How does cell volume influence the total light absorption efficiency of a mixed population of dinoflagellates with similar cell shapes and pigment compositions?

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Abstract: *In vivo* absorption spectra and spectrally reconstructed absorption spectra from HPLC-determined pigment concentrations were obtained and compared for five different mixtures of two dinoflagellates (large cells, *Prorocentrum micans* and small cells, *Prorocentrum minimum*) with similar cell shapes and pigment compositions but significant differences in pigment contents and cell volume. Although the large cells contained 4-fold higher chlorophyll *a* content than the small cells, the observed intracellular chl *a* contents per unit cell volume (C_i) increased with decreasing cell volume. Exponentially grown cells with the abovementioned characteristics were prepared for use in mixing experiments with 0, 25, 50, 75, and 100% of *P. minimum* and *vice versa* for *P. micans*. The *in vivo* absorption values determined at nine wavelengths (412, 440, 480, 510, 532, 555, 650, 676, and 700 nm) were significantly associated with the biomass with respect to either cell volume or chl *a* concentration and were inversely correlated with C_i ($p < 0.01$). The observed package effect [$Q_a^*(\lambda)$] indicated an overestimation of absorption spectra at all wavelengths by reconstruction. The fractional reduction of pigment absorption due to $Q_a^*(\lambda)$ was wavelength dependent regardless of the mixing ratio of the two populations. The results showed that the product of equivalent spherical diameter and C_i should be considered for quantitative studies of $Q_a^*(\lambda)$ in various chromatic-adapted species in cultures as well as in coastal waters.

Key words: absorption efficiency, chlorophyll *a*, package effect, reconstructed absorption spectra

Introduction

Photosynthetically active radiation is mostly absorbed by primary producers in the ocean ecosystem. Global marine primary production is estimated from the observation of field measurements and of ocean color data generated via remote sensing from satellites (Longhurst et al. 1995). While classical field measurements are too scarce to provide information on global change, remote sensing from satellites can continuously provide information temporally and spatially over a wide area. Satellite remote sensing techniques have been intensively investigated to develop methods for estimating global marine primary production (McClain et al. 2003).

To estimate primary production in the ocean, multiple

bio-optical models have been developed (Siegel et al. 1989, Behrenfeld & Falkowski 1997), most of which are dependent on the light absorption characteristics of surface water, among which the chl *a* specific absorption coefficient (a_{ph}^*) of phytoplankton is primarily considered (Bricaud & Stramski 1990, Sosik & Mitchell 1991, Babin et al. 1993). For laboratory cultures, a wide range of absorption spectra data has been accumulated for a large diversity of species (Sathyendranath et al. 1987, Bricaud et al. 1988, Berner et al. 1989, Nelson & Prezelin 1990, Schofield et al. 1991, Finkel 2001, Fujiki & Taguchi 2002, Leong & Taguchi 2006). Variability in the a_{ph}^* is primarily governed by (1) pigment composition, (2) pigment concentration, and (3) cell volume (e.g., Sathyendranath et al. 1987). The changes in pigment composition influence the spectral characterization of a_{ph}^* (Sathyendranath et al. 1987). Increasing pigment concentration results in a decrease in a_{ph}^* (Kirk 1994, Lutz et al. 2001), while an increase in cell

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volume results in a decreased a_{ph}^* (Finkel 2001, Fujiki & Taguchi 2002). Variation in cell volume is also caused by environmental factors such as irradiance and nutrient supply (Fujiki & Taguchi 2002). In the ocean, natural phytoplankton assemblages are composed of a variety of species that have remarkable differences in pigment composition, concentration, and cell size. The results of field studies have suggested that variation in a_{ph}^* is caused by absorption properties within phytoplankton assemblages (Yentsch 1989). The dominant cell size and taxonomic group can govern the absorption spectra of natural phytoplankton assemblages (e.g., Ciotti et al. 2002). Variations in the absorption of phytoplankton assemblages with variable cell sizes have been demonstrated with a model (Morel & Bricaud 1981), which has shown that a_{ph}^* decreases exponentially with increasing mean cell size in phytoplankton assemblages.

