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Photosynthetic pigments in 37 species (65 strains) of Haptophyta: implications for oceanography and chemotaxonomy

Manuel Zapata[1], S. W. Jeffrey[2,*], Simon W. Wright[3], Francisco Rodríguez[1], José L. Garrido[4], Lesley Clementson[2]

[1]Centro de Investigacións Mariñas (CIMA), Consellería de Pesca, Xunta de Galicia, Apartado 13, 36620 Vilanova de Arousa, Spain

[2]CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia

[3]Australian Antarctic Division, Kingston, Tasmania 7050, Australia

[4]Instituto de Investigacións Mariñas, Consejo Superior de Investigacións Cientificas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

ABSTRACT: The pigment compositions of 37 species (65 strains) of cultured haptophytes were analysed using improved HPLC methods. We distinguished 8 pigment types based on the distribution of 9 chlorophyll *c* (chl *c*) pigments and 5 fucoxanthin derivatives. All types contained chl c_2 and Mg-2,4-divinyl phaeoporphyrin a_5 monomethyl ester (MgDVP), fucoxanthin, diadinoxanthin and β,β-carotene. Pigment types were based on the following additional pigments: Type 1: chl c_1; Type 2: chl c_1 and chl c_2-*Pavlova gyrans*-type; Type 3: chl c_1 and chl c_2-monogalactosyl diacylglyceride ester (chl c_2-MGDG [18:4/14:0]); Type 4: chl c_1, chl c_3 and non-polar chl c_1-like; Type 5: chl c_1, chl c_3, chl c_2-MGDG [18:4/14:0] and 4-keto-fucoxanthin; Type 6: chl c_3, monovinyl chl c_3 (MV-chl c_3), chl c_2-MGDG [18:4/14:0], 19′-hexanoyloxyfucoxanthin and its 4-keto derivative, and traces of 19′-butanoyloxyfucoxanthin; Type 7: similar to Type 6, minus MV-chl c_3 but with chl c_2-MGDG [14:0/14:0] added; Type 8: similar to Type 6, minus MV-chl c_3 but with significant 19′-butanoyloxyfucoxanthin. Taxonomic associations ranged from single genera to multiple families – Type 1: Pavlovaceae, Isochrysidaceae and Pleurochrysidaceae; Type 2: Pavlovaceae; Type 3: Isochrysidaceae; Type 4: *Prymnesium* spp.; Type 5: *Ochrosphaera* spp.; Type 6: Nöelaerhabdaceae, notably *Emiliania* spp.; Type 7: *Chrysochromulina* spp.; Type 8: Phaeocystaceae, Prymnesiaceae and Isochrysidaceae. These pigment types showed a strong correlation with available phylogenetic trees, supporting a genetic basis for the pigment associations. The additional marker pigments offer oceanographers greater power for detecting haptophytes in mixed populations, while also distinguishing a greater proportion of them from diatoms.

KEY WORDS: Haptophyta · HPLC · Chlorophylls *c* · Fucoxanthins · Pigment types · Phylogeny · Oceanography

INTRODUCTION

Haptophyte microalgae are an important component of the world's oceanic phytoplankton (Okada & McIntyre 1977), blooming seasonally at polar, equatorial and subtropical latitudes (Brown & Yoder 1994). The calcite-covered coccolithophorids such as *Gephyrocapsa oceanica* and *Emiliania huxleyi* dominate subtropical and sub-polar latitudes (Westbroek et al. 1994), and are significant globally in providing a long-term sink for inorganic carbon (Van der Wal et al. 1995, Paasche 2002). They also produce volatile dimethyl sulphide, which produces cloud-condensation nuclei, increasing cloud cover and affecting regional climates (Malin et al. 1994). In addition, some species (e.g. *Chrysochromulina polylepis*) are highly toxic to fin-fishes (Moestrup 1994). Monitoring of these and other phytoplankton groups is essential in order to follow seasonal successions, impacts of global warming on the marine environment, and harmful ecological events.

While taxonomic monitoring of the 200 known haptophyte species by microscopy is possible (Jordan et al.

*Corresponding author: Email: shirley.jeffrey@csiro.au

1995, Heimdal 1997), it is so time-consuming that oceanographers routinely use photosynthetic pigment profiles as chemotaxonomic markers of phytoplankton groups (Jeffrey et al. 1997b). In order to interpret pigment data from field samples, however, a thorough knowledge of the pigment composition of each of the likely species groups of the phytoplankton populations is necessary. Unfortunately very few wide-ranging pigment surveys of algal classes have been published, exceptions being for diatoms (Stauber & Jeffrey 1988) and haptophytes (Jeffrey & Wright 1994). Dominant species in field samples should always be assessed microscopically in representative samples (Andersen et al. 1996, Wright & van den Enden 2000).

Knowledge of pigment characteristics of any group is always limited by the resolution of current separation methods. The haptophyte pigment study of Jeffrey & Wright (1994), which used the SCOR-UNESCO HPLC method of Wright et al. (1991), distinguished most of the marker carotenoids, but failed to resolve monovinyl and divinyl analogues of chlorophyll *c* (e.g. chlorophylls c_1 and c_2) and additional fucoxanthin derivatives such as 4-keto-19′-hexanoyloxyfucoxanthin (Egeland et al. 2000). Nevertheless 4 useful pigment subgroups of the class were determined. New advances in HPLC pigment technology in the past decade (Jeffrey et al. 1999 [review], Zapata et al. 2000) have allowed a new examination of the pigment composition of this important group of microalgae in the present work.

The recent methods of Garrido & Zapata (1997) and Zapata et al. (2000), in which polymeric C_{18} or monomeric C_8 columns were used with pyridine as solvent modifier, have allowed separation of 11 chlorophyll *c* pigments (including chlorophylls c_1 and c_2) across algal classes (Zapata et al. in press) and several new fucoxanthin derivatives. Structural determinations of 2 'non-polar' chlorophyll *c* pigments in *Emiliania huxleyi* and *Chrysochromulina polylepis* showed them to be, not phytylated chlorophyll *c* derivatives (Nelson & Wakeham 1989), but chlorophyll c_2-monogalactosyl diacylglycerol esters (Garrido et al. 2000, Zapata et al. 2001). The finding of a chlorophyll attached to a massive lipid side-chain is unique in the photosynthetic literature, and this advance may provide new clues to the photosynthetic mechanisms of these important marine species (Jeffrey & Anderson 2000).

Van Lenning et al. (2003) recently used the Zapata et al. (2000) technique to study the pigment content of 9 species of Pavlovaceae, finding 3 pigment types that corresponded with phylogenetic relationships (based on 18S rDNA) and morphological differences within the family.

In this paper, we re-examine the photosynthetic pigments of haptophyte cultures from 7 families (37 species; 65 strains) using the HPLC methods cited above. Algal cultures were selected from 7 haptophyte families — Pavlovaceae, Phaeocystaceae, Prymnesiaceae, Isochrysidaceae, Noëlaerhabdaceae, Pleurochrysidaceae and Hymenomonadaceae — and included many globally important species. Multiple isolates of single species or genera from different geographic regions (e.g. *Emiliania huxleyi*, *Phaeocystis antarctica* and *Chrysochromulina* spp.) were also analysed to determine pigment variability. Of the 50 pigments separated, 9 chlorophyll *c* pigments and 5 fucoxanthin derivatives were useful indicators of 8 haptophyte pigment types. This new information shows the diversity of chlorophyll *c* and fucoxanthin pigments in the photosynthetic apparatus of haptophyte microalgae, and should provide useful additional biomarkers for haptophytes in field studies and new clues to photosynthetic mechanisms and phylogenetic relationships.

MATERIALS AND METHODS

Algal cultures. Haptophyte cultures (37 species, 65 strains) were obtained from 3 sources: the CSIRO Algal Culture Collection (Jeffrey & LeRoi 1997, CSIRO 1998), the Australian Antarctic Division, and the Provasoli-Guillard National Centre for Culture of Marine Phytoplankton (CCMP). Strains, isolate information and culture conditions (media and growth temperatures) are listed in Table 1. Light irradiances were: 60 to 70 µmol quanta m^{-2} s^{-1} on 12 h:12 h light:dark cycles (CSIRO 42 strains, CCMP 14 strains) and 40 µmol quanta m^{-2} s^{-1} on 16:8 h light:dark cycles (Australian Antarctic Division, 10 strains of *Phaeocystis antarctica*).

Sample preparation. Cultures were examined by light microscopy before HPLC pigment analysis to ensure the cells were in excellent health and morphology. Cells were harvested 4 to 6 h into the light cycle from cultures in exponential growth phase. We filtered 10 ml of each culture onto 25 mm Whatman GF/F filters using less than 20 kPa vacuum. Filters were frozen immediately at –25°C, and analysed within 12 h.

Pigment extraction. Frozen filters were extracted under low light in Teflon-lined screw capped tubes with 5 ml 95% methanol using a stainless steel spatula for filter-grinding. The tubes were chilled in a beaker of ice and sonicated for 5 min in an Ultrasonics Australia bath. Extracts were then filtered through 25 mm diameter hydrophilic Teflon (PTFE) syringe filters (MFS HP020, 0.2 µm pore size) to remove cell and filter debris. An aliquot (0.5 ml) of the methanol extract was mixed with 0.2 ml of water and 200 µl was injected immediately into the HPLC. This procedure avoids peak distortion of early eluting peaks (Zapata & Garrido 1991) and prevents the loss of non-polar pigments prior to injection.

Table 1. Haptophyte species, strain codes, culture media and growth temperatures of 37 species (65 strains) examined. CS = CSIRO Culture Collection of Living Microalgae, Hobart, Australia; CCMP = Provasoli-Guillard National Center for Culture of Marine Phytoplankton, USA. Culture medium: f/2 and variations fE/2, f/2-Si (Guillard & Ryther 1962, Guillard 1975); G medium and dilutions G/2, GP5; GSe = G + selenium; GSe/2 (CSIRO modifications of GPM medium, Loeblich 1975); K = Keller medium (Keller et al. 1987; modified by CSIRO Algal Culture Group); L1, L2, L2* = L2 in diluted seawater (Guillard & Hargraves 1993)

