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Derivative analysis of spectral absorption by photosynthetic pigments in the western Sargasso Sea

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ABSTRACT

Concurrent measurements of the spectral absorption coefficient and photosynthetic pigmentation of natural particulates were performed to determine the principal pigments responsible for the absorption of spectral irradiance in seawater. The spectral absorption coefficient, $Ap(\lambda)$, was then analyzed by taking the second and fourth derivatives with respect to wavelength. The wavelength and magnitude of these derivative values provide useful information regarding the identification and quantification of phytoplankton pigments responsible for a given spectral signature. Linear relationships were examined and established between derivative values at selected wavelengths and concentrations of the major tetrapyrrole pigments, specifically chlorophylls *a*, *b*, and *c*. The correlation between derivative values near 526 nm and concentrations of photosynthetic carotenoids was poor and presumably caused by the broad absorption spectra of these pigments. A comparison of the measured particulate absorption coefficient with the absorption coefficient "reconstructed" for the phytoplankton component revealed that detritus can be a major source of light absorption. The method described here provides a rapid means of obtaining estimates of photosynthetic pigment concentrations in natural samples where absorption can be strongly influenced by detrital matter.

1. Introduction

The increased use of optical instruments to study phytoplankton processes in the sea dictates the need for a better understanding of light absorption by photosynthetic pigments. Variations in the optical properties of the upper ocean as measured from satellites, aircraft, ships, or moorings have been almost exclusively interpreted in terms of a single pigment, chlorophyll *a*. While such simple interpretations of variations in light absorption have proven useful, they are restrictive and may lead to erroneous conclusions. Two additional and important sources of variability in absorption come from contributions by detritus and photosynthetic accessory pigments. Particulate detrital matter has been found to make large contributions to absorption particularly in the blue and ultraviolet region of the spectrum (Yentsch, 1962; Kiefer and SooHoo, 1982; Kishino *et al.*, 1986; Maske and Haardt, 1987; Mitchell and Kiefer, 1988a).

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Moreover, in the blue-to-orange spectral region (460–640 nm), chlorophyll *a* contributes little and the photosynthetic accessory pigments are the dominant absorbing components.

To examine potential links between the spectral absorption properties and pigmentation of suspended particulate matter, two analytical techniques were applied to seawater samples collected from the Sargasso Sea. One method, high-performance liquid chromatography (HPLC), involves the measurement of chromatographically-separated photosynthetic pigments by absorption spectroscopy. The other method involves scanning particles retained upon a glass fiber filter spectrophotometrically, and calculating the absorption coefficient for this material. By regressing HPLC-determined pigment concentrations against 4th derivative values calculated for *in vivo* absorption at pigment-specific wavelengths, we show that measurements of particulate absorption can provide quantitative information on chlorophyll *a* and other accessory pigment concentrations. A comparison of the total particulate absorption spectrum ($Ap(\lambda)$) with that "reconstructed" for the phytoplankton component from the HPLC pigment data ($Aph(\lambda)$), revealed that detritus can be an important source of light absorption in open-oceanic waters. This study demonstrates one means by which varying contributions to absorption by detritus and photosynthetic pigments from assemblages of different taxonomic groups of phytoplankton can be assessed.

2. Materials and methods

a. Sampling. Suspended particles were collected onto glass fiber filters aboard the RV *Knorr* during April 1985 as part of the Biowatt program (Marra and Hartwig, 1984). Stations were occupied between 24 and 35N along 70W in the western Sargasso Sea (Fig. 1). Stations 4, 10 and 19 were long-term sample sites designed to examine temporal variations in physical and bio-optical parameters over time-scales of hours to days. Stations 5, 7 and 9 were short-term sample sites occupied to examine spatial variations in physical and bio-optical parameters along 70W. Hydrocasts were performed in the upper 200 m using a rosette sampler/Neil-Brown CTD system equipped with eleven 10-liter Niskin bottles. A Sea Martec fluorometer was interfaced with the CTD, allowing the vertical fluorescence structure to be examined during the downcast. Niskin bottles were tripped during the upcast at depths determined by the distributions of *in situ* fluorescence. Water samples (1 to 4 liters) for the absorption and pigment analyses were filtered through 25 mm GF/F glass fiber filters.

b. Absorption measurements. The diffuse transmittance of filters containing natural particulates was measured at sea with a Bausch & Lomb Spectronic 2000 spectrophotometer. In measuring diffuse transmittance, a wetted filter was used as a blank, and both the sample and blank were scanned by dual beams from 400 to 750 nm. The spectrum for diffuse transmittance was transformed into an absorption spectrum by normalization at 750 nm and applying a correction for (1) the volume of seawater

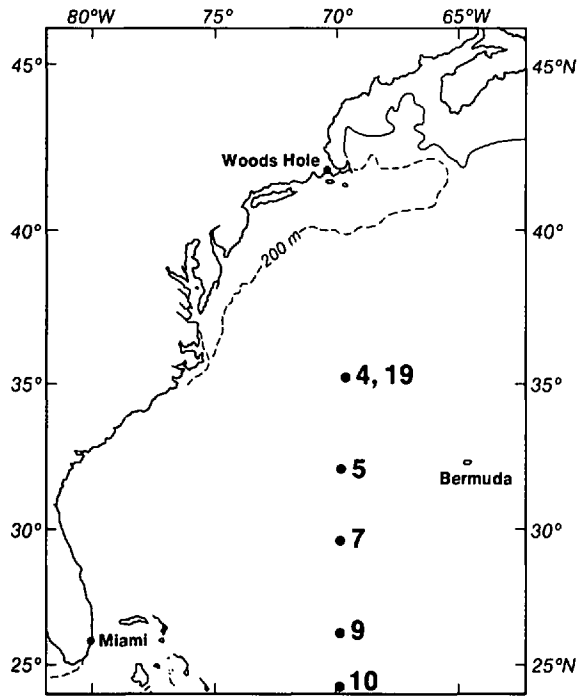

Figure 1. Map showing the locations of Biowatt stations occupied during April 1985. Station 4 was reoccupied after approximately two weeks and was designated as station 19.

