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Spectral absorption coefficient of photosynthetically active pigments in the equatorial Pacific Ocean (165°E–150°W)

C. DUPOUY,*‡ J. NEVEUX† and J. M. ANDRÉ*

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Abstract—Spectral absorption coefficients of total particulate material and detritus were measured throughout the euphotic zone along the equator between 165°E and 150°W and during time-series for each of these two longitudes in October 1994 (JGOFS–FLUPAC cruise). The sum of pigments obtained by spectrofluorometry (tChl*a*=DV−chl*a*+Chl*a*) was used for normalization (and was also compared to fluorometric and HPLC measurements as an intercalibration study). In order to assess the specific absorption coefficient of photosynthetically active pigments (a^*_{ps}) from the pigment-specific absorption coefficient for phytoplankton (a^*_{ph}), we made a multiple regression analysis of measured phytoplankton absorption spectra onto published *in vivo* spectra of pure pigments. This made it possible to calculate the concentrations of photoprotective carotenoids (tPPC) when HPLC measurements were not available and thus to subtract their contribution to absorption from the total phytoplanktonic absorption coefficient (a_{ph}). Methodological uncertainties in both coefficients used for calculating absorption coefficients and in pigment measurements are discussed. Pigments and absorption measurements made during the cruise enabled us to describe two typical trophic regimes in the equatorial Pacific ocean: oligotrophic waters of the "warm pool" west of 170°W and high-nutrient, low-chlorophyll waters (HNLC) of the upwelling east of 170°W. The vertical decreasing gradient of a^*_{ph} from the surface to the deep chlorophyll maximum (DCM) was due to a high tPPC/tChl*a* ratio at the surface and was higher in the oligotrophic (0.14–0.065 m^2 mg (tChl *a*)$^{-1}$ biomass dominated by *Prochlorococcus*, rich in zeaxanthin) than in the mesotrophic area (0.07–0.06 m^2 mg (tChl *a*)$^{-1}$ biomass dominated by picoeucaryotes). Below the DCM, a^*_{ph} reached a similar minimum value in both oligotrophic and mesotrophic areas. a^*_{ps} varied less than a^*_{ph} from the surface layer to the DCM in both oligotrophic and mesotrophic areas. The difference in a^*_{ph} and a^*_{ps} from west to east of the transect could be interpreted as a shift in the phytoplankton composition, with a dominance of procaryotes in the west and a dominance of eucaryotes in the upwelling area. Higher a^*_{ps} in well-lit typical oligotrophic waters indicated that phytoplankton communities dominated by *Prochlorococcus* might be more efficient for capturing light usable for photosynthesis than those present in the HNLC situation. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Modelling primary production of the ocean at a global scale from remote sensing pigment data and using light-dependent models of photosynthesis requires precise values of the pigment-specific absorption coefficient of phytoplankton (a^*_{ph}) and of the maximum photosynthetic quantum yield in addition to information on their regional variability

* Centre ORSTOM de Nouméa BP A5, 98948 Nouméa, New Caledonia.
† Observatoire Océanologique de Banyuls, Laboratoire d'Océanographie biologique (URA 2071) and Université Pierre et Marie Curie, BP 44, 66651 Banyuls, France.
‡ Corresponding author.

(Kiefer and Mitchell, 1983; Mitchell and Kiefer, 1988a,b; Platt and Sathyendranath, 1988; Balch *et al.*, 1989; Cleveland *et al.*, 1989; Sakshaug *et al.*, 1991; Morel, 1991; Bricaud *et al.*, 1995; Cleveland, 1995; Babin *et al.*, 1996). Bio-optical models may be improved by partitioning a^*_{ph} into components describing photosynthetically active (a^*_{ps}) and photoprotective (a^*_{ppc}) absorption by phytoplankton (Sosik and Mitchell, 1995; Lazzara *et al.*, 1996; Sosik, 1997; Allali *et al.*, 1997).

The Pacific Ocean equatorial region has two contrasting physical and trophic situations, the oligotrophic warm pool in the western part of the basin (150°E–160°E), and a zone of permanent nitrate enrichment due to upwelling in its central and eastern parts (140°W–90°W). Because this enrichment lasts the entire year and often extends as far west as 165°E (Radenac and Rodier, 1996), the estimation of its overall primary production is one of the major objectives of the JGOFS program. The equatorial upwelling is a case of HNLC (high nutrient low chlorophyll) waters, where picoplankton dominate and where nitrate greatly determines the size structure of the community (Le Bouteiller *et al.*, 1992). *In situ* primary production values reach 1 g C m^{-2} day^{-1} (Murray *et al.*, 1994; Le Bouteiller, 1995) even though chlorophyll biomass is evenly low along the equator (<0.4 mg m^{-3}, Le Bouteiller and Blanchot, 1992). The most recent estimate of primary production using a bio-optical model applied to ocean colour imagery was about 0.5 g C m^{-2} day^{-1} (Antoine *et al.*, 1996). As a first step to improving the application of this model, we defined (Dupouy *et al.*, 1993) the limits of the equatorial enrichment from nutrients observations at the surface ($NO_3+NO_2>0.1$ μM) and from the chlorophyll biomass detected by the satellite ($C_{sat}\geq 0.12$ mg m^{-3}, i.e. contained in the first optical depth (Gordon *et al.*, 1983)). The second step was to measure mean pigment-specific absorption coefficients for waters in and out of the upwelling.

Measurements of *in vivo* absorption spectra within tropical waters are scarce (Marra and Bidigare, 1994; Lindley *et al.*, 1995; Lazzara *et al.*, 1996; Allali *et al.*, 1997). Because of its simplicity at sea, the GF/F filter technique appears to be the easiest method, provided that an effective correction of the pathlength amplification factor through the filter, the "β effect", can be made (Mitchell, 1990; Bricaud and Stramski, 1990; Moore *et al.*, 1995). This technique yields absorption by total particles, $a_p(\lambda)$, which can be defined as:

$$a_p(\lambda) = a_{ph}(\lambda) + a_d(\lambda) \tag{1}$$

with

$$a_{ph}(\lambda) = a_{ps}(\lambda) + a_{ppc}(\lambda) \tag{2}$$

where the subscripts p, ph, d, ps and ppc denote particulate, phytoplankton, detritus, photosynthetic carotenoids and photoprotective carotenoids, respectively. $a_d(\lambda)$ must be determined separately either by direct measurement (Kishino *et al.*, 1985, 1986) or by using numerical decomposition methods (Morrow *et al.*, 1989; Roesler *et al.*, 1989; Bricaud and Stramski, 1990). $a_{ps}(\lambda)$ is the fraction of cellular absorption due to photosynthetically active pigments (without photoprotective carotenoids). This fraction can be deduced from measurements of *in vivo* fluorescence spectra (Maske and Haardt, 1987; Mitchell and Kiefer, 1988a,b; Sakshaug *et al.*, 1991; Sosik and Mitchell, 1991, 1995; Lazzara *et al.*, 1996). An alternative method is the use of mathematical reconstruction techniques based on HPLC pigment measurements (Sosik and Mitchell, 1991; Hoepffner and Sathyendranath, 1993; Nelson *et al.*, 1993; Lindley *et al.*, 1995).

To make comparisons over a range of chlorophyll biomass we present the absorption coefficients normalized to tChl a (e.g. $a_p^*(\lambda) = a_p(\lambda)/\text{tChl } a, \text{m}^2 \text{ mg (tChl } a)^{-1}$). The a_{ph}^* value is determined by total intracellular pigment concentration and cell size that influence the "package effect" (Kirk, 1975; Morel and Bricaud, 1981; Sathyendranath *et al.*, 1987). The a_{ps}^* value combines previous influences except those of non-photosynthetic (photoprotective) carotenoids. Recent results obtained on phytoplanktonic cultures show that the total intracellular pigment content and the proportion of photoprotective pigments can vary in response to light quantity and quality. This is true for large cells (Berner *et al.*, 1989; Sosik and Mitchell, 1991; Stramski and Reynolds, 1993) as well as for picoplankton (Kana and Glibert, 1987; Bidigare *et al.*, 1989; Stramski and Morel, 1990; Partensky *et al.*, 1993; Morel *et al.*, 1993; Moore *et al.*, 1995). The FLUPAC cruise gave us a unique opportunity to measure the absorption properties of essentially picoplanktonic cells along a zonal gradient of physical and biological conditions at the equator, and to interpret their variations in relation to pigment and cell composition.

The objectives of this paper are (1) to describe *in situ* absorption coefficients and pigments of phytoplankton in the photic zone from the west to the center of the Pacific Ocean (165°E to 150°W) in September–October 1994 during the equatorial JGOFS–FLUPAC cruise, (2) to compute with a spectral decomposition method the photosynthetically active part of the absorption coefficient spectra, and (3) to examine the influence of factors such as pigments and planktonic composition on the absorption properties of the phytoplankton community.

2. MATERIAL AND METHODS

Water collection

During the FLUPAC cruise on the R.V. *Atalante*, (24 September–26 October 1994) stations were occupied every degree along a meridional transect at 165°E from 15°S to 6°N and during a 6-day time-series at 165°E with a 3–4 hour regular sampling interval. Then, a transect at the equator was carried out at regular time intervals from 167°E to 150°W (alternatively at 12:00 a.m and pm, local time) and was followed by a second 7-day time-series at 150°W, as detailed in Le Borgne *et al.* (1995). PAR was measured by a QSP-200 in water and a QSR-240 on deck (Biospherical Instruments Inc.). Samples collected from 12 depths with Niskin bottles were analysed for JGOFS core parameters, among them nutrients (Oudot and Montel, 1988), chlorophyllous pigments by fluorometry (100 ml) and spectrofluorometry (500 ml). Water collected with NOEX bottles was used for total particulate absorption measurements (5 liters) and for HPLC pigment analysis (2 liters). Absorption measurements were performed at every station, except at 3:00 a.m., at about 7 depths from 0 to 180 m. A total number of 620 *in vivo* particulate spectra were measured on board.

