

## Testing the direct effect of CO<sub>2</sub> concentration on a bloom of the coccolithophorid *Emiliana huxleyi* in mesocosm experiments

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### Abstract

We studied the direct effects of CO<sub>2</sub> and related changes in seawater carbonate chemistry on marine planktonic organisms in a mesocosm experiment. In nine outdoor enclosures (~11 m<sup>3</sup> each), the partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) in the seawater was modified by an aeration system. The triplicate mesocosm treatments represented low (~190 parts per million by volume (ppmV) CO<sub>2</sub>), present (~410 ppmV CO<sub>2</sub>), and high (~710 ppmV CO<sub>2</sub>) pCO<sub>2</sub> conditions. After initial fertilization with nitrate and phosphate a bloom dominated by the coccolithophorid *Emiliania huxleyi* occurred simultaneously in all of the nine mesocosms; it was monitored over a 19-day period. The three CO<sub>2</sub> treatments assimilated nitrate and phosphate similarly. The concentration of particulate constituents was highly variable among the replicate mesocosms, disguising direct CO<sub>2</sub>-related effects. Normalization of production rates within each treatment, however, indicated that the net specific growth rate of *E. huxleyi*, the rate of calcification per cell, and the elemental stoichiometry of uptake and production processes were sensitive to changes in pCO<sub>2</sub>. This broad influence of CO<sub>2</sub> on the *E. huxleyi* bloom suggests that changes in CO<sub>2</sub> concentration directly affect cell physiology with likely effects on the marine biogeochemistry.

Large-scale changes in surface ocean chemical equilibrium and elemental cycling have occurred in the ocean's history and are expected to continue and intensify in the future. Prior to the industrial burning of fossil fuels, CO<sub>2</sub> concentration varied between 180 and 280 parts per million by volume (ppmV), with the lower values observed during glacial times. Since the middle of the 18th century, the atmospheric partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) has increased rapidly from 280 to 366 ppmV in 1998, and several future scenarios predict a further increase to 750 ppmV in 2100 (IPCC scenario IS92a; Houghton et al. 2001). The progressive increase in atmospheric CO<sub>2</sub> might affect the marine biosphere directly and indirectly. Indirect effects are expected through rising sea surface temperatures related to global warming, which might cause increased surface ocean stratification and mixed layer insulation and could also affect the rates of biological processes. Moreover, the increase of atmospheric temperature will likely modify global wind patterns, subsequently changing the dust input and the iron supply to the surface ocean.

Because of the rapid air-sea exchange of CO<sub>2</sub>, direct, and already determined, effects of increased atmospheric CO<sub>2</sub> concentration are the rise of CO<sub>2</sub> concentration in the surface ocean and a related shift in its chemical equilibrium (Chen and Millero 1979; Brewer et al. 1997). Several species of dissolved inorganic carbon (DIC) exist in seawater, namely CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, and CO<sub>3</sub><sup>2-</sup>, the proportions of which are largely determined by the total DIC concentration, pH, and total alkalinity (TA). TA is defined as the number of moles of [H<sup>+</sup>] equivalent to the excess of proton acceptors per kilo-

gram of seawater (Dickson 1981). At typical seawater pH of 8.2, about 90% of DIC is present as HCO<sub>3</sub><sup>-</sup>, 9% as CO<sub>3</sub><sup>2-</sup>, and <1% as CO<sub>2</sub>. When DIC increases through enhanced uptake of CO<sub>2</sub> from the atmosphere, the pH of seawater and the CO<sub>3</sub><sup>2-</sup> concentration decrease. The expected decrease in surface ocean pH by 0.7 units up to the year 2300 will exceed anything experienced in the past 300,000 yr of earth history (Caldeira and Wickett 2003).

This change of environmental factors is likely to affect the physiology of marine organisms and therefore has the potential to influence species composition and competition, as well as the cycling of biogeochemical key elements, such as C, N, and P.

Marine phytoplankton might be particularly sensitive to CO<sub>2</sub>-related changes in the carbonate chemistry because inorganic carbon assimilation is largely bound to CO<sub>2</sub> fixation by the carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase, which has a low affinity to CO<sub>2</sub> with half-saturation constants ranging between 30 and 70 μmol L<sup>-1</sup> (Badger et al. 1998). Many phytoplankton species are able to enhance their CO<sub>2</sub> supply to the site of carboxylation by CO<sub>2</sub> concentrating mechanisms (CCMs; Raven 1991, e.g., through active uptake of HCO<sub>3</sub><sup>-</sup> or the enhancement of HCO<sub>3</sub><sup>-</sup>, or both) to CO<sub>2</sub> conversion with the enzyme carbonic anhydrase (CA). Operation of the CCMs thereby ensures that photosynthetic carbon fixation is close to saturating primary production, even at the low CO<sub>2</sub> concentrations typically encountered in the surface ocean (i.e., 8–22 μmol L<sup>-1</sup>; Goerike and Fry 1994). However, an increase of CO<sub>2</sub> concentration might reduce the need for CCM activity and thereby lower the metabolic costs of the phytoplankton cells for inorganic carbon acquisition. Moreover, recent studies demonstrated that phytoplankton species and functional groups differ regarding the efficiency and regulation of their CCMs, indicating that changes in CO<sub>2</sub> availability might affect competition and succession of phytoplankton species (Burkhardt et al. 2001; Rost et al. 2003). A significant shift in the taxonomic composition of a phytoplankton assemblage exposed to different CO<sub>2</sub> levels was indeed observed in a recent study by Tortell et al. (2002). Differences in CO<sub>2</sub> uptake rates were further hypothesized to influence the production of extracellular organic matter, as demonstrated by the increase of transparent exopolymer particle (TEP) concentration with CO<sub>2</sub> concentration in an experimental study conducted with Baltic Sea phytoplankton (Engel 2002). The origination of

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TEPs from dissolved organic matter could indicate that the partitioning between the dissolved and the particulate organic carbon pool is sensitive to changes in CO<sub>2</sub> concentration.

A strong effect of CO<sub>2</sub>-related changes in the carbonate chemistry of seawater was recently demonstrated for calcification by marine phytoplankton. In laboratory experiments with nutrient-replete cultures of the marine coccolithophorids *Emiliania huxleyi* and *Gephyrocapsa oceanica*, it was shown that the ratio of particulate inorganic carbon (PIC) to particulate organic carbon (POC) production decreased with increasing CO<sub>2</sub> concentration (Riebesell et al. 2000; Zondervan et al. 2001, 2002). The change in [PIC]:[POC] ratios was attributed to a decrease in calcification rates, as well as an increase in organic carbon production. In nitrate-limited cultures of *E. huxleyi*, however, such a change in the ratio of calcification to organic carbon production was not observed (Sciandra et al. 2003). Together with foraminifera and corals, coccolithophorids are the major producers of calcium carbonate in the ocean. Because calcium carbonate formation is a chemical reaction that liberates CO<sub>2</sub> (i.e.,  $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$ ), the response of reduced calcification might provide a significant negative feedback to increasing CO<sub>2</sub> concentration.

Hence, CO<sub>2</sub>-related effects on the physiology of marine phytoplankton have the potential to affect carbon cycling by affecting both the rate of organic carbon assimilation and the amount of biological calcification. On a global scale, these effects could translate into changes in the efficiency of the biological carbon pumps—specifically, the soft tissue and carbonate pumps—and potentially affect global carbon sequestration (e.g., through changing of the “rain ratio” of PIC and POC). Because it has been proposed that the soft tissue pump is ultimately determined by the availability of inorganic nutrients, specifically nitrate (Dugdale and Goering 1967; Eppley and Peterson 1979), a CO<sub>2</sub>-related change of organic carbon sequestration would require a CO<sub>2</sub> effect on the C:N stoichiometry of the sinking particles. Apart from a few laboratory experiments, which indeed demonstrated a CO<sub>2</sub> effect on the stoichiometry of phytoplankton cells (Burkhardt et al. 1999; Gervais and Riebesell 2001; Urabe et al. 2003), it is still unknown whether CO<sub>2</sub> changes cause a systematic shift in the C:N:P stoichiometry of export production. From the potential linkages mentioned above, it is, however, evident that a mechanistic understanding of the influence of CO<sub>2</sub> effects on marine phytoplankton and of the consequences for biogeochemical cycling is essential to reliably assess ecosystem changes and the future concentration of CO<sub>2</sub> in the atmosphere.

To study the response of a natural phytoplankton bloom to different CO<sub>2</sub> concentrations, we designed an experimental, semienclosed system that enables the modification of seawater CO<sub>2</sub> concentration without changing TA, while maintaining an environment as natural as possible. Here, we present the setup of the experiment and give an overview on the development and biogeochemistry of a bloom of the marine coccolithophorid *E. huxleyi*, which developed during the exposure to three different CO<sub>2</sub> concentrations.

## Materials and methods

*Setup and sampling*—The study was conducted between 31 May and 25 June 2001 at the European Union Large Scale Facility (LSF) in Bergen, Norway. Nine polyethylene enclosures (~11 m<sup>3</sup>, 4.5 m water depth) were moored to a raft in the Raunefjorden (60.3°N, 5.2°E; for more details, see Williams and Egge 1998). The enclosures were filled with unfiltered, nutrient-poor, postbloom fjord water, which was pumped from 2 m depth adjacent to the raft. The enclosures were covered by gas-tight tents made of ethylene tetrafluoroethylene foil (Foiltec, Bremen, Germany), which allowed for 95% light transmission of the complete spectrum of sunlight, including ultraviolet A (UVA) and UVB.

