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Effects of the diatom-*Emiliania huxleyi* succession on photosynthesis, calcification and carbon metabolism by size-fractionated phytoplankton

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Abstract

Changes in species composition, photosynthesis, calcification and size-fractionated carbon metabolism by natural phytoplankton assemblages were monitored in three mesocosms under different nutrient conditions during May 1993. In the 3 enclosures, the decline of the diatom-dominated assemblages was followed by the development of a bloom of the coccolithophorid *Emiliania huxleyi*. Highest growth of *E. huxleyi* was observed in the mesocosm with a high N : P ratio, suggesting this species is a good competitor at low phosphate concentrations. The transition from diatom- to *E. huxleyi*-dominated assemblages brought about a sharp reduction of the phytoplankton standing stock and carbon-specific photosynthetic rate. The relative contribution of the smaller size fraction to total photosynthesis increased as the succession progressed. Calcification rate and *E. huxleyi* cell-specific calcite production were highest during the early stages of development of the *E. huxleyi* bloom. Distinct changes in the patterns of ^{14}C allocation into biomolecules were noticed during the diatom-*E. huxleyi* succession. The diatom-dominated assemblage showed high relative ^{14}C incorporation into low molecular weight metabolites (LMWM), whereas proteins and, specially, lipids accounted for the largest proportion of carbon incorporation in the *E. huxleyi* bloom. The patterns of photoassimilated carbon metabolism proved to be strongly dependent on cellular size, as protein relative synthesis was significantly higher in the smaller than in the larger size fraction, irrespective of the nutrient regime and the successional stage. These results are discussed in relation to the ecological and physiological features of small phytoplankton.

Introduction

The decline of the spring biatom bloom followed by the development of coccolithophorid assemblages is a well documented feature of phytoplankton succession in temperate coastal (Balch *et al.*, 1991) and oceanic areas (Holligan *et al.*, 1993). Among coccolithophorids, *Emiliania huxleyi* (Lohmann) Hay et Moler is the most widespread and abundant species, being thought to be the largest calcite producer on earth (Westbroek *et al.*, 1993). This organism has received increased attention in the last years, due to its ability to develop extensive coastal (Balch *et al.*, 1991) and oceanic blooms (Holligan *et al.*, 1983; Fernández *et al.*, 1993), which significantly influence the biogeochemical cycling of carbon (Robertson *et al.*, 1994) and sulphur (Malin *et al.*, 1993).

The use of experimental enclosures (mesocosms) has proved to be a useful tool in studying phytoplankton succession and in addressing the effects of environmental conditions (temperature, irradiance, nutrient supply) upon the patterns of abundance of different algal groups, including coccolithophorids (Morris *et al.*, 1983; Egge & Aksnes, 1992, among others). In mesocosm studies, it has been shown that diatoms dominate the phytoplankton community as long as

silicate levels are above *c.* 2 μm (Egge & Aksnes, 1992). Diatom growth appears to be limited at high nitrate to phosphate (N : P) supply ratios, suggesting that this group has a higher phosphorus requirement or a lower uptake affinity for phosphate than flagellates. On the other hand, *E. huxleyi* exhibited the highest growth rates when the N : P ratio was low, albeit it also bloomed at high N : P ratios (Egge, 1993).

The dynamics of *E. huxleyi* blooms are also linked to the existence of virus and virus-like particles (VLP). Viruses have been reported to terminate blooms of *E. huxleyi* in mesocosm experiments (Bratbak *et al.*, 1993). The viral activity was in turn related to the nutrient regime of the environment, as virus production was only significant when phosphate concentration was above 0.8 μmol l^{-1}.

The significance of succession in controlling phytoplankton dynamics and energetic transfer through the pelagic food web has often been recognized (Margalef, 1978; Smayda, 1980). In this connection, Fernández *et al.* (1992) reported major changes in the photosynthetic carbon metabolism of phytoplankton and the biochemical composition of particulate matter during the diatom-*Phaeocystis* sp. succession. These metabolic changes are believed to induce a shift in the trophic structure of the planktonic ecosystem from the classical food chain during the diatom bloom towards a dominance of the microbial loop at the end of the *Phaeocystic* bloom (Fernández *et al.*, 1992). A similar study on the diatom-*E. huxleyi* succession has not been undertaken so far, and therefore the effects of this process upon the flows of carbon into each cellular compartment and the biochemical composition of the phytoplankton assemblages remain unknown.

Cellular size has often been reported as a major factor in controlling the dynamics of nutrient uptake (Malone, 1980; Aksnes & Egge, 1991) as well as the strategies for energy utilization (Laws, 1975) and storage (Madariaga & Fernández, 1990). In this regard, size-fractionation of the sample has also been successfully used in order to gain a better understanding on the patterns of ^{14}C incorporation into each biochemical pool in natural phytoplankton assemblages (Taguchi & Laws, 1987; Howard & Joint, 1989; Jones *et al.*, 1990, among others). In this study, we adopted this approach to monitor the changes in the photosynthetic carbon metabolism of size-fractionated natural phytoplankton and the biochemical composition of particulate matter during the transition from diatom- to *E. huxleyi*-dominated assemblages in experimental enclosures. Our main goals were (i) to examine the effects of the species succession on the photosynthetic carbon metabolism of the phytoplankton assemblage and (ii) to evaluate the importance of microalgal cell size in determining the patterns of carbon allocation among different molecules.