Absorption measurements

Seawater was slowly filtered onto 25 mm glass-fiber filters (Whatman GF/F) at <25 hPa vacuum pressure. Whatmann GF/F were used for their retention efficiency since they retain 99% of *Prochlorococcus*, 100% of *Synechococcus* and 100% of picoeucaryotes (Blanchot and Rodier, 1996). Absorption spectra of the particles collected on the filters were measured

immediately (OD_{fp}) with a dual-beam Beckman DU-26 spectrophotometer. The filters were placed near the detector so that loss of light by scattering was minimized. The baseline was measured by using two GF/F filters soaked for 30 minutes in filtered (0.2 μm) seawater. In these conditions of water saturation, small variability (less than 1% maximal OD_{fp}) between filters was observed in the blue part of the spectrum. During sample measurement one of the blank filters was placed in front of the reference beam. The same soaked blank filter was used for the 7–11 samples of one station, and a new blank filter was taken for each station. All samples of a station were scanned within 1/2 hour to keep constant the optical quality of the blank. Optical densities were recorded from 800 to 350 nm with a resolution of 0.1 nm. A cubic spline function (FORTRAN NAG library) was used to smooth and resample spectra every 1 nm. A median filter was then applied to eliminate the few noisy residual peaks. Optical density at 790 nm was considered as a residual signal independent of the absorption by algal pigments and was subtracted from the whole spectrum. In order to test the linearity between optical density and collected chlorophyll biomass, we filtered a range of 1 to 7 liters seawater from a sample taken at the deep chlorophyll maximum. Above the optical density value of 0.35, corresponding to a filtered volume of 3 l (1.5 μg tChl*a*), we observed a saturation of $OD_f(440)$, reaching 20%. Only 25% of the $OD_{fp}(440)$ values measured during the FLUPAC cruise were above 0.35, and only 2 values exceeded the 0.2–0.4 OD_{fp} range as recommended by Mitchell (1990). After measurements of the particulate absorption spectra, the filters were frozen on board at −60°C and then stored (at the laboratory at −20°C) before analysis of the absorption due to the detrital part ($OD_{fd}(\lambda)$), as in Kishino *et al.* (1985). Filters were kept three hours in 95% methanol until complete extraction of the liposoluble pigments (no absorption signal at 673 nm), and the residual absorption spectra were recorded.

The measurements OD_f were converted to the equivalent absorption of a suspension OD_s using the quadratic equation proposed by Mitchell (1990):

$$OD_s = AOD_f + B(OD_f)^2 \quad (3)$$

which corrects for pathlength amplification due to scattering by the filter. Coefficients were determined by comparing optical densities of phytoplankton measured on the GF/F filters (affected by the β effect) and using the modified freeze transfer technique method (Allali *et al.*, 1995). Coefficients were 0.346 and 0.369 for A and B, respectively ($r = 0.94$, $n = 57$ spectra, Allali *et al.*, 1997). Similar coefficients were obtained when $OD_f(440)$ values greater than 0.35 were eliminated from the regression. These coefficients give significantly lower absorption values than the previously published ones (Mitchell *et al.*, 1990; Cleveland and Weidemann, 1993; Hoepffner and Sathyendranath, 1993; Arbones *et al.*, 1996). They are similar to the ones obtained for cultures of *Prochlorococcus*, such coefficients being attributed to a greater pathlength amplification factor in the filter by small cells (Moore *et al.*, 1995).

The package effect theoretically defined by Morel and Bricaud (1981) expresses the saturation of the Chl-specific absorption coefficient (a^*_{ph}) with the increase of the intracellular pigment content and the cell size. The package effect index was computed as the ratio of $a^*_{ph}(675)$ to $a^*_{sol}(675)$, which is the absorption of pigment material dispersed in solution. The value of 0.0207 $m^2\ mg^{-1}$ ($a^*_{sol}(675)$ in 90% acetone, Morel and Bricaud, 1981) was taken in this study. It was corrected from the influence of Chl*b* and DV-chl*b* at 675 nm, as in Bricaud *et al.* (1997). The blue to red ratio (B/R) of a^*_{ph} was computed as the ratio of the maximum absorption coefficient in the blue spectral range (440–450 nm) vs. the maximum

one in the red (670–680 nm). It reflects, at least for phytoplankton in cultures, the relative proportion of accessory pigments (non photosynthetic and photosynthetic pigments) to chlorophyll (Partensky *et al.*, 1993, Moore *et al.*, 1995), and is influenced by the package effect.

Pigments

Pigments were collected for each station by filtration on 47 mm (spectrofluorometry) and 25 mm (fluorometry) Whatman GF/F filters. In the fluorometric method, the extraction was done in 93% methanol without grinding of the filters, whereas in the spectrofluorometric method extraction was done in 90% acetone and included the grinding of the filters. The fluorometric analysis used fluorescence measurements before and after acidification (HCl 0.5 N) on methanolic extracts (Le Bouteiller *et al.*, 1992) with a Turner Model 112 fluorometer. The spectrofluorometric analysis used fluorescence measurements at 24 fixed excitation and emission wavelengths on acetonic extracts with a Perkin-Elmer MPF 66 spectrofluorometer operated in the ratio mode (Neveux and Lantoine, 1993). This latter technique enabled us to discriminate divinylchlorophylls *a* and *b* (DV-chl*a* and DV-chl*b*) associated with *Prochlorococcus marinus* (Chisholm *et al.*, 1988; Neveux *et al.*, 1989; Goericke and Repeta, 1992) from the other chlorophylls ("normal" monovinyl chlorophylls *a* and *b* and chlorophyll *c*) and from the phaeopigments deriving from all chlorophylls.

Additional information was obtained on some samples (Claustre, *unpublished results*) by using reversed phase HPLC pigment analysis (Vidussi *et al.*, 1996). At 23 oligotrophic stations and 20 mesotrophic stations, water from three depths (above, below and at the depth of the chlorophyll maximum (DCM)) was filtered on 25 mm Whatman GF/F, and filters were frozen in liquid nitrogen before analysis at the laboratory. Pigments were extracted in methanol. This technique allowed all chlorophylls and different carotenoids (photoprotective (tPPC) and photosynthetic (tPSC)) to be quantified. In this paper, measured concentrations of zeaxanthin for procaryotes and diadinoxanthin for picoeucaryotes were summed as total tPPC concentrations. Measured 19'HF and 19'BF for nano- and pico-flagellates, fucoxanthin for diatoms and peridinin for dinoflagellates were considered as total tPSC.

Cellular pigment content. In order to illustrate vertical variations in the cellular pigment content of both picoplankton groups *Prochlorococcus* and picoeucaryotes, the DV-chl*a* and Chl*a* concentrations were divided, respectively, by the total number of *Prochlorococcus* cells and the total number of picoeucaryotes counted by flow cytometry (*Synechococcus* cells is a relatively minor contributor to phytoplankton chl*a* biomass (< 1%) in the equatorial Pacific ocean, Blanchot and Rodier, 1996). The proportion of Chl*a* associated to total picoeucaryotes (0.8–3 μm) was considered to be constant i.e. 55% of the total Chl*a* concentration (Navarette, 1997).

Normalization of absorption coefficients

In the present study, the sum of Chl*a*+DV-chl*a* determined by spectrofluorometry ($tChla_{sp}$) was used to normalize absorption data since it was available for each station and depth and allowed chlorophyll concentrations to be determined free of influence by phaeopigments. However, the absorption results also were compared with those obtained

by more usual normalization (sum of Chl*a*+Phaeopigment *a* assessed by fluorometry o sum of Chl*a*+DV-Chl*a* determined by HPLC: tChla_{HPLC}) (see Section 4).

Spectral decomposition of phytoplankton absorption spectra

It has been shown that estimates of *in vivo* absorption spectra can be reconstructed from the concentrations in seawater of five pigment groups: tChl*a* (chl*a*+DV-chl*a*), tChl*b* (chl*b*+DV-chl*b*), chl*c*, photosynthetic carotenoids (tPSC), photoprotective carotenoids (tPPC) (Bidigare *et al.*, 1990). Such a reconstruction technique has been widely used (Sosik and Mitchell, 1991; Nelson *et al.*, 1993; Lindley *et al.*, 1995). Conversely, derivative analysis (Bidigare *et al.*, 1989) or decomposition in gaussian curves (Hoepffner and Sathyendranath, 1993) of the phytoplankton *in vivo* absorption spectrum has been attempted to estimate the contributing concentrations of pigments. We develop here a simple method to retrieve concentrations of the above five pigment groups (tChl*a*, tChl*b*, Chl*c*, tPSC, tPPC) by using a multiple linear regression analysis of *in vivo* phytoplankton absorption spectra ($a_{ph}(\lambda)$) knowing *in vivo* absorption spectra of pure pigments (*in vitro* absorption coefficients spectrally shifted to match *in vivo* coefficients) determined by Bidigare *et al.* (1990). This allows in particular the photoprotective and photosynthetic carotenoids concentrations to be assessed as only a few HPLC measurements were available during FLUPAC. The method assumes that the package effect is negligible. Retrieved chlorophylls were compared to our spectrofluorometric measurements ($N=378$) and retrieved photosynthetic and photoprotective carotenoids were compared with the few HPLC measurements ($N=73$). Results of linear regressions between measured and computed pigment concentrations are found in Table 1. For tChl*a*, the most significant absorber, the agreement is rather good, with values 5% lower on average than spectrofluorometric ones. For pigments with less contribution, there is a significant bias. Retrieved photosynthetic carotenoids are 4% lower and photoprotective ones are 31% higher than HPLC measurements. An interesting point is that, for tChl*a* as well as for both carotenoids, the relative difference between measured and retrieved pigments does not show any tendency over the vertical. For tChl*b* the agreement is good only when this pigment is in significant concentration, i.e. below the euphotic layer (for concentrations >0.1 mg m^{-3}). Our decomposition method failed to compute Chl*c*. Results of a test of the decomposition method are shown for Station 65. The vertical distribution of measured and retrieved pigments is shown in Fig. 1a. Spectra have been reconstructed (as

Table 1. Slopes, intercepts (Y-int) and correlation coefficients r *for linear regressions between measured and computed pigment concentrations. Chlorophyll concentrations are measured by spectrofluorometry (as in Neveux and Lantoine, 1993) and photosynthetic (tPSC) and photoprotective (tPPC) carotenoids are measured by HPLC (as in Williams and Claustre, 1991). Computed pigment concentrations result from the decomposition method of the absorption spectra of phytoplanton* a_{ph} *(see text)*

Measured vs. computed	Slope	*Y*-int	*r*
tChl*a*	*0.95*	−0.02	0.88
tChl*b*	0.57	0.05	0.74
tChl*c*	–	–	<0.5
tPPC	0.67	0	0.71
tPSC	*0.96*	0.02	0.81

