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Spectral absorption coefficients of living phytoplankton and nonalgal biogenous matter: A comparison between the Peru upwelling area and the Sargasso Sea

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Abstract

Absorption coefficients of total particulate (biogenous) matter were determined in the mesotrophic waters of the Peruvian upwelling and the oligotrophic waters of the Sargasso Sea. A method based on spectral criteria was used to partition these coefficients into absorption by living algal cells and by nonalgal (mainly detrital) material. This method was tested by independently measuring absorption by nonalgal material for samples from the Sargasso Sea.

The average Chl-specific absorption coefficients measured in the Sargasso Sea are significantly higher than in the Peruvian upwelling and also higher in the surface layer than in the deep Chl maximum layer. These systematic variations appear to be mainly due to differences in sizes and intracellular pigment concentrations of the cells, inducing differences in the amplitude of the package effect.

In the open ocean, particulate biogenous matter dominates the optical variability of the water column. This suspended matter includes two types of particles present in variable proportions and having different spectral properties: living pigmented algal cells, and weakly pigmented or unpigmented particles mainly derived from phytoplankton as well as other living heterotrophs such as bacteria. These two categories will hereafter be referred to as the "algal" and "nonalgal" fractions.

Unlike the absorption coefficients of the pigments extracted from natural popula-

tions of phytoplankton, which are easily measurable, much uncertainty still remains because of methodological difficulties about absorption coefficients of intact, living cells. These absorption coefficients must be known to interpret the inherent (absorption) or apparent (diffuse attenuation coefficient, color) optical properties of the ocean, to model the photosynthetic capacities and growth rates of natural phytoplankton populations (e.g. Kiefer and Mitchell 1983), and to "physiologically" model primary production (Morel 1989).

Absorption coefficients of biogenous matter can be inferred indirectly from apparent optical properties as measured at sea (Bukata et al. 1981; Prieur and Sathyendranath 1981). Such an approach, however, is limited to the surface layer and does not permit algal and nonalgal fractions to be discriminated. Direct (spectrophotometric) measurements of the spectral absorption coefficients of water samples have been attempted. Because particle concentrations in the natural environment are generally low, such determinations require preconcentration. According to Kirk (1980), particles can be concentrated onto a membrane filter and then resuspended in a small volume with an efficiency of ~90%. In general, however, recovery of particles may not be quantitative, and the procedure is hardly usable for

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J. Gostan and J. Neveux are acknowledged for providing the results concerning the (Chl *a* + pheo *a*) concentrations during the Pacipro and Chlmax cruises, and L. Prieur for helpful comments. We express our particular gratitude to A. Morel for constructive criticism of the manuscript and are indebted to K. Carder and two anonymous referees for comments and suggestions.

routine measurements at sea. Concentration may also be obtained by centrifugation—also with questionable efficiency (Weidemann and Bannister 1986).

The method initiated by Yentsch (1962), who collected marine particles on membrane filters cleared with cedar oil, was modified by Trüper and Yentsch (1967), who concentrated cultured bacteria on glass-fiber filters before measuring their absorption directly on the wet filter. This latter method is now widely used for natural phytoplankton populations (e.g. Yentsch and Phinney 1982; Dubinsky et al. 1984; Carder and Steward 1985; Lewis et al. 1985, 1988; Maske and Haardt 1987). Thus a large amount of data presently exists concerning spectral absorption by natural phytoplankton assemblages.

Unfortunately, most of these results cannot be used quantitatively because "pathlength amplification" due to multiple scattering inside the filter has been either ignored or set to an arbitrary value. This pathlength amplification or " β factor," evidenced by Butler (1962), is defined as the ratio of optical to geometrical pathlength. It is very close to 1 for dilute suspensions, provided that multiple scattering is negligible, i.e. that the optical thickness, cr (where r is the geometrical pathlength and c the attenuation coefficient) is <0.1 (van de Hulst 1957). For samples concentrated on stacked halves of filters, β was found to be as high as 6 (Kiefer and SooHoo 1982). From measurements on algal cultures, Kishino et al. (1985) determined β to vary between 2.5 and 4.7 with species and found good agreement between spectral patterns of absorption measured on the suspension and on the filter, suggesting nearly constant β with wavelength. Conversely, Mitchell and Kiefer (1984, 1988a) established an inverse relationship between the β factor and the optical density of the particles on the filter, with numerical coefficients dependent on the filter type. A similar relationship had already been observed by Clarke (1982) for aerosol particles concentrated on Nuclepore filters. It implies that β is spectrally variable for a given sample. The major mechanisms determining the amplitude of β remain poorly understood.

When evaluating absorption coefficients

of living algal cells only, a second methodological problem is discrimination between algae and other particles. In most of the above-mentioned studies, such discrimination was not attempted. Kiefer and SooHoo (1982) tried to separate, by multiple regression, the contribution to absorption of particles covarying with Chl *a* and that of particles covarying with pheopigments; the latter is supposed to represent absorption by small detrital particles present in the euphotic zone. Pheopigments are rapidly photo-oxidized (SooHoo and Kiefer 1982*a,b*), however, so these pigments are not reliable indicators of detrital concentration, as outlined by Mitchell (1987). From simultaneous measurements of absorption and fluorescence excitation spectra and a statistical relationship between these spectra for living phytoplankton, Mitchell (1987) partitioned absorption into fluorescing phytoplankton and nonfluorescing detritus contributions. Bidigare et al. (1989) "reconstructed" an absorption spectrum for living phytoplankton by using the published *in vivo* absorption coefficients of the main pigments and assuming a reduction of these coefficients by 30% due to the package effect. By subtracting this reconstructed spectrum from the experimental absorption spectrum of total particulate matter, they derived absorption by nonalgal particles.

The first study, to our knowledge, dedicated to experimental discrimination between absorption by pigmented and non-pigmented matter retained on a filter was made by Konovalov and Bekasova (1969). They attempted to remove the pigments either by washing with a mixture of organic solvents or by applying UV radiation. Recently, Kishino et al. (1985, 1986) measured the absorption coefficients of marine particles concentrated on a glass-fiber filter before and after immersion in methanol. After methanol extraction, the residual absorption is due to unpigmented particles but also to nonextractable pigments (phycobilins) and to bleached cells; likewise the difference of absorption before and after extraction is due not only to *in vivo* pigments extracted from living cells but also to (extractable) pheopigments and detrital carotenoids present in debris. Therefore, the algal

and nonalgal fractions cannot be identified unambiguously and quantified easily with this method.

The microphotometric technique recently developed by Iturriaga et al. (1988) permits determination of the efficiency factors for absorption, Q_a , of individual living or detrital particles. A partition of absorption into phytoplanktonic and detrital components can then be performed by multiple regression (Iturriaga and Siegel 1988) under the proviso that representative mean values of Q_a can be obtained for both (living and nonliving) populations. In practice, this technique requires tedious measurements on many individual particles and is restricted to particles $\geq 2.5 \mu\text{m}$ in diameter. Therefore, its application to routine field studies seems to be impractical, especially since the major fraction of absorbing particles in the ocean may be $< 2.5 \mu\text{m}$.

These methodological difficulties emphasize the need to develop indirect approaches as alternatives to experimental methods. We here present a method based on spectral criteria for evaluating the algal and nonalgal contributions to absorption. This method was applied to absorption coefficients of total particulate matter measured in two contrasting water types: mesotrophic waters of the Peruvian upwelling dominated by living phytoplankton in the upper layer, and oligotrophic waters of the Sargasso Sea containing a significant amount of biogenous detritus. At both sites, the terrigenous inorganic particle concentration is negligible (except in case of episodic atmospheric inputs), and the total absorption is attributable only to phytoplankton, other organisms, and their remains. At the first site, the respective contributions of algal cells and other particles were estimated only indirectly. During the second cruise the same method was applied, and in parallel these respective contributions were determined experimentally according to the direct method developed by Kishino et al. (1985). This method, albeit imperfect, presently seems to be the sole possibility for obtaining experimental values for algal and nonalgal absorption with reasonable precision and accuracy. The values determined with this direct method therefore allow the efficiency

of the spectral decomposition method to be tested.

The aim of this paper is thus to describe and evaluate the reliability of the indirect method for partitioning the overall absorption into contributions due to the algal and nonalgal fractions, to study the variability of the absorption coefficients of both algal cells and nonalgal particles in two different water types, and to compare these absorption coefficients with published data concerning natural or cultured phytoplankton and detrital particles.

Materials and methods

Sampling—Measurements in the Peruvian upwelling area were carried out during the Paciprod-J. Charcot cruise (first part, August–September 1986) on two transects in the area 16.15° – 8°S and 84.10° – 75.30°W ; 50 samples were collected at 10 stations at various depths between 0 and 400 m.

In the Sargasso Sea, measurements were made during the Chlomag-Suroît cruise (September–October 1987) along a 61°W transect from 20° – 30°N ; 64 samples were collected at 15 stations between 0 and 250 m. For both cruises, absorption measurements and pigment extractions were made nearly simultaneously.

Absorption measurements—Seawater volumes of 0.25–1.5 liters (Paciprod samples) or 3–10 liters (Chlomag samples) were filtered onto 25-mm glass-fiber filters (Whatman GF/F). Optical densities of total particulate matter, $\text{OD}_{\text{tp}}(\lambda)$, were measured directly on the wet filters by the method of Trüper and Yentsch (1967) with a double-beam recording spectrophotometer Perkin-Elmer 571 in the spectral range 380–750 nm. The filter was placed in front of a diffrusing window adjacent to an end-on photomultiplier of large surface area. An unused, wetted filter was taken as a reference blank and baseline variations were automatically corrected. The spectral resolution was 1 nm, and optical densities were digitized at 2.5-nm intervals. During a scan (< 4 min), the wetness of the filters remained essentially unchanged. Relative variations in wetness between the sample and reference filters were likely, however, to be the source of negative values observed in the infrared

for Pacipro samples. For Chlmax samples this drift was minimized by soaking the filters for 1–1.5 h in filtered seawater before use.

