TEP and DOC production during a bloom experiment with *Emiliania huxleyi* exposed to different CO₂ concentration

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Abstract

The role of transparent exopolymer particles (TEP) and dissolved organic carbon (DOC) for organic carbon partitioning under different CO₂ conditions was examined during a mesocosm study with the coccolithophorid *Emiliania huxleyi*. Nine outdoor enclosures (~11m³) were modified to simulate ‘glacial’, ‘present’ and ‘year 2100’ CO₂ environments and fertilized with nitrate and phosphate in order to favor bloom development of *E. huxleyi*. Extracellular organic carbon in form of DOC and TEP was determined over a period of 19 days, covering the pre-bloom and bloom period of *E. huxleyi*. In all of the mesocosms, an uptake of approximately 60% more dissolved inorganic carbon (DIC) than inferred from nitrate utilization and Redfield stoichiometry occurred and was largely traced to the particulate organic carbon (POC) pool. 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 approximately 33% of POC increase. Highest TEP production rates per cell were observed in the ‘year 2100’ CO₂ treatment. DOC concentration was highly variable over time and neither related to *E. huxleyi* abundance nor to TEP concentration. DOC concentration increased significantly over time in two of the ‘year 2100’ mesocosms, in one ‘present’ mesocosm, but in none of the ‘glacial’ mesocosm. Although our results showed that the role of DOC and TEP for carbon partitioning was fundamentally different during the *E. huxleyi* bloom, they indicate that production of both, DOC and TEP, may be sensitive to CO₂ concentration.
Introduction

An important mechanism for the regulation of atmospheric CO$_2$ concentration is the fixation of CO$_2$ by marine phytoplankton and the subsequent export of the organically bound carbon to the deeper ocean. Following the ideas of Redfield et al. (1963) and Eppley and Peterson (1979), the magnitude of organic carbon export is expected to depend on the availability of major nutritional elements in the surface ocean and can be estimated from nitrate uptake, using a C:N ratio of 106:16. However, the applicability of the ‘Redfield’ ratio for calculation of oceanic carbon fluxes has been challenged recently, since the draw down of dissolved inorganic carbon (DIC) exceeds the amount expected from nitrate removal and Redfield stoichiometry seasonally (Sambrotto et al. 1993; Michaels et al. 1994, Marchal et al. 1996, Thomas et al. 1999, Körtzinger et al. 2001). This observation was referred to as ‘carbon overconsumption’ by Toggweiler (1993). Since then a number of hypotheses were 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). To which extent these processes are responsible for the excess DIC uptake in the field has yet to be determined and may depend on the area or season considered. A question of particular importance in this context is the fate of excess carbon, i.e. to what extent it will be exported below the winter mixed layer and hence removed from exchange with the atmosphere for more than one year. Thus, it is of special interest whether a mechanism mediating carbon overconsumption has the capability to also account for a deep carbon export.
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 this assimilation of excess carbon is the release of extracellular organic matter (EOM) (Fig. 1) (Fogg, 1966, Wood and Van Valen, 1990). Although the mechanisms of EOM release has not been fully elucidated yet, it can be assumed that low molecular weight (LMW) substances, such as monomer or oligomer sugars penetrate the cell membrane by diffusion. The rate of this LMW-EOM leakage should 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. 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). Since EOM release is a possibility for the cell to dispose of excess photosynthesates under nutrient deplete conditions, EOM should not contain more than negligible amounts of the limiting element (Wood and Valen 1990). In this respect EOM release can be viewed as a cellular carbon overflow. Whether this cellular carbon overflow is responsible for carbon overconsumption in the field has yet to be determined. It is well known that EOM release 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 and Koeve 2000, Søndergaard et al. 2000), supporting the idea that DOC production results from a cellular carbon overflow. Yet, the deep export of DOC is principally restricted to subduction of surface waters, e.g. by thermohaline ventilation. Because this process operates on long times scales, i.e. months to years, much of the seasonal accumulated DOC will likely be degraded before it arrives at greater depths.
The major fraction of HMW-EOM are polysaccharides (Benner 2002), some of them contain acidic sugars that facilitate polysaccharide aggregation into particles, known as transparent exopolymer particles (TEP) (Alldredge et al. 1993, Leppard 1995, Engel et al. submitted). 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, as well as 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. 2002). 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 diatom blooms (Engel et al. 2002a). In contrast to DOC, TEP can participate in particle mediated processes such as marine snow formation and sinking (Alldredge et al. 1993, Passow et al. 2001) and have therefore the potential to account for a deep export of carbon on relatively short time scales.

