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Pigment composition, spectral characterization and photosynthetic parameters in *Chrysochromulina polylepis*

Geir Johnsen[1], Egil Sakshaug[1], Maria Vernet[2]

[1] Trondhjem Biological Station, The Museum, University of Trondheim, Bynesveien 46, N-7018 Trondheim, Norway
[2] Marine Research Division, A-018, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093, USA

ABSTRACT: The photobiological response of an isolate of the prymnesiophyte *Chrysochromulina polylepis*, obtained from a bloom in the Skagerrak in May–June 1988, was evaluated with respect to pigment composition, spectral dependence of light harvesting, and photosynthetic parameters of cultures grown at 75 to 120 μmol m^{-2} s^{-1} irradiance, 16 h day length and 15 °C. Results were compared to similarly grown cultures of the diatom *Skeletonema costatum* that appeared before and after the *C. polylepis* bloom. Chl *a*-specific absorption of light ($^{\circ}a_c$) and chl *a*-specific absorption of quanta transported to photosystem II, estimated by means of a scaled fluorescence excitation spectrum ($^{\circ}F$), were 1.7 to 2.1 times larger in *C. polylepis* than in *S. costatum* in the visible spectrum. *C. polylepis* harvested blue-green light (450 to 500 nm) particularly efficiently. This is related to a high proportion of 19'-hexanoyloxyfucoxanthin and chl c_3 relative to chl *a*. Nonetheless, both *C. polylepis* and *S. costatum* absorb light more efficiently in 'clearest' blue ocean water than in 'clearest' green coastal water according to calculations based on spectrally corrected absorbed quanta transported to photosystem II ($^{\circ}\bar{F}$). Carbon-specific light absorption was about the same in the 2 species since the chl *a*:C ratio in *S. costatum* was twice as high as in *C. polylepis*. *C. polylepis* had a much smaller maximum carbon uptake (P_m^B) than *S. costatum*. Differences between the 2 species in terms of photosynthetic parameters, pigment composition, and spectral characteristics normalized to chl *a*, carbon, and cell are discussed.

INTRODUCTION

The bloom of *Chrysochromulina polylepis* in the Skagerrak in 1988 was first observed after a diatom bloom dominated by *Skeletonema costatum* along the Swedish coast in the first week of May (Granéli et al. 1989). For the next 2 wk *C. polylepis* bloomed in the Skagerrak and was transported along the southern coast of Norway. The population of *C. polylepis* was observed at the pycnocline, often situated at 5 to 10 m depth. The maximum cell concentrations were 40 to 80 × 10^6 cells l^{-1} (Aksnes et al. 1989, Lindahl & Dahl 1989). During the bloom period the weather was exceptionally bright and sunny, the waters were extremely stratified, and the temperature in the surface layer was 6 to 12 °C (Aksnes et al. 1989, Skjoldal & Dundas 1991). The bloom harmed marine life and aquaculture (Dahl et al. 1989). When the *C. polylepis* bloom terminated near Arendal (Norway), a bloom of *S. costatum* developed near the surface (Skjoldal & Dundas 1991).

It has been suggested that prymnesiophyte blooms in the Kattegat/Skagerrak and along the southern coast of Norway occur when limitation by silicate prevents diatoms from forming large stocks (Aksnes et al. 1989, Kaartvedt et al. 1990). The light regime, however, may also play a role, particularly in the initial phase of a bloom before nutrients become limiting. During May, irradiance levels were above the mean values for that month measured in earlier years, and the largest positive anomaly occurred from 6 to 12 May, which coincided with the first registration of *Chrysochromulina polylepis* (Skjoldal & Dundas 1991).

Waters in the Skagerrak and along the Norwegian coast differ from oceanic waters in that their colour is blue-green to green, mainly due to high concentrations of humic substances (Jerlov 1976). Spectral irradiance may have an impact on species distribution.

To evaluate the photobiological response of *Chrysochromulina polylepis*, with respect to light, including its spectral distribution, we investigated pigment compos-

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ition, spectral dependence of light harvesting, and photosynthetic parameters of moderately shade-adapted cultures. These results were then compared to results for *Skeletonema costatum*, grown in the same light regime and temperature.

MATERIAL AND METHODS

Culture conditions. The *Chrysochromulina polylepis* (Manton & Parke 1962) strain was isolated from a bloom that occurred in May–June 1988 outside Hvaler (outer Oslofjord, Norway: 59°00'N, 10°45'E). *C. polylepis* was maintained in f/2 medium (Guillard & Ryther 1962) at 15°C, 34 ppt salinity and 16 h day length. Scalar irradiance (E_o, PAR) was 75 or 120 µmol m^{-2} s^{-1} (provided by 4 Philips TL 40W/55 fluorescent tubes). E_o was measured with a QSL-100 quantum sensor (Biospherical Instruments). Pigment and chemical composition as well as light absorption characteristics did not differ at the 2 irradiances, and we here report the pooled results. *Skeletonema costatum*, clone Skel-5, isolated from the Trondheimsfjord (Norway) in 1969, was grown at 75 µmol m^{-2} s^{-1}.

