

This discussion paper is/has been under review for the journal Biogeosciences (BG).  
Please refer to the corresponding final paper in BG if available.

# Effects of long-term high CO<sub>2</sub> exposure on two species of coccolithophores

**M. N. Müller<sup>1,2</sup>, K. G. Schulz<sup>1</sup>, and U. Riebesell<sup>1</sup>**

<sup>1</sup>Leibniz Institute of Marine Sciences, Marine Biogeochemistry, Düsternbrooker Weg 20,  
24105 Kiel, Germany

<sup>2</sup>LOV, UMR 7093, Station Zoologique, BP 28, 06234 Villefranche-sur-mer, France

Received: 4 November 2009 – Accepted: 6 November 2009 – Published: 24 November 2009

Correspondence to: M. N. Müller (mueller@obs-vlfr.fr)

Published by Copernicus Publications on behalf of the European Geosciences Union.

**BGD**

6, 10963–10982, 2009

**Long-term CO<sub>2</sub>  
effects on  
coccolithophores**

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Abstract

The physiological performance of two coccolithophore species, *Emiliania huxleyi* and *Coccolithus braarudii*, was investigated during long-term exposure to elevated  $p\text{CO}_2$  levels. Mono-specific cultures were grown over 152 (*E. huxleyi*) and 65 (*C. braarudii*) generations while  $p\text{CO}_2$  was gradually increased to maximum levels of  $1150\text{ }\mu\text{atm}$  (*E. huxleyi*) and  $930\text{ }\mu\text{atm}$  (*C. braarudii*) and kept constant thereafter. Rates of cell growth and cell quotas of particulate organic carbon (POC), particulate inorganic carbon (PIC) and total particulate nitrogen (TPN) were determined repeatedly throughout the incubation period. Increasing  $p\text{CO}_2$  caused a decrease in cell growth rate of 9% and 29% in *E. huxleyi* and *C. braarudii*, respectively. In both species cellular PIC:TPN and PIC:POC ratios decreased in response to rising  $p\text{CO}_2$ , whereas no change was observed in the POC:TPN ratios of *E. huxleyi* and *C. braarudii*. These results are consistent with those obtained in shorter-term high  $\text{CO}_2$  exposure experiments following abrupt perturbations of the seawater carbonate system, indicating that for the strains tested here i) a gradual  $\text{CO}_2$  increase does not alleviate  $\text{CO}_2/\text{pH}$  sensitivity, and ii) observed  $\text{CO}_2$  sensitivities are persistent over multiple generations.

## 1 Introduction

Emissions of anthropogenic  $\text{CO}_2$  since the beginning of the industrial revolution have lead to an increase in atmospheric carbon dioxide concentration, resulting in a fraction of the anthropogenic  $\text{CO}_2$  being absorbed by the ocean. The ongoing oceanic uptake of anthropogenic  $\text{CO}_2$  is steadily altering the seawater carbonate chemistry and has lead to a reduction in surface ocean pH by 0.12 units over the past 200 yr (Alley et al., 2007). This process, termed ocean acidification, seriously alters the physiological performance of pelagic and benthic organisms (Fabry et al., 2008). Due to their ecological and biogeochemical importance and because they are easily grown in cultures, coccolithophores are among the best studied organisms with respect to

**BGD**

6, 10963–10982, 2009

### Long-term $\text{CO}_2$ effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



their response to ocean acidification (reviewed by Rost et al., 2008; Zondervan, 2007). Results from these studies indicate considerable species- and even strain-specific differences in CO<sub>2</sub> sensitivity.

When exposed to elevated pCO<sub>2</sub> levels as projected for the course of this century, *Emiliania huxleyi*, *Gephyrocapsa oceanica* and *Calcidiscus leptoporus* respond by decreasing calcification to varying degrees, corresponding to a decrease in the particulate inorganic to organic carbon ratio, PIC:POC (Riebesell et al., 2000; Zondervan et al., 2002; Langer et al., 2006; Feng et al., 2008; Langer et al., 2009). *Coccolithus braarudii*, on the other hand, seems to be insensitive to elevated pCO<sub>2</sub> (Langer et al., 2006) with respect to calcification. Differences in pCO<sub>2</sub> sensitivity between species were also observed with respect to organic carbon production. While *E. huxleyi* and *G. oceanica* increased POC production by up to 50% under elevated pCO<sub>2</sub> (Riebesell et al., 2000; Zondervan et al., 2002; Feng et al., 2008; Shi et al., 2009), no change with increasing pCO<sub>2</sub> was observed in *C. braarudii* and *C. quadriperforatus* (Langer et al., 2006). Recently, Iglesias-Rodriguez et al. (2008) reported an increase of up to 100% in both calcification and organic carbon production per cell in response to elevated pCO<sub>2</sub>. This trend reverses when accounting for the large difference in cell size between pCO<sub>2</sub> treatments as observed in previous experiments on *E. huxleyi*, yielding a slight decrease in calcification with increasing pCO<sub>2</sub> when normalizing the data to cellular carbon content (Riebesell et al., 2008b).

