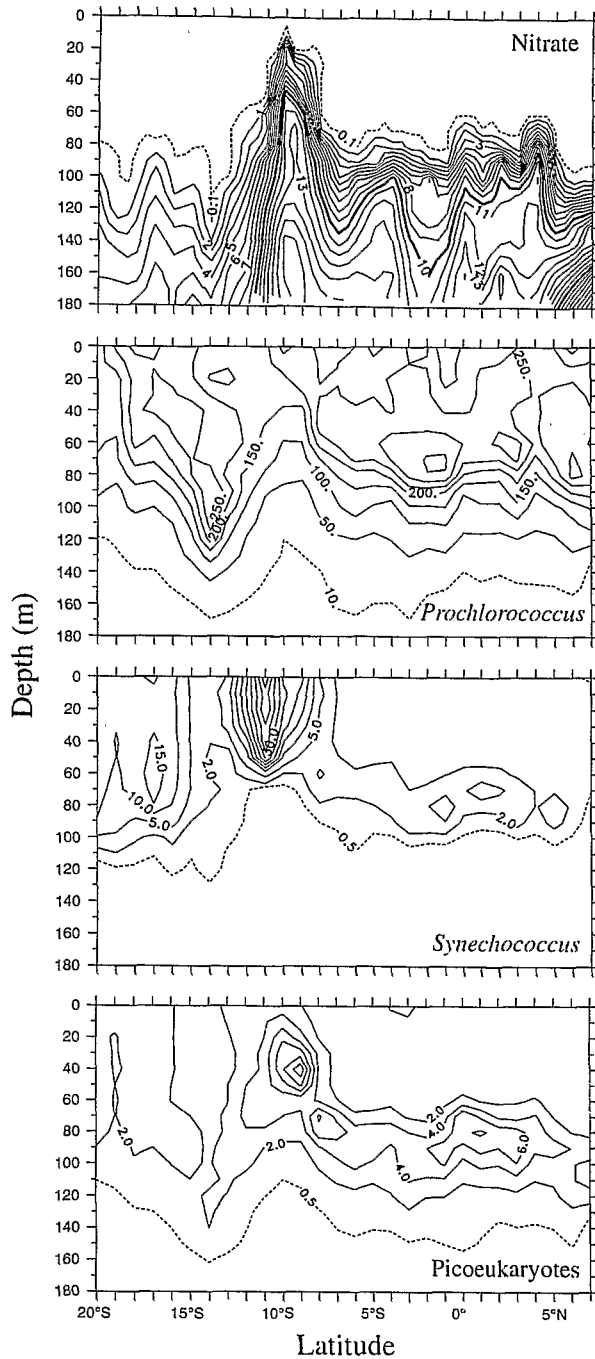Fig. 2.   Meridional sections of nitrate concentration (μM) and *Prochlorococcus*, *Synechococcus* and picoeukaryote cell abundances (10^3 cells ml^{-1}), from 20°S to 7°N at 165°E in August 1992.
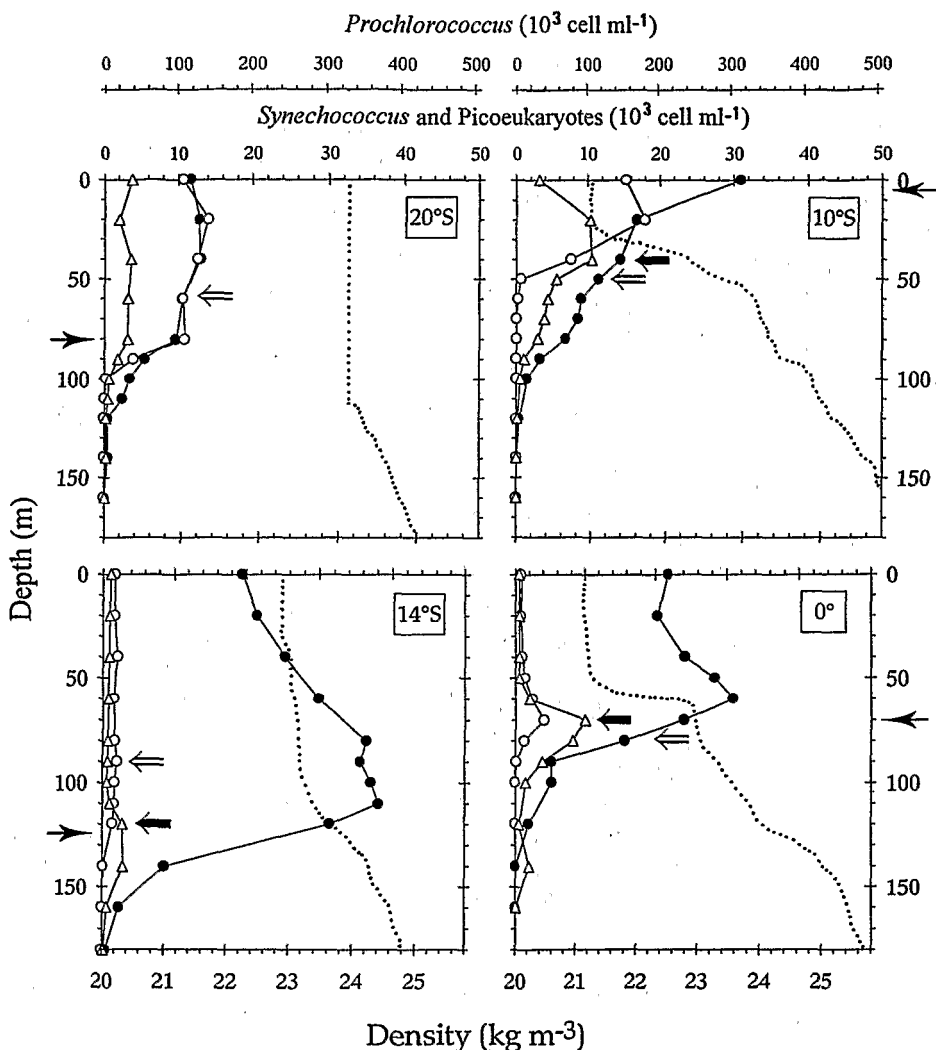
Fig. 3. Typical depth profiles of *Prochlorococcus* (●), *Synechococcus* (○) and picoeukaryote (△) abundance at four selected stations (20°S, 14°S, 10°S, 0°). Arrows indicate the depths of the chlorophyll maximum (DCM, →), 1% light (⇒) and the top of the nitracline (→). Dotted lines represent density.

