

# Transparent exopolymer particles and dissolved organic carbon production by *Emiliana huxleyi* exposed to different CO<sub>2</sub> concentrations: a mesocosm experiment

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**ABSTRACT:** The role of transparent exopolymer particles (TEP) and dissolved organic carbon (DOC) for organic carbon partitioning under different CO<sub>2</sub> conditions was examined during a mesocosm experiment with the coccolithophorid *Emiliana huxleyi*. We designed 9 outdoor enclosures (~11 m<sup>3</sup>) to simulate CO<sub>2</sub> concentrations of estimated 'Year 2100' (~710 ppm CO<sub>2</sub>), 'present' (~410 ppm CO<sub>2</sub>) and 'glacial' (~190 ppm CO<sub>2</sub>) environments, and fertilized these with nitrate and phosphate to favor bloom development. Our results showed fundamentally different TEP and DOC dynamics during the bloom. In all mesocosms, TEP concentration increased after nutrient exhaustion and accumulated steadily until the end of the study. TEP concentration was closely related to the abundance of *E. huxleyi* and accounted for an increase in POC concentration of 35 ± 2 % after the onset of nutrient limitation. The production of TEP normalized to the cell abundance of *E. huxleyi* was highest in the Year 2100 treatment. In contrast, DOC concentration exhibited considerable short-term fluctuations throughout the study. In all mesocosms, DOC was neither related to the abundance of *E. huxleyi* nor to TEP concentration. A statistically significant effect of the CO<sub>2</sub> treatment on DOC concentration was not determined. However, during the course of the bloom, DOC concentration increased in 2 of the 3 Year 2100 mesocosms and in 1 of the present mesocosms, but in none of the glacial mesocosms. It is suggested that the observed differences between TEP and DOC were determined by their different bioavailability and that a rapid response of the microbial food web may have obscured CO<sub>2</sub> effects on DOC production by autotrophic cells.

**KEY WORDS:** *Emiliana huxleyi* · Transparent exopolymer particles · TEP · Dissolved organic carbon · DOC · Carbon overconsumption · CO<sub>2</sub> · Redfield ratios · Mesocosms

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## INTRODUCTION

An important mechanism for the regulation of atmospheric CO<sub>2</sub> concentration is the fixation of CO<sub>2</sub> by marine phytoplankton and the subsequent export of the organically bound carbon to the deeper ocean. The magnitude of oceanic carbon sequestration is traditionally expected to depend on the availability of major nutritional elements in the surface ocean, and is esti-

mated from nitrate uptake using a C:N ratio of 106:16 (Redfield et al. 1963, Eppley & Peterson 1979). However, the draw-down of dissolved inorganic carbon (DIC) can exceed the amount expected from nitrate removal and Redfield stoichiometry (Sambrotto et al. 1993, Michaels et al. 1994, Marchal et al. 1996, Thomas et al. 1999, Körtzinger et al. 2001). This was first referred to as 'carbon overconsumption' by Toggweiler (1993). Since then, a number of hypotheses have been

raised to explain carbon overconsumption, including the underestimation of new production due to unaccounted for biological  $N_2$ -fixation (Michaels et al. 1996, Hood et al. 2001), the temporary accumulation of carbon-rich dissolved organic matter (DOM) (Kähler & Koeve 2001), preferential nutrient recycling (Thomas et al. 1999) or the formation of carbon-rich extracellular particles known as transparent exopolymer particles (TEP) (Engel et al. 2002a).

Considering carbon cycling at the cellular level, it is well known that the uptake of carbon continues when nutrient acquisition limits cell division but not primary production. One consequence of the excess assimilation of carbon is the extracellular release (ER) of organic matter (Fig. 1) (Fogg 1966). Although the mechanisms of ER have not yet been fully elucidated, it can be assumed that low molecular weight (LMW) substances, such as monomer or oligomer sugars and amino acids, penetrate the cell membrane by diffusion (Fogg 1966). The rate of this leakage of LMW substances should therefore depend on the concentration gradient between the inner and outer cell. The release of high molecular weight (HMW) substances by diffusion is not possible, and has to be accomplished by active exudation processes. Polysaccharides, for example, are synthesized in the vesicles of the Golgi apparatus and secreted to the outer cell by exocytosis (see review by Leppard 1995). Exudates are expected to contain minimal amounts of limiting elements, because exudation has been hypothesized to be a process by which algal cells dispose of excess photosynthates under conditions of nutrient limitation (Wood & Van Valen 1990). In this respect, exudation can be viewed as a cellular carbon overflow. Whether this cellular carbon overflow is linked to carbon overconsumption in the field has yet to be determined. It is well known that ER by autotrophic cells is an important source for

dissolved organic carbon (DOC) in the upper ocean (Alluwihare et al. 1997), and the production of DOM with high C:N ratios has frequently been observed (Williams 1995, Kähler & Koeve 2001, Søndergaard et al. 2000). Yet, the deep export of DOC is principally restricted to subduction of surface waters, e.g. by thermohaline ventilation. Because this process operates over long times scales, i.e. months to years, much of the seasonally accumulated DOC is likely to be degraded before it arrives at greater depths.

The major fraction of HMW-ER is made up of polysaccharides (Benner 2002). Some of these contain acidic sugars that facilitate polysaccharide aggregation into particles known as TEP (Alldredge et al. 1993, Leppard 1995). TEP are therefore naturally rich in carbon but poor in nitrogen (Engel & Passow 2001, Mari et al. 2001). Especially when nutrients become limiting, TEP occur in phytoplankton cultures, during experimental phytoplankton blooms, and in natural environments (see Passow 2002 for review) and are therefore regarded as a result of the cellular carbon overflow (Engel 2002, Engel et al. 2002a). Because they represent a fraction of the particulate organic matter (POM), a relative increase of TEP can induce a shift in POC:PON ratios during phytoplankton blooms (Engel et al. 2002a). TEP can participate in particle-mediated processes such as marine snow formation and sinking (Alldredge et al. 1993, Passow et al. 2001), and therefore have the potential to account for a deep export of carbon on relatively short time scales.

According to the carbon-overflow model, primary production should control ER under conditions of nutrient limitation and, in fact, ER has been related to primary production in the ocean (Baines & Pace 1991). Consequently, factors influencing primary production will also influence ER. This has been shown for light (Wood & Van Valen 1990), but may also apply to  $CO_2$ ,