Most acidification experiments with coccolithophores were done applying an abrupt perturbation of seawater carbonate chemistry, either by bubbling with CO<sub>2</sub> enriched/depleted air (Feng et al., 2008; Iglesias-Rodriguez et al., 2008) or by adding acid/base (Riebesell et al., 2000; Zondervan et al., 2002; Langer et al., 2006), and preacclimating the cultures before starting the experiment (commonly 7–10 generations). After the acclimation time, experimental high CO<sub>2</sub> exposure time ranged between 1–2 (Iglesias-Rodriguez et al., 2008) and 7–10 generations (Riebesell et al., 2000; Zondervan et al., 2002; Langer et al., 2006; Feng et al., 2008). This results in a maximum of 20 cell divisions under high pCO<sub>2</sub> conditions. The physiological re-

**BGD**

6, 10963–10982, 2009

## Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



sponse to high  $p\text{CO}_2$  exposure of *E. huxleyi* within one cell generation was recently investigated by Barcelos e Ramos et al. (2009) and was found to be consistent with the observations gained from longer-term experiments. However, it is presently unknown whether the observed  $\text{CO}_2$  sensitivities of coccolithophores are persistent over long-term high  $p\text{CO}_2$  exposure. Here, we report on semi-continuous batch culture experiments with two coccolithophore species (*E. huxleyi* and *C. braarudii*), which were grown over multiple generations (152 and 65 generations, respectively) under gradually increasing  $p\text{CO}_2$  levels.

## 2 Methods

### 2.1 Cultures

*Coccolithus braarudii* RCC 1200, formerly known as *C. pelagicus* (Saez et al., 2003), was obtained from the Roscoff Culture Collection. *Emiliania huxleyi* was isolated in 2005 during the PeECE III mesocosm study (Riebesell et al., 2008a) in the Raune Fjord (Norway). Both cultures were grown at  $16^\circ\text{C}$  in  $0.2\text{ }\mu\text{m}$  filtrated North Sea water with a salinity of 33 and  $f/20$  nutrient additions (Guillard, 1975), corresponding to  $88\text{ }\mu\text{mol l}^{-1}$  nitrate and  $3.6\text{ }\mu\text{mol l}^{-1}$  phosphate, at a photon flux density of  $140\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$  (Philips TL-D 90 DeLuxePro, 36W/950) under a 14:10 light:dark cycle.

### 2.2 Experimental setup

Coccolithophores were grown in duplicates under semi-continuous batch culture conditions (as described above) in 280 ml autoclaved polycarbonate Erlenmeyer flasks.  $p\text{CO}_2$  was adjusted by additions of HCl or NaOH to the media. Cultures were allowed to grow for about 5–10 generations corresponding to a dissolved inorganic carbon (DIC) consumption of 5 to 10%. At this stage exponentially growing cultures were sampled for

**BGD**

6, 10963–10982, 2009

## Long-term $\text{CO}_2$ effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



DIC, pH, cell number, total particulate and particulate organic carbon (TPC and POC), and total particulate nitrogen (TPN) before being diluted with fresh media (carbonate system already adjusted) to a concentration of 100 and 50 cells ml<sup>-1</sup> (*E. huxleyi* and *C. braarudii*, respectively). The media in the control flasks (low *p*CO<sub>2</sub>) was adjusted to a pH<sub>total</sub> of 8.21±0.05, resulting in a *p*CO<sub>2</sub> of 260 μatm±20 with a corresponding calcite saturation state (Ω) of 5.6±0.4. At the beginning of the experiment all flasks started under conditions described above and then the treatment flasks were slowly acidified over several generations (79 and 28 generations for *E. huxleyi* and *C. braarudii*, respectively) to the target *p*CO<sub>2</sub> values (1150 μatm±140 and 930 μatm±180 for *E. huxleyi* and *C. braarudii*, respectively) and kept at these levels until the end of the experiment.

## 2.3 TPC, POC, PIC, TPN

Two sub-samples from each flask were filtrated onto precombusted (525°C for 7 h) GF/F filters and frozen at -20°C. For POC analysis filters were fumed over HCl for 24 h to remove all inorganic carbon and afterwards all filters were measured on a Euro EA Elemental Analyser (Ehrhardt and Koeve, 1999). PIC (particulate inorganic carbon) was calculated from the difference of TPC and POC.

Cell quotas of carbon and nitrogen increase during the light phase whereas the cell density stays constant because of synchronized cell division in the dark phase (Müller et al., 2008). Because samples were taken at different times of the light phase, normalizing the data on a per cell basis generates a bias in the data due to sampling time. We therefore present the data as cellular ratios of PIC:POC, PIC:TPN and POC:TPN, which show no temporal trend over the course of the light phase (Fig. 1).

**BGD**

6, 10963–10982, 2009

## Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## 2.4 Cell counts

Cell number was determined with a Coulter Counter (Z Series). Samples were measured three times and the mean was used to calculate the growth rate ( $\mu$ ) as

$$\mu = \frac{(\ln c_1 - \ln c_0)}{t_1 - t_0} \quad (1)$$

- 5 where  $c_0$  and  $c_1$  are the cell concentrations at the beginning ( $t_0$ ) and end of the incubation period ( $t_1$ ).

## 2.5 Carbonate system

The carbonate system was monitored by DIC and pH measurements. DIC was analyzed after Stoll et al. (2001) using an automated segmented-flow analyzer (Quattro) equipped with an auto-sampler ( $\pm 10 \mu\text{mol kg}^{-1}$  accuracy and five  $\mu\text{mol kg}^{-1}$  precision) and pH was measured using a “Metrohm 713 pH-Meter”, equipped with pH and reference electrodes and temperature sensor. Sensor and electrodes were stored in filtrated seawater at  $16^\circ\text{C}$  to match the ionic strength of the sampled water. pH measurements were periodically checked by calculating pH from measurements of total alkalinity (Dickson, 1981) and DIC of filtrated seawater using the program CO2sys (version 1.05 by E. Lewis and D. W. R. Wallace) with dissociation constants for carbonic acid after Roy et al. (1993). Calculated pH values closely agreed with pH measurements with a maximum deviation of  $\pm 0.02$ . Here we present pH values on the total scale.

