Primary production during nutrient-induced blooms at elevated CO$_2$ concentrations

J. K. Egge$^1$, T. F. Thingstad$^1$, A. Engel$^2$, R. G. J. Bellerby$^3$, and U. Riebesell$^4$

$^1$Department of Biology, University of Bergen, 5020 Bergen, Norway
$^2$Alfred Wegener Institute (AWI) for Marine and Polar Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
$^3$Bjerknes Centre for Climate Research, Univ. of Bergen, Allégaten 55, 5007 Bergen, Norway
$^4$Leibniz Institut für Meereswissenschaften, IFM-GEOMAR, Kiel, Germany

Received: 14 November 2007 – Accepted: 14 November 2007 – Published: 27 November 2007

Correspondence to: J. K. Egge (jorun.egge@bio.uib.no)
Abstract

Mesocosms experiments (PeECE II and PeECE III) were carried out in 9 transparent mesocosms. Prior to the experimental period, the seawater carbonate system was manipulated to achieve three different levels of CO$_2$. At the onset of the experimental period, nutrients were added to all mesocosms in order to initiate phytoplankton blooms. Rates of primary production were measured by in-situ incubations using $^{14}$C-incorporation and oxygen production/consumption. Particulate primary production by $^{14}$C was also size fractionated and compared with phytoplankton species composition. Nutrient supply increased the primary production rates, and a net autotrophic phase with $^{14}$C-fixation rates up to 4 times higher than initial was observed midway through the 24 days experiment before net community production returned to near-zero and $^{14}$C-fixation rates relaxed back to lower than initial. We found a trend in the $^{14}$C-based measurements towards higher cumulative primary production at higher pCO$_2$, consistent with recently published results for DIC removal (Riebesell et al., 2007). There were found differences to the size fractionated primary production response to CO$_2$ treatments. The plankton composition changes throughout the bloom, however, resulted in no significant response until the final phase of the experiment where phytoplankton growth became nutrient limited, and phytoplankton community changed from diatom to flagellate dominance. This opens for the two alternative hypotheses that such an effect is associated with mineral nutrient limited growth, and/or with phytoplankton species composition. The lack of a clear net heterotrophic phase in the last part of the experiment supports the idea that a substantial part of production in the upper layer was not degraded locally, but either accumulated there or was exported vertically.

1 Introduction

In the upper photic zone where primary production is limited by mineral nutrients (e.g. N, P or Fe), the microbial food web can be seen as a set of cycles of the limiting
element, grossly described by the import-export and the regenerated cycles (Dugdale and Goering, 1967). Onto this set of nutrient cycles, the C-cycle is linked via more or less flexible stoichiometric relationship in the different trophic levels and their interactions. Relatively small alterations in either element cycles or stoichiometric C:nutrient coupling may have consequences for the ocean’s C-cycle, particularly if the net result is a change the C:limiting element ratio of the exported material, and/or in the quality of this matter in a manner affecting the relative depths at which C and limiting elements are released from sinking to non-sinking forms (Thingstad, 1993).

Increased atmospheric CO$_2$ leads to both an increased pCO$_2$ and a lowered pH (Riebesell, 2004). It is an a priori possibility that both of these environmental changes may affect either the cycling of the limiting element, and/or its stoichiometric coupling to C. In either case, this would be expected to lead to changes in the rate of fixation of C into organic material and in the processes producing and consuming oxygen.

CO$_2$ is often quoted as being a non-limiting factor for primary production in seawater (Raven and Jonston, 1991; Clark and Flynn, 2000) The RUBISCO enzyme has however a relatively low affinity for CO$_2$ (Raven and Jonston, 1991) and this has led to a discussion of the possibility that increased CO$_2$-levels may stimulate primary C-fixation (Riebesell, 2004). Should this occur without a proportional change in the cycle of limiting elements, the consequence is a change in the stoichiometric relationships in the microbial food web. Based on measurements of removal of inorganic-C and nitrate, the PeECE-experiments have shown such an effect (Riebesell et al., 2007).

