

# Changes in pigmentation, bio-optical characteristics and photophysiology, during phytoflagellate succession in mesocosms

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*Pigmentation, bio-optical characteristics and photophysiology, were studied in mesocosms with different N:P ratios. No significant difference in biomass or species composition was seen under different nitrogen to phosphorus ratios (N:P), but a temporal succession of different flagellate groups was observed in all mesocosms. An initial bloom of prymnesiophytes containing chlorophyll (Chl) c and 19' hexanoyloxy-fucoanthin (19' HOF) was followed by prasinophytes containing Chl b. Electron microscope analysis confirmed the presence of genera such as Chrysochromulina (Prymnesiophyceae), Tetraselmis and Pyramimonas (Prasinophyceae). Traces of prasinoxanthin in the pigment samples showed that smaller prasinophytes were also present. Chl b influenced the photophysiology of the prasinophytes resulting in higher Chl a-specific absorption, but a greater difference between absorption and scaled fluorescence excitation spectra indicated that light absorbed by Chl b is associated with photosystem I (PSI). Since a larger fraction of the light was absorbed by chlorophyll in PSI and/or photoprotective carotenoids, the light-saturated Chl a-specific rate of photosynthesis ( $P_m^B$ ) and maximum light utilization coefficient ( $\alpha^B$ ) decreased when [Chl b] increased. The highest  $P_m^B$  values were seen when the ratios of fucoxanthins to Chl a were high, indicating that prymnesiophytes might be more efficient in light harvesting and electron transport through photosystem II (PSII) by fucoxanthins and Chl c. Our results therefore indicate different light acclimation strategies in prasinophytes versus prymnesiophytes, which may be reflected in the successional appearance of these communities in the natural environment. We also suggest that grazing by ciliates and rotifers caused periodic decreases in phytoplankton biomass, which in turn gave rise to the phytoflagellate succession observed in the mesocosms.*

## INTRODUCTION

Phytoplankton species succession is a common feature of coastal waters, and the dynamic succession of larger groups such as diatoms and dinoflagellates is described from many ecosystems [e.g. (Sakshaug, 1972)]. The succession of smaller flagellates in natural plankton communities has largely been overlooked mainly due to methodological limitations. Studies of natural phytoplankton communities in mesocosms have become a valuable tool in marine microbial ecology studies. The use of these enclosures makes nutrient manipulation possible at a larger experimental level and can provide a link

between laboratory culture and field work (Duarte *et al.*, 1997; Larsen *et al.*, 2001). Flagellates of the Prasinophyceae (Chlorophyta) and Prymnesiophyceae (Haptophyta) are common in the coastal waters around Scandinavia, as well as in other areas of the global oceans.

The chlorophyll (Chl) *c*-containing prymnesiophytes such as *Chrysochromulina* and *Prymnesium* are well known from laboratory and field work, and it has been shown that they can be toxic [e.g. (Johansson and Granéli, 1999; Johnsen *et al.*, 1999)]. Small and sometimes fragile Chl *b*-containing prasinophytes have been underestimated by microscopic counts (Roy *et al.*, 1996) and cell counts can underestimate prasinophyte abundance

compared with pigment analysis (Buma *et al.*, 1992). There is a lack of information on chlorophytes in marine environments due to the inadequacy of light microscopy (LM) in detecting nanoplankton (Thomsen and Buck, 1998). Chemotaxonomy based on the analysis of different combinations of pigments in phytoplankton is a valuable identification tool and recent research strives to improve chromatographic methods that can separate and identify common pigments in field samples (Wright *et al.*, 1991; Jeffrey *et al.*, 1999; Zapata *et al.*, 2000). 'Type 3' prymnesiophytes such as *Chrysochromulina* spp. are characterized by the marker pigment 19' hexanoyloxyfucoxanthin (19'HOF) and the absence of 19' butanoyloxyfucoxanthin (19'BOF) (Mackey *et al.*, 1996). Several carotenoid markers can be used to identify the different Chl *b*-containing phytoflagellates. Neoxanthin, violaxanthin and  $\beta,\epsilon$ -carotene indicate prasinophytes of the 'group 1 type', where large species such as *Pyramimonas* spp. and *Tetraselmis* spp. are found. Prasinoxanthin is the principal carotenoid in small prasinophytes of 'group 3', where genera such as *Pseudoscurfieldia*, *Micromonas* and *Mantoniella* are included (Egeland, 1996). Cellular pigment concentration in different phytoplankton groups under varying conditions is documented from many culture studies [see (Jeffrey *et al.*, 1997) and references therein].

Photosynthetic parameters of phytoplankton can be species-specific, and are also affected by external variables such as light regime (intensity and spectral quality of irradiance, and day length), temperature and nutrients (Sakshaug *et al.*, 1997). Changes in nutrient concentrations often provoke phytoplankton species succession, which covary with photophysiology and bio-optical adaptations (Sosik and Mitchell, 1991; Bricaud *et al.*, 1995; Sosik and Mitchell, 1995; Lutz *et al.*, 1998; Stuart *et al.*, 2000). The light-limited slope ( $\alpha^B$ ) of the photosynthesis-irradiance relationship is mainly dependent on spectral irradiance (Falkowski, 1981; Sakshaug *et al.*, 1997). Laboratory experiments have also shown that  $\alpha^B$  is influenced by nutrients and can increase under high nutrient conditions and decrease under limiting nutrient conditions (Welschmeyer and Lorenzen, 1981; Cleveland and Perry, 1987). By comparison, the maximum photosynthetic rate  $P_m^B$ , is not spectrally dependent (Pickett and Mayers, 1966) and in the absence of nutrient limitation, the reactions that determine  $P_m^B$  are enzymatically controlled and temperature dependent (Harris, 1978). In the absence of significant changes in temperature, the acclimation of phytoplankton to different light regimes occurs mainly through changes in  $\alpha^B$  and can be either due to physiological adaptation (Perry *et al.*, 1981) or changes in species composition (Gallegos, 1992).

