ELSEVIER

Journal of Marine Systems 9 (1996) 57–66

JOURNAL OF MARINE SYSTEMS

Intracellular carbon partitioning in the coccolithophorid *Emiliania huxleyi*

Emilio Fernández [a], Emilio Marañón [b], William M. Balch [c]

[a] *Dpto. Recursos Naturais e Medio Ambiente, Facultade de Ciencias do Mar, Campus Lagoas-Marcosende, Universidade de Vigo, E-36200 Vigo, Spain*

[b] *Dpto. Biología de Organismos y Sistemas, Campus del Cristo, Universidad de Oviedo, E-33071 Oviedo, Spain*

[c] *Bigelow Laboratory, McKown Point, West Boothbay Harbor, ME 04575 USA*

Received 26 May 1995; accepted 24 November 1995

Abstract

Experiments carried out with the coccolithophorid *Emiliania huxleyi* maintained in batch cultures showed that the patterns of photosynthetic carbon metabolism characteristic of this species are: (1) carbon incorporation into proteins only represents about 20% of total carbon fixation into organic carbon, (2) protein synthesis in darkness is a significant and growth-dependent process, (3) most of the carbon fixed photosynthetically (45–60%) flows towards the lipid fraction, (4) the relative contribution of lipid-C to cellular biomass is directly related to the amount of calcite-C present as coccoliths, (5) half of the carbon incorporated into polysaccharides during the light period is respired during the night, (6) dark ^{14}C losses during the night generally represent 10–13% of gross photosynthesis, and (7) the release of dissolved organic carbon is related to growth stage and accounts for 2–6% of the total amount of carbon incorporated photosynthetically. Most of these patterns of carbon partitioning were validated in natural phytoplankton assemblages dominated by *E. huxleyi* during sampling conducted in the Norwegian fjords. The results are interpreted and discussed in terms of their potential ecological and biogeochemical significance.

1. Introduction

Emiliania huxleyi (Lohm.) Hay et Mohler is the most abundant coccolithophorid species and has been the subject of exhaustive experimental work during the past decades directed towards the understanding of the mechanisms involved in the process of carbon fixation into coccoliths, especially in relation to photosynthesis (e.g. Paasche, 1964; Sikes et al., 1980; Nimer et al., 1994; Paasche and Brubak, 1994; Sekino and Shiraiwa, 1994). Comparatively little attention has been devoted to its ecophysiological characteristics, particularly in relation to organic carbon metabolism, chemical composition and growth rate (e.g. Fernández et al., 1994a).

Knowledge of the patterns of photosynthetic carbon partitioning has proved to be a valuable tool in assessing the physiological state of phytoplankton both in culture and natural conditions (e.g. Morris, 1981; Hama et al., 1988; Fernández et al., 1992, 1994b). This kind of information gives insight into the metabolic strategies adopted by the different species as a response to environmental changes, that will ultimately determine the biochemical composition of phytoplankton cells and, to a large extent, will modulate the interaction between primary pro-

PII S0924-7963(96)00016-4

duction and the chemistry of surface waters resulting from within-species and between-species variability in the relative rates of macromolecular synthesis (e.g. Laws, 1991).

The aim of this paper is to summarize the most relevant results obtained by our research group in a series of experiments with cultures and also during studies with natural populations designed to understand and quantify the main flows of photosynthetically incorporated carbon associated with *E. huxleyi*-dominated assemblages. Patterns of carbon partitioning are interpreted in terms of their potential implications for bloom development and biogeochemical cycling of carbon in surface waters.

2. Methods

2.1. Culture experiments

Axenic cultures of the coccolithophorid *Emiliania huxleyi* (strain Bigelow Laboratory No. 88E) were grown in 1000 ml glass culture flasks and maintained in an incubator at 16°C under short (10 h light, 14 h dark) or long (16 h light, 8 h dark) photoperiod regimes. Culture medium was prefiltered (0.4 μm) nutrient-depleted water from the Gulf Stream collected off Miami (Florida, USA), enriched with modified K medium as detailed in Fernández et al. (1994a). Irradiance in the incubator was 200 μE m^{-2} s^{-1} provided by cool-white fluorescent lamps. Triplicate culture vessels were maintained for each experimental treatment and harvested daily inmediately before the beginning of the light period for the estimation of cell abundance. Samples for the determination of the cellular chemical composition and the patterns of ^{14}C incorporation into photosynthetic end-products were collected from each of the 3 culture vessels during the exponential and stationary phases of growth.