The affinity for CO$_2$ differs among phytoplankton species (Rost et al. 2003), and some phytoplankton species are able to change their CO$_2$ supply by CO$_2$-concentrating mechanisms (CCM) (Raven, 1991). The efficiency and regulation of CCM differs among phytoplankton species and functional groups. Changes in CO$_2$ availability might therefore affect competition and succession of phytoplankton species (Burkhardt et al., 2001; Rost et al., 2003). A shift in dominance between *Phaeocystis* and diatoms has been observed in a natural plankton community where CO$_2$ has been manipulated (Tortell et al., 2002). Changes in phytoplankton composition may affect primary pro-
duction, and the effect of increased CO$_2$ on primary production has been investigated theoretically as well as experimentally. Some papers report small, if any, effect (Clark and Flynn, 2000; Tortell et al., 2002) whereas other papers document increased primary production with increasing CO$_2$ (Heine and Sand-Jensen, 1997; Schippers et al., 2004; Riebesell et al., 2007).

3 mesocosm experiments, in 2001, 2003 and 2005 have been carried out in the framework of the Pelagic Ecosystem CO$_2$ Enrichment study (PeECE). The aim of these experiments has been to study the effects of elevated levels of CO$_2$ on the planktonic community and on sea water chemistry (Delille et al., 2005; Engel et al., 2004, 2005; Rochell-Newall et al., 2004; Grossart et al., 2006). Blooms of the coccolithoporid Emiliania huxleyi and/or diatoms were initiated by nutrient addition, and the plankton community was monitored for about 3 weeks. Primary production has been measured during all PeECE experiments. No differences in primary production were observed in the 2001 experiment where CO$_2$ concentration in the mesocosms was manipulated to 180, 370 and 700 µatm (Delille et al., 2005). The same CO$_2$ concentrations were also used in the 2003 experiment, but elevated to 350, 700 and 1050 µatm CO$_2$ in 2005 (Schulz et al., 2007)

This paper reports primary production results from PeECE III, with a comparison of the corresponding data from (PeECE II conducted in 2003. $^{14}$C and O$_2$ measurements were used for estimating production in both experiments, but different techniques were used for O$_2$-detection (Winkler titration and optodes in PeECE II and PeECE III, respectively). Primary production in size-fractions: 0.2–1, 1–5, 5–10 and >10 µm, was measured only during the 2005 experiment.
2 Materials and methods

2.1 Set up and sampling

The PeECE III mesocosm experiment was carried out at Marine Biological Station, University of Bergen, Norway between May 16 and June 10, 2005 (see Table 1). 9 mesocosms (volume 27 m$^3$) made of polyethylene were filled with unfiltered, nutrient-poor post-bloom water from the fjord, and manipulated to achieve 3 different levels of CO$_2$ in triplicate mesocosms. The levels of CO$_2$ at the start of the experimental period were 350 µatm (1 × CO$_2$), 700 µatm (2 × CO$_2$) and 1050 µatm (3 × CO$_2$). Nutrients, as nitrate and phosphate, were added to the mesocosms in order to achieve an increase in growth and biomass of osmotropic organisms. For further details concerning the set-up of the experiment see Schulz et al. (2007). The PeECE II experiment was carried out between 4–24 May 2003, where CO$_2$ concentration in the nine mesocosms were adjusted to 190 µatm (glacial), 370 µatm (present) and 750 µatm (future) CO$_2$, in triplicates. While in 2005 low concentrations of silicate were available in the fjord water used to fill the mesocosm, allowing for an initial bloom of diatoms followed by coccolithophore dominance, silicate was added in excess in 2003. For further details see Grossart et al. (2006).

2.2 $^{14}$C Primary production

Primary production was measured using the $^{14}$C method, according to Steemann-Nielsen (1952) and Gargas (1975). Plastic bottles (76 ml) (NUNC Easyflask) spiked with approximately 4 µCi were incubated in situ between 10:00 and 14:00 h. Dark uptake was measured bottles wrapped in aluminium foil. Bottles were incubated in the sea outside the mesocosms, at the irradiance level corresponding to middle dept of the upper layer of the mesocosms (see Schulz et al., 2007). The incubation depth was determined base on light profiles inside and outside the mesocosms. A Li-Cor Li 1000, Datalogger with Li 190SA-Quantum sensor and Li-192SA Underwater Quantum
Sensor was used both for profiling and logging. In 2005, triplicate incubation was made from all 9 mesocosm, while in 2003 only two mesocosms per treatment were analysed. In addition to short time incubation, 24 h incubations were also conducted in 2005. For these incubations 118 mL glass bottles were used, and only one mesocosm per treatment was sampled, M2, M5 amd M8. These samples were either filtrated onto Sartorius membrane filters or onto Nuclepore filters of 0.2, 1, 5 and 10 µm. After filtration were all filters treated with fuming HCl in order to remove inorganic $^{14}$C. Scintillation solution (Ecosint O) was then added, and the samples were stored overnight before counted in a Packard Tri Carb Liquid Scintillation Analyser, model 1900 A. Calculations were done according to Gargas (1975). Daily primary production, based on 4 h incubation was calculated as function of incoming irradiance during incubation period (4 h) and total irradiance for the 24 h. Calculation of primary production was done according to Gargas (1975).