Pigments. For pigment extraction, cultures were concentrated on Whatman GF/C glass fibre filters at 50 mb differential pressure. The filters were then extracted for 20 h at 4 °C in darkness in 90 % acetone bubbled with N_2 (*Chrysochromulina polylepis*) or according to Hallegraeff (1981) for *Skeletonema costatum*. Extracts were cleared by filtration through Whatman GF/C filters and injected into the HPLC column without further treatment. The chlorophyll *a* (chl *a*) concentration was also estimated spectrophotometrically according to Jeffrey & Humphrey (1975) with the same pretreatment as above. Spectrophotometrical estimation of chl *a* was used for normalizing the light absorption spectra and chl *a*:C ratios and were in agreement with the HPLC values.

Pigments were analyzed by high-performance liquid chromatography (Merck & Hitachi L-6200 HPLC) on a SPHERI-5 RP-18 reverse-phase C-18 column (Brownlee Labs 25 cm × 4.6 mm, 5 µm particles) by elution in a low-pressure gradient system consisting of a linear gradient from 100 % A to 100 % B in 10 min and maintaining B for another 15 min. Solvent A (1 l) consisted of 80:20 methanol:water (v:v) where 100 ml of distilled water was prepared with 1.5 g tetrabutylammonium acetate and 0.96 g ammonium acetate as ion-pairing agent (Mantoura & Llewellyn 1983). Solvent B consisted of 60:40 methanol:ethyl acetate (v:v). Chlorophylls and carotenoids were monitored by absorption at 440 nm and quantified by calibration of the column with pigments isolated by thin-layer chromatography from a culture of the diatom *Thalassiosira nordenskioeldii*. For chlorophylls we used the extinction coefficients of Jeffrey & Humphrey (1975) and Jeffrey & Wright (1987). For carotenoids, extinction coefficients were as follows – fucoxanthin and its derivatives: 160 l g^{-1} cm^{-1} at 450 nm; diadinoxanthin, diatoxanthin and their derivatives: 250 l g^{-1} cm^{-1} at maximum absorption (446 to 453 nm) (Jeffrey 1968, Davies 1976). Absorption spectra of the eluted pigments were recorded in the eluent on a Hitachi Spectrophotometer Model U-2000 fitted with a flow-through cell and compared to published spectra for identification (Davies 1976, Wright & Shearer 1984). A more detailed analysis of the carotenoids of *Chrysochromulina polylepis* has been published elsewhere

Table 1. *Chrysochromulina polylepis*. Spectral characteristics and cellular contents of pigments. Parentheses denote shoulders in the absorption spectra

Pigment	Reference time (min)	Absorption maxima (eluent)	Cellular concentration (pg $cell^{-1}$)	% Total carotenoid (w:w)
Chlorophyll c_3	6.7	457, 587, 625	0.19	
Chlorophyll c_2[a]	7.7	447, 586, 633	0.16	
Fucoxanthin	9.9	450, (468)	0.09	13.0
19'-hexanoyloxyfucoxanthin	10.3	447, 470 449[b], 473	0.45	65.2
9' *cis* hexanoyloxyfucoxanthin	11.0	448, 468	0.03	4.3
Diadinoxanthin	11.4	(423), 446, 476	0.08	11.6
Diatoxanthin	11.9	453, 480	0.01	1.5
19'-hexanoyloxyparacentrone 3-acetate	12.1	(422), 446, 471	0.01	1.5
Chl *a*	14.7	433, 618, 665	0.93	
β-Carotene	20.2	(428), 454, 479 (422)[c], 447, 472	0.02	2.9

[a] May include chl c_1; [b] absorption maxima in ethanol; [c] in ether

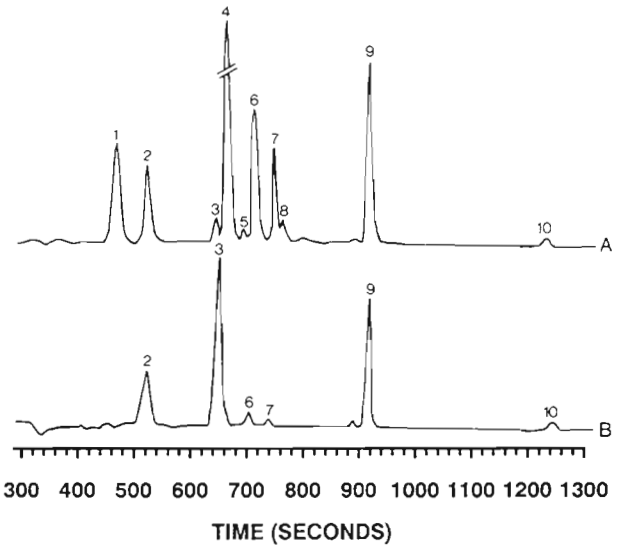

Figure 1. Chromatogram (absorbance at 440 nm) of pigment extract obtained from (A) *Chrysochromulina polylepis* and (B) *Skeletonema costatum*. Peak identifications: 1 = chl c_3, 2 = chl c_{1+2}, 3 = fucoxanthin, 4 = 19'-hexanoyloxyfucoxanthin, 5 = 9'*cis* hexanoyloxyfucoxanthin, 6 = diadinoxanthin, 7 = diatoxanthin, 8 = 19'hexanoyloxyparacentrone 3-acetate, 9 = chl *a*, 10 = β-carotene

(Bjerkeng et al. 1990). Our system separates chl c_3 from chl c_2 well (1 min between peaks; Table 1), but chl c_2 and c_1 would coelute if the latter were present (Fig. 1).