picoeukaryotes contrasted clearly with that of *Prochlorococcus*. The two last groups were abundant when nitrate was detectable, i.e. around the 10°S rising zone and, to a lesser extent, in the vicinity of the deep nitracline between 8°S and 7°N (Figs 2 and 3). In the convergence zone, they remained low over the whole water column. *Synechococcus* were light-dependent and their distribution displayed a spectacular increase around 10°S (up to 64×10^3 cells ml^{-1}) when both favorable light and nitrate conditions existed. Inversely, they declined sharply around the 1% light level (Fig. 3). Picoeukaryotes as well as *Prochlorococcus* are less light dependent and could thrive in the poorly lit layers. As

already observed by epifluorescence microscopy (Blanchot *et al.*, 1992), the depth-distribution of maximum picoeukaryote abundance and chlorophyll biomass are closely related (Fig. 3). In the Pacific Ocean (this paper) and in the Atlantic Ocean (Olson *et al.*, 1990a; Partensky *et al.*, in press) the lowest prochlorophyte abundance is observed when *Synechococcus* are maximal. This general trend is perhaps due to unknown biological reasons, such as growth inhibitors produced by some organisms (*Synechococcus* or others) that are present when nitrate is not deficient.

The only exception encountered to this general pattern of phytoplankton abundance distribution was observed in the southernmost region of the transect at 20°–16°S (Figs 2 and 3). In this particular case, significant *Synechococcus* concentrations were observed in the nitrate-depleted layers and also under the 1% light level. Picoeukaryotes were also more abundant than in the other nutrient-depleted zones, whereas *Prochlorococcus* were less abundant. Such an observation may be related to the very weak pycnocline and deep mixing (below the photic zone) typical of this zone in winter (Radenac and Rodier, in press). Therefore, we will consider two regions: the first one from 15°S to 7°N corresponding to the Typical Tropical Structure (Herbland and Voituriez, 1979) with a two-layer ecosystem, and the second one extending from 20°S to 16°S with a one-layer ecosystem.

The integrated water column abundance of the three fluorescing groups over 160 m (Fig. 4) confirmed the huge dominance of *Prochlorococcus* from 20°S to 7°N along 165°E. The presence of *Prochlorococcus* throughout the entire water column induced large integrated cell numbers ($2.3 \pm 0.7 \times 10^9$ cells cm^{-2}). The integrated abundance of *Synechococcus* was always close to or greater than that of picoeukaryotes. The depth-median distribution of cells (Fig. 4) showed that picoeukaryotes always had the deepest median position, except south of 16°S. In this particular region, the median depths of the three fluorescing cell types were closely related and reflected homogenous distributions. Out of this region, no general trend in depth-median distribution appeared between the two prokaryotic groups: *Synechococcus* had the most superficial position in the convergence zone and in the rising area, although the median depth of *Prochlorococcus* was shallower north of 9°S.

