VARIATIONS IN SPECIFIC ABSORPTION COEFFICIENTS AND TOTAL PHYTOPLANKTON IN THE GULF OF CALIFORNIA

EDUARDO MILLÁN-NÚÑEZ
Secretaría de Marina
Estación de Investigación Oceanográfica de Ensenada
Boulevard Costero y Calle Sanginés
22800, Ensenada, Baja California
México
emillan@cicese.mx

J. RUBÉN LARA-LARA
División de Oceanología, CICESE
Apartado Postal 2732
Ensenada, Baja California
México
rlara@cicese.mx

JOAN S. CLEVELAND[1]
Center for Hydro-Optics and Remote Sensing
San Diego State University
6505 Alvarado Road, Suite 206
San Diego, California 92120-5005

ABSTRACT

From 15 to 19 October 1994, an oceanographic cruise was carried out in the Gulf of California. Phytoplankton biovolume, pigment concentrations, and taxonomy were examined as functions of location and light depth. The specific absorption coefficient of phytoplankton (a^*_{ph}) showed variability in magnitude and spectral shapes between stations and with depth. The a^*_{ph} values ranged from 0.020–0.056 $m^2(mg\ chl\ a)^{-1}$ at 440 nm to 0.013–0.020 $m^2(mg\ chl\ a)^{-1}$ at 674 nm. Spectra of phytoplankton belonging to the same taxonomic group tended to have similar shape. At stations where the environmental conditions favored the presence of microphytoplankton populations (cells >20 μm), the lowest a^*_{ph} were found. Of all the variables studied, pigments, particularly the photoprotective pigment zeaxanthin, had the highest correlation with a^*_{ph}. Changes in pigment composition and cellular concentration were responsible for over 70% of the variability of the specific absorption at 440 nm. Including biovolume per cell in a multiple regression improved the model to explain up to 80% of a^*_{ph} variations. The work described here concurrently examined pigment packaging, measured as the cellular concentration of chlorophyll a and as the phytoplankton cell volume, and the confounding effect of the blue-absorbing accessory pigments on the specific absorption coefficient. The a^*_{ph} varied as a function of all three variables, indicating the importance of both taxonomic variations (size and accessory pigments) as well as responses to environmental variations.

INTRODUCTION

Particulate material, including phytoplankton, is responsible for most of the light scatter and absorption in the ocean. The characterization of particle variability provides information about light attenuation, potential primary production, and the phytoplankton pigment biomass. Changes in the optical characteristics of water masses have been associated with biochemical processes that are related to the energy used in photosynthesis (Yentsch 1960; Morel and Prieur 1977; Mitchell and Kiefer 1988a; and Bricaud et al. 1995).

Several authors have reported the distribution of light absorption by particles in the ocean (Maske and Haardt 1987; Yentsch and Phinney 1989; Nelson et al. 1993; and Cleveland 1995), concluding that there is a nonlinear relation between the phytoplankton light-absorption coefficient and the chlorophyll concentration. This relation is a function of the phytoplankton environment, particle form and size, and concentration of pigments and detritus (Morel and Bricaud 1981; Spinrad and Brown 1986; and Bricaud et al. 1995). These authors have argued that cell size and the photosynthetic pigment composition of the phytoplankton are partly responsible for the variability of the specific light-absorption coefficient.

Several analytical models have been developed to estimate primary productivity as a function of the in vivo phytoplankton light absorption (e.g., Kiefer and Mitchell 1983). In these models, the a^*_{ph} values are taken as constants; however, recent studies have shown both horizontal and vertical variations in a^*_{ph} (e.g., Sosik and Mitchell 1995).

The objective of this research was to study the factors that cause variability of the phytoplankton-specific light-absorption coefficient in the Gulf of California. In particular, pigment composition was examined as a possible cause of variable a^*_{ph} at specific wavelengths.

MATERIALS AND METHODS

From 15 to 19 October 1994, an oceanographic cruise was made aboard the R/V *A. Humboldt H-03* in the Gulf of California. Seawater samples were collected from five depths: 100%, 32%, 10%, 3%, and 1% of surface irradiance E_o (fig. 1).

Photosynthetically available radiation (PAR) was measured with a light sensor (Biospherical Instruments, Inc. PNF-300). Temperature and salinity were measured with a CTD recorder. Phosphate concentrations were measured with a Bausch & Lomb (Spectronic 1001) spectrophotometer, following Strickland and Parsons (1972). Phytoplankton species and abundances were analyzed with a Carl Zeiss inverted microscope with 16× and 40× objectives. Phytoplankton cell volume was estimated following relationships developed by Strathmann (1967) and Edler (1979).

[1]Present address: Office of Naval Research, 800 N. Quincy Street, Arlington, Virginia 22217.

[Manuscript received January 30, 1998.]

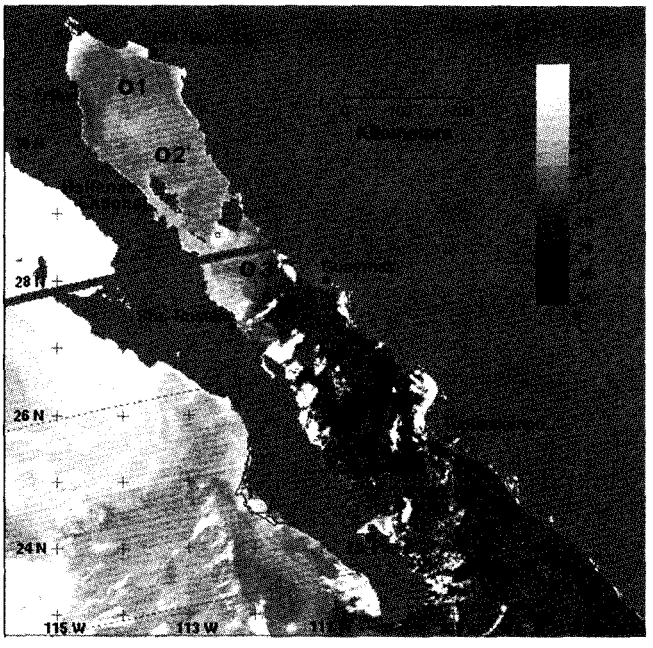

Figure 1. Satellite image of sea-surface temperature in the Gulf of California, and sampling stations 1–5.