When cells with various cell volumes co-exist and are

well mixed, the effect of cell volume on a_{ph}^* can provide information regarding the light absorption of phytoplankton assemblages, particularly at high concentrations. By using species within the same genus with a similar pigment composition, the influence of cell volume can be solely resolved for the package effect. In the field, a measurable package effect (Berner et al. 1989) was principally observed in samples taken from the subsurface chl *a* maximum layer and in association with populations of large diatoms or dense concentrations of prymnesiophytes as described by Nelson et al. (1993). A similar package effect can be observed in dinoflagellates. *Prorocentrum minimum* (Pavillard) Schiller and *Prorocentrum micans* Ehreberg are primarily observed in coastal waters in temperate and tropical regions (Kimor et al. 1985, Smayda 1990, Grzebyk et al. 1997). These dinoflagellates bloom together and are consistently present in late summer and fall (Hornor 2002, Kraberg et al. 2010). To describe the ecological

Table 1. Definition of symbols and abbreviations used in text.

a_d (λ)	absorption coefficient of methanol-extracted particles (m^{-1})
a_p (λ)	absorption coefficient of particles (m^{-1})
a_{ph} (λ)	absorption coefficient of cells (m^{-1})
a'_{ph} (λ)	absorption coefficient of cells reconstructed by the method proposed by Bidigare et al. (1990) (m^{-1})
a_{ph}^* (λ)	absorption coefficient of total cellular chl <i>a</i> (m^2 [mg chl <i>a</i>] $^{-1}$)
$\bar{a}_{ph}^*$ (λ)	spectral average of the chl <i>a</i> specific absorption coefficient
a_{min}^* (λ)	absorption coefficient of cellular chl <i>a</i> of <i>Prorocentrum minimum</i> (m^2 [mg chl <i>a</i>] $^{-1}$)
a_{mic}^* (λ)	absorption coefficient of cellular chl <i>a</i> of <i>Prorocentrum micans</i> , (m^2 [mg chl <i>a</i>] $^{-1}$)
β -caro	β -carotene
chl <i>a</i>	chlorophyll <i>a</i>
chl <i>c</i>	chlorophyll <i>c</i>
C_i	intracellular <i>i</i> th pigment content ($kg\ m^{-3}$)
C_{min}	chlorophyll <i>a</i> concentration of <i>Prorocentrum minimum</i> ($mg\ m^{-3}$)
C_{mic}	chlorophyll <i>a</i> concentration of <i>Prorocentrum micans</i> ($mg\ m^{-3}$)
C_t	intracellular total pigment content ($kg\ m^{-3}$)
DD	diatoxanthin
DT	diatoxanthin
ESD	equivalent spherical diameter (m)
<i>h</i>	height (m)
<i>l</i>	length (m)
μ	growth rate (d^{-1})
<i>N</i>	cell density
N_t	cell density in cells mL^{-1} at the beginning (t_0) of the exponential phase
N_0	cell density in cells mL^{-1} at the end (t_1) of the exponential phase
OD_s (λ)	optical density of particles in suspension (m^{-1})
OD_f (λ)	optical density of filtered total particles (m^{-1})
perid	peridinin
Q_a (λ)	cell absorption efficiency (Bricaud et al. 1983)
Q_a^* (λ)	package effect
Q'_a (λ)	specific absorption efficiency (Morel and Bricaud 1981)
ρ' (λ)	absorption index, product of a_{ph} (λ) and <i>d</i>
<i>S</i>	filtered clearance area (m^2)
<i>V</i>	filtered volume (m^3)
<i>w</i>	width (m)

significance of both species, a determination of their bio-optical properties may be useful to divide them into groups of two sizes as discussed by Devred et al. (2006).

The objective of this study was: (1) to compare the effect of cell volume on *in vivo* absorption spectra [$a_{ph}(\lambda)$] and spectrally reconstructed *in vivo* absorption spectra [$a'_{ph}(\lambda)$] based on observations of different mixing ratios of the dinoflagellates *P. micans* and *P. minimum*, which have different cell volumes but similar cell shapes and pigment compositions under similar culture conditions; and (2) to assess the impact of variability in the package effect [$Q_a^*(\lambda)$] on the total cellular absorption spectra.