2.2. Field studies

Sampling of natural phytoplankton populations was conducted during a mesocosms experiment carried out from 12 to 24 May 1993 in a bay adjacent to Raunefjorden, 20 km south of Bergen, western Norway, and throughout a series of visits to Fauskangerpollen and Nordasvannet fjords on board R/V *Hans Brattström* which took place between 19 and 27 May 1993. The mesocosm experimental design and sampling strategy during the cruises are described in Marañón et al. (1996) and Bratbak et al. (1995), respectively.

2.3. Incubation procedures and analytical methods

The experimental, analytical and incubation procedures followed for the determination of phytoplankton abundance, the concentration of particulate proteins, carbohydrates and lipids, and for the calculation of the rates of ^{14}C incorporation into proteins, polysaccharides, lipids and low molecular weight metabolites (LMWM) were as described in Fernández et al. (1994a).

The determination of photosynthate labelling patterns was performed by inoculating 20 ml (culture experiments) or 70 ml (natural populations) seawater samples with 370 kBq of $H^{14}CO_3^-$. Cultured populations were incubated either under 16h:8h or 10h:14h light–dark cycle for 24 h. Natural samples were placed in an incubator provided with an artificial light source (Osram Powerstar HQI-T 400 W/DH) and maintained under 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 carried out under a 16h:8h light–dark cycle and lasted 24 h.

The percentage of night ^{14}C reallocation for each biochemical pool was determined according to:

$$\%\ \text{reallocation} = (D_{24} - D_{16-10}) \times 100 / D_{16-10}$$

where D_{24} is ^{14}C incorporation over the 24 h (L–D) incubation and D_{16-10} is ^{14}C incorporation over the 16 h or 10 h incubation under continuous light.

Cell-specific growth rates were determined from cell counts performed in a Palmer-Maloney counting cell. Carbon-specific growth rates were calculated from "net" daily carbon incorporation (i.e. total daily carbon incorporation minus dark ^{14}C losses) and the organic carbon content of the cells. The concentration of calcite-C was measured as in Fernández et al. (1993). Calcification rates were measured using the ^{14}C method (Paasche, 1963) as in

Fernández et al. (1993). The rates of dissolved organic carbon production during 24 h incubations were measured following the method by Mague et al. (1980).

3. Results

The cellular concentrations of the main biochemical constituents as well as the flows of carbon into

