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Patterns of carbon and nitrogen uptake during blooms of *Emiliania huxleyi* in two Norwegian fjords

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Abstract. Blooms of the coccolithophorid *Emiliania huxleyi* were monitored in two land-locked fjords, Fauskangerpollen and Nordåsvannet (Western Norway), in May 1993. The chemical composition of particulate matter, size-fractionated photosynthesis, calcification, nitrogen uptake rates and the patterns of macromolecular synthesis were examined during the peak and decline of *E.huxleyi* blooms. The temporal evolution of the bloom in Fauskangerpollen was defined by a gradual decrease in cell abundance and cell-specific calcification rates, and by increasing numbers of empty coccospheres and the ratio detached coccoliths/living cells. A large proportion of the nitrogen required for microplankton growth was supplied by ammonium and dissolved organic compounds such as urea and, as a consequence, the f-ratios were low (~0.2). In general, nitrogen uptake patterns were consistent with ambient concentrations of nitrogenous species. The photosynthetic carbon metabolism of *E.huxleyi*-dominated phytoplankton assemblages was characterized by high carbon allocation into lipids and relatively low carbon incorporation into protein as compared with diatom-dominated assemblages. Consequently, the organic C/nitrogen uptake ratio determined stoichiometrically was significantly higher (up to 10.8) when coccolithophorids were dominant than in diatom-based or mixed-phytoplankton assemblages. These carbon incorporation patterns were reflected in differences in the chemical composition of particulate matter.

Introduction

The coccolithophorid *Emiliania huxleyi* (Lohm.) Hay et Mohler is a species known to form extensive blooms in coastal and oceanic environments (e.g. Holligan *et al.*, 1983, 1993; Balch *et al.*, 1991; Van der Val *et al.*, 1995). Recent investigations have stressed the significance of this species in the biogeochemical cycling of carbon (Holligan *et al.*, 1993; Robertson *et al.*, 1994) and sulphur (Malin *et al.*, 1993; Matrai and Keller, 1993), derived from its capability of synthesizing external structures of calcium carbonate (coccoliths) and considerable amounts of the climate-related volatile compound dimethyl sulfide.

In spite of the increasing efforts in recent years devoted to the investigation of *E.huxleyi* bloom dynamics, knowledge on the temporal evolution of these blooms under natural conditions is still limited. It has been shown that at the time of being detected by remote sensing, they are in a declining or even collapsing stage (Fernández *et al.*, 1993). Moreover, little is known about the basic ecophysiological characteristics of these populations, such as the patterns of carbon and nitrogen assimilation under sea-truth conditions (see, for example, Balch *et al.*, 1992; Fernández *et al.*, 1993).

Emiliania huxleyi blooms succeeding the spring diatom bloom are known to be

recurrent features in Norwegian fjords (e.g. Braarud *et al.*, 1974; Erga, 1989) and have been reported since early this century (Gran, 1912). These blooms are sometimes restricted to subsurface waters underneath a brackish surface layer (Kristiansen *et al.*, 1994b), but discoloration of surface waters due to extremely high densities of this organism is also quite common (Berge, 1962).

Fjords, and particularly land-locked fjords, are excellent locations for studying the dynamics of *E.huxleyi* populations because it is possible to monitor almost unaltered water bodies during time scales relevant for the evolution of phytoplankton blooms. This singular characteristic is even more pronounced when these blooms take place in subsurface layers as exchange processes between land-locked and the main fjord are then more restricted (Gaarder and Bjerkan, 1934). The objectives of this research were: (i) to describe changes in standing stocks and physiological rates associated with the development (decline) of a bloom of *E.huxleyi* in a land-locked fjord; (ii) to determine rates of calcification and nitrogen uptake by phytoplankton populations in the fjords; (iii) to find out differences in the patterns of photosynthetic carbon metabolism between *E.huxleyi*-dominated and diatom-dominated phytoplankton assemblages.

Method

Sampling and study sites

Sampling was conducted on board R/V 'Hans Brattström' in Fauskangerpollen (60°30'N, 5°02'W; 19, 24 and 26 May, 1993) and Nordåsvannet (60°20'N, 5°20'W; 27 May 1993). Both study sites are land-locked fjords situated on the west coast of Norway. Fauskangerpollen, located ~20 km west of Bergen, is connected with the main fjord through a 40 m wide, 4 m deep entrance and consists of a single basin with a maximum depth of 92 m. Nordåsvannet is situated ~7 km south of Bergen and consists of an inner basin 90 m deep which is connected with the outer fjord by two parallel channels, each ~12 m wide and with a maximum depth of 2–4 m.

Water samples for the determination of salinity, nutrients, chlorophyll *a* phytoplankton abundance, particulate carbon and nitrogen, proteins, carbohydrates, lipids, and carbon and nitrogen incorporation rates were collected with 5 l Niskin bottles. The samples were then transferred to 5 l acid-washed polypropylene carboys and kept refrigerated in darkness until transport to the laboratory. The vertical extinction of light was calculated from the reading of the Secchi disk depth.

Hydrographic and standing stock variables

Salinity was determined with an inductive salinometer, and dissolved nitrate and soluble reactive phosphorus in a Scalar autoanalyzer within 24 h, according to Grashoff *et al.* (1983). Ammonium and urea were analyzed within 2–4 h after sampling following the methods of Solórzano (1969) and McCarthy (1970), respectively.

Chlorophyll *a* was measured on two size fractions, 0.2–5 μm, by filtration of

100 ml water subsamples through Poretics polycarbonate filters, freezing of the filter and extraction in 90% acetone at 4°C overnight. Chlorophyll *a* fluorescence was then measured with a Turner Design fluorometer previously calibrated with a solution of pure chlorophyll *a*. Identification and counting of phytoplankton cells were performed by examining samples preserved both in Lugol's iodine and buffered formalin with an inverted microscope. Coccolithophorids were counted only in the formalin-preserved samples. Samples preserved in Lugol's iodine solution were not taken on the second visit to Fauskangerpollen (24 May) and, as a consequence, the abundance of small flagellates on that date could not be estimated. Cell numbers were converted to carbon biomass as described in Holligan *et al.* (1984).

