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Short-term response of the coccolithophore *Emiliania huxleyi* to abrupt changes in seawater carbon dioxide concentrations

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Abstract

The response of the coccolithophore *Emiliania huxleyi* to rising CO_2 concentrations is well documented in acclimated cultures where cells are exposed to the CO_2 treatments for several generations prior to the experiment. Extended acclimation times have generally been applied because of the lack of information about time required to reach a new physiological "equilibrium" (acclimation) in response to CO_2-induced changes in seawater carbonate chemistry. Here we show that *Emiliania huxleyi*'s short-term response (hours to 1 day) to increasing CO_2 is similar to that obtained with acclimated cultures under comparable conditions in earlier studies. At CO_2 concentrations ranging from glacial (190 μatm) to projected year 2100 (750 μatm) levels, calcification decreased and organic carbon fixation increased within 8 h after exposing the cultures to the changed CO_2 conditions. This led to a decrease in the ratio of $CaCO_3$ to organic carbon production. Our results show that *Emiliania huxleyi* rapidly alters the rates of various essential processes in response to changes in seawater carbonate chemistry, establishing a new physiological (acclimation) "state" within a matter of hours. If this relatively rapid response applies to other phytoplankton species, it may simplify interpretation of studies with natural communities (e.g. mesocosm studies and ship-board incubations), where often it is not feasible to allow for a pre-conditioning phase before starting experimental incubations.

1 Introduction

Until the year 2100 atmospheric CO_2 concentration is expected, for a "business-as-usual" CO_2 emission scenario, to almost triple from pre-industrial values (IPCC, 2007), with a concomitant 45% decrease of CO_3^{2-} ion concentrations and a drop of 0.4 pH units in the surface ocean. Substantial effort has been undertaken to understand phytoplankton responses to these changes, with different laboratory approaches including incubations with dilute (Burkhardt et al., 1999; Riebesell et al., 2000a; Rost et al.,

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2003) and dense monoclonal batch cultures (Iglesias-Rodriguez et al., 2008), semi-continuous (Barcelos e Ramos et al., 2007; Fu et al., 2007; Xia and Gao, 2003) and chemostat (Sciandra et al., 2003) cultures, as well as ship-board incubations (Tortell et al., 2002; 2008) and mesocosm field experiments of natural populations (Delille et al., 2005; Engel et al., 2005; Riebesell et al., 2007).

Particular attention has been given to coccolithophores, a group of calcifying marine phytoplankton which was found to exhibit distinct sensitivity to ocean acidification. Members of this group, which is considered responsible for a significant fraction of the pelagic biogenic carbonate precipitation (Milliman, 1993), responded to CO_2 induced seawater acidification by changing cellular calcification rates. The best studied and probably most productive coccolithophore, *Emiliania huxleyi*, has generally been found to decrease its calcification rate in response to elevated CO_2 concentrations under nutrient and light replete conditions (Feng et al., 2008; Riebesell et al., 2000b; Zondervan et al., 2001).

All laboratory work on CO_2/pH sensitivity of *Emiliania huxleyi* so far have used cultures pre-exposed (acclimated) to the experimental CO_2 treatment. While a common acclimation period applied in these studies corresponds to about 9 to 12 generations (Riebesell et al., 2000b; Zondervan et al., 2002; Feng et al., 2008), the actual time needed for acclimation to elevated CO_2 is unknown. Acclimation period refers to the time necessary for individual cells to establish a new physiological "state" in response to a change in the environmental condition.

In cases where an individual's phenotypic plasticity (acclimation) and the population's genotypic variability are insufficient to maintain competitive fitness under changing environmental conditions, a species' survival may depend on its ability to adapt (Bell and Collins, 2008). Projecting a species' long-term response to environmental change therefore requires knowledge about both its acclimation and adaptation potential. Phenotypic plasticity responses to a changing environment may delay, favour or even speed up adaptive evolution (Ghalambor et al., 2007), further complicating attempts of predicting pathways of species evolution and ecosystem development un-

der changing environmental conditions. With regard to *Emiliania huxleyi* it is unknown what role the observed phenotypic plasticity will have in its response to the future CO_2 concentrations, neither if its natural populations will have the potential to adapt to the high CO_2 ocean. It is known that this species has high genetic variability, as reported for *Emiliania huxleyi* blooms (Medlin et al., 1996), but no evolutionary study with this species has been performed to date.

Studies with natural communities are a valuable approach to address questions related to species interactions in response to climate change. Indeed, diatom community's shifts in response to elevated CO_2 concentrations were described in phytoplankton assemblages from the Equatorial Pacific (Tortell et al., 2002) and Southern Ocean (Tortell et al., 2008). In recent mesocosm experiments the most pronounced CO_2 related effect was rather on inorganic carbon uptake and organic carbon loss from the upper water column (Schulz et al., 2008). These types of experiments are often conducted without prior acclimation of the enclosed communities to the CO_2 treatments. The time needed for phytoplankton physiology to respond to abrupt and drastic changes in seawater carbonate chemistry and to what extent this involves a temporary stress response are presently unknown. Considering the importance of studying the potential effects of rising CO_2 on natural communities (e.g. in mesocosm and shipboard incubations) and the relatively limited incubation time in these studies, a better understanding of the relevant time-scales in physiological processes of acclimation is urgently needed.

Thus, in this study *Emiliania huxleyi's* response to an abrupt change in CO_2 concentrations was followed during 26 h and the results were compared to those obtained for acclimated cultures in earlier studies. Furthermore, by following short-term cellular responses we investigate the acclimation time necessary for phytoplankton suddenly exposed to elevated CO_2.

