JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 104, NO. C2, PAGES 3311-3321, FEBRUARY 15, 1999

Phycoerythrins in the southern tropical and equatorial Pacific Ocean: Evidence for new cyanobacterial types

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Abstract. Quantitative and qualitative investigations of phycoerythrins (PE) were achieved in the central southern tropical and equatorial Pacific during the Flux dans l'ouest du Pacifique equatorial (FLUPAC: September-October 1994) and Oligotrophie en Pacifique (OLIPAC: November **1994)** cruises. We observed mainly high-phycourobilin (PUB) PE related to small Synechococcus spp. (0.6-l .4 μm). This PE was characterized by fluorescence excitation peaks at 496 and 550 nm in 50% glycerol. Highest concentrations (0.1-0.2 $\mu g\ L^{-1}$) were recorded either in the mixed layer (nutrient-enriched area) or at depth in the vicinity of the 0.1 μM NO_3^- isopleth (oligotrophic waters). Maximum abundance of Synechococcus did not exceed $3\ 1 \times 10^3$ cell mL^{-1}. No subpopulations of Synechococcus were evidenced by flow cytometry. Nevertheless, in a few samples, two new PE spectral types were observed. The first type was a high-PUB PE with two fluorescence excitation peaks at 494 and 564 nm. It appears to be attributable to nonmotile round cells, 2-3 μm in size and easily detected by flow cytometry, likely cyanobacteria. They were only observed in very oligotrophic waters south of 15°30'S along 150°W in the top 60 m. In this area, nitrate, nitrite, and ammonia were undetectable above 120 m, while phosphate was always recorded. This suggests that these larger cyanobacteria may fix dinitrogen (N_2) to supply their growth and therefore possibly play a significant role in oceanic new production. The second type, a high-phycoerythrobilin (PEB) PE was observed in three samples obtained at or near the equator. It displayed two fluorescence excitation peaks at 496 and 536 nm. The organisms that contained this PE type were not identified. These data suggest that PE is more diverse in oceanic waters than was previously assumed.

1. Introduction

Phycobiliproteins are water-soluble proteineous pigments (phycoerythrin, phycoerythrocyanin, phycocyanin, and allophycocyanin) encountered in cyanobacteria and eukaryotic algae (Cryptophyceae, and Rhodophyceae). They contribute to photosynthesis by harvesting solar energy and transferring it to Chlorophyll *a* *[French and Young,* 1952]. They may also serve as cellular nitrogen reserves *[Barlow and Alberte, 1985; Wyman et al., 1985; Heathcote et al.,* 1992], although this latter role is subject to controversy *[Yeh et al.,* 1986]. In marine pelagic ecosystems their characteristics and distribution have not been fully investigated, although they have been suggested to be useful as chemotaxonomic and biomass markers *[Stewart and Farmer, 1984; Lantoine and Neveux,* 1997]. Such markers could provide informations on the structure and functioning of phytoplankton community and eventually might help to delineate biogeochemical regimes [*Exton et al.,* 1983]. Regarding their spectral absorption characteristics, phycoerythrins (PE) appear different in cyanobacteria, Cryptophyceae, and Rhodophyceae [*O'Carra and O'hEocha,* 1976]. Such variations are also observed between species within each of these classes *[Wood et al., 1985; Hill and Rowan,* 1989]. Phycoerythrins are the main phycobiliproteins found in oceanic waters, usually associated to cyanobacteria [*Waterbury et al., 1986; Campbell and Carpenter,* 1987, *Lantoine and Neveux,* 1997]. They contain two different prosthetic groups in various proportions: phycourobilin (PUB) and phycoerythrobilin (PEB). Several methods have been developed to obtain concentration of either bulk PE *[Moreth and Yentsch,* 1970; *Algarra et al., 1988; Wyman, 1992; Lantoine and Neveux,* 1997] or of various categories of phycobiliproteins *[Stewart and Farmer,* 1984]. Nevertheless,

Paper number 98JC02000.
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relatively few quantitative data on bulk PE in the field are available today (Mediterranean Sea [*Algarra et al.,* 1988; *Lantoine,* 1995], Atlantic Ocean [*Glover et al.,* 1988a; *Lantoine and Neveux,* 1997], and Pacific Ocean *[Bidigare and Ondrusek, 19961).* Qualitative information is even more scarce. Our present views on oceanic PE result from studies of various PE-containing marine species that have been isolated in culture [*Alberte et al.,* 1984; *Ong et al.,* 1984; *Wood et al.,* 1985]. Flow cytometry has allowed the mapping of areas of occurrence for high and low PUB *Synechococcus* in the North Atlantic Ocean and Caribbean Sea *[Olson et al.,* 1988]. Some direct measurements have been recently reported in the Atlantic Ocean *[Lantoine and Neveux,* 1997], the Mediterranean Sea *[Lantoine,* 1995], and the Black Sea *[Shalapenok and Shalapenok,*1997]. The present paper provides data on the quantitative and qualitative distribution of PE in the southern tropical and equatorial Pacific Ocean sampled in September-November 1994. We also examine the relationship between the PE concentration and the abundance and orange fluorescence of *Synechococcus* cells obtained by flow cytometry.

2. Material and Methods

2.1. Phycoerythrin Determination

Vertical profiles were sampled at several stations during the Flux dans l'ouest du Pacitique equatorial (FLUPAC: September 23 to October 29, 1994) and Oligotrophie en Pacitique (OLIPAC: November 3 to December 1, 1994) cruises (Figure 1). Three transects were completed: the first from 15°S to 6°N along 165°E, the second along the equator from 165°E to 150°W, and the third from 16°S to 1°N along 150°W. Water was sampled with a rosette composed by 12 L Niskin bottles and coupled with a SeaBird conductivity-temperature-depth (CTD) probe. At each depth, 3 L of seawater were filtered onto a 47 mm Whatman GF/F glass fiber filter. Filters were stored during 2-8 days in liquid nitrogen before analysis. In previous studies *[Lantoine and Neveux,* 1997] PE were extracted by thawing and grinding the filters in 0.1 M (*p*H = 6.5) phosphate buffer. As PE extraction may be difficult for some strains *[Glover et al.,* 1988a], we decided to grind the filters in glass tubes containing 6 mL of 50% glycerol in phosphate buffer, following the so-called in vivo method of *Wyman* [1992]. Glycerol enhances the in vivo PE fluorescence by uncoupling the energy transfer between PE and Chlorophyll *a* in intact cells. The extracts were vigorously shaken and then centrifuged at 3500 rpm. After transferring the supernatant into a 1 cm square measuring cuvette, fluorescence was measured on a Perkin Elmer MPF 66 spectrofluorometer using an OBEY program *[Lantoine and Neveux,* 1997]. The program first performed an excitation scan from 450 to 560 nm (emission: 575 nm) and then, depending on the excitation maximum found, an emission scan from 530 to 700 nm (excitation: 495 nm) or from 550 to 700 nm (excitation: 530 nm). Excitation and emission slits were set respectively to 5 and 10 nm. An additional measurement at fixed wavelengths ($\lambda_{excitation}$ = 610 nm; $\lambda_{emission}$ = 650 nm; integration time = 2 s) was performed to check the presence of phycocyanin. Excitation and emission blank spectra were performed as follows: a new GF/F filter, impregnated with 0.2 pm pore size filtered seawater, was ground into 6 mL of a 50% glycerol solution. After centrifugation at 3500 rpm the blank spectra were recorded and subtracted from the raw spectra of the PE extracts. Equal concentrations of PE dissolved either in phosphate buffer or in 50% glycerol showed very similar fluorescence excitation properties (Figure 2a). Therefore we calculated PE concentrations using the areas under the fluorescence excitation spectra, the PUB/PEB ratio (495/548 nm ratio), and the' formulae determined for phosphate buffer solutions *[Lantoine and Neveux,* 1997].