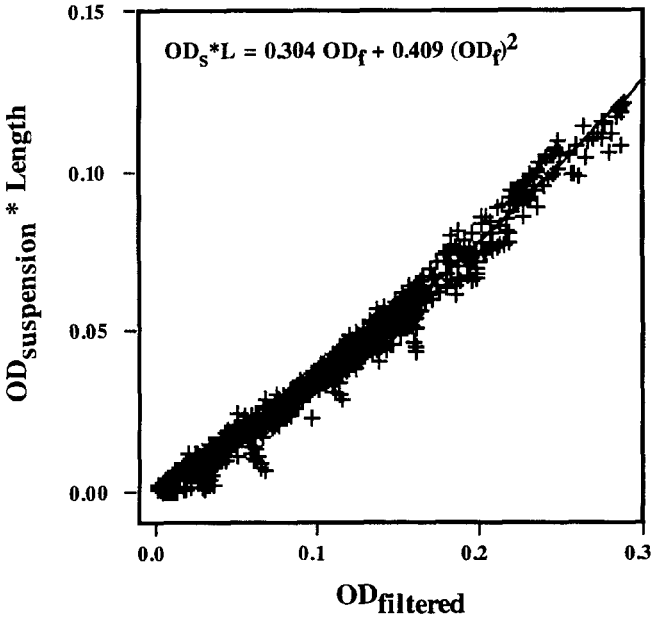

Figure 2. Relationship between the optical density (OD) of cells measured in suspension and on a filter from laboratory culture.

Absorption Measurements

Samples for measurements of phytoplankton light absorption were collected on Whatman GF/F filters and immediately frozen in liquid nitrogen. In the laboratory, the filters were defrosted, then saturated with filtered seawater. Spectral absorption was measured with a Hewlett Packard 8452 diode array spectrophotometer equipped with an integrating sphere (Labsphere RSA-HP-84), following the technique described by Mitchell (1990) and Cleveland and Weidemann (1993). The filtered samples were scanned between 400 and 750 nm with a spectral resolution of 2 nm. The filters were rinsed (~20 minutes) with hot methanol to remove pigments, and a second reading was made to estimate the detritus absorption (Kishino et al. 1985). Phytoplankton pigment absorption was estimated by subtracting the detritus absorption from the total particulate absorption.

We corrected the absorption spectra for the path-length amplification factor (β) by using the algorithm empirically derived from laboratory cultures by Charles Trees (fig. 2) for the HP diode array spectrophotometer. This path-length amplification adjusts optical density for filtered samples $OD_{filt}(\lambda)$ to equal optical density for an equivalent sample in suspension $OD_{sus}(\lambda)$.

$$OD_{sus} = 0.3038\ OD_{filt} + 0.4086\ (OD_{filt})^2$$

The specific light-absorption coefficient, a^*_{ph}, m^2(mg chl a)$^{-1}$, was obtained by dividing the phytoplankton absorption coefficient by the chlorophyll a concentration measured in acetone extracts with a Turner Designs 10-005 fluorometer (Yentsch and Menzel 1963).

HPLC Pigment Analyses

Samples for high-performance liquid chromatography (HPLC) pigment analysis were collected on Whatman GF/F filters and frozen in liquid nitrogen for analysis in the laboratory. Chlorophylls and carotenoids were separated by means of the Wright et al. 1991 method. A spectra-focus UV2000 detector with autosampler As3000, with a reverse-phase Radial-PAK C_{18} column (Spherisorb, 25 micron, 25 cm) was used with a flow rate of 1 ml minute^{-1} to separate and quantify the pigments as they eluted off the column. Before injection, a 1,050 µl aliquot of sample extract was mixed with 3 ml of water to help separate dephytolalid pigment compounds. We used canthaxanthin as an internal standard to correct for volume changes during the extract process.

Comparisons of HPLC and standard fluorimetric methods for the determinations of chlorophyll a are shown in figure 3. We found reasonable agreement between chlorophyll a measured by HPLC and fluorimetric methods ($r^2 = 0.89$; $p = 0.05$).

RESULTS

Figure 1 shows the typical distribution of sea-surface temperature during our study. There were two main features: North of the large islands, the temperature varied from 29.5° to 31.0°C. In the southern part of the gulf, a cold front with temperatures from 22.0° to 24.0° was established. In general, these conditions remained in the gulf throughout the entire cruise.

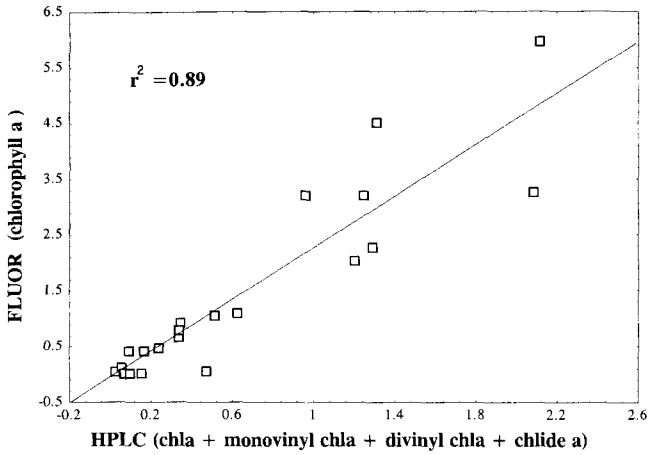

Figure 3. Comparison between HPLC and fluorometrically measured chlorophyll a in the Gulf of California, October 1994 (r^2 = 0.89; p = 0.05).

TABLE 1
Spatial Distribution of the Main Bio-Optical Variables in the Gulf of California, October 1994

Irradiance	100%	32%	10%	3%	1%
Station 1					
Depth (m)	1	6	14	23	33
Chl a[a] fluor.	1.085	1.162	1.089	1.068	0.921
a^*_{ph} (440 nm)	0.054	0.045	0.047	0.041	0.042
a^*_{ph} (674 nm)	0.023	0.020	0.021	0.018	0.020
Station 2					
Depth (m)	1	6	12	18	25
Chl a[a] fluor.	2.03	2.70	2.34	1.25	0.585
a^*_{ph} (440 nm)	0.026	0.021	0.022	0.023	0.022
a^*_{ph} (674 nm)	0.012	0.010	0.011	0.015	0.013
Station 3					
Depth (m)	1	5	10	16	25
Chl a[a] fluor.	2.764	3.204	—	1.487	0.585
a^*_{ph} (440 nm)	0.020	0.017	—	0.016	0.029
a^*_{ph} (674 nm)	0.013	0.010	—	0.011	0.019
Station 4					
Depth (m)	1	11	26	42	60
Chl a[a] fluor.	0.438	0.479	0.503	0.625	0.184
a^*_{ph} (440 nm)	0.056	0.040	0.036	0.036	0.040
a^*_{ph} (674 nm)	0.016	0.015	0.014	0.017	0.018
Station 5					
Depth (m)	1	7	22	35	45
Chl a[a] fluor.	0.479	0.682	0.804	0.495	0.406
a^*_{ph} (440 nm)	0.031	0.028	0.030	0.037	0.047
a^*_{ph} (674 nm)	0.020	0.013	0.015	0.025	0.027

[a] $mg\ m^{-3}$

Variability of a^*_{ph} Values

The vertical distribution of the phytoplankton-specific light-absorption coefficient at 440 nm for stations 1 to 5 (fig. 4) ranged from 0.016 to 0.056 at 440 nm. At 674 nm, a^*_{ph} ranged from 0.010 to 0.027 m^2(mg chl a)$^{-1}$ (table 1). The spatial variability of the phytoplankton-specific light-absorption coefficient between stations showed the maximum differences for stations 1, 4, and 5 at 100%, 32%, and 10% of E_o; stations 2 and 3 were very similar at these light depths. The specific absorption spectrum for station 4 at the 1% light depth was noisy and high, but this may have been an artifact of the low chl a concentrations and the small volume filtered.

