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In vivo absorption characteristics in 10 classes of bloom-forming phytoplankton: taxonomic characteristics and responses to photoadaptation by means of discriminant and HPLC analysis

Geir Johnsen[1], Oddgeir Samset[2], Lois Granskog[1], Egil Sakshaug[1]

[1]Trondhjem Biological Station, The Museum, University of Trondheim, Bynesveien 46, N-7018 Trondheim, Norway
[2]SINTEF Industrial Mathematics, NTH, N-7034 Trondheim, Norway

ABSTRACT: The spectral light absorption characteristics (400 to 700 nm) of 10 main classes, covering 31 species, of bloom-forming phytoplankton (diatoms, dinoflagellates, prymnesiophytes, euglenophytes, prasinophytes, raphidophytes, cryptophytes, chlorophytes, chrysophytes and cyanobacteria) have been examined. The survey is based on *in vivo* chlorophyll (chl) *a*-specific light absorption spectra [$^{\circ}a_c(\lambda)$, 400 to 700 nm] of low- and high-light adapted monocultures grown in the laboratory. Pigments were isolated by means of high-performance liquid chromatography (HPLC) to obtain visible spectra of isolated pigments to identify peaks and shoulders of the *in vivo* absorption spectra. A total of 217 $^{\circ}a_c(\lambda)$ spectra were log-transformed and normalized at 675 nm [$a_{log}(\lambda)$] to minimize photoadaptational effects on the spectral characteristics due to differences in pigment composition and the package effect. These $a_{log}(\lambda)$ spectra were analyzed by stepwise discriminant analysis to determine sets of optimum wavelengths for classification. Discrimination and classification were most effective when low- and high-light adapted phytoplankton were grouped separately. A set of only 3 wavelengths (481, 535, 649 nm) chosen on the basis of discriminant analysis classified, according to the jackknife technique, 93% of the $a_{log}(\lambda)$ spectra. By using combinations of 4 (481, 535, 586, 649 nm) or 5 (481, 535, 586, 628, 649 nm) chosen wavelengths, 97 to 99% of the spectra were classified correctly. For pooled data (low- and high-light adapted cells), 60 to 86% of the spectra were correctly identified using a combination of 3 to 5 selected wavelengths, indicating that variations due to photoadaptation were not entirely removed by log-transforming and scaling of the spectra at 675 nm. By using the above combination of 3 wavelengths, 4 main groups of phytoplankton were clearly separated, depending mainly on their accessory chlorophylls, i.e. chl *b* (prasinophytes, euglenophytes, chlorophytes), chl c_1 and/or c_2 (diatoms, dinoflagellates, prymnesiophytes, chrysophytes, raphidophytes, cryptophytes), chl c_3 (toxic prymnesiophytes and dinoflagellates) and no accessory chlorophylls (cyanobacteria). The wavelengths employed here correspond to the peaks and shoulders of the *in vivo* absorption spectra. We conclude that different phytoplankton classes may be identified during blooms on the basis of *in situ* bio-optical measurements at 3 to 5 appropriately chosen wavelengths.

KEY WORDS: Bio-optical taxonomy · *In vivo* light absorption · Pigment composition · Photoadaptation · Phytoplankton classes · Discriminant analysis

INTRODUCTION

Marine phytoplankton have evolved a large variety of light-harvesting pigment systems. The 3 main pigment groups that determine the bio-optical properties are the chlorophylls, the carotenoids and the phycobiliproteins (Rowan 1989). Identification of phytoplankton on the basis of *in vivo* light absorption spectra thus depends on the pigment composition of the cells, as modified by variations in the photoadaptational response, and how many wavelengths are measured (cf. SooHoo et al. 1986, Bidigare et al. 1987, 1989a, 1990a, b, Sathyendranath et al. 1987, Hoepffner & Sathyendranath 1991).

Different species of photosynthetic phytoplankton contain different accessory chlorophylls in addition to

the ubiquitous chlorophyll (chl) *a*. Whereas chl *a* exhibits *in vivo* absorption maxima at 440 and 675 nm, chl *b* exhibits peaks at ca 470 and 650 nm (Bidigare et al. 1990b). Chl *c* represents a mixture of slightly spectrally distinct components: chl c_1, chl c_2, chl c_3 and magnesium 2,4-divinyl phaeoporphyrin a_5 monomethyl ester (Mg-D). These have *in vivo* absorption maxima at approximately 460 to 470, 586 and 635 nm (Rowan 1989, Bidigare et al. 1990a, Johnsen et al. 1992, Johnsen & Sakshaug 1993).

The carotenoids of eukaryotic phytoplankton and cyanobacteria represent more than 100 different pigments which absorb mainly in the blue to green parts of the visible spectrum (Straub 1987, Bjørnland & Liaaen-Jensen 1989, Rowan 1989). The most important light-harvesting carotenoids are fucoxanthin and its 19'-acyloxy-derivatives as well as peridinin; the latter being specific to the majority of dinoflagellates (cf. Liaaen-Jensen 1978, Jeffrey 1980, 1989, Bjørnland & Liaaen-Jensen 1989, Rowan 1989).

The 4 major types of phycobiliproteins are phycocyanin, phycoerythrin, allophycocyanin, and phycoerythrocyanin (the major light-harvesting pigments in the cyanobacteria and cryptophytes) (Rowan 1989).