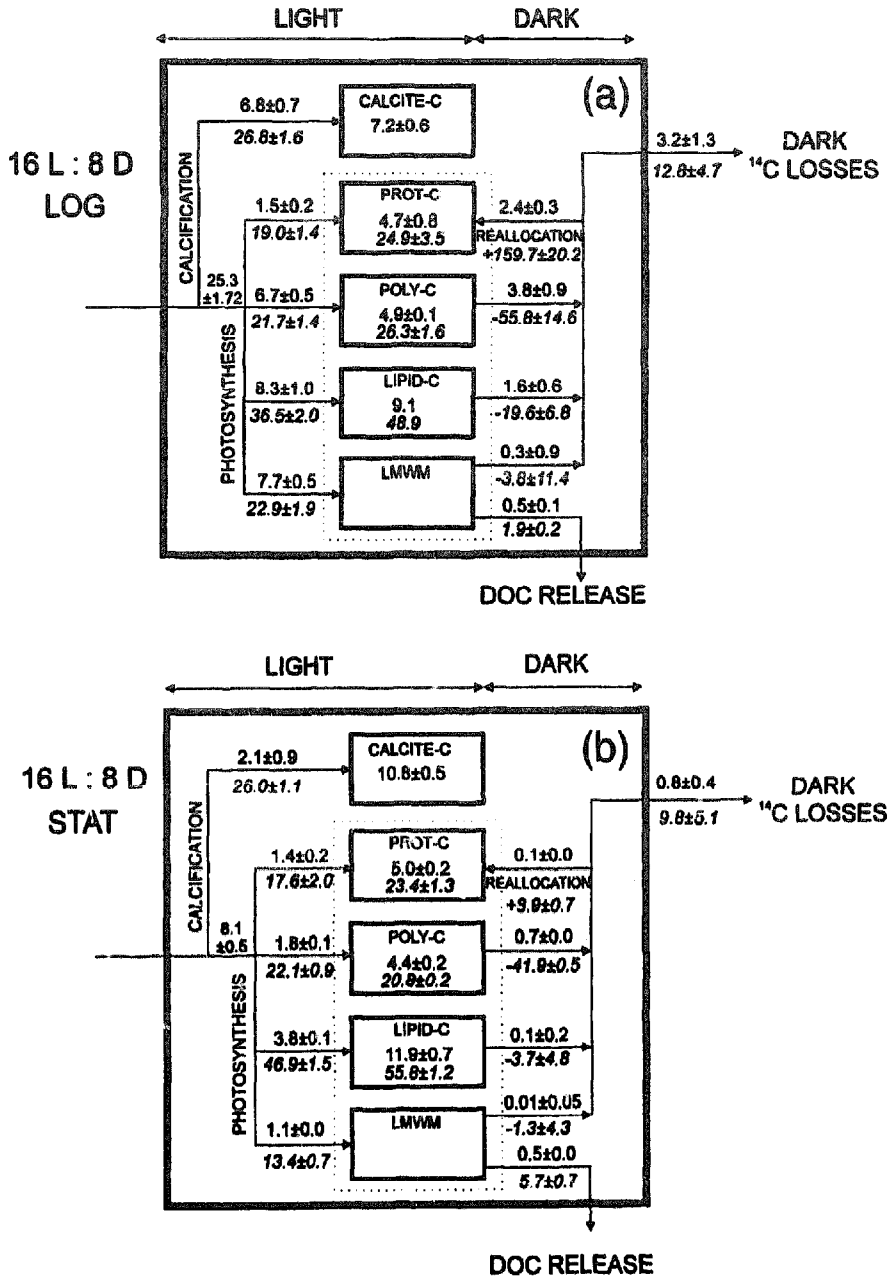

Fig. 1. Cellular carbon partitioning of *Emiliania huxleyi* cells growing under a 16L:8D photoperiod. A. Cells growing exponentially. B. Cells during the stationary phase of growth. The non-italic figures indicate the concentrations of the major cellular chemical constituents (pg C $cell^{-1}$): calcite (*CALCITE-C*), proteins (*PROT-C*), polysaccharides (*POLY-C*) and lipids (*LIPID-C*) and rates of daily carbon incorporation (pg C $cell^{-1}$ d^{-1}) into the biochemical pools quoted above and also into low molecular weight metabolites (*LMWM*). Night reallocation of carbon incorporated during the light period is also indicated. Numbers in *italics* represent the relative contribution of the pool to total carbon biomass or total carbon incorporation into the organic fraction. The percentages of dark ^{14}C losses and of dissolved organic carbon (*DOC*) release indicate the relative contribution of these processes with respect to gross photosynthesis.

such pools are represented in Figs. 1 and 2 for *Emiliania huxleyi* cells growing in batch cultures under long (16L:8D; Fig. 1) and short (10L:14D; Fig. 2) photoperiods during the exponential (Figs. 1A and 2A) and stationary (Figs. 1B and 2B) phases of growth.

3.1. Carbon cell quotas, photosynthesis and calcification rates

Cell-specific growth rates during the exponential growth phase, as determined from microscopic counts, were 0.81 and 0.68 d^{-1} for the 16L:8D and