2.3 Oxygen and Oxygen production

In 2003 dissolved oxygen was determined based on the Winkler method using an automated titration (Titrino, Metrohm) and potentiometric endpoint detection. Samples were collected from the mesocosms at 9.00 using a Ruthner water sampler and transferred without air bubbles to 100 ml BOD bottles through a silicon tube. A total of 6 bottles were filled from each mesocosm. Two bottles were immediately fixed with 1 ml of manganese chloride solution and 1 ml of potassium iodate (0.003 M) and mixed. Another two bottles were placed in photoresistant plastic foil and incubated together with the remaining two bottles in the fjord at about 1m depth for 9 h. The samples were removed in the evening (~19.00), fixed immediately, mixed well and stored for more than 12 but less than 24 h in the dark. After dissolving the precipitated with sulfuric acid (5 M) the iodine was titrated with 0.02 N thiosulfate solution. Calculation of the oxygen concentration was performed after Dickson (1994).

In 2005, BOD bottles were incubated for 24 h and oxygen was measured using the OxyMini optode system (World Precision Instruments). The instrument was two-point
calibrated according to the manual and used with automatic temperature compensation. Oxygen concentration was determined individually in each BOD bottle both before and after incubation.

3 Results

Initial particulate primary production rates, based on the $^{14}$C method (4 h incubations), ranged from 0.33 to 0.37 $\mu$mol C L$^{-1}$ h$^{-1}$ in 2005 (Fig. 1). After addition of nutrients, a rapid production increase was observed in all treatments. Maximum rates were observed on day 8, ranging from 1.6 to 1.8 $\mu$mol C L$^{-1}$ h$^{-1}$. Two weaker but distinct peaks were observed on day 12 and day 20 before the production rates decreased to levels lower than initial. In the second half of the experiment there was a tendency of increasing production with increasing CO$_2$. Although not statistically significant, this trend is visible from ca. Day 10 in the cumulative production (Fig. 1b). In the PeECE II experiment in 2003, average primary production ranged from 0.28 to 0.52 $\mu$mol C L$^{-1}$ h$^{-1}$.

Certainly, present CO$_2$ concentration (370 $\mu$atm) gave slightly higher production than past (190 $\mu$atm) and future (750 $\mu$atm), which also can be seen in gross production, leaving no consistent increasing or decreasing trend with increasing CO$_2$ (Table 2). For comparison, average primary production during PeECE III was somewhat higher, ranging from 0.57 to 0.62 $\mu$mol C L$^{-1}$ h$^{-1}$.

In 2005, the highest gross production, as measured by oxygen incubation, was observed on day 6 in 1$\times$ and 2$\times$ CO$_2$, with 56 and 58 $\mu$mol O$_2$ L$^{-1}$ d$^{-1}$ respectively, whereas the corresponding value, 58 $\mu$mol O$_2$L$^{-1}$ d$^{-1}$ in 3$\times$ CO$_2$ was observed a few days later (Fig. 2). For all treatments, maximum net community production was observed on day 6, and after Day 10 no net production was observed in the system in any of the treatments. With this method, CO$_2$ did not have any clear effects, neither on timing nor on scaling of production or respiration.

While there was no detectable variation in primary production with CO$_2$-level in the PeECE II (2003) experiment, there was a difference in phytoplankton community com-
position. In PeECE III (2005), we therefore decided to carry out fractionated primary production. The fractions 0.2–1, 1–5, 5–10 and >10 \( \mu \text{m} \) contained on average 29, 18, 12 and 41%, respectively, of total primary production. All fractions showed an increase in production at the onset of the experiment, but during the first week production was dominated by organisms in the fraction >10 \( \mu \text{m} \). On day 6, 70% of the total production was found in this fraction, thereafter the contribution from the >10 \( \mu \text{m} \) fraction decreased rapidly (Fig. 3). A tendency of higher production, although not significant, was observed in 3\( \times \), followed by 1\( \times \) and 2\( \times \) (Fig. 4). Cumulative production indicated that the differences were more pronounced after day 6 (data not shown). A distinct, but much smaller peak was observed in the fraction 5–10 \( \mu \text{m} \) on day 10. Average production in the fraction 5–10 \( \mu \text{m} \) was 0.9, 0.8 and 0.6 \( \mu \text{mol C L}^{-1} \text{d}^{-1} \) in 3\( \times \), 2\( \times \) and 1\( \times \) \( \text{CO}_2 \), respectively (Fig. 4).