Taxon	Strain code	Culture medium	Growth temp (°C)	Geographic origin	Original designation or synonym
CLASS PAVLOVOPHYCEAE					
Order Pavlovales					
Family Pavlovaceae					
Diacronema vlkianum Prauser	CS-266	GSe	17.5	Ryde, Isle of Wight, UK	Plymouth 244, CCAP914/1
Diacronema sp.	CCMP1610	L2*	15	Chesapeake Bay, USA	
Pavlova gyrans Butcher	CCMP608	L2	17	Helfard, Cornwall, UK	MPPAV, Plymouth 93
	CS-213	f/2	17.5	Helfard, Cornwall, UK	Plymouth 93, CCMP608
Pavlova lutheri (Droop) Green	CS-23	f/2	17.5	Halifax, Canada	
	CS-182	f/2	17.5	Finland	CCMP1325, MONO
Pavlova pinguis Green	CS-286	GSe	17.5	Madeira, Atlantic Ocean	Plymouth 471, CCAP 940/2
	CS-375	GSe	15	Pipeclay Lagoon, Tasmania, Australia	PRPL01
Pavlova sp.	CS-50	f/2	17.5	Sargasso Sea	Woods Hole Tr.fl, CCMP613
	CS-63	f/2	17.5	Port Phillip Bay, Victoria, Australia	SPECK 16.3
Rebecca (*Pavlova*) *salina* (Carter) Green	CS-49	f/2	17.5	Sargasso Sea	Woods Hole S1, CCMP1233
CLASS PRYMNESIOPHYCEAE					
Order Phaeocystales					
Family Phaeocystaceae					
Phaeocystis antarctica Karsten	A 1-3	GP5	3	Antarctica (63° 11′S, 85° 43′E)	
	A 1-4	GP5	3	Antarctica (68° 47′S, 73° 30′E)	
	DE 10	GP5	3	Davis, Antarctica	
	DE 12.1	GP5	3	Davis, Antarctica	
	MSIA-1	GP5	3	Ice, Prydz Bay, Antarctica	
	MSIA-2	GP5	3	Ice, Prydz Bay, Antarctica	
	RG 1.2	GP5	3	Davis, Antarctica	
	RG 2.2	GP5	3	Davis, Antarctica	
	T 4.1	GP5	3	Antarctica (68° 39′S, 72° 21′E)	
	T 9.1	GP5	3	Antarctica (68° 39′S, 72° 21′E)	
Phaeocystis cf. *pouchetii* (Hariot) Lagerheim	CS-165	GSe	17.5	Port Hacking NSW, Australia	
Phaeocystis globosa Scherffel	CCMP627	L2	17	Gulf of Mexico	1209
Order Prymnesiales					
Family Prymnesiaceae					
Chrysochromulina camella Leadbeater & Manton	CS-268	GSe	15	49° 10′N, 6° 10′W	Plymouth 297
Chrysochromulina ericina (Parke) Manton	CCMP282	L1	17	Gulf of Maine, USA	8610C3
	CCMP283	L1	24	Gulf of Maine, USA	8610G
Chrysochromulina hirta Manton	CS-228	GSe	15	–	Polar Institute Japan
Chrysochromulina kappa Parke & Manton	CCMP288	L1	17	Bigelow Laboratory, West Boothbay Harbor, USA	3D
Chrysochromulina polylepis Manton & Parke	CCMP286	L2	17	Kristineberg, Sweden	ED2
Chrysochromulina strobilus Parke & Manton	CS-231	GSe	15	–	Polar Institute Japan
Chrysochromulina sp.	CS-410	GSe	17.5	Pipeclay Lagoon, Tasmania, Australia	
Imantonia rotunda Reynolds	CCMP457	L2	15	38° 42′N, 72° 22′W	IID2
	CS-194	fE/2	17.5	Gulf of Mexico	CCMP456
Prymnesium parvum Carter	CCMP708	L2	15		PRYM
Prymnesium patelliferum (Green, Hibberd & Pienaar)	CS-345	GSe	17.5	Cawthron Institute, New Zealand	CAWP 12
Prymnesium patelliferum	CS-288	GSe	17.5	Dorset, UK	Plymouth 527, CCMP709
	CS-376	GSe	15	Pipeclay Lagoon, Tasmania, Australia	
Prymnesium sp.[a]	CS-458	GSe	17.5	Serpentine River, Western Australia	

(Table continued on next page)

Table 1 (continued)

Taxon	Strain code	Culture medium	Growth temp (°C)	Geographic origin	Original designation or synonym
Order Isochrysidales					
Family Isochrysidaceae					
Chrysotila lamellosa Anand emend. Green & Parke	CS-272	GSe	17.5	UK	Plymouth 408, CCAP 918/1
Cricosphaera carterae (Braarud & Fagerland) Braarud[b]	CS-40	G	17.5	–	F.T. Haxo Cr. cart.
Dicrateria inornata Parke	CCMP355	L2	15	–	DICRAT
	CS-254	f/2-Si	17.5	–	CCMP355
	CS-267[c]	GSe	17.5	Plymouth, UK	Plymouth B, CCAP 915/1
	CCMP1323	L2	15	Isle of Man, UK	Plymouth I, CCAP 927/1
	CS-22	f/2	17.5	Halifax, Canada	
Isochrysis sp.	CS-177	f/2	17.5	Tahiti, Society Islands	CCMP1324, T-ISO
Pseudoisochrysis paradoxa Ott	CS-186	f/2	17.5	York River, Virginia, USA	CCMP715, VA12
Family Noëlaerhabdaceae					
Emiliania huxleyi (Lohmann) Hay & Mohler	CCMP370	L2	15	Oslo fjord, Norway	451B
Emiliania huxleyi	CCMP373	L2	15	Sargasso Sea	BT-6
	CS-57	f/2	17.5	Sargasso Sea	BT-6
	CS-275-2	GSe	15	60°N 20°W, Iceland Basin	Plymouth G1779Ga
	CS-279	GSe	15	24° 27′N, 20° 24′W	Plymouth DNN53/74/6
	CS-282	GSe	15	32°N 62°W, Sargasso Sea	Plymouth M181
	CS-283	GSe	15	Durban, South Africa	Plymouth M186
	CS-284	GSe	15	Sargasso Sea	Plymouth MCH-1,CCMP375
	CS-363	G/2	15	Pipeclay Lagoon, Tasmania	
	CS-369	K	15	Pipeclay Lagoon, Tasmania	
	CS-370	K	15	Pipeclay Lagoon, Tasmania	
Gephyrocapsa oceanica Kamptner	CS-335	GSe/2	17.5	Jervis Bay, NSW, Australia	
Order Coccolithales					
Family Pleurochrysidaceae					
Pleurochrysis aff. *carterae* (Braarud & Fagerland) Christiansen	CS-287	GSe	17.5	Port Erin, Isle of Man, UK	Plymouth 156, CCMP646 CCAP 961/5
Pleurochrysis roscoffensis Chadfaud & Feldman	CCMP1588	L2	15	Narragansett Bay, USA	CO791C
Family Hymenomonadaceae					
Ochrosphaera neapolitana Schussnig	CS-285	GSe	17.5	Salcombe, UK	Plymouth 162, CCAP 923/1
Ochrosphaera verrucosa Schussnig	CCMP594	L2	15	Puerto Penasco, Mexico	UW 390, Norris 20-6-11
UNKNOWN					
Haptophyte (unidentified)	CS-124	G/2	25	Coral Sea	
	CS-260	fE/2	25	Dunk Island, Queensland, Australia	

[a] Now identified as *P. parvum* (G. M. Hallegraeff pers. comm.)
[b] *C. carterae* is also known as *Pleurochrysis carterae* (Braarud & Fagerland) Christensen
[c] *Dicrateria inornata* Parke (CS-267) shows pigment pattern indistinguishable from that of *Imantonia rotunda* (CS-194)

HPLC pigment analyses. We used 2 HPLC methods: the C_8 method of Zapata et al. (2000), which was used for all haptophyte cultures, and the C_{18} method of Garrido & Zapata (1997), which was used for a subset of the cultures. The chromatographic equipment for the C_8 method was a Waters 600 pump and a Waters 996 diode-array detector (samples analysed at CSIRO Marine Research, Hobart, Australia). The stationary phase was a C_8 column (Waters Symmetry, 150 × 4.6 mm, 3.5 µm particle size, 100 Å pore size) thermostated at 25°C either by means of a column oven, or a 25°C circulating water bath. Mobile phases were: A = methanol:acetonitrile: aqueous pyridine solution (0.25 M pyridine, pH adjusted to 5.0 with acetic acid) in the proportions 50:25:25 (v/v/v), and B = acetonitrile: acetone (80:20 v/v). A segmented linear gradient was (time in min, % B): 0 min, 0%; 18 min, 40%; 22 min, 100%; 38 min, 100%. Initial conditions were re-established by reversed linear gradient (4 min). Flow rate was 1 ml min^{-1}.

The C_{18} HPLC method of Garrido & Zapata (1997) was used to analyse 12 haptophyte species (14 strains) from

CCMP, cultured at the Instituto de Investigacións Mariñas. The HPLC equipment in the Spanish laboratory was a Waters Alliance HPLC System with a 2690 separations module, a Waters 996 photodiode array detector (350 to 750 nm; 1.2 nm optical resolution) interfaced to a Waters 474 scanning fluorometer (samples analysed at Centro de Investigacións Mariñas, Spain). The stationary phase was a polymeric C_{18} column (Vydac 201 TP54, 250 × 4.6 mm, 5 µm particle size, 300 Å pore size) thermostated at 27°C by a column oven. Mobile phases were: A = methanol:acetonitrile:aqueous pyridine solution (0.25 M pyridine, pH adjusted to 5.0 with acetic acid) in the proportions 45:35:20 (v/v/v), and B = acetonitrile:acetone (60:40, v/v). A segmented linear gradient was programmed as follows (time in min, %B): 0 min, 0%; 28 min, 60%; 32 min, 100%; 38 min, 100%. Initial conditions were re-established by reversed linear gradient (4 min). Flow rate was 1.2 ml min^{-1}.

The 2 HPLC techniques achieve separations primarily by differences in hydrophobic interactions of the pigments with the stationary phase, and the polymeric C_{18} method has an additional shape-dependent mechanism that allows separation of pigments with very similar molecular structures (see Garrido & Zapata 1997). Using both systems allowed comparison of unknown pigments with pigment standards under differing conditions (Bjørnland 1997, Jeffrey & Mantoura 1997b).

Pigment identification. Pigments were identified either by co-chromatography with authentic standards obtained from SCOR (Scientific Commitee for Oceanic Research) reference cultures and diode-array spectroscopy (see Zapata et al. 2000) or by liquid chromatography – mass spectrometry. After checking for peak purity, spectral information was compared with a library of chlorophyll and carotenoid spectra from pigments prepared from standard phytoplankton cultures (SCOR cultures, see Jeffrey & Wright 1997). For both known and novel compounds, electrospray mass spectra (ES-MS) were obtained with a Thermo Quest-Finnigan Navigator mass spectrometer coupled to a Thermo Quest liquid chromatograph with a Waters Symmetry C_{18} (150 × 2 mm, 3.5 µm particle size, 100 Å pore size) column. Each pigment was injected using 95% aqueous methanol as mobile phase at a flow rate of 200 µl min^{-1}. Mass spectra of carotenoids were acquired in positive ion mode (insert probe capillary voltage = 4 kV, probe temperature = 200°C, cone voltage = 30 V).

Pigment nomenclature and abbreviations were as suggested by SCOR Working Group 78 (Jeffrey & Mantoura 1997a), noting that MgDVP is also known as divinyl-protochlorophyllide (DV-Pchlid) (Zapata et al. in press). For nonpolar chlorophyll *c*-like pigments whose molecular structures have recently been elucidated, the nomenclature was chl c_2-MGDG [18:4/14:0] for the major compound from *Emiliania huxleyi* (Garrido et al. 2000), and chl c_2-MGDG [14:0/14:0] for the major compound from *Chrysochromulina polylepis* (Zapata et al. 2001). Fatty acids in these chl *c*-MGDG pigments are designated as 'total number of C atoms:number of double bonds'. For chlorophylls whose molecular structure is unknown, the pigment name includes a reference to the most likely chl *c* chromophore (chl c_1, c_2 and c_3-like), as well as the species in which the pigment was initially detected (e.g. chl c_2-like *Pavlova gyrans*-type, nonpolar chl c_2-like *Chrysochromulina hirta*-type).