2 Material and methods

2.1 Experimental setup

Monospecific cultures of the coccolithophore *Emiliania huxleyi* (strain isolated during 2005 mesocosm experiment in Bergen by M. N. Müller) were grown at a constant CO_2 concentration (approximately 495 μatm, with a corresponding pH_{total} value of 7.7) for a total of about 20 generations (3 consecutive semi-continuous batch cultures). These pre-cultures were continuously aerated with 0.2 μm filtered ambient (room) air (Rena Air50 aquarium pump), which allowed to grow the cultures to the cell abundance needed as inocula to start the experiment (2.1×10^6 cell ml^{-1}), without major shifts in the CO_2 level. However, while aeration replenishes dissolved inorganic carbon (DIC), calcification reduces total alkalinity (TA), resulting in a decrease in pH and carbonate saturation state (minimum of about 7.7 pH and 0.9 Omega, with a corresponding 495 μatm CO_2) at constant pCO_2. Thus, the carbonate system from both the last pre-culture and the experiment were monitored through TA and DIC measurements. Both pre-cultures and experimental cultures were grown in 0.2 μm sterile filtered North Sea water, at 15°C, a photon flux density of 150 μmol m^{-2} s^{-1} (supplied from cool white fluorescent bulbs, Philips TLD 36 W/54) and a 14/10 h light/dark cycle. Nutrient enrichment followed *f*/2 (Guillard, 1975; Guillard and Ryther, 1962) for the pre-cultures and *f*/20 (88 μmol l^{-1} nitrate and 3.6 μmol l^{-1} phosphate) for the experiment. The carbonate system of the media was adjusted shortly before the day of the experiment by addition of 1 molar NaOH or HCl, simulating well CO_2-induced changes in seawater carbonate chemistry (Schulz et al., 2009). For the experiment, cells were inoculated just before the beginning of the light phase to a starting concentration of about 3.5×10^4 cells ml^{-1} in each of the 4 CO_2 treatments ranging from minimum approximately 182 to maximum 1591 μatm. This corresponded to pH_{total} values ranging from 8.36 to 7.47 with a concomitant 8.5-fold increase in CO_2, a 1.1-fold increase in bicarbonate (HCO_3^-), a 7-fold decrease in carbonate (CO_3^{2-}) concentrations and a calcite saturation state rang-

ing from 7.6 to 1.1 (Table 1). The cell abundance chosen assures that less than 2% DIC was taken up by the cells during the experiment. After carefully mixing the culture inocula with the manipulated media, each CO_2 treatment was subdivided into smaller bottles for the determination of carbon fixation rate, carbonate chemistry, cell numbers and diameter and F_v/F_m. Additionally, samples were taken for scanning electron microscopy. Sampling occurred 2 h, 4 h, 8 h, 14 h, 24 h and 26 h after the start of the first light phase.

2.2 Carbonate system

CO_2 concentrations were calculated from temperature, salinity, phosphate, DIC and TA concentrations using CO2sys (Lewis and Wallace, 1998), with the equilibrium constants given in Roy et al. (1993). DIC was measured photochemically (Stoll et al., 2001) using an automated segmented-flow analyzer (Quaatro) equipped with an autosampler (+/−10 μmol kg^{-1} accuracy and 5 μmol kg^{-1} precision). DIC measurements were calibrated with certified reference material (Dickson standard). Alkalinity was measured according to Dickson et al. (2003) in duplicate (minimum) through potentiometric titration, using a Metrohm Titrando 808 with about 24 μmol kg^{-1} accuracy (calibration with Dickson standard) and 3.5 μmol kg^{-1} precision.

2.3 Carbon fixation

For each data point (time after onset of light: 2 h; 4 h; 8 h; 14 h; and 26 h) 6×65 ml culture flaks were spiked with 100 μl of a 1.85×10^{12} Bq H^{14} CO_3^- solution, of which 4 flasks were incubated under experimental conditions and 2 were kept in the dark. Radioactive label was added to the samples just before the light phases of both experiment days. Duplicate subsamples for total (25 ml) and organic (40 ml) particulate carbon, plus the corresponding darks were filtered onto cellulose acetate (0.45 μm) filters under low pressure (200 mbar). After filtration, 1 ml HCl (0.1 molar) was added to the particulate organic carbon filter (organic carbon fixation) for 30 s, assuring the

dissolution of all calcium carbonate (see Müller et al., 2008). Both filters were rinsed with 0.2 μm filtrated seawater to remove the excess of radioactive dissolved inorganic carbon. Lumagel Plus (Universal LSC cocktail) was then added to the filters in scintillation vials and the radioactivity measured in a Liquid Scintillation Analyser (Tri-Carb 2900TR, Packard). Particulate inorganic carbon fixation (calcification) was calculated as the difference between total carbon (not acidified filters) and organic carbon (acidified filters) fixation.

3 Cell diameter and numbers

Cell abundance and diameter were determined immediately after sampling at each time point by using a Coulter Counter Z series (Beckmann Coulter). Cell division rate (μ) was calculated according to:

$$\mu = (\ln \mathrm{Ce} - \ln \mathrm{Ci})/\Delta t \quad (1)$$

where Ce and Ci refer to end and initial cell concentrations, respectively and Δt to the duration of the experiment in days.

3.1 Maximum photochemical quantum yield of photosystem II (F_v/F_m)

Photosynthetic efficiency was determined as F_v/F_m by using a PAM (PhytoPAM, Phyto-ED Walz, PPAA0138) after a 20 min dark incubation.

3.2 Scanning electron microscopy (SEM)

SEM samples were fixed with formaldehyde (1% final concentration) at each time point. Samples were then filtrated onto polycarbonate filters (0.45 μm pore size) under low pressure (<200 mbar), dried for 12 h at 60°C and glued on aluminium stabs. The filters

were coated with gold-palladium and photographs of the most representative specimens taken with a CamScan-CS-44 (Scanning electron microscope) at the Institute of Geosciences of the Christian Albrechts University in Kiel.