Methods

Mesocosms experiment

The mesocosms experiment was conducted between 10 and 24 May 1993 in a bay near Raunefjorden, 20 km south of Bergen, western Norway. The enclosures had a total volume of 11 m^3, were made of polyethylene and were open to the air. Homogeneization of the water mass inside the enclosures was ensured by pumping water from the bottom of the bags to the surface at a rate of *c.* 40 l min^{-1}. 10% of the total water volume was daily renewed with water from 1 m depth outside the bags (for further details see Egge & Aksnes, 1992). In order to compensate for the loss due to flowing, 10% of the initial nutrient concentration was daily added to the bags.

Bags were filled on 10 May with non-filtered seawater from 1 m depth. One of the bags (bag L, for low nitrate to phosphate [N : P] ratio) was enriched with nutrients ($NaNO_3$ and K_2HPO_4) in order to provide initial nutrient concentrations of *c.* 10 μM nitrate and 3.5 μM phosphate. Another bag (bag H, for high N : P ratio) was supplied with nutrients to give initial concentrations to 10 μM nitrate and 0.2 μM phosphate. A third enclosure (bag C, for control) was not enriched with nutrients and served as a control. 10% of initial nutrient concentration were daily added to each bag in order to compensate for the nutrient loss through flowing.

Sampling was conducted early in the morning (7:00–8:00 A.M.) every two days. Water samples for standing stock measurements and flow rate experiments were drawn from 0.5 m depth using a plastic bucket and immediately prefiltered through a 200 μm sieve.

Analytical methods

Samples for the determination of nitrate, phosphate and silicate concentrations were kept at 4 °C until analysis, which was conducted during the sampling day. The analysis was done using a Skalar autoanalyzer and

following the methods described in Grasshoff *et al.* (1983).

Chlorophyll *a* concentration was determined fluorometrically on two size fractions, 0.2–5 μm and > 5 μm, after filtration of 100 ml water aliquots through Poretics polycarbonate filters, freezing of the filter and extraction in 90% acetone overnight. Identification and counting of microplankton were carried out with an inverted microscope using samples preserved both in Lugol's iodine and buffered formalin. Coccolithophorids were counted in the formalin-preserved samples. Cell counts were transformed to estimated carbon biomass as in Holligan *et al.* (1984).

Particulate inorganic carbon (PIC) concentration was determined after filtration of 0.5 l water through Whatman GF/F filters, which were frozen at -20 °C until further analysis. PIC concentration was calculated on the basis of the measured calcium content, assuming that all the particulate calcium was in the form of calcium carbonate. Calcium concentration was measured by flame atomic absorption spectrometry using an air-acetylene flame at 422.7 nm wavelength, as in Fernández *et al.* (1993). The amount of particulate carbon in the form of proteins (Protein- C), carbohydrates (Carbohydrate-C) and lipids (Lipic-C) were determined in duplicate as in Fernández *et al.* (1992). The carbon content by weight of each pool was assumed to be 53% for proteins (Laws, 1991) and 40 and 83% for carbohydrates and lipids respectively (Fraga & Pérez, 1990).

The experiments for the determination of ^{14}C incorporation into different cellular compounds started early in the morning (between 7:30 and 8:30 A.M.). Duplicate 70 ml acid-cleaned polycarbonate bottles were filled with the sample, inoculated with 370 kBq (10 μCi) of $NaH^{14}CO_3$ and then suspended at 1 m depth from a buoy placed in the vicinity of the experimental enclosures. At the end of the incubation, which lasted 24 hours, samples were sequentially filtered through 5 μm and 0.2 μm Poretics polycarbonate filters.

The amount of ^{14}C incorporated 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) was determined as in Fernández *et al.* (1994). Acidification during the first steps of this procedure proved to dissolve any calcium carbonate present in the sample, therefore allowing complete removal of all ^{14}C incorporated by the cells through calcification. Total daily primary production (total photosynthesis) was calculated as the sum of the ^{14}C incorporation into each fraction. Preliminary experiments showed no significant differences between the sum of the ^{14}C-activity in the 4 fractions and total activity as measured in non-fractionated samples (t-Test, $p > 0.2$, $n = 10$).

Calcification rates were measured in duplicate in bags H and L using the ^{14}C method (Paasche, 1963), according to the procedure described in Fernández *et al.* (1993). Inoculation, incubation and filtration of the samples were carried out as indicated above for the incorporation of ^{14}C into macromolecules.

Statistical analysis

Differences in the patterns of macromolecular synthesis between enclosures and size fractions, as well as the effect of time were tested using univariate analysis of variance with repeated measures (ANOVAR, Potvin *et al.*, 1990). The significance of the observed differences in selected variables between phytoplankton assemblages was determined using one-way ANOVA. Homogeneity of variances was tested by means of the Cochran's test. Student-Newman-Keuls test was used in order to compare the means of different groups at the 0.05 significance level.

Results

Nutrients, chlorophyll a and phytoplankton biomass

Nutrient levels did not show major changes throughout the experiment in any of the experimental enclosures (Table 1). Nitrate concentration was in the range 6–8 μM in bag L and ranged from 10 to 12 μM in bag H, being almost undetectable during most of the experiment in bag C. Phosphate concentration was *c.* 0.2 μM in bags C and H, whereas it took much higher values in bag L (*c.* 3 μM). Silicate levels were low (< 0.8 μM) in all the enclosures. Ammonia concentration was determined on several occasions during the experiment (Svein Kristiansen, pers. comm.), typical values being below 0.6 μM.