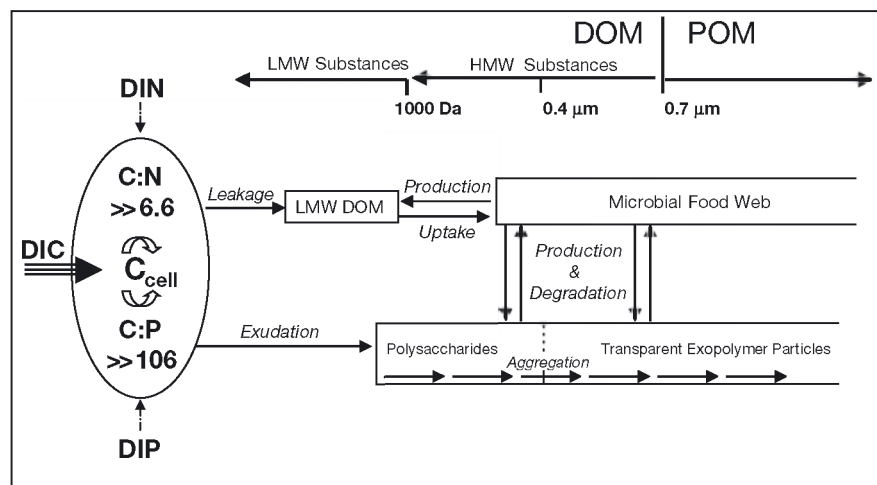


Fig. 1. Conceptual model showing potential pathways of organic matter released by an autotrophic cell. Under the assumption that assimilation of dissolved inorganic carbon (DIC) greatly exceeds uptake of dissolved inorganic nitrogen (DIN) or phosphorus (DIP), a fraction of the organic carbon accumulating intracellularly is released from the cell by leakage and exudation. Depending on its quality, extracellular organic carbon can enter the microbial food web or aggregate into particles, such as transparent exopolymer particles. For further details see 'Introduction'. DOM: dissolved organic matter; POM: particulate organic matter; LMW: low molecular weight; HMW: high molecular weight

since CO<sub>2</sub> concentration can limit primary production of marine phytoplankton (Riebesell et al. 1993, Chen & Durbin 1994, Rost et al. 2003). Many bloom-forming phytoplankton species are able to enhance their CO<sub>2</sub> supply by carbon concentration mechanisms (CCM) (Raven 1991) and thereby saturate primary production even at low CO<sub>2</sub> concentrations typical for the ocean, i.e. 8 to 22 µmol l<sup>-1</sup> (Goerike & Fry 1994). This active regulation of carbon uptake results in an apparent insensitivity of primary production to CO<sub>2</sub> concentrations under oceanic conditions (Goldman 1999). However, CCM may be down-regulated at times of nutrient exhaustion, because they require nitrogen for enzyme biosynthesis and depend on phosphorus for ATP supply (Beardall & Giordano 2002). In this case, primary production could be CO<sub>2</sub>-limited even at oceanic CO<sub>2</sub> concentrations, because the major carboxylating enzyme RUBISCO has a low affinity for CO<sub>2</sub> (Badger et al. 1998). This would explain a direct relationship of TEP production and diffusion-controlled CO<sub>2</sub> uptake rates, as suggested by Engel (2002). Hence, as the timing of carbon overflow coincides with a CO<sub>2</sub> concentration already reduced by phytoplankton growth and a low CO<sub>2</sub> uptake capacity of the phytoplankton cell, changes in seawater CO<sub>2</sub> concentration could potentially influence primary production rates and, in consequence, ER.

We investigated this hypothesis during a mesocosm experiment with *Emiliana huxleyi* exposed to 3 different CO<sub>2</sub> concentrations. In particular, our objectives were to identify (1) the temporal changes of extracellular products, i.e. TEP and DOC, during an *E. huxleyi* bloom, (2) the role of TEP and DOC in storage of excess carbon, and (3) the influence of seawater CO<sub>2</sub> concentrations on TEP and DOC production.

## MATERIALS AND METHODS

**Set-up and sampling.** The study was conducted at the Large Scale Facilities (LSF) in Bergen, Norway, as part of the outdoor-mesocosm project 'Biological responses to carbon dioxide-related changes in seawater carbonate chemistry during a bloom of the coccolithophorid *Emiliana huxleyi*'. A detailed description of the experimental set-up will be reported elsewhere (A. Engel et al. unpubl.). Briefly, 9 polyethylene enclosures (~11 m<sup>3</sup>, 4.5 m water depth) were moored to a raft in a fjord near Bergen, Norway (for more details see Williams & Egge 1998). The bags were filled with unfiltered, nutrient-poor, post-spring bloom fjord water, which was pumped from 2 m depth adjacent to the raft. The enclosures were covered by gas-tight tents made of ETFE (ethylene tetrafluoroethylene) foil, which allowed 95% light transmission of the complete spectrum of sunlight. Atmospheric and seawater pCO<sub>2</sub>

were manipulated to achieve 3 different CO<sub>2</sub> levels in triplicate, corresponding to (1) approximately Year 2100 (according to the Intergovernmental Panel on Climate Change 'business as usual' scenario, IS92a) (Mesocosms 1 to 3), (2) present (Mesocosms 4 to 6) and (3) glacial (Mesocosms 7 to 9) atmospheric CO<sub>2</sub> levels. To promote the development of a coccolithophorid bloom, nitrate and phosphate were added at a ratio of 30:1, yielding initial concentrations of 15 µmol l<sup>-1</sup> NO<sub>3</sub> and 0.5 µmol l<sup>-1</sup> PO<sub>4</sub>. After nutrient addition and throughout the study, the water was gently mixed by means of an airlift pump (for details see Egge & Aksnes 1992), using the same air as for gassing the tents. Over a period of 3 wk, samples were taken daily from each mesocosm by gentle vacuum-pumping of 20 l through a siphon at 0.5 m depth. After Day 16, large visible particle aggregates (>0.5 cm; 'marine snow') appeared in the mesocosms and were sufficiently abundant on Day 17 to be collected manually with a syringe in the upper 0.5 m of the water column.

**Biological and chemical analyses.** Nitrate and nitrite were determined from GF/F-filtered and poisoned (0.1% HgCl<sub>2</sub>) samples with an autoanalyzer (AA II) at the AWI laboratory. Phosphate and ammonium were measured on the day of sampling using the methods of Koroleff & Grasshof (1983). Particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined by elemental analysis (ANCA SL 20-20, Europa Scientific) from 1 l (Days 0 to 12) and 0.5 l (Days 13 to 19) samples filtered gently (200 mbar) through precombusted glass-fiber filters (GF/F, Whatman). Particulate organic phosphorus (POP) was determined colorimetrically (Koroleff & Grasshof 1983) after persulfate oxidation from 0.5 to 1.0 l samples filtered onto GF/F filters. All filters were prepared in duplicate and stored at -20°C until analysis.

TEP were detected by staining with Alcian Blue (Fig. 2), a cationic copper phthalocyanine dye that complexes carboxyl (-COO<sup>-</sup>) and half-ester sulfate (OSO<sub>3</sub><sup>-</sup>) reactive groups of acidic polysaccharides. The amount of Alcian Blue adsorption per sample volume is a measure of TEP concentration and was determined colorimetrically according to the method of Passow & Alldredge (1995) from 50 to 100 ml samples filtered onto 0.4 µm Nuclepore filters. All filters were prepared in 2 replicates. The carbon content of TEP was determined following the approach of Engel & Passow (2001). Aliquots of 5 l (pooled samples of Mesocosms 1-3, 4-6 and 7-9, respectively) were collected on 7 d throughout the bloom and filtered through precombusted glass-fiber filters. TEP were concentrated from the filtrate during circulation through a tangential flow-filtration (TFF) system with a 0.16 µm membrane for 24 h at 10°C. The fraction >0.16 µm was concentrated from 5 l to a final volume of 1 to 2 l. The concen-