In most cases the major *in vivo* absorption signature in blue (400 to 500 nm), and especially in red (580 to 700 nm), is caused by the chlorophylls (chl *a*, *b* and *c*; Bidigare et al. 1990a, b). The major light-harvesting carotenoids, i.e. the fucoxanthins and the 19'-acyloxy-fucoxanthins together with peridinin and prasinoxanthin absorb *in vivo* mainly at 450 to 550 nm (Prézelin & Boczar 1986, Bidigare et al. 1987, 1990b, Sathyendranath et al. 1987, Johnsen et al. 1992). Phycobiliprotein-dominated pigment systems of many cyanobacteria and cryptophytes absorb green to orange light (480 to 600 nm) efficiently (Prézelin & Boczar 1986, Bidigare et al. 1987, 1989b).

As more is learned about the molecular basis for the bio-optical differences between classes of phytoplankton, spectral characteristics of the different types of pigment-proteins and their respective pigment composition can form a useful basis for the determination of the phytoplankton composition in natural waters (Kirk 1983, Prézelin & Boczar 1986, Rowan 1989). With this respect, instruments for *in situ* measurements must be calibrated against data for phytoplankton grown under controlled conditions in the laboratory to understand how environmental factors affect the *in vivo* absorption characteristics in different phytoplankton classes. We consider here only the effect of light, i.e. photoadaptive effects and sets of wavelength optimized for identification of spectra, although temperature and nutrient effects also play a role.

Our survey covers 31 species of important toxic and non-toxic bloom-forming phytoplankton, representing 10 phytoplankton classes, grown under low- and high-light conditions. The major objective was to determine sets of wavelengths that highlight, as accurately as possible, the bio-optical differences between light absorption spectra and, thus, the species.

In order to distinguish species-specific spectral *in vivo* absorption signatures, we have used discriminant analysis, a multivariate statistical technique (Mardia et al. 1979, Anderson 1984, Krzanowski 1988). This technique separates distinct sets of observations and classifies new observations relative to the previously defined groups. The predictions obtained from the discriminant analysis has formed the basis for selection of convenient sets of wavelengths, for which the appropriateness can be checked independently by the use of jackknife technique. Such information derived from laboratory cultures may be of value for the identification of different phytoplankton groups *in situ* on the basis of data from multi-wavelength transmissometers connected to buoys, as a supplement or an alternative to the traditional use of microscopy.

MATERIAL AND METHODS

Cultures. The different isolates were obtained from the culture collection of Trondhjem Biological Station, University of Oslo, Plymouth Culture Collection and the Provasoli-Guillard Centre for Culture of Marine Phytoplankton (Table 1). The cultures were grown in f/2 medium (Guillard & Ryther 1962), with addition of silicate (diatoms), soil extract (euglenophytes and cyanobacteria) and selenium (prymnesiophytes) at 15 °C (except 0 °C for *Synedra hyperborea* and 20 °C for *Gyrodinium aureolum* and *Gymnodinium galatheanum*) and a salinity of 33‰ (Table 1). Scalar irradiance (E_0, 400 to 700 nm, PAR) was measured inside the culture flasks filled with distilled water by means of a QSL-100 quantum sensor (Biospherical Instruments). The cultures were grown in cool-white fluorescent light with similar spectral output (Philips TLD 18W/95 and TL 40W/55). The light regimes for the different cultures were as follows; day lengths of 12 or 24 h with low light (LL, 15 to 75 µmol m^{-2} s^{-1}) and high light (HL, 170 to 500 µmol m^{-2} s^{-1}; Table 1). The cultures were allowed to adapt to the experimental light regime for 6 to 12 generations before sampling was carried out during the exponential growth phase.

Pigments. Subsamples were concentrated on Whatman GF/C or GF/F (small cells) glass-fibre filters at 50 mb differential pressure and immediately extracted at 0 to 4 °C for 20 h (Johnsen & Sakshaug 1993) using a mixture of methanol and acetone (3:7, vol:vol) (Hertzberg & Liaaen-Jensen 1966). The extracts were refiltered through a Whatman GF/C or GF/F filter

Table 1. Overview of the 10 classes of phytoplankton (31 species) grown under high light (170 to 500 µmol m^{-2} s^{-1}) and low light (15 to 75 µmol m^{-2} s^{-1}) used in this survey for measuring spectral *in vivo* absorption characteristics [$a_{log}(\lambda)$]. Note: M, V, 1, 3 and 4 were only grown in low light. Isolate sources are TBS: Trondhjem Biological Station; UO: University of Oslo; PCC: Plymouth Culture Collection; P-G: Provasoli-Guillard Centre for Culture of Marine Phytoplankton