Total chlorophyll *a* concentration decreased steadily during the experiment in all the bags, changing from initial values of 3–5 mg m^{-3} towards less than 1 mg m^{-3} at the end of the sampling period (Fig. 1). Variations in the concentration of chlorophyll *a* followed a similar trend in both size fractions. The relative con-

Table 1. Nitrate (NO_3^-), phosphate (HPO_4^{2-}) and silicate (SiO_2) concentration (μM) in bags C, L and H during the sampling period.

	Bag C			Bag L			Bag H		
Date	NO_3^-	HPO_4^{2-}	SiO_2	NO_3^-	HPO_4^{2-}	SiO_2	NO_3^-	HPO_4^{2-}	SiO_2
14th	0.00	0.23	0.54	7.37	3.06	0.43	9.42	0.23	0.54
16th	0.00	0.22	0.71	5.74	2.89	—	10.90	0.20	0.71
18th	0.00	0.22	0.72	6.12	3.41	—	11.13	0.23	0.78
20th	0.00	0.21	0.44	7.64	3.85	0.36	11.92	0.22	0.47
22nd	0.05	0.14	0.64	6.60	3.34	0.26	11.29	0.14	0.56
24th	0.05	0.15	0.56	6.95	3.55	0.18	11.15	0.15	0.54

tribution of the larger (> 5 μm) size fraction to total chlorophyll *a* ranged from 50 to 70% during most of the experiment in the 3 enclosures, taking lower values at the end of the sampling period.

In agreement with chlorophyll *a* distribution, estimated total phytoplankton carbon decreased in the 3 bags from 120–140 mgC m^{-3} at the start of the experiment to final values of 40–60 mgC m^{-3} (Fig. 2). Diatoms were the most abundant group at the beginning of the experiment, making up more than 50% of total phytoplankton carbon. *Skeletonema costatum* (Greville) Cleve and, to a lesser extent, *Chaetoceros debilis* Cleve were the most abundant diatom species. As the experiment progressed, diatom abundance decreased sharply. This taxonomic group had completely disappeared from all the enclosures by the end of the sampling period. As diatoms declined, *E. huxleyi* started to grow actively, and reached its biomass maximum by 20 May in bags L and H and 2 days later in bag C. The largest values of *E. huxleyi* biomass (72 mg C m^{-3} on 20 May) were measured in bag H, whereas the magnitude of the *E. huxleyi* bloom was lower in bag L. The abundance of other undetermined flagellates (below 5–7 μm in size) showed the opposite pattern, taking higher values (above 60 mgC m^{-3}) in bag L than in bags H and C.

PIC and biochemical composition of particulate matter

PIC concentration was low on the first sampling days (< 10 mgC m^{-3}), but increased as the *E. huxleyi* population developed (Fig. 3). The largest PIC values were measured in bags L and H (56 and 40 mgC m^{-3}, respectively) at the middle of the experiment, concurring with the highest *E. huxleyi* biomass. In bag C, PIC concentration increased steadily throughout the experiment reaching final values of *c.* 20 mgC m^{-3}.

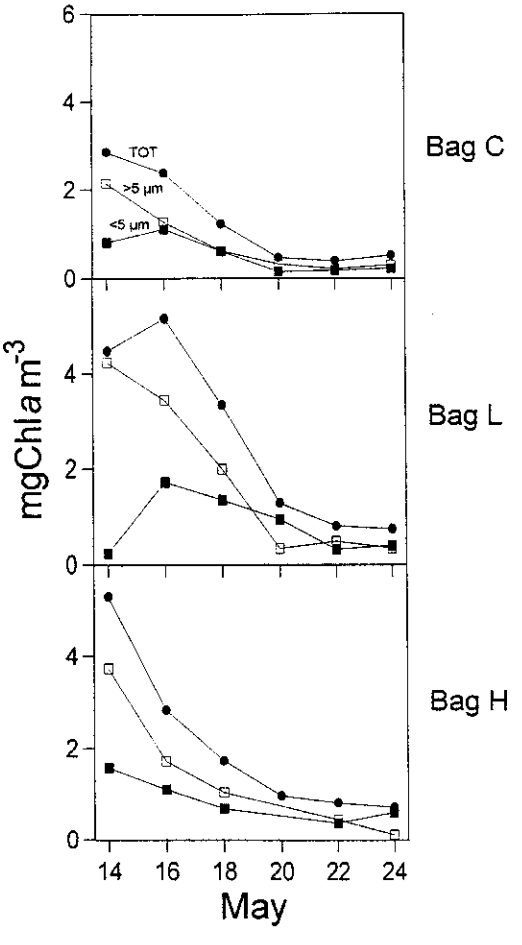

Fig. 1. Temporal variation of total chlorophyll *a* concentration (TOT, ●), chlorophyll *a* concentration in the smaller size fraction (< 5 μm, ■) and chlorophyll *a* concentration in the larger size fraction (> 5 μm, □) in each enclosure during the sampling period. Units are mg m^{-3}.