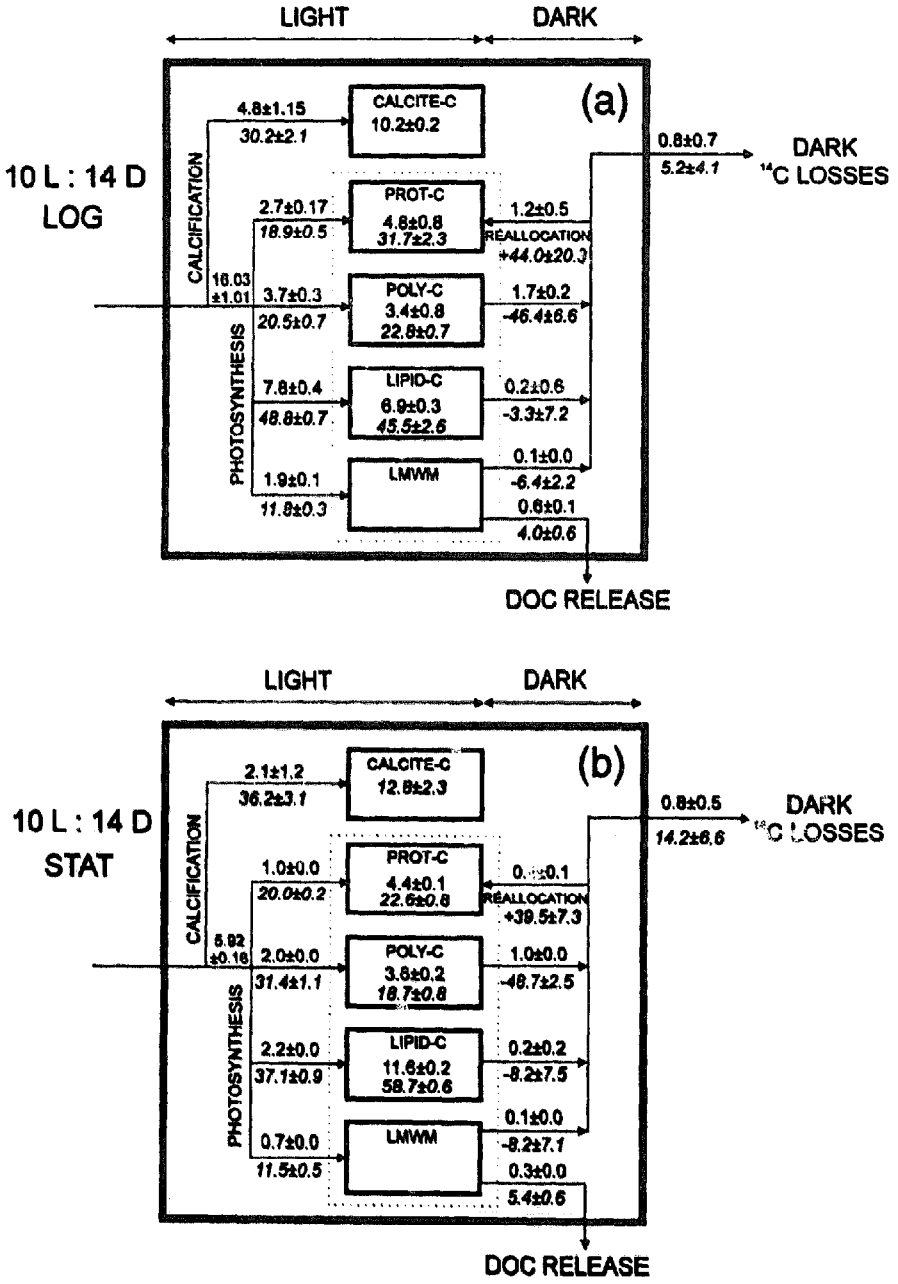

Fig. 2. Cellular carbon partitioning of *Emiliania huxleyi* cells growing under a 10L:14D photoperiod. A. Cells growing exponentially. B. Cells during the stationary phase of growth. See Fig. 1 for explanation.

Table 1
Cell-specific growth rate (μ_{cell}; d^{-1}), organic carbon cell quota (C-biomass; pg C $cell^{-1}$), daily "net" photosynthesis ("net" photo; pg C $cell^{-1}$ d^{-1}) and carbon-specific growth rate (μ_{carb}; d^{-1}) of *E. huxleyi* populations growing at different rates under short and long photoperiods

Photoperiod	Growth stage	μ_{cell}	C-biomass	"net" photo	μ_{carb}
16L:8D	Exponential	0.81	18.7	21.6	0.80
	Stationary	–	21.4	7.3	0.24
10L:14D	Exponential	0.68	15.0	15.2	0.69
	Stationary	–	19.6	5.1	0.18

10L:14D photoperiods, respectively (Table 1). The organic carbon content of *E. huxleyi* cells measured during the experiments was variable ranging from 15.0 to 21.4 pg C $cell^{-1}$ and showed an increasing trend with growth rate during the exponential growth phase. The organic carbon cell quota was also higher when cellullar division almost ceased than when growth was still active (Table 1). Carbon-specific growth rates were in perfect agreement with those estimated from cell counts, evidence of the reliability of the analytical procedures used in this study.

3.2. Inorganic carbon production

The amount of calcite-C per cell varied from 7.2 to 12.8 pg calcite-C $cell^{-1}$ and was inversely related to growth rate during the exponential phase (Figs. 1 and 2). The calcite-C content increased during the stationary phase for both photoperiod conditions tested. The rates of carbon incorporation into the organic and inorganic cellular fractions were related and, consequently, the contribution of calcification to total carbon fixation did not display large variations, ranging from 26 to 36% (Figs. 1 and 2). In spite of this, the calcification to photosynthesis ratio was slightly higher in cells growing under the short photoperiod treatment (10L:14D).

3.3. Carbon incorporation into macromolecules

3.3.1. Low molecular weight metabolites

The LMWM compartment is a complex pool made up of a large number of compounds of diverse nature and, as a consequence, its measurement is not an easy task. The amount of LMWM has not been quantified in this investigation, this is the main cause of the slight differences observed between the relative contribution of the major biochemical compounds and their corresponding relative rates of synthesis as determined from ^{14}C incorporation experiments. Carbon flow into LMWM was generally about 12% of photosynthetic carbon incorporation, except in exponentially growing cells under a 16L:8D photoperiod when it represented an abnormally high value of 22.9% of total carbon fixation (Fig. 1A). Day–night changes in the amount of labelled carbon as LMWM were not very important. Percentages of dark reallocation for this pool were always lower than −8%.

