JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 104, NO. C2, PAGES 3297–3310, FEBRUARY 15, 1999

Diel variability of photosynthetic picoplankton in the equatorial Pacific

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Abstract. The diel variability in cell abundance, light scatter, and pigment fluorescence of three autotrophic picoplankton groups (*Prochlorococcus*, *Synechococcus*, picoeukaryotes) measured by flow cytometry was investigated in surface waters of the equatorial Pacific Ocean (5°S, 150°W) during 5 days with about 1 hour temporal resolution. The diel variability of vertical profiles was examined at the same station on days 2 and 4. *Prochlorococcus* division rate was also estimated from cell cycle measurements. A more limited data set was obtained at a station located in very oligotrophic waters (16°S, 150°W). All three picoplankton populations exhibited very marked diel variability. Cell division was highly synchronized but not phased identically for all three populations: *Synechococcus* divided first, followed 2 hours later by *Prochlorococcus* and 7 hours later by picoeukaryotes. Cells grew in size only once the sun had risen, but growth did continue in the dark for a short period. Growth processes occurred in parallel at the top and the bottom of the mixed layer, inducing uniform profiles for cell abundance and scatter. For chlorophyll fluorescence, in contrast, prokaryotes displayed opposite patterns during the light period between surface (decrease due to very strong quenching) and depth (increase). This created steep vertical gradients during the day that vanished at night because of convective mixing. In the top 25 m, strong light intensities (including UV radiation) had very pronounced detrimental effects on prokaryotes, especially on *Prochlorococcus*, inducing fluorescence quenching, slowed down growth, and retardation of DNA synthesis.

1. Introduction

The regular succession of light and darkness is one of the most obvious environmental signals on Earth. However, its effect on pelagic oceanic ecosystems has been surprisingly little studied, in contrast, for example, to coastal or benthic ecosystems. Many oceanic phenomena display a 24 hour periodicity. This is true, in particular, of physical processes such as surface layer mixing: solar heating induces a temporary thermocline near the surface during the day that disappears at night [*Gardner et al.*, 1995]. Photochemical processes are also obviously influenced by the diel cycle [e.g., *Doney et al.*, 1995]. However, biology-related parameters are those that display the clearest periodical patterns. For example, *Siegel et al.* [1989] showed that beam attenuation exhibits very clear diel oscillations with minima at dawn and maxima at dusk. Chlorophyll fluorescence also presents diel periodicity: at surface, minima occur at midday, and maxima occur at night, while patterns are inverted at depth [*Stramska and Dickey*, 1992; *Dandonneau and Neveux*, 1997]. In oligotrophic regions such as the oceanic gyres the diel variability of these parameters is often larger than the temporal variability observed over scales of days or months or than mesoscale spatial variability.

It has been known for almost a century [*Gough*, 1905] that the division of phytoplankton in the sea occurs only at specific periods of the day. In laboratory cultures placed under photoperiodic conditions [*Chisholm*, 1981] most cellular processes such as photosynthesis, cell growth, or cell division are phased. The underlying mechanism for such phasing is still unclear, and two alternative hypotheses have been proposed [*Edmunds*, 1988]. Each cellular process could be directly and independently entrained by the light stimulus. Alternatively, cells could possess an endogenous clock that is set by light and that would, in turn, drive many cellular processes according to a specific time program, resulting in phase shifts between processes that need to occur in sequence (e.g., the cell must increase in size before dividing). Although the "clock" hypothesis is supported by evidence drawn from a small set of organisms that grow well in the laboratory and that display highly synchronized behavior such as *Euglena* or *Chlamydomonas* [*Edmunds and Adams*, 1981]; other evidences point to the direct entrainment hypothesis [*Spudich and Sager*, 1980; *Vaulot and Chisholm*, 1987]. Very recently, the balance has tipped toward the clock hypothesis as an important corpus of elegant work [*Johnson et al.*, 1996] has uncovered the genetic basis of a potential clock in the photosynthetic prokaryote *Synechococcus*. In strain PCC 7942 a large number of genes are expressed with a clear diel pattern, which persists even when cells are transferred to constant light conditions, a clear sign of the presence of an endogenous clock. The clock genes themselves are, however, still uncharacterized.

Observations of the synchronization of natural phytoplankton populations are scarce. Pioneer studies in the seventies demonstrated that the cell division of large phytoplankton cells such as diatoms or dinoflagellates is

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Paper number 98JC01333.
0148-0227/99/98JC-01333$09.00

phased to the light-dark cycle [*Swift and Durbin,* 1972; *Smayda,* 1975]. This phenomenon has been utilized to obtain rough estimates of cell division rates in marine waters (mitotic index method) [*McDuff and Chisholm,* 1982]. Recent improvements using cell cycle measurements provided much more accurate estimates [*Carpenter and Chang,* 1988] and revealed the very high synchrony of some oceanic populations [*Vaulot et al.,* 1995].

In the present paper we report data on the diel behavior of photosynthetic picoplankton (i.e., the smaller size fraction of phytoplankton, roughly below 2 μm) obtained mainly at one station located at 5°S 150°W in the equatorial Pacific. Surface populations were sampled during 5 days at relatively high frequencies (roughly once per hour) and analyzed onboard the ship by flow cytometry. We also analyzed the diel variability of the vertical structure of picoplankton populations during two different days with a lower frequency (once every 2 hours). This data set reveals the complexity of picoplankton diel patterns. Each parameter displays its own characteristic behavior that differs depending on the population and depth considered. Comparisons with a smaller data set obtained at 16°S in more oligotrophic waters indicate that the diel patterns are also affected by nutrient availability. This complexity explains, in part, why diel patterns are difficult to observe if only global parameters, such as chlorophyll, that encompass several populations are measured and if insufficient sampling resolution is used.

2. Methods

2.1. Sample Collection.

In November 1994 the OLIPAC cruise, a component of the Joint Global Ocean Flux Study (JGOFS)-France program called "Etudes de Processus dans l'Océan Pacifique Equatorial (EPOPE)", investigated a transect along 150°W, between 16°S (near Tahiti) and 1° N, on board the French research vessel NO Atalante. General oceanographic conditions during the cruise are described in *Stoens et al.* [this issue]. Two stations were occupied at 5°S and 16°S, respectively, each during 5 days for a detailed investigation of diel variability. Water was pumped continuously from ~5 m below the surface, and samples were taken roughly every hour. Moreover, on the second and fourth days at these fixed stations, detailed vertical profiles were sampled every 2 hours from 0600 to 2200.

2.2. Flow Cytometry.

For the analysis of cell abundance, light scatter, and pigment fluorescences, samples were kept in the dark at 4°C for a maximum of 6 hours. During this period, minimal change in flow cytometry measured parameters take place [*Jacquet et al.,* 1998b]. Samples were then analyzed onboard the ship with a flow cytomer (FACSort, Becton Dickinson, San Jose, California) as previously described [*Partensky et al.,* 1996; *Marie et al.,* 1998]. The sample delivery rate was determined carefully each day. For each sample the volume analyzed was computed from the delivery rate and the duration of the analysis. The 0.95 μm yellow green (YG) beads (Polysciences, Warrington, Pennsylvania) were used as internal standards. For the analysis of the cell cycle, samples were preserved with 0.1% glutaraldehyde, immediately frozen in liquid nitrogen, and stored at -80°C. Back in the laboratory, samples were thawed, stained for DNA with SYBR-I Green (Molecular Probes, Eugene, Oregon) and analyzed as previously described [*Marie et al.,* 1997].

2.3. Data Analysis.

Flow cytometry data were analyzed with the Cytowin freeware (available through http://www.sb-roscoff.fr/Phyto/cyto.html) [*Vaulot,* 1989]. Three cell populations were discriminated on the basis of scatter and fluorescence signals: *Prochlorococcus* had low scatter, low red fluorescence, and no orange fluorescence; *Synechococcus* had intermediate scatter and orange and red fluorescence; picoeukaryotes had large scatter and red fluorescence but little or no orange fluorescence. In surface samples the red fluorescence of *Prochlorococcus* decreased sharply during the middle of the day (see Section 3) such that in some cases a small fraction (20%-30%) of the population fell below the level of detection. Under such circumstances, abundance and fluorescence values were estimated by fitting a Gaussian curve to the visible part of the red fluorescence *Prochlorococcus* histogram and extrapolating it to the undetected cells (this function is implemented in the current version of Cytowin). For picoeukaryotes all cells that had measurable red fluorescence were included, and no attempt was made to discriminate subpopulations. All cell parameters are reported relative to the 0.95 μm beads. DNA distribution analysis was performed by MultiCYCLE (Phoenix Flow Systems, San Diego, California), which provided estimates of the fraction of the population in each of the cell cycle phases (G_1, beginning of cell cycle; S, DNA synthesis; and G_2, end of cell cycle). The cell division rate was computed from time series of S and G_2 fractions using the method of *Carpenter and Chang* [1988] as described by *Vaulot et al.* [1995]. Fourier filter smoothing was performed with the Origin 4.1 software package (Microcal Software Inc., Northampton Massachusetts).