Protein-C and lipid-C accounted for a large proportion of the total particulate organic carbon throughout the experiment in the 3 enclosures, whereas

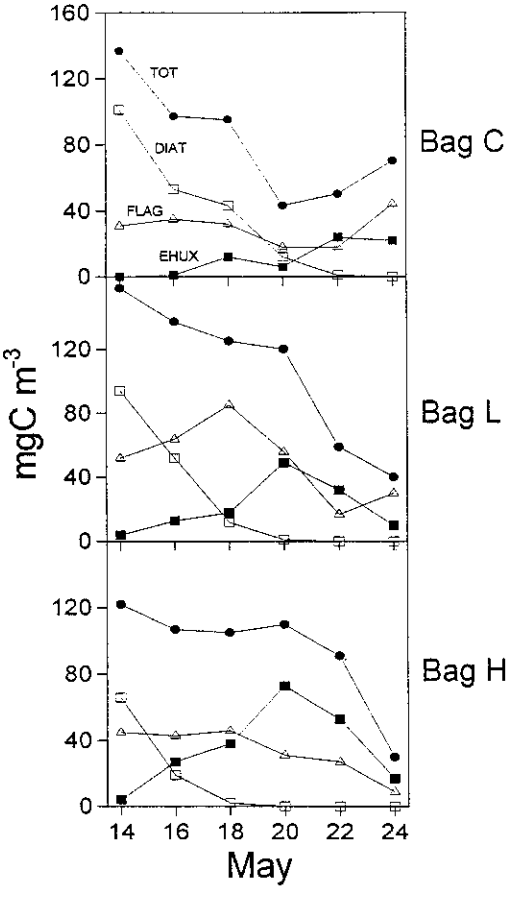

Fig. 2. Temporal variation of diatom (DIAT, □), flagellate (FLAG, △), *E. huxleyi* (EHUX, ■) and total (TOT, ●) phytoplankton estimated carbon biomass (mgC m^{-3}) in each enclosure during the sampling period.

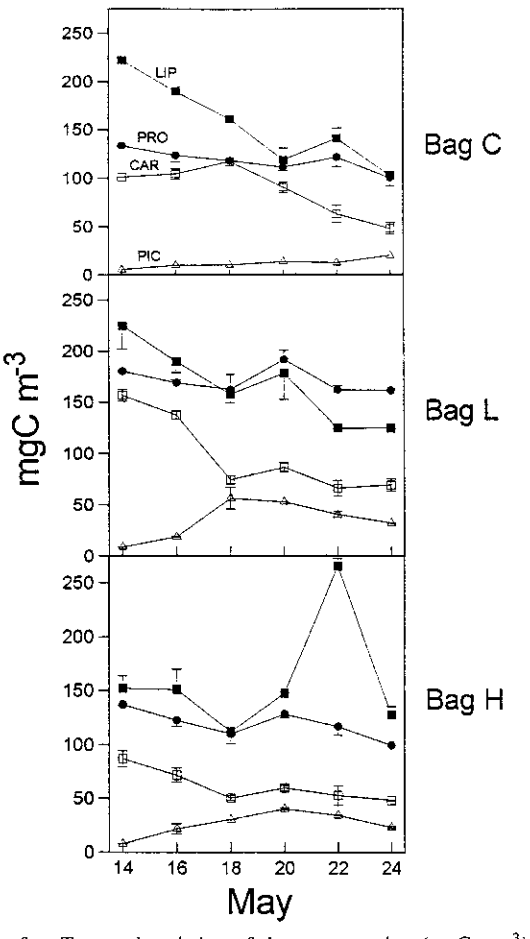

Fig. 3. Temporal variation of the concentration (mgC m^{-3}) of protein-C (PRO, ●), carbohydrate-C (CAR, □), lipid-C (LIP, ■) and particulate inorganic carbon (PIC) (PIC, △) in each enclosure during the sampling period. Bars represent ± 1 standard deviation ($n = 2$).

carbohydrate-C were always less important (Fig. 3). Lipids were the most abundant pool (150–220 mgC m^{-3}) at the beginning of the experiment in the 3 enclosures, representing about 40–50% of total organic carbon. The relative importance of this pool increased noticeably in bag H, where it reached values of up to 60%, just after the maximum of *E. huxleyi* biomass. Although protein-C concentration decreased during the sampling period, the relative contribution of this pool to total organic carbon increased from *c.* 30% to *c.* 40% in bags C and L, and remained unchanged in bag H. The concentration of carbohydrate-C decreased in all the bags during the experiment, and its relative contribution to total organic carbon was in the range 20–30% throughout the experiment.

The relative contribution of PIC to total particulate carbon, estimated as the sum of the 3 biochemical pools and PIC, ranged from *c.* 5% at the beginning of the experiment to maximum values of 15% during the last days of the sampling period. The PIC/POC ratio generally took lower values in bag C than in bags L and H.

Photosynthesis and calcification

The temporal variation of total photosynthesis during the experiment followed the same decreasing pattern in the 3 enclosures (Table 2), paralleling the changes in chlorophyll *a* concentration and diatom biomass (Figs 1–2). Photosynthetic rates ranged from initial rates of 50–80 mgC $m^{-3}d^{-1}$ at the beginning of the experiment to final values of *c.* 20 mgC $m^{-3}d^{-1}$.

Table 2. Total photosynthesis (Pho, mgC $m^{-3}d^{-1}$), calcification (Cal, mgC $m^{-3}d^{-1}$), calcification per *E. huxleyi* cell (Cal/cell, pgC $cell^{-1}d^{-1}$) and calcification to photosynthesis ratio (Cal/Pho) in the 3 enclosures during the experiment. Calcification data are not available for bag C. Values of the calcification to photosynthesis ratio refer to the larger (> 5 μm) size fraction (see Methods).