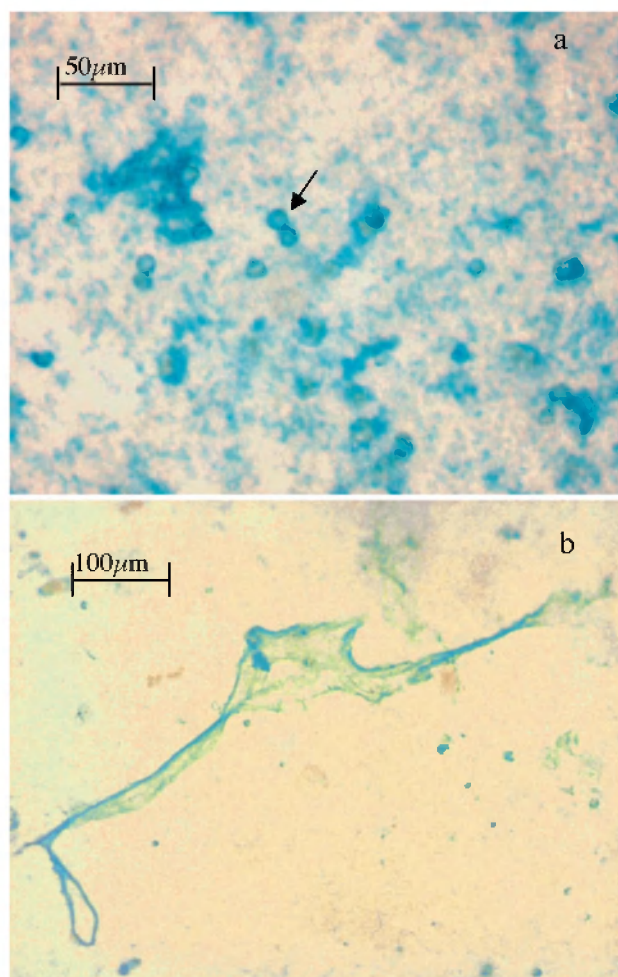


Fig. 2. Microscopic view of material sampled from a mesocosm on 0.4 μm membrane filter and stained with the polysaccharide-specific dye Alcian Blue. (a) Circular cells of *Emiliana huxleyi* (arrow) silhouetted against transparent exopolymer particles (TEP), which typically appear as blue-stained particles of fractal structure; because the coccoliths of *E. huxleyi* are coated with an acidic polysaccharide, the cell surface is stained with Alcian Blue also. (b) Large web-like TEP that appeared later during *E. huxleyi* bloom, often entangled with cells and detritus

trated samples were analyzed for carbon, nitrogen and TEP concentration. TEP were measured colorimetrically from 100 to 200 ml, POC and PON were determined from 0.8 to 1.6 l, as described above. All material in contact with the sample was either autoclaved or acid (10% HCl)-rinsed. Blank glass-fiber filters were prepared for each filtration series.

We collected 10 ml of samples for DOC analysis in glass ampoules after filtration through precombusted GF/F filters. The samples were poisoned with 100 μl of 85% H<sub>3</sub>PO<sub>4</sub>, flame-sealed immediately after collection, and stored until measurement at 4°C in the dark. The

DOC analysis was performed using high-temperature combustion on a Shimadzu TOC-5000 total organic carbon (TOC) analyzer. A 4-point calibration curve was constructed for each measurement day using potassium phthalate standards prepared fresh in UV-treated Milli-Q water. The standards covered the range 0 to 200 μmol C l<sup>-1</sup> and were run for every 8th sample to account for temporal changes during the analysis. The instrument blank was assessed using 2 external standards (certified reference standards, CRMs) obtained from the Bermuda Biological Station for Research. The machine blank was between 8 and 12 μmol l<sup>-1</sup> C for all samples and was subtracted from the measurements. All DOC concentrations reported are the average of 3 injections from each sample. The standard deviation between the 3 injections was <1%. When a higher deviation occurred, the sample was run again. No DOC samples were collected on Day 19.

Cell counts of *Emiliana huxleyi* were performed with a FACSCalibur flow-cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm and with a standard filter set-up. The algae were analyzed from fresh samples at high flow rate (~70 μl min<sup>-1</sup>) with the addition of 1 μm fluorescent beads (Molecular Probes). *E. huxleyi* cells were discriminated on the basis of their forward or right-angle light scatter and chlorophyll fluorescence. Listmode files were analyzed using CYTOWIN (Vaulot 1989).

**Statistical treatment of data.** Average values are given by the statistical mean ( $\bar{x}$ ) and its standard variation (SD). To determine the significance of the coefficient of correlation ( $r^2$ ) a *t*-test according to Fisher (see Sachs 1974) was performed. The effect of the CO<sub>2</sub> treatment on a biological or chemical variable was tested by the analyses of variance or covariance of data (ANOVA, ANCOVA). Statistical significance was accepted for  $p < 0.05$ .

## RESULTS

### General bloom development

On Day 1 of the experiment, seawater CO<sub>2</sub> concentration was adjusted to  $713 \pm 6$  ppm CO<sub>2</sub> in the Year 2100 scenario of Mesocosms 1 to 3, to  $414 \pm 11$  ppm CO<sub>2</sub> in the present Mesocosms 4 to 6 and to  $190 \pm 2.4$  ppm CO<sub>2</sub> in the glacial Mesocosms 7 to 9. During the study period, primary production accounted for a decrease of dissolved inorganic carbon (DIC) of  $161 \pm 5$ ,  $168 \pm 16$  and  $173 \pm 8$  μmol C l<sup>-1</sup> in the Year 2100, present and glacial treatments, respectively. More detailed information about the seawater carbonate chemistry during the experiment will be given elsewhere (B. Delille et al. unpubl.).

In all mesocosms, biomass growth induced a rapid decline in inorganic nutrients after the first week. Phosphate dropped from an initial concentration of  $0.48 \pm 0.02 \mu\text{mol l}^{-1}$  to below the detection limit after Day 10. Nitrate started at  $15.3 \pm 0.2 \mu\text{mol l}^{-1}$  and was not detectable after Day 13. Ammonium was undetectable throughout the study. The abundance of *Emiliania huxleyi* cells increased exponentially after Day 3 in all mesocosms. Despite the depletion of inorganic nutrients between Days 10 and 13, cell growth of *E. huxleyi* continued until Day 17, yielding an average net growth rate of  $0.45 \pm 0.20 \text{ d}^{-1}$ , equivalent to a cell doubling every 1.5 d. For comparison, maximum growth rates for nutrient-replete cultures of *E. huxleyi* were determined with  $\mu_{\text{max}} = 0.76 \text{ d}^{-1}$  (Riegman et al. 2000). Maximum cell abundance of *E. huxleyi* varied from  $1.54 \times 10^7$  to  $5.56 \times 10^7 \text{ cells l}^{-1}$  in the 9 mesocosms. Average cell abundance of *E. huxleyi* decreased after Day 19. Therefore, only data obtained before the collapse of the bloom will be presented here.

The initial POC concentration in the mesocosms was  $17.2 \pm 2.0 \mu\text{mol l}^{-1}$  and increased to  $136 \pm 38 \mu\text{mol l}^{-1}$  on Day 17. PON and POP started at  $2.0 \pm 0.15$  and  $0.02 \pm 0.004 \mu\text{mol l}^{-1}$ , reaching  $11.5 \pm 0.60$  and  $0.25 \pm 0.02 \mu\text{mol l}^{-1}$  on Days 13 and 14, respectively, and declining steadily thereafter. During the first 2 wk, changes in POC concentration were closely related to changes in PON and POP concentrations, with  $d[\text{POC}]/d[\text{PON}]$  equal to  $6.48 \pm 0.3 \text{ mol mol}^{-1}$  and  $d[\text{POC}]/d[\text{POP}]$  equal to  $338 \pm 13 \text{ mol mol}^{-1}$ . The former value is in accordance with the expected Redfield C:N ratio of 6.6. The latter, however, was about twice as high as the Redfield C:P ratio of 116 and underlines the exceptional ability of *Emiliania huxleyi* to grow with low cellular P (Riegman et al. 2000). During this time, changes in PON were closely related to POP, resulting in a  $d[\text{PON}]/d[\text{POP}]$  of  $48.0 \pm 1.3 \text{ mol mol}^{-1}$ . After Day 13, POC production was clearly decoupled from POP and PON. While POC concentration more than doubled, PON and (even more pronounced) POP concentrations decreased, leading to a steep rise in  $[\text{POC}]:[\text{PON}]$  and  $[\text{POC}]:[\text{POP}]$  ratios (Fig. 3). The decoupling of POC from PON production coincided with DIN exhaustion (Fig. 4), indicating that a large amount of carbon was assimilated under nutrient-deplete conditions and accumulated in the POC pool.