3. Results

Picoplankton populations, analyzed by flow cytometry, revealed the three types of cells usually encountered in oceanic waters: *Prochlorococcus*, *Synechococcus*, and picoeukaryotes (Figure 1). At the first station occupied at 5°S and located in equatorial Pacific waters, populations displayed typical mesotrophic features [e.g., *Partensky et al.,* 1996], i.e., *Prochlorococcus* concentrations around 150 x 10^3 cell mL^{-1} and relatively high *Synechococcus* and eukaryote concentrations at surface, of the order of 25 and 5 x 10^3 cell mL^{-1}, respectively (Figure 1). Very clear diel patterns were observed for all three populations in surface waters (Figures 2-4). The second station at 16°S was situated in very oligotrophic waters. *Synechococcus* and picoeukaryote concentrations were an order of magnitude lower than at 5°S, while *Prochlorococcus* remained at similar levels (Figure 1). All populations extended much deeper down the water column. At surface, *Prochlorococcus* cells could not be detected (Figure 1a) because their fluorescence was too weak, and the low abundance of *Synechococcus* and picoeukaryotes induced noisy diel patterns (not shown). Therefore, in the following we concentrate on the patterns observed at 5°S and only discuss 16°S data for comparison purposes.

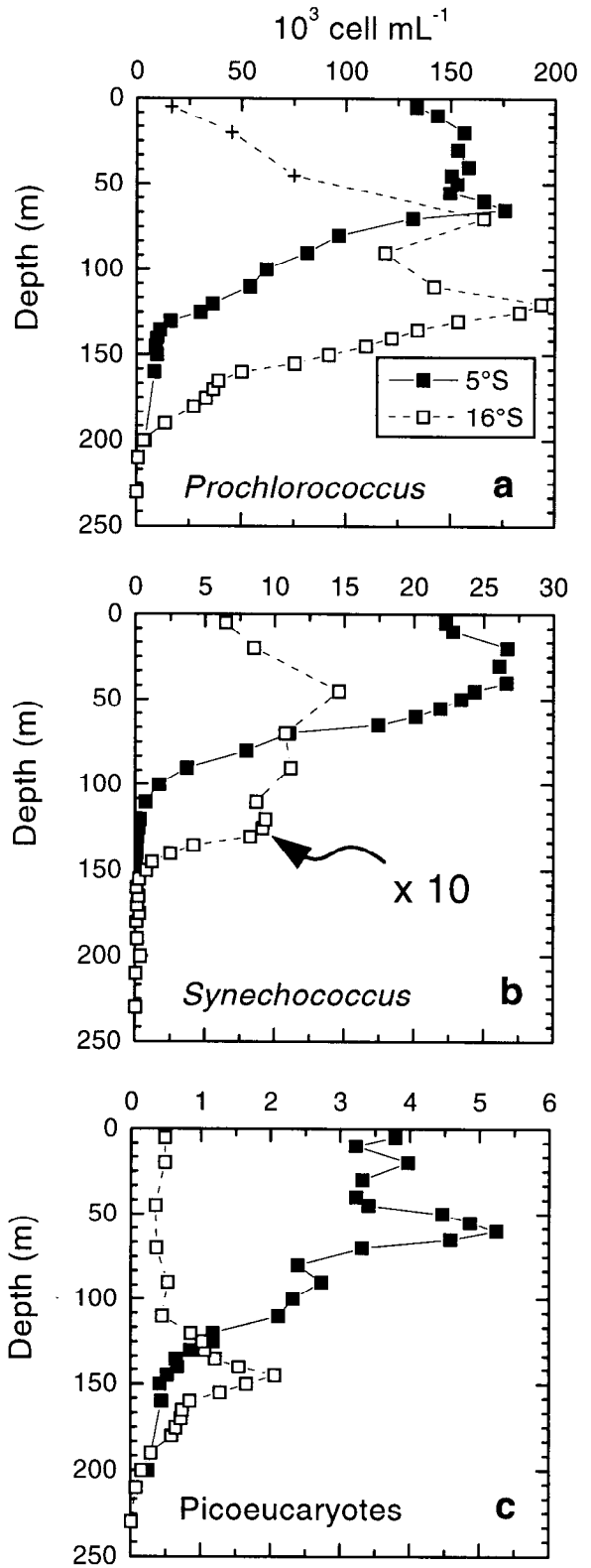

Figure 1. Depth distributions of picoplankton concentrations measured by flow cytometry at 5°S and 16°S during the OLIPAC cruise (150°W, November 1994). (a) *Prochlorococcus*. At 16°S, *Prochlorococcus* chlorophyll fluorescence was too weak at the top three depths, and cell concentrations were severely underestimated (crosses). (b) *Synechococcus*. Note that the concentration scale at 16°S has been multiplied by 10 (i.e., the actual range is from 0 to 3 x 10^3 cell mL^{-1}). (c) Picoeukaryotes.

3.1. Picoplankton Abundance in Surface at 5°S

At 5°S all three populations displayed well-marked diel oscillations in abundance (Figure 2). Patterns were noisier for populations with lower abundance (i.e., for which fewer cells were analyzed), such as the picoeukaryotes. In general, population increased at night and decreased during the day. For example, *Prochlorococcus* abundance began to increase around 1600 and reached a maximum at 0200 in the night. This increase was clearly linked to cell division that occured during that period [*Vaulot et al.*, 1995; *Liu et al.*, 1997]. Once division had stopped at 0200, cell concentrations remained invariant until dawn, indicating that loss processes were not active until the sun came up. *Synechococcus* pattern was nearly identical, except that both the beginning of the increase and the maximum occurred ~2 hours earlier than for *Prochlorococcus*. This suggests that *Synechococcus* divided earlier than *Prochlorococcus*. Picoeukaryote patterns were also dephased with respect to *Prochlorococcus*, but this time with a delay (not an advance) of ~5 hours, as cell concentration began to rise only ~2200 and peaked at dawn. We interpret this as a retardation of picoeukaryote division compared to *Prochlorococcus*. The decrease of picoeukaryote cell concentration appeared to be biphasic, slow until 1 hour prior to dawn and faster after that.

Over the 5 days sampled the relative ranges of variation in abundance were very similar for *Prochlorococcus* and picoeukaryotes and smaller for *Synechococcus* (Table 1). The long-term evolution of *Prochlorococcus* and *Synechococcus* was comparable, increasing until dawn of November 21 and then decreasing sharply (Figure 2). This evolution mirrored that of the permanent mixed layer depth which increased until November 20 down to 80 m and then returned to 40 m at the end of the sampling period (Figure 2d). Picoeukaryote abundance remained stable until November 21 and then dropped abruptly, similarly to prokaryotes (Figure 2c).

3.2. Light Scatter and Pigment Fluorescence at 5°S

For all three picoplankton populations, side scatter, a proxy for cell size, increased mainly during the day and decreased at night (Figure 3). However, subtle differences were registered between the various populations. For *Prochlorococcus*, cell enlargement was obviously triggered by dawn. Scatter increased only at a moderate rate until 1600 and then more rapidly until it reached its maximum between 2000 and 2200, to drop sharply thereafter. Scatter decrease at night is obviously due to cell division, as cell volume is divided by 2 after this event. For *Synechococcus*, maximum scatter was reached earlier ~1800, which is easily explained by the advance in *Synechococcus* cell division relative to *Prochlorococcus* (see above). Also, on some days (e.g., November 21) scatter did not increase immediately after dawn but lagged by a couple of hours. For picoeukaryotes, scatter maximum was, in contrast, delayed until the middle of the night because of the retardation of cell division (see above). The range of scatter variation was much larger for *Prochlorococcus* than for the other two populations (Table 1). In contrast to cell abundance, there was no clear overall trend over the 5 days sampled (Figure 3).

Chlorophyll fluorescence displayed in surface a pattern almost opposite to that of light scatter (Figure 4). For all populations it began to decrease as soon as the sun rose and