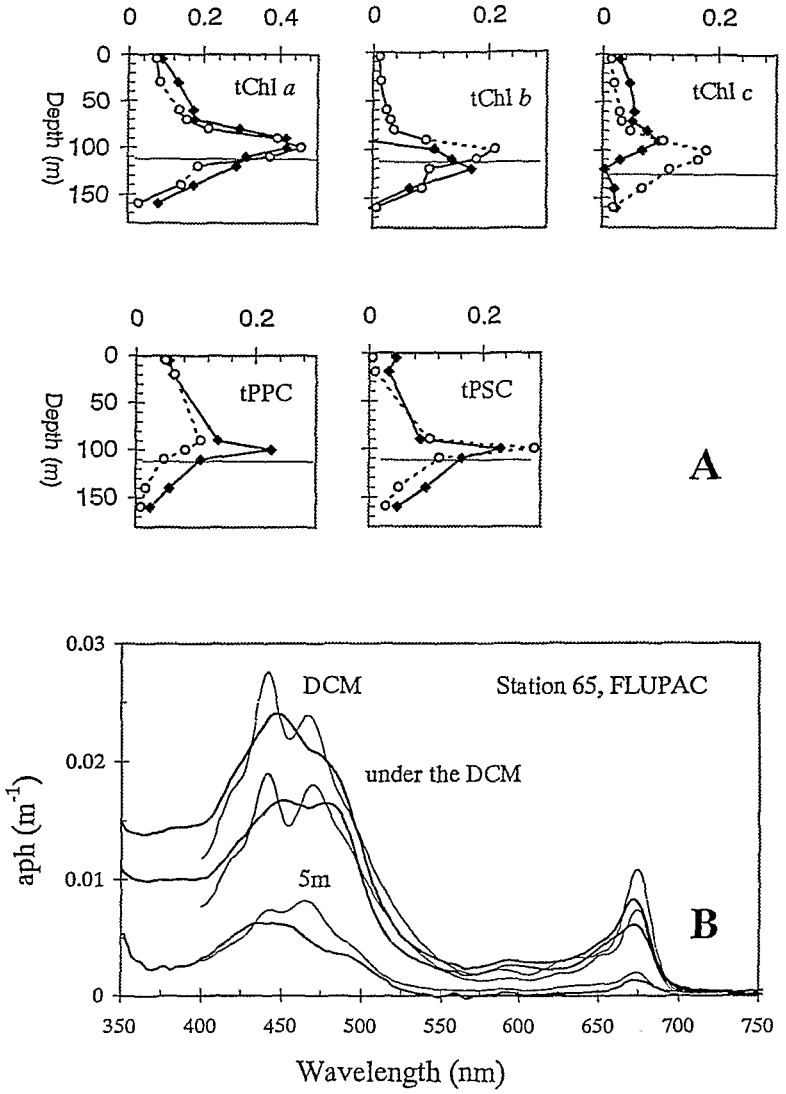

Fig. 1. Station 65 (A) Vertical profiles of computed pigment concentrations as determined with the decomposition method (full diamonds) compared to measured pigment concentrations (open circles): spectrofluorometrically determined-chlorophylls and HPLC determined photoprotective carotenoids (tPPC) and photosynthetic carotenoids (tPSC) (B) Measured (full line) and reconstructed (dashed line) phytoplankton absorption spectra [$a_{ph}(\lambda)$, m^{-1}] from tChl*a*, tChl*b*, tChl*c*, tPSC, tPPC given by the decomposition method and pure pigment spectra by Bidigare *et al.* (1990) at three typical depths.

linear combinations of the 5 pigment concentrations computed from the decomposition method and of the pure pigment spectra) and compared with measured ones at a few depths (Fig. 1b). The difference observed at the blue peak is surprisingly small when considering both errors in absolute *in vivo* absorption coefficients of pigments and in the neglected

package effect, which is greatest at this wavelength (Sosik and Mitchell (1991); Nelson *et al.* (1993)).

This decomposition method consequently was successful in retrieving four of the main pigment groups, tChl*a*, tChl*b*, photosynthetic and photoprotective carotenoids, as it provides concentrations with an uncertainty of about 30%, which is about the uncertainty observed when comparing concentrations of pigments obtained by different methods (see our discussion). Assuming that Chl*c* is well measured by spectrofluorometry, its retrieval is not achieved by the decomposition method, probably because of the large overlapping of its blue peak with the one of Chl*b*, together with its low signal in the red part of the spectrum. Although obviously imperfect, the proposed method is good enough to estimate pigments and in particular the photoprotective carotenoids concentrations. The absorption due to the photosynthetically active pigment $a_{ps}(\lambda)$ was achieved as:

$$a_{ps}(\lambda) = a_{ph}(\lambda) - t\text{PPC}^{*}\alpha_{\text{PPC}}(\lambda) \quad (4)$$

where $\alpha_{\text{PPC}}(\lambda)$ represents the *in vivo* specific absorption of the photoprotective carotenoids and (tPPC) their concentration obtained by the decomposition method. Since our tPPC concentrations are greater by 31% than HPLC determinations, our a^{*}_{ps} values represent lower-bound estimates.

RESULTS

General environmental characteristics

Physical and chemical conditions encountered during the FLUPAC cruise have been discussed in detail in other papers (Eldin *et al.*, 1997; Menkes *et al.*, submitted). Mean distributions of cell abundance and pigment concentrations are to be found in Blanchot *et al.* (submitted). These aspects are briefly discussed below. Oligotrophic waters west of 170°W, represented by station 65 (Fig. 2a), are characteristic of the warm pool. Nitrate was undetectable above the pycnocline at 80–90 m. The 1% light level was located at 110 m. The cell vertical distribution of the three picoplanktonic groups showed maxima clearly above their pigment maxima. The maximal cell abundance for *Prochlorococcus* (1.9×10^5 cells ml^{-1}) was at 70 m, above that of the DV-chl*a* (90 m). The DV-chl*b* peaked much deeper (110 m). The maximal cell abundance of *Synechococcus* (4×10^3 cells ml^{-1}) and picoeucaryotes (3×10^3 cells ml^{-1}) were at 90 m, above the Chl*a*, *b* and *c* peaks (100 m). (Fig. 2b). Consequently, the tChl*b*/tChl*a* ratio abruptly increased from 0.2 to 0.5 at 90 m, depth of the DCM (Fig. 2c). Phaeopigments *a* represented 10% of the tChl*a*. The mean tPPC concentration (10 stations) mainly composed of zeaxanthin decreased from the surface down to the euphotic depth (0.075 to 0.05 mg m^{-3}) while diadinoxanthin was less concentrated and constant from the surface to the 1% light depth (not shown).

For typical upwelled waters of the HNLC situation (Station 77, Fig. 3a), east of 170°W, the principal pycnocline was as deep as 120 m and nitrate varied between 2 and 4 μM throughout the whole euphotic layer. The 1% light level was at 85 m. The distribution of the three picoplanktonic groups has a broad sub-surface maximum around 20–40 m depth (*Prochlorococcus*, *Synechococcus*, picoeucaryotes: 1.5×10^5, 1×10^4 and 6×10^3 cells ml^{-1}). All photosynthetic pigment concentrations had maxima between 40 and 60 m in the upper mixed layer (Fig. 3b) except that of the DV-chl*b*, which peaked at 80 m (0.15 mg m^{-3}) and decrease linearly to 0.06 mg m^{-3} by 140 m. Phaeopigments *a* represented less than 10% of

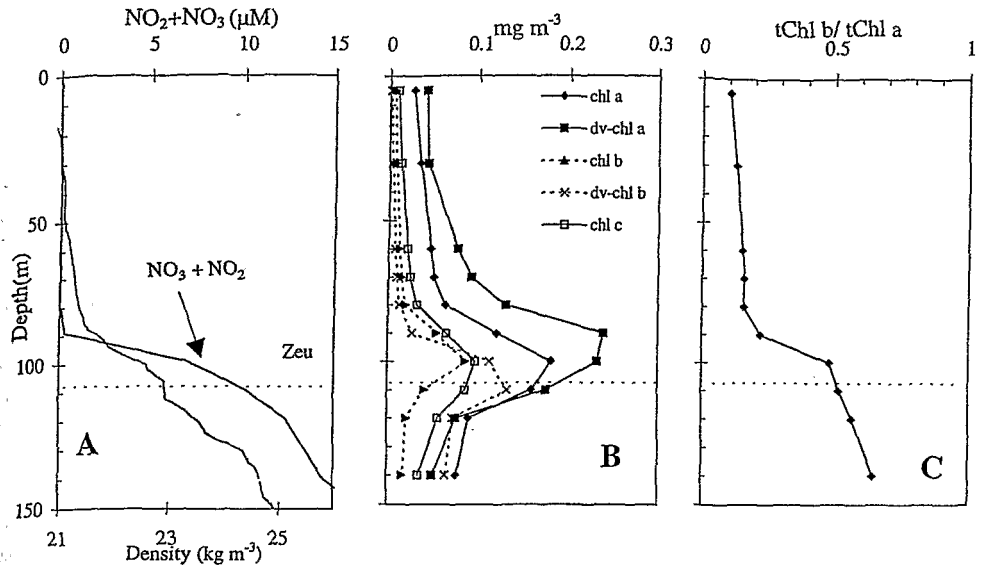

Fig. 2. Vertical distributions at a typical oligotrophic station during the FLUPAC cruise (10 October 1994); station 65, Equator, 175°62'E, local time: 11 h 42 (A) $NO_3 + NO_2$ concentration at the 0.1 µM limit of detection, and density excess (σ_t) (B) Chlorophyllous pigments as measured by spectrofluorometry (C) ratio of the tChl*b*/tChl*a* concentrations. The dashed line indicates the bottom of the euphotic zone Z_{eu}, i.e. where PAR is reduced to 1% of the value just under the surface.

