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RESEARCH ARTICLE

F. Rodríguez · M. Chauton · G. Johnsen · K. Andresen
L.M. Olsen · M. Zapata

Photoacclimation in phytoplankton: implications for biomass estimates, pigment functionality and chemotaxonomy

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Abstract Chl *a* and C-normalized pigment ratios were studied in two dinophytes (*Prorocentrum minimum and Karlodinium micrum*), three haptophytes (*Chrysochromulina leadbeateri, Prymnesium parvum* cf. *patelliferum*, *Phaeocystis globosa*), two prasinophytes (*Pseudoscourfieldia marina*, *Bathycoccus prasinos*) and the raphidophyte *Heterosigma akashiwo*, in low (LL, 35 µmol photons m^{-2} s^{-1}) and high light (HL, 500 µmol photons m^{-2} s^{-1}). Pigment ratios in LL and HL were compared against a general rule of photoacclimation: LL versus HL ratios ≥1 are typical for light-harvesting pigments (LHP) and <1 for photoprotective carotenoids. Peridinin, prasinoxanthin, gyroxanthin-diester and 19′-butanoyloxy-fucoxanthin were stable chemotaxonomic markers with less than 25% variation between LL versus HL Chl *a*–normalized ratios. As expected, Chls exhibited LL/HL to Chl *a* ratios >1 with some exceptions such as Chl c_3 in *P. globosa* and MV Chl c_3 in *C. leadbeateri*. LL/HL to Chl a ratios of photosynthetic carotenoids were close to 1, except Hex-fuco in *P. globosa* (four-fold higher Chl *a* ratio in HL vs LL). Although pigment ratios in *P. globosa* clearly responded to the light conditions the diadinoxanthin-diatoxanthin cycle remained almost unaltered at HL. Total averaged pigment and LHP to C ratios were significantly higher in LL versus HL, reflecting the photoacclimation status of the studied species. By contrast, the same Chl *a*-normalized ratios were weakly affected by the light intensity due to co-variation with Chl *a*. Based on our data, we suggest that the interpretation of PPC and LHP are highly dependent on biomass normalization (Chl *a* vs. C).

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F. Rodríguez
Centro Oceanográfico de Canarias, Ctra. San Andrés s/n,
38120 S/C Tenerife, Spain

M. Chauton · G. Johnsen (✉) · K. Andresen · L.M. Olsen
Department of Biology, Norwegian University of Science
and Technology, Bynesveien 46, 7018 Trondheim, Norway
E-mail: geir.johnsen@bio.ntnu.no
Tel.: +47-735-91581
Fax: +47-735-91597

M. Zapata
Centro de Investigacións Mariñas, Xunta de Galicia,
Pedras de Corón s/n, 36620 Vilanova de Arousa, Spain

Introduction

The assessment of phytoplankton populations constitutes a major task in many oceanographic studies, due to their important role in the pelagic food webs (Fenchel 1988) and their implications in the biogeochemical budgets and the global climate system (Bains et al. 2000). The photoacclimation index Chl *a* to C (Sakshaug et al. 1997) is subject to variation since cellular Chl *a* (in contrast to C) is highly variable, in a given species as a function of light climate. High-light (HL) acclimated cells usually exhibit low Chl *a* to C ratios, whereas low-light (LL) adapted cells have high ratios (overall range Chl *a*:C 0.003–0.1 (µg:µg); Falkowski et al. 1985; Cloern et al. 1995) because they accumulate pigments to enhance their light absorption efficiency per C biomass (Johnsen and Sakshaug 1993). Therefore, the availability of C and Chl *a*–normalized pigment ratios in different culture conditions are relevant physiological information for primary production models to translate pigment data into pigment-specific biomass estimates of phytoplankton (Lefèvre et al. 2001).

Phytoplankton cells adjust their pigment content and ratios under different light and nutrient conditions (Falkowski and Raven 1997; Goericke and Montoya 1998; Schlüter et al. 2000; Henriksen et al. 2002), so that for nutrient-replete balanced growth a species-specific response pattern can be predicted (Johnsen and Sakshaug 1993). For example, co-variation between photosynthetic pigments and Chl *a* is common, since photosynthetic pigments in most Chromophytes are embedded in light-harvesting complexes associated with photosystem II (Johnsen et al. 1997). Also, decreasing

the size of the light-harvesting antennae and associated pigments in HL conditions (Anderson et al. 1995) can determine light-harvesting pigments (LHP) to Chl *a* ratios in LL versus HL, which are typically ≥1 (Schlüter et al. 2000; Henriksen et al. 2002). In turn, increasing the photoprotective carotenoids (PPC) in HL conditions to prevent photo-oxidative damages to the photosynthetic apparatus is a common mechanism in microalgae (Demmig-Adams et al. 1999), in such cases the Chl *a* ratios in LL versus HL are usually <1.

To our knowledge, a comprehensive dataset for Chl *a* and C-normalized pigment ratios has only been reported for prymnesiophytes and diatoms in logarithmic and stationary growth phases (Llewellyn and Gibb 2000). In the present work we report the pigment content normalized to C and Chl *a* of eight phytoplankton species in two light conditions to study the light-dependent (photoacclimation) effects on these pigment ratios. The implications of these results, for both chemotaxonomic and biomass studies, are further discussed.