Pigment quantification. HPLC calibration by external standards was performed using chlorophyll and carotenoid standards isolated from microalgal cultures (Zapata et al. 2000). The molar extinction coefficients (ε; l mol^{-1} cm^{-1}) provided by Jeffrey (1997b) were used for pigment quantification. For chl *c*-like pigments whose molar extinction coefficients are not available (i.e. chl c_3, MV-Chl c_3, and chl c_2-like *Pavlova gyrans*-type) the mean of the extinction coefficients for chl c_1 and c_2 at the blue absorption band (see Jeffrey et al. 1997a) was used. The nonpolar chls *c* were quantified by using the molar extinction coefficient of the appropriate chl c_2 or chl c_1 chromophore. For fucoxanthin-related compounds (i.e. acyloxy and 4-keto derivatives), the molar extinction coefficient for fucoxanthin was used, following the recommendations of Jeffrey et al. (1997a), even though the absorption spectra of fucoxanthin-derivatives differ slightly from those of the parent compounds. Thus pigment to chl *a* ratios are expressed on a molar basis (mol mol^{-1}).

RESULTS

Chromatographic resolution and pigment identities

The elution order of pigments by the Zapata et al. (2000) method is shown in Table 2 together with retention times and visible absorption maxima in eluent. Of the 44 pigments, 25 were well-known chlorophylls and carotenoids, and had previously been characterised (Jeffrey & Wright 1987, Bjørnland & Liaaen-Jensen 1989, Fookes & Jeffrey 1989, Jeffrey 1989, Jeffrey et al. 1997b, Helfrich et al. 1999, Egeland et al. 2000, Zapata et al. 2000). Structures of chlorophyll c pigments may be found in Jeffrey (1997a) and Zapata et al. (in press), and structures of algal carotenoids in Bjørnland (1997) and Egeland et al. (2000). We also detected 7 unknown pigments with chlorophyll *c*-like spectra and 12 unknown pigments with carotenoid-like spectra in trace quantities. Pigments used to discriminate haptophyte pigment types (see next subsection) are given in boldface in Table 2.

One important unknown carotenoid isolated from *Ochrosphaera verrucosa* was tentatively identified as 4-keto-fucoxanthin. Electrospray mass spectra (ES-MS) of

Table 2. Elution and visible absorption characteristics of pigments in eluent from haptophyte cultures using C_8 HPLC method (Zapata et al. 2000). Wavelengths in parentheses denote shoulders. Pigments in boldface are those used to discriminate pigment types

Peak no.	Pigment	Abbreviation	Time (min)	λ maxima in eluant (nm)		
	(Solvent front)		1.93			
1	**Chlorophyll c_3**	chl c_3	7.19	459	591	(629)
2	**Chlorophyll c_2-like from *Pavlova gyrans***	chl c_2 *P. gyrans*-type	7.71	459	586	635
3	**Monovinyl chlorophyll c_3**	MV chl c_3	7.87	452	586	631
4	Unknown chlorophyll *c*	unk-chl *c*	9.20	450	583	631
5	Chlorophyllide *a*	chlide *a*	10.46	430	581	663
6	**Mg-2,4-divinyl phaeoporphyin a_5 monomethyl ester**[a]	MgDVP	10.50	439	577	628
7	**Chlorophyll c_2**	chl c_2	11.05	453	586	635
8	**Chlorophyll c_1**	chl c_1	11.74	449	583	633
9	Methyl-chlorophyllide *a*	Me-chlide *a*	12.46	430	581	663
10	Unknown 19′-butanoyloxyfucoxanthin-like	unk-car 1	16.25		448	472
11	**4-keto-fucoxanthin**[b]	4-k-fuco	17.29		453	
12	**19′-butanoyloxyfucoxanthin**	but-fuco	17.63		447	471
13	**Fucoxanthin**	fuco	18.27		450	
14	Unknown fucoxanthin derivative	unk-car 2	19.15		447	471
15	**4-keto-19′-hexanoyloxyfucoxanthin**	4-k-hex-fuco	20.18		448	472
16	Violaxanthin	violax	20.30	417	441	471
17	**19′-hexanoyloxyfucoxanthin**	hex-fuco	20.91		447	471
18	Unknown carotenoid λ_{max}447	unk-car 3	21.85		447	475
19	Diadinochrome	diadchr	21.91	(406)	429	457
20	**Diadinoxanthin**	diadino	22.54	(422)	448	477
21	cis-fucoxanthin	c-fuco	23.31		442	
22	Unknown carotenoid λ_{max}448	unk-car 4	23.84		448	471
23	Unknown carotenoid λ_{max}447	unk-car 5	24.38		447	470
24	Diatoxanthin	diato	25.66	(426)	454	482
25	Non-polar chlorophyll c_2-like from *Chrysochromulina hirta*	np-chl c_2 *C. hirta*-type	26.84	455	584	633
26	Unknown carotenoid λ_{max}447	unk-car 6	26.95	(420)	447	472
27	Unknown carotenoid λ_{max}442	unk-car 7	28.00	418	442	471
28	Unknown carotenoid λ_{max} 445	unk-car 8	28.00	(419)	445	471
29	Unknown carotenoid λ_{max}446	unk-car 9	28.00	(419)	446	473
30	Unknown carotenoid λ_{max} 448	unk-car 10	28.92	426	448	477
31	Non-polar chlorophyll c_2-like from *Emiliania huxleyi*	np-chl c_2 *E. huxleyi*-type	30.09	454	584	631
32	Non-polar chlorophyll c_2-like from *Emiliania huxleyi*	np-chl c_2 *E. huxleyi*-type	30.39	454	584	631
33	Non-polar chlorophyll c_2-like from *Emiliania huxleyi*	np-chl c_2 *E. huxleyi*-type	30.62	454	584	631
34	**Chlorophyll c_2 monogalactosyldiacylglyceride ester from *Emiliania huxleyi***	chl c_2-MGDG [18:4/14:0]	30.82	454	584	631
35	**Non-polar chlorophyll c_1-like from *Prymnesium parvum***	np-chl c_1 *P. parvum*-type	31.08	451	579	632
36	Chlorophyll *a* allomer	chl *a* allomer	31.27	430	615	662
37	**Chlorophyll *a***	chl *a*	31.48	431	617	662
38	**Chlorophyll c_2 monogalactosyldiacylglyceride ester from *Chrysochromulina polylepis***	chl c_2-MGDG [14:0/14:0]	31.83	454	584	631
39	Chlorophyll *a* epimer	chl *a*′	32.06	430	615	664
40	Phaeophytin *a*	phytin *a*	33.21	409	608	665
41	**β,ε-carotene**	βε-car	33.64	(422)	448	476
42	**β,β-carotene**	ββ-car	33.80	(426)	454	481
43	cis-β,β-carotene	c-ββ-car	34.11	(426)	452	478

[a]Also known as 3,8-divinyl protochlorophyllide (DV-Pchlid; Helfrich et al. 1999)
[b]Tentative identification

the compound and that of fucoxanthin are shown in Fig. 1A,B together with their visible absorption spectra (Fig. 1C,D). Fig. 1A presents the mass spectrum of fucoxanthin in positive-ion mode (molecular weight = 658.92) showing signals to the sodium derivative $[M + Na]^+ = 681.7$, and the protonated derivative $[M + H]^+ = 659.6$ molecular ions, and major mass fragments at $641 = [M + H - 18]^+$ and $581 = [M + H - 18 - 60]^+$. In comparison, the unknown fucoxanthin derivative (Fig. 1B) was 14 U heavier, $695 = [M + Na]^+$; $673 = [M + H]^+$; $655 = [M + H - 18]^+$; $595 = [M + H - 18 - 60]^+$, suggesting that the unknown derivative is a ketofuco-xanthin. Visible absorp-

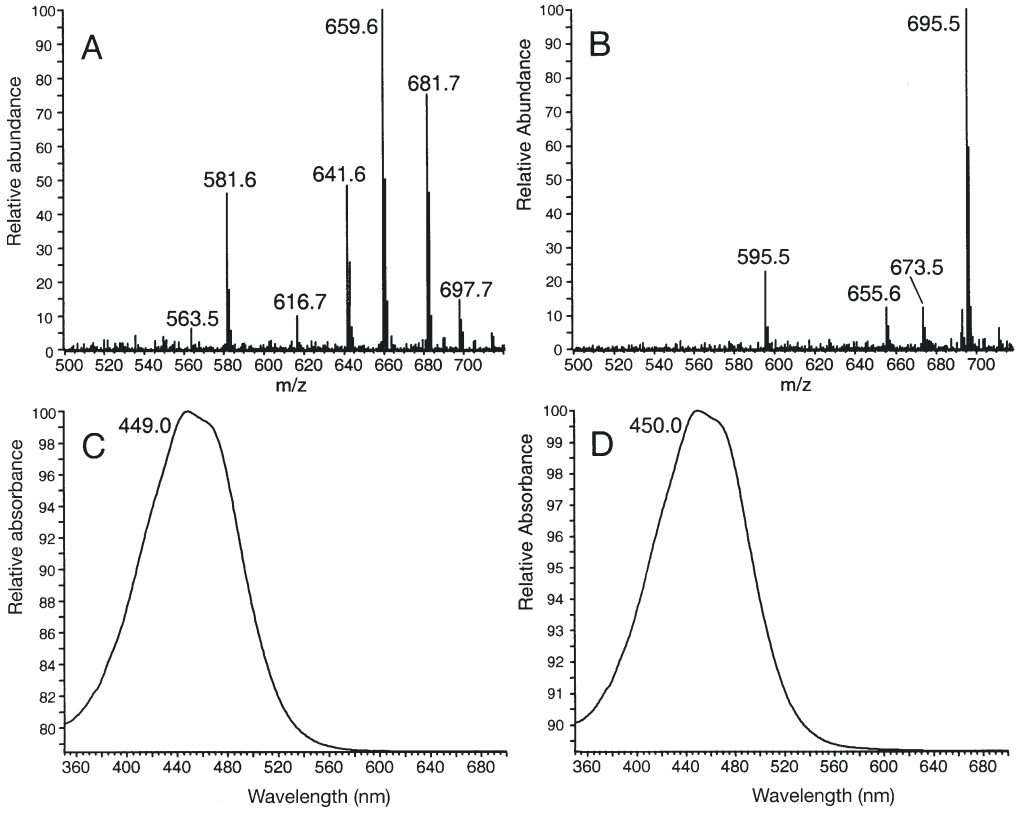

Fig. 1. Mass spectra (A, B) and visible spectra (C, D) of fucoxanthin and an unknown carotenoid (tentatively identified as 4-keto-fucoxanthin) isolated from *Ochrosphaera verrucosa*. Mass spectra were obtained by electrospray mass spectrometry in positive-ion mode

tion spectra of the 2 compounds (Fig. 1C,D) showed that the unknown derivative was indistinguishable from fucoxanthin, except for a 1 nm bathychromic shift of the wavelength of maximum absorption, suggesting the keto group does not affect the chromophore. The position C-4 for the keto substituent is suggested by analogy with the keto derivative of 19′-hexanoyloxyfucoxanthin (Egeland et al. 2000). Further confirmation of this tentative structure by NMR (nuclear magnetic resonance) techniques is needed.