Class	No. of species examined	Species	Source	Growth conditions: Scalar irradiance (µmol m^{-2} s^{-1})	Day length (h)	Temperature (°C)
Diatoms	6	1 = *Chaetoceros* cf. *gracilis*	TBS	75 only	24	15
		2 = *Phaeodactylum tricornutum*	P-G	15 & 500	24	15
		3 = *Skeletonema costatum*	TBS	75 only	24 & 12	15
		4 = *Synedra hyperborea*	TBS	35 only	24	0
		5 = *Thalassiosira nordenskioeldii*	TBS	75 & 330	12	15
		6 = *Thalassiosira pseudonana*	P-G	35 & 500	24	15
Dinoflagellates	6	7 = *Ceratium lineatum*	TBS	75 & 330	12	15
		8 = *Gonyaulax spinifera*	TBS	75 & 330	12	15
		9 = *Gymnodinium galatheanum*	TBS	30 & 170	12	20
		A = *Gyrodinium aureolum*	TBS	30 & 170	12	20
		B = *Katodinium rotundatum*	TBS	75 & 330	12	15
		C = *Prorocentrum minimum*[a]	TBS, P-G	35 & 500	24	15
Prymnesiophytes	9	D = *Chrysochromulina* sp. A	UO	35 & 500	24	15
		E = *Chrysochromulina leadbeateri*	UO	35 & 500	24	15
		F = *Chrysochromulina polylepis*	UO	35 & 500	24	15
		G = *Chrysochromulina* sp. B	TBS	75 & 330	12	15
		H = *Emiliania huxleyi*	UO	35 & 500	24	15
		I = *Isochrysis galbana*	TBS	35 & 500	24	15
		J = *Pavlova lutheri*	TBS	35 & 250	24	15
		K = *Phaeocystis* cf. *pouchetii*[a]	TBS, PCC	35 & 500	24	15
		L = *Prymnesium patelliferum*	UO	35 & 500	24	15
Prasinophytes	4	M = *Bathycoccus prasinos*	UO	35 only	24	15
		N = *Micromonas pusilla*	UO	35 & 500	24	15
		O = *Pseudoscourfieldia marina*	UO	35 & 500	24	15
		P = *Pyramimonas* sp.	TBS	75 & 330	12	15
Euglenophytes	1	Q = *Eutreptiella gymnastica*	UO	35 & 500	24	15
Chlorophytes	1	R = *Dunaliella marina*	TBS	35 & 500	24	15
Chrysophytes	1	S = *Pseudopedinella pyriformis*	UO	35 & 500	24	15
Raphidophytes	1	T = *Heterosigma akashiwo*	UO	35 & 500	24	15
Cryptophytes	1	U = *Rhodomonas baltica*	UO	35 & 250	24	15
Cyanobacteria	1	V = *Synechococcus* sp.	UO	35 only	24	15

[a]Two strains

before analysis. Chl *a* concentration and purity were estimated spectrophotometrically together with HPLC (high-performance liquid chromatography) for normalizing of *in vivo* absorption spectra to chl *a* (Jeffrey & Humprey 1975, Johnsen et al. 1992). Pigment analyses were carried out with a Merck & Hitachi L-6200 HPLC equipped with a SPHERI-5 RP-18 reverse-phase C-18 column (Brownlee Labs 25 cm × 4.6 mm, 5 µm particles; Johnsen et al. 1992). Extracts of 50 to 100 µl were injected into the HPLC and detection performed at 440 nm in a Hitachi Spectrophotometer Model U-2000 fitted with a flow-through cell using the solvent system presented by Mantoura & Llewellyn (1983). For spectral identification of isolated pigments, the HPLC pump was stopped at the maximum absorption peak and scanned from 350 to 800 nm at 0.5 nm intervals ('stop-flow technique'). Several authentic standards of pigments for identification and calibration of the HPLC were obtained from *Chrysochromulina polylepis* (Johnsen et al. 1992) and 3 species of dinoflagellates (Johnsen & Sakshaug 1993). Standards of peridinin, neoxanthin, prasinoxanthin, alloxanthin, lutein, echinenone and α-carotene (TLC-isolated) were donated by the Carotenoid Group at the Institute of Organic Chemistry, University of Trondheim-NTH. Other pigments obtained from the cultures used here were isolated and characterized by means of HPLC, using the stop-flow technique to measure spectral absorption (carotenoids and chlorophylls) and fluorescence excitation (chlorophylls only) characteristics (Johnsen &

Sakshaug 1993). Absorption peaks and shoulders (i.e. small peaks) (350 to 800 nm) of these standards were in agreement with reported absorption characteristics of the same purified pigments in corresponding solvents (cf. Hertzberg & Liaaen-Jensen 1966, Liaaen-Jensen 1978, Jeffrey 1989, Rowan 1989, Johnsen et al. 1992, Johnsen & Sakshaug 1993).

***In vivo* absorption spectra.** Chl *a*-specific light absorption [$^{\circ}a_c(\lambda)$, m^2 mg^{-1} chl *a*, 400 to 700 nm] was measured on a Hitachi 150-20 spectrophotometer using Whatman GF/C or GF/F glass-fiber filters and the correction algorithm and method of Mitchell & Kiefer (1988); for details, see Johnsen & Sakshaug (1993). A total of 217 absorption spectra were obtained and subsequently log transformed (natural logarithm) and scaled to 0 at 675 nm [$a_{log}(\lambda)$].

Discriminant analysis. A stepwise discriminant analysis (Krzanowski 1988, Tabachnick & Fidell 1989) was performed using the 217 $a_{log}(\lambda)$-spectra reduced to 85 variables, i.e. every third nanometer from 400 to 652 nm. Data for wavelengths > 652 nm were excluded from the analysis. The statistical analysis has been carried out using all absorption spectra with all light regimes included (pooled data), or with the spectra separated into groups representing either LL- or HL-adapted cells (see Table 3). In the stepwise discriminant analysis, calibration of the models (determination of the linear combinations on selected wavelengths; Table 3) and predictions (classifications) were performed on the same observations, i.e. predictions were carried out on the basis of observations already used in the calibration.

On the basis of the results from the stepwise discriminant analysis, we have selected sets of wavelengths and verified their appropriateness by means of the jackknife technique, i.e. predictions were carried out independent of the calibrations (Mardia et al. 1979, Tabachnick & Fidell 1989). In this technique one observation is removed and calibration performed on the rest of the data set before prediction is done on the observation which was first removed. This was repeated for all the observations in the data set (see Table 4).

Both the discriminant analysis and jackknife technique were performed on a Macintosh IIci equipped with 8 MB RAM using SPSS Release 4.0 for Macintosh.