The atmospheric and seawater pCO<sub>2</sub> were manipulated to achieve three different CO<sub>2</sub> levels in triplicate: (1) high pCO<sub>2</sub>, approximately simulating the atmospheric CO<sub>2</sub> concentration in year 2100 (mesocosms 1–3), representing IPCC's “business as usual” scenario IS92a; (2) present pCO<sub>2</sub> (mesocosms 4–6), representing the present day environment; and (3) low pCO<sub>2</sub> (mesocosms 7–9), representing the glacial atmosphere. The setup of the experimental system was designed by the Institute for Environmental Process Engineering (IUV, Bremen, Germany) and is displayed in Fig. 1. With the use of rotary vane compressors (Becker, DT-4.8) different CO<sub>2</sub> levels in seawater were achieved by aerating the water and fumigating the tents with air enriched with either pure CO<sub>2</sub> (purity 2.7), natural air, or air depleted in CO<sub>2</sub>. The latter was obtained by running natural air through a CO<sub>2</sub> absorber (Na<sub>2</sub>CO<sub>3</sub> platelets with a pH indicator). After 4 d of CO<sub>2</sub> adjustment, the desired pCO<sub>2</sub> levels in water were reached (day 0) and the CO<sub>2</sub> aeration of the water column was stopped.

To promote the development of a coccolithophorid bloom, NaNO<sub>3</sub> and Na<sub>2</sub>HPO<sub>4</sub> were added on day 0 (6 June 2001) at a ratio of 30:1, yielding initial concentrations in the seawater of 15.3 μmol L<sup>-1</sup> nitrate and 0.5 μmol L<sup>-1</sup> phosphate. After nutrient addition and throughout the study, the water was gently mixed by means of an airlift (for more details, see Egge and Asknes 1992) with the same air as for gassing the tents. The gassing of the tents was continued to keep the pCO<sub>2</sub> of the overlying atmosphere at a constant level. On each day, conductivity-temperature-depth (CTD; SAIV A/S, model SD204) casts monitored the temperature and salinity profiles within the enclosures. The nutrient-initiated bloom was monitored over a 19-day period, during which samples and profiles were taken on a daily basis before midday (0900–1000 h). Samples were taken from 0.5 m depth by gentle vacuum pumping of a 20-liter volume. Immediately after arrival to the onshore laboratory, subsamples for the different analyses were taken. At the end of the experiment, we sampled the material, which settled during the experiment, from mesocosms 1, 4, and 7 by pumping 6 liters of water from the bottom of the enclosures and discarding the first 3 liters.

*Measurements and analyses*—An overview of all variables determined during the study is given in Table 1. Methods for the determination of variables, which are also included in separate papers, are described only briefly.

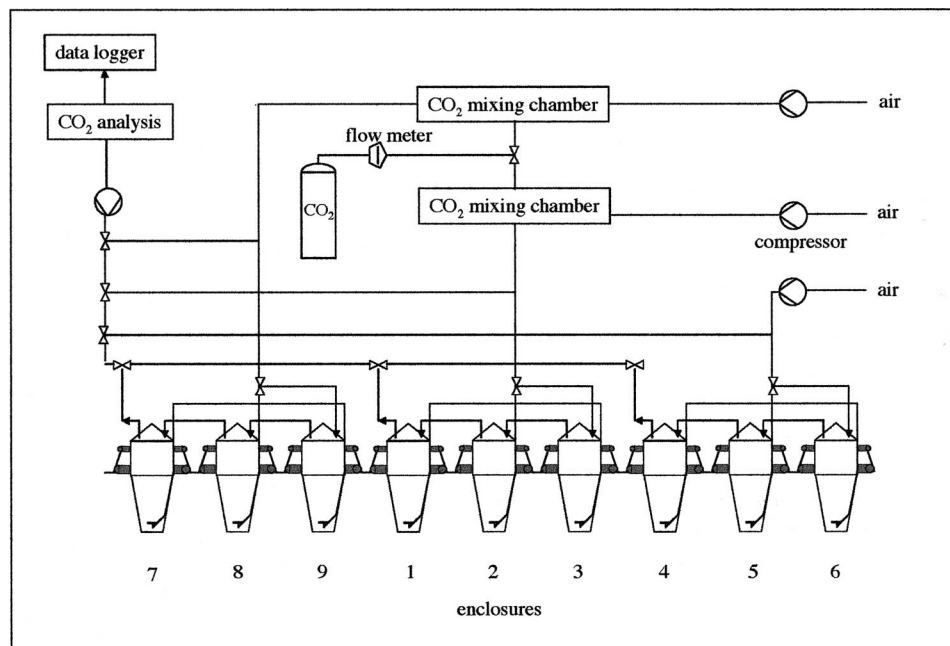


Fig. 1. Schematic representation of the aeration systems for CO<sub>2</sub> manipulation of seawater inside the mesocosms. Surrounding air was either pumped directly to the mesocosms (present mesocosms 4–6) or enriched with CO<sub>2</sub> in a mixing chamber (high-CO<sub>2</sub> mesocosms 1–3), or depleted in CO<sub>2</sub> through adsorption onto Na<sub>2</sub>CO<sub>3</sub> platelets (low-CO<sub>2</sub> mesocosms 7–9). The air was injected into the water just above the bottom of the mesocosms, where it was released through a pipe system equipped with a nozzle at its end. To fumigate the atmosphere within the tents, all mesocosms of one treatment were connected. The CO<sub>2</sub> concentration of the air used for aeration of water and atmosphere was measured regularly by a CO<sub>2</sub> gas analyzer.

Irradiance was continuously measured every 10 min throughout the study with a Li-Cor cosine sensor (LI-192SA), mounted on top of the floating mesocosm laboratory. Depth profiles of irradiance inside the mesocosms (on average every 3 d) were measured with a Li-Cor spherical sensor (LI-193SA). The atmospheric pCO<sub>2</sub> inside the tents were monitored up to three times a day by a multicomponent continuous gas analyzer (Rosemount analytical BINOS 100 2M) connected to a portable compact gas conditioning unit (MandC Products PSS-5). The pCO<sub>2</sub> in seawater was measured by means of an equilibrator (Frankignoulle et al. 2001) coupled to an infrared analyzer (Li-Cor 6262). The system was calibrated routinely with air standards with nominal mixing ratios of 0, 350, and 800 ppmV of CO<sub>2</sub> (Air Liquide Belgium and Hydrogas). Temperature at the inlet of the pump and in the equilibrator was measured simultaneously with a Li-Cor thermosensor. For each measurement of CO<sub>2</sub>, samples for TA were taken. TA was measured by the classical Gran electrotitration method (Gran 1952) on 100-ml GF/C-filtered samples. The reproducibility of measurements was 3 μmol kg<sup>-1</sup>. The pCO<sub>2</sub> was then corrected for temperature changes with TA and the dissociation constants of Roy et al. (1993). DIC was calculated from seawater pCO<sub>2</sub> and TA. For both DIC computation and CO<sub>2</sub> temperature compensation, CO<sub>2</sub> speciation was calculated with the use of the CO<sub>2</sub>SYS Package (Lewis and Wallace 1998), the CO<sub>2</sub> acidity constants of Roy et al. (1993), the CO<sub>2</sub> solubility coefficient of Weiss (1974), and the borate acidity constant of Dickson

(1990). The total borate molality was calculated with the Uppström (1974) ratio to salinity. The uncertainty on the DIC computation is estimated to be 5 μmol kg<sup>-1</sup>. The pCO<sub>2</sub> of seawater was normalized to a temperature of 12°C (error 3 μmol CO<sub>2</sub>).

Chlorophyll *a* (Chl *a*) was determined on 100-ml samples filtered on 0.45-μm cellulose nitrate filters, frozen and extracted in 90% acetone at 4°C overnight as described by Parsons et al. (1984). Chl *a* concentration was measured with a Turner Design Fluorometer (model 10-AU). A standard solution of pure Chl *a* was used for calibration. Nitrate and nitrite were determined from GF/F-filtered and preserved (0.1% HgCl<sub>2</sub>) samples with an autoanalyzer (AA II) within 4 months. Phosphate and ammonium were measured on the day of sampling by the methods of Koroleff and Grasshof (1983). Total particulate carbon (TPC) and particulate organic nitrogen (PON) were determined by elemental analysis from 1 liters (days 0–12) or 0.5 liter (days 13–19) samples filtered gently (200 mbar) through precombusted (24 h, 500°C) glass fiber filters (GF/F, Whatman). Particulate inorganic carbon (PIC) was determined by subtracting particulate organic carbon (POC) from TPC. For determination of POC, filters were fumed for 2 h with saturated HCl to remove all particulate inorganic carbon and dried for 2 h at ~50°C. TPC, POC, and PON were subsequently measured on a Europa Scientific ANCA SL 20-20 mass spectrometer. Particulate organic phosphorus (POP) was determined colorimetrically (Koroleff and Grasshof 1983) after persulfate

Table 1. Biological and chemical variables determined during the mesocosm study.

Variable	Method	Reference
Algal cell density	Flow cytometry	This study
Alkalinity	Gran electrotitration	Delille et al. unpubl. data
Alkenone concentration	Gas chromatography	A. Benthien unpubl. data
Alkenone $\delta^{13}\text{C}$	GCirmMS	A. Benthien unpubl. data
Bacterial abundance	Microscopy	Rochelle-Newall et al. 2004
<sup>14</sup> C primary production	Radiography	Delille et al. unpubl. data
Chromophonic DOM	Spectrophotometry	Rochelle-Newall et al. 2004
Chl <i>a</i>	Fluorometry	This study
Coccosphere/coccolith size	SEM	This study
Coccolith weight	Image analysis	This study
pCO <sub>2</sub>	Infrared analyzer (Li-Cor <sub>7</sub> 6262)	Delille et al. unpubl. data
DOC	Shimadzu TOC analyzer	Engel et al. 2004a
DIC	Calculated from TA, pH, and CO <sub>2</sub>	Delille et al. unpubl. data
Light intensity	Li-Cor spherical sensor (LI-193SA)	This study
Mono-/polysaccharides	Colorimetric analysis with TPTZ	Engel et al. 2004b
Net primary production	Winkler titration	Delille et al. unpubl. data
Nutrients	Autoanalyzer	This study
O <sub>2</sub>	Winkler titration	Delille et al. unpubl. data
pH	Sensor	This study
PIC	CHN analyzer (TPC-POC)	This study
POC	CHN analyzer	This study
PON	CHN analyzer	This study
POP	Acid combustion	This study
$\delta^{13}\text{C}$ POC	Mass spectrometry	Riebesell, unpubl.
Salinity	CTD	This study
Temperature	CTD	This study
TEP	Colorimetric analysis, microscopy	Engel et al. 2004a
Viral density	Flow cytometry	Rochelle-Newall et al. 2004

oxidation from 0.5–1.0-liter samples filtered onto GF/F filters. All filters were prepared in duplicate and stored at –20°C until analysis.

