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High rates of lipid biosynthesis in cultured, mesocosm and coastal populations of the coccolithophore *Emiliania huxleyi*

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ABSTRACT: The patterns of carbon incorporation into proteins, polysaccharides, lipids and low molecular weight metabolites and the resulting cellular biochemical composition were examined within cultured and natural populations of the coccolithophore *Emiliania huxleyi*. The flows of carbon incorporated through photosynthesis were primarily directed towards the synthesis of lipids (40 to 60%), mainly neutral lipids, whereas relative carbon incorporation into proteins was low (ca 20%), regardless of irradiance levels or growth stage. Actively dividing *E. huxleyi* cells showed higher rates of carbon incorporation into protein during darkness than during the previous light period, whereas under energy-limited growth conditions proteins produced during the light period were catabolized in darkness. The observed ^{14}C labelling patterns were consistent with both the measured biochemical composition of *E. huxleyi* cells growing in cultures under the same conditions and with that of coccolithophore-dominated natural phytoplankton assemblages. The relative decrease in cellular density caused by a lipid-rich composition could be significant for the buoyancy of cells and, consequently, for the dynamics of blooming events. Furthermore, the high-lipid, low-protein metabolism characteristic of this species implies an increase of carbon uptake relative to nitrogen that would, to some extent, counteract the enhancing effect of calcification on the partial pressure of CO_2 typical of blooms of *E. huxleyi*.

KEY WORDS: *Emiliania huxleyi* · Photosynthetic carbon metabolism · Biochemical composition · Lipids

INTRODUCTION

Emiliania huxleyi (Lohm.) Hay et Mohler is the most abundant coccolithophore species, being possibly the major producer of calcium carbonate in the biosphere (Westbroek et al. 1985). 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 & Keller 1993) reflects its capability to develop extensive blooms in coastal and oceanic environments (e.g. Holligan et al. 1983, Balch et al. 1991, Fernández et al. 1993, Brown & Yoder 1994) and explains why *E. huxleyi* is currently the subject of intensive investigation at both the cellular and ecological levels (Westbroek et al. 1993).

Relevant physiological research on *Emiliania huxleyi* has been centered primarily on the determination of carbon sources and the biochemical regulatory mechanisms involved in the process of carbon fixation through photosynthesis and calcification (Sikes et al. 1980, Sikes & Wheeler 1982, Nimer & Merrett 1992, Nimer et al. 1994), on the molecular basis of coccolith synthesis (de Jong et al. 1983), and also on the effect that diverse environmental variables exert upon the mechanisms of coccolith formation (see review in Klaveness & Paasche 1979). There is a lack of knowledge, however, on the environmental regulation of the fluxes of organic carbon in *E. huxleyi* and also on the wide range of ecophysiological processes related to cellular growth. Information of this kind is needed for

the development of dynamic models which take account of the physiological mechanisms involved in the formation, maintenance and collapse of blooms of this species, as well as on its biogeochemical implications.

In this work we have focused on the study of the patterns of photosynthetic carbon metabolism measured both in culture conditions and in naturally occurring blooms of *Emiliania huxleyi*, with the aim of determining the rates of carbon incorporation into the major cellular organic constituents and the derived cellular biochemical composition typical of this species. To our knowledge, these are the first results of this type reported for coccolithophorids.

METHODS

Culture experiments. Axenic cultures of a calcifying strain of *Emiliania huxleyi*, isolated from the Gulf of Maine (Bigelow Laboratory No. 88E), USA, were grown in 1000 ml glass culture vessels, maintained in an incubator at 16 °C under a 16 h light, 8 h dark photoperiod. The culture medium was prefiltered (0.4 µm), nutrient-poor sea water from the Gulf Stream collected off Miami, Florida, USA, enriched with a modified K medium (Keller et al. 1987). This modification consisted of (1) a 75% reduction in the final concentrations with respect to K medium and (2) the omission of ammonia and silicate. Inoculation of cells was conducted after autoclaving both glassware and medium. All experiments were started with cultures in the exponential phase of growth and a cellular density of 2 to 3×10^4 cells ml^{-1}. Irradiance in the incubator was 200 µE m^{-2} s^{-1} provided by cool-white fluorescent lamps. Low irradiance conditions (50 µE m^{-2} s^{-1}) were obtained by using neutral density plastic screens. Triplicate culture vessels were maintained in parallel under the low and high irradiance levels with cellular growth rates of 0.81 ± 0.04 and 0.49 ± 0.01 d^{-1}, respectively. Cells were harvested daily from each of the 6 culture vessels for the estimation of cell abundance. Samples for the determination of the biochemical composition and the patterns of ^{14}C incorporation into photosynthetic end-products were collected also from each of the 6 culture vessels during the logarithmic and stationary phases of growth.