Phytoplankton Abundances

We found 15 genera of diatoms with 46 species; stations 2 and 3 showed the highest number of genera (table 2). The average biovolume (μm^3) for diatoms ranged from 19.52 to 22.29 ln biovolume l^{-1}. Stations 2 and 3 showed the highest cell abundance (~90×10^3 and ~80×10^3 cells l^{-1}, respectively; fig. 5). *Chaetoceros* spp. were the most abundant, with a cellular volume of ~280 μm^3, equivalent to a ~10 μm diameter. However, *Rhizosolenia* spp. showed the maximum cellular volume of ~13.4×10^6 μm^3, equivalent to a ~295 μm diameter. In general, stations 1, 4, and 5 showed abundances below 5×10^3 cells l^{-1}.

Photosynthetic Pigments

Photosynthetic pigments were classified into five groups in accordance with Bidigare et al. 1990: (1) "CHLA" chlorophyll a, chlorophyllide a, and phaeopigment a (not including phaeophorbide); (2) "CHLB" chlorophyll b, phaeopigment b; (3) "CHLC" chlorophyll c and c_2; (4) "PSC" photosynthetically active carotenoids, including fucoxanthin, 19' butanoyloxyfucoxanthin, 19' hexanoyloxyfucoxanthin, and prasinoxanthin; and (5) "PPC" photoprotectant carotenoids, including diadinoxanthin, alloxanthin, and zeaxanthin/lutein (table 3). This method does not make it possible to separate zeaxanthin from lutein (they have the same retention time), but there is evidence suggesting that zeaxanthin dominates over lutein in the ocean (Everitt et al. 1990), so we assumed that all the absorption at that particular retention time was due to zeaxanthin. However, these values should be interpreted with care. If chlorophyll b concentrations were low, then the presence of lutein would be minimal.

As expected, the concentration of chlorophyll a exceeded that of any other pigment, with an average range from 0.192 to 1.256 mg m^{-3}, while the ratio zeaxanthin/chl a varied from 0.066 to 0.374. Fucoxanthin pigment showed the same pattern as chlorophyll a, in the ranges of the average ratios (0.19 to 0.692) and concentrations from 0.05 to 0.87 mg m^{-3}.

Shape of the Absorption Spectra

The absorption, at all depths at which measurements were taken, was averaged (fig. 6a). From the averaged absorption spectra, two groups of stations were identified: group 1 includes stations 1, 4, and 5; group 2 includes

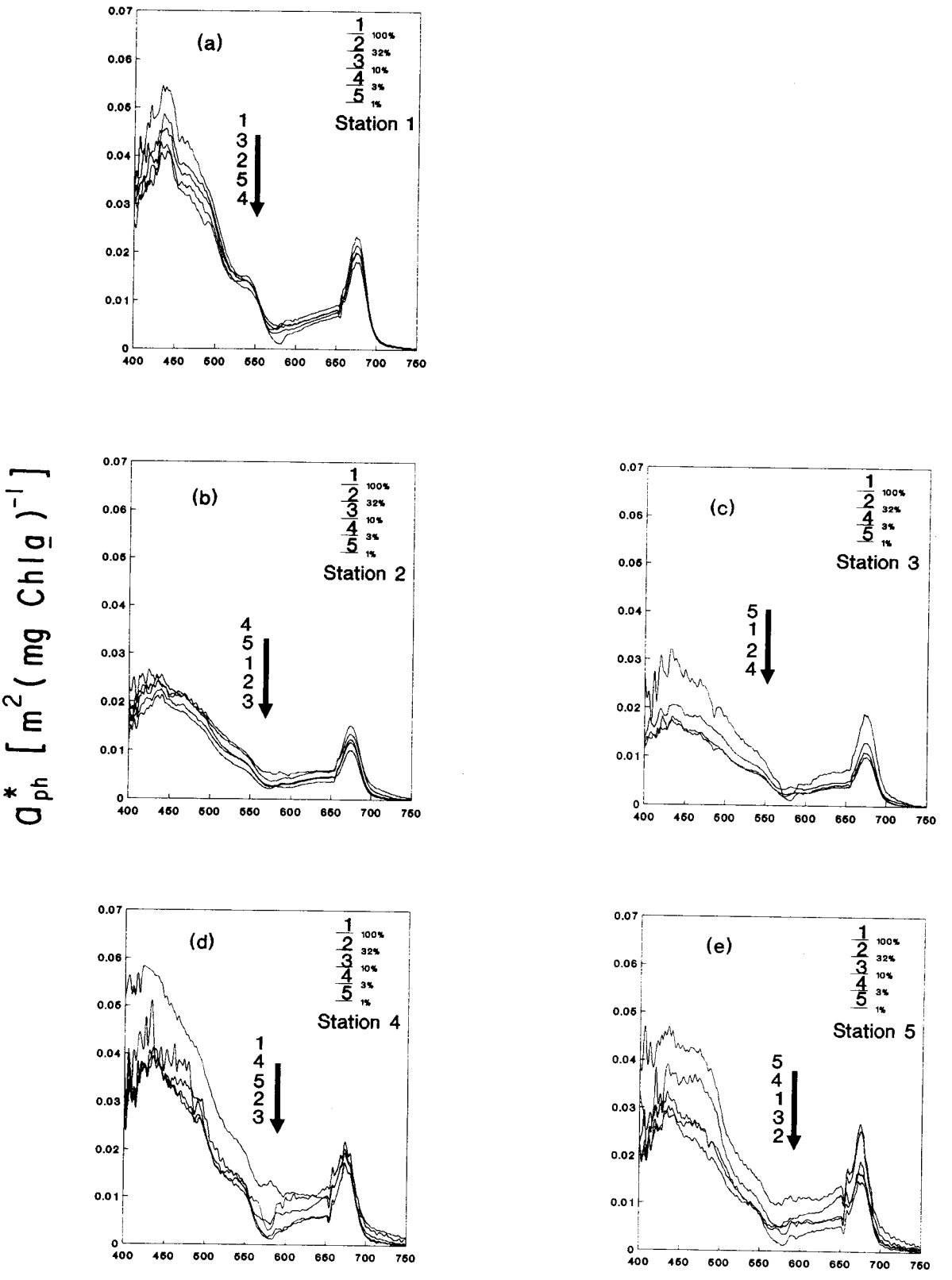

Figure 4. Spectral variability of the specific light-absorption coefficient of phytoplankton (a^*_{ph}) m^2(mg chl a)$^{-1}$ at five light depths (100%, 32%, 10%, 3%, and 1% of E_o): *a*, station 1; *b*, station 2; *c*, station 3; *d*, station 4; and *e*, station 5. Numbers to the left of the arrows indicate the position sequence of the spectral curves.