4 Results

After 8 h of exposure to the experimental CO_2 levels (~190 to 1500 μatm) cumulative organic carbon fixation in *Emiliania huxleyi* showed an increasing trend with CO_2 concentration (Fig. 1a). The opposite trend, a decrease with increasing CO_2 was obtained for cellular calcification (Fig. 1b). Due to a stronger decrease in calcification compared to the increase in organic carbon fixation the cumulative total carbon fixation decreased with rising CO_2 (Fig. 1c). Carbon fixation rates were also determined for each period between 2 consecutive sampling points. From 4 to 8 h after the inoculation, organic carbon fixation rate increased 35% from the lowest to the highest CO_2 level (Fig. 2a). For the same period of time, this corresponded to a 19% decrease in the calcification rate from 190 μatm to approximately 800 μatm and 44% from 190 to 1500 μtam (Fig. 2b). Total carbon fixation rates increased during the whole light phase. After 26 h, at the beginning of the new light phase, carbon fixation rates were again at the low levels measured at the start of the experiment (Fig. 2). At this point, organic carbon fixation rates slightly increased and calcification rates slightly decreased from 190 to 1500 μtam.

The ratio of calcification to organic carbon fixation (Calcification/OC_{fix}) decrease with rising CO_2 (Fig. 1d) became evident about 8 h after the inoculation. This trend is maintained even after the start of the next light phase, even though with a smaller slope and absolute values. Scanning electron microscopy after 8 h and 26 h reveals some under-calcified coccoliths on cells exposed to high CO_2 concentration (Fig. 3). The under-calcified coccoliths are mostly in the layer closest to the cells surface, as is expected for newly produced coccoliths. After 8 h the under-calcified coccoliths of the 1500 μtam CO_2 treatment were mostly observed in smaller cells, because it is on those

that the most recently formed layer becomes visible. The 800 μtam CO_2 treatment showed only slight under-calcification both 8 and 26 h after the manipulation.

Cell division rate decreased from 1.01 at ~190 μtam to 0.90 at ~1500 μtam (Fig. 4). Cell size increased during the light phase, with a weak trend of decreasing cell diameters with increasing CO_2 concentrations at the end of the light phase (Fig. 5a). This trend was reversed after cell division. F_v/F_m increased during the light phase and was lower at low CO_2, maintaining the same trend at the beginning of the following light phase (Fig. 5b).

5 Discussion

5.1 From short-term to acclimated response

The effect of increasing CO_2 concentrations in the ocean has generally been assessed by the physiological response of acclimated phytoplankton cultures (from days to weeks). However, virtually nothing is known about their short (within 24 h) and long-term (months to years) response.

5.1.1 Calcification

Our results showed that within hours after the high CO_2 exposure the calcification response of non-acclimated *Emiliania huxleyi* is similar to that observed in acclimated cultures (Riebesell et al., 2000b), under the same light irradiance (150 μmol m^{-2} s^{-1}), temperature (15°C), similar CO_2 range (~190 to 800 μatm) and L/D cycle (in this study 14/10 while other 16/8). In fact, after 8 h we found a 19% decrease in calcification with rising CO_2 concentrations which compares well with the 15.7% found by Riebesell et al. (2000b). In terms of the absolute values, calcification was slightly higher in this compared to the previous study.

Remarkably the decrease in calcification could be seen with scanning electron microscopy already after short-term exposure to high CO_2. Cells grown under elevated

CO_2 levels showed increased numbers of incomplete or under-calcified coccoliths. However, because newly formed coccoliths are positioned at the cell surface and were therefore hidden by a second layer of coccoliths formed under pre-experimental conditions (approx. 1 coccolith per hour, Paasche, 2002), a systematic analysis of the degree of calcification and the frequency of malformations was not possible in this short-term incubation.

5.1.2 Organic carbon fixation and F_v/F_m

As previously reported, elevated CO_2 stimulated organic carbon fixation, although the effect was almost 3-fold higher than observed in an earlier study (Riebesell et al., 2000b). In agreement with the increase of organic carbon fixation rates under enhanced CO_2 conditions there was an increase of the maximum photochemical quantum yield of photosystem II (F_v/F_m) during the first 14 h. After cell division, cells exposed to the "present" CO_2 condition seemed to have the highest F_v/F_m. Interestingly, in cells subjected to a decrease in the CO_2 concentration F_v/F_m decrease within a short period of time and did not recover in the next 26 h. F_v/F_m is lower when the electrons can not be transported as fast as their production. In this case, a decrease in organic carbon fixation rate due to a change in CO_2 supply might be faster then the re-organization of the Calvin-Benson Cycle substrates, with consequent "clogging" of the electron transport chain.

5.1.3 Calcification/OC_{fix}

There was a decrease in the Calcification to OC_{fix} ratio (already 8 h after manipulation) like in previous studies with acclimated cultures within a similar CO_2 range (Riebesell et al., 2000b; Zondervan et al., 2001). However, there was an overall higher Calcification/OC_{fix} which can be explained by higher calcification rates in this study, since the organic carbon fixation was quite similar to that in a previous study (Riebesell et al., 2000b). Interestingly, the decrease of the Calcification/OC_{fix} ratio after the start

of the next light phase had a less pronounced slope with rising CO_2.

5.1.4 Diel cycle

The diurnal variation of cellular calcification and organic carbon fixation was higher than the differences encountered between the CO_2 levels ranging from ~190 to 1500 μatm. This highlights the importance of the timing of sampling during experiments. For most of the time considered, both our study and Zondervan et al. (2002) show higher organic carbon fixation and lower calcification at enhanced CO_2 concentrations. Unlike Zondervan et al. (2002), however, Calcification/OC_{fix} did not continuously decrease during the light phase, but increased in all CO_2 treatments towards the end of the light phase. An explanation for the disparity in results might be that here an extra sampling point was taken closer to the dark phase.