Field studies. The mesocosms experiment was carried out from 12 to 24 May 1993 in a bay adjacent to Raunefjorden, 20 km south of Bergen, western Norway. The enclosures were made of polyethylene, with a volume of 11 m^3 and open to the air (for details see Egge & Aksnes 1992) The bags were filled with water from 1 m depth, and supplied with nutrients at different N:P ratios. Stirring was achieved by an airlift system that pumped water from the bottom of the enclosures to the surface at a rate of ca 40 l min^{-1} Each day, 10% of the enclosed water was continuously renewed with water from 1 m depth. Only the results from the bag with a N:P ratio of 16:0.2, in which an almost monospecific bloom of *Emiliania huxleyi* took place, are shown in this paper.

A naturally occurring bloom of *Emiliania huxleyi* was subsequently sampled on board RV 'Hans Brattström' at a station located in Nordåsvannet fjord (western Norway) on 27 May 1993. At this station, water samples were taken for the determination ashore of salinity, nutrients, phytoplankton cell counts, particulate biochemical composition and flows of carbon into photosynthetic end-products.

Incubation procedures and analytical methods. The abundance of *Emiliania huxleyi* in cultures was determined using a Palmer-Maloney counting cell. Natural microplankton samples were preserved in Lugol's iodine solution and buffered formaldehyde. For each sample, microplankton cells were identified, enumerated and the biomass estimated as described by Holligan et al. (1984).

The concentrations of particulate proteins, carbohydrates and lipids were determined following the methods of Lowry et al. (1951), Dubois et al. (1956) and Barnes & Blackstock (1973), respectively. The carbon content by weight of each biochemical pool was assumed to be 53% for proteins, based on the average amino acid composition of microalgae (Laws 1991), and 40 and 83% for carbohydrates and lipids, respectively, as calculated from the mean elemental composition of each biochemical pool (Fraga & Pérez 1990).

In laboratory experiments, photosynthetic carbon incorporation into cellular constituents was determined by transferring two 10 ml aliquots drawn from each of the 6 culture vessels into acid-washed, 20 ml glass scintillation vials, and inoculating them with $NaH^{14}CO_3$ to provide a final activity of 0.5 to 0.6 µCi ml^{-1} (18.5 to 22.2 kBq ml^{-1}), which was measured immediately after addition of the isotope. The vials were immediately placed in the same incubator as the original cultures and the 2 aliquots incubated for 16 and 24 h, respectively, to examine the overnight redistribution of radioactivity within intracellular metabolic products. Incubations started immediately after lights were switched on early in the morning. In field studies, triplicate 70 ml acid-cleaned polycarbonate bottles were filled with the sample, radioactively labelled as detailed above with 10 µCi (370 kBq) of $NaH^{14}CO_3$ and suspended at 1 m depth from a buoy placed in the vicinity of the enclosures. In both cases, sampling was conducted early in the morning (between 07:00 and 09:00 h). During the fjord cruise, water samples collected were transferred into the incubation bottles, inoculated as described above and placed in an artificial-light incubator that simulated a range of irradiance

levels corresponding to the irradiances experienced by the algae at the sampling depths. Samples were cooled with circulating water. Incubations carried out with water from the mesocosm and the fjord lasted 24 h. At the end of the incubation period, samples were filtered onto 0.45 µm (laboratory experiments), 0.2 µm (fjord study) or 5 µm polycarbonate filters (mesocosm study). A larger pore size was selected for the mesocosm study with the aim of partially overcoming the 'noise' added by species other than *Emiliania huxleyi*. Filters were exposed for 3 to 5 min to concentrated HCl fumes. This decontamination treatment removed any $NaH^{14}CO_3$ remaining in the filter as well as the $Ca^{14}CO_3$ synthesized by coccolithophorids through calcification. Filters were then transferred into plastic scintillation vials and stored frozen at −20 °C until further analysis.

The organic material retained on the filters was separated into 4 major biochemical constituents: proteins, polysaccharides, lipids and low molecular weight metabolites (LMWM). The fractionation was based on a serial extraction routine modified from Li et al. (1980) and Lohrenz & Taylor (1987), as described in E. Marañón, E. Fernández & R. Anadón (unpubl.), which partitions the original sample into methanol/water-soluble compounds (LMWM), chloroform-soluble compounds (lipids), hot-trichloroacetic-acid-insoluble compounds (proteins) and hot-trichloroacetic-acid-soluble compounds (polysaccharides and nucleic acids). Parallel experiments showed that 93 to 100% (n = 24) of the ^{14}C was recovered as compared with independently measured total carbon fixation. The amount of radioactivity in the samples was measured by liquid scintillation counting and quenching corrected by the channels ratio method. The percentage of night ^{14}C reallocation for each biochemical pool was determined according to:

$$\% \text{ reallocation} = (D_{24} - D_{16}) \times 100/D_{16}$$

where D_{24} is ^{14}C incorporation over the 24 h incubation and D_{16} is ^{14}C incorporation over the 16 h incubation under continuous light.

Lipids were separated by silica gel chromatography using the same solvent phases as in Smith & D'Souza (1993), with the exception of the methanol phase (phase 6) that was not used. The technique enables fractionation of the sample into 3 lipid classes: (1) neutral lipids, eluted with a chloroform + 1% methanol phase, (2) polar lipids (largely glycolipids) eluted with 1:1 chloroform:acetone and pure acetone phases and (3) phospholipids, eluted with 2:1 chloroform:methanol and 1:1 chloroform:methanol phases. Each elution was allowed to dry and the radioactivity measured as described above. Recovery values for ^{14}C ranged between 85 and 95%.