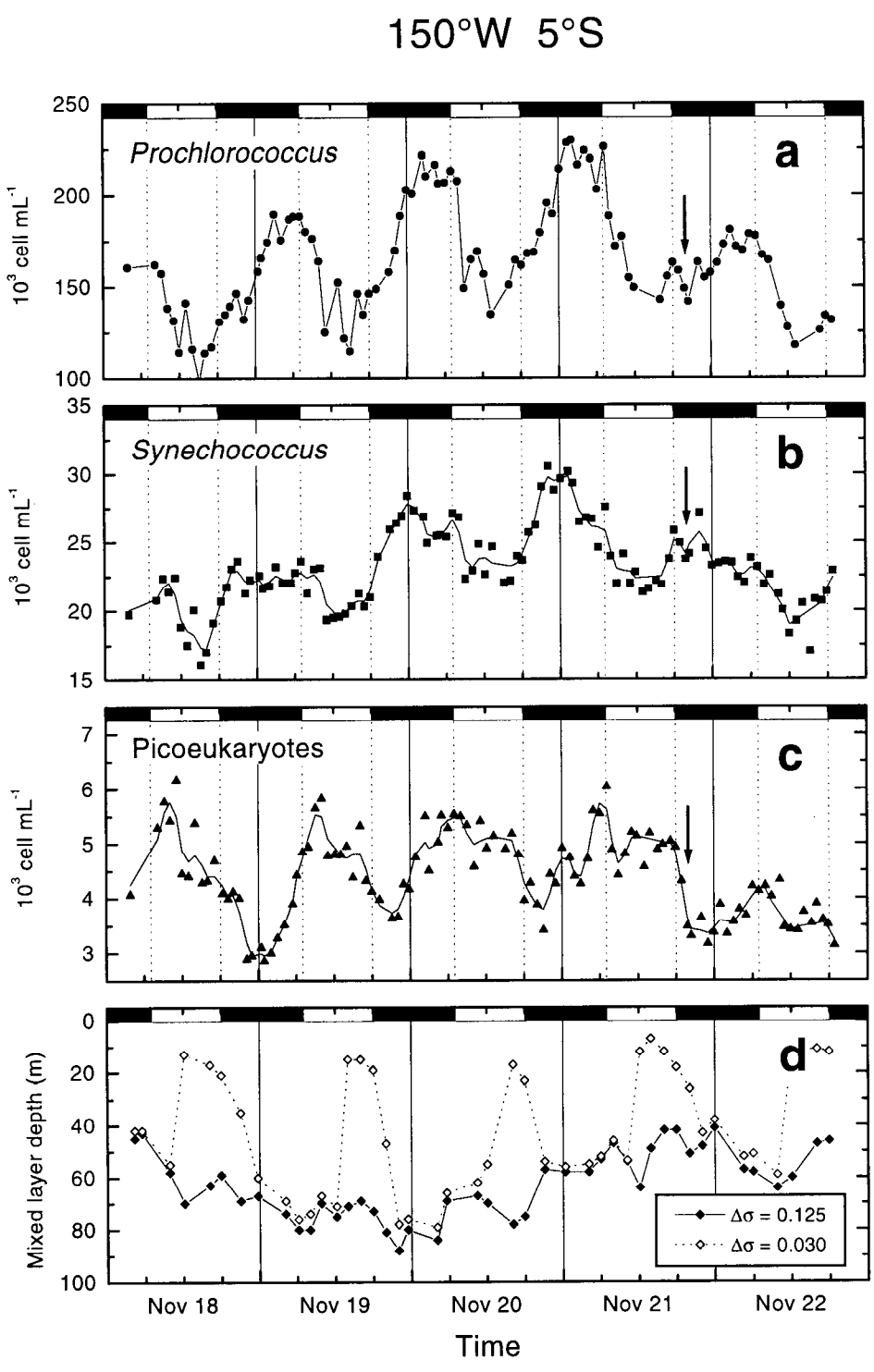

Figure 2. Diel variation of picoplankton concentration measured by flow cytometry and mixed layer depth at 5°S in surface during the OLIPAC cruise (150°W, November 1994). (a) *Prochlorococcus*. (b) *Synechococcus*. The solid line corresponds to low-pass Fourier filter smoothing to eliminate frequencies higher than 0.5 h^{-1}. (c) Picoeukaryotes. Solid line as in Figure 2b. (d) Mixed layer depth estimated by a density difference with the surface of either 0.125 (solid line) or 0.03 (dashed line). The former corresponds to the permanent mixed layer, and the latter corresponds to the diurnal surface mixed layer. Vertical arrows correspond to a suspected advective event.

increased back only past midday. *Prochlorococcus* displayed a very ample and reproducible pattern with a nearly three-fold variation. The morning decrease inversely mirrored the solar irradiance increase, while afternoon recovery processed at a slower rate. A secondary minimum was observed around midnight, lying between two nearly equal maxima. The first midday minimum is clearly linked to fluorescence quenching due to the high irradiances experienced by cells in the surface layer, while the second minimum is associated with division. *Synechococcus* fluorescence decrease during the day was not

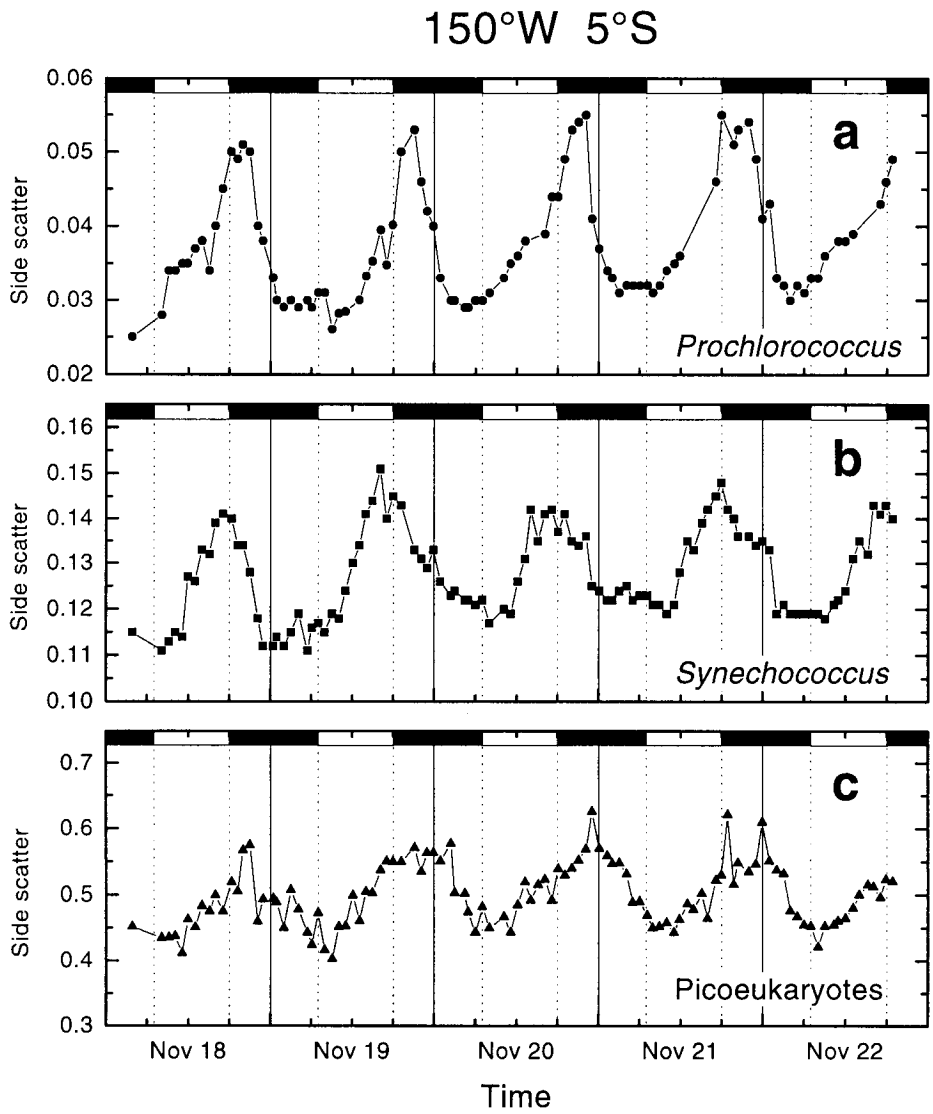

Figure 3. Diel variation of picoplankton side scatter measured by flow cytometry and normalized to 0.95 μm beads at 5°S in surface during the OLIPAC cruise (150°W, November 1994): (a) *Prochlorococcus*, (b) *Synechococcus*, and (c) picoeukaryotes.

as rapid as that for *Prochlorococcus*. Moreover, it did not display a clear secondary night minimum. Instead, fluorescence recovery was biphasic, rapid in the first part of the night and then slower. These differences result probably, in part, from the advance of *Synechococcus* division compared to *Prochlorococcus* division. Finally, for picoeukaryotes, fluorescence oscillations were much less pronounced (Figure 4d and Table 1), although qualitatively similar to those observed for the procaryotes (decrease during the day and recovery at night).

Phycoerythrin orange fluorescence, only present in *Synechococcus*, exhibited a very interesting pattern. Usually phycoerythrin and chlorophyll fluorescence are very well correlated over large spatial and temporal scales [*Neveux et al.*, this issue]. However, these parameters are somewhat uncoupled at the daily scale. In particular, the daily variation of phycoerythrin was narrower than that of chlorophyll (Table 1). Moreover, although both parameters displayed parallel trends most of the day, phycoerythrin always presented a minimum around 2100 clearly linked to cell division (Figure 4c, arrowheads).

3.3. Vertical Variability of Cell Parameters at 5°S

The patterns we described above were recorded at surface. Are these patterns identical throughout the water column? To address this question, we sampled the water column on days 2 and 4 every 2 hours from 0600 to 2200. Since both days yielded comparable patterns we only focus on the data of November 19, 1994. Also, picoeukaryotes, because of their low abundance, did not produce clear patterns and are not discussed further.