	Bag C	Bag L				Bag H			
Date	Pho	Pho	Cal	Cal/cell	Cal/Pho	Pho	Cal	Cal/cell	Cal/Pho
14th	51.1±4.6	81.8±4.8	4.6	16.8	0.07	71.1	1.5	4.3	0.03
16th	36.9±1.7	55.7±0.6	—	—	—	33.3	16.1±2.4	7.6±1.1	0.65±0.10
18th	14.7±1.3	44.4±2.1	—	—	—	28.6±1.7	—	—	—
20th	11.8±0.3	27.0±2.1	13.0±4.1	9.1±2.9	0.68±0.22	24.6±0.8	13.4±4.4	2.6±0.9	0.71±0.23
22nd	14.7±0.3	15.5±1.1	—	—	—	14.3±0.8	7.5±4.0	2.1±1.1	0.94±0.54
24th	25.2	17.3±0.6	3.5	7.3	1.09	17.1±0.2	2.5	4.4	0.42

Significant rates of carbon incorporation into coccoliths were measured in bags L and H (Table 2). Maximum rates of calcification were *c.* 15 mgC $m^{-3}d^{-1}$ and accounted for about 40% of total carbon incorporation. In both bags, the rates of calcification per *E. huxleyi* cell were relatively high during the first half of the experiment (16.8 pgC $cell^{-1}d^{-1}$ in bag L on 14 May and 7.6 pgC $cell^{-1}d^{-1}$ in bag H on 16 May), previous to the peak of the *E. huxleyi* bloom, then decreasing at the end of the sampling period. In contrast, maximum values of the calcification to photosynthesis ratio (about 1) were measured during the last days of the experiment, coinciding with the decline of the *E. huxleyi* bloom.

Size-fractionated photosynthesis and photosynthesis to chlorophyll a ratio

The decrease in total photosynthesis that took place during the first half of the experiment in the 3 enclosures (Table 2) was related to a sharp reduction in the photosynthetic rates of the larger size fraction (Fig. 4). Photosynthetic rates in this size fraction varied from 45–65 mgC $m^{-3}d^{-1}$ at the beginning of the experiment to final values of *c.* 10 mgC m^{-3} d^{-1}. In contrast, photosynthetic rates in the smaller size fraction did not show major temporal variations throughout the sampling period, taking values around 10 mgC $m^{-3}d^{-1}$. Thus, the relative contribution of the larger size fraction to total photosynthesis was about 80% on the first sampling days. At the end of the experiment, primary production of the smaller size fraction was similar to or even higher than that of the larger size fraction. Both the temporal changes in photosynthetic rate during the experiment and the observed differences between size fractions proved to be highly significant (ANOVAR, $p = 0.0001$).

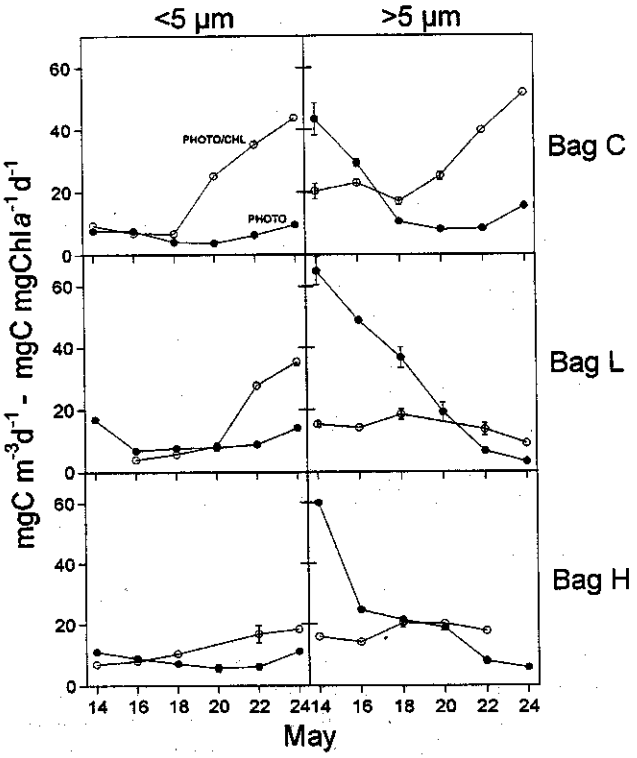

Fig. 4. Temporal variation of total photosynthetic rate (PHOTO, mgC $m^{-3}d^{-1}$, ●) and photosynthetic rate referred to chlorophyll *a* (PHOTO/CHL, mgC $mgChl^{-1}d^{-1}$, ○) in the smaller (< 5 μm) and the larger (> 5 μm) size fraction in each enclosure during the sampling period. Bars represent ± 1 standard deviation ($n = 2$).

In bag C, the photosynthesis to chlorophyll *a* ratio increased sharply towards the end of the experiment in both size fractions (Fig. 5). A slight increase of this ratio was also measured in the smaller size fraction in bags L and H, whereas no major changes were noticed in the larger size fraction.