filtered, (2) the clearance area of the filter, and (3) the multiple pathlength effect, β (Mitchell and Kiefer, 1988b). The spectra presented in this study represent the absorption ($Ap(\lambda)$; m^{-1}, base e) due to particulates in a collimated light field. Absorption spectra were smoothed prior to derivative analysis using a low pass Blackman window filter to eliminate frequencies with periods shorter than 4 nm (MacMillan Software's Asyst).

c. HPLC pigment analysis. The GF/F filters used for the spectral absorption coefficient measurements were cut in half. One-half of each filter was extracted in 2 ml 90% acetone for 24–48 hr in the dark (−10°C), while the remaining filter half was sent frozen to USC for the analysis of detrital pigments. Following extraction, the samples were centrifuged for 5 min to remove cellular debris. Chlorophyll and carotenoid pigments were separated at sea using a Spectra-Physics Model SP8100 liquid chromatograph and Radial-PAK C_{18} column (0.8 × 10 cm, 10 μ particle size; Waters Assoc.) at a flow rate of 10 ml min^{-1}. Samples were prepared for injection according to the method outlined by Mantoura and Llewellyn (1983). A two-step solvent program was used to separate the acetone-extractable phytoplankton pigments (Fig. 2).

The specific algal pigments identified and quantified during the cruise include:

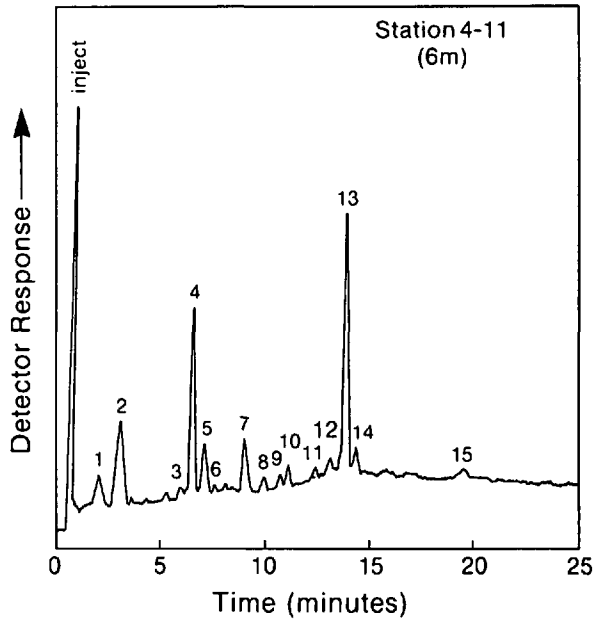

Figure 2. Reverse-phase HPLC chromatogram for station 4-11 (6 m). The chlorophyll and carotenoid pigments were detected by absorption spectroscopy at a wavelength of 436 nm. Peak identities are: (1) chlorophyllide *a*; (2) chlorophyll *c*; (3) 19′-butanoyloxyfucoxanthin; (4) fucoxanthin; (5) 19′-hexanoyloxyfucoxanthin; (6) prasinoxanthin; (7) diadinoxanthin; (8) unknown xanthophyll (alloxanthin?); (9) zeaxanthin (plus lutein); (10) unknown xanthophyll; (11) chlorophyll *b*; (12) chlorophyll *a* allomer; (13) chlorophyll *a*; (14) chlorophyll *a*′; and (15) carotene.

chlorophylls *a*, *b* and *c*; peridinin; fucoxanthin; 19′-hexanoyloxyfucoxanthin; prasinoxanthin; diadinoxanthin; and zeaxanthin (plus lutein). The HPLC method employed is not capable of separating zeaxanthin from lutein. After injection (500 μl sample), mobile phase A (80:15:5; methanol:water:ion-pairing agent) was ramped to mobile phase B (methanol) over a 12 min period. Mobile phase B was then pumped for 13 min for a total analysis time of 25 min. Individual peaks were detected and quantified (by area) with a Waters Model 440 Fixed Wavelength Detector (436 nm) and a Hewlett-Packard Model 3392A integrator, respectively.

Calibration standards were obtained from Sigma Chemical Co. (chlorophylls *a* and *b*) or purified by two-dimensional thin-layer chromatography (Jeffrey, 1981). Concentrations of the pigment standards were determined spectrophotometrically in 1 cm cuvettes using published extinction coefficients (Jeffrey and Humphrey, 1975; Mantoura and Llewellyn, 1983). The concentration of the prasinoxanthin standard was estimated with the β-carotene extinction coefficient and 19′-hexanoyloxyfucoxanthin concentrations were calculated with the fucoxanthin response factor. Known quantities of each pigment were injected and resultant peak areas were used to calculate

individual standard response factors (ng pigment area^{-1}). Pigment concentrations of natural particulate extracts were calculated with these response factors and knowledge of the extraction and sample volumes to yield concentrations in units of ng pigment per liter of seawater filtered (ng L^{-1}). Peak identities were periodically checked during the cruise by injection of extracts prepared from algal cultures of known pigment composition. HPLC injections of acetone-extracts prepared from ten samples collected in duplicate during the cruise yielded coefficients of variation (C.V., standard deviation/mean times 100%) of 3.2, 8.8 and 10% for chlorophylls *a*, *b* and *c*, respectively, for analyte concentrations >50 ng liter^{-1}.

d. Reconstruction of phytoplankton absorption spectra. The measurement of phytoplankton absorption is confounded by the presence of particulate, nonphototrophic absorbing matter (Kiefer and SooHoo, 1982; Lewis *et al.*, 1985; Kishino *et al.*, 1986; Iturriaga and Siegel, 1988). In the approach used here, phytoplankton absorption coefficients were estimated using the methods described by Bidigare *et al.* (1987) to avoid absorption contributions by nonphytoplankton components.

