Response of primary production and calcification to changes of $p\text{CO}_2$ during experimental blooms of the coccolithophorid \textit{Emiliania huxleyi}

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\textit{Global Biogeochemical Cycles In press}

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Abstract

Primary production and calcification in response to different partial pressures of CO₂ (\(P\text{CO}_2\)) ('glacial', 'present' and 'year 2100' atmospheric CO₂ concentrations) were investigated during a mesocosm bloom dominated by the coccolithophorid *Emiliania huxleyi*. The day-to-day dynamics of net community production (NCP) and net community calcification (NCC) were assessed during the bloom development and decline by monitoring dissolved inorganic carbon (DIC) and total alkalinity (TA), together with oxygen production and \(^{14}\text{C}\) incorporation. When comparing 'year 2100' with 'glacial' \(P\text{CO}_2\) conditions we observed: 1) no conspicuous change of net community productivity (NCP); 2) a delay in the onset of calcification by 24 to 48 h, reducing the duration of the calcifying phase in the course of the bloom; 3) a 40% decrease of NCC; 4) enhanced loss of organic carbon from the water column. These results suggest a shift in the ratio of organic carbon to calcium carbonate production and vertical flux with rising atmospheric \(P\text{CO}_2\).

1. Introduction

In the context of rising \(P\text{CO}_2\) in the atmosphere and concomitant increase of \(p\text{CO}_2\) in the oceans, the response of marine organisms and ecosystems to elevated \(p\text{CO}_2\) has received little attention compared to terrestrial plants and ecosystems. This is partly due to the fact that photosynthesis by marine phototrophs is, generally, not considered to be carbon-limited due to the large pool of DIC in seawater, mostly in the form of bicarbonate. Indeed, if some studies have shown that marine autotrophic communities are often insensitive to \(p\text{CO}_2\) changes, several studies have shown that some seagrasses [Zimmerman et al., 1997], macroalgae [Gao et al., 1993], diatom [Riebesell et al., 1993; Chen and Durbin, 1994], coccolithophorid [Nimer and Merrett, 1993; Hiwatari et al., 1995; Riebesell et al., 2000; Zondervan et al., 2001] and cyanobacteria [Qiu and Gao, 2002] species exhibit higher rates of
photosynthesis under CO₂ enrichment. In their review of the effects of CO₂ concentration on marine plankton, Wolf-Gladrow et al. [1999] pointed out the apparent discrepancy between ample CO₂ supply from the bulk medium combined with the capacity for direct utilization of HCO₃⁻ in many marine phytoplankton on the one hand and the sensitivity of both phytoplankton growth rate and elemental composition to CO₂ concentration on the other. They argue that one of the factors to be considered when trying to resolve this discrepancy is the low affinity for CO₂ of the primary carboxylating enzyme RuBisCO and that the sensitivity of marine phytoplankton to CO₂ is best viewed as a co-limitation of CO₂ in concert with light availability and other limiting factors such as nutrients. Irrespective of which mechanism is responsible for the sensitivity of some phytoplankton species to pCO₂, the topic has received comparatively little attention considering its potential importance for carbon export and sequestration and its potential negative feedback to rising atmospheric CO₂.

The response of calcifying organisms and communities to elevated pCO₂ appears to be more straightforward. Biogenic precipitation of calcium carbonate is generally described by the following equation:

\[ \text{Ca}^{2+} + 2 \text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O} \]  

(1)

Thus, calcification acts as a source of CO₂ to the water column and counteracts the photosynthetic uptake of CO₂. The net effect of these two antagonistic processes on CO₂ is dependant on the ratio of NCC to NCP (C:P). Moreover, the release of CO₂ by NCC is modulated by the buffering capacity of the carbonate system [Frankignoulle, 1994]. Any variation of the C:P ratio will have significant implications on biogeochemical fluxes, and particularly on the sign and strength of the overall feedback of coccolithophorids to rising atmospheric PCO₂. Furthermore, changes of the C:P ratio affect the density and sinking rate of coccolithophorid cells and debris and, therefore, the magnitude of carbon export. Hence
Buitenhuis et al. [2001] suggested that a decrease of the C:P ratio induces a decrease of carbon export and affects the overall uptake of CO₂ by coccolithophorids.

Generally, the rate of calcification decreases with rising $p$CO₂ and diminishing CaCO₃ saturation state ($Ω$). This response is nowadays well documented for reef-building corals and coralline algae [Gattuso et al., 1999]. Mesocosm experiments have recently established the link between the decrease in calcification rate of coral reefs with rising CO₂, and the concomitant drop of aragonite $Ω$ [Langdon et al., 2000; Leclercq et al., 2002; Langdon et al., 2003]. In the same way, Bijma et al. [1999] observed that the decrease of $Ω$ causes a decrease in the calcification rate by foraminifera. Riebesell et al. [2000] and Zondervan et al. [2001; 2002] observed a similar response with coccolithophorids, which may be the largest contributors to marine pelagic calcification. These authors showed, in batch cultures of *Emiliania huxleyi* and *Gephyrocapsa oceanica*, that the production of particulate organic carbon (POC) increases with increasing CO₂ and is additionally depending on the total irradiance and the photoperiod length. Also, the production of particulate inorganic carbon (PIC) decreases, leading to the decrease of the C:P ratio. It therefore seems that depression of calcification or/and C:P ratio at elevated $p$CO₂ is a general feature among marine calcifying organisms.

Previous studies of the response of coccolithophorids to increasing $p$CO₂ were most often made in batch cultures by manipulating the carbonate system through the addition of acid or base [Nimer et al., 1993; Buitenhuis et al., 1999; Zondervan et al., 2001;2002]. The aim of identifying the influence of increasing CO₂ on calcification has driven these authors to eliminate environmental interactions and to grow cultures under optimal conditions which do not perfectly reflect *in situ* conditions. Furthermore, the manipulation of the carbonate system through the addition of acid and base also alters TA, whereas oceanic TA will not change significantly in the next decades. For instance, an increase of $p$CO₂ induced by acid addition
leads to a decrease in calcite and aragonite saturation states 20% higher and an increase in HCO$_3^-$ concentration 60% lower than would be observed for the same increase of $p$CO$_2$ induced by CO$_2$ addition. However, calcification appears to be controlled by $\Omega$ than by $p$CO$_2$ per se [Langdon et al., 2000; Leclercq et al., 2002]. Moreover, HCO$_3^-$ was suggested to be the substrate for calcification of *E. huxleyi* [Buitenhuis et al., 1999]. Therefore, the control of the carbonate system using gas addition may be more suited to reproduce the future changes of carbonate chemistry than acid/base addition techniques.