Because of the low PE concentrations observed during the first transect at 165°E, we improved the sensitivity of the assay by increasing the excitation and emission slit widths (10 and 20 nm, respectively). In order to avoid artifacts associated with background cellular fluorescence it was necessary to shift the measuring emission wavelength from 575 to 585 nm. These changes in the optical setup were taken into account in the calculation of PE concentration by dividing the measured excitation area by 2.1.

Qualitative information was difficult to obtain for PE concentrations $<0.010\ \mu g\ L^{-1}$. In such cases the first excitation peak near 495 nm was generally visible, but a significant scattering signal was superimposed on top of the second peak (550 nm) which was then ill-defined. Moreover, significant contamination of the spectra was also noted below 470 nm. In these cases, PE concentrations were assessed from the fluorescence at 495 nm, assuming similar excitation spectra and PUB/PEB ratio to those observed at the depth of the PE maximum of the same station.

In some samples we compared the PE concentration estimated either with phosphate buffer *[Lantoine and Neveux,* 1997] or phosphate buffer:glycerol (50:50) *[Wyman,* 1992] extraction. The latter procedure led clearly to 1.5-2 times higher values of PE and consequently would be preferred for quantitative PE estimation. Qualitative spectral information, however, was similar among both procedures (Figure 2b).

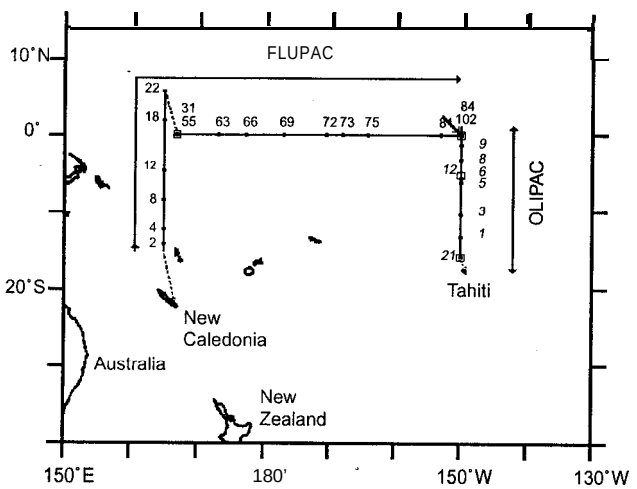

Figure 1. Ship track and sampling stations for phycoerythrins (PE) measurements. Station numbers of the Oligotrophie en Pacitique (OLIPAC) cruise are noted in italics. Open squares represent long-term stations.

2.2. Chlorophyll Determination

Chlorophylls and phaeopigments including divinyl Chlorophylls *a* (dv-Chl *a)* and *b* (dv-Chl *b*) were assessed by the spectrofluorometric method of Neveux *and Lantoine [* 1993] using a MPF 66 spectrofluorometer. Fluorescence was meas-

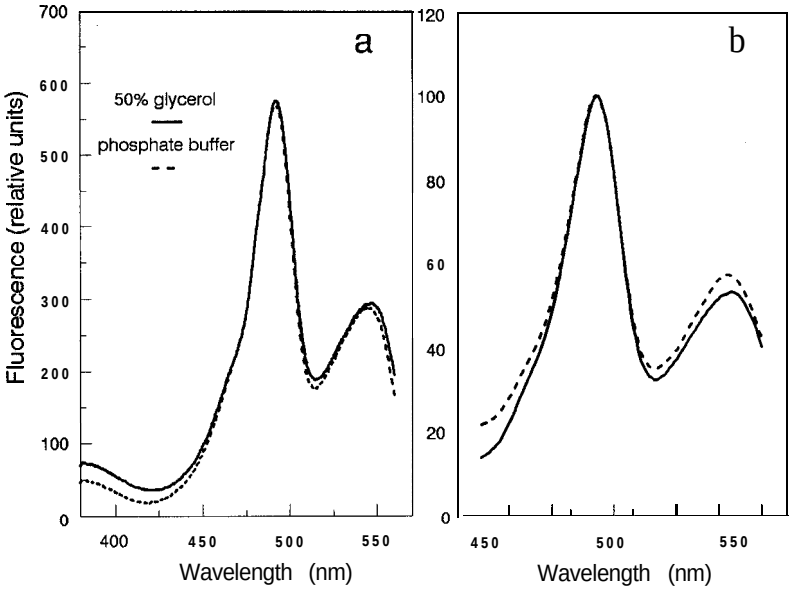

Figure 2. (a) Comparison of the fluorescence excitation spectrum obtained by a half-half dilution either in phosphate buffer or in glycerol of a PE solution in phosphate buffer. (b) Normalized fluorescence excitation spectra of a natural sample (OLIPAC, station 13, 45 m) extracted in phosphate buffer or in phosphate buffer:glycerol (50:50).

ured at 24 fixed excitation and emission wavelengths with an OBEY program, and the pigment concentrations were determined by least squares approximation.

2.3. Flow Cytometry

Analyses were performed at all stations on freshly collected samples by using either of two similar flow cytometers (Becton Dickinson), a fluorescence activated cell sorter scan (FACScan) during the FLUPAC cruise and a fluorescence activated cell sorter sorter (FACSorter) during the OLIPAC cruise. For each cell the forward and right angle light scatters as well as the fluorescence emitted by PE (orange) and Chlorophyll *a* or dv-Chl a (red) were measured. Fluorescent microspheres (Polysciences) of 2.02 μm (FACScan) and 0.95 μm (FACSort) diameter were used as internal reference. Data were processed as described by *Partensky et al. [* 1996].

To compare the data sets from both cruises with PE concentrations, we normalized the FLUPAC data to 0.95 μm beads by multiplying the orange (PE) and red (Chl *a* and dv-Chl *a)* fluorescence by 7.81 and 5.7, respectively, which correspond to the ratios of the orange and red fluorescence of the beads (2.02/0.95 μm).