***In vivo* spectral characteristics.** Chl *a*-specific light absorption ($^\circ a_c$) was measured on Whatman GF/C filters according to Mitchell & Kiefer (1988). Chl *a*-specific fluorescence excitation spectra were measured at an emission wavelength of 730 nm (Neori et al. 1988) and were quantum-corrected by means of the dye Basic Blue 3 (Kopf & Heinze 1984). They were then scaled by matching of the red peak of the fluorescence excitation spectrum at 676 nm to the corresponding absorption peak of $^\circ a_c$ to provide estimates of specific absorption of quanta transported to photosystem II, where O_2 is released [$^\circ F$, m^2 (mg chl *a*)$^{-1}$; Sakshaug et al. 1991]. $^\circ F$ may be interpreted as an action spectrum for photosynthesis (Haxo 1985, Neori et al. 1988). $^\circ a_c$ and $^\circ F$ were measured in duplicate at 1 nm intervals.

The integrated values of $^\circ\bar{a}_c$ and $^\circ\bar{F}$ (400 to 700 nm) and thus the photosynthetic efficiency ($\alpha^B = \phi_{max}\,{}^\circ\bar{F}$, where ϕ_{max} = maximum quantum yield) depend on the spectral distribution of ambient light and were calculated according to Morel et al. (1987):

$$^\circ\bar{a}_c \text{ or } ^\circ\bar{F} = \left[\int_{400}^{700} X(\lambda) \cdot E_o(\lambda)\mathrm{d}\lambda\right] / E_o(\mathrm{PAR}) \qquad (1)$$

where $^\circ\bar{a}_c$ = absorbed quanta, spectrally corrected; $^\circ\bar{F}$ = absorbed quanta transported to photosystem II, spectrally corrected; $X(\lambda) = {}^\circ a_c(\lambda)$ or $^\circ F(\lambda)$; $E_o(\lambda)$ = *in situ* spectral irradiance; E_o(PAR) = integrated irradiance.