RESULTS

The $a_{log}(\lambda)$ spectra yielded different peaks and shoulders depending on the pigment composition (Tables 1 & 2, Figs. 1 to 3). As an example, the prymnesiophyte *Pavlova lutheri* has its main *in vivo* absorp-

Table 2. Relationship between *in vivo* absorption maxima and predominant pigments at various wavelengths, assuming spectral shift from *in vitro* to *in vivo* according to Bidigare et al. (1990b). Wavelengths are those identified/selected by the discriminant analysis (see Table 3) and verified by the jackknife technique (see Table 4). /: presence of one or the other pigment; parentheses indicate pigments that are uncommon in a phytoplankton class. Pigments are Mg-D: magnesium 2,4-divinyl phaeoporphyrin a_5 monoester; DD: diadinoxanthin; F: fucoxanthin; P: peridinin; 19'F: 19'-acyloxyfucoxanthins; PR: prasinoxanthin; V: violaxanthin; Z: zeaxanthin; N: neoxanthin; L: lutein; AX: alloxanthin; β: β-carotene; PE: Cr-phycoerythrin 545; PUB: phycourobilin; PEB: phycoerythrobilin. Species as in Table 1

Phytoplankton classes	Absorption maxima			
	481 nm[a]	535 nm[b]	586 nm[c]	649 nm[d]
Diatoms	DD, F	F	Chl c_{1+2}	–
Dinoflagellates	DD, P/(F)	P/(F)	Chl $c_{2(3)}$	–
Prymnesiophytes	DD, F, 19'F	F, 19'F	Chl c_{2+3}	–
Prasinophytes	PR/L, V, Z, chl *b*	PR	Chl *a* + *b*, Mg-D	Chl *b*
Euglenophytes	DD, N, chl *b*	–	Chl *a* + *b*	Chl *b*
Chlorophytes	V, Z, L, chl *b*	–	Chl *a* + *b*	Chl *b*
Chrysophytes	DD, F	F	Chl c_2	–
Raphidophytes	F, V, Z	F	Chl c_{1+2}	–
Cryptophytes	AX	PE	Chl c_2	–
Cyanobacteria	PUB, Z, β	PEB	–	–

[a] These pigments have their respective *in vivo* absorption maxima at ca 470 to 490 nm (cf. Prézelin & Boczar 1986, Bidigare et al. 1990b, Johnsen & Sakshaug 1993). Photoprotective carotenoids, such as diadino- and zeaxanthin, have their respective *in vivo* absorption maxima at ca 490 nm (Bidigare et al. 1989b, G. Johnsen unpubl.)

[b] This wavelength denotes primarily the absorption valley from the main light-harvesting carotenoids or phycobiliproteins in the respective phytoplankton class (cf. Bidigare et al. 1990b, Johnsen & Sakshaug 1993)

[c] All chlorophylls absorb light at this wavelength, yet the *in vivo* weight-specific absorption coefficients of chl c_1, c_2 and c_3 are ca 2 times higher than those of chl *a* and chl *b* (Bidigare et al. 1990b). For chl c_3 in dinoflagellates, see Johnsen & Sakshaug (1993)

[d] The *in vivo* weight-specific absorption coefficient of chl *b* is ca 3 times higher than all chl *c*s and chl *a*

tion peaks at 437, 490 and 676 nm, which correspond to chl *a* (*in vivo* peaks at 437 and 676 nm; Prézelin & Boczar 1986, Bidigare et al. 1990b) and diadinoxanthin (*in vivo* peaks at 490 nm; G. Johnsen unpubl.), whereas shoulders at 415, 460, 535, 585 and 630 nm are due mainly to absorption by chl *a* (415 nm), chl c_{1+2} together with fucoxanthin and diadinoxanthin (460 nm), fucoxanthin (535 nm) and, finally, chl *a* and chl c_{1+2} at 585 and 630 nm (cf. Prézelin & Boczar 1986, Johnsen & Sakshaug 1993; Figs. 1 & 3, Table 2).

The *in vivo* spectral characteristics of phytoplankton (Figs. 1 & 3) are also modified by the light regime (LL vs HL conditions). This variability, which is manifested both in the magnitude and the spectral shape of $a_{log}(\lambda)$ is due to variations in pigment composition as well as the package effect (intracellular self shading; Fig. 3; Kirk 1983, Hoepffner & Sathyendranath 1991). Photoadaptation-dependent variability thus may impede the identification of algal species on the basis of bio-optical characteristics. To minimize this problem, as well as to minimize the signals from chl *a*, we have log-transformed the spectra, scaled them at the red peak (675 nm) as well as discarded data for wavelengths >652 nm in the mathematical treatments. The log-transformation enhanced, relatively, absorption in the information-rich region where small absorption peaks and shoulders are related to accessory pigments (580 to 652 nm); the scaling at 675 nm minimized the package effect, while the exclusion of data for wavelengths >652 nm removed some of the taxonomically irrelevant information from chl *a* without removing information from chl *b* (red peak maximum at 650 nm; Fig. 3, Table 2).

The original spectra [$^{\circ}a_c(\lambda)$] yielded information about absorption at no less than 300 wavelengths, and the corrected versions [$a_{log}(\lambda)$] at 85 wavelengths. A crucial question in practical terms is how far the number of wavelengths can be reduced with no appreciable loss of taxonomically relevant information. The stepwise discriminant analysis selects wavelengths with high prediction success for the corrected spectra. This analysis indicates that a set of only 3 wavelengths, optimized for the classification of the spectra, can identify 93 to 97% of the spectra when they are grouped into LL and HL spectra, respectively. Two sets of either 3 or 5 wavelengths identified 72 and 92% of the spectra respectively, when pooled irrespective of photoadaptational status (Table 3). These results imply that few optimized wavelengths are necessary for identification; however, they also imply that our corrections did not remove variations due to photoadaptation entirely.

