

# Chromoprotein- and pigment-dependent modeling of spectral light absorption in two dinoflagellates, *Prorocentrum minimum* and *Heterocapsa pygmaea*

Geir Johnsen\*, Norman B. Nelson, Raffael V. M. Jovine, Barbara B. Prézelin

Department of Biological Sciences and The Marine Science Institute, University of California, Santa Barbara, California 93106, USA

**ABSTRACT:** Pigment- and chromoprotein-dependent spectral models, designed to accurately reconstruct whole cell absorption spectra for photosynthetic dinoflagellates, were assessed. Measured spectral absorption properties (400 to 700 nm) included signatures from whole cells, dispersed thylakoid fragments (unpacked absorption), isolated chromoproteins and individual pigments from high ( $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and low ( $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) light-adapted cells of the dinoflagellates *Prorocentrum minimum* and *Heterocapsa pygmaea* grown in continuous light at 15 °C. For model verification, we also developed a procedure to measure unpackaged cell absorption, free of solvent and light-scattering effects. Maximum measured chl *a*-specific absorption at 675 nm appears to be closer to 0.027 than a predicted value of  $0.0203 \text{ m}^2 \text{ mg}^{-1}$  chl *a* based on absorption from chl *a* in 90% acetone. The percent fractional absorption of 'in vivo' weight-specific absorption coefficients of individual pigments relative to total weighted absorption (all pigments) was estimated to indicate the light-harvesting capabilities of the different pigments as a function of photoadaptive status and water color. Correspondingly, the weighted absorption of each pigment fraction has been estimated in theoretical white light and in 'clearest' green coastal and blue oceanic waters. Independent of water color, peridinin was by far the most important light-harvesting pigment, followed by chl  $c_2$  and chl *a*. The photoprotective diadinoxanthin absorbed most efficiently in the blue part of the visible spectrum. Results indicate that the chromoprotein model (1) overcame spectral distortions inherent in more general pigment-dependent models and, when combined with corrections for pigment packaging effects, (2) provided accurate spectral estimates of *in vivo* absorption coefficients and (3) worked equally well for dinoflagellate species with or without the major light-harvesting peridinin-chlorophyll-protein complex, PCP. Findings are discussed in the context of modeling of bio-optical characteristics in dinoflagellates, their photoecology and implications for the *in situ* optical monitoring of red tides.

**KEY WORDS:** Modeling of *in vivo* light absorption · Pigments · Chromoproteins · Fractional absorption of pigments · Measured and modeled package effect · Photoadaptation · Dinoflagellates

## INTRODUCTION

Many dinoflagellate species form toxic and/or anoxic blooms (red tides) which have a negative impact on fisheries, water quality, tourism and human health (Smayda 1990). The global frequency of red tides

appears to be increasing and the rise may be related to increasing organic pollution of coastal waters, estuaries and wetlands (Bjergskov et al. 1990, Smayda 1990, Smayda & White 1990). The characteristic brick-red color of most red tides is due to photosynthetic pigments, most notably the unique dinoflagellate carotenoid peridinin. Information on the regional dynamics of red tides, including their distribution, abundance, photo-physiological state and possible rates of production, can be derived from knowledge of their *in vivo*

\*Present address: Trondhjem Biological Station, The Museum, University of Trondheim, Bynesveien 46, N-7018 Trondheim, Norway

light absorption properties (Prézélin 1987). In the case of bio-optical *in situ* measurements (e.g. moorings) or remote sensing, one also needs to consider the optical properties of the water itself, dissolved colored matter, detritus and phytoplankton (Kirk 1992, Johnsen et al. 1994). The main goal of the present study was to quantify the *in vivo* spectral absorption properties of 2 red tide dinoflagellates and to determine the relationship between their whole cell optical properties and their pigment and chromoprotein (pigment-protein complexes) composition. With such knowledge, predictive bio-optical algorithms for natural red tides might be developed successfully.

Chlorophyll *a* (chl *a*)-specific *in vivo* absorption is an important parameter for calculation of the total amount of light absorbed and utilized by phytoplankton. However, there are methodological problems regarding direct measurements of *in vivo* absorption, caused by particle scattering and absorption by detritus. One way to avoid this problem, and thus making laboratory approaches workable in field situations, is to reconstruct *in vivo* absorption on the basis of pigment and chromoprotein composition (Bidigare et al. 1987, 1990, Nelson & Prézélin 1990). There is more than one approach to reconstruct phytoplankton absorption, and the accuracy of results depends on the approach chosen. One approach to modeling *in vivo* light-absorption characteristics is to reconstruct '*in vivo*' weight-specific absorption coefficients (400 to 700 nm) of the different major pigments on the basis of *in vitro* spectra in organic solvents (Bidigare et al. 1987, 1989, 1990). Such spectra are scaled by using their respective weight-specific absorption coefficients in a given organic solvent. The problem is that organic solvents cause spectral shifts relative to the corresponding *in vivo* characteristics where the pigments are embedded in proteins (chromoproteins). To correct for this difference, the *in vitro* absorption spectra have been empirically spectrally shifted to mimic corresponding *in vivo* absorption peaks (Bidigare et al. 1987). An alternate approach is based on '*in vivo*' weight-specific absorption coefficients (400 to 700 nm) of isolated chromoproteins (Nelson & Prézélin 1990). The spectral absorption coefficients of the chromoproteins differ from the pigment-based coefficients, because the protein attachments to the pigments alter both absorption peaks and shoulders as well as the absorption coefficients. We present here 2 models based on '*in vivo*' absorption coefficients of pigments and chromoproteins which can discriminate between photosynthetic and photoprotective pigments. This exercise is important for the evaluation of different approaches one might choose to estimate total light absorption and light utilization for photosynthesis (Bidigare et al. 1992), identification of phytoplankton-class specific optical characteristics

(Johnsen et al. 1994), and modeling the impact on underwater light fields by red tide dinoflagellates (Kirk 1992).

Lastly, the predictive accuracy of the above approaches would be lost if no account of pigment packaging was considered. The package effect refers to the reduction of the light absorption of a suspension of pigmented particles (e.g. living phytoplankton cells) relative to that of the same amount of pigments in solution (dispersed thylakoid fragments = thylakoid micelles = 'unpacked absorption'; Kirk 1983). To model this effect, possibly common in highly concentrated populations (bloom conditions), measurements of unpacked absorption are first necessary for quantitative comparison of the fractional absorption of the different light-harvesting components. Estimates of unpacked absorption have been made earlier by measuring absorption in suspensions of disrupted cells by the use of ultrasonication or passage of cells through an X-press pressure cell (Kirk 1983, Geider & Osborne 1987), by detergent solubilization (Berner et al. 1989, Sosik & Mitchell 1991), or by theoretical calculations based on *in vivo* absorption and cell dimensions (Morel & Bricaud 1981, Sathyendranath et al. 1987). In the present study we introduce a new and apparently improved method for measuring unpacked absorption, free of particle-scattering and solvent-induced spectral shifts.

## MATERIAL AND METHODS

**Culture conditions.** The Provasoli-Guillard Center for Culture of Marine Phytoplankton (CCMP) provided a culture of *Prorocentrum minimum* (Pavillard), Schiller strain CCMP 1329 ('EXUV'). *Heterocapsa pygmaea* (Loeblich et al. 1981) was originally obtained from a subculture in the collection at Scripps Institution of Oceanography (SIO code PY-33, a.k.a. *Glenodinium* sp., L. Provasoli, M. Bernard strain) and maintained in culture at the University of California, Santa Barbara (UCSB code 5M29). A subculture of UCSB code 5M29 has been relocated to the CCMP, where it is designated CCMP1322.

Culture conditions were similar to those used by Johnsen & Sakshaug (1993). Cells were grown in Fernbach flasks containing 2.0 l of seawater with F/2 enrichment (Guillard & Ryther 1962) and incubated at 15°C. Continuous 'white' light was provided by a rack of 12 Philips F5 T8/Cool White fluorescent tubes (15 W). Once a day, cells were gently mixed. Scalar irradiances ( $E_0$ , 400 to 700 nm, PAR) were 35  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (low light, LL) and 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (high light, HL). Experimental measurements were made with exponentially growing cultures that had been adapted

for several generations by serial dilution with fresh media.

**Pigments.** Pigments were extracted in acetone (Johnsen & Sakshaug 1993), and chl *a* concentration was estimated using the chromophyte equation of Jeffrey & Humphrey (1975) for quantification of chl *a* in 90% acetone. Optical density was recorded on a DW-2000 (SLM-Aminco Inc., Urbana, IL, USA) spectrophotometer operated in the split-beam mode. The pigment composition (including chl *a*) of whole cells was quantified by high performance liquid chromatography (HPLC) according to Johnsen & Sakshaug (1993). Refiltered pigment extracts were injected into a Merck & Hitachi L-6200 HPLC pump equipped with a SPHERI RP-18 column (Brownlee Labs). Detection was performed in a Hitachi U-2000 spectrophotometer (all pigments) and a Hitachi F-3000 spectrofluorometer (chlorophylls only). *In vitro* absorption spectra (350 to 800 nm) of major pigments (chl *a*, chl *c*<sub>2</sub>, peridinin and diadinoxanthin) were measured by the HPLC stop-flow technique (Johnsen et al. 1992) using a Hitachi U-2000 spectrophotometer. Published extinction coefficients of the individual pigments were used for calibration of the HPLC column.