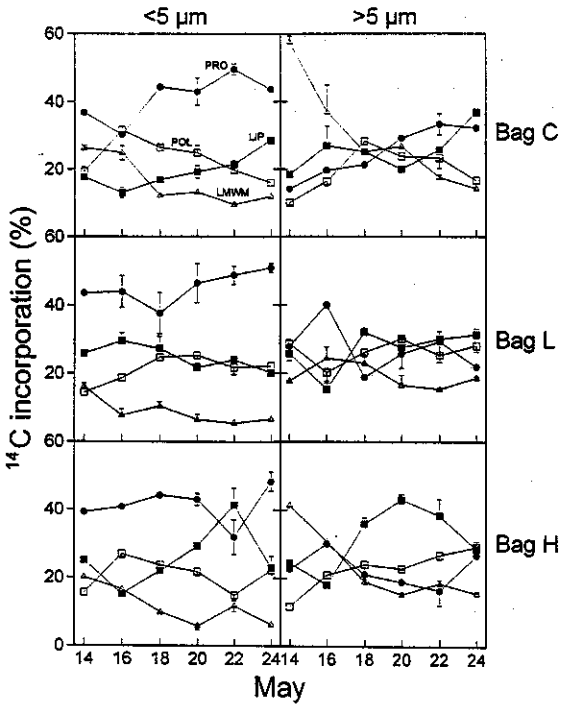

Fig. 5. Temporal variation of the percentage of ^{14}C incorporated into proteins (PRO, ●), polysaccharides (POL, □), lipids (LIP, ■) and LMWM (LMWM, Δ) in the smaller (< 5 µm) and the larger (> 5 µm) size fraction in each enclosure during the sampling period. Bars represent ± 1 standard deviation ($n = 2$).

Size-fractionated ^{14}C-partitioning

Significant differences were measured in the patterns of ^{14}C incorporation between both size fractions (Fig. 5). In the smaller size fraction, relative incorporation of carbon into protein was constantly high (*c.* 40%), whereas the percentage of ^{14}C incorporated into LMWM was less than 20% and decreased throughout the sampling period even reaching values as low as 10% by the end of the experiment. In contrast, relative carbon incorporation into proteins in the larger size fraction was below 30% during most of the sampling time. These differences in the relative carbon incorporation into proteins and LMWM between size fractions were statistically significant (ANOVAR, $p = 0.0001$). Carbon partitioning in the larger size fraction was roughly equitable, no pool being consistently dominant.

The percentage of carbon incorporated into lipids showed an increase in both size fractions in bag C and remained almost unchanged in bag L. Highest values of relative lipid synthesis (around 40%) were measured in the larger size fraction in bag H, coincident with the development of an *E. huxleyi*-dominated phytoplankton assemblage. Taking the results from the 2 size fractions in conjunction, the relative synthesis of proteins and lipids showed an increasing trend as the experiment progressed in the 3 enclosures, whereas the percentage of ^{14}C incorporated into LMWM followed the opposite trend.

Variations in the patterns of carbon partitioning during the phytoplankton succession

A summary of the characteristic patterns of photosynthesis, calcification and carbon metabolism for diatom-dominated, mixed and *E. huxleyi*-dominated assemblages is presented in Table 3. Total chlorophyll *a* concentration and photosynthesis were significantly higher during the declining phase of the diatom bloom, taking much lower values during the *E. huxleyi* bloom. As succession progressed, the relative contribution of the larger size fraction to total photosynthesis decreased significantly. Rates of calcification per *E. huxleyi* cell took higher values when the phytoplankton assemblage was still dominated by diatoms and the *E. huxleyi* bloom was in an early stage of development. In contrast, the calcification to photosynthesis ratio increased during the experiment and was highest (*c.* 1) in the *E. huxleyi*-dominated assemblage. The diatom-dominated assemblage showed a lower relative concentration of protein-C (30.2 ± 0.8), as compared with the other two assemblages. The percentage of carbon incorporation into LMWM was relatively high in the diatom-dominated assemblage (41.8 ± 5.6), whereas it took significantly lower values as the succession progressed. The variation in the relative synthesis of proteins followed the opposite trend, the lowest values (21.5 ± 2.2) being found in the diatom-dominated assemblage.

Discussion

The enclosure of natural phytoplankton populations in mesocosms did not substantially alter the patterns of species succession typical of Norwegian coastal waters during late spring and summer (Braarud *et al.*, 1974). The decline of the diatom-dominated assemblage as a result of the low levels of silicate was paralleled by the development of a bloom of the coccolithophorid *Emiliania huxleyi* (Fig. 2). The use of mesocosms allowed the study of early growth stages of a natural *E.*

Table 3. Average values (± standard error) of selected variables for diatom-dominated, *Emiliania huxleyi*-dominated and mixed assemblages in the 3 enclosures throughout the experiment. Dominance of each taxonomic group was established on the basis of a relative contribution to the total phytoplankton biomass higher than 50%. The number of observations for each assemblage is shown in parentheses. p indicates the significance of the observed differences between assemblages (one-way ANOVA). The last column shows the results of the Student-Newman-Keuls test ($p \leq 0.05$) carried out to test for differences between all paired assemblages (D, diatom; M, mixed; E, *E. huxleyi*). n.s., not significant.