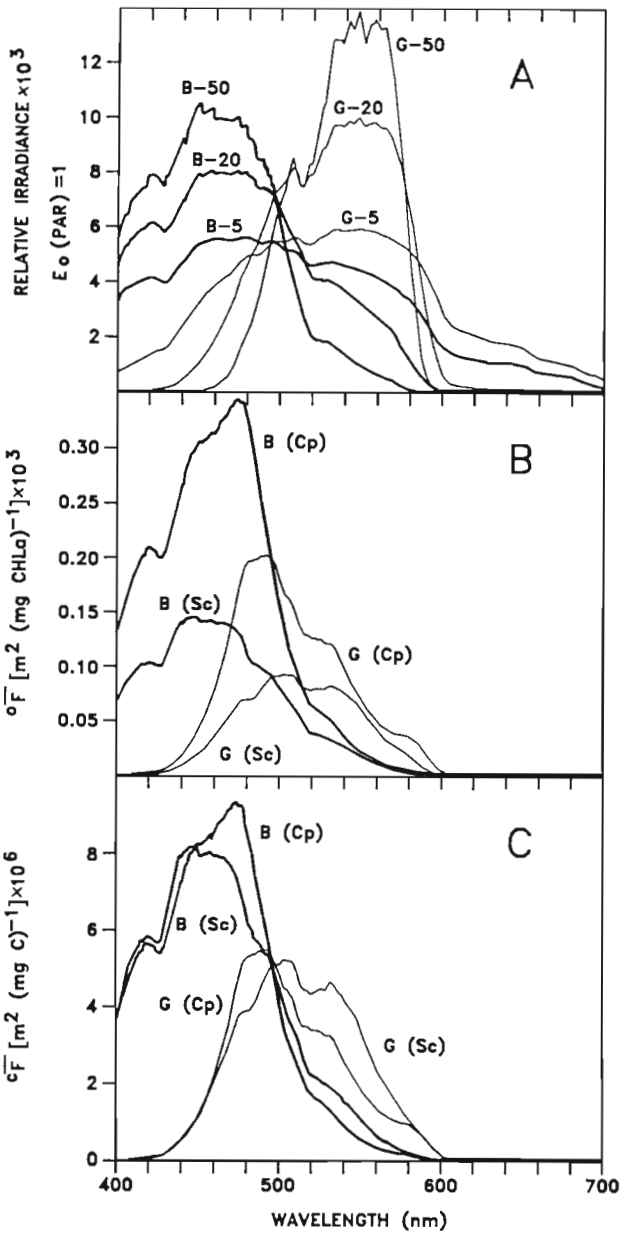

Fig. 2. (A) Relative spectral irradiance [$\times 10^3$, E_o (PAR) = 1] at 5, 20 and 50 m depth in 'clearest' green (G) and blue waters (B). (B) Spectrally corrected chl *a*-normalized absorption of quanta transported to photosystem II [$^\circ F(\lambda)\ E_o(\lambda)$, m^2 (mg chl *a*)$^{-1} \times 10^3$] in 'clearest' green and blue water at 20 m depth assuming infinitesimal chl *a* concentration. E_o(PAR) = 1. (C) As (B), but carbon-normalized [$^C F(\lambda)\ E_o(\lambda)$, m^2 (mg C)$^{-1} \times 10^6$]. Cp: *Chrysochromulina polylepis*; Sc: *Skeletonema costatum*

Calculations of spectral irradiance vs depth (Fig. 2A) were carried out on the basis of spectral vertical diffuse attenuation coefficients for 'clearest' green water in the Trondheimsfjord on 6 March (< 0.1 mg chl *a* m^{-3}) and

'clearest' blue ocean water (Smith & Baker 1981) using the daylight spectrum (cloudless sky) in Trondheim (63° N) at noon on 6 March. Spectral irradiance in 'clearest' green water was measured with a Li-Cor 1800-UW spectroradiometer (400 to 700 nm) at 1 nm intervals and is very comparable with the colour of Skagerrak water (R. Dalløkken pers. comm.).

Cellular carbon and nitrogen were analyzed in a Carlo Erba Elemental Analyzer, model 1104, after treatment with fuming hydrochlorid acid.

Photosynthetic parameters. *P* vs *I* experiments were performed at 15 °C in 'blue-green' light provided by 4 Philips TLM 115W/33RS fluorescent tubes; for spectral characteristics, see Johnsen & Hegseth (1991). Two prefiltered ampoules with $NaH^{14}CO_3$ activity of 370 kBq ml^{-1} (New England Nuclear, NEC-086S) were added to 80 ml algal culture. Duplicate 1 ml samples were incubated 4 h into the light phase for 1 h in 20 ml scintillation vials (Zinsser polyethylene) in a modified 'Photosynthetron' (Lewis & Smith 1983) with 20 different irradiances (7 to 565 µmol m^{-2} s^{-1}; Sakshaug et al. 1991). This technique involves measurement on unfiltered samples and therefore does not discriminate between particulate and dissolved phases of ^{14}C. *P* vs *I* parameters were computed according to Platt et al. (1980) by means of the curvilinear least-square regression program LSQUARE (see Table 3 for definitions and units).

RESULTS

Pigments

Predominant accessory pigments of *Chrysochromulina polylepis* are chlorophyll c_3 (Jeffrey & Wright 1987) and chl c_2 in addition to 19'-hexanoyloxyfucoxanthin as the major carotenoid, while fucoxanthin contributes 13 % of total carotenoids (Table 1, Fig. 1). In contrast, fucoxanthin is the major accessory pigment in *Skeletonema costatum* (Pennington et al. 1988; Fig. 1, Table 2). The pigment composition of *C. polylepis* is similar to that of the Prymnesiophyte *Emiliana huxleyi* (Wright & Jeffrey 1987), but differs from the Plymouth isolate of *C. polylepis* because 2 recently found pigments in our strain (Bjerkeng et al. 1990) are absent in that strain (Riley & Wilson 1967). Similar to the findings of Riley & Wilson (1967), our strain exhibits minor amounts of a carotenoid eluted after diatoxanthin (Table 1, Fig. 1; at 12.1 min) with absorption maxima at 422, 446 and 471 nm. This carotenoid was identified as 19'-hexanoyloxyparacentrone 3-acetate (Bjerkeng et al. 1990). Also, a minor carotenoid which is eluted before diadinoxanthin was found (Table 1, Fig. 1; at 11.0 min). It has absorption maxima at 448 and 468 nm and has been identified as 9'*cis* hexanoyloxyfucoxanthin (Bjerkeng et al. 1990).

Table 2. Cellular contents of pigments in *Skeletonema costatum*; chl *c*:chl *a* and fucoxanthin:chl *a* ratios in *Chrysochromulina polylepis* and *S. costatum*

Pigment	Cellular conc. (pg $cell^{-1}$)	% Total carotenoid (w:w)
Chl c_{1+2}	0.23	
Fucoxanthin	0.43	89
Diadinoxanthin	0.03	6.0
Diatoxanthin	0.01	2.4
Chl *a*	0.83	
β-Carotene	0.01	2.6
	C. polylepis	*S. costatum*
Chl *c*:chl *a*[a]	0.38	0.28
Fucoxanthin:chl *a*	0.58[b]	0.52

[a] Chl *c* = total chl *c* for each species
[b] 19'-hexanoyloxyfucoxanthin is included

In vivo spectral characteristics

The *in vivo* chl *a*-specific absorption spectrum ($^{\circ}a_c$) of *Chrysochromulina polylepis* exhibits major peaks at 439, 468, 592, 639 and 675 nm, which correspond to chl *a* (439, 639 and 675 nm) and chl c_2+c_3 (468, 592, and in part 639 nm; Fig. 3A). A shoulder at 500 to 540 nm probably corresponds to *in vivo* absorption by the carotenoids 19'-hexanoyloxyfucoxanthin and fucoxanthin, which may also contribute significantly at 468 nm.