5.2 Cell division rate and diameter

While cell division of *Emilinia huxleyi* was not found to be affected by elevated CO_2 concentrations in previous studies (Buitenhuis et al., 1999; Clark and Flynn, 2000; Rost et al., 2002) a slight decrease in cell division rate with rising CO_2 was observed in this investigation. This difference may be due to the broader range of CO_2 levels applied here. We do not expect the CO_2 effect on cell division rate to be a short-term stress response caused by changing the CO_2 manipulation procedure (aeration in the pre-cultures and non-aeration in the experiment) or other factors derived from the experimental procedure because cell division rate of the 410 μatm treatment (1.01 d^{-1}) was similar to that of the pre-cultures (1.02 d^{-1} ±0.09, 4 replicates) exposed to similar CO_2 conditions. Moreover, a similar effect on cell division rate was also found during a long-term (>100 generations) high CO_2 exposure by M. N. Müller (personal communication, 2009), indicating that the observed response was unrelated to the abrupt change in CO_2 concentrations applied in this approach. The opposite trend in cell division rate with rising CO_2 concentration has been observed in other phytoplankton groups, such

as diatoms (Riebesell et al., 1993) and the cyanobacterium *Trichodesmium* (Barcelos e Ramos et al., 2007; Hutchins et al., 2007; Levitan et al., 2007). The apparent difference in specific growth rate responses between various taxonomic groups may be related to the process of calcification, but further investigation is needed to clarify this.

In this study, the cell diameter decreased with increasing CO_2 concentration during the first 14 h. This is most likely due to a more pronounced decrease in calcification than the increase of organic carbon fixation with a consequent decrease in the cellular total carbon. After the dark period, when most cells had divided, on average cells exposed to elevated CO_2 levels had a larger cell diameter. This may be due to the slightly lower cell division rate of high CO_2 exposed cells resulting in a larger number of cells which had not yet undergone cell division. Lower cell diameters at the beginning of the experiment in all treatments may have resulted from higher coccolith detachment due to aeration of the pre-culture.

5.3 CO_2 and pH, a combined effect

Rising CO_2 concentration in the ocean also changes pH, $[HCO_3^-]$ and $[CO_3^{2-}]$, so it is hard to separate the potential effect of each parameter individually. Maintaining a high concentration of CO_2 at the site of carboxilation to ensure efficient operation of the CO_2 fixing enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) is an energy demanding processe. A CO_2 increase in the surrounding environment of a cell is likely to decrease the net diffusive efflux of CO_2, reducing the energy needed to maintain high CO_2 inside the cell. The lower energetic cost may be used to increase organic carbon fixation. As for calcification, the decrease in the calcite and aragonite saturation states has been connected to the observed decrease in calcification in foraminifera (Bijma et al., 1999) and corals (Langdon et al., 2000; Leclercq and Gattuso, 2002; Leclercq et al., 2000). As coccolithophore calcification occurs intracellularly and there is no evidence of CO_3^{2-} utilization or any known CO_3^{2-} transporters, the observed response may rather reflect sensitivity to a decrease in pH, associated with increased energetic costs of transporting protons generated during calcification

outside the cell.

Based on the observed increase of organic carbon production at high CO_2 concentration one might expect a concomitant increase in cell division rate, but a slight decrease was observed instead. This effect on cell division rate could be a direct consequence of changing seawater pH, affecting cellular acid-base regulation. In a study on 3 red-tide dinoflagellates Hansen et al. (2007) concluded that growth is mostly affected by pH and that inorganic carbon only plays a minor role under low initial dissolved inorganic carbon concentrations and high pH.

Whatever parameter or combination of parameters influences the different cellular rates, here the cells showed a fast physiological adjustment potentially at the expense of intracellular regulation of DIC content and pH. This possibly happened at the regulation level of both transporters in the membrane and electron chain, and/or enzymes.

5.4 Short (acclimated) to long-term experiments, stepping stones in the understanding of the effect of future climate change

Experiments done with acclimated cells often looked at how the individuals of a clonal culture respond to the projected changes in CO_2 concentrations. *Emiliania huxleyi* acclimated (already after hours) to increasing CO_2 concentrations decreased calcification and increased organic carbon fixation rates in several studies (this study; Feng et al., 2008; Riebesell et al., 2000b; Zondervan et al., 2001). While evolutionary adaptation to increasing CO_2 concentrations has so far not been addressed in *Emiliania huxleyi,* helpful information can be obtained from work done with the plant *Arabidopsis thaliana* (Lau et al., 2007), the alga *Chlamydomonas* (Collins and Bell, 2004) and natural populations from CO_2 springs (Collins and Bell, 2006). Both species referred and natural populations from CO_2 springs showed phenotypic changes with increased CO_2 treatments, but no adaptation (e.g. correlations between CO_2 treatment and genetic patterns, heritability). Still, these phenotypic changes might favour or even speed up adaptive evolution. The lack of indications for adaptation reinforces, on the one hand, the importance to further study phenotypic plasticity changes (acclimation) with rising

CO_2 and, on the other hand, to re-evaluate the long-term experimental designs. Some long-term experiments consider that after 1000 generations there is enough genetic variability so that the culture is not clonal anymore and, therefore, can be treated as a population (Collins et al., 2006). Nevertheless, future long-term experiments could allow for more genetic variability by using several clones, preferentially freshly isolated from the same location, and/or inducing sexual reproduction. It is also important to include some CO_2 variability in these experimental setups, since phytoplankton in its natural environment will not evolve under constant CO_2 concentration, but to an average higher concentration with abrupt changes through time. The daily and seasonal changes of CO_2 concentration will be even more pronounced in the future, due to decreasing ocean buffer capacity. Moreover, one has to start considering in both acclimated and long term experiments, that phytoplankton will be exposed to a combined CO_2, temperature and potentially nutrient composition/availability change.

