

The response of photosynthetic absorption coefficients to irradiance in culture and in tidally mixed estuarine waters

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Abstract

The accuracy of models for primary production and light propagation depends on correct assignment of absorption to photosynthetic pigments. The phytoplankton absorption coefficient is comprised of two components: photosynthetic and photoprotective absorption coefficients. A method based on the fluorescent excitation of chlorophyll *a* is used to quantify the photosynthetic absorption coefficient for phytoplankton grown in culture and sampled from Puget Sound, Washington. The difference spectrum between total phytoplankton and photosynthetic absorption should be equivalent to photoprotective absorption. For cultures, the difference spectra exhibit peaks near 460 and 490 nm and broad-band absorption between 400 and 450 nm. However, for field samples an additional pronounced peak is observed around 440 nm, similar in shape to the chlorophyll *a* Soret peak. If the 440-nm peak were associated with photosystem I chlorophyll *a*, the photosynthetic absorption coefficient will be underestimated by <15% for these samples.

Variability in both coefficients is predictable as a function of irradiance. The photosynthetic coefficient varies inversely with growth irradiance, and the photoprotective coefficient varies directly with irradiance. This direct relationship with irradiance accounts for much of the variability in the spectral shape of the total phytoplankton absorption coefficient. The ratio of the photosynthetic absorption coefficient to the total phytoplankton absorption coefficient increases as a function of decreasing irradiance for cultures and for field samples collected from stratified regions of the water column. This ratio is a photoadaptive parameter that can serve to integrate physiological response to irradiance and has the potential to provide estimates of mixed layer dynamics.

Modeling light propagation through the water column and determining a photon budget for the euphotic zone requires an estimate of the phytoplankton absorption coefficient. The phytoplankton absorption coefficient often is the most variable optical component of the water column. The composition and quantity of the phytoplankton assemblage affect both the spectrum and the magnitude of the absorption coefficient. Phytoplankton adapt the composition and concentration of photosynthetic and nonphotosynthetic pigments in response to variations in irradiance intensity and spectral composition (Yentsch and Yentsch 1979; SooHoo et al. 1986; Mitchell and Kiefer 1988; Johnsen and Sakshaug 1993). The accuracy of photon budgets and of bio-optical models for ocean productivity is improved by the incorporation of spectral information into the irradiance and absorption coefficient measurements (e.g., Bidigare et al. 1987, 1992; Smith et al. 1989). The accuracy also is improved by

information as to whether photosynthetic or photoprotective pigments are responsible for the absorption.

The methodology for measuring phytoplankton absorption coefficients in the field has evolved over several decades (Fig. 1). Yentsch (1962) proposed concentrating dilute field samples onto glass fiber filters to increase the accuracy of absorption measurements. After accounting for corrections to the methodology (i.e., Shibata et al. 1954; Trüper and Yentsch 1967; Kiefer and SooHoo 1982; Mitchell and Kiefer 1988; Bricaud and Stramski 1990; Mitchell 1990; Roesler 1992; Cleveland and Wiedemann 1993), this spectrophotometric technique yields the total particle absorption coefficient that includes absorption by phytoplankton, detritus, bacteria, microzooplankton, and nonorganic particles. Chemical (Kishino et al. 1985; Maske and Haardt 1987), particle-specific (Itturiaga and Siegel 1988), and modeling (Bidigare et al. 1987; Morrow et al. 1989; Roesler et al. 1989; Cleveland and Perry 1994) methods were developed to separate an absorption coefficient for pigments associated with phytoplankton from the particle absorption coefficient. An estimate of the pheophytin absorption coefficient can be calculated independently (Roesler et al. 1989; Cleveland and Perry 1994; Sosik and Mitchell 1995). The remaining components are the photosynthetic and photoprotective pigments.

Two methods have been proposed to quantify the photosynthetic absorption coefficient. The first method models the photosynthetic absorption coefficient using the concentrations of photosynthetic pigments that have been measured using high performance liquid chromatography and packaging the pigments using the phytoplankton absorption coefficient that has been measured spectrophotometrically (Babin et al. 1996; Allali et al. 1997). The second method uses

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Fig. 1. Absorption coefficients directly measured (rectangles), independently calculated (diamond), or calculated by difference (circles).

the chlorophyll *a* (Chl *a*) fluorescence excitation spectrum to identify photosynthetic pigments associated with photosystem II (Neori et al. 1986, 1988). The spectrum is then scaled to the phytoplankton absorption coefficient that has been measured spectrophotometrically (Maske and Hardt 1987; Sakshaug et al. 1991). The fluorescence method has also been used on the chromoproteins and thylakoid micelles of cells to provide information on the amount of light harvested by the components and the efficiency with which the light is utilized (Johnsen et al. 1997).

Changes in the amount and ratios of photosynthetic and photoprotective pigments are acclimation responses to irradiance (Falkowski 1980) that may be affected by nutrient concentrations (Herzig and Falkowski 1989; Sosik and Mitchell 1991). The photosynthetic portion appears to be the less variable component of the phytoplankton absorption coefficient; therefore, variation is largely attributable to changes in the photoprotective component (Sosik and Mitchell 1995; Allali et al. 1997). Field studies have shown a general trend of the chlorophyll-specific phytoplankton absorption coefficient decreasing with optical depth due to decreased photoprotective pigments and increased pigment packaging; however, the cause of variation among phytoplankton assemblages is largely unclear (Lutz et al. 1998). With the availability of remote sensing and in situ instrumentation that can simultaneously measure absorption and fluorescence spectra (Cowles et al. 1993, 1996; Bricaud et al. 1995), it will be possible to apply the fluorescence excitation method to separate the photosynthetic and nonphotosynthetic absorption coefficients to in situ measurements.

In this study, spectral photosynthetic and nonphotosynthetic absorption coefficients were determined for phytoplankton assemblages in Puget Sound. Absorption coefficients for field samples were compared with the coefficients measured using cultures that were adapted to specified irradiance conditions to study the influence of irradiance on

the proportioning of photosynthetic and nonphotosynthetic absorption within the phytoplankton absorption coefficient. Documenting the variability of the photosynthetic and nonphotosynthetic absorption coefficients and determining the reasons for their variability is important for extrapolating parameters used to estimate photon budgets and productivity over large spatial scales.

Methods

Cultures—Cultures of the prymnesiophyte *Isochrysis galbana*, the dinoflagellate *Amphidinium carterae*, and two diatoms, *Nitzschia closterium* and *Thalassiosira weissflogii*, were maintained in exponential growth in modified medium (Perry et al. 1981) at 18°C on a light:dark cycle of 16:8 h. Cultures were adapted to four irradiances (700, 300, 25, and 5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for a minimum of 10 generations before experimentation. Prior to measurements, samples were placed in the dark for 30 min and then stored on ice in the dark to prevent cellular adaptation and growth until completion of all measurements (<2 h).

Field samples—Field samples were collected at various sites within the main basin of Puget Sound, Washington, during the summers of 1993 and 1994. Conductivity, temperature, and pressure were measured with a SEACAT SBE19 Profiler (Sea-Bird Electronics). Water samples were taken within the euphotic zone from the surface to 15 m. Field samples were stored in dark bottles on ice for transport to the laboratory (3–4 h).

Chl *a* and pheophytin *a* concentrations—Concentrations of Chl *a* and pheophytin *a* (Pheo *a*) were measured fluorometrically in triplicate by filtration of water samples through Whatman GF/F filters. Filters were extracted in 90% acetone at -20°C for 24 h, sonicated for 7 min, and measured both before and after acidification on a fluorometer (Turner Designs) (Holm-Hansen et al. 1965). The fluorometer was calibrated using a Chl *a* standard (Sigma).

Fluorescence excitation spectra—Fluorescence spectra $F(\lambda_e, 730)$; see (Notation table for list of symbols) were measured on a Spex Fluorolog 2 using excitation wavelengths 400–700 nm at 2-nm intervals (5-nm bandwidth); emission was monitored at 730 nm (10-nm bandwidth; Maske and Hardt 1987; Neori et al. 1988). Spectra were corrected for variation in xenon lamp output using Basic Blue 3 as a quantum counter (Kopf and Heinz 1984) and corrected for instrument optics according to Culver et al. (1994). Samples were stirred during measurement. For cultures, the excitation spectrum of sterile IMR medium served as a blank, and three replicates were measured. Field samples were warmed to room temperature before measurement. The excitation spectrum of seawater filtered through a GF/F filter was used as a blank for water and dissolved substances, and 6–15 replicates were measured. The mean spectrum was smoothed using a locally weighted regression procedure (LOWESS; Cleveland 1979; SYSTAT; version 5.2: SYSTAT).