The scaled fluorescence excitation spectrum $^{\circ}F$ of *Chrysochromulina polylepis* exhibits a maximum at 472 nm, which corresponds to light-energy transport from 19'-hexanoyloxyfucoxanthin, chl c_2 and in particular chl c_3, which have maximum absorption at 457 nm in solution (Table 1, Fig. 3B).

On a chl *a*-normalized basis, *Chrysochromulina polylepis* absorbs light far more efficiently than *Skeletonema costatum*: $^{\circ}a_c$ (676 nm) is 0.019 m^2 (mg chl *a*)$^{-1}$ in the former vs 0.013 in the latter (Fig. 3A). Corresponding values for 440 nm are 0.044 and 0.020 m^2 (mg chl *a*)$^{-1}$ (Fig. 3A).

Photosynthetic parameters

Chrysochromulina polylepis exhibits a maximum photosynthetic rate (P_m^B) of 1.3 mg C (mg chl *a*)$^{-1}$ h^{-1}. This rate compares well to *in situ* assimilation num-

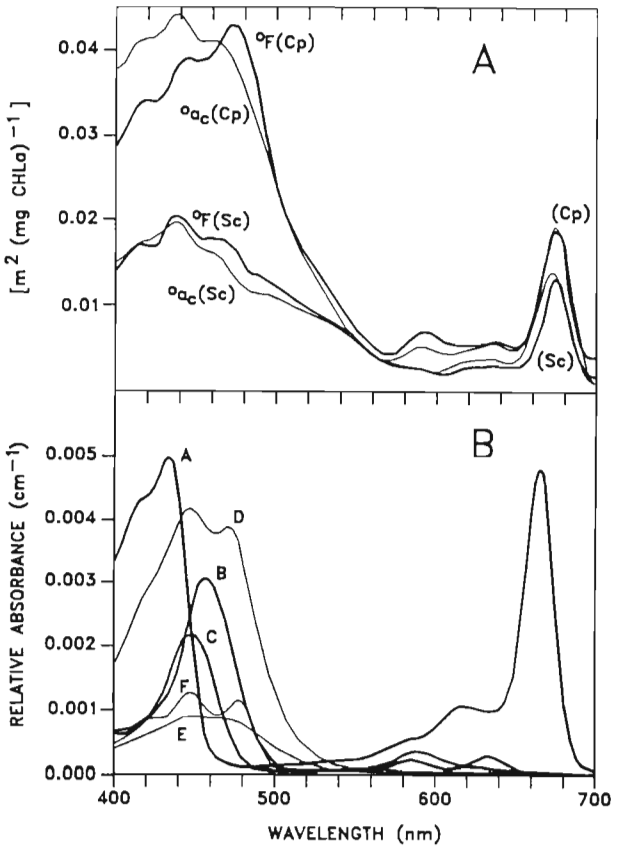

Fig. 3. *Chrysochromulina polylepis* (Cp) and *Skeletonema costatum* (Sc). (A) Chl *a*-specific absorption ($°a_c$) and scaled fluorescence excitation spectra ($°F$). (B) *In vitro* absorbance of the individual pigments isolated from *C. polylepis* and scaled to the cellular concentration presented in Table 1: (A) chl *a*, (B) chl c_3, (C) chl c_2, (D) 19'-hexanoyloxyfucoxanthin, (E) fucoxanthin, (F) diadinoxanthin (solvent; eluent from the HPLC column)

bers (P^B) of 0.3 to 1.9 mg C (mg chl *a*)$^{-1}$ h^{-1} for *C. polylepis* at the pycnocline in the southern Skagerrak on 30 May to 1 June 1988 (Nielsen et al. 1990). P_m^B for *Skeletonema costatum*, however, is much higher [4.7 mg C (mg chl *a*)$^{-1}$ h^{-1}; M. Gilstad & G. Johnsen unpubl.]. Photosynthetic efficiency (α^B) and the photoadaptation index (I_k) for *C. polylepis* in incubator illumination was 0.027 mg C (mg chl *a*)$^{-1}$ h^{-1} (µmol m^{-2} s^{-1}) and 48 µmol m^{-2} s^{-1}, respectively. The α^B value in *S. costatum* was almost the same as that for *C. polylepis* [0.023 mg C (mg chl *a*)$^{-1}$ h^{-1} (µmol m^{-2} s^{-1})], while I_k was 4.4 times higher in the former (211 µmol m^{-2} s^{-1}). In *C. polylepis*, the photoinhibition index (β^B) and the photoinhibition irradiance (I_b) were 0.9×10^{-3} mg C (mg chl *a*)$^{-1}$ h^{-1} (µmol m^{-2} s^{-1}), and 1700 µmol m^{-2} s^{-1}, respectively (Table 3). Thus, in contrast to *S. costatum*, no photoinhibition of photosynthesis was registered (Table 3).