In the ocean, EOM release was found to be related to primary production (Baines and Pace, 1991). Since primary production of marine phytoplankton is sensitive to CO₂ concentrations (Rost et al. 2003), one might speculate that EOM release is not only responsible for, but also mediates a CO₂ effect on organic carbon production. We investigated these hypotheses during a mesocosm bloom study with *Emiliana huxleyi*, exposed to three different CO₂ concentrations, focusing on a) the temporal changes in EOM concentration, respectively DOC and TEP, b) the role of DOC and TEP for storage of excess carbon during an *E. huxleyi* bloom and c) the influence of seawater CO₂ concentrations on DOC and TEP production.
Material & Methods

Set-up and sampling.

This study was conducted at the EU-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 (Zondervan et al. in prep.). Briefly, nine polyethylene enclosures (~ 11 m³, 4.5 m water depth) were moored to a raft in a fjord (for more details see Williams & Egge, 1998). The bags were filled with unfiltered, nutrient-poor, post-bloom fjord water, which was pumped from 2m depth adjacent to the raft. The enclosures were covered by gas-tight tents made of ETFE foil, which allowed for 95% light transmission of the complete spectrum of sunlight. The atmospheric and seawater pCO₂ were manipulated to achieve 3 different CO₂ levels in triplicate, corresponding to approximately year 2100, assuming the IPCC's 'business as usual' scenario IS92a- (mesocosms 1-3), present (mesocosms 4-6) and glacial atmospheric CO₂ levels (mesocosms 7-9), respectively (Delille et al., in prep.). To promote the development of a coccolithophorid bloom, nitrate and phosphate were added in a ratio of 30:1 yielding initial concentrations of 15 µmol L⁻¹ NO₃ and 0.5 µmol L⁻¹ PO₄. After nutrient addition, the water was gently mixed by means of an airlift (for more details see Egge & Asknes, 1992), using the same air as for gassing the tents. Over a period of three weeks 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 particle aggregates (> 0.5 cm; 'marine snow') appeared in the mesocosms and were abundant enough to be collected manually with a syringe in the upper 0.5m of the water column on day 17.
**Biological and chemical analyses.** Nitrate and nitrite were determined from GF/F-filtered and poisoned (0.1% HgCl₂) samples with an autoanalyser (AA II) at the home 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 mass spectrometry (ANCA SL 20-20, Europa Scientific) from 1 L (day 0-12) and 0.5 L (day 13-19) 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 0.5-1.0 L samples filtered onto GF/F filters. All filters were prepared in duplicates and stored at –20°C until analysis.

Samples for DOC analysis were collected in glass ampoules after filtration through precombusted GF/F filters. The samples were poisoned with 85% H₃PO₄, 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) analyser. A four 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⁻¹. In order to assess the instrument blank we used two external standards (Certified Reference Standards, CRM’s) obtained from the Hansell Laboratory, Bermuda Biological Station. The machine blank was between 8 – 12 µmol L⁻¹ C for all samples and was subtracted from the measurements. All DOC concentrations reported are the average of three injections from each sample.

TEP are detected by staining with Alcian Blue (Fig. 2), a cationic copper phthalocyanine dye that complexes carboxyl (–COO⁻) and half-ester sulfate (OSO₃⁻) reactive groups of
acidic polysaccharides. The amount of Alcian Blue adsorption per sample volume is a
measure for TEP concentration and was determined colorimetrically according to Passow
and Alldredge (1995) from 50-100 ml samples filtered onto 0.4 µm Nuclepore filters. All
filters were prepared in triplicate. The carbon content of TEP was determined following the
approach of Engel and Passow (2001). Three aliquots of 5 L (pooled samples of
mesocosms 1-3, 4-6 and 7-9, respectively) were collected on 7 days throughout the bloom
and filtered through precombusted glass fiber filters. TEP were generated from the filtrate
during 24 h of circulation through a Tangential Flow Filtration (TFF) system with a 0.16
µm membrane. The fraction >0.16 µm was concentrated from 5 L to a final volume of 1-2
L. The concentrated samples were analyzed for carbon, nitrogen and TEP concentration.
TEP were measured colorimetrically (Passow & Alldredge 1995) from 100-200 ml, with at
least 2 replicates each. POC and PON were determined from 0.8-1.6 L as described above.
All materials in contact with the sample were either autoclaved or acid (10% HCl) rinsed.
Blank glass fiber filters were prepared for each filtration series. In 10 samples the carbon
concentration was below the detection limit of 30 µg L⁻¹. For the remainder of the
samples, the slope of the regression of POC concentration versus colorimetrically
determined TEP concentration (in µg Xanthan Equivalents (Xeq.) L⁻¹) was calculated with:

\[ \text{[POC, µg]} = 0.39±0.08 \times \text{[TEP, µg Xeq.]} \ (r^2=0.73, n=11, p<0.005) \]

No correlation was observed between TEP and PON, indicating that nitrogen is not a major element of TEP as
suggested before (Engel & Passow 2001).