*In vivo* Chl *a*-specific absorption coefficients,  $a_\phi^*(\lambda)$ , (Morel *et al.*, 1987) combined with spectral *in vivo*

fluorescence excitation (Haxo, 1985) give scaled PSII fluorescence,  $F_{PSII}^*(\lambda)$ , (Johnsen *et al.*, 1997). A close match between  $a_\phi^*(\lambda)$  and  $F_{PSII}^*(\lambda)$  indicates that a large fraction of light energy has been transferred to PSII, but scaling of  $F_{PSII}^*(\lambda)$  to  $a_\phi^*(\lambda)$  in field samples is tentative since we lack information on this method of estimating 'true PSII absorption' for most algal groups.

We followed a succession of phytoflagellates and this work presents photophysiological features of Prasinophyceae (Chlorophyta) versus Prymnesiophyceae (Haptophyta) dominated communities, as they appeared in our mesocosms. In this paper we study changes in pigment composition, photosynthetic efficiency and bio-optical characteristics during a phytoflagellate succession in mesocosms with different nutrient additions. We discuss changes in these parameters in relation to nutrients, light and grazing.

## METHOD

### Mesocosm bags and nutrient/Chl *a* analysis

The experiment was performed in Trondheim (Norway), from August 23 to September 8 1999 [experimental days (ED) 1-17] in mesocosms made from white, spectrally neutral polyethylene (150  $\mu\text{m}$  thick,  $\text{O}$  1.08 m, 1.5 m deep, effective volume approx 1 m<sup>3</sup>) placed in an outdoor salt-water basin (500 m<sup>3</sup>). All mesocosms were filled with prefiltered (150  $\mu\text{m}$  mesh size) sea water from the basin, which was nutrient depleted due to an earlier phytoplankton bloom since water circulation in the basin had been cut off for a period of nearly two months. On the first day of the experiment we inoculated the mesocosms with a natural community dominated by flagellates.

At the time of inoculation [N] and [P] were close to 0 mmol m<sup>-3</sup>, [Si] 0.7 mmol m<sup>-3</sup>, salinity 33.14  $\pm$  0.08 (SD) p.s.u. and Chl *a* concentration ([Chl *a*]) 0.35  $\mu\text{g L}^{-1}$ . Temperature in the mesocosms varied from 13 to 14°C during the experiment. One mesocosm was used as control and received no treatment (Bag 5), and a total of 10 mesocosms were enriched with different levels of nitrogen (as NO<sub>3</sub>-N) and phosphorus (as PO<sub>4</sub>-P). Different nutrient loadings did not induce any significant variation in biomass levels or species composition, and a selection of mesocosms representing the nutrient loading categories 'low N:P', 'Redfield ratio' and 'high N:P' were included here. The selected mesocosms had the following N:P ratios: Bag 1, 1.6; Bag 2, 16; Bag 3, 160; and Bag 4, 320. The water in all mesocosms was mixed carefully with a plastic disc on a rod every morning before sampling, and again when nutrients were added at the end of the day. Water for nutrient (NO<sub>3</sub>, NO<sub>2</sub>, PO<sub>4</sub>, Si) and [Chl *a*] analysis was sampled every day at 10 a.m., and analysed in a Skalar

'San plus Systems' autoanalyser, using the standard methods provided with the instrument. The difference between the daily measured levels of N and P in the mesocosms, and the desired nutrient levels for mesocosms 1–4 (as N:P) was calculated, and nutrients were added every evening to replenish levels in all mesocosms except for the control. Three replicate samples of [Chl *a*] were filtered onto GF/F and extracted in 100% methanol for at least 4 h at 4°C in darkness. Optical density (OD, = absorbance) of the refiltered sample was measured on a Hitachi 150–20 (dual-beam) spectrophotometer, with methanol as reference. [Chl *a*] was calculated from readings at 665 nm, using an extinction coefficient of 74.5 L g<sup>-1</sup> cm<sup>-1</sup> (Mackinney, 1941). The coefficients of variation for Chl *a* samples were 0–2.6%.

### Phytoplankton and heterotrophic carbon analysis

Phytoplankton identification and enumeration on samples fixed with Lugol's iodine were performed with an inverted light microscope. Prasinophycean and prymnesiophycean cells (and possibly other cells with similar morphology and size) were grouped together as 'flagellates'. Further identification of flagellates was performed on sub-samples preserved with glutaraldehyde and analysed by scanning electron microscopy (SEM).

Duplicate samples for measurements of particulate organic carbon and nitrogen (POC and PON) were analysed on a Fisons Instruments autoanalyser, model NA 1500 NC. Regression analysis of POC versus [Chl *a*] gave an intercept of 168.72 µg L<sup>-1</sup> ( $P \ll 0.01$ ) which suggests non-autotrophic and detrital particulate carbon (Sakshaug *et al.*, 1983). Heterotrophic particulate carbon (POC<sub>Het</sub>) was calculated from the number of heterotrophic dinoflagellates, ciliates and rotifers present, and their estimated biovolumes, using the volume:C conversion factors of Menden-Deuer and Lessard (Menden-Deuer and Lessard, 2000). The carbon content of rotifers was calculated using a conversion factor of 96 ng C ind<sup>-1</sup>, which was based on a simplified calculation of volume ( $V = 0.12 \times \text{length}^3$ , average body length 200 µm) and a volume:C correction factor of 0.1 pg C µm<sup>-3</sup> (Bottrell *et al.*, 1976). The sum of POC<sub>Het</sub> was subtracted from total POC.