In vivo absorption spectra ($Aph(\lambda)$; m^{-1}, base e) for the phytoplankton component of natural particulate samples collected at station 4-11 (20 and 101 m) were "reconstructed" from knowledge of the HPLC-determined pigment concentrations and their specific absorption coefficients:

$$Aph(\lambda) = \sum_{i=1}^{5} a_i(\lambda)\, C_i \tag{2.1}$$

where $a_i(\lambda)$ are the specific spectral absorption coefficients for the major absorbing pigment groups (m^2 mg^{-1}) and C_i are the pigment concentrations (mg m^{-3}) at a specific depth.

Since diatoms were a major biomass component at this station (F. Reid, pers. comm.), we have utilized corresponding spectral absorption data available in the literature. The absorption contributions provided by water soluble phycobilin pigments were not included since the cyanobacteria were a minor biomass component at station 4 (Iturriaga and Marra, 1988). Specific absorption coefficients for the diatom pigments (chlorophyll *a*, fucoxanthin, chlorophyll *c* and nonphotosynthetically-active carotenoids) were determined from the deconvoluted "*in vivo*" absorption spectra published for the major pigments isolated from *Phaeodactylum tricornutum* (Mann and Myers, 1968). Their absorption spectra (m^{-1}) were digitized (400–700 nm) in 2-nm increments and normalized to pigment content (mg m^{-3}) to yield estimates of specific absorption, a_i (m^2 mg^{-1}). The concentration of chlorophyll *a* was estimated to be 13.9 μg ml^{-1} from their absorption spectrum of a 90% acetone extract of this diatom (Mann and Myers, 1968) and an extinction coefficient of 87.67 L g^{-1} cm^{-1} (Jeffrey and Humphery, 1975). The estimated spectral absorption coefficients for chlorophyll *a*

are within ~25% of the values determined by Thornber (1969) for the chlorophyll *a*-protein complex isolated from the blue-green alga, *Phormidium luridum*.

Concentrations of the remaining diatom pigments (chlorophyll *c*, fucoxanthin, diadinoxanthin and β-carotene) could not be easily determined from their available data and were estimated from accessory pigment-to-chlorophyll(ide) *a* ratios determined by HPLC for the same clone of *P. tricornutum* (Culture Collection of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine). Since the chlorophyllase activity of diatoms is activated by harvesting and/or extraction techniques (Barrett and Jeffrey, 1964; Suzuki and Fujita, 1986; Jeffrey and Hallegraeff, 1987), it was assumed that the chlorophyllide *a* present in the *P. tricornutum* extracts was originally in the form of its parent compound, chlorophyll *a*. For this reason, accessory pigments were normalized to chlorophyll(ide) *a* (i.e., chlorophyll *a* equivalents calculated as [chlorophyll *a*] + 1.45[chlorophyllide *a*]). The HPLC determined accessory pigment-to-chlorophyll(ide) *a* ratios (w:w) were in good agreement with previously published values (Abaychi and Riley, 1979) for this species of phytoplankton. The concentrations of nonphotosynthetically-active carotenoids were calculated by summing the diadinoxanthin and β-carotene concentrations (cf. Mann and Myers, 1968).

The specific absorption coefficients (400–700 nm) for chlorophyll *b* were calculated by subtracting the chlorophyll *a* contribution to the absorption spectrum published for the light harvesting chlorophyll *a*/*b*-protein complex (Thornber and Alberte, 1977), and normalizing to chlorophyll *b* concentration. The "total" absorption spectrum of this complex was partitioned into contributions by chlorophyll *a* and *b*. The concentrations of chlorophyll *a* and *b* represented by this spectrum were calculated with the chlorophyll *a*-specific molar extinction coefficient (158 L $mmol^{-1}$ cm^{-1}, 437 nm) and chlorophyll *b*:*a* ratio (1:1) published for this complex (Kan and Thornber, 1976). The spectral absorption of chlorophyll *a* was estimated from the spectral properties of the P700-chlorophyll *a*-protein complex (Thornber, 1969; Prezelin and Alberte, 1978). After subtraction, the remaining spectral absorption was normalized to chlorophyll *b* concentration to yield estimates of specific absorption for chlorophyll *b*.

The absorption (m^{-1}) due to individual pigments (chlorophylls and carotenoids) was calculated by multiplying pigment concentrations (C_i) by their respective specific absorption coefficients (a_i). Pigment-specific absorption coefficients are not implicit constants and can vary with the degree of "pigment packaging" within the cell (cf. Morel and Bricaud, 1981; Kirk, 1983). Morrow (1988) employed microphotometric techniques to investigate "pigment packaging" effects on the absorption efficiencies of single cells collected during this cruise. Single cells representing the Chlorophyceae (green algae), Prymnesiophyceae (coccolithophores) and Bacillariophyceae (diatoms) were collected within the euphotic zone of stations occupied along 70W (Fig. 1). The results showed that intracellular chlorophyll *a* concentrations decreased exponentially with increasing cell diameter (1–15 μm), indicating that pigment packaging had only

minor effects on phytoplankton absorption. While 3-fold increases in cellular absorption efficiency were observed in cells collected at the base of the euphotic zone, <20% changes in cellular absorption could be ascribed to "package" effects at a given depth horizon. As a first approximation, the phytoplankton absorption coefficients (400–700 nm) were reduced thirty percent to compensate for "pigment packaging" effects (cf. Morel *et al.*, 1987; Morel, 1988).

3. Results

a. Phytoplankton pigments. Phytoplankton pigment concentrations, compositions and vertical distributions showed considerable between-station variability in the western Sargasso Sea during April 1985 (Fig. 3). At station 4-11, the chlorophyll *a* maximum was found at ~40 m, near the depth of the 1% light level. The dominant accessory pigments measured at this station were chlorophyll *c*, fucoxanthin and diadinoxanthin (Fig. 3A), confirming the microscopic observations which showed that diatoms were a major biomass component in the upper 50 m (F. Reid, pers. comm.). Chlorophyll *b* and prasinoxanthin concentrations, by contrast, were considerably lower and showed weak maxima at a depth of 60 m. The photosynthetic accessory pigments, chlorophyll *c* and fucoxanthin, both had subsurface maxima which were coincident with the chlorophyll *a* maximum; diadinoxanthin, on the other hand, decreased in concentration with increasing depth, suggestive of its role as a photoprotectant (cf. Prezelin and Boczar, 1986; Bidigare *et al.*, 1987).