Phytoplankton counts 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 setup. The cells were analyzed from fresh samples at high flow rate (~70  $\mu\text{l min}^{-1}$ ) with the addition of 1- $\mu\text{m}$  fluorescent beads (Molecular Probes). Autotrophic groups were discriminated on the basis of their forward or right angle light scatter (FALS, RALS) and chlorophyll (and phycoerythrin for *Synechococcus* and cryptophyte populations) fluorescence. Listmode files were analyzed with CYTOWIN (Vaulot 1989). The sizes of coccoliths and coccospheres of *E. huxleyi* were determined by scanning electron microscopy (JEOL JSM 6300 SEM). Samples were taken from mesocosms 1, 4 and 7 at days 13, 15, and 17. Every sample (20 ml) was filtered on a 47-mm aerosol-grade 0.4- $\mu\text{m}$  pore-size Nuclepore filter. To enhance the homogeneity of the particle distribution, a paper filter was placed underneath the Nuclepore filter during filtration. Every filter was rinsed three times with 0.05- $\mu\text{mol L}^{-1}$  NH<sub>4</sub>HCO<sub>3</sub> buffer to remove crystallized salts. The filters were placed in petri dishes and air dried. Samples were coated with a 50-nm carbon layer to prevent charging during the analysis. From every sample, 200 coccoliths and 200 coccospheres of *E. huxleyi* were measured for their size, calculated as the equivalent spherical diameter from measurements of the coccolith area. The weight of coccoliths sampled on days 0, 4, 9, 11, 14, and

16 from each of the mesocosms was determined by a morphometric method. For this, 1 liter was filtered on cellulose Micronsep (MSI) membrane (0.45  $\mu\text{m}$  effective pore size, 47 mm diameter). The membrane was cut and mounted between slide and coverslip with Canadian Balsam. The slides were scanned by an automatic light microscope (Leica DMRBE) at  $\times 500$  magnification with cross-polarized light. One hundred view fields were randomly grabbed by a digital camera and analyzed by SYRACO (Dollfus and Beaufort 1999). On average, 1,800 coccoliths of *E. huxleyi* per sample were recognized. Because coccoliths are made of calcium carbonate, they appear bright in cross-polarized light. The brightness of a thin birefringent calcite object in cross-polarized light depends on its thickness. By measuring the total brightness (the sum of the gray value of all pixels composing the coccolith image), the weight of a coccolith was determined by a transfer function between brightness and calcite weight, derived from pure calcite of known thickness (Beaufort unpubl.).

*Statistical treatment of data*—Average values are given by the statistical mean ( $\bar{x}$ ) and its standard deviation (SD). Mean values were compared by means of a *t*-test. Significance of the correlation coefficient ( $r^2$ ) against  $H_0$ ;  $\rho = 0$  was tested by a Student's *t*-test according to Sachs (1974):

$$\hat{t} = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} \quad (1)$$

Degree of freedom is  $n - 2$ .  $H_0$  ( $r^2 = 0$ ) is rejected for  $\hat{t} \geq \hat{t}_{n-2}$ ;  $p$ . The influence of the  $\text{CO}_2$  treatment on biological or chemical variables was determined by means of the analysis of variance. The effect of the  $\text{CO}_2$  treatment on a linear relationship between two biological or chemical variables was tested by comparing the slope ( $b$ ) of the linear regression  $F(x) = b(x) + a$ , calculated for each treatment separately, with a  $t$ -test (Sachs 1974).

$$\hat{t} = \frac{|b_1 - b_2|}{\sqrt{\frac{S^2_{y_1x_1}(n_1 - 2) + S^2_{y_2x_2}(n_2 - 2)}{n_1 + n_2 - 4} \left( \frac{1}{Q_{x_1}} + \frac{1}{Q_{x_2}} \right)}} \quad (2)$$

Degree of freedom is  $n_1 + n_2 - 4$ .  $Q_x$  is the sum of the squared residuals of  $x$ , and  $S_{yx}$  is the residual variance of  $y$ .  $H_0(b_1 = b_2)$  is rejected for  $\hat{t} \geq \hat{t}_{n_1+n_2-4}$ ;  $p$ . Significance level of each test was  $p < 0.05$ .

## Results

**The physical environment**—Day length during the study period was between 20.3 and 22.7 h and increased from the beginning of the study until day 15 (midsummer). Average photosynthetically active radiation (PAR) during daytime was  $598 \pm 213 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and ranged from 127 to  $836 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Irradiance at 3.5 m depth inside the mesocosms was always above 1% of surface irradiance. Water temperature increased steadily over the course of the experiment from  $\sim 10^\circ\text{C}$  at day 0 to  $\sim 13^\circ\text{C}$  at day 19. Daily CTD casts showed virtually no variation of salinity with depth and only minor temperature variation ( $< 0.2^\circ\text{C}$ ), indicating even, vertical mixing of the nonparticulate parameters in the mesocosms.

**General bloom development**—At the beginning of the experiment, seawater  $\text{pCO}_2$  was adjusted to  $713 \pm 6.0 \text{ ppmV}$  in the high- $\text{pCO}_2$  scenario of mesocosms 1–3, to  $414 \pm 11 \text{ ppmV}$  in the present- $\text{pCO}_2$  mesocosms 4–6, and to  $190 \pm 2.4 \text{ ppmV}$  in the low- $\text{pCO}_2$  mesocosms 7–9. Because of biological activity, seawater  $\text{pCO}_2$  decreased strongly during bloom development (Fig. 2a). This was most pronounced in the high- $\text{pCO}_2$  treatment, largely because of the lower buffering capacity of the carbonate system at higher  $\text{pCO}_2$ . Parallel to the decrease in  $\text{pCO}_2$ , the pH of seawater increased in all treatments by  $0.27 \pm 0.04$ ,  $0.18 \pm 0.04$ , and  $0.12 \pm 0.03$  units in the high-, present-, and low- $\text{pCO}_2$  treatments, respectively (Fig. 2b). The decrease in DIC by organic carbon fixation (i.e., the decrease in total DIC minus the amount of inorganic carbon removed through calcification:  $\Delta[\text{DIC}^*] = [\Delta\text{DIC}] - [\frac{1}{2}\Delta\text{TA}]$ ) was similar in the three treatments and amounted to  $161 \pm 10 \mu\text{mol C L}^{-1}$  in the high- $\text{pCO}_2$  mesocosms, to  $168 \pm 28 \mu\text{mol L}^{-1}$  in the present- $\text{pCO}_2$  mesocosms and to  $156 \pm 32 \mu\text{mol L}^{-1}$  in the low- $\text{pCO}_2$  mesocosms. Detailed information about the changes in the seawater carbonate chemistry during the bloom will be given elsewhere (Delille unpubl.).

Net growth of the phytoplankton community was detectable after day 5 in all of the mesocosms and induced an increase in Chl *a* concentration from about  $1 \mu\text{g L}^{-1}$  pre-bloom concentration to values between 6.5 and  $12.5 \mu\text{g L}^{-1}$

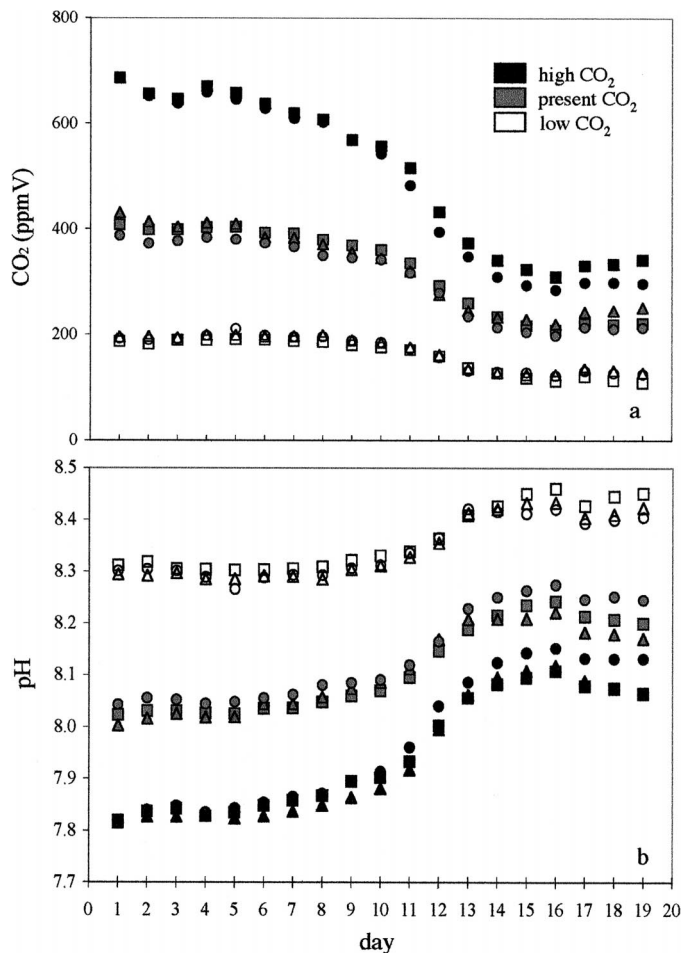


Fig. 2. (a) Changes in seawater  $\text{pCO}_2$  and (b) pH occurred in the course of the study in all of the mesocosms and were most pronounced in the high- $\text{CO}_2$  treatment. Black: high- $\text{CO}_2$  treatment; circle, mesocosm 1; square, mesocosm 2; triangle, mesocosm 3. Grey: present- $\text{CO}_2$  treatment; square, mesocosm 4; triangle, mesocosm 5; circle, mesocosm 6. White: low- $\text{CO}_2$  treatment; square, mesocosm 7; triangle, mesocosm 8; circle, mesocosm 9.

at the height of the bloom on day 14 (Fig. 3). Thereafter, Chl *a* concentration declined steadily and became more variable between mesocosms. The decline of the phytoplankton bloom was accompanied by particle aggregation, leading to the appearance of amorphous marine snow on day 16.

