Spatial variations in the chlorophyll-specific absorption coefficients of phytoplankton and photosynthetically active pigments in the equatorial Pacific

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Abstract. Chlorophyll-specific absorption coefficients of particles, \( a_p^*(\lambda) \), and of phytoplankton, \( a_{pl}^*(\lambda) \), were determined using the glass-fiber filter technique along 150°W in the equatorial Pacific (13°S–1°N). A site-specific algorithm for correcting the path length amplification effect was derived from field measurements. Then a decomposition technique using the high-performance liquid chromatography pigment information and taking into account the package effect was used to partition \( a_{ps}^* \) into the contributions of photosynthetic pigments (\( a_{ps}^* \)) and nonphotosynthetic pigments (\( a_{nps}^* \)). Both \( a_{ps}^* \) and \( a_{nps}^* \) values were observed to decrease from the oligotrophic waters of the subequatorial area (13° 1°S) to the mesotrophic waters of the equatorial area (1°S–1°N) and from the surface to deep waters. The \( a_{ps}^* \) variations were primarily, but not exclusively, caused by changes in the concentrations of nonphotosynthetic pigments. The level of pigment packaging was also variable both horizontally and vertically, as a result of changes in populations and photoacclimation. In comparison with \( a_{ps}^* \), \( a_{nps}^* \) exhibited a reduced range of variation with depth and along the latitudinal gradient. The variations in \( a_{nps}^* \) originating from the package effect were partly compensated by variations in the concentrations of photosynthetic pigments. We extended this analysis to include data collected in other areas with different trophic states. The \( a_{ps}^* \) values varied over a factor of 4 at 440 nm, instead of 8 for \( a_{ps}^* \), for chlorophyll \( a \) concentrations covering 2 orders of magnitude (0.02 to 2 mg m\(^{-3}\)). In agreement with a previous study performed off California with a different method [Sosik and Mitchell, 1995], we conclude that \( a_{ps}^* \) is less dependent on environmental parameters than \( a_{ps}^* \). In addition, our results provide evidence that the variability in \( a_{ps}^* \) cannot be neglected. The use of \( a_{ps}^* \) instead of \( a_{nps}^* \) with light-photocatalysis models (in conjunction with a quantum yield for carbon fixation defined with respect to the photosynthetically active absorbed amount of quanta) presents the advantage of removing the variability associated with nonphotosynthetic pigments.

1. Introduction

The absorption coefficients of phytoplankton account for a large part of the optical variability in the open ocean. They are therefore key input parameters when modeling various phenomena such as light propagation within the ocean and oceanic color [e.g., Gordon et al., 1988; Morel, 1988], carbon fixation by phytoplankton [Kiefer and Mitchell, 1983], and the heating rate of the upper ocean [Morel and Antoine, 1994]. Over the last 10 years the variability in absorption properties of phytoplankton has been extensively documented for both cultures in the laboratory [e.g., Briceaud et al., 1983, 1988; Mitchell and Kiefer, 1988a; Berner et al., 1989; Stramski and Morel, 1990; Ahn et al., 1992] and natural populations [e.g., Mitchell and Kiefer, 1988b; Yentsch and Phinney, 1989; Briceaud and Stramski, 1990; Hopfner and Sathyendranath, 1992; Babin et al., 1993; Briceaud et al., 1995; Cleveland, 1995]. These various studies have shown that the variability in the absorption coefficients of phytoplankton per unit chlorophyll (chl) concentration (chl-specific absorption, \( a_{ps}^* (\lambda) \)) is driven by both the packaging effect and the pigment composition, in varying proportions. On one hand, the packaging effect depends on the cell size and intracellular pigment concentration [Kirk, 1975; Morel and Briceaud, 1981], which in turn vary with the trophic state of waters and the photoadaptive state of populations. On the other hand, for a given chlorophyll content the relative abundance of accessory pigments is highly variable with the species and with their photoadaptive and nutritional status.

Among these pigments the nonphotosynthetic carotenoids (essentially, zeaxanthin, diadinoxanthin, diatoxanthin, and β carotene; see, e.g., Bidigare et al. [1987]) do not transfer excitation energy to chlorophyll \( a \) within the antennae, so that the energy absorbed by these pigments is not usable for photosynthesis [e.g., Butler, 1978]. It is therefore relevant, particularly for light-photocatalysis models, to make a distinction between that part of the absorption coefficient which is associated with photosynthetically active pigments (\( a_{ps}^* (\lambda) \)) and that associated with nonphotosynthetic pigments (\( a_{nps}^* (\lambda) \)):

\[
a_{ps}^*(\lambda) = a_{ps}^*(\lambda) + a_{nps}^*(\lambda)
\]

The recent study by Sosik and Mitchell [1995] of the variations in \( a_{ps}^* \) and \( a_{nps}^* \) in mesotrophic and eutrophic waters off California led to the conclusion that \( a_{ps}^* (\lambda) \) varies only weakly horizontally and vertically in these waters. Such results suggest that \( a_{nps}^* (\lambda) \) could be much less dependent on environmen-
tial parameters than $a^*_{ph}(\lambda)$ and therefore better suited to
global light-photosynthesis models, (2) the relative abundance
of nonphotosynthetic pigments would be the dominant source of
variability in $a^*_{ph}(\lambda)$, and (3) the packaging effect would
have only a minor influence upon the spatial variations of
$a^*_{ph}(\lambda)$.