Evolution of cell abundance at surface (5 m) for bottle samples was roughly similar to that of the pumped water (Figures 5a-5b). The observed differences could stem from slight variations in the sampling depth of the pumped samples due to ship motion. *Prochlorococcus* exhibited a pronounced decrease in the middle of the day. This depression is not seen below 25 m or for *Synechococcus* at any depth. At all depths, population increased markedly in late afternoon (*Synechococcus*) or early night (*Prochlorococcus*). For *Synechococcus* the increase is clearly delayed by ~2 hours at surface compared to deeper samples. Cell concentrations

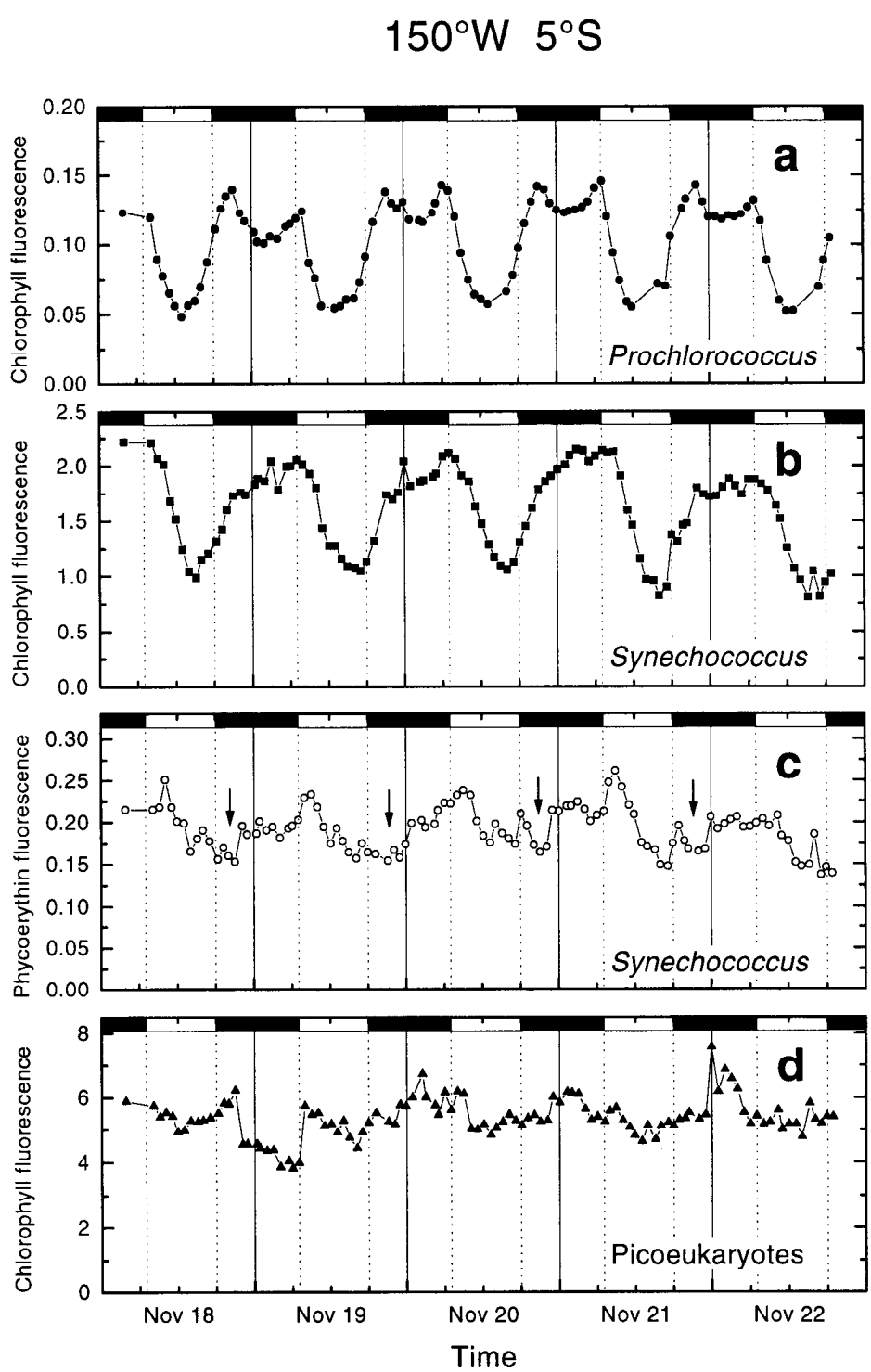

Figure 4. Diel variation of picoplankton chlorophyll and phycoerythrin fluorescences measured by flow cytometry and normalized to 0.95 μm beads at 5°S in surface during the OLIPAC cruise (150°W, November 1994): (a) *Prochlorococcus* chlorophyll, (b) *Synechococcus* chlorophyll, (c) *Synechococcus* phycoerythrin (see text for meaning of arrows), and (d) picoeukaryote chlorophyll.

display little vertical gradients in the mixed layer (Figures 5c-5d), although they are always slightly depressed at surface, especially for *Prochlorococcus* at midday. Clear oscillations in the depth of the thermocline are evident from the variability of the depth at which concentrations begin to drop sharply.

Daily side scatter evolution was very similar throughout the water column (Figure 6). For *Prochlorococcus*, for example it increased constantly until dusk. The rate of increase was however slightly larger at depth than at surface (Figure 6a). Moreover, the maximum side scatter was reached later at surface than at depth, a delay that paralleled that of division (see above). The similarity in trends at different depths resulted in nearly uniform vertical distributions over the mixed layer (Figure 6c), with a slight maximum near 30 m during the afternoon. Nocturnal mixing

Table 1. Relative Range of Variation of Parameters at 5°S During 5 Days as Estimated From the Ratio (Maximum-Minimum)/Mean

Population	Abundance	Side Scatter	PE Fl.	Chl. Fl.
Prochlorococcus	79	95	n.a.	96
Synechococcus	61	31	65	84
Picoeukaryotes	75	55	n.a.	69

In percent, $n = 98$; PE, Phycoerythrin; Chl., Chlorophyll; Fl., fluorescence; and n.a., not applicable.

(see below) completely erased the vertical structure within the mixed layer at 0600. For *Synechococcus*, maximum scatter was reached earlier than for *Prochlorococcus* (Figure 6b) as established for surface samples, and the delay between the occurrence of the maximum in surface and at depth was only 2 hours, versus 4 hours for *Prochlorococcus*.

At 5 m, chlorophyll fluorescence displayed exactly the same pattern as observed for surface samples, namely a midday depression (Figures 7a-7b). However, the pattern at 25 m was different from that at 5 m, with no midday depression but, instead, a late afternoon increase. At 55 m, fluorescence displayed a very sharp increase until 1400 followed by a decrease past 1800. These opposite patterns at surface and at depth created a very strong vertical gradient within the mixed layer during the afternoon (Figure 7c). By 2200 this gradient was already less pronounced and had completely vanished at the end of the night before dawn. *Synechococcus* fluorescence displayed a similar vertical behavior, although the midday increase at depth was significantly less marked than for *Prochlorococcus*.

3.4. *Prochlorococcus* Cell Cycle and Division Rate at 5°S

Cell DNA staining with the novel dye SYBR Green I [*Marie et al.*, 1997] allowed us to estimate *Prochlorococcus* cell division rate from cell cycle data. The other picoplankton populations were not abundant enough to perform a similar analysis. As shown previously at the equator [*Vaulot et al.*, 1995], *Prochlorococcus* cell cycle was highly synchronized. Below 50 m the fraction of cells in the S phase was maximum at 1600, and the fraction in G_2 was maximum at 1800 (data not shown). Between 50 and 25 m, the S phase was retarded by ~2 hours and, above, by 4 hours. The estimated division rate reached maximum values of 0.8-0.9 d^{-1} between 35 and 45 m and showed a clear depression in surface by up to 30% (Figure 8). On November 19, a secondary maximum was recorded at 90 m. On that date the depth of thermocline oscillated markedly between 60 and 85 m (see above). This

