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Calcification in coccolithophores: Effects of environmental conditions and paleoproxy calibrations

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Der Dekan

Dedicated to my family ’Erhard, Marion, Mark, Mareike and Tian’

Hiermit erkläre ich an Eides statt, dass die vorliegende Dissertation, abgesehen von der Beratung meiner wissenschaftlichen Betreuer, nach Inhalt und Form meine eigene Arbeit ist. Desweiteren erkläre ich, dass mit dieser Arbeit noch kein Promotionsversuch meiner Seite unternommen wurde.

Marius N. Müller

Contents

1 Abstract

The present thesis investigates the influence of various environmental parameters on the physiology of coccolithophores, focusing on the process of biogenic calcification in regard to variations in the ocean's calcium and magnesium concentrations, as well as to changes in the macronutrient concentrations and in the seawater carbonate chemistry.

Oceanic calcium and magnesium concentrations changed drastically since the appearance of coccolithophores 225 million years ago. By producing massive depositions of calcareous sediments during the Cretaceous (145-65 Ma), coccolithophores played an active role in this major change in ocean chemistry. A recent hypothesis claims that the low seawater Mg/Ca ratio at that era (≈ 1 compared to 5 in the present ocean) induced enhanced reproduction and calcification of coccolithophores. The results presented within this thesis reveal, however, that this hypothesis is not valid for the two coccolithophore species *Emiliania huxleyi* and *Coccolithus braarudii*. Physiological rates (division rate, photosynthesis, calcification and chlorophyll *a* production) were actually reduced (by up to 50%) in *E. huxleyi* at Cretaceous Mg/Ca ratios due to an increased seawater calcium concentration, whereas the response in the larger species *C. braarudii* was less pronounced. Furthermore, a decreased chlorophyll *a* production was associated with an increase of the magnesium to calcium ratio in the calcitic cover of the investigated coccolithophores. The calcium and magnesium isotopic signals of the coccoliths were determined and the physiological influence is discussed. The conclusions drawn emphazise the relevance of the physiology for magnesium based paleoproxies in coccolithophores, and it is revealed

that the seawater calcium concentration is the main factor influencing coccolithophorid physiology rather than the seawater magnesium concentration.

On short time scales (< 1000 years) the oceans' calcium and magnesium concentrations are constant, so that other environmental parameters primarily influence coccolithophorid calcification, like nutrient supply and light availability in the surface mixed layer.

Under nutrient and light replete conditions calcification in *E. huxleyi* occurs in a specific phase of the cellular division cycle, namely the G1 phase. When cellular growth was limited by nitrogen, cells decreased in size, remained in the G1 phase and showed a moderate increase in the cell-specific calcite content. Limitation of growth through phosphorus, however, caused an enlargement in cell size and a dramatic increase in cellular calcite content. Light limitation, by prolonging the time cells spent in the G1 phases, reduced the growth rate with a corresponding increase in the cellular calcite content. These results help to explain the differing responses of coccolithophorid growth and calcification to nitrogen, phosphorus and light limitation in the recent ocean.

The ongoing anthropogenic release of CO_2 to the atmosphere since the industrial revolution leads to major shifts in the carbonate chemistry of the ocean. Invasion of atmospheric CO_2 into the upper ocean consequently increases the aquatic CO_2 concentration and decreases the ocean's *p*H, termed as ocean carbonation/acidification. The effect of ocean acidification was tested on two coccolithophore species with regard to the immediate (hours to a day) and the long-term (days to months) response. The results were compared to previous published studies and revealed a consistent picture. A decreased cellular calcification rate, as reported in the literature, was measured after 8 hours to high CO_2 exposure and remained impaired over months of clonal reproduction. Furthermore, it was indicated that a gradual CO_2 increase did not alleviate observed *p*H/CO_2 sensitivities. Organic carbon production per biomass, on the other hand, remained constant or decreased (*E. huxleyi* and *C. braarudii*, respectively) under long-term high CO_2 exposure. These observations are consistent with those obtained from short-term experiments.

Oceanic production of organic carbon and $\mathrm{CaCO_3}$ by marine flora and fauna 'play' a major role in the Earth's cycling of carbon and calcium. Coccolithophores, as a major phytoplankton group, are involved in both cycles and are relevant in the regulation of the seewater calcium carbonate saturation state since the Mesozoic.

2 General introduction

Coccolithophores (one major group of marine phytoplankton) are unicellular protists, that belong to the phylum 'haptophyta' and are characterized by calcitic plates (coccoliths) surrounding the cell. Furthermore, the coccolithophores are morphologically subdivided by the architecture of the coccoliths into two groups, the holococcolithophores and heterococcolithophores, whereby the holococcolithophores are certainly the haploid (sexual stage with one chromosome set) life stage within the life cycle of the heterococcolithophores (two homologous copies of the chromosome set, Young et al. (2005)). Heterococcoliths are precipitated intracellular in a coccolith vesicle, actively transported and released to the cells surface. In contrast, evidence supports that the more simple structered holococcoliths are precipitated at the cells' surface (Young et al. 1999). However, in both ways the cell biologically controls the crystal growth direction, leading to a variety of coccolith morphostructures.

Since the evolution of coccolithophores (≈ 225 million years ago), most of the 4000 morphological diversified species are extinct today and about 280 species are described in the recent ocean, whereby 90 are holococcolithophores (Young et al. 2005). Only two species out of these 190 (excluding holococcolithophores) are capable to form massive blooms in the oceans' surface water, namely *Emiliania huxleyi* and *Gephyrocapsa oceanica*.

2.1 Coccolithophores and biogeochemical cycles

The ocean is a crucial part in the biogeochemical cycles of the Earth's elements and represents a considerable interface between the atmosphere and the lithosphere. Coccolithophores, as a major planktonic group, are involved in the cycling of several elements by uptake and incorporation into organic and inorganic matter or by gaseous release (e.g. O_2 and dimethyl sulfide). Biologically active elements (C, O, N, P, S, Fe) are cycled on short (days to a millenium) and geological time scales. Elements with a high oceanic residence time, like Ca and Mg (residence time of: 1 and 13 million years, respectively), are mainly modified in their oceanic concentration over geological time scales due to changes in seafloor spreading rates. In the following the carbon and the calcium cycles are emphasized. Both cycles are tightly linked to each other over geological time scales.

2.1.1 The carbon cycle

For the last 225 million years coccolithophores play a multiple role in the fate of inorganic and organic carbon in the Earth's system. Global carbon is stored in different reservoirs, which are coupled with each other on varying time scales. The biggest carbon reservoir on earth is the lithosphere with $600\,\mathrm{million\,Pg\,C}$, followed by the ocean with $40.000\,\mathrm{Pg\,C}$ (mainly in the form of DIC, 'dissolved inorganic carbon'), both reservoirs are connected by the sediment which contains $150\,\mathrm{Pg\,C}$. The terrestial biosphere contributes $2.000\,\mathrm{Pg\,C}$ to the global budget and the atmosphere adds $750\,\mathrm{Pg\,C}$, mainly in the form of carbon dioxide (CO_2). The concentration of CO_2 in the present atmosphere averages 380 parts per million and is steadily increasing due to anthropogenic activities such as fossil fuel combustion, deforestation, cement production and changes in land use by a rate of about $3.3\,\mathrm{Pg\,C\,year^{-1}}$. On short time scales (days to a millenium) the lithosphere is negligible for the fluxes of carbon in the system and thus the ocean plays a dominant role (replacing the lithosphere as the biggest carbon reservoir) in the fate of carbon. Coccolithophores contribute to the photosynthetic fixation of CO_2 in the euphotic zone of the upper ocean and the export of organic material into the deep ocean, which causes a draw

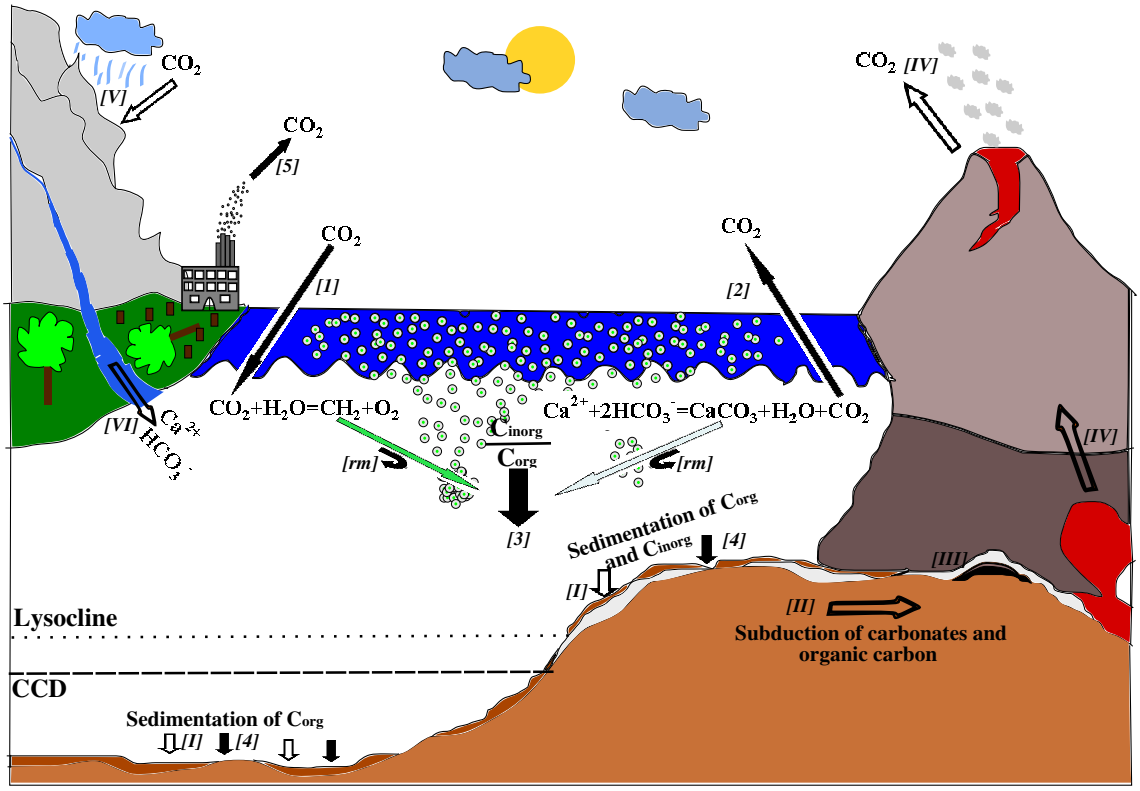

Figure 2.1: *Scheme of the global carbon cycle in relevance to the ocean. On short time scales the carbon cycle can be followed by the filled arrows: [1] Organic carbon pump; [2] inorganic carbon counter pump; [3] rain ratio; [4] sedimentation of organic and inorganic carbon; [5] anthropogenic release of* CO_2*. On geological time scales the carbon cycle can be followed by the open arrows: [I] Sedimentation of organic and inorganic carbon; [II] subduction of carbonates and organic carbon; [III] formation of carbonate rocks and fossil fuels; [IV] volcanism and release of* CO_2 *into the atmosphere; [V] weathering of carbonate rocks; [VI] river input of* HCO_3^-, Ca^{2+} *and nutrients. See text for details.*

down of CO_2 in the surface ocean, termed as the organic carbon pump (Fig. 2.1 *[1]*). On the other hand the biogenic production and export of calcium carbonate counteracts the photosynthetical draw down of CO_2 by altering the marine carbonate system towards a net release of CO_2 *[2]*. Parts of the biologically bound matter are remineralised and become available again for phytoplankton in the surface ocean *[rm]*. The strength of the CO_2 draw down from the upper into the deep ocean is described by the rain ratio ($C_{inorg} : C_{org}$ *[3]*). Thus, the rain ratio alters the flux of CO_2 between the atmosphere and the surface ocean by export of carbon to the deep ocean. Coccolithophores play an important role for the rain ratio, not only because of particulate organic and inorganic production but also

due to a ballast effect of accumulating coccolith-calcite with organic matter (Armstrong et al. 2002; Klaas and Archer 2002). Calcite enriched particles have an increased sedimentation speed and an elevated potential to sink to the deep ocean before they can be remineralised. As a consequence thereof, calcitic marine snow may increase the strength of the organic carbon pump (Hofmann and Schellnhuber 2009). Sedimentation of calcium carbonate dominantly occurs above the calcite compensation depth (CCD). It represents the depth at which the rate of calcium carbonate accumulation equals the rate of calcium carbonate dissolution. Dissolution of $\mathrm{CaCO_3}$ starts at the lysocline ($\approx 300-800\,\mathrm{m}$ above the CCD), where the water is undersaturated in respect to $\mathrm{CaCO_3}$. This is described by a calcite saturation state (Ω) below 1. Accumulation and formation time of sediments by coccolithophorid material varies from $10\,\mathrm{mm}$ to $100\,\mathrm{mm}$ per thousand years (Baumann et al. 2004).

Marine sediments form the interface between the ocean and the lithosphere. Continuous deposition and burial of calcerous material *[I]* occurs at $\approx 48\,\%$ of the ocean's floor (Rothschild 2005), even calcerous sediments are limited to depths above the CCD. Plate tectonic movements push the seafloor under continental margins at the site of subduction zones *[II]*. Forced deeper into the earth, the former marine sediments form carbonate rocks, mainly limestone, dolomite ($\mathrm{CaCO_3}$, $\mathrm{CaMg(CO_3)_2}$, respectively) and fossil fuels *[III]*. $\mathrm{CO_2}$ is released from the lithosphere primarily by volcanic activity *[IV]* and partly by non-volcanic degassing (Mörner and Etiope 2002). Tectonic forces uplift previously buried carbonate rocks to the Earth's surface where they are exposed to weathering. Chemical weathering of carbonate rocks withdraws $\mathrm{CO_2}$ from the atmosphere *[V]* and releases the ingredient ions (e.g. $\mathrm{Ca^{2+}}, \mathrm{HCO_3^-}, \mathrm{Si^{4+}}, \mathrm{Mg^{2+}}$) by rivers to the ocean, where the ions are utilized by phytoplankton. Humans interfere directly with the natural carbon cycle through the emission of $\mathrm{CO_2}$ from fossil fuel *[5]*, which originated over geological times.

2.1.2 The calcium cycle

Calcium is the fifth most abundant dissolved ion in modern seawater and termed as conservative, which implies that the concentration in seawater is not affected by changes in temperature, *p*H and pressure. The oceanic calcium cycle was believed to be in a steady state over geological time, but publications within the last decades revealed that this is not the case (De La Rocha and DePaolo 2000; Fantle and DePaolo 2005; Griffith et al. 2008; Lowenstein et al. 2001). The biogeochemical cycle of calcium is tightly linked to the inorganic carbon cycle and coupled to processes that control oceanic alkalinity and atmospheric CO_2 (see also Fig. 2.1). The concentration of calcium in the ocean is controlled by the balance of several processes, that are summarized in Table 2.1. Formation and burial of biogenic mediated calcium carbonate removes calcium from the ocean (1). Hydrothermal processes (2) and dolomitization (3) are a source of Ca^{2+} as well as the weathering of Ca-bearing rocks and subsequent riverine input (4+5).

Table 2.1: *Biogeochemical processes involved in the fate of calcium. See text for details.*

1	biogenic calcification	$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2$
2	hydrothermal processes	$Mg^{2+} + \mathrm{Ca\,basalt} \rightarrow Ca^{2+} + \mathrm{Mg - basalt}$
3	dolomitization	$2CaCO_3 + Mg^{2+} \rightarrow MgCa(CO_3)_2 + Ca^{2+}$
4	carbonate weathering	$CaCO_3 + H_2O + CO_2 \rightarrow Ca^{2+} + 2HCO_3^-$
5	silicate weathering	$CaSiO_3 + H_2O + 2CO_2 \rightarrow SiO2 + Ca^{2+} + 2HCO_3^-$

2.2 Coccolithophores in the paleo-ocean

The face of the Earth underwent major changes in geographic distribution and shape of land mass throughout the Mesozoic and Cenozoic era (the last 250 Ma). In the Late Jurassic, with Africa and North America drifting apart, the Atlantic Ocean began to expand. During the Cretaceous the Atlantic was extend by the Southern Atlantic Ocean forming a third ocean besides the Pacific and the Tethys ocean. India began its race towards Eurasia, creating the Indian ocean, while the Atlantic still continued to increase its size (Fig.: 2.2).

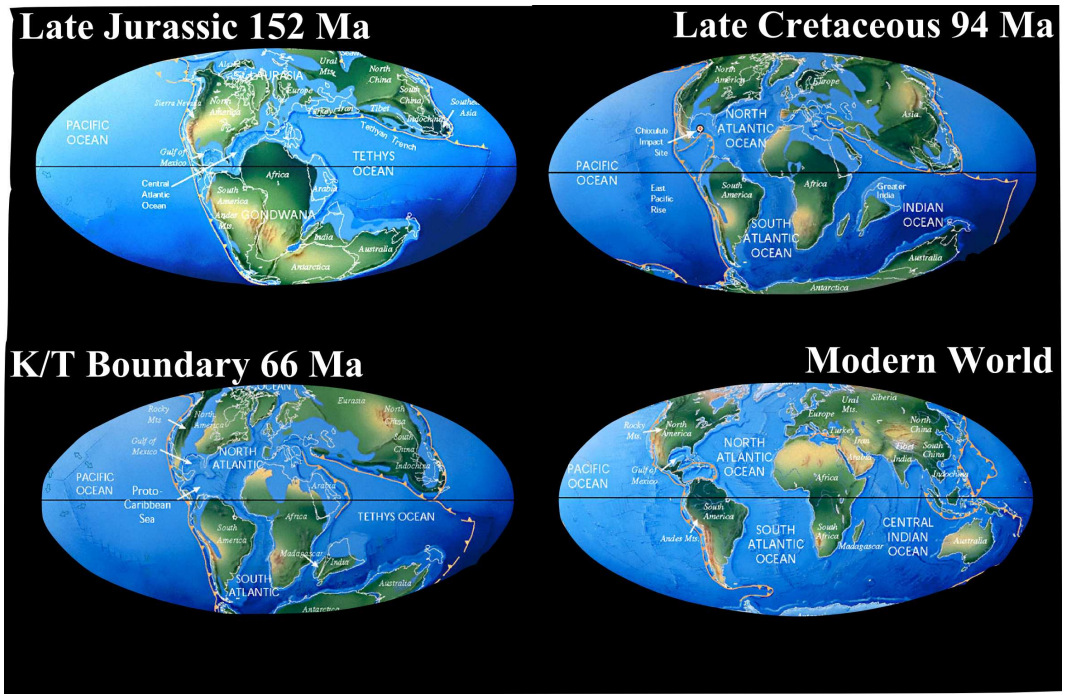

Figure 2.2: *Changes in land mass and ocean distribution since the rise of coccolithophores at the Jurassic/Cretaceous boundary. For a detailed explanation of the pictures please consider the 'Paleomap Project' by Christopher R. Scotese at http://www.scotese.com.*

The sediment record of coccolithophores goes back to the Triassic and experienced a remarkable increase in diversity during the Cretaceous (Fig.: 2.3). This increase in species diversity and abundance during the late Cretaceous is associated with a widespread and large deposition of calcerous material in the Earth's crust, which is visible today in calcerous rocks, like the white cliffs of Dover or in the chalk cliffs on Rügen. Even though coccoliths have been present in the sediment since 225 Ma, the first appearance of pelagic carbonate dominanted by nannofossil (coccoliths and calcerous flagelates) is dated to the Jurassic/Cretaceous boundary in the western Tethys ocean (Baumgartner 1987). The most obvious events in coccolithophore diversity on geological time are certainly the increase of diversity during the Cretaceous and the mass extinction event at the K/T (Cretaceous/Tertiary) boundary, where only 10% of all Late Cretaceous species remain present in the subsequent sediments (Bown et al. 2004; Bown 2005*b*). However, the species diversity of the sediment record is probably underestimated, since resent studies confirm

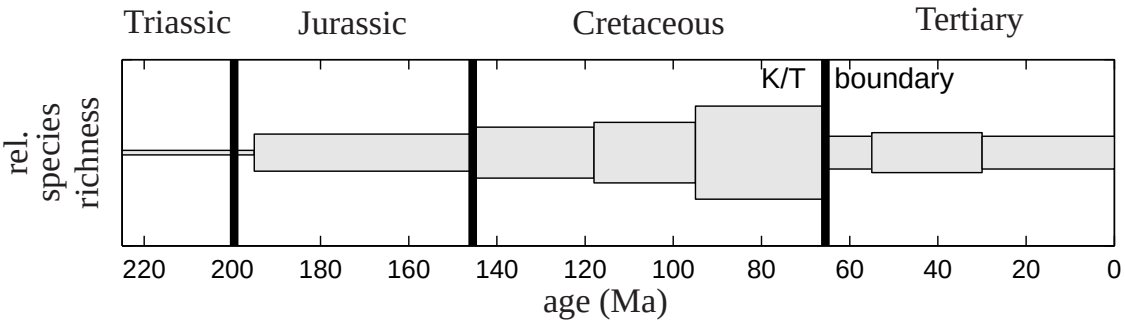

Figure 2.3: *Simplified coccolithophore relative species richness (indicated by the grey boxes) over the last 225 million years. For a detailed species diversity see Bown et al. (2004).*

that most small species (coccoliths diameter $< 2.5\,\mu\mathrm{m}$) are partly lost in present sediment traps and in Holocene (<1 Ma) core samples due to dissolution and diagenesis (Andruleit et al. 2004; Baumann et al. 2005). Additionally, well preserved sediment samples show a higher diversity of coccolithophores (Crudeli and Young 2003). Therefore, exact numbers of cretaceous coccolithophore species should be considered with care and used only as an indication for coccolithophore diversity.

2.2.1 Environmental conditions during the Cretaceous

The oceanic environment during the Cretaceous was quite different to the conditions coccolithophores currently face in the modern ocean. First of all the sea level was about $150\,\mathrm{m}$ higher than today (Ridgwell 2005), creating more shallow seas, and oceanic water temperature was $6 - 12^{\circ}\mathrm{C}$ warmer (Barrera and Johnson 1999). Warm and well stratified shallow seas during the Cretaceous are favorable conditions for a fast and massive deposition of coccolithophorid calcite, but since it is evident that seawater chemistry altered throughout geological time, it has been suggested that the changes in seawater $[\mathrm{Ca}^{2+}]$ and Mg/Ca ratio (Fig.: 2.4) are more important for coccolithophorid calcite production (Erba 2006; Brennan et al. 2004).

Seawater Na^{+} and Mg^{2+} concentrations were lower than in the modern ocean, whereas the Ca^{2+} concentration was approximately three times higher resulting in a Mg/Ca ratio

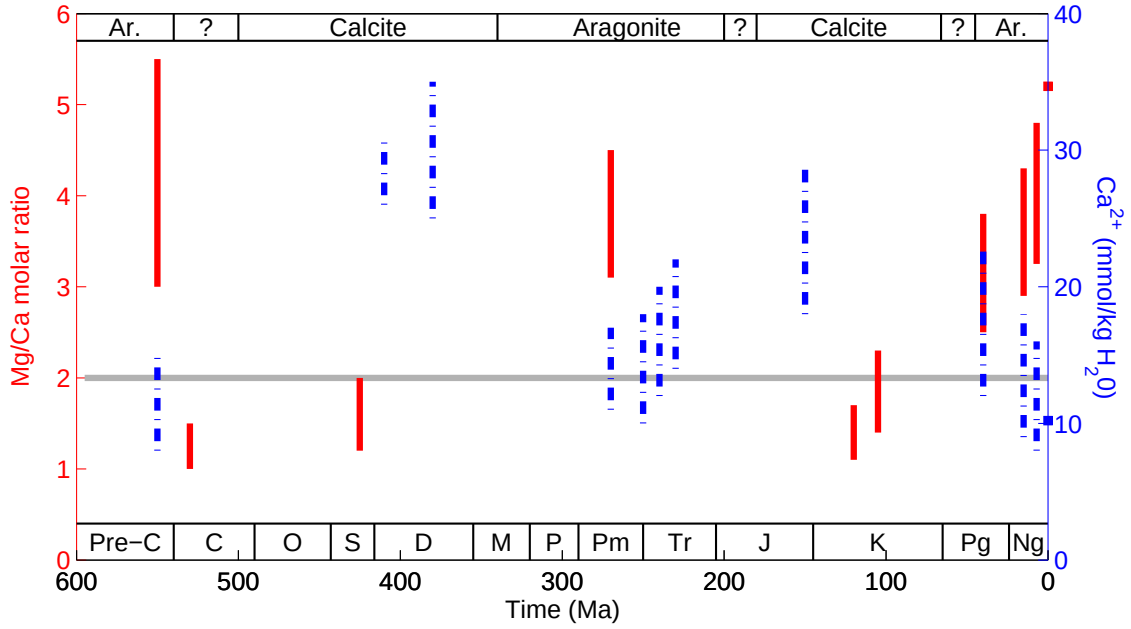

Figure 2.4: *Seawater Ca abundance (blue lines) and Mg/Ca ratio (red lines) over Phanerozoic time, as well as the principal time intervals dominated by 'calcite' and 'aragonite' seas. Aragonite and high-Mg calcite are favored when the Mg/Ca mole ratio is above 2; calcite is favored at ratios below 2. Fluid inclusions measurements were plotted after Lowenstein et al. (2001); Horita et al. (2002).*

between 1 and 2.3 during the Cretaceous (Lowenstein et al. 2001). Inorganic calcium carbonate precipitation from a mother solution of Mg/Ca ratios below 2 actually provokes calcite formation, whereas the more soluble polymorph aragonite and high Mg-calcite are favored at ratios above 2 (Folk 1974). This classifies the ocean into 'calcite' and 'aragonite' seas over geological time (Fig.: 2.4).