From day 6 onwards we observed a decreasing trend in production in the smallest fraction (0.2–1 \( \mu \text{m} \)). The production in fraction 1–5 \( \mu \text{m} \) increased somewhat in the same period (Fig. 3). This was particularly the case in 1\( \times \) and 2\( \times \) \( \text{CO}_2 \), and on the very last day of the experiment, production in 3\( \times \) \( \text{CO}_2 \) was significantly lower than in the two other treatments (ANOVA, \( P < 0.05 \)). The largest differences between treatments were found in the smallest size fraction 0.2–1 \( \mu \text{m} \), where average production for the whole experimental period was 1.3 \( \mu \text{mol C L}^{-1} \text{d}^{-1} \) in the 1\( \times \) \( \text{CO}_2 \) treatment and increased with increasing \( \text{CO}_2 \) to 1.7 and 2.1 \( \mu \text{mol C L}^{-1} \text{d}^{-1} \) in 2\( \times \) and 3\( \times \) \( \text{CO}_2 \) respectively (Fig. 4).

4 Discussion

4.1 Effects of \( \text{CO}_2 \) on total primary production

Based on in situ measurements of dissolved inorganic carbon, determined significantly higher carbon consumption at elevated \( \text{CO}_2 \) (Riebesell et al., 2007; Bellerby et al., 2007). Over the course of the experiment excess DIC drawdown accumulated to
approximately 40 µmol kg\(^{-1}\) higher carbon consumption at 3×CO\(_2\) relative to 1×CO\(_2\) treatments. Plotting our \(^{14}\)C-data as cumulative production, we found (Fig. 1b) a somewhat smaller but comparable (22 µmol-C L\(^{-1}\)) difference in particulate primary production. The \(^{14}\)C-based results thus support the increase in C-fixation with increased CO\(_2\)-level proposed by Riebesell et al. (2007). The standard deviation (±18 µmol-C L\(^{-1}\)) of the \(^{14}\)C-based difference in particulate primary production is, however, insufficient for the effect to be statistically significant based on these measurements alone. Higher primary production as a result of elevated CO\(_2\) has also been reported by others (Hein and Sand-Jensen, 1997; Schippers et al., 2004). On the other hand, Clark and Flynn (2000) found neither the rate nor the extent of primary productivity to be significantly limited by DIC concentration.

Riebesell et al. (2007) also reported a difference in in-situ O\(_2\) concentrations supporting the hypothesis of a net stimulatory effect of elevated CO\(_2\) levels on C-fixation. We could not detect a similar trend in our O\(_2\) light/dark-bottle measurements. Our results also indicate a higher O\(_2\) production, in all treatments, relative to drawdown of DIC (Riebesell et al., 2007). The reason for this apparent discrepancy between in situ measurements and incubated samples is unknown, but one may suspect disturbances of auto- and/or heterotrophic processes during the 24 h confinement in the 125 ml bottles used in O\(_2\) incubation. We also have an unexplained low ratio (ca 1:4) of particulate \(^{14}\)C-fixation to gross oxygen production in our 24 h incubations. Using a 15 h (sun rise to sun set) incubation, Gazeau et al. (2007) found a near 1:1 (mol C: mol O\(_2\)) ratio between \(^{14}\)C incubations and gross production. Theoretically, significant production of \(^{14}\)C-DOC (not measured in this study) could help to balance the carbon fixation:O\(_2\)-production stoichiometry in our measurements.