Such conclusions need to be reexamined for a broader range
of oceanic conditions and especially for the oligotrophic waters
encountered in the open ocean. In particular, the packaging
effect was often assumed to be insignificant in oligotrophic
waters because of the predominance of tiny cells [Chavez, 1989;
Lindley et al., 1995], although it was never experimentally
quantified. Also, Sosik and Mitchell [1995] identified the light
environment and nutrient availability as the main sources of the
variability in $a^*_{ph}$. Further interpretation of their data has
been, however, limited by the lack of information concerning
the pigment and taxonomic compositions in the studied areas.
Such information is essential to understand the direct causes of
variation in $a^*_{ph}$.

Very few studies have investigated the respective roles of
$a^*_{ps}(\lambda)$ and $a^*_{bps}(\lambda)$ in the variability of $a^*_{ph}(\lambda)$, because the
estimate of these two coefficients raises some methodological
problems. Various methods were proposed to estimate $a^*_{ps}$ and
$a^*_{bps}$. The first one is based on a spectral reconstruction tech-
nique [Bidigare et al., 1989]. The in vivo weight-specific absorp-
tion coefficients of the major pigments are reconstructed on
the basis of the in vitro (high-performance liquid chromatog-
raphy (HPLC) generated) spectra in organic solvents, with the
absorption maxima shifted to match the corresponding in vivo
peaks. This approach is limited because the packaging effect,
which is likely to modify the amplitude and spectral shape of
the absorption spectrum, cannot be taken into account. The
second method is based on the fluorescence excitation spec-
trum. It was suggested that excitation spectra for dichloro
phenyl-methyl-urea (DCMU)-enhanced in vivo fluorescence
can be used to provide information about the absorbed energy
that is effectively transported to photosystem (PS) II [e.g.,
Sukhshaev et al., 1991]. The fluorescence excitation spectrum,
obtained in relative values and quantum corrected, is scaled to
the chl a-specific absorption spectrum by matching the fluo-
rescence peak at 670 nm to the corresponding absorption peak
[Sukhshaev et al., 1991; Johnsen and Sukhshaev, 1993]. Sosik and
Mitchell [1995] applied this method to derive $a^*_{ph}(\lambda)$ for
field samples and introduced a correction for absorption by
aepigments. Note that this approach may also be limited in
some cases, as it assumes an equal distribution of pigments
between PS I and PS II, which may be not valid for some
phyacobiliprotein-containing species such as cryptophytes
and cyanobacteria [Neori et al., 1988]. Finally, Babin et al. [1996]
proposed a modification of the reconstruction technique to
estimate $a^*_{bps}$ and $a^*_{bps}$. They quantified at each wavelength
the relative contributions of each pigment to $a^*_{bps}$ from the
weight-specific absorption coefficients of the various pigments
and their concentrations, as measured by HPLC. Then they
derived $a^*_{ps}(\lambda)$ and $a^*_{bps}(\lambda)$ from $a^*_{bps}(\lambda)$ by summing these contributions
for photosynthetic and nonphotosynthetic pigments (see
(3)-(5)). This approach is thus based on the actual $a_{bps}$ spec-
trum and offers the advantage that the package effect is ac-
counted for.

We used this latter technique to assess the relative contribu-
tions of $a^*_{ps}$ and $a^*_{bps}$, in forming $a^*_{bps}$, for waters of the
equatorial Pacific Ocean. This oceanic region is subject to an
increasing interest because of its role in the global carbon cycle
(see, e.g., Murray et al. [1995, and references therein]) and
offered the opportunity to observe various situations along a
meridian transect, from the oligotrophic waters near the South
Pacific gyre to the mesotrophic waters of the equatorial up-
welling. The major goal of this study was to determine the
spatial (vertical and horizontal) patterns of variability in $a^*_{ph},$
$a^*_{ps},$ and $a^*_{bps}$, so as to answer the following questions: (1)
which part of the variability in $a^*_{ph}$ is attributable to nonpho-
synthetic pigments, and (2) what is the importance of the
packaging effect in oligotrophic and mesotrophic waters? We
also examined the variations of these coefficients obtained
during earlier cruises, so as to determine whether the conclu-
sions of the present study can be extended to other parts of the
world's ocean with different trophic states.

2. Materials and Methods
2.1. Data Collection

Sampling was conducted in November 1994 during the Oli-
gotrophie en Pacifique (OLIPAC) cruise (Etude de Processus
dans l'Ocean Pacifique Equatorial (EOPOPE)—Joint Global
Ocean Flux Study (JGOFS) France program) in the equatorial
Pacific. Stations were occupied along a transect at 150°W be-
tween 13°S and 1°N (Figure 1). Hydrocasts were performed
using a rosette sampler/Seabird conductivity-temperature-
depth (CTD) system equipped with twenty-four 12-L Niskin
bottles. A Sea Tech fluorometer was interfaced with the CTD,
allowing vertical fluorescence profiles to be measured. Samples
for absorption measurements were usually collected twice a
day at 10 depths in the upper 200 m. Pigment and flow-
cytometric determinations were performed on the same casts
at 12 depths.