For most of the calcium carbonate precipitating marine organisms it is well documented that the seawater Mg/Ca ratio has a direct influence on the skeletal elemental composition (Stanley 2008). A current hypothesis links these conditions in seawater chemistry, triggered by mantle convection and plate tectonics (Horita et al. 2002), to the massive chalk depositions during the Cretaceous (Stanley et al. 2005).

2.3 Coccolithophores in the modern ocean

C. G. Ehrenberg was the first who used the term 'coccoliths' in 1836 to describe small chalk particles from the island of Ruegen in the Baltic Sea. Today, we know that these coccoliths are the eponymous cover of coccolithophores. Since the possibility to culture coccolithophores in the lab was established (Provasoli et al. 1957), a huge amount of controlled experiments have been conducted, mainly based on a few species, namely *Emiliania huxleyi*, *Pleurochrysis carterae*, *Coccolithus pelagicus* and *Calcidiscus leptoporus*. Coccolithophores are widely distributed in the ocean and therefore face a variety of environmental conditions in nature. Especially, the 'lab-rat' *Emiliania huxleyi* is reported to appear from the Bering Sea (Merico et al. 2003) to the Southern Ocean (Cubillos et al. 2007). *Emiliania huxleyi* is currently the best known coccolithophore and physiological responses on various environmental parameters were previously summarized by Paasche (2002).

In the next section an overview on the response in cellular calcification of coccolithophores to changing environmental conditions (e.g. light, temperature, nutrients and marine carbonate system) will be given. Biogenic calcification in coccolithophores cannot be examined individually, because calcification is an energy demanding process and therefore dependent on a energy supply by photosynthesis at daytime and respiration in the night. Whether this dependency or interconnection between photosynthesis and calcification is also valid in the opposite direction, in other words that calcification supports or influences photosynthesis on a cellular level, is currently under scientific discussion (Rost and Riebesell 2004). Nevertheless, there is increasing evidence that calcification does not promote or influence photosynthesis directly, what is nicely summarized in Trimborn et al. (2007). In this regard, calcification in coccolithophores will be elucidated in combination with other physiological parameters (e.g. photosynthesis and division rate).

2.3.1 Calcification under changing environmental conditions at the cellular level

Light

Irradiance in the euphotic zone is controlled by the intensity of sun radiation, the albedo and the angel in between the sea surface and the sun. The latter varies because the earth axis is tilted (by an angle of 23.5 degrees) towards the sun, creating the seasons in annual regularity. Seasonal variation and influence becomes more pronounced at higher latitudes, making it more variable in environmental conditions and causing a latitudinal diversity gradient in marine life.

Light and coccolithophores. In regard to irradiance, coccolithophores are an exceptional phytoplankton group by lacking photoinhibition to high light ($> 750\,\mu\mathrm{mol\,photons\,m^{-2}s^{-1}}$) conditions. *E. huxleyi* and *Pleurochrysis* spp. are known to photosynthesize and calcify at light intensities, where other phytoplankton groups (e.g. diatoms and dinoflagellates) are inhibited (Israel and Gonzales 1996; Nimer and Merrett 1993; Nanninga and Tyrrell 1996). This relevant ability is frequently observed during blooms of *E. huxleyi* in well stratified and shallow surface oceanic waters under high light conditions (Nanninga and Tyrrell 1996). These observations have led to the discussion of whether the surrounding coccolithosphere could be a protective shield against photoinhibition. Uncalcified cells of *E. huxleyi*, however, were tested tolerant against increased light intensity, thus arguing against a photo-protection by coccoliths (Nanninga and Tyrrell 1996).

A projected rise in ocean surface temperature by $2^{\circ}\mathrm{C}$ to $6^{\circ}\mathrm{C}$ (Meehl et al. 2007) is likely to increase ocean stratification and therefore shallowing the euphotic zone. The manner in which an increased light availibility will change coccolithophore productivity is a hard task to assess, because a concomitant decrease in nutrient supply from deeper waters will additionally affect future productivity. Overall effects will probably vary in time and region.

Coccolith production or coccolithogenesis is a process recorded to be strongly light dependent (Paasche 1966; Sikes and Wilbur 1980; Dong et al. 1993) but interestingly

a significant amount of up to 15% is reported to occure in the dark (Nimer and Merrett 1992; Paasche and Brubak 1994; Sekino and Shiraiwa 1996). This discrepancy was hypothetically linked by Paasche (2002) to the cell division cycle, where the author supposed that coccolithogenesis is triggered by a certain phase of the cell cycle.

Nitrogen and phosphorus

Nitrogen is the most abundant element in the earth's atmosphere (78.08%) and the biggest marine reservoir is represented by the atmosphere-derived dinitrogen ($\mathrm{N_2}$) with about $1 \cdot 10^4\,\mathrm{Pg\,N}$ (Gruber 2008), which is only available for nitrogen-fixing organisms (cyanobacteria). Aerob assimilation of nitrogen into organic compounds by phytoplankton occurs preferably from ammoniun ($\mathrm{NH_4^+}$) and nitrate ($\mathrm{NO_3^-}$), whereas the latter one represents the dominant bioavailable reservoir in the ocean ($5.8 \cdot 10^2\,\mathrm{Pg\,N}$).

Phosphorus has only an insignificant atmospheric reservoir and oceanic input is controlled by fluvial, hydrothermal and low-temperature-weathering input (Froelich et al. 1982). The dominant and bioavailable phosporus species in the ocean is phosphate ($\mathrm{PO_4^{3-}}$).

Nitrate and phosphate are distributed in the global ocean at a ratio of $\approx 16 : 1$ (Redfield 1934) and both nutrients increase proportionately with depth due to the consumption by phytoplankton in the euphotic zone. Deep water upwelling, diffusion and partly remineralisation of organic matter refill the upper mixed layer of the ocean with nutrients. Whether nitrate or phosphate forms the limiting factor for primary production in the ocean was long time under scientific debate (Smith 1983), but new findings indicate that nitrate is the limiting factor (with the exception of high-nutrient and low-chlorophyll areas) on short time scales and phosphate regulates oceanic productivity on geological time scales (Tyrrell 1999).

Nitrate, phosphate and coccolithophores. The cosmopolitan coccolithophore *E. huxleyi* is a poor competitor for nitrate compared to diatoms (Riegman et al. 1992) but has an extraordinary high affinity for orthophosphate and is able to use organic phosphate (Riegman et al. 2000), displaying a high competitive ability in phosphate limited areas. However, this assumption was recently questioned by new observations indicating blooms

of *E. huxleyi* at low nitrate:phosphate ratios (Lessard et al. 2005).

Under nutrient replete conditions the calcification to photosynthesis ratio varies between 0.5 and 1.2 (Paasche 2002), whereas higher ratios are observed under nitrate and phosphate limitation. Both limitations result in a stop of reproduction, and a remarkable increase in cell size was reported for phosphate limitation (Paasche and Brubak 1994; Paasche 1998; Riegman et al. 2000). Nevertheless, it has to be mentioned that the increased calcification to photosynthesis ratio under nitrate or phosphate limitation is not an effect of an increased cellular calcification rate; rather a decreased photosynthetic activity and a less reduced (nitrate limitation) or unchanged (phosphate) calcification rate is responsible for the increase. The underlying cellular mechanisms leading to an elevated calcification:photosynthesis ratio under macro-nutrient limitation are hypothetically linked to dissipating absorbed light energy when not consumed by cell division (Paasche 2002). However, this hypothesis remains questionable.

Carbonate system

The marine carbonate system is one of the most complex chemical systems on short and geological time scales, but has received an increasing interest by marine biologist in the recent years due to ocean acidification. A complete chemical description of the marine carbonate system is beyond the scope of this work. Therefore, the carbonate system will be described in terms of biological relevance and ongoing ocean acidification; the interested reader is recommended to further consult Zeebe and Wolf-Gladrow (2001) and Wolf-Gladrow et al. (2007).

Inorganic carbon is predominatly present in three forms: Carbon dioxide (CO_2), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. The sum of the three components is termed as dissolved inorganic carbon (DIC).

$$\mathrm{DIC} = [\mathrm{CO_2}] + [\mathrm{HCO_3^-}] + [\mathrm{CO_3^{2-}}] \tag{2.1}$$

The relative abundance of each species to each other is a function of seawater *p*H (Fig. 2.5), indicating that HCO_3^- is the most abundant carbon species in the modern surface

ocean.

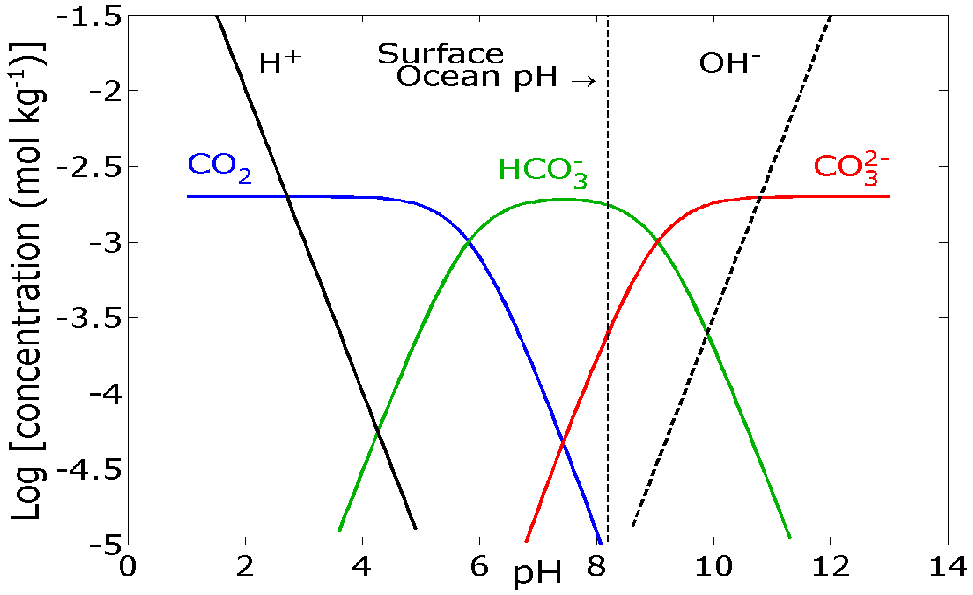

Figure 2.5: *Typical concentrations of dissolved carbonate species in seawater as a function of pH. At temperature of 15°C and a salinity of 35. With a surface water* $p\mathrm{H}_{\mathrm{total\,scale}}$ *of 8.20, the three species are distributed as 0.5%, 89% and 10.5%* (CO_2, HCO_3^- *and* CO_3^{2-}*, respectively). Picture from Zeebe (2009)*

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The next important parameter to describe the carbonate system is the total alkalinity (TA). The concept of TA is well defined as: '...the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with dissociation constant $\mathrm{K} \leq 10^{-4.5}$, at 25°C and zero ionic strength) over proton donors (acids with $\mathrm{K} > 10^{-4.5}$) in one kilogram of sample.' (Dickson 1981).

$$\begin{aligned} \mathrm{TA} \;=\; & \overbrace{[HCO_3^-] + 2[CO_3^{2-}] + [B(OH)^{4-}] + [OH^-]}^{mc} + [HPO_4^{2-}] + 2[PO_4^{3-}] \\ & + [SiO(OH)_3^-] + [NH_3] + [HS^-] \overbrace{-[H^+]}^{mc} - [HSO_4^-] \\ & - [HF] - [H_3PO_4] \end{aligned} \tag{2.2}$$

Certainly, additional minor acid or base species contribute to TA, but they only have a

very small influence on TA and can be neglected. The main contributors (mc) within the concept of ocean TA are parenthesized in Eq. (1.2).

All six parameters of the carbonate system (CO_2, HCO_3^-, CO_3^{2-}, DIC, TA and *p*H) can be calculated from any two of them (using equations from Zeebe and Wolf-Gladrow (2001)) and applying the stoichiometric equilibrium constants, which are dependent on temperature, salinity and pressure.

Ocean acidification and coccolithophores. Emissions of anthropogenic CO_2 since the industrial revolution have led to an increase in atmospheric carbon dioxide which is partly absorbed by the ocean. The ongoing oceanic uptake of anthropogenic CO_2 alters the carbonate chemistry of the ocean by increasing DIC, $[CO_2]$ and $[HCO_3^-]$, keeping TA constant and decreasing $[CO_3^{2-}]$ as well as seawater *p*H. In fact, ocean *p*H has dropped by 0.12 in the last 200 years (Caldeira and Wickett 2003). This process is termed ocean acidification and such rapid changes have not been experienced by marine life (Raven et al. 2005). Since ocean acidification decreases the surface ocean calcium carbonate saturation state (Ω), biological mediated calcium carbonate faces unavoidable dissolution as soon as Ω drops below 1. This is predicted to occure first in the Southern Ocean (Orr et al. 2005) and more rapidly for the polymorph aragonite than for calcite (precipitated by coccolithophores).

The in situ effect of ocean acidification on coccolithophores is difficult to measure since combined effects of climate change influence coccolithophorid physiology and calcite production in nature. First approaches to measure in situ effects are still in development and the results have to be considered very carefully, if associated to ocean acidification (Halloran et al. 2008; Cubillos et al. 2007). Nevertheless, it is important to link controlled laboratory and mesocosm studies together with natural observations. In the last years numerous lab and field studies were conducted and are presenting, so far, a relatively consistent picture on the physiological response of coccolithophore to changing seawater carbonate chemistry.

If exposed to pCO_2 values predicted for the year 2100 ($\approx 750\,\mu\mathrm{atm}$) the two bloom-forming coccolithophore species (*E. huxleyi* and *G. oceanica*) will decrease calcification

rate per cell with a concomintant increase in photosynthesis, which will lead to a reduction in the cellular particulate inorganic to organic carbon ratio (Riebesell et al. 2000; Zondervan et al. 2002; Sciandra et al. 2003; Feng et al. 2008). Interestingly, some coccolithophore species (*C. quadriperforatus* and *C. braarudii*) seem to be insensitive to ocean acidification within the tested range of pCO_2 values or display an optimal performance peaking at ambient CO_2 concentrations (Langer et al. 2006*a*). However, whether these observations are an effect of species specific plasticity and/or experimental resolutions remain an open question and has to be subject to further species diverse laboratory studies (MonacoRP 2008).

2.4 Element ratios and calcium fractionation in coccolithophore calcite

Coccolithophores found in the sediments provide multiple indicators for past oceanographic and environmental conditions, both in the organic (U^K_{37} and $\varepsilon^{13}C$ alkenones) and the inorganic ($CaCO_3$) fraction. The calcite in the sediments provides appropriate material for analyses of elemental ratios (e.g. Sr/Ca, Mg/Ca, Li/Ca and Ba/Ca) and of stable isotope composition (e.g. $\delta^{18}O$, $\delta^{13}C$, $\delta^{44}Ca$ and $\delta^{26}Mg$). Some of those measurements are related to physical-chemical variables like past sea surface temperature (SST), atmospheric pCO_2 or sea-ice cover, whereas others are linked to biological productivity. However, all isotope and element ratio measurements differ in the composition from what is expected from analysis of inorganic calcite equilibrium precipitations. This difference or offset is commonly termed as the 'vital effect'. Hence, paleo-proxy measurements based on biogenic precipitated calcite have to be calibrated in controlled laboratory experiments to extract reliable information about past environmental conditions.

In the following the incorporation of Sr, Ca and Mg into coccolithophore calcite will be introduced as well as the current understanding of Ca fractionation.

2.4.1 Sr and Mg incorporation

During inorganic and biogenic precipitation of calcium carbonate (Eq.: 2.3), the calcium ion can be replaced by another divalent cation like Sr^{2+} or Mg^{2+} (resulting in strontianite or magnesite, respectively). Strontianite is a rather rare mineral whereas magnesite is commonly found in dolomite rocks.

$$Ca^{2+} + CO_3^{2-} = CaCO_3 \tag{2.3}$$

The partial incorporation of the trace element (Tr) (Sr^{2+} or Mg^{2+}) into the biogenic mediated calcite of coccolithophores is described by the partition coefficient D_{Tr}, which is calculated by the partition of the Tr between calcite (c) and seawater (s), normalized to the distribution of calcium in seawater and the precipitated calcite (eq.: 2.4).

$$D_{Tr} = \frac{[Tr/Ca]_c}{[Tr/Ca]_s} \tag{2.4}$$

Sr/Ca ratio in coccolithophores. The biological processes leading to the offset or 'vital effect' in coccolith Sr/Ca ratio compared to inorganic calcite equilibrium precipitation is not well understood. Nevertheless, the Sr/Ca ratio in coccolithophorid calcite is used as an indicator for nutrient-stimulated growth of coccolithophores, what is supported by field and laboratory studies (Stoll and Ziveri 2004; Rickaby et al. 2002).

The seawater Sr/Ca ratio changed throughout Mesozoic and Cenozoic time (Lear et al. 2003), but evidence from laboratory studies shows that this does not influnce the partition coefficient D_{Sr} (Langer et al. 2006*b*). Even if there are uncertainties about the Sr^{2+} incorporation and the mechanisms behind it, it is clear that a tight control by the physiology is present since D_{Sr} values of coccolith calcite are about a magnitude higher than values of inorganic precipitated calcite.

Mg/Ca ratio in coccolithophores.An elevated Mg^{2+} content in calcite results in a higher solubility (Mucci and Morse 1984). The percentage of Mg^{2+} relative to the Ca^{2+} ion defines high Mg-calcite ($> 4\,mol\%$) or low Mg-calcite ($< 4\,mol\%$). The Mg/Ca ratio of

inorganic precipitated calcite is positively correlated to the Mg/Ca ratio of the mother solution (seawater) (Morse and Bender 1990).Similar relationship has been observed in calcite of corraline red algae, echinoderms and foraminifera (Ries 2004; Segev and Erez 1994). Additionally, laboratory experiments indicate that corals and calcareous green algae, which precipitate aragonite at ambient seawater Mg/Ca ratio of 5.2, are changing the crystal structure of their skeleton towards calcite at low seawater Mg/Ca ratios (Stanley 2008).

Coccolithophores are known to precipitate low Mg-calcite ($< 1\,\mathrm{mol}\%$) at ambient seawater Mg/Ca ratio (Siesser 1977), which is confirmed by more recent laboratory and field studies (Stoll et al. 2001 2007). In contrast, results from Stanley et al. (2005) indicate that two coccolithophore species (*Pleurochrysis carterae* and *Ochrosphaera neopolitana*) precipitate high Mg-calcite ($> 15\,\mathrm{mol}\%$) at ambient Mg/Ca ratio.

2.4.2 Ca isotope fractionation

The atomic nucleus contains protons and neutrons. While the number of protons defines the element and the sum of the protons and neutrons the atomic mass, the number of neutrons defines the isotope of that element. Stable isotopes do not decay into other elements. In contrast, radioactive isotopes are unstable and decay into other elements (e.g. $^{14}\mathrm{C}$ to $^{14}\mathrm{N}$).

Calcium has six stable isotopes with different natural abundances: $^{40}\mathrm{Ca} = 96.941\%$, $^{42}\mathrm{Ca} = 0.647\%$, $^{43}\mathrm{Ca} = 0.135\%$, $^{44}\mathrm{Ca} = 2.086\%$, $^{46}\mathrm{Ca} = 0.004\%$ and $^{48}\mathrm{Ca} = 0.187\%$ (Coplen et al. 2006). The less abundant stable isotopes of calcium have two or several additional neutrons, and thus are heavier than the more common stable isotope $^{40}\mathrm{Ca}$.

Heavy and light stable isotopes participate in chemical, biological and geochemical processes, but the chemical kinetics for heavy and light stable isotopes are different during chemical or biological reactions. The heavier isotopes react more slowly than the lighter isotopes leading to isotopic fractionation between the reactant and the product. Composition of stable calcium isotopes (indicated by the δ) is discribed by the ratio of the heavy isotope to light isotope in the sample over the ratio of a standard (Eisenhauer et al.

(2004), eq.: 2.5).

$$\delta^{44}\mathrm{Ca}\,(\mathrm{in}\,‰) \;=\; \left(\frac{\left(\frac{^{44}\mathrm{Ca}}{^{40}\mathrm{Ca}}\right)_{\mathrm{sample}}}{\left(\frac{^{44}\mathrm{Ca}}{^{40}\mathrm{Ca}}\right)_{\mathrm{standard}}} - 1 \right) \cdot 1000 \tag{2.5}$$

Commonly, the sample material is measured against the SRM 915a standard and the isotope fractionation is expressed relative to the isotopic composition of the mother solution (e.g. seawater) with the 'big delta' (eq.: 2.6).

$$\Delta^{44}\mathrm{Ca}\,(‰) \;=\; \frac{\delta^{44}\mathrm{Ca}_{\mathrm{sample}} - \delta^{44}\mathrm{Ca}_{\mathrm{seawater}}}{1 + \delta^{44}\mathrm{Ca}_{\mathrm{seawater}}/10^3} \;\approx\; \delta^{44}\mathrm{Ca}_{\mathrm{sample}} - \delta^{44}\mathrm{Ca}_{\mathrm{seawater}} \tag{2.6}$$

Coccolithophores have contributed a significant amount (up to 90%) to calcareous sediments since the Jurassic (Berger and Roth 1975). Ca isotope fractionation in coccolithophorid calcite plays a critical role in the reconstruction of the oceanic Ca budget since the amount of calcium and its isotopic composition in seawater are determined by the balance between the input and output fluxes (see also 2.1.2 and De La Rocha and DePaolo (2000); Fantle and DePaolo (2005); Griffith et al. (2008)).

$\Delta^{44}\mathrm{Ca}$ *in coccolithophores.* Controlled lab experiments on calcium isotope fractionation in coccolithophores are relativley rare. Therefore, it is not clear which exact mechanisms lead to the observed 'vital effects' during coccolithogenesis or calcite production. Gussone et al. (2007) introduced a positive temperature dependence of $\Delta^{44}\mathrm{Ca}_{\mathrm{coccolith}}$ for five coccolithophore species. This temperature relation was previously presented for inorganic precipitated calcite and explained by a crystallization rate effect (Lemarchand et al. 2004). However, direct conversion of inorganic crystallization rates ($\mathrm{mol\,area^{-2}\,time^{-1}}$) to biological calcification rates (e.g. $\mathrm{mol\,cell^{-1}\,time^{-1}}$) is not trivial and to my knowledge a satisfying attempt is still lacking. Langer et al. (2007) presented data indicating that an increase of cellular growth rate with associated enhanced calcification rate by light did not affect $\delta^{44}\mathrm{Ca}_{\mathrm{coccolith}}$. Suggestions are made that calcium isotope fractionation probably occurs mostly at $\mathrm{Ca^{2+}}$ channels and plasma membrane transporters (e.g. $\mathrm{Ca^{2+}}$ ATPases) during the transport of $\mathrm{Ca^{2+}}$ from seawater to the side of precipitation, the coc-

colith vesicle (Gussone et al. 2006).