Cell counts of *E. 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 analysed from fresh samples at high flow rate (~70 µl
min⁻¹) 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 analysed using CYTOWIN (Vaulot 1989) and WinMDI (version 2.7, Trotter).

**Statistical treatment of data**

Average values are given by the statistical mean ($\bar{x}$) and its standard variation (SD). In order to determine the significance of a regression coefficient, i.e. the slope of a linear relationship ($d[y]/d[x]$), a $t$-test according to Sachs (1974) was performed. Significance was accepted for values of $p<0.1$.

**Results**

**General bloom development**

At day 1 of the experiment, seawater CO$_2$ concentrations were adjusted to 741±47 ppmV in the 'year 2100' scenario of mesocosms 1-3, to 414±11 ppmV in the 'present' enclosures 4-6 and to 190±2.4 ppmV in the 'glacial' mesocosms 7-9. More detailed information about the seawater carbonate chemistry during the experiment will be given elsewhere (Delille et al. in prep.). Increase of biomass was detectable after day 3 in all of the mesocosms and after day 10 the phytoplankton community was dominated by number by *E. huxleyi*. In opposite to the simultaneously increase in biomass, inorganic nutrients declined rapidly. Phosphate dropped from an initial concentration of ~ 0.5 µmol L$^{-1}$ to below detection limit after day 10. Nitrate started at ~ 15.5 µmol L$^{-1}$ and was not detectable after day 13. Ammonia was undetectable during the whole study. Maximum concentrations of POP and PON were observed on day 13 and 14, respectively, and declined steadily thereafter. Cell abundance of *E. huxleyi* increased exponentially after day 3, but maximum cell concentrations varied considerably between mesocosms (Zondervan et al., in prep.). Cell growth was on average
0.45 d⁻¹, equivalent to a cell doubling every 2.2 days. This value is considerably lower than maximum growth rates, observed for nutrient replete cells (µmax=0.76 d⁻¹) (Riegmann et al. 2000) and indicates that *E. huxleyi* was growth limited during the bloom development. Average cell abundance decreased after day 19, possibly due to viral lysis (Dellile et al., in prep.). Therefore, only data before the collapse of the bloom (day 19) will be presented here.

**Transparent exopolymer particles (TEP)**

Mean TEP concentration at the beginning of the study was 166±34.8 µg Xeq. L⁻¹, stayed rather constant until day 10 and increased rapidly thereafter in all of the mesocosms (Fig. 3a-c). Between mesocosms, variability of TEP production was small at the beginning of the experiment, but high during the late bloom, yielding a wide range of TEP concentrations on day 19. During the study, TEP accounted for an increase of POC concentration on the order of 40.8±17.5 µmol L⁻¹. TEP concentrations were closely related to the cell abundance of *E. huxleyi* with \(\frac{d[TEP]}{d[cell]}\) yielding 32.3±0.92 pg Xeq. cell⁻¹ or 1.05±0.03 pmol TEP-C cell⁻¹ or 12.6±0.36 pg TEP-C cell⁻¹ (Fig. 4). The production rate of TEP-C per cell can therefore be estimated with 5.7 pg TEP-C cell⁻¹ day⁻¹.

Slight but significant differences in the ratio \(\frac{d[TEP]}{d[cell]}\) were observed between the mesocosms. The highest increase of TEP concentration with cell abundance occurred in the ‘year 2100’ mesocosms 1 and 3 (table 1). In the ‘present’ and ‘glacial’ treatments TEP production per cell was lower and more uniform.