Data on total particulate carbon and nitrogen are not reliable due to methodological problems during sample preparation, and therefore are not presented in this paper. The concentration of proteins, carbohydrates and lipids was determined as described in Fernández *et al.* (1992) using as standards bovine serum albumin, glucose and cholesterol, respectively. Concentrations were converted to carbon equivalents by assuming the carbon content of each biochemical pool to be 53% of total weight for proteins (Laws, 1991), and 40 and 83% for carbohydrates and lipids, respectively (Fraga and Pérez, 1990). These conversion factors have previously been demonstrated to show a very small variability in natural phytoplankton populations (Fraga and Pérez, 1990; Laws, 1991). Parallel estimates of the organic carbon content in natural phytoplankton measured by elemental analysis and the sum of the carbon content of proteins, carbohydrates and lipids calculated as described above did not show significant differences (Fernández *et al.*, 1994b). Experiments undertaken with cultures of *E.huxleyi* (Fernández *et al.*, 1996a) revealed that both estimates were linearly related with a slope not differing significantly from unity [particulate organic carbon (POC) (estimated) = 0.97 (± 0.13) POC (measured) –0.41 (± 1.65) $r = 0.80$, $P < 0.001$]. Total particulate nitrogen standing stock was estimated by dividing total particulate proteins by 6.25 (Packard and Dortch, 1975).

Nitrogen uptake measurements

Uptake rates of ammonium, nitrate and urea were determined on water samples taken from the 5 l carboys using ^{15}N isotopes (Kristiansen and Paasche, 1989); 0.5 μmol l^{-1} of ammonium (95 atom-% ^{15}N), nitrate (96.8 atom-% ^{15}N) and urea (99 atom-% ^{15}N) were added to separate incubation bottles covered with neutral-density screens. The bottles were placed in deck incubators cooled by circulating surface seawater. Incubations started between 14:00 and 15:00 h (local time), and lasted 3 h (24 and 27 May) or 6 h (26 May). After incubation, the samples were filtered through pre-combusted Whatman GF/F glass fiber filters (no fractionations), which were dried at 60°C and later analyzed by emission spectrometry (Kristiansen and Paasche, 1989). Reduced incubation times (3–6 h) and ^{15}N additions of 0.5 μmol l^{-1} were used because we expected short turnover times of dissolved nitrogen. The uptake rates may have been slightly overestimated because of the isotope additions.

Carbon incorporation measurements

Triplicate 20 ml acid-washed glass vials were filled with water from each 5 l carboy at the laboratory 3–4 h after sampling and were then inoculated with 370 kbq (10 μCi) of $NaH^{14}CO_3$, placed in an incubator provided with an artificial light source (Osram Powerstar HQ1-T 400 W/DH) and cooled by circulating water. Samples were incubated at a range of irradiances simulating the irradiance experienced by the cells at the sampling depth. The irradiance level corresponding to surface samples was set at 650 μE m^{-2} s^{-1}. Attenuation of light was achieved by using neutral-density glass filters. Incubations were performed under a 16 h:8 h light–dark cycle and lasted 24 h.

For the determination of carbon incorporation into photosynthetic end products, the contents of the incubation vials were filtered sequentially through 5 and 0.2 μm Poretics polycarbonate filters, and stored at –20°C until further analysis. Rates of ^{14}C incorporation into methanol/water-soluble compounds (low-molecular-weight metabolites; LMWM), chloroform-soluble compounds (lipids), hot trichloroacetic acid (TCA)-soluble compounds (polysaccharides and nucleic acids) and hot TCA-insoluble compounds (proteins) were determined as in Marañón *et al.* (1995a). During the first steps of the extraction, coccoliths are dissolved and the ^{14}C fixed through calcification completely removed. Carbon incorporation for each size fraction was calculated as the sum of the radioactivity measured in each biochemical pool. In previous experiments using this technique, between 93 and 100% (n = 24) of the ^{14}C has been recovered relative to total carbon fixation. Carbon (C) to nitrogen–assimilation ratios were calculated by assuming: (i) an average molar C/N ratio of 3.28 for proteins (Laws, 1991) and 3.75 for nucleic acids (Fraga and Pérez, 1990); (ii) a free amino acid-N to protein-N ratio of 0.05 (Dortch *et al.*, 1984); (iii) a negligible amount of radioactive carbon as free nucleotides (~0.5% of total incorporated carbon); and (iv) fixation of 4% of the ^{14}C incorporated into nucleic acids (Fraga and Pérez, 1990). Variations in the ratio amino acid-N/protein-N did not cause large changes in the C/N assimilation ratios as they represented a small proportion of the carbon incorporated into the LMWM fraction which, in turn, generally accounts for <20% of the total carbon incorporation in this study.

Rates of carbon incorporation into calcium carbonate cellular structures (coccoliths) were determined using the ^{14}C method (Paasche, 1963), basically following the procedure described in Fernández *et al.* (1993). Inoculation and incubation procedures were as described above for the incorporation of carbon into macromolecules.

Results

Physical and chemical conditions

A sharp pycnocline was present in Fauskangerpollen between 3 and 5 m depth (Figure 1), overlying a subsurface layer characterized by temperatures between 5 and 10°C and salinities >25 psu. This layer maintained its thermohaline properties rather unaltered throughout the study. In Nordåsvannet, the thermohaline

structure was quite similar to that described above, but the pycnocline was less distinct. The 1% surface irradiance was located at ~8–10 m in both fjords.

The patterns of vertical distribution of inorganic nutrients were similar in Fauskangerpollen and Nordåsvannet (Table I). Nitrate concentrations were very low or negligible in the brackish layer, and not detectable in the subsurface layer, then increasing below 12–15 m. Ammonium and urea were, however, present with values ranging from 0.3 to 0.9 μM in the upper 10 m. Phosphate concentrations