RESULTS

Culture experiments

Daily patterns of carbon incorporation into end products of photosynthesis were examined during the exponential and stationary phases of growth in cultures of *Emiliania huxleyi*. Cell-specific carbon incorporation rates and the percentages of carbon incorporated into each biochemical pool are shown in Table 1 and Fig. 1A, respectively.

As expected, the rates of carbon incorporation into protein were lower with decreasing irradiance (Table 1). The relative incorporation of carbon into protein did not show significant differences between the 2 irradiances (200 and 50 µE m^{-2} s^{-1}) and growth phases tested (ANOVA: *F*-ratios 0.007 and 2.345; p = 0.933 and 0.141, respectively), with percentages of incorporation around 20% of the total photosynthetically incorporated carbon (Fig. 1A).

The most significant result obtained with cultures was that large amounts of carbon were fixed into the lipid fraction regardless of the irradiance level and growth phase. Cell-specific rates of carbon incorporation into lipids varied from 2.56 pg C $cell^{-1}$ d^{-1} in cells growing exponentially under low irradiance to more than 6.69 pg C $cell^{-1}$ d^{-1} during the stationary phase of high-irradiance growing cells (Table 1), and accounted for 30 to 50% of the total photosynthetically fixed carbon. Differences between growth phases were significant with more carbon flowing into lipids when cell division slowed or ceased (ANOVA: *F*-ratio = 53.5; p = 0.0001) (Fig. 1A). Relative carbon incorporation into LMWM followed an opposite trend to that into lipids. Only 10 to 15% of the carbon was fixed into polysaccharides (Fig. 1A).

Table 1. *Emiliania huxleyi*. Daily (24 h) cell-specific rates (± SE, n = 3) of carbon incorporation (pg C $cell^{-1}$ d^{-1}) into photosynthetic end-products for batch cultures (strain 88-E) maintained at high (200 µE m^{-2} s^{-1}) or low (50 µE m^{-2} s^{-1}) irradiance levels during the logarithmic (LOG) and stationary (STAT) phases of growth. Cellular growth rates during the logarithmic phase were 0.81 ± 0.04 and 0.49 ± 0.01 d^{-1} for high and low irradiance cultures, respectively. LMWM: low molecular weight metabolites

Treatment	Proteins	Lipids	Polysaccharides	LMWM
LOG-200	3.96 ± 0.64	6.69 ± 0.68	2.98 ± 0.18	7.47 ± 0.51
LOG-50	1.74 ± 0.27	2.56 ± 0.13	0.77 ± 0.06	1.88 ± 0.09
STAT-200	1.67 ± 0.22	3.64 ± 0.08	1.04 ± 0.07	1.09 ± 0.04
STAT-50	1.07 ± 0.16	2.89 ± 0.14	0.67 ± 0.06	0.83 ± 0.09