Materials and Methods

Strain and Pre-cultures

The Dinophyceae *Prorocentrum micans* (NIES-218) and *Prorocentrum minimum* (NIES-237) were obtained from the microbial culture collection at the National Institute for Environmental Study (NIES), Japan. These species were maintained at 20°C and a salinity 35 in enriched f/2 medium (Guillard & Ryther 1962) without silicate using aged filtered sea water collected from Manazuru Bay, Japan. An irradiance of 600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was provided for growth using cool fluorescent tubes on a 12 h light : 12 h dark cycle. Cells were preconditioned in a semi-continuous culture by being inoculated into daughter cultures for three growth cycles every three days to acclimate the cells to the growth conditions. The inoculation volume of *P. micans* and *P. minimum* was determined from the initial cell density, approximately 2000 and 2500 cells mL^{-1} , respectively.

Experimental design

Prorocentrum micans and *Prorocentrum minimum* were grown for 7 days under similar culture conditions to generate pre-cultures in three bottles for each species. The cells were harvested and used for the mixing of the two species on day 2 after inoculation. This date corresponded to the exponential phase of growth, which was determined by assessing the cell density. The mixing ratios based on total cell volume of *P. micans* and *P. minimum* were assumed as follows. The five different mixing proportions of *P. minimum* were set at 0% (mixing experiment 1), 25% (mixing experiment 2), 50% (mixing experiment 3), 75% (mixing experiment 4), and 100% (mixing experiment 5), using a similar total cell density of the sum of two populations. Since the chl *a* concentration is significantly associated with cell volume in laboratory cultures, the total chl *a* concentration (C) was calculated as the sum of the concentrations of $C_{\min}$ and C_{mic} of the two populations,

$$C = C_{\min} + C_{\text{mic}} \quad (1)$$

where $C_{\min}$ and C_{mic} are the chl *a* concentrations ($\text{mg chl } a \text{ m}^{-3}$) of the two phytoplankton populations (*P. minimum* and *P. micans*) with different cell sizes. Therefore, a total

absorption of coefficient (m^{-1}) due to summation of the two species, $a_{ph}(\lambda)$ can be determined as,

$$a_{ph}(\lambda) = a_{\min}^*(\lambda)C_{\min} + a_{\text{mic}}^*(\lambda)C_{\text{mic}} \quad (2)$$

where $a_{\min}^*(\lambda)$ and $a_{\text{mic}}^*(\lambda)$ are the specific absorption coefficients ($\text{m}^2 [\text{mg chl } a]^{-1}$) of the two phytoplankton populations (*P. minimum* and *P. micans*) with different cell sizes, respectively.

Cell density

The cells in each 20-mL subsample were centrifuged at a constant speed of 4,000 rpm for ten minutes. The supernatant was discarded, and the sedimented pellets were resuspended in 2.5-mL tubes with fresh filtered sea water. Then, the samples were centrifuged again at a constant speed of 10,000 rpm for five minutes and concentrated to 0.20 mL. The cell density of each subsample was measured on an inverted microscope (Olympus, IMT-2, Japan) at 100 $\times$ magnification using a hemocytometer (Erma Inc. Japan).

Growth rate

The growth rate [$\mu \text{ (d}^{-1})$] was calculated from the cell density using the following equation (Guillard 1973):

$$\mu = \ln(N_t/N_0)/(t_1 - t_0) \quad (3)$$

where N_t and N_0 are the cell density in cells mL^{-1} at the beginning (t_0) and end (t_1) of the exponential phase, respectively.

Cell volume

It is crucial to estimate the cell volume in a study of light absorption, therefore, the shape of *Prorocentrum micans* and *Prorocentrum minimum* was assumed as ellipsoid. The length (l), width (w), and height (h) of 30–50 cells per sample were measured using a micrometer with an ocular ruler at 400 $\times$ magnification under a compound microscope (Olympus, CH-2, Japan). The cell volume was calculated using the equation for an ellipsoid described by Hillebrand et al. (1999):

$$V = 4\pi lwh/3 \quad (4)$$

Equivalent spherical diameter (ESD) was calculated from the estimated cell volume.