Notation

Symbol	Description
$a_{\text{der}}(\lambda)$	Spectral detritus absorption coefficient (m^{-1})
$a_{\text{I}}(\lambda)$	Spectral absorption coefficient for photosystem I (m^{-1})
$a_{\text{II}}(\lambda)$	Spectral absorption coefficient for photosystem II (m^{-1})
$a_{\phi}(\lambda)$	Spectral phytoplankton with pheophytin <i>a</i> absorption coefficient (m^{-1})
$a_{\text{part}}(\lambda)$	Spectral particle absorption coefficient (m^{-1})
$a_{\text{pheo}}(\lambda)$	Spectral pheophytin <i>a</i> absorption coefficient (m^{-1})
$a_{\text{phy}}(\lambda)$	Spectral phytoplankton absorption coefficient (corrected for pheophytin <i>a</i> absorption) (m^{-1})
$a_{\text{phy}}^*(\lambda)$	Spectral chlorophyll- <i>a</i> -specific phytoplankton absorption coefficient ($\text{m}^2 [\text{mg Chl } a]^{-1}$)
$a_{\text{pp}}(\lambda)$	Spectral photoprotective absorption coefficient (m^{-1})
$a_{\text{ps}}(\lambda)$	Spectral photosynthetic absorption coefficient (m^{-1})
$a_{\text{ps}}^*(\lambda)$	Spectral chlorophyll- <i>a</i> -specific photosynthetic absorption coefficient ($\text{m}^2 [\text{mg Chl } a]^{-1}$)
$a_{\text{ps}}:a_{\text{phy}}$	Photosynthetic fraction of phytoplankton absorption coefficient (see Eq. 3) (dimensionless)
Chl <i>a</i>	Chlorophyll <i>a</i> (mg m^{-3})
$d(\lambda)$	Difference between $a_{\text{phy}}(\lambda)$ and $a_{\text{ps}}(\lambda)$ from 400 to 550 nm (m^{-1})
$\bar{d}(\lambda)$	Spectrally averaged $d(\lambda)$ (400–500 nm) (m^{-1})
$E_{\text{g}}(\lambda)$	Spectral scalar irradiance of growth lights ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
E_{o}	Scalar total irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
$F(\lambda_{\text{e}}, 730)$	Fluorescence intensity at 730-nm emission (relative dimensions)
λ	Wavelength (nm)
λ_{e}	Excitation wavelength (nm)
n	Number of sites
Pheo <i>a</i>	Pheophytin <i>a</i> (mg m^{-3})
PAR	Photosynthetically active radiation ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
PUR	Photosynthetically usable radiation ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
σ_i	Density (kg m^{-3})
UV	Ultraviolet

Phytoplankton absorption coefficients—Particle absorption coefficients, $a_{\text{part}}(\lambda)$, were measured using the quantitative filter technique (Yentsch 1962; Mitchell and Kiefer 1988) with GF/F filters. Samples were measured with a dual beam spectrophotometer (SLM-Amico DW2) using air as a reference and corrected for filter path length amplification effects based on the method of Roesler (1992). The optical density at 750 nm was subtracted from the entire spectrum to correct for scattering (Roesler et al. 1989; Bricaud and Stramski 1990; Mitchell 1990).

Cultures were free of nonphytoplankton particles, and filters were not subjected to further procedures (Culver and Weiss unpubl. data). Filters for field samples first were extracted for 30 min in warm methanol to remove chlorophylls, pheopigments, and carotenoids (Kishino et al. 1985) and then extracted for 10 min in warm water to remove phycolipid pigments (Roesler 1992). Filters were scanned again to determine detritus absorption coefficients, $a_{\text{det}}(\lambda)$. The difference between $a_{\text{part}}(\lambda)$ and $a_{\text{det}}(\lambda)$ was attributed to $a_{\phi}(\lambda)$, the absorption coefficient of phytoplankton pigments.

In turn, $a_{\phi}(\lambda)$ is composed of $a_{\text{phy}}(\lambda)$, the absorption coefficient for pigments in intact cells, and $a_{\text{pheo}}(\lambda)$, the absorption coefficient for pheopigments (Fig. 1). Chl *a* and Pheo *a* are the major contributors to $a_{\phi}(\lambda)$ at red wavelengths; the contribution of chlorophyll *b*, when present, can be removed based on absorption at 646 nm (Cleveland and Perry 1994). A relative Pheo *a* absorption spectrum was generated by acidifying pure Chl *a* (Sigma) in 90% acetone with 10% HCl (Vernet and Lorenzen 1987). Peak absorption wavelengths for the extract were shifted to match in vivo values (410 nm in extract to 416 nm in vivo; 666 nm in extract to 678 nm in vivo; Vernet and Lorenzen 1987; Roesler et al. 1989). Absolute values for $a_{\text{pheo}}(\lambda)$ were calculated by scaling the relative Pheo *a* absorption spectrum to $a_{\text{pheo}}(676)$. Weight-specific absorption coefficients for Chl *a* and Pheo *a* acetone extracts were used to calculate $a_{\text{pheo}}(676)$ (Roesler et al. 1989; Cleveland and Perry 1994). This method assumed that the ratio of pigment-specific absorption coefficients at 676 nm for extracts of Chl *a* and Pheo *a* (1 : 0.58; Lorenzen and Jeffrey 1980) was the same as the ratio for in vivo pigment absorption. Measurements of $a_{\phi}(676)$, Chl *a*, and Pheo *a* concentrations were used to calculate $a_{\text{pheo}}(676)$ as

$$a_{\text{pheo}}(676) = a_{\phi}(676) \frac{0.58[\text{Pheo } a]}{[\text{Chl } a] + 0.58[\text{Pheo } a]} \quad (1)$$

Photosynthetic absorption coefficients—The coefficient $a_{\text{phy}}(\lambda)$, calculated as the difference between $a_{\phi}(\lambda)$ and $a_{\text{pheo}}(\lambda)$, was resolved into the absorption coefficient associated with photosynthetic pigments, $a_{\text{ps}}(\lambda)$, and the absorption coefficient associated with photoprotective pigments, $a_{\text{pp}}(\lambda)$. Fluorescence excitation spectra were converted from relative fluorescence units to absolute absorption units (per meter) according to

$$a_{\text{ps}}(\lambda) = F(\lambda_{\text{e}}, 730) \frac{a_{\text{phy}}(676)}{F(676, 730)} \quad (2)$$

(Sakshaug et al. 1991; Johnsen et al. 1992; cf. Johnsen and Sakshaug 1993; Sosik and Mitchell 1995).

The contribution of the photosynthetic absorption coefficient to the total phytoplankton absorption coefficient, $a_{\text{ps}}:a_{\text{phy}}$, was quantified by integrating each spectrum from 400 to 550 nm, the wavelengths most affected by photoprotective pigments. The ratio was calculated as

$$a_{\text{ps}}:a_{\text{phy}} = \frac{\int_{400}^{550} a_{\text{ps}}(\lambda) d\lambda}{\int_{400}^{550} a_{\text{phy}}(\lambda) d\lambda} \quad (3)$$

A difference spectrum for 400–550 nm, $d(\lambda)$, was calculated by subtracting $a_{\text{ps}}(\lambda)$ from $a_{\text{phy}}(\lambda)$. The effects of pigment concentration and magnitude of the absorption coefficient were removed by normalizing $d(\lambda)$ to its spectral average, $\bar{d}(\lambda)$.

Irradiance—For cultures, photosynthetically active radiation (PAR) was measured using a 4π PAR sensor ($E_{\text{o}}[\text{PAR}]$; Biospherical Instruments QSL-100). The spectrum of the

fluorescent bulbs was measured using a remote cosine sensor (LiCor 1800). Spectral irradiance of the bulbs, $E_g(\lambda)$, was calculated from $E_o(\text{PAR})$ and a normalized spectrum. For the culture experiments, the photosynthetically usable radiation (PUR) portion of the growth irradiance, $E_o(\text{PUR})$, was calculated by weighting $E_g(\lambda)$ by a nondimensional form of $a_{ps}(\lambda)$ such that

$$E_o(\text{PUR}) = \int_{400}^{700} E_g(\lambda) \frac{a_{ps}(\lambda)}{a_{ps}(\lambda_{\max})} d\lambda \quad (4)$$

(Morel 1978).