### Pigment analysis

Water samples of 600–1500 ml for HPLC analysis were filtered onto GF/F and frozen at -20°C, and prior to analysis the filters were immersed in 2 ml of 7:3 acetone/methanol and mixed on a Vortex table mixer before overnight extraction in the dark. Samples were analysed on a Hewlett-Packard Series 1100 HPLC with reversed phased C18 column and eluents as follows [(Egeland *et al.*, 1995) with some modifications]: 0 min 3:7 1 M ammonium acetate in water:methanol, 15 min

7:3 methanol:acetone, 25 min 3:5:2 methanol:acetone:hexane, 27.5 min 4:6 acetone:hexane, 30 min pure methanol, 32.5 min 3:7 1 M ammonium acetate in water:methanol.

### Spectral absorption and fluorescence

For comparison of *in vivo* spectral absorption and fluorescence, the optical density (OD) of live cells collected on GF/F was measured on a Hitachi 150–20 (dual-beam) spectrophotometer with a clean filter wetted with filtered sea water as a reference. Three replicate spectra were measured from 350 to 800 nm, and the average OD from 750 to 800 nm was subtracted from the whole spectrum to correct for light scattering. OD on filters (OD<sub>f</sub>) was converted to OD in suspension (OD<sub>s</sub>) with a second order polynomial expression (Mitchell, 1990):

$$\text{OD}_s = m_1 \times \text{OD}_f + m_2 \times (\text{OD}_f)^2 \quad (1)$$

The increased path length of light in glass fibre filters and algal material was corrected with β-correction factors 'm1' and 'm2' from laboratory cultures; m1 = 0.570 and m2 = 0.236 from *Tetraselmis* sp. were used to correct spectra from ED 12, and m1 = 0.508 and m2 = 0.134 from *Prymnesium* sp. were used for spectra from ED 8 and 17. Absorption ( $a$ , m<sup>-1</sup>) was calculated from OD<sub>s</sub>:

$$a = (2.3 \times \text{OD}_s) \times (S/V) \quad (2)$$

where  $S$  is the clearance area of the GF/F filter (mm<sup>2</sup>) and  $V$  is the volume (ml) of the filtered sample (Mitchell and Kiefer, 1984). Correction for absorption by detritus was performed numerically (Bricaud and Stramski, 1990), and the resulting spectra  $a_i(\lambda)$  were subtracted from the absorption spectra before normalization to spectrophotometrically derived [Chl *a*], to give  $a_\phi^*(\lambda)$ .

*In vivo* fluorescence excitation was measured on concentrated samples in suspension after addition of DCMU (final concentration 50 µM). Measurements were performed on a Hitachi F-3000 spectrofluorometer, equipped with a Schott IR 695 filter in front of the photonmultiplier. Excitation scans were made from 350 to 700 nm and emission was monitored at 730 nm (Neori *et al.*, 1988), using 5 nm bandwidth on both excitation and emission light sources. The average of two replicate spectra was quantum-corrected with a spectrum of the dye Basic Blue 3 (Kopf and Heinze, 1984). Quantum corrected spectra were re-scaled to  $a_\phi^*(\lambda)$  in the lowest absorption area, to give scaled fluorescence spectra [ $F_{PSII}^*(\lambda)$ ] with no overshoot.

### P-E experiments

P-E experiments were conducted in linear incubators illuminated at the front by 50 W, 12 V tungsten halogen

lamps [see (Arbones *et al.*, 2000) for details of incubator design] maintained at 13–14°C using a temperature controller.  $E_{PAR}$  (see Table I for explanations) at the position of each incubation bottle was measured using a Li-Cor cosine sensor LI-190SA, and the flask at the end of the incubator was covered with aluminium foil and used to measure dark  $^{14}C$  fixation. After 2–3 h of incubation, the suspended material was filtered through 25 mm Whatman GF/F filters and exposed to concentrated HCl fumes for 12 h, before  $^{14}C$  disintegration per minute on the filters was determined on a Packard Tri-Carb 2500 TR liquid scintillation analyser using the external standard and the channel ratio methods to correct for quenching.

$P_m^B$  and  $\alpha^B$  were estimated by fitting the data to the model of Webb *et al.* (Webb *et al.*, 1974) since no photo-inhibition was observed:

$$P^B = P_m^B [1 - \exp(-\alpha^B \cdot E_{PAR}/P_m^B)] \quad (3)$$

The spectral quality of the incident light did not change along the incubators so:

$$E_{incubator}(\lambda) = E(\lambda) \cdot E_{PAR} \quad (4)$$

$E_{PUR}$  at each position in the incubator and for each sampling depth was estimated according to Dubinsky (Dubinsky, 1980):

$$E_{PUR} = \int_{400}^{700} a_{\phi}^*(\lambda) \cdot E_{incubator}(\lambda) d\lambda \quad (5)$$

$\phi_m$  was determined by fitting  $P^B$  to  $E_{PUR}$ :

$$P^B = P_m^B [1 - \exp(-\alpha_{PUR}^B \cdot E_{PUR}/P_m^B)] \quad (6)$$

and

$$\phi_m = 0.0231 \cdot [Chl a] \cdot \alpha_{PUR}^B \quad (7)$$

The factor 0.0231 converts milligrams of carbon to moles,  $\mu\text{mol}$  of photons to moles and hours to seconds. The light saturation parameter for light absorbed by phytoplankton is  $E_k = P_m^B/\alpha_{PUR}^B$  ( $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ).