### Transparent exopolymer particles

The carbon content of TEP (TEP-C) produced during the *Emiliania huxleyi* bloom was estimated from the analysis of the particulate matter obtained by ultrafiltration according to the method of Engel & Passow (2001). A linear relationship between TEP and POC

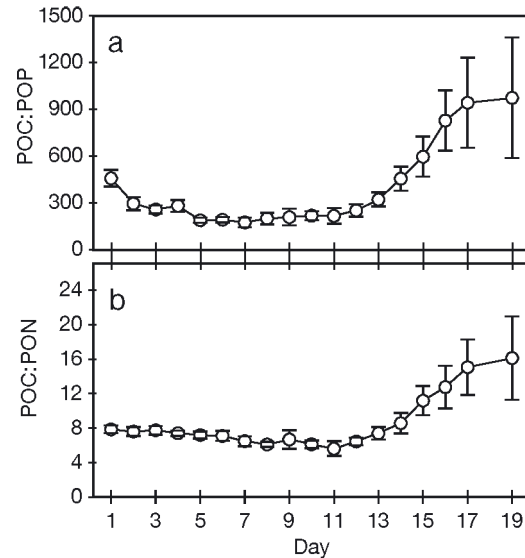


Fig. 3. Molar  $[\text{POC}]:[\text{PON}]$  and  $[\text{POC}]:[\text{POP}]$  ratios in mesocosms toward the end of the *Emiliania huxleyi* bloom. Ratios are means ( $\pm 1 \text{ SD}$ ) of 9 mesocosms. POC, PON: particulate organic carbon and nitrogen, respectively

concentration was observed, yielding  $[\text{POC}, \mu\text{mol}] = 0.033 \pm 0.007 [\text{TEP}, \mu\text{g Xanthan equivalents} = \text{Xeq.}] + 1.8$  ( $r^2 = 0.73$ ,  $n = 11$ ,  $p < 0.005$ ). The slope of the regression is a first-order approximation of the increase in POC with an increase in TEP, and will be used here to convert colorimetrically determined TEP concentrations into carbon units. The PON concentration of the ultrafiltrate ranged between  $0.08$  and  $0.3 \mu\text{mol l}^{-1}$ , resulting in molar  $[\text{POC}]:[\text{PON}]$  ratios between 7.5 and

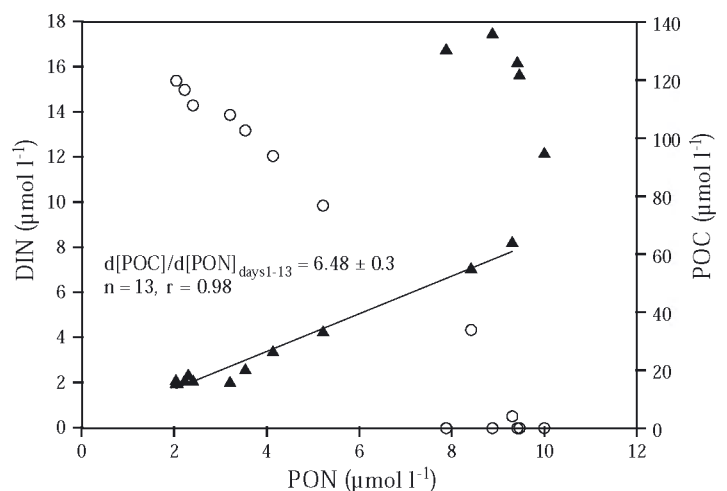


Fig. 4. Changes in POC concentration ( $\blacktriangle$ ) in mesocosms as a function of PON concentration ( $p < 0.001$ ). ( $\circ$ ) DIN concentrations. Data are means of 9 mesocosms. Regression was calculated for data obtained from Days 1 to 13 and yielded no significant y-offset

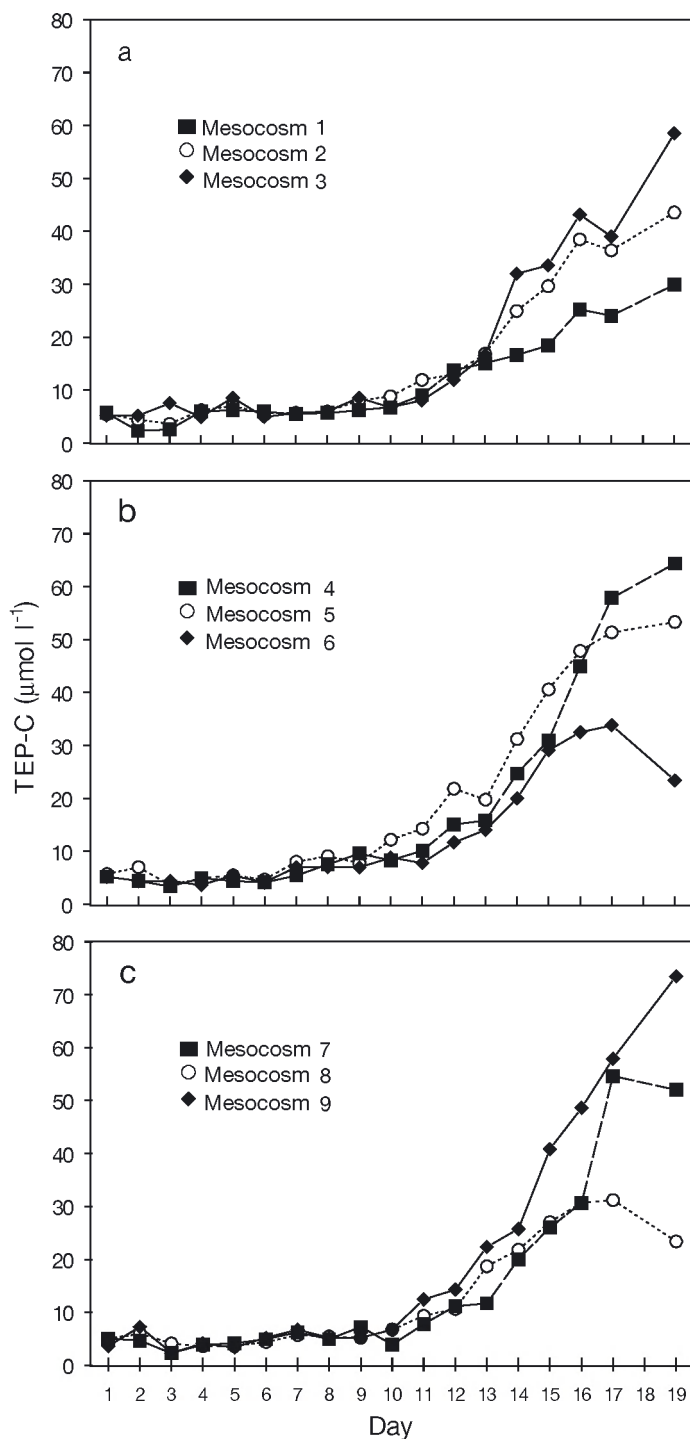


Fig. 5. TEP concentration in mesocosms during the *Emiliana huxleyi* bloom experiment in (a) Year 2100, (b) present, and (c) glacial treatments

42 (average ratio  $19.8 \pm 2.9$ ). However, no correlation was observed between TEP and PON ( $p = 0.77$ ), indicating that nitrogen is not a major element of TEP as suggested by Engel & Passow (2001). This assumption

is in accordance with earlier elemental analyses showing that nitrogen is not contained in the acidic polysaccharides released by *E. huxleyi* (Fichtinger-Schepmann et al. 1979).