This drift was corrected for Pacipro samples by re-estimating the optical density at 750 nm, $OD_{fp}(750)$, for each sample with an empirical law derived from Chlmax data:

$$OD_{fp}(750) \approx 0.6[OD_{fp}(380) - OD_{fp}(505)].$$

The rationale for such a relationship is that $OD_{fp}(750)$ as well as $OD_{fp}(380) - OD_{fp}(505)$ are attributable mostly to detritus, as will be discussed later. Measurements on unused filters showed that the drift was quasi-neutral throughout the spectrum, so the optical densities measured for each Pacipro sample were shifted at all wavelengths by setting $OD_{fp}(750)$ to its estimated value.

After the measurement of $OD_{fp}(\lambda)$, and only for Chlmax samples, the spectral absorption by nonalgal material was measured separately with the method of Kishino et al. (1985). The filter was placed in absolute methanol at 4°C for 45–60 min in order to extract pigments. The bleached filter was dried and then soaked again in filtered seawater for 1 h. The same treatment was applied in parallel to the reference filter. Then the filters were placed in precisely the same geometrical position as before and optical densities of the bleached material, $OD_{fd}(\lambda)$, were recorded. The efficiency of the methanol treatment in extracting pigments was estimated by Kishino et al. (1985) to be at least 90–95%. Incomplete extraction results in a slight overestimate of absorption by nonalgal material and thus in an underestimate of absorption by living algal cells.

The optical density of pigments can be assumed to be zero at 750 nm, so the $OD_{fd}(750)$ value was adjusted to $OD_{fp}(750)$, and the $OD_{fd}(\lambda)$ values were shifted accordingly at all wavelengths. Then the optical densities $OD_{fp}(\lambda)$ or $OD_{fd}(\lambda)$ were transformed into absorption coefficients $a_p(\lambda)$ or $a_d(\lambda)$ according to the relationship

$$a_{p/d}(\lambda) = 2.3 OD_{fp/d}(\lambda)s/[V_f \beta(\lambda)] \quad (1)$$

where s is the clearance area of the filter, V_f the volume of filtered seawater, and $\beta(\lambda)$ the pathlength amplification factor (Mitchell

Significant symbols

λ	Wavelength, nm
OD_s	Optical density of sample in suspension
OD_f	Optical density of sample on filter
OD_{fp}	Optical density of total particulate matter on filter
OD_{fd}	Optical density of detrital (or nonalgal) matter on filter
β	Pathlength amplification factor
s	Clearance area of the filter, m^2
V_f	Volume of filtered seawater, m^3
a_p	Absorption coefficient of total particulate matter, m^{-1}
a_d	Absorption coefficient of nonalgal matter, m^{-1}
a_{ext}	Absorption coefficient of extracted pigments, m^{-1}
a_{ph}	Absorption coefficient of living phytoplankton, m^{-1}
a_{ph}^*	Absorption coefficient of living phytoplankton per unit of (Chl a + pheo a) concn, m^2 [mg (Chl a + pheo a)] $^{-1}$
S	Exponent in the spectral absorption law for detrital matter, nm^{-1}
d	Particle diameter, μm
c_i	Intracellular (Chl a + pheo a) concn, $kg m^{-3}$
λ_{rm}	Wavelength of the red absorption maximum, nm
$h^*(\lambda_{rm})$	Amplitude of the red absorption maximum normalized by the (Chl a + pheo a) concn, m^2 [mg (Chl a + pheo a)] $^{-1}$

and Kiefer 1984). The term $V_f \beta/s$ represents the actual pathlength of light passing the sample. (Definitions and units are given in list of symbols.)

The absorption coefficients of viable phytoplankton, $a_{ph}(\lambda)$, were obtained by subtracting the absorption by the bleached matter, $a_d(\lambda)$, from the absorption by total particulate matter, $a_p(\lambda)$. Finally these coefficients were converted into "Chl-specific" coefficients [$a_{ph}^*(\lambda)$], i.e. divided by the (Chl a + pheophytin a) concentration. For pigment determinations, aliquots of each water sample were filtered on GF/F filters. They were analyzed fluorometrically (Turner 512) on methanol extracts for Pacipro samples and spectrofluorometrically on acetone extracts for Chlmax samples. During the Chlmax cruise, a comparison between acetone and methanol extraction efficiencies revealed no systematic bias (J. Neveux pers. comm.). Spectrophotometric measurements (on acetone extracts) were also performed on some replicate samples of both

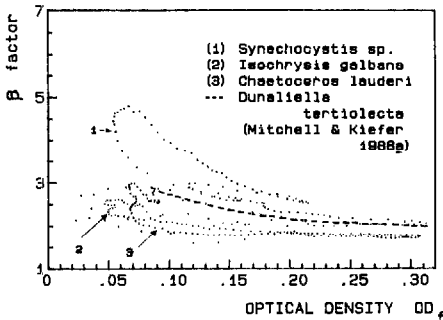


Fig. 1. Variations of the pathlength amplification factor β occurring inside GF/F filters vs. optical density of the sample on the filter for different phytoplankton cultures. Curves 1–3 were obtained by comparing spectral absorption coefficients measured on GF/F filters and on dilute suspensions. Points are spaced by 2.5-nm intervals in wavelength; the hysteresis effect is manifested as characteristic loops. The curve obtained by Mitchell and Kiefer (1988a) is plotted for comparison (dashed line). Data from Mitchell and Kiefer were obtained with GF/C filters; this curve was plotted by assuming that the optical density measured on a GF/F filter is on average 1.3 times higher than on a GF/C filter (Mitchell 1987).

cruises in order to obtain information on the carotenoid:Chl *a* ratio.

Fluorometric determinations were made by calibrating the fluorometer with pure Chl *a* (Sigma); spectrofluorometric measurements were performed with the method of Neveux and Panouse (1987). When spectrophotometry was used, Chl *a* and *c* concentrations were determined according to Jeffrey and Humphrey (1975), carotenoid concentrations according to Richards and Thompson (1952), and relative proportions of Chl *a* and pheophytin *a* according to Lorenzen (1967).

It must be emphasized that some uncertainty persists for the Chl concentrations determined within the deep maximum layer in the Sargasso Sea. It is due to the presence in this layer of small, red-fluorescing cells probably belonging to the division of Prochlorophyta, which have been identified by flow cytometry (Neveux et al. 1989). These cells contain a divinyl Chl *a*-like pigment with a fluorescence excitation maximum shifted by about 5 nm toward higher wavelengths compared to that of Chl *a*. When we consider their high number density within the deep Chl maximum (one order of magnitude higher than that of other types

of cells), their presence could result in an underestimate of the Chl *a* concentration of up to 30% (J. Neveux pers. comm.).

Estimation of the pathlength amplification factor β —For cultured species, β can easily be determined by comparing the spectral optical densities of a given sample measured on a filter (OD_f) and on the suspension (OD_s). If the suspension is sufficiently dilute so that multiple scattering is negligible, β is simply equal to $OD_f \times s \times r / (OD_s \times V_f)$, where r is the pathlength in the suspension (see Eq. 1).

At weak OD_f (<0.20–0.25) β varies significantly among species (Fig. 1), i.e. presumably according to the absorption and scattering properties of particles. Part of the variability may also be due to imprecision in estimating β in regions of low absorption (low OD_s and OD_f signals). Furthermore, even for a given species, the relationship between β and OD_f is equivocal: β values differ at the same OD_f values over varying wavelengths, which one could refer to as a “hysteresis effect” for different spectral rates of change in absorption. When $OD_f > 0.2$, curves for different species are in better agreement with β values varying weakly around 2.

This trend is also confirmed by β determinations performed on 10 Chlmax samples. Particles were first collected onto a 0.2- μ m Nuclepore filter and resuspended in a small volume of filtered water ($1/600$ to $1/2,200$ of the initial volume) by direct rubbing with a glass rod and 2–3 min of gentle ultrasonication (Kirk 1980; Davies-Colley 1983); the absorption spectrum of the suspension, $OD_s(\lambda)$, was measured. A given volume of this concentrate was then refiltered onto a GF/F filter and the absorption spectrum $OD_f(\lambda)$ measured on the filter. This procedure for determining β was designed to avoid errors due to incomplete resuspension of the particulate material in the original sample. Furthermore, the resuspension procedure does not introduce any apparent qualitative changes in the particulate community because OD_f spectra for particulate matter in the original and resuspended sample have very similar shapes. β values, based on data in the range 400–500 nm, were between 1.7 and 2 and varied only weakly with λ [be-

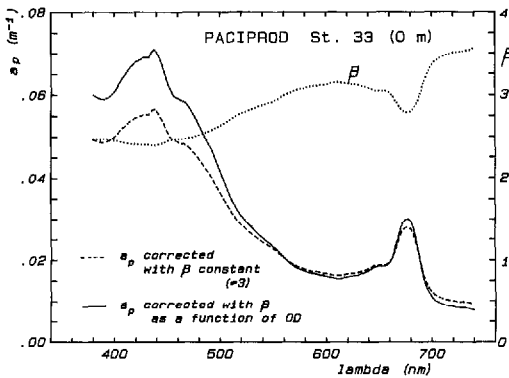


Fig. 2. Influence of the spectral variations of β on the spectral values of absorption by particulate matter, a_p . The dashed-line spectrum was obtained by assuming β constant ($=3$). The solid-line spectrum was obtained by computing β according to Eq. 2. The corresponding values of β are shown as a dotted line.

yond 500 nm, the instability of the signal from the spectrophotometer operated at sea did not allow reliable measurement of $OD_s(\lambda)$. Interestingly, the β values for the samples from the mixed layer and aphotic zone tended to be slightly lower than those for the deep Chl maximum.

At this stage the complicated behavior of β could not be incorporated for correcting the measured absorption spectra, so an average relationship, β vs. OD_f with values around 2 in the OD_f range 0.20–0.30 has been adopted. We believe that adopting such a relation is a reasonable first approximation, especially at sufficiently high OD_f (>0.20 – 0.25). It must be kept in mind, however, that at low OD_f , $a_p(\lambda)$ might be seriously in error due to uncertainty in β . The relationship determined by Mitchell and Kiefer (1988a) which appears on Fig. 1 as intermediate among the different curves was chosen and fitted to the function

$$\beta(\lambda) = 1.63 OD_f(\lambda)^{-0.22}, \quad (2)$$

which was used to convert the measured $OD_{f/d}(\lambda)$ values into $a_{p/d}(\lambda)$ values (see Eq. 1). Spectral variations of β have a significant influence on $a_p(\lambda)$ values (Fig. 2).