***In vivo* absorption, corrected for scattering.** Absolute absorption coefficients (see Table 1 for symbols) of whole cell suspensions were determined using the DW-2000 spectrophotometer equipped with an integrating sphere with an internal and centrally located sample holder custom built for the DW-2000 by Lab-sphere, Inc. The integrating sphere neutralizes wavelength-dependent scattering which causes errors with conventional methodology. Calibration and performance properties of the sphere have been detailed elsewhere (Nelson & Prézelin 1993). When using the sphere, the spectrophotometer was operated in the dual wavelength mode with a reference wavelength of 750 nm. Measured optical densities were corrected for absorption amplification and converted to chl *a*-specific absorption coefficients,  $a^*_{ph}(\lambda)$ , following procedures described in Nelson & Prézelin (1993).

**Measured unpacked absorption.** We devised an approach which disrupted thylakoid membranes in a sucrose buffer into stable micelles. The micelles contained all cellular chromoproteins and were devoid of cell debris. To avoid chromoprotein degradation, all sample handling was done in dim light at 0 to 4°C. Cells were harvested by 10 min centrifugation in a Sorvall Superspeed RC2-B refrigerated centrifuge at  $13500 \times g$ . The pellet was quick frozen in liquid nitrogen for 3 min and stored at -70°C. Frozen pellets were thawed in HEPES buffer (50 mM HEPES pH 8, 200 mM sucrose, 250 mM NaCl, 14 mM MgCl<sub>2</sub>) and disrupted by 3 passes through a French press at  $8.3 \times 10^7$  Pa. Cell breakage of >99% was confirmed micro-

scopically. The recovered slurry was loaded onto an 80% sucrose cushion and centrifuged at  $12000 \times g$  for 20 min. Cell debris was found as a pellet in the sucrose cushion, while the supernatant contained >95% of the sample chl *a*. Gel electrophoresis, HPLC of extracted pigments, and fluorescence excitation techniques were used to confirm that the supernatant contained intact chromoproteins, no free pigments and that pigment ratios were identical to those for pigment extracts from whole cells (data not shown).

No light scattering in the micelle preparation was detectable in the visible spectrum. We define the light absorption properties of these dispersed micelles as 'unpacked' absorption [ $a^*_{mu}(\lambda)$ , m<sup>2</sup> mg<sup>-1</sup> chl *a*; Table 1]. Absorption spectra of the clear supernatant were measured in a 1 cm quartz cuvette using the SLM Aminco DW-2000 spectrophotometer operating in the split-beam mode. Determination of spectral shifts due to solvent effects and variations in absorption coefficients was done by taking absorption spectra of these micelles before and after extraction in 90% acetone (400 to 700 nm).

**Individual chromoproteins and their unpacked absorption coefficients.** The chl *a*-specific absorption coefficients of isolated chromoproteins [ $a^*_c(\lambda)$ , m<sup>2</sup> mg<sup>-1</sup> chl *a*] of *Prorocentrum minimum* and *Heterocapsa pygmaea*, i.e. chl *a*-chl *c*<sub>2</sub>-peridinin-protein (ACP), photosystem (PS) I, PS II and peridinin-chl *a*-protein (PCP) were isolated and quantified by the use of dodecyl maltoside solubilization of thylakoid membranes and sucrose gradient centrifugation (modified from Hiller et al. 1993, Iglesias-Prieto et al. 1993). Absorption measurements of these chromoproteins in HEPES buffer [ $a^*_c(\lambda)$ ] were made in quartz cuvettes using the DW2000 spectrophotometer in split-beam mode. The mean chl *a*-specific absorption coefficients for ACP, PCP and PS I ( $a^*_c$ , 673 to 680 nm) were used for scaling of the corresponding mean *in vivo* weight-specific absorption coefficients for these chromoproteins used in the model (Figs. 1 & 2B). Since we only had small amount of PS II, no determination of [chl *a*] was made. We therefore assumed the  $a^*_c$  for PS II at 676 nm to be 0.020 m<sup>2</sup> mg<sup>-1</sup> chl *a* (same value as chl *a* in 90% acetone).

**Individual pigments and their unpacked absorption coefficients.** Absorption spectra of alcohol-extracted and purified chl *c*<sub>2</sub>, peridinin and diadinoxanthin were spectrally shifted to match the absorption peaks and shoulders observed *in vivo* using the approach described by Bidigare et al. (1990). Then, the resulting pigment spectra were scaled to their respective weight-specific absorption coefficients to give *in vivo* weight-specific absorption coefficient spectra of the individual pigments [ $a^*_i(\lambda)$ , m<sup>2</sup> mg<sup>-1</sup>; Table 2, Figs. 1 & 2A]. The *in vivo* weight-specific absorption coefficients

Table 1. Definitions of bio-optical nomenclature and references to their use in equations in the text

$a^*_i(\lambda)$	Measured specific absorption coefficient of individual pigment ( $\text{m}^2 \text{mg}^{-1}$ , 400 to 700 nm, PAR). Eq. (1)
$a^*_c(\lambda)$	Measured specific absorption coefficient of individual chromoproteins ( $\text{m}^2 \text{mg}^{-1}$ chl a, PAR). Eq. (1)
$a^*_{ph}(\lambda)$	Measured chl a-specific absorption coefficient of phytoplankton (= <i>in vivo</i> , packed absorption; $\text{m}^2 \text{mg}^{-1}$ chl a, PAR). cf. Eq. (3)
$a_{ph}(\lambda)$	Absorption coefficient of phytoplankton ( $\text{m}^{-1}$ , PAR). Eq. (1)
$a'_{ph}(\lambda)$	Reconstructed absorption coefficient of phytoplankton ( $\text{m}^{-1}$ , PAR). Eq. (1)
$a^*_{mu}(\lambda)$	Measured unpacked absorption coefficient (micelles of dispersed thylakoid fragments; $\text{m}^2 \text{mg}^{-1}$ chl a, PAR). Eq. (5)
$a^*_{pu}(\lambda), a^*_{pp}(\lambda)$	Modeled unpacked [ $a^*_{pu}(\lambda)$ ] and packed [ $a^*_{pp}(\lambda)$ ] absorption based on the pigment model (p). ( $\text{m}^2 \text{mg}^{-1}$ chl a, PAR). Eqs. (1 to 6)
$a^*_{cu}(\lambda), a^*_{cp}(\lambda)$	Modeled unpacked [ $a^*_{cu}(\lambda)$ ] and packed [ $a^*_{cp}(\lambda)$ ] absorption based on the chromoprotein model (c). ( $\text{m}^2 \text{mg}^{-1}$ chl a, PAR). Eqs. (1 to 6)
$\bar{a}^*_\lambda$	Spectrally weighted (400 to 700 nm) chl a-specific absorption of phytoplankton ( $\text{m}^2 \text{mg}^{-1}$ chl a): x denotes measured unpacked ( $\bar{a}^*_{mu}$ ) and packed (= <i>in vivo</i> , $\bar{a}^*_{ph}$ ) absorption. Modeled packed (p) and unpacked (u) absorption; pigment-model ( $\bar{a}^*_{pu}, \bar{a}^*_{pp}$ ) and chromoprotein model ( $\bar{a}^*_{cu}, \bar{a}^*_{cp}$ ). Eq. (7)
$\bar{a}^*_{AF}$	Spectrally weighted fractional absorption of individual pigments ( $\text{m}^2 \text{mg}^{-1}$ pigment, PAR). Eqs. (2 & 7)
$AF_i(\lambda)$	Fractional absorption of individual pigment <i>i</i> ( $\text{m}^2 \text{mg}^{-1}$ , PAR). Eq. (2)
$\rho'(\lambda)$	Absorption index (dimensionless). Eqs. (4 & 5)
$Q_a(\lambda)$	Cell absorption efficiency factor (dimensionless). Eqs. (3 to 6)
$Q^*_a(\lambda)$	Specific absorption efficiency (= package effect), dimensionless. Eq. (6)
$c_i$	Concentration of pigment <i>i</i> ( $\text{mg m}^{-3}$ ). Eq. (1)
$c_{chla}$	Concentration of cellular chl a ( $\text{mg m}^{-3}$ ). Eq. (5)
$d$	Cell diameter (spherical equivalent, $\mu\text{m}$ ). Eq. (5)
$G$	Geometrical cross-section of spherical cells ( $\text{m}^2$ ). Eq. (3)

for chl a (Bidigare et al. 1990) were originally based on measurement in 100% acetone (blue:red ratio of 1.30, absorption at 675 nm =  $0.020 \text{ m}^2 \text{mg}^{-1}$  chl a). The absorption coefficients of chl a (400 to 700 nm) were therefore multiplied by 1.33 in order to simulate the

maximum value for measured unpacked absorption at 675 nm, i.e.  $0.027 \text{ m}^2 \text{mg}^{-1}$  chl a, based on the values from measured unpacked absorption coefficients of dispersed thylakoid micelles and isolated chromoproteins (Figs. 2 & 3).