DISCUSSION

Pigments

A comparison with other bloom-forming prymnesiophytes from western Europe demonstrates that *Chrysochromulina polylepis* is similar to *Emiliana huxleyi*, *Phaeocystis pouchetii* and *Prymnesium parvum* in having chl c_3 as well as chl c_2 (Jeffrey & Wright 1987, Fawley 1989), and to *E. huxleyi* and *Corymbellus aureus* (Gieskes & Kraay 1986) in the dominance of 19'-hexanoyloxyfucoxanthin. It differs, however, from *E. huxleyi* by not having a phytol-containing chl *c* derivative (Nelson & Wakeham 1989).

As expected for shade-adapted *Chrysochromulina polylepis*, the photosynthetically active pigments (chl *a*, c_2, and c_3, 19'-hexanoyloxyfucoxanthin and fucoxanthin) are abundant while carotenoids believed to be involved in photoprotection (diadinoxanthin and diatoxanthin; Haxo 1985, Vernet et al. 1989) are low with 13 % by weight of the total carotenoid in the cell (Table 1). Diadino- and diatoxanthin contributed 8 % of total carotenoids in shade-adapted *Skeletonema costatum*, and 2.5 to 15 % in shade-adapted Barents Sea diatoms (Sakshaug et al. 1991, G. Johnsen unpubl.; Table 2, Fig. 1).

We found a chl *a* content of 0.93 pg cell^{-1} in *Chrysochromulina polylepis*. This indicates that our experiments are representative of cells living at the pycnocline. This chl *a* content belongs to the high range of data for the Skagerrak bloom in May/June 1988 (0.15 to 1.0 pg cell^{-1}; Dahl et al. 1989, Lindahl & Dahl 1989, Nielsen et al. 1990). The chl *a* content in *Skeletonema costatum* is comparable to that of *C. polylepis*, i.e. 0.83 pg cell^{-1}. This value is comparable to the chl *a* content (0.85 pg cell^{-1}) according to earlier studies of the same clone of *S. costatum* grown at 70 µmol m^{-2} s^{-1}, 15 °C, and 10 h daylength (Sakshaug & Andresen 1986).

In vivo spectral characteristics

The fluorescence maximum at 472 nm (Fig. 3A) is not evident in $°a_c$ and may represent a particularly efficient transfer of light-energy from 19'-hexanoyloxyfucoxanthin, chl c_2 and chl c_3 to reaction-center chl *a* in photosystem II. Other fluorescence excitation peaks coincide with the absorption peaks. Comparison of the spectral characteristics of the isolated pigments with $°F$ indicate that almost all of the measured chloroplast pigments of *Chrysochromulina polylepis* transfer light-energy to photosystem II. This is in agreement with Haxo's survey of photosynthetic effectiveness in the similar pigmented *Emiliania huxleyi*, based on spectral absorption, fluorescence excitation and O_2-action spectrum (Haxo 1985).

Table 3. *Chrysochromulina polylepis* and *Skeletonema costatum*. Photosynthetic parameters in 'blue-green' incubator light and cell chemistry (data for *S. costatum*: M. Gilstad & G. Johnsen unpubl.). Photosynthetic parameters: P_m^B, realized maximum uptake; P_s^B, theoretical maximum uptake; α^B, photosynthetic efficiency; β^B, photoinhibition index; I_k ($= P_m^B/\alpha^B$), photoadaptation index; I_m optimum irradiance; I_b, photoinhibition irradiance. $^\circ\bar{a}_c$: spectrally corrected absorbed quanta; $^\circ\bar{F}$: absorbed quanta transported to photosystem II (see Eq. 1). Superscript ^{C} denotes carbon-normalized coefficients

Parameter	*C. polylepis*	*S. costatum*	Unit
P_s^B	1.5	–	mg C (mg chl *a*)$^{-1}$ h^{-1}
P_m^B	1.3	4.7	mg C (mg chl *a*)$^{-1}$ h^{-1}
α^B	0.027	0.023	mg C (mg chl *a*)$^{-1}$ h^{-1} (µmol m^{-2} s^{-1})$^{-1}$
$\beta^B \times 10^3$	0.90	0	mg C (mg chl *a*)$^{-1}$ h^{-1} (µmol m^{-2} s^{-1})$^{-1}$
I_k	48	211	µmol m^{-2} s^{-1}
I_m	191	–	µmol m^{-2} s^{-1}
I_b	1700	–	µmol m^{-2} s^{-1}
$^\circ\bar{a}_c$, $^\circ\bar{F}$	0.012	0.0071	m^2 (mg chl *a*)$^{-1}$
Chl *a*:C	0.027	0.056	w:w
N:C	0.12	0.17	at.:at.
$^C\bar{a}_c$, $^C\bar{F}$	0.32	0.40	m^2 (mg C)$^{-1}$
^CP_s	0.041	–	h^{-1}
^CP_m	0.035	0.26	h^{-1}
$^C\alpha$	0.00073	0.0013	h^{-1} (µmol m^{-2} s^{-1})$^{-1}$

$^\circ a_c$ and $^\circ F$ differ relatively little whether *Chrysochromulina polylepis* or *Skeletonema costatum* are concerned – presumably as a result of the small content of photoprotective pigments, namely diadino- and diatoxanthin, in shade-adapted cells (Vernet et al. 1989, Sakshaug et al. 1991). While $^\circ F$ of *C. polylepis* exhibits a maximum at 472 nm, $^\circ F$ of *S. costatum* exhibits a maximum at 440 nm. Thus *C. polylepis* is somewhat more specialized than *S. costatum* in absorption of blue-green light (Figs. 2B, C & 3A).