Indirect estimation of relative contributions of algal and nonalgal material to overall particulate absorption—When absorption by detrital matter is not directly measured (Paciproduct cruise), an indirect

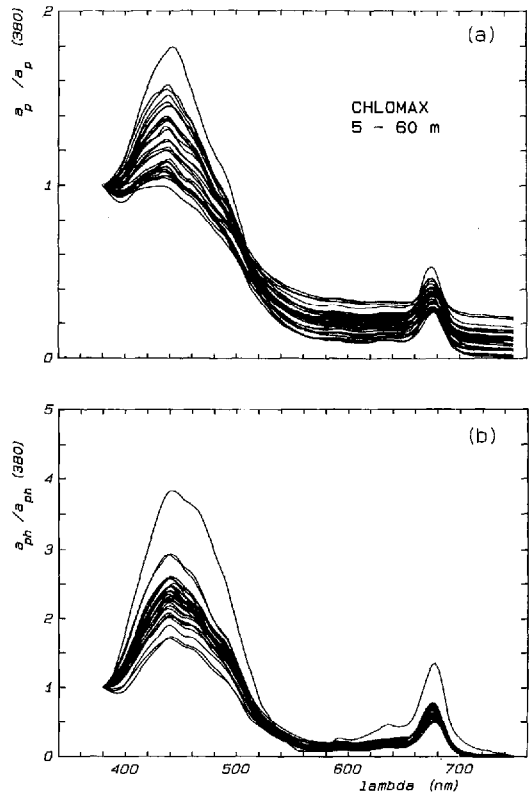


Fig. 3. Spectral values of absorption coefficients of total particulate matter, $a_p(\lambda)$, and living phytoplankton, $a_{ph}(\lambda)$, measured in the surface mixed layer (0–60 m) of the Sargasso Sea during the Chlmax cruise and normalized at 380 nm. The spectral values of a_p were measured on GF/F filters and corrected for pathlength amplification. The spectral values of a_{ph} were obtained by also measuring the absorption coefficients of nonalgal particulate matter, $a_d(\lambda)$, and then subtracting $a_d(\lambda)$ from $a_p(\lambda)$.

method must be used to assess the respective roles of the algal and nonalgal fractions. Our method makes use of the shape of the $a_p(\lambda)$ spectrum. On one hand, it is apparent (Fig. 3a) that $a_p(\lambda)$ values exhibit (variable) ascending values toward the short wavelengths (on which the absorption signatures of phytoplanktonic pigments are superimposed). They result in a violet-to-green ratio, e.g. $a_p(380 \text{ nm}) : a_p(500 \text{ nm})$, varying from 1.2 to 1.75 (Fig. 3a). On the other hand, when considering absorption by only living cells, a_{ph} (Fig. 3b), the same violet-to-green ratio is lower (~ 1) and (except in one sample) remarkably constant. The same observations are also valid for the yellow-

to-red ratio (say, 580 nm:700 nm). This finding suggests that the generally ascending values of a_p toward short wavelengths are due to detrital matter and that a partition of total absorption between the algal and nonalgal fractions could be possible on the basis of the information contained in the slope of the a_p spectra.

Criteria for selecting the wavelengths involved in these ratios are that the absorption by accessory pigments must be minimal, that the values of these ratios must be close to 1 for living phytoplankton, and that the wavelengths in each ratio must be sufficiently spaced to estimate the a_p slope with sufficient accuracy.

The rationale for having a minimal absorption by accessory pigments is that these ratios must be free as far as possible from the influence of variations in pigment composition, whatever the population. There is no wavelength, obviously, where this condition can be strictly fulfilled. Nevertheless, for narrow spectral domains around 380, 510, 580, and 700 nm, variations in the carotenoid:Chl *a* ratio should have a weak effect on both ratios because carotenoids do not absorb light around 580 and 700 nm and absorb only weakly around 380 and 510 nm. Chl *b* and *c* also have negligible absorption at these wavelengths. The presence of phycobilin pigments (e.g. phycoerythrins with an absorption maximum between 545 and 565 nm, O'Carra and O'hEocha 1976) might modify both ratios. In spite of the ascertained presence of *Synechococcus* cells in the upper layer (Neveux et al. 1989), absorption bands of phycoerythrin were hardly detectable in the experimental a_{ph} spectra (Fig. 3b) and cannot significantly influence the ratios considered. Practically, small variations in these ratios only weakly influence the retrieved values of $a_d(\lambda)$ and $a_{ph}(\lambda)$ (see later).

The rationale for choosing ratios close to 1 is that they must not be affected by the discreteness or package effect, i.e. the flattening effect that can be induced by an increase of either cell size or of intracellular pigment concentration (Morel and Bricaud 1981). Provided that the absorption coefficients have the same values for the two wavelengths in question, their ratio is not

modified by a flattening of the absorption spectrum.

The ratios best filling the above conditions for the spectra shown in Fig. 3b ($N = 31$) are

$$a_{ph}(505):a_{ph}(380) = 0.99 \text{ (SD 0.10)} \quad (3a)$$

and

$$a_{ph}(580):a_{ph}(692.5) = 0.92 \text{ (SD 0.16)}. \quad (3b)$$

Besides the postulate that the general increase of a_p toward short wavelengths is due only to nonalgal material, an assumption is required concerning the spectral dependency of absorption by this material. The following exponential expression is adopted:

$$a_d(\lambda) = A \exp(-S\lambda) \quad (4)$$

where A and S are variable. This formulation is consistent with the results of previous studies where the similarity between the spectral absorption properties of detrital particles and those of dissolved yellow substance has been emphasized (Yentsch 1962; Kirk 1980; Davies-Colley 1983). This assumption is again fully supported by the shape of the a_d spectra measured for Sargasso Sea samples (Fig. 4a) as well as by the a_p spectra observed for deep samples from Peruvian waters when algae are scarce (Fig. 4b).

By combining the above assumptions into the relationship

$$a_d(\lambda) = a_p(\lambda) - a_{ph}(\lambda),$$

we obtain the following equations:

$$A \exp(-380 S) - A \exp(-505 S) = a_p(380) - a_p(505) - 0.01a_{ph}(380); \quad (5a)$$

$$A \exp(-580 S) - A \exp(-692.5 S) = a_p(580) - a_p(692.5) + 0.08a_{ph}(692.5). \quad (5b)$$

Therefore, under the proviso that A (or equivalently a_d at a given wavelength) and S vary independently, which is evidenced by Fig. 5 for experimental data from the Sargasso Sea, the contributions of algal and nonalgal materials to overall absorption can be estimated for each sample by solving the following system of equations (equivalent

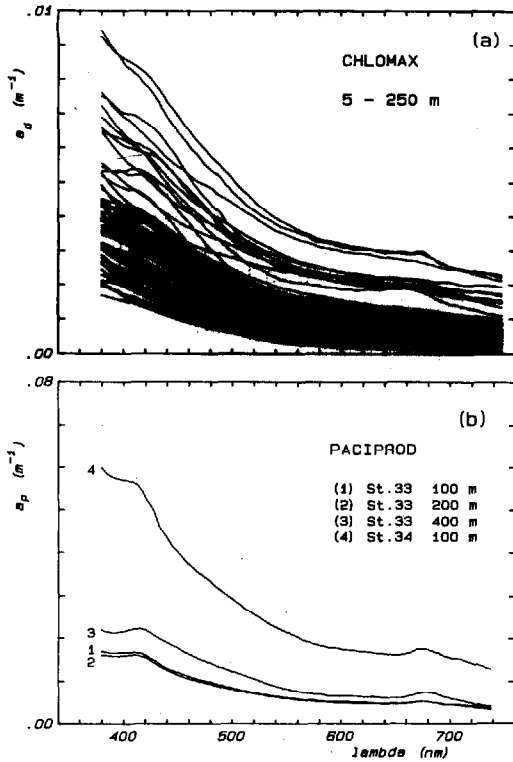


Fig. 4. Spectral values of absorption by nonalgal particulate matter $a_d(\lambda)$ measured for Sargasso Sea samples and total particulate matter $a_p(\lambda)$ obtained for deep samples from the Peruvian upwelling area.

to Eq. 5a and b) for A and S :

$$0.99A \exp(-380 S) - A \exp(-505 S) = 0.99a_p(380) - a_p(505), \quad (5c)$$

$$A \exp(-580 S) - 0.92A \exp(-692.5 S) = a_p(580) - 0.92a_p(692.5); \quad (5d)$$

and then computing at each wavelength (e.g. Fig. 6):

$$a_d(\lambda) = A \exp(-S\lambda) + a_p(750) - A \exp(-750 S), \quad (6a)$$

$$a_{ph}(\lambda) = a_p(\lambda) - a_d(\lambda). \quad (6b)$$

The term $[a_p(750) - A \exp(-750 S)]$ in Eq. 6a means that, as for experimental data, the computed values of $a_d(\lambda)$ are shifted at all wavelengths in such a way that $a_d(750)$ equals $a_p(750)$.

One advantage of this method is that the spectral shapes of a_{ph} and a_d are allowed to vary because the system is constrained only at two pairs of wavelengths. This flexibility

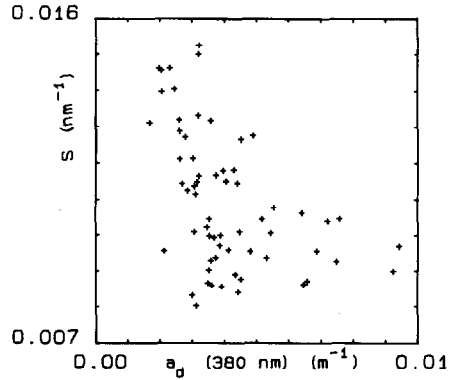


Fig. 5. Variations of the exponent S in the spectral absorption law of nonalgal material, $a_d(\lambda)$, measured for Sargasso Sea samples vs. the amplitude of absorption at 380 nm, $a_d(380)$. The values of S have been estimated from the decrease of the experimental absorption values between 380 and 700 nm.

is necessary for reconstructing $a_{ph}(\lambda)$ properly, even in a restricted region. Although $a_{ph}(505) \approx a_{ph}(380)$ for most of the samples, the ratio $a_{ph}(440) : a_{ph}(380)$, for instance, undergoes large variations (by a factor of 2.3) due to the combined influences of the package effect and pigment composition (Fig. 3b).