Pigments

Subsamples for intracellular pigment analyses (10–20 mL) were filtered onto 25 mm GF/F glass fiber filters (Whatman, USA) and preserved in the dark at -80°C until analysis. The filtered samples were fragmented using an ultrasonic disintegrator (SMT, Japan) with 2 mL of 90% acetone, after which the pigments on the filter were extracted for 24 h in the dark at -20°C . The extracted liquids were filtered through a filter with a pore size of $0.2 \mu\text{m}$ (Millex, Millipore, Germany). Subsequently, the filtered extracts were assayed via high-performance liquid chro-

matography (HPLC, Beckman, USA: 168 Diode Array Detector, C18 reversed phase Ultrasphere 3- μ m column) using a solvent gradient system described by Head & Horne (1993). Integrated HPLC peak areas were quantified using standards for chlorophyll *a* (chl *a*), chlorophyll *c* (chl *c*), peridinin (perid), diadinoxanthin (DD), diatoxanthin (DT), and β -carotene (β -Caro) obtained from the International Agency for 14C determination.

Light absorption

Subsamples for light absorption determinations (10–20 mL) were filtered onto 25-mm GF/F glass fiber filters (Whatman, USA) and preserved in the dark at -80°C until analysis (Sosik 1999). Light absorption was measured using a dual beam UV-Vis spectrophotometer (Shimadzu, UV-2450, Japan) following the quantitative filtered technique (QFT) method described by Mitchell & Kiefer (1988). A blank filter that was wetted with filtered seawater was used as a reference. Absorption spectra were recorded every nm from 250–800 nm. The absorption spectra were corrected by the average optical density at 730–760 nm (Babin & Stramski 2002). For the conversion of the corrected optical density of the filtered total particles (OD_f) to the particles in suspension (OD_s), the following general equation was used (Cleveland & Weideman 1993):

$$OD_s(\lambda) = 0.378OD_f(\lambda) + 0.523 (OD_f(\lambda))^2 \quad (5)$$

The optical density of the suspension (OD_s) was transformed into the absorption coefficient of particles in sample (a_p) using the following equation:

$$a_p(\lambda) = 2.303OD_s(\lambda)SV^{-1} \quad (6)$$

where 2.303 converts from decimal to natural logarithm, S is the filtered clearance area and V is the filtered volume. After taking OD_f measurements, the pigments on the filter were extracted with 100% methanol for 24 h (Kishino et al. 1985). Then, the de-colored filters were moistened with filtered sea water and the optical density of the non-pigmented particles (a_d) was measured using a spectrophotometer (Shimadzu, UV-2450, Japan). The absorption coefficients of detritus were calculated from the OD_f values of the de-colored filter. The difference between the a_d and a_p values was considered to be the light absorption coefficient of phytoplankton (a_{ph}),

$$a_{ph}(\lambda) = a_p(\lambda) - a_d(\lambda) \quad (7)$$

Finally, a_{ph} was converted to chl *a* specific absorption coefficient (a_{ph}^* ; $\text{m}^2 [\text{mg chl } a]^{-1}$) by dividing the a_{ph} value by the concentration of chl *a* ($\text{mg chl } a \text{ m}^{-3}$),

$$a_{ph}^*(\lambda) = a_{ph}(\lambda) / \text{chl } a \quad (8)$$

The spectral average chl *a* specific absorption coefficient [$\bar{a}_{ph}^*(\lambda)$] was calculated by integrating the absorption wavelengths measured from 400–700 nm and dividing by the number of wavelengths measured:

$$\bar{a}_{ph}^*(\lambda) = \frac{\int_{400}^{700} a_{ph}^*(\lambda) d(\lambda)}{\int_{400}^{700} d(\lambda)} \quad (9)$$

The absorption spectra of cells [$a'_{ph}(\lambda)$] were reconstructed at 2-nm intervals from 400–750 nm using the method described by Bidigare et al. (1990) and calculated using the following equation:

$$a'_{ph}(\lambda) = \sum_{i=1}^n C_i a_i^*(\lambda) \quad (10)$$

where C_i is the HPLC-determined concentration of pigment group *i* at the mixing ratios in the mixing experiments (mg m^{-3}) and $a_i^*(\lambda)$ is the specific absorption coefficient of pigment group *i* at wavelength λ ($\text{m}^2 \text{ mg}^{-1}$).