In summary, short/acclimated and long-term experiments provide complementary information about the phytoplankton response to increasing CO_2 or to a combined effect. Ideally, while the short-term approach identifies species phenotypic plasticity, long-term experiments aim to help understanding the adaptation potential to the future ocean.

6 Conclusions

With this work we were able to show that the response of acclimated cultures to rising CO_2 corresponds to establishing a new physiological "equilibrium" through the change of rates of various essential processes, which *Emiliania huxleyi* cells appear to achieve in less than 24 h. This implies that the cellular adjustment to increasing CO_2 concentrations is independent of cell division. If this relatively rapid response applies to other phytoplankton species, it might simplify the interpretation of studies with natural communities (e.g. mesocosm studies and ship-board incubations), where often it is not feasible to allow for a pre-conditioning phase before starting experimental incubations.

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References

Barcelos e Ramos, J., Biswas, H., Schulz, K. G., LaRoche, J., and Riebesell, U.: Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*, Global Biogeochem. Cy., 21, GB2028, doi:2010.1029/2006GB002898, 2007.

Bell, G. and Collins, S.: Adaptation, extinction and global change, Evolutionary Applications, Blackwell Publishing Ltd., 3–16, 2008.

Bijma, J., Spero, H. J., and Lea, D. W.: Reassessing foraminiferal stable isotope geochemistry: impact of the oceanic carbonate system (experimental results), in: Use of proxies I paleoceonagraphy- Exemples from the South Atlantic, edited by: Fischer, G. and Wefer, G., Springer-Verlag, 1999.

Buitenhuis, E. T., De Baar, H. J. W., and Veldhuis, M. J. W.: Photosynthesis and calcification by *Emiliania huxleyi* (Prymnesiophyceae) as a function of inorganic carbon species, J. Phycol., 35, 949–959, 1999.

Burkhardt, S., Zondervan, I., and Riebesell, U.: Effect of CO_2 concentration on C:N:P ratio in marine phytoplankton: A species comparison, Limnol. Oceanogr., 44, 683–690, 1999.

Clark, D. R. and Flynn, K. J.: The relationship between the dissolved inorganic carbon concentration and growth rate in marine phytoplankton, Proc. R. Soc. Lond., 267, 953–959, 2000.

Collins, S. and Bell, G.: Evolution of natural algal populations at elevated CO_2, Ecol. Lett., 9, 129–135, 2006.

Collins, S., Sültemeyer, D., and Bell, G.: Changes in C uptake in populations of *Chlamydomonas reinhardtii* selected at high CO_2, Plant, Cell Environ., 29, 1812–1819, 2006.

Delille, B., Harlay, J., Zondervan, I., Jacquet, S., Chou, L., Wollast, R., Bellerby, R. G. J., Frankignoulle, M., Borges, A. V., Riebesell, U., and Gattuso, J.-P.: Response of primary production and calcification to changes of pCO_2 during experimental

blooms of the coccolithophorid *Emiliania huxleyi*, Global Biogeochem. Cy., 19, GB2023, doi:10.1029/2004GB002318, 2005.

Dickson, A. G., Afghan, J. D., and Anderson, G. C.: Reference materials for oceanic CO_2 analysis: a method for the certification of total alkalinity, Mar. Chem., 80, 185–197, 2003.

Engel, A., Zondervan, I., Aerts, K., Beaufort, L., Benthien, A., Chou, L., Delille, B., Gattuso, J.-P., Harlay, J., Heemann, C., Hoffmann, L., Jacquet, S., Nejstgaard, J., Pizay, M. D., Rochelle-Newall, E., Schneider, U., Terbrueggen, A., and Riebesell, U.: Testing the direct effect of CO2 concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments, Limnol. Oceanogr., 50, 493–507, 2005.

Feng, Y. Warner, M. E:, Zhang, Y., Sun, J., Fu, F.-X., Rose, J. M., and Hutchins, D. A.: Interactive effects of increased pCO2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae), European J. Phycol., 43, 87–98, 2008.

Fu, F.-X., Warner, M. E., Zhang, Y., Feng, Y., and Hutchins, D. A.: Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine Synechococcus and Prochlorococcus (cyanobacteria), J. Phycol., 43, 485–496, 2007.

Ghalambor, C. K., McKay, J. K., Carroll, S. P., and Reznick, D. N.: Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments, Funct. ecol., 21, 394–407, 1975.

Guillard, R. R. L.: Culture of phytoplankton for feeding marine invertebrates, Plenum Press, 1975.

Guillard, R. R. L. and Ryther, J. H.: Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea*, Cleve. Can. J. Microbiol., 8, 229–239, 1962.

Hansen, P. J., Lundholm, N., and Rost, B.: Growth limitation in marine red-tide dinoflagellates: effects of pH versus inorganic carbon availability, Mar. Ecol. Progress Ser., 334, 63–71, 2007.

Hutchins, D. A., Fu, F.-X., Zhang, Y., Warner, M. E., Feng, Y., Portune, K., Bernhardt, P. W., and Mulholland, M. R.: CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present and future ocean biogeochemistry, Limnol. Oceanogr., 52, 1293–1304, 2007.

Iglesias-Rodriguez, M. D., Halloran, P. R.,Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R. H., Tyrrell, T., Gibbs, S. J., von Dassow, P., Rehm, E., Armbrust, E. V., and Boessenkool, K. P.: Phytoplankton calcification in a high-CO_2 world, Science, 320, 336–340, 2008.

IPCC: Climate change 2007: The physical basis, in: Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp., 2007.

Langdon, C., Takahashi, T., Sweeney, C., Chipman, D., Goddard, J., Marubini, F., Aceves, H., Barnett, H., and Atkinson, M. J.: Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef, Global Biogeochem. Cy., 14, 639–654, 2000.