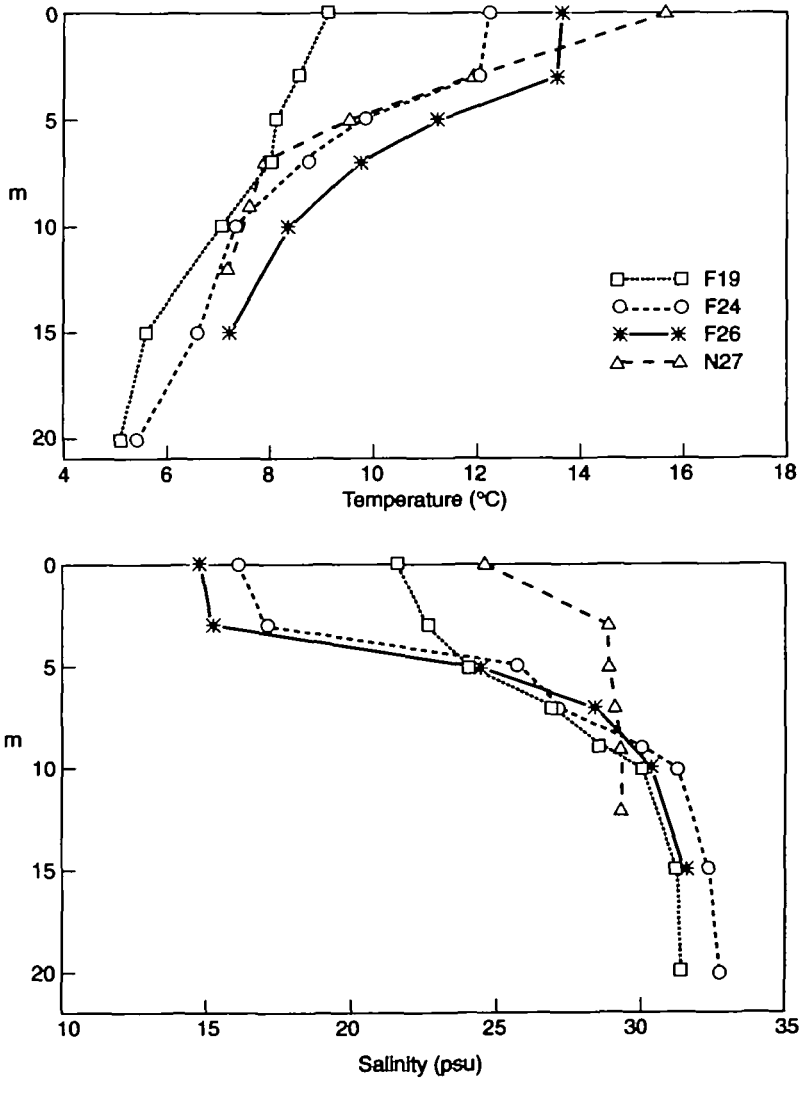

Fig. 1. Vertical distribution of temperature and salinity on 19 May (F19), 24 May (F24) and 26 May (F26) in Fauskangerpollen, and on 27 May (N27) in Nordåsvannet.

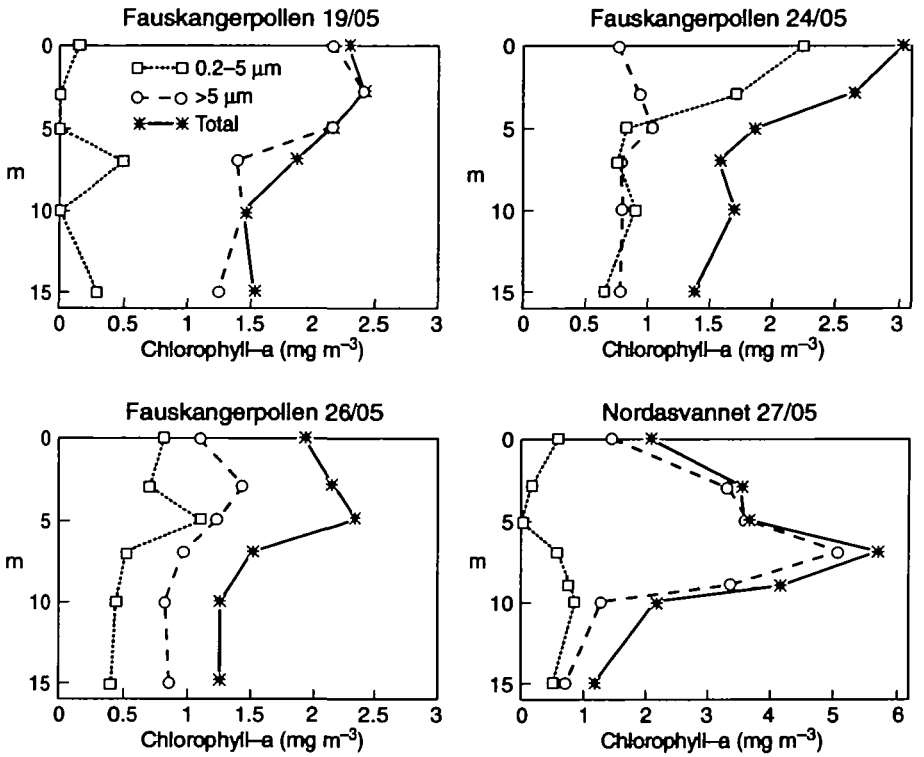

Fig. 2. Vertical distribution of size-frationated chlorophyl *a* (0.2–5 µm, >5µm and total) from the four cruises. Note the differences in the *x*-axis for Nordåsvannet.

were low but measurable. The NO_3/PO_4 ratio was close to the Redfield ratio in the brackish and deep layers, and very low just below the pycnocline. Silicate concentrations ranged from 0.5 to 1.8 µM and were higher in the brackish layer than in subsurface waters in Fauskangerpollen.

Phytoplankton standing stocks and bloom development

In Fauskangerpollen, maximum chlorophyll *a* concentrations were 2–3 mg m^{-3} in the brackish layer and 1–1.5 mg m^{-3} in the subsurface layer (Figure 2). The contribution of the <5 µm size fraction was negligible during the first visit to Fauskangerpollen on 19 May, then increased to 60–70% of total chlorophyll *a* concentration on 24 and 26 May. This was linked to an inflow of warmer and fresher water between 19 and 26 May. The chlorophyll *a* concentration in Nordåsvannet reached up to 5.5 mg m^{-3} at 7 m, mostly represented by phytoplankton cells >5 µm (~90%).

The surface and subsurface water bodies were characterized by different phytoplankton assemblages (Figure 3). Diatoms, mainly *Skeletonema costatum*, and flagellates dominated the autotrophic biomass in the brackish layer, whereas *E.huxleyi* made up a significant proportion of the total phytoplankton biomass in

Table I. Concentrations (μM) of dissolved nitrate, ammonium, urea, phosphate and silicate at Fauskangerpollen (Fausk) and Nordåsvannet (Nordås)

