Resolving phytoplankton photoprotective:photosynthetic carotenoid ratios on fine scales using in situ spectral absorption measurements

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Abstract
Temporal changes in phytoplankton pigments and spectral absorption were evaluated during June 1998 in East Sound, Orcas Island, Washington. High-resolution vertical profiles of in situ spectral absorption were obtained with a Wet Labs ac-9 (nine-wavelength absorption and beam attenuation meter), and pigment concentrations were determined for discrete water samples using high-performance liquid chromatography (HPLC). Fucoxanthin was the most abundant carotenoid, indicating the dominance of diatoms. We computed a slope index to evaluate changes in shapes of the in situ particulate absorption coefficient (a_\text{sph}) spectra, a_\text{sph} slope = (a_\text{sph}(488) - a_\text{sph}(532)) / (a_\text{sph}(488) - a_\text{sph}(532)). A clear linear relationship was seen between ratios of photoprotective:photosynthetic carotenoids (PPC:PSC) and these a_\text{sph} slopes. While pigment package effects may alter the absorption spectra, in our data set we still found a significant relationship between pigment ratios and in situ a_\text{sph} slopes. Retrieval of this relationship was facilitated by the low and relatively constant detrital absorption coefficient (a_\text{ph}) values in our study area. Similar relationships were found between PPC:PSC ratios and the estimated phytoplankton absorption coefficient (a_\text{ph}) spectra. High PPC:PSC ratios and steeper a_\text{sph} slopes were associated with high-light levels. Our results suggest that in situ absorption measurements can be used to estimate PPC:PSC ratios in areas where the a_\text{sph} contribution is low or can be estimated. These variations in pigment ratios and spectral absorption reflect photoacclimation responses and/or changes in phytoplankton species composition and suggest in situ absorption measurements may be used to estimate pigmentation changes over fine temporal and spatial scales.

Rapid, in situ assessment of phytoplankton physiological condition has been a goal of plankton ecologists for many decades. The recent development and field use of multi-wavelength biooptical instrumentation has brought us closer to that goal and provides the opportunity to quantify the relationship between in situ observations and traditional, discrete sample analyses of physiological condition. The work described in this paper was motivated by that opportunity and, in particular, tested the effectiveness of measurements of in situ absorption spectra as indicators of the photosynthetic state of the phytoplankton assemblage.

Pigments are widely used to characterize phytoplankton physiological state, species identity, and biomass in marine and freshwater environments (Falkowski and Raven 1997). Light harvesting pigments in the photosystem absorb light that impinges on chloroplasts within the cell. This absorbed light energy has three main fates, (1) carbon assimilation via photosynthesis, (2) dissipation as fluorescence, or (3) dissipation as heat. Photoprotective carotenoid (PPC) pigments help prevent damage to the chloroplast from excess light energy, while photosynthetic carotenoid (PSC) pigments are involved in transfer of energy to reaction centers during photosynthesis. Therefore, PPC:PSC ratios may serve as indicators of energy transfer pathways within phytoplankton cells.

Variations in the relative proportions of carotenoid accessory pigments also alter the shape of the phytoplankton absorption coefficient (a_\text{ph}) spectrum. Changes in the a_\text{ph} spectra have been used to differentiate low-light and high-light adapted cultures for a variety of different phytoplankton taxa (SooHoo et al. 1986; Johnsen et al. 1994). The slope of the a_\text{ph} spectra from 490 to 530 nm (normalized to 676 nm) has been found to be steeper in high-light compared to low-light adapted cultures (Johnsen et al. 1994). Such a_\text{ph} spectral variations are due to physiological changes in cellular pigment ratios and pigment packaging (intracellular self-shading; Duysens 1956). Under high light, increases in PPC, decreases in PSC, and a reduction in pigment packaging may cause the a_\text{ph} spectral slopes to become steeper, while the reverse
is true for low-light conditions. The in situ $a_p$ spectra also can reflect variations in taxonomic composition (Johnsen and Sakshaug 1996) and absorption by pigmented heterotrophic organisms.

Research over the past few decades has shown that changes in pigments and absorption coefficients, reflecting changes in phytoplankton physiology and species composition, are tied to fluctuations in the physical and chemical environment (irradiance, stratification, mixing, nutrients). Field studies have shown that variations in PPC and PSC potentially can be used to evaluate changes in light and mixing (Claustre et al. 1994; Moline 1998). In laboratory experiments, increases in the photoprotective pigments, diatoxanthin and diadinoxanthin, were associated with both increases in irradiance and decreases in nutrients (Latasa 1995).

These previous studies used discrete sample analyses to evaluate the photoadaptive state of specific samples. Common methodology for particulate absorption measurements (the quantitative filter technique, QFT, Yentsch 1962; Mitchell and Kiefer 1988), requires filtration of discrete water samples and analysis with a bench-top spectrophotometer to obtain $a_p$. The QFT allows estimation of the detrital absorption coefficient ($a_d$) following extraction of pigments from the filter (Kishino method, Kishino et al. 1985; Roesler 1992), thus providing an estimate of the phytoplankton absorption coefficient ($a_{ph}$). In contrast to this discrete sample analysis, the use of in situ multiwavelength optical instrumentation such as the Wet Labs ac-9 now allows the absorption coefficients for particulate ($a_p$) spectra to be estimated directly within the water column, enabling vertical profiles of $a_p(\lambda)$ to be easily obtained. Coupled with the measurement of phytoplankton pigment concentration using high-performance liquid chromatography (HPLC) on discrete samples, in situ absorption measurements now may provide a tool for assessing fine-scale variations in the photoadaptive state of phytoplankton. In addition, the nonintrusive nature of in situ optics provides an advantage over discrete water sample collection since one can eliminate the sampling artifacts during preservation, handling, and laboratory analysis. Finally, coincident measurements of physical parameters such as temperature ($T$) and salinity ($S$) allow a better correlation of biological and physical properties within the water column.