In the mesocosms, the average TEP concentration was  $5.5 \pm 1.1 \mu\text{mol C l}^{-1}$  at the beginning of the study, remaining constant until Day 10, and increasing rapidly thereafter (Fig. 5). Variations in TEP concentrations among the replicates of each treatment increased during the study and exceeded differences between the average values of the 3 treatments ( $\bar{x}_{1,2,3}$ ) during the late-bloom phase (Days 10 to 19; SD of Mesocosms 1 to 9 =  $17 \mu\text{mol C l}^{-1}$ , SD of  $\bar{x}_{1,2,3} = 0.99 \mu\text{mol C l}^{-1}$ ).

In all mesocosms, TEP concentration was closely related to the cell abundance of *Emiliana huxleyi* ( $p < 0.001$ , Fig. 6), yielding an average ratio of  $d[\text{TEP-C}]/d[\text{cell}]$  of  $1.05 \pm 0.03 \text{ pmol C cell}^{-1}$ . The daily production rate of TEP per cell was therefore estimated as  $0.68 \pm 0.02 \text{ pmol C cell}^{-1} \text{ d}^{-1}$ . Differences in the ratio  $d[\text{TEP}]/d[\text{cell}]$  were observed among the mesocosms, with the highest values in the Year 2100 Mesocosms 1 and 3 (Table 1). Taking into account the influence of cell abundance on TEP concentration, we tested the influence of the  $\text{CO}_2$  treatment on TEP production by ANCOVA. This analysis showed that TEP production in the Year 2100 treatment was significantly different from the present treatment ( $p < 0.05$ , ANCOVA,  $\text{df} = 89$ ) and from the glacial treatment ( $p < 0.005$ , ANCOVA,  $\text{df} = 89$ ). In the present and glacial mesocosms, lower and more uniform values for  $d[\text{TEP}]/d[\text{cell}]$  were observed. Neither treatment differed significantly from the other in terms of TEP production ( $p = 0.40$ , ANCOVA,  $\text{df} = 89$ ).

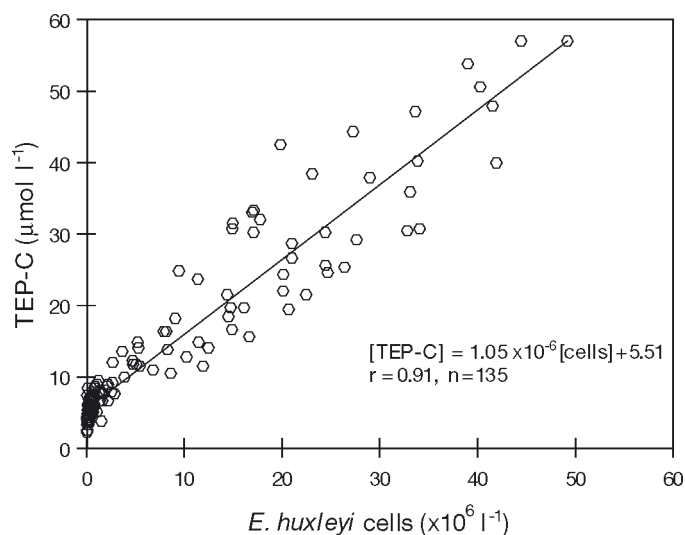


Fig. 6. TEP concentration in mesocosms as a function of abundance of *Emiliana huxleyi* ( $p < 0.001$ ). Data from all mesocosms on Days 3 to 17

Table 1. Parameters for linear regressions between transparent exopolymer particle (TEP) concentration and *Emiliania huxleyi* abundance for each mesocosm. Analysis included data from Days 3 to 17 ( $n = 15$ ) for each mesocosm

Treatment Mesocosm	$a \pm \text{SD}$	$b \pm \text{SD}$	$r^2$	$p <$
Year 2100				
1	$1.75 \pm 0.12$	$4.94 \pm 0.61$	0.95	0.001
2	$0.93 \pm 0.05$	$5.54 \pm 0.81$	0.96	0.001
3	$1.65 \pm 0.08$	$5.43 \pm 0.82$	0.97	0.001
Present				
4	$1.10 \pm 0.09$	$4.70 \pm 1.58$	0.92	0.001
5	$0.96 \pm 0.08$	$6.24 \pm 1.63$	0.92	0.001
6	$1.29 \pm 0.11$	$5.33 \pm 1.03$	0.92	0.001
Glacial				
7	$1.16 \pm 0.07$	$3.42 \pm 0.98$	0.96	0.001
8	$1.12 \pm 0.13$	$4.77 \pm 1.44$	0.84	0.001
9	$1.05 \pm 0.04$	$3.88 \pm 0.82$	0.98	0.001

The total increase in TEP concentration during the course of the bloom was  $40.8 \pm 17.5 \mu\text{mol C l}^{-1}$  averaged over all mesocosms. Whereas TEP and POC concentrations were unrelated during the first 10 d of the study, a strong relationship ( $p < 0.001$ ) was observed after the onset of nutrient (specifically phosphate) depletion, indicating that TEP was responsible for an average of  $35 \pm 2.2\%$  of the POC increase (Fig. 7).

### Dissolved organic carbon (DOC)

Considerable short-term fluctuations were observed in DOC concentrations during the course of the experiment (Fig. 8). A significant increase in DOC concentration with time was observed for the Year 2100 Mesocosm 1

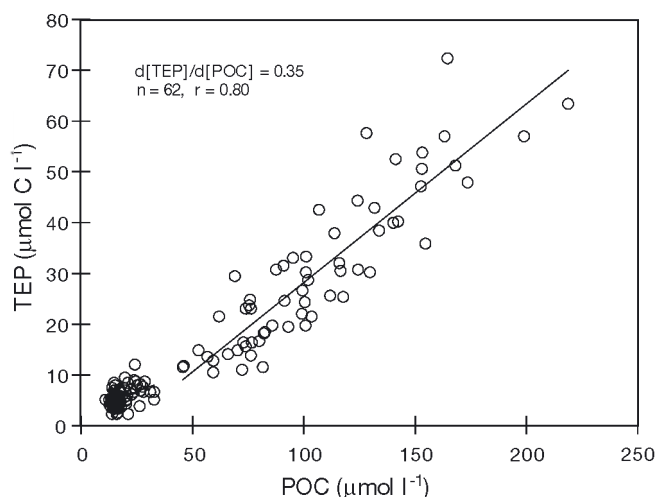


Fig. 7. TEP concentration in mesocosms as a function of POC concentration. Data from all mesocosms on Days 1 to 19