2.2. Absorption Measurements

Seawater samples (2–2.5 L) were collected and then filtered
through Whatman GF/F glass-fiber filters (25 mm) under low
vacuum pressure (<0.5 atm). Filter supports with a small clear-
ance area (1.2 cm²) were used to minimize the sample volume
necessary to measure sufficiently high optical densities. Optical densities of the particulates retained on the filter were measured using the glass-fiber filter technique [Trüper and Yentsch, 1967]. The procedure was identical to that described by Bre  
cau and Stramski [1990], except that a Perkin-Elmer Lambda 19 dual-beam spectrophotometer, equipped with an integrating sphere, was used. Spectral values of the absorption coefficient were recorded every 1 nm from 350 to 750 nm. The spectrophotometer automatically corrected for the baseline which was stored prior to analyses. All spectra were set to 0 at 750 nm to minimize differences between sample and reference filters. Such an approximation, which would be questionable for detrital samples, is justified here because total absorption was largely dominated by living phytoplankton (see Figures 4a, 4b, 4e, and 4f). The optical densities measured on the filter, OD(λ), were corrected for the path length amplification effect using a site-specific algorithm, as described in the Appendix (equation (8)). Then the absorption coefficients a_ρ(λ) (m⁻¹) were computed from the corrected optical densities, OD(λ), according to

\[ a_ρ(λ) = 2.3 \ OD(λ)/V \]

where \( S \) is the clearance area of the filter (m²) and \( V \) is the filtered volume (m³). Each \( a_ρ(λ) \) spectrum was then partitioned into phytoplanktonic \( (a_{p(λ)}) \) and non-algal \( (a_{p(λ)}) \) components using the numerical approach developed by Bre  
cau and Stramski [1990]. Finally, the absorption coefficients of living phytoplankton \( (a_{p(λ)}) \) were converted into chl a-specific absorption coefficients \( (a_{p(α)}(λ)) \), by normalizing to the sum of chlorophyll a and divinyl chlorophyll a (DV chl a) concentrations, denoted Tchl a. The phaeophytin a concentration, which never exceeded 2% of the Tchl a concentration, was not included in Tchl a.

2.3. Partitioning Absorption into Photosynthetic and Nonphotosynthetic Components

The chl a-specific absorption spectrum of phytoplankton, \( a_{p(α)}(λ) \), was further partitioned into its photosynthetic and nonphotosynthetic components, \( a_{p(α)}(λ) \) and \( a_{n(α)}(λ) \) (see (1)), using the relationships [Babin et al., 1996]

\[ a_{n(α)}(λ) = a_{p(α)}(λ) F_{n(α)}(λ) \]
\[ a_{p(α)}(λ) = a_{p(α)}(λ) [1 - F_{n(α)}(λ)] \]

\[ F_{n(α)}(λ), \text{ which represents the fraction of absorption attributable to nonphotosynthetic pigments at each wavelength, was quantified as follows:} \]

\[ F_{n(α)}(λ) = \left[ \sum a_{n(α)}(λ)C_i \right] / \left[ \sum a_{p(α)}(λ)C_i \right] \]

where \( a_{p(α)}(λ) \) is the unpacked in vivo specific absorption coefficient (m² mg⁻¹) for the pigment i at the wavelength \( λ \) and \( C_i \) is its concentration (mg m⁻³). The numerator of (5) represents absorption by nonphotosynthetic pigments, while the denominator represents absorption by all pigments. The \( a_{p(α)}(λ) \) coefficients were determined by scaling the HPLC-generated absorption spectrum of each pigment to its weight-specific extinction coefficient at a given wavelength and shifting the absorption maxima to match the in vivo maxima, following the procedure of Bidigare et al. [1980]. Extinction coefficients of individual pigments were taken from Goericke and Repeta [1993] and, for a carotene, from Bidigare et al. [1990].

2.4. Pigment Analyses and Other Measurements

Samples for pigment measurements were collected on Whatman GF/F filters and either analyzed immediately or stored in liquid nitrogen for later analysis. Pigments were quantified using HPLC according to the procedure described by Vadas et al. [1996]. The algal pigments identified and quantified include the following photosynthetic pigments: chlorophyll a, b, c, DV chl a, divinyl chlorophyll b (DV chl b), peridinin, 19'-hexanoyloxyfucoxanthin (19'-HF), 19'-butanoyloxyfucoxanthin (19'-BF), prasinoxanthin, fucoxanthin, a carotene, and three nonphotosynthetic pigments: zeaxanthin, diatoxanthin, and diadinoxanthin. The β carotene, which is a nonphotosynthetic pigment, was not discriminated from a carotene, which results in underestimating \( a_{p(α)}(β) \) and overestimating \( a_{p(α)}(a) \). Analyses previously performed on oligotrophic waters in the tropical North Atlantic, however, showed that the β carotene-to-zeaxanthin ratio was <10% throughout the water column, so that the error on these coefficients is thought to be very limited.