Irradiance in Puget Sound was measured as detailed by Culver and Perry (1997). Spectral upwelling and downwelling irradiance measurements from 380 to 750 nm were collected at 2-nm resolution (12-nm bandpass) using an underwater spectroradiometer (Model LI-1800UW, LiCor). The instrument package was equipped with upwelling and downwelling cosine PAR sensors (LiCor) and a 4π PAR sensor (LiCor) shielded to measure upwelling or downwelling scalar irradiance. The system carried tilt and pressure sensors for verification of measurement angle and depth. Variations in cloud cover were monitored by a cosine PAR sensor (LiCor) attached to the highest point of the vessel; in-water measurements were scaled to the above-water PAR measurements to correct for variations in incident solar irradiance. $E_o(\text{PUR})$ was calculated according to Eq. 4 except that $E_o(\lambda)$ at the depth of sampling was used in place of $E_g(\lambda)$.

Results

Absorption coefficients for cultures—In general, ratios of blue (440 nm) to red (676 nm) absorption were lower at low irradiance because of packaging effects associated with higher cellular concentrations of pigments (Duysens 1956) and

lower absorption by photoprotective carotenoids (Table 1). Values for Chl *a*-specific absorption coefficients, $a_{phy}^*(674)$, generally were lower at low irradiance because of enhanced pigment packaging at low light; however *N. closterium* deviated from some of these patterns. For all species grown at high light, $a_{phy}^*(\lambda)$ and $a_{ps}^*(\lambda)$ differed in the blue-green regions of the spectra where accessory pigments absorb (e.g., Fig. 2, see fig. 5 of Culver et al. 1994, for *N. closterium*). At low irradiances, $a_{phy}^*(\lambda)$ and $a_{ps}^*(\lambda)$ were more similar because of the low contribution by photoprotective carotenoids.

A greater proportion of $a_{phy}^*(\lambda)$ could be attributed to photosynthetic pigments at low $E_o(\text{PUR})$ (Table 2; Fig. 3), with the exception of *I. galbana*. The slope of $a_{ps}:a_{phy}$ vs. $E_o(\text{PUR})$ tended to be more similar for *A. carterae* and *N. closterium*. The linear regression using data from all species was

$$a_{ps}:a_{phy} = -5.5 \times 10^{-4} E_o(\text{PUR}) + 0.83 \quad (5)$$

(95% confidence limits of -3.1×10^{-4} to -8.0×10^{-4} ; Sokal and Rohlf 1981).

The magnitude of $d(\lambda)$, the difference between $a_{phy}(\lambda)$ and $a_{ps}(\lambda)$, indicates the amount of photoprotective pigments, and the shape of $d(\lambda)$ reflects the composition of photoprotective pigments. For all species, $d(\lambda)$ was higher per milligram of Chl *a* for cultures grown under high light. Normalized spectra, $d(\lambda)/\bar{d}(\lambda)$, were similar within a species, indicating a similar composition of photoprotective pigments regardless of light level. Spectra for all species showed peaks in absorption between 400 and 420 nm, a shoulder between 450 and 460 nm, and a peak at 490 nm (Fig. 4). The height of the 490 nm peak relative to the rest of the spectrum varied among species (Fig. 4D). The ensemble mean and variance spectra for all species at all light levels (Fig. 4E) show the

Table 1. Photosynthetic fraction of phytoplankton absorption coefficient for cultures; $a_{ps}(\lambda)$ is photosynthetic absorption coefficient; $a_{phy}(\lambda)$ is phytoplankton absorption coefficient corrected for pheopigment absorption; $a_{phy}^*(674)$ is chlorophyll-specific phytoplankton absorption coefficient. $E_g(\text{PAR})$ is growth irradiance, and $E_o(\text{PUR})$ is irradiance normalized to $a_{ps}(\lambda)$.

Species	$E_g(\text{PAR})$	$E_o(\text{PUR})$	$a_{ps}:a_{phy}$	$a_{ps}(440):$ $a_{ps}(676)$	$a_{phy}(440):$ $a_{phy}(676)$	$a_{phy}^*(674)$ (m^{-1})
	(μmol photons $\text{m}^{-2} \text{ s}^{-1}$)	(μmol photons $\text{m}^{-2} \text{ s}^{-1}$)				
<i>Amphidinium carterae</i>	700	357	0.58	1.20	2.10	0.011
	300	154	0.73	1.30	1.78	0.011
	25	15	0.79	1.17	1.45	0.006
	5	3	0.85	0.98	1.22	0.005
<i>Nitzschia closterium</i>	700	272	0.63	1.30	1.96	0.019
	3000	130	0.74	1.24	1.59	0.024
	25	12	0.88	1.19	1.32	0.016
	5	2	0.89	1.30	1.41	0.010
<i>Thalassiosira weissflogii</i>	700	307	0.80	1.40	1.70	0.012
	300	142	0.77	1.24	1.57	0.011
	25	13	0.85	1.20	1.38	0.008
	5	3	0.83	1.08	1.35	0.005
<i>Isochrysis galbana</i>	700	253	0.68	1.78	2.23	0.017
	300	114	0.77	1.58	1.89	0.015
	25	11	0.73	1.26	1.68	0.017
	5	3	0.83	1.06	1.18	0.005