The homogeneity of the cells should be considered as uniformly packed by pigments (Morel & Bricaud 1981). Large cells contain large amounts of photosynthetic pigments and are subject to the “package effect” (Berner et al. 1989). The absorption efficiency of cell *i* at a given wavelength [$a_{ph}(\lambda)$] in a suspension with a specific number of cells (N) for a suspension with a specific volume (V) is approximated by the following equation (11),

$$a_{ph}(\lambda) = \frac{1}{V} \sum_{i=1}^N Q_a(\lambda) i G_i \quad (11)$$

where $Q_a(\lambda)_i$ is the absorption efficiency for a cell *i* at wavelength (λ) (Bricaud et al. 1983) and G_i is the geometric cross-sectional area of cell *i* (Iturriaga & Siegel 1989). The fractional reduction of pigment absorption due to the package effect [$Q_a^*(\lambda)$] can be calculated for a given wavelength as the quotient of $a_{ph}(\lambda)$ and $a'_{ph}(\lambda)$ using the following equation (12) (Nelson & Prezelsin 1990),

$$Q_a^*(\lambda) = \frac{a_{ph}(\lambda)}{a'_{ph}(\lambda)} \quad (12)$$

Simultaneously, the $a'_{ph}(\lambda)$ can be obtained from the $a_{ph}(\lambda)$ of the ethanol extraction (Jeffery et al. 2011, Fujiki & Taguchi 2002).

Statistical analysis

Statistical differences in the variables at a given wavelength between treatments were analyzed using an ANOVA.

Results

Cell density and volume

The experimental cells used in this study were prepared from rapidly growing *Prorocentrum micans* (0.30 d^{-1}) and *Prorocentrum minimum* (0.45 d^{-1}). When the cell density of *P. micans* and *P. minimum* on day 2 reached a similar density, 4.52 ± 0.595 and $4.507 \pm 0.542 \times 10^6$ cells mL^{-1} , respectively (Fig. 1A), the cell volume of *P. micans* ($41.1 \pm 4.6 \times 10^6 \mu\text{m}^3 \text{ mL}^{-1}$) was approximately 8-fold great-

er than that of *P. minimum* ($4.89 \pm 0.75 \times 10^6 \mu\text{m}^3 \text{mL}^{-1}$) (Fig. 1B), resulting in a 2-fold difference in the ESD (Fig. 1C). The mixing ratios of *P. minimum* based on the cell volume were 0% (mixing experiment 1), $23 \pm 2\%$ (mixing experiment 2), $48 \pm 5\%$ (mixing experiment 3), $75 \pm 4\%$ (mixing experiment 4), and 100% (mixing experiment 5).

Pigments

Six pigments (Chl *a*, chl *c*, perid, DD+DT, and β -caro) were detected from *Prorocentrum micans* and *Prorocentrum minimum*. The chl *a* content per cell ($10.3 \pm 2.56 \text{ pg cell}^{-1}$) of *P. micans* was approximately 4.0-fold higher than that of *P. minimum* ($2.56 \pm 0.30 \text{ pg cell}^{-1}$), while the

total pigment content of *P. micans* ($21.6 \pm 2.1 \text{ pg cell}^{-1}$) was approximately 3.5-fold greater than that of *P. minimum* ($6.26 \pm 0.53 \text{ pg cell}^{-1}$). The (total and chl *a*) pigment content per cell (5.82 ± 0.76 and $2.39 \pm 0.37 \text{ kg m}^{-3}$) for *P. minimum* were approximately 2.1- and 2.4-fold higher than those observed for *P. micans* (2.38 ± 0.31 and $1.14 \pm 0.13 \text{ kg m}^{-3}$), respectively (Fig. 2). The relative abundance of (total and chl *a*) pigment content per cell [C_i (kg m^{-3})] for *P. micans* and *P. minimum* were not significantly different between the different mixing ratios. The photosynthetic pigments (chl *a* and chl *c*) accounted for 56.6–59.4% of total cellular pigments. The accessory pigments (perid) accounted for 22.4–28.5% of cellular total pigments, while the other photoprotective pigments (DD+DT and β -caro) accounted for 13.7–19.4% of the pigment content per cell (Fig. 2).