**Dissolved organic carbon (DOC)**

High day to day variations were observed for DOC concentrations in the course of the experiment (Fig. 5a-c). A significant increase in DOC concentration with time \(\frac{d[DOC]}{dt}\),
t: days 1-18) was observed for the ‘year 2100’ mesocosms 1 (1.30±0.44 µmol L⁻¹ d⁻¹) and 2 (1.11 ±0.17 µmol L⁻¹ d⁻¹) and for the ‘present’ mesocosm 4 (1.27±0.30 µmol L⁻¹ d⁻¹). In the others and in all of the ‘glacial’ mesocosms no significant increase of DOC occurred within the 18 days of observation (no samples were taken on day 19), indicating that degradation counterbalanced the production of DOC. DOC concentration was not related to the abundance of \( E. \textit{huxleyi} \) cells \((p=0.13)\) (Fig. 6). Furthermore, no significant relationship between TEP-C and DOC concentrations were observed \((p=0.10)\).

**Particulate carbon dynamics**

During the first two weeks, changes in POC concentration were closely related to changes in PON and POP concentrations, with \( d[\text{POC}]/d[\text{PON}] \) equal to 6.8±0.3 mol mol⁻¹, a value which is in accordance with the expected Redfield value of 6.6. In contrast, changes of POC relative to POP were more than twice as high as the Redfield value, yielding \( d[\text{POC}]/d[\text{POP}] \) equal to 338±13 mol mol⁻¹, which underlines the exceptional ability of \( E. \textit{huxleyi} \) to grow with low cellular P \((\text{Riegman et al., 2000})\). At the same time, changes in PON were tightly related to POP resulting in \( d[\text{PON}]/d[\text{POP}] \) of 48.0 ±1.3 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 of \([\text{POC}]:[\text{POP}]\) and \([\text{POC}]:[\text{PON}]\) concentration ratios (Fig. 7a, b). The decoupling of POC from PON production coincided with DIN exhaustion (Fig. 8), indicating that a large amount of carbon was assimilated under nutrient deplete conditions and accumulated in the POC pool. Contribution of TEP-C to POC production was roughly 16% \((p<0.05)\) within the first 10 days of the study, but was responsible for approximately 35% of the POC increase \((p<0.001)\) after nutrient depletion (Fig. 9).
**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 and TEP-C comprised about 38-55% of POC. The [POC]:[PON] ratios were accordingly large and ranged between 9.9 and 35. Microscopic examination showed that marine snow was mainly composed of a TEP matrix with particles entangled. The [PON]:[POP] ratios of marine snow ranged between 59 and 119, which, compared to the range of 26-65 for [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. 2002) and can be explained by increased activities of the ectoenzyme alkaline phosphatase in marine snow (Smith et al 1992, Grossart & Simon 1998).

**Discussion**

**TEP dynamics during the E. huxleyi bloom**

Although there has been no documentation about TEP production by coccolithophorids hitherto, the production, composition and release of an acidic polysaccharide is well documented for *E. huxleyi* (De Jong et al. 1976, Van Emburg et al. 1986, Nanninga et al. 1986). 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, an acidic sugar, which can mediate the aggregation of CP chains, because the carboxyl group of one D-galacturonic acid can align to another by divalent cation (Ca$^{2+}$) bridging (Leppard 1995).
CP has been isolated from coccoliths of *E. huxleyi* (De Jong et al. 1976), but also found as an dissolved polysaccharide in culture media (Nanninga et al. 1996). It is assumed that CP plays a role in the biomineralisation process and probably also in the agglutination of coccoliths in the coccosphere (Van Emburg et al. 1986). From microscopy of TEP filters, we, in fact, noticed that in addition to TEP the surface of *E. huxleyi* coccoliths was stained by Alcian Blue (Fig. 2). Since the colorimetric method can not discriminate between acidic polysaccharides contained in TEP or in CP coating the coccoliths, the latter will be included in the result. In order to estimate the contribution of this coating CP to TEP concentration, we determined the Alcian Blue adsorption and cell number in five samples prepared by diluting a nutrient replete *E. huxleyi* culture with 0.2 µm filtered artificial seawater. Alcian Blue adsorption of cells was equivalent to 2.59±0.40 pg Xeq. Cell⁻¹ (Fig. 10) and hence small compared to the TEP to Cell ratio within the mesocosm study. Thus the strong relationship between the abundance of *E. huxleyi* and TEP concentration during the mesocosm study indicated a tight coupling between the release of dissolved CP and TEP formation. This interpretation is supported by model calculations showing that the dynamics of dissolved polysaccharide and TEP concentrations during this study follow aggregation kinetics (Engel et al. submitted). It is striking that the amount of TEP-C produced per cell (12.6±3.6 pg TEP-C cell⁻¹) can exceeded the amount of organic carbon stored intracellular, e.g. 7.83±1.26 pg C cell⁻¹ as determined by Riegman et al. (2000). Nanninga et al. (1996) measured the release of 0.56 pg CP cell⁻¹ in a culture of calcifying *E. huxleyi*, which is equivalent to 0.22 pg C cell⁻¹ if a carbon content of CP of 39% (weight:weight) (Fichtinger-Schepman et al. 1979) is assumed. Hence, the authors concluded that the release of CP contribute little to organic carbon production during an *E. huxleyi* bloom. However, the authors calculated the production rate only for dissolved CP that was released by nutrient saturated cells, even though they observed that CP production increased after cell division terminated. Our study showed 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 form of polysaccharides can be of high importance for organic carbon cycling and may, under the condition of nutrient limitation, even exceed POC production by cell growth.