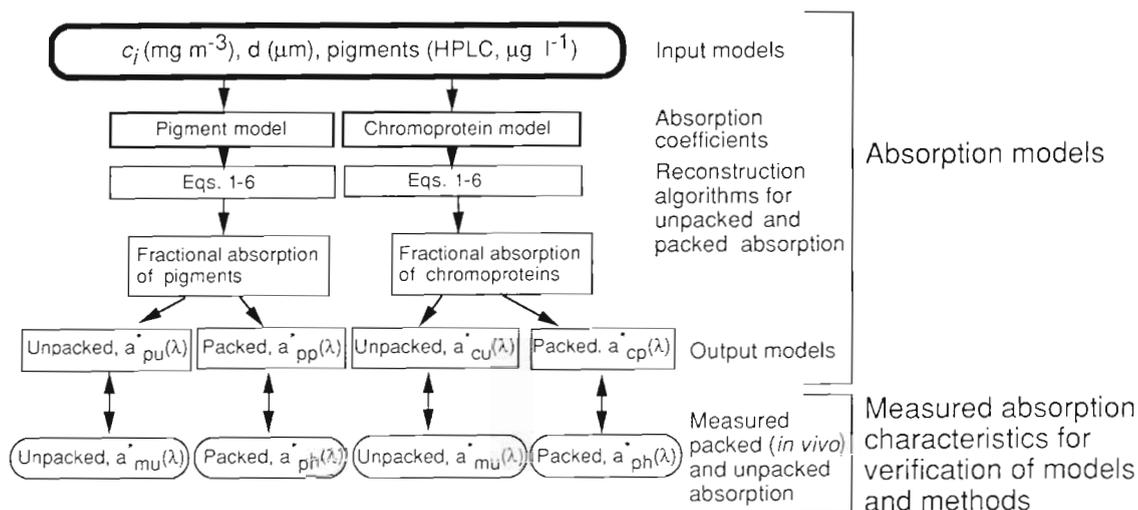


Fig. 1. Flow chart of the different methods and models for examination of unpacked and packed absorption characteristics in high and low light-adapted cells of *Prorocentrum minimum* and *Heterocapsa pygmaea*. Round- and square-cornered boxes are measured and modeled absorption, respectively (see 'Material and methods')

To model unpacked light absorption of whole cells of *Prorocentrum minimum*, the unpacked *in vivo* weight-specific absorption coefficients of individual pigments were used to reconstruct unpacked absorption spectra [ $a'_{ph}(\lambda)$ ,  $m^{-1}$ ; Figs. 1 & 2A]. This is based on  $a^*_i(\lambda)$  and their volume-based concentrations ( $c_i$ ,  $mg\ m^{-3}$ ; cf Bidigare et al. 1987, Nelson & Prézélin 1990 and Table 1):

$$a'_{ph}(\lambda) = \sum_{i=1}^n a^*_i(\lambda) c_i \quad (1)$$

and  $a^*_{ph}(\lambda) = a'_{ph}(\lambda)/chl\ a$  ( $m^2\ mg^{-1}\ chl\ a$ ).

**Fractional absorption of pigments.** Fractional unpacked absorption [ $AF_i(\lambda)$ ] of the different *in vivo* weight-specific pigment absorption coefficients [ $a^*_i(\lambda)$ ] was scaled by multiplying the different pigment-specific absorption coefficients by the corresponding pigment  $i$  to chl  $a$  ratio (w:w) (Figs. 2A & 4):

$$AF_i(\lambda) = a^*_i(\lambda) \frac{[\text{pigment } i]}{[\text{chl } a]} \quad (2)$$

The sum of fractional absorption spectra of individual pigments yields the absorption spectrum of reconstructed unpacked absorption spectra (Figs. 3 & 4; Eqs. 1 to 5).

**Chromoprotein modeling.** The computation scheme for reconstruction of unpacked absorption spectra based on *in vivo* weight-specific absorption coefficients of different chromoproteins [ $a^*_{mu}(\lambda)$ ] is similar as for the reconstruction of unpacked *in vivo* absorption spectra (Eq. 1) from individual pigments  $a^*_i(\lambda)$ , except that the component spectra are different [ $a^*_c(\lambda)$ ; Table 1]. In addition,  $a^*_i(\lambda)$  for diadinoxanthin

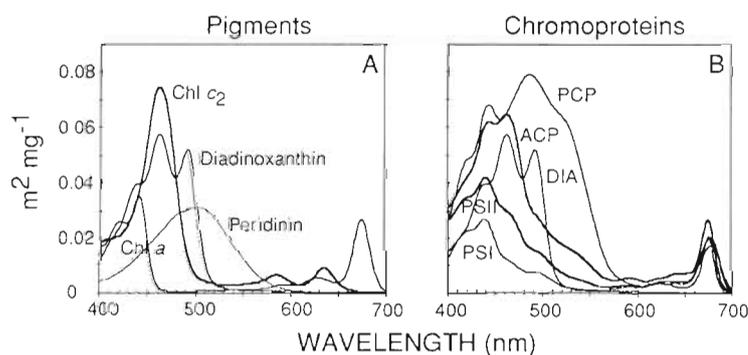


Fig. 2. (A) *In vivo* weight-specific absorption coefficients of the main pigments (chl  $a$ , chl  $c_2$ , peridinin and diadinoxanthin) in dinoflagellates. (B) Mean *in vivo* weight-specific absorption coefficients of chromoproteins isolated from low light-adapted cells of *Prorocentrum minimum* (PS I, PS II, ACP) and *Heterocapsa pygmaea* (PCP). The coefficients for diadinoxanthin are also included in the chromoprotein model for bio-optical modeling of high light-adapted cells (see 'Material and methods')

is included to model the impact of this photoprotective carotenoid in HL-adapted cells. The chl  $a$  concentration of each chromoprotein ( $mg\ m^{-3}$ ) was used for scaling of the different coefficients. Diadinoxanthin in the ACP chromoprotein used in the model comprised 5% of the total cellular pigment concentration per weight. When the cellular concentration of diadinoxanthin was higher than 5% of total pigments, absorption by the excess diadinoxanthin was approximated by the  $a^*_i(\lambda)$  diadinoxanthin from the pigment model.

**Modeling the package effect.** To model the package effect in LL- and HL-adapted cells we used an algorithm developed for homogeneous spherical cells (Morel & Bricaud 1981, Geider & Osborne 1987, Nelson & Prézélin 1990) (Fig. 1). This algorithm is derived from the concept that the absorption coefficient [ $a_{ph}(\lambda)$ ,

Table 2. Pigments and their respective wavelength shifts to mimic *in vivo* absorption spectra. The solvent for all pigments, except chl  $a$  (100% acetone), was from the HPLC eluent (60:40 methanol:ethyl acetate, v:v). The pigment-specific extinction coefficients ( $E$ , at their respective absorption maxima) were used to estimate *in vivo* weight-specific absorption coefficients of chl  $a$ , chl  $c_2$ , peridinin and diadinoxanthin ( $m^2\ mg^{-1}$ )

Pigments	Wavelength shifts (nm)		$E$ ( $l\ g^{-1}\ cm^{-1}$ )	Source for $E$
	$\lambda_{max}$ <i>in vitro</i>	$\lambda$ shift <i>in vivo</i>		
Chl $a^a$	662 (acetone)	>550: +14	88.2	Jeffrey & Humphrey (1975)
	432	<550: +8		
Chl $c_2$	632 (HPLC)	>550: +4	40.4	Jeffrey (1972)
	583	<550: +14		
	446			
Peridinin	475 (HPLC)	>375: +28	134.0	Jeffrey & Haxo (1968)
Diadinoxanthin	475 (HPLC)	>375: +15	250.0	Davies (1976)

<sup>a</sup>Modified *in vivo* absorption spectrum of chl  $a$  from Bidigare et al. (1990), absorption is assumed 1.33 times higher from 400 to 700 nm than originally reported (see 'Material & methods')

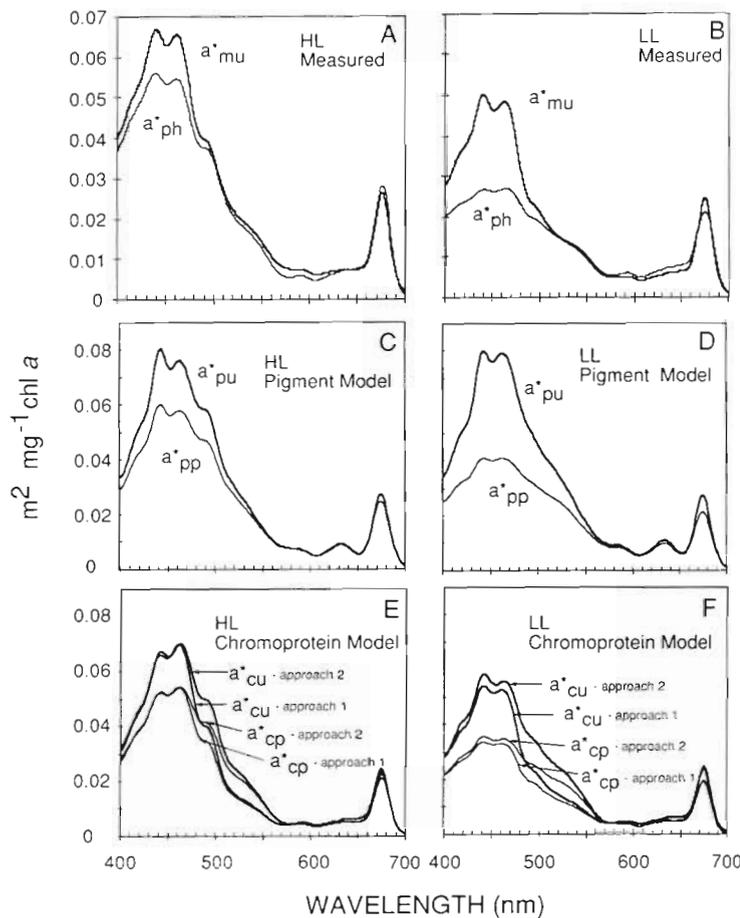


Fig. 3. *Prorocentrum minimum*. Measured and modeled unpacked and packed absorption spectra of high light (HL)- and low light (LL)-adapted cells. (A) Measured packed absorption [a<sup>\*</sup><sub>ph</sub>(λ)] and unpacked absorption [a<sup>\*</sup><sub>mu</sub>(λ)] in HL-adapted cells. (B) Same as (A), but in LL-adapted cells. (C) and (D) are modeled unpacked and packed absorption using the pigment model [a<sup>\*</sup><sub>pu</sub>(λ), a<sup>\*</sup><sub>pp</sub>(λ)] in HL- and LL-adapted cells, respectively. (E) and (F) are modeled unpacked and packed absorption using the chromoprotein model approach 1 and 2 [a<sup>\*</sup><sub>cu</sub>(λ), a<sup>\*</sup><sub>cp</sub>(λ)] in HL- and LL-adapted cells, respectively