TABLE 2
Spatial Variability by Stations of Average Cell Volume l_n ($\mu m^3\ l^{-1}$) for Diatoms in the Gulf of California

Diatom genus (average)	Station 1	Station 2	Station 3	Station 4	Station 5
Nitzschia	13.92	13.57	14.92	13.38	13.64
Rhizosolenia	20.01	20.98	22.17	21.15	19.46
Chaetoceros	11.87	15.87	14.66	11.86	11.71
Skeletonema	10.34	11.53	12.08	—	—
Corethron	15.24	16.52	17.39	—	14.85
Bacteriastrum	10.26	12.10	11.55	11.20	—
Thalassiothrix	12.55	19.00	17.92	16.05	14.65
Leptocylindrus	15.31	16.78	17.02	—	15.85
Navicula	11.89	11.70	16.80	10.40	14.51
Ditylum	18.08	19.35	18.77	—	—
Coscinodiscus	15.69	19.72	18.36	13.57	14.21
Pseudoeunotia	—	17.68	18.25	—	—
Hemiaulus	—	16.37	15.69	—	—
Actinoptychus	—	17.60	17.21	—	13.47
Guinardia	—	—	18.09	—	—

TABLE 3
Spatial Variability by Station of Average Chlorophyll and Carotenoid Concentrations (mg m^{-3}) in the Gulf of California

Pigments (average)	Station 1	Station 2	Station 3	Station 4	Station 5
Chlorophyllide a	0.1203	0.2647	0.2969	0.1085	0.1808
Chlorophyll c	0.0691	0.4120	0.5943	0.0388	0.0919
19' But-fucoxant	0.0297	0.0369	0.2398	0.0285	0.0597
Fucoxanthin	0.1495	0.8704	0.7695	0.0531	0.0692
19' Hex-fucoxant	0.1301	0.1067	0.0617	0.0457	0.0522
Prasinoxanthin	0.0294	0.1217	0.1142	0.0190	0.0080
Diadinoxanthin	0.0130	0.0479	0.0547	0.0077	0.0113
Alloxanthin	0.0138	0.0111	0.0344	—	—
Zeax/Lutein	0.2853	0.0923	0.0757	0.0533	0.0470
Chlorophyll b	0.1209	0.0681	0.1040	0.0628	0.0681
Chlorophyll a	0.7621	1.2569	1.1524	0.1920	0.2384
Phaeopigment b	0.0929	0.1910	0.2448	0.0170	0.1227
Phaeopigment a	0.0102	0.0245	0.0262	0.0030	0.0071
Divinyl chl*a*	0.0160	0.0170	0.0130	0.0130	0.0530

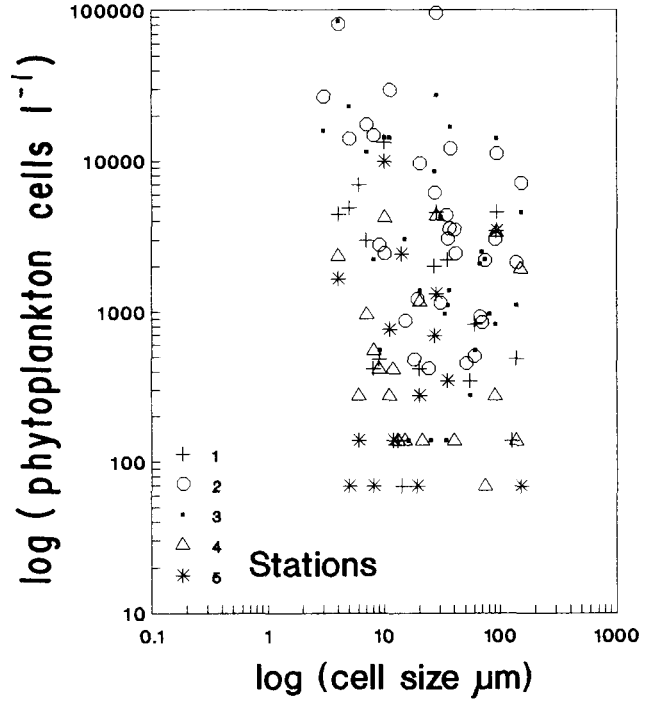

Figure 5. Abundances and cell sizes of phytoplankton by stations for the Gulf of California.

stations 2 and 3. The average spectral shapes, by stations, were determined from phytoplankton absorption normalized to 440 nm ($a_{ph}(\lambda)/a_{ph}440$ nm). The main difference between the two groups was the absorption at wavelengths 480–550 nm and 661 nm. The spectral shoulder observed near 545 nm has been reported as characteristic of cyanobacteria (Bidigare et al. 1989; Moore et al. 1995; Sathyendranath et al. 1996; and Jeffrey et al. 1997). Likewise, the absorption at 661 nm is characteristic of the divinyl chl a pigment and corresponds to the prochlorophytes group (Jeffrey et al. 1997).

In table 4 we show two algorithms, or empirical models, relating specific absorption of phytoplankton with the concentration of key pigments and with cell size. The best model ($r^2 = 0.80$; $p = 0.05$) for a^*_{ph} at 440 nm was achieved when the ratios zeax/chl a, chl a/biovolume, and biovolume cell^{-1} were included. For $a^*_{ph}(674)$, the best model ($r^2 = 0.67$; $p = 0.05$) was achieved when the ratios chl a/biovolume and zeax/chl a were considered.