It is long-standing knowledge that the concentration of particulate carbohydrates greatly increases in the course of phytoplankton blooms (Mc Allister et al. 1961, Barlow 1982). However, these changes were attributed to an intracellular increase of sugars, hitherto. This study showed that the extracellular formation of TEP may explain much of this particulate carbohydrate increase, at least during *E. huxleyi* blooms.

**DOC dynamics during the *E. huxleyi* bloom**

DOC in seawater is produced by various mechanisms, including exudation by photoautotrophic cells (Fogg 1983), enzymatic solubilization of particles (Cho and Azam, 1988, Karner and Herndl, 1992), cell lysis (Fuhrmann, 1999) or sloppy feeding by metazoa (Copping and Lorenzen, 1980, Nagata 2000). In cell cultures it has been observed that exponentially growing *E. huxleyi* produce about 0.12 pmol DOC cell⁻¹ day⁻¹ (Biddanda and Benner, 1997), which would have resulted in a daily production of approximately 2.5 µmol DOC L⁻¹ between day 12 and day 17 during this study. However, we did not find a relationship between cell abundance and DOC production, but a high variability of DOC concentrations over time, indicating that production and consumption of DOC was tightly coupled during all stages of the *E. huxleyi* bloom. The largest fraction of DOC released by *E. huxleyi* are carbohydrates (Biddanda and Benner, 1997). Because a large fraction of released polysaccharides accumulated in TEP, we assume that the changes in DOC concentration rather reflected the turn-over of labile, LMW carbohydrates and a slow
accumulation of refractory substances.

**Influence of TEP and DOC on carbon dynamics**

Carbon uptake and flow to the various pools of organic carbon was examined during the bloom of *E. huxleyi* and is summarized in table 2. Comparison of observed carbon flows with those expected from changes of nitrogen concentration and Redfield stoichiometry allows the assessment of ‘carbon overconsumption’ during the bloom and the relative importance of the different carbon pools for the storage of excess carbon.

As derived from the decrease in DIC concentrations (data will be given by Zondervan et al.) about 60% ‘carbon overconsumption’ occurred within the first 18 days of the study. The largest fraction of the excess carbon was traced in the POC pool. Approximately 4.6% of the excess carbon accumulated in the DOM pool, which is an upper estimate, since DON was not determined. Carbon contained in TEP explained the fate of 15.8% of overconsumed carbon and was responsible for more than 50% of excess POC. These results are in accordance with the observations of Engel et al. (2002), who observed that the largest fraction of excess carbon was channeled to TEP during a mesocosm diatom bloom. Compared to the mesocosm study of Engel et al. (2002), this study further showed that a significant production of TEP and relatively high contributions of TEP-C to POC concentrations are not exclusive to eutrophic systems with high phytoplankton biomass but can occur in low nutrient environments, which are more comparable to oceanic conditions. This conclusion is supported by observations from the Baltic Sea, where the relative contribution of TEP-C to POC was observed to be higher in the nutrient poor central Baltic Sea during summer, than at the coastal Baltic Sea in the course of the spring bloom (Engel et al., 2002b).
Almost half of the carbon and nitrogen, which had been taken up during the present study, were not recovered in the POM and DOC pool. We assume that this loss of organic matter was most likely due to sedimentation of particles, which was also indicated by a huge amount of ‘sediment’ pumped from the bottom of the enclosures at the end of the experiment. The loss of carbon relative to the loss of nitrogen, made up for a C:N ratio of 13.6, which is an lower estimate, since some of the missing nitrogen may have entered the DON pool. Accordingly, we can estimate a minimum ‘sedimentation’ of excess carbon of ~25%. This clearly shows that high C:N ratios of sinking material during a phytoplankton bloom can be explained by a selective enrichment of carbon, as a consequence of ‘carbon overconsumption’. High C:N ratios of the lost particles were in agreement with high C:N ratios of marine snow, observed during this study. As for marine snow, we can assume that TEP was partly responsible for the excess carbon in the sinking matter. However, C:N ratios of POM, from which TEP-C was subtracted, were considerably above the Redfield ratio also. This may be explained by intracellular carbon storage, which was observed for E. huxleyi in culture experiments (Riegm an et al. 2000). Another process potentially responsible for the formation of POM with high C:N ratios would be the preferential remineralisation of nitrogen from detrital particles. Because nitrogen regeneration supports primary production simultaneously with nitrate, 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). Thus, TEP production and preferential remineralisation of PON in the euphotic layer potentially have the same impact of 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 preferential nitrogen recycling on POC:PON ratios may even be of equal importance. However, it is the special property of TEP to accelerate marine snow formation and therewith to provide a vehicle for a deep export of particulate
Sensitivity of TEP and DOC production to CO₂ concentration