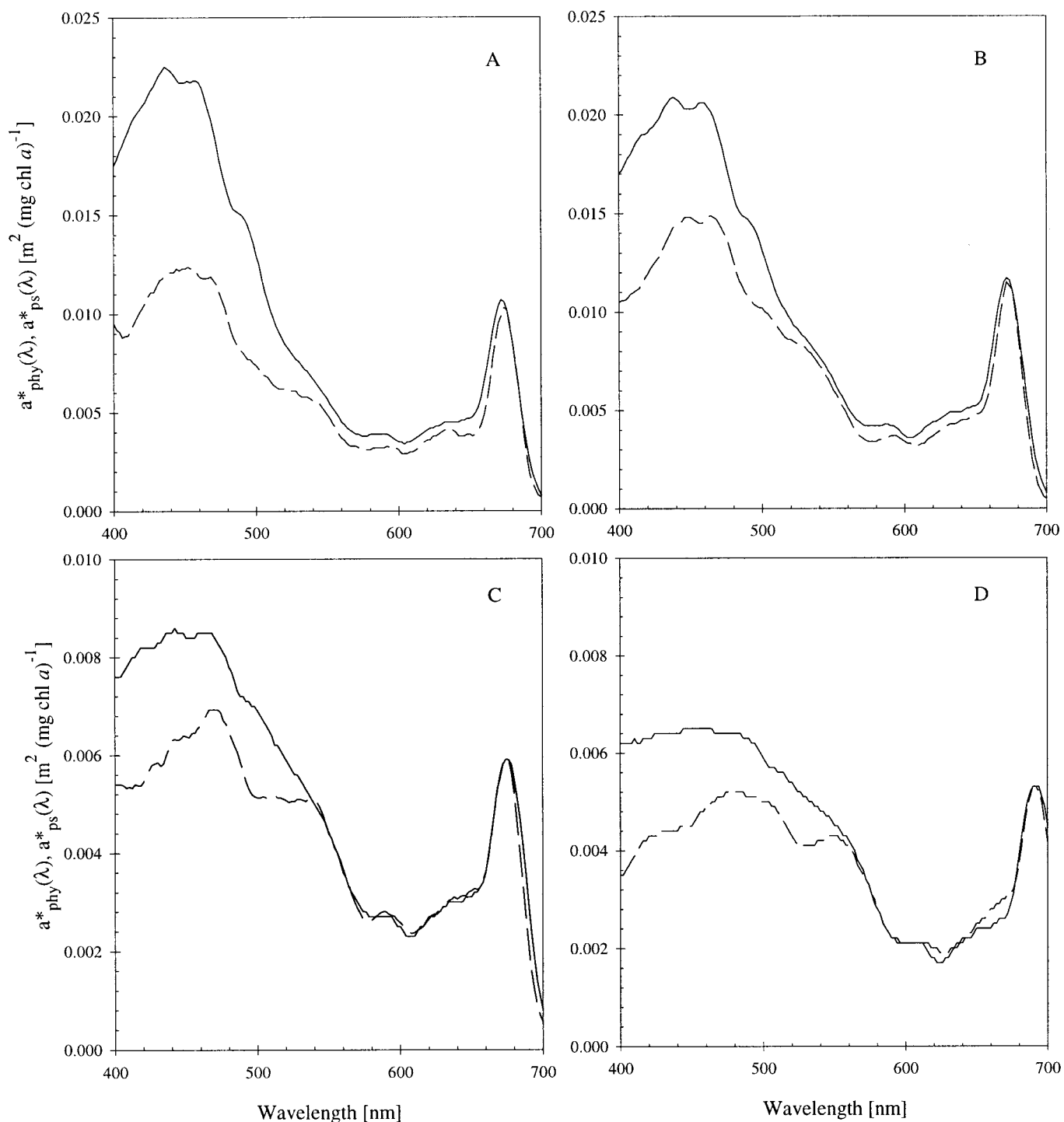


Fig. 2. Spectra for Chl-*a*-specific phytoplankton absorption coefficients, $a_{\text{phy}}^*(\lambda)$ (solid line), and Chl-*a*-specific photosynthetic absorption coefficients, $a_{\text{ps}}^*(\lambda)$ (dashed), for *A. carterae* grown at (A) 700, (B) 300, (C) 25, and (D) 5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Note change in scale for low light samples.

similarity among species. The spectrum for β -carotene adjusted for in vivo absorption (Bidigare et al. 1990) is shown in Fig. 4F for comparison between the spectral shapes of $d(\lambda)$ and a known photoprotective carotenoid.

Puget Sound phytoplankton absorption coefficients—Phytoplankton assemblages in Puget Sound typically were dominated by large single-celled diatoms, such as *Pseudonitzschia* spp. and *Ditylum* spp., and chain-forming diatoms,

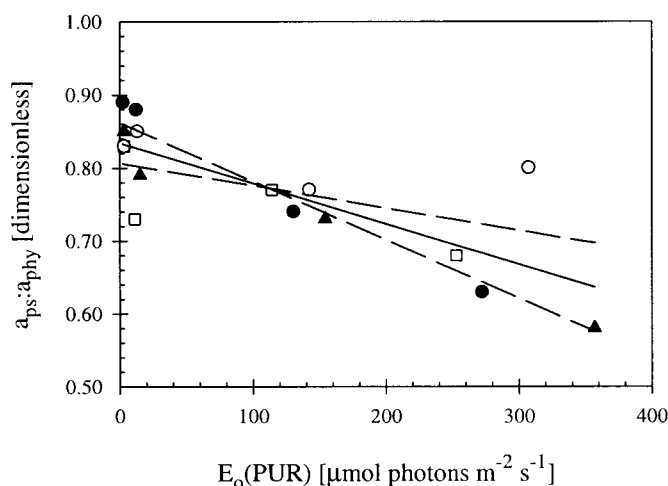


Fig. 3. Ratio of photosynthetic absorption coefficient to total phytoplankton absorption coefficient, $a_{ps}:a_{phy}$, vs. $E_o(\text{PUR})$ for *N. closterium* (●), *T. weissflogii* (○), *A. carterae* (▲), and *I. galbana* (□). Solid line shows Model II linear regression; dashed lines are 95% confidence limits on slope (Eq. 5, data listed in Table 2).

such as *Chaetoceros* spp. On one occasion, the assemblage contained high numbers of the dinoflagellate *Alexandrium* spp. (R. Horner pers. comm.). Chl *a* concentrations ranged from 2.06 to 23.7 mg m⁻³. Pheo *a* concentrations ranged from 0.45 to 5.22 mg m⁻³ and were 2.3–50.7% by weight of the total Chl *a* plus Pheo *a* concentration.

An example of the absorption spectra collected for a sample is shown in Fig. 5. The high values for $a_{part}(\lambda)$ in the blue wavelengths relative to the red is typical of samples containing detritus; however, only 2–13% (mean = 5%, $n = 37$) of $a_{part}(676)$ was detritus. The $a_{phy}(676)$ as a percentage of $a_{part}(676)$ was 74–98% (mean = 88%); $a_{phy}(676)$ as a percentage of $a_{part}(676)$ was 68–96% (mean = 84%).

Spectra of $a_{phy}^*(\lambda)$ show a prominent Soret Chl *a* absorption band with accessory pigments contributing to absorption at blue and green wavelengths (Fig. 6). The lack of phycobilipigment absorption indicated that cryptomonads and cyanobacteria were not abundant. Blue (440 nm) to red (676 nm) ratios ranged from 1.49 to 2.81 (mean = 2.09). Values for $a_{phy}^*(676)$ ranged from 0.004 to 0.02 m² (mg Chl *a*)⁻¹ and did not vary predictably with $E_o(\text{PUR})$ measured at the time of collection. Spectra of $a_{ps}^*(\lambda)$ (Fig. 7) showed lower mean absorption than $a_{phy}^*(\lambda)$ at blue and green wavelengths. Blue (440 nm) to red (676 nm) ratios for $a_{ps}^*(\lambda)$ ranged from 0.78 to 1.78 (mean = 1.26; Fig. 8).

Normalized $d(\lambda)$ spectra (Fig. 9) differed from those of the cultures (see Fig. 4). A peak and high variance near 440 nm was present in field samples but not in cultures. Secondary peaks near 460 and 490 nm were similar to peaks found for culture samples.

Puget Sound photosynthetic absorption and irradiance—Depth profiles for many of the stations were characterized by a 1–4-m lens of warmer ($\Delta 1$ – 3°C), fresher ($\Delta -4.0$ to -0.1 ppt) water at the surface overlying a mixed layer of varying depth. Ranges in temperature from 13°C to 15°C and in salinity from 28.5 to 30.0 ppt were characteristic of

the euphotic zone beneath the surface lens. Some stations also had additional water masses of cooler, more saline water below the upper mixed layer. The mixed layer was defined as a region in which density (σ_t) changed by <0.10 kg m⁻³ within 1 m. A stratified region was defined as a warm, low-salinity surface lens or as a subsurface layer in which σ_t changed by >0.10 kg m⁻³ within 1 m. Figure 10 shows a station with alternating mixed and stratified regions.

Values of $a_{ps}:a_{phy}$ for samples from stratified regions, where the irradiance would be expected to remain more or less constant relative to incident irradiance, followed a trend similar to that observed for cultures grown under constant irradiance. For stratified field samples, the relationship between $a_{ps}:a_{phy}$ and $E_o(\text{PUR})$ can be described as

$$a_{ps}:a_{phy} = -4.6 \times 10^{-4} E_o(\text{PUR}) + 0.90 \quad (6)$$

(Fig. 11A). The slope for cultures (-5.5×10^{-4} ; Eq. 5) did not differ significantly from the slope for stratified samples (-4.6×10^{-4} ; 95% confidence limits of -3.1×10^{-4} to -6.1×10^{-4}), although the y-intercepts were different. Measurements of $a_{ps}:a_{phy}$ for samples from mixed layers fell below the regression line and were not correlated with $E_o(\text{PUR})$ (Fig. 11B).

Discussion

Absorption coefficients derived from fluorescence excitation spectra—Light-harvesting complexes, composed of Chl *a* and accessory pigments, funnel exciton energy into the dedicated clusters of Chl *a* molecules that constitute the reaction centers of photosystems I and II. At room temperature, $F(\lambda_e, 730)$ is primarily a response to energy absorbed by accessory pigments and Chl *a* molecules in the light harvesting complex and photosystem II, $a_{II}(\lambda)$; therefore, the accessory pigments and Chl *a* that are associated with photosystem I are not represented by $F(\lambda_e, 730)$ (review by Govindjee and Satoh 1986; Owens 1991). This limitation is important to acknowledge when working with phytoplankton assemblages that contain cyanobacteria or cryptomonads, which have a large portion of Chl *a* associated with photosystem I (Haxo and Blinks 1950; Fork and Mohanty 1986).