During the prebloom and the early exponential growth phase, phytoplankton composition was dominated by number of autotrophic flagellates (mainly *Micromonas* spp.) and picocyanobacteria *Synechococcus* spp. Highest abundance of autotrophic flagellates was observed on day 7, with an average value of  $6.7 \times 10^7 \text{ cells L}^{-1}$ . *Synechococcus* spp. peaked on day 5, with an average abundance of  $1.1 \times 10^7 \text{ cells L}^{-1}$ . Both species did not show a response to the  $\text{CO}_2$  treatment (ANOVA,  $p = 0.51$ ). The average abundance of *E. huxleyi* in all mesocosms was  $0.09 \times 10^6 \pm 0.04 \times 10^6 \text{ cells L}^{-1}$  initially. As the bloom progressed, *E. huxleyi* clearly became the dominant species (Fig. 4). Maximum cell abundance of *E. huxleyi* was reached between days 17 and 18 and varied from  $15 \times 10^6$  to  $56 \times 10^6 \text{ cells L}^{-1}$  among

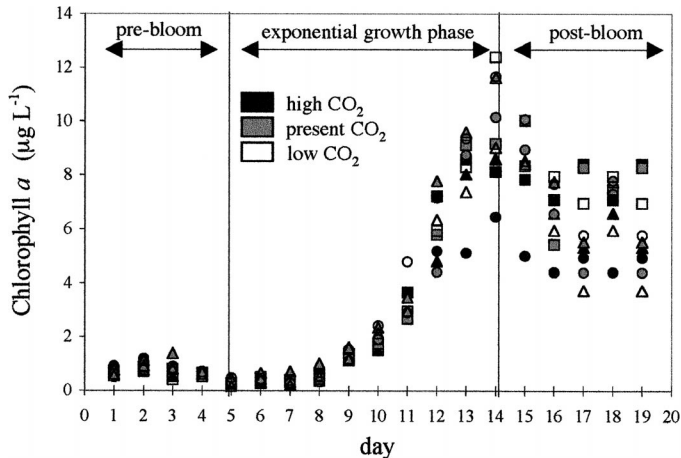


Fig. 3. The development of Chl *a* concentration during the experiment indicated three phases of the phytoplankton bloom: a pre-bloom phase from days 1 to 5, exponential growth of the phytoplankton from days 5 to 14, when nutrient concentrations were replete, and a postbloom phase after day 14, when nitrate and phosphate were below detection limits. Symbols as in Fig. 2.

all mesocosms (Fig. 5a). Initial PIC concentration was  $1.2 \pm 1 \mu\text{mol L}^{-1}$  and increased clearly after day 9 following the course of the *E. huxleyi* population (Fig. 5b). Highest PIC concentrations ranging from 17 to  $56 \mu\text{mol L}^{-1}$  were measured on day 19.

The sizes of coccospheres and of individual coccoliths of *E. huxleyi* were determined on days 13, 15, and 17 by scanning electron microscopy (SEM; Fig. 6). Because only one mesocosm was sampled from each treatment (mesocosms 1, 4, and 7), no information can be given on the variability of coccosphere and coccolith sizes within the CO<sub>2</sub> treatments. Nevertheless, a large number of individual size measurements ( $n = 600$  for each treatment) indicated that the size of coccospheres was significantly different between the treatments (ANOVA,  $p < 0.001$ ). Thereby, the average coccosphere size was significantly larger in the low-pCO<sub>2</sub> mesocosm, at  $5.90 \pm 0.47 \mu\text{m}$ , than in the present- and high-pCO<sub>2</sub> mesocosms, at  $5.75 \pm 0.46$  and  $5.72 \pm 0.61$ , respectively ( $t$ -test,  $p < 0.001$ ). Furthermore, the average sizes of individual coccoliths in the three different mesocosms were  $3.58$

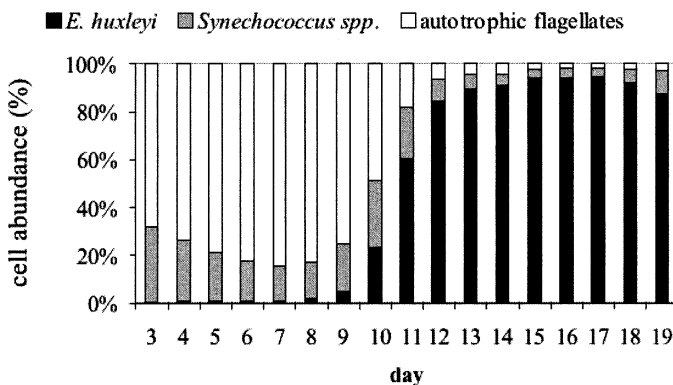


Fig. 4. Mean relative abundance of the major phytoplankton species during the study period.

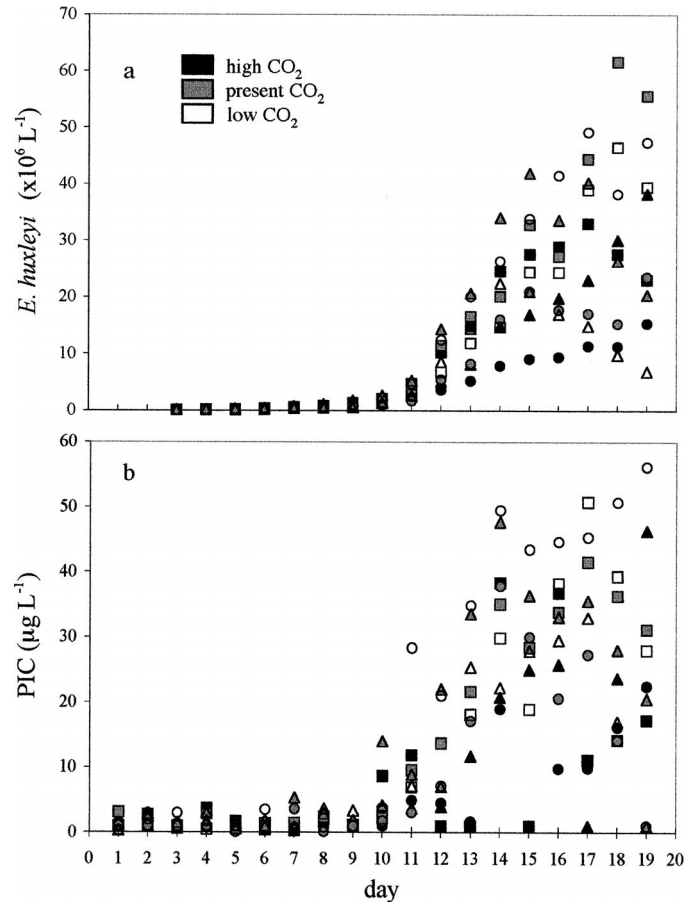


Fig. 5. (a) Growth of the *E. huxleyi* population was accompanied by (b) an increase of particulate inorganic carbon (PIC). Symbols as in Fig. 2.

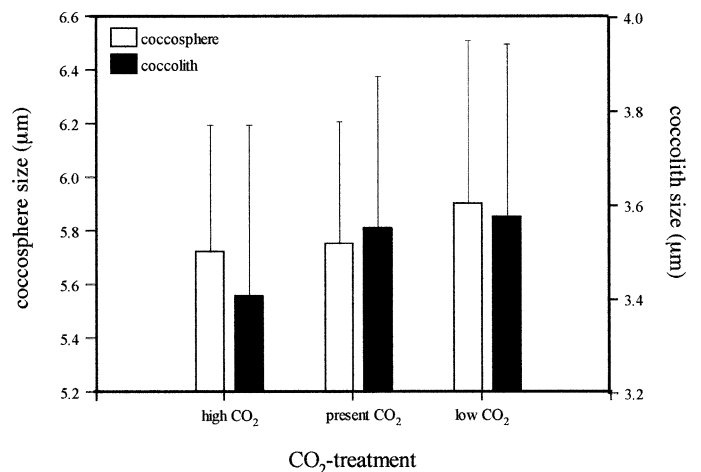


Fig. 6. Average size of coccospheres and coccoliths of *E. huxleyi* determined by SEM from samples of mesocosms 1, 4, and 7 collected on 3 days during the bloom. Number of measurements:  $n = 600$  each; error bars = +1 SD.

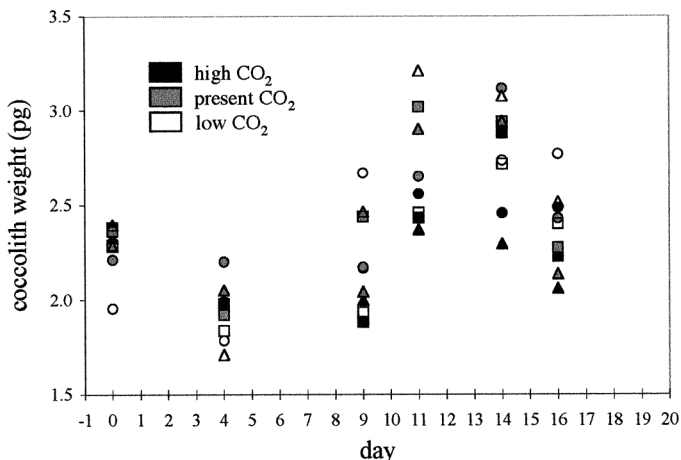


Fig. 7. The weight of *E. huxleyi* coccoliths changed in the course of the bloom. Each data point represents the mean value of at least 1,800 measurements.

$\pm 0.36$ ,  $3.55 \pm 0.32$ , and  $3.41 \pm 0.37 \mu\text{m}$  in the low-, present-, and high- $\text{pCO}_2$  mesocosms, respectively, and significantly different from each other (ANOVA,  $p < 0.001$ ). Again the average size decreased significantly with increasing  $\text{pCO}_2$  ( $t$ -test,  $p < 0.001$ ). No difference in the visual appearance of coccoliths between the  $\text{pCO}_2$  treatments was determined by SEM (i.e., coccospheres and coccoliths of *E. huxleyi* in the high- $\text{pCO}_2$  treatment were not excessively malformed).