Our results show that in the intertropical western Pacific *Prochlorococcus* are by far the most abundant group along the whole transect. More paradoxical is the fact that the highest abundances were observed in the nitrate-depleted layer, which suggests that the population of *Prochlorococcus* is weakly sensitive to nitrate depletion. Our observations are very similar to those reported by Campbell and Vaulot (1993) in the central north Pacific in terms of cell abundance and distribution structure. Inversely, the vertical distribution types found in the western Pacific differ from those described in other tropical oligotrophic zones, which present a minimum at the surface and a maximum tracking the nitracline. If we exclude the cases where low surface concentrations were probably due to the lack of sensitivity of the epifluorescent microscope (Ishizaka *et al.*, 1994) or of the Fcm system used (Shimada *et al.*, 1993), we can hypothesize that the strains present in the Pacific Ocean have different (less dependent) nitrate requirements than the ones described, with much more sensitive apparatus (EPICS V, FACScan), in the Atlantic Ocean (Olson *et al.*, 1990a) or in the Mediterranean, where Vaulot and Partensky (1992) reported that *Prochlorococcus* were not actively dividing below a nitrate level of 0.48 μM. Vaulot *et al.* (1995) showed that at 0°–140°W the growth rates of *Prochlorococcus* were very high during an El Niño event (and were not nitrate limited). Our observations on the numerical dominance of *Prochlorococus* in nitrate-depleted waters seem to confirm the presence of different stains in the Atlantic and Pacific Oceans.
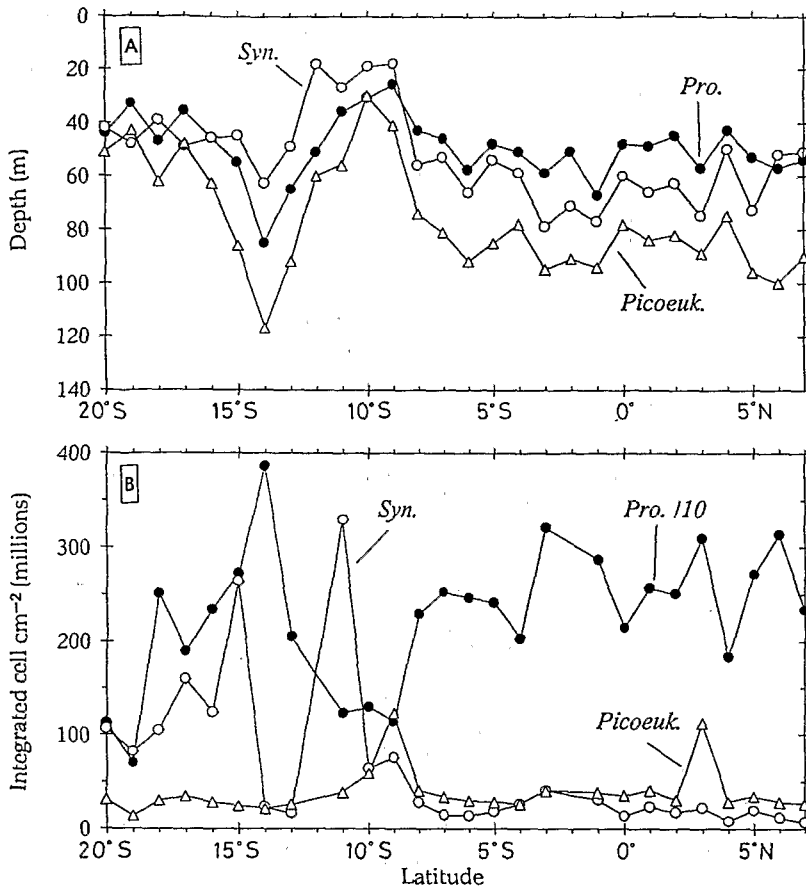
Fig. 4. (A) Median depth for *Prochlorococcus* (Pro., ●), *Synechococcus* (Syn., ○) and picoeukaryotes (Picoeuk, △), and (B) integrated abundances (0–160 m) expressed in 10^6 cells cm^{-2}, along 165°E. The integrated abundances of *Prochlorococcus* are divided by 10 (Pro./10).