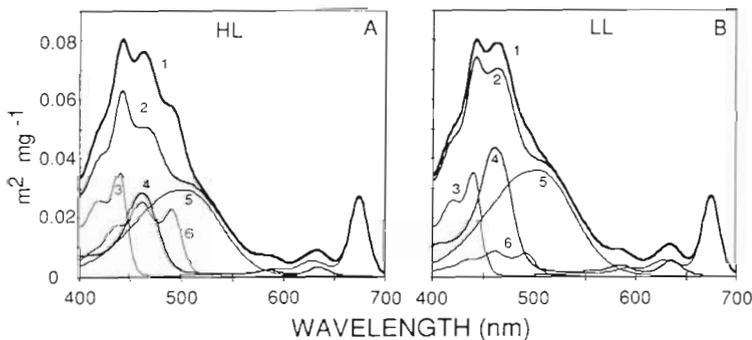


Fig. 4. *Prorocentrum minimum*. Fractional unpacked absorption (pigment model) of individual pigments and the effect of the photoprotective carotenoid diadinoxanthin in (A) high light- and (B) low light-adapted cells (cf. Eq. 2). 1. total pigments; 2. photosynthetic pigments (total pigments – diadinoxanthin); 3. chl a; 4. chl c<sub>2</sub>; 5. peridinin; 6. diadinoxanthin

m<sup>-1</sup>] of an algal suspension of identical cells is given by the product of the number of cells (N) in a given volume (V, m<sup>-3</sup>), the absorption efficiency factor [Q<sub>a</sub>(λ), dimensionless] and the geometric cross-section (G, m<sup>2</sup>; Table 1):

$$a_{ph}(\lambda) = \frac{N}{V} Q_a(\lambda) G \quad (3)$$

A model of Q<sub>a</sub>(λ) has been described by van der Hulst (1957) and Morel & Bricaud (1981):

$$Q_a(\lambda) = 1 + \frac{2e^{-\rho'(\lambda)}}{\rho'(\lambda)} + 2 \frac{e^{-\rho'(\lambda)} - 1}{\rho'^2(\lambda)} \quad (4)$$

where Q<sub>a</sub>(λ) is a function of the absorption index ρ'(λ) (dimensionless; Morel & Bricaud 1981). The absorption index is the product of the cell diameter (d, spherical equivalent, m) and a<sub>cm</sub>(λ) which, in turn, is the product of the intracellular chl a concentration (c<sub>chl a</sub>, mg m<sup>-3</sup> cell volume) and unpacked absorption a<sup>\*</sup><sub>mu</sub>(λ) (m<sup>2</sup> mg<sup>-1</sup>; Table 1):

$$\rho'(\lambda) = a^*_{mu}(\lambda) d c_{chl a} \quad (5)$$

The fractional reduction of absorption due to the package effect [Q<sup>\*</sup><sub>a</sub>(λ) = specific absorption efficiency, dimensionless] is computed as (Morel & Bricaud 1981):

$$Q^*_a(\lambda) = \frac{3}{2} \frac{Q_a(\lambda)}{\rho'(\lambda)} \quad (6)$$

Packed (*in vivo*) absorption spectra were computed as the product of modeled unpacked absorption and Q<sup>\*</sup><sub>a</sub>(λ) (Eqs. 4 to 6).

**Spectrally weighted absorption.** Spectrally weighted absorption ( $\bar{a}_x$ , 400 to 700 nm, m<sup>2</sup> mg<sup>-1</sup>) of the different types of absorption spectra,  $\bar{a}_x$ ,  $\bar{a}_{AF}$  [termed a(λ) in Eq. (7); Table 1] were calculated according to Morel et al. (1987):

$$\bar{a}_x = \int_{400}^{700} a(\lambda) E_o d\lambda / E_o(\text{PAR}) \quad (7)$$

For this computation, spectral irradiance for blue oceanic waters and green coastal waters at 20 m depth were taken from Johnsen et al. (1992). Theoretical white light (similar response from 400 to 700 nm) was used for comparison of 'weighted' absorption between measured unpacked and packed (= *in vivo*) and modeled unpacked and packed absorption using the pigment- and chromoprotein models.



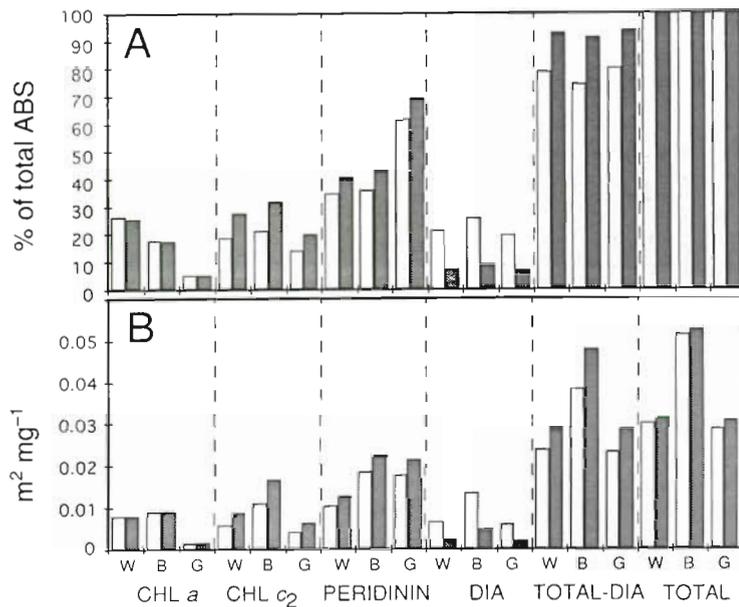


Fig. 6. *Prorocentrum minimum*. High light- (light colored bars) and low light- (dark colored bars) adapted cells. (A) % fractional unpacked absorption of individual pigments of total absorption in theoretical white light (W), clearest blue oceanic (B) and green coastal waters (G) at 20 m depth (spectral irradiances given in Johnsen et al. 1992). (B) Weighted absorption of unpacked individual pigments in white, blue and green waters. For further explanation see Eq. (2). DIA: diadinoxanthin; TOTAL-DIA: total pigments minus diadinoxanthin

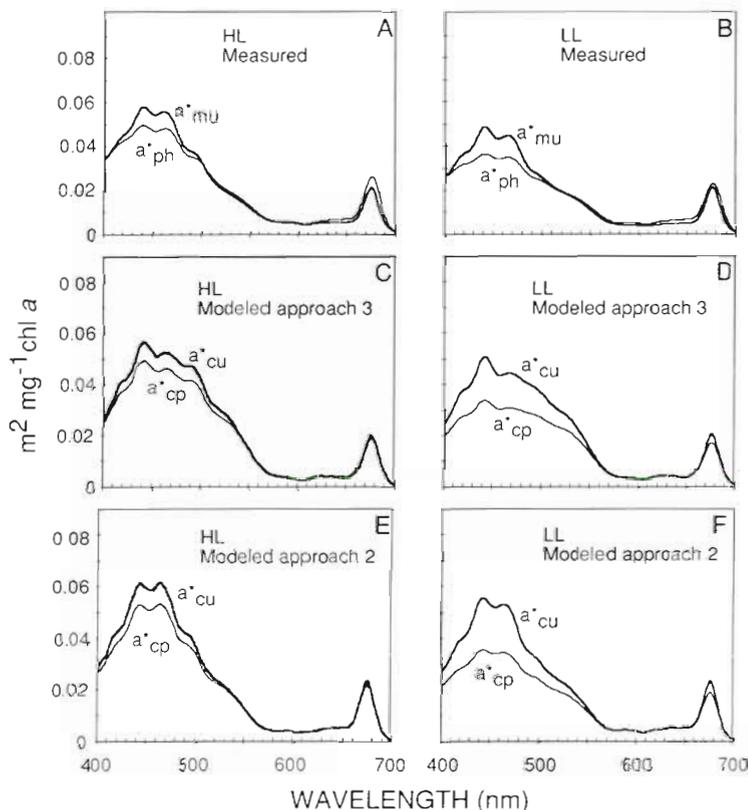


Fig. 7. *Heterocapsa pygmaea*. Measured and modeled unpacked and packed absorption spectra of high light (HL)- and low light (LL)-adapted cells. (A) Measured packed absorption [ $a_{ph}^*(\lambda)$ ] and unpacked absorption [ $a_{mu}^*(\lambda)$ ] in HL-adapted cells. (B) Same as (A), but in LL-adapted cells. (C) and (D) is modeled absorption using the chromoprotein model [ $a_{cu}^*(\lambda)$ ,  $a_{cp}^*(\lambda)$ ] approach 3 in HL- and LL-adapted cells. (E) and (F) is modeled absorption using the chromoprotein model approach 2 in HL- and LL-adapted cells, respectively

415 to 420 nm (chl *a*), 440 nm (chl *a*, peridinin, diadinoxanthin), 460 nm (chl *c*<sub>2</sub>, peridinin, diadinoxanthin), 490 nm (diadinoxanthin, peridinin), 540 nm (peridinin), 590 nm (chl *c*<sub>2</sub>, chl *a*), 625 nm (chl *a*, chl *c*<sub>2</sub>), 635 nm (chl *c*<sub>2</sub>, chl *a*) and 675 nm (chl *a*) (Figs. 2 to 7). In *P. minimum* and *H. pygmaea*  $a_{ph}^*$  (675 nm) was 0.028 and 0.027 in HL-adapted cells, compared to 0.021 and 0.023  $m^2 mg^{-1}$  chl *a* in LL-adapted cells, respectively (Figs. 3 & 7).