Fig. 2 shows representative chromatograms from each of the 8 haptophyte pigment types found using the C_8 HPLC method. The polar chl *c* pigments eluted before the fucoxanthin derivatives while the non-polar chl *c* pigments eluted near chl *a*. Fig. 3 shows 4 selected haptophyte species separated by the polymeric C_{18} method of Garrido & Zapata (1997). Using this method, fucoxanthin derivatives preceded the polar chl *c* pigments whereas the non-polar chl c_2-MGDG derivatives eluted after chlorophyll *a* in the hydrophobic region of the chromatogram. The different pigment resolution of these methods allowed additional confirmation of peak identities.

Haptophyte pigment composition and definition of pigment types

Quantitative data for all strains are shown as molar pigment to chl *a* ratios, grouped together in similar pigment types, Type 1 being the most simple and Type 8 the most complex (Table 3). Chl *c* distribution patterns across haptophyte families and those of the fucoxanthin derivatives are summarised separately in Tables 4 & 5, respectively. Pigment types were allocated on the basis of increasing diversity of chl *c* and fucoxanthin pigments, noting that chl c_2, MgDVP, fucoxanthin, diadinoxanthin and β,β-carotene were common to all pigment types.

Table 4 shows that MgDVP and chl c_2 were present in all pigment types and were accompanied by chl c_1 (Types 1 to 5); chl c_3 (Types 4 to 8); chl c_2-MGDG [18:4/ 14:0] (Types 3 to 8); with each of the remaining 4 chl *c* pigments found in only 1 pigment type i.e. chl c_2-like *Pavlova gyrans*-type (Type 2); MV-chl c_3 (Type 6); non-polar chl c_1-like pigment (Type 4) and chl c_2-MGDG [14:0/14:0] (Type 7).

Table 5 shows that fucoxanthin occurred in all pigment types and was present as the only fucoxanthin pigment in Types 1 to 4. It was accompanied by

4-keto-fucoxanthin in Type 5; with 19′-hexanoyloxy-fucoxanthin, 4-keto-hexanoyloxyfucoxanthin and traces of 19′-butanoyloxyfucoxanthin in Types 6 and 7; and with significant quantities of 19′-butanoyloxyfucoxanthin, co-dominant with 19′-hexanoyloxyfucoxanthin and 4-keto-hexanoyloxyfucoxanthin, in Type 8.

Quantitative pigment data

Quantitative abundances of chl *c* and fucoxanthin pigments derived from data in Table 3 are shown as molar percentages of total chl *c* and total fucoxanthin in Tables 6 & 7.

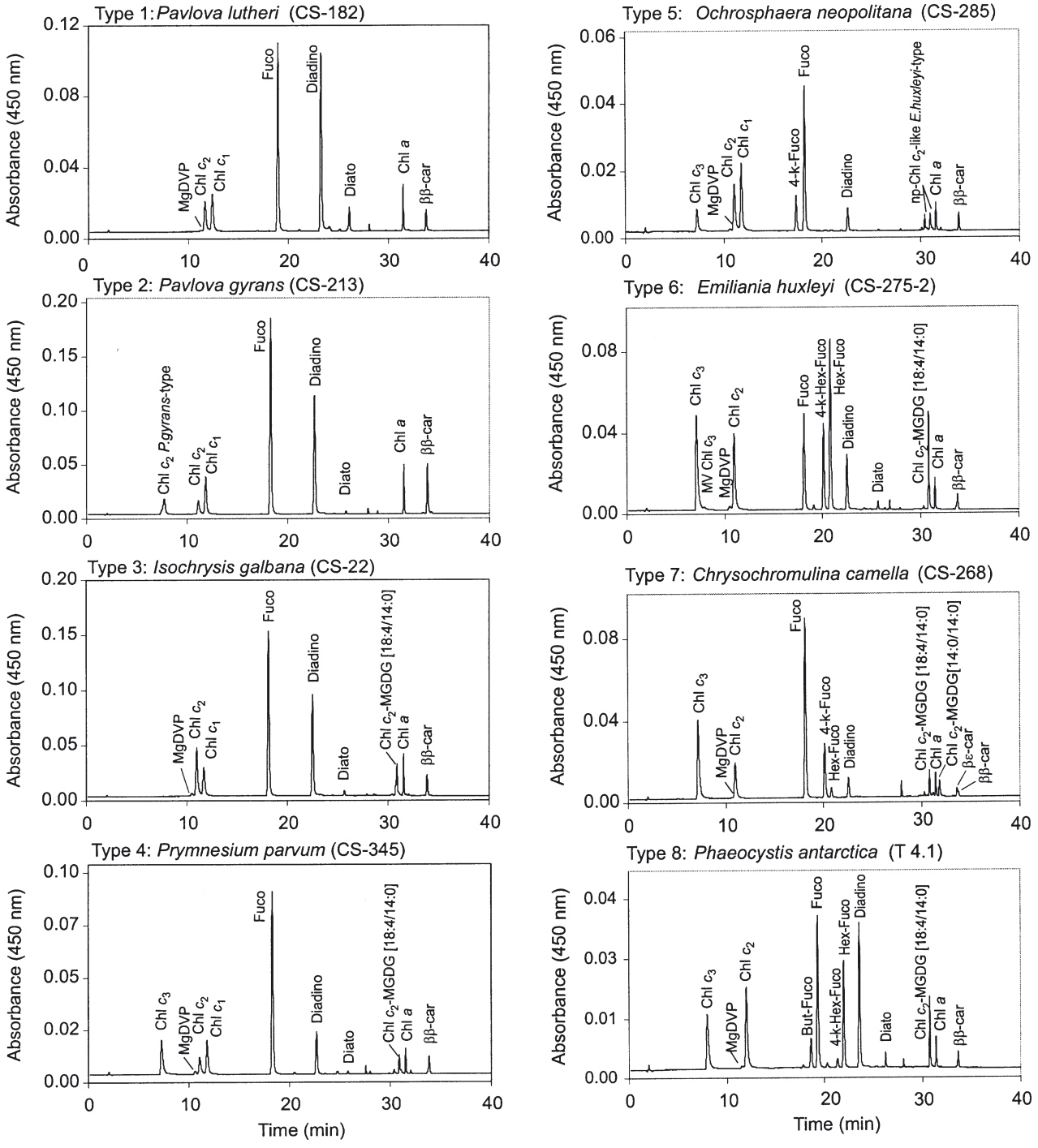

Fig. 2. HPLC chromatograms (C_8 method) of haptophyte species representing each pigment type. Detection by absorbance at 450 nm. Peak identifications as in Table 2

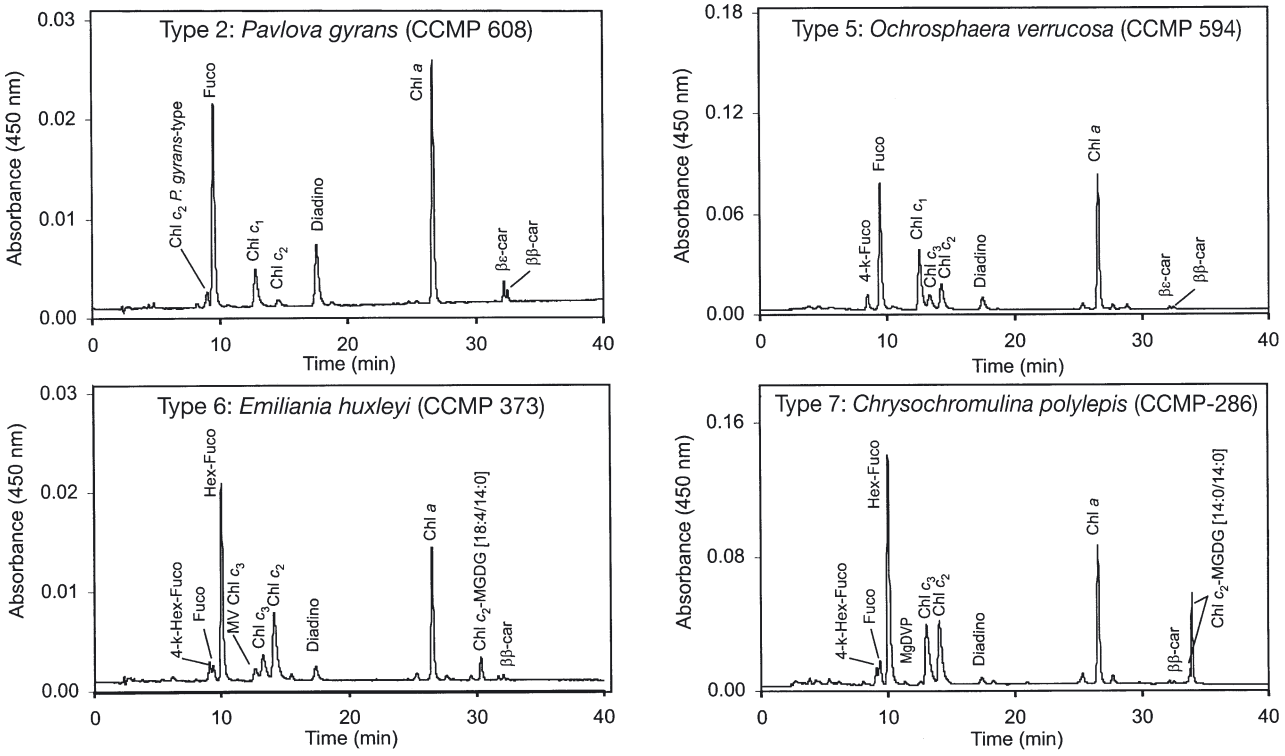

Fig. 3. HPLC chromatograms (polymeric C_{18} method) of haptophyte species belonging to selected pigment types. Detection by absorbance at 450 nm. Peak identifications as in Table 2

Chlorophyll *c* pigments. The 9 chl *c* pigments (quantified as molar percentages of total chlorophyll *c*) ranged from major to trace across the species. Chl c_1, c_2 and c_3 were always of major importance, each ranging from one-third to one-half of the total chl *c* (Table 6). Chl c_2 occurred in all haptophyte pigment Types (1 to 8), while chl c_1 occurred in pigment Types 1 to 5 and chl c_3 in Types 4 to 8. Chl c_1 and c_3 co-occurred in haptophyte pigment Types 4 and 5. The chl c_2-like *Pavlova gyrans*-type pigment was also of major significance, and reached 22 to 36% of the total chlorophyll *c* in haptophyte pigment Type 2. The newly discovered minor pigment chl c_2-MGDG [18:4/14:0] reached 6 to 15% of the total chl *c* in pigment Types 3 to 8, while the remaining 3 pigments, MV-Chl c_3, chl c_2-MGDG [14:0/14:0] and MgDVP, occurred only in trace quantities at 1 to 5% of the total chl *c*, in pigment Types 6, 7 and 1 to 8, respectively.

Fucoxanthin derivatives. Variations in relative abundance of the 5 fucoxanthin derivatives across haptophyte species were less dramatic than those of chl *c*, but they were no less significant (Table 7). Fucoxanthin was always abundant in all strains and was the only fucoxanthin derivative in haptophyte pigment Types 1 to 4. In pigment Type 5, fucoxanthin co-occurred with the minor pigment 4-keto-fucoxanthin which represented 7 to 20% of the total fucoxanthins.