	Assemblage				
	Diatom	Mixed	*E. huxleyi*	p	SNK
Variable	(4)	(6)	(5)		
Chl a (mg m^{-3})	3.7 ±0.7	1.5 ±0.4	0.7 ±0.1	0.0031	D>M=E
Protein-C (%)	30.2 ±0.8	37.3 ±1.6	39.2 ±2.0	0.0192	D<M=E
Carbohydrate-C (%)	25.8 ±1.5	24.2 ±2.0	17.2 ±1.3	0.0171	D=M>E
Lipid-C (%)	43.7 ±2.8	38.7 ±1.0	43.8 ±3.6	n.s.	D=M=E
Photosynthesis (mgC $m^{-3}d^{-1}$)	60.2 ±9.1	31.6 ±5.3	17.2 ±1.9	0.0171	D>M=E
Photosynthesis > 5 μm (%)	81.9 ±1.5	70.8 ±1.4	55.5 ±5.7	0.0001	D>M>E
Calcif/cell (pgC $cell^{-1}d^{-1}$)	10.6 ±6.2	8.4 ±0.7	2.4 ±0.3	n.s.	D=M=E
Calcif/photo	0.051±0.023	0.41±0.001	0.59±0.01	0.0001	D<M<E
Protein synthesis (%)	21.5 ±2.2	32.9 ±2.1	36.5 ±4.6	0.0222	D>M=E
Poly. synthesis (%)	16.8 ±3.0	25.0 ±1.2	22.8 ±0.5	0.0149	D<M=E
Lipid synthesis (%)	22.8 ±1.5	22.4 ±2.2	30.8 ±3.5	0.0320	E>M
LMWM synthesis (%)	41.8 ±5.6	21.0 ±1.4	12.3 ±1.2	0.0001	D>M>E

huxleyi assemblage, a situation not often encountered under sea-truth conditions, due to the fact that these blooms become detectable by satellite imagery when they are in an advanced state of development (Balch *et al.*, 1991; Fernández *et al.*, 1993).

The dynamics of species succession were similar in bags L and H, where *E. huxleyi* biomass declined sharply after 20 May (Fig. 2). Nitrate levels were high in both enclosures and phosphate concentration did not appear to limit *E. huxleyi* growth, as this species is known to form heavy blooms (around 100 10^6 cell l^{-1}) under phosphate concentrations lower than 0.2 μmol l^{-1} (Egge, 1993). Therefore, it is unlikely that nutrient limitation was the reason for the termination of the bloom in bags L and H. One possible explanation for the decline of the bloom could be given by the high abundance of microzooplankton (mainly the cilliates *Strombidium* spp. and *Lohmaniella* spp.) found during the last days of the experiment (Cabal & Harris, in prep.). Microzooplankton have previously been shown to graze efficiently on *E. huxleyi* blooms in oceanic waters (Holligan *et al.*, 193). Grazing by microzooplankton could also explain the fact that flagellate abundance did not show any increase in bag L, despite the high nitrate and phosphate concentrations available in this enclosure.

The high numbers of virus-like particles (VLPs) recorded in enclosures H and L during the last days of the experiment (Bratbak *et al.*, in prep.) suggest that the *E. huxleyi* bloom could have been terminated by viral activity, as it has been previously reported for other mesocosms blooms (Bratbak *et al.*, 1993). The same inverse relationship between *E. huxleyi* and VLP abundance was found during the decline of a coccolithophore bloom in a Norwegian fjord (Fernández *et al.*, submitted). The possible impact of viral activity on *E. huxleyi* growth agrees with the observation that the bloom of this species was more conspicuous in bag H than in bag L (73 mgC m^{-3} compared to 49 mgC m^{-3} on 20 May). Viral activity on *E. huxleyi* has been shown to be reduced when phosphate concentration is below 0.8 μmol l^{-1} (Bratbak *et al.*, 1993). Therefore, the higher growth of *E. huxleyi* in bag H, as compared to bag L, could be related to a lower viral activity in that enclosure, where phosphate levels were below 0.3 μmol l^{-1} throughout the experiment. In addition, the larger magnitude of the *E. huxleyi* bloom in bag H is in accordance with recent observations indicating that this species is a better competitor than other flagellates at low phosphate concentrations (Riegman *et al.*, 1992; Egge, 1993).

The ratio between total chlorophyll a and total phytoplankton biomass decreased markedly as species suc-

cession is progressed (Figs 1–2), which is in agreement with previous observations both in coastal (Balch *et al.*, 1991) and oceanic (Fernández *et al.*, 1993) systems showing low chlorophyll *a* levels during *E. huxleyi* blooms. During the peak of the *E. huxleyi* bloom, values of the ratio between total photosynthesis and *E. huxleyi* biomass were in the range 0.4–0.6 mgC $mgC^{-1}d^{-1}$, i.e., similar to or slightly lower than those found by Balch *et al.* (1992) and Fernández *et al.* (1993) in coastal and oceanic coccolithophorid blooms. Moreover, photosynthesis per chlorophyll *a* measured when *E. huxleyi* biomass was maximum (*c.* 20 mgC $mgChl\ a^{-1}d^{-1}$, bags L and H, 20 May) (Fig. 4) agreed with the values reported for an actively growing *E. huxleyi* population during the 1991 North Atlantic bloom (Fernández *et al.*, 1993). Our results show that the shift from a diatom-dominated assemblage to an *E. huxleyi*-dominated assemblage results in a sharp reduction of the phytoplankton standing stock and the rates of photosynthesis on a cellular carbon basis (Figs 2 and 4), which is in accordance wit the higher growth potential characteristic of diatoms as compared with non-diatom species (Chan, 1980; Furnas, 1990; Aksnes & Egge, 1991).