Changes in coccolith weight occurred in the course of the bloom, yielding the lowest weights on day 4 and the highest weights between days 11 and 14 (i.e., at the height of the exponential growth phase; Fig. 7). Within each treatment, no relationships between coccolith weight and  $\text{CO}_2$  or  $\text{CO}_3$  concentration were determined. Between the treatments, significant differences in the weight of *E. huxleyi* coccoliths were observed (ANOVA,  $p < 0.001$ ), with an average coccolith weight of  $2.27 \pm 0.25 \text{ pg}$  in the high- $\text{pCO}_2$  treatment, which was lower than in the present- $\text{pCO}_2$  treatment at  $2.45 \pm 0.37 \text{ pg}$  ( $t$ -test,  $p < 0.01$ ) and also lower than in the low- $\text{pCO}_2$  treatment at  $2.45 \pm 0.49 \text{ pg}$  ( $t$ -test,  $p < 0.05$ ).

Cell growth induced a rapid decline of inorganic nutrients after the first week. Nitrate dropped from an initial concentration of  $15.3 \pm 0.2 \mu\text{mol L}^{-1}$  to below the detection limit after day 13 (Fig. 8a). Phosphate started at  $0.48 \pm 0.02 \mu\text{mol L}^{-1}$  and was not detectable after day 11 (Fig. 8b). Ammonium was undetectable during the whole study. Particulate organic phosphorus (POP) and nitrogen (PON) concentrations followed the course of Chl *a* concentration and reached maximum values between days 13 and 14, with  $0.2\text{--}0.3 \mu\text{mol POP L}^{-1}$  and  $10\text{--}12 \mu\text{mol PON L}^{-1}$  (Fig. 9a,b). PON production ceased directly after nitrate depletion, whereas approximately one third of net POP production occurred within the 2 d after phosphate fell below the detection limit. Average POC concentration was  $17.2 \pm 2.0 \mu\text{mol L}^{-1}$  at the beginning of the study and increased continuously until day 17 (Fig. 9c). Variability of POC concentration among the replicate mesocosms was low initially, but increased after the onset of nutrient depletion, yielding a large range of

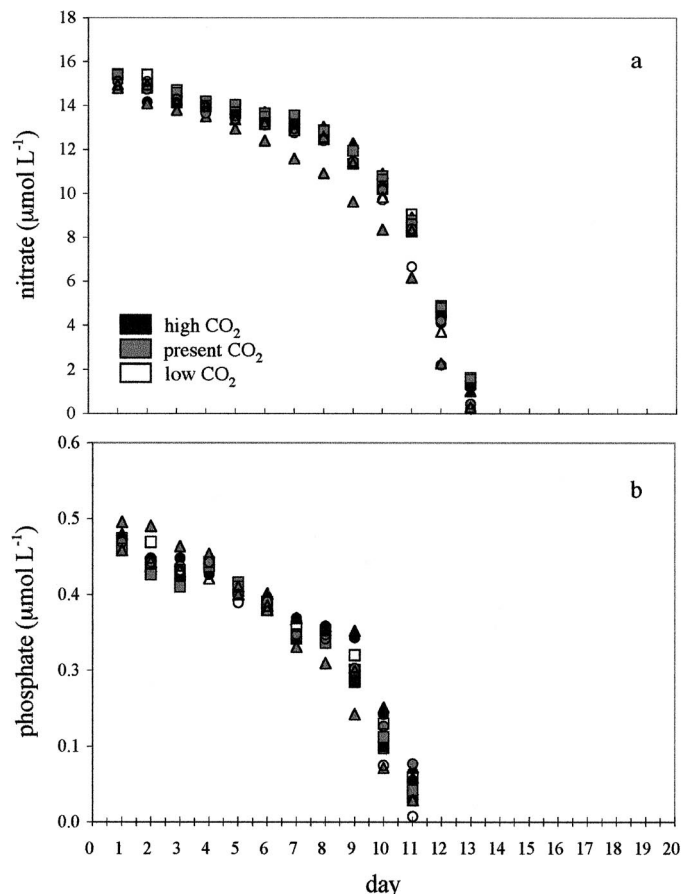


Fig. 8. Biomass growth led to rapid decrease of the inorganic nutrients (a) nitrate and (b) phosphate. Nitrate was below detection limit ( $0.1 \mu\text{mol L}^{-1}$ ) after day 13, phosphate after day 11 (limit  $0.05 \mu\text{mol L}^{-1}$ ). Symbols as in Fig. 2.

maximum POC concentrations per mesocosm from 69 to  $219 \mu\text{mol L}^{-1}$  at the end of the study.

*Normalization of production rates and elemental stoichiometry*—In general, the variability of concentrations of particulate components among the replicate mesocosms was either of equal magnitude or even larger than between the treatments. Hence, no statistically significant differences between the  $\text{CO}_2$  treatments were observed during the study regarding absolute concentrations of the particulate components mentioned. However, particle concentration during the course of the bloom was likely affected by small differences in the initial distribution of cells and zooplankton, as well as by secondary processes such as grazing or particle sinking with potentially different rates between the mesocosms. To test whether  $\text{CO}_2$  had an effect on the rates of phytoplankton development, the relationships between biological and chemical variables were examined more closely. Whenever possible, a normalization was performed by calculating uptake or production ratios; that is,  $(dx/dt)/(dy/dt) = \Delta[x] : \Delta[y]$ . The results are listed in Table 2. Because production rates were calculated from changes in standing stocks, the derived ratios address net changes. Particles that settled to the bottom of the mesocosms were not quantified. Elemental ratios of



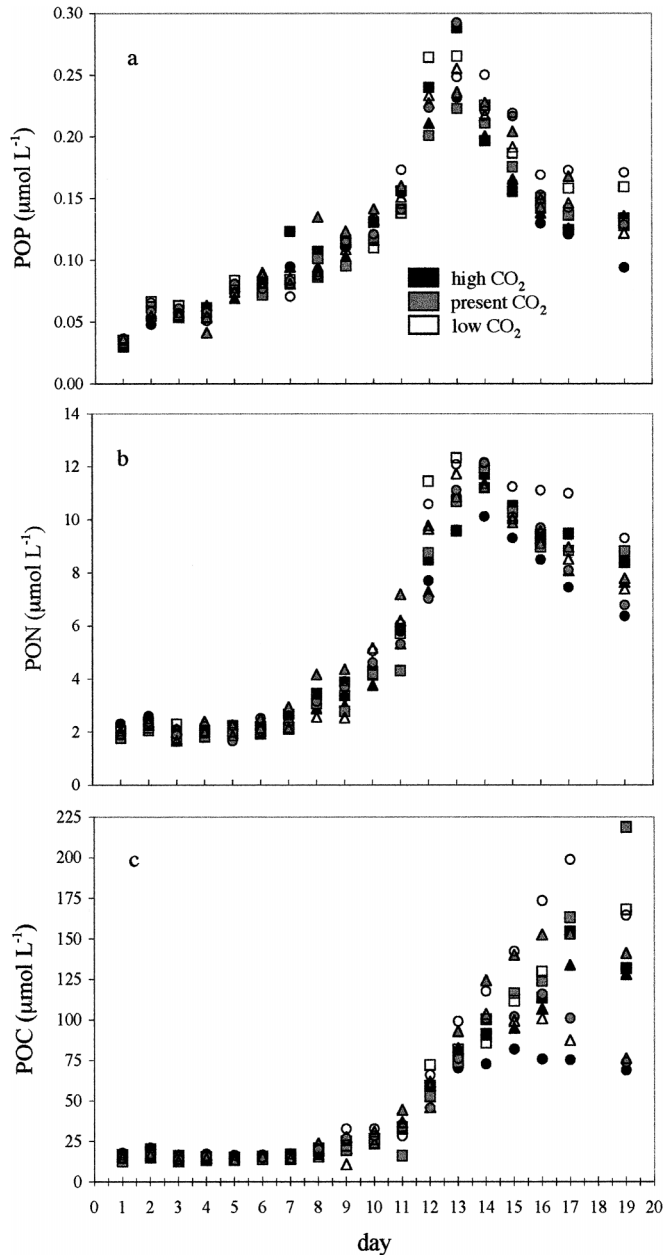


Fig. 9. Development of the particulate organic elements (a) phosphorus (POP), (b) nitrogen (PON), and (c) carbon (POC) in the course of the study. Symbols as in Fig. 2.

particulate matter therefore refer only to the material suspended in the water column.

The net specific growth rate ( $\mu$ ) of *E. huxleyi* was calculated for each treatment as the average of the daily growth rates:  $\mu_t = [\ln(C_i) - \ln(C_{i-1})]/[t_i - t_{i-1}]$ , with  $\ln(C_i)$  and  $\ln(C_{i-1})$  being the natural logarithms of cell concentrations on two consecutive days. Until the peak of the bloom (day 14), the net specific growth rate was  $0.50 \pm 0.26 \text{ d}^{-1}$  ( $n = 36$ ) in the low-pCO<sub>2</sub> treatment,  $0.47 \pm 0.26 \text{ d}^{-1}$  ( $n = 36$ ) in the present-pCO<sub>2</sub> treatment, and  $0.43 \pm 0.19 \text{ d}^{-1}$  in the high-pCO<sub>2</sub> treatment ( $n = 36$ ). Although a systematic CO<sub>2</sub> effect

on the net specific growth rates of *E. huxleyi* was not determined (ANOVA,  $p < 0.1$ ), the average growth rate in the low-pCO<sub>2</sub> treatment was significantly higher than in the high-pCO<sub>2</sub> treatment ( $t$ -test,  $p < 0.04$ ). This result is in accordance with a higher net production of PIC relative to POC at low pCO<sub>2</sub> ( $t$ -test,  $p < 0.06$ ). PIC and POC production were linearly related to *E. huxleyi* cell abundance in all mesocosms ( $t$ -test,  $p < 0.001$ ). Although  $\Delta[\text{POC}]:\Delta[\text{Cell}]$  ratios showed no differences between the CO<sub>2</sub> treatments, with an average ratio of  $3.9 \pm 0.53 \text{ pmol cell}^{-1}$ , the  $\Delta[\text{PIC}]:\Delta[\text{Cell}]$  ratio at low pCO<sub>2</sub> was significantly higher than in the present-pCO<sub>2</sub> treatment ( $t$ -test,  $p < 0.001$ ) and higher than in the high-pCO<sub>2</sub> treatment. However, because of a large variability of data within the high-pCO<sub>2</sub> treatment, the latter result was of lower significance ( $t$ -test,  $p < 0.06$ ).