## RESULTS

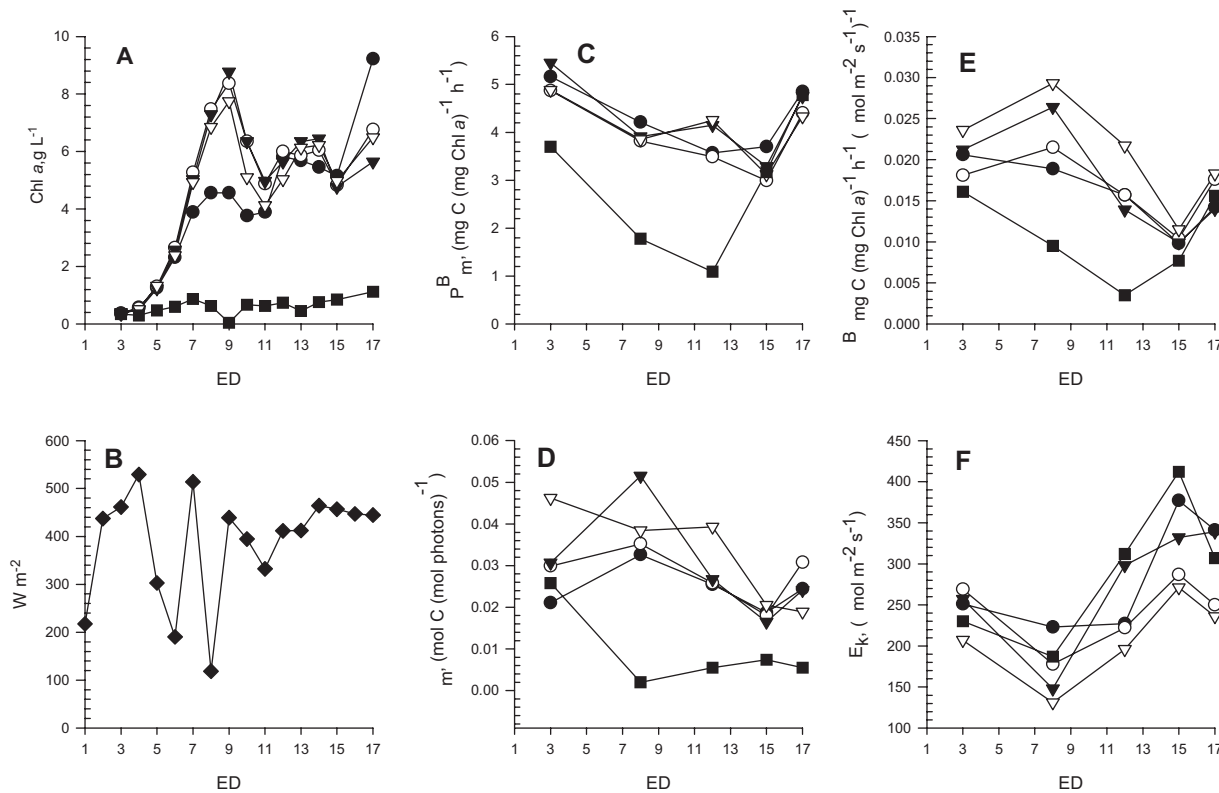
[Chl *a*] increased exponentially from ED 3 in the experimental mesocosms 1–4 (Figure 1A, Table II) reaching nearly  $8 \mu\text{g L}^{-1}$  in mesocosms 2, 3 and 4, and then decreasing from ED 9 to  $<5 \mu\text{g Chl } a \text{ L}^{-1}$  on ED 11 followed by an increase a few days later. On ED 17 [Chl *a*] was highest ( $9.2 \mu\text{g L}^{-1}$ ) in mesocosm 1. In the control Bag 5, [Chl *a*] was below  $1 \mu\text{g L}^{-1}$  until the last day of the experiment, ED 17.

Pyranometric measurements from the experimental period showed that total insolation above sea level on clear days was as high as  $530 \text{ W m}^{-2}$  (at 12:00 on the August 26) and the whole experimental period was characterized by clear skies and high levels of insolation (Figure 1B). Maximum PAR at the sea surface was approximately  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the light regime was similar in all bags. For example in mesocosm 2 on ED 12: irradiance at the water surface was  $\sim 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and at the bottom of the bag

Table I: Symbols, units and definitions used in connection with determination of photosynthetic parameters, and bio-optical measurements

Symbol	Units	Explanation
$a$	$\text{m}^{-1}$	Absorption coefficient
$a_{\phi}^*(\lambda)$	$\text{m}^2 (\text{mg Chl } a)^{-1}$	Chlorophyll <i>a</i> -specific absorption of phytoplankton, 400–700 nm
$\alpha^B$	$\text{mg C } (\text{mg Chl } a)^{-1} \text{ h}^{-1} (\mu\text{mol m}^{-2} \text{s}^{-1})^{-1}$	Maximum light utilization coefficient
$E_k$	$\mu\text{mol quanta m}^{-2} \text{s}^{-1}$	Light saturation parameter
$E(\lambda)$	$E_{400-700 \text{ nm}} = 1 \mu\text{mol m}^{-2} \text{s}^{-1}$	Relative mean spectrum of the tungsten-halogen lamps
$E_{PAR}$	$\mu\text{mol quanta m}^{-2} \text{s}^{-1}$	Photosynthetically available radiation, 400–700 nm
$E_{PUR}$	$\mu\text{mol m}^{-3} \text{s}^{-1}$	Photosynthetically usable radiation, 400–700 nm
$E_{incubator}(\lambda)$	$\mu\text{mol quanta m}^{-2} \text{s}^{-1}$	Spectral irradiance at each location in the incubator
$F_{PSII}^*(\lambda)$	$\text{m}^2 (\text{mg Chl } a)^{-1}$	PSII-scaled fluorescence excitation spectrum, 400–700 nm
OD, OD <sub>f</sub> , OD <sub>s</sub>	(dimensionless)	Optical density, OD on filters, OD in suspension
$P_m^B$	$\text{mg C } (\text{mg Chl } a)^{-1} \text{ h}^{-1}$	Light-saturated Chl <i>a</i> -specific rate of photosynthesis
$P^B$	$\text{mg C } (\text{mg Chl } a)^{-1} \text{ h}^{-1}$	Chlorophyll-specific rate of photosynthesis
$\phi_m$	$\text{mol C } (\text{mol photons})^{-1}$	Maximum quantum yield of carbon fixation

For further details see Sakshaug *et al.* (Sakshaug *et al.*, 1997).