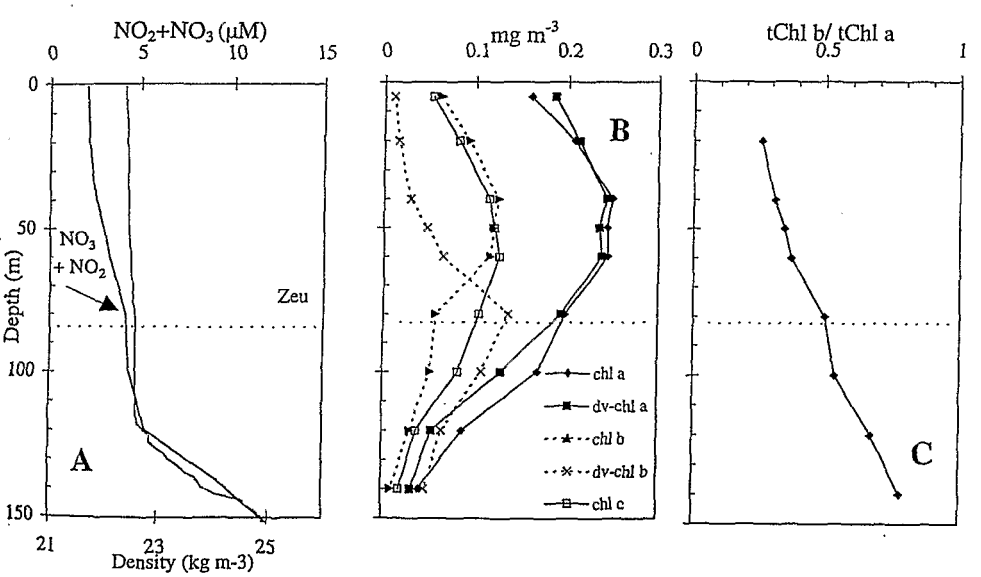

Fig. 3. Vertical distributions at a typical mesotrophic station during the FLUPAC cruise (16 October 1994); station 77, Equator, 160°60'W, local time: 10 h 42 (A) $NO_3 + NO_2$ concentration at the 0.1 µM limit of detection, and density excess (σ_t) (B) Chlorophyllous pigments as measured by spectrofluorometry (C) the ratio of tChl*b*/tChl*a* concentrations. The dashed line indicates the bottom of the euphotic zone Z_{eu}, i.e. where PAR is reduced to 1% of the value just under the surface.

tChl*a*. The tChl*b*/tChl*a* ratio was 0.2 at 20 m and increased linearly with depth to a value of 0.8 at 140 m (Fig. 3c). The photoprotective carotenoids decreased from the surface down to the euphotic depth. This decrease was essentially associated with zeaxanthin (0.1 to 0.02 mg m^{-3}), since the diadinoxanthin was quite uniform from the surface to the 1% light depth (not shown).

Contributions of detritus, photoprotective and photosynthetic pigments to a_p^*

tChl*a*-specific particulate absorption spectra from 167°E to 150°W are shown separately for the well-lit surface layer (5–20 m) and for the deep layer below the DCM (<0.5% PAR: 100–120 m) in Figs 4A–D. Mean spectra of a_p^* in the two layers differ in absolute values as well as in shape. In the well-lit surface layer, they show a blue maximum near 440 nm, while at depth, they exhibit an additional secondary peak at 480 nm and a broader red absorption band. These spectral modifications result from the relative increase with depth of the photosynthetic accessory pigments (mainly tChl*b* and photosynthetic carotenoids: Fig. 2b and 3b) to tChl*a* ratio related to chromatic photoadaptation of algae to blue-green low light, as observed previously (Bricaud and Stramski, 1990; Partensky *et al.*, 1996; Lazzara *et al.*, 1996: Allali *et al.*, 1997). Detrital absorption spectra for oligotrophic region (Fig. 4A) can be fitted with exponential functions as:

$$a_d(\lambda) = \lambda^{(-C)} \quad (5)$$

with a mean value of C of 0.009 nm^{-1} (Fig. 4A). For the mesotrophic region, the $a_d^*(\lambda)$ can not be fitted (Fig. 4B). Specific absorption spectra by phytoplankton (a_{ph}^*) differ from the spectra of a_p^* mainly in the blue, since the largest detritus contribution was in this range. A few a_{ph}^* spectra recorded in the euphotic layer showed a significant absorption maximum at 390 nm, as had been observed already in samples of open ocean waters (Kishino *et al.*, 1986; Yentsch and Phinney, 1989; Nelson and Robertson, 1993). The specific absorption coefficient of photosynthetically active pigment, a_{ppc}^* differs spectrally from a_{ph}^*, due to the influence of photoprotective carotenoids on a_{ph}^* at the blue wavelengths, and more in the surface waters than in deeper waters (Fig. 4C and 4D).

Mean tChl*a*-specific absorption coefficients a_{ph}^*, a_{ps}^* and a_{ppc}^* at 440 nm were higher in surface than in deep waters (Table 2), whereas the mean a_d^* coefficient exhibited only slight

Table 2. Mean, S.D. and sample number (N) for $a_p^*(440)$, $a_d^*(440)$, $a_{ph}^*(440)$, $a_{ps}^*(440)$ *and* $a_{ppc}^*(440)$ *for surface samples (5–20 m) and for samples collected below the DCM (<100 m for mesotrophic waters, and <110–120 m for oligotrophic waters). Percentages at* $\lambda = 440$ *nm for each component of the total* a_p^* *and of the total* a_{ph}^*. *Data collected during the FLUPAC cruise in the equatorial Pacific Ocean. Values of* a_{ps}^* *issued from the decomposition method*

	Surface (5–20 m)						Below the DCM (100-110-120 m)			
	$a_p^*(440)$	$a_d^*(440)$	$a_{ph}^*(440)$	$a_{ps}^*(440)$	$a_{ppc}^*(440)$	$a_p^*(440)$	$a_d^*(440)$	$a_{ph}^*(440)$	$a_{ps}^*(440)$	$a_{pps}^*(440)$
Mean	0.098	0.014	0.085	0.054	0.032	0.075	0.016	0.059	0.046	0.013
S.D.	0.027	0.005	0.022	0.016	0.006	0.022	0.008	0.015	0.013	0.002
% a_p^*	100	14	86	54	32	100	22	78	61	17
% a_{ph}^*	–	–	100	63	37	–	–	100	77	23
N	75	67	67	67	67	67	60	60	60	60

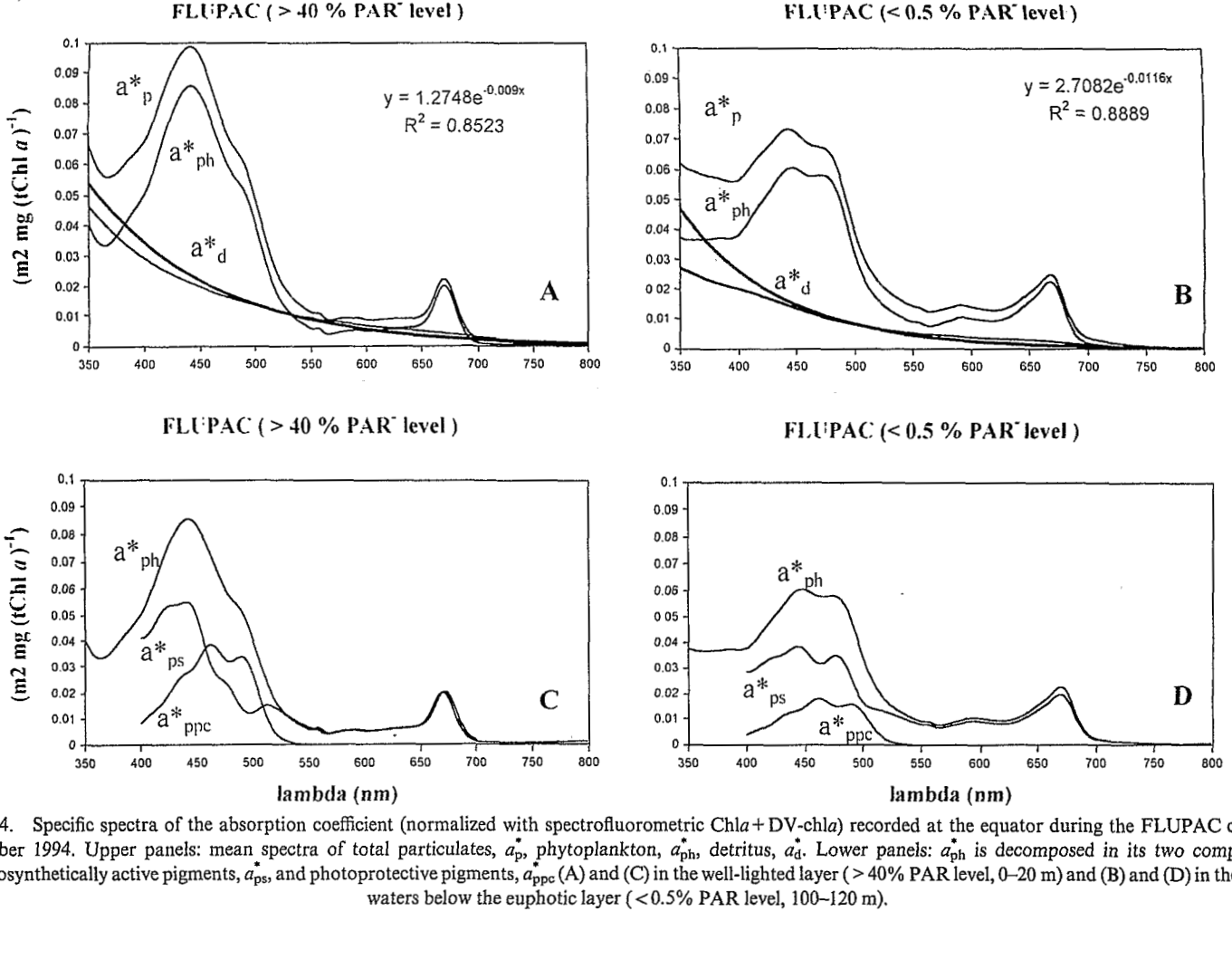

Fig. 4. Specific spectra of the absorption coefficient (normalized with spectrofluorometric Chl*a*+DV-chl*a*) recorded at the equator during the FLUPAC cruise in October 1994. Upper panels: mean spectra of total particulates, a_p^*, phytoplankton, a_{ph}^*, detritus, a_d^*. Lower panels: a_{ph}^* *is decomposed in its two components:* photosynthetically active pigments, a_{ps}^*, and photoprotective pigments, a_{ppc}^* (A) and (C) in the well-lighted layer (> 40% PAR level, 0–20 m) and (B) and (D) in the deeper waters below the euphotic layer (< 0.5% PAR level, 100–120 m).

variations. At the surface, the mean contributions to $a^*_p(440)$ of the different absorbing component $a^*_d(440)$, $a^*_{ps}(440)$ and $a^*_{ppc}(440)$ were 14%, 54% and 32%, respectively. At depth, the relative contribution of a^*_{ph} was lower than at the surface (78%), and these of $a^*_d(440)$ and $a^*_{ps}(440)$ higher (22% and 61% respectively) (Table 2). By integrating values of the different contributions to a^*_p over the whole visible spectrum (400–700 nm), we observed that at high light levels (>40% PAR^-, about 5–20 m), a^*_{ph}, a^*_{ps}, and a^*_d represented 82%, 48% and 18%, while at low light levels (<0.5% PAR^-) they represented 76%, 65%, and 25%, respectively. The relative contribution of a^*_{ps} and a^*_{ppc} to $a^*_{ph}(400–700)$ were 63% and 37% at the surface, and 78% and 22% in the deep layer, respectively. This indicates that in the well-lit surface waters of the photic zone the photoprotective carotenoids have a relatively stronger influence on absorption than photosynthetic pigments.