The drawdown of DIC for organic carbon fixation normalized to cell abundance,  $\Delta[\text{DIC}^*]:\Delta[\text{Cell}]$ , was highest in the high-pCO<sub>2</sub> treatment ( $t$ -test,  $p < 0.001$ ), whereas no significant differences in this respect were observed between the present- and the low-pCO<sub>2</sub> treatments. Normalized to nitrate, the drawdown of DIC\* uptake until day 13 was significantly higher at high pCO<sub>2</sub> than at present ( $t$ -test,  $p < 0.01$ ) and low pCO<sub>2</sub> ( $t$ -test,  $p < 0.001$ ). Thereby, the uptake ratios  $\Delta[\text{DIC}^*]:\Delta[\text{NO}_3^-]$  in the high- and present-pCO<sub>2</sub> treatments were above the Redfield C:N ratio of 6.6 (Redfield et al. 1963), but below in the low-pCO<sub>2</sub> treatment. In all mesocosms, the net production of Chl *a* was linearly related to the uptake of nitrate ( $p < 0.001$ ) during the exponential growth phase (days 5–13). Thereby, the ratio  $\Delta[\text{Chl } a]:\Delta[\text{NO}_3^-]$  was lowest at high pCO<sub>2</sub> and significantly different from the respective ratios in the present- and low-pCO<sub>2</sub> treatments ( $p < 0.005$ ).

Despite the higher DIC\* uptake, the accumulation of POC decreased as a function of increasing pCO<sub>2</sub>. This was indicated by differences in the ratio of POC accumulation to DIC\* drawdown,  $\Delta[\text{POC}]:\Delta[\text{DIC}^*]$ , which were significant between high and present pCO<sub>2</sub> ( $t$ -test,  $p < 0.001$ ) and between present and low pCO<sub>2</sub> ( $t$ -test,  $p < 0.05$ ). Specifically, normalized to the uptake of DIC\*, the accumulation of POC was 24% lower in the high-pCO<sub>2</sub> than in the low-pCO<sub>2</sub> treatment. Similar to POC, the accumulation of PON relative to the drawdown of DIC\* (i.e.,  $\Delta[\text{PON}]:\Delta[\text{DIC}^*]$ ) was also significantly smaller at high pCO<sub>2</sub> than at present ( $t$ -test,  $p < 0.001$ ) and low pCO<sub>2</sub> ( $t$ -test,  $p < 0.001$ ). The accumulation of POP (i.e.,  $\Delta[\text{POP}]:\Delta[\text{DIC}^*]$ ) was not significantly different between the high- and present-pCO<sub>2</sub> treatments but was slightly higher in the low-pCO<sub>2</sub> mesocosms ( $t$ -test,  $p < 0.05$ ).

In accordance with the  $\Delta[\text{DIC}^*]:\Delta[\text{NO}_3^-]$  uptake ratios, the highest  $\Delta[\text{POC}]:\Delta[\text{PON}]$  ratios were observed at high pCO<sub>2</sub>, significantly higher than those at low pCO<sub>2</sub> ( $p < 0.05$ ), although not significantly different from those in the present-pCO<sub>2</sub> mesocosms. On the contrary, the  $\Delta[\text{POC}]:\Delta[\text{POP}]$  ratios were lowest in the high-pCO<sub>2</sub> mesocosms ( $t$ -test,  $p < 0.005$ ). Differences in the elemental stoichiometry of the treatments were also observed for the production of PON relative to POP. Again, high-pCO<sub>2</sub> mesocosm behavior was significantly different from that in the present- and low-pCO<sub>2</sub> mesocosms ( $t$ -test,  $p < 0.001$ ), having the lowest PON to POP production ratio.

Table 2. The stoichiometry of elements and particles during the bloom was determined by regression analysis of  $y = ax + b$ . DIC\* is the concentration of DIC corrected for changes in alkalinity and salinity. Degree of freedom is  $n - 2$ . No samples were taken on day 18.

Ratio $\Delta y : \Delta x = a$	CO <sub>2</sub> treatment	$\Delta t$ (days)	a	$\pm$ SD	$r^2$	df
$\Delta[\text{DIC}^*] : \Delta[\text{Cell}]$ (pmol : cell)	High	3–17	–5.5	0.49	0.77	43
	Present	3–17	–4.1	0.28	0.84	43
	Low	3–17	–3.6	0.30	0.77	43
$\Delta[\text{DIC}^*] : \Delta[\text{NO}_3^-]$ (mol : mol)	High	1–13	8.10	0.17	0.98	37
	Present	1–13	8.11	0.34	0.93	37
	Low	1–13	6.01	0.51	0.78	37
$\Delta[\text{POC}] : \Delta[\text{DIC}^*]$ (mol : mol)	High	1–13	–0.56	0.02	0.95	37
	Present	1–13	–0.66	0.03	0.92	37
	Low	1–13	–0.74	0.06	0.82	37
$\Delta[\text{PON}] : \Delta[\text{DIC}^*]$ (mol : mol)	High	1–13	$-7.4 \times 10^{-2}$	$0.2 \times 10^{-2}$	0.97	37
	Present	1–13	$-8.5 \times 10^{-2}$	$0.4 \times 10^{-2}$	0.94	37
	Low	1–13	$-11 \times 10^{-2}$	$0.7 \times 10^{-2}$	0.82	37
$\Delta[\text{POP}] : \Delta[\text{DIC}^*]$ (mol : mol)	High	1–13	$-1.9 \times 10^{-3}$	$8.6 \times 10^{-5}$	0.84	37
	Present	1–13	$-1.9 \times 10^{-3}$	$9.5 \times 10^{-5}$	0.86	37
	Low	1–13	$-2.0 \times 10^{-3}$	$20 \times 10^{-5}$	0.74	37
$\Delta[\text{PIC}] : \Delta[\text{POC}]$ (mol : mol)	High	1–19	0.23	0.03	0.60	34
	Present	1–19	0.24	0.02	0.80	42
	Low	1–19	0.29	0.02	0.84	42
$\Delta[\text{PIC}] : \Delta[\text{Cell}]$ (pmol : cell)	High	3–19	0.94	0.37	0.53	28
	Present	3–19	0.84	0.10	0.63	38
	Low	3–19	1.10	0.11	0.76	35
$\Delta[\text{Chl } a] : \Delta[\text{NO}_3^-]$ ( $\mu\text{g} : \mu\text{mol}$ )	High	5–13	–0.58	0.03	0.94	25
	Present	5–13	–0.66	0.03	0.95	25
	Low	5–13	–0.64	0.02	0.98	24
$\Delta[\text{POC}] : \Delta[\text{PON}]$ (mol : mol)	High	1–13	7.0	0.2	0.97	37
	Present	1–13	6.8	0.3	0.95	37
	Low	1–13	6.6	0.3	0.96	37
$\Delta[\text{POC}] : \Delta[\text{POP}]$ (mol : mol)	High	1–13	260	16	0.87	37
	Present	1–13	280	21	0.82	37
	Low	1–13	308	19	0.87	37
$\Delta[\text{PON}] : \Delta[\text{POP}]$ (mol : mol)	High	1–13	37.1	1.9	0.91	37
	Present	1–13	42.2	2.1	0.92	37
	Low	1–13	47.2	1.8	0.95	37
$\Delta[\text{PON}] : \Delta[\text{NO}_3^-]$ (mol : mol)	High	1–13	0.60	0.02	0.98	37
	Present	1–13	0.67	0.02	0.97	37
	Low	1–13	0.71	0.03	0.95	37

The uptake ratio of nitrate and phosphate was not significantly different between the treatments, yielding a mean  $\Delta[\text{NO}_3^-] : \Delta[\text{PO}_4^{3-}]$  of  $15.8 \pm 0.41$  ( $r^2 = 0.94$ ,  $n = 99$ ). Calculating  $\Delta[\text{POP}] : \Delta[\text{PO}_4^{3-}]$  and  $\Delta[\text{PON}] : \Delta[\text{NO}_3^-]$  ratios revealed that the net yield of phosphorus in POP was similar in all mesocosms but that the net yield of nitrogen in PON decreased with pCO<sub>2</sub> ( $t$ -test,  $p < 0.001$ ).

In all mesocosms, the phosphorus yield in particulate organic matter was much lower than the nitrogen yield. Hence, the production ratio of  $\Delta[\text{PON}] : \Delta[\text{POP}]$  differed clearly from the  $\Delta[\text{NO}_3^-] : \Delta[\text{PO}_4^{3-}]$  uptake ratio (Fig. 10). In contrast, the average concentration ratio  $[\text{PON}] : [\text{POP}]$  between days 1 and 14 was  $37.5 \pm 10$  and not differ significantly from the concentration ratio  $[\text{NO}_3^-] : [\text{PO}_4^{3-}]$  between days 1 and 11 at  $39 \pm 13$ .