Sub-populations

On several occasions during the transect, bimodal distributions of red fluorescence histograms indicated well marked sub-populations of *Prochlorococcus* (Fig. 5). These sub-populations were clearly identified in the southern zone at the bottom of the euphotic zone, because the two sub-populations were numerous enough to be clearly seen. In the northern part of the transect (north of 15°S), the two populations still existed, but their concentrations were very different and so the two peaks could not be discriminated: the larger peak overlapped the smaller peak. The two sub-populations appeared only with the cytogram (red fluorescence vs scatter), where the two patches of cells (a big one and a small one) could be distinguished. These results agree with those reported in the central Pacific (Campbell and Vaulot, 1993) and in the oligotrophic eastern Atlantic (Partensky *et al.*, in press). For picoeukaryotes, the presence of several populations separated by fluorescence versus scatter was noted at 17 out of 29 stations. The co-occurrence of different clusters of picoeukaryotes is not surprising owing to the existence of numerous species in the open
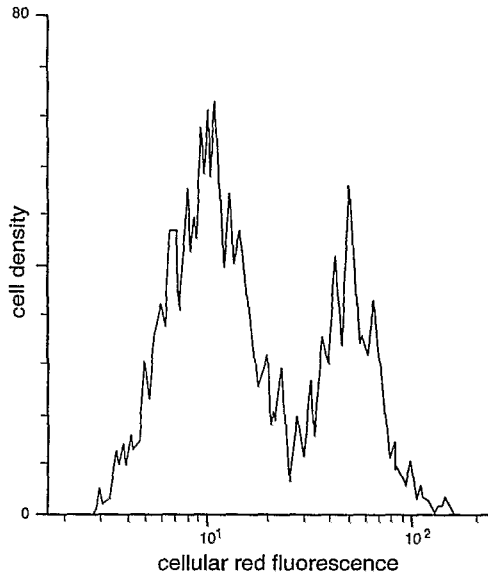
Fig. 5. A typical bimodal red fluorescence histogram for *Prochlorococcus* (20°S–165°E, 100 m). The cellular red-fluorescence is expressed on a log scale in arbitrary units.

waters. In contrast, no sub-population of *Synechococcus* was directly evidenced by chlorophyll fluorescence histograms. Another method based on the green/orange fluorescence ratio was used in order to discriminate between different strains of *Synechococcus*, according to their phycourobilin content (Ong *et al.*, 1984; Wood *et al.*, 1985; Olson *et al.*, 1988). Even this more "refined" emission approach did not allow us to distinguish between distinct sub-populations. Every population of *Synechococcus* encountered along the transect was found to have the same green/orange fluorescence ratio, equal to 0.036, from the surface down to 180 m. The only exception was observed at 8°S, in the vicinity of the rising zone, where the ratio increased from 0.034 ± 0.003 in the upper layers (0, 20, 40 m) to 0.060 ± 0.001 in the deep layers. The constancy does not suggest the presence of several well-marked populations as evidenced in the Atlantic by Olson *et al.* (1990b).

Fluorescence

The mean cellular red fluorescences of the three phytoplankton groups are presented in regard to irradiance profile and nitrate availability (Fig. 6). Several facts emerge from these vertical profiles. Firstly, the increase of fluorescence with depth (normalized to surface value) associated with photo-adaptation occurs at different light intensities in the presence and in the absence of nitrate. Thus, photo-adaptation begins at 2–5% Io (40–60 m) when the nitracline is relatively shallow (0° and 10°S) and at <1% Io (80–100 m) when the oligotrophic conditions ($NO_3 < 0.1 \, \mu M$) extend down to 120 m. Secondly, at a given irradiance, the presence of nitrate promotes red fluorescence for each group, but this increase is higher for photoprokaryotes than for photoeukaryotes. The overall average of the ratio between cellular fluorescence in the presence versus in the absence of nitrate is
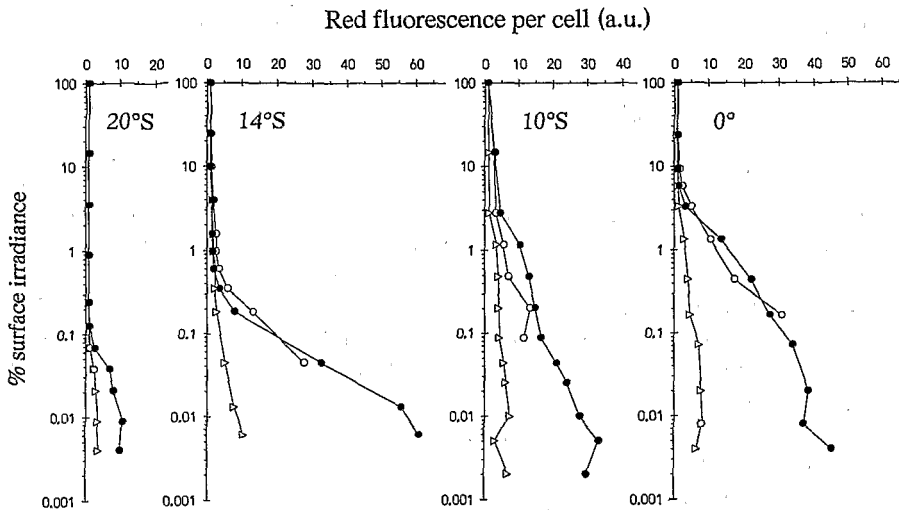
Red fluorescence per cell (a.u.)

Fig. 6. Increase in mean cellular red-fluorescence for *Prochlorococcus* (●), *Synechococcus* (○), and picoeukaryotes (△) as a function of % surface irradiance. Fluorescence values are normalized to surface values as defined by 100% irradiance. a.u., arbitrary units.