Variations with depth of the tChla-specific absorption properties, pigment ratios and intracellular pigment content in oligotrophic and mesotrophic waters

In order to obtain representative mean profiles of extreme trophic conditions encountered during the FLUPAC cruise, we averaged the data for absorption and intracellular pigment content from ten highly stratified stations having a tChl*a* (DV-chl*a* + Chl*a*) maximum between 90 and 100 m ($n = 10$, oligotrophic conditions–warm pool) and data from eight stations in the upwelled waters characterized by a broad tChl*a* maximum just below the surface mixed layer (50 m, mesotrophic conditions–upwelling). Mean profiles are presented in Figs 5 and 6. The same averaging was done for pigment ratios, but the station numbers were reduced (few HPLC measurements).

In the oligotrophic waters, $a^*_{ph}(440)$ was relatively constant in the first 40 m, then it decreased from 0.11 to 0.06 m^2 mg $(tChla)^{-1}$ at the base of the 90–100 m DCM (Fig. 5a). Similarly, $a^*_{ps}(440)$ decreased from 0.06 to 0.04 m^2 mg $(tChla)^{-1}$, as $a^*_{ppc}(440)$ represented decreasing proportions of $a^*_{ph}(440)$ (47% to 28%). The blue-to-red ratio of a^*_{ph} decreased from 5 in the surface layer to 2.5 below the DCM (see transect Fig. 7c). The package effect index did not vary significantly with depth (1.04 ± 0.05, not shown). The tPPC (diadinoxanthin + zeaxanthin)/tChl*a* ratio reached its maximum (1.3) in the upper layer (0–40 m), and decreased to 0.4 at the base of the DCM (Fig. 5b). This decrease was mainly due to the decrease in the zeaxanthin/DV-chl*a* ratio in *Prochlorococcus* (2.2 to 0.5), whereas the ratio of diadinoxanthin/Chl*a* was more uniform (Fig. 5b). The mean cellular content in DV-chl*a* and *b* of *Prochlorococcus* was relatively constant (0.56 fg $cell^{-1}$ and 0.017 fg $cell^{-1}$, respectively) within the whole 90 m euphotic layer. It began to increase for cellular DV-chl*a* at 90 m and for cellular DV-chl*b* at 110 m, (Fig. 5c) as the result of photoacclimation processes (Partensky *et al.*, 1996). Cellular Chl*a* content of picoeucaryotes was about 40–45 fg $cell^{-1}$ in the euphotic layer and abruptly increased to about 200 fg $cell^{-1}$ at the DCM (Blanchot *et al.*, 1997).

In the mesotrophic area, $a^*_{ph}(440)$ decreased with depth more slowly than in oligotrophic waters, from 0.060 m^2 mg $(tChla)^{-1}$ at the surface to 0.046 m^2 mg $(tChla)^{-1}$ at 70 m, depth of the base of the DCM (Fig. 6a). Along the vertical, $a^*_{ps}(440)$ was more uniformly distributed as $a^*_{ppc}(440)$ represented 30% to 23% of $a^*_{ph}(440)$. The blue to red ratio of a_{ph} decreased from 3 at the surface to 2 at 90 m (see transect Fig. 7c). The package effect again showed an uniform distribution and was higher (0.94 ± 0.05) than in oligotrophic waters (not shown). The tPPC/tChl*a* ratio showed a smoother gradient (from 0.6 in the surface layer to 0.3 at the base of the DCM), mainly influenced by the changes in the zeaxanthin/

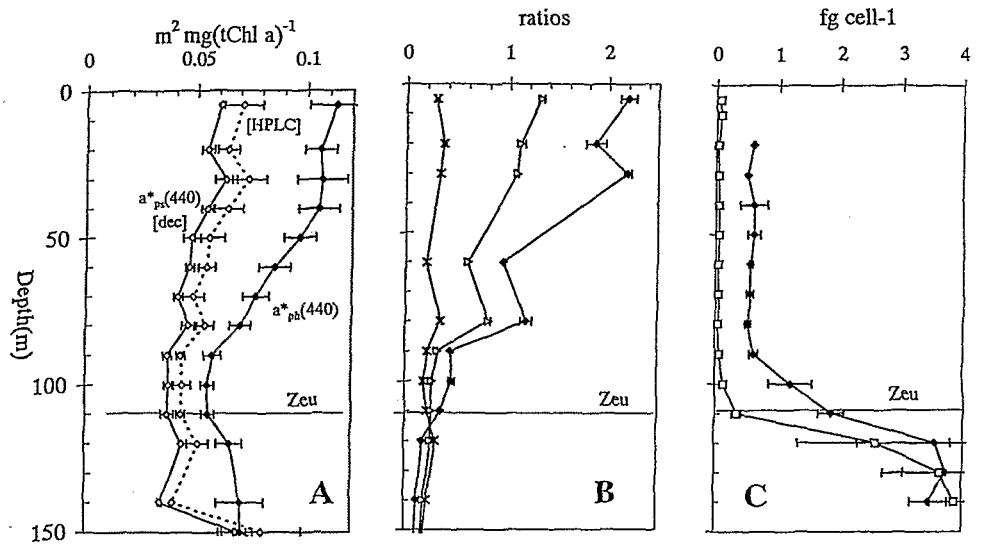

Fig. 5. Mean vertical profiles in typical oligotrophic waters with a DCM at 100 m ($n = 11$ stations) of (A) the specific absorption coefficient for phytoplankton, $a^*_{ph}(440)$ and the specific absorption coefficient by photosynthetically active pigments $a^*_{ps}(440)$ computed from the decompostion method (corrected and uncorrected) (B) the ratio of tPPC against tChl*a* (the total photoprotective pigments from procaryotes and eucaryotes as measured by HPLC against tChl*a* (Chl*a*+DV-chl*a*) (open triangles), the ratio of zeaxanthin to DV-chl*a* (full diamonds), and the ratio of diadinoxanthin to Chl*a* (crosses) (C) Intracellular content of DV-chl*a* (full diamonds) and DV-chl*b* (open squares) for *Prochlorococcus*.

DV-chl*a* ratio (Fig. 6b). Intracellular zeaxanthin concentration of *Prochloroccus* was highly variable from one station to another and particularly at 20 m (0.5 to 1.5 fg cell^{-1}). The mean intracellular DV-chl*a* increased gradually down to 70 m (1–2 fg cell^{-1}), then to 4 fg cell^{-1} below the base of the euphotic zone (Fig. 6c). The relative increase at this depth (ten fold) in intracellular DV-chl*b* (0.7 to 6) was even stronger than in the oligotrophic area (Fig. 6c). Cellular Chl*a* of picoeucaryotes was about 17–30 fg cell^{-1} and increased to 60–180 fg cell^{-1} below the DCM (Navarette *et al.*, 1997).

Decreases in specific-pigment absorption coefficients a^*_{ph} and a^*_{ps} in oligotrophic as well as in mesotrophic situations were associated with increases in the intracellular pigment content with depth, both due to photoacclimation (e.g. Veldhuis and Kraay, 1993; Goericke and Repeta, 1992; Moore *et al.*, 1995) and to the presence of two genetically distinct *Prochlorococcus* populations with regards to the DV-chl*a* and DV-chl*b* content (e.g. Campbell and Vaulot, 1993; Partensky *et al.*, 1993). There was no significant vertical variation in the package effect index, though intracellular pigment largely increased with depth. There is an indication from the flow cytometry forward scattering (FS) that "cell size" of picoplankton also could increase with depth (Navarette *et al.*, 1997), which could make the cell optical properties rather similar along the vertical.

The FLUPAC equatorial transect

The FLUPAC transect along the equatorial line between 167°E and 150°W shows that the

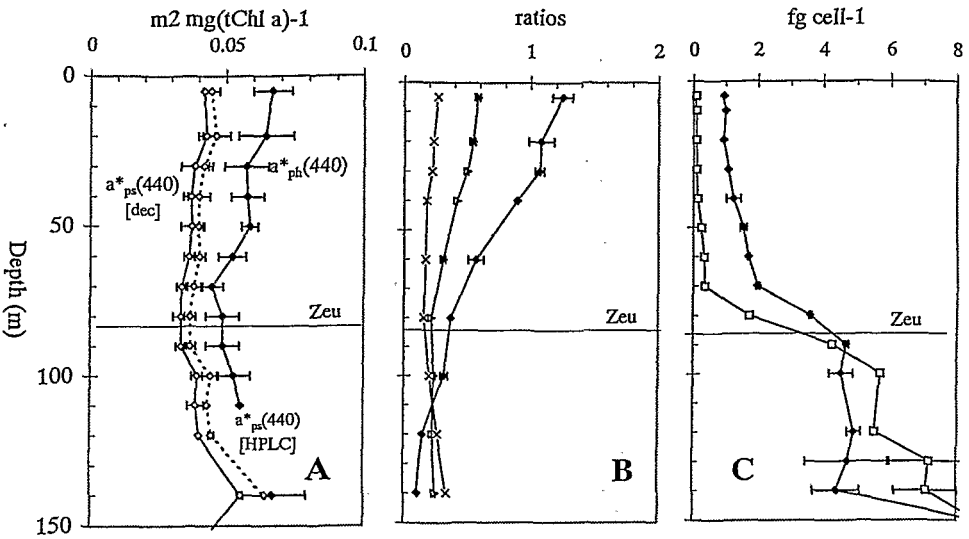

Fig. 6. Mean vertical profile in typical mesotrophic waters with a DCM at 50 m ($n=10$) of (A) the specific absorption coefficient of phytoplankton at 440 nm, $a^*_{ph}(440)$ and the specific absorption coefficient by photosynthetically active pigments computed from the decomposition method $a^*_{ps}(440)$ (corrected and uncorrected) (B) ratio of tPPC against tChl*a* (the total photoprotective pigments from procaryotes and eucaryotes as measured by HPLC) against tChl*a* (Chl*a* + DV-chl*a*) (open triangles), the ratio of zeaxanthin to DV-chl*a* (full diamonds) and the ratio of diadinoxanthin to Chl*a* (crosses), (B) Intracellular content of DV-chl*a* (full diamonds) and DV-chl*b* (open squares) for *Prochlorococcus*.