To expand the remote sensing utility of fluorescence excitation from an estimate of only $a_{II}(\lambda)$ to estimates of $a_{ps}(\lambda)$ and $a_{pp}(\lambda)$ also, a very simplistic model can be invoked where photosynthetic absorption is the sum of $a_{II}(\lambda)$ and absorption by photosystem I, $a_I(\lambda)$:

$$a_{ps}(\lambda) = a_{II}(\lambda) + a_I(\lambda). \quad (7)$$

The method for determining $a_{ps}(\lambda)$ and $a_{pp}(\lambda)$ from $F(\lambda_e, 730)$ and $a_{phy}(\lambda)$ is based on the following assumptions: (1) that photosynthetic pigments are capable of efficiently transferring energy to reaction centers and inducing Chl *a* fluorescence; (2) that both photosystems I and II have similar pigment composition and organization, and (3) that $a_{phy}(\lambda)$ is comprised solely of absorption by photosynthetic and photoprotective pigments,

$$a_{phy}(\lambda) = a_{ps}(\lambda) + a_{pp}(\lambda), \quad (8)$$

so that the difference spectrum constitutes the photoprotective spectral absorption coefficient:

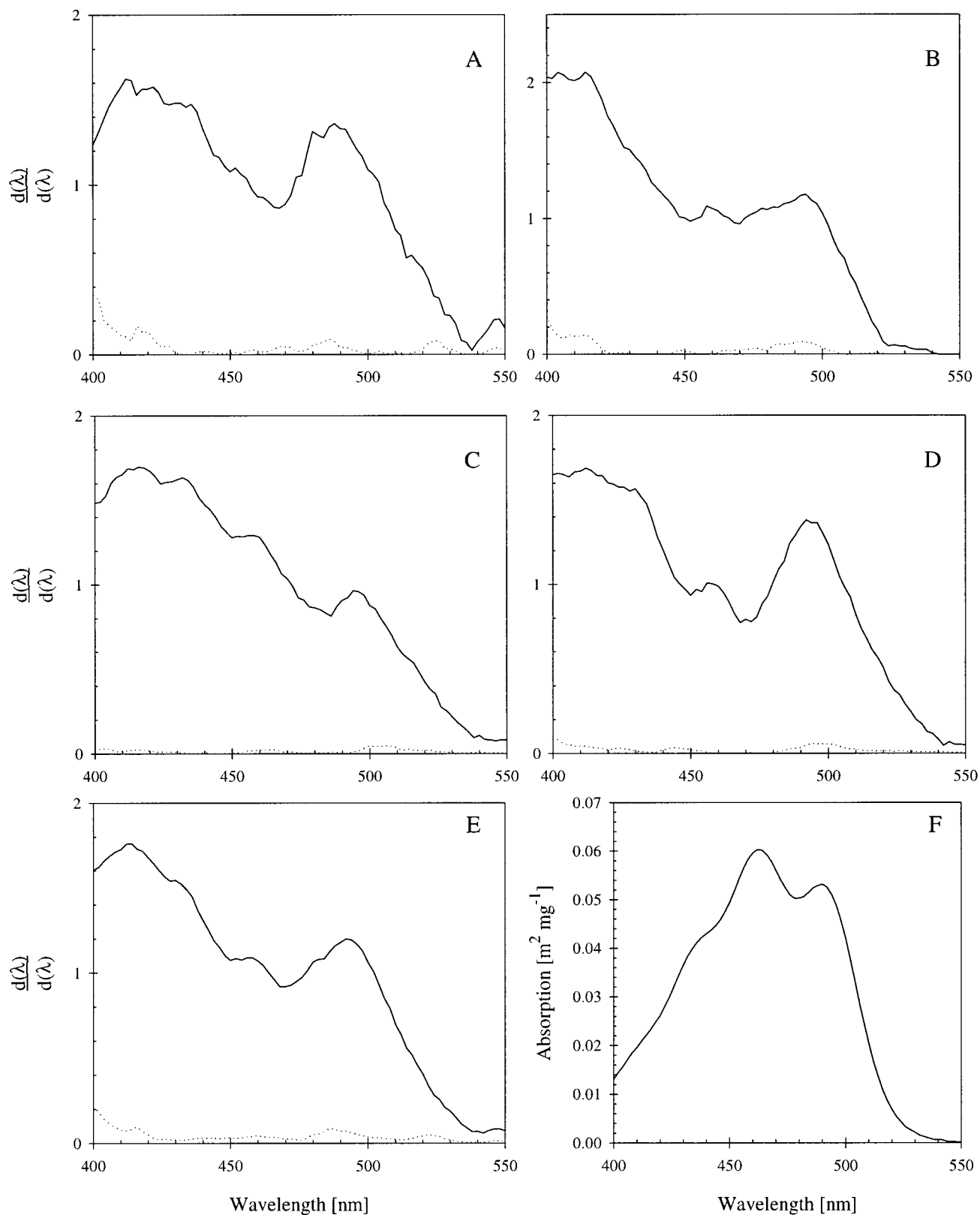


Fig. 4. Mean (solid curve [relative units]) and variance (dashed curve) of difference spectra, $d(\lambda)$, normalized to the spectral average, $\bar{d}(\lambda)$, for cultures grown at four light levels: (A) *N. closterium*, (B) *T. weissflogii*, (C) *A. carterae*, (D) *I. galbana*, and (E) for all cultures at all light levels. (F) In situ weight-specific absorption spectrum for the photoprotective pigment β -carotene, from Bidigare et al. (1990).

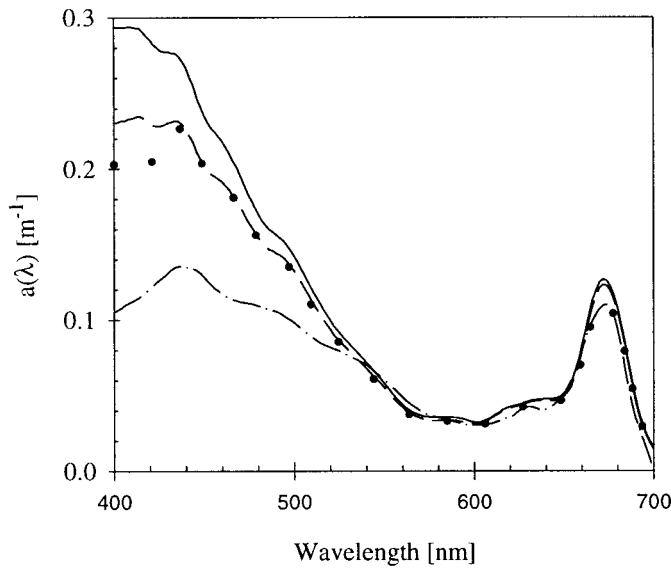


Fig. 5. Absorption coefficients for a surface sample collected at Port Madison, Washington, on 20 August, 1993; particle, $a_{\text{part}}(\lambda)$ (solid curve), phytoplankton plus pheopigments, $a_{\phi}(\lambda)$ (dashed curve), phytoplankton pigments, $a_{\text{phy}}(\lambda)$ (dotted curve), and photosynthetic, $a_{\text{ps}}(\lambda)$ (dot-dash curve).

$$d(\lambda) = a_{\text{phy}}(\lambda) - a_{\text{ps}}(\lambda) = a_{\text{pp}}(\lambda). \quad (9)$$

Deviations in the shape of the culture and field $d(\lambda)$ spectra from the absorption spectrum of a typical photoprotective pigment indicate that at least one of these assumptions has been violated or that the pigment composition in field samples is very different from that predicted from data based on laboratory cultures.

If the energy transfer efficiency, composition, and packaging of pigments is similar for the light-harvesting and reaction center complexes of both photosystems II and I, then the spectral shapes of $a_{\text{II}}(\lambda)$ and $a_{\text{I}}(\lambda)$ will be similar. In this case, $F(\lambda_c, 730)$ will represent both spectral shapes, $a_{\text{ps}}(\lambda)$ can be derived accurately from $F(\lambda_c, 730)$, and $d(\lambda)$ will be equal to $a_{\text{pp}}(\lambda)$. If the energy transfer efficiency, composition, or packaging of pigments in the light harvesting and reaction center complex of photosystem II differ from those of photosystem I, then $F(\lambda_c, 730)$ will represent only $a_{\text{II}}(\lambda)$, and $a_{\text{ps}}(\lambda)$ and $a_{\text{pp}}(\lambda)$ will be in error. Johnsen et al. (1997) found that 9–19% of Chl *a* is associated with nonfluorescent photosystem I in two dinoflagellates. When the photosystem I Chl *a* concentration can be estimated, then absorption by the light-harvesting complexes and reaction centers of photosystem II, $a_{\text{II}}(\lambda)$, can be estimated by adjusting the scaling factor at 676 nm for $F(\lambda_c, 730)$ from a value of 1.0 (cf. Eq. 2) to a lower value that represents only the absorption coefficient of photosystem II Chl *a* (Johnsen and Sakshaug 1996; Johnsen et al. 1997). Accuracy of estimates of $a_{\text{II}}(\lambda)$ can be improved by accounting for the variation in light energy transfer from the light harvesting complexes associated with photosystem II to Chl *a*. By measuring each of these components, Johnsen et al. (1997) found that the scaling factor can vary from 0.81 to 0.89.