There are almost no previous studies about the direct effects of CO₂ on carbon exudation, extracellular carbon dynamics or concentrations. In incubation experiments with natural plankton harvested from the central Baltic Sea, Engel (2002) observed that final TEP concentrations were related to initial seawater CO₂ concentration. Similar results were obtained during incubation experiments with monospecific cultures of T. weissflogii and a non-calcifying strain of E. huxleyi (Heemann 2002, Heemann et al., in prep.).

During this study TEP production rates normalized to cell number were significantly higher in two of the three high CO₂ mesocosms, than in the ‘present’ and ‘glacial’ CO₂-treatments. This indicates that a direct effect of CO₂ on polysaccharide exudation and TEP formation, as suggested from fully enclosed systems and culture experiments, may also emerge in more complex plankton communities, like in the mesocosms. Release of carbohydrates from autotrophic cells contributes also to the build up of the DOC pool, and the observation that DOC increased in the ‘year 2100’ and ‘present’ treatment, but not in the ‘glacial’ treatment may point to a stimulating CO₂ effect on phytoplankton exudation also. The hypothesis that in particular carbon release by marine phytoplankton is sensitive to CO₂ concentration can be supported by theoretical arguments. First, as primary production is the first order process to control organic carbon release under nutrient limiting conditions, factors influencing primary production will consequently affect organic carbon release. For marine phytoplankton, an influence of CO₂ on primary production is well known (Riebesell et al. 1993, Rost et al. 2003). Second, the onset of organic carbon release, e.g. at the end of a phytoplankton bloom, is the time when seawater CO₂
concentration has already been reduced through phytoplankton growth. Many bloom forming phytoplankton species are capable to enhance CO₂ supply by carbon concentrations mechanisms (CCM) (Raven 1991) and therewith saturate primary production even at low CO₂ concentrations in the ocean; i.e. 8-22 µmol L⁻¹ (Goerike & Fry 1994). This active regulation of carbon uptake results in an apparent insensitivity of primary production to CO₂ concentrations at oceanic conditions (Goldman 1999). However, CCMs may be down-regulated at times of nutrient exhaustion, because they require the biosynthesis of enzymes and depend on ATP supply (Beardall & Giordano 2002). In this case, primary production could be CO₂ limited even at oceanic CO₂ concentrations, because the major carboxylating enzyme RubisCo, has a low affinity for CO₂ (Riebesell et al. 1993). This would explain a direct relationship of TEP production and diffusion controlled CO₂ uptake rates as suggested by Engel (2002). Hence, as the timing of carbon overflow potentially coincides with reduced CO₂ concentration and a low CO₂ uptake capacity of the phytoplankton cell, changes in seawater CO₂ may directly influence primary production rates and in consequence carbon release.