The composition and distributions of phytoplankton pigments measured at station 10-49 were markedly different. The depth of the chlorophyll *a* maximum increased to ~140 m, slightly deeper than the 1% light level. The quantitatively important accessory pigments measured within the chlorophyll *a* maximum were chlorophyll *b* and 19′-hexanoyloxyfucoxanthin, reflecting the presence of green algae (or prochlorophyte-like phytoplankton?; Chisholm *et al.*, 1988) and prymnesiophytes, respectively (Fig. 3B). The presence of 19′-butanoyloxyfucoxanthin at this station (data not shown) suggests that chrysophyte-like phytoplankton may be an important biomass component as well (Liaaen-Jensen, 1985; Gieskes and Kraay, 1986; Hooks *et al.*, 1988). The subsurface zeaxanthin (plus lutein) maximum was coincident with the depth (i.e., 100 m) at which *Synechococcus* spp. abundance was highest (Iturriaga and Marra, 1988).

Station 4 was reoccupied after two weeks and designated as station 19. During this brief period there was a major shift in phytoplankton composition as evidenced by HPLC pigment analysis (Fig. 3C). While the chlorophyll *a* profiles at these two stations were similar (Fig. 3A and C), the distributions and concentrations of accessory pigments were markedly different. Fucoxanthin showed a ~twofold decrease in concentration, and 19′-hexanoyloxyfucoxanthin, chlorophyll *b*, prasinoxanthin, and peridinin all showed >twofold increases. These data suggest that the diatom bloom

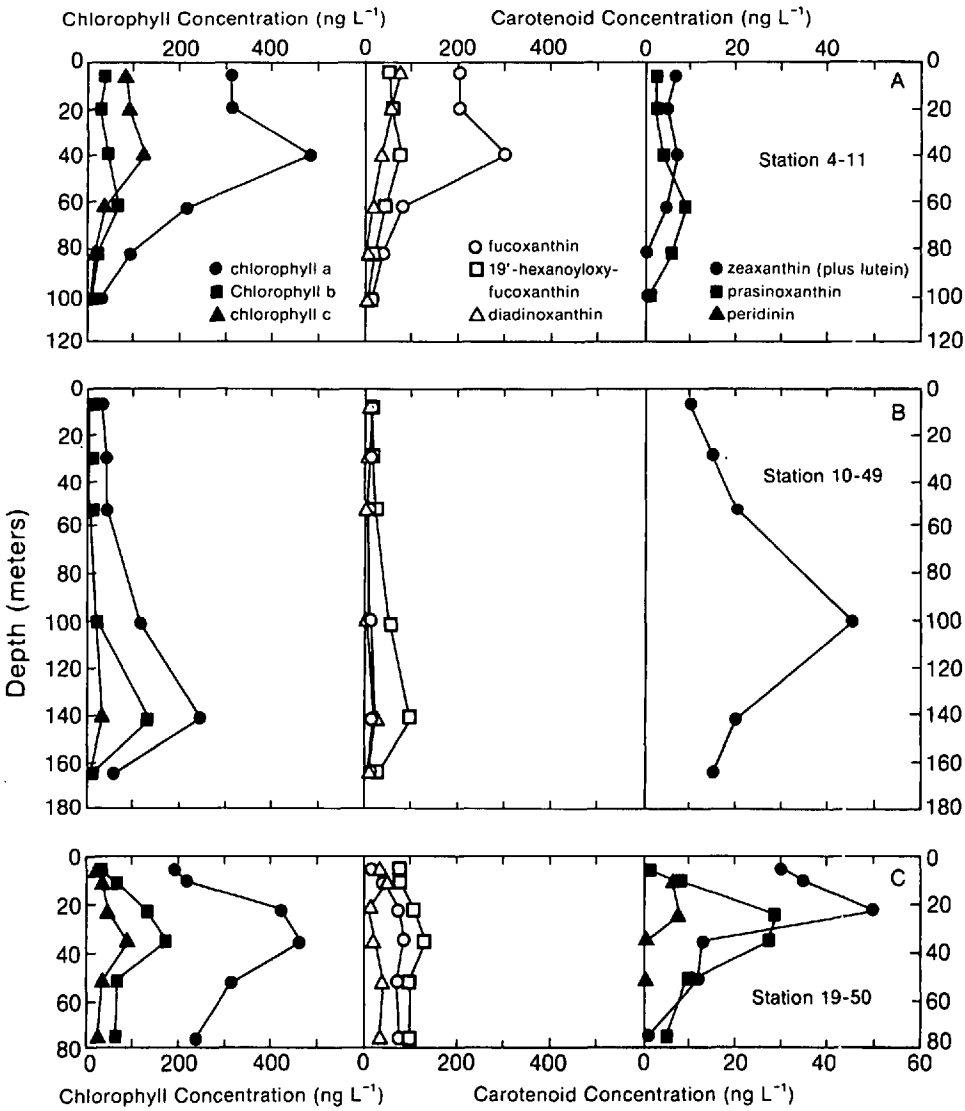

Figure 3. Chlorophyll and carotenoid pigment profiles for (A) station 4-11 occupied on 4 April 1985; (B) station 10-49 occupied on 14 April 1985; and (C) station 19-50 occupied on 22 April 1985 (see Fig. 1 for station locations). Concentrations of peridinin at stations 4-11 and 10-49, and prasinoxanthin at station 10-49, respectively, were below the limit of HPLC quantification.

initially observed at station 4 was replaced by a considerably more diverse phytoplankton assemblage consisting of prymnesiophytes, green algae (including prasinophytes), dinoflagellates, as well as diatoms. The zeaxanthin (plus lutein) maximum was located at ~25 m. The fact that the *Synechococcus* spp. maximum also occurred near this depth (Iturriaga and Marra, 1988) provides circumstantial evidence that the "zeaxan-