4.69 ± 1.43, 2.45 ± 0.65 and 1.94 ± 0.47 for *Prochlorococcus*, *Synechococcus* and picoeukaryotes, respectively. An exception to this general pattern was observed in the southern region (20°S). Since a deep mixing takes place between photic and aphotic layers, the cells are not able to photo-adapt, and the increase of red fluorescence remains the lowest of the transect.

We observed at all the selected stations that the increase of fluorescence with depth of *Prochlorococcus* (normalized to surface fluorescence) was much more important than the increase of fluorescence of the other groups. There are likely two main reasons. The first one is the very good ability of *Prochlorococcus* to photoacclimate to low light intensities, as reported by Olson *et al.* (1990c), with high increase of divinyl chlorophyll *a* and *b* per cell (Goericke and Repeta, 1993; Moore *et al.*, 1995; Partensky *et al.*, in press). The second one is the the the presence of different strains in the field with dim strains in upper well-lit layers and bright strains in the poorly illuminated layers as suggested by Fig. 5 and proved by DNA analysis near Hawaii (Campbell and Vaulot, 1993). In our study, we measured the *in vivo* fluorescence. The red fluorescence is known to emanate from the Chl *a* of photosystem II (Owens, 1991; Furuya and Li, 1992), but at our excitation wavelength (488 nm), a great amount of energy is absorbed by accessory pigments, mainly Chl *b*, and transferred to Chl *a* (Falkowski and Kiefer, 1985; Furuya and Li, 1992). As the time of transfer from Chl *b* to Chl *a* (max = 2 ps, Eads *et al.*, 1989) is shorter than the time of analysis (0.5 ms), the measured fluorescence depends in fact on the sum of the light absorptions of the two chlorophylls at 488 nm, as reported by Veldhuis and Kraay (1990), Moore *et al.* (1995) and Partensky *et al.* (1993, in press). Therefore a part of the increase of the red fluorescence is due to a change in the Chl *a*/Chl *b* ratio, with deep cells having relatively more Chl *b* than surface cells. A part of the fluorescence increase with depth is linked to photo-acclimatization (Olson *et al.*, 1990c).

The percentage contributions of the three groups to total *in vivo* fluorescence of the three

groups are presented in Fig. 7 (combined contribution of red fluorescence from all three groups weighted by cell number). *Prochlorococcus* made a major contribution to total fluorescence (up to 70%) in the nitrate-depleted layer, but their relative abundance was lower in the nitracline and below, where picoeukaryotes generally prevailed independently of the light level. *Synechococcus* was a minor contributor (from 10 to 20%), and below the 1% light level the presence of *Synechococcus* was negligible. As mentioned for numerical abundance, the observations made from 15°S to 7°N were no longer valid in the southern area (20°S). At this particular station, picoeukaryotes were more fluorescent than *Prochlorococcus* in the nitrate-depleted upper layer and the mean proportion of *Synechococcus* to total fluorescence was ∼40%. It is inferred that the contribution to fluorescence was quite similar for the three groups in the southern region (*Pro.* 31%, *Syn.* 31%, picoeuk. 39%), while the percentage contribution of *Synechococcus* was low in the equatorial region compared to the two other groups (*Pro.* 47%, *Syn.* 4%, picoeuk. 49%).

The picoeukaryotes represented a majority at the deep Chl *a* maximum (DCM), thus implying they contribute largely to the DCM formation (Fig. 8). *Prochlorococcus* dominated at the DCM only when it was located above the nitracline, as in the convergence zone (14°S), which agrees with the results on cell abundance. Considering all the stations presenting a well-defined DCM (15°S–7°N), the contributions of the three groups at the DCM was $50 \pm 12\%$ for picoeukaryotes, $38 \pm 13\%$ for *Prochlorococcus* and only $12 \pm 8\%$ for *Synechococcus*. The fluorescence per cent contribution of *Prochlorococcus* at the DCM is similar to that reported for chlorophyll pigment in the Atlantic Ocean by McManus and Dawson (1994). In spite of variable proportions of cell types in the total
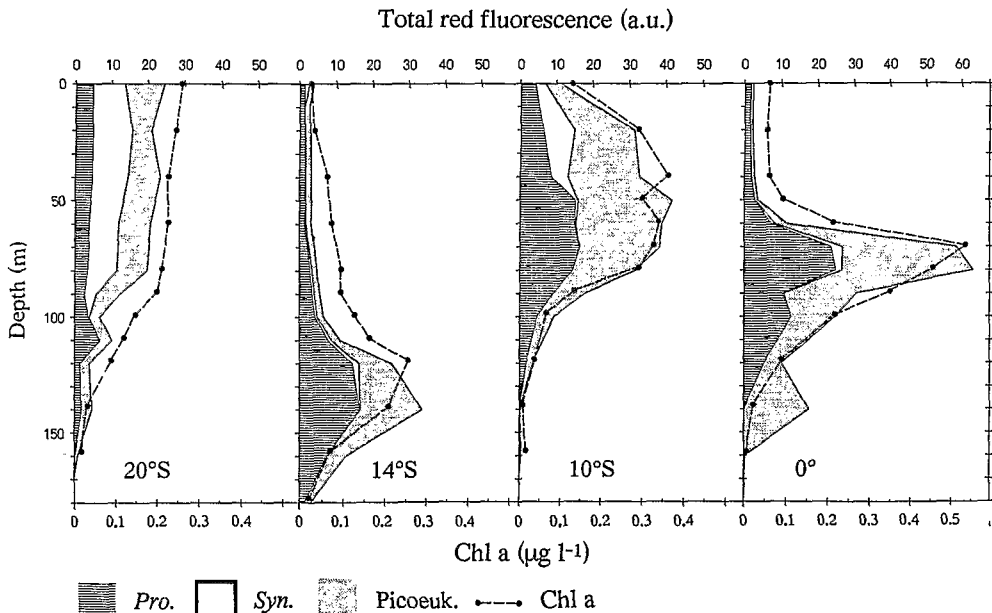