Note also that this method could be simplified by replacing Eq. 5d by

$$A \exp(-750 S) = a_p(750),$$

which would be equivalent to the assump-

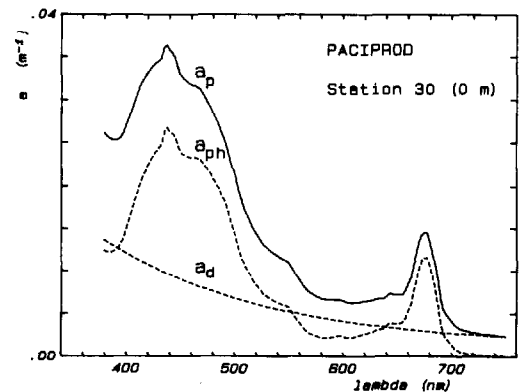


Fig. 6. Decomposition of the spectral values of absorption coefficients of total particulate matter $a_p(\lambda)$ into absorption coefficients of nonalgal particles $a_d(\lambda)$ and of living phytoplankton $a_{ph}(\lambda)$ shown for a sample from the Paciprod cruise. The spectral values of a_d have been adjusted so that $a_d(750)$ equals $a_p(750)$, i.e. $a_{ph}(750) = 0$.

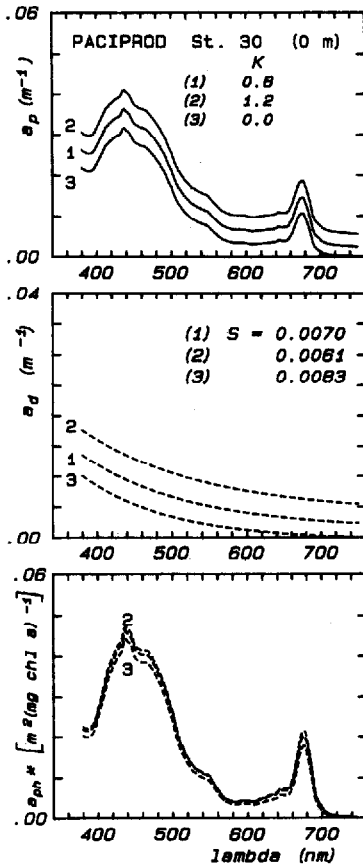


Fig. 7. Influence of variations in the infrared background value of absorption by particulate matter $a_p(\lambda)$ on the spectral values of absorption by detrital matter $a_d(\lambda)$ and of specific absorption by living phytoplankton $a_{ph}^*(\lambda)$. Background optical density $OD_{fp}(750)$ is computed with the equation $OD_{fp}(750) = K[OD_{fp}(380) - OD_{fp}(505)]$ where OD_{fp} is the optical density of the sample on the filter. Values of K are indicated.

tion $a_{ph}(750) = 0$. The advantage of such a modification would be reduction of the number of assumptions because the use of the yellow-to-red ratio would become unnecessary. This approach cannot be applied in the present work because in the procedure used to estimate the infrared absorption background (Paciproduct samples), $a_p(750)$ and $[a_p(380) - a_p(505)]$ are directly linked.

Results and discussion

Propagation of possible errors in the computed values of $a_d(\lambda)$ and $a_{ph}(\lambda)$ —For Paciproduct samples, the $a_d(\lambda)$ and $a_{ph}(\lambda)$ values were computed from optical densities mea-

sured on total particulate matter with different assumptions: infrared absorption was not directly measured but re-estimated with optical densities measured in the violet and green parts of the spectrum; β was assumed to vary as a function of OD_f according to Eq. 2; the spectral ratios $a_{ph}(505):a_{ph}(380)$ and $a_{ph}(580):a_{ph}(692.5)$ were assumed to be constant from one sample to another; and $a_d(\lambda)$ was assumed to be exponentially decreasing with increasing λ (plus a constant background). Note that only the latter two assumptions are inherent to our decomposition method. Because deviations from these diverse assumptions may affect both $a_d(\lambda)$ and $a_{ph}^*(\lambda)$ nonlinearly, it is necessary to examine to what extent their values are sensitive to possible errors. For Chlmax samples, where $a_{ph}(\lambda)$ was experimentally determined, only the second assumption must be considered.

Influence of background variations in infrared absorption—The infrared background of a_p spectra was estimated for Paciproduct samples by assuming that $OD_{fp}(750)$ is proportional to $OD_{fp}(380) - OD_{fp}(505)$. The proportionality factor, K , evaluated from Chlmax samples (0.60) is somewhat variable with a standard deviation of 0.26 ($n = 63$). A variation in the infrared background modifies the values of $OD_{fp}(\lambda)$ and consequently of $\beta(\lambda)$ over the entire spectrum. In practice, however, variations in K even if assumed (arbitrarily) to be ± 0.60 ($\pm 100\%$ of the mean) would have repercussions mainly on the infrared background of $a_d(\lambda)$, while the exponent (S) in $a_d(\lambda)$ as well as the spectral values of $a_{ph}^*(\lambda)$ would be little modified (Fig. 7).

Influence of variations in the relation between β and optical density—As expected, the choice of the $\beta(OD_f)$ function has a drastic influence on the $a_p(\lambda)$ values and therefore on the $a_{ph}(\lambda)$ values as well as the $a_d(\lambda)$ values (in magnitude and steepness) (Fig. 8a). Practically, the influence of the β function on the a_{ph}^* values is dependent on the range of variation of OD_f considered and therefore varies from one sample to another. Consequently, the different functions β vs. OD_f have been applied individually to each sample, and an average specific absorption spectrum has been computed for each func-

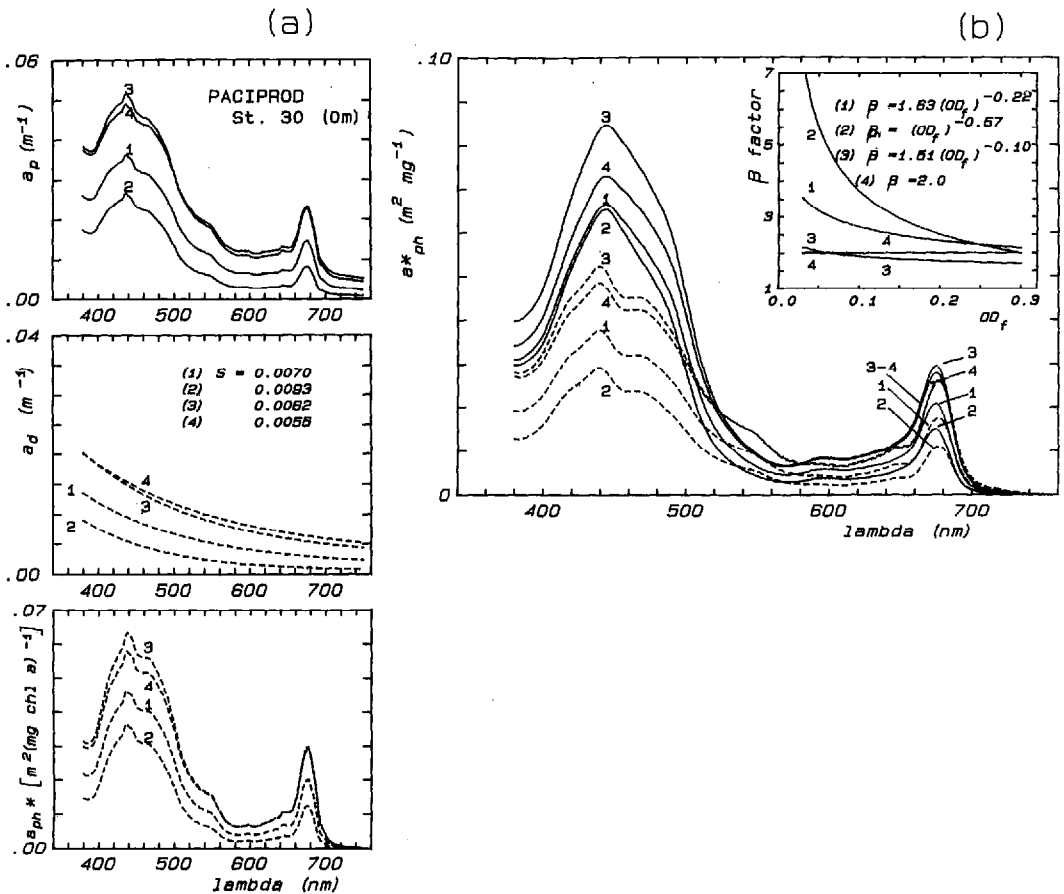


Fig. 8. a. Spectral values of absorption by total particulate matter (a_p) and detrital matter (a_d) and of specific absorption by living phytoplankton (a_{ph}^*) for four different curves of β vs. OD_f . These curves, chosen to cover the large range of variation observed for β on monospecific algal cultures (see Fig. 1), are shown in panel b. The corresponding values of the exponent S in $a_d(\lambda)$ are indicated. b. Mean spectral values of (Chl a + phaeo a)-specific absorption by living phytoplankton (a_{ph}^*) for the Paciproduct cruise (dashed lines) and for the Chlomag cruise (solid lines) for the same curves of β vs. OD_f as in panel a.

tion and for both cruises (Fig. 8b). As expected, the highest a_{ph}^* curves are obtained with the curve (3) that provides the lowest β values over most of the spectrum. For the samples from the Peruvian upwelling, considering that β varies according to function 3 instead of function 1 of Fig. 8 enhances the specific absorption values by about 37% in the blue (around 440 nm) and by about 27% around 675 nm. Conversely, considering that β varies strongly with OD_f (function 2) decreases the specific values by about 23% around 440 nm and by 35% around 675 nm.