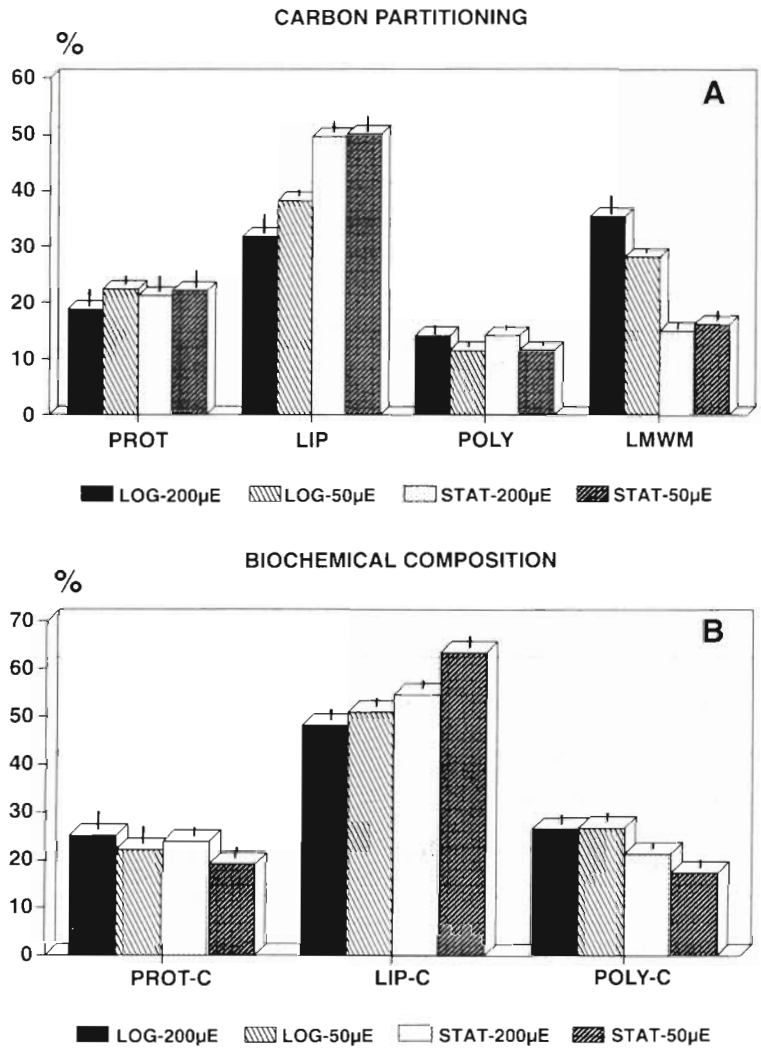

Fig. 1. *Emiliania huxleyi*. (A) Percentages of daily (24 h) ^{14}C incorporation into proteins, lipids, polysaccharides and low molecular weight metabolites (LMWM) and (B) relative concentrations of protein-C, lipid-C and polysaccharide-C, measured for independent batch cultures (strain 88E; Gulf of Maine) growing in modified K medium at 16 °C and a 16 h light:8 h dark photoperiod under low (50 µE m^{-2} s^{-1}) and high (200 µE m^{-2} s^{-1}) irradiance during the logarithmic (LOG) and stationary (STAT) phases of growth. Cellular growth rates during the logarithmic phase were 0.81 ± 0.04 and 0.49 ± 0.01 d^{-1} for high and low irradiance cultures, respectively. Error bars represent standard errors (n = 3)

In this case, differences in relative carbon incorporation between treatments were not statistically significant (ANOVA; $p > 0.05$).

Table 2. *Emiliania huxleyi*. Cellular content (± SE, n = 3) (pg C $cell^{-1}$) of protein-C, lipid-C and polysaccharide-C in coccolithophores (strain 88-E) growing under high (200 µE m^{-2} s^{-1}) or low (50 µE m^{-2} s^{-1}) irradiance levels during the logarithmic (LOG) and stationary (STAT) phases of growth. Cellular growth rates during the logarithmic phase were 0.81 ± 0.04 and 0.49 ± 0.01 d^{-1} for high and low irradiance cultures, respectively

Treatment	Protein-C	Lipid-C	Polysaccharide-C
LOG-200	4.7 ± 0.8	9.1	4.9 ± 0.1
LOG-50	3.3 ± 0.6	7.9 ± 1.2	4.0 ± 0.5
STAT-200	5.0 ± 0.2	11.9 ± 0.7	4.4 ± 0.2
STAT-50	3.0 ± 0.2	10.2 ± 0.4	2.7 ± 0.2

The patterns of ^{14}C labelling of the major biochemical pools described above were consistent with measurements of the biochemical composition of *Emiliania huxleyi* cells growing under the same conditions (Table 2, Fig. 1B). A large proportion of the cellular carbon, between 48 and 64 %, was present in the cells in the form of lipids, whereas protein-C and polysaccharide-C each accounted for approximately 20 % of the total carbon.

Day-night changes in ^{14}C photosynthate labelling patterns were also measured (Table 3). Labelled carbon was incorporated into protein during the night except during the stationary phase of low-irradiance growing cells. During the exponential growth phase, the amount of carbon flowing into protein in darkness was similar or even higher than that measured in the light period. About 50 % of the carbon fixed into polysaccharides during the light phase was either respired or reallocated in darkness in all the treatments tested. Dark losses of car-