2.5 Outline of the thesis

This thesis describes the physiological response of coccolithophores to changing seawater conditions. The seawater chemistry was experimentally altered by varying: the calcium to magnesium ratio, the macronutrients concentrations and the carbonate system.

- **Section 3.1**: Manuscript I deals with calcification in regard to the cell division cycle. It is clarified that calcification in *E. huxleyi* is tightly linked to the G1 phase of the cell cycle and elucidates the different effects of light and nutrient limitations (nitrate and phosphate) on calcification.

- **Section 3.2**: Manuscript II investigates the immediate physiological response of *E. huxleyi* to changes in the seawater carbonate chemistry. *E. huxleyi* has after 8 hours the same response to a reduction/increase in seawater *p*H/$\mathrm{CO_2}$ as reported for an acclimation time of 10 generation ($\approx 10\,\mathrm{days}$). This finding helps to evaluate and assess field experiments dealing with the response of coccolithophores to changing seawater chemistry.

- **Section 3.3**: Manuscript III reports the physiological response of two coccolithophore species (*E. huxleyi* and *C. braarudii*) to a reduction/increase in seawater *p*H/$\mathrm{CO_2}$ under long-term cultivation. Cultures grown over more than 65 generations under elevated $p\mathrm{CO_2}$ show reduced calcification and diminished growth rate. In summary, a gradual $p\mathrm{CO_2}$ increase does not alleviate $\mathrm{CO_2}$/*p*H sensitivity and the observed effects are persistent over multiple generations.

- **Section 3.4**: Manuscript IV describes the effect of changes in the seawater Mg/Ca ratio on coccolithophorid (*E. huxleyi* and *C. braarudii*) physiology. The seawater $\mathrm{Ca^{2+}}$ concentration is the main factor inducing changes in physiological rates, whereas variations in the $\mathrm{Mg^{2+}}$ concentration have minor effects. It is elucidated that cellular rates (like chlorophyll *a* production) may have a significant influence on the magnesium incorporation into the calcite lattice.

3 Special topics

Contribution to each publication/ manuscript:

- **Cell devision cycle**: The lab experiments were realised by myself with advise of A. N. Antia and J. LaRoche. The manuscript was written by myself in cooperation with the coauthors. *Published in Limnology and Oceanography.*

- **Short-term *p*$\mathrm{CO_2}$ exposure**: The lab experiment was realised by J. Barcelos e Ramos and myself. The manuscript was written by J. Barcelos e Ramos in cooperation with myself and U. Riebesell. *Published to Biogeosciences Discussion.*

- **Long-term *p*$\mathrm{CO_2}$ exposure**: The lab experiments were realised by myself with advise of K. G. Schulz. The manuscript was written by myself in cooperation with the coauthors. *To be submitted to Biogeosciences.*

- **Response to seawater** $[\mathrm{Mg^{2+}}]$ **and** $[\mathrm{Ca^{2+}}]$: The lab experiments were realised by myself. The manuscript was written by myself in cooperation with the coauthors. *To be submitted to Cosmochemica et Geochemica Acta.*

3.1 Manuscript I: Cell devision cycle

Limnol. Oceanogr., 53(2), 2008, 506–512

Influence of cell cycle phase on calcification in the coccolithophore *Emiliania huxleyi*

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Abstract

Calcification of the cosmopolitan coccolithophore species *Emiliania huxleyi* was investigated in relation to the cell division cycle with the use of batch cultures. With a 12 : 12 h light : dark cycle, the population was synchronised to undergo division as a cohort, simultaneously passing through the G1 (assimilation), S (DNA replication), and G2+M (cell division and mitosis) phases. Cell division was followed with the use of quantitative DNA staining and flow cytometry. Simultaneously, carbon-14 (^{14}C) assimilation in organic and inorganic carbon as well as cell abundance, size, and organic nitrogen content were measured at 2-h intervals. In additional experiments, changes in calcification and cell cycle stages were investigated in nitrogen-, phosphorus-, and light-limited cultures. Calcification occurred only during the G1 cell cycle phase, as seen by the very tight correlation between the percentage of cells in G1 and calcification during the dark period. When growth was limited by nitrogen, cells decreased in size, remained in the G1 phase, and showed a moderate increase in the cell-specific calcite content. Limitation of growth by phosphorus, however, caused a significant increase in cell size and a dramatic increase in cellular calcite. Light limitation, by slowing the growth rate, prolonged the time cells spent in the G1 phase with a corresponding increase in the cellular calcite content. These results help explain the differing responses of coccolithophorid growth to nitrogen, phosphorus, and light limitation.

Coccolithophores are unicellular photosynthetic algae that produce platelets of calcium carbonate, called coccoliths, that surround the cells. They are the dominant planktonic calcifiers in the present ocean and are responsible for up to 80% of global oceanic calcification (Deuser and Ross 1989; Fabry 1989) of 0.8–1.4 Pg of $CaCO_3$-C y^{-1} (Feely et al. 2004). The cosmopolitan species *Emiliania huxleyi* in particular forms huge seasonal blooms that extend over >100,000 km^2 (Brown and Yoder 1994), making it an important player in the marine environment.

Calcification plays a substantial role in the marine carbon cycle in that formation and export of calcium carbonate reduce alkalinity in the surface ocean and cause a net release of CO_2 to the atmosphere, counteracting the CO_2 drawdown by photosynthesis. Calcification thus decreases the efficiency with which the oceans' ''biological pump'' takes up atmospheric CO_2 (Antia et al. 2001). Hence, variations in the ratio of calcification to photosynthesis (C : P) and ratio of particulate inorganic carbon to particulate organic carbon (PIC : POC) leaving the surface ocean are important in determining the efficiency of biogenic carbon sequestration by the ocean. Additionally, because coccolith formation is negatively affected by decreasing seawater pH, changes in the abundance of calcifiers is expected because of ongoing ocean acidification (Riebesell 2004; Delille et al. 2005) with unknown effects on marine ecosystems.

Although there is no consensus as to why coccolithophores calcify (Harris 1994; Young 1994; Bratbak et al. 1996), several factors that influence the rate of calcification, as well as the ratio of calcification to photosynthesis, have been identified. In numerous controlled laboratory experiments and mesocosm and field studies, changes in calcification and the PIC : POC ratios were seen to change with dependence on light, nutrient availability, growth rate, and strain diversity (summarized in Paasche 2002). Elevated bulk calcite production and cell-specific calcium carbonate quota are found particularly under high light conditions and when phosphorus rather than nitrogen limits growth (Paasche and Brubak 1994; Riegmann et al. 2000; Zondervan 2007). Higher cell–calcite quotients also result from nitrogen limitation, but to a lesser extent (Paasche 1998).

The physiological reasons underlying these observations are unclear. Calcification is energy consuming, fueled by photosynthesis in the light and respiration in the dark (Sekino and Shiraiwa 1996). Though *E. huxleyi* calcifies primarily during the light phase of the diel cycle, cells that have been decalcified by acidification can build coccoliths when incubated in the dark, albeit at a much slower rate (Sekino and Shiraiwa 1996). In his comprehensive review of the coccolithophore *E. huxleyi*, Paasche (2002) speculated that calcification is linked to the cell division cycle, with calcification being primarily a G1 (gap 1, assimilation) process and thus reduced in the dark when dividing cells pass through the S (DNA synthesis) and G2+M (gap 2 [cell division] + mitosis) phases of division. This argument is supported by the observation that the coccolith vesicle is not present during nuclear division and is reconstituted after mitosis (van Emburg 1989). If calcification is related to the G1 phase of the cell cycle, processes arresting cells in G1, such as nutrient limitation, would cause an increase in bulk calcification.

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3.2 Manuscript II: Short-term pCO_2 exposure

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Biogeosciences Discussions

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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.,

3.3 Manuscript III: Long-term *p*CO_2 exposure

Influence of high CO_2 on two coccolithophore key species under long-term cultivation

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running head: long-term CO_2 effect on coccolithophores

Abstract

The physiological performance of two coccolithophore species, *Emiliania huxleyi* and *Coccolithus braarudii*, was investigated during long-term exposure to elevated pCO_2 levels. Mono-specific cultures were grown over 152 (*E. huxleyi*) and 65 (*C. braarudii*) generations while pCO_2 was gradually increased to maximum levels of $1150\,\mu\mathrm{atm}$ (*E. huxleyi*) and $930\,\mu\mathrm{atm}$ (*C. braarudii*) and kept constant thereafter. Rates of cell division and cell quotas of particulate organic carbon (POC), particulate inorganic carbon (PIC) and total particulate nitrogen (TPN) were determined continuously throughout the incubation period. Increasing pCO_2 caused a decrease in cell growth rate of 9% and 29% in *E. huxleyi* and *C. braarudii*, respectively. In both species nitrogen based PIC production and cellular PIC:POC ratio decreased in response to rising pCO_2, whereas no change in nitrogen based POC production was observed in *E. huxleyi*. *C. braarudii*, on the other hand, displayed a reduction in POC production under a constant high pCO_2 level. These results are consistent with those obtained in shorter-term high CO_2 exposure experiments following abrupt changes in carbonate chemistry, indicating that for the strains tested here i) a gradual CO_2 increase does not alleviate CO_2/pH sensitivity, and ii) observed CO_2 sensitivities are persistent over multiple generations.

Introduction

Emissions of anthropogenic CO_2 since the beginning of the industrial revolution have lead to an increase in atmospheric carbon dioxide concentration, resulting in a proportion of the anthropogenic CO_2 being absorbed by the ocean. The ongoing oceanic uptake of anthropogenic CO_2 is steadily altering the carbonate chemistry of the ocean which has experienced a reduced oceanic *p*H by 0.12 units over the last 200 years (Caldeira and Wickett 2003). This process is termed ocean acidification and has serious effects on pelagic and benthic calcifiers with diverging physiological responses. Larvae of the brittle star *Ophiothrix fragilisis* have a highly enhanced mortality rate and reduced calcification due to a drop of 0.2 units in seawater *p*H (Dupont et al. 2008) whereas the adult species *Amphiura filiformis* can cope with increased seawater acidity by thickening its calcite skeleton but at substantial physiological costs (Wood et al. 2008). Reduced calcification has also been observed in warm water corals (Marubini et al. 2008; Langdon and Atkinson 2005; Gatusso et al. 1998) and in foraminifera (de Moel et al. 2009; Bijma et al. 1999).

Coccolithophores as unicellular primary producers (standing at the base of the food web) were tested to changing seawater carbonate chemistry in several laboratory experiments with species-specific results. Under nutrient and light replete conditions *Emiliania huxleyi*, *Gephyrocapsa oceanica* and *Calcidiscus leptoporus* are decreasing particulate inorganic carbon production per cell to different degrees within the tested pCO_2 range (Riebesell et al. 2000; Zondervan et al. 2002; Langer et al. 2006; Feng et al. 2008) and *Coccolithus braarudii* seems to be insensitive to elevated pCO_2 (Langer et al. 2006). A similar insensitivity was observed in the particulate organic carbon production per cell of *C. leptoporus* and *C. braarudii* (Langer et al. 2006). *E. huxleyi* and *G. oceanica*, on the other hand, increase $POC_{prod_{cell}}$ by up to 20% under elevated pCO_2 (Riebesell et al. 2000; Zondervan et al. 2002; Feng et al. 2008). Recently, Iglesias-Rodriguez et al. (2008) observed both in particulate organic and inorganic carbon production of *E. huxleyi* an increase of up to 100%.

All acidification experiments with coccolithophores were done following an abrupt change of the seawater chemistry, either by bubbling with CO_2 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 allowing an acclimation time before starting the experiment. Actual experimental time differs in the literature from 1-2 generations (Iglesias-Rodriguez et al. 2008) up to 7-10 generations (Riebesell et al. 2000; Zondervan et al. 2002; Langer et al. 2006; Feng et al. 2008). This results in 20 divisions as a maximum under high pCO_2 conditions. The physiological response of *E. huxleyi* within one generation was recently investigated by Barcelos e Ramos et al. (2009). However, if the observed CO_2 sensitivities are persistent over more than 20 generations is unknown. Here, we report a culture experiment with two coccolithophore species (*E. huxleyi* and *C. braarudii*), which were grown over multiple generations under high pCO_2 (152 and 65 generations, respectively).

Methods

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. 2008) in the Raune Fjord (Norway). Both cultures were grown at 16 °C in $0.2\,\mu\mathrm{m}$ filtrated North Sea water with a Salinity of 33 and f/20 nutrient additions (Guillard 1975), corresponding to $88.0\,\mu\mathrm{mol\,L^{-1}}$ nitrate and $3.6\,\mu\mathrm{mol\,L^{-1}}$ phosphate, at a photon flux density of $140\,\mu\mathrm{mol\,quanta\ m^{-2}\ s^{-1}}$ (Philips TL-D 90 DeLuxePro, 36W/950) under a 14:10 h light:dark cycle.

Experimental setup. Coccolithophores were grown in duplicates under semi-continuous batch culture conditions (as described above) in $280\,\mathrm{mL}$ autoclaved polycarbonate Erlenmeyer flasks. $p\mathrm{CO_2}$ 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 DIC, *p*H, 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 $50\,\mathrm{cells\,mL^{-1}}$ (*E. huxleyi* and *C. braarudii*, respectively). The media in the control flasks (low $p\mathrm{CO_2}$) was adjusted to a $p\mathrm{H_{total}}$ of 8.21 ± 0.05, resulting in a $p\mathrm{CO_2}$ of $260\,\mu\mathrm{atm} \pm 20$ 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 $p\mathrm{CO_2}$ values ($1150\,\mu\mathrm{atm} \pm 140$ and $930\,\mu\mathrm{atm} \pm 180$ for *E. huxleyi* and *C. braarudii*, respectively) and kept at these levels until the end of the experiment.

TPC, POC, PIC, TPN. Two sub-samples from each flask were filtrated onto precombusted (525°C for 7 hours) GF/F filters and frozen at $-20\,^{\circ}\mathrm{C}$. For POC analysis

filters were fumed over HCl for 24 hours 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 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 (μ) as

$$\mu = \frac{(\ln \mathrm{c}_1 - \ln \mathrm{c}_0)}{\mathrm{t}_1 - \mathrm{t}_0} \tag{1}$$

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

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 (Quaatro) equipped with an auto-sampler ($\pm 10\,\mu\mathrm{mol\,kg^{-1}}$ accuracy and $5\,\mu\mathrm{mol\,kg^{-1}}$ precision) and pH was measured using a 'Metrohm 713 *p*H-Meter', equipped with *p*H and reference electrodes and temperature sensor. Sensor and electrodes were stored in filtrated seawater at 16 °C to match the ionic strength of the sampled water. *p*H measurements were periodically checked by calculating *p*H 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 *p*H values closely agreed with *p*H measurements with a maximum deviation of ± 0.02. Here we present *p*H values on the total scale.

Scanning electron microscopy. $5\,\mathrm{mL}$ samples were taken periodically from the control and high $\mathrm{CO_2}$ treatment and fixed with formaldehyde (1% end concentration). Subsequently, the samples were filtered onto polycarbonate filters ($0.2\,\mu\mathrm{m}$ pore size), dried at $60\,^{\circ}\mathrm{C}$ for 24 hours and then sputter-coated with gold-palladium. Pictures were

taken with a CamScan-CS-44 scanning electron microscope at the Institute of Geosciences of the Christian-Albrecht-University in Kiel.

Rate normalization. Rates of particulate organic and inorganic carbon production were normalized to total particulate nitrogen (TPN). Cellular nitrogen quota increases over the course of the day due to a steady accumulation of particulate material (Fig. 1A) but no diel trends are observed in the cellular ratios of PIC:TPN and POC:TPN (Fig. 1B+C). In this regard, a rate normalization to TPN remains unaffected by the hour of sampling. Production rates of POC and PIC were calculated as

$$\mathrm{POC}_{\mathrm{prod_N}}\,,\mathrm{PIC}_{\mathrm{prod_N}} = \frac{\mathrm{molC\,cell^{-1}}}{\mathrm{molN\,cell^{-1}}} \cdot \mathrm{growth\,rate}\,(\mu). \qquad (2)$$

For differentiation between production rates based on TPN (present study) and cell number (as reported in the literature), we will abbreviate the latter as $\mathrm{POC}_{\mathrm{prod_{cell}}}$ and $\mathrm{PIC}_{\mathrm{prod_{cell}}}$.

Results

Emiliania huxleyi. *E. huxleyi* was cultured for 98 days, corresponding to 152 generations in the control treatment (low pCO_2) and 144 generations in the high pCO_2 treatment. Cellular division in the control treatment stabilized at a rate of $\mu = 1.10 \pm 0.06\,\mathrm{d}^{-1}$ throughout the course of the experiment. During the gradual increase from low to high pCO_2, no detectable change in growth rate was visible, whereas after reaching the maximum pCO_2 level of $1150\,\mu\mathrm{atm}$ the growth rate decreased to $\mu = 1.00 \pm 0.06\,\mathrm{d}^{-1}$ up until the end of the experiment (Fig. 2B). $\mathrm{PIC}_{\mathrm{prod_N}}$ was relatively constant at $5.2 \pm 1.1\,\mathrm{molC\,molN^{-1}\,day^{-1}}$ under low pCO_2 but with the onset of high pCO_2 the rate decreased consistently compared to the control on any given day by $2.2 \pm 0.7\,\mathrm{molC\,molN^{-1}\,day^{-1}}$ (Fig. 2C). $\mathrm{POC}_{\mathrm{prod_N}}$ was consistent in both treatments (low and high pCO_2) and revealed in a mean rate of $10.5 \pm 1.8\,\mathrm{molC\,molN^{-1}\,day^{-1}}$ (Fig. 2D). The PIC:POC ratio was considerably lower under constant high pCO_2 driven by the decrease in $\mathrm{PIC}_{\mathrm{prod_N}}$ (Fig. 2E). Coccolith morphology of *E. huxleyi* did not display a visible difference between pCO_2 treatments (Fig. 4A+B). On day 80, pCO_2 accidentally dropped below $870\,\mu\mathrm{atm}$, which was followed by an immediate increase in cell growth. As soon as pCO_2 was elevated above $1000\,\mu\mathrm{atm}$, the cell growth rate decreased again to the previous level.

Coccolithus braarudii. *C. braarudii* was cultured for 66 days corresponding to 65 generations in the control treatment and 51 generations in the high pCO_2 treatment. After transition to constant high pCO_2 on day 31 the growth rate decreased from initially 0.69 ± 0.04 to $0.49 \pm 0.06\,\mathrm{d}^{-1}$, whereas it remained at the initial level in the control treatment (Fig. 3B). Both $\mathrm{PIC}_{\mathrm{prod_N}}$ and $\mathrm{POC}_{\mathrm{prod_N}}$ were obviously decreased under high pCO_2 from 6.1 ± 1.1 to 1.0 ± 0.3 and from 7.5 ± 1.2 to $4.3 \pm 1.0\,\mathrm{molC\,molN^{-1}\,day^{-1}}$, respectively (Fig. 3C+D). As a consequence of the stronger decline in $\mathrm{PIC}_{\mathrm{prod_N}}$ than in $\mathrm{POC}_{\mathrm{prod_N}}$ the PIC:POC ratio was reduced by $\approx 70\%$ (Fig. 3E) and clear signs of malformation were observed on individual coccoliths under constant high pCO_2 conditions (Fig. 5D).

Discussion

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 natural ocean acidification changes the DIC concentration at constant TA. However, biologically important parameters ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-} and $[H^+]$) are changed in similar ways by manipulating TA at constant DIC (Schulz et al. 2009). For example, manipulating seawater with salinity of 35 at 15°C with pCO_2 of $380\,\mu atm$ and DIC of $2100\,\mu mol\,kg^{-1}$ by *i*) aeration with CO_2 enriched air (TA constant) or *ii*) acid addition (DIC constant) to a pCO_2 of $1000\,\mu atm$ would result in the following percentage changes of biologically relevant parameters. $CO_{2(aq)}$: +164% (*i* and *ii*); HCO_3^-: +12% (*i*) and +4% (*ii*); CO_3^{2-}: -52% (*i*) and -59% (*ii*); $[H^+]$: -4.5% (*i*) and -5% (*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).

Growth rate. A decrease of 9% was observed in *E. huxleyi*'s growth rate at a pCO_2 of $1150\,\mu atm$. Previous studies detected no change in growth rates at lower pCO_2 levels (Riebesell et al. 2000; Zondervan et al. 2002; Feng et al. 2008). The latter studies exposed *E. huxleyi* to a maximum pCO_2 of $900\,\mu atm$ and additionally, a minor decrease of 9% is likely to be superimposed by uncertainties in the measurements of singular samples during short term experiments. Recent results by Barcelos e Ramos et al. (2009); Langer et al. (2009) indicate a decreased growth rate at a pCO_2 of $> 1000\,\mu atm$ in short term experiments. Interestingly, at day 80 where pCO_2 accidently dropped to $870\,\mu atm$ the growth rate increased to control values (Fig. 3B) and afterwards on day 86 with pCO_2 at $1150\,\mu atm$ the growth rate decreased again. A similar instant effect of pCO_2 on the growth rate of *E. huxleyi* was reported to occur within one generation (Barcelos e Ramos et al. 2009).

Under constant high pCO_2 the growth rate of *C. braarudii* was reduced by 29%. At

comparable pCO_2 values, Langer et al. (2006) observed no significant reduction in the growth rate of *C. braarudii*. This differnce might be induced by the long-term culturing under constant high pCO_2 but other factors like differences in the experimental temperature and the light intensity cannot be excluded. Nevertheless, recent results indicate that *C. braarudii* has a decreasing growth rate within 15 generations if exposed to $pCO_2 > 1400\,\mu\mathrm{atm}$ (Krug 2009).

PIC$_{\mathrm{prod_N}}$. Under constant high pCO_2 *C. braarudii* and *E. huxleyi* decreased their PIC production per total particulate nitrogen by $\approx 80\%$ and $\approx 40\%$, respectively. A reduction in calcification rates of *E. huxleyi* by increasing the pCO_2 level was previously observed in several studies (Riebesell et al. 2000; Zondervan et al. 2002; Feng et al. 2008; Langer et al. 2009; Barcelos e Ramos et al. 2009). The latter studies reported calcification rates per cell number to decrease by 5 to 30%. Conversion of $\mathrm{PIC_{prod_{cell}}}$ to $\mathrm{PIC_{prod_N}}$ of *E. huxleyi* form literature experiments (Zondervan et al. 2002) results in a similar decrease of $\mathrm{PIC_{prod_N}}$ (Fig. 5A) as seen under the current study. Depending on the light intensity *E. huxleyi* reduced $\mathrm{PIC_{prod_N}}$ by 25 to 45% (Fig. 5A). Therefore, independent of the implemented rate normalization *E. huxleyi* shows a decreasing trend in calcification under high pCO_2. However, the calcification response of *E. huxleyi* to high pCO_2 can vary with experimental condition, set up and species strains (summarized in Ridgwell et al. (2009).

We observed a decrease in $\mathrm{PIC_{prod_N}}$ of *C. braarudii* under high pCO_2, what was mainly driven by the reduction in growth rate (Fig. 3B) and is in contrast to results of Langer et al. (2006). In the latter study, *C. braarudii* was insensitiv to a similar elevated pCO_2 level, but the experimental light intensity was about 2.5 times higher than in the present study. From Fig. 5A, where an increased light intensity amplifies the pCO_2 effect on $\mathrm{PIC_{prod_N}}$ of *E. huxleyi* , we would expect that a pCO_2 effect on $\mathrm{PIC_{prod_N}}$ of *C. braarudii* would be more pronounced in the study of Langer et al. (2006). However, the opposite is observed and this might be due to the long-term exposure to constant high pCO_2.