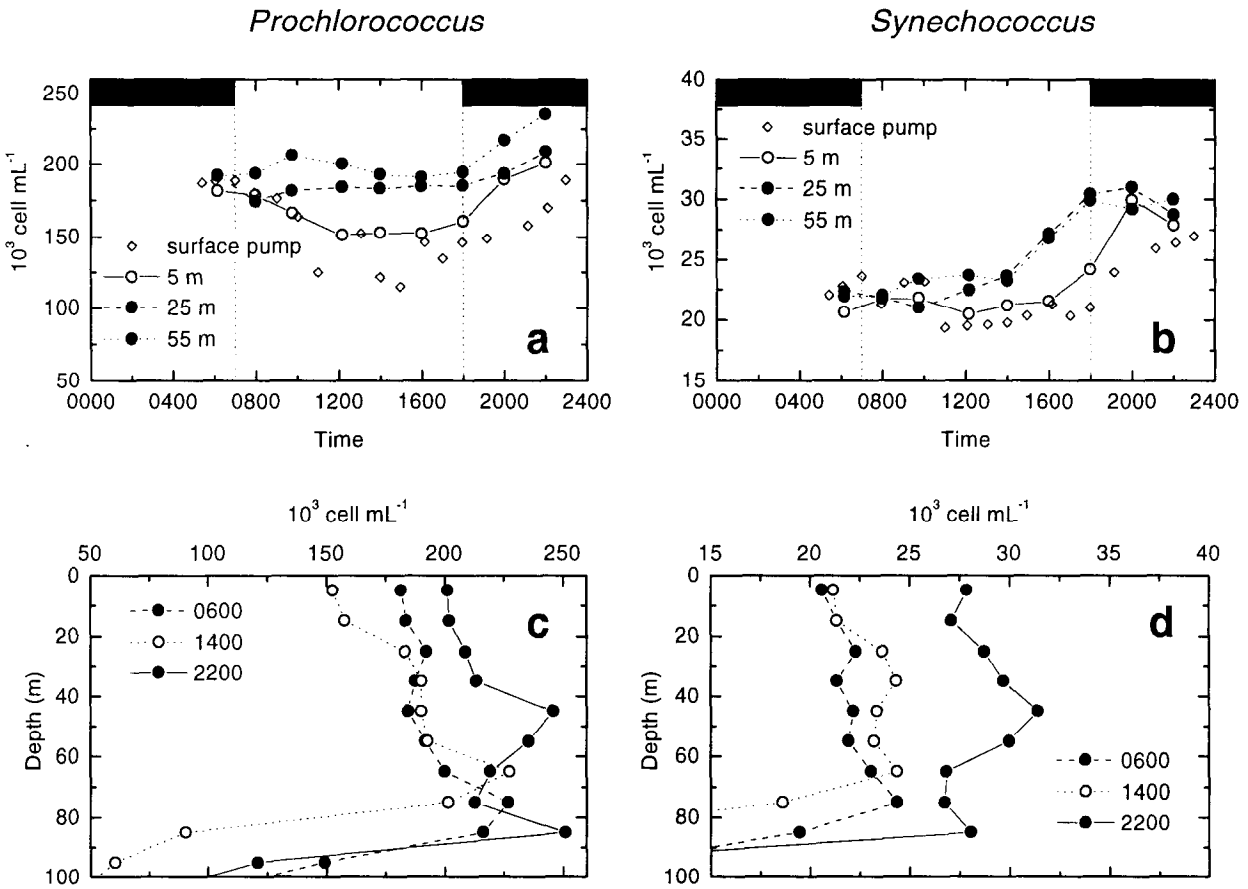

Figure 5. Vertical variation of *Prochlorococcus* and *Synechococcus* cell concentration at 5°S, 150°W on November 19, 1994 (OLIPAC cruise): (a) Diel variability at selected depths of *Prochlorococcus* concentration, (b) idem for *Synechococcus*, (c) depth variation of *Prochlorococcus* concentration at specific times, and (d) idem for *Synechococcus*.

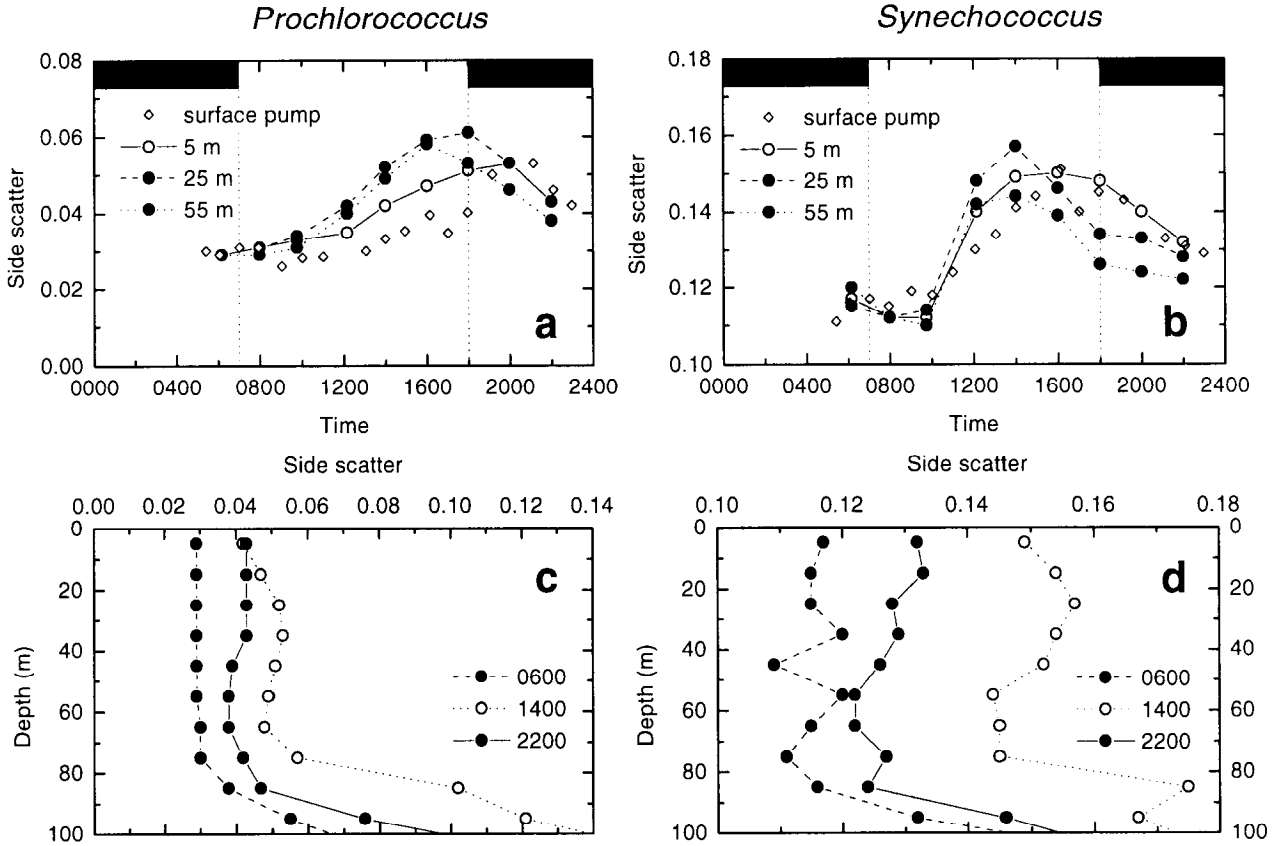

Figure 6. Vertical variation of *Prochlorococcus* and *Synechococcus* side scatter measured by flow cytometry and normalized to 0.95 μm beads at 5°S, 150°W on November 19, 1994 (OLIPAC cruise). Legend as in Figure 5.

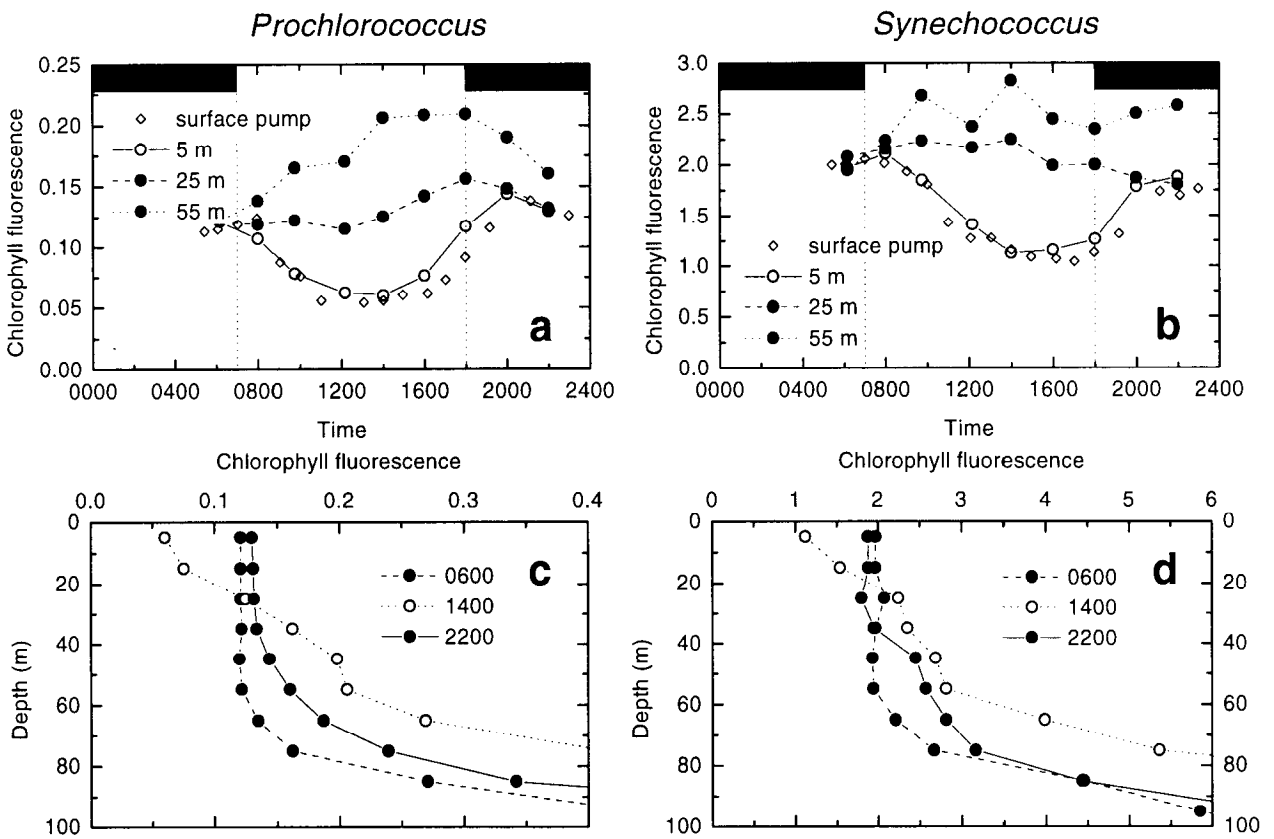

Figure 7. Vertical variation of *Prochlorococcus* and *Synechococcus* red chlorophyll fluorescence measured by flow cytometry and normalized to 0.95 μm beads at 5°S, 150°W on November 19, 1994 (OLIPAC cruise). Legend as in Figure 5.