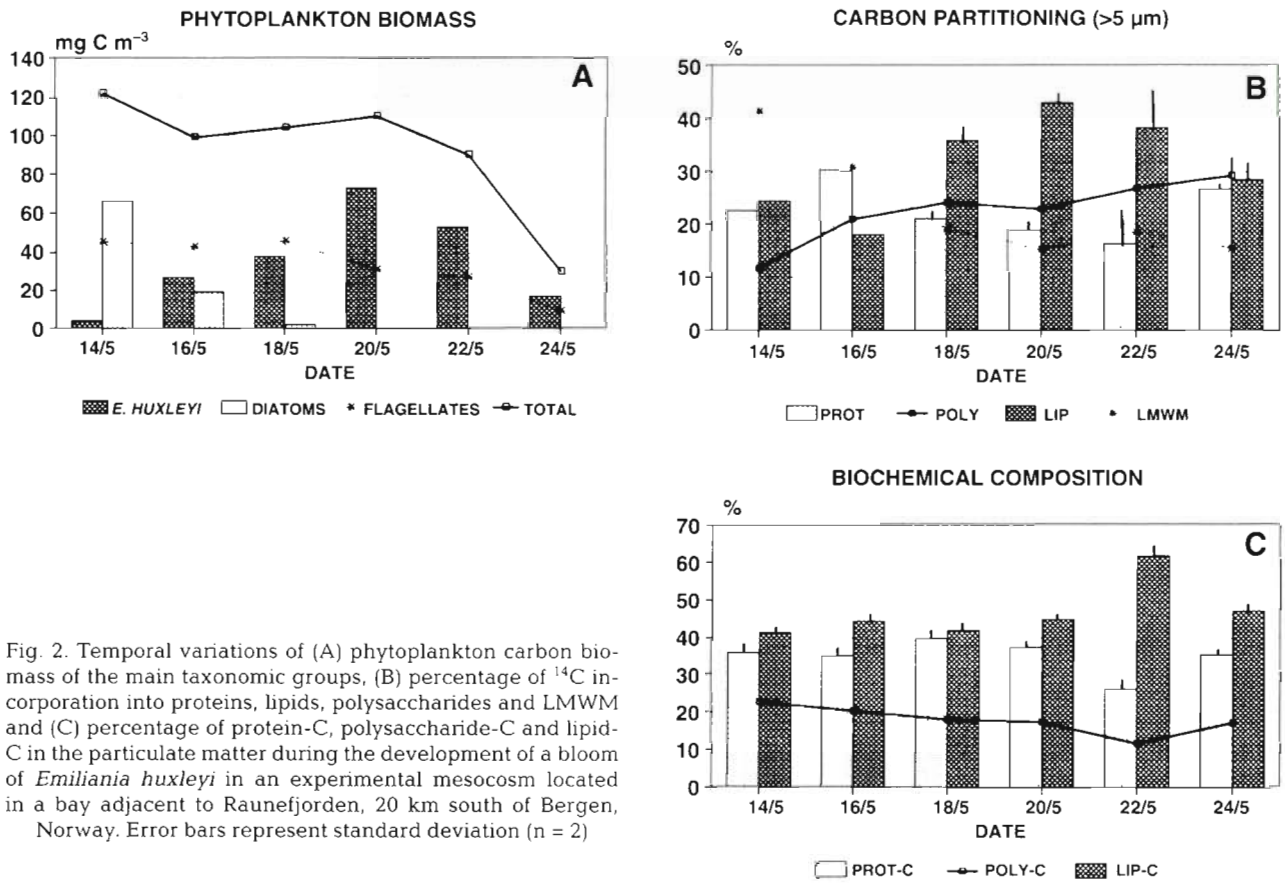

Fig. 2. Temporal variations of (A) phytoplankton carbon biomass of the main taxonomic groups, (B) percentage of ^{14}C incorporation into proteins, lipids, polysaccharides and LMWM and (C) percentage of protein-C, polysaccharide-C and lipid-C in the particulate matter during the development of a bloom of *Emiliania huxleyi* in an experimental mesocosm located in a bay adjacent to Raunefjorden, 20 km south of Bergen, Norway. Error bars represent standard deviation (n = 2)

bon in the lipid and LMWM fractions were less pronounced than in the case of polysaccharides, with percentages of dark reallocation ranging from 1 to 20%.

Field studies

Carbon incorporation into the major biochemical constituents was also studied during a bloom of *Emiliania huxleyi* which developed in an experimental mesocosm (Fig. 2). *E. huxleyi* started to grow during the initial phase of the experiment, following the decline of a diatom assemblage (Fig. 2A). By 20 May, it reached maximum biomass values of about 75 mg C m^{-3} corresponding to cell densities of up to 5000 cells ml^{-1}. By that date, *E. huxleyi* made up a large proportion of total phytoplankton biomass (67%), declining thereafter until the end of the experiment. Flagellate abundance remained constant throughout the experiment, their estimated biomass being slightly higher than 40 mg C m^{-3} during most of the period studied. Temperature in the enclosure ranged from 8.8 to 11.4°C. Nitrate concentration was always above 9.5 µM, whereas phosphate levels varied from 0.24 to 0.14 µM.

Incorporation of ^{14}C into lipids followed the same trend as that described for the development of the *Emiliania huxleyi* bloom (Fig. 2B). Percentages of incorporation were about 20% at the early stages of the study, then increasing up to 40% coinciding with the peak in *E. huxleyi* biomass. By contrast, the percentage of

Table 3. *Emiliania huxleyi*. Percentage (± SE, n = 3) of dark reallocation of the major biochemical pools in batch cultures (strain 88-E) maintained at high (200 µE m^{-2} s^{-1}) or low (50 µE m^{-2} s^{-1}) irradiance levels during the logarithmic (LOG) and stationary (STAT) phases of growth. Cellular growth rates during the logarithmic phase were 0.81 ± 0.04 and 0.49 ± 0.01 d^{-1} for high and low irradiance cultures, respectively. For details on calculations see 'Methods'

Treatment	Proteins	Lipids	Polysaccharides	LMWM
LOG-200	159.7 ± 20.2	−19.6 ± 6.8	−55.8 ± 14.6	−3.8 ± 11.4
LOG-50	118.7 ± 50.5	−0.9 ± 16.8	−42.8 ± 5.8	−21.9 ± 2.9
STAT-200	8.9 ± 0.7	−3.7 ± 4.8	−41.9 ± 0.5	1.3 ± 4.3
STAT-50	−14.1 ± 0.3	−7.2 ± 4.7	−42.3 ± 4.5	−2.7 ± 9.3