**Fig. 1.** (A) Biomass as [Chl *a*] in mesocosms during the experimental period. (B) Pyranometric measurements of insolation, daily maximum. (C) Light-saturated Chl *a*-specific rate of photosynthesis ( $P_m^B$ ). (D) Maximum quantum yield,  $\phi_m$ . (E) Maximum light utilization coefficient ( $\alpha^\beta$ ). (F) Light saturation parameter,  $E_k$ . Symbols in all figures (except B): filled circle, mesocosm 1; open circle, mesocosm 2; filled triangle, mesocosm 3; open triangle, mesocosm 4; filled square, mesocosm 5 (control bag).

$>300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and [Chl *a*] was  $6 \mu\text{g L}^{-1}$  (Table II), indicating that light was not a limiting factor.

Diatom numbers were low until ED 12 and then increased in all mesocosms (Table II). The dominant species were *Pseudo-nitzschia* sp. and *Cylindrotheca closterium*. Highest dinoflagellate numbers were found on ED 8 in mesocosms 1, 3 and the control mesocosm 5, when cells from Gymnodiniales and Gonyaulaceae, and *Heterocapsa triquetra* were the most abundant. *Ceratium* spp., *Prorocentrum micans* and gonyaulaceans were dominant at the end of the experiment. Flagellate cell counts peaked on ED 8 and were the most dominant phytoplankton group during the whole experiment. Electron microscope analysis of selected samples showed the presence of prymnesiophytes (*Chrysochromulina* spp.) and prasinophytes (possibly *Pyramimonas* sp. and *Tetraselmis* sp.). Cryptophyte standing stocks increased strongly during the first exponential growth phase between ED 3–9, when they reached the highest densities ( $1.1$  and  $2.6 \times 10^5 \text{ cells L}^{-1}$ ) and subsequently disappeared from all mesocosms (Table II).

The number of small ciliates ( $<20 \mu\text{m}$ ) was initially low ( $200\text{--}400 \text{ L}^{-1}$ ), and increased in mesocosms 1, 3 and 4 towards the end of the experimental period (up to

$74\,000 \text{ L}^{-1}$  in mesocosm 3 on ED 17), while in mesocosm 2 no small ciliates were found on ED 17. The number of larger ciliates ( $20\text{--}50 \mu\text{m}$ ) was initially high ( $12\,000 \text{ L}^{-1}$ ), then decreased towards the middle period of the experiment and increased towards the end of the experiment in all mesocosms except mesocosm 1 where no ciliates in this size class were detected in the final sampling. High numbers of rotifers were found in some samples, such as mesocosm 2 on ED 12. The ratios of heterotrophic carbon ( $\text{POC}_{\text{Het}}$ ) to total carbon (POC) varied from 0.01 to 0.57 (w:w, Table II). Highest ratios were seen on ED 12, and were related to an increasing numbers of ciliates and in some cases high numbers of rotifers.

Chl *b*:Chl *a* and Chl  $c_1+c_2$ :Chl *a* ratios were similar in the experimental mesocosms on each sampling day, but varied with time. Chl *b*:Chl *a* ratio doubled from ED 8 to ED 12, and decreased to less than 0.1 on ED 17 (Table II). Chl  $c_1+c_2$ :Chl *a* was lowest on ED 12, when Chl *c* was  $<7\%$  of [Chl *a*]. A nonpolar Chl *c* (Garrido *et al.*, 2000) was found in all four experimental mesocosms on ED 17. On ED 8, 19'HOF was high whereas fucoxanthin levels were low in all mesocosms. On ED 12 data were only available for mesocosms 2 and 3 and

Table II: Cell numbers, [Chl *a*], accessory pigments, organic carbon and photosynthetic parameters