## 2.6 Scanning electron microscopy

5 ml samples were taken periodically from the control and high  $\text{CO}_2$  treatment and fixed with formaldehyde (1% end concentration). Subsequently, the samples were filtered onto polycarbonate filters ( $0.2 \mu\text{m}$  pore size), dried at  $60^\circ\text{C}$  for 24 h and then sputter-coated with gold-palladium. Pictures were taken with a CamScan-CS-44 scanning

10968

**BGD**

6, 10963–10982, 2009

**Long-term  $\text{CO}_2$   
effects on  
coccolithophores**

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



### 3 Results

#### 3.1 *Emiliania huxleyi*

5 Cells were cultured for 98 d, corresponding to 152 generations in the control treatment (low  $p\text{CO}_2$ ) and 144 generations in the high  $p\text{CO}_2$  treatment. Cellular division in the control treatment stabilized at a rate of  $\mu=1.10\pm0.06\text{ d}^{-1}$  after a couple of weeks into the experiment. During the gradual increase from low to high  $p\text{CO}_2$ , no change in growth rate was detectable. After reaching the maximum  $p\text{CO}_2$  level  
10 of  $1150\text{ }\mu\text{atm}$  the growth rate decreased to  $\mu=1.00\pm0.06\text{ d}^{-1}$  and remained at this value until the end of the experiment (Fig. 2a, b). The PIC:TPN ratio was relatively constant at  $4.9\pm1.0\text{ mol C mol N}^{-1}$  under low  $p\text{CO}_2$  but with the onset of high  $p\text{CO}_2$  the ratio decreased and was consistently lower compared to the control by an average value of  $1.8\pm0.7\text{ mol C mol N}^{-1}$  (Fig. 2c). No consistent difference between  $p\text{CO}_2$   
15 treatments was observed in POC:TPN with a mean ratio of  $10.0\pm1.4\text{ mol C mol N}^{-1}$  (Fig. 2d). The PIC:POC ratio was considerably lower under constant high  $p\text{CO}_2$  with a mean value of  $0.33\pm1.3\text{ mol C mol C}^{-1}$  compared to  $0.56\pm1.3\text{ mol C mol C}^{-1}$  at low  $p\text{CO}_2$  (Fig. 2e). Coccolith morphology of *E. huxleyi* did not display a visible difference between  $p\text{CO}_2$  treatments (Fig. 4a, b). Between day 73 and 80,  $p\text{CO}_2$  accidentally  
20 dropped to  $870\text{ }\mu\text{atm}$ , which was followed by an immediate increase in cell growth. As soon as  $p\text{CO}_2$  was elevated above  $1000\text{ }\mu\text{atm}$ , the cell growth rate decreased again to the previous level.

## 3.2 *Coccolithus braarudii*

Cells were cultured for 66 d corresponding to 65 generations in the control treatment and 51 generations in the high  $p\text{CO}_2$  treatment. After transition to constant high  $p\text{CO}_2$  on day 31 the growth rate decreased from initially  $0.69 \pm 0.04$  to  $0.49 \pm 0.06 \text{ d}^{-1}$ , whereas it remained at the initial level in the control treatment (Fig. 3a, b). Both PIC:TPN and POC:TPN displayed similar trends as seen in *E. huxleyi*. PIC:TPN decreased from  $8.9 \pm 1.6$  to  $2.1 \pm 0.6 \text{ mol C mol N}^{-1}$  under high  $p\text{CO}_2$ , whereas no change was detected in the POC:TPN ratio (Fig. 3c,d). Mean values of the POC:TPN ratio under low and high  $p\text{CO}_2$  were calculated as  $10.9 \pm 1.5$  and  $9.3 \pm 2.0 \text{ mol C mol N}^{-1}$ , respectively. The PIC:POC ratio was reduced by  $\approx 70\%$  to a mean value of  $0.28 \pm 0.11 \text{ mol C mol C}^{-1}$  (Fig. 3e) and clear signs of malformation were observed on individual coccoliths under constant high  $p\text{CO}_2$  conditions (Fig. 4d).

## 4 Discussion

### 4.1 Carbonate system manipulation

Manipulation of the carbonate system by acid/base addition changes the total alkalinity (TA) at a constant dissolved inorganic carbon (DIC) concentration, whereas “ongoing ocean acidification” changes the DIC concentration at constant TA. However, biologically important parameters ( $[\text{CO}_{2(\text{aq})}]$ ,  $[\text{HCO}_3^-]$ ,  $[\text{CO}_3^{2-}]$  and  $[\text{H}^+]$ ) undergo similar changes by manipulating TA at constant DIC compared to manipulating DIC at constant TA in the  $p\text{CO}_2$  range applied here (Schulz et al., 2009). For example, manipulating seawater with a salinity of 35 at  $15^\circ\text{C}$ ,  $p\text{CO}_2$  of  $380 \mu\text{atm}$  and a DIC concentration of  $2100 \mu\text{mol kg}^{-1}$  by i) aeration with  $\text{CO}_2$  enriched air (TA constant) or ii) acid addition (DIC constant) to a  $p\text{CO}_2$  of  $1000 \mu\text{atm}$  would result in the following percentage changes of biologically relevant parameters.  $[\text{CO}_{2(\text{aq})}]$ : +164% (i and ii);  $[\text{HCO}_3^-]$ : +12% (i) and +4% (ii);  $[\text{CO}_3^{2-}]$ : -52% (i) and -59% (ii);  $[\text{H}^+]$ : +135% (i) and +152%

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





(ii). Calculations were done using the program CO2sys (version 1.05 by E. Lewis and D. W. R. Wallace) using dissociation constants for carbonic acid after Roy et al. (1993).