Materials and methods

Algal cultures

Two dinoflagellates (*Prorocentrum minimum*; isolated by Karl Tangen (KT), strain KT-79A, held at culture collection both at University of Oslo (UiO), and Norwegian University of Science and Technology (NTNU at Trondhjem Biological Station, TBS), *Karlodinium micrum* (=*Gymnodinium galatheanum*; KT, Trondheim Biological Station (TBS), one raphidophyte (*Heterosigma akashiwo*; UiO), three haptophytes (*Chrysochromulina leadbeateri*; UiO), *Prymnesium parvum* cf. *patelliferum* (Green, Hibberd and Pienaar) A. Larsen stat. nov. (Larsen 1999) (clone Ryfylke, TBS, denoted *P. parvum* for simplicity in MS), *Phaeocystis globosa* (PLY540, Plymouth Culture Collection) and two prasinophytes (*Pseudoscourfieldia marina* and *Bathycoccus prasinos*, UiO), were grown in two replicate cultures of each species in f/2 medium (Guillard and Ryther 1962). Scalar irradiance (E_0, 400–700 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 a cool-white fluorescent light with a similar spectral output (Philips TLD 18W/95 and TL 40W/55). Light regimes for the different cultures were as follows: continuous light with either LL (35 µmol m^{-2} s^{-1}) or HL (500 µmol m^{-2} s^{-1}). Cultures were acclimated to these conditions for more than eight generations before sampling to ensure that the cells in a given culture obtained the same photoacclimation status. The growth of the cultures was monitored by in vivo fluorescence (Turner Designs). The samples were measured with and without 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to ensure that the cultures were in the exponential phase, according to Sakshaug (1984). Variable volumes (25–40 ml depending on culture cell density) were harvested by gentle filtration onto Whatman GF/F glass fibre filters. Cultures were filtered in the culture room in a given growth light regime and frozen immediately to ensure that the epo/de-epoxidation state in the cells reflect the experimental LL or HL condition (Lohr and Wilhelm 1999). Samples were kept at -20°C for up to one week before HPLC analysis.

Pigment analyses

Assessment of the pigment composition was performed using a Hewlett-Packard HPLC 1100 Series system, equipped with a quaternary pump system and diode array detector. Pigments were separated on a Waters Symmetry C_8 column (150×4.6 mm, 3.5 µm particle size) using the HPLC method of Zapata et al. (2000). Frozen filters from algal cultures were extracted in a Teflon-lined screw-capped tube with 5–7 ml 95% methanol using a spatula for filter grinding. The tube was then placed in a beaker with ice and water and placed in an ultrasonic bath for 5 min. All sample preparations were done under subdued light. Extracts were then filtered through Millipore 0.45 µm filters to remove cell and filter debris and 200 µl of the final extract were injected in the HPLC system. Chlorophylls and carotenoids were detected by absorbance at 440 nm and identified by a diode array detector ($\lambda = 350$–750 nm, 1.2 nm spectral resolution). HPLC calibration was performed using chlorophyll and carotenoid standards purchased from SIGMA (Aldrich, UK) and The International Agency for ^{14}C determination, DHI Water & Environment (Denmark). The specific extinction coefficients (α: l g^{-1} cm^{-1}) provided by Jeffrey et al. (1997) were used for pigment quantification. For Chl *c* pigments whose α are unknown, α for Chl c_2 was used (e.g. Chl c_3, MV Chl c_3, Chl c_2-MGDG). For fucoxanthin derivatives as 4-keto-19′hexanoyloxy-fucoxanthin (4keto-hex-fuco), α values for fucoxanthin were applied. Thus, pigment ratios either Chl *a* or C-normalized correspond with mass ratios (µg:µg).

Particulate organic carbon and nitrogen

Triplicate samples of algal cultures for measurements of particulate organic carbon (POC) and nitrogen (PON) were filtered onto Whatman GF/F precombusted glass fibre filters (450°C for 4 h) and frozen at −20°C until analysis. Filters were thawed, acidified with fuming HCl and packed in tin capsules, before measurements in a Fisons Instruments autoanalyser, model NA 1500 NC. Generally, N to C (at:at) values were close to the Redfield ratio 0.151 (average in LL and HL 0.159 ± 0.023), indicating that there was no nitrogen limitation at the time of sampling.

Statistical analyses

Data on Chl *a* and C ratios in LL versus HL conditions were compared using a paired *t* test. All statistical analyses were performed using STATISTICA for Windows, release 5.0, StatSoft, Inc. (1995).

Results

Pigment composition

The HPLC chromatograms on the eight studied species in HL conditions are given in Fig. 1. The pigment ratios normalized to Chl *a* in LL and HL treatments are detailed in Table 1. The most stable class/pigment group-specific pigment markers (< 25% difference between LL vs HL Chl *a* ratios) were peridinin (Perid), prasinoxanthin (Prasino), gyroxanthin-diester (Gyro) and 19′-butanoyloxy-fucoxanthin (But-fuco), respectively.

Briefly, the main Chl *c* pigment in most studied species was Chl c_2. *C. leadbeateri* synthesized monovinyl (MV) Chl c_3 in HL conditions (Fig. 1c; see also Zapata et al. 2001) and displayed three distinct non-polar Chl *c* pigments, the most retained with chromatographic properties identical to Chl c_2-monogalactosyl-diacylglicerol (MGDG) [14:0/14:0] (Zapata et al. 2001, 2004). *P. globosa* (Fig. 1a) and *P. parvum* (Fig. 1e) posessed Chl c_2-MGDG [14:0/18:4] (Garrido et al. 2000; Zapata et al. 2004).