The $d(\lambda)$ spectra from cultures show absorption peaks near

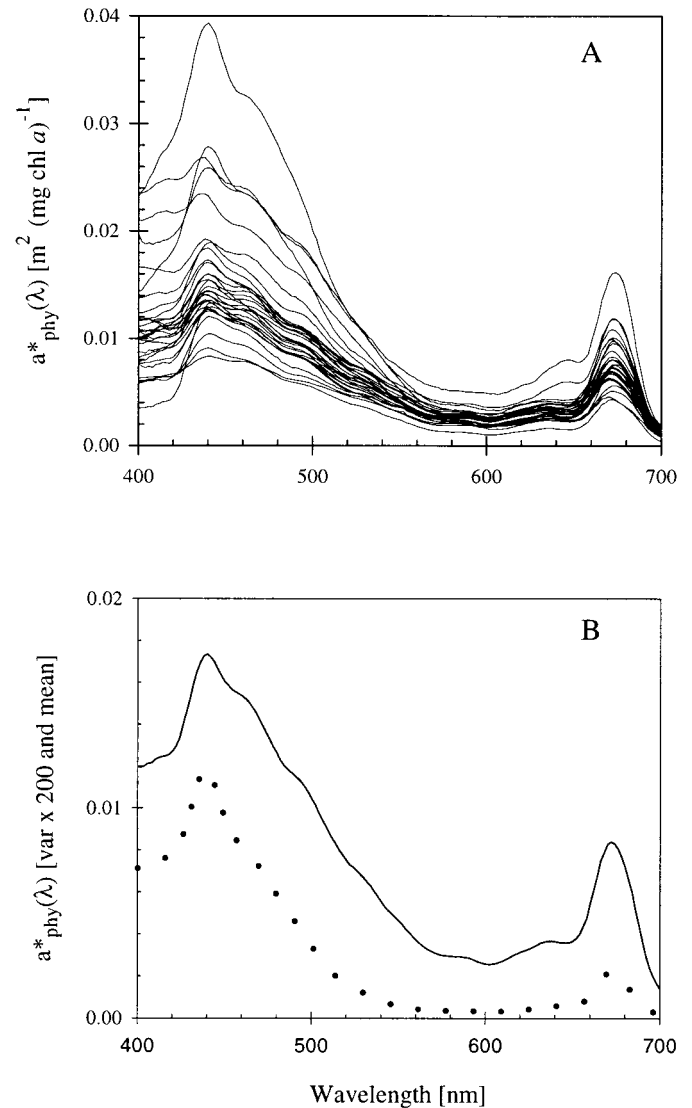


Fig. 6. (A) Spectra of Chl-*a*-specific phytoplankton absorption coefficient, $a_{\text{phy}}^*(\lambda)$, for all Puget Sound samples ($n = 37$). (B) Mean (solid curve [$\text{m}^2 (\text{mg Chl } a)^{-1}$]) and variance (dashed curve) of $a_{\text{phy}}^*(\lambda)$.

460 and 490 nm that are characteristic of photoprotective pigment absorption (Fig. 4A–E vs. 4F; Johnsen et al. 1997, Stuart et al. 1998). In addition, there is significant and variable absorption at blue wavelengths that cannot be explained by the β -carotene-type absorption spectrum (Fig. 4F). One explanation for the blue absorption is the inclusion of absorption by additional substances in $d(\lambda)$. Methanol extracts of cultures show that detritus contributes <5% to absorption at 400 nm (Culver and Weiss unpubl. data), indicating that the contribution of $a_{\text{det}}(\lambda)$ to $d(\lambda)$ was very small. Fluorometric measurements of Pheo *a* concentration were near zero; therefore, the contribution of $a_{\text{pheo}}(\lambda)$ was negligible. There is no distinguishable feature in $d(\lambda)$ that suggests Chl *a* absorption is included. Other possible explanations for absorption at wavelengths <440 nm include the presence of pigments that protect against ultraviolet radiation

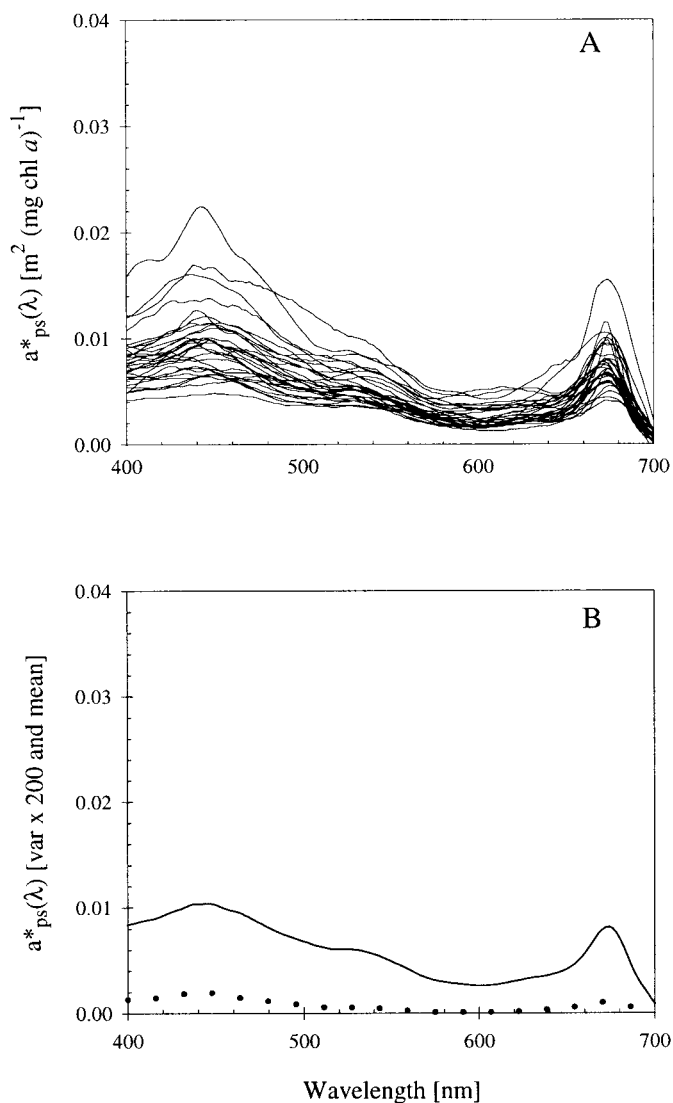


Fig. 7. (A) Spectra of Chl-*a*-specific photosynthetic absorption coefficient, $a_{ps}^*(\lambda)$, for all Puget Sound samples ($n = 37$). (B) Mean (solid curve [$\text{m}^2 (\text{mg Chl } a)^{-1}$]) and variance (dashed curve) of $a_{ps}^*(\lambda)$.

(UV) or of other chromophoric intracellular organic matter. The absorption coefficients of UV-absorbing compounds are maximal at UV wavelengths and decline to negligible values near 420 nm (Vernet et al. 1989). Dissolved or colloidal organic matter absorbs strongly in the UV and blue wavelengths (Bricaud et al. 1981); hence, intracellular concentrations of organic matter could contribute to absorption. The general agreement between $a_{phy}(\lambda)$ and $a_{ps}(\lambda)$ for cultures grown at low irradiances suggests that the fluorescence excitation method provides a good estimate of $a_{ps}(\lambda)$ and that $d(\lambda)$ represents $a_{pp}(\lambda)$.