3.3.2. Proteins

E. huxleyi cells were characterized by a relatively low protein-carbon content, representing between 23 and 32% of the amount of organic carbon per cell (Figs. 1 and 2), and displayed a reduced variability between treatments. The flow of carbon into proteins during the light period showed an even more homogeneous pattern, with a relative ^{14}C incorporation into this pool varying from 18 to 20% of total carbon fixation, regardless of photoperiod or growth stage. Significant differences between treatments were detected, however, in the patterns of night carbon reallocation into proteins. Thus, carbon incorporation into proteins in darkness represented up to 159% of the amount of carbon flowing into this pool during the light period for the 16L:8D photoperiod whereas it was only 44% for the 10L:14D. Percentages of dark ^{14}C reallocation were markedly reduced during the stationary phase of growth.

3.3.3. Carbohydrates

The carbohydrate-C content of *E. huxleyi* ranged from 3.4 to 4.9 pg C $cell^{-1}$, representing between 19 and 26% of the total cellular organic carbon quota. This relative contribution decreased with growth rate and also during the stationary phase of growth (Figs.

Table 2
Rates of photosynthesis (Photo) and calcification (Calc) and patterns of carbon incorporation into proteins (Prot), polysaccharides (Pol), lipids (Lipi) and low molecular weight metabolites (LMWM) of diatom-dominated and *E. huxleyi*-dominated phytoplankton assemblages sampled in experimental mesocosms, Fauskangerpollen fjord (Fausk.) and Nordasvannet fjord (Nordas). Values in parentheses represent standard errors

Date	Location	*E. huxleyi* (mg C m^{-3})	*E. huxleyi* (%)	Diatoms (%)	Photo (mg C m^{-3} d^{-1})	Calc (mg C m^{-3} d^{-1})	Prot (%)	Pol (%)	Lipi (%)	LMWM (%)
14/05/93	Mesocosm H2	4.0	3.3	54.1	59.9	1.5	22.6	11.7	24.4	41.4
20/05/93	Mesocosm H2	73.0	66.4	0.0	18.8	13.4	18.9	22.9	43.1	15.2
					(0.5)	(4.4)	(0.8)	(0.2)	(1.6)	(0.5)
24/05/93	Fausk., 3 m	8.8	6.4	84.6	74.5	6.6	33.5	23.2	21.1	22.1
					(9.2)	(0.9)	(3.5)	(1.6)	(0.6)	(2.7)
24/05/93	Fausk., 10 m	42.8	64.0	8.9	5.1	1.6	38.8	26.5	20.1	14.6
					(0.8)	(1.8)	(2.5)	(1.1)	(0.6)	(0.7)
26/05/93	Fausk., 3 m	4.6	2.7	61.8	37.2	2.5	30.2	24.1	24.0	21.7
					(5.4)	(1.3)	(6.8)	(4.0)	(2.7)	(1.6)
27/05/93	Nordas., 9 m	163.0	84.0	0.0	18.4	0.22	20.2	27.7	38.5	13.6
					(2.9)	(0.07)	(2.1)	(2.1)	(2.8)	(1.1)

1 and 2). The flows of carbon into polysaccharides during the illuminated period were similar for all situations (ca. 21%) with the exception of the lowest growth rate where it increased to 37% of total carbon fixation into the organic fraction. Polysaccharides are a highly mobile pool as they were always lost in darkness, with percentages of reallocation representing −45% to −55%, indicating that about half of the carbon incorporated into carbohydrates during the light period was respired or reallocated into proteins during the night regardless of photoperiod or growth stage.

3.3.4. Lipids

Lipids always represented a large proportion of cellular organic carbon in *E. huxleyi*. Their relative contribution to total carbon biomass was always higher than 45% during the exponential phase, increasing up to values as high as 55–58% when cellular division ceased. Variations in the contribution of lipid-C to total carbon between treatments were closely related to parallel changes in the amount of calcite-C per cell. The higher the amount of carbon as coccoliths in the cell the higher the magnitude of the lipid pool compared with the total organic fraction. As expected from the cellular biochemical composition noted above, a high proportion of the amount of carbon incorporated photosynthetically is directed towards the synthesis of lipids. In general, lipids were not intensely mobilized in darkness, with percentages of reallocation ranging form −3 to −8%. An exception to this pattern was apparent during the exponential phase (16L:8D photoperiod; Fig. 1A) where carbon incorporated into lipids was a significant source of the total amount of carbon respired in darkness and/or to the night synthesis of proteins.