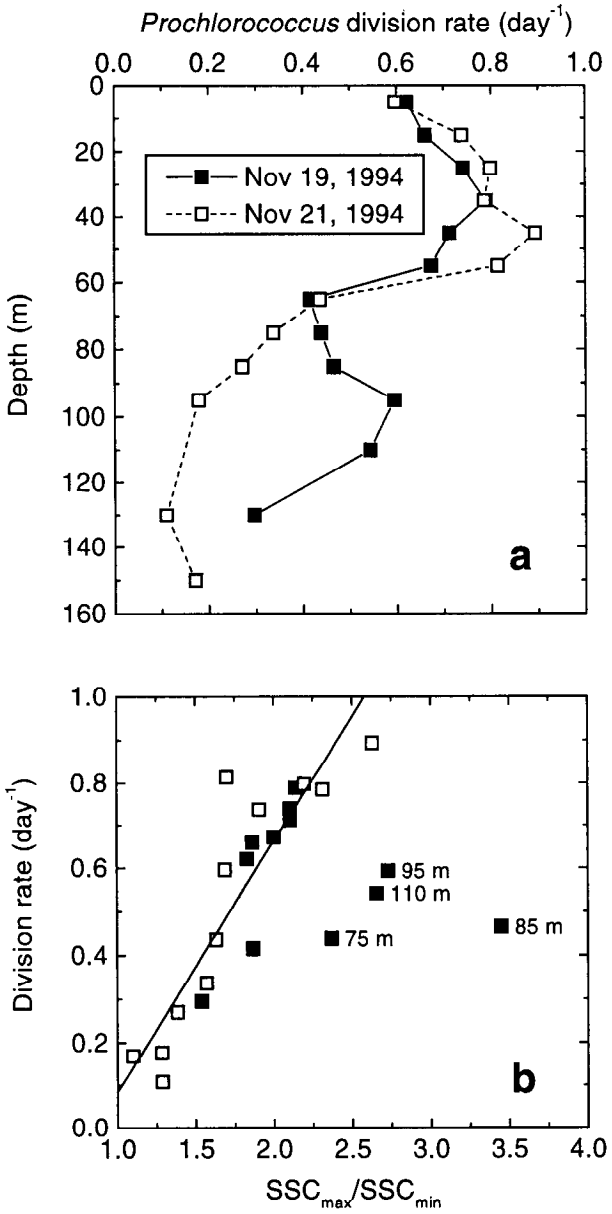

Figure 8. (a) Vertical distribution of *Prochlorococcus* cell division rate estimated from cell cycle data at 5°S on November 19 and 21, 1994. (b) Relationship between *Prochlorococcus* cell division rate estimated from cell cycle data and the ratio of the maximum to minimum side scatter (SSC_{max}/SSC_{min}) at 5°S. The straight line corresponds to the linear regression of all data except the four labeled depths of November 19 (r^2=0.78 and n=20).

probably entrained surface populations down below the mixed layer as evidenced by the presence of two *Prochlorococcus* populations with different chlorophyll content (data not shown).

Binder et al. [1996] observed a linear relationship between the diel increase of forward scatter and the minimum division rate (as estimated from a single cell cycle distribution at dusk). We found a similar relation between side scatter increase and the more precise estimate of the division rate obtained from the time series of cell cycle distributions (Figure 8b). However, on November 19, data from four depths below the thermocline exhibited significantly larger side scatter increases, relative to their division rate. Using forward scatter instead of side scatter did not improve the observed relationship (data not shown).

3.5. Patterns at 16°S

At 16°S, only cyanobacteria patterns could be recorded in surface because *Prochlorococcus* cells were not fluorescent enough and picoeukaryotes were too few (Figure 1). For *Synechococcus*, fluorescence intensities were much weaker than at 5°S, but the general diel evolution was surprisingly similar despite the environmental differences (Figure 9a). In particular, it showed clear signs of midday depression. The only marked difference with 5°S was the occurrence of a clear dip in chlorophyll fluorescence around midnight, reminiscent of what was observed for *Prochlorococcus* at 5°S and probably linked to *Synechococcus* division. Later at night, chlorophyll fluorescence increase was sharper than at 5°S.

At 16°S a second population of unicellular cyanobacteria of 2-3 μm was present. Although they were almost 100 times brighter and their fluorescence excitation and emission characteristics were different from that of *Synechococcus* [*Neveux et al.*, this issue], the diel patterns of the two prokaryotes were remarkably similar (Figure 9b). The only reproducible difference between the patterns of the two populations was observed between 1800 and 2400, as the larger cyanobacteria did not recover as rapidly as *Synechococcus*.

4. Discussion

In oligotrophic oceans the abundance of picoplankton populations is surprisingly invariant across vast regions as well as over seasonal and annual temporal scales [e.g., *Campbell et al.*, 1997]. This clearly indicates that growth and loss processes are balanced over these large scales. It also makes population dynamics very difficult to study at these scales. In contrast, recent studies, including the data presented in this paper, suggest much larger variations at the daily scale that are created by temporary imbalances between growth and loss processes. In fact, the key to interpreting large-scale patterns probably lies in the detailed understanding of the factors that regulate daily growth and loss since very small differences between these two terms drive the long-term evolution of cell populations. In the following discussion we will first examine how the individual cell parameters respond to the diel cycle and then turn to the overall population dynamics.

4.1. Size and Fluorescence Diel Patterns

Light scattered by individual cell as measured by flow cytometry is a function of both cell size and refractive index [*Morel*, 1991]. However, for a given cell type, either forward or side scatters (corresponding to collecting angles around 0° and 90°, respectively) appear to be good proxies for biomass. For example, a strong correlation has been demonstrated between side scatter and cell size for a range of eukaryotic strains [*Simon et al.*, 1994] and between forward scatter and cell carbon for *Nannochloris* and *Synechococcus* [*DuRand and Olson*, 1996].

The two major processes implicated in side scatter patterns are cell growth and cell division. We focus here on the first process, with cell division being addressed later. For the three populations examined here, cellular growth was clearly triggered in the morning by light, proceeded throughout the

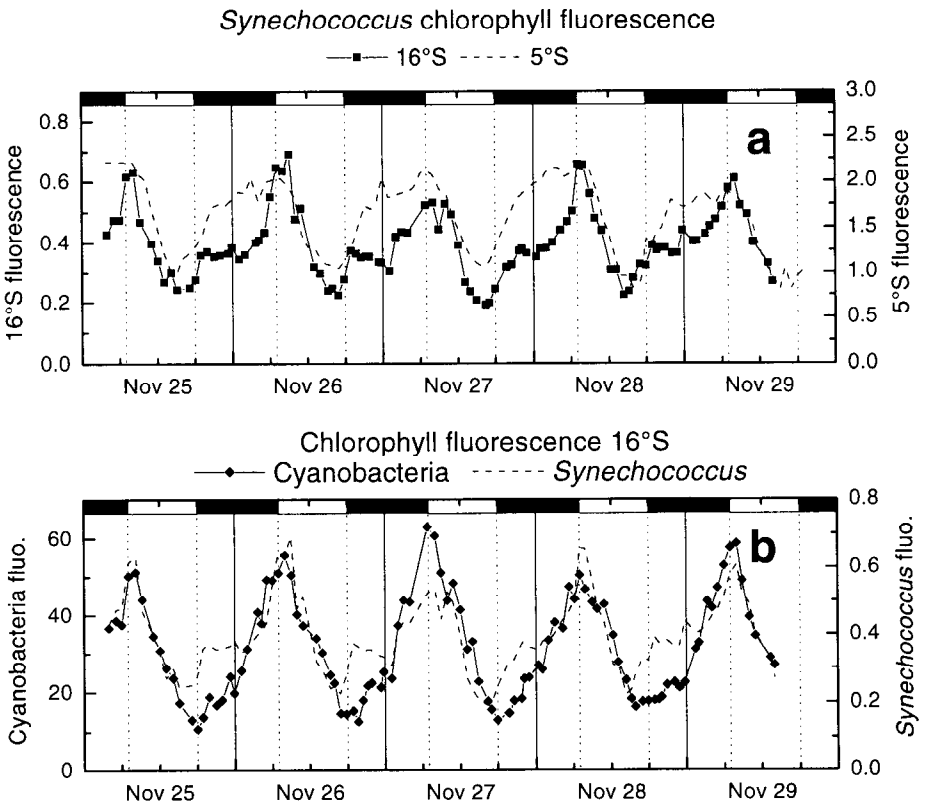

Figure 9. (a) Diel variation of *Synechococcus* chlorophyll fluorescence measured by flow cytometry and normalized to 0.95 μm beads at 16°S in surface during the OLIPAC cruise (150°W, November 1994). The dashed line corresponds to the pattern observed at 5°S (see Figure 4b). (b) As in Figure 9a but for the uncharacterized cyanobacteria of size 2-3 μm observed at 16°S. The dashed line corresponds to the pattern observed for *Synechococcus* at 16°S (see Figure 9a).