Cruise	Depth (m)	Nitrate	Ammonium	Urea-N[a]	Phosphate	Silicate	Phytoplankton assemblage
Fausk-1	0	0.24	–	–	0.19	0.27	Diatoms
(19 May)	3	0.00	–	–	0.20	0.22	Diatoms
	5	0.00	–	–	0.21	0.31	Mixed
	7	0.00	–	–	0.21	0.34	*E.huxleyi*
	10	0.00	–	–	0.22	0.42	*E.huxleyi*
	15	0.00	–	–	0.23	0.45	Mixed
	20	5.23	–	–	0.70	0.74	Mixed
Fausk-2	0	0.62	0.36	0.20	0.08	1.81	Diatoms
(24 May)	3	0.18	0.27	0.39	0.08	1.03	Diatoms
	5	0.00	0.25	0.22	0.12	0.45	Mixed
	7	0.00	0.36	0.41	0.13	0.52	*E.huxleyi*
	10	0.00	0.23	0.11	0.14	0.55	*E.huxleyi*
	15	0.88	0.38	0.63	0.2	2.97	Mixed
	20	5.23	–	–	0.63	5.87	Mixed
Fausk-3	0	0.00	0.33	0.43	0.09	0.65	Diatoms
(26 May)	3	0.00	0.38	0.33	0.08	0.48	Diatoms
	5	0.00	0.38	0.77	0.12	0.47	Mixed
	7	0.03	0.48	0.77	0.12	0.54	Mixed
	10	0.00	0.54	0.87	0.14	0.56	Mixed
	15	0.00	0.62	1.91	0.16	0.81	Mixed
Nordås	0	0.00	0.33	–	0.13	0.38	Mixed
(27 May)	3	0.00	0.40	–	0.14	0.52	*E.huxleyi*
	5	0.00	0.47	–	0.15	0.50	*E.huxleyi*
	7	0.00	0.54	0.13	0.31	0.53	*E.huxleyi*
	9	0.00	0.42	–	0.17	0.48	*E.huxleyi*
	12	0.10	0.40	–	0.26	0.62	Mixed
	15	3.34	1.17	–	0.48	1.61	Mixed

[a] 1 M urea = 2 M urea-N.

the subsurface layer. *Emiliania huxleyi* biomass decreased gradually in Fauskangerpollen: from 100 mg C m^{-3} on 19 May to 25 mg C m^{-3} on 26 May. In Nordåsvannet, diatoms were not abundant (<20%), whereas *E.huxleyi* biomass represented >87% of the total phytoplankton biomass at depths greater than 3 m. The maximum *E.huxleyi* biomass was found at 7 m (355 mg C m^{-3}).

Figure 4 shows the vertical distribution of a series of variables directly related to the development of *E.huxleyi* populations. The distribution of *E.huxleyi* cell number paralleled that of biomass. In Fauskangerpollen, maximum cell densities decreased with time from 8×10^3 to 2.5×10^3 cells ml^{-1} throughout the study. The vertical distribution of free coccoliths mirrored that of living cells, although maximum abundances were found at slightly deeper layers on 24 and 26 May. The maximum densities of free coccoliths were $350–450 \times 10^3$ coccoliths ml^{-1}; a decrease in the number of free coccoliths was not noticeable during the survey. The vertical distribution of empty *E.huxleyi* coccospheres showed, in general, maximum densities (400–1000 coccospheres ml^{-1}) associated with the maxima of living cells. An exception to this pattern was observed on 26 May, when a pronounced maximum of 2300 empty coccospheres ml^{-1} was measured at 10 m. The

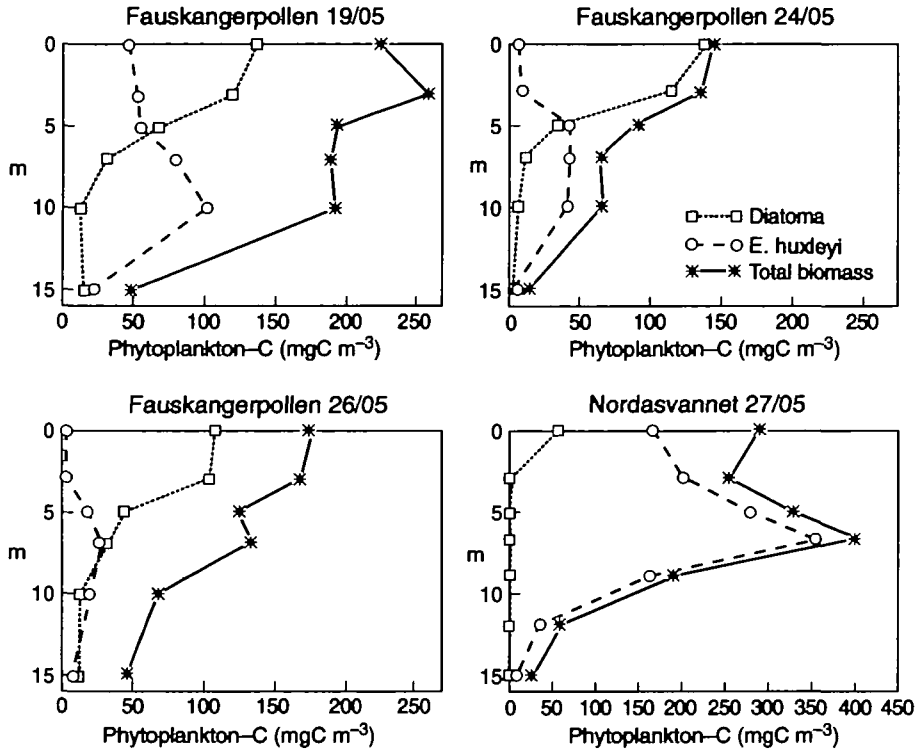

Fig. 3. Vertical distribution of total phytoplankton biomass (Total biomass), diatom biomass (diatoms) and *E.huxleyi* biomass (*E.huxleyi*) in the four cruises. Note the differences in the *x*-axis for Nordåsvannet.

ratio between the abundance of free coccoliths and living cells showed a gradual increase (from 60 to 250) in the intermediate layer during the three cruises. In Nordåsvannet, *E.huxleyi* reached very high densities of up to 28 × 10^3 cells ml^{-1}, the number of free coccoliths was 654 × 10^3 ml^{-1}, and the ratio between the abundance of free coccoliths and living cells showed values lower than those found in Fauskangerpollen.

Chemical composition of particulate matter

The protein/carbohydrate ratio was close to one in the brackish layer and increased downwards to 1.5–1.8 in the deep layer, in association with higher nitrate concentrations (Figure 5). The percentage of lipid carbon relative to total particulate carbon, estimated as the sum of lipids, carbohydrates and proteins, and the contribution of *E.huxleyi* to total phytoplankton biomass were related (r = 0.66, P < 0.05, n = 24). The relative contribution of lipid carbon to total carbon biomass reached values as high as 60%, paralleling peaks of coccolithophorids.