Building on the pioneering batch culture experiments, the aim of the present study was to follow the development and decline of a bloom of a natural plankton community dominated by the coccolithophorid *E. huxleyi* exposed to various $p$CO$_2$ under more natural conditions in large seawater volumes. This allowed us to investigate the $p$CO$_2$ related effects at the community level and to examine their impacts on the dynamics of the bloom. This was achieved by employing large seawater enclosures containing natural assemblages of bacterioplankton, phytoplankton and micro-zooplankton kept under ambient light and temperature conditions and subjected to atmospheric CO$_2$ concentrations simulating the 'glacial', 'present' and 'year 2100' atmospheric $PCO_2$ conditions (respectively 180, 370 and 700 ppmV). Inorganic nutrient concentrations and the carbonate chemistry were adjusted prior to the onset of the bloom, and were allowed to evolve without further regulation as would occur in the mixed surface layer of a stratified water column during the course of a bloom. The day-to-day response of inorganic and organic carbon production by the enclosed communities was assessed using O$_2$ production and $^{14}$C uptake during incubations together with the monitoring of daily changes in TA and DIC.
2. **Material and methods**

*Overall description of the experiment*

The experiment was carried out between 31 May and 25 June 2001 at the Marine Biological Field Station (Raunefjorden, 60.3° N, 5.2° E) of the University of Bergen, Norway. Nine enclosures made of polyethylene bags of 2 m diameter and volume of 11 m³ were used. The bags were secured to the sides of a raft equipped with a small laboratory. Each bag was filled with unfiltered nutrient poor (post-spring bloom) water pumped at a depth of 2 m in the fjord on 1 June. The following provides a short description of the experimental set up, for more details we refer to a companion paper by Engel et al. [2004a].

The tops of the mesocosms were covered with ethylene tetrafluoroethylene films (95% transmission for photosynthetically active radiation) forming a tent over more than 90% of the mesocosm surface area. The atmospheric $P_{CO_2}$ underneath the tents was controlled by injecting a continuous stream of gases with a known $CO_2$ content. Three levels of $pCO_2$ (180, 370 and 700 ppmV) were used with 3 replicates each; they will be referred to as 'glacial' (Mesocosm (M)7, M8 and M9), 'present' (M4, M5 and M6) and 'year 2100' (according to the Intergovernmental Panel on Climate Change “business as usual” scenario IS92a; M1, M2 and M3). In contrast to similar laboratory experiments carried out on phytoplankton cultures, in which seawater $pCO_2$ was maintained at constant values throughout the experiment, in this study seawater $CO_2$ was manipulated only at the start of the experiment before initiation of the bloom. This was achieved by bubbling $CO_2$-free, ambient or $CO_2$-enriched air at the bottom of the mesocosms until 6 June (hereafter referred to as 'day 0'). From day 0 until the end of the experiment, the carbonate system was allowed to evolve naturally while maintaining only the atmosphere underneath the tents covering each mesocosm at 'glacial', 'present' and 'year 2100' atmospheric $PCO_2$ conditions. The seawater $pCO_2$ values reached on 6 June are given in Table 1. To promote the development of the coccolithophorid bloom,
nitrate and phosphate were added to each mesocosm on 'day 0' at initial concentrations of about 17 µmol L\textsuperscript{-1} NO\textsubscript{3}\textsuperscript{-} and 0.5 µmol L\textsuperscript{-1} PO\textsubscript{4}\textsuperscript{3-}. The water enclosed in the mesocosms was gently homogenized using an airlift system consisting of a plastic tube in which a gas stream, having a PCO\textsubscript{2} identical to that of the atmosphere confined above the mesocosm, produced an upward motion of water in the tube. The flow rate was very low in order to avoid significant gas exchange between the air stream and seawater.

Each mesocosm was sampled every day between 9:00 and 11:00 – while daylight last from around 4:30 to 23:00 - to measure the abundances of various phytoplankton groups, bacteria and viruses, the concentration of nutrients, as well as parameters of the carbonate system (TA and pCO\textsubscript{2}). Elapsed time is referred to as "d\textsubscript{x}" where x is the number of days since "d\textsubscript{0}".

**Seawater partial pressure of CO\textsubscript{2} and temperature**

Measurements of pCO\textsubscript{2} were carried out using an equilibrator coupled to an infra-red gas analyzer (IRGA, Li-Cor® 6262). Seawater flows into the equilibrator (3 L min\textsuperscript{-1}) from the top and a closed air loop (3 L min\textsuperscript{-1}) ensured circulation through the equilibrator (from the bottom to the top), a desiccant (Drierite®) and the IRGA [Frankignoulle et al., 2001]. The barometric pressure inside the equilibrator was kept equal to atmospheric pressure. Both the barometric pressure and temperature were monitored in the air loop. The IRGA was calibrated daily with air standards with nominal mixing ratios of 0, 350, 800 ± 0.3 ppmV of CO\textsubscript{2} supplied by Air Liquide Belgium® and Hydrogas®. The equilibration time of the system was less than 3 min [Frankignoulle et al., 2001]. The system was kept running twice this time before recording and averaging the values given by the IRGA and temperature sensors over a 30 s period.

*In situ* and equilibrator temperatures were measured simultaneously using Li-Cor® sensors. Differences in temperature were less than 0.5°C. TA measurements were made with each measurement of pCO\textsubscript{2} and used to temperature correct pCO\textsubscript{2} using dissociation constants of Roy et al. [1993]. The uncertainty of pCO\textsubscript{2} is estimated to ±3 ppmV.
Total alkalinity, dissolved inorganic carbon and salinity

TA was measured using the classical Gran potentiometric method [Gran, 1952] on 100 ml GF/C filtered samples. The reproducibility of measurements was ±3 µmol kg\(^{-1}\).

Dissolved inorganic carbon (DIC) was calculated from pCO\(_2\) and TA. CO\(_2\) speciation was calculated using the CO2SYS Package [Lewis and Wallace, 1998], the CO\(_2\) acidity constants of Roy et al. [1993], the CO\(_2\) solubility coefficient of Weiss [1974] and the borate acidity constant of Dickson [1990]. The total borate molality was calculated using the Uppström [1974] boron to salinity ratio. The uncertainty on the DIC computation is estimated to ±5 µmol kg\(^{-1}\).

TA was corrected for the drawdown of nitrate and phosphate associated with phytoplankton nutrient utilization. According to the classical Redfield-Ketchum-Richards reaction of biosynthesis [Redfield et al., 1963; Richards, 1965]

\[
106\text{CO}_2 + 16\text{NO}_3^- + \text{H}_2\text{PO}_4^- + 17\text{H}^+ + 122\text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O})_106(\text{NH}_3)_16\text{H}_3\text{PO}_4^- + 138\text{O}_2\]

one mole of H\(^+\) is consumed for each mole of NO\(_3^-\) or H\(_2\text{PO}_4^-\) consumed through biosynthesis, increasing TA by one mole. TA corrected for primary production (TA\(_{\text{corrected}}\)) can therefore be computed from measured TA (TA\(_{\text{measured}}\)) using the relation:

\[
\text{TA}_{\text{corrected}} = \text{TA}_{\text{measured}} - \Delta\text{NO}_3^- - \Delta\text{H}_2\text{PO}_4^- \quad (3)
\]

where \(\Delta\text{NO}_3^-\) and \(\Delta\text{H}_2\text{PO}_4^-\) denote the decreases of \text{NO}_3^- and \text{H}_2\text{PO}_4^- since the reference day \((d_1)\). Correction for nutrient uptake accounts for less than 13 % of the TA changes.