Our goals in this work were to determine the extent to which in situ $a_p$ measurements can estimate phytoplankton accessory pigment composition over fine scales and to evaluate the resulting impacts on phytoplankton ecology at these scales. Specifically, we wished to (1) compare PPC and PSC concentrations from HPLC analyses of discrete water samples with in situ absorption spectra for estuarine phytoplankton assemblages, (2) demonstrate how in situ $a_p$ and $a_{ph}$ measurements can be used to evaluate PPC : PSC ratios over fine scales, (3) provide examples of the relationship of PPC : PSC ratios and absorption measurements to physical forcing mechanisms (light, nutrients, and mixing), and (4) quantify the potential effects of $a_p$ and package effect variations on the relationship between PPC : PSC ratios and $a_p$ and $a_{ph}$ spectra.

Materials and methods

Sampling site—Data collection occurred from 14 to 24 June 1998 from the R/V Henderson, moored near the head of East Sound (148°40.62′N and 122°53.45′W), a fjord type inlet of Orcas Island, Washington (Fig. 1). The depth of the water column varied from 20 to 22 m depending on tidal stage.

In situ measurements of hydrography and biooptics—A vertical time series of temperature data (Fig. 2) was obtained with a thermistor chain located ~100 m east of the R/V Henderson. Vertical profiles of temperature, salinity, fluorescence, and spectral absorption were obtained with a free-falling optical instrument package. This package included a high-resolution conductivity–temperature–depth (CTD) sensor (SBE911, Seabird), a fluorometer (Wetstar, Wet Labs), and two nine-wavelength in situ spectral absorption and beam attenuation meters (ac-9, Wet Labs). A 0.2-μm filter (maxicapsule, Gelman) was attached to the intake port of one of the ac-9s to measure the absorption by dissolved materials. Wavelengths for in situ absorption measurements were 412, 440, 488, 510, 532, 555, 650, 676, 715 nm. The ac-9s were calibrated every 2–4 d during the study using the pure water calibration technique (Twardowski et al. 1999).

Collection of discrete water samples—Water samples were collected once or twice per day (Fig. 2) within and outside vertical intervals of high particle concentration. Samples for pigment composition, QFT absorption, nutrients, and phytoplankton taxonomy were either siphoned from depth, collected with a separate 5-liter Niskin bottle, or sampled with a rosette system of bottles (20 cm in height; 500-ml capacity) deployed with the optical instrumentation package. $T$ and $S$ signatures from CTD measurements were obtained for both water samples and in situ optical measurements and then used to match depths of water sample collection with optical measurements. Water samples and optical measurements were collected within 2.5 h of each other with the majority (80%) collected within 1 h.

Phytoplankton pigment analyses—For phytoplankton pigment determinations, water samples (0.5 to 1 liters) were filtered onto 25-mm glass fiber filters (GF/F filters, Whatman) and frozen in liquid N$_2$. Pigments were extracted overnight in cold 90% acetone, sonicated, and quantified using reverse-phase HPLC (UltraspHERE C18 column, dual wavelength SpectraSystem UV2000 absorption detector) following a modified mobile solvent protocol (Wright and Jeffrey 1997). Calibrations were done with external standards. Quantifiable pigments included chlorophylls ($a$, $b$, $c_1/c_2$), chlorophyllide $a$, PSC (19-hexanoyloxyfucoxanthin, 19-butanoyloxyfucoxanthin, fucoxanthin, peridinin), and PPC (alloxanthin, $\beta$ carotene, diadinoxanthin, diatoxanthin, lutein/zeaxanthin, violaxanthin). To use chlorophyll $a$ as a biomass reference level in pigment ratios, we computed the sum of chlorophyll $a$ (Chl $a$) and chlorophyllide $a$, noted as Tchl $a$.

The output voltage of the Wetstar fluorometer on the profiling package was converted to Chl $a$ equivalents using fluorometric analyses of extracted Chl $a$ and pheopigments from
discrete water samples collected with the rosette sampler. Samples were filtered and extracted in cold 90% acetone and analyzed with a Turner Model AU-10 fluorometer (Parsons et al. 1984). Chl a concentration from extracted samples was linearly correlated with the in situ fluorometer voltage ($r^2 = 0.89$, $p < 0.0001$).

**Discrete sample absorption spectra analyses**—Discrete water sample $a_p$, $a_{ph}$, and $a_d$ spectra were obtained following methods described in Culver and Perry (1999). A dual beam spectrophotometer (SLM-Amico DW2) was used to measure $a_p$ spectra using the QFT (Yentsch 1962; Mitchell and Kiefer 1988). Phytoplankton pigments were removed using methanol (Kishino et al. 1985), and filters were rescanned to measure the $a_d$ spectra. The $a_{ph}$ spectra were determined by subtracting $a_d$ from $a_p$ spectra.

**Nutrient analyses**—Nutrient samples were immediately frozen after collection and analyzed within 6 months for total nitrate, nitrite, ammonium, phosphate, and silicate. Nutrient concentrations were determined with a Technicon autoanalyzer following standard colorimetry protocols (UNESCO 1994).

**In situ particulate absorption spectra**—The total in situ absorption coefficient ($a_t$) spectrum consists of absorption coefficients for water ($a_w$), particulates ($a_p$), and dissolved constituents ($a_g$). Pure water absorption is removed in the calibration methodology, so that the ac-9 measures $a_p + a_g$, denoted $a_{pg}$. Corrections for the temperature dependence of pure water absorption and variations in salinity were applied (Pegau et al. 1997), while the scattering error in our $a_p$ measurements was removed by subtracting $a_{pg} - 715$ nm from all wavelengths (Zaneveld et al. 1994). Data from the two ac-9s were used to estimate $a_p$ by subtraction of $a_g$ from $a_{pg}$. A time-lag correction for a slower flow rate was applied to the filtered ac-9 data in order to align the particulate and dissolved measurements.