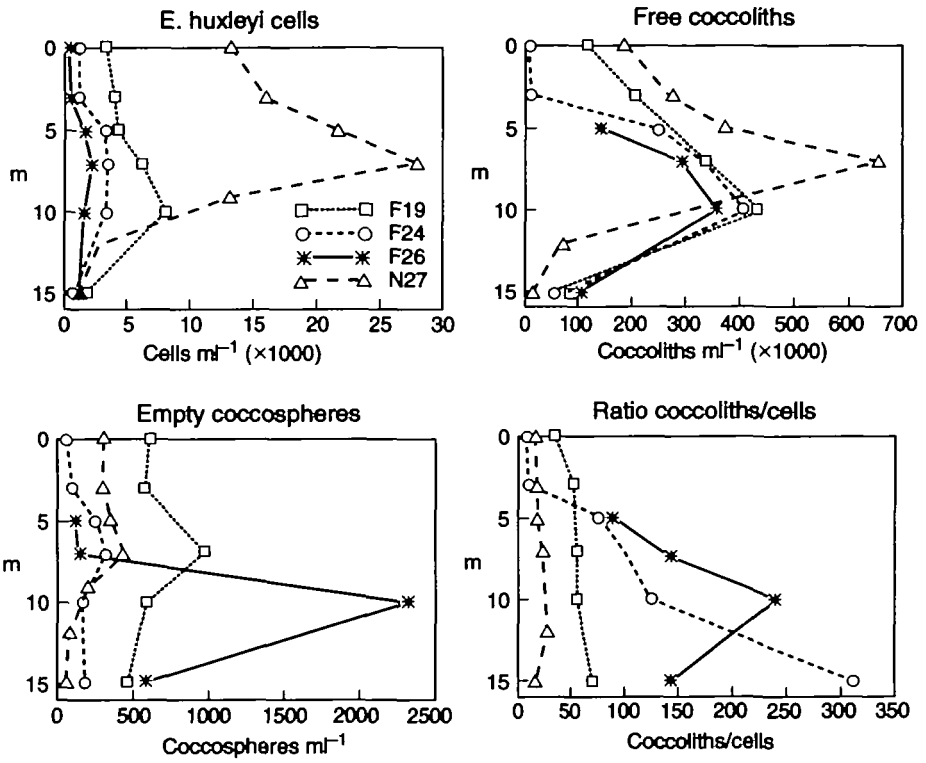

Fig. 4. Vertical distribution of the abundance of *E.huxleyi* cells, *E.huxleyi* free coccoliths, *E.huxleyi* empty coccospheres and the ratio free coccoliths/living cells on 19 May (F19), 24 May (F24) and 26 May (F26) in Fauskangerpollen, and on 27 May (N27) in Nordåsvannet.

Nitrogen uptake rates

Uptake rates of nitrate, ammonium and urea were measured at all the sampling depths in Fauskangerpollen on 24 and 26 May, and at the depth of the *E.huxleyi* maximum in Nordåsvannet (Table II). The highest nitrogen uptake rates were measured in the brackish layer, coinciding with maximum values of chlorophyll *a* and phytoplankton carbon biomass. The sample from 15 m on May 24 was collected within the upper part of the nutricline (Table I), and therefore uptake rates of dissolved inorganic nitrogen compounds were also high. Most of the nitrogen taken up by phytoplankton cells was in the form of ammonium, with urea also being an important nitrogenous source. The f-ratio (ratio between nitrate uptake rate and nitrate + ammonia + urea uptake rate) was generally low and ranged from 0.1 to 0.3, being slightly higher in the brackish layer than in coccolithophore-dominated waters.

Organic carbon production

Maximum rates of primary production were found at the base of the brackish layer (Fauskangerpollen) or just underneath (Nordåsvannet) (Figure 6), reaching

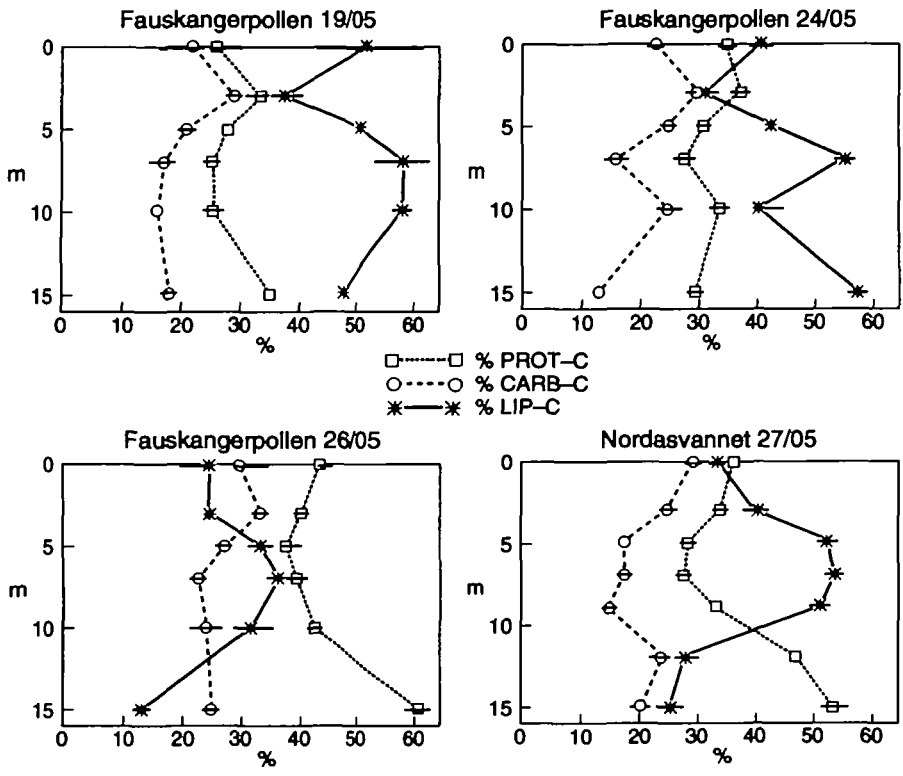

Fig. 5. Vertical distribution of the relative contribution of protein-C (%PROT-C), carbohydrate-C (%CARB-C) and lipid-C (%LIP-C) to total particulate organic carbon (estimated as the sum of the three biochemical constituents) during the four cruises.

values of up to 100 mg C m^{-3} day^{-1} (Fauskangerpollen, 24 May). However, rates were very low at the surface in spite of the elevated chlorophyll *a* and phytoplankton biomasses. Carbon incorporation rates at subsurface depths dominated by *E.huxleyi* were ~10 mg C m^{-3} day^{-1} in Fauskangerpollen, increasing to values of up to 50 mg C m^{-3} day^{-1} in Nordåsvannet. Phytoplankton cells >5 μm accounted for most of the primary production in the two fjords; the contribution of the <5 μm size fraction was negligible during the first visit to Fauskangerpollen on 19 May.