3.4. Dark carbon losses and dissolved organic carbon release

The rates of ^{14}C losses in darkness typically varied between 10 and 13% of gross photosynthesis (Figs. 1 and 2), with the exception of cells growing exponentially under a 10L:14D photoperiod where this value was notably lower (5.2%). It should be mentioned in this context that errors associated with these estimates are generally high, as they are computed from the corresponding reduction in the amount of carbon experienced by each biochemical pool during the dark period.

The release of dissolved organic carbon (DOC) by axenic cultures of *E. huxleyi* was relatively low, never exceeding 6% of gross photosynthesis (Figs. 1 and 2). The lowest relative contribution of DOC to total carbon incorporation into the organic fraction was determined for the more actively growing cells (1.9%), whereas it increased during the stationary phases of growth for the two photoperiods tested.

3.5. Carbon partitioning in natural phytoplankton populations

Extrapolation of the results drawn from culture experiments was sought by comparing the patterns of carbon incorporation into natural phytoplankton populations dominated either by diatoms or *E. huxleyi* (Table 2). Analysis of the partitioning between photosynthesis and calcification in natural conditions is difficult to achieve as it depends on the contribution of photosynthesis by non-coccolithophorid species and also on the physiological state of the coccolithophorids. Thus, the relative contribution of calcification to total carbon fixation in *E. huxleyi*-dominated populations is generally very variable (see Table 2) ranging from about 1% to values close to 50%.

The patterns of photosynthetic carbon metabolism measured in natural phytoplankton populations largely reflect the results obtained previously in cultures. In general, coccolithophorid-dominated assemblages showed a low percentage of carbon incorporated into proteins whereas a comparatively higher fraction of the recently photoassimilated carbon flowed towards the lipid pool with respect to diatom-dominated assemblages (Table 2).

4. Discussion

The results obtained in this investigation indicate that the main patterns of photosynthetic carbon partitioning in cultures and natural populations of *Emiliania huxleyi* are (1) a higher than average rate of carbon incorporation into the lipid fraction, (2) a low relative rate of protein synthesis compared with other

phytoplankton species, (3) dark reallocation of proteins is a significant process dependent on growth rate, (4) typically, carbon incorporation into LMWM is about 12% of total carbon incorporation into particulate organic carbon. About half of the carbon fixed into this pool is released as dissolved organic carbon.

The high percentage of carbon incorporated into lipids and, consequently, the high contribution of this biochemical pool to cellular carbon biomass has been suggested to exert a relevant effect upon the maintenance of the cells in the euphotic zone (Fernández et al., 1994a). These authors performed some simple calculations showing that the decrease in density associated with this high-lipid metabolism would be about 6%. Given the small size of *E. huxleyi*, this slight reduction in cellular density would eventually result in a sinking rate decrease of about 20% which is likely to be a significant figure in terms of bloom maintenance and development.

The relative contribution of lipid carbon to total cellular organic carbon appears to be directly linked to the amount of carbon present as coccoliths and therefore to the process of calcification (Figs. 1 and 2). Experiments carried out with continuous cultures of *E. huxleyi* reported comparable results (Fernández et al., in press b) and were able to establish a statistically significant direct relationship between the inorganic carbon cell quota and the percentage of lipid-C to total organic carbon. This metabolic behaviour might be interpreted as a cellular strategy tending to reduce sinking rates when the relative contribution of calcite-C to total cellular carbon reaches maximum values.

The coccolithophorid *Emiliania huxleyi* is not only characterized by a high lipid concentration but also by a complex and characteristic lipidic composition (Volkman et al., 1980; Pond and Harris, in press). Alkenones and alkenoates, polyunsaturated fatty acids related to chlorophyll membranes and flagella and a series of compounds such as methyl and ethylketones, whose function remains unknown, are the main components of this biochemical pool (Pond and Harris, in press). The significance of this lipid composition for the reproductive success of zooplankton populations and ultimately for the transfer of energy through the food web remains unclear and deserves further investigation.