In general, the relationships of POC to PON and POP, respectively, were tight only until nitrate exhaustion on day 13. Thereafter, a decoupling of the dynamics of the nutritional elements occurred, leading to a steep rise of  $[\text{POC}] : [\text{PON}]$  ratios (Fig. 11). As Fig. 11 shows, the behavior of the  $[\text{POC}] : [\text{PON}]$  ratio was independent of the resource ratio  $[\text{DIC}] : [\text{NO}_3^-]$ , which increased nonlinearly during the experiment by two orders of magnitude. During the period of nitrate exhaustion, the variability of  $[\text{POC}] : [\text{PON}]$  ratios among the replicate mesocosms increased strongly ( $[\text{POC}] : [\text{PON}]_{\text{days 14–19}}$ ,  $\text{SD}_{\text{max}} = 5$ ). As a consequence, the maximum POC concentration of the bloom was neither predictable from PON or nitrate concentrations nor replicable under the same environmental conditions (i.e., within one treatment). Because of the decoupling of elemental dynamics, no rela-

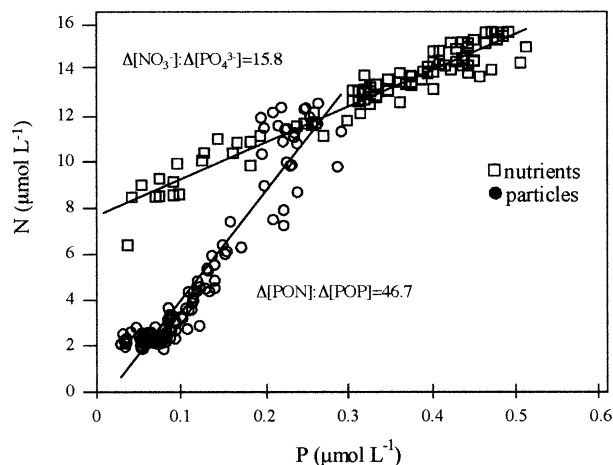


Fig. 10. Differences between the uptake ratio of  $[\text{NO}_3]:[\text{PO}_4]$  and the accumulation ratio of  $[\text{PON}]:[\text{POP}]$  were determined until the height of the bloom (day 14).

tionships between particulate elements existed during the postbloom phase; hence, no CO<sub>2</sub> effects on elemental stoichiometry could be determined for that time.

## Discussion

Testing the direct effect of CO<sub>2</sub> concentration and of CO<sub>2</sub>-induced changes in seawater carbonate chemistry on natural plankton communities is a difficult task. Sufficiently large differences of CO<sub>2</sub> concentration within a manageable region are difficult to find in the ocean. Our knowledge about the influence of CO<sub>2</sub> on marine phytoplankton therefore stems primarily from laboratory studies and bottle experiments in which CO<sub>2</sub> concentration has been modified either through acid or base addition or has been controlled by aeration. Although these kinds of experiments have the advantage of being highly controllable and reproducible, the dynamics of a natural environment are not well simulated, and important environmental factors, such as particle sinking and zooplankton grazing, are excluded. Moreover, most previous experiments have not addressed the effects of CO<sub>2</sub> on the development and decline of a natural phytoplankton bloom.

To overcome these problems, we have developed an experimental setup that manages and controls the pCO<sub>2</sub> in outdoor seawater mesocosms. The desired CO<sub>2</sub> concentrations representing environments with high, present day, and low CO<sub>2</sub> were achieved within a few days, and perturbation of the chemical equilibrium thereafter was limited to that induced by biological activity within the mesocosm itself. The reproducibility of single components of seawater chemistry within the three replicates of each CO<sub>2</sub> treatment was high, as demonstrated by low variability in pCO<sub>2</sub>, pH, alkalinity, and inorganic nutrients within the treatments. By covering the mesocosms with air-tight domes and continuous fumigation of the tents, the CO<sub>2</sub> partial pressure in the overlying air was maintained at a constant level throughout the study. Hence, the experimental setup provided the basic parameters necessary to study the effects of CO<sub>2</sub> changes on a phytoplankton bloom.

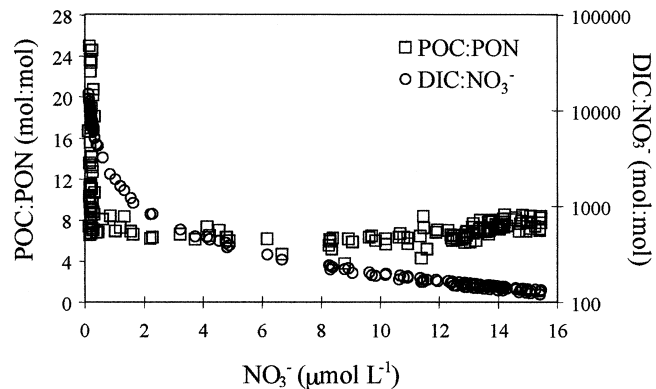


Fig. 11. The ratios of  $[\text{POC}]:[\text{PON}]$  and  $[\text{DIC}]:[\text{NO}_3^-]$  determined from daily samples of all nine mesocosms as a function of nitrate concentration.

During this study, a phytoplankton bloom dominated by the coccolithophorid *E. huxleyi* occurred simultaneously in all of the nine mesocosms. After an initial lag phase of 5 d, exponential growth of the phytoplankton community started, as evidenced in changes in chlorophyll, nutrients, and biomass. This bloom declined after nitrate concentration became exhausted. A postbloom phase characterized by declining Chl *a*, PON, and POP concentrations ensued in the last 5 d of the experiment. When comparing bloom development in the various mesocosms, the most obvious feature was the large variability of particulate concentrations among the replicate mesocosms that obscured clear CO<sub>2</sub> treatment-related effects. Normalization of production rates calculated for the nutrient-replete, exponential growth phase eliminated a large part of natural variability. After nitrate depletion, the biogeochemistry of the bloom became chaotic, in the sense that particulate organic carbon, nitrogen, and phosphorus concentrations were no longer predictable from each other and were highly variable even among the replicates of one treatment (i.e., under the same environmental conditions).

CO<sub>2</sub> treatment-related effects were identified for the growth and calcification rates of *E. huxleyi* and for the stoichiometry of particulate C, N, and P during this study. Calcite formation by *E. huxleyi* was clearly affected by the carbonate chemistry of the seawater and decreased with CO<sub>2</sub> concentration and increased respectively with CO<sub>3</sub> concentration and pH. This was consistently indicated by a variety of measurements showing that in the low-CO<sub>2</sub> treatment (i.e., under lowered CO<sub>2</sub> concentration), the  $\Delta[\text{PIC}]:\Delta[\text{Cell}]$  and  $\Delta[\text{PIC}]:\Delta[\text{POC}]$  ratios were highest, the coccospheres and coccoliths largest, and the weight of individual coccoliths highest. These results are furthermore in accordance with an observed decrease in the rate of calcification with increasing CO<sub>2</sub> concentration, as derived from changes in the seawater alkalinity by Delille et al. (unpubl.). Although differences in  $\Delta[\text{PIC}]:\Delta[\text{POC}]$  ratios could also result from an effect of CO<sub>2</sub> on cell growth and organic carbon production by *E. huxleyi*, the differences in  $\Delta[\text{PIC}]:\Delta[\text{Cell}]$  ratios and in the size and weight of coccoliths observed between the treatments is direct evidence that calcification itself was affected by the changes of the carbonate chemistry. A direct influence of CO<sub>2</sub>-related effects on biological calcification has previ-

ously been demonstrated for corals (Gattuso et al. 1999). This study shows that  $\text{CO}_2$  directly affects calcification by *E. huxleyi* during bloom development, as previously suggested in culture studies (Zondervan et al. 2001, 2002).

Another difference between the treatments was detected for the net specific growth rate of *E. huxleyi*, which decreased with  $\text{pCO}_2$  and might have influenced differences in net PIC accumulation. Because net specific growth rates were derived from changes in cell concentration, we have to speculate whether cell division rate was directly affected by the  $\text{CO}_2$  treatment or whether different loss rates (i.e., by grazing, sinking, or autolysis) occurred. Nitrate and phosphate uptake rate ratios, as well as Chl *a*-normalized  $^{14}\text{C}$ -POC primary production rates were similar in all mesocosms (Delille et al. unpubl.) and do not indicate a reduced biomass production at higher  $\text{CO}_2$ . Reduced calcification rates and direct effects of low pH on cell physiology nevertheless might have affected cell division rates. It has been previously demonstrated that many coastal marine phytoplankton species are directly sensitive to the pH of seawater and that the growth rate of some phytoplankton species has an optimum pH, below and above which the rate of cell division decreases (Hinga 2002). However, bottle experiments with a culture strain of *E. huxleyi* did not show such a decrease of cell growth rates with increasing  $\text{CO}_2$  (Zondervan et al. 2002). If we assume that the lower net specific growth rate in the high- $\text{CO}_2$  treatment mirrored cell division rates and was not caused by an enhancement of loss processes, we can conclude that the  $\text{CO}_2$  effect on calcification was even stronger than apparent from changes in [PIC]:[POC] ratios. This conclusion is consistent with an earlier study conducted with a culture strain of *E. huxleyi*, which demonstrated that a reduction of growth rates results in increased [PIC]:[POC] ratios (Riegman et al. 2000). Because the opposite was observed during this study (i.e., highest calcification was observed at highest net specific growth rates), the effect of the  $\text{CO}_2$  treatment on calcification must have been large enough to counteract and even exceed the growth effect.

The net production of POC and PON relative to the drawdown of  $\text{DIC}^*$  and nitrate, respectively, was significantly affected by  $\text{CO}_2$  treatment and decreased with increasing  $\text{CO}_2$ . Because the  $\Delta[\text{POC}]:\Delta[\text{POP}]$  and  $\Delta[\text{PON}]:\Delta[\text{POP}]$  ratios were significantly lower in the high- $\text{pCO}_2$  than in the present- and low- $\text{pCO}_2$  treatments, the data rather point to a selective loss of POC and PON at higher  $\text{pCO}_2$  than to reduced cell growth. This loss of carbon and nitrogen has resulted either from an enhanced release of carbon and nitrogen into the dissolved organic matter (DOM) pool or from a higher sedimentation rate of phosphorus-depleted particles. The average increase of DOC was  $11 \pm 8 \mu\text{mol C L}^{-1}$ , equal to approximately 18% of the  $\text{DIC}^*$  taken up during the study. Because a significantly different accumulation of DOC between the  $\text{CO}_2$  treatments was not detected (Engel et al. 2004a), DOC accumulation does not explain the differences in  $\Delta[\text{POC}]:\Delta[\text{DIC}^*]$  ratios between the treatments. Particle sinking was therefore most likely responsible for the differences in the loss of particulate organic elements between the treatments, at least for carbon. The cause of a higher POC sedimentation rate with increasing  $\text{pCO}_2$  remains an open question. Although enhanced sedimentation

of particles, such as cells and detritus, might have been responsible for the observed differences in net growth rates, an enhanced sedimentation of *E. huxleyi* cells, potentially depleted in phosphorus, at higher  $\text{pCO}_2$  cannot fully explain the reduced fraction of POC formation relative to  $\text{DIC}^*$  uptake in these mesocosms. *E. huxleyi*-derived organic carbon accounted for only 32% of POC, assuming a maximum carbon quota of  $1.3 \text{ pmol cell}^{-1}$ , as determined by Zondervan et al. (2001). Net growth rates differed by  $\sim 14\%$ , equal to 4.5% of POC. In contrast, differences between POC accumulation normalized to  $\text{DIC}^*$  drawdown were on the order of 16% and 22% of POC. Thus, differences in net growth or loss rates of *E. huxleyi* were not large enough to quantitatively account for the different loss of POC.