Production in the 0.2–1 µm size-fraction was relatively high (29% of total production), and there is a tendency of increasing production in this size-fraction with increasing CO\(_2\). There was an increase in *Synechococcus* abundance in the last part of the experiment (Paulino et al., 2007). *Synechococcus* cells are in the size-range from 0.8 to 1.5 µm (Johnson and Sieburt, 1979), and parts of the population may therefore have
passed through 1 µm filters. The even smaller Prochlorophytes (<1 µm), known to contribute to primary production at lower latitudes (Li, 1994; John et al., 2007), are not common in Norwegian coastal waters and were not observed during the experiment. 14C found in the 0.2–1 µm size-fraction may also be due to bacterial uptake of labelled carbon released from phytoplankton, an option suggested by Li et al. (1993). The deviating trends observed for bacterial production (Allgaier et al., 2007) and 14C uptake in this fraction do not lend immediate support for this. Nevertheless, bacterial activity could still explain the comparatively high rate of 14C incorporation in the 0.2–1 µm size-fraction, if uptake of 14C-labeled DOC was accomplished by only a fraction of the bacterial community. Thus, neither the contribution of phytoplankton nor of heterotrophic bacteria to the radiolabel found in the 0.2–1 µm size-fraction can be ruled out based on these results. We note, however, that the highest primary production in this fraction was measured on Day 6, coinciding with the minimum in abundance of small organisms potentially passing a 1 µm filter (Paulino et al., 2007). An alternative possibility would be that 14C-labelled organic material released from phytoplankton passed the 1 µm filter but was (partly) retained on the 0.2 µm filters. An increasing production of TEP with increasing CO2 concentration has previously been shown (Engel, 2002; Engel et al., 2004; Rochelle-Newall et al., 2004). The CO2 effect may thus be linked to the mechanism of carbon overproduction under nutrient limited conditions (Engel, 2002) in accordance with the impression one gets from Fig. 1b where all treatments behave similarly until nutrients are depleted.

Our 14C-based measurements did not include DO14C, allowing for the possibility of a conversion of the over-consumption of DIC into DOC by e.g. excretion or leakage from phytoplankton cells. Statistically significant CO2 treatment effects on the concentration of DOC, however, were not detected in any of the PeECE experiments (Rochelle-Newall et al., 2004; Grossart et al., 2006; Schulz et al., 2007), and the Nuclepore filters

used in this experiment are supposed to have low absorption of $^{14}$C-DOC (Karl et al., 1998).

Daily measured concentration of DOC, however, does not reveal the pattern of production and consumption (turnover) of DOC. A higher bacterial production, indicating a higher DOC consumption, was observed in the treatment with highest CO$_2$ level in PeECE II, but this was not the case in PeECE III (Grossart et al., 2006; Allgaier et al., 2007$^1$).

If the effect of increased pCO$_2$ is an increase in the production of organic-C under conditions of mineral nutrient limited phytoplankton growth, this will only have a feedback effect on atmospheric CO$_2$ if the extra material is not respired by bacteria in the photic zone. It has been suggested that the ability of bacteria to consume labile DOC is highly dependent on the state of growth rate limitation in the bacteria (Thingstad et al., 1997) with C-limited bacteria in principle consuming all accessible organic material, while mineral nutrient limitation of bacterial growth may lead to accumulation of the otherwise degradable organic material. A net effect on C-sequestration may therefore depend not only on the physiological responses in phytoplankton, but also vary with ecological status and limiting factor for bacterial growth in the photic zone (Tanaka et al., 2007). The lack of any net heterotrophic phase in PeECE III shows that organic material produced during net autotrophy was not degraded by bacteria in the upper layer, but either accumulated or was exported vertically. This supports the interpretation of Riebesell et al. (2007) of a high export of organic material through the pycnocline in this experiment. This accumulation/export, combined with the observation of a CO$_2$ effect on bacterial production in PeECE II (Grossart et al., 2006), but not in PeECE III (Allgaier et al., 2007$^1$), highlights the need to better understand the ecological mechanisms regulating bacterial growth rate limitation in order to understand the net effects of any increased C-fixation at high pCO$_2$. 

4.2 Primary production related to osmotroph succession

All PeECE mesocosm studies were carried out in May–June, but nutrient additions, phytoplankton community composition, and other conditions were different (Table 2). By manipulating nutrient concentrations and stoichiometry, blooms of different phytoplankton groups, e.g. *Emiliania huxleyi* and/or diatoms were introduced. In 2001, nitrate and phosphate were added in a ratio of 34:1 (atomic) in order to induce blooms of the coccolithophorid *Emiliania huxleyi*, which towards the end of the experiment reached cell numbers ranging from 20 to $40 \times 10^3$ cells ml$^{-1}$ (Rochelle-Newall et al., 2004). In 2003, nitrate and phosphate were initially supplied in a lower N:P ratio (18:1) with silicate in excess (N:Si=1:1.33) in order to initiate diatom blooms. Diatoms, but also *E. huxleyi* became dominant during this experiment and different species compositions were observed within different CO$_2$ treatment (Grossart et al., 2006).