At present, there is a severe lack of information on the cycling of dissolved organic carbon (DOC) in *E. huxleyi* blooms. The results presented in this paper show that DOC release in axenic cultures of this species only represented 2–6% of the amount of carbon incorporated photosynthetically depending on growth conditions. This figure, although apparently low, could be significant over spatial and temporal scales typical of coccolithophorid blooms. A simple simulation of bloom development assuming an initial inoculum of 50 cells ml^{-1} growing at 0.5 doublings d^{-1}, a maximum cell abundance of 12,000 cell ml^{-1} at the peak of the bloom, and an average DOC release rate of 0.5 pg C cell d^{-1} (Figs. 1 and 2), would represent about 3–4 $\mu mol\ l^{-1}$ DOC after 20 days. This value, however, is probably an underestimation of DOC production rates actually occurring in nature as low molecular weight compounds appear to constitute a significant fraction of cellular biomass, but mainly because the effect of grazing (Harris, 1994) and viral mortality (Bratbak et al., 1993) might be the main contributor to DOC production under natural conditions. If the concentration of labile DOC were significantly enhanced during and following the peak of *E. huxleyi* blooms, these events could potentially alter the trophodynamic efficiency of the pelagic ecosystem by triggering bacterial production and hence, the microbial trophic loop. In support of this hypothesis, bacterial abundance was found to be very high after blooms of this species developed in mesocosms (M. Levasseur et al., pers. commun.) and also following a large scale bloom in the North East Atlantic (A. Pomroy, pers. commun.).

A direct consequence of the patterns of photosynthetic carbon metabolism characteristic of *E. huxleyi* for the biogeochemistry of surface oceanic waters is that phytoplankton assemblages dominated by this species have a greater capacity to take up carbon from seawater on a nitrogen basis due to the low proportion of carbon that is incorporated into the protein pool. Simple calculations based on the stoichiometry of biomass production and the patterns of carbon partitioning determined experimentally, indicate that the carbon to nitrogen molar uptake ratio in cultures of *E. huxleyi* varies around 15, i.e. twice the value of the Redfield ratio, and even in *E. huxleyi*-dominated natural phytoplankton assem-

blages this ratio can rise up to 10–11, as calculated for blooms sampled in Norwegian fjords (Fernández et al., in press a). These estimates clearly illustrate that changes in phytoplankton community structure involving shifts from diatom-dominated to coccolithophorid-dominated populations, would potentially result in major alterations in the chemistry of surface waters and in the composition of exported particulate matter due to the joint effect of calcification (Holligan et al., 1993; Purdie and Finch, 1994; Robertson et al., 1994) and to an organic carbon metabolism primarily based on the synthesis of large amounts of lipids which tended partially to counteract the elevated cellular sedimentation rate imposed by coccoliths (Fernández et al., 1994a).

Acknowledgements

This work was partly funded by the European Commission under contract EHUX MAS-CT92-0038. We are grateful to Kay Kilpatrick and Jennifer Fritz (RSMAS, Univ. of Miami) for their help with culture procedures and to Jorum Egge and Berit Heimdal (Department Marine Biology and Fisheries, University of Bergen) and Roger Harris (PML) for excellent support of the work in the mesocosms and at sea. A postdoctoral fellowship from the Spanish Ministry of Science and Education and by a grant from ONR (N00014-91-J-1048) provided funding for E.F., and W.M.B. was suppported by grants from NASA (BAGW-2426), ONR (N00014-91-J-1048) and NSF (OCE-9022227). The work of E.M. was funded by the postgrade fellowship from the Spanish Ministry of Science and Education. This paper is EHUX contribution no. 55.

References

Bratbak, G., Egge, J.K. and Heldal, M., 1993. Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. Mar. Ecol. Progr. Ser., 93: 39–48.

Bratbak, G., Levasseur, M., Michaud, S., Cantin, G., Fernández, E., Heimdal, B.R. and Heldal, M., 1995. Viral activity in relation to *Emiliania huxleyi* blooms: a mechanism of DMSP release? Mar. Ecol. Progr. Ser., 128: 133–142.

Fernández, E., Serret, P., Madariaga, I. de, Davies, A.G. and Harbour, D.S., 1992. Photosynthetic carbon metabolism and biochemical composition of spring phytoplankton assemblages enclosed in microcosms: the diatom–*Phaeocystis* sp. succession. Mar. Ecol. Progr. Ser., 90: 89–102.

Fernández, E., Boyd, P., Holligan, P.M. and Harbour, D.S., 1993. Production of organic and inorganic carbon within a large scale coccolithophore bloom in the North Atlantic ocean. Mar. Ecol. Progr. Ser., 97: 271–285.

Fernández, E., Balch, W.M., Marañón, E. and Holligan, P.M., 1994a. High rates of lipid biosynthesis in cultured, mesocosm and coastal populations of the coccolithophore *Emiliania huxleyi*. Mar. Ecol. Progr. Ser., 114: 13–22.