**Calculation of slopes from absorption spectra**—To evaluate changes in the shape of the $a_{ph}$ and $a_p$ spectra from 488 to 532 nm, we normalized the absorption data to 676 nm. The slopes of the normalized absorption curves from 488 to 532 nm were computed:

$$a_{slope} = \frac{(a_{488} - a_{532})}{(a_{676})}$$

where $a_x$ is denoted as $a_{ph}$ or $a_p$. 
The ac-9 slope measurements were averaged over 1-m intervals (~50 data points at a profiler descent rate of 0.12 m s⁻¹). The $a_p$ and $a_d$ slopes from the ac-9 and discrete samples were then compared to the PPC : PSC ratios from HPLC. A steeper slope was assumed to indicate an increase in relative amounts of PPC and/or a decrease in PSC based on the wavelength of maximum absorption and spectral shape of these pigment groups. The peak in vivo absorption for PPC is ~460 nm with specific absorption dropping near zero (0.001 m² mg⁻¹) at ~540 nm (Bidigare et al. 1990). In comparison, the peak in vivo absorption for PSC is ~490 nm, dropping near zero at ~590 nm. Chlorophyll c (Chl c) absorbs within the wavelengths of interest (488 to 532 nm), so absorption slopes also were compared to Chl c : PSC, Chl c : PPC, and Chl c : Tchl a ratios. We used model II linear regression analyses for all comparisons.

Detrital absorption ($a_d$) estimation—We investigated three methods to estimate and remove $a_d$ from the ac-9 $a_p$ measurements to obtain $a_p$. Method 1 used the $a_d$ results from specific discrete water samples collected close in time and depth to the ac-9 samples and analyzed with the QFT (and Kishino method). Method 2 used a mean $a_d$ spectrum derived from all QFT data combined. Method 3 involved modeling the $a_d$ shape and magnitude following methods in Roesler et al. (1989), using the equation

$$a_d(\lambda) = (a_{440}) e^{-(\lambda - 440)}$$

where $a_{440} = a_p - a_d$. We measured $a_{440}$ with the ac-9 but needed to estimate $a_d$ and the exponent, $s$, to apply this method. We assumed that $a_d$ drops exponentially from blue to red wavelengths, little detrital absorption is expected in the red region of the spectrum). We then assumed $a_{p,440} = 1.61 \times a_{p,676}$, where 1.61 was the mean blue:red value from QFT results. We used $s = 0.0065$ nm⁻¹, since it gave $a_p$ slopes insignificantly different from slopes found using QFT $a_d$ from specific samples (95% CI for regression line slope, $s = 0.0060$ to 0.0072 nm⁻¹).

We then calculated ac-9 $a_p$ slopes based on the $a_p(\lambda)$ that resulted from each of the three $a_d$ correction methods outlined above. The linear regressions between $a_p$ slopes and PPC : PSC ratios were not significantly different using any of the methods ($p > 0.05$) after removal of one outlier (which had no comparable QFT data). For our data set consisting of ac-9 data and discrete pigment samples, we used the QFT $a_d$ from specific discrete water samples (method 1) to calculate $a_p$ slopes for all samples, except for one outlier. Since QFT data were limited in number, some QFT $a_d$ data were used for more than one ac-9/HPLC sample pair (QFT, $n = 21$; HPLC, $n = 35$, excluding outlier). For the single outlier and for fine-scale vertical profiles of ac-9 derived $a_p$ slopes, we used method 3 (or a variation of this method with a different $s$) to estimate $a_d$ and subsequently $a_p$ slopes, since comparable QFT $a_d$ data were unavailable, particularly for deep samples.

The relative importance of $a_d$ to the $a_p$ spectra was evaluated by comparing ratios of $a_{412}$ : $a_{440}$ from ac-9 measurements. Detritus has higher absorption at 412 nm than at 440 nm, while phytoplankton show the opposite trend. A ratio of $a_{412} : a_{440}$ greater than 0.96 was assumed to indicate the presence of detritus, since QFT samples were never found to have $a_{p,412} : a_{p,440}$ ratios exceeding 0.96. We used this indicator to identify depths within specific vertical
profiles that may have had high \( a_d \) relative to \( a_p \), when discrete sample \( a_d \) data were unavailable.

**Package effects**—We examined the effects of packaging on our data set by reconstructing unpackaged \( a_p \) spectra from phytoplankton pigment concentrations (determined by HPLC) using methods in Bidigare et al. (1990). We calculated the unpackaged phytoplankton absorption coefficient (\( a_p(\lambda) \)) from

\[
a_p(\lambda) = \sum_{i=1}^{c} c_i a_i^*(\lambda)
\]

where \( c_i \) is the concentration of pigment \( i \) (mg m\(^{-3}\)) and \( a_i^*(\lambda) \) is the specific absorption coefficient of pigment \( i \) (m\(^2\) mg\(^{-1}\)) at wavelength (\( \lambda \)). The percent loss of pigment absorption due to the package effect (\( Q_a^* \), Morel and Bricaud 1981) can be calculated as in Nelson et al. (1993)

\[
Q_a^*(\lambda) = \frac{\text{measured } a_p \text{ (includes packaging)}}{\text{reconstructed } a_p \text{ (unpackaged)}}
\]

We used \( Q_a^*(676) \) to compare package effects. Absorption at 676 nm is due almost entirely to Tchl \( a \) and thus is not confounded by possible errors resulting from misidentified or missing pigments (phycochlorophylls) in the blue green region of the spectrum (Nelson et al. 1993).

**Photosynthetically available radiation (PAR)**—Irradiance measurements were obtained from a tethered spectral radiometer buoy (TSRB, Satlantic; see Cullen et al. 1997), deployed 30 m from the R/V *Henderson* from midmorning to late afternoon during 18–20 June and 22–24 June 1998. Downward irradiance (\( E_d \)) just above the surface was measured at 6 Hz at seven wavelengths (412, 443, 490, 555, 670, 684, 700 nm).