As for the first PeECE experiment, the aim in PeECE III was to introduce *E. huxleyi* blooms. Only nitrate and phosphate in a 25:1 ratio were therefore added to the mesocosms. The initial water did, however, contain about 3 $\mu$mol L$^{-1}$ silicate, favouring diatom growth (Schulz et al., 2007). Due to these start conditions the phytoplankton community became dominated by diatoms followed by a weak bloom of *E. huxleyi* and other nano- and pico-sized phytoplankton (Paulino et al., 2007). The nutrient environment during the experiment can be divided into 5 phases (Tanaka et al., 2007). During the first 6 days all nutrients were detectable and silicate was the first nutrient that was depleted (Phase I). Phosphate was depleted on day 11 (Phase II) and nitrate on day 15 (Phase III). Between day 16 and 20 all nutrients were depleted (Phase IV) before increased turnover times for phosphorus indicated regeneration of nutrients from day 21 (Phase V) (Tanaka et al., 2007).

The highest particulate primary production was observed in the first two phases. At the time of silicate depletion (Day 6), 70% of the total production was observed in the largest size fraction (>10 $\mu$m), and the same fraction used 50–70% of the $^{33}$PO$_4$-uptake (Tanaka et al., 2007). Pigment analysis showed that diatoms dominated among larger
algae during the two first phases (Schulz et al., 2007). As net production was hardly observed after Phase II, diatoms were probably the main contributors to the net primary production during the PeECE III experiment. Higher production was observed at 3× than at 1× and 2× CO₂ in the >10 μm fraction. The difference was not significant, and mainly observed after Day 6. Since photosynthetic carbon fixation rate of all diatoms tested so far are at or close to saturation at present CO₂ levels (Riebesell, 2004), we did not expect to see much effect in this group. In a field incubation experiment, Tortell et al. (2002) observed no change in primary production, but a relative shift in species composition between 150 and 750 ppm CO₂. In their experiment, diatoms became more abundant, *Phaeocystis* biomass decreased with increasing CO₂. Also the ratio between consumed nitrate and consumed silicate, N:Si, decreased with decreasing CO₂. In our experiment, neither consumption of nutrients, nor diatom abundance was affected by CO₂ levels (Schulz et al., 2007).

Most species of marine phytoplankton tested to date can use HCO₃⁻ in addition to CO₂ as a source for inorganic carbon (Tortell et al., 1997; Burkhard et al., 2001; Rost et al., 2003; Giordano et al., 2005). For the two diatoms *Thalassiosira weissflogii* and *Phaeodactylum tricornutum*, Burkhardt et al. (2001) showed that photosynthesis was unaffected by pCO₂ levels ranging from 36 to 1800 ppmv CO₂, but the uptake of HCO₃⁻ was more important as source of inorganic C at low CO₂ levels than at high. If this is a general trend among diatoms, it can also explain why primary production in our experiment was less affected by CO₂ levels in the period when diatoms dominated the phytoplankton community.