Cell number densities for Prochlorococcus, Synechococcus, and picocockaryots were determined using a FACSort flow cytometer. Nutrient concentrations were measured with a Technicon Autoanalyzer. Micromolar and nanomolar determinations of nitrites and nitrates were made following the protocols described by Tréguer and Le Corre [1973] and Rainville et al. [1990], respectively. Vertical profiles of the photosynthetically available radiation (PAR) were obtained by integrating between 400- and 700-nm downwelling irradiance spectra measured with a spectroradiometer LICO R LI-1800 UW equipped with a cosine collector. The euphotic depth, \( Z_{eup} \), was determined as the depth where PAR is 1% of its value just below the surface. The optical depth, \( ξ \), was computed as

\[ ξ = 4.6 Z/Z_{eup} \]

where \( Z \) represents the depth.

3. Results

3.1. Characteristics of the Investigated Area

Figure 2 illustrates the spatial patterns of seawater potential density, (NO_3 + NO_2) concentrations, and (chlorophyll a + DV chl a) concentrations along the south-north transect. The depth of the upper mixed layer increases drastically, from approximately 40 to 130 m, when going toward the equator (Figure 2a). The euphotic depth, \( Z_{eup} \), continuously decreases, from 98 m at 11°S to 65 m at 1°N. The (NO_3 + NO_2) concentrations in surface waters range from nanomolar values in subeutropical waters to an average value of 3 μM close to the equator (Figure 2b). The Tchl a concentration shows a maximum around 80 m at 11°S (with a peak value of 0.35 mg m⁻³), which progressively broadens and slopes up toward 30–70 m in the equatorial area (Figure 2c). The Tchl a concentration remains weak in the whole area, with values never exceeding 0.36 mg m⁻³. The areal Tchl a content (integrated over the water column) increases from 26 to 42 mg m⁻² along the transect, with a steep gradient around 1°S. This gradient was used to delimit two areas differing with respect to their trophic status: the oligotrophic subeutropical area (15°–2°S) and the mesotrophic equatorial area (1°S–1°N).

Flow-cytometric analyses show that Prochlorococcus cells remain numerically predominant over the whole area and their abundance decreases only slightly when going toward the
three absorption coefficients exhibit the same trend to decrease with increasing depth, with a strong vertical gradient in the subequatorial area, while in the equatorial area the vertical patterns are much less accentuated (Figures 3a, 3b, and 3c). In contrast with the other coefficients, $a^x_{ps}(440)$ varies within a narrow range, both horizontally and vertically (Figure 3d). Slightly enhanced values are observed above the deep chlorophyll maximum (40–70 m) from 13° to 2°S. This structure disappears close to the equator. Values of $a^x_{np}(440)$ are systematically higher than those of $a^x_{ps}(440)$, albeit the difference between these coefficients is reduced in the surface waters between 13° and 2°S.

As vertical patterns in $a^x_p$, $a^x_{ph}$, $a^x_{ps}$, and $a^x_{np}$ are thought to be primarily influenced by the light regime, mean and standard deviation values of the various coefficients (at 440 nm) were computed as functions of the optical depth $\xi$ for the two areas identified above (Figure 4). In the subequatorial area, $a^x_p(440)$ and $a^x_{ph}(440)$ are high in the upper layer and gradually decrease with increasing depth, on average from 0.14 to 0.07 m$^{-2}$ mg$^{-1}$ Tchla$^{-1}$ for $a^x_p$ and from 0.13 to 0.06 m$^{-2}$ mg$^{-1}$ for $a^x_{ph}$ (Figures 4a and 4b). Note the very weak contribution of detritus to total absorption, which essentially originates from living phytoplankton. In turn, the major part (about 80%) of the $a^x_{ps}(440)$ variations is related to changes in $a^x_{np}(440)$, which decreases by 1 order of magnitude along the vertical (Figure 4c). Absorption by nonphotosynthetic pigments represents almost half the total phytoplanktonic absorption at 440 nm near the surface, whereas it becomes a negligible fraction (<10%) below the euphotic zone. In contrast to $a^x_{np}$, $a^x_{ps}(440)$ shows a weak decrease with depth (0.07–0.05 m$^{-2}$ mg$^{-1}$; Figure 4d). A small part (about 20%) of the variations in $a^x_{ps}(440)$ can, however, be attributed to this decrease.

In the equatorial area, $a^x_p(440)$ and $a^x_{ps}(440)$ are lower in the upper layer than in the subequatorial area (Figures 4e and 4f). These coefficients also show a gradual decrease downward (from 0.08 to 0.05 m$^{-2}$ mg$^{-1}$ for $a^x_p$), although less pronounced than in subequatorial waters, and converge toward similar values at six–eight optical depths. The $a^x_{np}(440)$ coefficient is about twice lower in the upper layer of the equatorial area than in the subequatorial area and decreases down to a similar value at depth (Figure 4g). The fraction of phytoplanktonic absorption (at 440 nm) originating from nonphotosynthetic pigments drops to ~30% near the surface and is less than 10% at the bottom of the euphotic zone. As in the subequatorial area, $a^x_p(440)$ shows low variability throughout the water column, with also a slight decrease with depth (Figure 4f). Values are slightly lower than in the subequatorial waters near the surface (~0.06 instead of 0.07 m$^{-2}$ mg$^{-1}$) and virtually identical at depth.