The \( E_d(\lambda) \) from TSRB data was integrated from 400 to 715 nm to estimate PAR above the water surface. Subsurface irradiance was obtained using

\[
E_s(z) = E_d(0)e^{-K_d z}
\]

where \( E_s(z) \) is the downward irradiance at depth \( z \) in meters, \( E_d(0) \) is the downward irradiance just below the water surface, and \( K_d \) is the average vertical attenuation coefficient from 0 to \( z \). We assumed 5% loss of light at the air–water interface during calm conditions. \( K_p(\lambda) \) was approximated by \( K_d(\lambda) \), the vertical attenuation coefficient for net downward irradiance, where \( K_p(\lambda) = a_p(\lambda)/\mu b(\lambda) \), and \( \mu b(\lambda) \) is the average cosine for the light field. We used \( a_p(\lambda) \) values estimated from ac-9 data and average mixed layer \( \mu b(\lambda) \) values from subsurface measurements made with a Satlantic SeaWiFS profiling multichannel spectral radiometer and an ac-9 from a nearby vessel in East Sound (A. Barnard pers. comm.). Subsurface PAR values were derived from the integral (400–715 nm) of estimated \( E_s(z) \). To estimate prior light exposure, subsurface PAR values were averaged over the depth of mixing. Mixing was assumed to occur over a depth range that had a sigma–theta (density anomaly) differential <0.01 kg m\(^{-3}\).

**Results**

**Slopes of absorption spectra in relation to pigments**—Clear linear relationships were found between PPC : PSC ratios and normalized \( a_p \) and \( a_d \) slopes from ac-9 measurements (\( r^2 = 0.93, p < 0.001 \); Fig. 3a,c) and from QFT analysis of discrete samples (\( r^2 = 0.81 \) to 0.82, \( p < 0.001 \); Fig. 3b,d). The relationship between PPC : PSC ratios and ac-9 \( a_d \) slopes was robust throughout the entire range of values. There were only four data points in the higher (>0.5 g : g) PPC : PSC range; however, removal of these points did not significantly alter the linear regression (\( p < 0.05 \)), but reduced the \( r^2 \) to 0.80. The PPC : PSC ratios for the entire HPLC data set (two replicates per sample) had coefficients of variation (CV) of 0.1 to 15.1% with a mean CV of 4.5% for all samples. For the colocated water parcels, ac-9 \( a_d \) slopes had CVs ranging from 20 to 76% with a mean CV of 40%. The derived ac-9 \( a_d \) slopes had CVs ranging from 28 to 128% with a mean of 47%.

The regression line slopes and intercepts for \( a_p \) and \( a_d \) slopes and PPC : PSC ratios were not significantly different from each other (\( p < 0.05 \)) for either ac-9 derived data (Fig. 3a,c) or QFT data (Fig. 3b,d). These results suggest that detrital absorption had an insignificant effect on \( a_d \) slopes for our data set, with the exclusion of a single outlier (plus symbol in Fig. 3c). This conclusion was supported by the low \( a_d \) relative to \( a_p \) seen in QFT samples (Fig. 4).

For the outlier, we calculated \( a_d \) and subsequently the \( a_p \) slope using a variation of the method 3 \( a_d \) correction (see Methods), assuming \( s = 0.011 \) nm\(^{-1}\), since \( s = 0.0065 \) nm\(^{-1}\) (as applied to the other samples) did not yield a sufficient \( a_d \) correction. This higher \( s \) (steeper exponential slope) allowed the outlier sample point to fall close to the regression line for the PPC : PSC ratio and \( a_p \) slope relationship (Fig. 3a). This single deep sample (18.1 m) from 23 June 1998 appeared to contain high \( a_d \) based on high ratios of \( a_d : 412 : a_p : 440 \) (1.12 for this outlier compared to a range of 0.87 to 1.03 for the other data points shown in regressions). This correction implies that the \( a_d \) shape and magnitude were different (steeper exponential slope and higher magnitude) for this outlier compared to the remaining samples (all but one collected at shallower depths).

**Slopes compared to \( a_p \) ratios, Tchl \( a \), and other pigment ratios**—We compared the ac-9 \( a_p \) slope calculations and the more straightforward ratios of \( a_d : 488 : a_p : 676 \) and \( a_p : 488 : a_p : 532 \) and found that the PPC : PSC ratios had a stronger correlation with \( a_p \) slopes (\( r^2 = 0.93 \)) than with these \( a_p \) ratios (\( r^2 = 0.84 \) and 0.70, respectively).

A comparison of Tchl \( a \) to \( a_d \) slopes showed that Tchl \( a \) had a much weaker relationship to \( a_d \) slopes than was found for PPC : PSC ratios (\( r^2 = 0.45 \) compared to 0.93). Removing the four lowest Tchl \( a \) values (and also steepest \( a_d \) slopes) from the analysis yielded an even weaker relationship (\( r^2 = 0.20 \)). In contrast, strong linear relationships were found between ac-9 \( a_d \) slopes and ratios (g : g) of PPC : total pigments (chlorophylls, PSC, and PPC) and PPC : total carotenoids (\( r^2 = 0.90 \) and 0.93, respectively, \( p < 0.001 \)). Weak linear relationships were seen between absorption slopes and ratios.
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Fig. 3. Relationship of PPC : PSC ratios from HPLC analysis to normalized absorption slopes, slope = \((a_{488} - a_{532})/(a_{676} \times (488 - 532 \text{ nm}))\), from measurements of (a) in situ ac-9 phytoplankton absorption coefficients \((a_{ph})\), (b) discrete sample QFT \(a_{ph}\), (c) in situ ac-9 particulate absorption coefficients \((a_{p})\), and (d) discrete sample QFT \(a_{p}\). Open symbols are near surface samples (<5 m), and closed squares are deep samples (>5 m). Circles indicate surface mixed layer extends deeper than 5 m; diamonds indicate surface mixed layer <5 m; triangles indicate a continuously stratified surface layer. The plus sign (+) indicates a single outlier that appeared to contain high levels of detritus (see text). Model II linear regressions shown for \(n = 36\) samples in panel a, \(n = 35\) samples (excludes the outlier) in panel c, and \(n = 21\) samples in panels b and d.
Fig. 4. Mean \( a_p \) and \( a_d \) spectra (solid lines) for QFT samples collected concurrently with HPLC samples, \( n = 21 \). Standard errors indicated by dashed lines.