The most intriguing feature in the $d(\lambda)$ spectra from field samples is the peak at 440 nm that strongly resembles the Soret absorption of Chl *a* (Fig. 9). This Chl-*a*-like peak is most prominent in samples from the bottom of the euphotic zone, although not all deep samples show this peak. The variance in $d(\lambda)$ is greatest for wavelengths < 430 nm, and

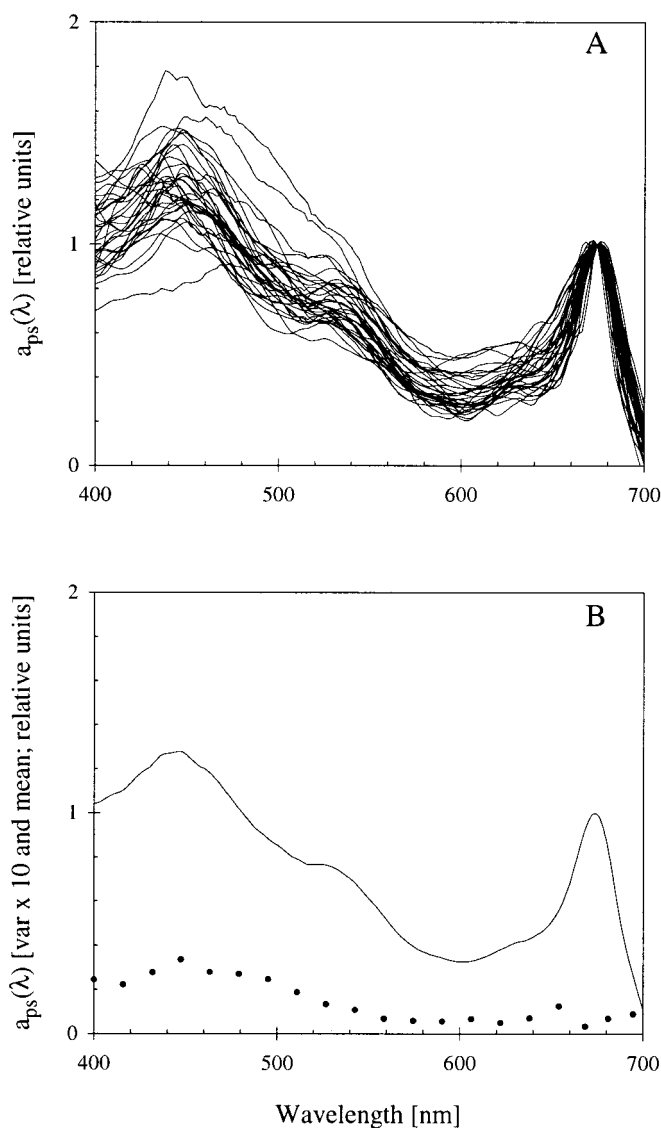


Fig. 8. (A) Spectra of photosynthetic absorption coefficients normalized to the red Chl *a* peak, 676 nm, for all Puget Sound samples ($n = 37$). (B) Mean (solid curve) and variance (dashed curve) of normalized spectra.

in some samples, low values at these wavelengths accentuate the prominence of the 440-nm peak. These lower values are affected by the correction for Pheo *a* absorption. The samples with the more prominent peaks have low concentrations of Pheo *a* relative to Chl *a*. For all 37 Puget Sound samples, Pheo *a* contributes between 3.7% and 43.6% (mean = 19.5%) to a_{ϕ} at the 416-nm Pheo *a* absorption peak. In contrast, the Pheo *a* contribution is negligible at 440 nm, where Pheo *a* absorption is very low. If Pheo *a* absorption is not removed from the phytoplankton absorption spectrum, a 440-nm peak still would be present in $d(\lambda)$, although it would be less prominent.

An explanation for the 440-nm peak is a possible difference in the organization or composition of pigments between the complexes of the two photosystems. Characterization of chlorophyll-protein complexes suggests that accessory ca-

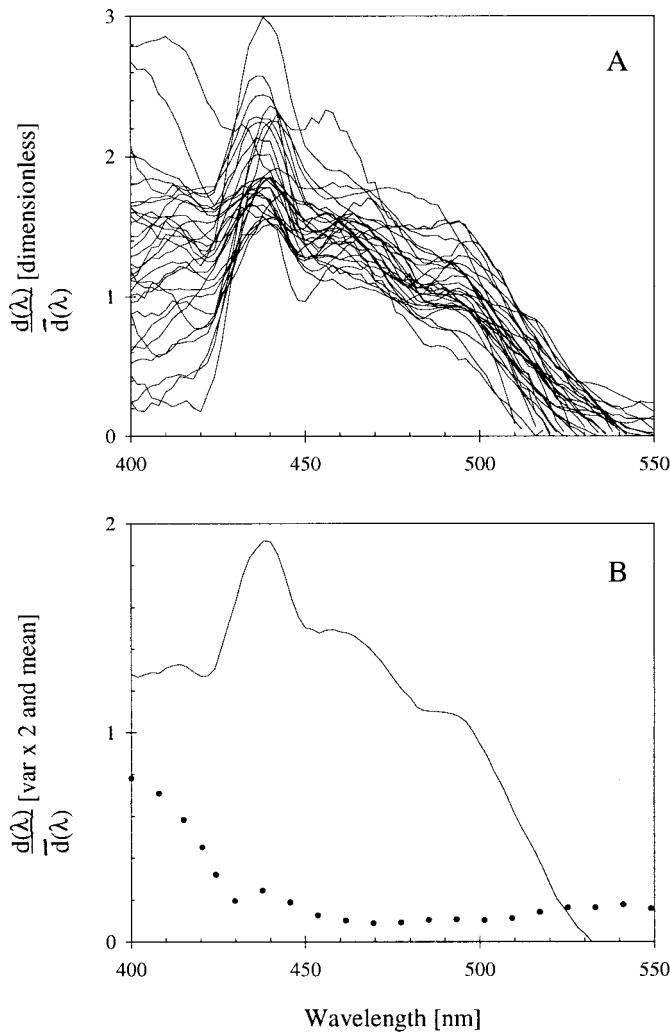


Fig. 9. (A) Difference spectra, $d(\lambda)$, normalized to the spectral average, $\bar{d}(\lambda)$, for all Puget Sound samples ($n = 37$). (B) Mean (solid curve) and variance (dashed curve).

rotenoids and any phycobilipigments primarily are associated with the light-harvesting complexes of photosystem II (review by Fork and Mohanty 1986; Boczar and Prézelin 1989; Boczar et al. 1990). The energy transfer efficiencies may be $<100\%$ for these complexes (Johnsen et al. 1997), thereby affecting the shape of the fluorescence excitation spectrum (Lutz et al. 1998). Photosystem I primarily contains Chl a , which may or may not be packaged similarly to the Chl a in photosystem II, and may contain accessory chlorophylls (Cogdell 1988; Boczar and Prézelin 1989; Johnsen et al. 1997). If the 440-nm peak is due to Chl a , then $a_{ps}(\lambda)$ is underestimated and $a_{pp}(\lambda)$ is overestimated. For Puget Sound samples, the peak averaged $<15\%$ of $d(\lambda)$; therefore, $a_{ps}(\lambda)$ would be underestimated by $<15\%$ in the region from 420 to 450 nm.

Another explanation for the 440-nm peak observed in $d(\lambda)$ from Puget Sound is absorption by photoprotective pigments, particularly UV-protecting compounds. Spectra similar to Puget Sound $d(\lambda)$ can be generated by combining the in situ absorption spectrum for β -carotene (Bidigare et al.

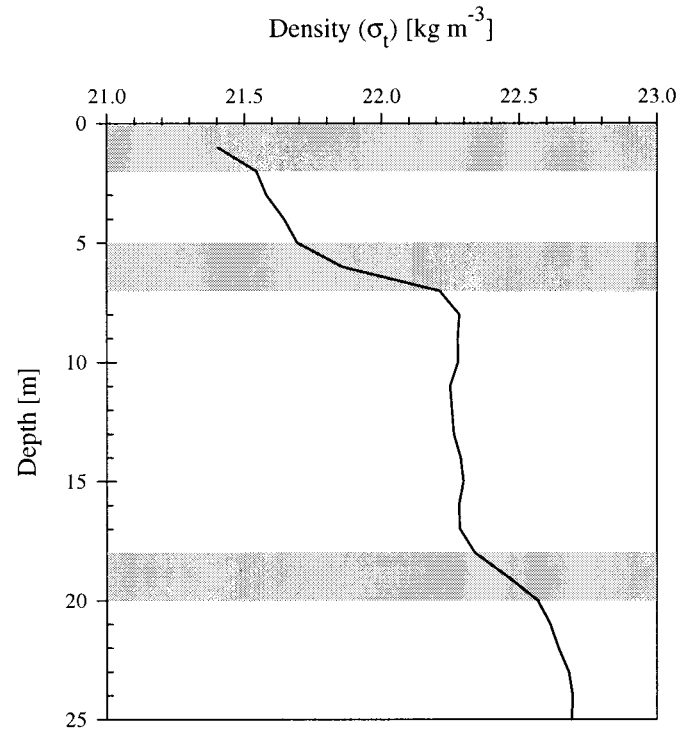


Fig. 10. Density profile (σ_t) from Puget Sound (Port Madison; 2 August, 1994); depth regions are classified as stratified (shaded) or mixed according to criteria outlined in text.