DISCUSSION

During our study, the phytoplankton-specific absorption coefficient varied both between stations and within stations. Two of the five stations showed that a^*_{ph} was higher for the deeper samples. The values for $a^*_{ph}(440)$ were more variable than the values for $a^*_{ph}(674)$. The values for $a^*_{ph}(440$ nm) for stations 1, 4, and 5 were higher (~45%) than the values for stations 2 and 3. Stations 2 and 3 were located near the large islands (fig. 1); this region of the gulf has the highest ratios of chlorophyll c and fucoxanthin to chlorophyll a, indicating that diatoms dominate. This region is characterized by intense mixing (winds and tides), and typically has high primary productivity rates and blooms of microphytoplankton (cells >20 µm). Stations 1 and 5 had higher ratios of chlorophyll a/biovolume, fewer diatom genera, and lower ratios of chlorophyll c and fucoxanthin to chlorophyll a, compared to stations 2 and 3. In general, stations 2 and 3, which exhibited the lowest specific absorption coefficient, are located in a region where the environment favors the flourishing of larger species within the phytoplankton community. Our findings agree with previous conclusions about the ecological implications of the variability of absorption efficiencies of natural phytoplankton communities (Lewis et al. 1985; Mitchell and Kiefer 1988b; Yentsch and Phinney 1989; Bricaud

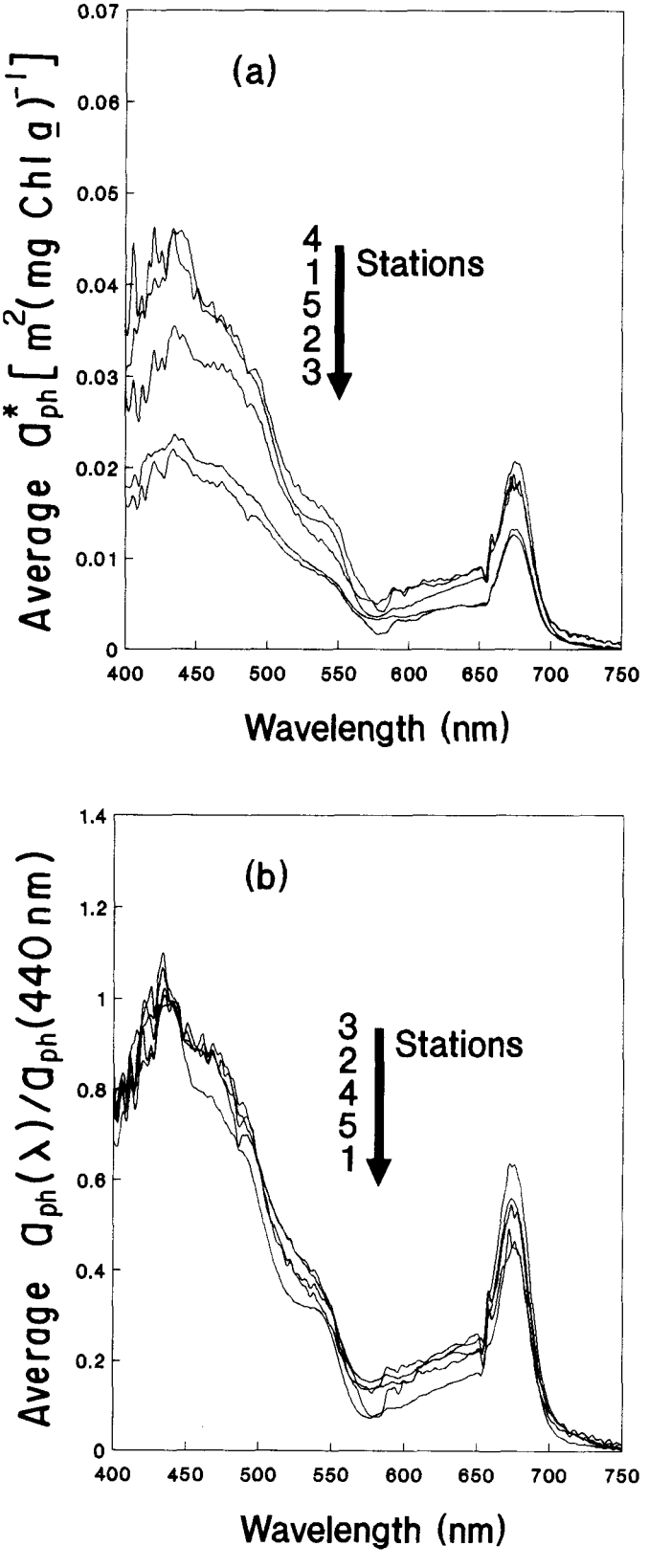

Figure 6. Average phytoplankton absorption spectra by stations: *a*, specific absorption coefficient; *b*, absorption phytoplankton coefficient normalized at 440 nm. The numbers to the left of the arrows show the position sequence of the spectral curves.

TABLE 4
Summary of Stepwise Regression Analysis for the Phytoplankton-Specific Light-Absorption Coefficient a^*_{ph} (440 and 674 nm) as a Dependent Variable

Variables	Partial cor.	R^{2a}	F^b	*P* level
440 nm				
Zeax/chla	0.778	0.578	20.07	.000624
Chla/biovol	0.716	0.708	13.76	.002642
Biovol/cel	0.563	0.800	6.05	.028801
674 nm				
Chla/biovol	0.800	0.504	22.75	.000334
Zeax/chla	0.683	0.671	11.35	.004994

[a]Multiple coefficient of determination.
[b]Variance ratio of the multiple regression.

and Stramsky 1990; Hoepffner and Sathyendranath 1992; Babin et al. 1993).

One of the main objectives of our study was to elucidate the factors causing variability in the phytoplankton absorption coefficient in the gulf. For instance, several studies (theoretical and laboratory) have reported that the absorption coefficient is not constant, and have argued that the variability is caused by the flattening of the absorption spectra due to particle effect (size, shape, and optical density of the particles) and the pigment composition (Duysens 1956; Morel and Bricaud 1981; Dubinsky et al. 1986; Sathyendranath et al. 1987; Mitchell and Kiefer 1988b; Sosik and Mitchell 1991; Kirk 1994).

Morel and Bricaud (1981) argued that the packing of chlorophyll into a cell physically changes the probability that an individual chlorophyll molecule will absorb a passing photon. As chlorophyll per cell increases at constant cell volume, the specific absorption coefficient decreases. This pattern might occur if a single species were increasing its pigments, perhaps due to photoadaptation, but not changing its size. However, in a natural phytoplankton community where the size spectrum comprises diverse species, it is uncertain whether chlorophyll per cell volume will remain constant as cell size varies. Our data (fig. 7) showed that chlorophyll a per volume [ln(chlorophyll a/biovolume)] decreased as cell size [ln(biovolume cell^{-1})] increased. This pattern indicated that, at least during our sampling, chlorophyll per cell volume was not independent of cell size, as assumed in Morel and Bricaud's (1981) calculations. Because this particular model restriction was not met by the field data, it is not necessary to expect a^*_{ph} to vary inversely with cell size. And, in fact, it did not: When plotted as a function of ln(biovolume cell^{-1}), neither $a^*_{ph}(674)$ nor $a^*_{ph}(440)$ showed any discernable pattern (data not shown), and the relationships were not statistically significant.