day, but did also continue in the dark during the first part of the night for *Prochlorococcus* and picoeukaryotes. Such daytime growth clearly agrees with observations in the field [*DuRand and Olson,* 1996] or in culture [*Stramski et al.,* 1995; *Sciandra et al.,* 1997]. This is consistent with the view that cells start to accumulate carbon once photosynthesis has begun but also can probably continue building up organic carbon or grow in size in the dark for a short amount of time. This pattern is especially clear for *Prochlorococcus* at surface (Figure 3). Cell growth appeared to be quasi instantaneously triggered by light at dawn and to continue for at least 3 hours in the dark. After that, division became the dominant process, although some cells may have continued to increase their size for a short period. The slower growth observed during the first half of the light period could well have resulted from photoinhibition, as reflected in both chlorophyll fluorescence and cell division (see below). Once division had ceased past midnight, no growth took place during the second part of the night. In contrast to *Prochlorococcus*, cell growth was not always immediately initiated at dawn for *Synechococcus* and picoeukaryotes but could exhibit a delay of a couple of hours. Whether this represents a slower response from the cellular machinery to photosynthetic carbon uptake or a higher growth irradiance requirement is an open question. Growth processes were surprisingly uniform across depth in the mixed layer. At dawn the homogenization due to nocturnal mixing (see below) resulted in a nearly uniform vertical profile of side scatter from the surface down to the bottom of the mixed layer at 75 m (Figure 6c). Thereafter, side scatter increased at virtually uniform rates across the mixed layer such that vertical profiles displayed no gradient although displaced toward higher values as the day progressed. Populations closer to the surface exhibited a slightly slower rate of scatter increase for *Prochlorococcus* (but not for *Synechococcus*). The only other noticeable difference occurring with depth was the timing of the maximum that was retarded as depth increased, probably as a consequence of the retarded division (see below).

Three processes were at play to shape the pattern of chlorophyll cell fluorescence: quenching, chlorophyll synthesis, and cell division. Again, we only focus on the two former phenomena since the latter will be discussed below. In the case of *Prochlorococcus*, since cell division only took place after dusk (see above) and since no drop in the concentration of divinyl chlorophyll *a* per cell could be detected at midday (e.g. on November 19, values were 0.31 fg cell^{-1} at 0600, 0.32 fg cell^{-1} at 1200, and 0.28 fg cell^{-1} at 1800) (H. Claustre and C. Cailliau, personal communication, 1997), fluorescence quenching was probably the dominant process inducing midday fluorescence decrease. Past midday, quenching was obviously rapidly reversed at surface when cells were exposed to decreasing light intensities (Figure 4). The observed fluorescence decrease could be due either to non-photochemical quenching (e.g., due to the non-photosynthetically active pigment zeaxanthin) or to photodamage to photosystem II (PSII) reaction centers. In either case the deactivation/repair mechanism probably required active growth [*Falkowski and Kolber,* 1995] as

suggested by the absence of reversal when cells were maintained in the dark at 4°C (data not shown). The asymmetry of the fluorescence curve with respect to its midday minimum (Figure 4) suggests that recovery processes were not instantaneous, as would have been the case if rapid non-photochemical quenching processes (such as state transitions occurring in cyanobacteria [*Fujita et al.,* 1994]) were dominant, but had a timescale of the order of a couple of hours, compatible with slower non-photochemical quenching processes such as zeaxanthin protection or PSII core de novo synthesis following photodamage [*Oquist et al.,* 1995]. At surface, UV-inflicted damages were also probably very important, especially since cells were trapped in a narrow surface mixed layer during the day (see below). UV may be, in fact, more detrimental to the photosynthetic apparatus than visible light [*Cullen et al.,* 1992; *Lao and Glazer,* 1996]. The fact that midday quenching was more reduced and processed at a slower rate for *Synechococcus* as well as for the larger cyanobacteria encountered at 16°S suggests that these prokaryotes may be better protected than *Prochlorococcus* against excess visible and UV light. This hypothesis is reinforced by the fact that *Synechococcus* divides closer to midday than *Prochlorococcus* at surface (see below) and could be explained by the much thicker thylakoid layer in the former genus (e.g., compare *Synechococcus* and *Prochlorococcus* electron microscopy pictures by *Chisholm et al.* [1988]). The virtual absence of fluorescence quenching for picoeukaryotes is probably not a matter of cell size (the 2-3 μm cyanobacteria observed at 16°S have the same size than picoeukaryotes but display the same quenching as *Synechococcus*) and may stem from the fundamentally different and probably more elaborate photoprotective mechanisms in eukaryotes, such as the xantophyll cycle [*Demmig-Adams and Adams,* 1992]. At 5°S, photosynthetic prokaryotes contribute to ~70% of total cellular fluorescence measured by flow cytometry, suggesting that the very strong diel cycle of bulk red fluorescence observed with continuous flow-through or in situ moored fluorometers [*Stramska and Dickey,* 1992; *Dandonneau and Neveux,* 1997] could be mostly due to photosynthetic prokaryotes in contrast to what happens for the diel variation of beam attenuation to which eukaryotes contribute most [*DuRand and Olson,* 1996]. Quenching only concerns the top 20 m of the water column (Figure 7a). At 25 m, no midday depression is visible, and at 55 m (Figure 7a), there is a clear increase in chlorophyll fluorescence during the day that parallels that of light scatter and is a likely consequence of daytime chlorophyll synthesis, as is usually observed in cultures exposed to noninhibiting growth irradiances [*Stramski and Reynolds,* 1993; *Stramski et al.,* 1995; *Sciandra et al.,* 1997]. In contrast to light scatter, chlorophyll synthesis during the light period is not invariant with depth (if this were the case, we would have a uniform profile below 25 m) but increases from top to bottom as would be expected if cells photoacclimate in response to reduced light at depth. The two opposing phenomena of chlorophyll synthesis and fluorescence quenching result in the creation of a very steep gradient at midday (1400) with a nearly four-fold difference between the surface and 55 m. This gradient suggests that both phenomena occur on a shorter timescale than vertical mixing. Vertical mixing is probably much reduced by the creation around 1200 of a secondary thermocline in the top 10-20 m (Figure 2d). This diurnal shallow mixed layer breaks down after dusk, and mixing resumes down to 40-80 m, resulting in a nearly uniform vertical profile of all cell properties the next morning. It should be noted, however, that on November 19 at 0600, scatter was uniform throughout the 80 m deep mixed layer, while chlorophyll fluorescence was only uniform from the surface down to 55 m and increased below. Most likely, photoacclimation took place between 55 and 80 m, and vertical mixing was not strong enough to completely erase it.

4.2. Population Dynamics

The population dynamics of a given group of organisms is the complex result of many interacting phenomena, either physical or biological. For the very small picoplankton (below 2 μm), physical processes include mostly advection and diffusion since settling is virtually negligible. Biological processes can be either intrinsical, such as cell division or cell death, or extrinsical such as viral mortality or grazing. In turn, these biological processes are driven by external forcing such as nutrient or light availability. At timescales above 1 day it is very difficult to examine these phenomena independently. In order to uncouple them it is necessary to act experimentally on the samples, for example, by diluting them with filtered water if one wants to measure grazing mortality [*Landry and Hassett,* 1982]. Such manipulations usually disturb dramatically the delicate balance of the microbial food web. In contrast, the temporal uncoupling that takes place during the diel cycle allows one to analyze some of these phenomena without handling the samples, one drawback being that the uncoupling is never total, which leaves room for uncertainties in data interpretation.

Cell cycling and cell division are the phenomena more amenable to analysis because they can be very precisely visualized using cell DNA staining. However, such analysis is only feasible for populations that are numerous enough (typically well above 10,000 cell mL^{-1}). In the present case, only *Prochlorococcus* cell cycle data were obtained. They confirm previous reports of the good synchrony and relatively high division rate of *Prochlorococcus* in equatorial and tropical Pacific waters [*Vaulot et al.,* 1995; *Liu et al.,* 1997]. As previously reported, the division rate is depressed at surface by up to 30%, most likely as a result of high irradiances. Two lines of evidence suggest that UV wavelengths are mostly responsible for this. First, the depth to which the phenomenon extends coincides with that where UV has been shown to affect bacteria and phytoplankton [*Smith et al.,* 1992; *Jeffrey et al.,* 1996]. Second, the most obvious cellular response observed near the surface is the retardation of entry in S phase, a well-known response of cells to UV-inflicted damages [*Setlow et al.,* 1963; *Buma et al.,* 1996]. In fact, *Synechococcus* division was also retarded at surface since its light scatter reached its daily maximum 2 hours later at 5 m than at 25 m (Figure 6c). Increase in the duration of the S phase in response to high solar irradiances was also recently observed in surface waters of the Mediterranean Sea [*Jacquet et al.,* 1998a].