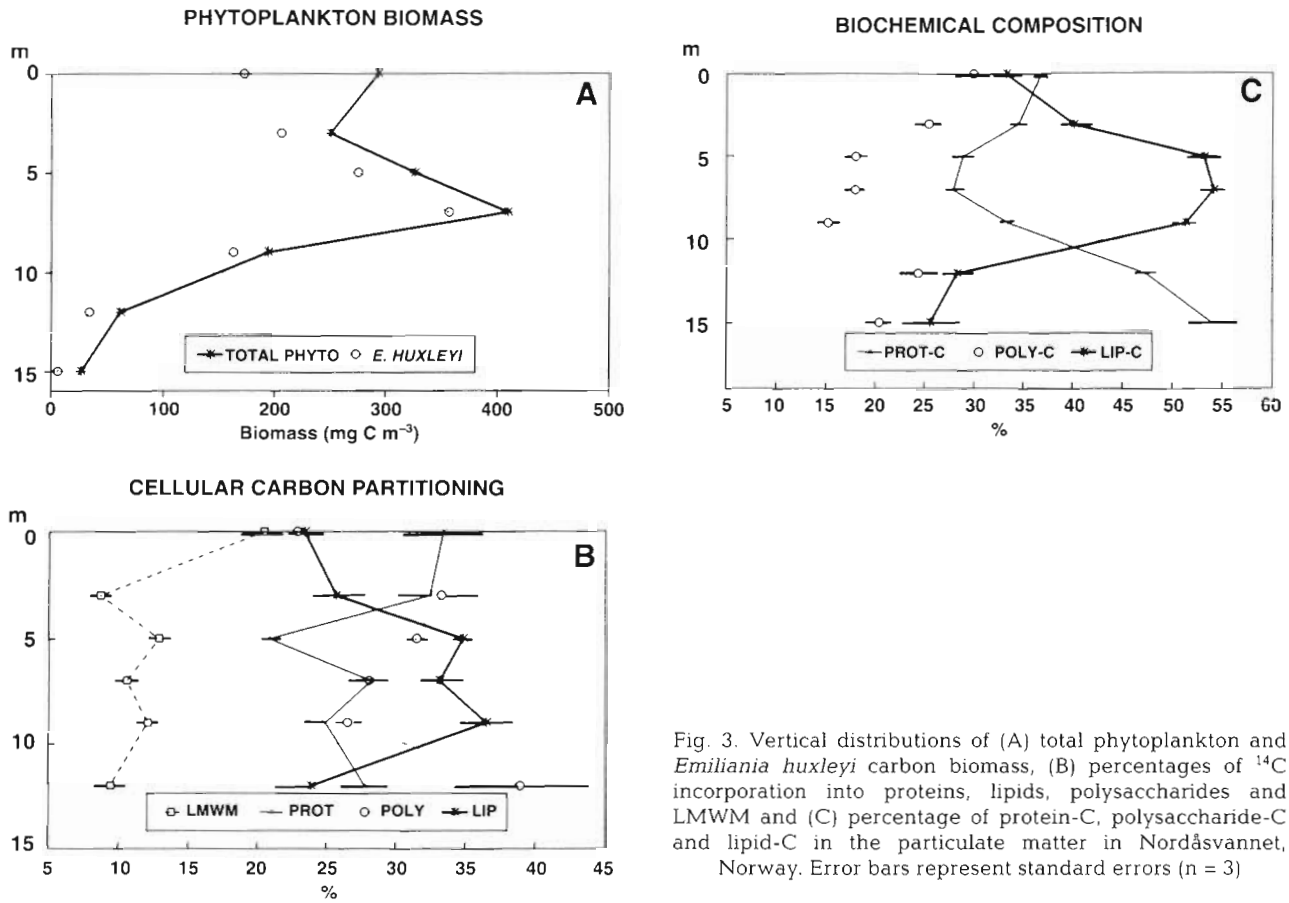

Fig. 3. Vertical distributions of (A) total phytoplankton and *Emiliania huxleyi* carbon biomass, (B) percentages of ^{14}C incorporation into proteins, lipids, polysaccharides and LMWM and (C) percentage of protein-C, polysaccharide-C and lipid-C in the particulate matter in Nordåsvannet, Norway. Error bars represent standard errors (n = 3)

carbon incorporated into proteins was lower (around 20%) during the coccolithophorid bloom. Relative carbon incorporation into polysaccharides displayed a steady increase throughout the experimental period, whereas incorporation into LMWM followed the opposite trend. The correspondence between the patterns of daily ^{14}C incorporation and chemical composition was less good than in the case of cultures (Fig. 2C), as expected given the contribution of non-phytoplankton material to the composition of particulate matter. Nevertheless, a maximum in the relative contribution of lipid-C to total particulate carbon was measured during the declining phase of the coccolithophorid bloom;

Table 4. Percentage (± SE, n = 3) of ^{14}C incorporation into different lipid classes from *Emiliania huxleyi*-dominated assemblages sampled at 7 m and 12 m in Nordåsvannet fjord, Norway (see Fig. 3)

Lipid fraction	7 m	12 m
Neutral lipids	74.7 ± 2.4	63.2 ± 2.6
Glycolipids	2.7 ± 0.4	11.1 ± 2.0
Phospholipids	22.6 ± 2.0	25.75 ± 2.0

the time lag can be explained in terms of the transfer and accumulation of lipids within the planktonic food web.