Similar variations are observed for samples from the Sargasso Sea with a reduced range of variation of a_{ph}^* from curve 2 to

curve 3 (Fig. 8b), first because measurements were generally made at higher OD_f and second because the a_{ph} values were experimentally determined and not computed [for Paciproduct samples the $\beta(OD_f)$ curve influences a_{ph} values also through the decomposition method]. We expect that the actual dependence of β vs. OD_f is less variable than shown in Fig. 8b for two reasons. First, in experiments performed on field samples from the Mediterranean Sea (Y.-H. Ahn pers. comm.) or from the Sargasso Sea, none of these samples has revealed as strong a dependence of β on OD_f as observed on pure cultures (curves 2). Second, the highest $a_{ph}^*(\lambda)$ curves (3 and 4, corresponding to β

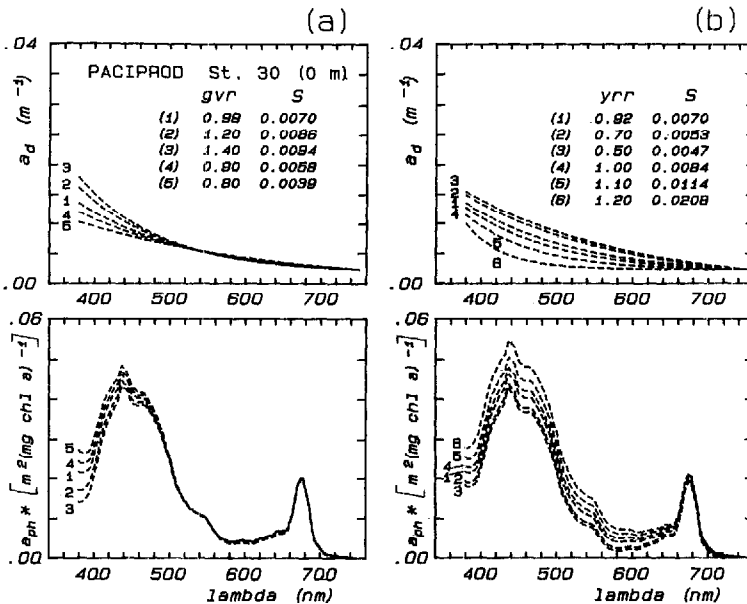


Fig. 9. a. Spectral values of absorption by detrital matter (a_d) and of specific absorption by living phytoplankton (a_{ph}^*) obtained by decomposing a total absorption spectrum, assuming different values of the green-to-violet ratio $a_{ph}(505):a_{ph}(380)$. The values of this ratio (gvr) and those obtained for the exponent S in $a_d(\lambda)$ are indicated. b. As in panel a for different values of the yellow-to-red ratio $a_{ph}(580):a_{ph}(692.5)$. The values of this ratio (yrr) and those obtained for the exponent S in $a_d(\lambda)$ are indicated.

constant or weakly varying with OD_p) exhibit absorption peaks around 675 nm that exceed the theoretical maximum, considering that the height of this peak in vivo should not exceed that of Chl *a* in acetone solution (see Bricaud et al. 1983), i.e. 0.0206 m² mg⁻¹. Therefore, the corresponding $\beta(OD_p)$ functions are probably unrealistic, at least for the samples considered. It remains possible that variations of β induce significant variations in $a_d(\lambda)$ and $a_{ph}^*(\lambda)$, which should be kept in mind in all studies concerning absorption by particulate matter or living phytoplankton derived from measurements on filters.

Influence of variations in the green-to-violet and yellow-to-red ratios for living phytoplankton—The ratios $a_{ph}(505):a_{ph}(380)$ and $a_{ph}(580):a_{ph}(692.5)$ have been found to be nearly constant (respectively 0.99 and 0.92) for samples from the surface layer in the Sargasso Sea (Eq. 3). They are anticipated to vary from one area to another, however, with the dominant species. From measurements on pure, pheopigment-free cultures (Bricaud and Morel 1986; Bricaud

et al. 1988) these ratios are found to be 0.81 (SD 0.10) and 1.01 (SD 0.14) ($N = 10$; phycobilin-containing species are excluded from these averages). Variations in the green-to-violet ratio from 0.8 to 1.4 modify a_{ph}^* from <15% around 440 nm and lead to insignificant variations in the green and red parts of the spectrum (Fig. 9a). A decrease of the yellow-to-red ratio from 0.92 to 0.5 modifies a_{ph}^* by only a few percent throughout the spectrum (Fig. 9b). An increase of this ratio from 0.92 to 1.2, however, is responsible for significant enhancement of a_{ph}^* in the blue and green parts of the spectrum (by ~20% around 440 nm). In practice, for each sample the effect of such variations becomes significant when one of the ratios approaches the corresponding ratio for a_p [i.e. $a_p(505):a_p(380)$ or $a_p(580):a_p(692.5)$]; this problem can be detected by noting anomalous (close to 0 or ≈ 0.020) values of S . All variations of both ratios have an insignificant effect on absorption beyond 660 nm.

Comparison between computed and experimental values of $a_d(\lambda)$ and $a_{ph}^(\lambda)$ for Sargasso Sea samples*—The decomposition

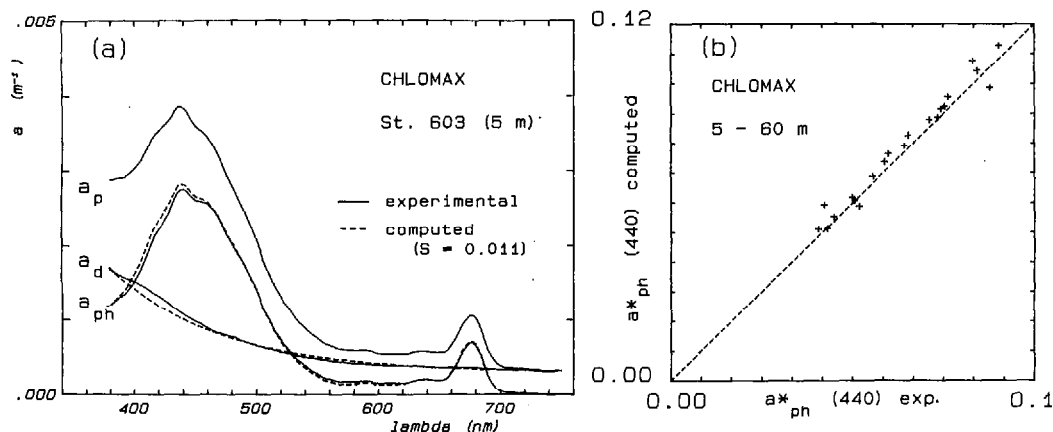


Fig. 10. a. Comparison of the spectral values of absorption by nonalgal material, $a_d(\lambda)$, and by living phytoplankton, $a_{ph}(\lambda)$, as experimentally obtained with the method of Kishino et al. (1985) (solid lines), and computed by applying the indirect decomposition method to $a_p(\lambda)$ (dashed lines; see text) for a surface-layer sample of the Sargasso Sea. The value obtained for the exponent S in $a_d(\lambda)$ is indicated. Both experimental and computed values of $a_d(\lambda)$ have been adjusted so that $a_d(750)$ equals the measured value of $a_p(750)$. b. (Chl a + pheo a)-specific absorption coefficient of phytoplankton at 440 nm, $a_{ph}^*(440)$ (in $\text{m}^2 \text{mg}^{-1}$) as computed by applying the decomposition method to $a_p(\lambda)$ vs. the same coefficient experimentally obtained with the method of Kishino et al. (1985) for the surface-layer samples (5–60 m) of the Sargasso Sea.

method was tested on the Chlomag surface layer samples for which direct determinations of $a_d(\lambda)$ were made. The experimental and computed values of $a_d(\lambda)$ and therefore of $a_{ph}(\lambda)$ were found to be in excellent agreement for most of the samples (Fig. 10). The mean square error on $a_{ph}^*(440)$ is $0.0039 \text{ m}^2 \text{mg}^{-1}$, i.e. 3–8% of $a_{ph}^*(440)$. Because the spectral criteria used in the decomposition were established on these samples, the agreement between computed and experimental values suggests that, for this set of data, absorption by detrital matter is properly represented as the sum of a term exponentially decreasing with increasing λ and a constant background.

The partition of the a_p spectra was also tested on samples from the Sargasso Sea taken in or under the deep Chl maximum (100–200 m). In this layer, phytoplankton populations as identified by flow cytometry are different from those present in the surface layer (mainly cells belonging to the division of Prochlorophyta instead of cyanobacteria and larger eucaryotic cells; see Neveux et al. 1989), and a_{ph} for these samples exhibits a distinct behavior (see Fig. 16). The ratios $a_{ph}(505) : a_{ph}(380)$ and $a_{ph}(580) : a_{ph}(692.5)$ are on average equal to 0.78 (SD 0.12, $N = 33$) and 1.37 (SD 0.60),

which represents significant divergence from the assumptions made for partitioning a_p . For these samples, then, the discrepancies between computed and experimental spectra result from deviations both in the assumptions concerning the spectral behavior of a_d and in those concerning the a_{ph} ratios.

As expected, small deformations occur in the shapes of the recomputed a_{ph} and a_d spectra (Fig. 11a). Nevertheless, application of the decomposition method leads to acceptable agreement between measured and computed values of $a_{ph}^*(440)$ (Fig. 11b), with a mean square error of $0.0032 \text{ m}^2 [\text{mg} (\text{Chl } a + \text{pheo } a)]^{-1}$, i.e. 4–13% of $a_{ph}^*(440)$. We believe therefore that the use of the same assumptions for partitioning a_p in the Peruvian upwelling area and in the Sargasso Sea is justified as an approximation because changes in the pigment composition of phytoplankton populations seem to have only small effects on the retrieval of a_{ph} . Further, the exponential spectral dependency of absorption by nonalgal particles does not appear to be a serious constraint as long as the exponent S is allowed to vary. The predominant source of uncertainty in the computed values of $a_{ph}^*(\lambda)$ and $a_d(\lambda)$ remains the β factor.