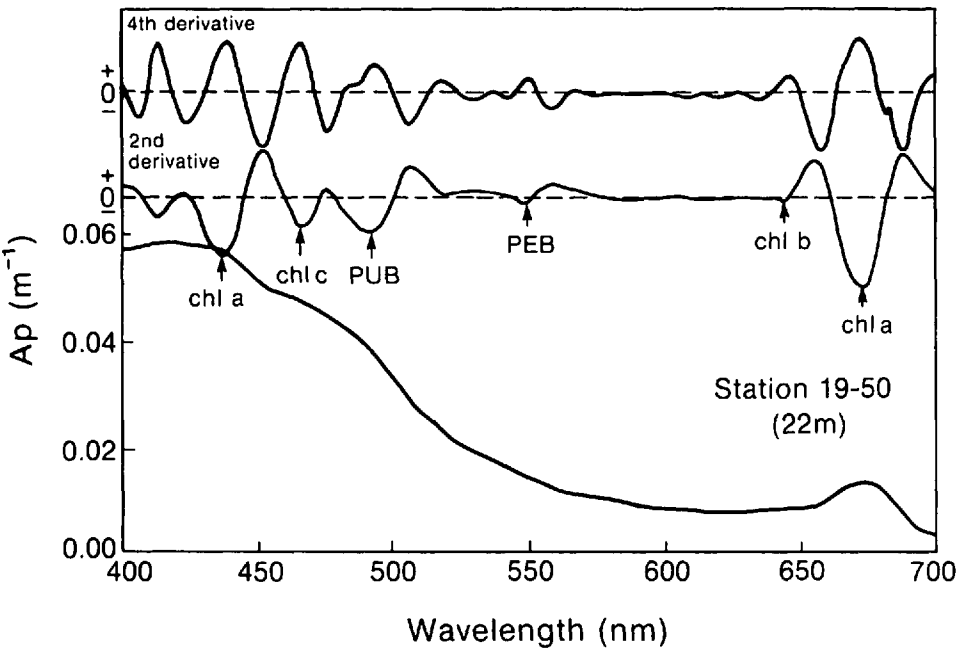

Figure 4. Representative spectral absorption coefficients and their derivatives (2nd, 4th) measured at station 19-50 (22 m). The arrows indicate the derivative positions for the major phytoplankton pigments.

thin plus lutein" peak separated by HPLC is dominated by zeaxanthin and not lutein (cf. Guillard *et al.*, 1985).

b. Spectral absorption and derivative analysis. The spectral absorption coefficients obtained during this cruise and those obtained from other geographic regions can be generally characterized as relatively structureless (e.g., Fig. 4); they lack the sharply defined peaks and shoulders that are apparent in spectra obtained from phytoplankton cultures. This lack of spectral definition could be due to large contributions from detrital matter, which is generally in an oxidized state and lacks well-defined resonances (Kiefer and SooHoo, 1982). It could also be caused by the presence of diverse photosynthetic pigments with overlapping bands of absorption.

The spectral absorption coefficient, $Ap(\lambda)$, is an estimate of an inherent optical property, the volume absorption coefficient of particles found within the seawater. The product of this coefficient and the incident scalar irradiance provides an estimate of the flux of light absorbed by particles within a unit volume of seawater. Provided that the efficiency of light absorption by pigments within these particles is not significantly affected by their packaging, the value of the absorption coefficient at a given wavelength should be proportional to the concentration of pigments that absorb at that wavelength.

Table 1. Summary of phytosynthetic pigment absorption maxima determined by derivative analysis (this study) and those available in the literature (published).

Pigment group	λ_{max} (this study)	λ_{max} (published)	Reference
Chlorophylls			
chlorophyll *a*	440, 675 nm	438, 675 nm	Prezelin and Alberte (1978)
chlorophyll *b*	483, 650 nm	470, 652 nm	Kan and Thornber (1976)
chlorophyll *c*	467, 630 nm	460, 640 nm	Mann and Myers (1968)
Carotenoids			
fucoxanthin	521–531 nm	460–530 nm	Mann and Myers (1968)
diadinoxanthin (+carotene)	—	425–500 nm	Mann and Myers (1968)
Phycoerythrin			
phycoerythrobilin	548 nm	543 nm	Ong *et al.* (1984)
phycourobilin	495 nm	492 nm	Ong *et al.* (1984)

The coincident measurement of $Ap(\lambda)$ generally reflects both changes in the quantity and composition of phytoplankton pigments from station-to-station as well as variations with depth at a given station. In order to quantify these relationships, derivative transformations were performed on the digitally-stored absorption spectra. Derivative analysis provides information regarding the convexity and concavity of a given absorption curve, and is useful for separating the secondary absorption peaks and shoulders produced by algal pigments in regions of overlapping absorption. The minima of the 2nd and the maxima of the 4th derivative occur close to or at wavelengths where there are absorption peaks attributable to photosynthetic pigments.

We have found that while the spectrum for the 2nd derivative is very useful for qualitative identification of pigments, the magnitude of the 2nd derivative does not provide a reliable measure of the concentration of photosynthetic pigments. This problem arises because the absorption contributed by overlapping pigments affects the value of the 2nd derivative of the pigment of interest. A good example of this effect was apparent for chlorophyll *b* at 650 nm, where the curvature contributed by chlorophyll *a* is a source of variability in the value of the 2nd derivative. The 4th derivative maxima were found to be less affected by overlapping pigments and thus provided a better estimate of pigment concentration.

By comparing measured wavelength positions for the absorption minima (2nd derivative) and maxima (4th derivative) with published values we were able to identify the major contributing pigments (Table 1). We wish to point out that several of the smaller minima and maxima have not been identified, and that we have not yet examined the possibility of determining the contribution by pheophorbide *a* (and its derivatives) to absorption at 675, 438, 418 and 410 nm. Our identification of the two

bilipigments was based upon a comparison of the derivative spectra with the fluorescence intensity of phycoerythrin measured on the same sample. The derivative at 495 and 545 nm measured for these samples was found to covary closely with the fluorescence at 575 nm produced by broad band excitation at 500 nm.