Mesocosm	1			2			3			4			5		
N:P	1.6			16			160			320			(control)		
Experimental day	8	12	17	8	12	17	8	12	17	8	12	17	8	12	17
Flagellates ( $\times 10^6$ L <sup>-1</sup> )	6.39	2.14	8.78	8.17	1.72	10.85	15.48	4.7	5.75	4.6	0.8	10.44	0.45	0.56	4.04
Diatoms ( $\times 10^3$ L <sup>-1</sup> )	0.75	4.44	96.4	2.16	6.65	82.1	3.24	4.5	37.4	0.53	9.54	44.98	0.09	4.98	
Dinofl. ( $\times 10^3$ L <sup>-1</sup> )	156.8	40.5	11.88	32.6	48.38	12.34	168.2	2.34	10.84	29.37	56.31	7.34	248	0.7	0.94
[Chl <i>a</i> ], ( $\mu$ g L <sup>-1</sup> ) <sup>a</sup>	4.56	5.82	9.22	7.47	6.00	6.76	7.26	5.64	5.64	6.85	5.03	6.49	0.63	0.74	1.12
19'HOF:Chl <i>a</i> (w:w)	0.32		0.25	0.47	0.13	0.46	0.41	0.10	0.30	0.37		0.30			<sup>b</sup>
Fuco:Chl <i>a</i>	0.07		0.33	0.08	0.10	0.43	0.07	0.12	0.26	0.07		0.33	0.10	<sup>b</sup>	0.46
Chl <i>b</i> :Chl <i>a</i>	0.30	0.60	0.07	0.22	0.41	0.09	0.22	0.49	0.08	0.25	0.49	0.06		<sup>b</sup>	
Chl <i>c</i> <sub>1+2</sub> :Chl <i>a</i>	0.04	0.03	0.07	0.04	0.03	0.07	0.04	0.03	0.04	0.04	0.03	0.06	0.08	<sup>b</sup>	
POC, $\mu$ g L <sup>-1</sup>	358.4	523.0	595.5	433.4	975.0	590.4	443.5	585.3	647.2	538.5	580.9	730.9	177.2	195.3	279.7
(STD, <i>n</i> = 2)	(33.8)	(7.6)	(43.7)	(78.5)	(133.3)	(19.3)	(24.4)	(51.2)	(21.4)	(33.5)	(43.6)	(109.5)	(18.2)	(5.0)	(0.6)
POC <sub>Het</sub> ( $\mu$ g L <sup>-1</sup> )	13.7	231.4	30.8	5.3	553.7	44.2	13.2	221.2	70.6	6.3	229.3	47.9	18.2	63.8	36.3
Chl <i>a</i> :C (w:w) <sup>c</sup>	0.013	0.020	0.016	0.017	0.014	0.012	0.017	0.015	0.010	0.013	0.014	0.010	0.004	0.006	0.005
$a_{\phi}^*(675)$ (m <sup>2</sup> mg Chl <i>a</i> <sup>-1</sup> )	0.024	0.029 <sup>d</sup>	0.021 <sup>e</sup>	0.022	0.027	0.021	0.023	0.025	0.021	0.023 <sup>e</sup>	0.025 <sup>d</sup>	0.021			
$\alpha^{B,f}$	0.019	0.016	0.014	0.022	0.016	0.018	0.026	0.014	0.014	0.029	0.022	0.018	0.010	0.004	0.016
$P_m^{B,g}$	4.21	3.57	4.85	3.82	3.49	4.40	3.92	4.15	4.75	3.85	4.25	4.33	1.78	1.09	4.78
$E_k$ ( $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )	223	227	341	178	222	250	148	298	339	131	196	236	187	312	307

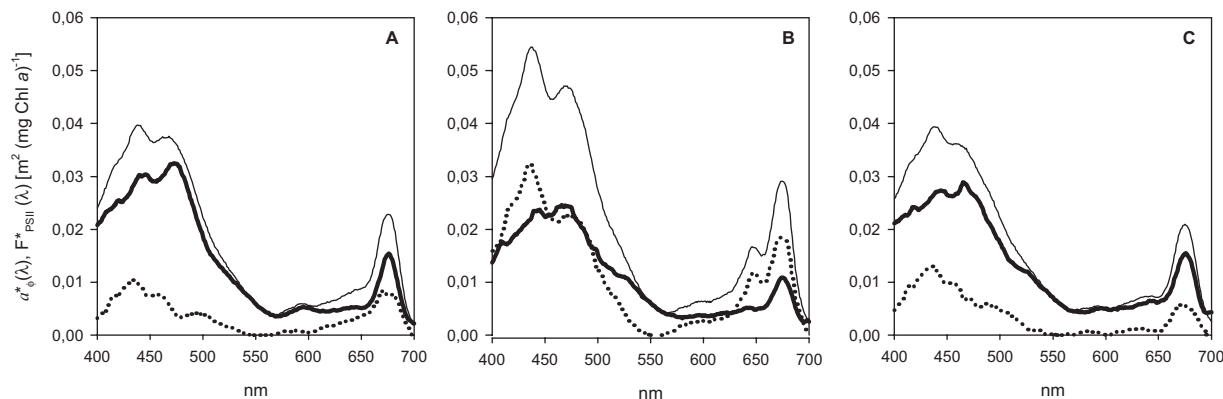
Chl *a* (HPLC), particulate organic carbon (POC) and standard deviation, heterotrophic C (POC<sub>Het</sub>), ratio of Chl *a*:C, Chl *a* specific light absorption coefficients [ $a_{\phi}^*(675)$ ] at red peak, maximum light utilization coefficient ( $\alpha^B$ ), light-saturated Chl *a*-specific rate of photosynthesis ( $P_m^B$ ), and light saturation parameter ( $E_k$ ). Superscripts denote <sup>a</sup>measured spectrophotometrically, <sup>b</sup>HPLC sample missing, <sup>c</sup>C = POC-POC<sub>Het</sub>, <sup>d</sup>674 nm, <sup>e</sup>676 nm, <sup>f</sup>[mg C (mg Chl *a*)<sup>-1</sup> h<sup>-1</sup> ( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], <sup>g</sup>[mg C (mg Chl *a*)<sup>-1</sup> h<sup>-1</sup>].

19'HOF:Chl *a* and fucoxanthin:Chl *a* levels were almost equal. On ED 17 in mesocosm 2 the proportion of light harvesting carotenoids increased to 50% of the Chl *a* content (Table II). Our analysis also showed the presence of prasinoxanthin in the samples from day 12. Violaxanthin was found in samples from ED 8 and 17. Traces of alloxanthin were found in all experimental mesocosms on ED 8, and in mesocosms 2 and 3 on the last sampling day ED 17.  $\epsilon,\epsilon$ -carotene was found in samples from ED 12.