2.4. Cultures

Synechococcus strains max 42 and max 01 were isolated from the Sargasso Sea in 1987 by D. Vaulot and C. Courties. Synechococcus strain DC2 (CCMP 1334, equivalent to Woods Hole Collection (WH) 7803) and *Prochlorococcus* strain CCMP 1375 originated from the Guillard Provasoli National Center for Culture of Marine Phytoplankton (CCMP, Boothbay Harbor, Maine). Synechococcus was cultured in f/2 medium *[Guillard and Ryther,* 1962] and *Prochlorococcus* as described by *Scanlan et al.* [1996].

2.5. Nutrients

Nutrient analyses ($NO_3^- + NO_2^-$; PO_4^{3-}) were performed immediately onboard with a Technicon Autoanalyzer. ($NO_3^- + NO_2^-$) concentrations below 1.5 μM were determined with a nanomolar sensitivity method *[Oudot and Montel, 1988; Raimbault et al.,* 1990].

3. Results

3.1. Physical and Chemical Conditions

Physical and chemical conditions encountered during the FLUPAC and OLIPAC cruises are discussed in details in other papers *[Eldin et al., 1997; Stoens et al.,* this issue]. During the FLUPAC cruise, along 165°E and along the equator (west of 166°W), highly stratified waters with oligotrophic conditions in the surface layer (NO; $+NO_2^- < 0.1$ μM) prevailed. Rapid transition from nitrate-depleted to nitrate-replete surface waters occurred between 166°W and 165°W. $NO_3^- + NO_2^-$ increased drastically from <0.1 μM to above 1 μM in the 60 m deep mixed layer. Concentrations increased continuously east of 168°W reaching more than 3 μM at 150°W. The OLIPAC cruise showed that the enriched area (NO; $+NO_2^- > 1$ μM) along 150°W extended southward to 7% An important depleted surface layer occurred (NO; $+NO_2^-$ <0.1 μM) between 11°S (O-SO m) and 16°S (O-120 m). Along 150°W, phosphate surface concentrations were always detectable (>0.12 μM in surface waters). However, values <0.1 μM were observed west of 178°W (data not shown).

Oligotrophic waters were characterized by a deep chlorophyll maximum, whereas enriched waters showed relatively homogeneous chlorophyll concentrations from the surface down to 60-80 m. The relative proportion of dv-Chl *a* decreased from oligotrophic to nutrient-rich waters.

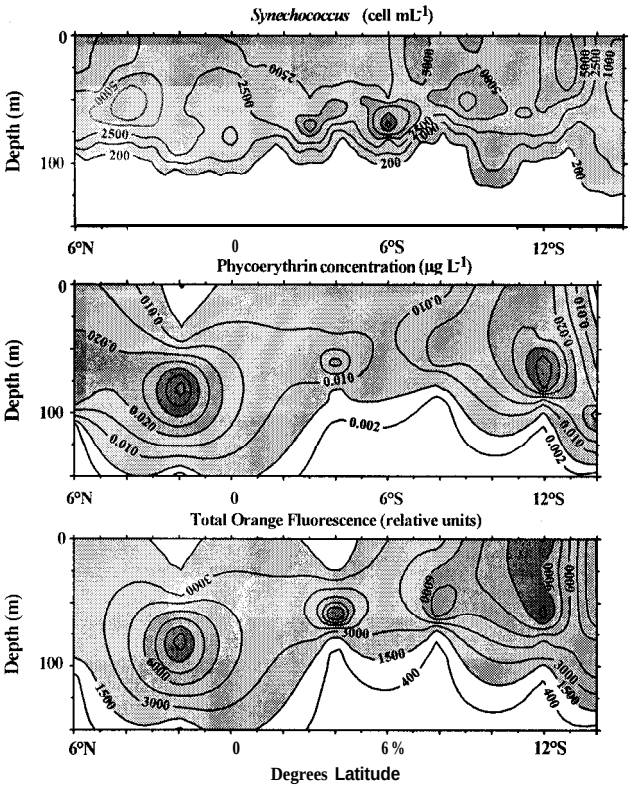

Figure 3. Distributions of *Synechococcus,* PE concentration, and total orange fluorescence of *Synechococcus* cells along 165°E (September 24 to October 1, 1994). Sampling was realized with higher frequency for *Synechococcus* than for PE concentration. Orange fluorescence data were plotted using only samples for which PE was measured.

3.2. *Synechococcus* Distribution

3.2.1. Transect along 165°E. In the surface layer (O-50 m), cell abundance always remained below 10 x 10^3 cell mL^{-1} (Figure 3). Variations were generally associated with fluctuations in the depths of the nitrate and phosphate isopleths. The lowest values (<2.5 x 10^3 cell mL^{-1}) were found over the entire water column at 14°-15°S, where the 0.1 µM isopleths were very deep (100-I 10 m). The rising of the isopleths coincided with an increase in cell abundance at the surface which was higher when the 0.1 µM phosphate isopleth reached the surface (5-9 x 10^3 cell mL^{-1} between 13° and 7°S). Locally, a sharp maximum was observed near the top of the nitracline (60-70 m; three stations) with cell abundance reaching 20 x 10^3 cell mL^{-1} at 6°S.

3.2.2. **Transect along the equator.** Two contrasting situations were observed according to the different trophic conditions (Figure 4). West of 172°W, oligotrophy and stratification prevailed in surface waters. *Synechococcus* abundance was low (<2.5 x 10^3 cell mL^{-1}) with a slight maximum at depth. The maximum deepened westward and finally disappeared beyond 173°E. Even when the 0.1 µM phosphate isopleth reached the surface, the 0.1 µM nitrate isopleth was always near or below 80 m, as observed along 165°E in regions with the lowest *Synechococcus cell* abundance. East of 172°W, abundance maxima were located in the mixed layer, and the highest values (20-31 x 10^3 cell mL^{-1}) were observed between 167" and 164°W. Surprisingly, east of this region, although surface nitrate concentration kept increasing, *Synechococcus* abundance decreased.

3.2.3. Transect **along 150°W.** North of 13°S, the *Synechococcus* vertical cell distribution was homogeneous and abundance was maximal in the surface mixed layer (Figure 5). The highest values (20-27 x 10^3 cell mL^{-1}) were observed between 2°30' and 8°S. Low concentration of *Synechococcus* (<2.5 *x* 10^3 *cell* mL^{-1}) appeared between 13° and 16°S when the 0.1 µM nitrate isopleth deepened down to 100-120 m. At 16°S, flow cytometric analyses (Figure 6) showed the presence of an orange fluorescing (0.5-I x 10^3 cell mL^{-1}) population of larger cell (around 2-3 µm in diameter) than the small *Synechococcus (0.6-1.4* pm). They were determined by epifluorescence microscopy to be nonmotile round cells emitting orange fluorescence, closely resembling cyanobacteria.