The sets of optimized wavelengths derived from discriminant analysis pose a practical problem in that they differ depending on whether the data sets are pooled

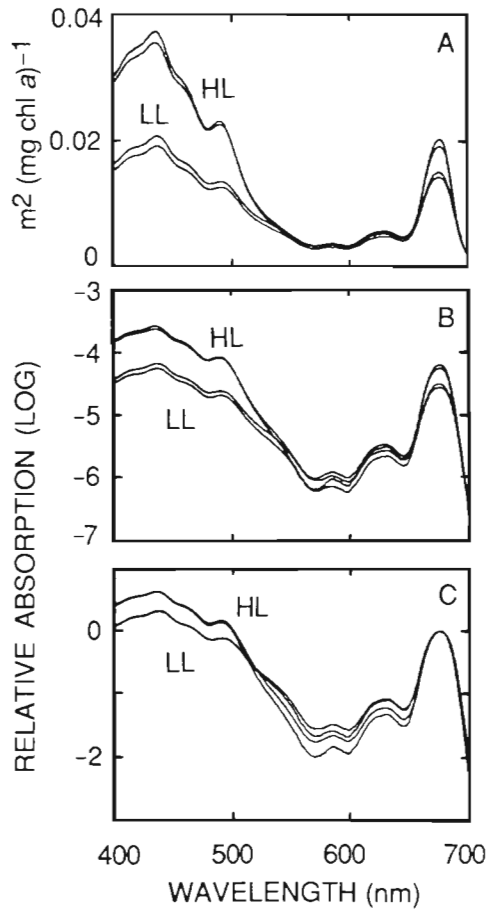

Fig. 1. *In vivo* absorption spectra from low-light (LL, 35 µmol m^{-2} s^{-1}) and high-light (HL, 250 µmol m^{-2} s^{-1}) adapted cells of the prymnesiophyte *Pavlova lutheri* grown at 24 h day length at 15°C. (A) Chl *a*-specific absorption spectra, [$^{\circ}a_c(\lambda)$, m^2 mg^{-1} chl *a*] of HL- and LL-adapted cells (n = 2). (B) Same as (A), but log-transformed. (C) Same as (B), but scaled at 675 nm [$a_{log}(\lambda)$]

or treated separately as LL and HL sets (Table 3). To overcome this, sets appropriate for both pooled HL and LL spectra have to be employed. Noting that many of the wavelengths in Table 3 are close neighbours, thus yielding insignificantly different information (data not shown), we have chosen 649, 628, 586, 535 and 481 nm (the latter representing the range 475 to 493 nm) and tested the relevance of sets of these wavelength combinations by means of the jackknife technique. These wavelengths represent approximately the *in vivo* absorption maxima of important accessory pigments (Table 2).

The jackknife technique shows that the sets of chosen wavelengths (Table 4) identify about the same percent of the transformed spectra as sets with a corresponding number of wavelengths derived from stepwise discriminant analysis, provided that the spectra are separated into LL and HL groups (93 to 99%,