However, as far as total concentration of TEP and DOC are concerned, we could not determine any significant difference between the CO₂ treatments during this study. Seemingly, the standing stocks of TEP and DOC were determined by mechanisms other than production. For DOC, it is known that total concentration strongly depends on the response of the microbial food web. This comprises a plethora of possible predator-prey interactions, which may not at all be influenced by CO₂ concentration. The concentration of TEP may be ‘top down’ controlled either, but the role of heterotrophs for the fate of TEP is not well understood. Obernosterer and Herndl (1995) showed that exopolymers released by phytoplankton under phosphate limitation are rather resistant to bacterial decomposition. In a similar way TEP produced under nutrient deficiency in the mesocosm may also escape
bacterial degradation. The role of zooplankton grazing on TEP production is under debate. Passow and Alldredge (1999) documented that euphausids graze on TEP; Prieto et al. (2000), however, showed that copepods may not. On the other hand, a well known process for the fate of TEP is the aggregation of TEP with solid particles (Logan et al. 1995, Engel 2002), such as organisms and debris, and the subsequent sinking to deeper waters (Alldredge et al. 1993, Passow et al. 2001). Since marine snow formation was observed in the course of this study, it is suggested that aggregation had the major regulating effect on TEP concentration and cell abundance during this study. Thus, the total amount of TEP produced during the bloom may have been influenced by CO₂ but may not have been detectable from suspended TEP concentration, because of aggregate sedimentation. One consequence would be that export of POC would as well become sensitive to CO₂. In order to evaluate, if this hypothesis is correct and applicable to the ocean, a series of additional studies need to be performed, which should elucidate the fate of TEP and DOC more properly. Additionally, more general questions will have to be solved, which address the temporal and regional importance of TEP production for carbon cycling and carbon export in the ocean. Also the role of bacteria for TEP degradation needs to be elucidated, since degradation may be an important loss process for TEP, specifically in ocean environments, where carbon rather than nitrogen or phosphorous are the limiting elements. Nevertheless, our results indicate that production of TEP by marine phytoplankton provides a potential link between CO₂ sensitive carbon assimilation and sequestration of ‘excess carbon’ to the deep ocean.
References


Engel, A. 2000. The role of transparent exopolymer particles (TEP) in the increase in apparent particles stickiness ($\alpha$) during the decline of a diatom bloom. J. Plankton Res., 22:


sedimentation of particulate matter. Continental Shelf res. 21, 327-346.


Figure legends

Fig. 1: Conceptual model showing various pathways of organic matter released by an autotrophic cell. Under the condition of nutrient exhaustion a fraction of the carbon accumulating intracellular is released from the cell by leakage and exudation processes. Depending on the quality, the extracellular organic carbon can enter the microbial food web or aggregate into particles, such as TEP. For further description, see text.

Fig. 2 a,b: Microscopic view on material sampled from the mesocosm on a 0.4 µm membrane filter and stained with the polysaccharide specific dye Alcian Blue. A) The bold, circular cells of *E. huxleyi* (arrow) are silhouetted against TEP, which typically appear as blue stained particles of fractal structure. Because the coccoliths of *E. huxleyi* are coated by an acidic polysaccharide the cells surface is stained with Alican Blue also. B) Large web-like TEP appeared later during the *E. huxleyi* bloom (day 19), often entangled with cells and detritus.

Fig. 3a-c: Increase of TEP concentration during the bloom experiment in a) the ‘year 2100’ (mesocosm: 1-3), b) the ‘present’ (mesocosm: 4-6) and c) the ‘glacial’ treatment (mesocosm: 7-9). Given are the colorimetrically determined TEP concentrations in µg Xanthan equivalents (µg Xeq.) L⁻¹.

Fig. 4: The concentration of carbon contained in TEP (TEP-C) was significantly related to the abundance of *E. huxleyi* (p<0.001).

Fig. 5a-c: Changes of DOC concentration in the course of the experiment in a) the ‘year 2100’ treatment (mesocosm: 1-3), with significant increase of DOC in the mesocosms 1(p<0.1) and 2 (p<0.001), b) the ‘present’ treatment (mesocosm: 4-6), with significant increase of DOC in mesocosm 4 (p<0.01) and c) the ‘glacial’ treatment (mesocosm: 7-9).

Fig. 6: No significant relationship between DOC concentration and cell abundance of *E. huxleyi* was observed (n=70, r²=0.17).

Fig. 7a,b: Increase of molar [POC]:[PON] (a) and [POC]:[POP] (b) ratios towards the end of the *E. huxleyi* bloom. Ratios are mean values of nine mesocosm, error bars indicate ± 1SD.
Fig. 8: During the experiment, a decoupling of POC (solid triangles) from PON concentration occurred, when dissolved inorganic nitrogen (DIN) concentrations (open circles) fell under the detection limit. Until DIN exhaustion, changes in POC concentration were directly related to changes in PON concentration (p<0.001).

Fig. 9: TEP concentrations relative to POC concentrations, before (open circles) and after the onset of nutrient (PO₄) depletion on day 11 (solid circles). After day 11 changes in TEP concentration were directly related to changes in POC concentration (p<0.001).