There were no significant differences in the photosynthetic parameters  $P_m^B$ ,  $\alpha^B$  and  $E_k$  between treatments, instead a temporal variation was observed (Figure 1C,E,F). On ED 8, 12 and 17  $\alpha^B$  varied from 0.014 to 0.029 mg C (mg Chl *a*)<sup>-1</sup> h<sup>-1</sup> ( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>,  $P_m^B$  from 3.5 to 4.9 mg C (mg Chl *a*)<sup>-1</sup> h<sup>-1</sup>, and  $E_k$  from 131 to 341  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> (Table II). Data on photosynthetic parameters from ED 3 and 15 have been included in statistical tests, and  $P_m^B$  was significantly higher on ED 3 compared with the other days ( $F_{4, 19} = 23.16$ ,  $P < 0.001$ ) when mean  $P_m^B$  was 5.1 mg C (mg Chl *a*)<sup>-1</sup> h<sup>-1</sup>.  $\alpha^B$  values for all mesocosms were significantly higher on ED 8 ( $F_{4, 19} = 12.06$ ,  $P < 0.001$ ) when mean  $\alpha^B$  was 0.024 mg C (mg Chl *a*)<sup>-1</sup> h<sup>-1</sup> ( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>.  $E_k$  was significantly higher on ED 15 ( $F_{4, 19} = 6.6$ ,  $P = 0.0028$ ; mean  $E_k$  317  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). In the control mesocosm,

$P_m^B$  and  $\alpha^B$  were lower until ED 15 when they reached the same values as in the other experimental mesocosms.  $\phi_m$  varied from 0.017 to 0.052 mol C (mol photons)<sup>-1</sup> in experimental mesocosms, and between 0.002 and 0.026 mol C (mol photons)<sup>-1</sup> in the control mesocosm (Figure 1D).

Chl *a*-specific absorption coefficients  $a_{\phi}^*(\lambda)$  were similar on each sampling day but varied as a function of time (Table II). One typical spectrum of each treatment is shown in Figure 2. Spectra from ED 8 showed increased absorption around 460–470 nm due to fucoxanthins and Chl *b*, 590 and 645 nm (Chl *c*'s and Chl *b*), as well as the Chl *a* peaks at 440 and 675 nm (Figure 2A). The distinct peaks at 468 and 646 nm seen on the spectrum from ED 12 are typical of Chl *b* absorption (Figure 2B). The spectrum on ED 17 showed that the phytoplankton community was again dominated by Chl *c*/fucoxanthin-containing algae in all mesocosms, and the signal from Chl *b* was absent (Figure 2C). Scaled fluorescence excitation spectra  $F_{PSII}^*(\lambda)$  showed distinct differences during the experimental period. On ED 8  $a_{\phi}^*(\lambda)$  and  $F_{PSII}^*(\lambda)$  were similar in shape especially between 480 and 600 nm (Figure 2A), while on ED 12 the spectra were markedly different and the difference spectrum was similar to an *in vivo* Chl *a*-Chl *b* PSI absorption spectrum (Figure 2B). On the last sampling day the spectra were



**Fig. 2.** Chl *a* specific absorption coefficients,  $a_{\phi}^*(\lambda)$  (thin, black line), scaled fluorescence excitation,  $F_{PSII}^*(\lambda)$  (thick, black line) and difference (dotted, black line) spectra. **(A)** Sample from mesocosm 6 on ED 8. **(B)** Sample from mesocosm 2 on ED 12. **(C)** Sample from mesocosm 4 on ED 17.

similar to those on ED 8, when  $a_{\phi}^*(\lambda)$  and  $F_{PSII}^*(\lambda)$  converged between 480 and 650 nm (Figure 2C).

## DISCUSSION

Different nutrient loadings did not induce differences in phytoplankton communities or biomass. There was an initial exponential increase in biomass ([Chl *a*] and cell numbers) in the experimental mesocosms, followed by a period of oscillating biomass levels (Figure 1A and Table II). Nutrient concentrations in the experimental mesocosms were not limiting (Guildford and Hecky, 2000), but the N:P ratios were far from optimal for phytoplankton growth especially for mesocosms 1, 3 and 4 which varied from 1.6 or >160. The fact that nutrients were not the limiting factor in most of our mesocosms is consistent with the similarity in species composition and biomass levels between mesocosms, since nutrient manipulation is known to cause structural changes in phytoplankton communities (Samuelsson *et al.*, 2002). Temperature did not exert a strong effect since it only varied between 13 and 14°C in all mesocosms.

Light is another important regulating factor for autotrophic organisms, and pyranometric measurements from the period showed that irradiance levels were high during the experimental period (Figure 1B). Both bio-optical measurements and photophysiology confirmed high light acclimation of cells (Figure 1C–F and Table II), while low amounts of photoprotective pigments such as zeaxanthin, diadinoxanthin and lutein indicated that irradiance levels were not inhibiting photosynthesis in the mesocosms (data not shown). Both sun height and day length were significantly reduced at this latitude (63°N) at this time of the year, and the duration of high irradiance was reduced on a daily basis.

There were no significant differences in the photosynthetic parameters  $P_m^B$ ,  $\alpha^B$  and  $E_k$  between N and P treatments, but there was a significant variation over time (Figure 1C–F). The observed changes in  $P_m^B$  and  $\alpha^B$  are possibly the result of light acclimation of phytoplankton to the light regime in each mesocosm, which varied as the biomass levels fluctuated. However, on ED 8 at the first biomass peak the [Chl *a*] in the nutrient-replete mesocosms 2–4 were nearly twice as high as in the low N:P mesocosm 1 (Figure 1A, Table II), and a difference in photosynthetic parameters due to increased attenuation of downwelling light could be expected. No significant difference in  $P_m^B$  and  $\alpha^B$  was found between mesocosms on ED 8, and change in these parameters over time covaried with phytoplankton succession.  $P_m^B$  was significantly higher on ED 3 when both 19'HOF and Chl *b*:Chl *a* were high, and then decreased on ED 12 when [Chl *b*] increased and increased when [fucoxanthins] increased on ED 17. There was a negative covariation between both  $P_m^B$  and  $\alpha^B$ , and Chl *b*. This indicated that although Chl *b* contributes to phytoplankton light absorption it does not increase the photosynthetic rate any further. High  $\alpha^B$  values are previously reported from works on small cells (Stuart *et al.*, 2000) and Johnsen (Johnsen, 1994) found  $\alpha^B$  between 0.01 and 0.029 mg C (mg Chl *a*)<sup>-1</sup> h<sup>-1</sup> ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )<sup>-1</sup> in diatoms and prymnesiophytes under different light adaptation, which agrees well with our results since higher  $\alpha^B$  was seen when Chl *c* and fucoxanthins were the main light harvesters.