## 4.2 Growth rate

5 An average decrease of 9% was observed in *E. huxleyi*'s growth rate in response to increasing  $p\text{CO}_2$  from 260 to 1150  $\mu\text{atm}$ . Previous studies detected no change in growth rates after exposure to elevated  $p\text{CO}_2$  (Riebesell et al., 2000; Zondervan et al., 2002; Feng et al., 2008). While this difference may be due to the fact that the maximum  $p\text{CO}_2$  of 900  $\mu\text{atm}$  in these earlier studies was somewhat lower than in the present experiment, the small difference in growth rate of 9% may also have been below the  
10 detection limit in the single end-point sampling approach used in previous studies. Recent results by Barcelos e Ramos et al. (2009) and Langer et al. (2009) indicate a reduced growth rate at a  $p\text{CO}_2 > 1000 \mu\text{atm}$  in short-term experiments. Interestingly, between day 73 and day 80, when  $p\text{CO}_2$  accidentally dropped to 870  $\mu\text{atm}$ , the growth rate increased to control values (Fig. 3b) and immediately returned to the lower value  
15 after  $p\text{CO}_2$  was raised to 1150  $\mu\text{atm}$ . A similar instantaneous effect of  $p\text{CO}_2$  on the growth rate of *E. huxleyi* was reported to occur within one cell generation (Barcelos e Ramos et al., 2009).

Under constant high  $p\text{CO}_2$  the growth rate of *C. braarudii* was reduced by 29%. At comparable  $p\text{CO}_2$  values, Langer et al. (2006) observed no significant reduction in the  
20 growth rate of *C. braarudii*. This difference might be induced by the long-term culturing under constant high  $p\text{CO}_2$ , but other potential factors such as differences in the experimental temperature and the light intensity cannot be excluded as being responsible. Recent results by Krug (personal communication) indicate a reduced growth rate of *C. braarudii* when exposed to  $p\text{CO}_2 > 1400 \mu\text{atm}$  for 15 generations.

**BGD**

6, 10963–10982, 2009

### Long-term $\text{CO}_2$ effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



### 4.3 PIC:TPN and PIC:POC

Under constant high  $p\text{CO}_2$  the ratios of PIC:TPN (and equally PIC:POC) in *E. huxleyi* and *C. braarudii* were reduced by  $\approx 42\%$  and  $\approx 70\%$ , respectively. The reduction of the PIC:POC ratio in *E. huxleyi* is a commonly observed response under high  $p\text{CO}_2$  which is driven by the decrease of the cellular PIC quota and increase in POC quota and ranges between 10 and 60% depending on  $p\text{CO}_2$  level, temperature and light intensity (Riebesell et al., 2000; Zondervan et al., 2002; Feng et al., 2008; Langer et al., 2009; Barcelos e Ramos et al., 2009). Since the POC:TPN ratio remains constant under different  $p\text{CO}_2$  values (as discussed in the next paragraph) the PIC:TPN ratio should display a similar behaviour as the PIC:POC ratio. Indeed, the PIC:TPN ratios are decreasing in response to elevated  $p\text{CO}_2$  values in both species under long-term high  $p\text{CO}_2$  cultivation (Figs. 2c and 3c), which is also seen in short-term experiments under different light intensities for *E. huxleyi* (Fig. 5a, data from Zondervan et al., 2002). *Coccolithus braarudii*, on the other hand, is reported to maintain constant PIC:POC and PIC:TPN ratios within  $p\text{CO}_2$  values ranging from 345 to 915  $\mu\text{atm}$  (Langer et al., 2006). However, more recently Krug (personal communication) measured in short-term experiments a significant decrease in both the PIC:POC and the PIC:TPN ratio when *C. braarudii* was exposed to  $p\text{CO}_2$  values above 1000  $\mu\text{atm}$ .

### 4.4 POC:TPN

Particulate organic carbon production was observed to slightly increase under high  $p\text{CO}_2$  and nutrient replete conditions in *E. huxleyi* (Zondervan et al., 2002; Feng et al., 2008; Barcelos e Ramos et al., 2009), whereas *C. braarudii* maintains a constant rate (Langer et al., 2006). The POC:TPN ratio, however, remains unchanged under short-term high  $p\text{CO}_2$  exposure in *E. huxleyi* (Feng et al., 2008) and *C. braarudii* (Langer, personal communication). These observations from short-term studies are well in agreement with those revealed from the current long-term study. Figure 5b displays the POC:TPN ratios of *E. huxleyi* (data from Zondervan et al., 2002) in comparison to

**BGD**

6, 10963–10982, 2009

## Long-term $\text{CO}_2$ effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



the present long-term data. The measured ratio of  $10.0 \pm 1.4 \text{ mol C mol N}^{-1}$  under long-term cultivation is slightly higher than ratios reported from short-term and mesocosms experiments, which vary between 6 and  $7 \text{ mol C mol N}^{-1}$  (Engel et al., 2005; Feng et al., 2008). POC:TPN ratios of  $\approx 10$  and higher were observed in *E. huxleyi* under nitrogen limitation (Engel et al., 2004; Sciandra et al., 2003). However, we can assure that in the present study *E. huxleyi* was not nitrogen limited since i) an initial nitrate concentration of  $88 \mu\text{mol l}^{-1}$  would be sufficient to supply exponential growth up to a cell density of  $5 \times 10^8 \text{ l}^{-1}$  which was never reached during this study, and ii) the measured growth rate of  $1.10 \pm 0.06 \text{ d}^{-1}$  is well in agreement with maximal growth rates under nutrient replete, similar temperature and light conditions (Buitenhuis et al., 2008). Therefore, we can rule out nitrogen limitation to be responsible for the higher POC:TPN ratio in the present study.