1990) with the absorption spectrum of a carotenoid-like pigment associated with high-UV lake environments (compound B, Leavitt et al. 1997). In this scenario, fluorescence excitation provides a good estimate of $a_{ps}(\lambda)$, and $d(\lambda)$ of the 440-nm peak could be addressed by including high performance liquid chromatography pigment analysis to determine the presence of unique carotenoid-like pigments and by measuring fluorescence excitation spectra at liquid nitrogen temperatures to distinguish the spectral composition of photosystem I and II light-harvesting complexes.

Photosynthetic absorption and irradiance for Puget Sound samples—The trend of decreasing $a_{ps}:a_{phy}$ with increasing $E_0(\text{PUR})$ for field samples collected from stratified regions of the water column was similar to the pattern for cultures (Fig. 11A). In contrast, there was no apparent pattern for field samples from mixed regions of the water column (Fig. 11B). Many of the phytoplankton sampled from low irradiance environments had $a_{ps}:a_{phy}$ ratios characteristic of phytoplankton adapted to much higher irradiances. Acclimation of photosynthetic characteristics to the local irradiance is opposed by the process of mixing (Lewis et al. 1984a,b; Cullen and Lewis 1988). Mixing subjects phytoplankton to variation in irradiance and results in a water column that is physiologically and physically homogeneous (Falkowski 1983, 1984; Lewis et al. 1984a,b). If the rate of mixing is high relative to that of photoacclimation, then $a_{ps}:a_{phy}$ will not be correlated with $E_0(\text{PUR})$; however, if the rate of mixing is low relative to photoacclimation, then $a_{ps}:a_{phy}$ will be

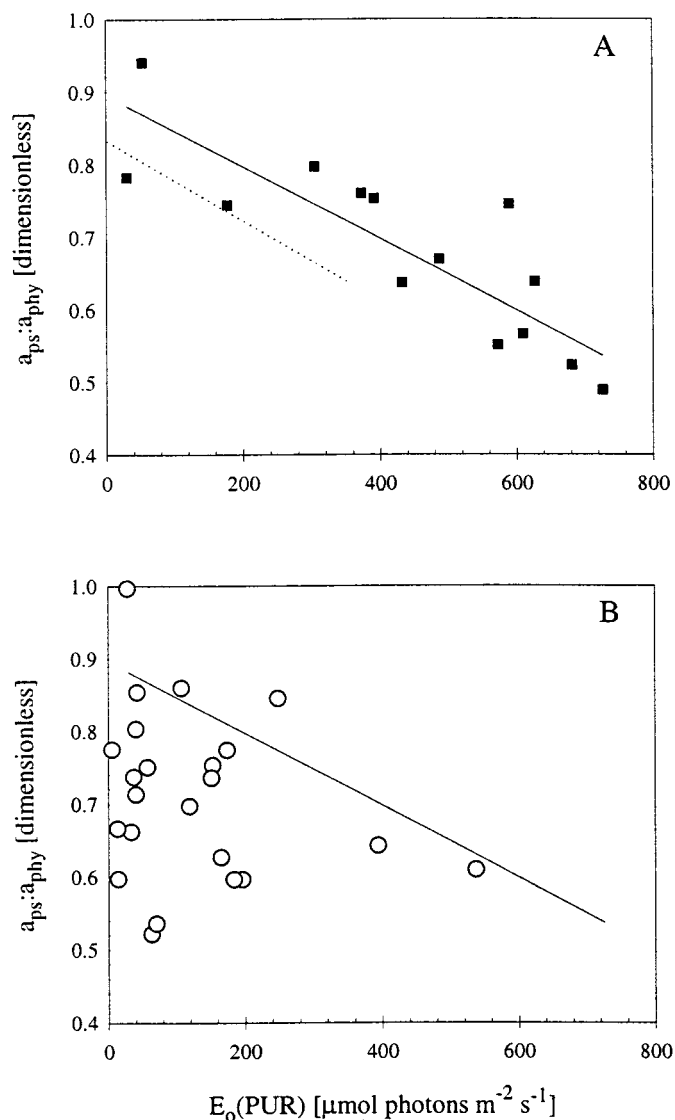


Fig. 11. Ratio of photosynthetic absorption coefficient to total phytoplankton absorption coefficient, $a_{ps}:a_{phy}$, vs. photosynthetically usable radiation, $E_o(\text{PUR})$, for (A) stratified regions (■) and (B) mixed regions (○) in Puget Sound. Solid line shows Model II linear regression (Eq. 6) for stratified samples ($n = 14$). Dotted line shows Model II linear regression for cultures (Eq. 5; see Fig. 3).

correlated with $E_o(\text{PUR})$. The lack of correlation between $a_{ps}:a_{phy}$ and $E_o(\text{PUR})$ in Fig. 11B indicates that these cells are mixing at rates that exceed photoacclimation rates.

In Puget Sound, winds and net seaward-flowing surface currents interact with river outflow to produce a freshwater lens near the surface (Winter et al. 1975; Bretschneider et al. 1985). The temporal stability of these lenses and the lower variation in irradiance encountered by cells within the lens allow acclimation of photosynthetic absorption to the local irradiance. The freshwater lens acts as a boundary and sets the minimum irradiance encountered by surface cells within the lens and the maximum irradiance encountered by subsurface cells below the lens. In many parts of Puget Sound, stratified regions also exist within the water column and can

span 2–5 m (e.g., Fig. 10); cells within these regions are exposed to restricted variations in irradiance.

Regions of mixing below the surface lens generally span 3–15 m (Fig. 10), and cells in these regions are subject to greater variations in irradiance. Subsurface currents in Puget Sound are correlated with tidal cycle (Bretschneider et al. 1985). If tides disrupt density gradients within the water column, then acclimation of $a_{ps}:a_{phy}$ occurs on time scales of <6 h. This acclimation time is consistent with estimates for the time scales for photoacclimation by *Thalassiosira pseudonana* of 6–8 h for maximal rates of photosynthesis and 2–6 h for photosynthetic efficiency (Lewis et al. 1984a), although time scales for acclimation of a given parameter may vary depending on whether irradiance is increasing or decreasing (Cullen and Lewis 1988).

Variation in other photoadaptive indices, such as photosynthetic parameters, vary with species composition in the field (Marra 1978) and may vary with nitrate limitation (Shimura and Fujita 1975). The strong association of $a_{ps}:a_{phy}$ with $E_o(\text{PUR})$ in stratified regions of Puget Sound, regardless of species composition and nutrient concentrations, indicates that $a_{ps}:a_{phy}$ is a powerful indicator of photoacclimation that reflects the recent light history of the cells.

The fluorescence excitation technique can be used with instrumentation that is now available for in situ measurement of spectral irradiance, absorption, and fluorescence excitation (Cowles et al. 1996) to provide an estimate $a_{ps}(\lambda)$ and $a_{pp}(\lambda)$ for profiles of bio-optically modeled primary production and for use in photon budgets. The major source of uncertainty is the potential misassignment of a portion of photosystem I absorption to photoprotective pigment absorption. The significant relationship between $a_{ps}:a_{phy}$ and $E_o(\text{PUR})$ observed for phytoplankton grown in the laboratory and sampled from stratified environments in the field indicates that $a_{ps}(\lambda)$ responds predictably to irradiance. The ratio of $a_{ps}:a_{phy}$ is an indicator of the integrated irradiance to which a phytoplankton assemblage is exposed and thus serves as an indicator of mixing or mixed layer depth.

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