Light absorption

The absorption spectrum [$a_{\text{ph}}(\lambda)$] of *Prorocentrum micans* and *Prorocentrum minimum* showed two absorption maxima at approximately 440 and 676 nm and decreased with mixing (Fig. 3A). There was a slight difference in the absorption spectrum between 400 and 460 nm, *i.e.*, the absorption in *P. minimum* was lower than that in *P. micans*. The highest absorption was observed for mixing experiment 1, whereas the lowest absorption was observed for mixing experiment 5 (Fig. 3B). However, the a_{ph}^* value was not significantly different between the five mixing ratios assayed in this study (Fig. 3C). The $a_{\text{ph}}(\lambda)$ values at 9 wavelengths (412, 440, 480, 510, 532, 555, 650, 676, and 700 nm) were significantly associated with cell volume ($\mu\text{m}^3 \text{mL}^{-1}$) (Fig. 4A), chl *a* concentration (mg m^{-3}) (Fig. 4B), and intracellular pigment content, C_i (kg m^{-3}) (Fig. 4C).

Spectrally reconstructed absorption spectra from HPLC-determined pigment concentrations [$a'_{\text{ph}}(\lambda)$] and measured *in vivo* absorption spectra [$a_{\text{ph}}(\lambda)$] ranged from the highest values at 440 nm to the lowest values at 700 nm

Fig. 1. Total cell density (A), cell volume (B), and ESD (C) in 5 mixtures of *Prorocentrum micans* (gray bar) and *Prorocentrum minimum* (black bar).

Fig. 2. Abundances of chl *a*, chl *c*, perid, DD+DT, and β -caro in *Prorocentrum micans* and *Prorocentrum minimum* based on intracellular pigment contents (C_i) in 5 mixtures of *P. micans* and *P. minimum*.

in the five mixing ratios. *Prorocentrum micans* alone exhibited the highest values, whereas *P. minimum* alone exhibited the lowest values in all mixing combinations. The $Q_a^*(\lambda)$ values were lower than unity at all mixing ratios assayed, indicating an overestimation of absorption spectra

Fig. 3. Normalized light absorption of cells (a_{ph}) at 676 nm (A), light absorption [$a_{ph}(\lambda)$] (B), and chl *a* specific absorption [$a_{ph}^*(\lambda)$] (C) in 5 mixtures of *Prorocentrum micans* and *Prorocentrum minimum*.

by reconstruction and resulting in the package effect at the 9 wavelengths, although the values were highly variable (Fig. 5A). The fractional reduction in the pigment absorption due to $Q_a^*(\lambda)$ exhibited a significant relationship with the 9 measured wavelengths ($p < 0.01$, Fig. 5B). The smallest package effects were observed at 676 nm, whereas the largest effects were observed at 440 nm.

Discussion

The non-sphericity of many phytoplankton cells has been well documented (Jonasz 1987). However, spherical approximations have often been used in field studies (e.g. Nelson et al. 1993) despite there being little detailed information on the shapes of cells in the samples. General meth-

Fig. 4. Light absorption [$a_{ph}(\lambda)$] at 412, 440, 480, 510, 532, 555, 650, 676, and 700 nm versus total cell volume (A), chl *a* concentrations (B), and intracellular chl *a* contents (C).

ods used to calculate the absorption properties of various shapes of cells have been developed by Kirk (1976). The spherical approximation method has proved to be satisfactory for cultures covering a wide variety of phytoplankton taxa (Morel & Bricaud 1981, Sathyendranath et al. 1987, Bricaud et al. 1988, Nelson & Prezelin 1990, Nelson et al. 1993), and the homogeneity of microalgal cells is ubiquitously determined among chronological groups (Jeffrey et al. 2011).