In general, we can confirm the observed trend in the POC:TPN ratios of short-term experiments and conclude that *E. huxleyi* increases the POC production per cell under long-term high  $p\text{CO}_2$  exposure within the tested range. However, since the POC:TPN ratio stays constant the bulk organic matter per available nitrogen of an exponentially growing *E. huxleyi* population will be equal under high and low  $p\text{CO}_2$ .

## 5 Conclusions

Since the studies of Riebesell et al. (2000) and Langer et al. (2006, 2009) species- and strain-specific performance of coccolithophores under elevated  $p\text{CO}_2$  levels is known from short-term experiments, typically involving 7–10 cell generations. Here, we discussed data from a multiple-generation experiment using two coccolithophore species which generally confirm the observed  $\text{CO}_2$  sensitivities obtained in short-term experiments. A gradual  $\text{CO}_2$  increase did not to alleviate the  $\text{CO}_2/\text{pH}$  sensitivity under the experimental conditions. In contrast to earlier studies we observed reduced growth rates in response to elevated  $p\text{CO}_2$ .

Coccolithophores and other phytoplankton groups will face a changing environment

**BGD**

6, 10963–10982, 2009

## Long-term $\text{CO}_2$ effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



in the future ocean. The question of genetic/physiological adaptation to changing environmental conditions is a challenge that needs to be addressed in future investigations. Experiments with a higher genetic variation induced by multiclonal culturing and sexual reproduction will probably provide a suitable tool to test for adaptation potential in the lab (Colegrave, 2002).

*Acknowledgements.* We thank K. Nachtigall for CN measurements, M. Meyerhöfer for DIC analysis and U. Schuldt for the SEM operation. We thank Ian Probert for providing *Coccolithus braarudii*. Jon Havenhand provided helpful comments on data evaluation and analysis. This work was funded by the Deutsche Forschungsgemeinschaft as part of the ESF project “Casiopiea”, by the EU-FP7 in the framework of the “European Project on Ocean Acidification” (EPOCA) under grant agreement 211384 and by the BMBF project “BIOACID” (BMBF, FKZ 03F0608A).



The publication of this article is financed by CNRS-INSU.

## References

Alley, R. B., Berntsen, T., Bindoff, N. L., Chen, Z., Chidthaisong, A., Friedlingstein, P., Gregory, J. M., Hegerl, G. C., Heimann, M., Hewitson, B., Hoskins, B. J., Joos, F., Jouzel, J., Kattsov, V., Lohmann, U., Manning, M., Matsuno, T., Molina, M., Nicholls, N., Overpeck, J., Qin, D., Raga, G., Ramaswamy, V., Ren, J., Rusticucci, M., Solomon, S., Somerville, R., Stocker, T. F., Stott, P. A., Stouffer, R. J., Whetton, P., Wood, R. A., and Wratt, D.: Summary for Policymakers, in: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Elsevier Inc., Cambridge and New York, 2007. 10964

10974

**BGD**

6, 10963–10982, 2009

## Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Barcelos e Ramos, J., Müller, M. N., and Riebesell, U.: Short-term response of the coccolithophore *Emiliania huxleyi* to abrupt changes in seawater carbon dioxide concentrations, *Biogeosciences Discuss.*, 6, 4739–4763, 2009, <http://www.biogeosciences-discuss.net/6/4739/2009/>. 10966, 10971, 10972
- 5 Buitenhuis, E. T., Pangerc, T., Franklin, D. J., Le Quere, C., and Malin, G.: Growth rates of six coccolithophorid strains as a function of temperature, *Limnol. Oceanogr.*, 53, 1181–1185, 2008. 10973
- Colegrave, N.: Sex releases the speed limit on evolution, *Nature*, 420, 664–666, 2002. 10974
- Dickson, A.: An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data, *Deep-Sea Res.*, 28, 609–623, 1981. 10968
- 10 Ehrhardt, M. and Koeve, W.: Determination of particulate organic carbon and nitrogen, in: *Methods of Seawater Analysis*, 3rd ed., edited by: Grasshoff, K., Kremling, K., and Erhardt, M., WILEY-VCH, 1999. 10967
- Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbrüggen, A., and Zondervan, I.: Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO<sub>2</sub> concentrations: a mesocosm experiment, *Aquat. Microb. Ecol.*, 34, 93–104, 2004. 10973
- 15 Engel, A., Zondervan, I., Aerts, K., Beaufort, L., Benthien, A., Chou, L., Delille, B., Gattuso, J.-P., Harley, J., Heemann, C., Hoffmann, L., Jacquet, S., Nejstgaard, J., Pizay, M.-D., Rochelle-Newall, E., Schneider, U., Terbrüggen, A., and Riebesell, U.: Testing the direct effect of CO<sub>2</sub> concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments, *Limnol. Oceanogr.*, 50, 493–504, 2005. 10973
- 20 Fabry, V., Seibel, B. S., Feely, R. A., and Orr, J. C.: Impacts of ocean acidification on marine fauna and ecosystem processes, *ICES J. Mar. Sci.*, 65, 414–432, 2008. 10964
- Feng, Y., Warner, M., Zhang, Y., Sun, J., Fu, F.-X., Rose, J., and Hutchins, D.: Interactive effects of increased pCO<sub>2</sub>, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae), *Eur. J. Phycol.*, 43, 87–98, 2008. 10965, 10971, 10972, 10973
- 25 Guillard, R.: Culture of phytoplankton for feeding marine invertebrates, in: *Culture of Marine Invertebrates*, edited by: Smith, W. and Chanley, M., Plenum, New York, 1975. 10966
- 30 Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R. H., Tyrell, T., Gibbs, S. J., von Dassow, P., Rehm, E., Armbrust, E. V., and Boessenkool, K. P.: Phytoplankton calcification in a high-CO<sub>2</sub> world, *Science*, 320, 336–340, 2008. 10965

## Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Langer, G., Geisen, M., Baumann, K.-H., Kläs, J., Riebesell, U., Thoms, S., and Young, J. R.: Species-specific responses of calcifying algae to changing seawater carbonate chemistry, *Geochem. Geophys. Geosy.*, 7, Q09006, doi:10.1029/2005GC001227, 2006. 10965, 10971, 10972, 10973
- 5 Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry, *Biogeosciences Discuss.*, 6, 4361–4383, 2009, <http://www.biogeosciences-discuss.net/6/4361/2009/>. 10965, 10971, 10972, 10973
- Müller, M. N., Antia, A. N., and LaRoche, J.: Influence of cell cycle phase on calcification in the coccolithophore *Emiliania huxleyi*, *Limnol. Oceanogr.*, 53, 506–512, 2008. 10967
- 10 Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. M.: Reduced calcification of marine plankton in response to increased atmospheric CO<sub>2</sub>, *Nature*, 407, 364–367, 2000. 10965, 10971, 10972, 10973
- Riebesell, U., Bellerby, R. G. J., Grossart, H.-P., and Thingstad, F.: Mesocosm CO<sub>2</sub> perturbation studies: from organism to community level, *Biogeosciences*, 5, 1157–1164, 2008a. 10966
- 15 Riebesell, U., Bellerby, R. G. J., Engel, A., Fabry, V. J., Reusch, T. B. H., Schulz, K. G., and Morel, F. M. M.: Phytoplankton calcification in a high CO<sub>2</sub> world (technical comment), *Science*, 322, 1466b, doi:10.1126/sciences.1161096, 2008b. 10965
- Rost, B., Zondervan, I., and Wolf-Gladrow, D.: Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions, *Mar. Ecol.-Prog. Ser.*, 373, 227–237, 2008. 10965
- 20 Roy, R., Roy, L., Vogel, K., Porter-Moore, C., Pearson, T., Good, C., Millero, F., and Campbell, D.: The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C, *Mar. Chem.*, 44, 249–267, 1993. 10968, 10971
- 25 Saez, A., Probert, I., Geisen, M., Quinn, P., Young, J., and Medlin, L.: Pseudo-cryptic speciation in coccolithophores, *P. Natl. Acad. Sci. USA*, 100, 7163–7168, 2003. 10966
- Schulz, K. G., Barcelos e Ramos, J., Zeebe, R. E., and Riebesell, U.: CO<sub>2</sub> perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations, *Biogeosciences*, 6, 2145–2153, 2009, <http://www.biogeosciences.net/6/2145/2009/>. 10970
- 30 Sciandra, A., Harlay, J., Lefèvre, D., Lemée, R., Rimmelin, P., Denis, M., and Gattuso, J.-P.: Response of coccolithophorid *Emiliania huxleyi* to elevated partial pressure of CO<sub>2</sub> under nitrogen limitation, *Mar. Ecol.-Prog. Ser.*, 261, 111–122, 2003. 10973

## Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Shi, D., Xu, Y., and Morel, F. M. M.: Effects of the pH/ $p\text{CO}_2$  control method on medium chemistry and phytoplankton growth, *Biogeosciences*, 6, 1199–1207, 2009, <http://www.biogeosciences.net/6/1199/2009/>. 10965
- 5 Stoll, M., Bakker, K., Nobbe, G., and Haese, R.: Continuous-flow analysis of dissolved inorganic carbon content in seawater, *Anal. Chem.*, 73, 4111–4116, 2001. 10968
- Zondervan, I.: The effect of light, macronutrients, trace metals and  $\text{CO}_2$  on the production of calcium carbonate and organic carbon in coccolithophores – A review, *Deep-Sea Res. Pt. II*, 41, 521–537, 2007. 10965
- 10 Zondervan, I., Rost, B., and Riebesell, U.: Effect of  $\text{CO}_2$  concentration on the PIC/POC ratio in the coccolithophore *Emiliana huxleyi* grown under light-limiting conditions and different daylengths, *J. Exp. Mar. Biol. Ecol.*, 272, 55–70, 2002. 10965, 10971, 10972, 10982