The largest minima in the 2nd derivative at station 19-50 (22 m) were contributed by chlorophylls *a* and *c*, as well as the bilipigment, phycourobilin (PUB); smaller 2nd derivative minima were contributed by phycoerythrobilin (PEB) and chlorophyll *b* (Fig. 4). The corresponding HPLC pigment data indicate the phytoplankton sampled at this depth were dominated by golden-brown algae (primarily prymnesiophytes), cyanobacteria and green algae (Fig. 3C).

The concentration of chlorophyll *a*, *b*, and *c* covaried with the value of the 4th derivative at 675, 650 and 467 nm, respectively (Fig. 5). These three wavelengths are the bands where the value of the 4th derivative for each chlorophyll was maximal. Samples were omitted when the derivative or the pigment concentration was below the limit of detection. The maxima in the 4th derivative for all spectra occurred within 1 nm of 675 for chlorophyll *a*; within 2 nm of 650 for chlorophyll *b*; and within 3 nm of 467 nm for chlorophyll *c*. Unfortunately, phycoerythrin determinations were not made during the cruise, thus, we are unable to present a cross plot of phycoerythrin concentration versus the 4th derivative at 495 nm.

Regression analysis of pigment concentration vs. derivative value was performed on these data (Table 2). The accuracy with which one can predict the pigment concentration from the value of the 4th derivative is best for chlorophyll *a* ($r = 0.89$), somewhat less for chlorophyll *b* ($r = 0.83$), and worst for chlorophyll *c* ($r = 0.69$). The correlation between chlorophyll *a* concentrations and 4th derivative values was higher when calculated for individual stations. For chlorophyll *b*, the correlation coefficient was highest when all stations were included in the regression analysis. In the case of chlorophyll *c*, the correlation coefficient was highest for station 4. It should be noted here that the red wavelengths used in the regression for chlorophyll *a* (675 nm) and chlorophyll *b* (650 nm) are characterized by very little absorption by other pigments. Hence, these peaks are "almost" solely attributable to chlorophylls *a* and *b*, respectively. In addition, since the relative absorption is less (i.e., in comparison to their soret bands), pigment packaging effects will be minimized (cf. Kirk, 1983). The poor correlation for chlorophyll *c* at 467 nm could be caused in part by the presence of other pigments which absorb at this wavelength.

c. Reconstructed phytoplankton absorption spectra. The "reconstructed" *in vivo* absorption spectra, $Aph(\lambda)$, for the phytoplankton component of two suspended particulate samples collected at station 4 are shown in Figure 6. The calculated chlorophyll *a*-specific absorption coefficients (672 nm) at 20 and 101 m are 0.014 and 0.016 $m^2\ mg^{-1}$, respectively. These coefficients are in excellent agreement with the

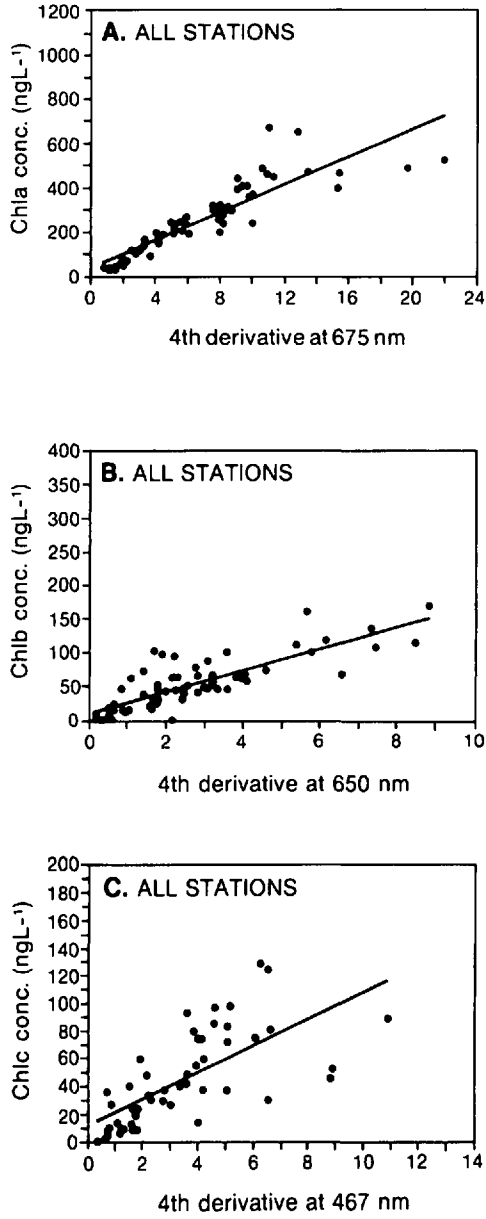

Figure 5. Cross plots of pigment concentrations (ng L^{-1}) and derivative values for (A) chlorophyll *a*; (B) chlorophyll *b*; and (C) chlorophyll *c* measured at Biowatt stations 4, 5, 7, 9, 10 and 19. Equations for regressions plotted are in Table 2.

Table 2. Summary of the fourth derivative positions of three algal chlorophyll pigments and the corresponding regression equations for the pigment concentration-derivative cross plots.