In both areas the variations in the magnitude of $a^x_{ps}(k)$ are accompanied by changes in the spectral shape (Figure 5). The coefficients, when normalized with respect to $a^x_{ph}(440)$, increase progressively around 410–480 nm and 650 nm from the surface down to the deeper layers. This feature can be ascribed to the increase in the relative concentration of Tchlb (−chl b + DChl b), with the Tchlb/Tchla ratio increasing from 0.1 near the surface to approximately 0.7 at $\xi \approx 7$ (see Figures 6b and 6t). In parallel the shoulder around 490 nm progressively vanishes at depth, in correspondence with the decrease in the relative concentration of zeaxanthin, the dominating nonphotosynthetic pigment (Figures 6a and 6e). The other accessory pigments are less variable along the vertical, so that the associated spectral changes are hardly detectable. Note finally that

![Figure 2. Spatial distributions of (a) seawater potential density, (b) nitrite and nitrate concentrations, and (c) Tchla (−chl a + DVis chl a) concentrations, along the meridian transect. South and north latitudes are indicated by negative and positive values, respectively.](image-url)
in the subequatorial area, the wavelength of maximal absorption is slightly shifted toward higher wavelengths (from 440 to 446–447 nm) from the surface down to the chlorophyll maximum, in relation with the increasing predominance of Prochlorococcus relatively to other populations [e.g., Bricaud and Stramski, 1990].

4. Discussion

4.1. Vertical and Intersite Variations in $a_{ps}^*$, $a_{psr}^*$, and $a_{ps}^*$

The decrease in $a_{ps}^*(440)$ along the vertical observed in both areas is consistent with previous observations made in other parts of the world’s ocean [Sosik and Mitchell, 1995; Lazzara et al., 1996; Babin et al., 1996]. The decrease in $a_{ps}^*$ surface values from oligotrophic to mesotrophic areas was also observed and related to the nutrient availability [Ventsch and Phinney, 1989; Sosik and Mitchell, 1995].

It is apparent from Figure 4 that vertical and intersite variations in $a_{ps}^*$ are, for the major part, accounted for by changes in absorption by nonphotosynthetic pigments and in particular by zeaxanthin. The zeaxanthin concentration (relative to Chl $a$) decreases with depth by 1 order of magnitude in subequatorial waters (Figure 6a) and still by a factor of 5 in the equatorial area (Figure 6c). Diadinoxanthin and diatoxanthin, with much lower and less variable concentrations along the vertical, have only a minor influence upon the $a_{ps}^*$ variations.

The residual variations observed in $a_{ps}^*$ suggest, however, that nonphotosynthetic pigments are not the sole factor driving the $a_{ps}^*$ variations. Moreover, the $a_{ps}^*$ variations with depth cannot be related to the dominating photosynthetic pigments (namely chl $b$ and DV chl $b$, 19'-HF and 19'-BF), which vary in an opposite way, i.e., increase with depth relatively to Tchl $a$ (Figures 6b and 6f). Therefore it is likely that the vertical distributions of $a_{ps}^*$, $a_{ps}^*$, and $a_{psr}^*$ are also influenced by the package effect. This effect increases when either the average cell size ($d$) or the absorption coefficient of the cellular material ($a_{com}(x)$) increases, with the result of depressing absorption at all wavelengths and “flattening” the spectrum [Morel and Bricaud, 1981].

In order to test the hypothesis of vertical variations in the package effect, the mean values of the “package effect index,” $Q_{ps}^*$, were computed as a function of the optical depth for both subequatorial and equatorial areas. For a given sample and a given wavelength, this index is defined as

$$Q_{ps}^* = a_{ps}^*/a_{sol}^*$$  \hspace{1cm} (7)

where $a_{sol}^*$ is the specific absorption coefficient of the same cellular matter ideally dispersed in a dissolved state. $Q_{ps}^*$ varies from 1 (no package effect) to 0 (maximal package effect) [Morel and Bricaud, 1981]. The computation of $Q_{ps}^*$ was performed at 675 nm to minimize the influence of pigment composition upon the package effect. The $a_{ps}^*(675)$ coefficient was taken equal to 0.020 m$^2$ mg$^{-1}$, and $a_{ps}^*(675)$ was corrected for the enhancement originating from the presence of chl $b$ and DV.
Figure 4. Mean variations of the absorption coefficient at 440 nm of particles, $a_{ph}^p(440)$ (Figures 4a and 4e); phytoplankton, $a_{ph}^p(440)$ (Figures 4b and 4f); nonphotosynthetic pigments, $a_{nps}^n(440)$ (Figures 4c and 4g); and photosynthetic pigments, $a_{ps}^p(440)$ (Figures 4d and 4h) versus the optical depth $\xi$ (top) in the subequatorial area ($13^\circ$ to $1^\circ$ S) and (bottom) in the equatorial area ($1^\circ$ S to $1^\circ$ N). Units are m$^2$ mg$^{-1}$. Averages correspond to 16 profiles (eight stations) for the subequatorial area and six profiles (three stations) for the equatorial area. Horizontal bars indicate the standard deviations.