19′-hexanoyloxyfucoxanthin almost always assumed dominance of, or co-dominance with, fucoxanthin when acyloxyfucoxanthins were present (pigment Types 6 to 8). The 4-keto derivative of 19′-hexanoyloxyfucoxanthin usually co-occurred with its parent compound as a minor pigment, representing 7.5 to 22.7% of the total fucoxanthins (Types 6 to 8). Finally, 19′-butanoyloxyfucoxanthin, present only in traces (0.2 to 1.1%) in haptophyte pigment Types 6 and 7, generally assumed major importance (up to 30%) in most strains belonging to haptophyte pigment Type 8 (e.g. *Phaeocystis* spp.).

Fig. 4 shows that a significant relationship ($p < 0.05$) existed between the total fucoxanthins and the total chl *c* pigments, normalised to chl *a*. The implication of these observations is unknown, but may indicate a stoichiometric relationship between chl *c* pigments and fucoxanthin derivatives in the light-harvesting complexes of the Haptophyta.

Detailed examination of Table 3 shows several exceptions to these generalizations — mainly some pigment absences which may represent concentrations below detection limits (e.g. MgDVP in haptophyte pigment Types 1, 2, 3, 6, 7 and 8).

Table 3. Pigment:chlorophyll (chl) *a* molar ratios of 37 species (65 strains) of haptophyte cultures. : below detection limits; *P. gyr* : *Pavlova gyrans*; chl c_2-MGDG [18/14], [14/14]: chl c_2-monogalactosyl diacylglyceride ester [18:4/14:0], [14:0/14:0], respectively. Other abbreviations as in Table 2

Pignent type Species	Strain code	chl c_3	chl c_2 *P. gyr* type	MV-chl c_3	MgDVP	chl c_2	chl c_1	chl c_2-MGDG [18/14]	chl c_2-MGDG [14/14]	but-fuco	fuco	4-k-hex-fuco	Hex-fuco
Type 1													
Chrysotila lamellosa	CS-272	–	–	–	0.003	0.039	0.034	–	–	–	0.282	–	–
Diacronema vlkianum	CS-266	–	–	–	–	0.034	0.053	–	–	–	0.312	–	–
Diacronema sp.	CCMP 1610	–	–	–	0.004	0.039	0.072	–	–	–	0.249	–	–
Pavlova lutheri	CS-23	–	–	–	–	0.036	0.036	–	–	–	0.266	–	–
	CS-182	–	–	–	0.001	0.042	0.048	–	–	–	0.317	–	–
Pleurochrysis roscoffensis	CCMP 1588	–	–	–	0.007	0.051	0.071	–	–	–	0.259	–	–
Pleurochrysis aff. *carterae*	CS-287	–	–	–	0.002	0.037	0.065	–	–	–	0.320	–	–
Type 2													
Pavlova gyrans	CCMP 608	–	0.033	–	0.004	0.016	0.078	–	–	–	0.288	–	–
	CS-213	–	0.025	–	0.001	0.018	0.045	–	–	–	0.316	–	–
Pavlova pinguis	CS-286	–	0.032	–	0.001	0.023	0.059	–	–	–	0.429	–	–
	CS-375	–	0.053	–	–	0.022	0.077	–	–	–	0.503	–	–
Pavlova sp.	CS-50	–	0.028	–	–	0.017	0.060	–	–	–	0.402	–	–
	CS-63	–	0.037	–	–	0.023	0.079	–	–	–	0.535	–	–
Rebecca (*Pavlova*) *salina*	CS-49	–	0.031	–	–	0.021	0.051	–	–	–	0.399	–	–
Type 3													
Cricosphaera carterae	CS-40	–	–	–	0.003	0.101	0.091	0.027	–	–	0.449	–	–
Dicrateria inornata	CCMP355	–	–	–	0.002	0.088	0.049	0.017	–	–	0.254	–	–
	CS-254	–	–	–	0.003	0.064	0.032	0.014	–	–	0.291	–	–
Isochrysis galbana	CCMP1323	–	–	–	–	0.100	0.053	0.016	–	–	0.270	–	–
	CS-22	–	–	–	0.004	0.075	0.042	0.022	–	–	0.321	–	–
Isochrysis sp.	CS-177	–	–	–	0.005	0.130	0.093	0.033	–	–	0.468	–	–
Pseudoisochrysis paradoxa	CS-186	–	–	–	0.002	0.051	0.032	0.033	–	–	0.257	–	–
Type 4													
Prymnesium parvum	CCMP 708	0.099	–	–	0.010	0.066	0.109	0.032	–	–	0.512	–	–
	CS-345	0.085	–	–	0.007	0.043	0.080	0.022	–	–	0.569	–	–
Prymnesium patelliferum	CS-376	0.090	–	0.003	0.005	0.056	0.110	0.030	–	–	0.455	–	–
	CS-288	0.078	–	0.003	0.007	0.041	0.084	0.023	–	–	0.411	–	–
Prymnesium sp.	CS-458	0.069	–	0.003	0.003	0.027	0.071	0.021	–	–	0.469	–	–
Haptophyte 124	CS-124	0.065	–	–	0.003	0.107	0.100	0.024	–	–	0.556	–	–
Type 5													
Ochrosphaera verrucosa	CCMP 594	0.052	–	–	0.009	0.111	0.215	0.016	–	–	0.351	0.038	–
Ochrosphaera neopolitana	CS-285	0.046	–	–	0.004	0.103	0.139	0.020	–	–	0.398	0.099	–
Haptophyte 260	CS-260	0.048	–	–	0.005	0.102	0.139	0.019	–	–	0.434	0.033	–
Type 6													
Emiliania huxleyi	CCMP370	0.178	–	0.009	0.006	0.269	–	0.090	–	0.008	0.032	0.062	0.638
	CCMP 373	0.193	–	0.008	0.006	0.225	–	0.090	–	–	0.008	–	0.739
	CS-57	0.207	–	0.050	0.002	0.241	–	0.094	–	0.007	0.006	–	1.507
	CS-275-2	0.205	–	0.007	0.007	0.152	–	0.090	–	0.003	0.252	0.313	0.593
	CS-279	0.221	–	0.005	0.005	0.171	–	0.097	–	–	0.179	0.145	0.950
	CS-282	0.132	–	0.015	–	0.176	–	0.074	–	–	0.011	–	1.143
	CS-283	0.199	–	–	0.006	0.147	–	0.090	–	–	0.358	0.199	0.490
	CS-284	0.156	–	0.013	0.006	0.147	–	0.091	–	–	0.189	0.100	0.835
	CS-363	0.162	–	–	0.008	0.182	–	0.089	–	–	0.553	0.129	0.292
	CS-369	0.163	–	0.016	0.007	0.173	–	0.089	–	–	0.297	0.121	0.669
	CS-370	0.162	–	0.015	0.007	0.163	–	0.091	–	–	0.375	0.077	0.567
Gephyrocapsa oceanica	CS-335	0.189	–	–	0.004	0.145	–	0.081	–	0.008	0.285	0.254	0.447
Type 7													
Chrysochromulina camella	CS-268	0.214	–	–	–	0.094	–	0.031	0.027	–	0.608	0.266	0.044
Chrysochromulina ericina	CCMP 282	0.346	–	–	0.045	0.400	–	0.285	–	0.006	0.384	0.674	1.315
	CCMP 283	0.219	–	–	0.012	0.179	–	0.163	–	–	0.319	0.425	0.527
Chrysochromulina hirta	CS-228	0.207	–	–	0.006	0.134	–	0.134	–	–	0.425	0.379	0.321
Chrysochromulina kappa	CCMP 288	0.178	–	–	0.008	0.193	–	0.067	–	0.007	0.747	0.190	0.261
Chrysochromulina polylepis	CCMP286	0.219	–	–	0.014	0.166	–	0.002	0.067	0.006	0.124	0.084	1.107
Chrysochromulina strobilus	CS-231	0.194	–	–	0.003	0.123	–	0.025	0.031	–	0.751	0.129	–
Chrysochromulina sp.	CS-410	0.221	–	–	0.003	0.131	–	0.013	0.046	0.011	0.203	0.262	0.775
Type 8													
Dicrateria inornata	CS-267	0.236	–	–	0.006	0.081	–	0.058	–	0.266	0.036	0.106	0.694
Imantonia rotunda	CS-194	0.204	–	–	0.002	0.074	–	0.048	–	0.223	0.042	0.141	0.496
	CCMP 457	0.274	–	0.025	0.009	0.119	–	0.077	–	0.117	0.293	0.085	0.336
Phaeocystis antarctica	A 1-3	0.110	–	–	–	0.239	–	0.033	–	–	0.102	0.055	0.296
	A 1-4	0.062	–	–	–	0.199	–	0.031	–	–	0.065	0.050	0.344
	DE 10	0.077	–	–	–	0.197	–	0.025	–	–	0.072	–	0.222
	DE 12.1	0.156	–	–	0.002	0.183	–	0.067	–	0.169	0.012	0.005	1.052
	MSIA-1	0.153	–	–	0.004	0.189	–	0.067	–	0.120	0.532	0.029	0.020
	MSIA-2	0.161	–	–	0.003	0.144	–	0.067	–	0.196	0.521	0.039	0.065
	RG 1.2	0.091	–	–	–	0.219	–	0.029	–	–	0.032	–	0.343
	RG 2.2	0.142	–	–	0.003	0.144	–	0.054	–	0.080	0.011	0.007	0.918
	T 4.1	0.123	–	–	0.004	0.170	–	0.063	–	0.111	0.398	0.035	0.384
	T 9.1	0.109	–	–	–	0.273	–	0.035	–	0.022	0.104	0.066	0.284
Phaeocystis pouchetii	CS-165	0.218	–	–	–	0.118	–	0.056	–	0.270	0.166	0.133	0.333
Phaeocystis globosa	CCMP 627	0.200	–	–	0.016	0.213	–	0.035	–	0.007	0.223	0.051	0.155

Table 4. Distribution of chlorophyll *c* pigments across haptophyte families, showing families with designated pigment type and type species with characteristic pigment patterns. n: no. of species (strains); other abbreviations as in Table 2

Pigment type Haptophyte family	Type species	n	Chlorophyll *c* pigments chl c_2 *P. gyrans*-type	chl c_3	MV chl c_3	MgDVP	chl c_2	chl c_1	chl c_2-MGDG [18:4/14:0]	np-chl c_1-like	chl c_2-MGDG [14:0/14:0]
Type 1											
Pavlovophyceae Isochrysidaceae Pleurochrysideceae	*Chrysotila lamellosa*	6 (7)				•	•	•			
Type 2											
Pavlovophyceae	*Pavlova gyrans*	5 (7)	•			•	•	•			
Type 3											
Isochrysidaceae	*Isochrysis galbana*	5 (7)				•	•	•	•		
Type 4											
Prymnesiaceae	*Prymnesium parvum*	4 (6)		•		•	•	•	•	•	
Type 5											
Hymenomonadaceae	*Ochrosphaera neopolitana*	3 (3)		•		•	•	•	•		
Type 6											
Noëlaerhabdaceae	*Emiliana huxleyi*	2 (12)		•	•	•	•		•		
Type 7											
Prymnesiaceae	*Chrysochromulina polylepis*	7 (8)		•		•	•		•		•
Type 8											
Phaeocystaceae Prymnesiaceae Isochrysidaceae	*Phaeocystis antarctica*	5 (15)		•		•	•		•		

Table 5. Distribution of fucoxanthin derivatives across haptophyte families. tr: trace; further details as in Table 4