Algal pigment	Derivative position	Station description	Regression analysis ($y = mx + b$; r, n)
Chlorophyll *a*	675 nm	All Stations	$y = 31.0x + 38.3$ (0.89, 63)
		Station 4	$y = 46.4x - 43.6$ (0.91, 29)
		Station 10	$y = 50.1x - 21.2$ (0.98, 12)
		Station 19	$y = 19.8x + 119.3$ (0.93, 11)
Chlorophyll *b*	650 nm	All Stations	$y = 16.2x + 10.3$ (0.83, 65)
		Station 4	$y = 15.4x + 8.7$ (0.74, 29)
		Station 10	$y = 36.1x - 8.3$ (0.67, 10)
		Station 19	$y = 14.3x + 20.1$ (0.76, 16)
Chlorophyll *c*	467 nm	All Stations	$y = 9.6x + 11.8$ (0.69, 51)
		Station 4	$y = 18.1x - 7.6$ (0.89, 27)
		Station 10	$y = 19.5x - 5.2$ (0.64, 9)
		Station 19	$y = 2.6x + 42.8$ (0.34, 9)

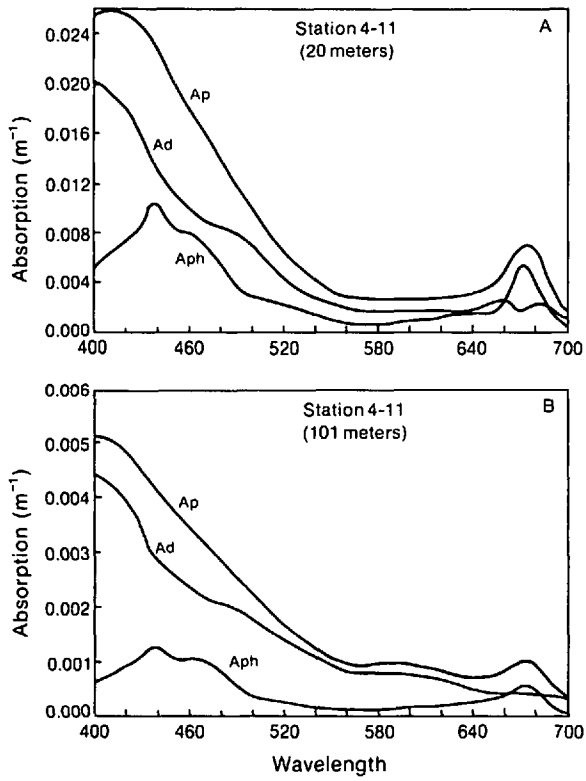

Figure 6. Comparison of the total particulate absorption coefficient (*Ap*; m^{-1}, base e) with the estimated phytoplankton pigment contribution (*Aph*; m^{-1}, base e) for (A) station 4, 20 m and (B) station 4, 101 m. The difference between these two spectra, *Ad* (m^{-1}, base e), represents the contribution by nonliving particulate matter (tripton) and detrital pigments.

mean values determined for phytoplankton collected in the northwestern Atlantic (0.014 to 0.020 m^2 mg^{-1}; Iturriaga and Siegel, 1988; Yentsch and Phinney, 1988).

The spectral reconstruction approach used here allows the absorption contributed by detrital pigments and nonliving particulates ($Ad(\lambda)$) to be estimated by difference:

$$Ad(\lambda) = Ap(\lambda) - Aph(\lambda). \tag{3.1}$$

To estimate the phytoplankton contribution to the total particulate absorption coefficient, the area under each "reconstructed" phytoplankton absorption spectrum was integrated with respect to wavelength and divided by the integrated absorption coefficient determined by the glass fiber filter method. These calculations suggest that phytoplankton accounted for only 37 and 23% of the total particulate absorption coefficient at 20 and 101 m, respectively. In comparison, 88 and 32% of the acetone-extractable absorbance (436 nm) associated with the 20 and 101 m samples, respectively, was identified and quantified using the HPLC method employed. The spectral absorption curves, $Aph(\lambda)$ and $Ad(\lambda)$, have the same features as the specific absorption coefficients determined for those pigments which covaried with chlorophyll *a* and pheopigments, respectively, in the coastal waters of Baja California (Kiefer and SooHoo, 1982). The spectral absorption coefficients reported here are within ~30% of the values determined by other Biowatt investigators using three alternative "deconvolution" approaches. A comparison of these different techniques is beyond the scope of this paper and will be presented elsewhere (Chamberlain, Kiefer, Morrow, Iturriaga, Siegel, Perry, Cleveland and Bidigare, in prep.).

4. Discussion

Faust and Norris (1985) first applied derivative spectroscopy to the determination of pigment concentrations in natural phytoplankton assemblages. They initially tested the method with unialgal and mixed cultures (Faust and Norris, 1982) and then applied the method to samples collected from Chesapeake Bay. Their method of analysis differs from ours in two respects. First, the values of spectral absorption were recorded in terms of the optical density of diffuse transmittance, log (1/transmission), rather than an absorption coefficient. Thus, their measurements include the effects of an extended light path caused by the scattering by the filter (Butler, 1964). Since this enhancement of absorption is wavelength dependent, the shape of their spectra for diffuse transmission will differ somewhat from that of the spectra for $Ap(\lambda)$. Second, the wavelengths they chose for prediction of pigment concentration do not occur at the wavelengths of peak absorption by photosynthetic pigments. Their concentration estimates for chlorophylls *a*, *c* and total carotenoids in field samples were based upon derivative values at 681.5, 677.0, and 572.5 nm, respectively. These wavelengths were chosen because they provided the best fit for the regression of concentration on the value of the 2nd derivative. It is also noteworthy that the correlation coefficients they obtained from over 100 samples from Chesapeake Bay exceeded 0.9 for each of the

three pigment types investigated. The following section, which summarizes the rationale for derivative analysis, may help to reconcile the differences between the two approaches.

The derivative analysis of spectral absorption is simply justified as follows. Consider the simple case of a solution of two pigments that share a spectral region where both contribute to absorption. If $[A]$ and $[B]$ are the concentrations of two pigments and $a(A, \lambda)$ and $a(B, \lambda)$ are the specific absorption coefficients at wavelength λ within the region of overlap, then the absorption coefficient is the sum of products:

$$Ap(\lambda) = [A]^*a(A, \lambda) + [B]^*a(B, \lambda) \tag{4.1}$$

and the derivative of any order is similarly the sum of the products of concentration and the derivative of the specific coefficients:

$$Ap(\lambda)' = [A]^*a(A, \lambda)' + [B]^*a(B, \lambda)'. \tag{4.2}$$

In the approach used here, we have attempted to obtain an estimate of pigment A concentration free of contributions from pigment B by finding a combination of wavelength and derivative order such that $a(B, \lambda)$ is equal or close to zero. In the case of photosynthetic pigments, the 4th derivative of the specific absorption coefficient is much larger at the wavelength of maximal absorption than it is at an adjacent wavelength. In Figure 4, this is evident in the sharpness in shape of the 4th derivative spectra when compared to either the spectra for the 2nd derivative or the absorption coefficient itself. At the wavelength corresponding to the maximum 4th derivative value for pigment A, $a(A, \lambda)' \gg a(B, \lambda)'$.