Lau, J. A., Shaw, R. G., Reich, P. B., Shaw, F. H., and Tiffin, P.: Strong ecological but weak evolutionary effects of elevated CO_2 on a recombinant inbred population of *Arabidopsis thaliana*, New Phytologist, 175, 351–362, 2007.

Leclercq, N. and Gattuso, J.-P.: Primary production, respiration, and calcification of a coral reef mesocosm under increased CO_2 partial pressure, Limnol. Oceanogr., 47, 558–564, 2002.

Leclercq, N., Gattuso, J. P., and Jaubert, J.: CO_2 partial pressure controls the calcification rate of a coral community, Glob. Change Biol., 6, 329–334, 2000.

Levitan, O., Rosenberg, G., Setlik, I., Setlikova, E., Grigel, J., Klepetar, J., Prasil, O., and Berman-Frank, I: Elevated CO_2 enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium,* Glob. Change Biol., 13, 531–538, 2007.

Lewis, E. and Wallace, D. W. R.: Program Developed for CO_2 System Calculations, ORNL/CDIAC-105, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, 1998.

Medlin, L. K., Barker, G. L. A., Campbell, L., Green, J. C., Hayes, P. K., Marie, D., Wrieden, S., and Vaulot, D.: Genetic characterisation of *Emiliania huxleyi* (Haptophyta), J. Mar. Syst., 9, 13–31, 1996.

Milliman, J. D.: Production and accumulation of calcium carbonate in the ocean: Budget of a nonsteady state, Glob. Biogeochem. Cy., 7, 927–957, 1993.

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.

Paasche, E.: A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification- photosynthesis interactions, Phycologia, 40, 503–529, 2002.

Riebesell, U., Burkhardt, S., Dauelsberg, A., and Kroon, B.: Carbon isotope fractionation by a marine diatom: dependence on the growth-rate-limiting resource, Mar. Ecol. Progress Ser.,

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193, 295–303, 2000a.
Riebesell, U., Schulz, K. G., Bellerby, R. G. J., Botros, M., Fritsche, P., Meyerhöfer, M., Neill, C., Nondal, G., Oschlies, A., Wohlers, J., and Zöllner, E.: Enhanced biological carbon consumption in a high CO_2 ocean, Nature, 450, 545–548, 2007.
Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V.: Carbon dioxide limitation of marine phytoplankton growth rates, Nature, 361, 249–251, 1993.
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_2, Nature, 407, 364–367, 2000b.
Rost, B., Riebesell, U., and Burkhardt, S.: Carbon acquisition of bloom-forming marine phytoplankton, Limnol. Oceanogr., 48, 55–67, 2003.
Rost, B., Zondervan, I., and Riebesell, U.: Light-dependent carbon isotope fractionation in the coccolithophorid *Emiliania huxleyi*, Limnol. Oceanogr., 47, 120–128, 2002.
Roy, R. N., Roy, L. N., Vogel, K. M., Porter-Moore, C., Pearson, T., Good, C. E., Millero, F. J., and Cambel, D. J.: 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.
Schulz, K. G., Riebesell, U., Bellerby, R. G. J., Biswas, H., Meyerhöfer, M., Müller, M. N., Egge, J. K., Nejstgaard, J. C., Neill, C., Wohlers, J., and Zöllner, E.: Build-up and decline of organic matter during PeECE III, Biogeosciences, 5, 707–718, 2008, http://www.biogeosciences.net/5/707/2008/.
Schulz, K. G., Barcelos e Ramos, J., Zeebe, R. E., and Riebesell, U.: CO_2 perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations, Biogeosciences Discuss., 6, 4441–4462, 2009, http://www.biogeosciences-discuss.net/6/4441/2009/.
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_2 under nitrogen limitation, Mar. Ecol. Prog. Ser., 261, 111–122, 2003.
Stoll, M. M. C., Bakker, K., Nobbe, G. H., and Haese, R. R.: Continuous-flow analysis of dissolved inorganic carbon content in seawater, Anal. Chem., 73, 4111–4116, 2001.
Tortell, P. D., Ditullio, G. R., Sigman, D. M., and Morel, F. M. M.: CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage, Marine Ecology Progress Series, 236, 37–43, 2002.
Tortell, P. D., Payne, C. D., Li, Y., Trimborn, S., Rost, B., Smith, W. O., Riesselman, C., Dunbar,

R. B., Sedwick, P., and DiTullio, G. R.: CO_2 sensitivity of Southern Ocean phytoplankton, Geophys. Res. Lett, 35, L04605, doi:10.1029/2007GL032583, 2008.

Xia, J. and Gao, K.: Effects of doubled atmospheric CO_2concentration on the photosynthesis and growth of *Chlorella pyrenoidosa* cultured at varied levels of light, Fisheries Science, 69, 767–771, 2003.

Zondervan, I., Rost, B., and Riebesell, U.: Effect of CO_2 concentration on the PIC/POC ratio in the coccolithophore *Emiliania huxleyi* grown under light-limiting conditions and different daylengths, J. Exp. Mar. Biol. Ecol., 272, 55–70, 2002.

Zondervan, I., Zeebe, R. E., Rost, B., and Riebesell, U.: Decreasing marine biogenic calcification: A negative feedback on rising atmospheric pCO_2, Global Biogeochem. Cy., 15, 507–516, 2001.

Table 1. Carbonate system data determined from total alkalinity (TA) and dissolved inorganic carbon (DIC) at 15°C, 34 salinity and 3.4 μmol l^{-1} phosphate using CO2sys (Lewis and Wallace, 1998) with the equilibrium constants given in Roy et al. (1993). While all TA values were measured, meaningful values of DIC could only be obtained for the initial water (start) due to storage problems of the remaining samples (calculated DIC in italic). Hence, DIC at 14 h and 26 h were estimated from organic and inorganic carbon fixation (see Fig. 1) and the assumption that respiration during the night and organic carbon fixation in the first 2 h of the following day were cancelling each other. Thus, DIC drawdown after 26 h was corrected for inorganic carbon fixation only from the difference in TA (14 to 26 h).