3.3. Phycoerythrin Distribution

PE distribution fairly well matched that of *Synechococcus cells* (Figures 3, 4, and 5). The major discrepancies reflected essentially the use of different sampling frequencies for the two parameters. *Synechococcus* red and orange fluorescence were highly correlated over the entire data set from both cruises *(r = 0.988,* and *n* = 151), suggesting that the pigment ratio (PE /Chl a) and the energy transfer efficiency from PE to Chl a were roughly similar for all samples. Therefore we only compared PE concentrations with orange fluorescence (Figures 3, 4, and 5). PE data were better correlated with the total in vivo orange fluorescence of *Synechococcus* cells (*r = 0.92,* and *n* = 15 1) than with cell number (*r* = 0.63). Maximum PE values >0.1 µg L^{-1} were observed in the enriched mixed layer (Figures 4 and 5). The vertical distribution of PE

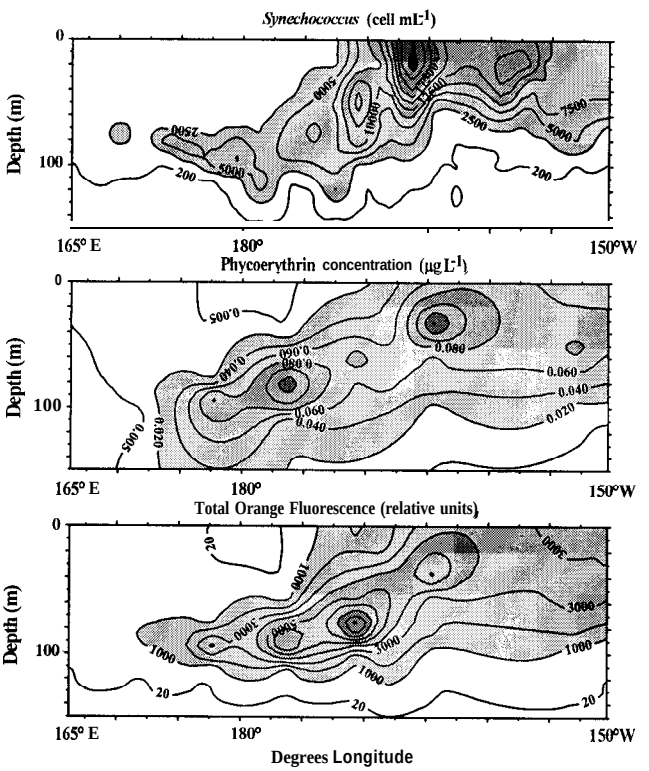

Figure 4. Similar to Figure 3 but along the equator (2-26 October, 1994).

concentration always showed a maximum at depth whatever the vertical distribution of Synechococcus cells was (Figures 3, 4, 5, and 7). For instance, at station 12 (OLIPAC) the intracellular PE concentration varied from 2.6 to 16 fg cell-‘, and the vertical distribution of the total orange fluorescence of Synechococcus cells was very similar to that of PE (Figure 7). The lowest values of intracellular PE were observed in surface oligotrophic waters (0.5-1.0 fg cell-‘) and the highest values in samples beyond 100 m depth (30-50 fg cell-‘). These values were in agreement with the ranges observed previously [*Glover* et *al.*, 1988a; *Lantoine and Neveux*, 1997]. In the enriched area, depth profiles showed that the PE maximum was generally at the level or slightly above the total Chl *a* (TChl a = Chl *a* + dv-Chl *a)* maximum. In oligotrophic conditions, the TChl *a* maximum was distinctly deeper than the PE maximum.

Among *Synechococcus,* those containing high-PUB PE (-96 out of the 110 usable excitation spectra) were dominant throughout the sampling area. The excitation peaks corresponding to the presence of PUB and PEB were located at 496 ± 1 and 549 ± 2 nm, respectively, while the emission peak was at 570 ± 2 nm. The PUB/PEB ratio (first /second excitation peak ratio) ranged from 1.5 1 to 2.00. Along 1 50°W it slightly increased northward. The vertical mean value ranged from 1.68 to I.73 south of 7°S and from I.78 to 1.85 between 7° and 1°S. Along the equator it was lower east of 167°W (1.55-1.65). It was clearly higher west of 170°W, reaching values close to 2 in the PE maxima. In this region, however, the low cell concentrations at the surface did not allow for precise estimates of the PUB/PEB ratio. In the enriched areas the vertical distribution of the PUB/PEB ratio was relatively homogeneous (Figure 7).

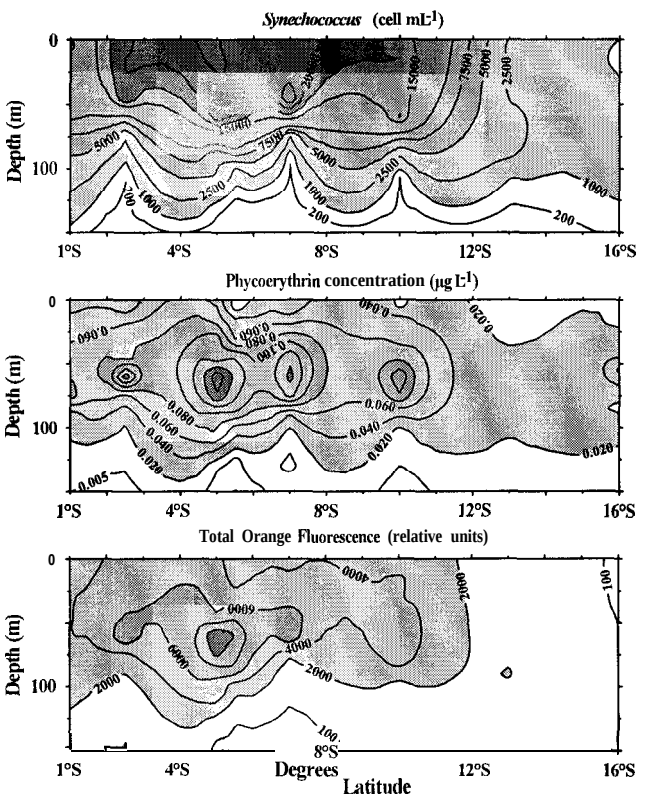

Figure 5. Similar to Figure 3 but along 150°W (5-29 November, 1994).