The light absorption of an algal population [$a_{ph}(\lambda)$] varies with cell size, although they have similar pigment concentrations (Morel & Bricaud 1981). Increasing cell volume facilitates pigment self-shading and a decrease in a_{ph}^* , which is estimated using the intracellular chl a contents (Kirk 1976, Finkel 2001). However, the a_{ph}^* value at a given wavelength was not significantly different between the five mixing ratios assayed in this study. When the proportion of large cells was increased, the packaging effect caused by the cell volume may facilitate a decrease in a_{ph}^* . In the present study, opposite trends in the relationship between cell volume or C_i values and the mixing ratios may cancel the package effect. Due to a combination of these two vari-

ables, the observed a_{ph}^* did not indicate significantly different effects in the mixing ratios used for the two species, e.g., 0.0112 ± 0.0006 and $0.0122 \pm 0.0023 \text{ m}^2 (\text{mg chl } a)^{-1}$, for *Prorocentrum micans* and *Prorocentrum minimum* alone, respectively. The results of the present study do not indicate any significant change in the a_{ph}^* value within the *ESD* size range evaluated in this study (Fig. 1C).

However, as wider ranges of cell sizes occur *in situ* in the ocean, the relationship between cell volume and the a_{ph}^* value can be affected by the pigment content per unit cell volume and pigment composition (Kirk 1975, Morel & Bricaud 1981). When light absorption of chl a [$a_{ph}^*(676)$] is compared with previously published data (Fujiki & Taguchi 2002, Leong & Taguchi 2006), it is significantly related to $ESD \cdot C_i$ ($p < 0.01$, Fig. 6A). The estimates obtained in the present study are observed at the far-left end of the regression line because of the relatively large size of the studied cells compared to those in other studies (Fujiki & Taguchi 2002, Leong & Taguchi 2006). The spectral package effect of (Q_a^*) at 676 nm, based on the same sets of data shown in Fig. 6A, are significantly correlated with $ESD \cdot C_i$ ($p < 0.01$, Fig. 6B), suggesting lower package effects at smaller $ESD \cdot C_i$ values. However, this result sug-

Fig. 5. Light absorption efficiency [$Q_a^*(\lambda)$] at 412, 440, 480, 510, 532, 555, 650, 676, and 700 nm for the 5 mixtures of *Prorocentrum micans* and *Prorocentrum minimum* (A) and wavelength (B).

Fig. 6. Relationship between light absorption [$a_{ph}^*(676)$] (A) or light absorption efficiency [$Q_a^*(676)$] and $ESD \cdot C_i$ (B). The open circle, reversed closed triangle, open triangle, closed square, open square, and closed diamond represent *Isochrysis galbana*, *Pleurochrysis carterae*, *Chaetoceros gracilis*, *Dunaliella tertiolecta*, *Thalassiosira weissflogii*, and *Coscinodiscus* sp., respectively (Fujiki & Taguchi 2002). The open diamond represents *Alexandria tamarensis* (Leong & Taguchi 2006), and the closed circle represents the present study.

gests that most estimates of a_{ph}^* are subject to the package effect, particularly in coastal waters. Cell volume should be considered for quantitative studies on the relationship between cell size and spectral light absorption. One possible application of the present study would involve intensive HPLC profiling, which provides chl *a* specific absorption, a_{ph}^* and size distribution (Brewin et al. 2011), combined with direct measurements of phytoplankton absorption using QFT methods (Mitchell & Kiefer 1988) or *in situ* spectrophotometry (Johnsen et al. 1994) to estimate the spectral $Q_a^*(\lambda)$. This approach would allow for correctly reconstructed spectra to be obtained for use in modeling absorption-based bio-optical productivity models in the ocean, although some models are successfully employed without using the absorption (e.g., Siegel et al. 1989). In particular, the addition of information on the package effect would improve current models.

Prorocentrum minimum and *P. micans* are primarily observed in coastal waters in temperate and tropical regions (Kimor et al. 1985, Smayda 1990, Grzebyk et al. 1997) blooming together and consistently being present in the late summer and fall (Horner 2002, Kraberg et al. 2010) in lit, shallow surface waters (Kimor et al. 1985). However, they occasionally reach higher than 30 mg chl *a* m⁻³ at the subsurface chlorophyll maximum (Pingree et al. 1982). Under field conditions, both species show a lower package effect than other groups of phytoplankton as the $Q_a^*(676)$ of the two species is larger than approximately 0.5, as shown in the present study.

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