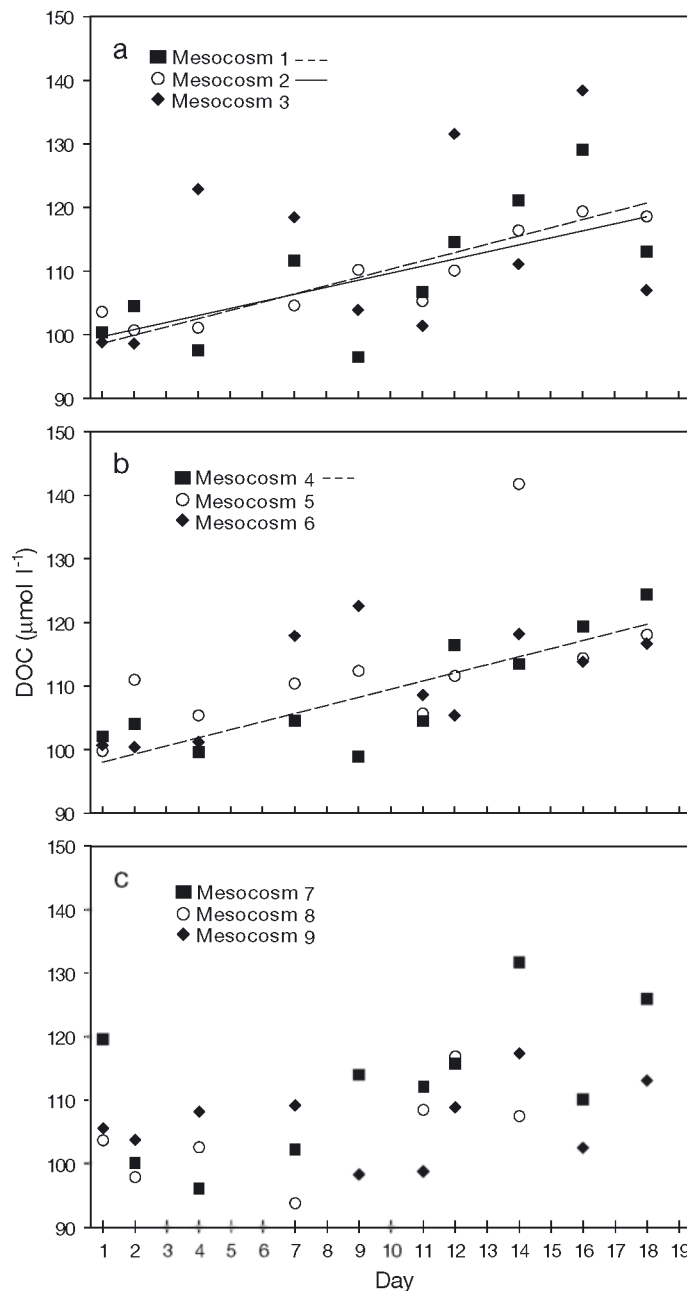


Fig. 8. Changes in DOC concentration in mesocosms during experiment in (a) Year 2100 treatment, with significant increase of DOC in Mesocosms 1 ( $n = 10$ ,  $r^2 = 0.58$ ) and 2 ( $n = 10$ ,  $r^2 = 0.84$ ), (b) present treatment, with significant increase of DOC in Mesocosm 4 ( $n = 10$ ,  $r^2 = 0.70$ ) and (c) glacial treatment

( $p < 0.05$ ,  $d[\text{DOC}]/dt = 1.30 \pm 0.44 \mu\text{mol l}^{-1} \text{d}^{-1}$ ) and Mesocosm 2 ( $p < 0.001$ ,  $d[\text{DOC}]/dt = 1.11 \pm 0.17 \mu\text{mol l}^{-1} \text{d}^{-1}$ ) and for the present Mesocosm 4 ( $p < 0.01$ ,  $d[\text{DOC}]/dt = 1.27 \pm 0.30 \mu\text{mol l}^{-1} \text{d}^{-1}$ ). In the other mesocosms no significant increase of DOC occurred during the 18 d observation, indicating that loss processes such as microbial degradation or aggregation into particles counterbalanced the production of DOC. Overall, DOC



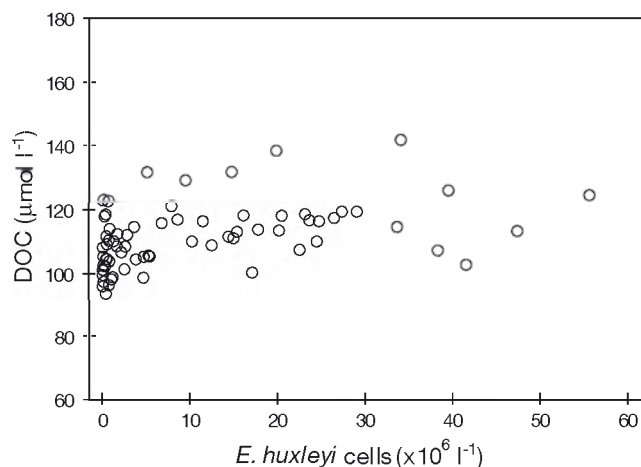


Fig. 9. Relationship between DOC concentration and cell abundance of *Emiliania huxleyi* in mesocosms ( $n = 70$ ,  $r^2 = 0.17$ ). Data from all mesocosms on Days 3 to 17

concentration was neither related to the abundance of *Emiliania huxleyi* ( $p = 0.13$ ,  $n = 70$ ) (Fig. 9) nor to TEP concentration ( $p = 0.06$ ,  $n = 79$ ). Thus, a single covariate influencing DOC concentration was not identified. No statistically significant  $\text{CO}_2$  effect on absolute DOC concentration was determined ( $p = 0.49$ , ANOVA).

### Marine snow formation

Amorphous marine snow appeared in the mesocosms on Day 16, and was sampled on Day 17. The amount of TEP within marine snow was high, comprising about 38 to 55 % of POC. The [POC]:[PON] ratios of marine snow were accordingly large, ranging between 9.9 and 35. Microscopic examination showed that the marine snow was mainly composed of a TEP matrix with entangled solid particles. The [PON]:[POP] ratios of marine snow ranged between 59 and 119, which, when compared to the range of 26 to 65 for the [PON]:[POP] ratios of suspended particles, indicate a preferential release of phosphorus from marine snow. This rapid enzymatic degradation of POP in marine snow has been observed previously (e.g. Smith et al. 1992, Engel et al. 2002b) and can be explained by increased activities of the ectoenzyme alkaline phosphatase (Smith et al. 1992, Grossart & Simon 1998).