DIC changes were corrected daily for air-sea exchange of CO\(_2\) using the air-sea gradient of CO\(_2\), the volume of the bags, and assuming that water in the bags was well homogenized and zero wind under the tents. Air-sea exchange of CO\(_2\) from the enclosed atmosphere to the water was computed using the algorithm for stagnant boundary layer thickness from Smith [1985], molecular diffusivity from Jähne et al. [1987] and chemical enhancement model from
*Hoover and Berkshire* [1969]. The formulation given by *Smith* [1985] was established using the stagnant film model and measurements from wind tunnels at low wind speed and corresponds better to our experimental set-up than other relations derived from *in-situ* measurements affected by additional turbulent processes such as currents and rain. Correction for air-sea exchange accounted for less than 5% of changes in DIC.

Normalized TA and DIC at a constant salinity (S=31) are denoted as TA$_{31}$ and DIC$_{31}$. Salinity were measured using a conductivity-temperature-pressure sensor (CTD SAIV A/S, model SD204).

**Flow cytometry sample processing and analysis**

Analyses were performed with a FACSCalibur flow cytometer (Becton Dickinson®) equipped with an air-cooled laser providing 15 mW at 488 nm and with standard filter set-up. The algae were analyzed from fresh samples at a high flow rate (~70 µl min$^{-1}$) with the addition of 1 µm 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. Enumeration of viruses was carried out on samples fixed with glutaraldehyde (0.5% final concentration) and frozen (in liquid nitrogen). Once thawed at 37°C, samples were diluted 10 to 100 times in Tris-EDTA (pH=8) buffer and heated for 10 min at 80°C after staining with the DNA dye SYBR® Green I (1/20 000 final concentration, Molecular Probes®, [Marie et al., 1999]). Counts were performed at medium rate (~30 µl min$^{-1}$). Viruses were discriminated on the basis of their RALS vs. green DNA-dye fluorescence. Listmode files were analyzed using CYTOWIN ([Vaulot, 1989], available at http://www.sb-roscoff.fr/Phyto/cyto.html#cytowin) and WinMDI (version 2.7, Trotter, available at http://www.bio.umass.edu/mcebfaas/flowcat.html#winmdi).
**Primary production from \(^{14}\)C incubations**

Subsurface seawater for incubation experiments was sampled in M1, M4 and M9 before sunrise. All water samples were pre-sieved through a 200 µm nylon mesh to remove large zooplankton. All incubations were carried out in 60 ml flasks inoculated with H\(^{14}\)CO\(_3\) (~20 µCi per 500 ml) and incubated *in situ* for 24 h at a depth of 1.5 m in the water of the fjord adjacent to the mesocosms. Concomitant incubations were made in dark bottles. After incubation, samples were filtered on Whatman® GF/F filters under gentle vacuum. Duplicate filters were collected for each sample incubated and rinsed with 0.2m-filtered seawater in order to remove excess DI\(^{14}\)C. One set of filters was treated with 100 µl HCl (0.01 N) to eliminate the radiocarbon incorporated into CaCO\(_3\). Primary production was estimated from \(^{14}\)C measurements after exposure of the filters to HCl while calcification was estimated by subtracting primary production from the total \(^{14}\)C collected on untreated filters.

**Net community production and respiration (O\(_2\) technique)**

Samples were collected in mesocosms M1, M2, M4, M5, M8 and M9 before sunrise and immediately distributed into 60 ml BOD bottles (overflowing > 150 ml). For each sampled mesocosm, four bottles were fixed immediately with Winkler reagents, three sets of 3 bottles were incubated *in situ* nearby the mesocosms at 0.5, 1.5 and 4 m, and 4 bottles were incubated in the laboratory in darkness at *in situ* temperature. The bottles incubated *in situ* were fixed at sunset and duration of the incubations was about 18 h (from about 05:10 until 23:00). The dark bottles incubated in the laboratory were fixed the next day between 08:00 and 10:00 and the duration of the incubations was 27 to 29 h.

The concentration of dissolved oxygen was determined using an automated Winkler titration technique with a potentiometric end-point detection [Anderson et al., 1992] using an Orion® 9778-SC electrode. Reagents and standardizations were similar to those described by Knap et al. [1996].
Net community production and net community calcification from DIC and TA changes

NCC can be estimated from the time course of TA corrected according to:

\[ \text{NCC} = -0.5 \times \frac{\Delta \text{TA corrected}}{\Delta t} \]  \hspace{1cm} (4)

where \( \Delta t \) denotes elapsed time.

Similarly, net community production (NCP\textsubscript{DIC}) of organic carbon can be computed from changes in DIC and TA according to:

\[ \text{NCP\textsubscript{DIC}} = -\frac{\Delta \text{DIC}}{\Delta t} + 0.5 \times \frac{\Delta \text{TA corrected}}{\Delta t} \]  \hspace{1cm} (5)

3. Results

3.1 *E. huxleyi* abundance, \( p\text{CO}_2 \), DIC\textsubscript{31} and TA\textsubscript{31}

A succession of distinct phytoplankton assemblages took place in the course of the experiment. The assemblage was first dominated by *Synechococcus* sp. and nanoflagellates [Jacquet, unpublished], and subsequently by *E. huxleyi* [Engel et al., 2004a]. From d\textsubscript{0} to d\textsubscript{7}, both *Synechococcus* sp. and nanoflagellate abundances increased to reach a maximum between d\textsubscript{5} and d\textsubscript{7}, depending on the mesocosm, with abundances ranging from 10 to 15x10\textsuperscript{3} cells ml\textsuperscript{-1} and 30 to 170x10\textsuperscript{3} cells ml\textsuperscript{-1} for *Synechococcus* sp. and nanoflagellates, respectively [Jacquet, unpublished]. This maximum cell abundance corresponded to chlorophyll-a (Chl a) concentrations ranging from 0.3 to 0.5 \( \mu \text{g L}^{-1} \) [Engel et al., 2004a]. The abundance of *Synechococcus* sp. and nanoflagellates subsequently decreased sharply to less than 3.2 and 7.0x10\textsuperscript{3} cell ml\textsuperscript{-1} respectively on d\textsubscript{10}, while *E. huxleyi* abundance remained below 2.7x10\textsuperscript{3} cell ml\textsuperscript{-1} from d\textsubscript{0} to d\textsubscript{10} (Fig. 1). During this coccolithophorid pre-bloom phase, small decreases of DIC\textsubscript{31} and \( p\text{CO}_2 \) were observed while TA\textsubscript{31} remained unchanged with similar values in all mesocosms.
On d_{10}, the decreases of DIC_{31} and pCO_{2} were larger and concomitant with the sharp increase of the abundance of *E. huxleyi*. The minimum pCO_{2} was observed on d_{16}. The magnitude of changes in pCO_{2} exhibited larges differences depending on the PCO_{2} conditions from d_{0} to d_{14} ranging from 386 ± 16 ppmV in the 'year 2100' mesocosms to 187 ± 13 ppmV in the 'present' mesocosms and 61 ± 2 ppmV in the 'glacial' mesocosms. However, until d_{14}, the differences in DIC_{31} patterns between mesocosms were not significant. From d_{12} onwards, TA_{31} decreased sharply in all mesocosms indicating the onset of calcification by coccolithophorids. Decreases of TA_{31} ranged from 30 to 95 µmol kg\(^{-1}\) between d_{13} and d_{14}.