A second factor that might be significant in this discussion of cell size is a technical limitation. Only

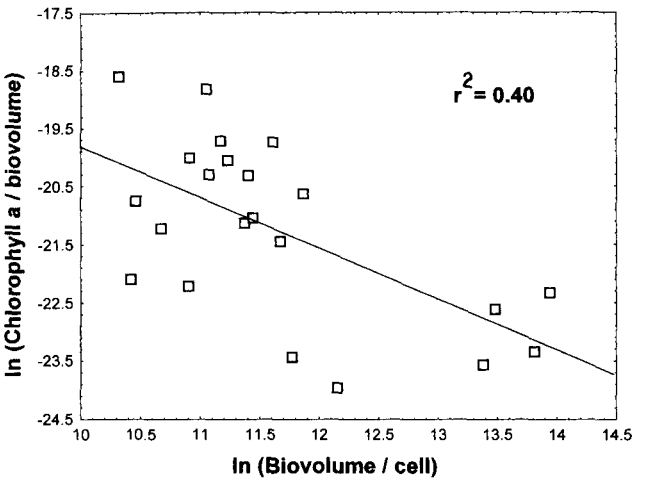

Figure 7. The chlorophyll a/biovolume ln(chlorophyll a/biovolume) as a function of the cell sizes ln(biovolume cell^{-1}) (r^2 = 0.40; p = 0.05).

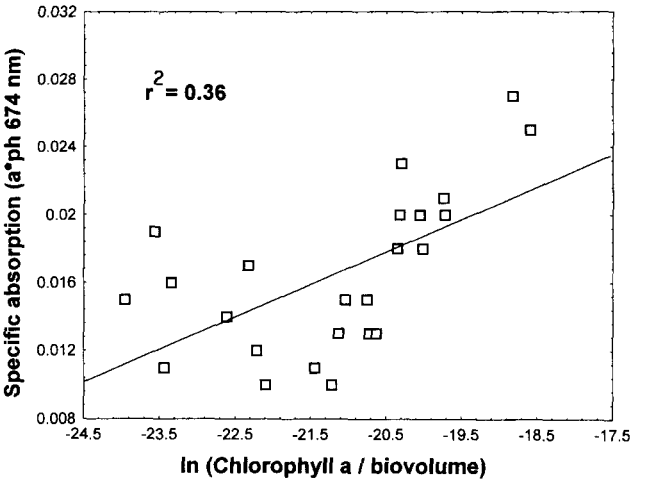

Figure 8. Specific light-absorption coefficients of phytoplankton a^*_{ph} m^2 (mg chl a)$^{-1}$ as a function of the ln(chlorophyll a/biovolume) at 674 nm (r^2 = 0.36; p = 0.05).

phytoplankton cells >5 μm can be counted and sized with the inverted microscope technique. However, the absorption and pigment measurements could include small cells (>0.7 μm). The observations that >60% of the countable phytoplankton cells in our samples were in the nanophytoplankton (<20 μm) fraction might suggest that the cells in the uncounted <5 μm fraction were abundant. This systematic bias may contribute to the absence of the expected pattern of decreasing specific absorption coefficient with increasing cell size; in this data set we were not able to find a significant slope (data not shown). However, when we used the ratio chlorophyll a/biovolume as an index of the size of the phytoplankton community, we found that the highest specific absorption coefficients (a^*_{ph}674 nm) corresponded to stations 1 and 5, where small-size cells were abundant (fig. 8).

As pointed out before, changes in pigment composition have also been associated with variations in phytoplankton-specific absorption coefficient. In the Gulf of California, the average ratios of zeaxanthin/chlorophyll a were high (up to 0.37). Of all the pigments studied (table 3), the highest correlation (r^2 = 0.58; p = 0.05) with a^*_{ph} was obtained between the ratio zeaxanthin/chlorophyll a and a^*_{ph}(440) (fig. 9). In general, the ratio zeaxanthin/chl a can be used as a marker pigment for cyanobacteria and prochlorophytes (Kana et al. 1988; Bidigare et al. 1989; Falkowski and Laroche 1991; Bricaud et al. 1995; Moore et al. 1995; and Sathyendranath et al. 1996). The distinctive divinyl chlorophyll a absorption peaks (~661 nm) of prochlorophytes were observed at stations 1, 4, and 5 (fig. 6a).

Cyanobacteria pigments are evident as absorption shoulders at 480 and 550 nm in the spectra from stations 1, 4, and 5 (fig. 6b), indicating that the cyanobacteria

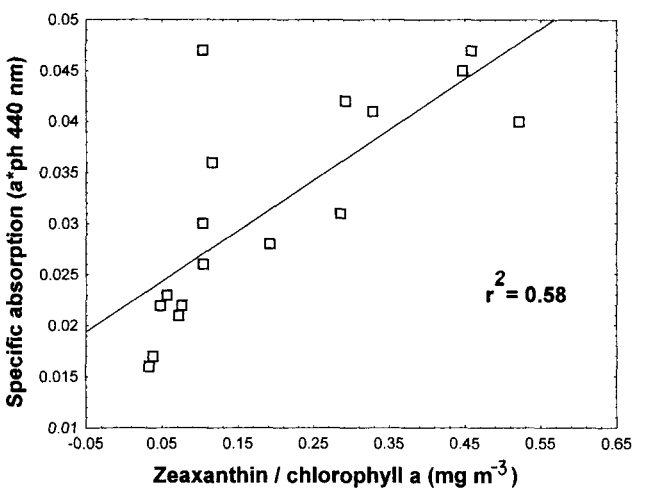

Figure 9. Specific light-absorption coefficient of a^*_{ph} m^2(mg chl a)$^{-1}$ in the blue band (440 nm) as a function of the ratio zeaxanthin/chlorophyll a (r^2 = 0.58; p = 0.05).

group was present. Even though we could not directly count cyanobacteria (typically about 1 μm in diameter) with the inverted microscope technique, the presence of these small cells is evidenced by these absorption and pigment data.

High a^*_{ph} is expected for small cells because of low or absent pigment-packaging effects; the presence of small cyanobacteria where zeaxanthin was high explains part of the pattern between a^*_{ph}(440) and zeaxanthin/chlorophyll a shown in figure 9. Futhermore, zeaxanthin manifests wavelength maxima at 454 and 480 nm (Jeffrey et al. 1997), typical of carotenoids; high at blue and blue-green wavelengths with a broad peak. When present, zeaxanthin is certainly responsible for some of the measured absorption at 440 nm, artificially increas-

ing $a^*_{ph}(440)$, which is normalized to chlorophyll a concentration only. Indeed, $a^*_{ph}(440)$ tended to be high where the pigment group PPC was high. The positive relation between $a^*_{ph}(440)$ and the ratio of zeaxanthin/chlorophyll a is probably due to the combined effects of pigment composition and cell size, and to the related errors in the β factor caused by these small phytoplankton on specific absorption.