Sample	Timing	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	pCO_2 (μatm)	pH_{free}	pH_{total}	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	CO_2 (μmol kg^{-1})	Omega for calcite
pre-culture	start	2328	2135	502	8.05	7.97	1971	145	19	3.5
pre-culture	end	1084	1029*	495	7.75	7.67	975	36	19	0.9
exp.	0 h	2558	2135	203	8.41	8.33	1822	305	8	7.3
exp.	0 h	2359	2135	432	8.11	8.04	1954	165	16	4.0
exp.	0 h	2228	2135	899	7.81	7.73	2017	85	34	2.0
exp.	0 h	2150	2135	1588	7.56	7.49	2027	48	60	1.2
exp.	14 h	2511	2071	182	8.44	8.36	1750	315	7	7.6
exp.	14 h	2305	2068	386	8.14	8.07	1882	172	15	4.1
exp.	14 h	2183	2069	750	7.87	7.79	1946	95	28	2.3
exp.	14 h	2106	2081	1435	7.60	7.52	1976	51	54	1.2
exp.	26 h	2490	2060	186	8.43	8.35	1746	307	7	7.4
exp.	26 h	2263	2047	418	8.12	8.03	1874	157	16	3.8
exp.	26 h	2174	2065	772	7.86	7.78	1944	92	29	2.2
exp.	26 h	2077	2066	1591	7.55	7.47	1961	45	60	1.1

*Estimated from TA and pH measured through potentiometric titration.

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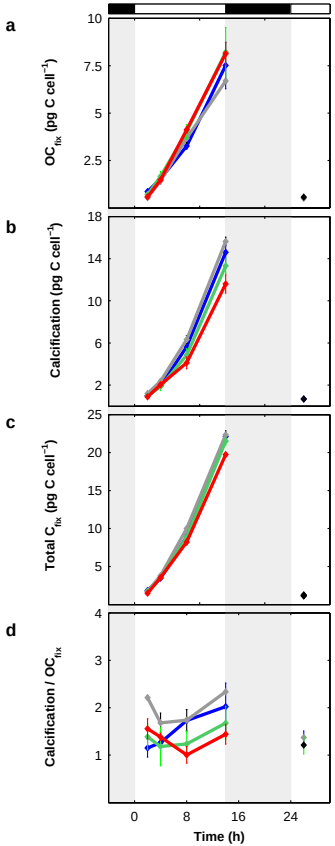

Fig. 1. Cumulative carbon fixation of *Emiliania huxleyi* through time. **(a)** organic carbon fixation per cell, **(b)** calcification per cell and **(c)** total carbon fixation per cell. 190 μatm CO_2 (blue), 410 μatm CO_2 (grey), 800 μatm CO_2 (green), 1500 μatm CO_2 (red). Data from the 26 h considers only a 2 h incubation period. Each CO_2 level has duplicate measurements. Vertical error bars represent the range of the data and the lines connect the averages of each time point. The white/black bar on top represents the light/dark diel cycle, vertical grey bars denote the dark phase.

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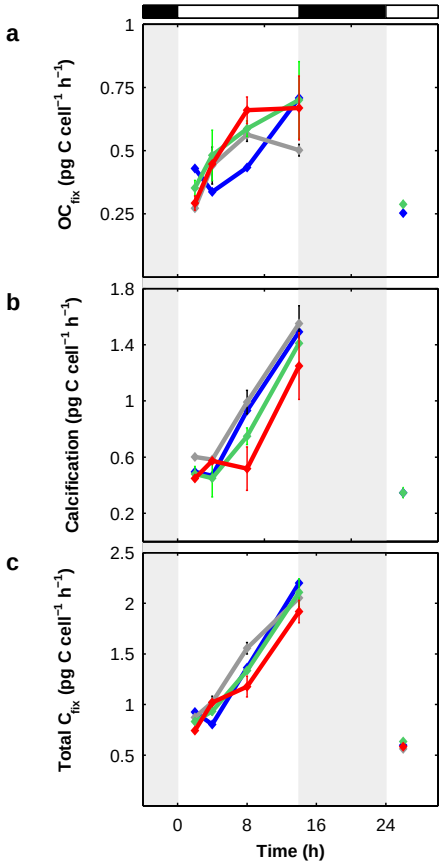

Fig. 2. Carbon fixation rates of *Emiliania huxleyi* determined for each period of time between consecutive sampling points. **(a)** organic carbon fixation per cell per h, **(b)** calcification per cell per h and **(c)** total carbon fixation per cell per h. For each period of time the data point marks the end of the incubation. Each CO_2 level has duplicate measurements. Vertical error bars represent the range of the data and the lines connect the averages of each time point. Line and color coding as in Fig. 1.