## DISCUSSION

### TEP dynamics during the *Emiliania huxleyi* bloom

Although TEP production by coccolithophorids has not previously been documented, the production, composition and release of an acidic polysaccharide is well

described for *Emiliania huxleyi* (De Jong et al. 1976, Van Emburg et al. 1986, Nanninga et al. 1996). Similar to TEP, this coccolithophorid polysaccharide (CP) was detected by staining with Alcian Blue (De Jong et al. 1976, Fichtinger-Schepman et al. 1979). The composition of CP includes mainly neutral sugars, such as manose, rhamnose and xylose. About 20 % of the total sugar content of CP is represented by D-galacturonic acid (De Jong et al. 1976, Fichtinger-Schepman et al. 1979), an acidic sugar which can mediate the aggregation of CP chains, since the carboxyl group of one D-galacturonic acid can align to another by divalent cation ( $\text{Ca}^{2+}$ ) bridging (Leppard 1995). CP has been isolated from coccoliths of *E. huxleyi* (De Jong et al. 1976), but has also been found as dissolved polysaccharide in culture media (Nanninga et al. 1996). It is assumed that CP plays a role in the biomineralization process and probably also in the agglutination of coccoliths in the coccosphere (Van Emburg et al. 1986). From microscopy of TEP filters, we noticed that in addition to TEP the surface of *E. huxleyi* coccoliths was stained by Alcian Blue (Fig. 2). Since the colorimetric method cannot discriminate between acidic polysaccharides contained in TEP or in CP coating the coccoliths, the latter were included in the measurements. In order to estimate the contribution of the coating CP to TEP concentration, we determined the Alcian Blue adsorption and cell number in 10 samples prepared by diluting a nutrient-replete *E. huxleyi* culture with 0.2  $\mu\text{m}$  filtered artificial seawater. Alcian Blue adsorption of cells was equivalent to  $2.59 \pm 0.40$  pg Xanthan equivalents  $\text{cell}^{-1}$  or  $0.085 \pm 0.013$  pmol C  $\text{cell}^{-1}$  (Fig. 10), and was hence small compared to the calculated  $d[\text{TEP}]/d[\text{cell}]$  ratio within the mesocosm study (i.e. 0.92 to 1.75 pmol C  $\text{cell}^{-1}$ , Table 1).

The strong relationship between the abundance of *Emiliania huxleyi* and TEP concentration during this study showed that ER by *E. huxleyi* is the main process controlling TEP concentration. It also indicated that the release of dissolved CP and its subsequent aggregation into TEP are tightly coupled. An important factor favoring the observed accumulation of TEP was the depletion of phosphate and nitrate concentrations, since nutrient limitation induces the release of polysaccharides by autotrophic cells on the one hand and limits the synthesis of hydrolytic enzymes by heterotrophic bacteria on the other. Moreover, Obernosterer & Herndl (1995) showed that exopolymers released by phytoplankton under phosphate limitation are fairly resistant to bacterial decomposition. Similarly, TEP produced under nutrient deficiency may also become recalcitrant to bacterial utilization. However, as we determined only the daily changes in TEP concentration, i.e. net production of TEP, we cannot rule out that a fraction of the freshly formed TEP was immediately



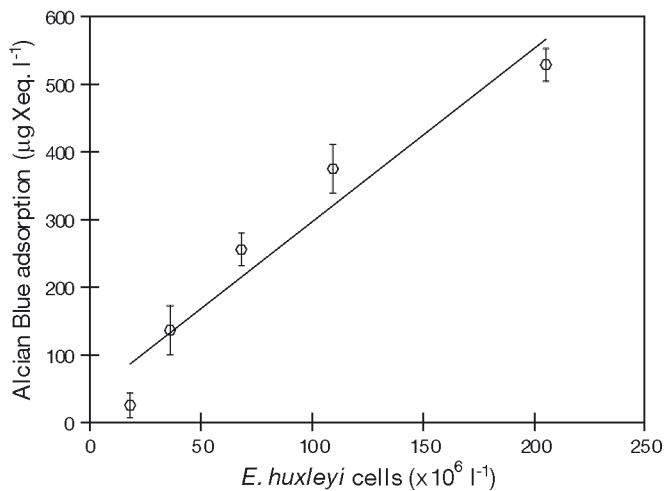


Fig. 10. *Emiliania huxleyi*. Relationship between Alcian Blue adsorption and abundance of cells harvested from a nutrient-replete cell culture ( $n = 10$ ,  $r^2 = 0.94$ ). No significant  $y$ -offset was determined. Adsorption of Alcian Blue onto cells is caused by acidic polysaccharide that covers coccoliths (see Fig. 1), but is small relative to adsorption of Alcian Blue by TEP observed during the mesocosm study

consumed by bacteria and other organisms. To solve this question, shorter-termed incubation or tracer experiments are needed.

It is striking that the average amount of TEP produced per cell during the course of the bloom ( $1.05 \pm 0.3 \text{ pmol C cell}^{-1}$ ) exceeded the amount of organic carbon stored intracellularly, e.g.  $0.65 \pm 0.11 \text{ pmol C cell}^{-1}$  as determined by Riegman et al. (2000). Nanninga et al. (1996) measured the release of  $0.56 \text{ pg CP cell}^{-1}$  in a culture of calcifying *Emiliania huxleyi*; this is equivalent to  $0.02 \text{ pmol C cell}^{-1}$  if a carbon content of CP of 39% (w:w) (Fichtinger-Schepman et al. 1979) is assumed. Hence, Nanninga et al. (1996) concluded that the release of CP contributes little to organic carbon production during an *E. huxleyi* bloom. However, they calculated the production rate only for dissolved CP that was released by nutrient saturated cells, even though they had observed that CP production increased after cell division ceased. Our study has shown that a much larger fraction of CP accumulated as particulate material, i.e. TEP. We therefore conclude that the amount of organic carbon released by *E. huxleyi* in the form of polysaccharides can be very important to organic carbon cycling and may, under certain conditions, even exceed POC production by cell growth.

It is long-standing knowledge that the concentration of particulate carbohydrates greatly increases during the course of phytoplankton blooms (McAllister et al. 1961, Barlow 1982). These changes are usually interpreted as an intracellular increase of sugars. This study has shown that much of this particulate carbohydrate increase can be explained by TEP formation.

## DOC dynamics during the *Emiliania huxleyi* bloom

Marine DOC is a very heterogeneous pool, and its total concentration is affected by a multitude of processes, including exudation by photoautotrophic cells (Fogg 1983), enzymatic solubilization of particles (Cho & Azam 1988, Karner & Herndl 1992), cell lysis (Fuhrmann 1999), sloppy feeding by metazoans (Copping & Lorenzen 1980, Nagata 2000), microbial uptake (Azam & Hodson 1977), photochemical decomposition (Mopper et al. 1991) and adsorption and coagulation processes (Leppard 1995). In laboratory cultures, it has been observed that exponentially growing *Emiliania huxleyi* produce about  $0.12 \text{ pmol DOC cell}^{-1} \text{ d}^{-1}$  (Bidanda & Benner 1997), which would have resulted in the production of approximately  $19 \mu\text{mol DOC l}^{-1}$  between Days 3 and 18 of this study. However, we did not find a relationship between cell abundance and net production of DOC. Instead, we observed a high variability in DOC concentrations over time, indicating that production and loss of DOC were tightly coupled during all stages of the *E. huxleyi* bloom. The increase in TEP concentration suggested that aggregation into particulate matter was the major sink for dissolved polysaccharides after the onset of nutrient depletion. Freshly produced DOC of low molecular weight can be taken up directly by heterotrophic bacteria, and presumably did not accumulate during the bloom either. Nevertheless, a slow accumulation of DOC occurred in some of the mesocosms, indicating either the formation of recalcitrant DOC or a severe limitation of heterotrophic bacterial activity.

## Role of TEP and DOC in storage of excess carbon

Carbon uptake and partitioning into different fractions of organic matter were examined during the bloom of *Emiliania huxleyi*. The results, averaged for all mesocosms, are summarized in Table 2. The comparison of observed carbon flows with those expected from changes of nitrogen concentration and Redfield stoichiometry allowed the assessment of carbon overconsumption during the bloom and the storage of excess carbon in the various elemental pools.