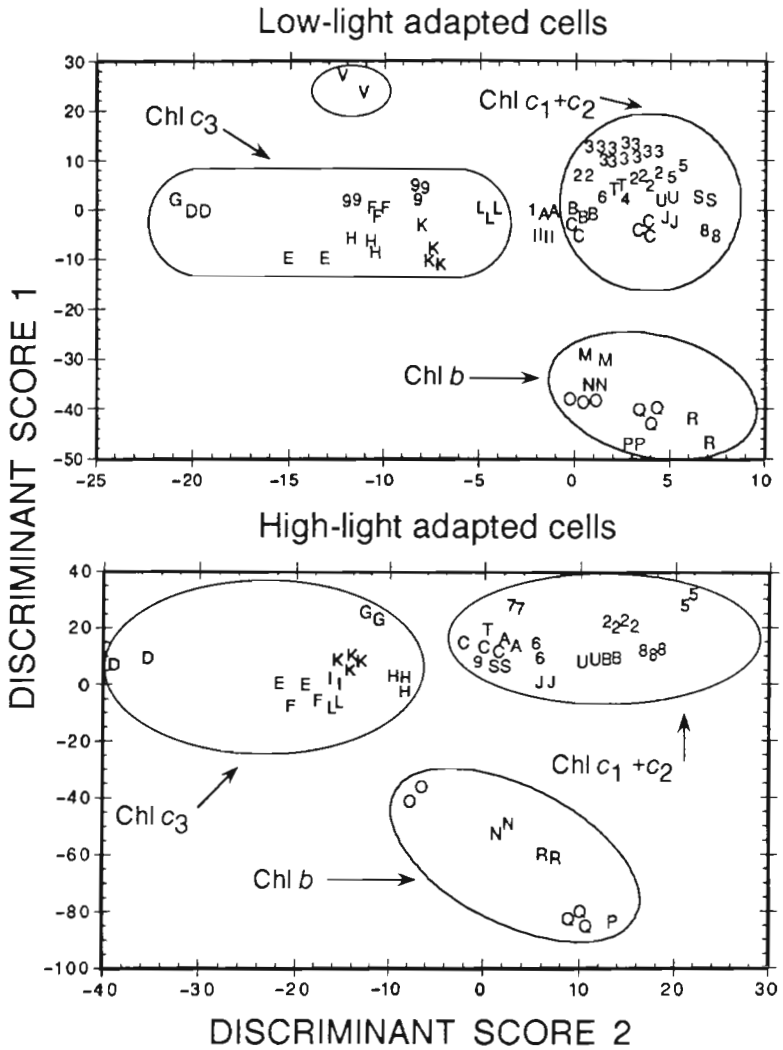

Fig. 2. Discriminant scores evaluated on $a_{log}(\lambda)$ obtained from 10 classes of phytoplankton (31 species, 1 to 9 and A to V; see Table 1) grown under low- and high-light conditions. Classification by means of 3 wavelengths: 481, 535 and 649 nm. Classes: diatoms (1-6), dinoflagellates (7 to 9, A to C), prymnesiophytes (D to L), prasinophytes (M to P), euglenophytes (Q), chlorophytes (R), chrysophytes (S), raphidophytes (T), cryptophytes (U), and cyanobacteria (V). The figure is divided into 4 main zones: phytoplankton without accessory chlorophylls (*Synechococcus* sp. which did not grow in high light), chl *b*, chl c_3 and chl c_{1+2} containing phytoplankton. For further information, see Tables 1 to 4. Note that these discriminant scores could be visualized in 3 dimensions, which would enhance the visual separation. Identical symbols denote replicates

depending on whether 3, 4 or 5 wavelengths are employed). The percent identified spectra on the basis of pooled sets, however, is somewhat lower for the sets of chosen wavelengths than for the sets of wavelengths derived from stepwise discriminant analysis. The stepwise discriminant analysis and the jackknife technique both agree that a set of 3 appropriate wavelengths is sufficient to identify the corrected spectra when they are grouped according to their photoadaptational status. For pooled data, a set of 5 wavelengths may be deemed sufficient, i.e. the set of 5 chosen wavelengths in Table 4 may identify 86% of the spectra correctly.

The species-specific differences in absorption can be visualized by the discrimination scores (the values of the linear combinations), using discriminant analysis. The set of the 3 chosen wavelengths which yielded the best discrimination, i.e. 481, 535 and 649 nm, separates the $a_{log}(\lambda)$ spectra into 4 main pigment-groups, depending on the type of accessory chlorophylls (chl c_1, chl c_2, chl c_3, Mg-D and chl *b*; Fig. 2). Group 1 represents chl *b*-containing phytoplankton (prasinophytes, euglenophytes and chlorophytes); Group 2 chl c_1 and/or c_2-containing phytoplankton (diatoms, prymnesiophytes, chrysophytes, raphidophytes, dinoflagellates and cryptophytes); Group 3 chl c_3-containing phytoplankton (prymnesiophytes and dinoflagellates) which possess chl c_2 and sometimes chl c_1; and Group 4 is phytoplankton which lack accessory chlorophylls (cyanobacteria; Fig. 2, Table 2).

The 4 pigment groups can be further divided into several subgroups down to the species level, e.g the chl *b*-group can be divided into Mg-D containing prasinophytes (which also contains prasinoxanthin; Fig. 2, species M, N, O; see Table 1 for species identification) and one for prasinophytes, chlorophytes and euglenophytes lacking Mg-D and prasinoxanthin (species P, Q, R). No large differences in discrimination were observed for LL- and HL-adapted species (Fig. 2). All the examined chl c_3-containing flagellates were grouped together by the discriminant analysis; these

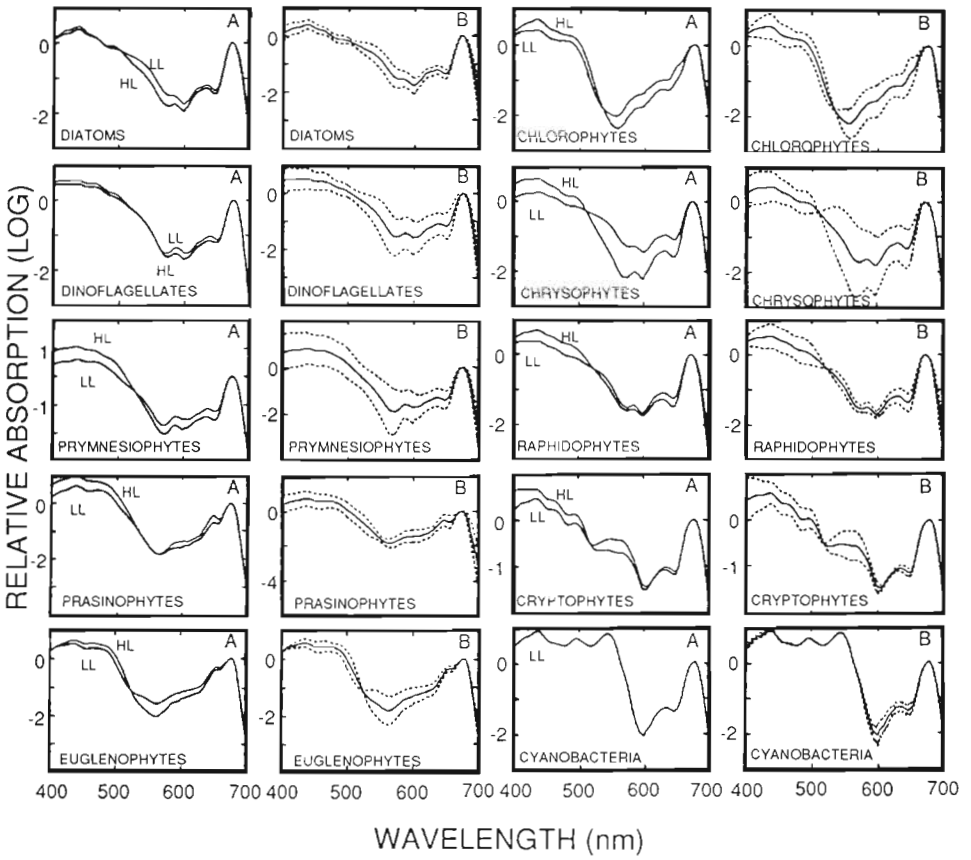

Fig. 3. (A) Mean log-transformed and red-peak (675 nm) scaled *in vivo* absorption spectra [$a_{log}(\lambda)$] in LL- and HL-adapted cells of 10 phytoplankton classes respectively. (B) Same as (A), but averaged for LL- and HL-adapted cells. Dotted lines: SD of the single observations. Species as in Table 1