An additional POC loss might have been provided by sinking of transparent exopolymer particles (TEP) because the formation rate of TEP normalized to cell abundance was significantly increased in the high- $\text{CO}_2$  mesocosms (Engel et al. 2004a). TEP originate from dissolved precursors and are known to favor particle aggregation (Engel 2000). Moreover, because TEP are mainly carbohydrates, they are relatively carbon rich and yield C:N ratios of  $\sim 26$  (Engel and Passow 2001, Mari et al. 2001). An enhanced formation of TEP at a high  $\text{CO}_2$  concentration could have resulted in enhanced aggregation of detrital particles with TEP and, subsequently, in the sedimentation of particles with an increased carbon and nitrogen content but depleted in phosphorus. The formation of aggregates with high TEP content was observed later during the bloom (Engel et al. 2004a). However, because of the high variability in particulate components, a statistically significant influence of TEP formation on the C:N ratios of particles could not be determined.

$\text{CO}_2$  effects on the C:N ratio are of special interest in evaluating the potential influence of  $\text{pCO}_2$  on the export fluxes of organic carbon in the ocean because export production is considered to be determined by the supply of nitrate to the surface ocean. Until the peak of the bloom, the  $\Delta[\text{DIC}^*]:\Delta[\text{NO}_3^-]$  uptake ratio was significantly higher under high than under present or low  $\text{pCO}_2$ . This indicates that the organic carbon yield of the nitrate-based production might increase with  $\text{pCO}_2$ . Comparing the uptake ratio  $\Delta[\text{DIC}^*]:\Delta[\text{NO}_3^-]$  with the yield of carbon in particulate matter showed that the loss of POC increased with  $\text{pCO}_2$ . When comparing the  $\Delta[\text{DIC}^*]:\Delta[\text{NO}_3^-]$  uptake ratio with the  $\Delta[\text{POC}]:\Delta[\text{PON}]$  accumulation ratio, we furthermore see that the C:N ratios of uptake processes were higher than the C:N ratios of particle formation under high and present  $\text{pCO}_2$ , but lower under low- $\text{pCO}_2$  conditions. Thus, an increased carbon uptake at present and high  $\text{pCO}_2$  seems to stimulate a preferential loss of carbon, whereas a reduced carbon uptake at low  $\text{pCO}_2$  apparently results in a selective accumulation of carbon in particles. It should be noted that the  $\Delta[\text{POC}]:\Delta[\text{PON}]$  ratio in the low- $\text{CO}_2$  treatment conformed to the Redfield ratio, whereas there was a trend toward higher C:N ratios with increasing  $\text{pCO}_2$ . Thus, our study indicates that with increasing  $\text{pCO}_2$  (1) the rate of particle loss is higher and (2) the elemental composition of sinking particles is shifted toward higher C:N ratios. Specifically, considering that, in the high- $\text{pCO}_2$  treatment, 60% of nitrate drawdown and 56% of  $\text{DIC}$  drawdown between days

1 and 13 accumulated as PON and POC, respectively (see  $\Delta[\text{PON}]:\Delta[\text{NO}_3^-]$  and  $\Delta[\text{POC}]:\Delta[\text{DIC}]$  in Table 2), the loss C:N ratio of carbon and nitrogen in this treatment would have been 8.4. In comparison, the corresponding values in the low-CO<sub>2</sub> treatment would be 71% and 74%, yielding a theoretical loss C:N ratio of 2.4.

No CO<sub>2</sub> effects were determined regarding the total uptake of nitrate and phosphate. For phosphate, this result was not surprising because phosphorus was the least available element and likely to become exhausted in all mesocosms. The drawdown of nitrate relative to phosphate followed Redfield stoichiometry, with an N:P ratio of approximately 16. Because nitrate and phosphate were added at a ratio of 30:1, a surplus of nitrate was left at the onset of phosphate exhaustion. The uptake of surplus nitrate and the development of the *E. huxleyi* population after the onset of phosphate exhaustion, as well as the ongoing POP formation between days 11 and 14, indicated the utilization of dissolved organic phosphorus (DOP). It is well documented that under P-limiting conditions, *E. huxleyi* has a high uptake affinity for DOP on the basis of production and release of the ectoenzyme alkaline phosphatase (Riegmann et al. 2000). The average concentration ratio of [N]:[P] in particles until the height of the bloom was similar to those of the inorganic nutrients, which apparently supports the hypothesis that unbalanced nutrient supply (relative to the Redfield ratio) results in unbalanced elemental stoichiometry of cells (Sternner and Elser 2002). However, it should be noted that the uptake ratio of nitrate and phosphate strongly deviated from the production ratio of PON and POP, and rather points to a balanced (i.e., Redfield-like) stoichiometry of growing (nutrient-assimilating) cells and simultaneously degrading POP from detritus. A rapid turnover of phosphorus was further indicated by the observation that apparently only 35% of the phosphorus assimilated as phosphate was stored in suspended particles. Compared with a nitrogen yield of 60–71% in PON, this indicated either sedimentation of POM with a lower N:P ratio than observed for the suspended POM or the release of organic phosphorus to the DOM pool. Sedimentation of relatively phosphorus-rich particles was very unlikely because samples of the sediment taken from the bottom of the mesocosms at the end of the study had a PON:POP ratio of  $168 \pm 43$ , clearly above the N:P ratio of suspended particles. Because P exhaustion was likely overcome by the utilization of organic phosphorus resources, nitrate has to be considered the ultimate limiting nutrient and therefore was likely to become depleted in all of the mesocosms.

We used high, present, and low CO<sub>2</sub> concentrations in this experiment, representing the future, present, and glacial atmospheric CO<sub>2</sub> conditions, respectively. Implications of the observed CO<sub>2</sub>-related effects for the understanding of changes in the biogeochemistry and ecology of phytoplankton blooms between glacial and future times should, however, be interpreted with caution. To identify the direct effect of CO<sub>2</sub> on the *E. huxleyi* bloom, it was necessary to keep all other environmental factors comparable among the mesocosms. As mentioned before, the oceanographic and chemical condition of the ocean does not remain steady from glacial to future times. Moreover, the response to the combined

effects of global change (e.g., CO<sub>2</sub> and temperature) could be different in magnitude than the response to changing CO<sub>2</sub> concentration alone (Reynaud et al. 2003). However, because of its high heat capacity and huge mass, relative changes in ocean surface temperatures on a global scale are proportionately smaller than the changes in surface ocean chemistry from the uptake of CO<sub>2</sub>. Moreover, the continuous increase in CO<sub>2</sub> concentration represents a change beyond the range of seasonal or spatial variability of environmental factors phytoplankton species usually encounter in the ocean. Nevertheless, to provide a detailed and realistic picture of global change for the marine ecosystem, the multiple effects of environmental change need to be addressed. The design and management of experiments that can cope with one or more variable factors, in addition to CO<sub>2</sub>, while maintaining a natural environment will be a challenging task for oceanography in the future.

It has been suggested that a decrease in the ratio of PIC:POC in sinking material could significantly affect the ocean's capacity to store atmospheric CO<sub>2</sub>, potentially acting as a negative feedback to increasing atmospheric CO<sub>2</sub> concentrations (Riebesell et al. 2000; Zondervan et al. 2001). This study supports the earlier observation of reduced calcification rates at high CO<sub>2</sub> concentrations and the conclusion that the carbonate pump could be particularly sensitive to CO<sub>2</sub>-related changes in seawater chemistry. The PIC:POC ratio of the sinking particles (i.e., the rain ratio) was not determined during this study but might have been even lower than the respective ratio in the suspended particles because of the suggested higher loss of POC at high pCO<sub>2</sub>. Moreover, if the reduction of the net specific growth rate of *E. huxleyi* is indeed representative of the natural environment under high CO<sub>2</sub>, the ability of this species to compete with other algae might become affected in the future. A community shift toward noncalcifying phytoplankton species could not only induce changes in the alkalinity of the ocean, as suggested by Riebesell et al. (2000), but could also reduce the amount of export production, which is correlated with CaCO<sub>3</sub> fluxes below 1,000 m water depth (Armstrong et al. 2002; Klaas and Archer 2002; Barker et al. 2003). On the other hand, if the observed differences in the loss of POC were associated with an enhancement of TEP-driven aggregation processes, the mode of sedimentation could shift toward a higher rate of aggregate sinking. A higher proportion of aggregates would increase the average sinking velocity of POM and, hence, increase the remineralization depth of POM, which in turn would lead to a deeper export of primary production.

We did not deal with any influence of pCO<sub>2</sub> changes on the heterotrophic food web during this study, apart from determining bacterial abundances, which were not different between the treatments (Rochelle-Newall et al. 2004). However, because the growth efficiency of heterotrophic organisms is controlled by the quality of food, the observed differences in the elemental stoichiometry of particle formation suggests that CO<sub>2</sub> could indirectly affect heterotrophic processes, such as growth rate, particle decomposition, and nutrient recycling.

Our study suggests that *E. huxleyi* is indeed sensitive to changes in CO<sub>2</sub> concentration, as was concluded from chang-

es in net specific growth rates and the calcification of cells, as well as from changes in the stoichiometry of elemental uptake, accumulation, and loss processes. This means that in addition to the expected indirect effects, changes in CO<sub>2</sub> concentration could directly influence biogeochemical cycling and carbon export in the ocean, as well as food web dynamics. To evaluate the direct effects of CO<sub>2</sub> concentration on export production, future studies will need to obtain information about the rates and timing of particle sinking, as well as about the elemental composition of the exported material.

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