**BGD**

6, 10963–10982, 2009

## Long-term $\text{CO}_2$ effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

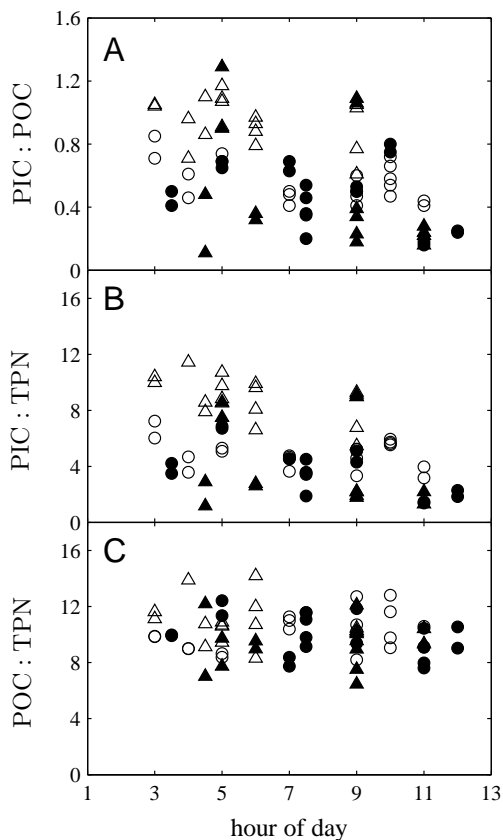
Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





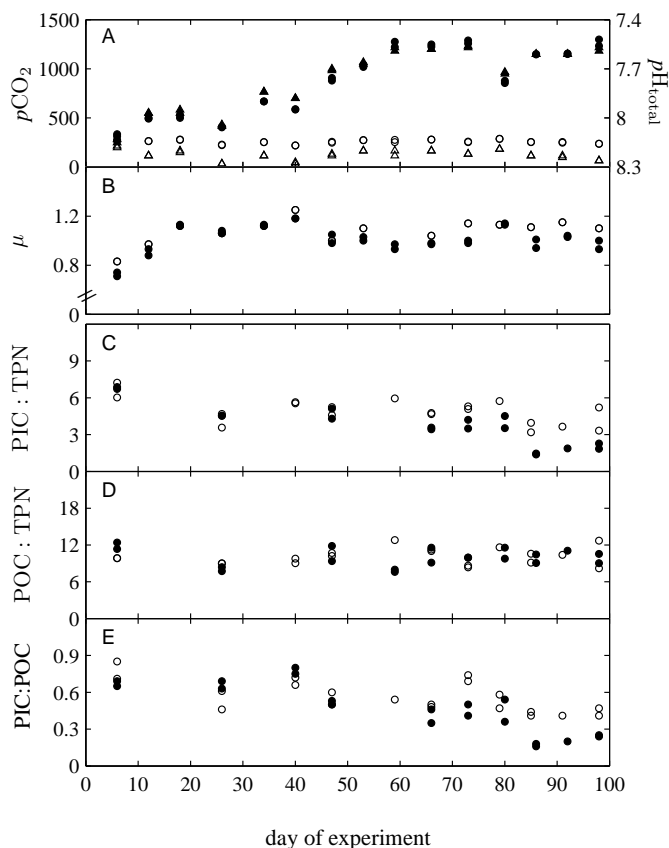
**Fig. 1.** Cell ratios over the hours of daily illumination of *E. huxleyi* (circle) and *C. braarudii* (triangle) under low  $p\text{CO}_2$  (open symbols) and high  $p\text{CO}_2$  (closed symbols) from all data points collected during the experiment. **(A)** PIC:POC ratio (mol C:mol C). **(B)** PIC:TPN ratio (mol C:mol N). **(C)** POC:TPN ratio (mol C:mol N).

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)



# Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.

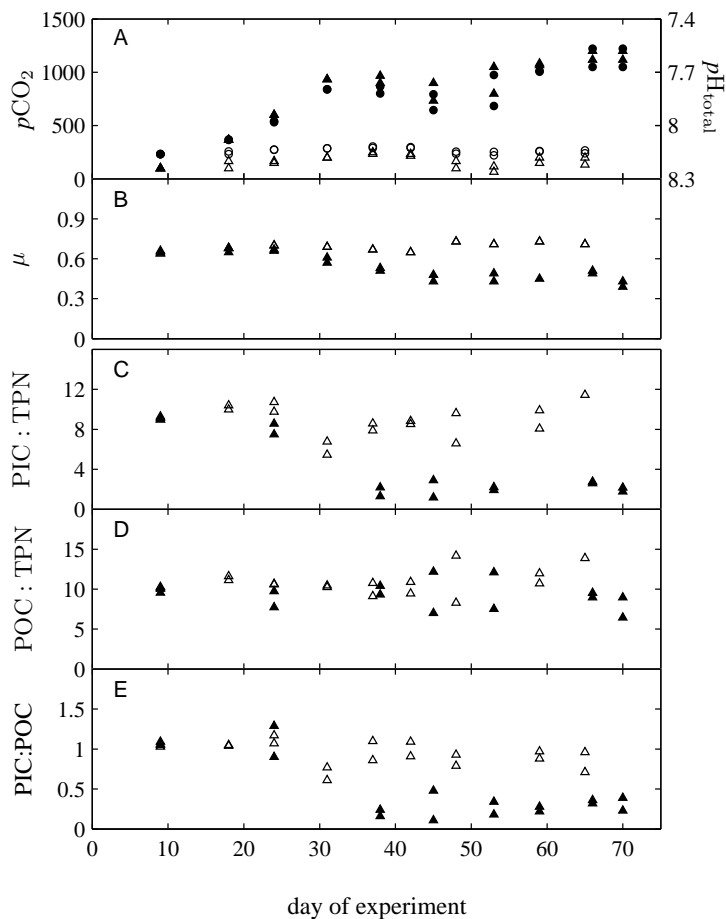


**Fig. 2.** Physiological responses of *Emiliana huxleyi* to elevated  $p\text{CO}_2$  over the course of the experiment (open and closed symbols represent the low  $p\text{CO}_2$  and high  $p\text{CO}_2$  treatments, respectively). **(A)**  $p\text{CO}_2$  (circles,  $\mu\text{atm}$ ) and pH (triangle) over experimental time. **(B)** growth rate ( $\text{d}^{-1}$ ). **(C)** PIC:TPN (mol C:mol N). **(D)** POC:TPN (mol C:mol N). **(E)** PIC:POC (mol C:mol C).