Whilst the increase in [Chl *b*] also increased  $a_{\phi}^*(\lambda)$  it caused a decrease in  $F_{PSII}^*(\lambda)$  and photosynthesis, and  $a_{\phi}^*(\lambda)$  and  $F_{PSII}^*(\lambda)$  varied as a function of the dominant algae groups in the three biomass peaks. In mesocosm 1 on ED 12 the difference between  $a_{\phi}^*(\lambda)$  and  $F_{PSII}^*(\lambda)$  indicated that approximately 60% of the cellular Chl *a* was associated with PSI, which implies that a significant fraction of the absorbed light was not reaching PSII

(Figure 2B). The shape of the difference spectrum revealed a signal very similar to Chl *a* + Chl *b* absorption, and a large fraction of Chl *a* and Chl *b* is bound to PSI in some prasinophytes (Smith and Alberte, 1991). By comparison, both Chl *c* and fucoxanthins are known to be efficient light harvesting pigments and are tightly connected with PSII (Owens, 1986; Lichtle *et al.*, 1995). This is reflected in the similarity of  $a_{\phi}^*(\lambda)$  and  $F_{PSII}^*(\lambda)$  spectra in the first and last biomass peaks (ED 8 and 17, Figure 2A and C).

Chl *a*-specific absorption in the red peak [ $a_{\phi}^*(675)$ ] varied from 0.021 to 0.029 m<sup>2</sup> mg Chl *a*<sup>-1</sup> during the experimental period. Values are related to cellular pigment composition and higher values indicate lower pigment packaging, which is typical of small cells. This is consistent with the cell counts and pigment analysis, which confirmed the dominance of flagellates in the mesocosms. Highest  $a_{\phi}^*(675)$  values were found in mesocosm 2 on ED12, when [Chl *b*] was high, and a small shift in the peak towards 674 nm was seen when [Chl *b*] was higher (Table II). Red peak shift to lower wavelength in small prasinophytes has been observed in other studies (Johnsen, unpublished results).

Some diatoms were found in the mesocosms, and their numbers increased towards the end of the experimental period (Table II) even though the mesocosms contained very little Si at the beginning of the experiment (0.7 mmol m<sup>-3</sup>, measured at the time of inoculation) and no Si was added. Paasche (Paasche, 1973) reported half saturation constants down to 0.8 µg at Si L<sup>-1</sup> for *Skeletonema costatum*, so growth of diatom cells on remineralized Si is a possibility in our experiment. Diatom cell numbers were more than twice as high in the P-rich mesocosms 1 and 2, than in mesocosms 3 and 4 where [P] was <1 mmol m<sup>-3</sup>. This has also been observed in previous studies where diatoms were found to be poor competitors at low phosphate concentrations (Egge, 1998).

Growth of phytoplankton standing stocks in the mesocosms followed a distinct pattern with an initial exponential development, then a sharp decrease before a period of oscillating biomass. A possible explanation for the turnover in phytoplankton communities could be grazing, since nutrients were added throughout the experiment and temperature and light conditions were not varying. Both ciliates and rotifers were seen in the mesocosms in sometimes high numbers, and grazing may explain the dynamic rise and fall of phytoplankton stocks in the mesocosms (Figure 1A). Larger ciliates were present at the start, and the number of small ciliates increased during the experimental period. High numbers of rotifers were also seen around ED 12 and probably also contributed to the sharp decrease in

phytoplankton biomass. Increasing grazer populations towards the middle of our experiment were also confirmed by high ratios of POC<sub>Het</sub>:POC, which on ED 12 were between 0.38 and 0.57 in experimental mesocosms. During the previous period (ED 8) and the final stage of the experiment (ED 17) this ratio was 0.11 or lower (Table II). The sharp decrease in heterotrophic standing stock could be related to a change in available food when phytoplankton communities changed, since many grazers depend on certain particle sizes to feed efficiently (Gismervik *et al.*, 1996). Nutrient enrichment increased the microplankton biomass and lengthened the food chain to include ciliates, while nutrient depletion resulted in lower biomass and a change towards pico- and nanoplankton, which in turn kept ciliate populations low (Samuelsson *et al.*, 2002). Thus the initial exponential increase in Chl *c*-containing flagellates in our mesocosms, followed by diatoms, could have supported an increasing population of ciliates before the change towards smaller Chl *b*-containing flagellates made ciliate feeding difficult and subsequently reduced the grazing by ciliates on phytoplankton.

## CONCLUSION

The effect of changes in phytoplankton community structure was greater than the effect of nutrient treatments on variations in bio-optical and photo-physiological characteristics of the phytoflagellate bloom. Grazing by ciliates and rotifers is suggested to cause the reductions in biomass levels and changes in community structure. The presence of Chl *b*-containing cells affected the photophysiology as seen by higher Chl *a*-specific absorption, a greater difference between  $a_{\phi}^*(\lambda)$  and scaled  $F_{PSII}^*(\lambda)$ , and correspondingly lower  $\alpha^B$  values. This indicated that light absorbed by Chl *b* is associated with PSI in the prasinophytes. The highest  $P_m^B$  values were seen when diatom and flagellate numbers increased towards the end of our experiment, and the Chl *c*<sub>1</sub>+*c*<sub>2</sub> and fucoxanthin to Chl *a* ratios were high. This indicated that diatoms and prymnesiophytes may be more efficient at light harvesting and transport to PSII by fucoxanthins and Chl *c*, as long as light is not limiting. Our results therefore point towards different light acclimation strategies in chromophytes and chlorophytes, which is reflected in their succession also in the natural environment.

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