At stations 2 and 3, there was less variability in the magnitude of a^*_{ph} with depth in the water column (fig. 4b, c), lower a^*_{ph}, and the lowest ratio of average zeaxanthin/chl a (0.073 and 0.066), with no indication of absorption by cyanobacteria pigments (table 3). We confirmed these observations by examining the exponential curve of methanol-extracted particulate absorption spectra (data not shown).

We used multiple regression analysis to explore whether cell size or pigment composition was more significant in the variability of the specific absorption coefficient (table 4). We found that the variability of $a^*_{ph}(440)$ was most strongly related first to the ratio of zeaxanthin/chlorophyll a ($r^2 = 0.58$; $p = 0.05$), second to the ratio chlorophyll a/biovolume, and finally to biovolume per cell, giving a final r^2 of 0.80; other variables were not significant in the model. Our results are in line with laboratory results of Sathyendranath et al. (1987), who studied the particle and pigment composition effect on the absorption spectra of eight species of phytoplankton. They were able to explain 44% of the variability of the absorption efficiency at 440 nm by changes in the pigment composition. When the pigment packaging was included with pigment composition, the model explained up to 96% of the variation in absorption efficiency.

Our data showed greater variability in a^*_{ph} at 440 nm than 674 nm. This difference results from strong absorption in the blue region by carotenoids; however, their concentrations were not considered in our calculations of the specific absorption coefficient. Variable contributions to $a^*_{ph}(440)$ by carotenoids will increase variability in $a_{ph}(440)$. In the red region (674 nm), the main absorption is due to chlorophyll a. Similar results have been reported by Sathyendranath et al. (1987) for laboratory cultures; Yentsch and Phinney (1989) for the western North Atlantic; Sosik and Mitchell (1991) for the California Current; and Sathyendranath et al. (1996) for the northwest Indian Ocean.

Kiefer and SooHoo (1982) measured values for the specific absorption coefficient in Gulf of California waters in March 1979 and reported a mean value of 0.022 $m^2(mg\ chl\ a)^{-1}$ for $a^*_{ph}(440)$. Yentsch and Phinney (1989) reported values for $a^*_{ph}(440)$ of ~0.082 and ~0.047 for $a^*_{ph}(670)$ for the southern part of the gulf in March 1988. Our October values for the central and northern gulf ranged from 0.020 to 0.056 $m^2(mg\ chl\ a)^{-1}$ for $a^*_{ph}(440)$ and from 0.013 to 0.020 $m^2(mg\ chl\ a)^{-1}$ for $a^*_{ph}(674)$. Yentsch and Phinney's values were higher than ours; they suggested that the high absorption values were due to increased concentration of UV-photoprotective pigments. The data available for the Gulf of California (this paper; Kiefer and SooHoo 1982; Yentsch and Phinney 1989) are not sufficient for interpreting seasonal or interannual variability.

For our data set, pigment composition was more important than cell size for determining the magnitude of $a^*_{ph}(440)$. However, in order to explain 80% of the variability in $a^*_{ph}(440)$, a variable related to cell size was required. Most of the previous field work on the variation of $a^*_{ph}(440)$ has focused on only one of these three variables at a time. Our results are significant because they illustrate the interaction of pigment composition and pigment packaging (through chlorophyll per cell volume and through cell size) in determining the magnitude of $a^*_{ph}(440)$.

CONCLUSION

The spectra of phytoplankton belonging to the same taxonomic group tended to have similar shapes. The specific absorption coefficient of phytoplankton was highly variable (spatial domain) in the gulf. Stations where the environment favors the development of microphytoplankton (cells >20 μm) presented the lowest specific absorption coefficients because of the increased pigment-packaging effect in these biggest cells. Pigments, particularly the photoprotectant pigment zeaxanthin, had the highest correlation with the absorption coefficient. Changes in pigment composition and cellular concentration were responsible for over 70% of the variability in the specific absorption coefficient at 440 nm, and—if biovolume per cell was included—the model explained up to 80% of the variance.

The significance of these results lies in the concurrent quantitative examination of several potential controls of the specific absorption coefficient: pigment packaging (i.e., the cellular concentration of chlorophyll a and the phytoplankton cell volume) and the confounding effect of blue-absorbing accessory pigments. The statistical significance of the resulting multivariate relationships indicates that all three of these factors can influence the specific absorption coefficient.

Chlorophyll per volume [ln(chl a/biovolume)] decreased as cell size (biovolume $cell^{-1}$) increased; theoretical analysis that holds one variable constant while examining the other does not account for the situation observed in these particular phytoplankton communities. The bigger cells did not maintain the same internal concentration of chlorophyll per cell volume as the smaller cells; therefore, figure 8 shows that as ln(chl

a/biovolume) decreased, indicating large cells, the a^*_{ph} decreased. Observations of a nonlinear relationship between $a_{ph}(440)$ or $a^*_{ph}(440)$ and chlorophyll a (e.g., Yentsch and Phinney 1989; Bricaud et al. 1995; Cleveland 1995) may indeed be due to a pigment-packaging effect related to cell size, as postulated. But our results and some of those in Cleveland 1995 show that this situation is not always the case. As previously pointed out by Hoepffner and Sathyendranath (1992), accessory pigmentation plays a significant role in the variability of $a^*_{ph}(440)$. Perhaps it is time for the community to define the specific absorption coefficient in terms that include these other pigments.

ACKNOWLEDGMENTS

We thank the crew and commander of R/V *A. Humboldt H-03*, Cap. de Frag. C. G. Rodríguez Toro from the Mexican Navy; Charles Trees for his friendship and great help with reanalysis and revisions of the manuscript; and José M. Domínguez and Francisco J. Ponce for the drawings. We thank also the technicians of the Estación Oceanográfica de Ensenada: Ramón Velázquez, Jorge Salas, and Leonardo A. Santamaría. We thank Jesús Paniagua and Jaime Farber for their participation during the cruise. This study was supported by the Dirección Adjunta de Desarrollo Científico of CONACyT, through research grant T9201-1111.

LITERATURE CITED

Babin, M., J. C. Therriault, L. Legendre, and A. Condal. 1993. Variation in the specific absorption coefficient for natural phytoplankton assemblages: impact on estimates of primary production. Limnol. Oceanogr. 38:154–177.

Bidigare, R. R., O. Schofield, and B. B. Prézelin. 1989. Influence of zeaxanthin on quantum yield of photosynthesis of *Synechococcus* clone WH7803 (DC2). Mar. Ecol. Prog. Ser. 56:177–188.

Bidigare, R. R., M. E. Ondrusek, J. H. Morrow, and D. A. Kiefer. 1990. In vivo absorption properties of algal pigments. Ocean Optics 10, Proc. Int. Soc. Optical Eng. 1302:290–302.