Specific absorption coefficients of living

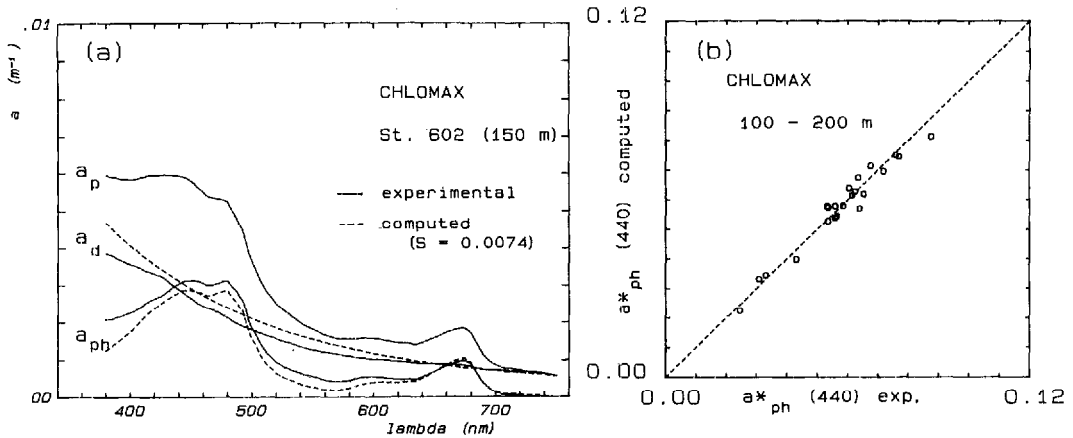


Fig. 11. a. As Fig. 10a, but for a sample from the deep maximum layer of the Sargasso Sea. For this sample the ratios $a_{ph}(505):a_{ph}(380)$ and $a_{ph}(580):a_{ph}(692.5)$ are 0.73 and 1.60. b. As Fig. 10b, but for the deep samples (100–200 m) of the Sargasso Sea.

phytoplankton for the Peruvian upwelling area—it is encouraging to note (Figs. 12 and 13) that in the spectral domain (around 675 nm) where the influences of pigment composition and package effect are both reduced, the mean absorption spectra for cultured phytoplankters and for natural populations of Peruvian waters exhibit similar values, $\sim 0.017 \text{ m}^2 [\text{mg}(\text{Chl } a + \text{pheo } a)]^{-1}$. The specific absorption spectra of cultures have been measured directly on algae in suspension, so this result attests a posteriori that the values of β have, on average, been properly evaluated for the field samples.

The standard deviation values shown in Fig. 13 show variability in specific absorption that can be attributed to three main causes. First, since absorption is normalized by the $(\text{Chl } a + \text{pheo } a)$ concentration, variations in the concentrations of accessory pigments (e.g. Chl *b*, *c*, carotenoids, phycobilins) may account for variations in the amplitude of a_{ph}^* , especially in the blue part of the spectrum. Second, because the relative proportions of Chl *a* and pheophytin *a* vary with station and depth and their respective absorption coefficients differ from one another at all wavelengths (see e.g. Mitchell 1987), normalization of absorption coefficients to the sum $(\text{Chl } a + \text{pheo } a)$ introduces some variability in a_{ph}^* . This problem is indicated by the exceptionally low a_{ph}^* values obtained at 50 m for station

34 (Fig. 12) where the pheophytin *a* concentration is 10-fold higher than the Chl *a* concentration. Third, variations in size (*d*) and intracellular pigment concentration (c_i) induce variations in the amplitude of a_{ph}^* , especially in the blue, due to the package effect (Morel and Bricaud 1981). This effect predicts that the absorption spectrum is “flattened” as c_i or *d* increases. Variations in *d* or c_i result from either a change in the species composition within the algal population or possibly from photoadaptation of cells inducing an increase of c_i in response to lower irradiance at greater depth.

Figure 14 shows that part of the variability (in the blue) is due to variations in the carotenoid content relative to Chl *a*. The ratio of carotenoid to $(\text{Chl } a + \text{pheo } a)$ varies from ~ 0.4 to 1.0 with station and depth for the Peruvian upwelling samples. These variations explain 35% of the variability in the blue-to-red ratio, $a_{ph}(440):a_{ph}(677.5)$. The fluctuations of the Chl *b*: Chl *a* and Chl *c*: Chl *a* ratios as well as those of the pheo *a*: Chl *a* ratio probably also contribute to this variability.

Photoadaptation of cells seems to influence the algal absorption properties in this area only weakly. Decrease of a_{ph}^* with increasing depth is not systematic and is observed only occasionally. At station 34, no clear tendency in the a_{ph}^* values is observed for the 0–50-m layer (Fig. 12). At many stations the optical variability is rather small

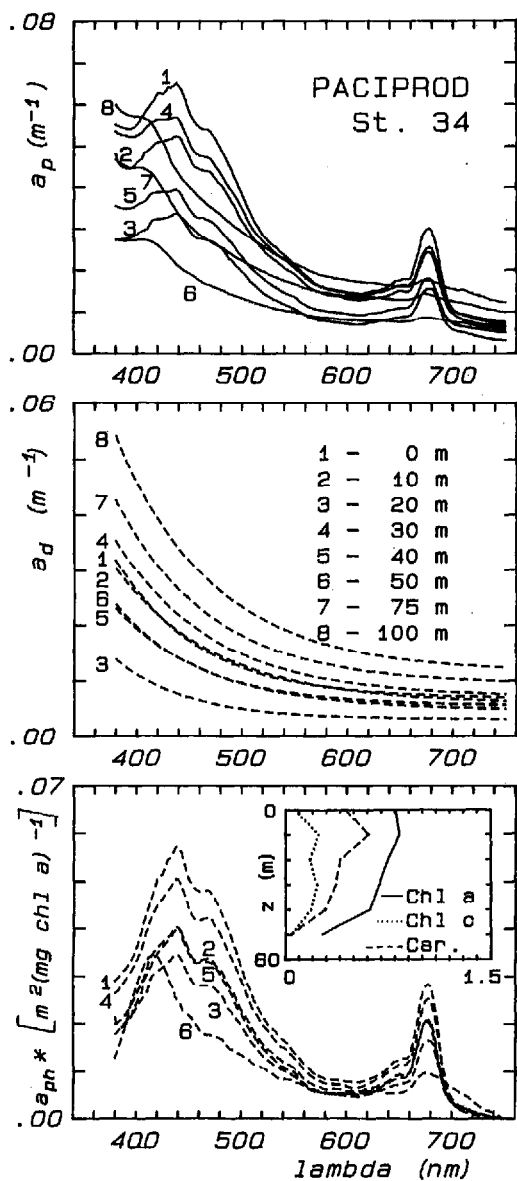


Fig. 12. Upper panels: Spectral values of absorption coefficients of total particulate matter a_p (solid lines) and of nonalgal particulate matter a_d (dashed lines) at different depths for Paciproduct station 34. The spectral values of a_d are obtained with the decomposition method (see text). Note the change in ordinate scale between a_p and a_d . Lower panel: Specific spectral values of absorption by living phytoplankton a_{ph}^* obtained by subtracting for each sample $a_d(\lambda)$ from $a_p(\lambda)$ and normalizing the values by the (Chl a + pheo a) concentration (at 75 and 100 m these concentrations were not available). Vertical profiles of (Chl a + pheo a), Chl c , and carotenoid concentrations (in mg m^{-3}) are shown in the inset. At most stations algal biomass

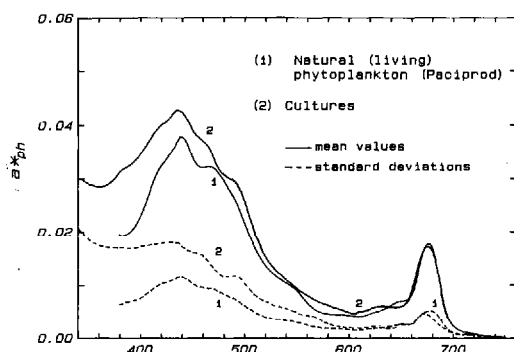


Fig. 13. Mean spectral values of (Chl a + pheo a)-specific absorption coefficients of living phytoplankton ($\text{m}^2 \text{mg}^{-1}$) obtained for the Paciproduct cruise (solid line; $N = 27$) and standard deviations (dashed line 1). Absorption spectra of deep samples with negligible pigment content or dominated by pheophytin were excluded from the average. Corresponding curves (2) obtained on healthy cultures ($N = 13$; see Bricaud 1989) are shown for comparison.

between 0 and 30–40 m, probably as a result of vertical mixing active throughout the surface layer as corroborated by the vertical density profiles as well as the pigment profiles (Fig. 12).

Specific absorption coefficients of living phytoplankton for the Sargasso Sea—The Sargasso Sea samples exhibit strong vertical

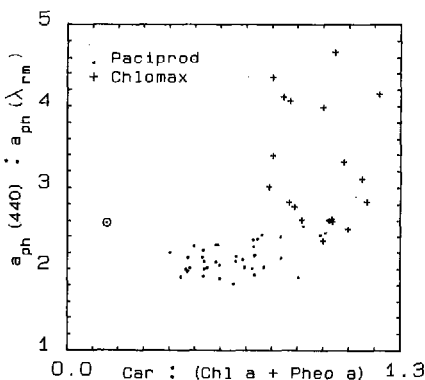


Fig. 14. Variations of the ratio of the absorption coefficients of living phytoplankton at 440 nm and at the wavelength of the red maximum (675 or 677.5 nm), $a_{ph}(440) : a_{ph}(\lambda_{rm})$, vs. the ratio of carotenoid to (Chl a + pheo a) concentrations. The circled point corresponds to a Paciproduct sample dominated by pheophytin (station 34, 50 m) with very low red absorption.

was moderately high, 0.3–1.4 $\text{mg (Chl } a + \text{ pheo } a) \text{ m}^{-3}$, and rather homogeneous between 0 and ~40 m before decreasing at greater depths.