Below 60 m, *Prochlorococcus* division rates decreased rapidly. However on November 19, a secondary maximum close to one division per day was observed at 95 m (Figure 8a). This day was characterized by the entrainment of surface populations down to the region below the thermocline, as evidenced by the presence of two separate *Prochlorococcus*

populations with different chlorophyll fluorescence at both 85 and 95 m (data not shown), as observed, for example, by *Campbell and Vaulot* [1993]. Since neither NO_3^- nor PO_4^{3-} were likely to be limiting in the surface layer (P. Raimbault, personnal communication, 1997), the increase in the division rate for the entrained populations could be due to exposure to the higher levels of iron found below the thermocline in equatorial waters [*Coale et al.*, 1996] to which *Prochlorococcus* responds [*Zettler et al.*, 1996].

The strong correlation between division rate and daily scatter increase (Figure 8b), first noted by *Binder et al.* [1996], probably reflects the fact that cells must reach a critical size before cell division can proceed [*Donachie*, 1993]. It must therefore be interpreted as a causal relation between the diel increase in scatter and division rate and not the converse. Looking more closely at this relation, the slope for the data set obtained above the thermocline is about half of the one below the thermocline (corresponding to division rates above and below 0.5 d^{-1}, respectively). Moreover, four points corresponding to the depths where *Prochlorococcus* populations were entrained below the thermocline on November 19 did not fit the overall relation, most likely because the scatter at dusk and dawn did not correspond exactly to the same populations. These facts suggest that some caution must be exercised before extrapolating scatter variation to division rate as well as when estimating division rates from cell cycle data since sampling at a fixed depth does not guarantee sampling always the same population.

For *Synechococcus* and picoeukaryotes, no cell cycle data could be reliably obtained, and therefore information on the timing of division must be inferred from the variation of cell parameters, assuming, in particular, that the period of cell concentration increase coincides with division, an hypothesis that is validated for *Prochlorococcus*, and conversely, that fluorescence and scatter decrease when cells divide. Our data confirm recent reports that both populations are also synchronized [*DuRand and Olson*, 1996; *Vaulot et al.*, 1996; *Blanchot et al.*, 1997]. However, the present data set reveals an interesting temporal dephasing. At surface, *Synechococcus* divides first around dusk, *Prochlorococcus* divides ~2 hours later, and picoeukaryotes divide ~7 hours later. These inferences drawn from cell concentrations are also confirmed by scatter and fluorescence patterns (Figures 3 and 4). Data from the Red Sea, where simultaneous cell cycle analyses of *Synechococcus* and *Prochlorococcus* could be performed, indicate a similar dephasing (M.J.W. Veldhuis, D. Vaulot and D. Marie, unpublished data, 1994). In contrast, data obtained at the equator itself [*DuRand and Olson*, 1996] did not show any dephasing between picoeukaryotes and *Prochlorococcus*, but picoeukaryotes observed at 0° [*DuRand and Olson*, 1996] and 5°S (present study) were probably taxonomically different. Discrepancies in timing could stem from differential sensitivity to damages due to high irradiances. However, this is contradicted by the fact that dephasing seems to persist with depths, for example, between *Synechococcus* and *Prochlorococcus*, as evidenced by the difference in the timing of the side scatter maxima at 55 m that occurs 2 hours later for *Prochlorococcus* (Figure 6). Alternatively, it could result from differences in the way cells assimilate inorganic carbon and synthesize material to prepare for the next round of division. For example, prokaryotes because of their simpler organization could metabolize more rapidly during the day, at the expense of having to wait for the return of the light period to resume growth. Eukaryotes may have evolved a more sophisticated strategy, restricting to the dark period all processes that do not strictly require light or that may be susceptible to photodamage such as DNA replication (oceanic eukaryotes may repair DNA damages more slowly than procaryotes [*Jeffrey et al.*, 1996]). These dephasings between populations could also be due to the specificity of their respective endogenous clocks. In contrast to these field observations, laboratory cultures exposed to 12:12 light-dark cycle of *Synechococcus* divide later than *Prochlorococcus* (S. Jacquet, unpublished, 1997), indicating that the relative timing of division of these two procaryotes is not immutable but varies with strains and environmental conditions.

We do not have direct data to compare division rates at 16°S with those at 5°S. However, from *Synechococcus* fluorescence patterns (Figure 9) we may hypothesize that more cells divided at 16°S because of the slight dip observed early at night, although it could also be due to a sharper synchronization.

Loss processes are more diverse and more difficult to investigate because they cannot be directly measured on cells. The very narrow range of cell abundance over large spatial and long temporal scales implies that growth and loss processes are balanced over these scales. Even at shorter timescales, such as the 5 days sampled at 5°S, this seems to hold since initial and final concentrations were very similar for all three photosynthetic populations (Figure 2). Diel variation of cell concentrations indicates that losses did not take place uniformly throughout the diel cycle for any given population. At surface, virtually no *Prochlorococcus* loss occurred between midnight (when division ceased) and sunrise, but it increased dramatically thereafter. This light-induced loss only occurred in the top 20 m (Figure 5a). It can probably be attributed, in part, to an underestimate of *Prochlorococcus* concentration because the midday decrease of its chlorophyll fluorescence pushes a fraction of the cells below the detection limit of the flow cytometer. For *Synechococcus*, in contrast, significant loss occurred throughout the night but not during the day (Figure 5). Finally, for eukaryotes, the loss rate was moderate during the day and much more drastic just after dusk. The two major biological removal processes for photosynthetic picoplankton are microzooplankton grazing and viral lysis. Although these two loss terms have been shown to be equivalent for heterotrophic bacteria [*Fuhrman and Noble*, 1995; *Steward et al.*, 1996], the latter has been shown to have little impact on photosynthetic organisms, at least on *Synechococcus* [*Waterbury and Valois*, 1993; *Suttle and Chan*, 1994], especially in stable environments where phage resistant strains have been selected. The difference in the decay rate and the timing of major loss periods among the different populations suggests that different predators graze each population and that their activity is not constant throughout the day. In the case of *Prochlorococcus*, *Liu et al.* [1997] have recently hypothesized that grazing is restricted to night hours just after division. Whether such synchrony is linked to the prey dynamics (for example, if protists preferentially graze on recently divided cells [*Sherr et al.*, 1992]), or to the endogenous cycle of the predator (for example, if grazing activity is much reduced when predator cells divide) is an open question. A third biological cause of cell loss could be UV-induced cell lysis observed, for example, for bacteria by

Müller-Niklas et al. [1995]. This could explain partly the decrease of *Prochlorococcus* abundance in the top 20 m after sunrise.

Physical processes are also important because they constantly disturb the delicate balance between growth and loss biological processes. While increased vertical mixing inputs limiting nutrients, such as iron, into the upper layer and therefore potentially boosts the division rate, it also increases the diffusion of cells out of the mixed layer. Advective events may also have considerable importance: one such event probably occurred on November 21 between 1800 and 2100 as reflected in the salinity data (not shown). This event caused abrupt cell loss, clearly visible for the picoeukaryotes (Figure 2, arrows) as well as for *Prochlorococcus* and *Synechococcus* populations, that increased much less than on previous days despite identical division rates, at least for *Prochlorococcus* (Figure 8).

Diel patterns of autotrophic picoplankton populations are very complex. Each population and each cellular parameter have their own behavior that is modulated by depth as well as nutrient levels. Their detailed analysis allows us to understand better the rate at which each population grows and should help in the future to interpret patterns observed in bulk measurements such as those recorded by conductivity-tempererature-depth (CTD) casts or moored instruments [*Claustre et al.*, this issue].

Acknowledgments. This work was part of the following programs: EPOPE (JGOFS-France) funded by CNRS-INSU, IFREMER, and ORSTOM, GDR 869 and JGOFS PROSOPE funded by CNRS-INSU, and MEDEA funded by the EU contract MAST CT 95-0016. We wish to thank Y. Dandonneau and B. Coste for coordinating very smoothly EPOPE and the OLIPAC cruise, respectively, as well as all crew and officers of the NO Atalante. We are grateful to J. Blanchot for the loan of a computer during the OLIPAC cruise, to N. Metzl and B. Brès for allowing us to sample from their continuous CO_2 measurement outlet as well as for collecting samples for us during long equatorial nights, to C. Bournot and D. Tailliez for the smooth operation of the CTD, to all the participants of the diel experiments and finally to Y. Dandonneau, M. Behrenfeld, F. Partensky and S. Jacquet for useful comments on a draft version of this paper.

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(Received August 29, 1997; revised April 3, 1998; accepted April 22, 1998)