#### Measured unpacked absorption

The locations of peaks and shoulders given in  $a_{mu}^*(\lambda)$  (measured unpacked absorption) in *Prorocentrum minimum* and *Heterocapsa pygmaea* were identical to the measured  $a_{ph}^*(\lambda)$  (whole cells), it appears that our approach generated no artificial spectral shifts in  $a_{mu}^*(\lambda)$ . Correspondingly, comparison of  $a_{mu}^*(\lambda)$  with  $a_{ph}^*(\lambda)$  indicates that the package effect was less pronounced in HL-adapted cells (10% in *P. minimum* and 3% in *H. pygmaea*) than in LL-adapted cells of both species (24% in *P. minimum* and 7% in *H. pygmaea*) (Tables 4 & 5, Figs. 3 & 7). The biggest difference between  $a_{mu}^*(\lambda)$  and  $a_{ph}^*(\lambda)$  was found in spectral regions where all pigments absorb in both *P. minimum* and *H. pygmaea*, i.e. 440 to 460 nm, indicating high pigment packaging.

Table 4. *Prorocentrum minimum*. Weighted absorption in theoretical white light ( $\bar{a}^*_x$ ,  $\text{m}^2 \text{mg}^{-1}$  chl *a*, 400 to 700 nm) in low light (LL)- and high light (HL)-adapted cells from measured and modeled absorption spectra with and without package effect. Percent change (%) reduction of weighted absorption coefficient due to the package effect. Measured packed absorption from whole cells ( $\bar{a}^*_{ph}$ ) is compared to the measured unpacked absorption spectra (dispersed thylakoid fragments,  $\bar{a}^*_{mu}$ ). Modeled absorption coefficients from the pigment model are compared before ( $\bar{a}^*_{pu}$ ) and after application of the package effect algorithm ( $\bar{a}^*_{pp}$ ). Weighted absorption coefficients from the chromoprotein model ( $\bar{a}^*_{cu}$ ,  $\bar{a}^*_{cp}$ ) are compared for 2 approaches representing varying amounts of chl *a* in the different chromoproteins (see 'Results')

	Low light			High light		
	Without packaging	With packaging	%	Without packaging	With packaging	%
<b>Measured</b>						
$\bar{a}^*_{ph}$	–	0.0145	24	–	0.0237	9.9
$\bar{a}^*_{mu}$	0.0190	–	–	0.0263	–	–
<b>Modeled</b>						
$\bar{a}^*_{pu}$ , $\bar{a}^*_{pp}$	0.0310	0.0204	34	0.0300	0.0250	17
$\bar{a}^*_{cu}$ , $\bar{a}^*_{cp}$ ; approach 1	0.0193	0.0143	26	0.0236	0.0201	15
$\bar{a}^*_{cu}$ , $\bar{a}^*_{cp}$ ; approach 2	0.0215	0.0157	27	0.0258	0.0218	16

### Pigment model

#### Unpacked absorption

The reconstructed unpacked absorption spectra  $a^*_{pu}(\lambda)$  in HL- and LL-adapted cells of *Prorocentrum minimum*, i.e. *in vivo* weight-specific absorption coefficients ( $\text{m}^2 \text{mg}^{-1}$ ) for chl *a*, chl *c*<sub>2</sub>, peridinin and diadinoxanthin, yielded peaks and shoulders similar to those observed in  $a^*_{mu}(\lambda)$  spectra (Figs. 1 to 3). In HL-adapted cells of both species, a shoulder at  $a^*_{pu}$  (490 nm) was caused by absorption of the photoprotective carotenoid diadinoxanthin (Fig. 3). Correspondingly, the  $a^*_{pu}(\lambda)$  spectra for LL-adapted cells exhibited a shoulder at 500 to 560 nm caused by peridinin, which was not clearly seen in HL.

### Fractional absorption of pigments

The fractional absorption spectra of individual pigments (Eq. 2; Fig. 4) in HL- and LL-adapted cells of *Prorocentrum minimum* indicate how the pigment composition (photosynthetic and photoprotective) changes as a function of growth irradiance. Absorption at 400 to 500 nm in HL-adapted cells is due to almost equivalent absorption by chl *a*, chl *c*<sub>2</sub>, peridinin and diadinoxanthin in different wavelength bands. Peridinin is the main absorbing pigment from 500 to 600 nm, with some contribution from diadinoxanthin (500 to 520 nm), whereas chl *c*<sub>2</sub> and chl *a* determine the absorption at 560 to 600 nm. In LL-adapted cells, chl *c*<sub>2</sub> and peridinin are the major light-harvesting pigments at 450 to 560 nm. Chl *a* contributes significantly from

Table 5. *Heterocapsa pygmaea*. Weighted absorption in theoretical white light ( $\bar{a}^*_x$ ,  $\text{m}^2 \text{mg}^{-1}$  chl *a*, 400 to 700 nm) in low light (LL)- and high light (HL)-adapted cells from measured and modeled absorption spectra with and without package effect. Percent change (%) reduction of weighted absorption coefficient due to the package effect. Measured packed absorption from whole cells ( $\bar{a}^*_{ph}$ ) is compared to the measured unpacked absorption spectra (dispersed thylakoid fragments,  $\bar{a}^*_{mu}$ ). Weighted absorption coefficients from the chromoprotein model ( $\bar{a}^*_{cu}$ ,  $\bar{a}^*_{cp}$ ) are compared for 2 approaches representing varying amounts of chl *a* in the different chromoproteins (see 'Results')

	Low light			High light		
	Without packaging	With packaging	%	Without packaging	With packaging	%
<b>Measured</b>						
$\bar{a}^*_{ph}$	–	0.0175	7	–	0.0221	3
$\bar{a}^*_{mu}$	0.0189	–	–	0.0227	–	–
<b>Modeled</b>						
$\bar{a}^*_{cu}$ , $\bar{a}^*_{cp}$ ; approach 2	0.0215	0.0161	25	0.0236	0.0196	17
$\bar{a}^*_{cu}$ , $\bar{a}^*_{cp}$ ; approach 3	0.0211	0.0160	24	0.0232	0.0212	9

400 to 450 and 590 to 700 nm relative to the other pigments. Because the diadinoxanthin content in LL-adapted cells was low, the effect on absorption of this pigment is minimal.

The percent fractional absorption of individual pigments to total weighted absorption [ $AF_i(\lambda)$ , Eqs. (2) & (7), Figs. 4 & 6] illustrates the light-harvesting capabilities of the different pigments. The  $AF_i(\lambda)$  values varied as a function of photoadaptive status and water color. Independent of water color, peridinin was by far the most important light-harvesting pigment (35 to 70% of total absorbed light), followed by chl  $c_2$  (15 to 30%) and chl  $a$  (5 to 25%). Chl  $a$  itself is most efficient in absorbing the white light regime. In contrast, chl  $c_2$  and peridinin absorb most efficiently in blue and green light regimes, respectively (Fig. 6).  $AF_i(\lambda)$  of diadinoxanthin is similar to chl  $a$  in theoretical white light, but absorbs more efficiently than chl  $a$  in blue and green light. In fact, for HL-adapted cells diadinoxanthin absorbs up to ~30% of the total light absorbed (blue light), in contrast to only ~8% in LL-adapted cells.

Weighted absorption of each pigment fraction or total pigments under different spectral irradiances ( $\bar{a}_{AF}$ , Eq. 7; Fig. 4) yields information on the amount of light absorbed ( $\text{m}^2 \text{mg}^{-1}$ ) by each pigment and reveals which ones are the most effective under different spectral conditions and photoadaptive status (Fig. 6). Chl  $a$  has a low light-harvesting capacity in green coastal waters in contrast to peridinin, which is the major absorbing pigment both in white, green and blue light regimes.