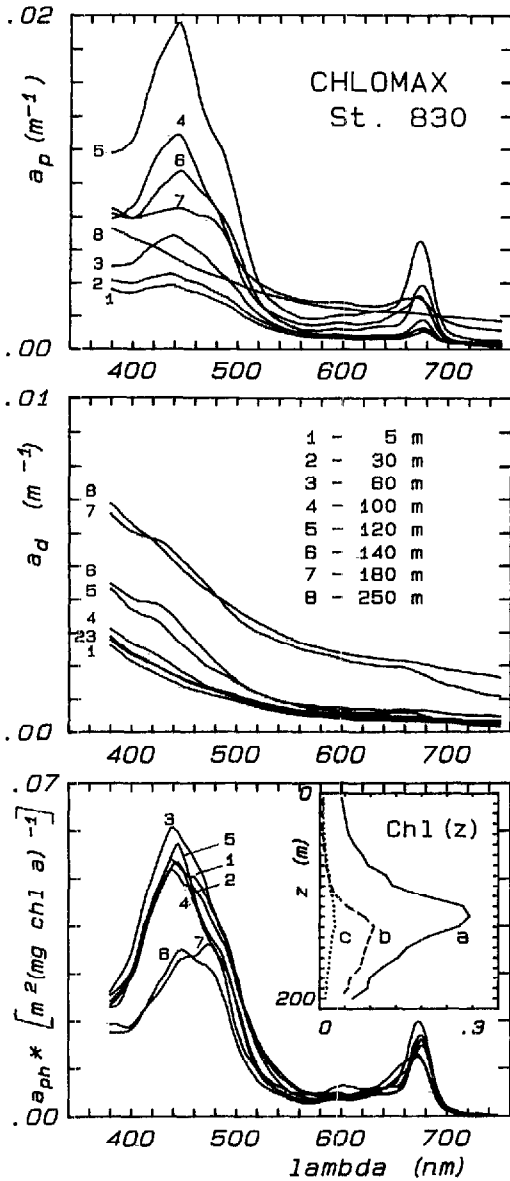


Fig. 15. Upper panels: Spectral values of absorption coefficients of total particulate matter a_p and of nonalgal particulate matter a_d as experimentally obtained at different depths for Chlomag station 830. Note the change in ordinate scale between a_p and a_d . Lower panel: Specific absorption coefficients of living phytoplankton a_{ph}^* obtained by subtracting for each sample $a_d(\lambda)$ from $a_p(\lambda)$ and normalizing the values by the (Chl a + pheo a) concentration (at 250 m this concentration was not measurable and the specific coefficients could not be computed). Vertical profiles of (Chl a + pheo a), Chl b , and Chl c concentrations (mg m^{-3}) are shown in the inset. At all stations a deep Chl maximum, 0.2–0.4 $\text{mg}(\text{Chl } a + \text{pheo } a) \text{ m}^{-3}$, was found

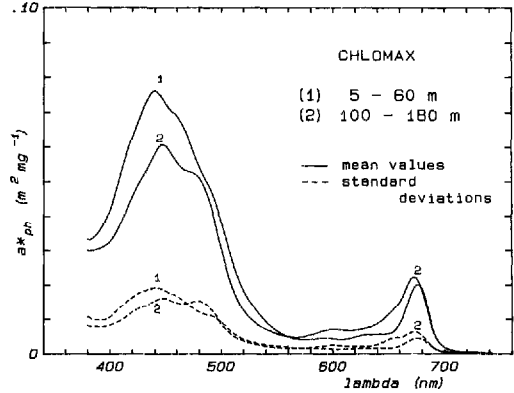


Fig. 16. Mean spectral values of (Chl a + pheo a)-specific absorption coefficients of living phytoplankton obtained for the Chlomag cruise (solid lines) in the surface mixed layer (curve 1; $N = 22$) and in the deep Chl maximum (curve 2; $N = 21$) with corresponding standard deviations (dashed lines). Note the change of ordinate scale from Fig. 13.

structure with a Chl maximum at 120–130 m (e.g. Fig. 15). The mean specific absorption coefficients were computed separately for the surface mixed layer (0–60 m) and for the layer of the deep Chl maximum (100–180 m) (Fig. 16). The rationale is that the corresponding spectra at all stations show distinct patterns in that layer, especially around 480, 595, and 650 nm. Absorption bands of Chl b (around 480 and 650 nm), weak in the surface layer, become apparent in the deep layer due to systematic increase of the Chl b : Chl a ratio below 70 m (from 10–15% in the surface layer to generally > 50% in the deep maximum; see Neveux et al. 1989 and inset in Fig. 15). The absorption band around 595 nm, clearly apparent only in the deep layer, is probably related to the slight increase of the ratio of Chl c to Chl a below 100 m (see Neveux et al. 1989). The blue absorption maximum is shifted from 440 nm in the surface layer to ~ 447 nm in the deep layer, consistent with the presence of prochlorophytes. According to Chisholm et al. (1988), the divinyl Chl a -like pigment contained in these cells presents (in diethyl ether) an absorption max-

between 100 and 130 m at the bottom of the seasonal thermocline under an oligotrophic ($< 0.1 \text{ mg m}^{-3}$) surface layer.

imum shifted by 8 nm toward greater wavelengths compared to the Chl *a* maximum. Note that the red peak is also slightly shifted, from ~677.5 nm in the surface layer to ~675 nm in the deep maximum layer.

The mean a_{ph}^* values obtained in Sargasso Sea waters are higher in the blue by ~60% (for the deep layer) or even 100% (for the surface layer) than those obtained for Peruvian waters. Figure 8b demonstrates that these differences are definitely significant even after taking into account uncertainty in the β factor, since they subsist whatever the $\beta(OD_p)$ function in each area. At the maximum around 675 nm, specific absorption values are slightly higher for Sargasso Sea waters (0.020 and 0.022 m² mg⁻¹ for curves 1 and 2) than for Peruvian waters (0.017 m² mg⁻¹).

Enhanced absorption in the Sargasso Sea compared to the Peruvian upwelling area, especially in the blue part of the spectrum, can be attributed to either or both of two phenomena: a higher concentration in accessory pigments, mainly carotenoids (Chl *b* and *c* concentrations are negligible in the surface layer), and a weaker package effect. A third possible cause of variability, i.e. the effect of normalizing a_{ph} by the sum (Chl *a* + pheo *a*), cannot be invoked because it would require that the proportion of pheopigments be systematically higher in Peruvian waters than in the Sargasso Sea. This situation was not observed and would contradict the common assertion that the proportion of living phytoplankton tends to increase with pigment content (e.g. Smith and Baker 1978).

The carotenoid:(Chl *a* + pheo *a*) ratio (Fig. 14) is generally higher in the Sargasso Sea (0.8–1.2) than in the Peru upwelling area (0.4–1.0). Enhanced blue absorption occurs, however, even for an identical carotenoid:Chl *a* ratio. For instance, when this ratio is equal to 0.9, the blue-to-red ratio varies between 2.6 and 4.0 for Sargasso Sea samples, instead of only 1.9–2.5 for Peruvian upwelling samples. A methodological artifact might be suspected because the global carotenoid concentration is evaluated with a single equation (involving the absorption coefficients of acetone extracts at 480 and 510 nm, Richards and Thompson 1952),

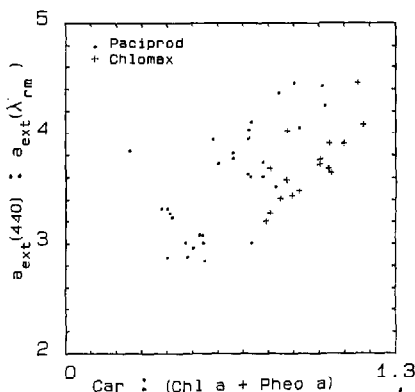


Fig. 17. Variations of the ratio of the absorption coefficients of acetone extracts at 440 nm and λ_{rm} (where λ_{rm} is the wavelength of the maximal absorption in the red, 662.5, or 665 nm), $a_{ext}(440):a_{ext}(\lambda_{rm})$, vs. the ratio of carotenoid to (Chl *a* + pheo *a*) concentrations for the Paciproed and Chlomax cruises.

although different types of carotenoids may be present with different absorption spectra. For instance, the *in vivo* absorption spectrum of nonphotosynthetic carotenoids extends farther toward 440 nm than fucoxanthinlike photosynthetic carotenoids (see Bidigare et al. 1987), thus leading to increased absorption at this wavelength. If so, the same tendency should be observed for acetone extracts, i.e. the blue-to-red ratio should be systematically higher for Sargasso Sea samples than for Peruvian upwelling samples. The opposite is observed (Fig. 17), so the high absorption values observed for the Sargasso Sea do not result solely from a high carotenoid content.

We conclude therefore that the higher absorption values observed in the Sargasso Sea are likely due to a weaker package effect than exists in the Peruvian upwelling waters, either because algal cells are of smaller size or because they have lower intracellular pigment concentrations. As indicated before, populations of small (0.4–0.8 μ m) prochlorophytes in the deep chlorophyll maximum and of small (0.5–2 μ m) cyanobacteria in the surface layer (together with larger cells) have been identified by flow cytometry during the Chlomax cruise (Neveux et al. 1989). Experiments on cultured cyanobacteria have shown further that, besides their small size, some species have a rather low intracellular Chl *a* concentration (~2 kg m⁻³) (Bricaud

Table 1. Values of the amplitude of the red absorption maximum normalized by the (Chl *a* + pheo *a*) concentration, $h^*(\lambda_{rm})$, defined as $a^*_{ph}(\lambda_{rm}) - [a^*_{ph}(657.5) + a^*_{ph}(697.5)]/2$ (where λ_{rm} is the wavelength of the maximum) estimated from different studies. For most of the studies (except ours and those of Kishino et al.) the values of a_{ph} were not available, but $h^*(\lambda_{rm})$ can be evaluated with the spectral variations of absorption by total particulate matter a_p instead of a_{ph} .