As derived from the decrease in DIC concentrations, about 37% carbon overconsumption occurred within the first 19 d of the study. The largest fraction of the assimilated carbon was traced to the POC pool. Carbon contained in TEP explained about 63% of excess POC. These results are in accordance with those of Engel et al. (2002a), who observed that TEP was a major sink for excess carbon during a mesocosm diatom bloom. Compared to the mesocosm study of Engel et al. (2002a), this study further showed that a significant production

Table 2. Total uptake and storage of carbon and nitrogen in different elemental pools during the study (Days 1 to 19), averaged over all mesocosms. Excess carbon calculated as carbon taken up or contained in excess of value expected from Redfield stoichiometry ( $\Delta N \times 6.6$ ). nd: not determined

Partition	$\Delta N$ ( $\mu\text{mol l}^{-1}$ )	$\Delta C$ ( $\mu\text{mol l}^{-1}$ )	C:N (mol:mol)	$\Delta N \times 6.6$ ( $\mu\text{mol l}^{-1}$ )	Excess C ( $\mu\text{mol l}^{-1}$ )
Uptake	$15.3 \pm 0.2$	$161 \pm 23$	$11 \pm 1.5$	$101 \pm 0.9$	$60 \pm 23$
POM	$5.9 \pm 1.0$	$114 \pm 48$	$19 \pm 8.8$	$39 \pm 6.6$	$75 \pm 48$
DOM	nd	$11 \pm 8$	–	–	$\leq 11 \pm 8$
TEP	nd <sup>a</sup>	$42 \pm 18$	–	–	$\leq 42 \pm 18$
(POM-TEP)	$\leq 5.9 \pm 1.0$	$72 \pm 51$	$\geq 12 \pm 8.9$	$\leq 39 \pm 6.6$	$\geq 33 \pm 51$

<sup>a</sup>TEP may adsorb nitrogen, but this was not determined during the study

of TEP and relatively high contributions of TEP to POC concentrations are not exclusive to eutrophic systems with high phytoplankton biomass, but can also occur in low-nutrient environments more comparable to oceanic conditions. This conclusion is supported by observations from the Baltic Sea, where the relative contribution of TEP to POC was observed to be higher in the nutrient-poor central Baltic Sea during summer than in the coastal Baltic Sea during the spring bloom (Engel et al. 2002b).

The average increase of  $11 \mu\text{mol DOC l}^{-1}$  during the bloom was small compared to the increase in POC and TEP-C. Moreover, a significant accumulation of DOC was observed in only 3 of 9 mesocosms. Because DON was not determined, only an upper limit for the amount of excess DOC could be calculated. However, even if there was no net production of DON, the role of DOC for storage of excess carbon was small.

Although we had reasons to assume that nitrogen was not an original component of TEP produced by *Emiliania huxleyi*, we cannot exclude that DON may later become incorporated into TEP by adsorption or aggregation processes. Therefore, the estimate for nitrogen contained in POM from which TEP was subtracted (POM-TEP) represents an upper boundary. Correspondingly, the calculated excess carbon of the non-TEP POM fraction and its C:N ratio are low estimates. However, even the minimum C:N ratio of non-TEP POM was considerably above the Redfield ratio. This can be explained by an intracellular storage of organic carbon, which was observed for *E. huxleyi* in culture experiments (Riegman et al. 2000). Another process potentially responsible for the formation of POM with high C:N ratios would be the preferential remineralization of nitrogen from detrital particles. Because nitrogen regeneration supports primary production, an underestimation of regenerated nitrogen could be responsible for carbon overconsumption, provided that the organic carbon produced in this way would enter the export flux (Thomas et al. 1999). In principle, TEP production and preferential reminerali-

zation of PON in the euphotic layer would have the same impact on POC:PON ratios and on carbon overconsumption. We can safely assume that neither process will occur exclusively, and this study indicates that the influence of TEP production and of preferential nitrogen recycling on POC production may even be of equal importance.

About 17% of the carbon that was taken up during the present study was not recovered in the POC and DOC pool. We presume that this loss of organic matter was due to sedimentation of particles, as also indicated by a sediment pumped from the bottom of the enclosures at the end of the experiment.

### Sensitivity of TEP and DOC production to $\text{CO}_2$

To the best of our knowledge, there are almost no previous studies on the direct effects of  $\text{CO}_2$  on ER. In incubation experiments with natural plankton harvested from the central Baltic Sea, Engel (2002) observed that final TEP concentrations were related to initial seawater  $\text{CO}_2$  concentration. Similar results were obtained during incubation experiments with monospecific cultures of *Thalassiosira weissflogii* and a non-calcifying strain of *Emiliania huxleyi* by Heemann (2002).

During this study, we could not determine any significant difference between the  $\text{CO}_2$  treatments as far as total concentrations of TEP and DOC were concerned. This was largely due to high variations among the replicates. With regard to TEP concentration, a large fraction of the variation could be explained by cell abundance. Normalized to cell abundance, TEP production was significantly higher in the high  $\text{CO}_2$  treatment Year 2100 than in the present and glacial treatments. This indicates that a direct effect of  $\text{CO}_2$  on polysaccharide exudation, as suggested from fully enclosed systems and culture experiments, may also emerge in larger and more natural systems, such as the mesocosms.

Although ER by autotrophic cells contributes to the build-up of the DOC pool, no relationship between DOC concentration and cell abundance was observed during this study. Consequently, no influence of  $\text{CO}_2$  on DOC production could be identified. Seemingly, the standing stock of DOC was determined by the response of the microbial food web rather than by primary production. The former comprises a plethora of possible predator-prey interactions, which may not be influenced by  $\text{CO}_2$  concentration. However, the

observation that DOC increased in some of the Year 2100 and present mesocosms, but in none of the glacial mesocosms may point to a stimulating effect of CO<sub>2</sub> on ER that influences the DOC concentration on time scales of weeks to months.

As a consequence of the higher TEP production under high CO<sub>2</sub> conditions, we would have expected an enhanced uptake of DIC and an increase in POC concentration in this treatment. This was not observed. One explanation could be that the increased TEP production stimulated particle aggregation, as observed previously by Logan et al. (1995) and Engel (2000), and accelerated sedimentation. During this study, formation and sedimentation of marine snow with a high TEP content occurred, supporting the assumption that TEP had a regulating effect on particle concentration. However, in order to discover if this hypothesis is correct and transferable to the ocean, additional studies need to be conducted to determine particle sedimentation and the fate of TEP more exactly. Also, the role of bacteria in TEP degradation needs to be elucidated, since degradation may be an important loss process for TEP, specifically in deep-ocean environments, where degradable organic matter rather than nitrate or phosphate is the limiting substance. Nevertheless, the results of this study suggest that production of TEP by marine phytoplankton can provide a potential link between CO<sub>2</sub>-sensitive carbon assimilation and sequestration of excess carbon to the deep ocean.

**Acknowledgements.** We are grateful to the staff of the Large Scale Facility (LFS) in Bergen, in particular to Clelia Booman, Jorun Egge, Agnes Aadnesen, Erling Heggoy and Jens Nejstgaard, who gave us a warm welcome and helped accomplish our study. Christoph Völker, Markus Schartau and Uta Passow are acknowledged for fruitful discussions. Three anonymous referees are acknowledged for their suggestions on improving this publication. This work was supported by the European Commission Human Potential Programm No. HPRI-1999-0056.

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