### Chromoprotein model

#### Unpacked absorption

The pigment composition of the main light-harvesting complex, ACP, is dominated by chl  $a$ , chl  $c_2$ , peridinin and diadinoxanthin. Photosystems I and II (PS I, PS II) are both dominated by chl  $a$ , and PCP contains chl  $a$  and peridinin (Hiller et al. 1993, Iglesias-Prieto et al. 1993). Three different approaches were used to quantify the possible distribution of total cellular chl  $a$  among the different photosynthetic chromoproteins [ $a^*_c(\lambda)$ ]. Approach 1 is based on measured averages of different chromoproteins in *Prorocentrum minimum*, i.e. 72% of the total chl  $a$  in ACP and 14% in both photosystems (unpubl. data). Approach 2 employs a 'mean value' for chromoproteins isolated from *P. minimum* and *Heterocapsa pygmaea*, i.e. 52% of the total chl  $a$  in ACP, 20% in PCP and 14% in each photosystem (unpubl. data). Approach 3 is based on the estimation of chl  $a$  in each chromoprotein complex according to Nelson & Prézélin (1990), i.e. ACP and PCP were

scaled using a molar ratio of 5:1 for chl  $c_2$ : $a$  (Boczar et al. 1980) and 4:1 for peridinin:chl  $a$  (Koka & Song 1977). The chl  $a$  content in PS I and II, assumed to be equally distributed in the 2 systems, was estimated by subtracting the chl  $a$  content in PCP and ACP from total chl  $a$ . For modeling approaches 1 to 3, the diadinoxanthin content in ACP is ~5% of total pigmentation by weight. This was the mean % of diadinoxanthin content to total pigments in this survey and in 3 species of LL-adapted dinoflagellates reported by Johnsen & Sakshaug (1993). The findings of 10% diadinoxanthin to total pigments in *H. pygmaea* are in agreement with reported values of up to 16% diadinoxanthin of total pigments in HL-adapted dinoflagellates (Johnsen & Sakshaug 1993). This implies that  $a^*(\lambda)$  for diadinoxanthin is included in the chromoprotein model only if the diadinoxanthin content is higher than 5% of total pigments (by weight).

There is a good agreement between measured unpacked absorption [ $a^*_{mu}(\lambda)$ ] and modeled unpacked absorption using  $a^*_{cu}(\lambda)$ . However, the use of different chl  $a$  contents in the different chromoproteins [ $a^*_c(\lambda)$ ] scaled to total chl  $a$  (approaches 1, 2 or 3) alters the reconstructed absorption characteristics and thus the spectrally dependent values for weighted absorption (Figs. 3 & 7).

By looking at weighted absorption  $\bar{a}^*_{cu}$  of *Prorocentrum minimum* in theoretical white light, approaches 1 and 2 overestimated absorption by 2 and 13%, respectively, compared to  $\bar{a}^*_{mu}$  in LL-adapted cells and gave an 11 and 2%, respectively, underestimation in HL-adapted cells (Table 4). The pigment model overestimated  $\bar{a}^*_{pu}$  relative to  $\bar{a}^*_{mu}$  with 63% in LL- and 14% in HL-adapted cells. Similar results were also observed for *Heterocapsa pygmaea*. In this species, the difference between  $\bar{a}^*_{mu}$  in LL- and HL-adapted cells was 20% compared to 38% in *P. minimum*. Comparison between  $\bar{a}^*_{cu}$  approach 2 and 3 to  $\bar{a}^*_{mu}$  in *H. pygmaea* in white light yielded results similar to those for *P. minimum* (Tables 2 to 4, Figs. 3 & 7).

#### Modeling of *in vivo* (packed) absorption

For *Prorocentrum minimum*, the modeling of *in vivo* (packed) absorption by the pigment- [ $a^*_{pp}(\lambda)$ ] and chromoprotein [ $a^*_{cp}(\lambda)$ ] models was compared with measured *in vivo* absorption [ $a^*_{ph}(\lambda)$ ; Table 4].  $\bar{a}^*_{pp}$  overestimated  $\bar{a}^*_{ph}$  by 40% in LL- and 5% in HL-adapted cells. Correspondingly,  $\bar{a}^*_{cp}$  approach 1 yielded results similar to  $\bar{a}^*_{ph}$  in LL- and 15% underestimation in HL-adapted cells. In contrast, modeled white light weighted *in vivo* absorption by *Heterocapsa pygmaea*, using the chromoprotein model approaches 2 and 3, underestimated  $\bar{a}^*_{ph}$  by 8 to 11% (Table 5).

## DISCUSSION

### Pigments

The 30 to 40% higher amount of peridinin to total pigments in *Heterocapsa pygmaea* compared to *Prorocentrum minimum* at both growth irradiances reflects the presence of PCP in *H. pygmaea* and its absence in *P. minimum* (Table 3). Our study indicates that diadinoxanthin is the second most abundant carotenoid in dinoflagellates (Johnsen et al. 1974, Johnsen & Sakshaug 1993) as it is in most chromophytes (Rowan 1989). In low light, both species increased the cellular content of light-harvesting pigments relative to high-light adapted cells (Prézelin 1976, 1981, Demers et al. 1991, Sakshaug et al. 1991, Johnsen & Sakshaug 1993).

These observations suggest that the abundance of photosynthetic chromoproteins, as well as the average optical cross-section for light absorption, was significantly greater in LL-adapted cells of *Heterocapsa pygmaea* than similarly cultured cells of *Prorocentrum minimum*. Interestingly, and perhaps photophysiologicaly related, was the observation that HL induction of the photoprotective carotenoid diadinoxanthin was especially pronounced in *P. minimum* cells. Thus similar changes in growth irradiance induced different responses in the 2 species. In white light, *H. pygmaea* cells appeared more sensitive to photoregulation of photosynthetic pigments, while *P. minimum* cells were more sensitive to photoregulation of photoprotective carotenoids. These clear differences in photophysiological regulation of pigmentation made these 2 experimental organisms ideal for testing and developing models of red tide bio-optical properties.

The high specific-absorption coefficients of chl  $c_2$  and diadinoxanthin relative to chl  $a$  and peridinin imply that the 2 former pigments will have a significant effect on the *in vivo* absorption characteristics, even when they make up < 10% of total pigments by weight (Figs. 2A & 6). Considering that the 2 dinoflagellates examined have up to 21 to 22% of chl  $c_2$  and 10 to 16% diadinoxanthin to total pigments (w:w), we may conclude that these pigments are important in light harvesting and photoprotection, respectively (Table 3, Fig. 6).

### Measured unpacked absorption

Our method appears to provide good direct measurements of absolute unpacked absorption coefficients [ $a^*_{mu}(\lambda)$ ] for both *Prorocentrum minimum* and *Heterocapsa pygmaea*. The key was the release of intact chromoproteins [ $a^*_{cu}(\lambda)$ ] from the cells while avoiding dissociation of pigments from the chromopro-

teins or distortion of *in vivo* spectral properties. We therefore avoided the use of denaturing detergents or organic solvents which may be suitable for some characterization studies of chromoproteins but which commonly lead to the release of free pigments and significant denaturation (Prézelin & Boczar 1986). The thylakoid fraction reported here represents a mixture of functional light-harvesting complexes and PS I and II (unpubl.).

The *in vivo* weight-specific absorption coefficient of chl  $a$  at ~675 nm is an important scaling factor for modeling of absorption characteristics in phytoplankton because only protein-bound chl  $a$  absorbs at this wavelength. Our data indicate that previous unpacked absorption values for chl  $a$  dissolved in 90% acetone have been underestimated at 675 nm. Maximum measured unpacked absorption at 675 nm [ $a^*_{mu}(675 \text{ nm})$ ] is closer to 0.027 than a predicted value of 0.020  $\text{m}^2 \text{mg}^{-1}$  chl  $a$  based on chl  $a$  in 90% acetone used in previous models (Geider & Osborne 1987, Nelson & Prézelin 1990) (Figs. 2 & 3). A value of  $a^*_{ph}(\lambda)$  of ~0.027  $\text{m}^2 \text{mg}^{-1}$  chl  $a$  has been reported in *Synechococcus* spp. (cyanobacteria; Bidigare et al. 1989, G. Johnsen unpubl.), diatoms, prymnesiophytes and dinoflagellates (Morel & Bricaud 1981, Nelson & Prézelin 1990) indicating cells with low package effect [small  $\rho'$  and high  $Q^*_a(\lambda)$  values]. When we scaled the *in vivo* weight-specific absorption coefficient of chl  $a$  [ $a^*_i(675 \text{ nm})$ ] to 0.027  $\text{m}^2 \text{mg}^{-1}$  chl  $a$ , we found a closer agreement between reconstructed and measured absorption than when using an  $a^*_i(675 \text{ nm})$  of 0.020  $\text{m}^2 \text{mg}^{-1}$  chl  $a$  (Figs. 2 & 3). Values of  $a^*_{mu}(675 \text{ nm})$  seem to be little affected by growth-irradiance, but are different between the 2 species examined, indicating species-specific differences in the proteins attached to chl  $a$ . HL- and LL-adapted cells of *Prorocentrum minimum* had  $a^*_{mu}(675 \text{ nm})$  of 0.027 and 0.025  $\text{m}^2 \text{mg}^{-1}$  chl  $a$ , respectively. Correspondingly, HL- and LL-adapted cells of *Heterocapsa pygmaea* had  $a^*_{mu}(675 \text{ nm})$  of 0.021  $\text{m}^2 \text{mg}^{-1}$  chl  $a$  for both irradiances. In contrast, values of  $a^*_{mu}(400 \text{ to } 550 \text{ nm})$  indicate a significant difference between LL- and HL-adapted cells for both species (Figs. 2, 3 & 7).