are mainly toxic and bloom-forming species (Johnsen et al. 1992, Johnsen & Sakshaug 1993). LL- and HL-adapted cells of the chl c_3-containing dinoflagellates *Gyrodinium aureolum* and *Gymnodinium galatheanum* (Johnsen & Sakshaug 1993) were grouped within or near the chl c_3-containing prymnesiophytes, respectively. This indicates that their optical properties are similar to important chl c_3-containing prymnesiophyte genera such as *Chrysochromulina*, *Prymnesium*, *Emiliania*, and *Phaeocystis* (species A and 9 in Fig. 2; Jeffrey 1989, Johnsen et al. 1992, Johnsen & Sakshaug 1993, G. Johnsen unpubl.). The chl c_{1+2}-containing *Chaetoceros* cf. *gracilis* (diatom) and *Isochrysis galbana* (prymnesiophyte lacking chl c_3) are also grouped at the border between chl c_3 and chl c_{1+2}-containing phytoplankton, indicating slightly different optical properties.

Table 3. Stepwise discriminant analysis. Identification of taxonomically important wavelengths based on $a_{log}(\lambda)$ obtained from 31 species of phytoplankton. % Pred: percent successful classifications. Data are grouped into low-light adapted cells (LL, 15 to 75 µmol m^{-2} s^{-1}), high-light adapted cells (HL, 170 to 500 µmol m^{-2} s^{-1}) and pooled data (HL + LL) by using up to 7 wavelengths (nm) in combination

Cum. no. of wavelengths used	HL + LL (n = 217)		LL (n = 147)		HL (n = 70)	
	nm	% Pred	nm	% Pred	nm	% Pred
1	487	14	649	50	475	69
2	652	47	628	74	652	96
3	640	72	640	93	538	97
4	544	86	535	99	640	100
5	628	92	481	100	523	100
6	586	98	517	100	628	100
7	493	99	493	100	505	100

Table 4. Jackknifing technique. Verification of the classification success of selected models (wavelengths) found by means of stepwise discriminant analysis (Table 3) using the jackknife technique. Data are grouped into low-light adapted cells (LL, 15 to 75 µmol m^{-2} s^{-1}), high-light adapted cells (HL, 170 to 500 µmol m^{-2} s^{-1}) and pooled data (HL + LL) by using from 3 to 5 wavelengths (nm) in combination

Wavelengths (nm)	% Prediction		
	HL + LL (n = 217)	LL (n = 147)	HL (n = 70)
481, 535, 649	60	93	93
481, 535, 586, 649	73	97	97
481, 535, 586, 628, 649	86	99	97

DISCUSSION

Our results demonstrate that *in vivo* light absorption characteristics and pigmentation agree closely, both in low light- and high light-adapted phytoplankton and that variations due to photoadaptation can be minimized albeit not entirely removed by our procedure involving log-transformation and scaling. However, 3 appropriately chosen wavelengths (481, 535, 649 nm) appear to be sufficient to identify groups, to some extent also species of phytoplankton. If we add 2 more wavelengths, i.e. 586 nm (indicating red peak absorption maxima of chl c_3) and 628 nm (indicates absorption peaks related to chl c_{1+2}; Johnsen et al. 1992, Johnsen & Sakshaug 1993) the prediction success increases from 93 to 99% for sets grouped according to photoadaptional status (Table 4). The prediction success for pooled data increased more, from 60 to 86% (Table 4).

We found ca 20 major pigments which we consider as bio-optically taxonomically important (cf. Rowan 1989), in particular the accessory chlorophylls, which separate the 10 different phytoplankton classes optically into 4 main groups (1 toxic and 3 non-toxic; Table 2, Figs. 2 & 3).

Several techniques have been used to classify absorption characteristics in phytoplankton, i.e. spectral derivative analysis (Bidigare et al. 1989a), multiple regression (Sathyendranath et al. 1987) and deconvolution of absorption spectra (Mann & Myers 1968, Hoepffner & Sathyendranath 1991). Our results are, for the most part, in accordance with these studies. Moreover, determination of *in vivo* weight-specific absorption coefficients for individual pigments or pigment-protein complexes has shown to be a promising and useful technique to trace pigment-specific signatures and to model the effect of packaging on the absorption characteristics of phytoplankton (Bidigare et al. 1987, Nelson & Prézelin 1990). Our studies indicate that additional transformation of data and the discriminant analysis on $a_{log}(\lambda)$ spectra together with spectral pigment fingerprinting, represented by HPLC data (Table 2), may also be useful in discriminating between species-specific absorption and in finding which sets of wavelengths are optimum for distinguishing between different phytoplankton taxa. As an example, all bloom-forming and toxic phytoplankton genera examined here have been found within or near the chl c_3 group, which in turn can be separated from other groups by the use of only 3 appropriate wavelengths (481, 535, 649 nm) if LL and HL data are grouped separately. Unfortunately, the chl c_3 group cannot be further separated into toxic and non-toxic subgroups because the optical characteristics of the species in this group examined by us were too similar.