Photosynthetic carbon incorporation into macromolecules

^{14}C labeling patterns of newly produced photosynthate were clearly different in the two fjords (Figure 7). In Fauskangerpollen, 40–50% of ^{14}C was fixed into proteins, in contrast to only 20–35% in Nordåsvannet. In general, the percentage of carbon incorporated into protein was low in *E.huxleyui*-dominated communities. Carbon incorporation into lipids was >35% of total incorporated carbon in Nordåsvannet, compared to 10–20% in Fauskangerpollen. At this sampling location, the relative carbon allocation into lipid increased slightly throughout the

Table II. Vertical distribution of hourly uptake rates of nitrate, ammonia and urea (mg N m^{-3} h^{-1}) in Fauskangerpollen (Fausk) on 24 and 26 May, and Nordåsvannet (Nordås) f-Ratios (nitrate uptake/nitrate + ammonium + urea daily uptake rates) are also shown

Cruise	Depth (m)	NO_3 uptake	NH_4 uptake	Urea-N uptake	f-ratio	Phytoplankton assemblage
Fausk-2	0	0.36	0.76	0.10	0.28	Diatoms
(24 May)	3	0.57	1.05	0.42	0.26	Diatoms
	5	0.49	1.00	0.42	0.23	Mixed
	7	0.27	0.56	0.29	0.22	*E.huxleyi*
	10	0.34	0.51	0.36	0.25	*E.huxleyi*
	15	0.65	0.51	0.49	0.36	Mixed
Fausk-3	0	0.14	0.37	0.14	0.20	Diatoms
(26 May)	3	0.14	0.37	0.14	0.20	Diatoms
	5	0.10	0.32	0.09	0.18	Mixed
	7	0.07	0.30	0.08	0.14	Mixed
	10	0.04	0.18	0.08	0.11	Mixed
	15	0.01	0.09	0.03	0.09	Mixed
Nordås (27 May)	7	0.18	1.66	0.19	0.08	*E.huxleyi*

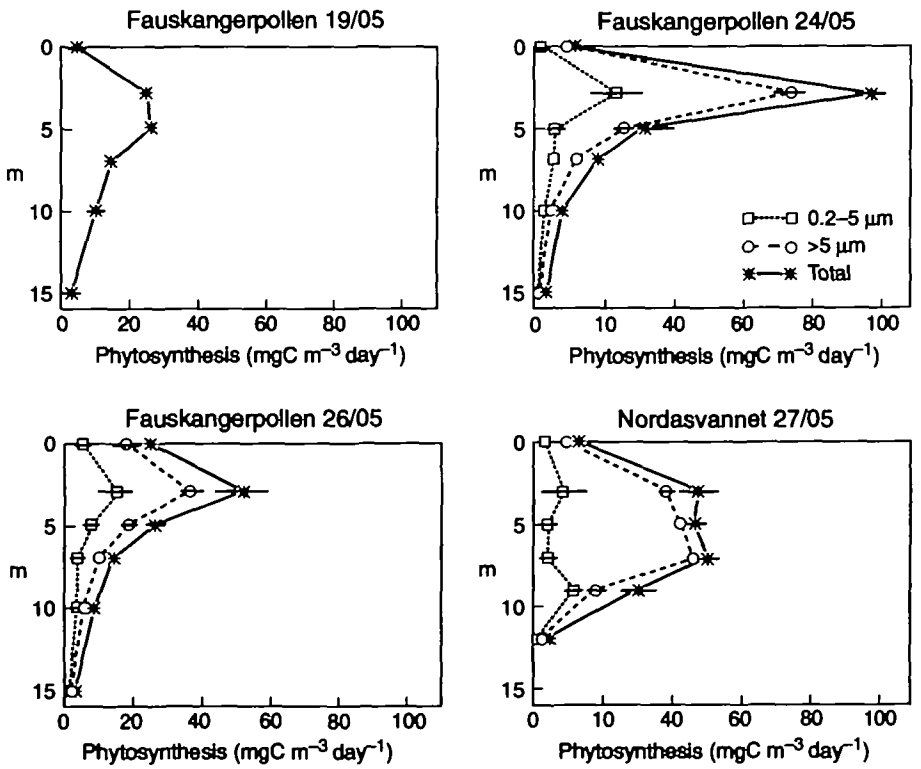

Fig. 6. Vertical distribution of size-fractionated primary production (0.2–5 µm, >5 µm and total) during the four cruises.

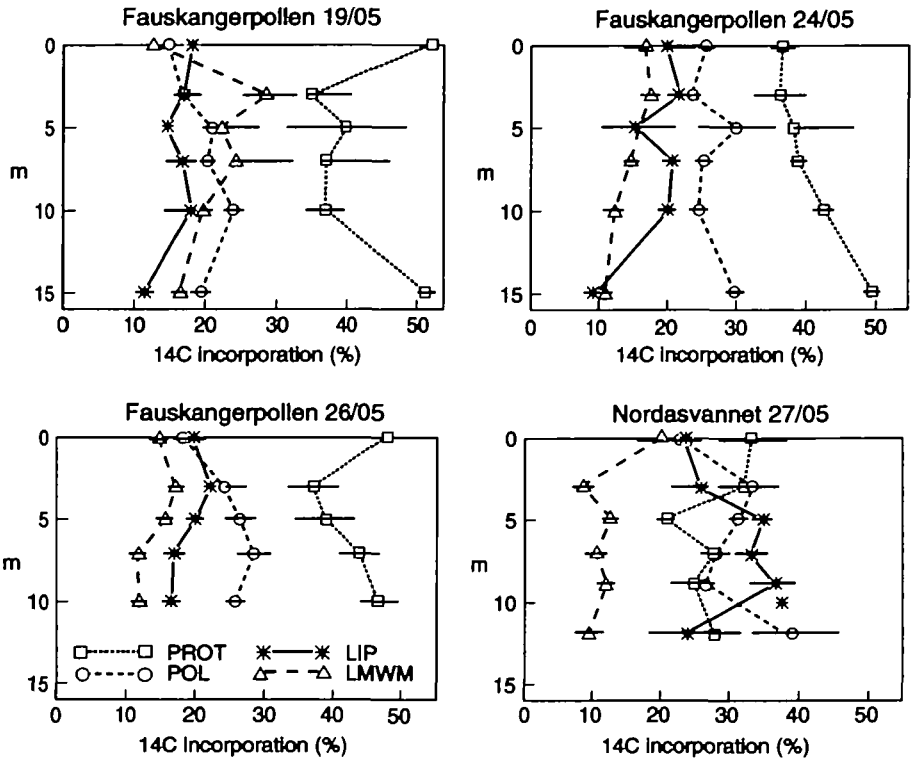

Fig. 7. Vertical distribution of the percentage of ^{14}C incorporated into proteins (PROT), polysaccharides (POL), lipids (LIP) and low-molecular-weight metabolites (LMWM) during the four cruises.

study period. Carbon incorporation into polysaccharides and LMWM was 15–30% of total carbon incorporation and did not show any distinct distribution pattern.