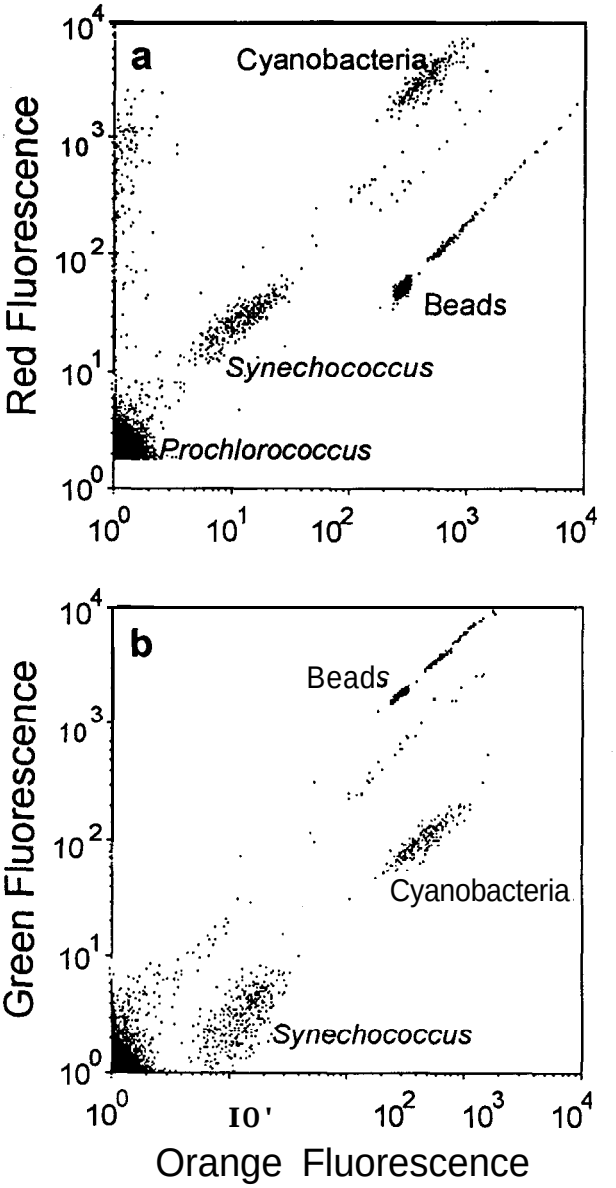

Figure 6. Comparison of flow cytometric signature of the larger coccoid cyanobacteria and *Synechococcus* (station 21, conductivity-tempereture-depth (CTD) 146, 45 m): (a) red versus orange fluorescence and (b) green versus orange fluorescence. Bead size is 0.95 pm.

Two types of unusual PE excitation spectra were observed in the more oligotrophic regions. First, at 16°S-150°W during the OLIPAC cruise the second excitation peak was shifted beyond 560 nm (Figure 8), while the first peak was at 494 nm. As the automated excitation scan stopped at 560 nm, it was necessary to complete it by recording the fluorescence beyond this wavelength in manual mode (emission 595 nm), which revealed the second excitation peak at 564 nm (Figure 9). The 494/564 nm ratio was -3, slightly higher than estimated with the routine procedure (2.5; see Figure 8). This difference resulted probably from the existence of a small residual scattering signal around 555-560 nm (even after the blank correction). The fluorescence emission maximum also showed a shift toward higher wavelength (576 nm) in comparison to that of *Synechococcus* (570 nm; Figure 8). This very high-PUB PE was clearly related to the population of 2-3 µm cya-

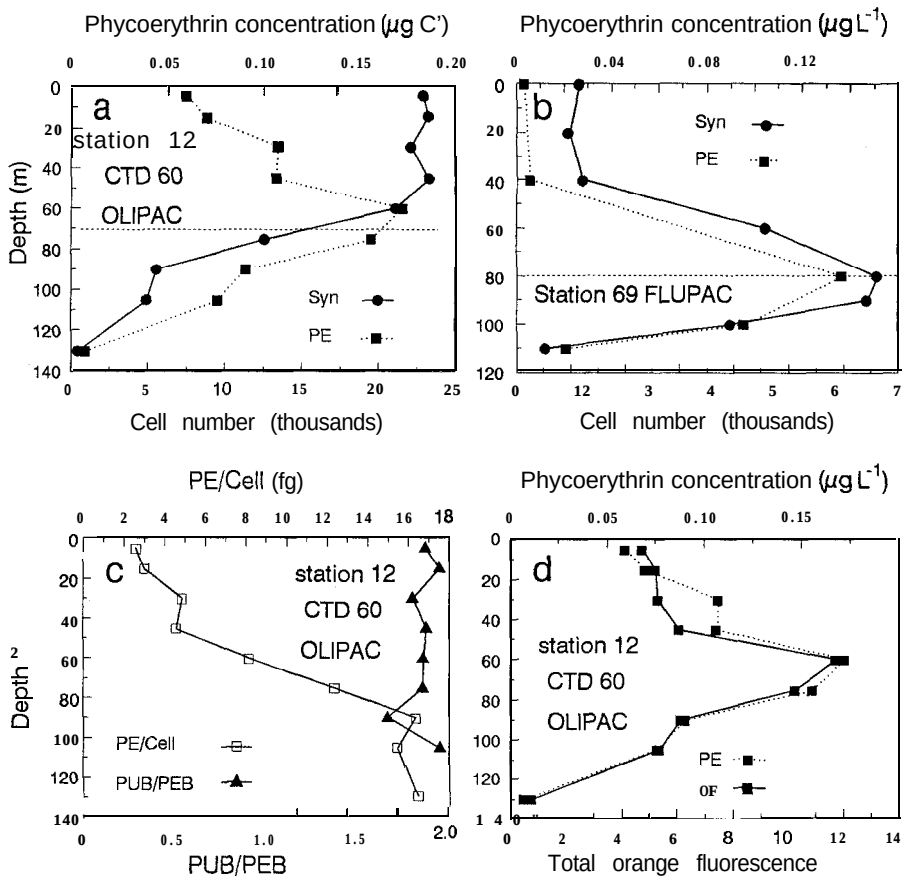

Figure 7. Depth profiles of *Synechococcus* (Syn) and PE concentration at (a) station 12 (OLIPAC) and (b) Station 69 of the Flux dans l'ouest du Pacifique equatorial (FLUPAC) cruise. (c) and (d) Depth profiles of PE concentration per cell, phycourobilin/phycoerythrobilin (PUB/PEB) ratio and total orange fluorescence (OF) of Synechococcus cells (mean cell fluorescence multiplied by cell number) at station 12 (OLIPAC). Horizontal dotted line corresponds to deep chlorophyll maximum.

nobacteria cells detected by their flow cytometric signature (Figure 6). These cells were only observed in the top 70 m (Figure 10). At this station, *Synechococcus* abundance was very low and exhibited a maximum at 70 m (2.5 x 10^3 cell mL^{-1}). The orange fluorescence per cell increased between 0 and 70 m in the same proportion (x 2.7) for both *Synechococcus* and the large coccoid cyanobacterial cells. As the orange fluorescence of the large cells was 25-30 fold higher than that of *Synechococcus,* more than 90% of the total orange fluorescence originated from the large cells in the upper 50 m (Figure 10).

Another unusual type of PE was observed in three samples taken during FLUPAC (Station 12: 4°S,165°E at 70 and 90 m; Station 31: 0°,167°E at 40 m). These samples were characterized by a second excitation peak located at 536 nm instead of 549 nm that was sometimes higher than the first peak at 498 nm (Figure 9). When such unusual PE excitation spectra were observed, flow cytometry did not detect any peculiar orange fluorescing population. A possible explanation could be the presence of irregularly distributed PE-containing organisms, represented either by relatively large cells or by colonies of cyanobacteria (filamentous or mucilaginous aggregates). This was clearly not *Oscillatoria* (=*Trichodesmium)* spp., although some variability in PE spectral characteristics was observed in this genus [*Fujita and Shimura,* 1974; *Shimura and Fujita, 1975; Haxo et al.,* 1987], no known strain exhibits a peak near 536 nm. The unusual PE could also belong to symbiotic cyanobacteria (phaeosomes) within large cells of Dynophysiales (Dinophyceae [Lucas, 1991]) or diatoms [*Magueet al.,* 1977] but not to known Cryptophyceae (see review by *Rowan [* 1989]).