As in several previous mesocosms experiments, blooms of *E. huxleyi* occurred in mesocosms fertilized with nitrate and phosphate (Egge and Heimdal, 1994; Engel et al., 2005). In the PeECE III experiment, however, the maximum numbers were low compared with previous observations, and the highest cell numbers were observed during Phase II (Paulino et al., 2007). The size fraction 5–10 μm has previously been shown to represent *E. huxleyi* quite well -when the bloom is dominated by this species (Egge, 1994, Engel et al., 2007). Maximum primary production rates in the 5–10 μm
fraction occurred on Day 10, when *E. huxleyi* numbers ranged from 4.4 to $4.7 \times 10^3$ cells ml$^{-1}$ (Paulino et al., 2007). Assuming *E. huxleyi* to be the dominant photoautotroph in this fraction, our measured production of $17–26 \mu g C L^{-1} d^{-1}$ corresponds to $4–5 \text{pg} C \text{cell}^{-1} d^{-1}$. This is in the same order of magnitude as reported from laboratory experiments ($7–10 \text{pg} C \text{cell}^{-1} d^{-1}$) (Skattebøl, 1995; Zondervan et al., 2001). The actual production of POC per *E. huxleyi* cell in the current experiment must have been considerably lower, as other species including other haptophyte species were more numerous and probably contributed more to the primary production in this fraction (Schulz et al., 2007; Paulino et al., 2007). Both increasing (Zondervan et al., 2001; Leonardos and Geider, 2005) and decreasing (Sciandra et al., 2003) production at elevated levels of CO$_2$ were reported for *E. huxleyi*. Average primary production in the fraction 5–10 µm showed a weak increase with increasing CO$_2$. The same trend was observed both for *E. huxleyi* numbers and the other nano-sized phytoplankton from the flowcytometer counts (Paulino et al., 2007, Fig. 4). In the last part of the PeECE I experiment, 2001, *E. huxleyi* reached maximum cell numbers up to 10 times higher than the present experiment, but elevated CO$_2$ had no conspicuous effect on primary production (Delille et al., 2005; Engel et al., 2005). Sciandra et al. (2003) observed decreased production of POC in cultures of *E. huxleyi* at elevated levels of CO$_2$ when nitrate was depleted. Nitrate was also depleted when the bloom peaked in 2001, and could have reduced the stimulating effect of elevated CO$_2$ on *E. huxleyi* production observed in other studies.

Production in the 1–5 µm fraction increased during the experiment and contributed 18 % of the total production. *Synechococcus* was probably an important contributor to the primary production in this fraction, and this genus became numerous towards the end of this experiment reaching cell numbers between 3 and $4 \times 10^5$ cell ml$^{-1}$ (Paulino et al., 2007). The development of primary production in this fraction mirrors the *Synechococcus* abundance, which increased markedly during the last week of the experiment. Cell numbers were higher in 1× CO$_2$ than in the two treatments with elevated levels of CO$_2$ (Paulino et al., 2007). A similar trend was observed towards the end of the
experiment in 2001 where the highest cell numbers of *Synechococcus* were observed in the treatment with lowest CO$_2$ (190 µatm) (Rochelle-Newall et al., 2004). Primary production in the 1–5 µm fraction was also highest in the 1× CO$_2$ treatment, but only on the very last day of the experiment. Analyses from FCM also showed that other picocyaotrophs (1–2 µm cells) indicated the opposite response to elevated CO$_2$, with the highest cell numbers in the 3× CO$_2$. These opposing effects will reduce the total effect in this size fraction even though *Synechococcus* numbers were 5 fold higher than other small picoeucaryotes during the last days of the experiment.

5 Concluding remarks

With our techniques, we did not observe significant effects of elevated CO$_2$ on daily primary production during the PeECE experiments. The trend found in cumulative $^{14}$C-based particulate primary production was, however, consistent with the over-consumption of DIC at high CO$_2$ reported by Riebesell et al. (2007) and Bellerby et al. (2007). Splitting the production into size-fractions gave more information, and when the dynamics of fractionated production was compared to species composition, the results indicate that in some groups or species primary production may be stimulated at elevated CO$_2$ levels. The 5–10 µm fraction showed a tendency towards increasing production with increasing CO$_2$ concentration, but as the production in this fraction was low, it did not contribute much to total production. Also, if two group of organism respond opposite to elevated CO$_2$ the effect on primary production will be reduced or eliminated, as suspected for *Synechococcus* and other picocyaotrophs the 1-5µm fraction.

The trend in $^{14}$C-based particulate primary production was only visible when inorganic nutrients were depleted. This could be due to the fact that the diatoms dominating in the first part were not affected by pCO$_2$ or that pCO$_2$ only affects primary production in nutrient stressed phytoplankton. Nutrient limitation may affect phytoplankton as well as bacteria, and the net outcome on community production may therefore well be differ-
ent in different experiments where the conditions controlling bacterial carbon demand may differ.

Experiments with duration of a few weeks do not include all possibilities in a potentially adaptive plankton community, extrapolation to long time scales should therefore be done with caution.

Acknowledgements. The staff at the Marine Biological Station, University of Bergen, in particular T. Sørlie and A. Aadnesen, and the Bergen Marine Research infrastructure (RI) are gratefully acknowledged for support in mesocosm logistics. C. Neill for providing TCO$_2$ data for primary production calculations, and Geophysical Institute, University of Bergen for providing data for global radiation. This study was supported by EU-TMR contract no HPRI-CT-2002-00181, and NFR project no. 158936/I10 Biodiversity patterns: Blooms versus stable coexistence in the lower part of the marine pelagic food web.