Effects of \( a_d \) magnitude and shape on slopes—The ac-9 \( a_p \) data and subsequent \( a_p \) slope calculations are influenced by phytoplankton and detrital absorption. In other coastal and oceanic environments containing high and/or variable detritus concentrations, it is critical to understand how the \( a_p \) spectra are affected by variations in \( a_d \) magnitude and shape. These variations will in turn influence the relationship between \( a_p \) slopes and PPC : PSC ratios. To this end, we examined how variations in magnitude and shape (exponential slope, \( s \)) of \( a_d \) spectra might affect the relationship of ac-9 derived \( a_p \) slopes to PPC : PSC ratios. Specific sample \( a_d \) values (QFT data) were used for all analyses. We varied the \( a_d \) magnitude by multiplying \( a_d \) by 1, 2, 4, 6, 10, 20 (yielding \( a_d ^{412} : a_p ^{412} \) ratios of 0.21, 0.32, 0.50, 0.58, 0.70, 0.82, respectively) and added these \( a_d \) values to prior estimates of ac-9 \( a_p \) values. We observed strong linear relationships between these new \( a_p \) slopes and PPC : PSC ratios up to 10 \( a_d \) (\( r^2 = 0.88 \) and 0.71 for 4 and 10 \( a_d \), respectively) (Fig. 5a). The linear relationship between \( a_d \) slopes and PPC : PSC ratios weakened at 20 \( a_d \) (\( r^2 = 0.44 \), data not shown). The intercepts were significantly different \(( p < 0.05)\) than seen for the original ac-9 \( a_p \) regression for \( s \leq 0.005 \text{ nm}^{-1} \) and \( s > 0.0125 \text{ nm}^{-1} \). The intercepts were not significantly different.

Finally, we varied both the magnitude and shape \((s)\) of the \( a_d \) spectra. Magnitudes of 1 and 4 \( a_d \) and \( s \) values of 0.004, 0.012 \text{ nm}^{-1} \) were used to calculate new \( a_d \) spectra for \( a_p \) slope estimates (Fig. 5c). Linear relationships between \( a_p \) slope and PPC : PSC ratios were found for all combinations of \( a_d \) magnitude and spectral shape, \( s \). At the higher values of \( s \), we observed greater differences in regression line intercepts between low and high \( a_d \) magnitudes.

The above analyses did not examine the effects of large variations in \( a_d \) between samples within one data set. We lacked the data to conduct such an analysis, but consideration of all points in Fig. 5c (as if the various \( a_d \) corrections were from a single data set) yields a linear association \(( p < 0.001, r^2 = 0.55)\) with significant differences in \( a_p \) slopes seen for PPC : PSC differences of 0.1 or greater (e.g., PPC : PSC ratios of 0.2 compared to 0.3, \( p < 0.04 \), two-sided \( t \) -test).

Effects of packaging on \( a_p \) slopes—Package effects result from a combination of intracellular pigment (composition and concentration) and cell size variations. These variations can reduce the optical cross section of the cell and alter the \( a_p \) and \( a_ph \) spectra and slopes. Therefore, we attempted to quantify the effects of pigment packaging on \( a_ph \) slope variability.

Cell size (ranging from 0.6 to >50 \text{ \mu m} \) diameter) can have an important influence on phytoplankton package effects between and within taxonomic groups (Morel and Bricaud 1981; Bricaud et al. 1983). The influence of cell size on packaging for diatoms was recently examined by Zinkel...
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Fig. 5. Relationship of PPC : PSC ratios from HPLC analysis to $a_p$ slopes derived by adding estimates of $a_d$ to ac-9 $a_{ac}$ values using (a) $a_d$ magnitudes of 4 and 10 times measured $a_{ac}$, (b) $a_d$ spectral slopes, $s$, of 0.004, 0.008, 0.012, and (c) varying magnitudes and spectral shapes ($s = 0.004$ and $0.012$ with 1 and 4 times measured $a_{ac}$). Linear regression lines (solid lines) for varying $a_d$ estimates are shown. Dashed lines show the linear regression of ac-9 $a_p$ slopes as in Fig. 3a. $a_p$ slope calculation as in Fig. 3.
Packaging effects for our data set were estimated using $Q_a(676)$ derived from our QFT data. These $Q_a(676)$ values ranged from 0.87 to 0.35 with a mean of 0.57. $Q_a(676)$ values were significantly higher for surface depths ($\leq 5$ m) than for depths $\geq 10$ m [mean $Q_a(676)$ values of 0.59 compared to 0.44; $t$-test, $p < 0.05$], suggesting that cells located near the surface had less packaging than cells located at depth. The trends in $Q_a(676)$ are similar for ac-9 derived $Q_a(675)$ data. For comparison, $Q_a(675)$ ranged from $\sim 0.98$ to 0.6 for a variety of diatom cultures reported in literature (Nelson et al. 1993). Bricaud et al. (1995) found $Q_a(675)$ values of $\sim 0.9$ to $< 0.3$ for samples with Tchl $a$ ranging from 1.5 to 20 $\mu$g L$^{-1}$ (overlapping the Tchl $a$ range in our study), with $Q_a(675)$ showing a general decrease with increasing Tchl $a$. We observed a weak trend of decreasing $Q_a(676)$, with increasing Tchl $a$ (although the scatter was large and the slope of the linear regression was not significantly different from zero). We found no significant relationships ($p > 0.05$) between $Q_a(676)$ and $a_{ph}$ slopes or PPC : PSC ratios.

Finally, we evaluated the variations in packaging on ac-9 $a_{ph}$ slopes by comparing measured $a_{ph}$ slopes to the $a_{ph}$ slopes derived from $a_{ph}$ (unpackaged) data using varying percentages of packaging. The $a_{ph}$ slopes with 0%, 50%, and 75% of their original packaging were on average 1.88, 1.48, and 1.25 times steeper than measured $a_{ph}$ slopes ($p < 0.05$, $t$-tests, Fig. 6). A comparison of these ac-9 $a_{ph}$ slopes to PPC : PSC ratios indicates that decreasing the package effect increases the magnitude of the regression line intercept but does not change the regression line slope appreciably. Similar results were obtained for reconstructed $a_{ph}$ slopes derived from QFT data. We found linear relationships between $a_{ph}$ slopes and PPC : PSC ratios for all packaging levels ($r^2 = 0.91$ to 0.93). As with the $a_d$ evaluations, these analyses did not address the effects of large variations in packaging between samples within one data set (as may occur in many oceanographic regions). If all packaging variations are considered at once (as if the various package effects shown in Fig. 6 were from a single data set), a linear association between ac-9 $a_{ph}$ slopes and PPC : PSC ratios is still found ($p < 0.001$; $r^2 = 0.54$).