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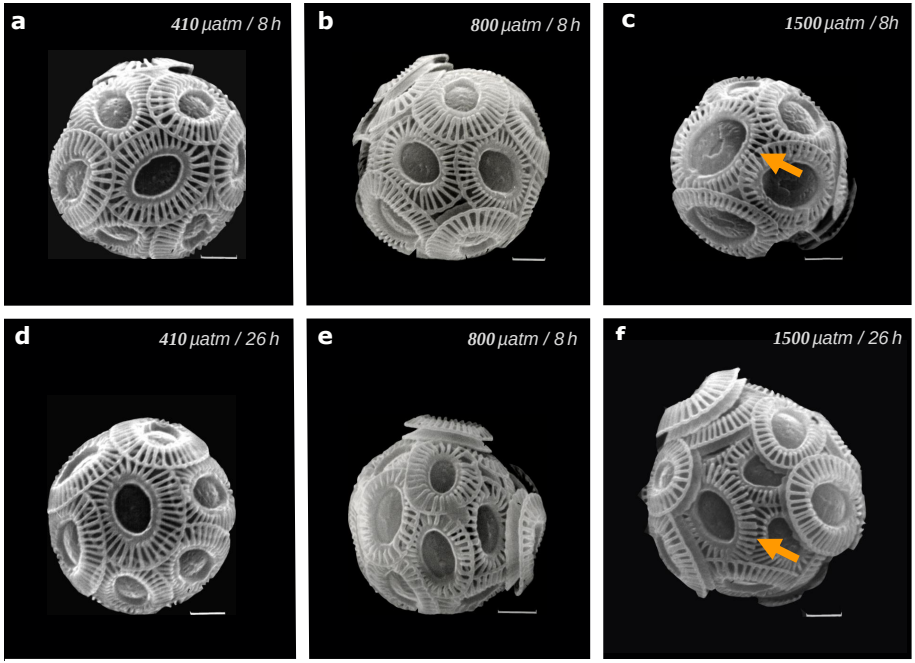

Fig. 3. Scanning electron microscope pictures of *Emiliania huxleyi* grown under different CO_2 concentrations after 8 h of exposure to **(a)** 410 μatm, (**b** and **e**) 800 μatm and **(c)** 1500 μatm and after 26 h of exposure to **(d)** 410 μatm and **(f)** 1500 μatm. The photos chosen are representative of the trend observed. Note the presence of under-calcified coccoliths under enhanced CO_2 conditions, especially visible in the connections between the elements forming the "outer ring" (orange arrows) and in the frequent enlargement of the central area. For the 800 μatm treatment both photographs correspond to cells exposed to the increase on CO_2 concentrations for 8 h, because no differences were found within the time considered (8 and 26 h) and the photographs taken after 26 h were not well focused. Scale bars correspond to 1 μm.

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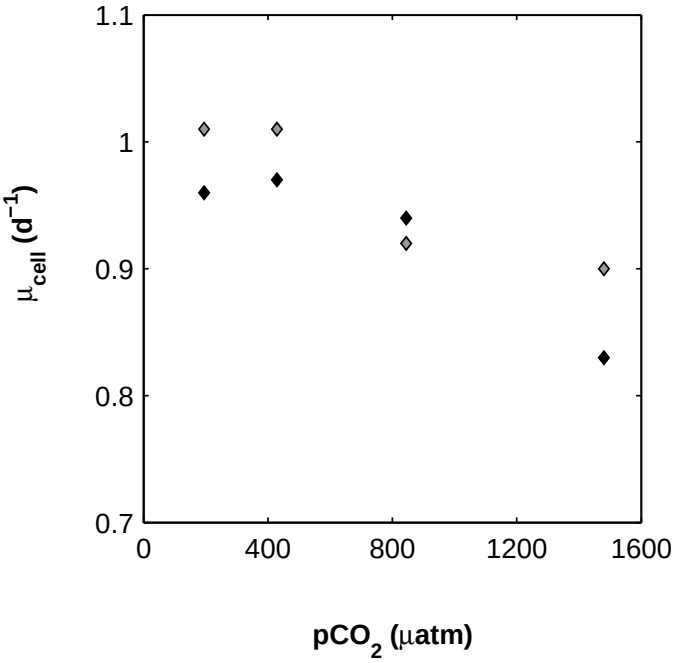

Fig. 4. Cell division rate based on cell counts (μ) of *Emiliania huxleyi* in relation to CO_2 levels (pCO_2). Black diamonds correspond to measurements done at the time of CO_2 manipulation and beginning of the light phase (0 h to 24 h), grey diamonds correspond to measurements done 2 h after that (2 h to 26 h).

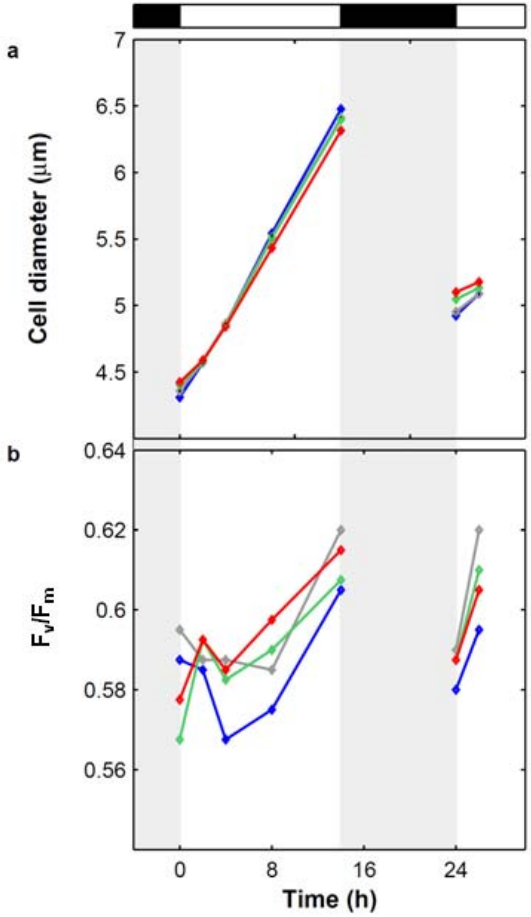

Fig. 5. **(a)** Cell diameter and **(b)** F_v/F_m of *Emiliania huxleyi* through time. In Fig. 6a each CO_2 level has 4 measurements from each bottle with vertical error bars representing standard errors. 190 μatm CO_2 (blue), 410 μatm CO_2 (grey), 800 μatm CO_2 (green), 1500 μatm CO_2 (red). The white/black bar on top represents the light/dark diel cycle, vertical grey bars denote the dark phase.

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