Bricaud, A., and D. Stramsky. 1990. Spectral absorption coefficients of living phytoplankton and nonalgal biogenous matter: a comparison between the Peru upwelling area and the Sargasso Sea. Limnol. Oceanogr. 35:562–582.

Bricaud, A., M. Babin, A. Morel, and H. Claustre. 1995. Variability in the chlorophyll-specific absorption coefficients of natural phytoplankton: analysis and parameterization. J. Geophys. Res. 100(7):13321–13332.

Cleveland, J. S. 1995. Regional models for phytoplankton absorption as a function of chlorophyll *a* concentration. J. Geophys. Res. 100(7):13,333–13,344.

Cleveland, J. S., and A. D. Weidemann. 1993. Quantifying absorption by aquatic particles: a multiple scattering correction for glass fiber filters. Limnol. Oceanogr. 38(6):1321–1327.

Dubinsky, Z., P. G. Falkowski, and K. Wyman. 1986. Light harvesting and utilization by phytoplankton. Plant Cell Physiol. 27:1335–1349.

Duysens, L. N. M. 1956. The flattening of the absorption spectrum of suspensions, as compared to that of solutions. Biochim. Biophys. Acta 19:1–12.

Edler, L. 1979. Recommendations for marine biological studies in the Baltic Sea, phytoplankton and chlorophyll. National Swedish Environmental Protection Board, 37 pp.

Everitt, D. A., S. W. Wright, J. K. Volkman, D. P. Thomas, and E. J. Lindstronm. 1990. Phytoplankton community compositions in the western equatorial Pacific determined from chlorophyll and carotenoid pigment distributions. Deep Sea Res. 37:975–997.

Falkowski, P. G., and J. LaRoche. 1991. Acclimation to spectral irradiance in algae. J. Phycol. 27:8–14.

Hoepffner, N., and S. Sathyendranath. 1992. Bio-optical characteristics of coastal waters: absorption spectra of phytoplankton and pigment distributions in the western North Atlantic. Limnol. Oceanogr. 37:1660–1679.

Jeffrey, S. W., R. F. C. Mantoura, and S. W. Wright. 1997. Phytoplankton pigments in oceanography: a guide to advanced methods. SCOR-UNESCO, Paris, 661 pp.

Kana, T. M., P. M. Glibert, R. Goericke, and N. A. Welschmeyer. 1988. Zeaxanthin and β-carotene in *Synechococcus* WH7803 respond differently to irradiance. Limnol. Oceanogr. 33:1623–1627.

Kiefer, D. A., and B. G. Mitchell. 1983. A simple, steady state description of phytoplankton growth based on absorption cross section and quantum efficiency. Limnol. Oceanogr. 28:770–776.

Kiefer, D. A., and J. B. SooHoo. 1982. Spectral absorption by marine particles of coastal waters of Baja California. Limnol. Oceanogr. 27(3):492–499.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems, 2d ed. New York: Cambridge Univ. Press, 509 pp.

Kishino, M., N. Takahashi, N. Okami, and S. Ichimura. 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bull. Mar. Sci. 37:634–642.

Lewis, M. R., R. E. Warnock, and T. Platt. 1985. Absorption and photosynthetic action spectra for natural phytoplankton populations: implications for productions in the open ocean. Limnol. Oceanogr. 30:794–806.

Maske, H., and H. Haardt. 1987. Quantitative in vivo absorption spectra of phytoplankton: detrital absorption and comparison with fluorescence excitation spectra. Limnol. Oceanogr. 32(3):620–633.

Mitchell, B. G. 1990. Algorithms for determining the absorption coefficient of aquatic particulates using the quantitative filter technique (QFT). *In* Ocean optics X, R. Spinrad, ed. Proceeding reprint: International Society for Optical Engineering, 1302, pp. 137–148.

Mitchell, B. G., and D. A. Kiefer. 1988a. Chlorophyll *a* specific absorption and fluorescence excitation spectra for light-limited phytoplankton. Deep-Sea Res. 35:639–663.

———. 1988b. Variability in pigment specific particulate fluorescence and absorption spectra in the northeastern Pacific Ocean. Deep-Sea Res. 35:665–689.

Moore, L. R., R. Goerike, and S. W. Chisholm. 1995. Comparative physiology of *Synechococcus* and *Prochlorococcus* influence of light and temperature on growth, pigments, fluorescence and absorptive properties. Mar. Ecol. Ser. 116:259–275.

Morel, A., and A. Bricaud. 1981. Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. Deep-Sea Res. 28:1375–1393.

Morel, A., and L. Prieur. 1977. Analysis of variations in ocean color. Limnol. Oceanogr. 22:709–722.

Nelson, N. B., B. B. Prézelin, and R. R. Bidigare. 1993. Phytoplankton light absorption and package effect in California coastal waters. Mar. Ecol. Prog. Ser. 94:217–227.

Sathyendranath, S., L. Lazzara, and L. Prieur. 1987. Variations in the spectral values of specific absorption of phytoplankton. Limnol. Oceanogr. 32:403–415.

Sathyendranath, S., T. Platt, V. Stuart, B. D. Irwin, M. J. W. Veldhuis, G. W. Kraay, and W. G. Harrison. 1996. Some bio-optical characteristics of phytoplankton in the NW Indian Ocean. Mar. Ecol. Prog. Ser. 132:299–311.

Sosik, H. M., and B. G. Mitchell. 1991. Absorption, fluorescence, and quantum yield for growth in nitrogen-limited *Dunaliella tertiolecta*. Limnol. Oceanogr. 36(5):910–921.

———. 1995. Light absorption by phytoplankton, photosynthetic pigments and detritus in the California Current system. Deep-Sea Res. 1717–1748.

Spinrad, R. W., and J. F. Brown. 1986. Relative real refractive index of marine microorganisms, a technique for flow cytometric estimation. Appl. Optics 25:1930–1934.

Strathmann, R. R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12:411–418.

Strickland, J. D., and T. R. Parsons. 1972. A practical handbook of seawater analysis. Bull. 167 Fish. Res. Board Can., Ottawa, 310 pp.

Wright, S. W., S. W. Jeffrey, R. F. Mantoura, C. A. Llewellyn, T. Bjornland, D. Repeta, and N. Welschmeyer. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar. Ecol. Prog. Ser. 77:183–196.

Yentsch, C. S. 1960. The influence of phytoplankton pigments on the colour of sea water. Deep-Sea Res. 7:1–9.

Yentsch, C. S., and D. W. Menzel. 1963. A method for the determination of phytoplankton chlorophyll and pheophytin by fluorescence. Deep-Sea Res. 10:221–231.

Yentsch, C. S., and D. A. Phinney. 1989. A bridge between ocean optics and microbial ecology. Limnol. Oceanogr. 34:1694–1705.