Figure 5. Examples of absorption spectra of phytoplankton, $a_{ph}^p(\lambda)$, measured at various depths (top) in the subequatorial area ($7^\circ$ S) and (bottom) in the equatorial area ($1^\circ$ N). The right panels show the same absorption spectra normalized at 440 nm, in order to evidence the spectral changes with depth.
Subequatorial area

Equatorial area

Figure 6. Mean vertical variations versus the optical depth ξ of the concentrations of the main nonphotosynthetic pigments (zeaxanthin and diadinoxanthin) relative to Tch1 a (Figures 6a and 6c), the concentrations of the main photosynthetic pigments, (chl b + 1V chl h) and (1V'-HF + 1V'-HF), relative to Tch1 a (Figures 6b and 6d), the package effect index Qa* (675) (sec 7) (Figures 6e and 6g), and the intracellular concentration of DV chl a (c_i) for Prochlorococcus cells (Figures 6d and 6h). Top and bottom panels correspond to averages for the subequatorial area (16 profiles) and the equatorial area (six profiles), respectively, as in Figure 4. Horizontal bars indicate the standard deviations. Note that in Figures 6b and 6d, the vertical profiles of chl c, α-carotene, peridinin, and fucoxanthin-to-Tch1 a concentration ratios were omitted for clarity as these ratios were <0.1 throughout the water column. The profile of the diatoxanthin-to-Tch1 a concentration ratio (<0.05) was also omitted in Figures 6a and 6c.

chl b using a baseline joining a_{ph}^{\alpha}(660) to a_{ph}^{\alpha}(700), as given by Bricaud et al. [1995].

The Qa* (675) values decrease gradually with depth, from about 0.90 to 0.62 in the subequatorial area and from 0.72 to 0.46 in the equatorial area (Figures 6c and 6g). In spite of the large standard deviations, analysis of variance showed that the observed vertical and inter-site variations are significant at a threshold of 5% (P < 0.0001). The package effect, practically negligible near the surface in the subequatorial area, becomes more noticeable in equatorial waters and progressively increases with depth in both areas. Note that any uncertainty in the a_{sol}^{\alpha}(675) value does not affect the relative variations of Qa* (675) with depth or from site to site. It is also worth recalling that in the blue part of the spectrum, where the a_{cm}(\lambda) values reach their maximum, the package effect is much stronger than at 675 nm.

As previously stated, the vertical variations in Qa* (675) originate either from vertical variations in the average cell size or from those in a_{cm}(675), or from both. The a_{cm}(675) coefficient is equal to the product a_{sol}^{\alpha}(675)c_i, where c_i is the intracellular Tch1 a concentration (i.e., the ratio of the Tch1 a concentration to the total cellular volume). Therefore the variations of a_{cm}(675) strictly follow those of c_i. These c_i values can be evaluated at least for the population of Prochlorococcus cells, from DV chl a concentrations and flow cytometric enumerations and assuming an average cell size of 0.6 μm [Morel et al., 1993]. Similarly to what was observed in other oligotrophic areas [Campbell and Vaulet, 1993; Morel et al., 1993; Lazzara et al., 1996], they reveal a strong increase, by about 1 order of magnitude, from the surface to the deeper layers (see Figures 6d and 6h). This increase results from a high photoacclimation ability of cells to low irradiances [e.g., Parnesky et al., 1993]. For other populations such as cyanobacteria and flagellates, c_i cannot be directly estimated because of uncertainties about their size and the distribution of chl a within these populations. Cellular red fluorescence of these populations measured by flow cytometry, however, is also observed to increase with depth by virtue of photoacclimation, although to a lesser extent than for Prochlorococcus (see also Campbell and Vaulet [1993]). Vertical variations in cell size, although not documented here, may also contribute to increase the package effect with depth [see Lazzara et al., 1996].

The site-to-site variations of the package effect (Figures 6c and 6g) also likely result from combined effects of cell size and cellular pigment contents. Although the c_i values for Prochlorococcus are on average higher in the equatorial area than in the subequatorial area (Figures 6d and 6h), variations in cell size are probably the dominant factor. This is particularly evidenced by the fucoxanthin/Tch1 a ratio, which is enhanced by more than a factor of 2 from 2°S to 1°N, indicating an increasing presence of large cells (diatoms) close to the equator.