Between d_{14} and d_{16}, the overall patterns of most parameters indicate the transition towards the decline of the bloom. On d_{14}, nutrients (NO\(_{3}^{-}\) and PO\(_{4}^{3-}\)) were exhausted in all mesocosms [Engel et al., 2004a]. From d_{16} onwards, pCO_{2} remained constant or increased slightly whereas DIC_{31} continued to decrease in most mesocosms.

DIC_{31} evolutions became confusing after d_{14}. DIC_{31} reached rapidly a plateau on d_{14} in M5, M6 and M8 ('glacial' mesocosm) whereas it continued to decrease significantly in M1 and M3, M4, M7 and M9 ('year 2100' mesocosms). Concomitantly, TA_{31} also began to differ greatly between mesocosms. In most cases, TA_{31} reached a plateau after a large and continuous drop. However, it should be noted that TA_{31} decreased at a high rate until the end of the experiment in M1 and M3 ('year 2100' conditions).

Interestingly, within these two mesocosms viruses specific to *E. huxleyi* were either absent or present in low abundance. The collapse of nutrient-induced *E. huxleyi* blooms as we observed in 7 of the 9 mesocosms, have also been commonly reported in similar experiments. It has been attributed to viral lysis by a virus identified as EhV [Bratbak et al., 1996; Jacquet et al., 2002; Castberg et al., 2002] which belongs to the genus *Coccolithovirus* proposed by Schroeder et al. [2002] within the family of algal viruses *Phycodnaviridae*. 
3.2 Net community production of organic carbon

NCP (Fig. 2a) assessed from oxygen incubations (NCP$_{O_2}$) and NCP$_{O_2}$ normalized against Chl a concentrations (Fig. 2c), i.e. the net community productivity (NCP$_{O_2}$), exhibited similar patterns for all the mesocosms during the pre-bloom period (d$_0$ to d$_{10}$) and the peak of the bloom (d$_{10}$ to d$_{15}$). They increased sharply from d$_7$ onwards to reach maximum values on d$_{12}$ coinciding with the marked increase of abundance of *E. huxleyi* (Fig. 1). From d$_{16}$ onwards, mesocosms showed different NCP$_{O_2}$ patterns which do not seem to be related to $PCO_2$ conditions.

Net primary production (Fig. 2b) estimated by the uptake of $^{14}C$ (NPP$_{_{14}C}$) exhibited similar pattern to those of NCP$_{O_2}$. From d$_{14}$ onwards, NPP$_{_{14}C}$ appears to be lower in M1 ('year 2100') compared to M4 ('present') and M9 ('glacial'). However, similarly to NCP$_{O_2}$, NPP$_{_{14}C}$ (Fig. 2d) was similar in all the mesocosms from d$_0$ onwards.

Community respiration (Fig. 2e) assessed from oxygen incubations decreased during the pre-bloom period then increased during the peak of the bloom to reach a maximum value ranging from 40 to 75 mmol O$_2$ m$^{-2}$ d$^{-1}$ on d$_{14}$. It subsequently remained between 30 and 60 mmol O$_2$ m$^{-2}$ d$^{-1}$. No obvious differences of community respiration were found in the various $PCO_2$ conditions.

3.3 Ca$^{14}$CO$_3$ production rate

Calcification by *E. huxleyi* estimated from $^{14}C$ in situ incubations started on d$_0$ in M4 and M9 and on d$_{11}$ in M1 (Fig. 3a), suggesting that the onset of calcification was delayed under 'year 2100' $PCO_2$ conditions. The highest values were reached on d$_{13}$ in M4 and M9 (16 and 20 µmol CaCO$_3$ kg$^{-1}$ d$^{-1}$, respectively). Calcification was higher in M9 than in M4, while it remained low in M1. This is consistent with the larger decreases of TA$_{31}$ observed in M4 and M9 compared to M1. During the peak of the bloom, M1 exhibited lower rates of calcification
normalized to Chl a than M4 and M9 (Fig. 3b), suggesting a lower competence of *E. huxleyi* to calcify under 'year 2100' *PCO₂* conditions, while net primary productivity seemed unaffected (see 3.2).

**3.4 Molar respiration ratio**

We compared net community production values obtained from O₂ incubations with values estimated from DIC and TA changes by plotting NCP<sub>O₂</sub> versus NCP<sub>DIC</sub> (Fig. 4). Model II linear regression gives a slope of 1.45 ± 0.12 and a correlation coefficient of 0.68 (p<0.0001, n=54, 4). The slope of the linear regression corresponds to the so-called molar respiration ratio (R.R.). Indeed, if we consider the respiration as the reverse of the Redfield-Ketchum-Richard equation (Eq. (2)), the complete oxic degradation of phytoplankton theoretically requires 138 moles of dissolved O₂/106 moles of organic carbon (C) leading to a molar respiration ratio (O₂/C) of 1.30. The R.R. obtained in the present study agrees well with the estimates of Hedges et al. [2002] based on phytoplankton elemental composition using nuclear magnetic resonance which ranges from 1.41 to 1.47, depending on the geographic area considered. The consistency of these results validates the use of NCP<sub>DIC</sub> derived from DIC and TA and enables therefore a comprehensive day-to-day comparison of NCP and NCC.

**3.5 Timing of organic and inorganic carbon production**

NCP<sub>DIC</sub> as a function of NCC are shown in the figure 4 where each point corresponds to a daily measurement. Connecting day-to-day estimates provides an overview of the temporal evolution of NCP<sub>DIC</sub> relative to NCC. During the pre-bloom period NCP<sub>DIC</sub> increased steadily (upward displacement along the Y-axis), firstly due to the rising abundances of *Synechococcus* sp. and nano-flagellates, and subsequently to the onset of the bloom of *E. huxleyi*. The increase of NCP<sub>DIC</sub> was enhanced from d<sub>10</sub> onwards in all the conditions,
concomitantly with the beginning of the peak of the bloom period (Fig. 1) and leads to maximum values of NCP\textsubscript{DIC} on d\textsubscript{12} and d\textsubscript{13}. By d\textsubscript{15} the nutrients were exhausted, NCP\textsubscript{DIC} decreased markedly. NCC increases (displacement to the right along the X-axis) in a second phase, when the coccolithophorid bloom is well underway, proceeded from d\textsubscript{11} to d\textsubscript{19} and remained at a high level while NCP\textsubscript{DIC} decreased dramatically, which is consistent with observations in cultures of *E. huxleyi* [Dong et al., 1993]. The third phase was the collapse of the bloom with a dramatic decrease of both NCP\textsubscript{DIC} and NCC. This phase corresponds to the period during which the coccolithovirus abundance passes over a threshold value, estimated to be around $5 \times 10^6$ part ml\textsuperscript{-1} (dashed lines in Fig. 5). At the end of the experiment, NCP\textsubscript{DIC} and NCC show negative values due to elevated respiration and CaCO\textsubscript{3} dissolution, as suggested by Milliman et al. [1999]. This is consistent with the increase of both CR (Fig. 2c) and bacterial abundance determined by flow cytometry (Jacquet, unpublished).