gradient between the two oceanographic regimes was rather sharp (Fig. 7) due to physical conditions at that period (Eldin *et al.*, 1997; Menkes *et al.*, 1997). The C_{sat} reached the threshold value typical of the upwelling at 170°W. Within 2 degrees of longitude (Fig. 7a), tChl*a* doubled. Moreover, the mean proportion of DV-chl*a* against tChl*a* in the euphotic layer decreased from 65% to 55% when crossing 170°W, illustrating the increasing influence of picoeucaryotes in the total biomass in the HNLC-type waters (Fig. 7b). The zonal pattern of the $a_{ph}(440)/a_{ph}(675)$ ratio (B/R) along the equator shows also contrasted patterns when crossing 165°W. It was almost constant and highest (5) in the clearest waters typical of the warm pool, west of 165°W, while it was relatively low (3.5) in the richer waters of the upwelling, east of 165°W (Fig. 7c). The steepest gradient in the B/R ratio was found within a front of 2 degrees in longitude at 170°W. The zonal pattern of $a^*_{ps}(440)$ resembled the one of the B/R. Roughly, values higher than 0.05 m^2 mg $(tChla)^{-1}$ in $a^*_{ps}(440)$ correspond to values higher than 4 in the B/R ratio. Nevertheless, values of $a^*_{ps}(440)$ higher than 0.05 m^2 mg $(tChla)^{-1}$ are distinguishable only in the upper layer of the oligotrophic area (0–40 m). These apparent variations in the 0–40 m layer could be produced by the sampling time schedule, although no significant difference was found between results of midday and midnight stations along the equatorial transect. Note that high values below 120 m are related to the presence of high proportion of tChl*b* at lower light level, which is not taken into account in the normalization of the absorption coefficients (Fig. 7d). Recall that values of $a^*_{ps}(440)$ given here are lower by 31% than values that would have been computed if HPLC measurements were available (see Section 2).

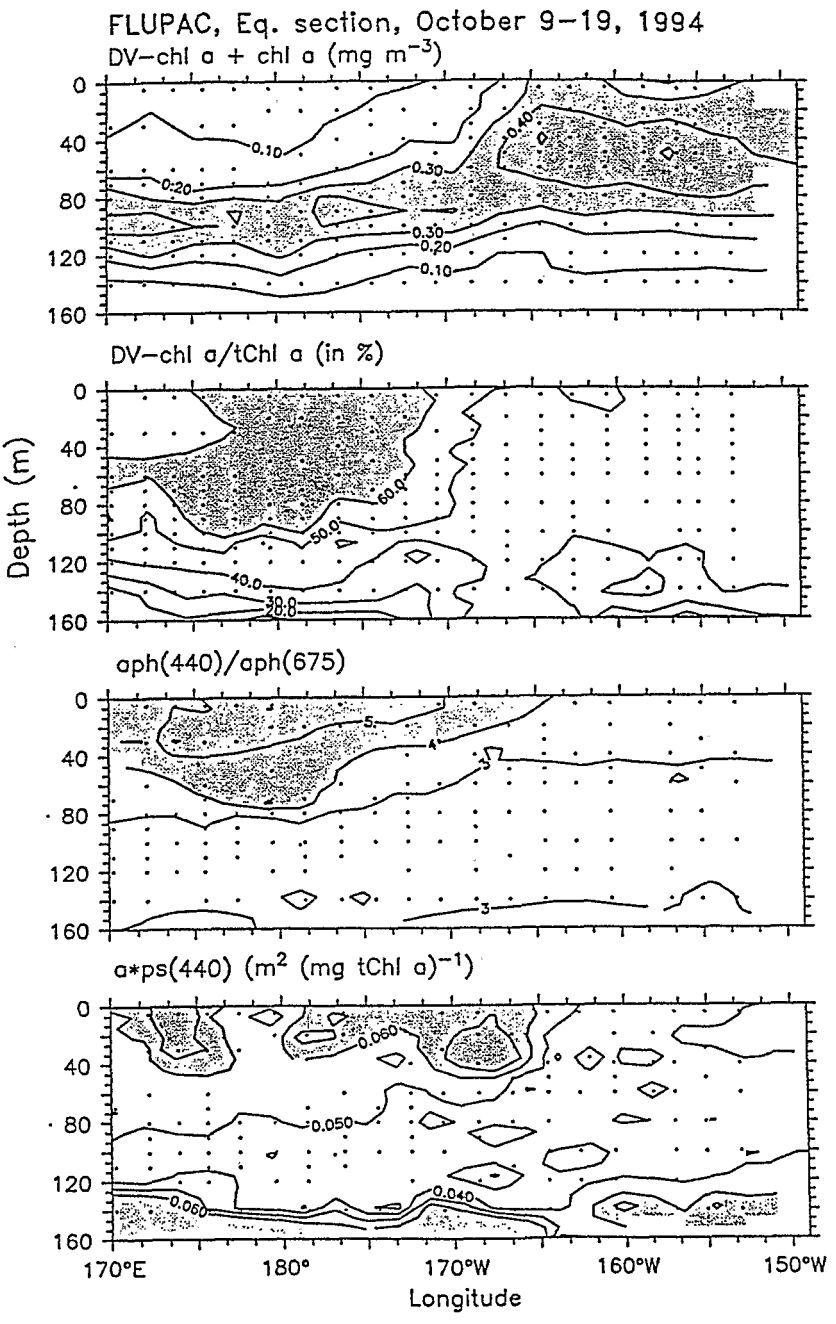

Fig. 7. Vertical sections during the equatorial transect of the FLUPAC cruise (October–November 1994) of (A) tChl*a* (Chl*a*+DV-chl*a* (mg mg^{-3}) (B) Relative biomass contribution of DV-chl*a* to tChl*a* (in %) (C) blue (440–450 nm) to red (670–680 nm) ratio of the absorption coefficient of phytoplankton, a_{ph} (D) absorption coefficient by photosynthetically active pigments, $a^*_{ps}(440)$ computed from the decomposition method. Note that the values of $a^*_{ps}(440)$ would be higher by 30% if they were corrected to match with HPLC data.

Diurnal variations of the specific absorption coefficient

During the 6 days of the second fixed station (in mesotrophic waters, at 150°W), four measurements of the absorption coefficient were performed during daylight hours. The blue to red ratio of a^*_{ph} increased significantly during the day in the 0–40 m layer, while no significant variation was observed below this depth. These increases of the B/R ratio between dawn and dusk (+15% at 5 and 20 m, +8% at 30 and 40 m) could be associated with a relative increase of the tPPC to chlorophyll ratio. Internal Chl*a* in eucaryotes increased from 03:00 to 19:00 (t-test, $p=0.05$) and internal changes of DV-chl*a* in *Prochlorococcus* cells were not significant. The diel variation in carotenoids concentrations was not available.

General dependency of a_{ph} and a_{ps} to pigments

The values of a_{ph} and a_{ps} are not related linearly with the tChl*a* concentration. There is a relative saturation, which is explained by an increase of the packaging effect related in the red to chlorophyll aggregation in cells and to accessory chlorophylls *b* and *c*, while in the blue it cannot be assessed independently of the total pigment composition including photoprotective and photosynthetic carotenoids (Bricaud *et al.*, 1995, Cleveland, 1995). The relationship between a^*_{ph} or a^*_{ps} and tChl*a*, though rather noisy can be described by a power law of the form: $a_{ph\ or\ aps}(\lambda) = A\ (\text{tChl}a)^B$ (Table 3; Fig. 8a). The small observed saturation probably originates in the higher package effect measured in the mesotrophic waters at the equator east of 170°W (p.e. of 6%). At 440 nm, the photosynthetic carotenoids also contribute to the saturation of a^*_{ps}. We found that the B/R ratio was well correlated to the ratio of $\text{tPPC}_{\text{HPLC}}/\text{tChl}a_{\text{SP}}$ (Fig. 8b), contrary to what was found by Bricaud and Stramski (1990) in oligo- and mesotrophic waters of the Sargasso Sea and Peruvian upwelling but similar to that found for *Prochlorococcus* cultures: Partensky *et al.*, 1993; Moore *et al.*, 1995). Thus, the B/R ratio of a_{ph} is a good indicator of the relative proportion of photoprotective carotenoids against chlorophylls in the equatorial waters of the Pacific ocean (and masks the possible impact of the package effect on the blue-to-red ratio).