**Stratification, light, and nutrients**—The thermistor chain record (Fig. 2) indicates that surface temperatures and mixed layer depths varied considerably over the 10-d survey period. Surface temperatures increased and surface mixed layers shoaled from 14 to 21 June 1998, with decreases in surface temperatures and deepening of surface mixed layers from 21 to 24 June 1998. Mean above surface PAR between 1000 and 1600 h was moderate on 18 June 1998 (800 $\mu$mol quanta...
Carotenoid ratios and in situ absorption

Fig. 7. Vertical profiles of density in sigma-theta (bold line), chlorophyll a calculated from in situ fluorescence (thin line), ac-9 $a_p$ slopes (open diamonds), and $a_{ph}$ slopes (closed diamonds) collected within a half hour of solar noon on (a) 20 June, (b) 22 June, (c) 23 June, and (d) 24 June 1998. The $a_{ph}$ slopes were derived from ac-9 $a_p$ data using the method 3 correction (see Methods text) assuming $a_{ph}^{440}:a_{ph}^{676} = 1.61$ and an exponential slope, $s$, based on ac-9 $a_p^{412}:a_p^{440}$ ratios. For samples with $a_p^{412}:a_p^{440}$ ratios $>0.96$ (typically located below 10–15 m), we used $s = 0.011 \text{ nm}^{-2}$. For all other samples we assumed $s = 0.0065 \text{ nm}^{-2}$. The $a_p$ and $a_{ph}$ slopes were calculated as in Fig. 3 and multiplied by negative 1,000 for scaling purposes. Error bars on $a_p$ and $a_{ph}$ slopes indicate ±1 SE. $a_p$ slopes that were significantly different (95% confidence level) from the 1-m interval directly above are indicated by an X. Significant differences were calculated only for depths with $a_p^{412}:a_p^{440}$ ratio $<0.96$ (i.e., samples that did not appear to contain high levels of detritus). The mean PAR value for the hour prior to sample collection is displayed on each panel. Note the large changes in $a_p$ and $a_{ph}$ slopes in panels a and b compared to c and d.

Vertical and temporal variations of $a_p$ slopes and pigment ratios—Steeper $a_p$ and $a_{ph}$ slopes and higher PPC : PSC ratios were observed more often near the surface than at depth (Fig. 3). Vertical ac-9 profiles of $a_p$ slopes and $a_{ph}$ slopes were used to document fine-scale variations in PPC : PSC ratios (Fig. 7). Deeper in the water column (below the main pycnocline), the greater magnitude $a_p$ slopes were likely due to higher $a_p$ in these waters (based on $a_p^{412}:a_p^{440}$ ratios). To estimate $a_{ph}$ slopes, we derived $a_p$ using method 3 (see Methods), with a slight variation for deep samples. We assumed that $s = 0.0065 \text{ nm}^{-1}$ in waters above the pycnocline with low $a_p$ and $s = 0.011 \text{ nm}^{-1}$ (as used for the single outlier) in deeper waters with high $a_p$ (see Fig. 7 legend). These estimated $a_{ph}$ slopes appear to be fairly low and constant below the pycnocline (with the possible exception of the 24 June profile), which suggests that PPC : PSC ratios were low and did not change appreciably in these deep waters. Note that these $a_{ph}$ slope estimates are dependent on the assumptions made for $a_p$ estimates (e.g., $s = 0.011 \text{ nm}^{-1}$ is an appropriate value for deep waters with high $a_p$).

The data from all four days (20, 22, 23, and 24 June 1998) show that $a_p$ and $a_{ph}$ slopes changed significantly at depths with large density gradients. For example, on 20 June 1998 (Fig. 7a), the $a_p$ slopes were significantly steeper at 3 m than...
4 m and at 6 m than 7 m (t-tests, \( p < 0.002 \)). Both of these transitions occurred over large density steps. A prediction of PPC : PSC ratios from these ac-9 \( a_p \) slopes (\( y = -55.43x - 0.292, r^2 = 0.94 \), model 1 linear regression) suggests that the PPC : PSC ratios were twice as high at 3 m than 4 m (difference of 0.46 g : g) and at 6 m than 7 m (difference of 0.24 g : g). Taxonomic data collected on 20 June 1998 revealed greater variation in species composition across the pycnocline than seen within waters above or below the pycnocline (D. Gifford pers. comm.).

Temporal variations in \( a_p \) slopes and pigment ratios can also be seen in the profiles shown in Fig. 7. Surface \( a_p \) and \( a_{ph} \) slopes were steeper on 20 and 22 June relative to 23 and 24 June 1998. The higher irradiance levels and shallower surface mixed layer depths on 20 and 22 June (Figs. 2, 7) likely contributed to these slope differences between the dates. The 2-m phytoplankton populations, for example, were exposed to average irradiances four times higher on 20 and 22 June than on 23 and 24 June 1998 (~950 compared to ~250 \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \)).

**Pigment ratios and \( a_p \) slopes in relation to light**—We compared prior light exposures for samples collected between 1100 h and 1600 h to pigment ratios and \( a_p \) slopes. Positive associations were seen between mean PAR for the hour prior to sample collection and (diaxanthin + diadinoxanthin) : PSC ratios, (diaxanthin + diadinoxanthin) : PPC ratios, PPC : PSC ratios, and \( a_p \) slopes (linear regression \( r^2 = 0.92, 0.98, 0.96, \) and 0.94, respectively; Fig. 8). Similar but slightly weaker relationships to pigment ratios and \( a_p \) slopes were seen for PAR averaged over 30 min or 2 h prior to sample collection (data not shown). Since the TSRB was not deployed until ~4 h after dawn, we did not have enough data to adequately assess cumulative irradiance effects from the start of the light period.