Fig. 7. Total red-fluorescence in arbitrary units (a.u.) for *Prochlorococcus* (Pro.), *Synechococcus* (*Syn.*) and picoeukaryotes (Picoeuk.), overlaid by Chl *a* concentration. The red fluorescences of each population are calculated from mean cellular fluorescences weighted by cell concentrations. Same stations as for Fig. 3.
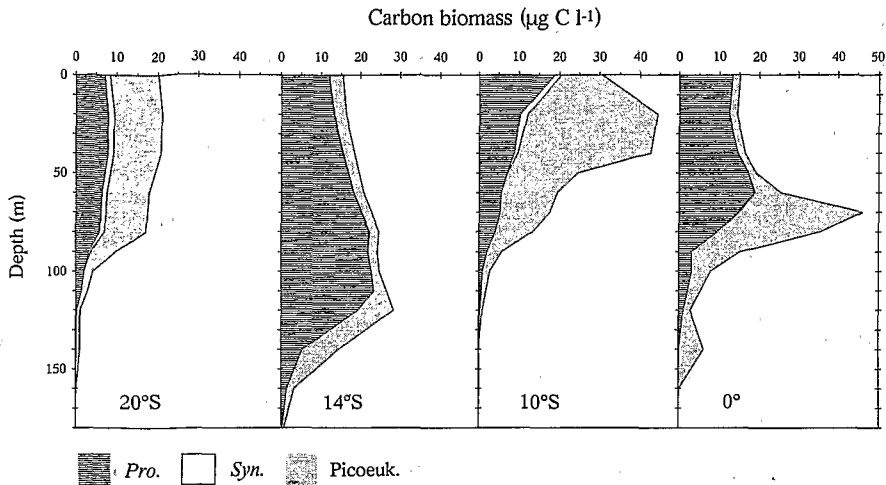
Carbon biomass (µg C l-1)

Fig. 8. Carbon biomass ($\mu g\, C\, l^{-1}$) for *Prochlorococcus* (*Pro.*), *Synechococcus* (*Syn.*) and picoeukaryotes (Picoeuk.). The carbon biomasses are calculated from cell concentrations and conversion factors (see text). Same stations as for Fig. 3.

fluorescence as described above, the total *in vivo* fluorescence depth profiles match that of Chl *a*, with a good co-incidence of the two maxima. Pooling the data from all stations, the relationship between Chl *a* and total *in vivo* fluorescence is well described by an exponential function: Chl $a = 0.113\, Fluo^{0.699}$ ($r = 0.75$; $n = 325$).

Carbon estimates

In contrast to *in vivo* fluorescence measurements, there is no direct method of estimation of carbon biomass derived from FCM measurements. One way to approach the problem is to consider the forward size scatter (FLS), expecting it to be directly related to cell size (Olson *et al.*, 1989), and therefore to cellular carbon (Strathmann, 1967; Li *et al.*, 1993). But, in fact for particles ($> 1\ \mu m$) of the same refractive index, the FLS increases linearly with the square of the diameter, and the light scattering intensity increases strongly with the refractive index (Steen, 1991). For spherical particles with diameter ($> 0.3\ \mu m$, $< 3\ \mu m$) and λ around $0.5\ \mu m$ the general trend for the variation of the FLS/SSC ratio with size is an increase proportional to the fourth power of the diameter $(d)^4$ (Morel, 1991). If the prokaryotic cells we are dealing with in such a theoretical way conform to these assumptions, our measurements would suggest that the *Synechococcus* are slightly bigger than the *Prochlorococcus* with a mean diameter ratio (1.2 ± 0.1). Considering a spherical shape for the prokaryotic cells, the volume of a *Synechococcus* is 1.7 times higher than the volume of a *Prochlorococcus*. For *Synechococcus* we computed the carbon cell content by simply multiplying the *Prochlorococcus* value by 1.7. Such an estimation assumes that the carbon biomass/volume ratio is similar for the two prokaryotes.