4. Discussion and Conclusions

Qualitative information on PE and more generally on phycobiliproteins in marine pelagic waters is relatively scarce. First, high-PEB [*Kursar et al.,* 1981; *Wood,* 1985], and then high-PUB [*Ong et al.,* 1984] PE-containing *Synechococcus* have been recognized in oceanic waters. In the open ocean, dual excitation beam flow cytometry showed that high-PUB *Synechococcus* are most frequently observed *[Olson et al.,* 1988]. This has been confirmed by excitation spectra obtained in oligotrophic waters *[Lantoine and Neveux,* 1997]. However, high-PEB *Synechococcus* could play a significant role in more coastal mesotrophic and eutrophic waters *[Olson et al.,* 1988; 1990] where cell and PE concentration can reach 400 x 10^3 cell mL^{-1} and 3 $\mu g\ L^{-1}$, respectively [*Partensky et al.,* 1996; *Lantoine and Neveux,* 1997].

Our results prove'that high-PUB PE-containing *Synechococcus* largely determined the distribution and the spectral characteristics of PE in oligotrophic and mesotrophic areas of the central equatorial and tropical Pacific. The lowest concen-

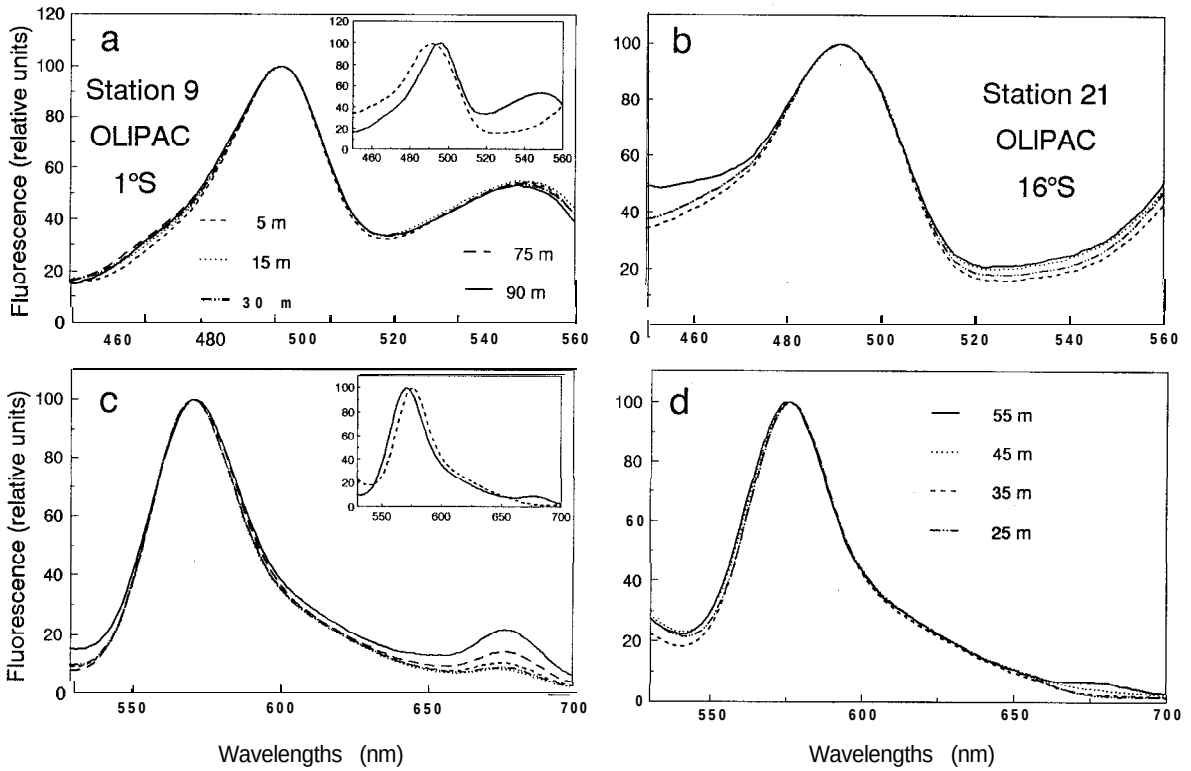

Figure 8. Spectral characteristics of phycoerythrins (PE) at two stations during OLIPAC. (a) Normalized excitation and (b) emission spectra at several depths of station 9 where Synechococcus cells were the sole PE-containing organisms. (c) and (d) Idem at station 21 where larger cyanobacteria (2-3 μm) dominated. Inserts compares the excitation (Figure 8a) and the emission (Figure 8c) spectra recorded from samples collected at the level of the PE maximum of the stations 9 (solid line) and 21 (dotted line).

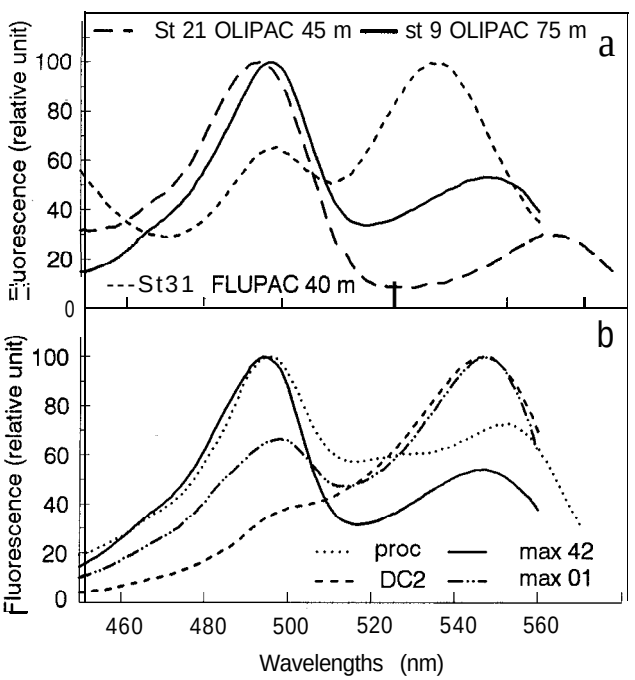

Figure 9. Comparison of the fluorescence excitation spectra of the usual and new phycoerythrin observed in the (a) equatorial Pacific with that of (b) cultured Synechococcus and *Prochlorococcus* (proc) (proc = Guillard Provasoli National Center for Culture of Marine Phytoplankton (CCMP) 1375) strains.

trations were observed throughout the water column in the more oligotrophic waters when the 0.1 μM nitrate isopleth was at or below 80 m as it was previously reported along 165°E *[Blanchot et al., 1992; Blanchot and Rodier,* 1996]. Surface abundance was clearly related to the rising of nitrate isopleths. Nitrate pulses into the euphotic zone of oligotrophic waters have been shown to promote the growth of *Synechococcus* 'marine populations [*Glover et al.,* 1988b, *Blanchot et al.,* 1992]. However, in the present study the maximum of *Synechococcus* abundance did not correspond to the highest nitrate concentration but rather to the 0.025-l μM range. The possibility that *Synechococcus* may fix molecular nitrogen in oceanic waters is still unresolved. None of the *Synechococcus* spp. strains belonging to marine cluster A as defined by *Waterbury and Rippka* [1989], i.e. open oceanic PE-containing strains, examined so far can fix nitrogen [Waterbury *et al,* 1986].