### Comparison of models

By extracting dispersed thylakoid fragments or chromoproteins in 90% acetone, spectral shifts and changes in spectral absorption coefficients occur, caused by breakage of the bonds between pigments and proteins. In addition, different types of organic solvents result in different spectral absorption characteristics of the pigment in question. For example the blue to red ratio of chl  $a$  is 1.15 and 1.30 in 90 and 100%

acetone, respectively (Rowan 1989, Johnsen & Sakshaug 1993). The reconstructed  $a_i^*(\lambda)$  in the pigment model overestimated absorption in the blue and underestimated it in the red (Fig. 5). This is most probably caused by solvent effects and overlapping of absorption from all major pigments, especially in the blue part of the spectrum. Whole-cell pigment extracts in 90% acetone in particular will cause a big overlap in absorption between chl *a* and  $c_2$  at the blue end of the spectrum. This is less pronounced in the reconstructed absorption spectrum of phytoplankton [ $a_{ph}^*(\lambda)$ ] because the different pigments have been spectrally shifted to mimic *in vivo* absorption spectra (Table 2). The advantage of the pigment model for reconstruction of *in vivo* absorption characteristics is that it can quite accurately determine the amount of photoprotective and photosynthetic pigments absorbing under different light regimes (Fig. 6), and requires only HPLC and cell dimension data as input. The modeled (unpacked) fractional absorption of individual pigments [ $AF^*(\lambda)$ ] illustrates the importance and plasticity of absorption characteristics and light utilization relative to changes in growth-light irradiance and color.  $AF_i^*(\lambda)$  is a useful tool to study how the absorption characteristics in a phytoplankton cell vary as a function of pigmentation and to see which pigments are important in light-harvesting and photoprotection under different spectral light regimes (cf. Prézelin 1987, Johnsen et al. 1992). This information can be used for a more precise prediction of primary productivity since we take into account the effect of photoprotective carotenoids, i.e. diadinoxanthin, by subtracting the contribution of the photoprotective carotenoid(s) from the rest of the light-harvesting pigments. Fractional absorption is also a useful tool to understand the dynamics between species-specific spectral characteristics for use in bio-optical taxonomy since the spectral signatures from the different pigments are known and can be modeled (Bidigare et al. 1990, Johnsen et al. 1994).

The modeled unpacked absorption coefficients based on  $a_c^*(\lambda)$  (absorption coefficients of individual chromoproteins) yield a closer agreement with measured unpacked absorption [ $a_{mu}^*(\lambda)$ ] than spectra derived from the pigment model (Figs. 2, 3 & 7). The weighted absorption coefficients for reconstructed packed and unpacked absorption in *Prorocentrum minimum* using the pigment model (Table 4) indicate ~30% (mean value for HL + LL) overestimation caused by the *in vitro* absorption coefficients. In contrast, the chromoprotein model approach 2 overestimated the measured unpacked and packed absorption coefficients by only ~3% (mean value for HL + LL; Table 4, Fig. 3). Approaches 2 and 3 yield similar results and are in accordance with measured values (Tables 4 & 5).

However, the contributions from diadinoxanthin at 490 nm and peridinin at 530 to 540 nm are slightly different in approaches 1 and 2 (Fig. 3). Chromoprotein model approach 3, which is based on constant molar ratios of ACP and PCP, also yielded reasonable values for weighted absorption compared to measured values (Table 5), whereas the spectral characteristics, due to a high amount of PCP relative to ACP, yield different spectral absorption characteristics compared to approaches 1 and 2 (Fig. 7). Thus the chromoprotein model approach 2 [ $a_{cu}^*(\lambda)$ ,  $a_{cp}^*(\lambda)$ ] yielded the absorption characteristics closest to measured absorption for both species [ $a_{mu}^*(\lambda)$ ,  $a_{ph}^*(\lambda)$ ].

The spectral characteristics of the PCP-lacking *Prorocentrum minimum* are mainly due to the ACP complex, in contrast to *Heterocapsa pygmaea*, which contains both ACP and PCP (Figs. 3 & 7) (Jovine et al. 1992, Iglesias-Prieto et al. 1993). Still, by using approach 2 for both species, good agreement between measured and modeled absorption characteristics was obtained. Since the main light-harvesting complexes (PCP and/or ACP) correspond to approximately 70% of cellular chl *a*, only minor absorption contributions from PS I and II are evident in both species.

By using these models, especially the chromoprotein model, we can model quite accurately *in vivo* absorption characteristics in dinoflagellates on the basis of weight-specific absorption coefficients of peridinin-containing dinoflagellates. The same approach can be used for other phytoplankton classes.

### Package effect

Variations in the package effect are mainly caused by differences in cell and chloroplast size/shape/morphology and in intracellular pigment concentration (van der Hulst 1957, Morel & Bricaud 1981, Kirk 1983, Geider & Osborne 1987, Nelson & Prézelin 1990, Sosik & Mitchell 1991, Johnsen & Sakshaug 1993). The models presented by us take into account most of these variables and can therefore discriminate between light absorption by photosynthetic and photoprotective pigments, thus making estimation of photosynthetically usable light possible. The values from measured unpacked  $a_{mu}^*(675 \text{ nm})$  and packed absorption (*in vivo*)  $a_{ph}^*(675 \text{ nm})$  are in agreement with 2 similarly grown HL-adapted strains of *Prorocentrum minimum* (strain EXUV and 79A; Johnsen & Sakshaug 1993). They reported a  $Q_a^*(675 \text{ nm})$  value of 0.92 to 0.98 in HL-grown cells, based on the van der Hulst approximation (Eq. 4), which is close to the theoretical value for no packaging [ $Q_a^*(\lambda) = 1.0$ ; cf. Morel & Bricaud 1981]. Measured chl *a*-specific absorption coefficients from HL-adapted cells of *P. minimum* (cells with no

significant package effect at 675 nm) were as follows:  $0.028 \text{ m}^2 \text{ mg}^{-1}$  chl *a* for  $a_{\text{ph}}^*(675 \text{ nm})$  and  $0.027 \text{ m}^2 \text{ mg}^{-1}$  chl *a* for  $a_{\text{mu}}^*(675 \text{ nm})$ , indicating close agreement. For HL-adapted *Heterocapsa pygmaea*,  $a_{\text{ph}}^*(675 \text{ nm})$  was 20% higher than  $a_{\text{mu}}^*(675 \text{ nm})$  and, correspondingly, 20% lower at 440 nm. The reason for the high  $a_{\text{ph}}^*(675 \text{ nm})$  is unknown since replicate measurements were in close agreement, indicating no methodological problems [ $a_{\text{ph}}^*(\lambda)$ :  $n = 3$ ,  $\text{CV} = \pm 1.0\%$ ;  $a_{\text{mu}}^*(\lambda)$ :  $n = 2$ ,  $\text{CV} = \pm 0.2\%$ ]. No degradation of chl *a* (or other pigments) was detected as checked by HPLC connected to absorption and fluorescence monitors (Johnsen & Sakshaug 1993).

There is a significant rise from 400 to 480 nm in  $a_{\text{mu}}^*(\lambda)$  relative to  $a_{\text{ph}}^*(\lambda)$  in HL- and, in particular, LL-adapted cells. In this spectral band the peaks at 440 nm (chl *a*, peridinin, diadinoxanthin) and 460 nm (chl *c*<sub>2</sub>, peridinin, diadinoxanthin) are regions where these pigments absorb at their respective maxima (Figs. 2 & 3). These spectral regions are in principle the regions with highest packaging effect, which is confirmed by comparing the maximum difference between  $a_{\text{ph}}^*(\lambda)$  and  $a_{\text{mu}}^*(\lambda)$  found at 440 and 460 nm. This is also in agreement with the calculations of  $Q_a^*(440 \text{ nm})$  reported by Johnsen & Sakshaug (1993).

### Conclusions

Our survey indicates that the unpacked and packed absorption characteristics in the 2 dinoflagellate species studied can be used as a useful approach to increase the understanding of bio-optical taxonomic characteristics and photoadaptational effects in phytoplankton. Our data can also be used as basic information for the interpretation of *in situ* sensed bio-optical data from multi-wavelength transmissometers and from remote sensing of ocean color (cf. Kirk 1983, Prézelin & Boczar 1986, Mitchell & Kiefer 1988, Schofield et al. 1991, Johnsen et al. 1994).

The pigment model can be used as a tool to visualize the spectral characteristics and light harvesting contributions from the individual pigments in cells grown under different light-regimes and to model *in vivo* absorption characteristics. Both models can in addition be used as a tool to understand and to model/reconstruct photosynthetic efficiency ( $\alpha^B$ ) with and without the impact of photoprotective pigments ( $\alpha^B = \phi_{\text{max}} \cdot \bar{a}_{\text{ph}}^*$ ; Bidigare et al. 1989, Sakshaug et al. 1991, Schofield et al. 1991).

**Acknowledgements.** Research was supported by the Norwegian Council for Scientific and Industrial Research (G.J.) and NSF funding to B.B.P. (OCE 8922935 and OCE 9301322). We thank Bernd Kroon, Allen Matlick, Egil Sakshaug and Oscar

Schofield for valuable comments and discussions during this work. This article is contribution number 259 from Trondhjem Biological Station.