The pigment content (pc) of *Prochlorococcus* was estimated indirectly by a size fractionation technique (Le Bouteiller *et al.*, 1992). At three stations in the equatorial region, screened water samples ($< GF/F$, $< 0.6\ \mu m$ and $< 1\ \mu m$) were subdivided into two sub-samples, one for Chl *a* measurement by fluorometry, the other for FCM analysis.

Results are presented in Table 1. On average, *Prochlorococcus* abundance in the <0.6-μm fraction accounted for 33% of the total *Prochlorococcus* population and contributed 99% of the total cell number $<0.6\,\mu$m (not shown). Owing to this huge dominance of *Prochlorococcus* in the <0.6-μm fraction, we considered that pc of *Prochlorococcus* could be reasonably estimated in the fraction $<0.6\,\mu$m by dividing the Chl *a* concentration by the *Prochlorococcus* cell number. The mean cellular pigment content through the water column was 1.1 fg Chl *a* cell^{-1} (extreme values: 0.2–6.4 fg Chl *a* cell^{-1}). Finally, carbon estimates were attempted as follows. For *Prochlorococcus* the conversion was made by multiplying the cellular pigment content 1.1 fg Chl *a* cell^{-1} obtained by size by the usual C/Chl *a* ratio of 55 g C/g Chl *a* (Ketchum and Corwin, 1965). This gives a carbon cell content of 61 fg C cell^{-1}, a value close to the 53–59 fg C per *Prochlorococcus* given by Li *et al.* (1992) and Campbell *et al.* (1994). For *Synechococcus*, we computed the carbon cell content by simply multiplying the *Prochlorococcus* value by 1.7, the volume ratio between the two prokaryotes reported above. Such an estimation assumes that the carbon biomass/biovolume ratio is similar for the two prokaryotes. The resulting value, 104 fg C cell^{-1}, is in good agreement with the carbon per cell (83 fg C cell^{-1}) calculated by Ishizaka *et al.* (1994), but it is much lower than the value of 250 fg C cell^{-1} given by Kana and Glibert (1987) for cells in culture. For picoeukaryotes a carbon cell content of 3110 fg C cell^{-1} was determined assuming a 3-μm diameter (Blanchot's microscopy observations, unpublished data), and a carbon volume content of 220 fg C μm^{-3} (Mullin *et al.*, 1966).

On the basis of the abundance profiles (Fig. 3) and using the estimated mean carbon cell contents, total carbon biomass profiles were tentatively computed and are presented in Fig. 8, along with the integrated values of biomass (Table 2). Our integrated values of biomass (extreme values 1733–3179 mg C m^{-2}) are higher than the mean values recorded by Campbell *et al.* (1994) near Hawaii: 1533 mg C m^{-2}. Note that these comparisons must be taken with caution insofar as mean factors were used to convert cell abundances to carbon profiles. Such a simple approach cannot resolve vertical variations perfectly.

If we consider the mean integrated carbon values of the one-layer ecosystems (20°S–16°S) and the two-layer ecosystems (5°S–5°N) the *Prochlorococcus*, *Synechococcus* and

Table 1.   *Mean percentages of Chl* a *(fluorometry) and cell abundance (FCM) in the* <0.6-μm, <1-μm *and* $<$GF/F *fractions for the* 120-m *water column, at three stations in the equatorial band at* 165°E (2°N, 0°, 2°S)

Size class	Latitude	Chl *a*	Cell abundance (% of total)		
			Prochlorococcus	*Synechococcus*	*Picoeukaryotes*
$<1\,\mu$m	2°N	59±32	90±8	85±13	34±14
	0°	52±23	99±1	89±15	40±11
	2°S	61±10	100	100	34±13
$<0.6\,\mu$m	2°N	18±7	36±8	1±3	16±11
	0°	16±7	34±9	1±3	13±20
	2°S	17±7	30±8	0	4±2
$<$GF/F	2°N	0	1	0	0
	0°	n.d.	n.d.	n.d.	n.d.
	2°S	n.d.	n.d.	n.d.	n.d.

n.d.: no data.

Table 2.   *Integrated values over 160-m column for C biomass (and percentage contribution to total particulate carbon) for each component of the phytoplankton community. Data from Fig. 8*

	Integrated biomass (mg C m^{-2})			
	20°S	14°S	10°S	0°
Prochlorococcus	688 (39%)	2489 (78%)	790 (29%)	1313 (54%)
Synechococcus	111 (6%)	24 (1%)	67 (3%)	14 (1%)
Picoeukaryotes	966 (55%)	667 (21%)	1850 (68%)	1088 (45%)
Total phytoplankton	1765 (100%)	3179 (100%)	2706 (100%)	2415 (100%)

picoeukaryotes represented 50%, 6% and 44%, respectively, of the total carbon in the southern region, and 59%, 1% and 40% in the equatorial area. Therefore, although the *Prochlorococcus* population was the most numerous (96% of the cell number) along 165°E, its importance was approximately equivalent to that of the picoeukaryotes in terms of carbon biomass. Inversely, *Synechococcus* represented only a few per cent in the TTS as reported near Hawaii by Campbell and Vaulot (1993), but was more noticeable in the southern region. It is important to notice that rigorous interpretations are very difficult owing to the uncertainty in carbon biomass estimation.