POC$_{\mathrm{prod_N}}$. Particulate organic carbon production rates per cell ($\mathrm{POC_{prod_{cell}}}$) are known to increase under high $p\mathrm{CO_2}$ to a certain degree 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). However, due to the severe reduction in growth rate *C. braarudii* clearly demonstrated a decreased $\mathrm{POC_{prod_N}}$ by $\approx 40\%$, whereas no changes were observed in *E. huxleyi* under long-term cultivation. Recently, several different strains of *E. huxleyi* were studied with regard to increasing $p\mathrm{CO_2}$ and displayed strain specific differences in $\mathrm{POC_{prod_{cell}}}$ (Langer et al. 2009). The latter study revealed that the increasing trend of POC production per cell of *E. huxleyi* (previously reported in Riebesell et al. (2000); Zondervan et al. (2002); Feng et al. (2008)) does not have to be uniform for particular strains of *E. huxleyi*. However, POC production normalized to total particulate nitrogen quota ($\mathrm{POC_{prod_N}}$) revealed no increasing trend within the tested $p\mathrm{CO_2}$ range (Fig. 2D). This is also seen in the study of Zondervan et al. (2002) under different light intensities (Fig. 5B) since the POC:TPN ratio does not change under elevated $p\mathrm{CO_2}$ (Feng et al. 2008). Therefore, under elevated $p\mathrm{CO_2}$ the uptake of DIC per cell is accelerated but since the POC:TPN ratio stays constant, the bulk DIC consumption per available nitrogen of an *E. huxleyi* population will be the same. These findings from laboratory studies are partly in contrast to mesocosm experiments, where clear evidence is given for an elevated $\mathrm{CO_2}$ draw down under high $p\mathrm{CO_2}$ during a phytoplankton community study (Riebesell et al. 2007). However, in this mesocosm study a mixed phytoplankton community dominated by diatoms and coccolithophores was investigated (Schulz et al. 2008), whereas previous results from a coccolithophore dominated mesocosm community study indicate no increased $\mathrm{CO_2}$ draw down at high $p\mathrm{CO_2}$ compared to ambient $p\mathrm{CO_2}$ (Engel et al. 2005).

Reporting production rates. Production rates of inorganic and organic carbon by coccolithophores can be reported per cell or per biomass, like total particulate nitrogen, cell volume or chlorophyll. Production rates on a per cell basis (calculated as: carbon quota per cell multiplied by the growth rate 'μ') crucially depend on i) the carbon cell

quota, ii) the growth rate and iii) the time of sampling, since under a light:dark cycle the carbon cell quota increases towards the end of the light phase and decreases throughout the dark phase, whereas the cell number stays constant in the light phase and increases during the dark phase. Under continuous light, on the other hand, the time of sampling does not influence the calculations (Müller et al. 2008). Therefore, production rates, which are calculated from samples of different time points during a light:dark cycle, cannot be compared to each other in terms of the absolute value. Additionally, it becomes more complicated if cell quota and growth rate is influenced by environmental parameters, like pCO_2.

For example, increased organic carbon quota of *E. huxleyi* induced by high pCO_2 (compared to ambient pCO_2) is more pronounced at the end of the light phase with respect to the absolute value. However, the percentage increase under high pCO_2 stays the same over the course of the day but may be covered at the beginning of the light phase by observational errors. Therefore, sampling time can greatly influence the outcome of an experiment, in especially when comparing only two pCO_2 values instead of a pCO_2 gradient. Hence, we recommend to report the time of sampling if rates are reported on a per cell basis.

Conclusions

Since the study of Riebesell et al. (2000), Langer et al. (2006) and Langer et al. (2009) species and strain specific performance of coccolithophores under elevated pCO_2 levels have been demonstrated in short-generation experiments. Here, we discussed data from a multiple-generation experiment using two coccolithophore species and can generally confirm the observed CO_2 sensitivities resulting from short-term experiments and additionally report decreased growth rates in response to elevated pCO_2. Furthermore, a gradual CO_2 increase seems not to alleviate CO_2/pH sensitivity under the experimental conditions.

Coccolithophores and other phytoplankton groups will face a changing environment

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

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Figure captions

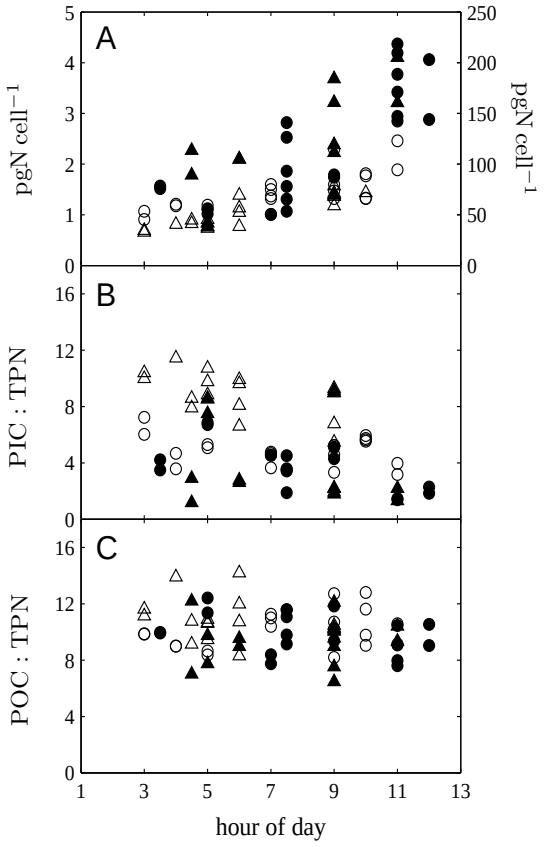
Fig.1: Cell nitrogen quota and consequent cell ratios over the hours of daily illumination of *E. huxleyi* (circle) and *C. braarudii* (triangle) under low pCO_2 (open markers) and high pCO_2 (closed markers) from all data points during the entire experiment. (A) pgN per cell of *E. huxleyi* (left y-axis) and *C. braarudii* (right y-axis). (B) PIC:TPN ratio (mol:mol). (C) POC:TPN ratio (mol:mol).

Fig.2: Physiological responses of *Emiliania huxleyi* to elevated pCO_2 over the course of the experiment (open and closed symbols represent the low pCO_2 and high pCO_2 treatments, respectively). (A) pCO_2 (circles, $\mu\mathrm{atm}$) and pH (triangle) regime over experimental time. (B) growth rate (d^{-1}). (C) $\mathrm{PIC}_{\mathrm{prod_N}}$ ($\mathrm{molC:molN\,d^{-1}}$). (D) $\mathrm{POC}_{\mathrm{prod_N}}$ ($\mathrm{molC:molN\,d^{-1}}$). (E) PIC:POC (mol:mol).

Fig.3: Physiological responses of *Coccolithus braarudii* to elevated pCO_2 over the course of the experiment (open and closed symbols represent the low pCO_2 and high pCO_2 treatments, respectively). Symbols as in Fig. 2.

Fig.4: Representative SEM photographs of the two coccolithophore species. Cells of *E. huxleyi* grown in the control treatment (A) and under high pCO_2 at day 73 (B). Cells of *C. braarudii* grown in the control treatment (C) and under high pCO_2 at day 66 (D).

Fig.5: Particulate carbon production of *E. huxleyi* as a function of pCO_2 ($\mu\mathrm{atm}$) at a 24:0 light:dark cycle under various light intensities: 15 (triangle), 30 (square), 80 (circle) and $150\,\mu\mathrm{mol\,quanta\ m^{-2}\ s^{-1}}$ (diamond). (A) $\mathrm{PIC}_{\mathrm{prod_N}}$ ($\mathrm{molC:molN\,d^{-1}}$). (B) $\mathrm{POC}_{\mathrm{prod_N}}$ ($\mathrm{molC:molN\,d^{-1}}$). Data calculated from Zondervan et al. (2002). Error bars represent 1SD (n=3).


A
B
C
pgN cell^{-1}
pgN cell^{-1}
PIC : TPN
POC : TPN
hour of day
0
1
2
3
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12
16
1
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7
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11
13

Fig. 1

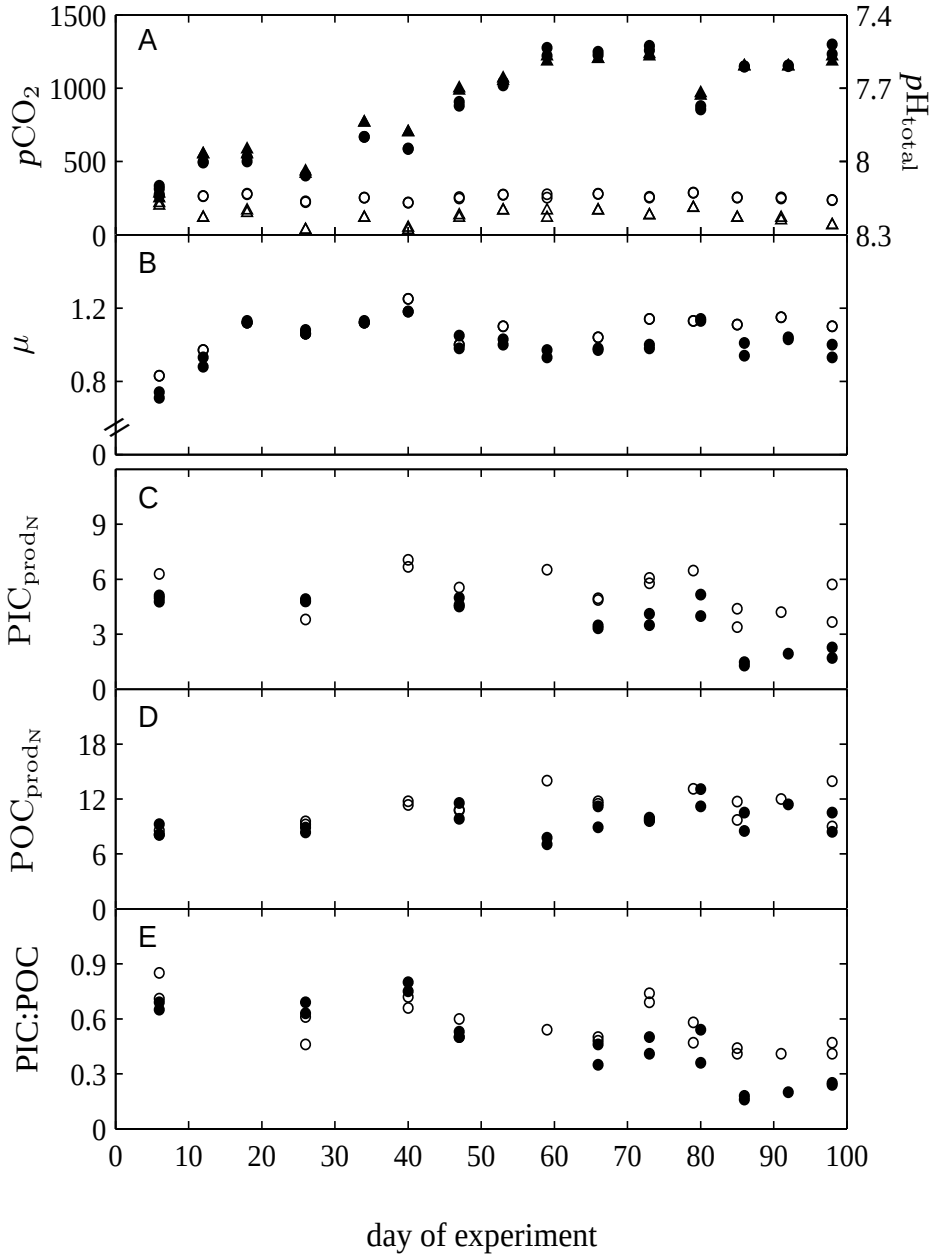
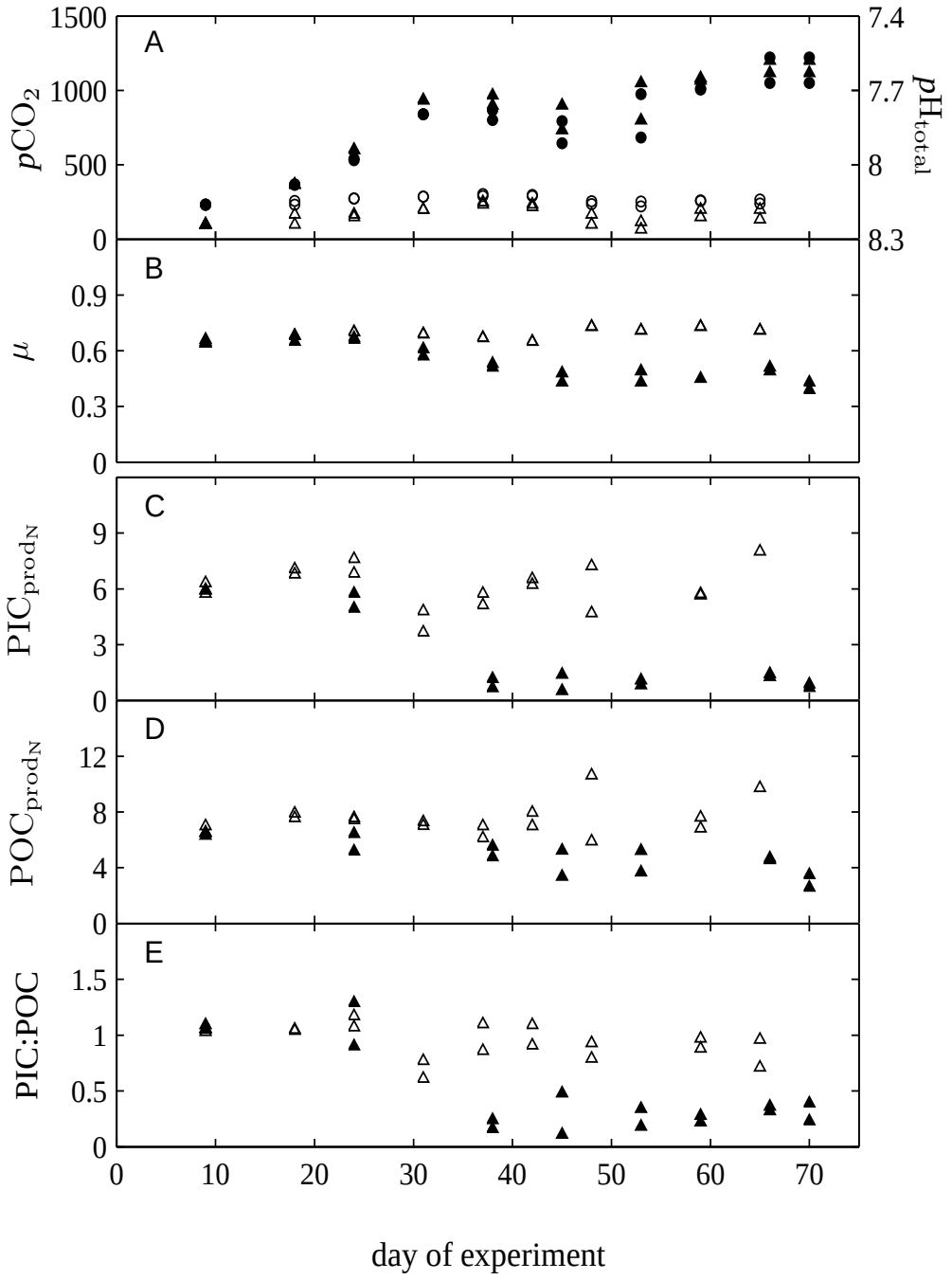

A
B
C
D
E
pCO_2
pH_{total}
μ
PIC_{prod_N}
POC_{prod_N}
PIC:POC
day of experiment

Fig. 2


A
B
C
D
E
pCO_2
pH_{total}
μ
PIC_{prod_N}
POC_{prod_N}
PIC:POC
1500
1000
500
0
7.4
7.7
8
8.3
0.9
0.6
0.3
9
6
3
12
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1.5
1
0.5
0
10
20
30
40
50
60
70
day of experiment

Fig. 3

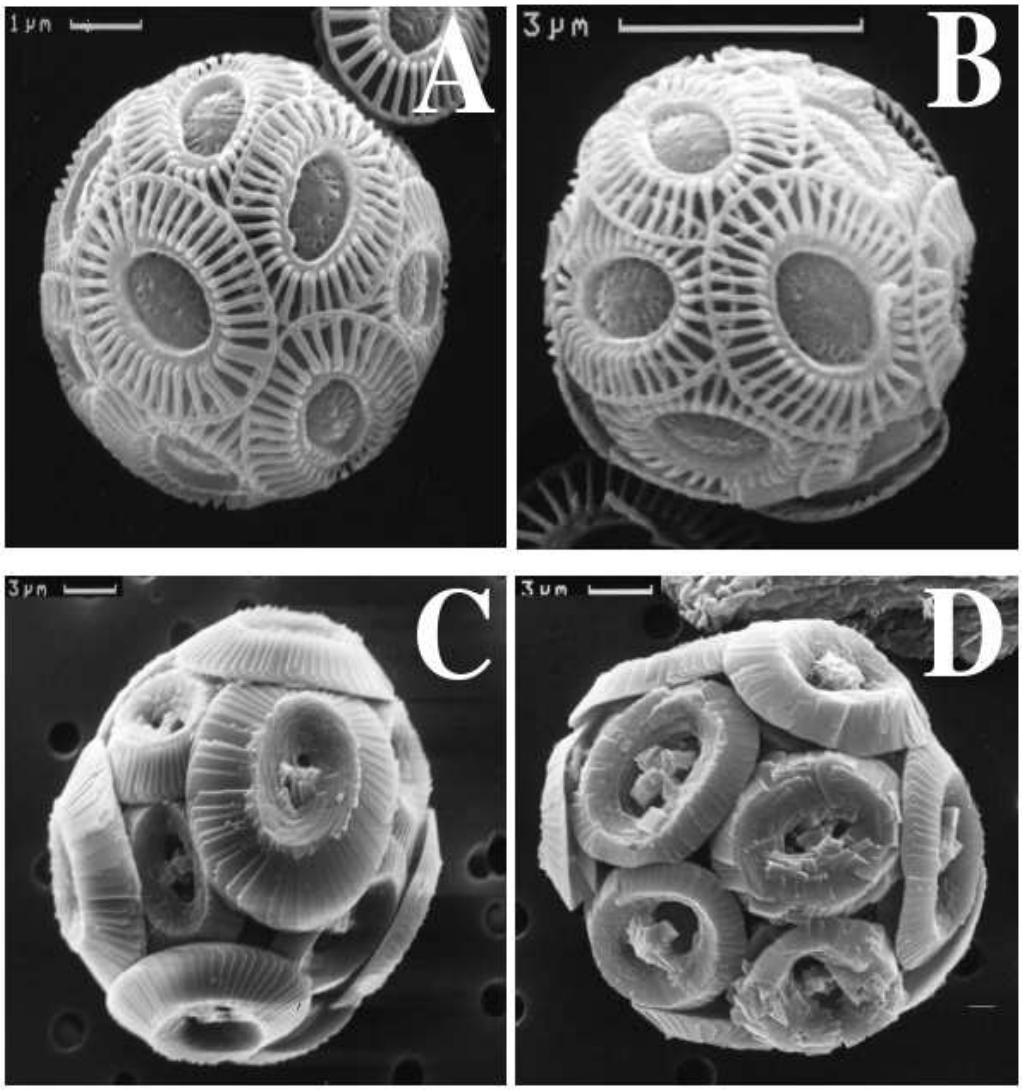

Fig. 4

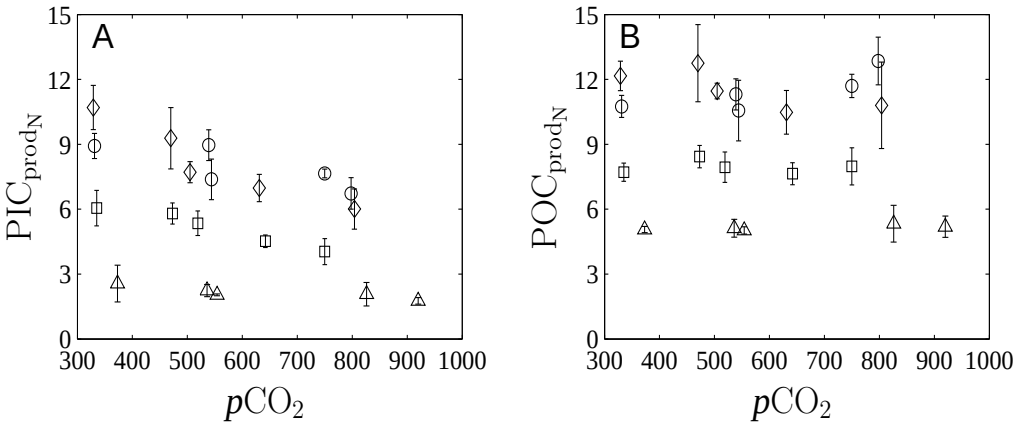
A
B
$\mathrm{PIC}_{\mathrm{prod_N}}$
$\mathrm{POC}_{\mathrm{prod_N}}$
$p\mathrm{CO}_2$
$p\mathrm{CO}_2$
0
3
6
9
12
15
300
400
500
600
700
800
900
1000

Fig. 5

3.4 Manuscript IV: Response to seawater $[Mg^{2+}]$ and $[Ca^{2+}]$

Response of cocccolithphores to changing seawater Mg^{2+} and Ca^{2+} concentrations: Mg^{2+}, Sr^{2+} incorporation and $\delta^{44/40}Ca$, $\delta^{26/24}Mg$ in calcite

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'order to be discussed'

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running head: Ca and Mg fractionation, seawater Mg/Ca ratio

Abstract

Seawater calcium, magnesium concentrations and consequently the Mg/Ca ratio have oscillated over geological time scales. We examined the response of two coccolithophore species (*Emiliania huxlexi* and *Coccolithus braarudii*) to changes in the seawater Mg/Ca ratio by either manipulating the calcium or the magnesium concentration. The physiological reponse was monitored by measurements of cell growth, particulate organic, inorganic carbon and chlorophyll *a* production. Additionally, coccolithophorid calcite was analysed in regard to elemental composition (Sr/Ca and Mg/Ca) and isotope fractionation of calcium and magnesium ($\Delta^{44/40}\mathrm{Ca}$ and $\Delta^{26/24}\mathrm{Mg}$). It revealed that physiological rates were substantially influenced by changes in the seawater calcium rather than the magnesium concentration within the range estimated to have occured in the last 250 million years. All physiological rates of *E. huxleyi* decreased at a calcium concentration above $25\,\mathrm{mmol L^{-1}}$, whereas *C. braarudii* displayed a higher tolerance to elevated seawater calcium concentrations. Elemental composition and isotope fractionation of magnesium in coccolithophorid calcite was essentially influenced by the physiology. This resulted in an increased Mg/Ca ratio in the calcite lattice at low seawater Mg/Ca ratios in both coccolithophore species. Calcium isotope fractionation was not altered by changes in the seawater Mg/Ca ratio. Magnesium isotopes were less fractionated in coccolithophorid calcite than in biogenic calcites of foraminifera, what may be explained by a tight connection between the organic and the inorganic magnesium isotope fractionation inside coccolithophores. These observations suggest a physiological control over magnesium elemental and isotope fractionation during the process of calcification in coccolithophores.

1 Introduction

Coccolithophores are unicellular marine phytoplankton, which appear in the sediment record since the late Triassic (Bown et al. 2004), about 225 million years ago. Coccolithophores surround themselves with a biogenically precipitated calcitic sphere and fixate carbon dioxide via photosynthesis. Therefore, this phytoplankton phylum has a major contribution to the earth's carbon and calcium cycle, both on short and geological time scales. The invention of pelagic biocalcification (e.g. in coccolithophores and foraminifera) had major impacts on the earth's climate and the regulation of marine carbonate chemistry (Ridgwell and Zeebe 2005). A major event in earth's history was the proliferation of planktic calcifiers during the Mid-Mesozoic, subsequently followed by a global decrease in the ocean's calcium concentration (Erba 2006). The decrease in seawater $[\mathrm{Ca}^{2+}]$ was accompanied by an increase of $[\mathrm{Mg}^{2+}]$ due to an overall slowdown in seafloor spreading rates and therefore elevating the seawater Mg/Ca ratio from ≈ 1 in the Cretaceous to the modern value of 5.2 (Lowenstein et al. 2001).

Seawater solutions with a Mg/Ca ratio greater 2 favour the precipitation of aragonite or high Mg-calcite ($> 4\% \, \mathrm{MgCO_3}$) during inorganic calcification, whereas at lower Mg/Ca ratios the polymorph calcite is precipitated (Folk 1974). Coccolithophores are reported to precipitate low Mg-calcite, containing less than 1% of $\mathrm{MgCO_3}$ at ambient seawater conditions (Siesser 1977; Stoll et al. 2007 2001*b*). Coccolith formation takes place in an intracellular coccolith vesicle (CV) connected to the Golgi apparatus (Young et al. 1999). Thus, calcification in coccolithophores is separated from seawater composition by several membranes and influx of seawater ions is probably highly controlled by membrane transport systems and ion channel regulations, reflecting a tight cellular control over calcite precipitation.