Light harvesting

Based on chl *a*-normalization, *Chrysochromulina polylepis* absorbs blue-green incubator light 1.7 times more efficiently than *Skeletonema costatum*, whether in terms of $^\circ\bar{F}$ or $^\circ\bar{a}_c$ (Table 3). This is related to the large amounts of 19'-hexanoyloxyfucoxanthin, chl c_2 and chl c_3 relative to chl *a* (Fig. 3). Since the fucoxanthin:chl *a* ratio in both species (fucoxanthin + 19'-hexanoyloxyfucoxanthin in *C. polylepis*) is about 0.5, the large difference in chl *a*-normalized spectra between species must be due to particularly efficient light-harvesting pigment-protein bonds in *C. polylepis*. The shoulder at 592 nm in both $^\circ a_c$ and $^\circ F$ obtained from *C. polylepis* indicates that chl c_{2+3} are efficient light-harvesting pigments (Figs. 2B, C & 3). *C. polylepis* is more efficient than *S. costatum* not only because these pigments transfer light-energy to photosystem II extremely efficiently, but also because it has 1.36 times more of total chl *c* per unit chl *a* (Fig. 3A, Table 2). *C. polylepis*, however, has an α^B value in blue-green incubation light which is only 1.2 times higher than in *S. costatum*.

The 1.4 times higher value of *Chrysochromulina polylepis* relative to *Skeletonema costatum* for $^\circ a_c$ at 676 nm, in which absorption by accessory pigments is negligible, indicates that the 'packaging' effect, i.e. self-shading in and between chloroplasts (Kirk 1983), is important in *S. costatum*. This is presumably related to the twice as high chl *a*:C ratio in *S. costatum* relative to *C. polylepis* (Table 3). The 2.2 times higher value for $^\circ a_c$ (440 nm) in *C. polylepis* compared to *S. costatum* reflects that accessory pigments are more important in live cells of *C. polylepis* than in live cells of *S. costatum* (Fig. 3A).

Light harvesting vs spectral irradiance

To demonstrate the effect of spectral distribution on algal photosynthesis in low light, we calculated $^\circ\bar{F}$, the absorbed quanta transported to photosystem II (Eq. 1), of *Chrysochromulina polylepis* and *Skeletonema costatum* in green and blue waters, assuming infinitesimal chl *a* concentrations (Table 4). It turns out that both species, in spite of being coastal strains, absorb light more efficiently in blue than in green water (Fig. 2B, C). Per unit chl *a*, *C. polylepis* absorbs quanta transported to photosystem II ($^\circ\bar{F}$) 1.96, 1.86 and 1.71 times more efficiently than *S. costatum* in green coastal water at 5, 20 and 50 m depth, respectively, and 2.07 to 2.13 times more efficiently than *S. costatum* in blue water (Table 4, Fig. 2B). α^B corresponds to the product of ϕ_{max},

Table 4. *Chrysochromulina polylepis* (*Cp*) and *Skeletonema costatum* (*Sc*). Spectrally corrected transport of absorbed quanta to photosystem II (Eq. 1), based on data from Fig. 2; $^{\circ}\bar{F}$ [m^2 (mg chl *a*)$^{-1}$], $^{c}\bar{F}$ [m^2 (mg C)$^{-1}$ $\times 10^3$] in green coastal waters (G) and blue oceanic waters (B; from Smith & Baker 1981) at 5, 20 and 50 m depth, assuming infinitesimal chl *a* concentration

Water, depth	$^{\circ}\bar{F}$ (*Cp*)	$^{\circ}\bar{F}$ (*Sc*)	$\frac{^{\circ}\bar{F}\ (Cp)}{^{\circ}\bar{F}\ (Sc)}$	$^{c}\bar{F}$ (*Cp*)	$^{c}\bar{F}$ (*Sc*)	$\frac{^{c}\bar{F}\ (Cp)}{^{c}\bar{F}\ (Sc)}$
G, 5 m	0.018	0.0092	1.96	0.49	0.52	0.94
G, 20 m	0.016	0.0086	1.86	0.43	0.48	0.90
G, 50 m	0.013	0.0076	1.71	0.35	0.43	0.81
B, 5 m	0.023	0.011	2.09	0.62	0.62	1.00
B, 20 m	0.029	0.014	2.07	0.79	0.79	1.00
B, 50 m	0.034	0.016	2.13	0.92	0.90	1.02