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[◀](#)
[▶](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

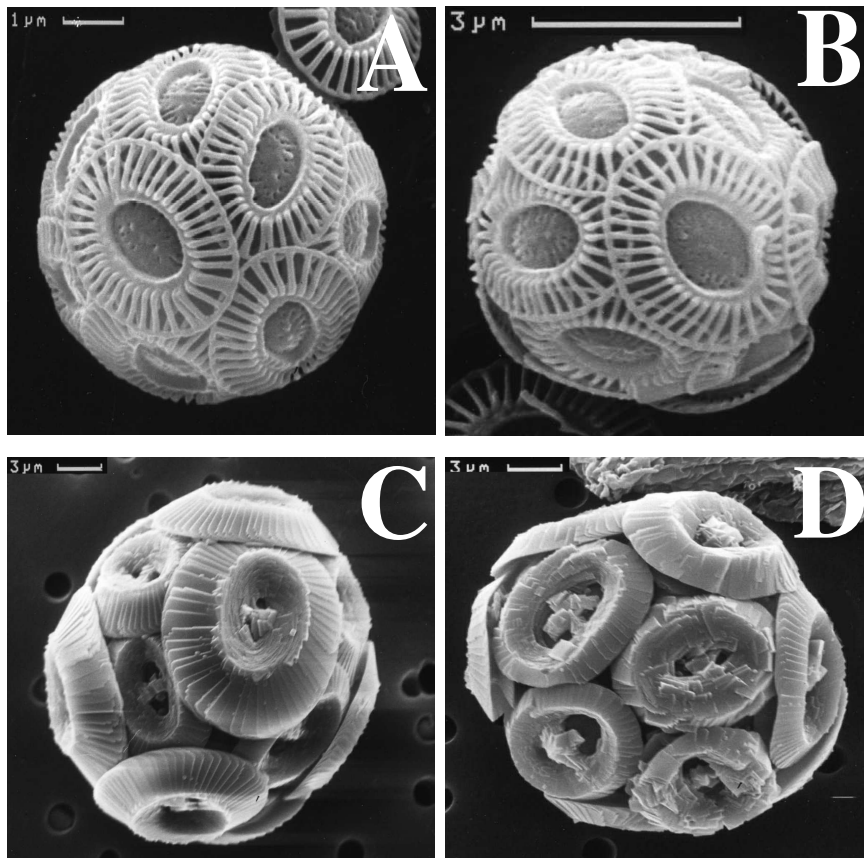

# Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.



**Fig. 3.** Physiological responses of *Coccolithus braarudii* to elevated  $p\text{CO}_2$  over the course of the experiment. Labels and symbols as in Fig. 2.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[◀](#)
[▶](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

**Fig. 4.** Representative SEM photographs of the two coccolithophore species. Cells of *E. huxleyi* grown in the control treatment (A) and under high  $p\text{CO}_2$  at day 73 (B). Cells of *C. braarudii* grown in the control treatment (C) and under high  $p\text{CO}_2$  at day 66 (D).

**BGD**

6, 10963–10982, 2009

## Long-term $\text{CO}_2$ effects on coccolithophores

M. N. Müller et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

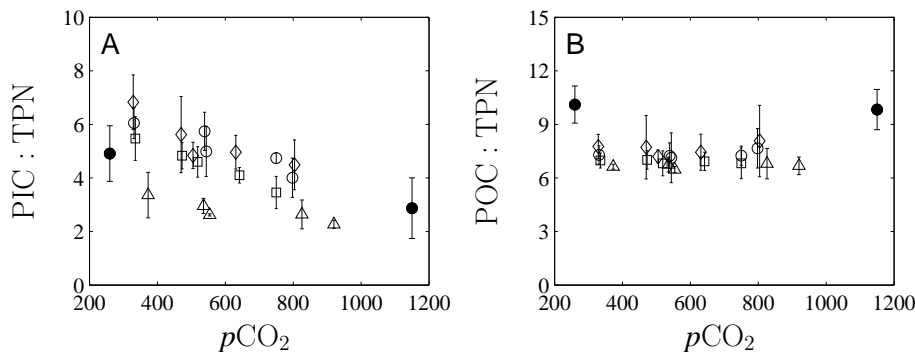
Printer-friendly Version

Interactive Discussion



# Long-term CO<sub>2</sub> effects on coccolithophores

M. N. Müller et al.



**Fig. 5.** Particulate carbon to nitrogen ratios of *E. huxleyi* as a function of  $p\text{CO}_2$  ( $\mu\text{atm}$ ) at a 24:0 light:dark cycle under various light intensities: 15 (triangle), 30 (square), 80 (circle) and  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (diamond). Open symbols represent data from Zondervan et al. (2002) (error bars represent 1SD,  $n=3$ ) and closed symbols indicate mean values of the present study with according standard deviations. **(A)** PIC:TPN (mol C:mol N). **(B)** POC:TPN (mol C:mol N).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