Data were obtained in the tropical Pacific during a temporary weakening of the 1991–1994 El Niño, in the absence of equatorial upwelling and in presence of an equatorial estward flow. Based on flow cytometry, our 1992 observations recognized the *Prochlorococcus* compartment and thus improved our understanding of the phytoplankton community structure over previous observations made during the 1987 El Niño (Blanchot *et al.*, 1992). The main result is that *Prochlorococcus* dominates numerically the phytoplankton community structure throughout the entire water column, independently of nitrate availability. Nevertheless, two regions were recognized by differences in the community structure. The first one extending from 15°S to 7°N, except at 10°S, corresponds to the Typical Tropical Structure (TTS) defined by Herbland and Voituriez (1979). A good example is given by the equatorial band, where a strong pycnocline divides the 0–180-m layer into two systems with different phytoplankton communities: the upper photic layer, which is nitrate-depleted, proves to be the kingdom of *Prochlorococcus* and the underlying zone, with significant nitrate concentrations, is dominated by picoeukaryotes. Otherwise, the contribution of *Prochlorococcus* to 180 m-integrated fluorescence and carbon biomass is essentially equivalent to that of picoeukaryotes. *Synechococcus* is a minor contributor that appears to be light- and nitrate-dependent. The similarity between these data and those reported for the subtropical Pacific Ocean north of Hawaii (station ALOHA) substantiates the suggestion by Campbell and Vaulot (1993) that the structure we observed in the so-called TTS is "probably typical of the whole subtropical Pacific wherever no deep mixing takes place". The second region extending south of 16°S functions differently: there is no well-defined pycnocline in the upper 180 m, and mixing takes place between the photic and aphotic layers. The concentration and the role of *Prochlorococcus* in the upper nitrate-depleted layer are reduced and the contribution of *Synechococcus* is more noticeable, which tends to prove their ability to take nitrate in at deep levels and then assimilate it in the photic zone as they are being "mixed".

The numerical abundance of prochlorophytes in the nitrate-free layers raises the

question: what kind of nitrogen do the prochlorophytes use for their primary production? Theoretical considerations indicate that even in very oligotrophic conditions (around 5 nM of nitrate), a cell of radius $0.35\,\mu$m could grow at a rate of 1 day^{-1} (Chisholm, 1992). However, considering our results, we assume that in the western tropical Pacific Ocean the prochlorophytes are better able to function on regenerated compounds than the other cell types and would contribute mainly to the regenerated production. The dominance of prochlorophytes in broad areas of the subtropical western Pacific raises a second question: what is the role of prochlorophytes in the carbon sink? The role of prochlorophytes in the sedimentation process is probably negligible for free cells because they are too small to sink, but it is possible that some cells can be trapped in quick-sinking aggregates as observed for cyanobacteria (Lochte and Turley, 1988). To our knowledge, nothing is known of their exportation through grazing and fecal pellet packaging. The packaging depends directly on the consumers and leads us to the third question: which organisms injest prochlorophytes? The answer will probably support the well established microbial food-web, and will give a pre-eminent importance to the grazers of very small phytoplankton (i.e. the heterotrophic and mixotrophic microflagellates, and the ciliates). Recently in the equatorial Pacific (2°N, 165°E), we observed no prochlorophytes but a great deal of *Synechococcus* (3×10^6 cells per pellet) in a few freshly produced appendicularian fecal pellets. Little is known about the prochlorophyte consumers, but grazing is usually considered as one of the most important factors controlling picophytoplankton abundance (Sanders and Porter, 1988; Nakamura *et al.*, 1993; Riegman *et al.*, 1993). Selective inhibitor techniques for measuring the grazing rates of *Prochlorococcus* show that their grazing mortality rate can exceed their growth rate (Liu and Campbell, 1994). Another possibility for control of the abundance of prokaryotic phytoplankton is viral infection, which can be responsible for a sizeable fraction of prokaryote mortality per day, up to 30% for cyanobacteria and up to 60% for marine heterotrophic bacteria (Proctor and Fuhrman, 1990). Most of the recent virus counts have been typically 10^6–10^7 cells ml^{-1} in ocean surface waters (Fuhrman and Suttle, 1993). However, the mortality is unknown in oligotrophic areas, which are less suitable than coastal waters for cyanophage infection and growth (Boehme *et al.*, 1993; Suttle and Chan, 1994).

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