which can be considered as constant in shade-adapted cells (Langdon 1988), and the spectrally corrected transport of light-energy to photosystem II ($^{\circ}\bar{F}$). As a consequence, a^B should vary in a linear proportion with $^{\circ}\bar{F}$ (Table 4; Johnsen & Hegseth 1991). Per unit carbon, however, absorption of light ($^{C}\bar{F}$ and $^{C}\bar{a}_c$) by *C. polylepis* is actually a little smaller than in S. costatum in green water while being equal in blue water (Table 4, Fig. 2C). Because growth is related to carbon-normalized parameters, a high value of the chlorophyll-normalized factor $^{\circ}\bar{F}$ can be deceptive in the context of growth rate; i.e. the high $^{\circ}\bar{F}$ of *C. polylepis* is offset by the high chl *a*:C ratio in *S. costatum*. One should bear in mind, however, that to compare with *S. costatum* is to compare with the most successful diatom species in nearshore Norwegian coastal waters and fjords (see Sakshaug & Andresen 1986). In fact, one may conclude that both species are efficient in absorption of blue as well as blue-green light (see Table 4).

Photosynthetic parameters

The photosynthetic parameters in *Chrysochromulina polylepis* indicate relatively high susceptibility to photoinhibition compared to shade-adapted diatoms (Richardson et al. 1983). High susceptibility to strong light, however, was not observed in field samples (Nielsen et al. 1990). They observed assimilation numbers from 0.6 to 6.7 mg C (mg chl *a*)$^{-1}$ h^{-1} for *C. polylepis* obtained from the pycnocline and illuminated at an irradiance corresponding to 2.5 m depth. Such differences in assimilation numbers are likely to be due to difference in incubation technique, temperature, photoadaptation (Sakshaug et al. 1991), or different isolates may be inherently different physiologically.

While shade-adapted *Chrysochromulina polylepis* has a low P_m^B and I_k well below the growth irradiance used in our experiments, in addition to an extremely low value for carbon-normalized maximum carbon uptake ($^{C}P_m$; Table 3), the opposite is true for shade-adapted *S. costatum*. This, and the high susceptibility of *C. polylepis* to photoinhibition, may imply that shade-adapted *C. polylepis*, in contrast to *Skeletonema costatum*, cannot tolerate short periods of strong light well. It may, however, compensate for this by phototaxis. Phototactic compensation is only possible in well-stratified water layers, such as during the bloom of *C. polylepis* in Kattegat/Skagerrak in 1988. Consequently, the 2 species, when shade-adapted, may differ primarily in their response to strong light.

Although not measured here, the growth rate of *Chrysochromulina polylepis* should be low. Our set of photosynthetic coefficients implies a gross growth rate (net rate, μ + respiration rate, r) of 0.46 d^{-1} at 75 µmol m^{-2} s^{-1} and 16 h day length, in contrast to 1.26 d^{-1} for *Skeletonema costatum* according to studies by Sakshaug et al. (1989). Correspondingly, the net growth rate (μ) of *C. polylepis* should be half or less than the value of about 0.8 d^{-1} for *S. costatum*, unless the species differ profoundly in terms of the respiration rate (Sakshaug & Andresen 1986).

CONCLUSIONS

The high efficiency of *Chrysochromulina polylepis* with respect to light harvesting in blue-green to green waters (Table 4) may have played a role in its success during the initial phase of the bloom in the Kattegat/Skagerrak in summer 1988. Moreover, low surface salinity, high surface temperature and the resulting stratification of the upper layers, as well as unusually sunny weather, may presumably have ensured close to ideal conditions for this species (Aksnes et al. 1989, Skjoldal & Dundas 1991). Similar conditions have also favoured another *Chrysochromulina* bloom, i.e. of the toxic *Chrysochromulina leadbeateri* mixed with *C. ericina*, *C. hirta* and peridinin-containing dinoflagellates in the surface layers in the Vestfjord/Tysfjord area in northern Norway in May–June 1991 (Johnsen 1991).

Our calculations indicate that *Skeletonema costatum* would grow faster than *Chrysochromulina polylepis*, particularly in the medium-to-low light near the pycnocline, considering the different carbon-specific photosynthetic efficiencies ($^{C}\alpha$) of the 2 species (Table 3). Because a majority of 'nuisance' species appear to grow slower than many diatoms in comparable light regimes (Brand & Guillard 1981), one might justifiably ask why *S. costatum* and other fast-growing species are absent during some phytoplankton blooms, rather than why *C. polylepis* and related species are successes.

This question, however, cannot be answered in terms of light strategies alone. Initial stocks of competitive species, for instance, may have been too small or absent, and a low supply of silicate may have controlled the diatom stock, at least late in the bloom of *C. polylepis* (Aksnes et al. 1989, Skjoldal & Dundas 1991). Moreover, whereas diatoms such as *Skeletonema* are bound to sink and to be grazed to a considerable extent, *C. polylepis* may stay near the pycnocline due to its motility while not being grazed appreciably due to its toxicity (Nielsen et al. 1990).

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