Under 'glacial' conditions (M7, M8 and M9), NCC started at the onset of the peak of the bloom (d\textsubscript{10}–d\textsubscript{11}) and then increased steadily in parallel to NCP\textsubscript{DIC} leading to an almost simultaneous maximum (only 1 d time lag). In contrast, in the 'year 2100' conditions (M1, M2 and M3), NCC began later (d\textsubscript{12} to d\textsubscript{13}), and suddenly, while NCP\textsubscript{DIC} had already reached its maximum. NCC subsequently increased very rapidly while NCP\textsubscript{DIC} was decreasing. The 'present' conditions exhibited an intermediate behavior between the 'year 2100' and 'glacial' conditions: the maximum level of NCP\textsubscript{DIC} was reached when NCC was already substantial but had not reached its maximum value.

Thus, if the overall pattern of NCP\textsubscript{DIC} prior to viral lysis is similar for all the conditions, the onset of NCC occurs sooner in the 'glacial' and 'present' conditions than in the 'year 2100' conditions. This is consistent with the calcification rates measured with the $^{14}$C in situ incubations. Furthermore under 'glacial' conditions NCC increases steadily from the very
beginning of the peak of the bloom in parallel to the exponential rise of \( \text{NCP}_{\text{DIC}} \), while in the 'year 2100' \( \text{NCC} \) occurs suddenly at the maximum of \( \text{NCP}_{\text{DIC}} \).

3.6 Mean community production and calcification rates

Changes in the standings stocks of total organic carbon (TOC) and PIC, associated with the bloom of \( E. \ huxleyi \), were assessed during the peak of the bloom (\( d_{10} - d_{15} \)) by integrating, respectively, daily \( \text{NCP}_{\text{DIC}} \) and \( \text{NCC} \) over time (Fig. 6). TOC standing stocks exhibit an almost linear evolution prior to the exhaustion of \( \text{PO}_4^{3-} \) and \( \text{NO}_3^- \) allowing the computation of mean TOC production rates. During this period bacterial abundance was low and no phytoplankton species other than \( E. \ huxleyi \) were present in significant numbers (Jacquet, unpublished) so TOC changes are mainly due to primary production by coccolithophorids. Likewise, since changes of PIC are linear from \( d_{11} \) until the coccolithovirus passed a threshold value of about \( 5 \times 10^6 \) part. ml\(^{-1} \), it is possible to compute the mean PIC production rates (Fig. 7) prior to viral lysis.

TOC increased steadily from \( d_{10} \) to \( d_{15} \) in all the mesocosms at rates ranging from 21.4 to 25.9 \( \mu\text{molC kg}\^{-1} \text{d}^{-1} \) (Fig. 6). TOC production rates are similar under the three \( \text{PCO}_2 \) conditions (Fig. 7). In contrast, the mean rate of PIC production (Fig. 7) was conspicuously lower in the 'year 2100' conditions (10.3 \( \pm \) 3.2 \( \mu\text{mol C kg}^{-1} \text{d}^{-1} \)) than in the 'present' (17.9 \( \pm \) 4.4 \( \mu\text{molC kg}^{-1} \text{d}^{-1} \)) and in the 'glacial' (17.9 \( \pm \) 2.5 \( \mu\text{mol C kg}^{-1} \text{d}^{-1} \)) conditions. The mean PIC/TOC production ratio (C:P ratio) of \( E. \ huxleyi \) is similar in the 'glacial' and 'present' conditions between 0.73 and 0.78, but fell to 0.45 in the 'year 2100' conditions.

3.7 Carbon losses

Carbon losses were calculated as the difference between TOC produced by photosynthesis (estimated from the integration of \( \text{NCP}_{\text{DIC}} \)) and the accumulation of POC in the water column (data from [Engel et al., 2004a]). Carbon losses during the peak of the bloom were
conspicuously higher in the 'year 2100' (48 ± 10 µmol C kg$^{-1}$ d$^{-1}$) than under the 'glacial' conditions (25 ± 16 µmol C kg$^{-1}$ d$^{-1}$) while TOC production remained similar (Fig. 7). If integrated over the d$_1$-d$_{15}$ period, this trend is enhanced, carbon losses being more than twice as high in the 'year 2100' (74 ± 14, µmol C kg$^{-1}$ d$^{-1}$) than under 'glacial' conditions (34 ± 16 µmol C kg$^{-1}$ d$^{-1}$)(data not shown).

4. Discussion

4.1 $p$CO$_2$ changes and buffering effect of the carbonate system

Until d$_{14}$, changes in DIC$_{31}$ followed the same trend and the same magnitude in all mesocosms while changes in $p$CO$_2$ were conspicuously different between the three conditions right from the beginning of the experiment (Fig. 1). Indeed, the magnitude of changes in $p$CO$_2$ from d$_0$ to d$_{14}$ were 6 times higher in the 'year 2100' conditions than in the 'glacial' conditions, while until d$_{14}$ differences in DIC$_{31}$ patterns between mesocosms were not significant since NCP$_{DIC}$ was roughly similar under the three PCO$_2$ conditions and NCC was negligible. Therefore, differences in the magnitudes of $p$CO$_2$ changes with regard to the PCO$_2$ conditions are not ascribable to biological processes and must be explained taking into account the buffering effect of the carbonate system.

The chemical buffer factor ($\beta=\Delta$CO$_2$/ΔDIC) describes the change in $p$CO$_2$ relative to the DIC change induced by an input/output of dissolved CO$_2$. It results from equilibrium dissociation reactions of the carbonate system and is a function of several physico-chemical conditions, among them on the $p$CO$_2$ itself. The evolution of the buffer factor, calculated for the initial conditions of the experiment, is given in Fig. 8. $\beta$ increases from 9.6 in the 'glacial' conditions to 16.6 in the 'year 2100' conditions. Subsequently, for the same removal of CO$_2$ by primary production, the consequent decrease of $p$CO$_2$ is 6 times higher in the 'year 2100'
condition ($\Delta p\text{CO}_2 = 116 \text{ ppmV}$ for $\Delta \text{DIC}= 20 \mu\text{mol kg}^{-1}$ and $\beta= 16.6$ at $p\text{CO}_2 = 700 \text{ ppmV}$, and $\text{DIC}= 2000 \mu\text{mol kg}^{-1}$) that under the 'glacial' conditions ($\Delta p\text{CO}_2 = 20 \text{ ppmV}$ for $\Delta \text{DIC}= 20 \mu\text{mol kg}^{-1}$ and $\beta= 9.6$ at $p\text{CO}_2 = 180 \text{ ppmV}$ and $\text{DIC}= 1740 \mu\text{mol kg}^{-1}$).