Pigment type Haptophyte family	Type species	n	Fucoxanthin derivatives but-fuco	fuco	4-k-hex-fuco	hex-fuco	4-k-fuco
Type 1							
Pavlovophyceae Isochrysidaceae Pleurochrysideceae	*Chrysotila lamellosa*	6 (7)		•			
Type 2							
Pavlovophyceae	*Pavlova gyrans*	5 (7)		•			
Type 3							
Isochrysidaceae	*Isochrysis galbana*	*5* (7)		•			
Type 4							
Prymnesiaceae	*Prymnesium parvum*	4 (6)		•			
Type 5							
Hymenomonadaceae	*Ochrosphaera neopolitana*	3 (3)		•			•
Type 6							
Noëlaerhabdaceae	*Emiliana huxleyi*	2 (12)	*tr*	•	•	•	
Type 7							
Prymnesiaceae	*Chrysochromulina polylepis*	7 (8)	*tr*	•	•	•	
Type 8							
Phaeocystaceae Prymnesiaceae Isochrysidaceae	*Phaeocystis antarctica*	5 (15)	•	•	•	•	

Pigment types and haptophyte taxa

The distribution of pigment types across the haptophyte classes, orders, families and genera are summarised in Table 8. Only 2 families of the 7 tested were characterised by a single pigment type: the Noëlaerhabdaceae (coccolithophorids; pigment Type 6) and the Hymenomonadaceae (pigment Type 5). In 2 cases, a single pigment type was restricted to a particular genus: *Prymnesium* (Type 4) and *Chrysochromulina* (Type 7). The remaining pigment types (Types 1, 2, 3, 6 and 8) were shared across several families and genera. *Diacronema*, *Pavlova*, *Chrysotila* and *Pleurochrysis* shared haptophyte Type 1 pigments (families Pavlo-

Table 6. Chl *c* pigments as mean percentages (range) of total chl *c* in haptophyte pigment types (data from Table 3). n: no. of strains; abbreviations as in Table 2

Pigment type	n	Chl *c* pigments							
		chl c_1	chl c_2	chl c_3	chl c_2-*P. gyrans* type	MV chl c_3	MgDVP	chl c_2-MGDG [18:4/14:0]	chl c_2-MGDG [14:0/14:0]
1	7	55 (45–62.5)	42 (34–51)	–	–	–	3 (0–5.5)	–	–
2	7	53 (49.5–59.5)	17 (12.2–20.4)	–	28 (21.7–35.9)	–	2 (0–3.0)	–	–
3	7	32 (28.3–41.0)	52 43.2–59.1)	–	–	–	2 (0–2.8)	14 (9.5–15.4)	–
4	6	35 (33.4–36.6)	21 (13.9–35.8)	31 (21.7–35.9)	–	1 (1.0–1.5)	2 (1.0–3.1)	12 (8.0–12.7)	–
5	3	47 (44.4–53.3)	31 (27.5–33.0)	14 (12.9–15.3)	–	–	2 (1.3–2.2)	6 (4.0–6.4)	–
6	12	–	39 (33.0–48.7)	39 32.2–45.1)	–	2 (1.0–8.4)	1 (0–1.8	19 (15.8–22.0)	–
7	8	–	33 (25.7–43.4)	45 (32.2–58.5)	–	–	2 (0–4.2)	15 (3.1–28.4)	5 (0–14.3)
8	15	–	46 (21.2–68.1)	40 (21.2–62.1)	–	–	2 (0–3.4)	12 (7.5–17.9)	–

vaceae, Isochrysidaceae and Pleurochrysidaceae); *Pavlova* and *Rebecca* shared Type 2 pigments (family Pavlovaceae); *Cricosphaera carterae* (CS-40), *Dicrateria inornata* (CCMP 355, CS-254), *Isochrysis* and *Pseudoisochrysis* shared Type 3 pigments (family Isochrysidaceae), *Emiliania* and *Gephyrocapsa* shared Type 6 pigments (family Noëlaerhabdaceae), and *Dicrateria inornata* (CS-267), *Imantonia* and *Phaeocystis* strains shared Type 8 pigments (families Phaeocystaceae, Prymnesiaceae and Isochrysidaceae); 3 *Dicrateria* strains occurred across 2 pigment types, raising questions as to the true taxonomic identity of these strains. These examples show the variations in specificity of pigment types encountered in haptophyte taxa.

Table 7. Fucoxanthin pigments as mean percentages (range) of total fucoxanthins in haptophyte pigment types (data from Table 3). n: no. of strains; abbreviations as in Table 2

Pigment type	n	Fucoxanthin pigments				
		fuco	4-k-fuco	hex-fuco	4-k-hex-fuco	but-fuco
1	7	100	–	–	–	–
2	7	100	–	–	–	–
3	7	100	–	–	–	–
4	6	100	–	–	–	–
5	3	88 (80.1–93.0)	12 (7.0–19.9)	–	–	–
6	12	20.3 (0.4–56.8)	–	68.5 (46.8–99.1)	11.0 (7.6–27.0)	0.2 (0.3–1.1)
7	8	39.7 (9.4–84.8)	–	37.3 (0.7–83.8)	22.7 (6.3–33.7)	0.3 (0.2–0.9)
8	15	25.9 (1.1–90.4)	–	55.0 (2.9–84.4)	7.5 (0–15.6)	11.6 (1.6–30.0)

Variation of pigments across strains of same species

Emiliania huxleyi and *Phaeocystis antarctica* were 2 species tested for variations in pigment composition across strains; 11 strains of *E. huxleyi* showed a high coherence to pigment Type 6 composition (see Table 3), and *P. antarctica* (10 strains) closely matched haptophyte pigment Type 8 composition. However, significant variations in ratios of fucoxanthin and its acyloxy derivatives were found in 2 strains *E. huxleyi* and 2 strains of *P. antarctica* (Fig. 5). Again, several minor pigments were not detected in some strains, probably being present in quantities below limits of detection e.g. MV-Chl c_3, MgDVP and 4-keto-19′-hexanoyloxyfucoxanthin in *E. huxleyi* strains, and MgDVP, 19′-hexanoyloxyfucoxanthin and 4-keto-19′-hexanoyloxyfucoxanthin in some strains of *P. antarctica*.

DISCUSSION

New pigment types in the Haptophyta

The present work has shown that 9 chlorophyll *c* pigments and 5 fucoxanthin derivatives are key discriminators of 8 pigment types in 37 species (65 strains) of Haptophyta. The HPLC methods used (Garrido & Zapata 1997, Zapata et al. 2000) allowed improved resolution of both polar and non-polar

chlorophylls and carotenoids, compared to that of the widely used Wright et al. (1991) method, and other methods published in the last decade (see Jeffrey et al. 1999). The Wright et al. (1991) method distinguished 4 useful haptophyte pigment types based on the presence/absence of chl c_3, 19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin (Jeffrey & Wright 1994). These pigment types have been applied successfully in oceanographic studies to distinguish pigment (but not taxon) differences in haptophyte field populations (e.g. Mackey et al. 1996, 1998, Wright & van den Enden 2000).

With 13 pigments now available for targeting haptophytes, resolution to families and even some genera in mixed phytoplankton populations is now possible using single pigments or pigment suites (see Tables 4, 5 & 8). The 9 chl *c* pigments formed 8 clear distribution patterns across the 65 strains (Table 4), while the fucoxanthin derivatives formed 4 distribution patterns across the strains (Table 5).

We will first discuss the validity of these pigment types in the light of current phylogenetic knowledge and then examine the extent of variability within the types under standard culture conditions, and how they may be modified in the natural environment. Finally we will consider the application of these pigment types as new markers for haptophytes in oceanographic field studies.

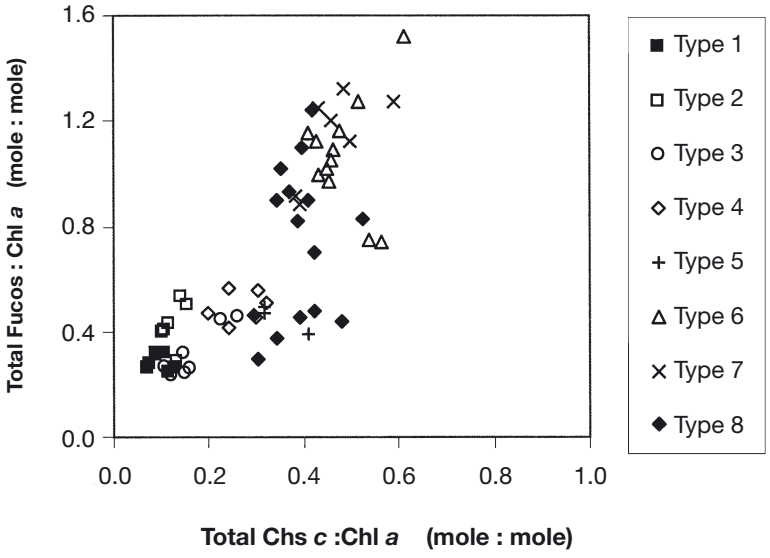

Fig. 4. Relationship between molar ratios of total fucoxanthins (Total fucos) and total chl *c* pigments to chl *a* across all haptophyte pigment types (data from Table 3)

Pigment types and haptophyte phylogeny

Two lines of evidence suggest a strong genetic component to the differences we observed in pigment patterns within the Haptophyta. First, the cultures had been isolated from a wide range of locations that included most ocean basins (Table 1). All strains were grown under standard culture conditions for subtropical, temperate or polar species (e.g. light, day length and temperature) to minimise variations that might

Table 8. Distribution of pigment types across haptophyte taxa; +: present; –: absent

Order	Family	Genus	Pigment types							
			1	2	3	4	5	6	7	8
Class Pavlovaceae										
Pavlovales	Pavlovaceae	*Diacronema*	+	–	–	–	–	–	–	–
		Pavlova	+	+	–	–	–	–	–	–
		Rebecca	–	+	–	–	–	–	–	–
Class Prymnesiophyceae										
Phaeocystales	Phaeocystaceae	*Phaeocystis*	–	–	–	–	–	–	–	+
Prymnesiales	Prymnesiaceae	*Chrysochromulina*	–	–	–	–	–	–	+	–
		Imantonia	–	–	–	–	–	–	–	+
		Prymnesium	–	–	–	+	–	–	–	–
Isochrysidales	Isochrysidaceae	*Chrysotila*	+	–	–	–	–	–	–	–
		Cricosphaera	–	–	+	–	–	–	–	–
		Dicrateria	–	–	+	–	–	–	–	+
		Isochrysis	–	–	+	–	–	–	–	–
		Pseudoisochrysis	–	–	+	–	–	–	–	–
	Noëlaerhabdaceae	*Emiliania*	–	–	–	–	–	+	–	–
		Gephyrocapsa	–	–	–	–	–	+	–	–
Coccolithales	Pleurochrysidaceae	*Pleurochrysis*	+	–	–	–	–	–	–	–
	Hymenomonadaceae	*Ochrosphaera*	–	–	–	–	+	–	–	–