Both calcification rate and cell-specific calcite production showed maximum values during the developing stages of *E. huxleyi* growth, then decreasing as the bloom peaked and eventually collapsed (Fig. 2, Table 2). Accordingly, PIC synthesis rates took the highest values (0.4–0.5 d^{-1}) before maximum *E. huxleyi* abundance was attained. Maximum cell-specific rates of inorganic carbon production in this study were in the range 7–9 pgC $cell^{-1}d^{-1}$ (excluding the value of 16.8 pgC $cell^{-1}d^{-1}$ in bag L on 14 May), i.e. similar to the highest calcification rate reported for *E. huxleyi* cultures (9.6 pgC $cell^{-1}d^{-1}$, Balch *et al.*, 1992) and higher than the values found by Fernández *et al.* (submitted) and Kristiansen *et al.* (1994) [*c.* 4 pgC $cell^{-1}d^{-1}$] in sea-truth blooms. In both bags, the changes in calcification rate per *E. huxleyi* cell followed a decreasing trend as the bloom progressed (Table 2). These results provide a confirmation with natural *E. huxleyi* populations to previous observations in cultures which indicated that calcification is maximal during the exponential growth phase (Balch *et al.*, 1992), whereas the rate of calcite production is sharply reduced in the later stages of bloom development.

The patterns of carbon partitioning among different molecules changed considerably during the diatom-*E. huxleyi* succession (Fig. 5). The diatom-dominated assemblage was characterized by a high relative ^{14}C incorporation into low molecular weight metabolites (LMWM). This is a recurrent feature of the declining phase of diatom blooms (Morris & Skea, 1978; Li & Harrison, 1982; Madariaga & Fernández, 1990), which is explained by a reduction in the rates of macromolecular synthesis. In contrast, the *E. huxleyi*-dominated assemblage showed higher rates of carbon incorporation into proteins and, specially, lipids, as 31% of the total ^{14}C was channeled into the lipid fraction (Table 3). High rates of carbon incorporation into lipids have been previously reported for cultures of *E. huxleyi*, as well as in natural populations in the Norwegian fjords (Fernández *et al.*, 1994). In the present study, the relative contribution of *E. huxleyi* to the total phytoplankton biomass showed a highly significant correlation with the percentage of ^{14}C incorporated into lipids ($r^2 = 0.376$, $n = 18$, $p = 0.0068$). When *E. huxleyi* was clearly dominant, as in the last days of the experiment in bag H (Fig. 2), the increased carbon incorporation into lipids was reflected in a higher C-lipid concentration (Fig. 3). The increase in the lipid cellular content has been estimated to reduce cellular density of *E. huxleyi* by 6%, which would imply a 20% reduction in sinking rate (Fernández *et al.*, 1994). This effect, together with the small size characteristic of this species, would increase the residence time of the cells within the euphotic zone and therefore be relevant in understanding the dynamics of bloom formation and development.

Although the relative contribution of each size fraction to the total phytoplankton biomass remained relatively constant during most of the study (Fig. 1), the proportion of photosynthesis accounted for by the larger size fraction decreased significantly during the experiment as the *E. huxleyi* bloom developed (Fig. 4, Table 3). These data are coincident with the observations by Joint *et al.* (1993), who found a close association between the dominance of netplankton and the existence of biomass and productivity maxima during late spring in the northeast Atlantic. Similar results have been reported for a seasonal cycle in the Celtic Sea (Joint *et al.*, 1986), during early summer in the southern North Sea (Madariaga & Joint, 1994) and during the early spring bloom in Dutch coastal waters (Riegman *et al.*, 1993). If our findings prove to be valid for natural ecosystems, then the diatom-*E. huxleyi* succession may be considered as a mechanism whereby small phytoplankton increase their relative contribution to total microalgal biomass and productivity.

The patterns of ^{14}C incorporation among different cellular compounds proved to be size-dependent.

The smaller phytoplankton was characterized by very high relative synthesis of proteins (around 40–50%) throughout the study, whereas no pool was significantly dominant in the larger size fraction (Fig. 5). Increased carbon incorporation into proteins in small size microalgae has been previously found both in microcosms (Madariaga & Fernández, 1990) and natural conditions (Howard & Joint, 1989; Jones *et al.*, 1990), irrespective of the taxonomic composition of the phytoplankton assemblage. Reduced cellular size and the lack of vacuoles possibly prevent small cells from accumulation of large amounts of storage compounds. Cellular growth and division have been shown to be more dependent on the synthesis of proteins than on the synthesis of polysaccharides or lipids (Morris, 1981; Di Tullio & Laws, 1983). Therefore, the ability of small cells to channel most of the energy flow towards the synthesis of proteins agrees with the generally observed pattern of increasing maximum growth rate with decreasing cell size (see Chisholm, 1992). This metabolic strategy would be of advantage under growth-limiting conditions, as proteins are indispensable for the maintenance of basic cellular functions. The dominance of small cells with a preferential synthesis of proteins during phases of low phytoplankton growth rate would be in accordance with the observed maintenance of protein synthesis under adverse environmental conditions (Morris, 1981; Barlow, 1984; Marañón *et al.*, in press).

In summary, the results presented here show that the transition from the declining phase of the diatom bloom towards the development of an *E. huxleyi*-dominated assemblage resulted in a reduction of phytoplankton biomass and carbon-specific photosynthetic rates, together with an enhancement of the relative contribution of the smaller size fraction to total productivity. The late stage of the diatom bloom was characterized by a high percentage of carbon allocated into LMWM, whereas the development of the *E. huxleyi* bloom induced an increase in the relative carbon incorporation into proteins and lipids. The patterns of carbon metabolism were strongly size-dependent, and the smaller size fraction was always characterized by an enhanced carbon incorporation into proteins. Confirmation of these observations in open-ocean blooms appears now as the next step towards a full understanding of the effects of the coccolithophorid-diatom succession upon the patterns of carbon metabolism in natural phytoplankton assemblages.

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