Two recent studies dealt with the effects of changing the major ion composition

(Ca^{2+} and Mg^{2+}) of seawater in regard to coccolithophorid physiology (Trimborn et al. 2007; Herfort et al. 2004). Both studies found that variations in the Ca^{2+} concentration of seawater had no effect on organic carbon production ($\mathrm{POC}_{\mathrm{prod}}$), but caused an apparent response in inorganic carbon production ($\mathrm{PIC}_{\mathrm{prod}}$), with increasing $\mathrm{PIC}_{\mathrm{prod}}$ when $[\mathrm{Ca}^{2+}]$ was raised from 0 to $10\,\mathrm{mmol\,L}^{-1}$. Herfort et al. (2004) additionally reported a reduced $\mathrm{PIC}_{\mathrm{prod}}$ in *E. huxleyi* at $[\mathrm{Ca}^{2+}]$ of $50\,\mathrm{mmol\,L}^{-1}$. In a different approach, Stanley et al. (2005) provided evidence that a low seawater Mg/Ca ratio (e.g. increased $[\mathrm{Ca}^{2+}]$ and decreased $[\mathrm{Mg}^{2+}]$) increased cellular growth rate and inorganic carbon production ($\mathrm{PIC}_{\mathrm{prod}}$) in three coccolithophore species (*Pleurochrysis carterae*, *Coccolithus neohelis* and *Ochrosphaera neopolitana*). Additionally, two of these species were reported to precipitate high Mg-calcite ($> 15\%$) at ambient seawater ion composition ($[\mathrm{Ca}^{2+}] = 10\,\mathrm{mmol\,L}^{-1}$; $[\mathrm{Mg}^{2+}] = 50\,\mathrm{mmol\,L}^{-1}$).

All studies on coccolithophorid response to variations in the major ion composition of seawater focus on either the physiology (Trimborn et al. 2007; Herfort et al. 2004) or the coccolith elemental composition (Stanley et al. 2005; Langer et al. 2007). In this study, we present controlled laboratory experiments on two coccolithophore species, *Emiliania huxleyi* and *Coccolithus braarudii*, which have a sediment record of about 270 thousand years and 65 million years, respectively. Our observations elucidate the physiological response of both species to changing $[\mathrm{Ca}^{2+}]$ and $[\mathrm{Mg}^{2+}]$, and the concomitant influence on coccolith Sr/Ca and Mg/Ca ratios and calcium isotope fractionation ($\Delta^{44/40}\mathrm{Ca}$). Furthermore, a data set on coccolith magnesium isotope fractionation ($\Delta^{26/24}\mathrm{Mg}$) is presented.

2 Methods

2.1 Culture setup

Monoculture of *Coccolithus braarudii* RCC 1200 was obtained from the Roscoff Culture Collection. *Emiliania huxleyi* was isolated in 2005 during the PeECE III mesocosm study (Riebesell et al. 2008) in the Raune Fjord (Norway). Before the start of the experiments coccolithophore cultures were acclimatized for about 20 generations under the experimental conditions and it was assured by cell counts that all cultures were in the exponential growth phase at the initiation of the experiments. Coccolithophore species were cultured at 15°C in artificial seawater (all salts were dried at 60°C before dissolution in the media, Kester et al. (1967)) with nutrient addition according to f/20 (Guillard 1975), corresponding to $35.2\,\mu\mathrm{mol\,L^{-1}\,NO_3}$ and $1.44\,\mu\mathrm{mol\,L^{-1}\,PO_4}$. All experiments were conducted in duplicates under dilute batch culture conditions in autoclaved and acid cleaned polycarbonate bottles at a photon flux density of $125\,\mathrm{to}\,200\mu\mathrm{mol\,quanta\,m^{-2}\,s^{-1}}$. Cultures were allowed to grow exponentially under experimental conditions for about 7 to 10 generations, what corresponds to a dissolved inorganic carbon (DIC) consumption of maximal 10%. At the end of the experiments, culture samples were taken for cell number, total particulate carbon (TPC), particulate organic carbon (POC) and chlorophyll *a* (Chl*a*).

2.2 $\mathrm{Mg^{2+}}$-matrix experiment: Altering the Mg/Ca ratio via $\mathrm{Mg^{2+}}$

E. huxleyi was tested in regard to changing $[\mathrm{Mg^{2+}}]$ with keeping the $[\mathrm{Ca^{2+}}]$ constant. Artificial seawater preparation was done as described above with varying the $[\mathrm{Mg^{2+}}]$ at a constant $[\mathrm{Ca^{2+}}]$ of $9.8 \pm 0.1\,\mathrm{mmolL^{-1}}$. Salinity was kept constant at 35 by altering the addition of NaCl. $[\mathrm{Mg^{2+}}]$ was adjusted at 5.5, 9.8, 18.9, 46.4 and $91.6\,\mathrm{mmolL^{-1}}$, what corresponded to a Mg/Ca of 0.6, 1.0, 1.9, 4.8 and 9.5, respectively.

Mg^{2+}-matrix experiment was carried out under a light intensity of $125\,\mu\mathrm{mol\,quanta\,m^{-2}\,s^{-1}}$. Starting media was adjusted to a $p\mathrm{H}_{\mathrm{total\,scale}}$ of 8.2 ± 0.1 and DIC was measured at the beginning and the end of the experiment to assure DIC consumption of less than 10%.

2.3 Ca^{2+}-matrix experiments: Altering the Mg/Ca ratio via Ca^{2+}

Artificial media preparation was followed as described above with modifications to alter the media Mg/Ca ratio. Two set of experiments were conducted: (1) $[Mg^{2+}]$ was kept constant at $\approx 50\,\mathrm{mmol\,L^{-1}}$ and $[Ca^{2+}]$ was varied to receive Mg/Ca ratio ranging from 1 to 10 ($[Ca^{2+}]$ from 5 to 50 $\mathrm{mmol\,L^{-1}}$), termed experiment 1; (2) $[Mg^{2+}]$ was kept constant at $\approx 25\,\mathrm{mmol\,L^{-1}}$ and $[Ca^{2+}]$ was varied to receive Mg/Ca ratio ranging from 0.5 to 10 (Ca^{2+} from 2.5 to 50 $\mathrm{mmol\,L^{-1}}$), termed experiment 2. In both experimental setups the salinity was kept constant at ≈ 35 by altering the addition of $NaCl$ (see table 1 and 2 for a detailed listening of media content). The carbonate system was adjusted to a $p\mathrm{H}_{\mathrm{total\,scale}}$ of 8.2 ± 0.1.

Ca^{2+}-matrix experiments were carried out under a light intensity of $160\,\mu\mathrm{mol\,quanta\,m^{-2}\,s^{-1}}$, except for experiment 1 in which *E. huxleyi* received a higher light intensity of $200\,\mu\mathrm{mol\,quanta\,m^{-2}\,s^{-1}}$. Carbonate system was monitored at the start and at the end of the experiments by dissolved inorganic carbon (DIC) and total alkalinity (TA) measaurements. After sampling for physiological parameters (see 2.1), the remaining water of the duplicate bottles was pooled and centrifuged at $14000\,\mathrm{rpm}$ for 10 minutes, afterwards the sample pellet was dried at $60^{\circ}\mathrm{C}$ for 48 hours and stored at room temperature for further analytics. Furthermore, at the start of the experiments water samples were taken for elemental composition (Ca^{2+}, Sr^{2+} and Mg^{2+}), $\delta^{44/40}\mathrm{Ca}$ and $\delta^{26/24}\mathrm{Mg}$.

2.4 Sample preparation via organic removel

Dried sample pellets were immersed into $2\,\mathrm{ml}$ of 10% NaClO solution in Teflon vials, placed for ten minutes in an ultrasonic bath and then incubated for 24 hours at room temperature. The next day, sample solution was centrifuged in $2\,\mathrm{ml}$ Eppendorf vials for five minutes at $14.000\,\mathrm{rpm}$; the supernatant was discarded, and the pellets were resuspended in ultrapure $\mathrm{H_2O}$ (with a *p*H of 9-10, adjusted by addition of $\mathrm{NH_4OH}$ to prevent partial dissolution of calcite), centrifuged again and resuspended in water a second time. The solution was removed and the pellets were transferred back into Teflon vials. The whole organic removal process was repeated a total of 3 times. After the third time, the pellet was washed four times with ultrapure water. Finally, the calcite pellet was partitioned for $\delta^{44/40}\mathrm{Ca}$, $\delta^{26/24}\mathrm{Mg}$ and elemental composition analysis.

2.5 Analytical methods

2.5.1 Carbonate system

The carbonate system was monitored by DIC and TA measurements at the start and the end of the $\mathrm{Ca^{2+}}$-matrix experiments. DIC was analysed photochemically after Stoll et al. (2001*a*) and TA was measured by the potentiometric titration method after Dickson (1981) and calibrated with Dickson seawater standards. The carbonate system ($p\mathrm{H_{total\,scale}}$, $[\mathrm{CO_2}],\ [\mathrm{HCO_3^-}]\ \mathrm{and}\ [\mathrm{CO_3^{2-}}]$) was calculated by equations from Zeebe and Wolf-Gladrow (2001) with dissociation constants for carbonic acid after Roy et al. (1993) modified with sensitivity parameters for $[\mathrm{Na^+}]$, $[\mathrm{Mg^{2+}}]$ and $[\mathrm{Ca^{2+}}]$ (Ben-Yaakov and Goldhaber 1973). The calcite saturation state (Ω) was calculated with regard to Mg/Ca ratio as described in Tyrrell and Zeebe (2004) and remained above 1.6 in all experiments.

2.5.2 Carbon pool parameters and chlorophyll *a*

Sub-samples were taken for TPC, POC and chl*a* from each flask, filtrated onto precombusted GF/F filters (525°C for 7 hours) and frozen at $-20\,^{\circ}\mathrm{C}$. For POC analysis filters were fumed over HCl for 24 hours to remove all inorganic carbon and afterwards filters for TPC and POC were measured on an Euro EA Elemental Analyser (Ehrhardt and Koeve 1999). Particulate inorganic carbon was calculated from the difference of TPC and POC. Prior to analysis chlorophyll *a* filters were kept in the dark to avoid photo-oxidation and measurements were performed using the fluorimetric method after Welschmeyer (1994).

2.6 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 (μ) as

$$\mu = \frac{(\ln \mathrm{c}_1 - \ln \mathrm{c}_0)}{\mathrm{t}_1 - \mathrm{t}_0} \tag{1}$$

where c_0 and c_1 are the cell concentration at the start (t_0) and end (t_1) of the experiment, respectively.

2.7 Elemental composition analysis

Calcite samples were analysed for Ca^{2+} and the trace metals Sr^{2+} and Mg^{2+} via ICP-MS (Stoll et al. 2001*b*). Seawater element composition was determined via ICP-AES calibrated to the IAPSO seawater standard with a relative precision of $\pm 0.40\,\%$, $\pm 0.74\,\%$, $\pm 0.37\,\%$ and $\pm 0.13\,\%$ for Na^{+}, Ca^{2+}, Mg^{2+} and Sr^{2+}, respectively, at the Geochemistry Department of the IFM-GEOMAR. The partition coefficient of the trace metal, D_{Tr}, between calcite (c) and seawater (s) was calculated as:

$$\mathrm{D}_{\mathrm{Tr}} = \frac{[\mathrm{Tr}/\mathrm{Ca}]_{\mathrm{c}}}{[\mathrm{Tr}/\mathrm{Ca}]_{\mathrm{s}}} \tag{2}$$

Samples from the first *E. huxleyi* Ca-matrix experiment ($[Mg^{2+}] = 50\,mmol L^{-1}$) were unfortunately lost during sample preparation.

2.8 Ca^{2+} isotope analysis

The $^{44}Ca/^{40}Ca$ ratio of the coccolith was measured using thermal ionization mass spectrometry (TIMS) double-spike technique on a Finnigan Triton T1 at the Leibniz Institute of Marine Sciences (IFM-GEOMAR), Kiel (Heuser et al. 2002). The calcium isotope data are expressed relative to NIST SRM915a standard, which was measured 4 times in each analytical session and average precision resulted in 0.1-0.2‰. Ca^{2+} isotope ratios are expressed as $\Delta^{44/40}Ca$ values according to Eisenhauer et al. (2004) as:

$$\Delta^{44/40}Ca(‰) = \left(\frac{(^{44}Ca/^{40}Ca)_{sample}}{(^{44}Ca/^{40}Ca)_{SRM915a}} - 1\right) \cdot 1000 - \left(\frac{(^{44}Ca/^{40}Ca)_{seawater}}{(^{44}Ca/^{40}Ca)_{SRM915a}} - 1\right) \cdot 1000 \quad (3)$$

Samples were measured 3 to 7 times and are presented with 2 standard deviations (2σ).

2.9 Mg^{2+} isotope analysis

Chemical separation of Mg^{2+} in samples from the Ca-matrix experiments was carried out using the column chemistry of Pogge von Strandmann (2008), only the samples of the *E. huxleyi* experiment with constant $[Mg^{2+}]$ of $50\,mmol L^{-1}$ received a different chemistry (described below). Approximately $1500\,ng$ of Mg^{2+} was passed through Bio-Rad AG 50W-X12 resin ($200 - 400\,mesh$) using HNO_3 as the elusion acid. Magnesium isotopes of these samples were measured on a ThermoElectron Neptune MC-ICP-MS in the isotope laboratory of the Ruhr-Universität Bochum, Germany (Buhl et al. 2007). The sample was introduced into the Neptune via desolating nebulizers Apex (ESI) connected to Aridus (CETAC). Each analysis on the Neptune consisted of measurements of 45 ratios. A $250\,ppb$ solution and solution uptake of $100\,\mu L min^{-1}$ typically produced a beam intensity of $\approx 10\,V$ compared with a background of

$< 30\,\mathrm{mV}$. Each sample was analysed in a sequence of 5 repeats, bracketed by the DSM3 standard that has a concentration within $\pm 10\%$ of that of the sample. The background, measured before and after the sequence, was substracted. We report the magnesium isotopic composition of each sample as the mean of 5 repeats ($\pm 2\sigma$). Fourteen analyses of Cambridge-1 standard gave $^{25/24}\mathrm{Mg} = -1.35 \pm 0.03‰$ and $^{26/24}\mathrm{Mg} = -2.58 \pm 0.09‰$. Three analyses of CJp-1 (passed through cation exchange chemistry) gave $^{25/24}\mathrm{Mg} = -1.05 \pm 0.01‰$ and $^{26/24}\mathrm{Mg} = -1.99 \pm 0.11‰$, within error of a previously published value ($^{26/24}\mathrm{Mg} = -2.01 \pm 0.22‰$, Wombacher et al. (2009)). Mg^{2+} isotopic composition of the Mg^{2+}-salt used in preparing the culturing fluid was measured as $^{25/24}\mathrm{Mg} = +0.09 \pm 0.03‰$ and $^{26/24}\mathrm{Mg} = +0.13 \pm 0.04‰$.

Chemical separation of Mg^{2+} in samples from the Ca^{2+}-matrix experiments with constant $[\mathrm{Mg}^{2+}]$ of $50\,\mathrm{mmol L}^{-1}$ was carried out using the elusion scheme of Wombacher et al. (2009). Approximately $1000 - 1500\,\mathrm{ng}$ of Mg^{2+} was passed through Bio-Rad AG 50W-X8 resin ($200 - 400\,\mathrm{mesh}$) using HCl as the elusion acid. Magnesium isotopes of these samples were measured on a AXIOM MC-ICP-MS at the Leibniz Institute of Marine Sciences (IFM-GEOMAR) in Kiel, Germany. The sample was introduced into the Axiom via desolating nebulizers Apex (PFA-ST) connected to Aridus (CETAC). Each sample was analysed in a sequence of 3-5 repeats as described above. Three analyses of CJp-1 (passed through cation exchange chemistry) resulted in $^{26/24}\mathrm{Mg} = -2.01 \pm 0.10‰$. Mg^{2+} isotopic composition of the Mg^{2+}-salt used in preparing the culturing fluid was measured as $^{26/24}\mathrm{Mg} = +0.27 \pm 0.08‰$.

Mg^{2+} isotope ratios are expressed as $\Delta^{26/24}\mathrm{Mg}$ values ralative to the Mg^{2+}-salt measured on the same analytical session according to:

$$\Delta^{26/24}\mathrm{Mg}(‰) = \left(\frac{(^{26}\mathrm{Mg}/^{24}\mathrm{Mg})_{\mathrm{sample}}}{(^{26}\mathrm{Mg}/^{24}\mathrm{Mg})_{\mathrm{DSM3}}} - 1\right) \cdot 1000 - \left(\frac{(^{26}\mathrm{Mg}/^{24}\mathrm{Mg})_{\mathrm{seawater}}}{(^{26}\mathrm{Mg}/^{24}\mathrm{Mg})_{\mathrm{DSM3}}} - 1\right) \cdot 1000 \tag{4}$$

3 Results

3.1 Physiology

3.1.1 *Emiliania huxleyi*

Mg^{2+}*-matrix.* Cellular growth rate of *E. huxleyi* was constant (with less than 10% variation) for a [Mg^{2+}] range from 18.9 to $91.6\,\mathrm{mmol\,L^{-1}}$, but decreased by 30% when seawater [Mg^{2+}] was lowered from 18.9 to $5.5\,\mathrm{mmol\,L^{-1}}$ (Fig. 1A). PIC_{prod} and POC_{prod} were relatively constant (9.18 ± 1.25 and $13.10 \pm 1.30\,\mathrm{pgC\,cell^{-1}day^{-1}}$, respectively) over the tested [Mg^{2+}] (Fig. 1 B+C). Chla_{prod} was reduced by 45% at [Mg^{2+}] below $18.9\,\mathrm{mmol\,L^{-1}}$, but increased by $\approx 20\%$ due to elevation of seawater Mg^{2+} concentration from 18.9 to $91.6\,\mathrm{mmol\,L^{-1}}$ (Fig. 1D).

Ca^{2+}*-matrix.* Growth rate was consistently lower by $\approx 0.3\,\mathrm{d^{-1}}$ in experiment 1 ($[Mg^{2+}] = 50\,\mathrm{mmol\,L^{-1}}$) compared to experiment 2 ($[Mg^{2+}] = 25\,\mathrm{mmol\,L^{-1}}$; Fig. 2A); this appears to be a result of the higher illumination of $40\,\mu\mathrm{mol\,quanta\,m^{-2}\,s^{-1}}$ in the latter experimental setup. Elevating seawater [Ca^{2+}] from below 13 to above $25\,\mathrm{mmol\,L^{-1}}$ resulted in a decrease in the reproduction rate of *E. huxleyi* (by 25 and 45% in experiments 1 and 2, respectively). PIC_{prod} followed an optimum curve with a peak value of $6.9\,\mathrm{pg\,C\,cell^{-1}day^{-1}}$ at an ambient [Ca^{2+}] of $10\,\mathrm{mmol\,L^{-1}}$ (Fig. 2B). Lowest PIC_{prod} rates were observed at Ca^{2+} concentrations $\geq 25\,\mathrm{mmol\,L^{-1}}$. POC_{prod} was relatively constant (6.1 ± 0.61 and $4.9 \pm 0.17\mathrm{pg\,C\,cell^{-1}day^{-1}}$ in experiment 1 and 2, respectively) at [Ca^{2+}] $< 15\,\mathrm{mmol\,L^{-1}}$, whereas elevating seawater [Ca^{2+}] from 15 to above $25\,\mathrm{mmol\,L^{-1}}$ resulted in a decrease in POC_{prod} (by 45 and 50% in experiment 1 and 2, respectively; Fig. 2C). Chla_{prod} followed a similar trend as that of POC_{prod}. Nevertheless, Chla_{prod} in experiment 2 was consistently 70 to 90% higher than in experiment 1 over the tested Ca^{2+} concentrations (Fig. 2D).

In summary, the observed decreases in physiological parameters of *E. huxleyi* to elevated seawater [Ca^{2+}] are equally observed at Mg/Ca ratios ≤ 2 (Fig. 3).

3.1.2 *Coccolithus braarudii*

Ca^{2+}*-matrix.* The growth rate of *C. braarudii* was highest ($0.70 \pm 0.03\,d^{-1}$) at 5 to $25\,mmol\,Ca\,L^{-1}$ (Fig. 4A), whereas the lowest rate ($0.46 \pm 0.01\,d^{-1}$) was observed at [Ca^{2+}] of $46.7\,mmol\,L^{-1}$ and [Mg^{2+}] of $47.7\,mmol\,L^{-1}$ what corresponds to a seawater Mg/Ca ratio of 1 (Fig. 5A). PIC_{prod} was relatively constant at $142 \pm 15\,pg\,C\,cell^{-1}day^{-1}$ between [Ca^{2+}] ranging from 10 to $50\,mmol\,Ca\,L^{-1}$ and decreased to $106 \pm 3\,pg\,C\,cell^{-1}day^{-1}$ at $2.6\,mmol\,Ca\,L^{-1}$ (Fig. 4B). POC_{prod} was neither affected by changing [Ca^{2+}] nor [Mg^{2+}] over the tested range (Fig. 4C) and had a mean value of $220 \pm 15\,pg\,C\,cell^{-1}day^{-1}$. Chl$a_{prod}$ had maximal rates of $5.0 \pm 0.5\,pgChla\,cell^{-1}\,d^{-1}$ at $[Ca^{2+}] \leq 25\,mmol\,L^{-1}$ and decreased to $2.5 \pm 0.1\,pgChla\,cell^{-1}\,d^{-1}$ at high seawater $[Ca^{2+}]$ of $\approx 48\,mmol\,L^{-1}$ (Fig. 4D). In Summary, the rather constant behaviour of *C. braarudii* to variations in seawater Ca^{2+} concentrations resulted in a similar picture in regard to seawater Mg/Ca ratios (Fig. 5), whereas a clear decrease of Chla_{prod} was observed at Mg/Ca ratios below 2 (Fig. 5D).

3.2 Calcite analytics

3.2.1 Sr^{2+} and Mg^{2+} incoporation

The coccolith Sr/Ca ratio was linearly related to the seawater Sr/Ca ratio (Fig. 4A) and no change was observed in the partition coefficient between species and experiments (Fig. 6B). The slope of the regression line resulted in a D_{Sr} of 0.32.

Coccolithophores grown at various seawater Mg/Ca ratios resulted in low Mg-calcite (<1% of $MgCO_3$). At a seawater Mg/Ca ratio above 2, the Mg/Ca ratio in coccolith calcite stayed relatively constant in each experiment and D_{Mg} ranged from $0.1 \cdot 10^{-3}$ to $0.6 \cdot 10^{-3}$ (Fig. 7). At seawater Mg/Ca ratios below 2, both the coccolith Mg/Ca ratio and D_{Mg} increased remarkably to a maximum value 13.7 for D_{Mg} (Fig. 7B).

3.2.2 $\Delta^{44/40}Ca$ and $\Delta^{26/24}Mg$

Calcium isotope fractionation in *E. huxleyi* as well as in *C. braarudii* was not influenced by alteration of the seawater [Ca^{2+}] of Mg/Ca ratio (Fig. 8). Also no significant difference was observed in $\Delta^{44/40}\mathrm{Ca}$ between species and different treatments. Mean $\Delta^{44/40}\mathrm{Ca}$ of *E. huxleyi* and *C. braarudii* resulted in $-1.05 \pm 0.21\,‰$ ($\pm 2\sigma$) and $-1.06 \pm 0.11\,‰$, respectively. The observed variability in calcium isotope fractionation is comparable to the external reproducibility of the method.

$\Delta^{26/24}\mathrm{Mg}$ in coccolithophorid calcite was relatively stable for seawater Mg/Ca ratios ranging from 1 to 5, resulting in mean $\Delta^{26}\mathrm{Mg}$ of $-1.00 \pm 0.22\,‰$ ($\pm 2\sigma$) and $-0.77 \pm 0.08\,‰$ for *E. huxleyi* and *C. braarudii*, respectively (Fig. 9). This indicates a higher magnesium isotope fractionation in coccolith calcite of *E. huxleyi* at an ambient Mg/Ca ratio. We can rule out an analytical or instrumental offset since different cultures of *E. huxleyi* (Ca^{2+}-matrix experiment 1 versus 2) analysed on different machines (section 2.9) produced similar $\Delta^{26/24}\mathrm{Mg}$ values for tested seawater Mg/Ca ratios. Both species showed a higher degree of Mg^{2+} isotope fractionation at high seawater Mg/Ca ratio (corresponding to $[\mathrm{Ca}^{2+}] < 5\,\mathrm{mmol\,L^{-1}}$). Whereas, the lowest $\Delta^{26/24}\mathrm{Mg}$ value of $-2.11\,‰$ was measured in *E. huxleyi* at $[\mathrm{Ca}^{2+}] = 5.1$ and $[\mathrm{Mg}^{2+}] = 49.8\,\mathrm{mmol\,L^{-1}}$ (Fig. 9A).