The metabolic behavior typical of *E.huxleyi*-dominated assemblages is characterized by the co-occurrence of relatively high percentages of carbon incorporation into lipid and low carbon allocation into protein (Table III), and therefore significant differences were found in the ratio of carbon to nitrogen uptake into particulate organic matter depending on the phytoplankton species composition. The average C:N assimilation ratio for *E.huxleyi*-dominated assemblages was 10.8 as compared to mean values of 8.3 and 8.6 in diatom-dominated and mixed-phytoplankton assemblages, respectively.

Inorganic carbon production

Both carbon incorporation into coccoliths and the biomass of *E.huxleyi* decreased during the investigation in Fauskangerpollen (Figure 8). Absolute rates ranged from ~20 mg C m^{-3} day^{-1} on 19 May to <5 mg C m^{-3} day^{-1} on 26 May. Very high

Table III. Patterns of photosynthetic carbon incorporation and chemical composition of *E.huxleyi*-dominated (Ehux), diatom-dominated (Diat) and mixed phytoplankton assemblages (Mix) from Fauskangerpollen and Nordåsvannet. Dominance was defined as the relative contribution of a given taxonomic group to total phytoplankton biomass >50%. Values in parentheses represent standard errors. ANOVA was performed to test for differences between assemblages. *P* indicates significance of the ANOVA. Student–Newman–Keuls (SNK) tests (0.05 significance level) were processed to ascertain significant differences between all paired groups

Variable	*E.huxleyi*-dominated assemblage (n = 22)	Diatom-dominated assemblage (n = 15)	Mixed assemblage (n = 26)	*P*	SNK tests
% protein-C	29.6 (±1.0)	36.3 (±2.0)	40.3 (±2.2)	<0.001	Mix = Diat > Ehux
% lipid-C	51.7 (±1.8)	35.6 (±3.3)	36.7 (±2.8)	<0.001	Ehux > Mix = Diat
Protein/carbohydrate + lipid	0.52 (±0.02)	0.61 (±0.04)	0.78 (±0.07)	0.002	Mix > Diat = Ehux
% incorp. proteins	32.2 (±1.9)	45.9 (±2.6)	41.0 (±2.0)	<0.001	Diat = Mix > Ehux
% incorp. polysaccharides	27.2 (±1.1)	21.8 (±1.3)	27.0 (±1.8)	0.041	Ehux = Mix > Diat
% incorp. lipids	26.4 (±1.8)	18.3 (±1.1)	17.3 (±1.2)	<0.001	Ehux > Diat = Mix
% incorp. LMWM	14.1 (±1.2)	14.1 (±2.2)	14.7 (±1.1)	n.s.	
Prot/poly + lipid incorp. ratio	0.65 (±0.08)	1.2 (±0.09)	1.0 (±0.1)	<0.001	Diat = Mix > Ehux
Carbon:nitrogen incorp. ratio	10.8 (±0.6)	8.3 (±0.4)	8.6 (±0.5)	0.002	Ehux > Mix = Diat

calcification rates (55 mg C m^{-3} day^{-1}) were measured at the depth of maximum *E.huxleyi* abundance in Nordåsvannet. The maximum cell-specific calcification rates were generally 4–6 pg C $cell^{-1}$ day^{-1}. Underneath the brackish layer in Fauskangerpollen, rates ranged from 5.2 pg C $cell^{-1}$ day^{-1} on the first visit to ~1 pg C $cell^{-1}$ day^{-1} measured on 26 May. By then, an inverse trend between cell-specific calcification rate and the abundance of *E.huxleyi* was observed ($r = 0.82$, $P = 0.011$, n = 13; see Figure 4). The calcification to photosynthesis ratio for cells >5 μm reached maximum values (around one) coinciding with peaks of *E.huxleyi* abundance during the first visit to Fauskangerpollen and in Nordåsvannet, and also in deeper waters on 24 May in Fauskangerpollen.

Discussion

Evolution of the E.huxleyi bloom

Diatoms and flagellates were the dominant phytoplankton groups in the surface layer of Fauskangerpollen, whereas *E.huxleyi* developed in subsurface, nitrate-depleted waters. In Nordåsvannet, however, *E.huxleyi* was significant in the upper 3 m of the water column. An inverse relationship was found between salinity and *E.huxleyi* abundance in the brackish layer (r = 0.94, $P < 0.001$, n = 12), thus suggesting that *E.huxleyi* populations were washed out by the outflow of freshwater. The same conclusion was drawn by Kristiansen *et al.* (1994b) during a survey of a bloom in the Samnangerfjord. In Fauskangerpollen, the maximum abundance of *E.huxleyi* cells was ~8×10^3 cells ml^{-1}, a value very close to those previously found in fjords (Kristiansen *et al.*, 1994b) as well as in shelf-break (Holligan *et al.*, 1983)