Validation of these observations was sought during a field survey conducted on 27 May in Nordåsvannet fjord (Fig. 3). The physical structure of the water column was characterized by a low-salinity layer in the upper 3 m, whereas temperature decreased continuously in the upper 7 m. Nitrate concentration was undetectable in the upper 10 m, then increasing sharply downwards to 3.34 µM at 15 m. Phosphate and silicate levels ranged between 0.13 and 0.31 µM and 0.38 and 0.50 µM, respectively, throughout the upper water column. The biomass of phytoplankton was elevated in the nitrate-depleted layer, with a subsurface maximum at about 7 m (Fig. 3A). *Emiliania huxleyi* made up most of the total biomass of phytoplankton, this contribution being greatest (up to 87%) below the halocline. The diatom *Skeletonema costatum* was dominant in the upper low-salinity layer.

The vertical distribution of photosynthate partitioning observed in Nordåsvannet fjord is comparable to the main patterns described above for culture and mesocosm populations (Fig 3B). The percentage of carbon incorporation into lipids increased below the

halocline, where *Emiliania huxleyi* became dominant, to a maximum of 37%. The marked shift in relative carbon incorporation into LMWM was also related to the change in species composition associated with the halocline; in close agreement with the results obtained in the mesocosm experiment (Fig. 2B), much higher relative carbon incorporations into LMWM were measured in the upper layer, where diatoms were present. The percentages of carbon incorporated into protein and polysaccharides did not show any distinct vertical pattern. The distribution of the relative contribution of lipid-C to total particulate carbon mirrored the pattern of *E. huxleyi* abundance (Fig. 2C), with a maximum of ca 55% at 7 m. The vertical distribution of the contribution of protein-C was inversely correlated to that of lipid-C.

The partitioning of radioactive label among lipid classes was determined on subsurface phytoplankton assemblages (7 and 12 m) from Nordåsvannet (Table 4). Most of the carbon incorporated photosynthetically into lipids (63 to 75%) was directed towards the synthesis of neutral lipids at both depths. Phospholipids made up 20 to 25% of the total incorporation, whereas incorporation into glycolipids was comparatively low.

DISCUSSION

The results presented in this study conclusively demonstrate that the flows of carbon incorporated through photosynthesis in the bloom-forming coccolithophorid *Emiliania huxleyi* are primarily directed towards the production of lipids. Relative carbon incorporation into protein is as low as 20%, compared with typical percentages of incorporation around 40 to 50% reported for an extended series of species growing in cultures and also in natural phytoplankton populations (see reviews by Morris 1981, Laws 1991). It is interesting to note that these low percentages of carbon allocation into protein by *E. huxleyi* were measured even during active phases of growth. This finding suggests that the proportion of photosynthate allocated into protein is not necessarily a good estimator of relative growth rate for *E. huxleyi* as it appears to be for other phytoplankton species (Di Tullio & Laws 1983, 1986).

However, the patterns of dark carbon reallocation into proteins appears to be related to cellular growth rate (Table 3). Actively dividing *Emiliania huxleyi* cells showed rates of ^{14}C incorporation into protein in darkness even higher than during the previous light period whereas, under intense energy-limited growth conditions, the labelled carbon fixed into proteins during the light period was catabolized in darkness to maintain cellular metabolic processes. Furthermore, carbon reallocation into proteins, when measurable, was largely sustained by polysaccharide catabolism, as the lipid pool was not generally mobilized in darkness. This pattern suggests that the lipid pool in *E. huxleyi* is made up of 2 different fractions which behave in a relatively conservative way. These fractions are either related to structural functions or act as a long-term storage product with relatively low turnover. The results also allow us to conclude that dark carbon reallocation into proteins, in conjunction with cellular sorting techniques (Rivkin 1985), is potentially a reliable estimator of *in situ* relative growth rate in natural populations of *E. huxleyi*.

Typical values for carbon incorporated into lipids varied within the range 5 to 30%, with an average of 15 to 20% (see Wainman & Lean 1992 and references therein). These are in good agreement with the results of Shifrin & Chisholm (1981) who showed that the proportion of a cell that is lipid in nutrient-replete phytoplankton is, on average, on the order of 20%. In *Emiliania huxleyi*, however, both relative carbon incorporation and carbon content were high, ranging from 40 to 60%. Large stores of lipid are known to build up under severe nutrient stress by phytoplankton species belonging to different taxonomic classes (e.g. Shifrin & Chisholm 1981, Taguchi et al. 1987). However, it is also known that the production of this compound is regulated by environmental factors in a manner that is not always systematic, and can be quite species specific (Shifrin & Chisholm 1981). In this study, although the percentage of carbon incorporation into lipids increased as growth became nutrient limited, high carbon allocation to this pool was also measured in exponentially growing cells (Fig. 1A) and in early stages of natural blooms of *E. huxleyi* (Fig. 2B). It is, therefore, concluded that *E. huxleyi* cells are characterized by a high-lipid, low-protein metabolic behaviour regardless of growth stage.