### LITERATURE CITED

- Berner, T., Dubinsky, Z., Wyman, K., Falkowski, P. G. (1989). Photoadaptation and the 'package' effect in *Dunaliella tertiolecta* (Chlorophyceae). *J. Phycol.* 25: 70–78
- Bidigare, R. R., Ondrusek, M. E., Morrow, J. H., Kiefer, D. A. (1990). *In vivo* absorption properties of algal pigments. *Ocean Optics X. SPIE Vol. 1302*: 290–302
- Bidigare, R. R., Prézelin, B. B., Smith, R. C. (1992). Bio-optical models and the problem of scaling. In: Falkowski, P. G., Woodhead, A. D. (eds.) *Primary productivity and biogeochemical cycles in the sea*. Plenum Press, New York, p. 175–212
- Bidigare, R. R., Schofield, O., Prézelin, B. B. (1989). Influence of zeaxanthin on quantum yield of photosynthesis of *Synechococcus* clone WH7803 (DC2). *Mar. Ecol. Prog. Ser.* 56: 177–188
- Bidigare, R. R., Smith, R. C., Baker, K. S., Marra, J. (1987). Oceanic primary production estimates from measurements of spectral irradiance and pigment concentrations. *Global biogeochem. Cycles* 1: 171–186
- Bjergskov, T., Larsen, J., Moestrup, Ø., Munk Sørensen, H., Krogh, P. (1990). Toksiske og potentielt toksiske alger i danske farvande. *Fiskeriministeriets Industritilsyn*, Copenhagen
- Boczar, B. A., Prézelin, B. B., Markwell, J., Thornber, J. P. (1980). A chl *c*-containing pigment-protein complex from the marine dinoflagellate, *Glenodinium* sp. *FEBS Lett.* 120: 243–247
- Davies, B. H. (1976). Carotenoids. In: Goodwin, T. W. (ed.) *Chemistry and biochemistry of plant pigments*. Academic Press, New York, p. 38–155
- Demers, S., Roy, S., Gagnon, R., Vignault, C. (1991). Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments in *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae): a photoprotection mechanism. *Mar. Ecol. Prog. Ser.* 76: 185–193
- Geider, R. J., Osborne, B. A. (1987). Light absorption by a marine diatom: experimental observations and theoretical calculations of the package effect in a small *Thalassiosira* species. *Mar. Biol.* 96: 299–308
- Guillard, R. R. L., Ryther, J. H. (1962). Studies of marine plankton diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8: 229–239
- Hiller, R. G., Wrench, P. M., Gooley, A. P., Shoebridge, G., Breton, J. (1993). The major intrinsic light-harvesting protein of *Amphidinium*: characterization and relation to other light-harvesting proteins. *Photochem. Photobiol.* 57: 125–131
- Iglesias-Prieto, R., Govind, N. S., Trench, R. K. (1993). Isolation and characterization of three membrane-bound chlorophyll-protein complexes from four dinoflagellate species. *Phil. Trans. R. Soc. Lond. B* 340: 381–392
- Jeffrey, S. W. (1972). Preparation and some properties of crystalline chlorophyll *c*<sub>1</sub> and *c*<sub>2</sub> from marine algae. *Biochim. Biophys. Acta* 279: 15–33
- Jeffrey, S. W., Haxo, F. T. (1968). Photosynthetic pigments of dinoflagellates (Zooxanthellae) from corals and clams. *Biol. Bull.* 135: 149–165

- Jeffrey, S. W., Humphrey, G. G. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*<sub>1</sub> and *c*<sub>2</sub> in algae, phytoplankton and higher plants. *Biochem. Physiol. Pflanz.* 167: 191–194
- Johansen, J. F., Svec, W. A., Liaaen-Jensen, S., Haxo, F. T. (1974). Carotenoids of the Dinophyceae. *Phytochem.* 13: 2261–2271
- Johnsen, G., Sakshaug, E. (1993). Bio-optical characteristics and photoadaptive responses in the toxic and bloom-forming dinoflagellates *Gyrodinium aureolum*, *Gymnodinium galatheanum*, and two strains of *Prorocentrum minimum*. *J. Phycol.* 29: 627–642
- Johnsen, G., Sakshaug, E., Vernet, M. (1992). Pigment composition, spectral characterization and photosynthetic parameters in *Chrysochromulina polylepis*. *Mar. Ecol. Prog. Ser.* 83: 241–249
- Johnsen, G., Samsø, O., Granskog, L., Sakshaug, E. (1994). *In vivo* absorption characteristics in 10 classes of bloom-forming phytoplankton: taxonomic characteristics and responses to photoadaptation by means of discriminant and HPLC analysis. *Mar. Ecol. Prog. Ser.* 105: 149–157
- Jovine, R. V. M., Triplett, E. L., Nelson, N. B., Prézelin, B. B. (1992). Quantification of chromophore pigments, apoprotein abundance and isoelectric variants of peridinin-chlorophyll *a*-protein complexes (PCPs) in the dinoflagellate *Heterocapsa pygmaea* grown under variable light conditions. *Plant Cell Physiol.* 33: 733–741
- Kirk, J. T. O. (1983). *Light and photosynthesis in aquatic ecosystems*. Cambridge University Press, Cambridge
- Kirk, J. T. O. (1992). The nature and measurement of the light environment in the ocean. In: Falkowski, P. G., Woodhead, A. D. (eds.) *Primary productivity and biogeochemical cycles in the sea*. Plenum Press, New York, p. 9–30
- Koka, P., Song, P. (1977). The chromophore topography and the binding environment of peridinin-chlorophyll *a*-protein complexes from marine dinoflagellate algae. *Biochim. Biophys. Acta* 495: 220–231
- Loeblich, A. R. III, Schmidt, R. J., Sherley, J. L. (1981). Scanning electron micrography of *Heterocapsa pygmaea* sp. nov., and evidence for polyploidy as a speciation mechanism in dinoflagellates. *J. Plankton Res.* 3: 67–79
- Mitchell, B. G., Kiefer, D. A. (1988). Chlorophyll *a* specific absorption and fluorescence excitation spectra for light-limited phytoplankton. *Deep Sea Res.* 35: 639–663
- Morel, A., Bricaud, A. (1981). Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. *Deep Sea Res.* 28A: 1375–1393
- Morel, A., Lazzara, L., Gostan, J. (1987). Growth rate and quantum yield time response for a diatom to changing irradiance (energy and color). *Limnol. Oceanogr.* 32: 1066–1084
- Nelson, N. B., Prézelin, B. B. (1990). Chromatic light effects and physiological modeling of absorption properties of *Heterocapsa pygmaea* (= *Glenodinium* sp.). *Mar. Ecol. Prog. Ser.* 63: 37–46
- Nelson, N. B., Prézelin, B. B. (1993). Calibration of an integrating sphere for determining the absorption coefficient of scattering suspensions. *Appl. Optics* 32: 6710–6717
- Prézelin, B. B. (1976). The role of peridinin-chl *a*-proteins in the photosynthetic light adaptation of the marine dinoflagellate, *Glenodinium* sp. *Planta* 130: 225–233
- Prézelin, B. B. (1981). Light reaction in photosynthesis. In: Platt, T. (ed.) *Physiological bases of phytoplankton ecology*. *Can. Bull. Fish. Aquat. Sci.* 210: 1–43
- Prézelin, B. B. (1987). Photosynthetic physiology of dinoflagellates. In: Taylor, F. J. R. (ed.) *The biology of dinoflagellates. I. Dinoflagellata*. Botanical Monographs, Vol. 21. Blackwell Scientific Publ., Oxford, p. 174–223
- Prézelin, B. B., Boczar, B. A. (1986). Molecular bases of cell absorption and fluorescence in phytoplankton: potential applications to studies in optical oceanography. In: Round, F. E., Chapman, D. J. (eds.) *Progress in physiological research*, Vol. 4. Biopress Ltd, Bristol, p. 350–465
- Rowan, K. S. (1989). *Photosynthetic pigments of algae*. Cambridge University Press, Cambridge
- Sakshaug, E., Johnsen, G., Andresen, K., Vernet, M. (1991). Modeling of light-dependent algal photosynthesis and growth: experiments with the Barents Sea diatoms *Thalassiosira nordenskiöldii* and *Chaetoceros furcellatus*. *Deep Sea Res.* 38: 415–30
- Sathyendranath, S., Lazzara, L., Prieur, L. (1987). Variations in the spectral value of specific absorption of phytoplankton. *Limnol. Oceanogr.* 32: 403–415
- Schofield, O., Prézelin, B. B., Smith, R. C., Stegmann, P., Nelson, N. B., Lewis, M. R., Baker, K. S. (1991). Variability in spectral and non-spectral measurements of photosynthetic light utilization efficiencies. *Mar. Ecol. Prog. Ser.* 78: 253–271
- Smayda, T. J. (1990). Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: Granéli, E., Sundström, B., Edler, L., Anderson, D. M. (eds.) *Toxic marine phytoplankton*. Elsevier, Amsterdam, p. 29–40
- Smayda, T. J., White, A. W. (1990). Has there been a global expansion of algal blooms? If so, is there a connection with human activities? In: Granéli, E., Sundström, B., Edler, L., Anderson, D. M. (eds.) *Toxic marine phytoplankton*. Elsevier, Amsterdam, p. 516–517
- Sosik, H. M., Mitchell, B. G. (1991). Absorption, fluorescence, and quantum yield for growth in nitrogen-limited *Dunaliella tertiolecta*. *Limnol. Oceanogr.* 36: 910–921
- van der Hulst, H. C. (1957). *Light scattering by small particles*. John Wiley, New York

This article was submitted to the editor

Manuscript first received: January 13, 1994

Revised version accepted: August 10, 1994