4 Discussion

4.1 Physiology

4.2 Mg-matrix

Changes in seawater [Mg^{2+}] within the range of variability during the last 250 Ma (30 to $50\,mmol\,L^{-1}$ based on fluid inclusion of marine halite by Holland (2003)) caused no effect on the physiological rates of *E. huxleyi* (Fig. 1). Therefore, we attribute changes in the physiological rates of *E. huxleyi* during the two Ca^{2+}-matrix experiments to the seawater [Ca^{2+}]. This is also valid for *C. braarudii*, since no substantial changes were observed by doubling the seawater [Mg^{2+}] from 25 to $50\,mmol\,L^{-1}$ (Fig. 4, open markers compared to filled ones).

4.3 Ca-matrix

4.3.1 Growth rate

The offset in growth rate of *E. huxleyi* between the two experiments of the Ca^{2+}-Matrix of ≈ 0.3 units (Fig. 1A) is attributable to the higher light intensity (200 compared to $160\,\mu mol\,quanta\,m^{-2}\,s^{-1}$). In fact, the growth rate is light saturated at $\approx 200\,\mu mol\,quanta\,m^{-2}\,s^{-1}$ and 15°C in *E. huxleyi* (Paasche 2002). Regardless of the [Mg^{2+}], an increased [Ca^{2+}] of $\geq 25\,mmol\,L^{-1}$ (Mg/Ca ratio ≤ 2) caused a significant decline in the growth rate of *E. huxleyi* and a decreased concentration below $10\,mmol\,Ca\,L^{-1}$ (Mg/Ca ≥ 5) had no substantial effect. Constant growth rates at [Ca^{2+}] ranging from 2 to $10\,mmol\,L^{-1}$ were previously reported for *E. huxleyi* by Trimborn et al. (2007), whereas in the latter study *E. huxleyi* was not tested to higher [Ca^{2+}]. *C. braarudii* displayed a similar response to seawater Ca^{2+} concentrations by decreasing its cell division at $50\,mmol\,L^{-1}$ but it seems to be less sensitive to elevated [Ca^{2+}] since no

change in growth rate was observed at $25\,\mathrm{mmol\,L^{-1}}$ as seen in *E. huxleyi* (Fig. 2A).

The observed decrease in the growth rate of *E. huxleyi* and *C. braarudii* with elevated $[Ca^{2+}]$ over $25\,\mathrm{mmol\,L^{-1}}$ are in discrepancy with observations on different coccolithophore species obtained Stanley et al. (2005). The latter study reported an increase in growth rate of three species (*Pleurochrysis carterae*, *Coccolithus neohelis* and *Ochrosphaera neopolitana*) by lowering the Mg/Ca ratio from ambient (5.2) to Cretaceous (≈ 1) value, either by elevating $[Ca^{2+}]$ or decreasing $[Mg^{2+}]$. The current results indicate that *E. huxleyi* and *C. braarudii* show decreased growth rates at seawater Mg/Ca ratio ≤ 2. This discrepancy cannot be clarified but species specific responses may be an possible explanation.

4.3.2 PIC$_{prod}$

Inorganic carbon production of the investigated species was not influenced by doupling the seawater $[Mg^{2+}]$ from 25 to $50\,\mathrm{mmol\,L^{-1}}$. Nevertheless, *E. huxleyi* and *C. braarudii* displayed different patterns in case of $\mathrm{PIC_{prod}}$ within the tested range of $[Ca^{2+}]$. *E. huxleyi* displayed an optimal calcification rate at ambient $[Ca^{2+}]$ (Fig. 2B). Nimer et al. (1996), Trimborn et al. (2007) and Herfort et al. (2004) reported similar increasing trends for $\mathrm{PIC_{prod}}$ in *E. huxleyi* in the range from 1 to $10\,\mathrm{mmol\,Ca\,L^{-1}}$, and equal to the current study Herfort et al. (2004) observed a reduction by more than 50% in $\mathrm{PIC_{prod}}$ of *E. huxleyi* at a seawater $[Ca^{2+}]$ of $50\,\mathrm{mmol\,L^{-1}}$. *C. braarudii*, on the other hand, increased $\mathrm{PIC_{prod}}$ up to a seawater $[Ca^{2+}]$ of $10\,\mathrm{mmol\,L^{-1}}$ and remained constant with higher $[Ca^{2+}]$ (Fig. 3B). Both species achieved saturation of $\mathrm{PIC_{prod}}$ at a $[Ca^{2+}]$ of $10\,\mathrm{mmol\,L^{-1}}$. This suggests a well established and adjusted calcification mechanism to calcium concentrations of the modern ocean. Interestingly, *C. braarudii* maintains optimal calcification at far higher calcium concentration than does *E. huxleyi*. A similar response was also observed in other physiological rates and will be elucidated at the end of this section.

4.3.3 POC$_{prod}$

Elevating $[\mathrm{Ca}^{2+}]$ from low to ambient values had no effect on $\mathrm{POC}_{\mathrm{prod}}$ in *E. huxleyi* and *C. braarudii*, what was previously observed in lab experiments for *E. huxleyi* (Trimborn et al. 2007; Herfort et al. 2004). In contrast, Nimer et al. (1996) observed a decrease in photosynthetic fixation by lowering media $[\mathrm{Ca}^{2+}]$ below $10\,\mathrm{mmol\,L^{-1}}$. These diverse findings could be due to different experimental set ups or the method used for the determination of photosynthetic activity (Trimborn et al. 2007). However, further calcium elevation above $20\,\mathrm{mmol\,L^{-1}}$ affected $\mathrm{POC}_{\mathrm{prod}}$ in *E. huxleyi* drastically, lowering the rate by 45%. Nevertheless, *C. braarudii* seems to be able to cope with high seawater $[\mathrm{Ca}^{2+}]$. Additionally, the increased $\mathrm{PIC}_{\mathrm{prod}}$ at [Ca^{2+}] from 1 to $10\,\mathrm{mmol\,L^{-1}}$ and the concomitant steady $\mathrm{POC}_{\mathrm{prod}}$ rate in both species gives evidence that calcification does not promote photosynthetic carbon acquisition in coccolithophores.

4.3.4 Chl*a*$_{prod}$

Under nutrient replete and constant temperature conditions $\mathrm{Chl}a_{\mathrm{prod}}$ is negatively correlated to light irradiance (Geider et al. 1996) what is clearly reflected in the low $\mathrm{Chl}a_{\mathrm{prod}}$ of *E. huxleyi* at higher illumination (Fig. 2D). Regardless of the light intensity, *E. huxleyi* and *C. braarudii* showed decreased $\mathrm{Chl}a_{\mathrm{prod}}$ by $\approx 50\%$ when seawater [Ca^{2+}] was increased from 10 to $50\,\mathrm{mmol\,L^{-1}}$. The central atom of chlorophyll is Mg^{2+}, which is the most abundant divalent cation in cells, mostly bound to nucleotides, RNA, DNA, phospholipids and it also reacts as a cofactor for ATP in a number of enzymatic reactions (Legong et al. 2001). Free Mg^{2+} inside the cell is precisely regulated and kept in homeostasis (Beeler et al. 1997). High external [Ca^{2+}] are capable of interfering with Mg^{2+} uptake in higher plants by blocking the relevant ion channels (Moore et al. 1960) and altering cytosol Mg^{2+} homeostasis. It remains questionable if this could be an explanation for the observed reduction of $\mathrm{Chl}a_{\mathrm{prod}}$ in coccolithophores.

4.3.5 Cellular physiology and free Ca^{2+}

Cellular free [Ca^{2+}] within the cytosol is kept very low at $0.1\,\mu mol\,L^{-1}$ and maintained constant by transmembrane transports via Ca^{2+} channels and Ca^{2+} ATPases (Brownlee et al. 1995). The Ca^{2+} cation is used as a major signalling ion to regulate energy output and cellular metabolism within the cell. Therefore, a constant [Ca^{2+}] in the cell seems to be a crucial component in maintaining cellular processes, e.g. regulation of HCO_3^- uptake (Nimer et al. 1996) or even motility in cyanobacteria (Pitta et al. 1997). In both species investigated, PIC_{prod} displayed a positive correlation to increasing external [Ca^{2+}] form 2 to $10\,mmol\,L^{-1}$, whereas all other tested physiological parameters (growth rate, POC_{prod} and Chla_{prod}) remained constant.

Precipitation of calcite occurs in the coccolith vesicle, which is separated from the cytosol by a biomembrane (Young et al. 1999), and calculations indicate a [Ca^{2+}] range from $100\,\mu M$ to $10\,mM$ inside the vesicle (Anning et al. 1996). Ca^{2+} flux from external seawater to the site of precipitation (coccolith vesicle) has to pass through two biomembranes, first the cell membrane (downhill concentration gradient, $-\Delta G$) and the coccolith-vesicle membrane (uphill concentration gradient, $+\Delta G$). Coccolithophores are probably well adapted to the recent [Ca^{2+}] of the ocean and therefore might not have mechanisms to react rapidly to a changing external calcium concentration since the oceanic Ca^{2+} abundance is rather uniform and has not changed within a millennium. Hence, influx of Ca^{2+} depends on the abundance of Ca^{2+} channels on the cell's surface and the concentration gradient between cytosol and seawater. If we assume the abundance of Ca^{2+} channels to be constant per surface area, then only the concentration gradient will alter the influx of Ca^{2+} ions. A lower external Ca^{2+} abundance ($< 10\,mmol\,L^{-1}$) will reduce the influx into the cytosol. The diminished influx can be compensated by either reducing the outward Ca^{2+} transport from the cytosol to the external media (seawater) or reducing the transport of Ca^{2+} into the coccolith vesicle. Here, we found a decreased PIC_{prod}, what may be an evidence for a diminished flux in the latter transport mode (cytosol to coccolith vesicle). On the other hand, an elevated

seawater calcium concentration (above $10\,\mathrm{mmol\,L^{-1}}$) will increase the $\mathrm{Ca^{2+}}$ influx to the cytosol. High [$\mathrm{Ca^{2+}}$] concentrations in the cytosol can have toxic effects, primarily targeting mitochondria and the endoplasmatic reticulum (Kass and Orrenius 1999), and therefore may affect all physiological rates as seen in *E. huxleyi*. *C. braarudii* seems to be able to cope with an elevated external [$\mathrm{Ca^{2+}}$] to a certain degree. This can be explained by a smaller surface to volume ratio what would decelerate the process of toxification by an accumulation of $\mathrm{Ca^{2+}}$ ions in the cytosol.

4.4 Calcite analytics

4.4.1 Sr^{2+} and Mg^{2+} incoporation

The partition coefficient ($\mathrm{D_{Sr}}$) was independent of seawater Sr/Ca ratios and no species specific dependency was found between *E. huxleyi* and *C. braarudii*. The calculated partition coefficient of 0.32 is well within the range of reported values from culture experiments (Rickaby et al. 2002; Stoll et al. 2002; Langer et al. 2006). $\mathrm{D_{Sr}}$ values from inorganically precipitated calcite display an order of magnitude lower values ranging from 0.021 to 0.14 and have a clear dependence on crystal growth rates (Lorens 1981; Tesoriero and Pankow 1996; Tang et al. 2008). The underlying mechanisms leading to the observed offset between biologically mediated and inorganically precipitated calcite are still under discussion. One factor is probably the missing knowledge regarding the actual Sr/Ca ratio inside the coccolith vesicle solution. Strontium incorporation into inorganically and biologically mediated calcite is discussed in detail elsewhere (e.g. Langer et al. (2006); Stoll and Ziveri (2004); Tang et al. (2008)) and here, we focus on the Mg/Ca ratios in coccoliths.

Data on $\mathrm{Mg^{2+}}$ incorporation into coccolithophore calcite are rather rare. First measurements of coccolith calcite were done by Siesser (1977) and were reported to contain less than 1% of $\mathrm{MgCO_3}$. This is well in agreement with the values revealed from the current study and further supported by similar $\mathrm{MgCO_3}$ measurements of *C. leptoporus*, *G. oceanica* and *H. carteri* by Stoll et al. (2007 2001*b*). These results are in

disagreement with results from Stanley et al. (2005) demonstrating high Mg-calcite (>15%) in two coccolithophore species (*P. carterae* and *O. neopolitana*) at ambient seawater conditions. Furthermore, the authors indicate that these two species precipitate low Mg-calcite if cultured at seawater Mg/Ca ratio of 1 or lower as inferred for the Late Cretaceous and suggested that a low seawater Mg/Ca ratio promoted massive chalk deposition by coccolithophores during the Late Cretaceous. Our results indicate that the two species *E. huxleyi* and *C. braarudii* are able to maintain low Mg-calcite precipitation (<1%) when cultured at different seawater Mg/Ca ratios and do not increase their growth or calcification rates under a low seawater Mg/Ca ratio.

Inorganic partition coefficients of magnesium are about two magnitudes higher than values observed in this study at seawater Mg/Ca ratios above 2 (Mucci and Morse 1983), what implies a tight control over Mg^{2+} incorporation during calcite precipitation in coccolithophores.

We observed a remarkable increase of the Mg/Ca ratio in coccolithophorid calcite at seawater Mg/Ca ratios below one, what was accompanied by an interference of $\mathrm{Chl}a_{\mathrm{prod}}$ at high seawater calcium concentrations (Fig. 2D+3D). This is further indicated by the correlation of $\mathrm{Chl}a_{\mathrm{prod}}$ and the magnesium partition coefficient of the calcite lattice (Fig. 8). However, a direct correlation of $\mathrm{Chl}a_{\mathrm{prod}}$ to D_{Mg} is very unlikely because cellular Mg^{2+} is not only involved in cellular $\mathrm{Chl}a_{\mathrm{prod}}$. Mg^{2+} plays a crucial role in all energy demanding processes by acting as cofactor for ATP in a number of enzymatic reactions (Legong et al. 2001), whereas only 6% of cellular Mg^{2+} is bound to chlorophyll. Therefore, we postulate that an increasing cytosol [Ca^{2+}] disturbs cellular Mg^{2+} homeostasis as reflected by decreased physiological rates, primarily in $\mathrm{Chl}a_{\mathrm{prod}}$.

Furthermore, it seems that impaired physiological rates lead to a reduced control over the exclusion of the Mg^{2+} ion during calcite precipitation, what may be reflected in an increased Mg/Ca ratio. Nevertheless, the MgCO_3 content of calcite remains very low (<1%) at seawater Mg/Ca ratios below 2, even if the physiological control over coccolith precipitation is impaired.

4.4.2 $\Delta^{44/40}Ca$ and $\Delta^{26/24}Mg$

The mean $\Delta^{44/40}\mathrm{Ca}$ value revealed from all *E. huxleyi* experiments in the present study was $0.3\,‰$ greater than previously reported values for *E. huxleyi* (Langer et al. 2007; Gussone et al. 2006). However, $\Delta^{44/40}\mathrm{Ca}$ values from the *E. huxleyi* experiment at $50\,\mathrm{mmol\,Mg\,L^{-1}}$ (exp. 1) fall well within the reported range (excepting the value at ambient seawater [$\mathrm{Ca^{2+}}$]). Comparison of the mean $\Delta^{44/40}\mathrm{Ca}$ from the *E. huxleyi* experiment 1 (Fig. 6A, filled diamonds; without the value at $10\,\mathrm{mmol\,Ca\,L^{-1}}$) and from experiment 2 (Fig. 6A, open diamonds) would lead to an offset of $0.4\,‰$. Therefore, an influence of either seawater [$\mathrm{Mg^{2+}}$] or light intensity might be suggested. Since different light intensities have no influence on the $\mathrm{Ca^{2+}}$ isotope fractionation in *E. huxleyi* (Langer et al. 2007), the suggested offset between the two experiments appears to be driven by the change in the external [$\mathrm{Mg^{2+}}$]. This would be only valid for *E. huxleyi* since a similar offset driven by external [$\mathrm{Mg^{2+}}$] was not observed in *C. braarudii* (Fig. 6B). Our observation that $\Delta^{44/40}\mathrm{Ca}$ is constant in both *E. huxleyi* and *C. braarudii* over a wide range of seawater Mg/Ca ratios suggests that calcium isotope records derived from coccolithophores are essentially unaffected by variations in seawater Mg/Ca. This is confirmed by the findings of Langer et al. (2007), who reported no change in $\mathrm{Ca^{2+}}$ isotope fractionation over seawater [$\mathrm{Ca^{2+}}$] expected in the oceans over the last 500 million years.

Seawater Mg/Ca concentration raised since the Late Cretaceous from ≈ 1 to a modern value of 5.2. Within this range of seawater Mg/Ca ratios, $\Delta^{26/24}\mathrm{Mg}$ values were relatively constant in both coccolithophore species ($-1.00 \pm 0.22‰$ and $-0.77 \pm 0.08‰$ for *E. huxleyi* and *C. braarudii*, respectively). Additionally, $\mathrm{Mg^{2+}}$ isotope fractionation in *E. huxleyi* was not influenced by doubling the seawater [$\mathrm{Mg^{2+}}$] from 25 to $50\,\mathrm{mmol\,L^{-1}}$ (Fig. 9A).

The $\mathrm{Mg^{2+}}$ isotope fractionation in coccolithophores is smaller than reported for foraminifera and coral calcite (Table 3). Pathways of $\mathrm{Mg^{2+}}$ to the site of precipitation are quite different in coccolithophores compared to corals and foraminifera. Corals and

foraminifera are supposed to transport Mg^{2+}ions via specific vesicles or vacuoles derived from seawater to the site of calcification (Erez 2003; Cohen and McConnaughey 2003). Transportways of Mg^{2+} in coccolithophores are not known so far but it seems likely that they are transported similarly as in bacteria and higher plants via unique membrane channels from the external media to the cytosol (Legong et al. 2001). Transported to the cytosol, the Mg^{2+} ions are facing four possible fates: (1) to be incorporated into organic compounds, (2) to be further transported to the coccolith vesicle and integrated to the calcite lattice, (3) to remain in the cytosol as free $Mg^{2+}$and (4) to be transported back to the external media. Organic Mg^{2+} concentrations are reported to vary between $2.0 \cdot 10^{-15}$ to $1.0 \cdot 10^{-14}\,\mathrm{mol}$ per cell of *E. huxleyi* (Stoll et al. 2001*b*). Inorganic Mg^{2+} concentrations in *E. huxleyi* range from $3.0 \cdot 10^{-16}$ to $1.1 \cdot 10^{-15}\,\mathrm{mol}$ per cell (derived from this study). Cytosol Mg^{2+} abundance is not known for coccolithophores, but we assume a concentration of $2 - 10\,\mathrm{mM}$ as reported for plant cells (Epstein 1965). Therefore, the cytosol displays the smallest pool of Mg^{2+} in *E. huxleyi*, whereas organic bound Mg^{2+} reflects the largest part. Thus, organic isotope fractionation of Mg^{2+} will alter the isotopic composition of the cytosolic Mg^{2+} to a large extent, what will have a secondary influence on the $\delta^{26/24}\mathrm{Mg}$ composition of the coccolith. We calculated a $\delta^{26/24}\mathrm{Mg}$ value for the cytosol by applying a mass balance with the following assumptions: We assumed firstly, that the total sum of Mg^{2+} ions transported into *E. huxleyi* is not fractionated compared to seawater and secondly, that organic Mg^{2+} is fractionated by -0.712‰, relative to the seawater (cyanobacterial chlorophyll-*a*, Black et al. (2006)). Based on these assumptions and the cellular Mg^{2+} concentrations mentioned above, we calculated $\delta^{26/24}\mathrm{Mg}$ values for the cytosol of *E. huxleyi* to have a range from 3.30 to 3.35‰. Therefore, coccolith $\Delta^{26/24}\mathrm{Mg}$ would result in -4.30 to $-4.35\,‰$ if fractionated against the cytosol, consistent with reported $\Delta^{26/24}\mathrm{Mg}$ values of foraminifera calcite (Pogge von Strandmann 2008; Chang et al. 2004). This leads to the conclusion that isotopic magnesium composition of coccolithophore calcite is greatly influenced by the Mg^{2+} isotope fractionation of the organic cell compartments since organic bound Mg^{2+} can be up to 500 higher than

inorganically bound $\mathrm{Mg^{2+}}$ (Stoll et al. 2001*b*).

4.5 Conclusions

Over the last 225 million years seawater [$\mathrm{Mg^{2+}}$] and [$\mathrm{Ca^{2+}}$] have changed remarkably (Horita et al. 2002). Within the predicted changes we tested two coccolithophore species as to their physiological response and analysed the coccolith calcite. The evolutionary young species *E. huxleyi* and the 'older' species *C. braarudii* were both affected by changes in the seawater [$\mathrm{Ca^{2+}}$] rather than the [$\mathrm{Mg^{2+}}$]. *E. huxleyi* displayed a higher sensitivity than *C. braarudii* to increased seawater [$\mathrm{Ca^{2+}}$] by showing a decreasing growth rate, $\mathrm{PIC_{prod}}$, $\mathrm{POC_{prod}}$ and $\mathrm{Chl}a_{\mathrm{prod}}$at concentrations above $25\,\mathrm{mmol\,L^{-1}}$, whereas *C. braarudii* was only affected at $\approx 50\,\mathrm{mmol\,L^{-1}}$. $\mathrm{PIC_{prod}}$ in both species was reduced at a [$\mathrm{Ca^{2+}}$] lower than in the ambient ocean, whereas other physiological parameters remained constant. The physiological results agree well with previously published data on coccolithophores and we have obtained no evidence that a low seawater Mg/Ca ratio increases coccolithophorid growth and calcite production.

Strontium and magnesium content of coccolithophorid calcite are controlled by different mechanisms. The Sr/Ca ratio in coccoliths was linearly correlated to the seawater Sr/Ca ratio, whereas Mg/Ca ratio was greatly influenced by the physiology. $\mathrm{Mg^{2+}}$ isotope fractionation in coccolithophores is lower than reported for other calcifiers, which may be explained by the different calcification mechanisms of coccolithophores. Further research on $\mathrm{Mg^{2+}}$ isotope fractionation in organic and inorganic material would probably reveal new insights into coccolithophorid calcification mechanisms.

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Zeebe, R., and D. Wolf-Gladrow. 2001. CO_2 in seawater: Equilibrium, Kinetics, isotopes. Elsevier Science B.V., Amsterdam, 1st ed.

Table 1: Elemental concentration of relevant seawater (Ca, Mg and Sr) ingredients and corresponding ratios of the *Emiliania huxleyi* experiments (Ca-Matrix).

Ca [mmol/L]	**RSD** %	**Mg** [mmol/L]	**RSD** %	**Sr** [μmol/L]	**RSD** %	**Sr/Ca** [mmol/mol]	**Mg/Ca** [mol/mol]
Emiliania huxleyi experiment 1							
5.1	0.1	49.8	0.3	99.5	0.1	19.51	9.76
10.0	0.2	49.7	0.2	102.4	0.1	10.22	4.95
24.4	0.1	48.3	0.3	97.4	0.1	4.00	1.98
50.3	0.4	50.6	0.1	100.3	0.1	1.99	1.01
Emiliania huxleyi experiment 2							
2.6	0.6	25.3	0.7	98.8	0.2	37.97	9.71
5.2	0.6	24.9	0.1	94.0	0.1	17.94	4.76
13.0	0.1	25.7	0.5	98.4	0.1	7.60	1.98
24.7	0.4	25.2	0.6	99.1	0.1	4.01	1.02
51.0	0.4	26.4	0.2	102.4	0.2	2.01	0.52

Table 2: Elemental concentration of relevant seawater (Ca, Mg and Sr) ingredients and corresponding ratios of the *Coccolithus braarudii* experiments (Ca-Matrix).