**Dominant phytoplankton species**—The chemotaxonomic pigments with the highest concentrations were fucoxanthin, peridinin, and alloxanthin; these pigments were used to assess the relative abundances of diatoms, dinoflagellates, and cryptophytes (Jeffrey and Veski 1997), respectively. The fucoxanthin : Tchl \( a \) ratios, peridinin : Tchl \( a \), and alloxanthin : Tchl \( a \) ratios indicate that taxonomic composition varied temporally and as a function of depth (Table 1). Fucoxanthin : Tchl \( a \) ratios were typically an order of magnitude higher than other biomarkers, which indicates that diatoms were the most abundant species (Table 1). *Chaetoceros socialis*, a colonial diatom, frequently was the most numerous diatom sampled, although its relative proportion of the assemblage varied over depth, time, and location within East Sound (D. Gifford, J. Rines pers. comm.).

**Discussion**

The key findings of this study were the strong relationships found between the ac-9 \( a_{ph} \) and \( a_p \) slopes and the HPLC-derived PPC : PSC ratios (Fig. 3). These relationships suggest that absorption measurements from in situ instrumentation may be used to estimate PPC : PSC ratios in field phytoplankton assemblages. The relationship between ac-9 \( a_p \) slopes and pigment ratios was robust in East Sound except in deeper waters where \( a_p \) was likely high. The general applicability of this approach in other coastal and oceanic waters, however, is dependent upon consideration of the contributions of detrital absorption (\( a_d \)), pigment packaging, species composition, and irradiance to the shape of the absorption spectrum at specific depths. For both the \( a_p \) and packaging sensitivity analyses, we were restricted by the narrow range of values in our particular data set. Analysis with a wider ranging data set that possesses larger variations in \( a_p \) and packaging would allow us to more fully evaluate the impact of these variations. In addition, a more extensive data set would permit us to address the combined effects of variations in \( a_p \) and packaging on the relationship of \( a_{ph} \) and \( a_p \) slopes to PPC : PSC ratios.

Our analysis of variable contributions of \( a_p \) to our estimated \( a_p \) slopes suggests that over a large range of \( a_p \) magnitudes (\( a_p 4.12 \) to 0.5) and spectral shapes (\( s \) from 0.004 to 0.012 calculated over 440 to 676 nm range, Fig. 5c) it is possible to infer PPC : PSC ratios from \( a_p \) slopes derived from in situ absorption measurements. Ideally, \( a_p \) values should be determined using the QFT for a representative number of samples. Measured, mean, or modeled (Roesler et al. 1989; Cleveland and Perry 1994) values of \( a_p \) can then be subtracted from the ac-9 \( a_p \) spectra to estimate \( a_{ph} \) values and slopes for samples collected within similar water masses. We suggest that in situ absorption measurements always be made with coincident measurements of water mass properties and the local light field to permit assignment of groups of \( a_p \) slopes to particular water mass types, thus reducing the impact of variable \( a_p \) magnitudes and spectral shapes on the interpretation of estimated PPC : PSC ratios.

Variation in pigment packaging may further complicate the interpretation of pigment ratios from absorption data (Hoepffner and Sathyendranath 1991). Within the data set we analyzed, we had a range of package effects [indicated by \( Q_{a=676} \) of 0.35 to 0.9]. In spite of this variation, we were able to derive a clear linear relationship between PPC : PSC ratios and \( a_{ph} \) slopes (linear regression \( r^2 = 0.92, 0.98, 0.96, \) and 0.94, respectively; Fig. 8). Similar but slightly weaker relationships to pigment ratios and \( a_p \) slopes were seen for PAR averaged over 30 min or 2 h prior to sample collection (data not shown). Since the TSRB was not deployed until ~4 h after dawn, we did not have enough data to adequately assess cumulative irradiance effects from the start of the light period.
Carotenoid ratios and in situ absorption

Fig. 8. Relationship of mean in-water PAR for the hour prior to sample collection to (a) (diatoxanthin + diadinoxanthin): Tchl a, (b) (diatoxanthin + diadinoxanthin): PSC, (c) PPC: PSC, and (d) ac-9 a_p slopes. Samples collected between 1100 h and 1600 h Pacific Daylight Time on 18–20 June and 22–24 June 1998. PAR calculated as described in text. Symbols and a_p slope calculation as in Fig. 3.

toacclimation responses to variations in irradiance and provide clues to the light history of the phytoplankton community. If turnover (mixing) of the water column is slower than the time required for pigment synthesis, then indicators of photoacclimation such as (diatoxanthin + diadinoxanthin): Chl a ratios can show a vertical gradient within the water column (Moline 1998). Culver and Perry (1999) found that natural phytoplankton from stratified depths in Puget Sound exhibited photoacclimation effects (photosynthetic pigment absorption coefficient: total phytoplankton absorption coefficient increased as irradiance decreased), while cells in mixed layers did not show a discernable photoacclimation trend. In our study, higher (diatoxanthin + diadinoxanthin): Tchl a ratios and PPC: PSC ratios, steeper a_p and a_ph slopes, and higher prior (1 h) light exposures were seen in stratified surface waters than in deeper waters (Table 1, Fig. 8), which reflects the reduced vertical mixing associated with shallow stratification. Additional studies at intermediate- to high-light levels are required to quantify the time scales of response between irradiance, pigment ratios, and ac-9 a_p slopes and the intersection of these time scales with the longer time scales of species compositional changes within a water mass.

Variations in nutrient concentrations also can promote
changes in physiology (Geider et al. 1993) and phytoplankton species succession that influence the relative pigment concentrations of the phytoplankton assemblage. However, the absence of an appreciable change in surface N levels, with the exception of 20 June 1998, suggests that for the most part, light influenced pigmentation more than nutrients during our study period.