The carotenoid composition in *P. globosa*, *C. leadbeateri* and *K. micrum* (Fig. 1d) was characterized by several fucoxanthin (Fuco) derivatives. The two former species contained the rare carotenoid 4keto-hex-fuco. The detection of a violaxanthin-like pigment in *P. parvum* (Fig. 1e) has also been reported by Lohr and Wilhelm (1999).

Uriolide and micromonal were detected in *B. prasinos* (Fig. 1f) but not in *P. marina* (Fig. 1h). The latter result confirms previous results (Fawley 1992; Latasa et al. 2004), although uriolide and micromonal have been also reported in *P. marina* (Egeland et al. 1995, 1997).

Chl *a*-normalized pigment ratios

Low light versus high light pigment to Chl *a* ratios (Table 1) were checked against a general rule for photosynthetic or photoprotective functionality: LL/HL ratios ≥1 would be expected in photosynthetic pigments, whereas LL/HL <1 are typical from photoprotective carotenoids (diadinoxanthin (Diadino), diatoxanthin (Diato), antheraxanthin (Anther), zeaxanthin (Zea) and lutein (Lut), in our study). Any other pigments (Table 1), including those with known or only hypothetical light-harvesting role (as e.g. 4-keto-Hex-fuco or Gyro) were grouped as light-harvesting pigments in the following (Table 2, Fig. 2).

As expected, Chls exhibited LL/HL ratios >1 with the notable exception of Chl c_3 in *P. globosa* and MV Chl c_3 in *C. leadbeateri*. A chl *c*-like pigment (MgDVP) was detected in trace amounts in all the studied species, and in the prasinophytes *P. marina* and *B. prasinos* a two-and ten-fold increases in HL versus LL amounts were seen.

Photosynthetic carotenoids to Chl *a* ratios in LL versus HL were close to 1 (Table 1). Chl *a*-normalized fucoxanthin varied little in *H. akashiwo*, contrasting with up to two to three fold higher values in LL versus HL in *P. parvum*, *P. globosa* and *K. micrum*. 19(-Hexanoyloxy-fucoxanthin (Hex-fuco) and 4-keto-Hex-fuco to Chl *a* ratios also varied in their light responses among species. Hex-fuco was the dominant carotenoid in HL in *C. leadbeateri*, *P. globosa* and *K. micrum*, but the decrease of Fuco:Chl *a* was only apparent in *K. micrum* and *P. globosa*. In the latter species it was also observed a significant increase in Hex-fuco to Chl *a* ratios in HL, as in the case of photoprotective carotenoids.

Photoprotective carotenoids to Chl *a* ratios increased their respective total pigment pools in HL versus LL (Table 1). Violaxanthin (Viola):Chl *a* exhibited LL/HL >1 due to its transformation into antheraxanthin and zeaxanthin (Zea) in HL conditions. The behaviour of diadinoxanthin-diatoxanthin (DD) and violaxanthin-antheraxanthin-zeaxanthin (VAZ) cycles largely differed among species. For example, *P. minimum* synthesized much larger amounts of Diadino than Diato in HL, in contrast to *K. micrum*, *P. parvum* and *C. leadbeateri*. Again, *P. globosa* showed a distinct response and its DD cycle remained almost unaltered in LL versus HL (Fig. 1a).

Regarding the VAZ cycle, the prasinophyte *P. marina* displayed four-fold higher values of Zea (Table 1) than *B. prasinos*. Finally, although Lut was present only as trace values in LL, both prasinophytes exhibited significantly higher Lut to Chl *a* ratios in HL conditions (Fig. 2, Table 1).

C-normalized pigment ratios

Chl *a*:C ratios varied between two to five fold in each species (Table 2), the corresponding LL and HL means being significantly different (HL = 0.016 ± 0.005 and HL = 0.047 ± 0.028; $n = 8$; $P < 0.05$). LL and LL ratios for C- and Chl *a*-normalized total pigments (TP), light-harvesting (LHP) and photoprotective compounds (PPC) are shown in Fig. 2. The average of C-normalized TP and LHP ratios (aggregate of chlorophylls and photosynthetic carotenoids) in LL versus HL were 2.57 ± 1.05 and 4.42 ± 2.31, respectively. TP to C and LHP to C ratios were significantly higher in LL versus HL in contrast with the same Chl *a*-normalized TP and LHP ratios (Fig. 2b). The main reason were two to ten fold higher photosynthetic carotenoids (PSC; fucoxanthin and its derivatives, peridinin, prasinoxanthin and violaxanthin) to C ratios (data not shown) in contrast with similar PSC to Chl *a* ratios in LL versus HL (due to co-variation with Chl *a*; Table 1).