4.2. Variations of Photosynthetic Absorption
With the Tch1 a Concentration

Bricaud et al. [1995] and Cleveland [1995] observed a general inverse covariance between the a_{ph}^{\alpha} coefficients and the field
Tchl \(\alpha\) concentration. Such covariation was attributed mostly to (1) the contribution of nonphotosynthetic pigments to total absorption, which decreases with depth, and (2) the level of pigment packaging, which increases both from low-chlorophyll to high-chlorophyll waters (presumably because of the larger contribution of diatoms and dinoflagellates to biomass [see Claustre, 1994]) and from the surface to deeper waters because of photoacclimation. Partitioning \(a_{\text{ps}}^{*}\) into its two components \(a_{\text{ps}}^{\text{ph}}\) and \(a_{\text{ps}}^{\text{np}}\), allows the relative importance of these two causes to be estimated. In order to extend the Tchl \(\alpha\) range in this analysis, we have considered, in addition to the data presented above, three other cruises: in the western Mediterranean (Tomographie d’un Front (TOMOFRON T), in the Tropical North Atlantic (Eutrophie Mesotrophie Oligotrophie (EUMELI 3) cruise), and in the equatorial Pacific (Flux dans l’Ouest du Pacifique Equatorial (FLUPAC) cruise). The methods used for HPLC and optical measurements during these cruises were identical to those described here [see Briand et al., 1995] for details on the first two cruises and Allali et al. [1995b] for the latter one.

Figure 7 confirms that the \(a_{\text{ps}}^{*}(440)\) variations are reduced compared to those in \(a_{\text{ps}}^{\text{ph}}(440)\), by approximately a factor of 4 instead of 8 when the Tchl \(\alpha\) concentration is varying over 2 orders of magnitude (0.02–2 mg m\(^{-3}\)). The range of \(a_{\text{ps}}^{\text{ph}}(440)\) values (0.02–0.09 m\(^{2}\) mg\(^{-1}\)) compares well with that observed by Sosik and Mitchell [1995] (0.02–0.08 m\(^{2}\) mg\(^{-1}\), for Tchl \(\alpha\) concentrations varying between 0.1 and 2 mg m\(^{-3}\)). The large scatter which is observed in \(a_{\text{ps}}^{*}(440)\) versus Tchl \(\alpha\) relationship very likely originates from the variations in photosynthetic accessory pigments (chlorophylls \(b\) and \(c\), DV chl \(b\), and photosynthetic carotenoids), which are in varying proportions with respect to the Tchl \(\alpha\) concentration (Figures 6b and 6f; see also Figure 6 of Bricaud et al. [1995] for the TOMOFRON and EUMELI 3 cruises). In spite of this scatter a residual inverse covariation of \(a_{\text{ps}}^{*}(440)\) with the Tchl \(\alpha\) concentration is still observed. As the influence of nonphotosynthetic pigments has been removed, this weak covariation (10% of the variations in \(a_{\text{ps}}^{*}(440)\) are explained by a power function with a negative exponent) can be considered as originating from the package effect. Therefore, even if the variability of \(a_{\text{ps}}^{*}\) is reduced compared to \(a_{\text{ps}}^{\text{ph}}\), the assumption of a constant coefficient regardless of the depth and the trophic state of water remains a rough approximation.

5. Conclusions

We have used in this study a spectral decomposition technique to derive the photosynthetic absorption coefficients from the absorption coefficients of phytoplankton. This method presents the advantage of taking the package effect into account, in contrast to previous similar works [Bidigare et al., 1989]. It is acknowledged that the accuracy of the present method is potentially limited by uncertainties in the in vivo spectra of individual pigments, variations in energy transfer efficiency between pigments, and the omission of phycoerythrin proteins which were not measured by the HPLC technique. This latter limitation does not affect our estimates of \(a_{\text{ps}}^{*}\) at 440 nm (where absorption by phycoerythrin is negligible) but may be a difficulty when reconstructing a whole \(a_{\text{ps}}^{*}(\lambda)\) spectrum. Use of the fluorescence excitation spectra, as proposed by Sosik and Mitchell [1995], is affected by other limitations, such as low sensitivity of the method, the uncertainty upon the scaling of these spectra (see Johnson et al. [1993] for a discussion of this problem), and the hypothesis concerning the equal distribution of pigments between PI and PS II. A combined use of these two complementary techniques might help solving some of these questions.

The decomposition technique allowed us to assess the range and patterns of variability in the two components of absorption by phytoplankton, \(a_{\text{ps}}^{\text{ph}}\) and \(a_{\text{ps}}^{\text{np}}\), in relation with its pigment and taxonomic composition. While \(a_{\text{ps}}^{\text{ph}}(440)\) exhibits important vertical and site-to-site variations, driven by changes in pigment composition, species composition, and photoacclimation to the light regime, the natural variability in \(a_{\text{ps}}^{\text{ph}}(440)\) is significantly lower, showing that nonphotosynthetic pigments are actually the major source of variation in \(a_{\text{ps}}^{\text{ph}}\). Even in oligotrophic waters, however, the package effect is actually not negligible as often claimed, particularly below the mixed layer. The vertical and inter-site variations in \(a_{\text{ps}}^{*}(440)\) remain moderate (a factor of 4 for a Tchl \(\alpha\) concentration varying over 2 orders of magnitude) because this package effect is partly compensated by the inverse variations in the relative concentrations of photosynthetic accessory pigments.