Table 3. *Linear regression analysis (N=342) between the absorption coefficient by phytoplankton (a_{ph}) at 445 nm and 675 nm and tChl*a *as measured by spectrofluorometry for two algorithms of correction of the β effect: FLUPAC (this study) and Mitchell (1990). Linear regression analysis (N=342) between the absorption coefficient of photosynthetically active pigments (a_{ps}) and tChl*a *using the FLUPAC algorithm*

a_{ph}	445 nm			675 nm		
	A	*B*	*r*	*A*	*B*	*r*
tChl*a* (FLUPAC)	0.04	0.69	0.95	0.016	0.84	0.97
tChl*a* (Mitchell *et al.*, 1990)	0.055	0.73	0.95	0.019	0.88	0.97
a_{ps}	445 nm			675 nm		
	A	*B*	*r*	*A*	*B*	*r*
tChl*a* (FLUPAC)	0.027	0.68	0.89	0.016	0.85	0.94

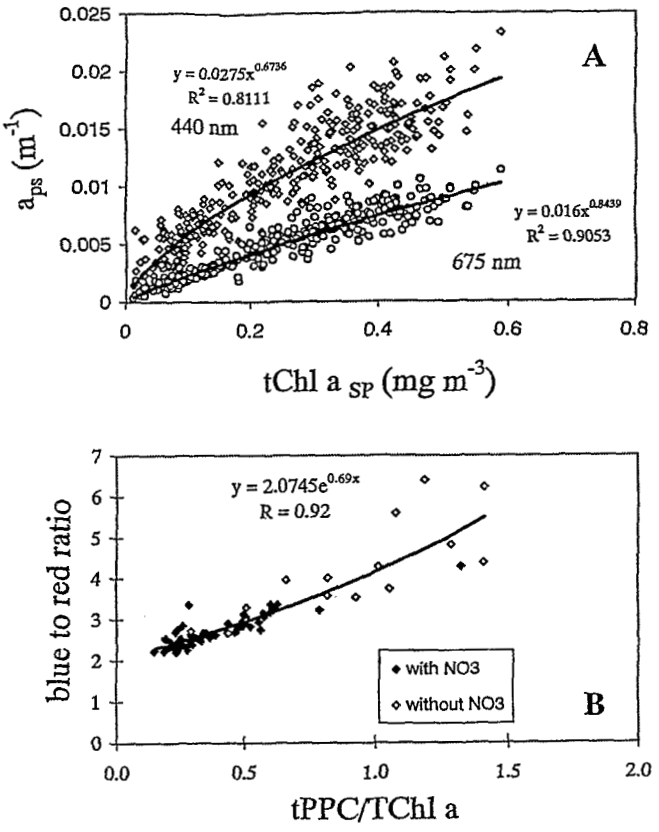

Fig. 8. (A) Relationship between the absorption coefficient of photosynthetically active pigments a_{ps} as computed from the decomposition method and the sum of pigments tChl*a* (Chl*a* + DV-chl*a*) as measured by spectrofluorometry at $\lambda = 440$ nm and $\lambda = 675$ nm. (B) Relationship between the blue-to-red ratio of the phytoplankton absorption coefficient, a_{ph}, and the ratio of photoprotective pigments (tPPC) to tChl*a* (Chl*a* + DV-chl*a*), for all samples of the FLUPAC cruise in the equatorial region where HPLC measurements and absorption were measured in parallel. Open symbols: without $NO_3 + NO_2$, full symbols: with $NO_3 + NO_2$.

4. DISCUSSION

The accuracy of the specific absorption coefficient values greatly depends on the algorithms used for the correction of the β effect on the GF/F filters, as well as on the methods used for pigment determination. This is crucial for primary production (*P*) estimates from light/photosynthesis models, as *P* is proportional to $a^*_{ph}(440)$ or $a^*_{ps}(440)$ (Morel, 1991; Sosik, 1997).

Methodological uncertainties in a_p and pigments

β-effect correction. $a_{ph}(440)$ varies by 40% depending on whether Mitchell's (1990) or the most recent algorithms (FLUPAC, this study, or Moore *et al.*, 1995) are used. Table 3 shows the influence of the β effect correction algorithm on the relation between $a_{ph}(675)$ and tChl*a*

for the FLUPAC data in the equatorial Pacific. Linear regressions at zero of $a_{ph}(675)$ t tChl*a* would give slopes of 0.019 and 0.022 with the FLUPAC and Mitchell's algorithms respectively. With the FLUPAC algorithm, the slope approaches the reference value o $a^*_{sol}(675)$ in 90% acetone ($=0.0207\ m^2\ mg^{-1}$) and would imply a mean package effect of 2% (estimated as 0 and 6% for the different trophic regimes), which is what is expected fo picoplankton dominated equatorial waters. With the Mitchell's algorithm, the slopes impl no package effect. If we consider now that $a^*_{sol}(675)$ is closer to $0.027\ m^2\ mg^{-1}$, based o recent laboratory studies with detergent solubilization (Berner *et al.*, 1989; Sosik an Mitchell, 1991) or cell breakage through a French press (Johnsen *et al.*, 1994), then slope determined for the FLUPAC data set at 675 nm are largely lower than $a^*_{sol}(675)$ and woul imply a package effect of 18 and 29% for the FLUPAC and Mitchell's algorithms respectively.

Normalizing pigments. The specific absorption coefficient is obtained by normalization o the phytoplankton absorption with the tChl*a* concentration obtained by spectrofluorometry and not with the fluorometric Chl*a* determined as in Holm-Hansen *et al.* (1965). Previous studies on absorption properties of phytoplankton used the normalization with the sum Chl*a*+Pheo*a*, since it was considered that phaeopigments were a large absorbing component originating from phytoplankton. However, it was demonstrated that in oceanic waters, most of the fluorometer-estimated phaeopigments were the consequence of a methodological bias (Neveux and De Billy, 1986; Gieskes and Kraay, 1986). In oligo- and mesotrophic waters as the ones studied here, this bias is due to the presence in the extracts of relatively large concentrations of DV-chl*a*, which has an acidification factor lower than this of Chl*a* (Goericke and Repeta, 1993), and also of Chl*b* and DV-chl*b* (since the formation of Pheo*b* and DV-pheo*b* after acidification leads to an increase in the fluorescence seen through the optical set of the fluorometer). Consequently, the fluorescence of the extract after acidification is overestimated compared with that obtained if only Chl*a* were present. This leads to an overestimation of the sum of Chl*a* and Pheo*a*, which affects essentially the Pheo*a* concentration. Spectrofluorometry and HPLC indeed show that the Pheo*a* concentration was considerably lower (few percent with regard to the total Chl*a* concentration) than previously estimated by fluorometry. During FLUPAC, we compared the chlorophyll concentrations obtained by the three different methods (fluorometry, spectrofluorometry and HPLC). Spectrofluorometric determination of tChl*a* ($tChla_{SP}$) can be compared to the fluorometric determination of the sum $(Chla+Pheoa)_{FL}$. Based on FLUPAC data, we found that:

$$tChla_{SP} = 0.588(Chla + Pheoa)_{FL},\ n = 516,\ r = 0.923 \quad (6)$$

The correlation is highly significant but it is clear that the fluorometric method overestimated the concentration of the sum Chl*a*+Pheo*a* (even if we included in $tChla_{SP}$ the weak concentration of Pheo determined by spectrofluorometry). The difference between $tChla_{SP}$ and $tChla_{HPLC}$ could reach 30%. This difference was explained partly by the two different calibration assumptions with spectrofluorometry and HPLC. HPLC analysis assumes that the specific absorption coefficient of Chl*a* and DV-chl*a* at the red absorption peak are identical. The spectrofluorometric method assumes that the specific fluorescence coefficient at the excitation and emission maxima of Chl*a* and DV-chl*a* are the same. In 90% acetone, the specific fluorescence equivalence assumption leads to a DV-chl*a* concentration 20% higher than the specific absorption equivalence assumption (Partensky *et al.*, 1996).

This difference is probably similar in 93% methanol. Nevertheless, some uncertainties exist in the value of the specific absorption coefficient of DV-chl*a* (Shedbalkar and Rebeiz, 1992; Goericke and Repeta, 1993). Based on the FLUPAC data, we found that:

$$\text{tChl}a_{\text{HPLC}} = 0.66\ \text{tChl}a_{\text{SP}} + 0.0175, r = 0.92, n = 43, \text{ with :} \quad (7)$$

$$\text{Chl}a_{\text{HPLC}} = 0.73\ \text{Chl}a_{\text{SP}} + 0.02,\ r = 0.91,\ n = 43 \quad (8)$$

$$\text{DV-chl}a_{\text{HPLC}} = 0.41\ \text{DV-chl}a_{\text{SP}} + 0.02,\ r = 0.72,\ n = 43 \quad (9)$$

It must noted that if $\text{tChl}a_{\text{HPLC}}$ were chosen as a reference with the FLUPAC algorithm, the slope of the linear regression a_{ph} vs. tChl*a* would have been 0.025, which is much higher than the reference $a^*_{\text{sol}}(675)$ but close to the reference $a^*_{\text{mu}}(675)$, and that if the sum Chl*a*+Pheo was taken as a reference, the slope would have been 0.014, which is much lower than both reference values.

Contribution of photoprotective carotenoids

No attempt was made to correct the tPPC concentrations computed for each station and depth with our decomposition method by a constant factor of 31% for matching the HPLC method (Table 1). This was done despite the large assumptions made for the decomposition method, i.e. that *in vivo* spectra by Bidigare *et al.* (1990) are reliable, that the sum of the 5 pigments indicated by these authors perfectly describes the pigment composition, and that the package effect (p.e.) does not affect the absorption coefficient. This last condition should be the case for small cells and for low absorption coefficients as shown by Bricaud *et al.* (1983); Bidigare *et al.* (1990) and Nelson *et al.* (1993), such conditions encountered in the equatorial Pacific during FLUPAC, where the p.e. did not exceed 5%.

Influence of detritus

The proportion of detritus can greatly influence the total absorption coefficient. Its assessment may be crucial to studies of primary production from ocean color reflectances (Garver *et al.*, 1994). The detrital-like absorption measured with the Kishino's extractive method comes from detrital chromophores associated with particles of unknown origin (Nelson and Robertson, 1993). Phycobiliproteins, which are photosynthetic water-soluble pigments, are not extracted by this procedure (Sosik and Mitchell, 1995). During FLUPAC, these shortcomings could be ignored since phaeopigments represented only 10% or less of tChl*a* and since phycoerythrins associated with cyanobacteria although present (Neveux *et al.*, submitted) did not contribute strongly to a_{ph}. Slopes around 0.01 nm^{-1} for detritus absorption spectra during FLUPAC were also characteristic of oligotrophic waters of the Sargasso Sea (0.008 to 0.015 nm^{-1}, Bricaud and Stramski, 1990). The proportions of $a^*_{\text{d}}(440)$ were 13% and 15% of $a^*_{\text{p}}(440)$ in the surface layer, and 20% and 25% below the euphotic layer, west and east of 170°W, respectively. The proportion of detritus could have been misestimated because of delayed laboratory measurements. As a comparison, the sum of detritus and CDOM (colored dissolved organic matter) can reach 50% at 440 nm, as was found in the Sargasso Sea at the BATS station (Siegel and Michaels, 1996). The proportion of detritus can reach 30% in surface samples and 50% in deeper ones (Morrow *et al.*, 1989) and could reach 60% in a region of very high detritus (Bidigare *et al.*, 1989). Nevertheless, it

has to be noted that these latter values result from decomposition methods, maybe not well adapted to oligotrophic regions. As a matter of fact, recent measurements of detritus on fresh samples by the Kishino's method (Zonal Flux cruise, 1995, *unpublished results*) gave a mean percentage for $a_d(440)$ not exceeding 14.5% of total $a_p(440)$.