Thus, owing to thermodynamic interactions of the carbonate system, the change in $p\text{CO}_2$ is significantly higher in the 'year 2100' condition than in the others conditions, even though the process originally responsible of these $p\text{CO}_2$ changes, i.e. the uptake of CO$_2$ by photosynthesis, appears to be roughly similar under the three $p\text{CO}_2$ conditions. Hence, one can note that in the future CO$_2$ rich world, other processes - temperature oscillations, upwelling of CO$_2$ rich waters or precipitation of calcium carbonate [Frankignoule et al., 1994] among other - will also contribute to the thermodynamic enhancement of the amplitude of $p\text{CO}_2$ changes from daily to seasonal time-scales. In the same way, a higher spatial heterogeneity of $p\text{CO}_2$ can be expected from local to global scales.

At the end of the experiment, from d$_{16}$ onwards, $p\text{CO}_2$ remained constant or slightly increased whereas DIC$_{31}$ continued to decrease in most mesocosms. The decoupling of $p\text{CO}_2$ and DIC$_{31}$, associated to a decrease of TA$_{31}$ indicates the larger influence of NCC compared to NCP on $p\text{CO}_2$ as observed in natural coccolithophorid blooms and mesocosm experiments [Robertson et al., 1994; Purdie and Finch, 1994; Buitenhuis et al., 1996].

4.2 Primary production and carbon export

The effect of $p\text{CO}_2$ on growth, productivity and calcification of the coccolithophorid $E. huxleyi$ is still a matter of debate. In our study, no conspicuous changes in primary productivity (both NCP$_{\text{O}_2}$ and NPP$_{\text{C}_4}$) related to $p\text{CO}_2$ conditions were observed during the peak of the $E. huxleyi$ bloom. Differences in the NCP and NPP observed during the bloom decline should be ascribed to the occurrence or not of viral lysis rather than to some $p\text{CO}_2$ related effects.
On the other hand, evidence of a higher loss of particulate organic carbon from the water column under 'year 2100' conditions (Fig. 7) emerges surprisingly. Since DOC concentrations were similar under all $PCO_2$ conditions [Rochelle-Newall et al., 2004], while grazing was negligible, enhanced carbon losses observed under 'year 2100' conditions are likely due to a higher rate of particle settling. In fact, when normalized to $E. huxleyi$ cell concentration, TEP production was highest under 'year 2100' conditions [Engel et al., 2004a], consistent with previous observations of enhanced TEP formation under elevated CO$_2$ [Engel, 2002]. TEP formation is typically seen as the result of carbon overproduction leading to exudation of polysaccharides by algal cells [Passow, 2002]. Aggregation of dissolved polysaccharides through a cascade of aggregation processes from the molecular scale up to the size of fast settling particles can lead to an enhancement of particle aggregation and subsequent export [Engel et al., 2004b]. The possible involvement of carbon-rich TEP as a mediator of enhanced particle settling in the 'year 2100' mesocosms is further supported by higher C:N ratio of suspended particles under high CO$_2$ conditions [Engel et al., 2004a].

4.3 Overview of the response of the C:P ratio to rising CO$_2$

In contrast to NCP$_{DIC}$, NCC exhibited a conspicuous decrease with increasing $PCO_2$. These responses lead to a drastic decrease of the PIC/TOC production ratio (C:P ratio) under elevated $PCO_2$. Also, calcification is delayed in the 'year 2100' condition, acting to reduce the overall amount of CaCO$_3$ produced during the experiment.

During the pre-bloom period ($d_0$ to $d_{10}$) and the peak of the bloom ($d_{10}$ to $d_{13}$), NO$_3^-$ and PO$_4^{3-}$ decreased rapidly from about 10 $\mu$mol L$^{-1}$ and 0.7 $\mu$mol L$^{-1}$, respectively, on $d_{10}$ to below 0.4 $\mu$mol L$^{-1}$ and 0.3 $\mu$mol L$^{-1}$ on $d_{13}$ with a mean photon flux density (PFD) around 650 $\mu$mol m$^{-2}$ s$^{-1}$ for 18:6 light period and a light attenuation coefficient at the bottom of the mesocosm (4 m) of about 80%. Therefore, the mesocosms were not light limited but were in an intermediate state towards NO$_3^-$ and PO$_4^{3-}$ depletion. When attempting to reconcile the
results of the present study to experiments reported in the literature and to draw a comprehensive picture of the response of primary production and calcification of *E. huxleyi* to elevated pCO$_2$, it is worth noting that the present knowledge is based on a mosaic of experiments with different growth conditions and involving several *E. huxleyi* ecotypes which give in some cases very different results (Table 2). However it emerges that when irradiance is not drastically reduced [Zondervan et al., 2002], elevated pCO$_2$ appears to be detrimental to calcification [Riebesell et al., 2000; Zondervan et al., 2001; 2002; Sciandra et al., 2003; this study] and this generally leads to a decrease of the C:P ratio (Table 2.). Such response of calcification to changes in seawater carbonate chemistry has also been observed in corals and foraminifera [Gattuso et al., 1998; Wolf-Gladrow et al., 1999; Langdon et al., 2000; Leclercq et al., 2002; Langdon et al., 2003; Reynaud et al., 2003]. The cause of such a decrease of calcification by *E. huxleyi* in response to elevated pCO$_2$ remains unclear. If it can be almost intuitive that a decrease of $\Omega_{\text{calc}}$ concomitant to an increase of oceanic pCO$_2$ can reduce biogenic calcification, i.e. an environmental control of the calcification as it has been reported for corals reefs, some authors have rather suggested an internal control of calcification by *E. huxleyi*. For instance, several studies have suggested that calcification could support photosynthesis [Sikes et al., 1980; Nimer and Merrett, 1992; Anning et al., 1996; Buitenhuis et al., 1999], acting as an effective low-cost energy pathway to directly supply the chloroplast with CO$_2$ in addition to direct CO$_2$ diffusion into the cell, and then rise the concentration of CO$_2$ in the chloroplast at the site of photosynthesis. However, recent studies have severely questioned this hypothesis [Sekino and Shiraiwa 1994; Herfort et al., 2002;2004; Rost and Riebesell 2004]. Some other metabolic benefits from calcification have been suggested like the "trash-can" function facilitating the use of HCO$_3^-$ in photosynthesis (see [Paasche, 2002] and [Rost and Riebesell, 2004] for reviews) and serving to remove excess Ca$^{2+}$ [Berry et al.,
Furthermore calcification could rid the cell of excess energy and therefore prevent damage of the photosynthetic machinery [Rost and Riebesell, 2004].

Some ecological implications have been also proposed. Hence, coccolith production could protect the integrity of the cell and maintain a suitable environment around the cell surface [Young, 1994; Paasche, 2002]. However, among these ecological benefits, the ability of coccospheres to prevent viral lysis [Young, 1994] is not supported by our experiment. Indeed coccolithoviruses were detected in the course of the exponential growth phase in seven of the nine mesocosms, but virus-induced lysis was not detected in the two mesocosms where calcification rates were the lowest (M1 and M3 – ‘year 2100’ conditions). Thus, the benefits of calcification for coccolithophores still remains an open question.