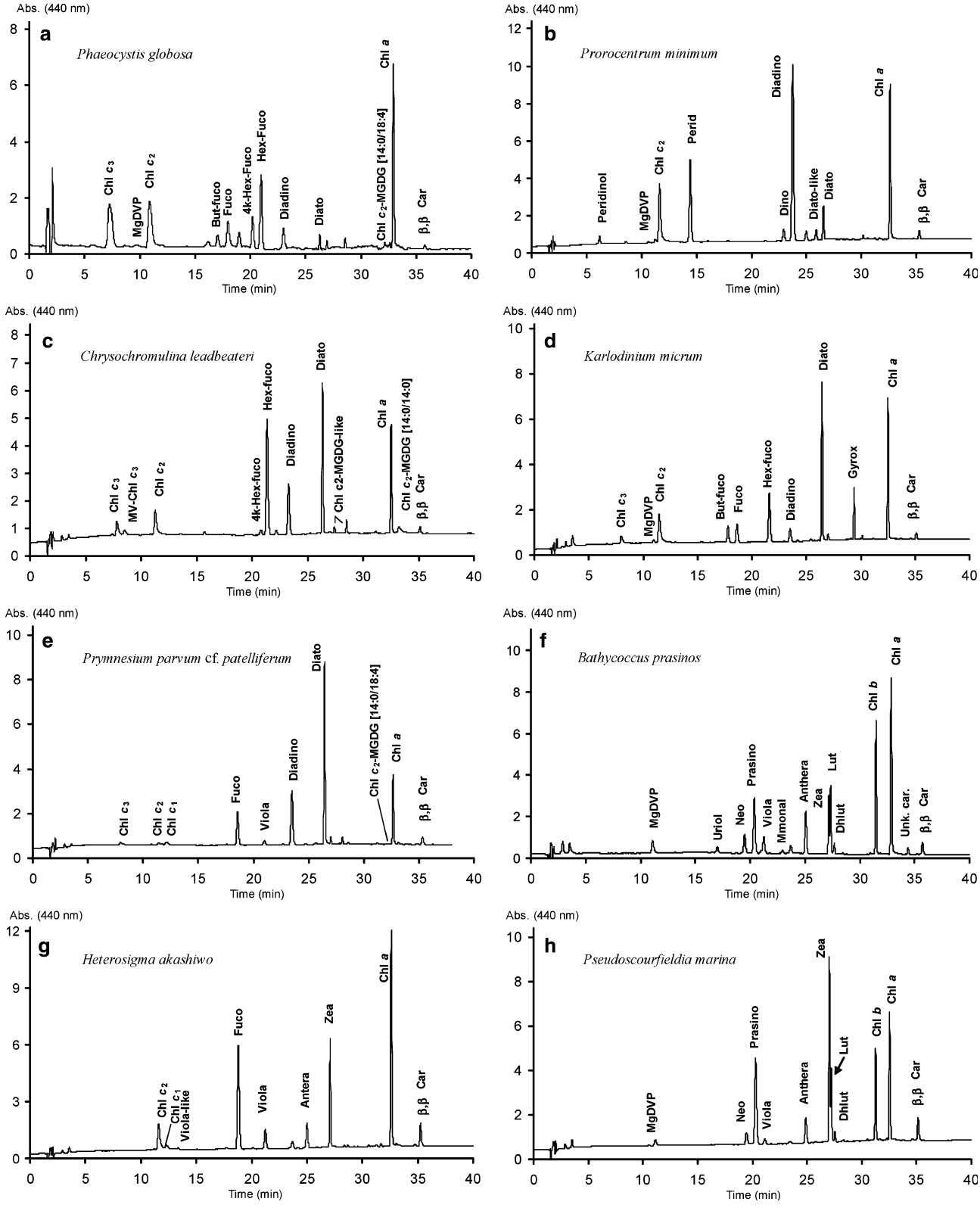

Fig. 1 Chromatograms (absorbance 440 nm) of the pigment composition of cultures of **a** *Phaeocystis globosa*, **b** *Prorocentrum minimum*, **c** *Chrysochromulina leadbeateri*, **d** *Karlodinium micrum*, **e** *Prymnesium parvum* cf. *patelliferum*, **f** *Bathycoccus prasinos*, **g** *Heterosigma akashiwo* and **h** *Pseudoscourfieldia marina*, acclimated to high light ($E = 500$ μmol m^{-2} s^{-1})

Table 1 Chl *a*-normalized ratios of accessory pigments in the algal isolates studied (means of two true replicates)

	Light	Chlorophylls								Carotenoids											
		Chl *c*3	MV Chl *c*3	MgDVP	Chl *c*2	Chl *c*1	Chl *c*2-MGDG[a]	Chl *b*	Chl *c*2-MGDG [b]	Perid	But-Fuco	Fuco	Prasino	Viola	Hex-Fuco	4k-Hex-fuco	Diadino	Diato	Zea	Gyro	Lut
Dinoflagellates																					
Prorocentrum	LL			*0.004*	0.293					0.320							*0.123*	*0.033*			
minimum	HL			*0.008*	0.165					0.285							*0.456*	*0.052*			
Karlodinium	LL	0.050		*0.004*	0.124						0.055	0.227			0.178		*0.024*	*0.026*		0.076	
micrum	HL	0.020		*0.008*	0.091						0.067	0.076			0.197		*0.040*	*0.285*		0.075	
Raphidophytes																					
Heterosigma	LL			*0.001*	0.068	0.015						0.289		0.023					*0.002*		
akashiwo	HL			*0.002*	0.060	0.011						0.273		0.016					*0.121*		
Haptophytes																					
Chrysochromulina	LL	0.130	*0.008*	0.008	0.164		0.045		0.053			0.004			0.797		*0.070*	*0.005*			
leadbeateri	HL	0.060	*0.023*	0.003			0.016		0.028			[c]			0.598		*0.197*	*0.365*			
Prymnesium	LL	0.064		0.007	0.058	0.072	0.020					0.490		0.002			*0.133*	*0.030*	*0.003*		
parvum	HL	0.018			0.009	0.016	0.004					0.263		0.011			*0.318*	*0.665*	*0.022*		
Phaeocystis	LL	*0.115*			0.190		0.013				0.039	0.215			*0.050*	*0.052*	0.043	[c]			
globosa	HL	*0.182*			0.167		[c]				0.040	0.095			*0.227*	*0.098*	0.044	*0.031*			
Prasinophytes																					
Pseudoscourfieldia	LL			0.057				0.849					0.216	0.014					*0.010*		*0.003*
marina	HL			0.024				0.445					0.278	0.008					*0.353*		*0.136*
Bathycoccus	LL			0.054				0.672					0.108	0.017					*0.009*		*0.001*
prasinos	HL			0.003				0.469					0.123	0.016					*0.077*		*0.088*