References


Primary production at elevated CO$_2$ concentrations

J. K. Egge et al.


Primary production at elevated CO₂ concentrations

J. K. Egge et al.


Table 1. Experimental period and CO₂ and nutrient manipulation of PeECE I, II and III carried out in 2001, 2003 and 2003, respectively. Temperature range and average global radiation (Geophysical institute, University of Bergen) is given.

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2003</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental period</td>
<td>31.05–25.06.</td>
<td>04.05–24.05.</td>
<td>16.05.–10.06.</td>
</tr>
<tr>
<td>CO₂ concentration</td>
<td>180, 370, 700 µatm</td>
<td>190, 370, 700 µatm</td>
<td>375, 750, 1150 µatm</td>
</tr>
<tr>
<td>Initial nutrient supply</td>
<td>17 µM N, 0.5 µM P</td>
<td>12 µM Si</td>
<td>15 µM N, 0.6 µM P</td>
</tr>
<tr>
<td>Temperature range</td>
<td>10–13°C</td>
<td>8–10°C</td>
<td>9–11.5°C</td>
</tr>
<tr>
<td>Average global radiation</td>
<td>17.46 MJ m⁻²</td>
<td>11.45 MJ m⁻²</td>
<td>12.81 MJ m⁻²</td>
</tr>
</tbody>
</table>
Table 2. Average primary production ($\mu$mol C L$^{-1}$ h$^{-1}$), gross production ($\mu$mol O$_2$ L$^{-1}$ d$^{-1}$) net production ($\mu$mol O$_2$ L$^{-1}$ d$^{-1}$) and net community respiration ($\mu$mol O$_2$ L$^{-1}$ d$^{-1}$) for the PeECE II experiment, 2003.

<table>
<thead>
<tr>
<th>CO$_2$ treatment</th>
<th>Primary production</th>
<th>Gross production</th>
<th>Net production</th>
<th>Community respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Past (190 ppmV)</td>
<td>0.28</td>
<td>18.37</td>
<td>-1.33</td>
<td>5.42</td>
</tr>
<tr>
<td>Past (190 ppmV)</td>
<td>0.40</td>
<td>16.02</td>
<td>1.95</td>
<td>4.26</td>
</tr>
<tr>
<td>Present (370 ppmV)</td>
<td>0.45</td>
<td>20.30</td>
<td>1.33</td>
<td>4.49</td>
</tr>
<tr>
<td>Present (370 ppmV)</td>
<td>0.52</td>
<td>20.77</td>
<td>3.94</td>
<td>3.89</td>
</tr>
<tr>
<td>Future (700 ppmV)</td>
<td>0.43</td>
<td>16.89</td>
<td>1.44</td>
<td>3.63</td>
</tr>
<tr>
<td>Future (700 ppmV)</td>
<td>0.44</td>
<td>18.41</td>
<td>0.49</td>
<td>4.05</td>
</tr>
</tbody>
</table>
Fig. 1. Development of $^{14}$C primary production through PeECE III; daily variation in $\mu$mol C l$^{-1}$ h$^{-1}$ (a) and cumulative production in $\mu$mol C l$^{-1}$ for the 24 days experimental period (b). Values are means ±SD of triplicate CO$_2$ treatments with 1× CO$_2$ (green), 2× CO$_2$ (grey) and 3× CO$_2$ (red).
Fig. 2. Gross- and Net community-production and Community respiration given as μmol O$_2$ L$^{-1}$ d$^{-1}$ based on oxygen incubations. Values are means ±SD of triplicate CO$_2$ treatments, colour code as in Fig. 1.
Fig. 3. Average $^{14}$C primary production ($\mu$mol C l$^{-1}$ d$^{-1}$), in the fractions 0.2–1, 1–5, 5–10 and >10 $\mu$m. One mesocosm of each CO$_2$ treatment (M2, M5 and M8) is investigated. Values are means±SD of triplicate incubations in each mesocosm, and colour code as in Fig. 1.
Fig. 4. Average $^{14}$C primary production ($\mu$mol C L$^{-1}$ d$^{-1}$) for the 24 days experimental period in the fractions 0.2–1, 1–5, 5–10, >10 $\mu$m and total. Colour code as in Fig. 1.