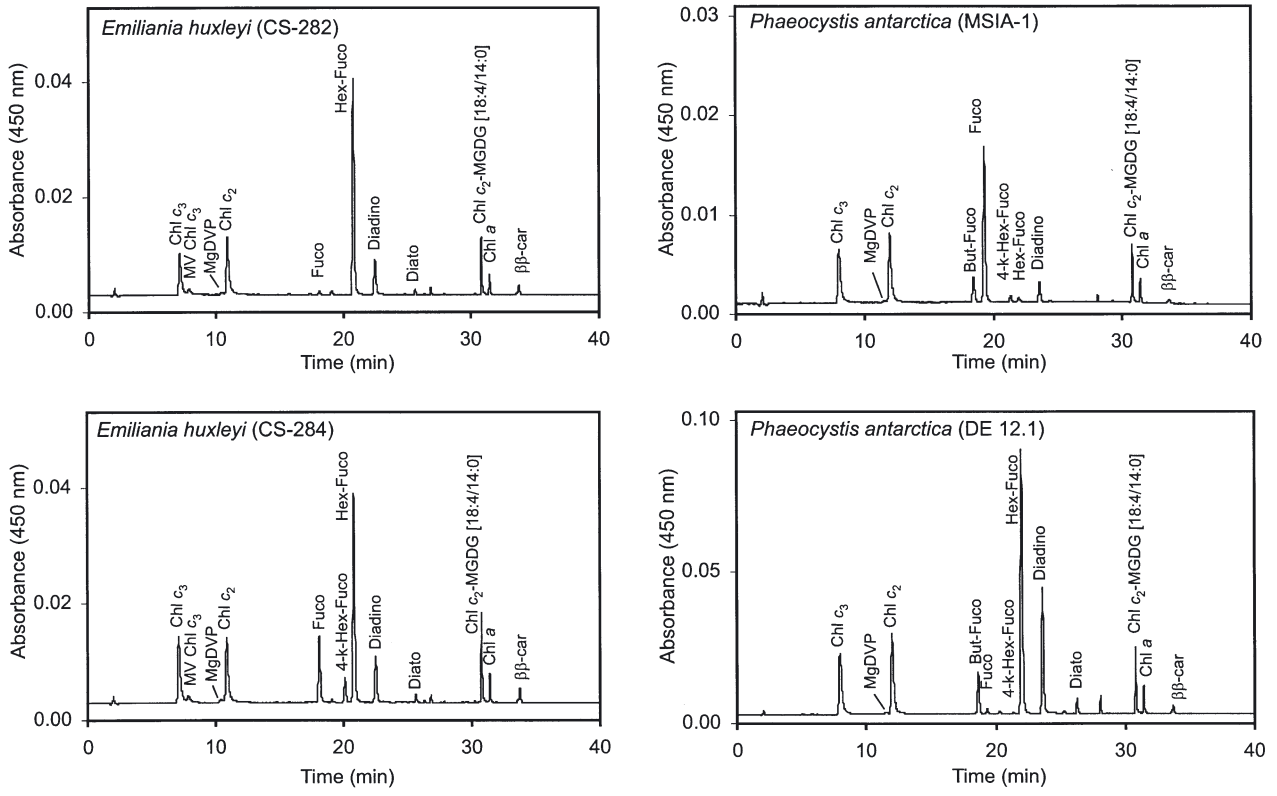

Fig. 5. Chromatograms (C8 HPLC method) showing differences in fucoxanthin and its acyloxyfucoxanthin derivatives in 2 strains of *Emiliana huxleyi* isolated from Sargasso Sea and 2 strains of *Phaeocystis antarctica* isolated from sea-ice (MSIA-1) and water column (DE12.1). Detection by absorbance at 450 nm. Peak identifications as in Table 2

otherwise occur between strains due to growth conditions. Analytical procedures and harvest times were also standardised. The pigment types observed are therefore less influenced by 'environmentally-induced' variability and should allow recognition of phylogenetic affinities among species.

Second, the 8 haptophyte pigment patterns identified here correlated closely with phylogenetic clades (Table 9) found in analysis of haptophyte 18S rDNA by Edvardsen et al. (2000). These authors established a tree using 25 identified haptophyte species (33 strains) in which 3 clades (A, [B1, B2], C) were within the Prymnesiophyceae, and 2 clades (D, E) were derived from amplified genes from phytoplankton taken from oligotrophic Pacific waters (presumably from closely related but unidentified members of Prymnesiophyceae). Members of the class Pavlovaceae formed a separate distinct group. (The Pavlovaceae were subsequently subdivided by Van Lenning et al. [2003], who found a tree structure that supported their 3 pigment types.)

Each pigment type was associated with only 1 clade, except for haptophyte pigment Type 8, which was found in 2 clades. Species containing pigment Type 5 were not included in the study of Edvardsen et al. (2000). Unfortunately, the 2 studies used many different species. Our 37 species (65 strains) coincided with only 15 of the 25 identified species used by Edvardsen et al. (2000), and only 1 strain in both studies was identical. Similar genetic analysis is required for our 65 strains in order to confirm the genetic basis for our haptophyte

Table 9. Associations of haptophyte pigment types (present data) with haptophyte clades identified by analysis of 18S rDNA by Edvardsen et al. (2000)

Clade	Typical genera	Pigment type
Pavlovaceae	*Pavlova*	2
Prymnesiophyceae	*Phaeocystis*	8
Clade A		
Clade B1	*Chrysochromulina*	7
	Prymnesium	4
	Imantonia	8
Clade B2	*Chrysochromulina* only	7
Clade C	*Pleurochrysis*	1
	Emiliania/Gephyrocapsa	6
	Isochrysis	3
Clade D	No cultured representatives	?
Clade E	No cultured representatives	?

pigment associations. In conclusion, we have some confidence in believing that our 8 haptophyte pigment types match phylogenetic trends among the species studied.

Possible pigment functions

Clear differences in relative quantities of the 9 chl *c* pigments and 5 fucoxanthin derivatives across the 65 strains (Tables 6 & 7) probably result from functional differences between them.

Chlorophyll *c* pigments. It is generally accepted that Chls c_1, c_2 and c_3 have a light-harvesting role (Anderson & Barrett 1986, Wilhelm & Wiedemann 1991, Green & Durnford 1996, Zapata et al. in press), and in our study these chl *c* pigments were always present as a major proportion of the total chl *c* (Table 6). The chl c_2-like *Pavlova gyrans*-type pigment, occurring in 35% of the total chl *c* pigments, may also have a light-harvesting role (Fawley 1989). The occurrence of MgDVP in trace quantities in most haptophytes (Table 3) may signify its role as a biosynthetic intermediate in chlorophyll synthesis (Porra 1997, Porra et al. 1997). When present in larger quantities (e.g. in some prasinophytes), it occurs with chl *a* and *b* in the chlorophyll protein complexes and has a light-harvesting role (Brown 1985). The function of 2 other trace pigments, MV-Chl c_3 and chl c_2-MGDG [14:0/14:0], is unknown.

The newly discovered minor pigment chl c_2-MGDG [18:4/14:0] may also have a light-harvesting role (J. L. Garrido pers. comm.) or it may function in the assembly of light-harvesting pigment complexes (Hoober & Eggink 2001). It may act as a transporter of chl c_2 from the MGDG-rich lipid bilayer of the inner chloroplast envelope membrane to its final location in the light-harvesting pigment protein complexes of the thylakoids (Jeffrey & Anderson 2000). For a more complete discussion of chl *c* chemistry, distribution and function, see Zapata et al. (in press).

Fucoxanthin derivatives. The light-harvesting roles of fucoxanthin and 19′-hexanoyloxyfucoxanthin were established by Sieferman-Harms (1985) and Haxo (1985), respectively. When present in significant quantities, 19′-butanoyloxyfucoxanthin may have a similar role. The function of the 4-keto derivatives is unknown. The universally distributed carotenoid pair diadinoxanthin and diatoxanthin, present in all haptophytes examined, have a well-established photoprotective function via the light-regulated epoxide cycle (Stransky & Hager 1970, Siefermann-Harms 1985, Demmig-Adams & Adams 1993, Moisan et al. 1998, Lohr & Wilhelm 1999). Further study is needed to understand the function and biosynthetic regulation of all these important marine pigments, and their consequent reliability as chemotaxonomic indicators in field oceanography.

Quantitative variation of chlorophyll *c* and fucoxanthins across strains and pigment types

The data in Tables 6 & 7 show that the patterns of relative abundance for chl *c* and fucoxanthins, respectively, are clear-cut, but there is considerable variation around the means for most pigments. While little is known of chl *c* variability in haptophytes or other taxa, variation in fucoxanthins has previously been observed in haptophytes.

Wright & Jeffrey (1987) gave a first indication of the variability of the relative proportions of fucoxanthin, 19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin in 4 different isolates of *Phaeocystis* spp. – 3 from the Southern Ocean (probably *P. antarctica*) and 1 from the East Australian Current (probably *P. globosa*; Medlin et al. 1994). This trend was confirmed in the present work, in which 11 strains of *Emiliana huxleyi* and 10 strains of *P. antarctica* were analysed (Tables 3 & 7).

While *Emiliana huxleyi* strains showed a strong coherence with haptophyte pigment Type 6, and those of *Phaeocystis antarctica* with pigment Type 8, variability in relative abundances of fucoxanthins and acyloxyfucoxanthins were indicated among the strains of both species (Tables 3 & 7, Fig. 5). These results do not deny the validity of the haptophyte pigment types, but point to the need to understand those factors that influence pigment variability within strains of the same species, isolated from different geographic areas, light fields or populations.

Confirmation of, and explanations for, fucoxanthin variability have been published in the past decade. Vaulot et al. (1994) observed 3 pigment clusters in 16 strains of *Phaeocystis* isolated mainly from temperate oceanic areas, supporting some of the present observations. In their *Phaeocystis* strains, both fucoxanthin and 19′-hexanoyloxyfucoxanthin were dominant or co-dominant, but 19′-butanoyloxyfucoxanthin in their study was never present except in minor or trace quantities. This pattern matches only 4 of our 11 *Phaeocystis* strains.

Jeffrey & Wright (1994) found 1 strain of *Phaeocystis* sp. from the East Australian Current had fucoxanthin and lacked 19′-hexanoyloxyfucoxanthin, similar to the finding of Breton et al. (1999) and Cottonec et al. (2001) with northern hemisphere *Phaeocystis* spp. None of the strains examined in the present work matched this pigment pattern.

Fucoxanthin/acyloxyfucoxanthin variability was produced by iron limitation aided by light stress in

1 Antarctic *Phaeocystis* strain (Van Leeuwe & Stefels 1998). Iron limitation caused increased synthesis of 19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin at the expense of fucoxanthin. Buma et al. (1991) found differences in the 19′-hexanoyloxyfucoxanthin to chl *a* ratios in *Phaeocystis* strains isolated from both Antarctic and Atlantic ocean regions, and pigment ratios were also affected by experimental differences in growth phase, temperature, morphological cell type (flagellates or colonies) and variations in day/night cycles. Stolte et al. (2000) also found that 19′-hexanoyloxyfucoxanthin was synthesised from fucoxanthin, with light acting as a modulating factor, in strains of *Emiliania huxleyi* grown under conditions of light, phosphate and nitrate limitation.

The relative importance of nutritional, environmental and genetic factors influencing fucoxanthin variability needs to be fully evaluated in order to define the reliability of fucoxanthins as indicators of algal types in the field.