HL ratios >25% relative to LL ratios of individual pigments in italics denote "photoprotective-like" trends

[a][14/0:18/4]

[b][14/0:14/0]

[c] < 0.001

The average of PPC to C ratios in LL/HL was 0.39 ± 0.23. The DD ratios from *P.globosa* (see Table 1) were not included to avoid larger SD values than the corresponding LL/HL average for PPC to C ratios, but discussed hereafter.

Discussion and conclusions

C versus Chl a ratios: LHP

Most chemotaxonomic markers such as peridinin, gyroxanthin-diester and prasinoxanthin co-varied with Chl *a* as previously reported (Johnsen and Sakshaug 1993; Millie et al. 1997; Schlüter et al. 2000; Böhme et al. 2002; Henriksen et al. 2002). Thus, although these pigments are useful chemotaxonomic markers, thanks to their co-variation with Chl *a*, the photoacclimation status can be better derived from their C-normalized ratios in different light conditions, as seen also in Chl *a* to C ratios (Table 2). The information on photoacclimation status derived from C-normalized pigment ratios is illustrated, for example, by the two to ten fold higher C-ratios of LHP in LL versus HL (Fig. 2, Table 2).

Chl *a*-normalized Diadino and Diato ratios were similar in *P. globosa* in LL and HL. In contrast, light intensity clearly influenced its Hex-fuco, Fuco and Chl c_3 to Chl *a* ratios. The higher Hex-fuco to Chl *a* and lower Fuco to Chl *a* in HL versus LL in *P. globosa* have been reported in other haptophytes (*Emiliania huxleyi* and *Phaeocystis* sp.; Schlüter et al. 2000; Stolte et al. 2000). An increase of Hex-fuco following nutrient (iron) limitation was found in *Phaeocystis* sp. (Van Leeuwe and Stefels, 1998). Our results in *P. globosa* show that light conditions influence the proportion of the different acyloxy-fucoxanthins in this species. This result has been explained by Stolte et al. (2000) as the synthesis of Hex-fuco from Fuco triggered by light, whereas influence of the light regime on the synthesis of 4-keto-hex-fuco has never been reported. Some haptophytes synthesize certain acyloxy-fucoxanthins with similar spectral properties to Fuco in HL, but the physiological basis for this process remains an open question for further photophysiological studies. A light-induced "fucoxanthin cycle" seems to operate in some species (e.g. *Emiliania huxleyi*, Stolte et al. 2000), but this is not a general rule such as with the DD and VAZ cycles. For example, in our experiment the Hex-fuco to Chl *a* in *C. leadbeateri* was even lower in HL versus LL as reported in *C. polylepis* by Henriksen et al. (2002).

The higher LL versus HL ratios of accessory chlorophylls (either Chl *a* or C-normalized) agree with their light-harvesting function. However, higher HL versus LL ratios to Chl *a* for Chl c_3 in *P. globosa* and MV Chl c_3 in *C. leadbeateri* demonstrate that this is not a general rule. In fact, slightly higher ratios of Chls *c* ($c_1 + c_2$) and c_3 to Chl *a* in HL versus LL have been observed previously (Schlüter et al. 2000). Light-dependent responses in Chls *c to* Chl *a* ratios are thus not straightforward but species-specific, while Chl *b* to Chl *a* ratios seem always to follow the expected rules (LL vs HL ratios ≥1). The erratic trends in the Chl *c*-like pigment MgDVP for Chl *c*-containing species are most probably related to its trace levels in these species and its role in the Chl biosynthetic pathway (Porra et al. 1997).

The function of different Chl *c* pigments might be somewhat different; Chl c_3 has been shown to be an efficient LHP (Johnsen and Sakshaug 1993) but the role of MV Chl c_3 is so far unknown. Chls c_2-MGDG have been suggested to play a light-harvesting function

Table 2 C-normalized ratios of nitrogen (N), chlorophyll *a* (Chl *a*), total pigments (TP), light-harvesting pigments (LHP) and photoprotective pigments (PPC) in the algal isolates studied (three replicates in the case of N to C ratios and two replicates in the other cases)

C-normalized ratios	Light	N:C	Chl *a*:C	TP:C	LHP:C	PPC:C
Dinoflagellates						
P. minimum	LL	0.153 ± 0.006	0.017	0.013	0.010	0.003
	HL	0.133 ± 0.002	0.008	0.007	0.003	0.004
K. micrum	LL	0.192 ND	0.029	0.021	0.019	0.002
	HL	0.186 ± 0.012	0.012	0.008	0.005	0.003
Raphidophytes						
H. akashiwo	LL	0.155 ± 0.004	0.067	0.027	0.025	0.001
	HL	0.179 ± 0.003	0.022	0.011	0.007	0.003
Haptophytes						
C. leadbeateri	LL	0.145 ± 0.026	0.023	0.029	0.027	0.002
	HL	0.181 ± 0.002	0.014	0.018	0.010	0.008
P. parvum	LL	0.136 ± 0.009	0.028	0.026	0.021	0.005
	HL	0.124 ± 0.003	0.014	0.018	0.004	0.014
P. globosa	LL	0.170 ± 0.019	0.099	0.088	0.084	0.004
	HL	0.175 ± 0.003	0.021	0.019	0.017	0.002
Prasinophytes						
P. marina	LL	0.165 ± 0.001	0.062	0.072	0.007	0.001
	HL	0.133 ± 0.005	0.016	0.021	0.012	0.008
B. prasinos	LL	0.144 ± 0.008	0.052	0.045	0.044	0.001
	HL	0.147 ± 0.019	0.021	0.018	0.014	0.004