Evidence available to date suggests that this metabolic pattern is not exceptional, as it has been shown in other phytoplankton species, most of them small phytoflagellates. Madariaga (1992), studying the patterns of ^{14}C allocation into photosynthetic end-products in different phytoplankton species, found that typical values of relative carbon incorporation into lipids ranged from 10 to 20%, except in the prymnesiophyte *Pavlova lutheri*, which displayed a value of 40% throughout the whole growth period, consistent with the chemical composition reported for this species (Emdadi & Berland 1989). *Isochrysis galbana*, also Prymnesiophyceae, showed as well a high percentage of lipid content (Thomson et al. 1993). Furthermore, investigations carried out both in enclosed experimental ecosystems (Morris et al. 1985) and in open ocean waters (Fernández et al. 1994) have shown that natural

populations dominated by small flagellates and coccolithophores are typically lipid-rich and protein-poor as compared to other taxonomic groups.

A large proportion of the carbon incorporated into the lipid fraction was found as neutral lipid (Table 4), confirming previous reports on the importance of this lipid fraction in phytoplankton blooms (e.g. Morris et al. 1983). It has been demonstrated, however, that the composition of neutral lipids in *Emiliania huxleyi* differs widely from that found in other microalgae. Thus, triacylglycerols only account for a minor proportion of this lipid fraction, whereas a series of very-long-chain alkenones are the main contributors to this pool (Volkman et al. 1980). It is well known that the amount of neutral lipid rises markedly in algal cultures as a consequence of aging or nitrogen limitation (Lewin 1962). In this case, however, although nitrate levels were below the limit of detection, both estimated doubling times (about 4 d in spite of the low irradiance levels) and cell-specific calcification rates of up to 2 pg C $cell^{-1}$ indicated that *E. huxleyi* cells were still growing, albeit slowly. This apparent discrepancy is likely to be attributable to the different biological function of the neutral lipid pool characteristic of this species.

The ecological implications of lipid accumulation by *Emiliania huxleyi* are uncertain. One consequence of a lipid-rich cellular composition is a reduction in cellular density. The significance of this effect for cell sinking rates was evaluated by assessing the relative differences in density accounted for by differences in the biochemical composition in a similar way to that described by Smayda (1970). Characteristically, phytoplankton cells are made up of 50% proteins, 30% carbohydrates and 20% lipids (see e.g. Morris 1981, Laws 1991). By comparison, for *E. huxleyi*, assuming that 25 to 30% of the cellular mass in actively growing cells is calcium carbonate in the form of coccoliths, as shown from culture work (Fernández et al. unpubl.), the decrease in density due to the high-lipid cellular composition was estimated to be about 6%. By applying Stokes' law, this reduction in cellular density can be directly translated into a sinking rate decrease of about 20%. In his classic review on phytoplankton sinking processes, Smayda (1970) concluded that fat accumulation is generally an unimportant suspension mechanism in phytoplankton. As an example, this author estimated the difference in cellular density of the high-lipid (40%), bloom-forming diatom *Coscinodiscus concinnus* as compared to typical cells, and showed that this characteristic chemical composition represented a decrease in density of only 3.5%, which, given the large size of this species, would imply a negligible change in absolute sinking rate. The estimations presented in this paper suggest, however, that in *E. huxleyi* the joint effect of a slight reduction in cellular density due to the effect of lipid storage and the small size characteristic of this species would account for a significant increase of the residence time of cells in the euphotic layer, in spite of the presence of high-density calcareous structures (coccoliths) and, therefore, could be relevant for the understanding of bloom formation and development. The significance of a reduction in cellular density would be that much greater for naked (non-calcifying), non-motile stage of *E. huxleyi* (N-cells), which may appear in the late phases of development of *E. huxleyi* blooms (Westbroek et al. 1993).

The photosynthetic carbon metabolism characteristic of *Emiliania huxleyi* populations has the potential to effectively alter the ratio of carbon to nitrogen uptake from sea water and, consequently, influence the cycling of carbon in surface oceanic environments. Thus, simple stoichiometric calculations based on the average elemental composition of the major biochemical compounds indicate that whilst the biosynthetic processes leading to the formation of 'normal' cells (see discussion above) would yield a C:N uptake ratio very close to the Redfield ratio, this ratio would be very much higher, approximately 14, in the case of a high-lipid, low-protein metabolism such as that described here for *E. huxleyi*. Such a pattern of organic carbon biosynthesis would, therefore, lead to an increase of carbon uptake relative to nitrogen that would, to some extent, counteract the enhancing effect of calcification on the partial pressure of CO_2 found in blooms of *E. huxleyi* (Holligan et al. 1993, Robertson et al. 1994). On the other hand, if, as some evidence suggests, this photosynthetic metabolism proved to be typical of a more general group of organisms, mainly involving small flagellates (Madariaga 1992, Fernández et al. 1994), it can be then postulated that changes in phytoplankton community structure would exert a significant modulating effect upon the dynamics of the inorganic carbon system in surface oceanic waters.

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