	$h^*(\lambda_{rm})$ $m^2 [mg (Chl\ a +\ pheo\ a)]^{-1}$	Measurement conditions
Carder and Steward 1985	0.018	On filter; β constant (unspecified value)
Kishino et al. 1985	0.019–0.028	On filter; β constant (=2.45)
Kishino et al. 1986	0.026	On filter; β constant (=2.45)
Weidemann and Bannister 1986	0.014–0.018	After filtration and resuspension
Haardt and Maske 1987	0.011–0.017	On suspension
Mitchell and Kiefer 1988 <i>b</i>	0.004–0.006	On filter; β function of optical density
Lewis et al. 1988	0.011	On filter; β constant (=2.45)
Our study	0.010–0.017	On filter; β function of optical density

et al. 1988); the generality of this character remains to be shown.

In the Sargasso Sea, Chl-specific absorption values decrease from the surface layer to the deep layer. When the possible underestimate of Chl *a* concentrations in the deep maximum layer (due to the presence of prochlorophytes) is considered, this decrease could be even stronger than observed in Figs. 15 and 16. Such a decrease could be due to an increase of c_i as a response to lower irradiance. The hypothesis that deep chlorophyll maxima are due to increased intracellular pigment concentration rather than increased cell number density has been set forth by several investigators (Kiefer et al. 1976; Beers et al. 1982; Mitchell and Kiefer 1988*b*). Besides, the population of small prochlorophytes described by Neveux et al. (1989) was found to have a pigment concentration much larger than the usual values for unicellular algae. Although a slight decrease of a_{ph}^* occurs inside the maximum (Fig. 15), the strongest decrease occurs just under the maximum (~140 m). This decrease seems therefore to result from photoadaptation of cells rather than population changes.

Our results concerning specific absorption can be compared (Table 1) to those previously published with regard to the maximal red amplitude, which in principle is not severely affected by either the package effect or pigment composition. The range of variation of this amplitude is, and was expected to be, rather large, considering the diversity of methods, the uncertainty on the β factor for measurements on filters, the

(weak but nonnegligible) influence of the package effect, and the effect of normalization by the (Chl *a* + pheo *a*) concentration. The discrepancy between our results and those obtained by Mitchell and Kiefer (1988*b*) in the North Pacific gyre (~0.004–0.006 $m^2\ mg^{-1}$) is more surprising because the same $\beta(OD)_f$ curve (derived from measurements on cultures) was used in both studies. This divergence remains largely inexplicable, although it might partly result from the behavior of β differing among regions, perhaps because of very different algal populations. The values obtained in this study do seem reasonable because the *in vivo* specific absorption coefficient of Chl *a* when there is no package effect should not be far from 0.020 $m^2\ mg^{-1}$ around 675 nm. We do not expect to observe field values markedly lower than this limiting value because the package effect has only a limited effect in the red part of the spectrum, and this effect should be especially weak for samples from the Sargasso Sea where cells are small.

Absorption by nonalgal (detrital) matter—For both cruises, the relative contribution (at 440 nm) of the detrital absorption to the overall absorption tends to decrease as the (Chl *a* + pheo *a*) concentration increases (Fig. 18), in agreement with the assertion made by Hobson et al. (1973) and Gordon and Morel (1973). The consistency of the $a_d(440):a_p(440)$ ratios between the Peruvian upwelling samples (for which the a_d values were indirectly estimated) and the Sargasso Sea samples (for which they were measured) indicates that reasonable values

of a_d have been obtained with the indirect method. The contribution of detrital matter to the overall absorption (at 440 nm) varies from 12 to 82% for the Sargasso Sea samples and (excluding deep samples) from 5 to 42% for the Peruvian upwelling samples. These high values emphasize the need for eliminating detrital absorption when attempting to assess absorption by natural phytoplankton populations. Ignoring this problem has probably led to many erroneous estimates of the in situ quantum yield for photosynthesis, as already pointed out by Kishino et al. (1986).

The spectral dependency of absorption by biogenous detrital matter has been shown to vary approximately exponentially, with a varying exponent. The range of variation of the exponent S was found to be $0.0024\text{--}0.017\text{ nm}^{-1}$ with a mean value of 0.0099 nm^{-1} (SD 0.0036 nm^{-1}) for the Peruvian upwelling samples and $0.008\text{--}0.015\text{ nm}^{-1}$ with a mean value of 0.0109 nm^{-1} (SD 0.0019 nm^{-1}) for the Sargasso Sea samples. No systematic variation of S with depth was detectable.

Experimental data concerning absorption by detrital matter are scarce; the first direct measurements apparently were made by Kishino et al. (1985) for the coastal waters of Japan. Fitting their spectra to an exponential law leads to values of S varying between 0.004 and 0.007 nm^{-1} . Some information can also be provided by the spectral values of total absorption for deep oceanic waters where the phytoplankton contribution has become negligible (no apparent absorption peaks). Spectra obtained by Yentsch (1962) for waters between 400 and 1,000 m off the New Jersey coast correspond to S varying from ~ 0.004 to 0.010 nm^{-1} . Spectra obtained by Okami et al. (1982) in Tokyo Bay lead to $S = 0.003\text{--}0.007\text{ nm}^{-1}$. The exponents obtained in our work therefore appear comparable (though on average slightly higher) with published data.

Conclusions

Correct assessment of the absorption coefficients of natural phytoplankton populations directly on filters requires that some methodological problems be solved. The

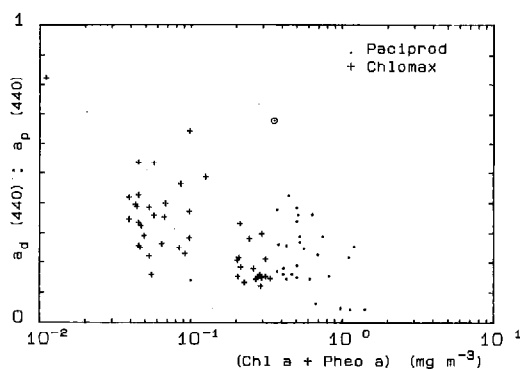


Fig. 18. Variations of the contribution (at 440 nm) of nonalgal particulate matter to total absorption as indicated by the ratio $a_d(440) : a_p(440)$ vs. the (Chl a + pheo a) concentration for the samples of the Paciproduct and Chlomap cruises. The values of $a_d(440)$ for the Chlomap cruise are those experimentally determined with the method of Kishino et al. (1985); those for the Paciproduct cruise were obtained by partitioning a_p into a_d and a_{pn} . The circled point corresponds to a Paciproduct sample (station 34, 50 m) dominated by pheopigments.

first problem is estimating the pathlength amplification factor β , often ignored in the past. Experimental studies show that β varies with optical density, and therefore from one sample to another, and for a given sample with wavelength. Independently, β probably varies with absorption and scattering properties of algal cells. These rather confusing variations can be greatly reduced, however, since β tends to be constant for sufficiently high optical densities ($>0.20\text{--}0.25$). We therefore recommend filtering sample volumes large enough to ensure that optical densities are higher than 0.2 at least within pigment absorption bands. At these optical densities a representative value of β is 2 with practically negligible spectral variation. This value can be useful as a first approximation until a thorough study identifies the parameters influencing β .

The second problem to overcome is discrimination between algal and nonalgal fractions of total particulate matter. Comparison between the experimental method of Kishino et al. (1985) and a numerical decomposition method based on spectral criteria results in satisfactory agreement. In spite of the assumptions made for partitioning the total absorption spectra, some degrees of freedom remain since the spectral

shape of absorption by viable phytoplankton, $a_{ph}(\lambda)$, and the exponent of the function representing absorption by nonalgal matter, $a_d(\lambda)$, are allowed to vary. Deviations from these assumptions generally have a limited influence upon the retrieval of $a_d(\lambda)$ and $a_{ph}(\lambda)$. Furthermore, the assumptions concerning the a_{ph} ratios can be adjusted for local conditions by making a few experimental determinations of $a_{ph}(\lambda)$. For routine measurements, this indirect method can be a useful alternative to the method of Kishino et al. (1985) which is time consuming.

Both methods have demonstrated that absorption by detrital particulate matter, $a_d(\lambda)$, constitutes a variable fraction of total absorption, $a_p(\lambda)$, and that this fraction is never insignificant even in eutrophic waters. Knowledge of $a_p(\lambda)$ is therefore quite insufficient for all applications concerning the photosynthetic capacities of natural phytoplankton populations and especially for estimating the in situ quantum yield for photosynthesis.

We must note that one problem has been ignored in previous studies and in ours. Some absorbing particles may pass through the filter and some absorbing matter may be released from algal cells damaged in filtration. These problems would result in underestimating absorption and possibly in some spectral distortion. The error will certainly vary among particulate populations, but we expect that in many oceanic situations absorption underestimation due to small, Chl-containing cells passing through the GF/F filter will extend to $\sim 10\%$ (Taguchi and Laws 1988). Such an error does not affect Chl-specific absorption coefficients because samples for pigment determinations were also filtered on GF/F filters.

The (Chl *a* + pheo *a*)-specific absorption values for living phytoplankton exhibit high variability at both sites studied due to variations in pigment composition (including pheopigments), size, and intracellular Chl *a* concentration. Values obtained in the Sargasso Sea are 60–100% higher (in the blue) than in the Peruvian upwelling area, probably mainly because of a weaker package effect. Systematic differences have also been evidenced in the Sargasso Sea between the surface layer and the deep maximum layer.

The overall variability observed for two contrasting water types remains small, however, probably due to an averaging effect, compared to the fourfold variability observed for cultured phytoplankters (e.g. Bricaud et al. 1988). Such systematic differences demonstrate nevertheless that assuming constant a_{ph}^* , whatever the trophic state of waters, is questionable. In particular, determining local values of the in situ quantum yield requires measurements of $a_{ph}^*(\lambda)$ simultaneously with irradiance measurements and photosynthesis-intensity (*P-I*) curves.

Such local studies are the way to establish on a more global basis an archive of the specific absorption properties of natural phytoplankton according to water type and the predominance of a given algal class. Such an archive could be used to refine models of oceanic reflectance as well as models of primary production by providing typical absorption coefficients specific to the situation and geographic area considered.

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