LHP included every pigment except the carotenoids Diadino, Diato, Anther, Zea and Lut which were considered as PPC

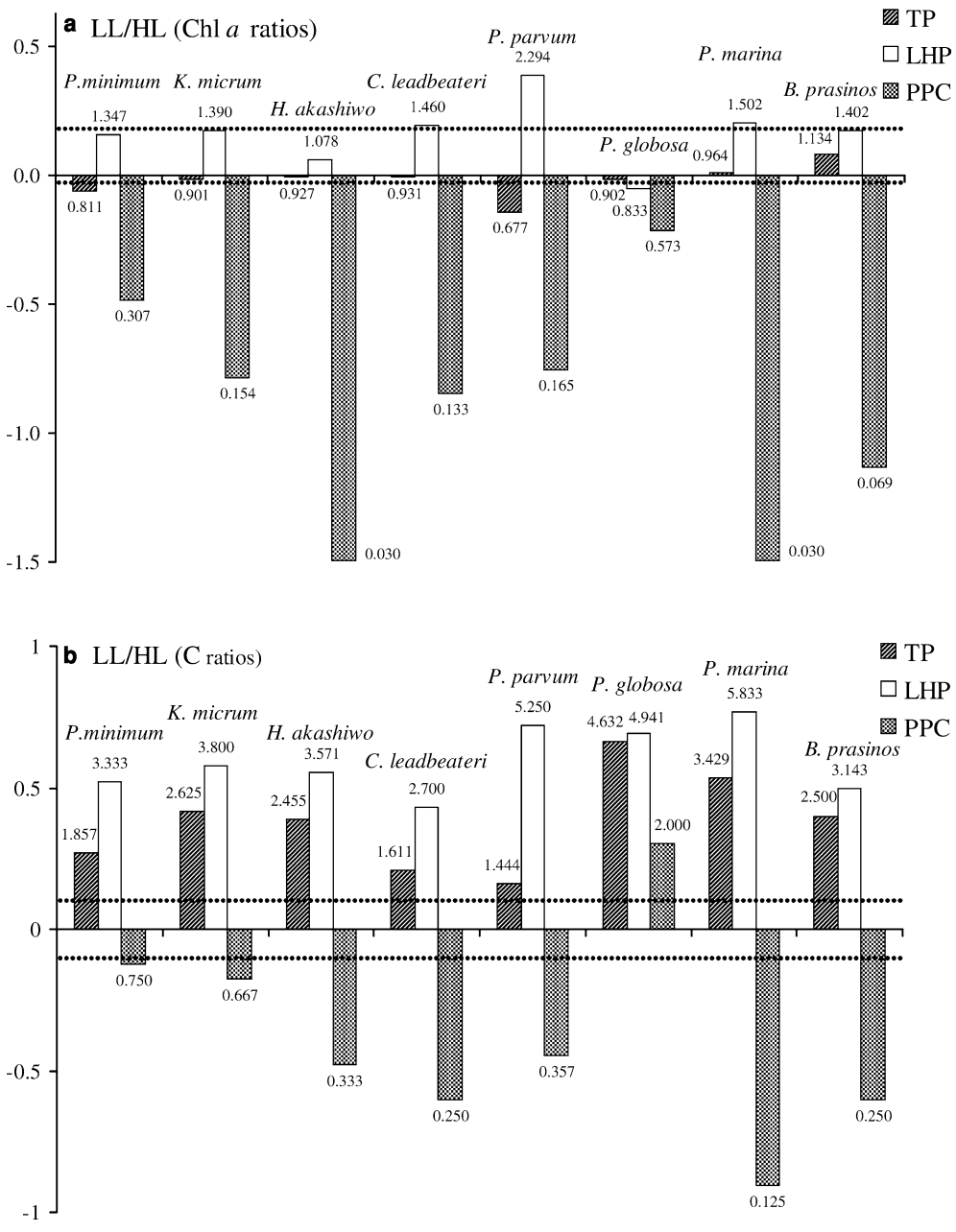

Fig. 2 Low-light/high-light (LL/HL) ratios (scale in $\log_{10}$: zero values indicate LL/HL = 1 and negative values LL/HL ratios < 1), of total pigments (TP), light-harvesting pigments (LHP) and photoprotective carotenoids (PPC) normalized to **a** Chl *a*, and **b** carbon. The actual values of each Chl *a* and C ratio are also shown beside the *bars*. *Dashed lines* stand for ±25% difference between LL versus HL ratios

and/or deliver Chl c_2 from its site of formation to the final location in the fucoxanthin-chlorophyll a/c protein antennae (Garrido et al. 2000). In the present study the light-dependent trends of Chl c_2-MGDG in *P. globosa* and *P. parvum* are similar to those of polar Chls *c* (i.e. LL/HL > 1) suggesting a light-harvesting function in these compounds.