Faust and Norris (1982, 1985), on the other hand, chose to empirically find wavelengths (via individual regressions performed for each wavelength) within the 2nd derivative spectra which minimize the unexplained variance. If individual pigment concentrations measured for natural waters covary (e.g., chlorophylls *a* and *c* in golden-brown algae), such an approach could be potentially misleading. The 2nd derivative position (677 nm) chosen by Faust and Norris (1985) for estimating chlorophyll *c* concentration, for example, is at a wavelength at which this chromophore is responsible for $\leq 5\%$ of the light absorbed by golden-brown algae (cf. Mann and Myers, 1968; Haxo, 1985). Chlorophyll *a*, on the other hand, is responsible for $\geq 95\%$ of the light absorbed at this wavelength. Thus, a strong correlation between 2nd derivative values at 677 nm and chlorophyll *c* concentrations could result from the covariation of chlorophyll *a* and *c* concentrations.

Wavelengths of absorption maxima for chlorophyll *a* determined in this study are in good agreement with values published for the P-700 chlorophyll *a*-protein complex isolated from the dinoflagellate *Glenodinium* sp. (Prezelin and Alberte, 1978). However, the wavelengths of maximal absorption for chlorophylls *b* and *c* were consistently greater (7–13 nm) and lower (2–10 nm) in the blue and red bands, respectively, when compared to literature values (Table 1). By comparison, wavelength

maxima measured for the biliproteins were 3–5 nm greater than those reported for phycoerythrin isolated from *Synechococcus* WH8103 (Ong *et al.*, 1984). Perhaps more important to our analysis of these data is the variation in λ_{max} observed for chlorophyll *b* between taxa of green algae (e.g. Brown, 1985). We suggest, but lack definitive evidence, that the anomalously low value for absorption at 650 nm by chlorophyll *b* (Table 2) at station 10 is due to the presence of a different suite of green algae (prochlorophyte-like phytoplankton?; Chisholm *et al.*, 1988) at this southern most, oligotrophic site.

In the analysis of natural particulates collected from the Sargasso Sea, the use of the 4th derivative always yielded stronger linear relationships between pigment concentration and derivative magnitude. Faust and Norris (1985) noted that the use of the 4th derivative provided additional sharpening of absorption bands, as well as accentuating weakly absorbing components. They also stated that none of the spectra obtained from their natural samples indicated the presence of chlorophyll *a* degradation products. Perhaps the presence of additional overlapping pigments (e.g., pheophorbide *a*) in natural samples collected from the Sargasso Sea dictates the use of the 4th derivative for accurately estimating pigment concentrations.

The variability apparent in the cross plots (Fig. 5) and regression analyses (Table 2) is not only affected by errors associated with the measurement of pigment concentrations and the absorption coefficient. Specifically, differences in the specific absorption coefficients of pigments measured *in vivo* or on glass fiber filters may be caused by spectral changes affected by differences in the pigment-protein complexes within the stock of particles. Another source of variability of specific absorption coefficient values is the packaging of chromophores within cells and detrital particles. Changes in either the concentration of pigments within the chloroplast, or the number of chloroplasts within the cell, or even the size of the chloroplast will effect the efficiency of light absorption. Such effects have been calculated for phytoplankton (Morel and Bricaud, 1981) and measured in cultures (Dubinsky *et al.*, 1986; Maske and Haardt, 1987; Sathyendranath *et al.*, 1987; Mitchell and Kiefer, 1988b). We are now examining in greater detail the wavelength dependence of pigment packaging and the influence of radiance distribution on the specific absorption coefficients for the major photosynthetic pigments.

The conspicuous absence of information regarding carotenoids in our analyses is probably due to two factors. We measured a large number of carotenoids during the study and many of them have similar spectral properties. Both the number and similarity makes identification within a spectrum difficult. Perhaps the more important factor is that the carotenoids are, in general, characterized by broad spectral absorption and relatively rounded absorption peaks (Table 1). Such features make them less accessible to derivative analysis since the method tends to detect only those regions where changes in absorption as a function of wavelength are rapid.

The shapes of the $Ap(\lambda)$ determined in this study as well as those from other

geographic sites (e.g., Yentsch, 1962) provide qualitative evidence that detrital material provides a large and variable contribution to light absorption in the water column. In addition, the decomposition of spectra by (1) the extraction of pigments with organic solvents (Kishino *et al.*, 1986; Maske and Haardt, 1987); (2) regression analysis of contributions to absorption by the phaeopigments (Kiefer and SooHoo, 1982); or (3) the approach employed here (Fig. 6) all provide indirect quantitative evidence of the importance of detrital absorption.

5. Conclusions

The measurement of the spectral absorption coefficient offers a rapid means of determining both the amount and general composition of particulate absorbing material within the water column. The time required to analyze a sample is mainly limited by the time it takes for vacuum filtration of 1 to 4 liters of seawater; this usually requires between 15 to 40 min under moderate to low vacuum. Scanning of the sample takes 2 min, and digital processing to obtain both $Ap(\lambda)$ and associated derivative spectra takes an additional 2 min with a microcomputer. Thus, one can obtain detailed information about spectral absorption by the phytoplankton and associated detrital stocks while the ship is on station. Derivative analysis of the spectral absorption coefficients of particles offers an alternative means of separating the absorption by specific photosynthetic pigments from that of detrital matter. The regression analyses indicate the concentrations of chlorophylls *a*, *b*, *c* (and presumably phycoerythrin) can be estimated with reasonable accuracy in samples where the absorption by detritus is significant and variable.

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