Natural variability along the zonal gradient at the equator

$a^*_{ph}(440)$. Along the equator, at $\lambda = 440$ nm, we found the maximum values of a^*_{ph} ($0.14\ m^2$ mg $(tChl a)^{-1}$) in the oligotrophic area where a high DV-chl*a*/tChl*a* (65%) occurred. This corroborates the high values of a^*_{ph} found in the Sargasso Sea ($a^*_{ph} = 0.08\ m^2$ mg $(tChl a)^{-1}$, Bricaud and Stramski, 1990), in the oligotrophic waters west of the California current ($a^*_{ph} = 0.1\ m^2$ mg $(tChl a)^{-1}$, Sosik and Mitchell, 1995), in eastern Atlantic and equatorial Pacific oligotrophic waters ($a^*_{ph} > 0.1\ m^2$ mg $(tChl a)^{-1}$, Lazzara *et al.*, 1996; Allali *et al.*, 1997), which are attributed to the relatively high abundance of procaryotes. In cultures, these procaryotes show high values of $a^*_{ph}(440)$ at high light levels (*Prochlorococcus*: 0.06 to $0.14\ m^2$ (mg DV-chl*a*)$^{-1}$) and *Synechococcus*: $0.1\ m^2$ (mg tChl*a*)$^{-1}$, Morel *et al.*, 1993; Moore *et al.*, 1995; Bricaud *et al.*, 1997), and also high B/R ratios (from 2 to 5 for *Prochlorococcus*, 1.2 to 2.5 for eucaryotic cells; Beeler-SooHoo *et al.*, 1986; Mitchell and Kiefer, 1988b, Berner *et al.*, 1989). In the mesotrophic area, where eucaryotes were relatively more abundant (ratio of DV-chl*a*/tChl*a* of 45%), $a^*_{ph}(440)$ was lower ($0.065\ m^2$ mg $tChl a)^{-1}$). These results agree well with values found in other mesotrophic areas (Lazzara *et al.*, 1996; Allali *et al.*, 1997) and for eucaryotes in culture (Mitchell and Kiefer, 1988a).

The highest values of a^*_{ph} also coincided with a higher proportion of photoprotective pigments in the well-lighted layer (1.3 instead of 0.6) and a high B/R ratio (around 5), in the oligotrophic part of the transect. These photoprotective pigments (mainly zeaxanthin) represented 44% and 35% of $a_{ph}(440)$ in the euphotic layer of the oligo- and mesotrophic waters, respectively, a percentage similar to the one reported by Lindley *et al.* (1995) in oligotrophic area of the equatorial Pacific at 140°W. Absorption by photoprotectants was higher by 40% during summer in the upper layer at the Biowatt site (Marra and Bidigare, 1994). As a higher tPPC/tChl*a* ratio (0.9 instead of 0.5) and a higher a^*_{ppc}/a^*_{ph} (60 and 40%) ratio are found at equivalent optical depths (at 4.6 Z/Z_{eu}, since 1% PAR is at an optical depth of 4.6), the difference in a^*_{ph} could be due to phytoplankton composition rather than to a difference in photoacclimation (together with the low intracellular chlorophyllous content in nutrient-depleted waters).

The pattern of variability presented here seems to be valid in the equatorial Pacific Ocean as our results converge with those found at 150°W on a latitudinal gradient between 1°N and 12°S one month later (JGOFS-OLIPAC cruise, Allali *et al.*, 1997).

$a^*_{ps}(440)$. The equatorial transect shows that the surface values of $a^*_{ps}(440)$ are higher in the oligotrophic part (see transect Fig. 7). Though this difference hardly can be considered as significant with regard to the attached uncertainties, it is independent of the method required to estimate the tPPC concentrations (values of $a^*_{ps}(440)$ obtained from HPLC or from the decomposition method differ by a constant factor which would not affect the longitudinal variation). Such variations in $a^*_{ps}(440)$ appear much smaller than that of $a^*_{ph}(440)$ as in Sosik and Mitchell (1995) and in Allali *et al.* (1997). The difference in the mean package effect index (associated with changes in both internal content and size) in oligo- and mesotrophic surface waters (1.04–0.94) could account for part of the variation

in a^*_{ps}. As a matter of fact, a higher intracellular pigment content and a higher forward scattering ($\times 3$) was observed for *Prochlorococcus* in mesotrophic waters ($\times 1.5$), but the opposite was observed for picoeucaryotes (the internal pigment content and the forward scattering (FS) decreased by a factor of 0.4 and 0.7 in mesotrophic waters, see Blanchot *et al.*, 1997). How these changes in FS and intracellular pigment content of both groups combine to produce a larger package effect in the mesotrophic waters has yet to be determined. The resulting higher $a^*_{ps}(440)$ of picoplankton in oligotrophic waters could come from the small size and lower cellular content (associated with a small package effect) of *Prochlorococcus*, generally recognized as favourable for capturing light (Raven, 1986), minimizing selfshading and absorbing nutrients (Kirk, 1994; Morel *et al.*, 1993; Moore *et al.*, 1995).

CONCLUSIONS

Methodological consistencies for the β effect correction are necessary. Intercalibrations in tChl*a* concentrations are also needed. This will make easier the interpretation of the variations in the pigment-specific absorption coefficient published by various authors in different marine environments more fruitful. The values of $a^*_{ph}(440)$ and $a^*_{ps}(440)$ given in this study for the equatorial Pacific ocean may be lower bounds as they were calculated with an algorithm that gives the lowest estimates of the absorption coefficients. However, they were normalized with the tChl*a* as measured by spectrofluorometry, which represents 60% of the classical sum of Chl*a* + Pheo obtained by fluorometry.

Despite the identified uncertainties in the methodology, the high values of the specific absorption coefficient of each component (particulate, phytoplankton) are consistent with the results of studies in tropical oceans where picoplankton predominates. We found higher values of $a^*_{ph}(440)$ outside of the upwelling region, where *Prochlorococcus* was dominant, as previously suggested.

Since the package effect was rather small, our simple spectral decomposition on the basis of the 5 pure pigment *in vivo* spectra defined by Bidigare *et al.* (1990) was helpful in providing separate estimates of the photosynthetic and non-photosynthetic carotenoids concentrations, when they were not available by direct HPLC measurements. This decomposition method allowed us to estimate a_{ps}, the photosynthetically active component of a_{ph}. Our results suggest that prochlorophyte-dominated picoplankton, as is the case in oligotrophic waters, would have a greater efficiency in capturing light usable for photosynthesis than eucaryotes-dominated picoplankton in the upwelling mesotrophic waters. Such variations should be confirmed by measuring the *in situ* absorption of each group of picoplankton, picoeucaryotes and procaryotes. One technique could be an *in situ* size fractionation on 0.8 μm pore size filters in order to separate efficiently *Prochlorococcus* from picoeucaryotes. How these different absorption properties are expressed in productivity indices would be an interesting task for the future.

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APPENDIX

Notation

λ	Wavelength	nm
OD_f	Optical density of the filter	
OD_s	Optical density of the suspension	
Chl a	Chlorophyll a concentration	$mg\ m^{-3}$
DV-chl a	Divinyl chlorophyll a concentration	$mg\ m^{-3}$
Chl*b*	Chlorophyll *b* concentration	$mg\ m^{-3}$
DV-chl*b*	Divinyl chlorophyll *b* concentration	$mg\ m^{-3}$
Chl*c*	Chlorophyll *c* concentration	$mg\ m^{-3}$
Pheo*a*	Phaeopigments *a* concentration	$mg\ m^{-3}$
tChl*a*	Sum of chlorophyll *a* and divinyl-chlorophyll *a* concentrations	$mg\ m^{-3}$
tChl*b*	Sum of Chl *b* and DV-chl *b* concentrations	$mg\ m^{-3}$
tPPC	Photoprotective carotenoids	$mg\ m^{-3}$
tPSC	Photosynthetic carotenoids	$mg\ m^{-3}$
α_{PPC}	*in vivo* absorption coefficient of photoprotective carotenoids (tPPC)	$m^2\ (mg)^{-1}$
β	Pathlength amplification factor	
$a_p(\lambda)$	Particulate absorption coefficient	m^{-1}
$a_d(\lambda)$	Absorption coefficient by detritus	m^{-1}
$a_{ph}(\lambda)$	Absorption coefficient of phytoplankton	m^{-1}
$a_{ps}(\lambda)$	Absorption coefficient of photosynthetically active pigments	m^{-1}
$a_{ppc}(\lambda)$	Absorption coefficient of photoprotective pigments	m^{-1}

$a^*_p(\lambda)$	tChl*a*-specific absorption coefficient of particulate matter	m^2 (mg Chl*a*+DV-chl*a*)$^{-1}$
$a^*_d(\lambda)$	tChl*a*-specific absorption coefficient by detritus	m^2 (mg Chl*a*+DV-chl*a*)$^{-1}$
$a^*_{ph}(\lambda)$	tChl*a*-specific absorption coefficient of phytoplankton m^2 (mg Chl*a*+DV-chl*a*)$^{-1}$	
$a^*_{ps}(\lambda)$	tChl*a*-specific absorption coefficient of photosynthetically active pigments	m^2 (mg Chl*a*+DV-chl*a*)$^{-1}$
$a^*_{ppc}(\lambda)$	tChl*a*-specific absorption coefficient of photoprotective pigments	m^2 (mg Chl*a*+DV-chl*a*)$^{-1}$
PAR^-	Photosynthetically available radiation just under the surface	µEinst m^2 s^{-1}
Z_{eu}	Depth of the euphotic zone (defined as 1% of PAR^-)	m
HNLC	High nutrient low chlorophyll	
σt	Density of sea water	kg m^{-3}
NO_3+NO_2	Concentrations of nitrate+nitrite	µM