Changes in PPC : PSC ratios can reflect physiological changes at the cellular level or indicate a shift in species composition with different light tolerances or nutrient requirements. During monospecific (or low species diversity) phytoplankton blooms, changes in the shape of the \(a_s\) spectrum indicate physiological acclimation rather than taxonomically diverse species. The phytoplankton assemblages in this study had differing species compositions (although diatoms were always the most abundant), photoacclimation responses, and/or light histories, all of which could influence \(a_s\) slopes and pigment ratios. For example, the high PPC : PSC ratios and steep \(a_s\) and \(a_p\) slopes seen in surface waters on 20 June 1998 likely resulted from photoacclimation as indicated by high diatoxanthin + diadinoxanthin : Tchl \(a\) ratios, in addition to taxonomic variation (presence of nondiatom species) as suggested by the relatively lower fucoxanthin : Tchl \(a\) ratios and relatively higher alloxanthin : Tchl \(a\) peridinin : Tchl \(a\) ratios (Table 1).

Finally, our results indicate that in situ \(a_s\) slope measurements may reveal significant differences in estimated PPC : PSC ratios on vertical scales of ~1 m. This fine-scale resolution, obtained with free-fall deployment methods, also allows estimates of \(a_s\) slopes and pigment ratios to be directly compared with parameters such as temperature, salinity, density, and fluorescence measured over the same vertical scales. These sharp vertical gradients in biooptical properties are consistent with other observations of fine-scale plankton blooms, changes in the shape of the PPC : PSC ratios, and ratios (wt : wt). Pigment types are abbreviated as follows: chlorophyll \(a\) (Chl \(a\)), diadinoxanthin : Tchl \(a\), peridinin (Peri), alloxanthin (Allo), diatoxanthin + diadinoxanthin (DiDd), \(\beta\) carotene (Bar), photoprotective carotenoids (PPC), photosynthetic carotenoids (PSC). Total pigment concentrations (total) were calculated as Tchl \(a + \ Chl \ r + PPC + PSC\). Dates and times are Pacific Daylight Time. Samples > 5 m shown in bold. Each data point represents the mean of two replicate samples unless indicated as no replicate (nr).

### Table 1. HPLC-determined pigment concentrations (\(\mu g L^{-1}\)) and ratios (wt : wt). Pigment types are abbreviated as follows: chlorophyll \(a\) + chlorophyllide \(\alpha\) (Tchl \(a\)), chlorophyll \(c\) : 2 (Chl \(c\)), fucoxanthin (Fuco), peridinin (Peri), alloxanthin (Allo), diatoxanthin + diadinoxanthin (DiDd), \(\beta\) carotene (Bar), photoprotective carotenoids (PPC), photosynthetic carotenoids (PSC). Total pigment concentrations (total) were calculated as Tchl \(a + Chl \ r + PPC + PSC\). Dates and times are Pacific Daylight Time. Samples > 5 m shown in bold. Each data point represents the mean of two replicate samples unless indicated as no replicate (nr).

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<th>Date</th>
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<th>Depth (m)</th>
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<th>Chl (c)</th>
<th>PP : PS</th>
<th>PP : total</th>
<th>PPC : Tchl (a)</th>
<th>PSC : Tchl (a)</th>
<th>Allo : Tchl (a)</th>
<th>DiDd : Tchl (a)</th>
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<td>0.083</td>
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### Data Analysis

- **Changes in Pigment Ratios**
  - PPC : PSC ratios increase with depth, suggesting a shift in species composition.
  - Changes in PPC : PSC ratios can reflect physiological changes at the cellular level or indicate a shift in species composition with different light tolerances or nutrient requirements.

- **Photoacclimation Responses**
  - Photoacclimation responses are consistent with other observations of fine-scale plankton blooms, changes in the shape of the PPC : PSC ratios, and ratios (wt : wt). Pigment types are abbreviated as follows: chlorophyll \(a\) + chlorophyllide \(\alpha\) (Tchl \(a\)), chlorophyll \(c\) : 2 (Chl \(c\)), fucoxanthin (Fuco), peridinin (Peri), alloxanthin (Allo), diatoxanthin + diadinoxanthin (DiDd), \(\beta\) carotene (Bar), photoprotective carotenoids (PPC), photosynthetic carotenoids (PSC). Total pigment concentrations (total) were calculated as Tchl \(a + Chl \ r + PPC + PSC\). Dates and times are Pacific Daylight Time. Samples > 5 m shown in bold. Each data point represents the mean of two replicate samples unless indicated as no replicate (nr).
tonic structure in East Sound (Dekshenieks et al. 2001; Rines et al. 2002; Alldredge et al. 2002), over the continental shelf (Cowles et al. 1993, 1998), and in the Baltic (Bjørnsen and Nielsen 1991).

In conclusion, our results suggest that absorption measurements from in situ instrumentation can be used to estimate PPC : PSC ratios in field phytoplankton assemblages in areas with low detrital concentrations or where the $a_p$ contribution can be adequately estimated. We show that the use of in situ optical instrumentation can provide a continuous vertical profile or temporal record of in-water optical properties, such as the normalized $a_p$ spectral slope (488 to 532 nm), that can detect changes in pigmentation on finer scales than possible with conventional discrete water sampling methods. Pigmentation and in situ absorption changes were observed in response to changes in light and stratification, with increases in PPC : PSC ratios and $a_p$ slopes associated with increases in irradiance and shoaling of the mixed layer. Such in situ–derived estimates of phytoplankton pigmentation changes may also provide insight into the recent light history of a particular phytoplankton population. For example, a time series of in situ absorption measurement could be used to estimate synthesis of PPC relative to PSC, given that advection effects are minimal or a single water mass can be monitored. While pigment package effects or $a_p$ variations may alter the absorption spectra, in our data set we still found a significant relationship between pigment ratios and in situ $a_p$ slopes. Further work is needed, however, to extend our understanding of the effects of packaging and $a_p$ on the relationship developed in this study. With careful consideration of the range of factors influencing in situ absorption, these measurements can provide valuable information for deciphering the spatial, temporal, and physical factors driving photoacclimation and species diversity in field phytoplankton populations. We look forward to additional comparisons of in situ $a_p$, HPLC-derived pigment composition, and $a_p$ estimates in other oceanic regions to confirm the general utility of $a_p$ and $a_p$ slopes to estimate PPC : PSC ratios.

References


Received: 17 December 2001
Accepted: 5 November 2002
Amended: 7 November 2002