Published data on the effect of elevated CO$_2$ on organic carbon production and C:P ratio by _E. huxleyi_ is even less clear (Table 2). Some papers report a decline of primary production at elevated $p$CO$_2$ and others an increase while no conspicuous change was observed in the present study. It is obvious that environmental parameters such as light and nutrients interact with $p$CO$_2$ since they play a major role in the energy status and/or metabolism. Furthermore, two experiments carried out in similar conditions can shows opposite trends regarding to changes of POC and PIC production with $p$CO$_2$ [Nimer et al., 1992; Buithenhuis et al., 1999] depending of the strain used. This underlines that attention must be paid to the ecotypes used in experiments or encountered in natural assemblages as also noted in a recent review on _E. huxleyi_ physiology [Paasche, 2002].

Among this mosaic of contrasting results, it must be pointed out that the two experiments carried out with natural communities under irradiance and nutrient concentrations close to environmentally realistic conditions [Riebesell et al., 2000; present study] converge to show that PIC production and the C:P ratio decrease markedly while POC production remains roughly constant with rising $p$CO$_2$. 

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Previous experiments were carried out in batch or continuous cultures and provide little information on the dynamics of calcification in natural conditions. Following the development and decline of a bloom demonstrates that the onset of calcification was delayed by 24 to 48h in the 'year 2100' compared to glacial CO$_2$ conditions. Unfortunately, since the bloom prematurely collapsed due to a massive viral infection, it is not possible to assess the overall duration of the calcification phase. However, we surmise that the delay in the onset of calcification under high PCO$_2$ could act to decrease the overall duration of the calcification phase. Such a reduction would lower the overall production of CaCO$_3$ in the full course of a coccolithophorid bloom.

### 4.4 Implications of the observed PCO$_2$ related effects on biogeochemical fluxes

The net effect of reduced calcification on air-sea CO$_2$ gradients and fluxes is the balance between two counteracting processes. First, the decrease in calcification reduces CO$_2$ release. Second, the changes in seawater carbonate chemistry induced by rising $p$CO$_2$ lead to an increase of the molar ratio of released CO$_2$ over calcium carbonate precipitation [Frankignoulle et al., 1995]. These two antagonistic processes seem to be balanced in coral reefs [Gattuso et al., 1999], but Zondervan et al. [2001] suggested that the response of pelagic calcification leans towards a negative feedback, that increases the retention of CO$_2$ in the ocean. However, the response of pelagic biogenic CaCO$_3$ fluxes to rising CO$_2$ also needs to consider export processes. Blooms of *E. huxleyi* may be more efficient carbon sinks than other phytoplankton blooms as a result of the higher density of sedimenting cells and zooplankton fecal pellets, due to the high density of calcite [Buitenhuis et al., 2001]. This is supported by the compilation of sediment trap data below 1000 m depth which shows that ballast minerals, and in particular calcium carbonate, drives the sinking of organic carbon to the deep ocean [Armstrong et al., 2001; Klaas and Archer, 2002]. Hence, a lower C:P ratio of coccolithophorids under 'year 2100' conditions could lead to a smaller ballast effect and to a
subsequent reduction of carbon export, thereby acting as a positive feedback to rising atmospheric CO$_2$.

In the present study, however, we actually observed the opposite, since carbon export by the *E. huxleyi* community, estimated as carbon losses, was higher under 'year 2100' conditions. Enhancement of carbon export through TEP production conspicuously overcomes the diminution of the ballast effect and turns the overall response of export of *E. huxleyi* to rising CO$_2$ concentration towards a negative feedback. This comes in addition to the decrease of the production of CO$_2$ as a consequence of the reduction of both rate and duration of calcification. Finally, if the enhancement of carbon export driven by higher TEP production under elevated $p$CO$_2$ is as significant for other phytoplanktonic groups as for coccolithophorids, then it would potentially represent a major negative feedback on rising atmospheric CO$_2$.

5. Conclusions

No conspicuous change of both net community productivity and net primary productivity of *E. huxleyi* was detected during the peak and the decline of a bloom of the coccolithophorid *E. huxleyi* for $p$CO$_2$ ranging from 175 to 600 ppmV. In contrast, the rate of net community calcification declined at elevated $p$CO$_2$, corroborating the observations of Riebesell et al. [2000], Zondervan et al. [2001], Zondervan et al. [2002] and Sciandra et al. [2003] on cultures of *E. huxleyi*. Furthermore, the onset of calcification is delayed by 24 to 48 h in the 'year 2100' conditions compared to 'glacial' conditions. The decrease of calcification rate combined with a rather constant organic carbon production led to a significant decrease of the C:P ratio.

When comparing previous reports on the response of organic and inorganic carbon production of *E. huxleyi* to increasing $p$CO$_2$ it appears that in non-saturating light and nutrient replete conditions, the increase in $p$CO$_2$ promotes organic carbon production [Riebesell et al.,
2000; Zondervan et al., 2001; 2002] unless $p$CO$_2$ becomes too high (above 1000 ppmV) [Nimer et al., 1994]. In contrast, under nutrient-limiting conditions organic carbon production remains constant [Riebesell et al., 2000; present study] or even decreases [Sciandra et al., 2003] with increasing $p$CO$_2$.

Since the changes of both organic and CaCO$_3$ production with rising $p$CO$_2$ are strongly influenced by light and nutrient conditions as well as by the level of $p$CO$_2$, one could expect even more complex response of the C:P ratio. On the whole, a decrease of the C:P ratio with increasing $p$CO$_2$ appears to be a general trend with the exceptions under severe light or nutrient limitations. However, it must be pointed out that in most studies, little attention has been paid to the ecotype of *E. huxleyi* or to species other than *E. huxleyi*. Information available to date [Zondervan et al., 2001; 2002] indicates differential responses depending on the species considered. Also, the interaction of increased temperature, which is significant in corals [Reynaud et al., 2003], has not been investigated in coccolithophorids. Any prediction of the future response of ocean biogeochemistry to elevated $p$CO$_2$ must therefore take into consideration the composition of the community as well as the interaction with various other environmental parameters which are also predicted to change. In this sense, it is relevant to note that increased surface temperature will lead to a higher stratification and lower nutrient inputs, so that the evolution of marine communities and ecosystems should be envisaged in a “high CO$_2$, low nutrient and warmer ocean” context.

Many open questions need to be settled to predict reliably the response of coccolithophorids to rising CO$_2$. However, it is worth noting that the two experiments carried out in environmentally realistic conditions converge satisfactorily and suggest that organic carbon production remains roughly constant with rising CO$_2$ while inorganic carbon production decreases drastically, reducing concomitantly the C:P production. Moreover, a delay in the onset of calcification under elevated $PCO_2$ conditions superimposed on a
decrease of CaCO$_3$ production rate is likely to reduce the overall production of CaCO$_3$ in the course of a coccolithophorid bloom.