While the knowledge of \(a_{\text{ps}}^{*}\) and \(a_{\text{ph}}\) variations remains essential when modeling light propagation within the ocean, the use of \(a_{\text{ps}}^{*}\) instead of \(a_{\text{ps}}^{\text{ph}}\) in primary production models may therefore present some advantages. On a sample-by-sample basis it is obviously strictly equivalent to use \(a_{\text{ps}}^{*}(\lambda)\) in conjunction with a quantum yield for carbon fixation defined with
respect to the amount of quanta absorbed by photosynthetic pigments or to use $a_{ph}^f(\lambda)$ and the "classical" quantum yield (i.e., defined with respect to the total amount of quanta absorbed by phytoplankton). For modeling purposes, however, the field variations of the input parameters are generally characterized independently [e.g., Morel et al., 1996], so that the two approaches are no more equivalent. The benefit of the approach using $a_{ph}^f$ is to reduce the natural variability of the absorption capacity of phytoplankton, by removing the "artificial" source of variability related to nonphotosynthetic pigments. In parallel, the variability of the quantum yield for carbon fixation, as computed with respect to photosynthetic absorption, should be also reduced, as already demonstrated by Babin et al. [1996] for waters of the tropical North Atlantic. Therefore the characterization of these two parameters for light-photosynthesis models should become easier and more accurate.

Appendix: Determination of a Site-Specific Algorithm for Correcting the Path Length Amplification Effect

When determining the absorption coefficients of natural phytoplanktonic populations with the GF/F technique, it is necessary to quantify the path length amplification factor ("\(B\) factor" [Butler, 1982]) resulting from multiple scattering within the filter and between the filter and particles. Several correction formulae have been proposed for this factor. From laboratory measurements, Mitchell [1990] established a quadratic relationship between optical densities measured on the filter (OD\(_f\)) and those measured on suspensions (OD\(_s\)) for an equivalent path length. Cleveland and Weidemann [1993], Tassan and Ferrari [1995], and Arbones et al. [1996], from measurements on various species, found similar relationships and emphasized that such a correction should be widely applicable, as little interspecific variability was observed. Recently, however, Moore et al. [1995] found, for Synechococcus and Prochlorococcus cultures, OD\(_s\) values strongly different from those provided by Mitchell's algorithm (30% and 50% lower, respectively, at OD\(_f\) = 0.4). They suggested that these differences might originate from the fact that small cells are more embedded in the filter than large ones, leading to modifications in filter-particle interactions. Whatever the cause, these results suggest that the algorithms proposed by Mitchell [1990] and Cleveland and Weidemann [1993] might not be applicable to oligotrophic or even mesotrophic waters, frequently dominated by Synechococcus and Prochlorococcus.

In order to check this hypothesis, a correction algorithm was derived from our field data. For six samples, two aliquots were filtered simultaneously on a Nuclepore (0.4 \(\mu\)m) and on a GF/F filter. The particles concentrated on the Nuclepore filter were transferred to a glass slide using liquid nitrogen freezing, according to the procedure described by Allali et al. [1995a], and optical densities were measured on the slide. As the path length amplification is avoided in this procedure, the measured optical densities are equal to OD\(_s\). Then, for each sample the OD\(_s\) values were compared to the OD\(_f\) values, scaled to the path length corresponding to OD\(_f\) measurements. Note that when establishing the OD\(_s\) versus OD\(_f\) relationship from measurements on cultures, Arbones et al. [1996] used the inverse procedure, i.e., scaled OD\(_f\) to the path length used for OD\(_s\) measurements (their equation (3)). As the path length amplification effect is considered to be primarily determined by the particle load on the filter (and therefore linked to a given OD\(_f\), value), this procedure is incorrect and provides a result similar to the procedure used here only in the quasi-linear domain of the OD\(_s\) versus OD\(_f\) relationship.

The empirical relationship obtained between the spectral values of OD\(_s\) and OD\(_f\) is given by a second-order polynomial:

$$OD_s = 0.264OD_f + 0.322OD_f^2 \quad r^2 = 0.967 \quad (8)$$

This relationship is intermediate (Figure 8) between those derived by Moore et al. [1995] for Synechococcus and Prochlorococcus, the two dominant populations in the investigated area. Therefore (8), which is to our knowledge the first algorithm for the correction of path length amplification established from field data, appears to confirm the results of Moore et al. [1995] obtained on cultures. Although this relationship was derived from only a few samples, concordant results were obtained from a more systematic study performed in a different area of the equatorial Pacific, along a meridian transect at 155°E and a transect along the equator from 167°E to 150°W (FLUPAC-Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) cruise [Le Borgne and Gesbert, 1995]). In this study the comparison between absorption spectra measured on GF/F filters [Dupouy-Douchement, 1995] and glass slides [Allali et al., 1995b] for 43 samples provided a relationship close to that obtained by Moore et al. [1995] for Synechococcus (Figure 8).

Note also that the algorithm previously derived by Bricaud and Stramski [1990] provides OD\(_s\) values significantly lower than those provided by Mitchell [1990] and Cleveland and Weidemann [1993] and is close to that corresponding to Synecho-

![Figure 8](image-url)
coccus. This suggests that Bricaud and Stramski's algorithm is appropriate for oligotrophic and mesotrophic waters, while the two other algorithms might be more adapted to eutrophic areas dominated by large cells.

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