Fernández, E., Marañón, E., Harbour, D.S. and Pingree, R.D., 1994b. Phytoplankton carbon incorporation patterns and biochemical composition of particulate matter in the eastern North-Atlantic subtropical region. J. Plankton Res., 16: 1627–1644.

Fernández, E., Marañón, E., Harbour, D.S., Kristiansen, S. and Heimdal, B.R., in press a. Patterns of carbon and nitrogen uptake during blooms of *Emiliania huxleyi* in two Norwegian fjords. J. Plankton Res.

Fernández, E., Fritz, J.J. and Balch, W.M., in press b. Chemical composition of the coccolithophorid *Emiliania huxleyi* under steady state growth. J. Exp. Mar. Biol. Ecol.

Hama, T.N., Handa, N., Takahashi, F., Whitney, F. and Wong, C.S., 1988. Change in distribution patterns of photosynthetically incorporated C during phytoplankton bloom in controlled experimental ecosystem. J. Exp. Mar. Ecol., 120: 39–56.

Harris, R.P., 1994. Zooplankton grazing on the coccolithophore *Emiliania huxleyi* and its role in inorganic carbon flux. Mar. Biol., 119: 431–439.

Holligan, P.M., Fernández, E., Aiken, J., Balch, W.M., Boyd, P., Burkill, P.H., Finch, M., Groom, S.B., Malin, G., Muller, K., Purdie, D.A., Robinson, C., Trees, C., Turner, S.M. and Van der Wal, P.A., 1993. A biogeochemical study of the coccolithophore *Emiliania huxleyi* in the North Atlantic. Global Biogeochem. Cycles, 7: 879–900.

Laws, E.A., 1991. Photosynthetic quotients, new production and net community production in the open ocean. Deep-Sea Res., 38: 143–167.

Mague, T.H., Friberg, E., Hugues, D.J. and Morris, I., 1980. Extracellular release of carbon by marine phytoplankton; a physiological approach. Limnol. Oceanogr., 25: 262–279.

Marañón, E., Fernández, E., Harris, R.P. and Harbour, D.S., 1996. Effects of the diatom—*Emiliania huxleyi* succession on photosynthesis, calcification and carbon metabolism by size-fractionated phytoplankton. Hydrobiologia, 317: 189–199.

Morris, I., 1981. Photosynthetic products, physiological state and phytoplankton growth. Can. Bull. Fish. Aquat. Sci., 210: 83–102.

Nimer, N.A., Brownlee, C. and Merret, M.J., 1994. Carbon dioxide availability, intracellular pH and growth rate of the coccolithophore *Emiliania huxleyi*. Mar. Ecol. Progr. Ser. 109: 257–262.

Paasche, E., 1963. The adaption of the carbon-14 method for the measurement of coccolith production in *Coccolithus huxleyi*. Physiol. Plant., 16: 186–200.

Paasche, E., 1964. A tracer study of the inorganic carbon uptake during coccolith formation and photosynthesis in the coccolithophorid *Coccolithus huxleyi*. Physiol. Plant. Suppl., 3: 1–82.

Paasche, E. and Brubak, S., 1994. Enhanced calcification in the coccolithophorid *Emiliania huxleyi* under phosphorus limitation. Phycologia, 33: 324–330.

Pond, D.W. and Harris, R.P., in press. The lipid composition of the coccolithophore *Emiliania huxleyi* and its possible ecophysiological significance. J. Mar. Biol. Assoc. U.K.

Purdie, D.A. and Finch, M.S., 1994. The impact of a coccolithophore bloom on the partial pressure of carbon dioxide in seawater enclosures in a Norwegian fjords. Sarsia, 79: 379–388.

Robertson, J.E., Robinson, C., Turner, D.R., Holligan, P.M., Watson, A., Boyd, P., Fernández, E. and Finch, M., 1994. The impact of a coccolithophore bloom on oceanic carbon uptake in the N.E. Atlantic during summer 1991. Deep-Sea Res., 41: 297–314.

Sekino, K. and Shiraiwa, Y., 1994. Accumulation and utilization of dissolved inorganic carbon by a marine unicellular coccolithophorid, *Emiliania huxleyi*. Plant Cell Physiol., 35: 353–361.

Sikes, C.S., Roer, R.D. and Wilbur, K.M., 1980. Photosynthesis and coccolith formation: Inorganic carbon sources and net inorganic reaction of deposition. Limnol. Oceanogr., 25: 248–261.

Volkman, J.K., Eglinton, G., Corner, E.D.S. and Forsberg, T.E.V., 1980. Long-chain alkenes and alkenones in the marine coccolithophorid *Emiliania huxleyi*. Phytochemistry, 19: 2619–2622.