The PUB/PEB ratio in *Synechococcus* exhibited more geographical variations than vertical variations. It varied in the same range in both oligotrophic (1.56-2.00) and mesotrophic waters (1.5 1- 1.95). In the enriched area ($NO_3^- + NO_2^- > 1\mu M$ at the surface) it was lower along the equator (1.5 l-l .67) than along 150°W between the equator and 7°S (1.64-I .95). In this type of high-nutrient low-chlorophyll (HNLC) waters, nutrients do not seem to favor the development of high-PEB *Synechococcus* as they do in other eutrophic or mesotrophic areas *[Olson et al.,* 1988; *Lantoine and Neveux,*1997]. This could be explained by the fact that coastal high-PEB strains have higher requirements for iron, which has been recently

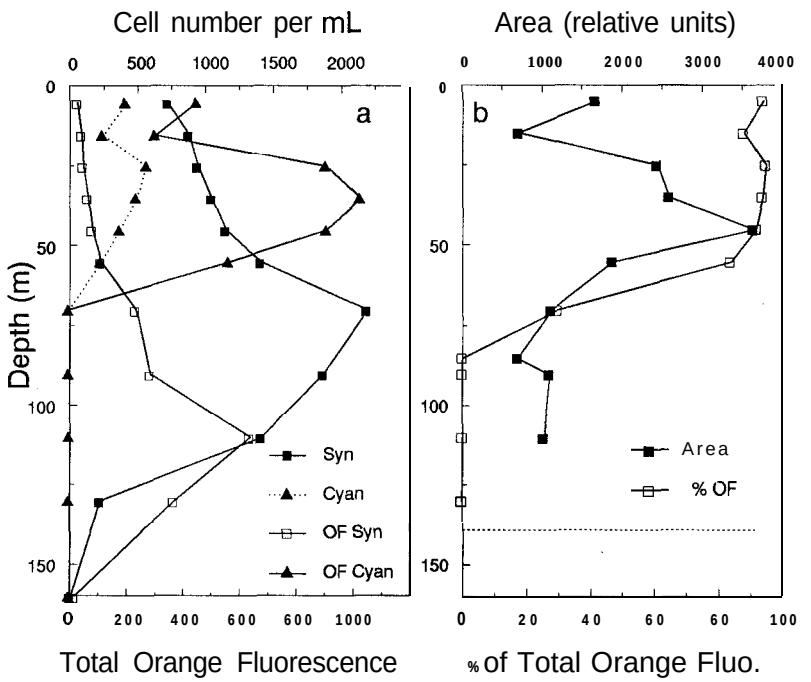

Figure 10. (a) Depth profiles of cell number and total orange fluorescence measured by flow cytometry (OF) far *Synechococcus* (Syn) and larger cyanobacteria (Cyan) at station 21 (OLIPAC: 16°S,150°W). (b) Depth profiles of the area below the phycoerythrin excitation spectrum between 450 and 560 nm of total phycoerythrin and of the percentage of total orange fluorescence associated with larger cyanobacteria. The horizontal dotted line corresponds to the deep chlorophyll maximum.

proved to limit phytoplankton growth in HNLC equatorial Pacific waters [*Coale et al.,* 1996]. Indeed, Brand [1991] showed that a Pacific strain of *Synechococcus* displayed a lower iron requirement than other *Synechococcus* isolates.

The observed PUB/PEB variability probably reflected the presence in various proportions of different *Synechococcus* genotypes having different PUB/PEB ratio *[Toledo and Palenik,* 1997] rather than qualitative changes of PE within the same genotype. However, the occurrence of subpopulations within *Synechococcus [Olson et al.,* 1988] was not evidenced by flow cytometry. Vertical variations could also be influenced by light since some strains can modify their PUB/PEB ratio according to changes in light conditions *[Wuterbury et al., 1986;* F. Lantoine and J. Neveux, unpublished data, 1995].

PE content (and orange fluorescence) per cell (for both *Synechococcus* and large cyanobacteria) increased with depth as a consequence of photoacclimation as already noted for *Synechococcus* in either mixed or stratified waters *[Clover et al.,* 1988a; *Wyman,* 1992; *Luntoine and Neveux,* 1997]. This explains why the correlation between PE and orange fluorescence was higher than the correlation with cell number.

The existence of unicellular cyanobacteria larger than *Synechococcus* in the open oligotrophic ocean had been reported previously in the central North Atlantic *[Glover et al.,* 1988a; *Li and Wood,* 1988] and in the surface waters of the southwestern *[Blanchot et al.,* 1990] and northwestern *[Ishizuku et al.,* 1994] tropical Pacific. At OLIPAC station 21 (16°S,150°W) they were clearly restricted to the top 70 m where temperature was between 26" and 29°C. Nitrate and ammonium were undetectable, even at the nanomolar level.

In contrast, phosphate concentrations were relatively high (LO.12 μM) throughout the water' column; on the basis of these observations, we speculate that the larger cyanobacteria may fix dinitrogen to support their growth. However, their relative low abundance probably reflected growth limitation by other essential elements like iron. Under low-iron conditions, cyanobacteria could improve iron fixation by producing extracellular siderophores and specific iron-siderophore membrane transport proteins as shown in a coastal marine strain (PCC 7002) of *Synechococcus [Trick and Wilhelm,* 1995]. Their compensation point could also be higher than in iron-replete conditions which could explain why they are restricted to the upper well-lighted layer *[Trick and Wilhelm,* 1995]. Restrictive growth temperature also could explain their limitation to the upper layer. These cells could be similar to those observed in the Pacific by one of us at 16°S,165°E during the Production Pélagique Pacifique (PROPPAC) 04 cruise *[Blunchot et al.,* 1990] and by *Ishizuku et al.* [1994] between 13° and 27°N along 175°E. In all these studies, very oligotrophic conditions prevailed (0.1 μM NO_3^- at 120 m), and large orange fluorescing cyanobacteria were counted by epifluorescence microscopy. At 16°S,165°E [*Blanchot et al.,* 1990] the size of cells varied from 2 up to 7 μm. They were only observed from the surface down to 60 m with a maximum abundance of 6 x 10^2 cell mL^{-1}. Unfortunately, no information was available on their PE characteristics.