Fig. 10: A direct relationship between Alcian Blue adsorption and abundance of E. huxleyi cells, harvested from a nutrient replete cell culture (n=10, r²=0.94). No significant y-offset was determined. Adsorption of Alcian Blue onto the cells is caused by an acidic polysaccharide that covers the coccoliths of E. huxleyi (see Figure 1), but is small relative to the Alcian Blue adsorption of TEP observed during the mesocosms study.
Exudation

Transparent Exopolymer Particles

Leakage

LMW Substances

HMW Substances

1000 Da

0.4 μm

0.7 μm

DOM

POM

Refractory DOM

Modification

Microbial Food Web

Production & Degradation

Production

Uptake

LMW DOM

Polysaccharides

Transparent Exopolymer Particles

Aggregation

C:N

>>6.6

C\text{cell}

C:P

>>106

DIN

DIC

C:N

DIN

C:P

DIN

Figure 1
Figure 3a-c
\[ [\text{TEP-C}] = 1.05 \times 10^{-6} \text{[cells]} + 5.51 \]
\[ r^2 = 0.91 \quad n = 135 \]

Figure 4
Figure 5a-c
Figure 6

DOC (µmol L\(^{-1}\))

E. huxleyi cells (x10^6 L\(^{-1}\))
Figure 7 a,b

Figure 8
\[ \Delta[\text{TEP-C}] = 0.35 \Delta[\text{POC}] \]
\[ r^2 = 0.80 \quad n = 62 \]

Figure 9
Fig. 10
Table 1. Parameter values for the linear regressions between TEP concentration and *E. huxleyi* calculated for each of the mesocosms. The analysis include data from days 3-17, n=15 for each mesocosm, SD: standard deviation.

<table>
<thead>
<tr>
<th>CO2-treatment</th>
<th>Mesocosm No.</th>
<th>Regression Statistics: $\text{[TEP, µg Xeq. L}^{-1}] = a \times 10^{-6} \text{[cell, L}^{-1}] + b$</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>$a \pm SD$</td>
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<tr>
<td>‘Year 2100’</td>
<td>1</td>
<td>53.1±3.53</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>28.2±1.56</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>49.9±2.43</td>
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<tr>
<td>‘Present’</td>
<td>4</td>
<td>33.2±2.73</td>
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<tr>
<td></td>
<td>5</td>
<td>29.0±2.40</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>39.1±3.21</td>
</tr>
<tr>
<td>‘Glacial’</td>
<td>7</td>
<td>35.1±2.04</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>33.8±4.03</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>31.8±1.18</td>
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</table>
Table 2: ‘Carbon overconsumption’ calculated as carbon taken up, or contained in the different elemental pools in excess to the expected value (DINx6.6) during the mesocosm study (days 1-18). Data are mean values calculated from all mesocosms except #8, where no data for DOC were available for day 18. The value (%) ‘Excess C’ indicates the fraction of excess carbon relative to the total C uptake. Therefore, excess carbon in the POC, DOC and ‘Loss’ fractions sum up to the amount of excess carbon uptake. It was assumed that N is not an inherent component of TEP, because no N was determined in the acidic polysaccharides released by *E. huxleyi*; n.d.: not determined.

<table>
<thead>
<tr>
<th>Days 1-18</th>
<th>ΔN (µmol L⁻¹)</th>
<th>ΔC (µmol L⁻¹)</th>
<th>C:N</th>
<th>ΔN x 6.6 (µmol L⁻¹)</th>
<th>Excess C (%)</th>
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<tbody>
<tr>
<td>Uptake</td>
<td>15.2</td>
<td>248.1</td>
<td>16.3</td>
<td>100.3</td>
<td>59.6</td>
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<td>Partitioning</td>
<td></td>
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<td></td>
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<tr>
<td>POM</td>
<td>6.4</td>
<td>117.2</td>
<td>18.3</td>
<td>42.2</td>
<td>74.9</td>
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<tr>
<td>DOM</td>
<td>n. d.</td>
<td>11.5</td>
<td>n.d.</td>
<td>n.d.</td>
<td>≤11.5, ≤4.6</td>
</tr>
<tr>
<td>TEP</td>
<td>-</td>
<td>39.2</td>
<td>-</td>
<td>-</td>
<td>39.2</td>
</tr>
<tr>
<td>(POM-TEP)</td>
<td>6.4</td>
<td>78.0</td>
<td>12.2</td>
<td>42.2</td>
<td>35.8</td>
</tr>
<tr>
<td>Calculated Loss</td>
<td>≤8.8</td>
<td>119.4</td>
<td>≥13.6</td>
<td>58.1</td>
<td>≥61.3, ≥24.7</td>
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</tbody>
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