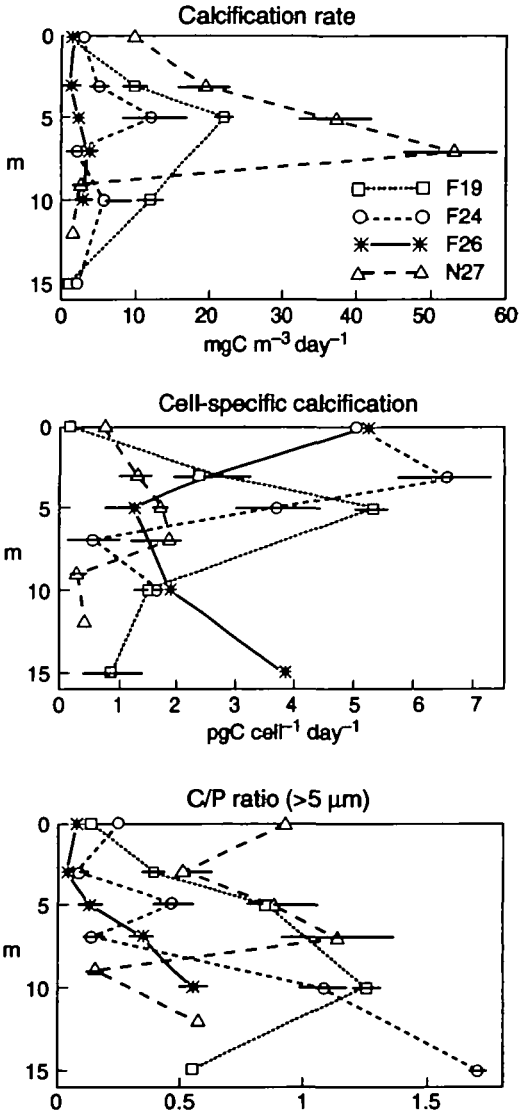

Fig. 8. Vertical distribution of the rates of calcification, cell-specific calcification and the calcification/photosynthesis ratio (C/P ratio) on 19 May (F19), 24 May (F24) and 26 May (F26) in Fauskangerpollen, and on 27 May (N27) in Nordåsvannet. The C/P ratio was calculated from the photosynthesis rate of phytoplankton cells larger than 5 µm, which contains *E.huxleyi*.

and oceanic environments (Holligan *et al.*, 1993). In Nordåsvannet, the abundance of *E.huxleyi* was higher (up to 27.8 × 10^3 cells ml^{-1}), but still ~4 times lower than the maximum cell numbers reported from fjords (Berge, 1962).

The results from Fauskangerpollen represent the peak and declining phases of a bloom of *E.huxleyi*. The almost unchanged thermohaline properties of

subsurface waters (Figure 1), and the very similar phytoplankton species composition observed during the three visits, suggest low mixing between waters from the main fjord and subsurface poll waters, thus allowing interpretation of the results from a successional perspective. Several arguments suggest that on the first sampling date in Fauskangerpollen, the *E.huxleyi* population reached its maximum density, with cells still growing actively. Maximum cell-specific calcification rates of ~5.2 pg C $cell^{-1}$ day^{-1} measured on this date are similar to calcification rates measured in *E.huxleyi* batch cultures under optimal growth conditions (Balch *et al.*, 1992) and in *E.huxleyi* blooms sampled in the North Sea (van der Wal *et al.*, 1995). These rates, however, are higher than a maximum measured in Samnangerfjord (3.48 pg C $cell^{-1}$ day^{-1}) (Kristiansen *et al.*, 1994b), and also slightly different to those reported for blooms monitored in mesocosms in spring 1992 and 1993 in Bergen, Norway (van der Wal *et al.*, 1994; Marañón *et al.*, 1995b).

Values of the calcification to photosynthesis ratio close to one, measured on 19 and 24 May in Fauskangerpollen, suggest that *E.huxleyi* cells were still in a growing phase. A calcification to photosynthesis ratio close to one has been found in actively growing cultures of *E.huxleyi* (e.g. Nimer and Merrett, 1992; Balch *et al.*, 1996). Increases in the calcification/photosynthesis ratio have also been shown to be related to phosphate-deprived *E.huxleyi* populations (van Bleijswijk *et al.*, 1994; Paasche and Brubak, 1994). Phosphate limitation, however, is not likely to account for the variability observed in this study as higher ratios were measured during periods of relatively high phosphate concentration (see Table I). The photosynthesis/calcification ratio is difficult to estimate under natural conditions due to the frequently high contribution of non-coccolithophore biomass. We have tried partially to overcome this problem by calculating the calcification/photosynthesis ratio using exclusively the uptake data for the >5 μm phytoplankton size fraction which contains *E.huxleyi* cells.

As the number of *E.huxleyi* cells and the rates of cell-specific calcification decreased in Fauskangerpollen, the ratio detached coccoliths/living *E.huxleyi* cells increased steadily from ~50 on 19 May to ~250 on 24 May. These maximum values are in the same range as those measured in other coastal environments at the time of bloom termination (Balch *et al.*, 1991; García-Soto *et al.*, 1995). A high concentration of empty coccospheres was also observed in the subsurface layer on 26 May when the *E.huxleyi* population was in a declining phase. The collapse of the *E.huxleyi* bloom in Fauskangerpollen is likely to be caused by viral infection, as has been inferred from the inverse relationship found between *E.huxleyi* numbers and the abundance of large virus-like particles in this fjord during the decline of the bloom (Bratbak *et al.*, 1995).

Patterns of carbon and nitrogen metabolism

A large proportion of the nitrogen taken up by the microplankton community sampled in this study was in the form of ammonium and also urea. This is consistent with ambient concentrations of the nitrogenous species, i.e. nitrate was generally negligible. These low f-ratios detected in this study, as low as 0.2, may be

typical during *E.huxleyi* blooms in the Norwegian fjords (Kristiansen *et al.*, 1994b). Nitrogen uptake rates were abnormally high compared to carbon incorporation rates. This disagreement arises from the different incubation times used (3–6 h for N, 24 h for C), overestimation of the nitrogen uptake rates (^{15}N additions) and because nitrogen incorporation is also influenced by bacterial activity (Kristiansen *et al.*, 1994a).

The photosynthetic carbon metabolism of *E.huxleyi*-dominated populations was characterized by very high carbon incorporation into lipids and relatively low carbon fixation into proteins as compared to the diatom-dominated populations (Figure 7 and Table III), especially in Nordåsvannet. These patterns of macromolecular synthesis were even more conspicuous when considering the chemical composition of particulate matter (Figure 5; Table III). Similar patterns of photosynthetic carbon metabolism were reported for cultures of these species (Fernández *et al.*, 1994a, 1996a,b) and also for natural phytoplankton populations dominated by either coccolithophorids (Marañón *et al.*, 1995) or other Prymnesiophyceae (Fernández *et al.*, 1994b).

A high inorganic carbon to nitrogen uptake ratio, resulting from the elevated lipid to protein synthesis ratio, and a low f-ratio seem to be characteristic for *E.huxleyi* blooms in Norwegian fjords. A direct consequence of this pattern of organic carbon biosynthesis is that naturally occurring phytoplankton populations dominated by *E.huxleyi* would take up more carbon relative to nitrogen from surface waters, regardless of the effect of calcification, as compared to diatom-dominated assemblages. These blooms are therefore good examples of the limitations involved in estimating net community production rates by scaling nitrate uptake by the Redfield ratio.

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