Ca	**RSD**	**Mg**	**RSD**	**Sr**	**RSD**	**Sr/Ca**	**Mg/Ca**
[mmol/L]	%	[mmol/L]	%	[μmol/L]	%	[mmol/mol]	[mol/mol]
Coccolithus braarudii experiment 1							
5.1	0.5	48.5	0.2	97.5	0.2	19.19	9.54
9.7	0.1	48.9	0.2	91.7	0.2	9.42	5.02
23.5	0.2	49.4	0.3	88.2	0.2	3.75	2.10
46.7	0.5	47.7	0.2	85.4	0.2	1.83	1.02
Coccolithus braarudii experiment 2							
2.6	0.9	25.9	0.7	98.8	0.1	37.67	9.86
5.2	0.2	25.4	0.1	95.7	0.2	18.54	4.92
12.3	0.6	25.5	0.2	97.0	0.2	7.89	2.08
24.2	0.2	25.8	0.2	100.2	0.1	4.14	1.06
48.8	0.3	25.8	0.4	99.7	0.1	2.04	0.53

Table 3: $\Delta^{26}\mathrm{Mg}$ values of different calcite precipitates at ambient seawater conditions.

$\mathrm{CaCO_3}$	$\Delta^{26}\mathrm{Mg}$	reference
corals	≈ -2.6	Pogge von Strandmann (2008)
foraminifera	-5.5 to -3.4	Chang et al. (2004)
foraminifera	-4.9 to -3.5	Pogge von Strandmann (2008)
coccolithophores	-1.0 to -0.8	this study

Figure captions

Fig.1: Response of *E. huxleyi* to changing seawater $[Mg^{2+}]$ with constant $[Ca^{2+}]$ at $10\,mmol\,L^{-1}$. **A**: Division rate; **B**: PIC production; **C**: POC production; **D**: Chl*a* production. Shaded area indicates the relevant $[Mg^{2+}]$ for comparison with the Ca^{2+}-Matrix.

Fig.2: Response of *E. huxleyi* to a changing seawater $[Ca^{2+}]$ with constant $[Mg^{2+}]$ at $50\,mmol\,L^{-1}$ (filled markers) and $25\,mmol\,L^{-1}$ (open markers). E= light intensity in $\mu mol\,quanta\,m^{-2}s^{-1}$. **A**: Division rate; **B**: PIC production; **C**: POC production; **D**: Chl*a* production.

Fig.3: Response of *E. huxleyi* to a changing seawater Mg/Ca ratios with constant $[Mg^{2+}]$ at $50\,mmol\,L^{-1}$ (filled markers) and $25\,mmol\,L^{-1}$ (open markers). E= light intensity in $\mu mol\,quanta\,m^{-2}s^{-1}$. **A**: Division rate; **B**: PIC production; **C**: POC production; **D**: Chl*a* production.

Fig.4: Response of *C. braarudii* to a changing seawater $[Ca^{2+}]$ with constant $[Mg^{2+}]$ at $50\,mmol\,L^{-1}$ (filled markers) and $25\,mmol\,L^{-1}$ (open markers). E= light intensity in $\mu mol\,quanta\,m^{-2}s^{-1}$. **A**: Division rate; **B**: PIC production; **C**: POC production; **D**: Chl*a* production.

Fig.5: Response of *C. braarudii* to a changing seawater Mg/Ca ratios with constant $[Mg^{2+}]$ at $50\,mmol\,L^{-1}$ (filled markers) and $25\,mmol\,L^{-1}$ (open markers). E= light intensity in $\mu mol\,quanta\,m^{-2}s^{-1}$. **A**: Division rate; **B**: PIC production; **C**: POC production; **D**: Chl*a* production.

Fig.6: Coccoltith Sr/Ca ratio (A) and corresponding exchange coefficient (B) of *E. huxleyi* (diamonds) and *C. braarudii* (circles) as a funtion of seawater Sr/Ca ratio at constant $[Mg^{2+}]$ of $50\,mmol\,L^{-1}$ (filled markers) and $25\,mmol\,L^{-1}$ (open markers). The slope of the linear regression curve yields a D_{Sr} of 0.32.

Fig.7: Coccoltith Mg/Ca ratio (A) and corresponding exchange coefficient (B) of *E. huxleyi* (diamonds) and *C. braarudii* (circles) as a funtion of seawater Mg/Ca ratio at constant $[Mg^{2+}]$ of $50\,mmol\,L^{-1}$ (filled markers) and $25\,mmol\,L^{-1}$ (open markers).

Fig.8: Calcium fractionation ($\Delta^{44}\mathrm{Ca}$) in calcite of *E. huxleyi* (A, diamonds) and *C. braarudii* (B, circles) to changing seawater $[\mathrm{Ca}^{2+}]$ at constant $[\mathrm{Mg}^{2+}]$ of $50\,\mathrm{mmol\,L^{-1}}$ (filled markers) or $25\,\mathrm{mmol\,L^{-1}}$ (open markers). Error bars indicate 2SD. E= light intensity in $\mu\mathrm{mol\,quanta\,m^{-2}s^{-1}}$.

Fig.9: Magnesium fractionation ($\Delta^{26}\mathrm{Mg}$) in calcite of *E. huxleyi* (A, diamonds) and *C. braarudii* (B, circles) to changing seawater Mg/Ca at constant $[\mathrm{Mg}^{2+}]$ of $50\,\mathrm{mmol\,L^{-1}}$ (filled markers) or $25\,\mathrm{mmol\,L^{-1}}$ (open markers). Error bars indicate 2SD. E= light intensity in $\mu\mathrm{mol\,quanta\,m^{-2}s^{-1}}$.

Fig.10: $\mathrm{D_{Mg}}$ as a funtion of Chl*a* production in *E. huxleyi* (diamonds) and *C. braarudii* (circles) at constant $[\mathrm{Mg}^{2+}]$ of $50\,\mathrm{mmol\,L^{-1}}$ (filled markers) and $25\,\mathrm{mmol\,L^{-1}}$ (open markers).

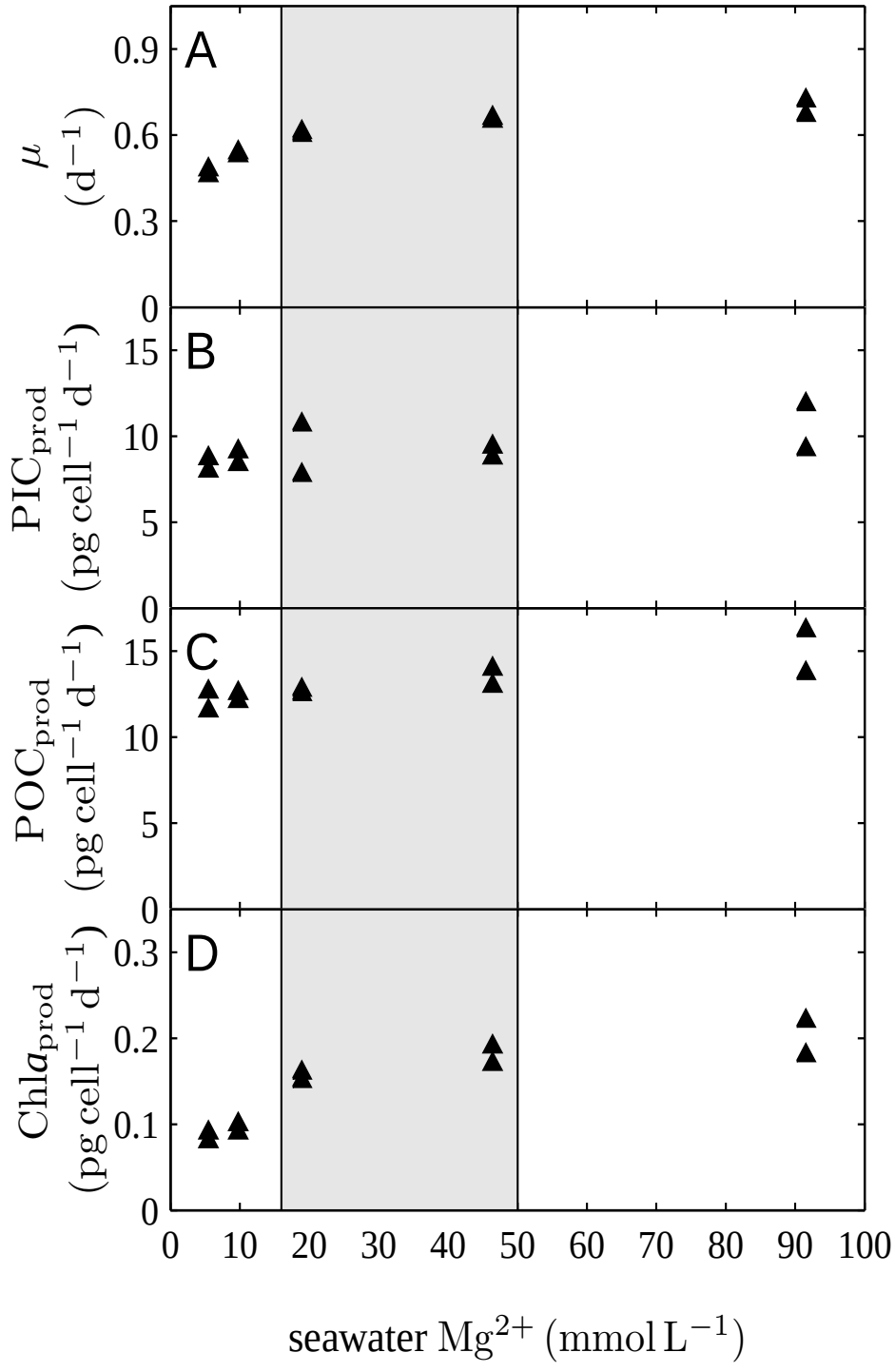
A
B
C
D
μ (d^{-1})
$\mathrm{PIC_{prod}}$ ($\mathrm{pg\,cell^{-1}\,d^{-1}}$)
$\mathrm{POC_{prod}}$ ($\mathrm{pg\,cell^{-1}\,d^{-1}}$)
$\mathrm{Chl}a_{\mathrm{prod}}$ ($\mathrm{pg\,cell^{-1}\,d^{-1}}$)
0.9
0.6
0.3
0
15
10
5
0
15
10
5
0
0.3
0.2
0.1
0
0 10 20 30 40 50 60 70 80 90 100
seawater $\mathrm{Mg^{2+}}$ ($\mathrm{mmol\,L^{-1}}$)

Fig. 1

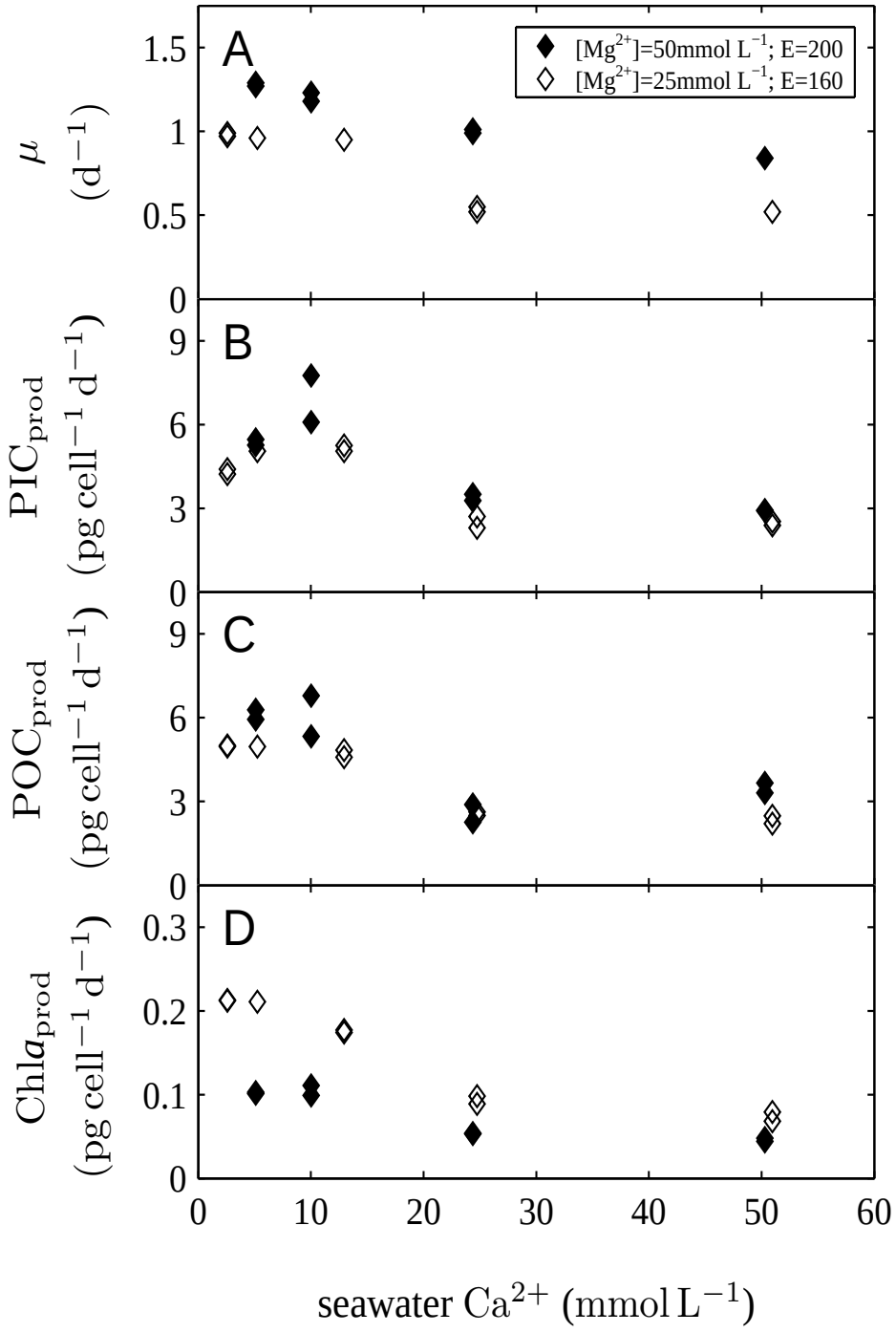
A
B
C
D
[Mg2+]=50mmol L−1; E=200
[Mg2+]=25mmol L−1; E=160
μ (d−1)
PICprod (pg cell−1 d−1)
POCprod (pg cell−1 d−1)
Chla prod (pg cell−1 d−1)
seawater Ca2+ (mmol L−1)

Fig. 2

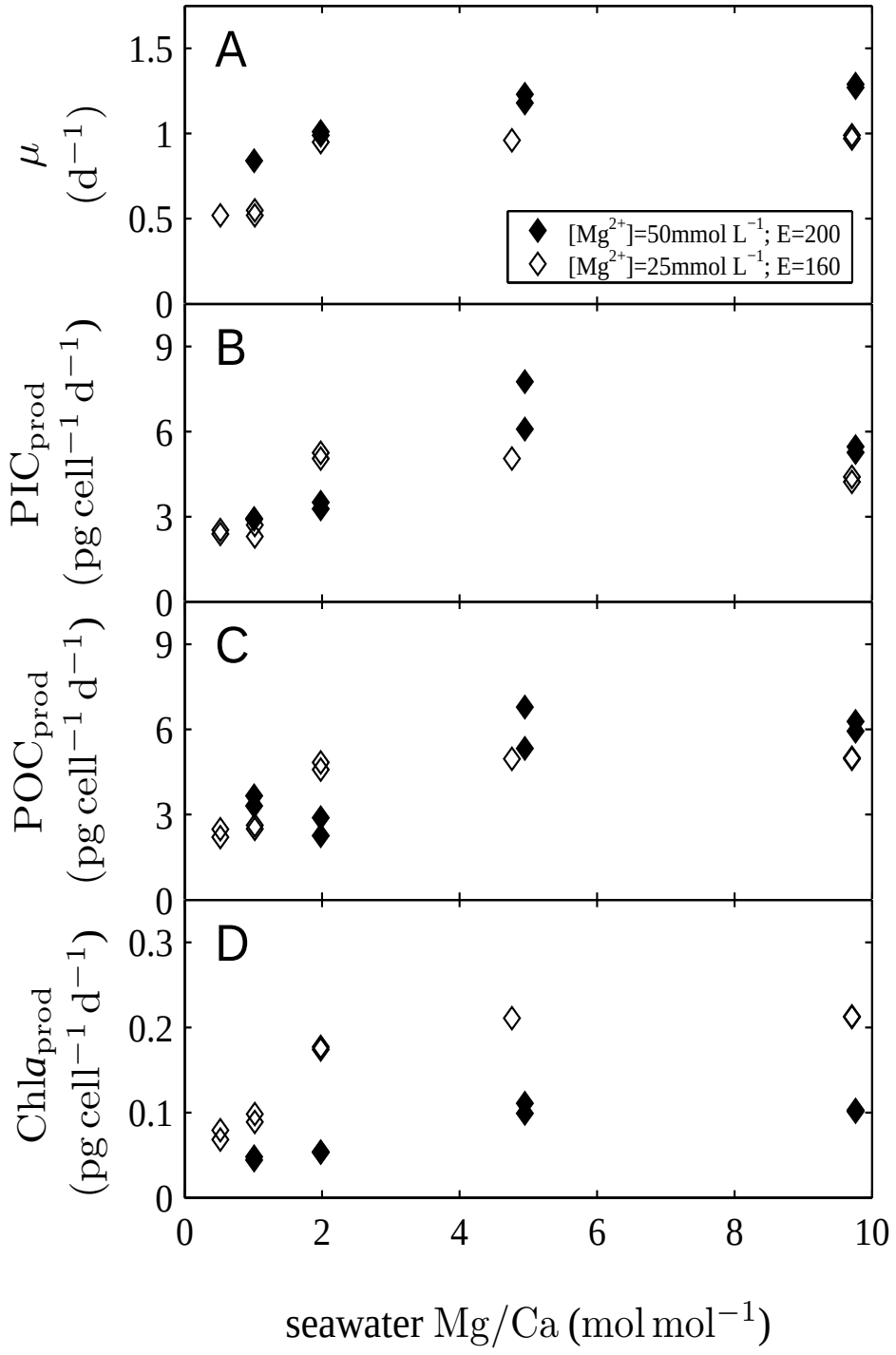
A
B
C
D
μ (d^{-1})
PIC_{prod} (pg cell^{-1} d^{-1})
POC_{prod} (pg cell^{-1} d^{-1})
Chla_{prod} (pg cell^{-1} d^{-1})
[Mg^{2+}]=50mmol L^{-1}; E=200
[Mg^{2+}]=25mmol L^{-1}; E=160
seawater Mg/Ca (mol mol^{-1})

Fig. 3

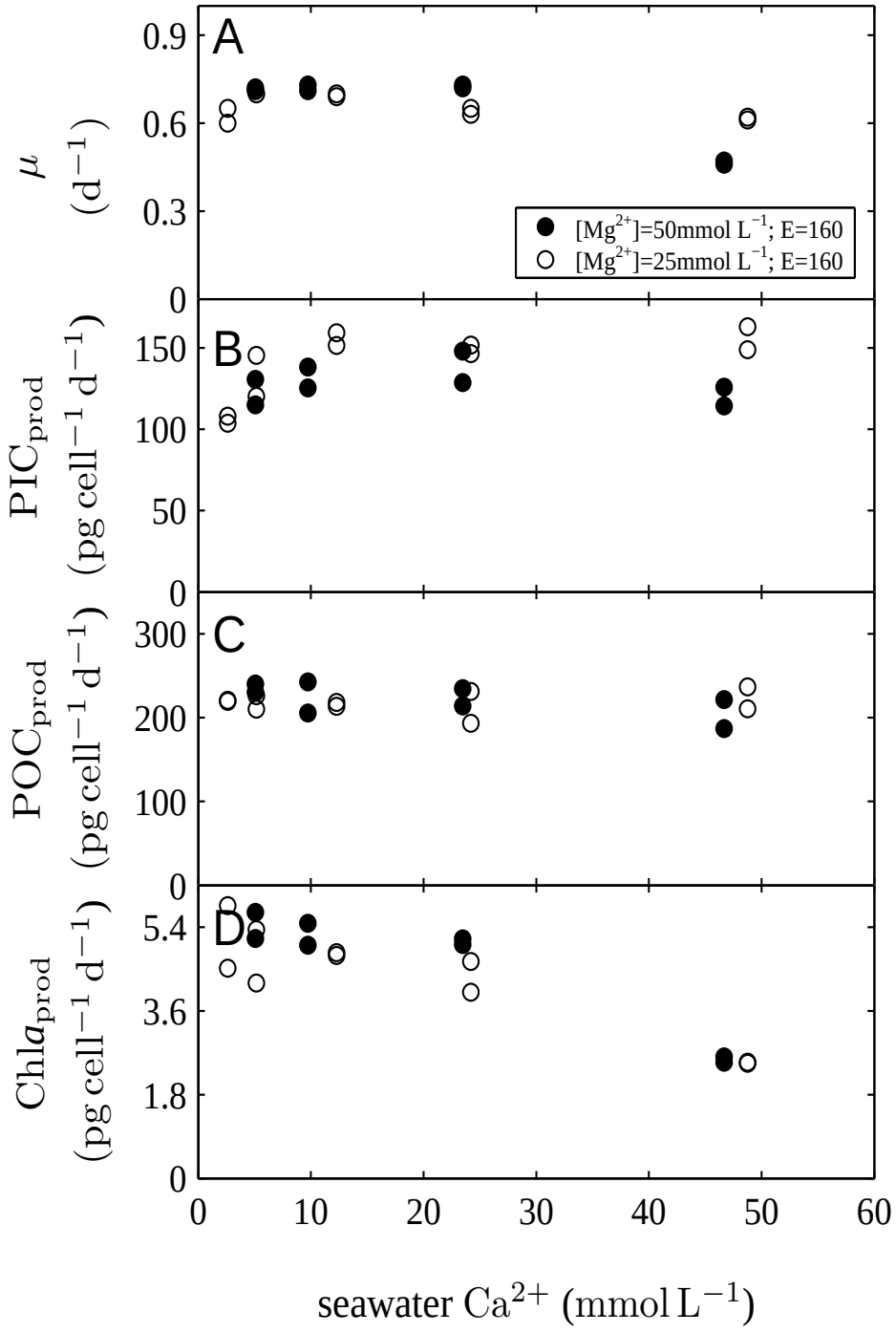
A
B
C
D
μ (d^{-1})
PIC$_{prod}$ (pg cell^{-1} d^{-1})
POC$_{prod}$ (pg cell^{-1} d^{-1})
Chla_{prod} (pg cell^{-1} d^{-1})
[Mg^{2+}]=50mmol L^{-1}; E=160
[Mg^{2+}]=25mmol L^{-1}; E=160
0.9
0.6
0.3
0
150
100
50
300
200
5.4
3.6
1.8
0
10
20
30
40
50
60
seawater Ca^{2+} (mmol L^{-1})