On the basis of a study of 5 phytoplankton species, SooHoo et al. (1986) concluded that photoadaptation affects the light absorption characteristics of phytoplankton more than the species-specific pigment signature. Three of the five species examined by them were also examined here, i.e. the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* and the prymnesiophyte *Pavlova lutheri*. These species have a similar pigment composition and thus, light absorption characteristics, when grown under similar light conditions (Figs. 1 to 3). In our study, the absorption spectra of the 3 species could be separated on the basis of only 3 wavelengths. We think this demonstrates the usefulness of the transformation and scaling of spectra employed here.

Admittedly, our data are based on unialgal laboratory cultures. However, such studies represent a necessary first step if *in situ* multi-wavelength bio-optical data are to be interpreted in terms of species composition. The use of 5 taxonomically relevant wavelengths (481 535, 586, 628, 649 nm) yielded near-optimal classification success, however, 3 of these wavelengths may be inconvenient in conjunction with remote sensing of ocean colour because of the high absorption coefficients of water relative to phytoplankton at 580 to 700 nm (cf. Kirk 1992). This may, to some extent, impede classification on the basis of remotely based data. Our approach, presumably, will be more useful for treating data from multi-wavelength attenuation sensors suspended from buoys. If communities are small and multi-specific, identification may be difficult. Yet, the more the phytoplankton community approaches a unialgal bloom, the easier it should be to identify the group to which the predominant species belong. Our 3 to 5 wavelength approach is therefore primarily relevant during phytoplankton blooms for which knowledge of species composition (e.g. toxic vs non-toxic species) may be of practical relevance in conjunction with 'algal warning' systems developed in conjunction with aquaculture.

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LITERATURE CITED

Anderson, T W. (1984). An introduction to multivariate statistical methods, 2nd edn. John Wiley, New York

Bidigare, R. R., Kennicutt, M. C. II, Ondrusek, M. E., Keller, M. D., Guillard, R. R. L. (1990a). Novel chlorophyll-related compounds in marine phytoplankton: distributions and geochemical implications. Energy Fuels 4: 653–657

Bidigare, R. R., Morrow, J. H., Kiefer, D. A. (1989a). Derivative analysis of spectral absorption by photosynthetic pigments in the western Sargasso Sea. J. mar. Res. 47: 323–341

Bidigare, R. R., Ondrusek, M. E., Morrow, J. H., Kiefer, D. A. (1990b). *In vivo* absorption properties of algal pigments. Ocean Optics 1302: 290–302

Bidigare, R. R., Schofield, O., Prézelin, B. B. (1989b). 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

Bjørnland, T., Liaaen-Jensen, S. (1989). Distribution patterns of carotenoids in relation to chromophyte phylogeny and systematics. In: Green, J. C., Leadbeater, B. S. C., Diver, W. L. (eds.) The chromophyte algae: problems and perspectives. Systematics Association Special Vol. 38. Clarendon Press, Oxford, p. 37–60

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

Hertzberg, S., Liaaen-Jensen, S. (1966). The carotenoids of blue-green algae. I. The carotenoids of *Oscillatoria rubescens* and an *Athrospera sp.* Phytochemistry 5: 557–563

Hoepffner, N., Sathyendranath, S. (1991). Effect of pigment composition on absorption properties of phytoplankton. Mar. Ecol. Prog. Ser. 73: 11–23

Jeffrey, S. W. (1980). Algal pigment systems. In: Falkowski, P. G. (ed.) Primary productivity in the sea. Brookhaven symposia in biology no. 31. Plenum Press, New York, p. 33–58

Jeffrey, S. W. (1989). Chlorophyll *c* pigments and their distribution in the chromophyte algae. In: Green, J. C., Leadbeater, B. S. C., Diver, W. L. (eds.) The chromophyte algae: problems and perspectives. Systematics Association Special Vol. No. 38. Clarendon Press, Oxford, p. 13–36

Jeffrey, S. W., Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pfl. 167: 191–194

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

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

Krzanowski, W. J. (1988). Principles of multivariate analysis. A user's perspective. Clarendon Press, Oxford

Liaaen-Jensen, S. (1978). Marine carotenoids. In: Scheuer, P. J. (ed.) Marine natural products. Chemical and biological perspectives. Academic Press, New York, p. 1–73

Mann, J. E., Myers, J. (1968). On pigments, growth, and photosynthesis of *Phaeodactylum tricornutum*. J. Phycol. 4: 349–355

Mantoura, R. F. C., Llewellyn, C. A. (1983). The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-pressure liquid chromatography. Analytica chim. Acta 151: 297–314

Mardia, K. V., Kent, J. T., Bibby, J. M. (1979). Multivariate analysis. Academic Press, London

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

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

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 phycological research, Vol. 4. Biopress, Ltd, Bristol, p. 350–465

Rowan, K. S. (1989). Photosynthetic pigments of algae. Cambridge University Press, Cambridge

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

SooHoo, J. B., Kiefer, D. A., Collins, D. J., McDermid, I. S. (1986). *In vivo* fluorescence excitation spectra of marine phytoplankton. I. Taxonomic characteristics and responses to photoadaptation. J. Plankton Res. 8: 197–214

Straub, O. (1987). Key to carotenoids, 2nd edn. Birkhäuser Verlag, Basel

Tabachnick, B. G., Fidell, L. S. (1989). Using multivariate statistics, 2nd edn. Harper & Row, New York

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