C versus Chl a ratios: PPC

As expected, the pool of C-specific DD cycle was higher in HL against LL (Table 1). The special case of *P. globosa* mentioned earlier can be explained by the kinetics of de-epoxidation triggered by higher light intensities in comparison with the other studied species. These interspecific differences in de-epoxidation states at identical irradiances, also evident in *B. prasinos* and *P. marina* (see e.g. Goss et al. 1998 for the incomplete VAZ cycle in *Mantoniella squamata*) should be kept in mind when using these xanthophylls to interpret the light regime and photoacclimation in the field (Table 1, cf. Riegman and Kraay 2001). The high zeaxanthin to Chl *a* ratios in HL particularly in *P. marina* (Table 1; Zea:Chl *a* = 0.144 reported by Henriksen et al. (2002) at 300 µmol m^{-2} s^{-1}) show that some prasinophytes could contribute in certain cases to the zeaxanthin values measured in natural samples. Microscopic cell counts would be essential to ensure that the dominant sources of zeaxanthin is in fact the cyanobacteria *Synechococcus*

such as elsewhere reported, and that it is not rivalled by green flagellates.

The increase of lutein to Chl *a* ratios in HL conditions in *B. prasinos* and *P. marina* has already been reported in previous studies of the prasinophycean *M. squamata* (e.g. Böhme et al. 2002). These results seem to confirm the photoprotective role of lutein in prasinophytes, as shown previously by Niyogi et al. (1997) in the chlorophycean *Chlamydomonas reinhardtii*.

Application of Chl a and C-normalized pigment ratios in the field

Carbon-specific biomass measurements of phytoplankton are difficult to obtain in situ due to obvious methodological limitations. Rather, the distribution of Chl *a* contributed by phytoplankton groups (using HPLC pigment analyses) and C (derived from light microscopy) has been recently compared in several studies (Garibotti et al. 2003; Llewellyn et al. 2005). The differences between both techniques and their associated errors explain a better agreement for Chl *a* and C in large diatom species (easy to recognize in cellular counts and to discern by pigments using fucoxanthin) but poorer results for other groups (dinoflagellates, prymnesiophytes, small flagellates; Garibotti et al. 2003; Llewellyn et al. 2005).

In our study, the light-dependent variation in TP and LHP to carbon ratios shows that C-normalized ratios reflect the photoacclimation status in phytoplankton. This is not always the case when examining the corresponding Chl *a*-normalized ratios since Chl *a* is a light-dependent variable. The co-variation of a main bulk of LHP with Chl *a* explains why Chl *a*-normalized ratios in different light conditions remain relatively unaltered, thereby masking the photoacclimation trends. This co-variation between LHP and Chl *a* explains their success to infer the relative contribution of pigmentary groups in the sea (Schlüter et al. 2000). Although, as in the case of carbon, a direct determination of pigment to Chl *a* ratios in field samples is not feasible, and we must rely on our initial guesses from cultures.

Based on our data, we suggest that the interpretation of PPC and LHP are highly dependent on biomass normalization (Chl *a* vs. C).

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Reference

Anderson JM, Chow WS, Park Y-I (1995) The big design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. Photosynth Res 46:129–139

Bains S, Richard D, Norris R, Courfield M, Faul KL (2000) Termination of global warmth at the Palaeocene/Eocene boundary through productivity feedback. Nature 407:171–174

Böhme K, Wilhelm C, Goss R, (2002) Light regulation of carotenoid biosynthesis in the prasinophycean *Mantoniella squamata*. Photochem Photobiol Sci 1:619–628

Cloern JE, Grenz C, Vidergar-Lucas L (1995) An empirical model of the phytoplankton chlorophyll:carbon ratio—the conversion factor between productivity and growth rate. Limnol Oceanogr 40:1313–1321

Egeland ES, Johnsen G, Eikrem W, Throndsen J, Liaaen-Jensen S (1995) Pigments of *Bathycoccus prasinos* (Prasinophyceae): methodological and chemosystematic implications. J Phycol 31:554–561

Egeland ES, Guillard RRL, Liaaen-Jensen S (1997) Additional carotenoid prototype representatives and a general chemosystematic evaluation of carotenoids in Prasinophyceae (Chlorophyta). Phytochemistry 44:1087–1097

Falkowski PG, Dubinsky Z, Wyman K (1985) Growth-irradiance relationships in phytoplankton. Limnol Oceanogr 30:311–321

Falkowski PG, Raven J (eds) (1997) Aquatic Photosynthesis. Blackwell, Oxford, UK

Fawley MW (1992) Photosynthetic pigments of *Pseudoscourfieldia marina* and select green flagellates and coccoid ultraphytoplankton: implications for the systematics of the Micromonadophyceae (Chlorophyta). J Phycol 28:26–31

Fenchel T (1988) Marine planktonic food chains. Ann Rev Ecol Syst 19:19–38

Garibotti IA, Vernet M, Kozlowski WA, Ferrario ME (2003) Composition and biomass of phytoplankton assemblages in coastal Antarctic waters: a comparison of chemotaxonomic and microscopic analyses. Mar Ecol Prog Ser 247:27–42

Garrido JL, Otero J, Maestro MA, Zapata M (2000) The main non-polar chlorophyll *c* from *Emiliania huxleyi* (Prymnesiophyceae) is a chlorophyll c_2-monogalactosyl-diacylglyceride ester: a mass spectrometry study. J Phycol 36:497–505