Comparison with previous surveys of Haptophyta

Table 10 highlights the advances in a comparison of the 8 haptophyte pigment types identified in the present work with the 4 of Jeffrey & Wright (1994). The earlier study could not distinguish the pigmentation of diatoms from that of 16 of 50 haptophyte strains studied (32%, their type 1). Most of these can now be distinguished by the presence of chl c_2 *Pavlova gyrans*-type and Chl c_2-MGDG [18:4/14:0] and fall within the new haptophyte pigment Types 2 and 3, respectively, with only 7 of the 65 haptophyte strains (11%) remaining in Type 1. Similarly, those taxa previously classified by Jeffrey & Wright (1994) as type 2 can now be further subdivided by the presence of chl c_2-MGDG [18:4/14:0] and non-polar chl c_1 (new Type 4) and 4-keto-fucoxanthin (new Type 5), respectively. The former type 3 of Jeffrey & Wright (1994) can now be subdivided into new Type 6 and new Type 7 on the basis of MV-chl c_3 and chl c_2-MGDG [14:0/14:0], respectively. Type 4 of Jeffrey & Wright (1994) could not be further subdivided (new haptophyte pigment Type 8), although chl c_2-MGDG [18:4/14:0] was recognised as an additional characteristic.

Application of haptophyte pigment signatures in oceanography

The additional pigments and pigment patterns identified in this study add power to biological oceanographic studies where one must detect algal pigment signatures in the presence of other taxa, some of which have potentially overlapping pigment compositions.

The recent analysis of 9 species from the Pavlovaceae by Van Lenning et al. (2003) found 3 pigment types: A, B, C. While not all species tested were common to our study, it is clear that their Pavlovophyceae pigment type A corresponds with our Type 1, and their type B with our Type 2, with no irregularities. Their type C was based on the presence of an additional pigment (thought to be the monovinyl form of chl c_2 *Pavlova gyrans*-type) that was found in a single species, *Exanthemachrysis gayraliae*, which unfortunately was not included in our survey. However this pigment appears to be a useful additional marker.

Several of the new marker pigments discussed above are restricted to particular taxa and may be useful for their detection in mixed populations. Of particular interest is MV-chl c_3, a minor pigment strongly associated with the globally important species

Table 10. Comparison of Jeffrey & Wrights' (1994) haptophyte pigment types (1 to 4), with Types 1 to 8 found in present work. tr: trace; further abbreviations as in Table 2

Jeffrey & Wright (1994)		Present work	
Type	Distinguishing pigments	Type	Distinguishing pigments
1	[chl c_1 + chl c_2][a], fuco	1	Identical to Jeffrey & Wright (1994) type 1
		2	Identical to Jeffrey & Wright (1994) type 1 + chl c_2 *P. gyrans*-type
		3	Identical to Jeffrey & Wright (1994) type 1 + chl c_2 MGDG [18:4/14:0]
2	[chl c_1 + chl c_2][a], chl c_3, fuco	4	Identical to Jeffrey & Wright (1994) type 2 + chl c_2 MGDG [18:4/14:0] + npchl c_2
		5	Identical to Jeffrey & Wright (1994) type 2 + chl c_2 MGDG [18:4/14:0] + 4-k-fuco
3	[chl c_2][a], chl c_3, fuco, hex-fuco[b], but-fuco (tr)	6	Identical to Jeffrey & Wright (1994) type 3 + MVchl c_2 + chl c_2 MGDG [18:4/14:0]
		7	Identical to Jeffrey & Wright (1994) type 3 + chl c_2 MGDG [18:4/14:0] + chl c_2 MGDG [14:0/14:0]
4	[chl c_2][a], chl c_3, fuco, hex-fuco[b], but-fuco	8	Identical to Jeffrey & Wright (1994) type 4 + chl c_2 MGDG [18:4/14:0]

[a][chl c_1 + chl c_2] were not resolved by Jeffrey & Wright (1994); table shows present understanding of previous results
[b]Present work shows that algae with hex-fuco also contain 4-k-hex-fuco

Emiliania huxleyi. This pigment and *E. huxleyi* cell counts were recently targeted in a Southern Ocean Transect (Wright unpubl.). Although cell numbers were low (263 cells ml^{-1} maximum), MV-chl c_3 was detected at a low concentration. However, an unknown co-chromatographing compound prevented reliable quantitation. MV-Chl c_3 may only be a useful marker in field samples under bloom conditions (when cell concentrations may exceed 10 000 ml^{-1}; Tyrrell & Taylor 1995).

Three other pigments, chl c_2 *Pavlova gyrans*-type, chl c_2-MGDG [14:0/14:0], and 4-keto-fucoxanthin, occur in higher concentrations than MV-chl c_3 and appear to be excellent indicators for members of the genera *Pavlova*, *Chrysochromulina* and *Ochrosphaera*, respectively. Absence of these pigments however is inconclusive, since the first 2 pigments were not detected in all members of their respective genera. Similarly, while 4-keto-fucoxanthin was not found outside the genus *Ochrosphaera*, it cannot be assumed to be universally present within the genus on the basis of only 2 species tested here.

Interpretation of field data is complicated by the fact that pigment ratios are variable, even under controlled growth conditions, and some markers are sometimes below detection limits or absent from their typical species. Some of the characteristic pigments described above were in low concentrations in algal cultures and may be insignificant in mixed field populations unless their source-species are in bloom. Pigment ratios are also strongly influenced by light intensity (and hence depth and season) and nutrient status.

Light intensities for culture growth in this study were kept constant (at 60 to 70 µmol quanta m^{-2} s^{-1}, except for *Phaeocystis antarctica*, 40 µmol quanta m^{-2} s^{-1}) so that genetic differences between strains could readily be observed. However phytoplankton in the field will experience a range of light intensities and adjust their pigment composition accordingly. The ratios determined in this paper will serve as a starting point for interpreting field samples, but the actual pigment ratios in the field will need to be retrieved from the data using a programme such as CHEMTAX (Mackey et al. 1996) after subdividing the data into depth layers to allow for differences in irradiance with depth.

While the unambiguous markers identified above may serve as indicators for the presence of certain taxa, determining the relative abundance of these and other groups in the planktonic community requires analysis of pigment suites (Jeffrey et al. 1999) representing the major species present. This cannot be done manually; it requires computer methods such as CHEMTAX (Mackey et al. 1996) to determine the pigment ratios for particular taxa and the relative abundances of those taxa in a set of field samples.

Endosymbioses and similarly pigmented non-haptophyte taxa

It is now generally accepted that the photosynthetic apparatus originated from a primary endosymbiosis between a cyanobacterium and a non-photosynthetic phagotrophic eucaryote (McFadden 2001 [review], Palmer 2003) that subsequently evolved to green, red and glaucophyte algal types (Moreira et al. 2000). Recent analyses of certain nuclear and chloroplast genes support the hypothesis of Cavalier-Smith (2002) that the chloroplasts of heterokonts, haptophytes, cryptophytes and dinoflagellates all arose from a common secondary endosymbiosis involving a red alga. Primary, secondary and tertiary symbioses with secondary plastid replacements, resulting in evolution of diverse pigment types, were convincingly demonstrated by Palmer (2003). For example, certain modern dinoflagellates that have evolved by secondary chloroplast replacement and tertiary endosymbioses have lost their original primitive red algal plastids and now have plastids of either chlorophyte or haptophyte origin (see Jeffrey & Vesk 1997, Tengs et al. 2000).

By this mechanism, pigment suites from haptophyte taxa may now be found in present-day oceans in some non-haptophyte taxa (Jeffrey & Vesk 1997). This can present difficulties in the interpretation of pigment profiles in the field. For example, Table 11 shows that most diatoms (examined by earlier methods) have the same pigment composition as those of haptophyte pigment Type 1, with chl c_1, c_2 and fucoxanthin as major pigments (Stauber & Jeffrey 1988). Chl c_3 also replaced chl c_1 in 5 tropical pennate diatoms. Type 2 haptophyte pigments have recently been found in both the toxic

Table 11. Haptophyte pigment types (present work) compared with those of similarly pigmented non-haptophyte algal taxa

Type	Non-haptophyte algal taxa with similar pigment pattern (representative species)
1	Diatoms (e.g. *Phaeodactylum tricornutum*)[a]
2	Diatoms (e.g. *Pseudo-nitzschia multiseries*, CCMP 1659)[b] Fucoxanthin-containing dinoflagellates (e.g. *Peridinium foliaceum*)[c]
3	No other algal group has this pigment pattern
4	No other algal group has this pigment pattern
5	No other algal group has this pigment pattern
6	No other algal group has this pigment pattern
7	Some fucoxanthin-containing dinoflagellates (e.g. *Karenia brevis*)[b]
8	No other algal group has this pigment pattern

[a]Stauber & Jeffrey (1988)
[b]Zapata et al. (1998)
[c]Zapata (unpubl.)

diatom *Pseudo-nitzschia multiseries* (Table 11), and the fucoxanthin-containing dinoflagellates *Peridinium* (=*Kryptoperidinium*) *balticum* and *P. foliaceum* (now either allocated in *Peridiniopsis* or *Durinskia*: Carty & Cox 1986). These dinoflagellates are known from electron microscopical studies and ribosomal RNA analysis to harbour a diatom endosymbiont (Tomas & Cox 1973, Jeffrey & Vesk 1976, Chesnick et al. 1997). A dinoflagellate with 19′-acyloxyfucoxanthins (*Karenia brevis*: Zapata et al. 1998) contains 19′-hexanoyloxyfucoxanthin, 4-keto-19′-hexanoyloxyfucoxanthin, chl c_3, chl c_2-MGDG [18:4/14:0] and chl c_2-MGDG [14:0/14:0], indicating the presence of an endosymbiont with haptophyte Type 7 pigments. A similar dinoflagellate, *Karlodinium* sp., has similar pigments but lacks chl c_2-MGDG [14:0/14:0].

Diatoms and fucoxanthin-containing dinoflagellates share haptophyte pigment Types 1 and 2 with members of the Pavlovaceae (Table 8), and dinoflagellates with 19′-acyloxyfucoxanthins share pigment Type 7 with *Chrysochromulina* species (Prymnesiaceae). At the present state of knowledge, haptophyte pigment Types 3, 4, 5, 6 and 8 are not known for other algal groups, and currently provide a unique 'tag' for those haptophyte taxa containing these pigment suites (see Tables 4, 5 & 8).

The complexity of pigment patterns and the complications of endosymbiotic plastids do not allow reliance on pigment data alone. To distinguish these similarly pigmented microalgae in field observations, it is essential that simultaneous microscopic examinations of representative phytoplankton samples are carried out (Thomsen et al. 1994, Wright et al. 1996).

Recommendations

Detection of the new diagnostic haptophyte pigments requires the use of high-resolution HPLC techniques (e.g. Zapata et al. 2000), since they cannot be adequately resolved by earlier techniques (e.g. Wright et al. 1991). Their potentially low concentrations require that sample collection and analysis are optimised for high sensitivity (fluorescence detection for chlorophylls) as well as maximum resolution (i.e. large filtration volumes, small filters, and small extraction and injection volumes). It must also be recognised that *cis*-carotenoids and chlorophyll degradation products may confuse the interpretation of minor pigments and methods should be optimised to minimize their formation. Measuring the response of pigment ratios to changing irradiances in the field will improve interpretation of ocean transects. Finally, cultured representatives of all other algal classes, should now be examined by the new HPLC methods, to determine whether the new pigments are restricted to the Haptophyta, or are more widely distributed.

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