The occurrence of these large cyanobacteria changed the size structure of the chlorophyll distribution. In the surface layer, more than 50% of Chl a was associated with cells larger than 1 μm, whereas in such oligotrophic areas a large dominance of the fraction <1 pm generally prevails, because of the presence of *Prochlorococcus* spp. *[Le Bouteiller et al.,* 1992]. The presence of an unusual PE in these large cyanobacterial cells in conjunction with flow cytometry analysis could provide a useful taxonomical marker to identify them and to de-

Table 1. Excitation and Emission Wavelength Maxima for the Different PE Discussed in This Paper

	Exc PUB (nm)	Exc PEB (nm)	PUB/PEB	Em PE (nm)	Reference
All samples (*Synechococcus*)	496	549	1.58-1.98	570	this study
Large cyanobacteria	494'	564'	3*	576'	this study
Three samples	498*	536'	0.65-1.20	570	this study
Trichodesmium	*495*	*547* and 565	?	573	*Fujita and Shimura [*1974]
Prochlorococcus	*496*	*552*	*1.38*	*568*	this study; *Hess et al. [*1996]

The abbreviations are defined as PE, phycoerythrins; Exc, excitation; Em, emission; PUB, phycourobilin; PEB, phycoerythrobilin.
*Indicates a departure from standard *Synechococcus* characteristics.

termine their spatial and temporal distribution. In some respects their ecological habitat resembles to this of *Trichodesmium* spp. [*Capone et al.,* 1997]. Further experimental study or the use of genetic markers (e.g., nitrogen fixation probes) will be necessary to determine whether they can fix dinitrogen. Their high-PUB PE is characterized by the predominance of the first fluorescence excitation peak at 494 nm over the second peak related to PEB at 564 nm. Until now, high-PUB-type PE have only been observed in cyanobacteria. In addition to some *Synechococcus* (e.g. WH8103 strain [*Ong et al.,* 1984]), N_2-fixing *Synechocystis* spp. isolated from coastal tropical waters by *Waterbury and Rippka [* 1989] present a very high-PUB PE. However, in all these species the second excitation peak of PE is around 547-550 nm *[Ong et al.,* 1984; *Swanson et al.,* 1991], distinctly at shorter wavelengths than that of the large cyanobacteria observed during OLIPAC.

The presence of PUB confers to all PE-containing organisms an absorption peak that shows relatively little variability in its position (496 ± 2 nm). The second peak related to the presence of PEB appears more variable depending on the conformation of the native PE. Schematically, PEB can lead to one or two absorption peaks (one of these two peaks can be reduced to a shoulder) in the range between 530 and 570 nm [*Rowan,* 1989]. For instance two absorption peaks originate from PEB in *Trichodesmium thiebautii (547* and 565 nm *[Shimura and Fujita,* 1975]) whereas only one peak is generally observed in *Synechococcus* spp. (around 547-551 nm) (see review by *Rowan* [1989]). The occurrence of PE with two peaks either at 494 and 564 nm (Station 21 OLIPAC) or at 496 and 536 nm (Station 31 FLUPAC) represents a potentially unique characteristic which, to our knowledge, has not been observed previously either in fhe field or in cultures. Figure 9 and Table 1 compare the novel PE with those found in various strains of small *Synechococcus* in culture. Almost all samples in the present studies have PE spectral characteristics similar to those of max 42, a strain isolated at depth from the Sargasso Sea. During these two cruises we have not found any evidence of high-phycocyanin cyanobacteria, confirming previous investigations in the open ocean *[Murphy and Haugen, 1985; Waterbury et al., 1986; Campbell and Carpenter, 1987; Lantoine and Neveux,* 1997]. The presence of PUB in the PE of oceanic cyanobacteria (filamentous or solitary) is considered as a chromatic adaptation to blue light which penetrates deeper than other wavelengths in the water column *[Wood,* 1985] and would allow the sustaining of photosynthesis down to 100-120 m in oligotrophic regions. If the high-PUB-containing *Synechococcus* can be effectively observed from the surface down to 120 m depth, the high-PUB larger cyanobacteria seem confined to shallower and better lit layers (O-70 m). This suggests that the ecological requirements and physiological properties of the two cell types may be different.

The diversity of PE in oceanic waters clearly appears to be more important than was previously assumed. Recently, low amounts of a new type of PE have been reported in *Prochlorococcus* spp. (strain CCMP 1375 *[Hess et al.,* 19961). Its fluorescence excitation spectrum shows two peaks at 496 and 552-553 nm and a shoulder near 527 nm (50% glycerol: Figure 9 and Table 1), and its emission spectrum shows a peak at 568 nm. In *Prochlorococcus* cultures, PE spectrum can only be detected, however, after concentrating cells illuminated by low light *[Hess et al.,* 1996]. We tried to measure *Prochlorococcus* PE spectrum during the OLIPAC cruise on samples collected at 130-140 m, depths at which the orange fluorescence of *Prochlorococcus* PE was clearly detected by flow cytometry *[Hess et al.,* 1996]. *Prochlorococcus* cells from 10 L of seawater were collected on GF/F after pretiltration through 0.6 μm (to eliminate *Synechococcus),* but no signal could be detected, most likely because the intracellular PE concentration and the cell abundance (13 x 10^3 cells in 10 L at 130 m) were too low.

Phycobiliprotein spectral characterization can constitute a useful tool for taxonomical and ecological studies. The difficulty of analyzing them could be overcome, at least partly, with a better knowledge of the diversity of PE in the ocean and of the effect of environmental factors on the PE characteristics of cultured strains. With such data at hand it would then be possible to determine the contribution of the different PE types from 3-D fluorescence spectra of bulk PE using least squares approximation or other spectral decomposition methods. The recognition of new types of PE should encourage efforts to isolate in culture the organisms that synthesize them. This step is essential to investigate further the physiology and the role of these organisms in marine ecosystems and to understand why the larger cyanobacteria observed at 16°S-150°W are apparently restricted to highly oligotrophic and illuminated waters.

Acknowledgments. The authors wish to thank the officers and crew of the R/V *Atalante* for their help during the EPOPE France-JGOFS studies, the chief scientists of FLUPAC, R. Le Borgne, and OLIPAC, B. Coste, as well as Y. Dandonneau for coordinating the EPOPE program and the guest editors for improving the final version of the manuscript. The chemical data are due to the courtesy of M. Rodier and P. Raimbault, and the CTD data are due to that of C. Bournot, D. Tailliez, G.Eldin, F. Gallois and J. Y. Panche. This work

was financed by the Université Pierre et Marie Curie, the Centre National de la Recherche Scientifique (INSU, UMR 7621), the Institut Français pour la Recherche et l'Exploration de la Mer (IFREMER) and the Institut Français de la Recherche Scientifique pour le Développement en Coopération (ORSTOM). DV and DM were partially supported by MAST contract CT95-0016 (MEDEA) as well as the ACC-SV7 BioSystematics network.

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(Received August 29, 1997; revised May 29, 1998;
accepted June 12, 1998.)