Goericke R, Montoya JP (1998) Estimating the contribution of microalgal taxa to chlorophyll *a* in the field-variations of pigment ratios under nutrient-and light-limited growth. Mar Ecol Prog Ser 169:97–112

Goss R, Böhme K, Wilhelm C (1998) The xanthophyll cycle of *Mantoniella squamata* converts violaxanthin into antheraxanthin but not to zeaxanthin: consequences for the mechanism of enhanced non-photochemical energy dissipation. Planta 205:613–621

Guillard RRL, Ryther JH (1962) Studies of marine plankton diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea*. (Cleve) Gran Can J Microbiol 8:229–239

Henriksen P, Riemann B, Kaas H, Sørensen HM, Sørensen HL (2002) Effects of nutrient-limitation and irradiance on marine phytoplankton pigments. J Plankton Res 24:835–858

Jeffrey SW, Mantoura RFC, Bjørnland T (1997) Data for the identification of 47 key phytoplankton pigments. In: Jeffrey SW, Mantoura RFC, Wright SW, (eds) Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO, Paris, pp 449–559

Johnsen G, Prézelin BB, Jovine RVM, (1997) Fluorescence excitation spectra and light utilization in two red tide dinoflagellates. Limnol Oceanogr 42:1166–1177

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

Larsen A (1999) *Prymnesium parvum* and *P. patelliferum* (Haptophyta)—one species. Phycologia 38:541–543

Latasa M, Scharek R, LeGall F, Guillou L (2004) Pigment suites and taxonomic groups in Prasinophyceae. J Phycol 40:1149–1155

Lefèvre N, Taylor AH, Gilbert FJ, Geider RJ (2001) Modeling carbon to nitrogen and carbon to chlorophyll *a* ratios in the ocean at low latitudes: evaluation of the role of physiological plasticity. Limnol Oceanogr 48:1796–1807

Llewellyn CA, Gibb SW (2000) Intra-class variablity in the carbon, pigment and biomineral content of prymnesiophytes and diatoms. Mar Ecol Prog Ser 193:33–44

Llewellyn CA, Fishwick JR, Blackford JC (2005) Phytoplankton community assemblage in the English Channel: a comparison using Chl *a* derived from HPLC-CHEMTAX and carbon derived from microscopy cell counts. J Plankton Res 27:103–119

Lohr M, Wilhelm C (1999) Algae displaying the diadinoxanthin cycle possess the violaxanthin cycle. Proc Natl Acad Sci USA 96:8784–8798

Millie D, Schofield O, Kirkpatrick G, Johnsen G, Tester P, Vinyard B (1997) Detection of harmful algal blooms using photopigments and absorption signatures: a case study of the Florida red tide dinoflagellate, *Gymnodinium breve*. Limnol Oceanogr 42:1240–1251

Niyogi KK, Björkman O, Grossman AR (1997) The roles of specific xanthophylls in photoprotection. Proc Natl Acad Sci USA 94:14162–14167

Porra RJ, Pfündel EE, Engel N (1997) Metabolism and function of photosynthetic pigments. In: Jeffrey SW, Mantoura RFC, Wright SW (eds) Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO, Paris, pp 85–126

Riegman R, Kraay GW, (2001) Phytoplankton community structure derived from HPLC analysis of pigments in the Faroe-Shetland Channel during summer 1999: the distribution of taxonomic groups in relation to physical/chemical conditions in the photic zone. J Plankton Res 23:191–205

Sakshaug E, Graneli E, Elbrachter M, Kayser H (1984) Chemical composition and alkaline phosphatase activity of nutrient-saturated and P-deficient cells of four marine dinoflagellates. J Exp Mar Biol Ecol 77:241–254

Sakshaug E, Bricaud A, Dandonneau Y, Falkowski P, Kiefer D, Legendre L, Morel A, Parslow J, Takahashi M (1997) J Plankton Res 19:1637–1670

Schlüter L, Møhlenberg F, Havskum H, Larsen S, (2000) The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios. Mar Ecol Prog Ser 192:49–63

Stolte W, Kraay GW, Noordeloos AAM, Riegman R (2000) Genetic and physiological variation in pigment composition of *Emiliania huxleyi* (Prymnesiophyceae) and the potential use of its pigment ratios as a quantitative physiological marker. J Phycol 36:529–539

van Leeuwe MA, Stefels J (1998) Effects of iron and light stress on the biochemical composition of Antarctic *Phaeocystis* sp II Pigment composition. J Phycol 34:496–503

Wright SW, van den Enden RL (2000) Phytoplankton populations off east Antarctica in relation to stratification/mixing regimes: CHEMTAX analysis of HPLC pigment profiles (BROKE survey, Jan-Mar 1996). Deep-Sea Res II 47:2363–2400

Zapata M, Rodríguez F, Garrido JL, (2000) Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed-phase C8 column and pyridine-containing mobile phases. Mar Ecol Prog Ser 195:29–45

Zapata M, Edvardsen B, Rodríguez F, Maestro MA, Garrido JL (2001) Chlorophyll c_2 monogalactosyldiacylglyceride ester (chl c_2?-MGDG) a marker pigment for C*hrysochromulina polylepis* species (Haptophyta). Mar Ecol Prog Ser 219:85–98

Zapata M, Jeffrey SW, Rodríguez F, Clementson L, Garrido JL, Wright SW (2004) Pigment variability in 37 species (65 strains) of Haptophyta: implications for phylogeny and oceanography. Mar Ecol Prog Ser 270:83–102