Acknowledgements

This work is dedicated to our invaluable colleagues and friends Roland Wollast and Michel Frankignoulle who left us on 28 July 2004 and 13 March 2005 respectively. We are grateful to Anja Engel, Emma Rochelle-Newall and Antoine Sciandra for fruitful discussions, to Marie-Dominique Pizay for technical assistance, to Jorun Egge for her welcome at the marine biological station of the University of Bergen and to Daniel Delille and two anonymous reviewers for their pertinent comments on the manuscript. Access to installations from the University of Bergen was funded by the Improving Human Potential Programme from the European Union (Contract No. HPRI-CT-1999-00056 "Bergen Marine"). A.V.B. and M.F. are, respectively, a post-doctoral researcher and a senior research associate at the Fonds National de la Recherche Scientifique. B.D and J.H. were supported by the Belgian Federal Office for Scientific, Technical and Cultural Affairs (Contracts n°EV/12/7E and n°EV/11/5A, respectively). This is MARE contribution n°XXX

References


Figures caption

Fig. 1. Evolution of seawater $p$CO$_2$, DIC, TA, abundance of *Emiliania huxleyi* and virus specific for *E. huxleyi* in the 9 mesocosms. TA (corrected for the uptake of NO$_3^-$ and PO$_4^{3-}$) and DIC are normalized to a constant salinity of 31.

2. (a) Net community production estimated from oxygen incubations (NCP$_{O_2}$), (b) net primary production estimated from $^{14}$C incubations (NPP$_{\mu C}$), (c) net community production normalized against chlorophyll a from oxygen incubations (NCP$_{\mu C}$), (d) net primary production normalized against chlorophyll a from $^{14}$C (NPP$_{\mu C}$) and (e) community respiration (CR) based on oxygen incubations in mesocosms 1 (filled squares, solid line), 2 (open squares, solid line), 4 (filled triangles, broken line), 5 (open triangles, broken line), 8 (filled circles, dotted line), and 9 (open circles, dotted line).

Fig. 3. $^{14}$C uptake of inorganic carbon (a) and normalized calcification (b) in mesocosms 1 (squares, solid line), 4 (broken line) and 9 (triangles, dotted line).

Fig. 4. Net community production computed from oxygen incubation (NCP$_{O_2}$) versus NCP computed from DIC and TA (NCP$_{DIC}$) with a model II regression line.

Fig. 5. Hysteresis showing the changes of net community production (NCP$_{DIC}$) and calcification (NCC) during the experiment. NCP$_{DIC}$ is plotted versus NCC and each data point corresponds to one day. Positive and negative Y-axis values indicate, respectively, a net gain and loss of organic carbon. Positive and negative X-axis values indicate, respectively, net production and dissolution of calcium carbonate. Time is running clockwise and dates of
some points are indicated ("d$_x$"). Dashed lines indicate when the *E. huxleyi* virus (*EhV*) abundance was above 5 $10^6$ part. ml$^{-1}$. A schematic shape is provided for each condition.

Fig. 6. Changes in the standing stocks of total organic carbon (squares) from d$_{10}$ until exhaustion of nutrients - with a regression line (dotted line) and corresponding slope and standard error (plain text) - and particulate inorganic carbon between d$_{11}$ to d$_{23}$ (circles) - with a regression line (thick line) and corresponding slope and standard error (bold text) -.

Regression line of particulate inorganic carbon were computed prior to viral lysis (*EhV*< 5 $10^6$ part. ml$^{-1}$, filled circles).

Fig. 7. Mean and standard deviation of total organic carbon (TOC) production between d$_{10}$ and d$_{15}$ (before nutrient depletion), mean particulate inorganic carbon (PIC) production from d$_{11}$ until viral lysis, mean PIC production:mean TOC production ratio (C:P) and carbon losses for the 3 PCO$_2$ conditions (light grey: 'glacial', dark grey: 'present', black 'year 2100').

Fig. 8. Buffer factor of the carbonate system for increasing partial pressure of CO$_2$ at the initial conditions of the experiment (salinity= 31.3, temperature= 10.0°C, TA= 2150 µmol kg$^{-1}$).
Table 1. $p\text{CO}_2$ (ppmV) and concentration of CO$_2$ (µmol kg$^{-1}$) of each mesocosm at $d_0$ and $d_{10}$ and $d_{15}$

<table>
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<tr>
<th>Mesocosm</th>
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Table 2. Changes in CaCO$_3$ production, organic carbon production and C:P ratio of *Emiliania huxleyi* with increasing $p$CO$_2$ reported in literature. In the "irradiance" column, numbers in brackets denote the daily light/day period in hours. "++","--","0" denote respectively increase, decrease and no significant changes while brackets denotes a trend not statistically significant. We limit our comparison to experiments which addressed the response of *E. huxleyi* to increase of $p$CO$_2$ or [CO$_2$] and the concomitant decrease of the calcite saturation state ($\Omega_{calc}$), within ranges of similar magnitude as predicted changes during the next hundred years.

<table>
<thead>
<tr>
<th>$E. huxleyi$ strain</th>
<th>Nitrate concentration (µmol L$^{-1}$)</th>
<th>Irradiance (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Organic production</th>
<th>CaCO$_3$ production</th>
<th>C:P ratio</th>
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<td>30% of surface irradiance</td>
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<td>Sciandra et al. [2003]</td>
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<td>170 (24/0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>This study</td>
<td>Norwegian natural assemblages</td>
<td>0.3 to 10</td>
<td>150 to 650 (16/8)</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig. 2

(a) NCP\(_{\text{O}_2}\) (mmol O\(_2\) m\(^{-2}\) d\(^{-1}\))

(b) NPP\(_{\text{C}}\) (µmol C kg\(^{-1}\) d\(^{-1}\))

(c) NCP\(_{\text{O}_2}\) (µmol O\(_2\) µg Chl-a\(^{-1}\) d\(^{-1}\))

(d) NPP\(_{\text{C}}\) (µmol C µg Chl a\(^{-1}\) d\(^{-1}\))

(e) Community respiration (mmolO\(_2\) m\(^{-2}\) d\(^{-1}\))

Day

pre-bloom peak bloom decline
Fig. 3

(a) 

^{14}C uptake of inorganic carbon (µmol CaCO_3 kg^{-1} d^{-1})

(b) 

Normalized calcification (µmol CaCO_3 µgChla^{-1} d^{-1})

Day

pre-bloom  peak  bloom decline
Fig. 4

\[ NCP_{\text{DIC}} \] (mmol C m\(^{-2}\) d\(^{-1}\))

\[ NCP_{\text{O2}} \] (mmol O\(_2\) m\(^{-2}\) d\(^{-1}\))

1.45 ± 0.124
Fig. 5
TOC production (µmol C kg⁻¹ d⁻¹)

- Glacial: 24.2 ± 1.9
- Present: 24.6 ± 0.4
- Year 2100: 22.9 ± 1.4

PIC production (µmol C kg⁻¹ d⁻¹)

- Glacial: 17.9 ± 2.5
- Present: 17.9 ± 4.4
- Year 2100: 10.3 ± 3.2

PIC : TOC production

- Glacial: 0.74 ± 0.05
- Present: 0.73 ± 0.17
- Year 2100: 0.45 ± 0.12

Carbon losses (µmol C kg⁻¹)

- Glacial: 25 ± 16
- Present: 36 ± 13
- Year 2100: 48 ± 10
$\beta = \frac{\Delta pCO_2}{\Delta DIC}$

Fig. 8