Fig. 4

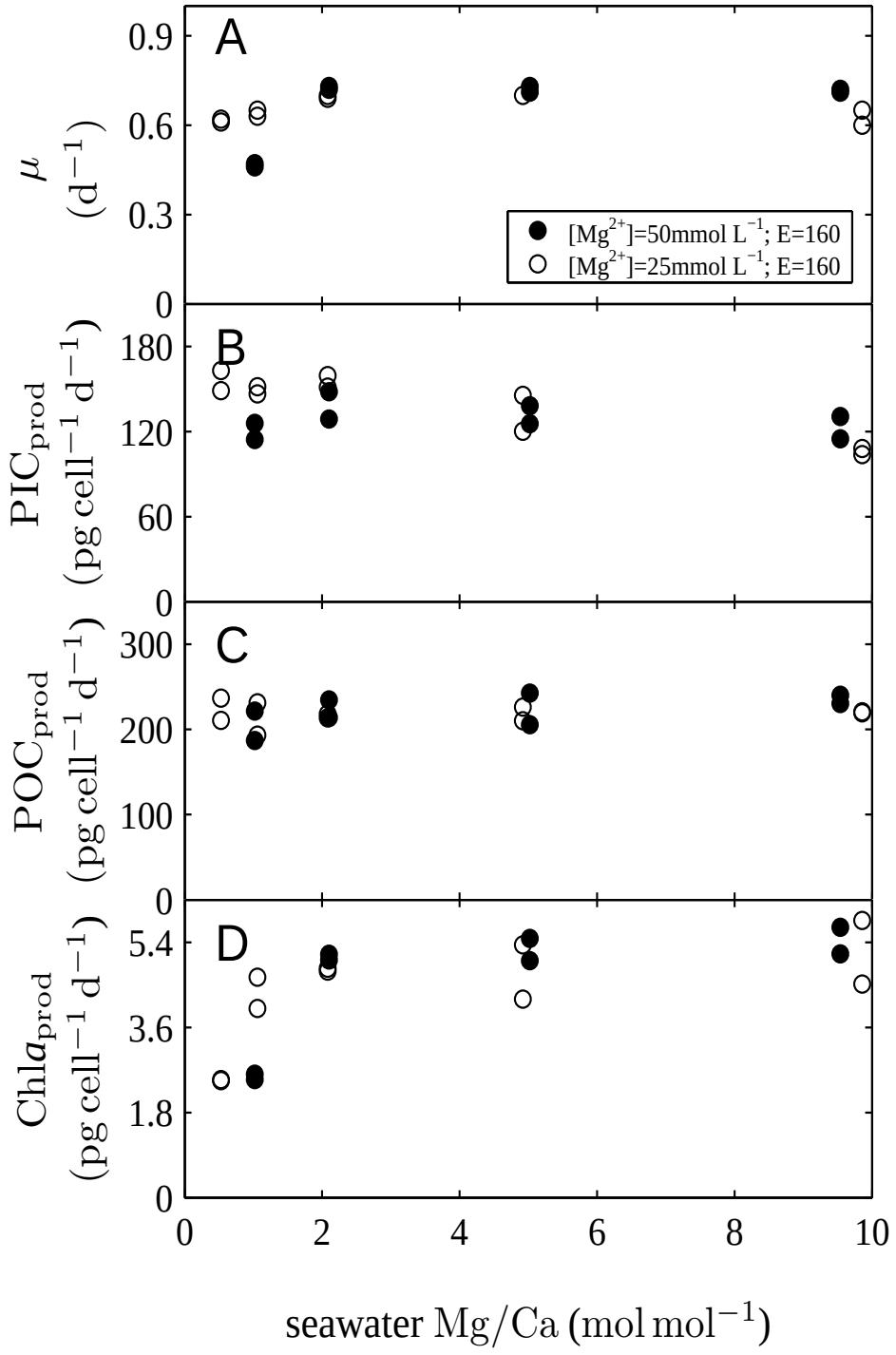
A
B
C
D
μ (d^{-1})
PIC$_{\mathrm{prod}}$ (pg cell^{-1} d^{-1})
POC$_{\mathrm{prod}}$ (pg cell^{-1} d^{-1})
Chla_{prod} (pg cell^{-1} d^{-1})
[Mg^{2+}]=50mmol L^{-1}; E=160
[Mg^{2+}]=25mmol L^{-1}; E=160
0.9
0.6
0.3
0
180
120
60
300
200
100
5.4
3.6
1.8
0
2
4
6
8
10
seawater Mg/Ca (mol mol^{-1})

Fig. 5

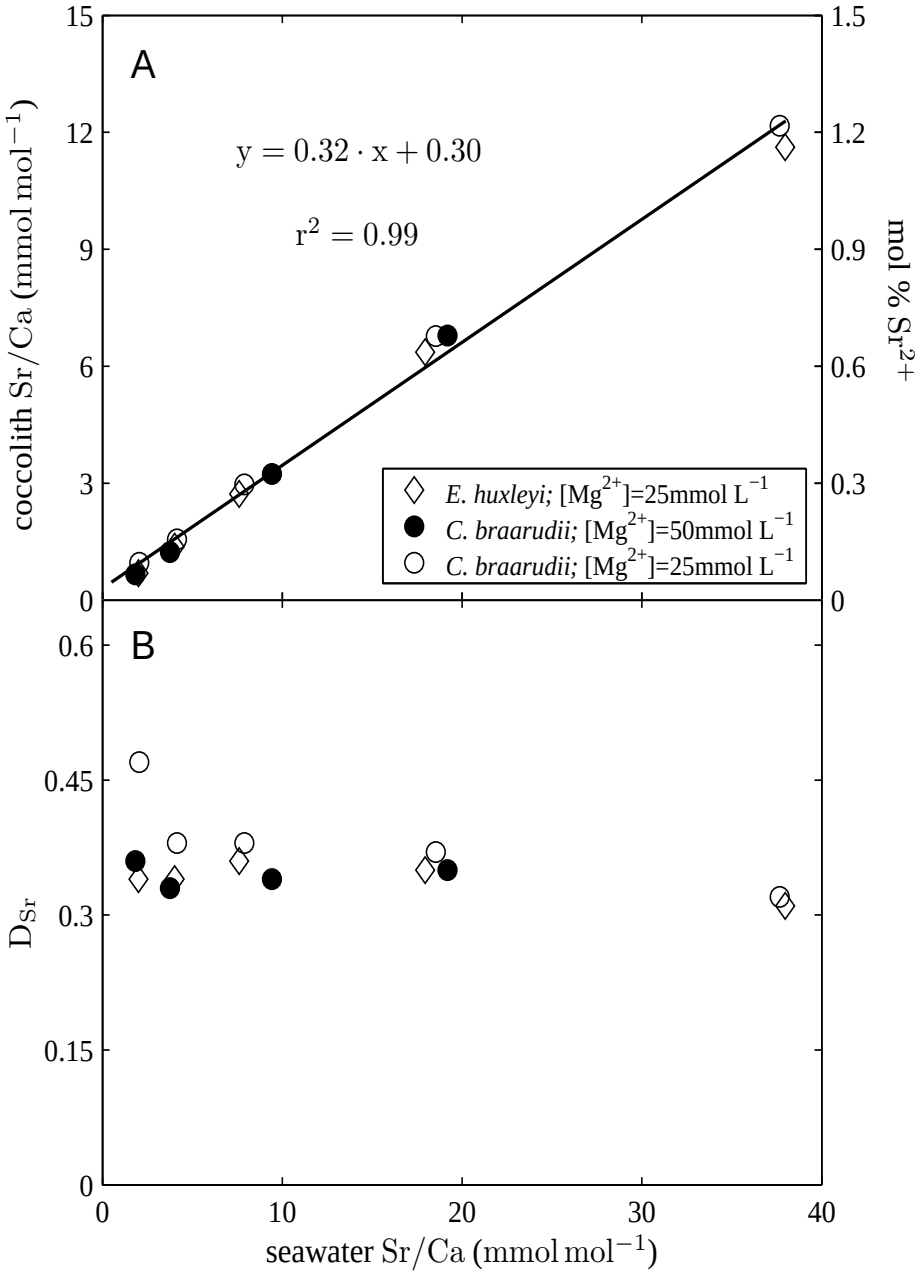
A
B
y = 0.32 · x + 0.30
r² = 0.99
E. huxleyi; [Mg2+]=25mmol L−1
C. braarudii; [Mg2+]=50mmol L−1
C. braarudii; [Mg2+]=25mmol L−1
coccolith Sr/Ca (mmol mol−1)
mol % Sr2+
DSr
seawater Sr/Ca (mmol mol−1)
0
3
6
9
12
15
0.3
0.6
0.9
1.2
1.5
0.15
0.45
10
20
30
40

Fig. 6

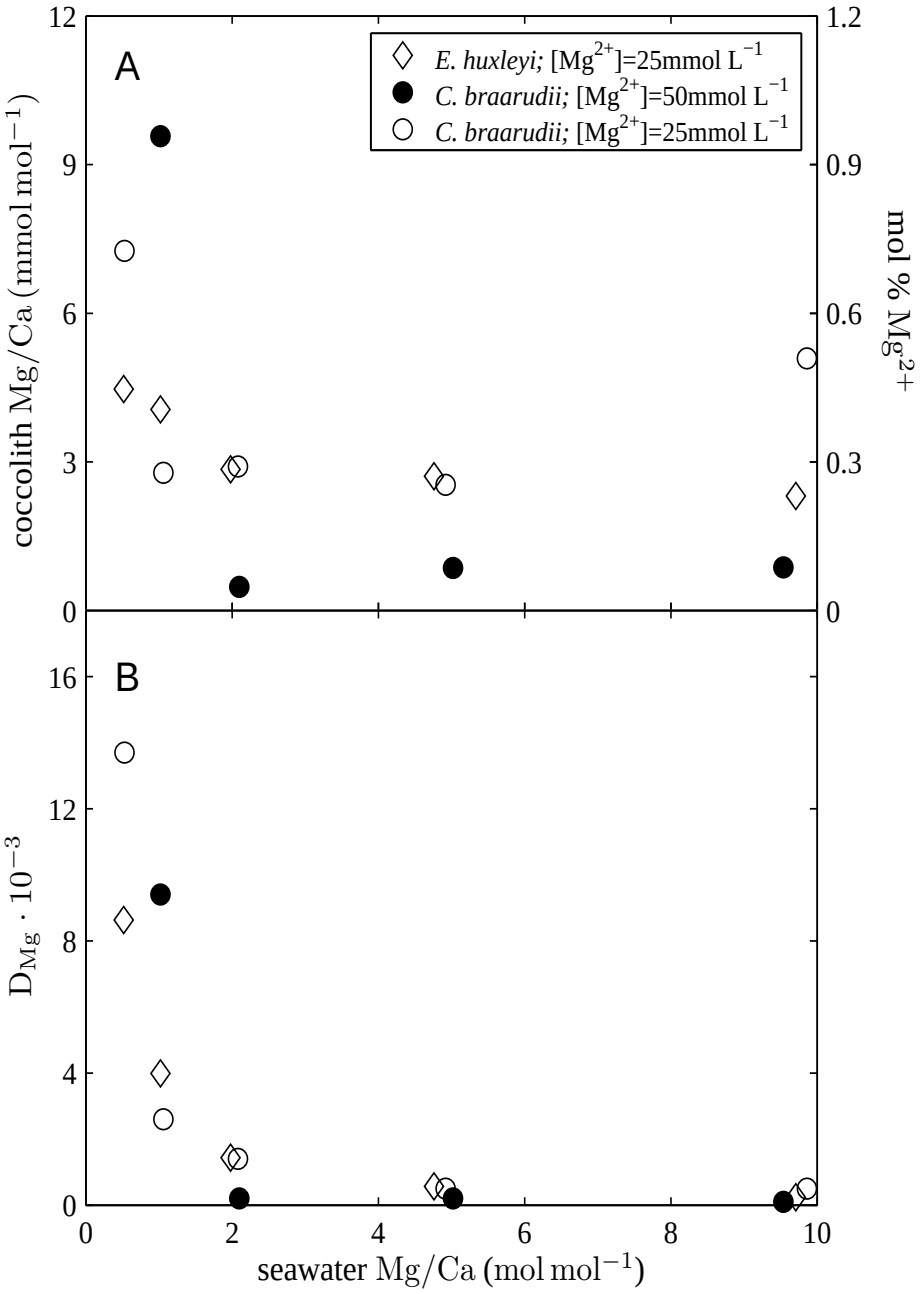
A
B
E. huxleyi; [Mg2+]=25mmol L−1
C. braarudii; [Mg2+]=50mmol L−1
C. braarudii; [Mg2+]=25mmol L−1
coccolith Mg/Ca (mmol mol−1)
mol % Mg2+
DMg · 10−3
seawater Mg/Ca (mol mol−1)
0
3
6
9
12
0
0.3
0.6
0.9
1.2
0
4
8
12
16
0
2
4
6
8
10

Fig. 7

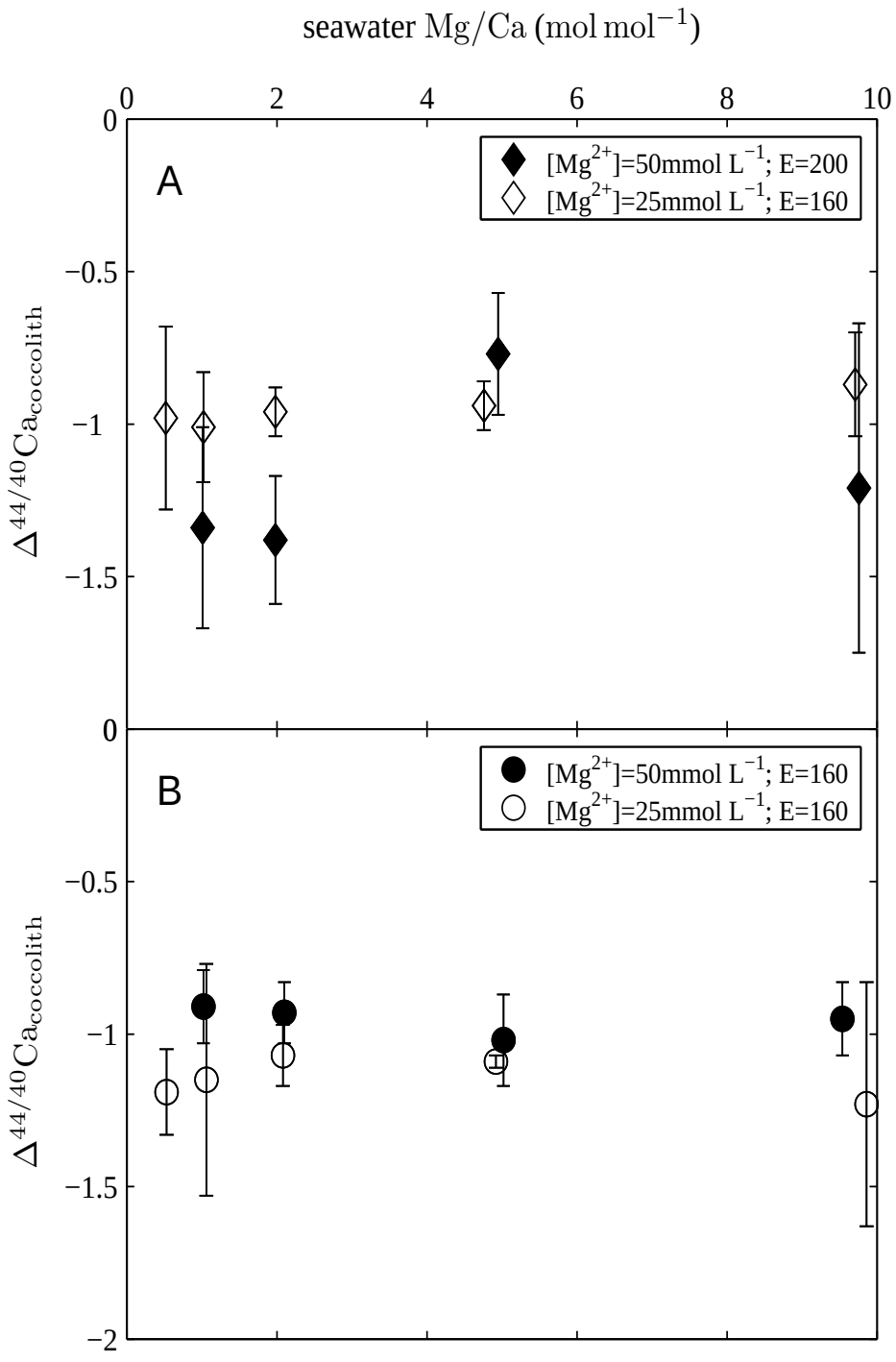
seawater Mg/Ca (mol mol^{-1})
0
2
4
6
8
10
A
[Mg^{2+}]=50mmol L^{-1}; E=200
[Mg^{2+}]=25mmol L^{-1}; E=160
$\Delta^{44/40}Ca_{coccolith}$
0
−0.5
−1
−1.5
B
[Mg^{2+}]=50mmol L^{-1}; E=160
[Mg^{2+}]=25mmol L^{-1}; E=160
$\Delta^{44/40}Ca_{coccolith}$
0
−0.5
−1
−1.5
−2

Fig. 8

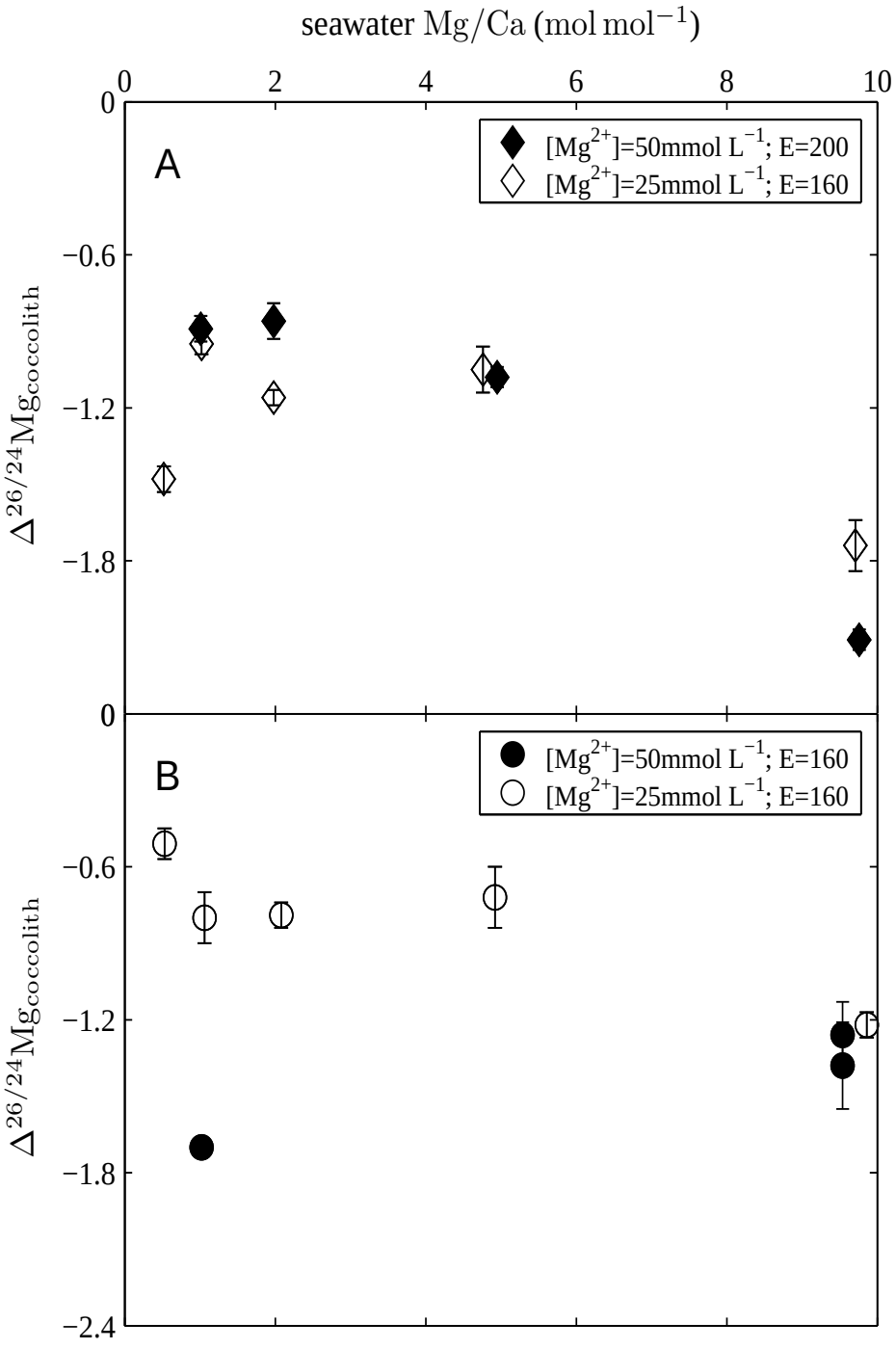
seawater Mg/Ca (mol mol^{-1})
0
2
4
6
8
10
A
[Mg^{2+}]=50mmol L^{-1}; E=200
[Mg^{2+}]=25mmol L^{-1}; E=160
$\Delta^{26/24}$Mg$_{coccolith}$
0
−0.6
−1.2
−1.8
B
[Mg^{2+}]=50mmol L^{-1}; E=160
[Mg^{2+}]=25mmol L^{-1}; E=160
$\Delta^{26/24}$Mg$_{coccolith}$
0
−0.6
−1.2
−1.8
−2.4

Fig. 9

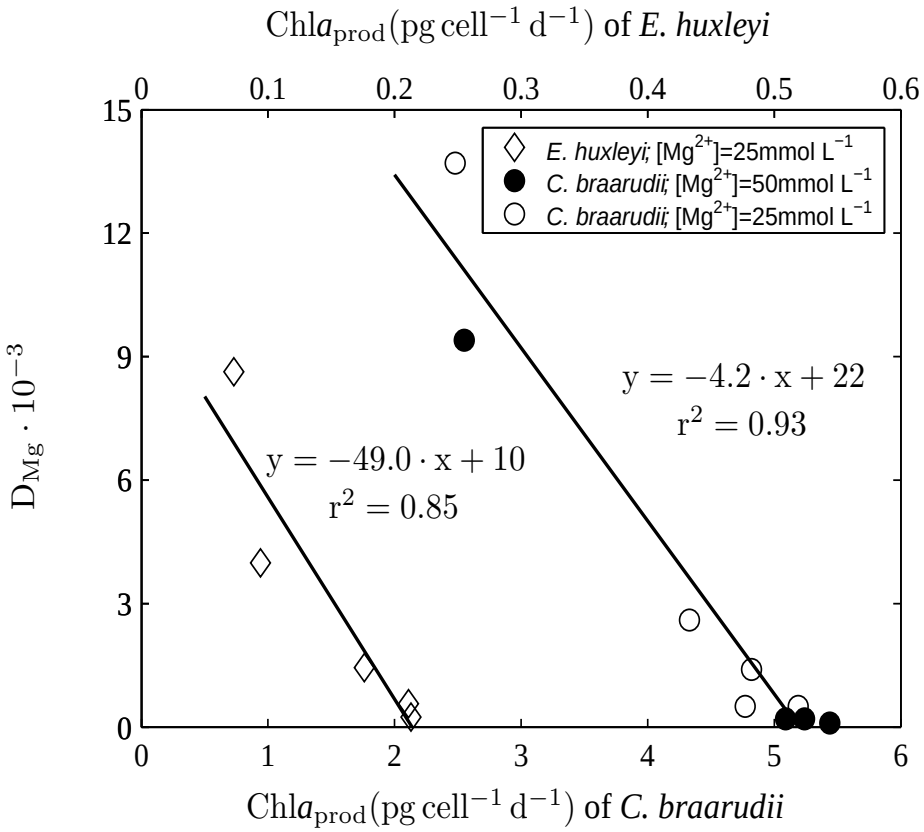
Chla_{prod}(pg cell^{-1} d^{-1}) of *E. huxleyi*
0
0.1
0.2
0.3
0.4
0.5
0.6
15
12
9
6
3
0
$D_{Mg} \cdot 10^{-3}$
E. huxleyi; [Mg^{2+}]=25mmol L^{-1}
C. braarudii; [Mg^{2+}]=50mmol L^{-1}
C. braarudii; [Mg^{2+}]=25mmol L^{-1}
$y = -4.2 \cdot x + 22$
$r^2 = 0.93$
$y = -49.0 \cdot x + 10$
$r^2 = 0.85$
0
1
2
3
4
5
6
Chla_{prod}(pg cell^{-1} d^{-1}) of *C. braarudii*

Fig. 10

4 Synthesis: Past, present and future of coccolithophores

All manuscripts within this thesis deal with the physiology of coccolithophores in regard to changing environmental conditions. Their physiological response is investigated, in regard to changes in the seawater calcium to magnesium ratio (which has varied over geological time scales), and to changes in the seawater nutrient concentrations of nitrogen and phosphorus. Furthermore, the effect of projected ocean acidification (changes in the seawater carbonate system) on coccolithophores is examined. In this chapter the major conclusions of the presented manuscripts will be summarized and put into a broader context.

4.1 Coccolithophores in the Cretaceous ocean

The geological record of coccolithophores experienced a remarkable increase in abundance and diversity during the Late Cretaceous period, reflected by massive coccolith depositions in the sediments.

The surface temperature of the Cretaceous ocean was about 6-12$^{\circ}\mathrm{C}$ higher than at present (Barron 1983), presumably driven by elevated atmospheric $\mathrm{CO_2}$ concentrations and increases in the poleward oceanic heat transport (Barron et al. 1993 1995). Additionally, the sea-level was $\approx 150\,\mathrm{m}$ higher due to increased rates of sea floor spreading (Ridgwell 2005).

The rise of calcareous nannoplankton (including coccolithophores) is hypothetically linked with the above mentioned 'greenhouse' conditions during the Mesozoic (250-65 Ma ago), whereas the increase in abundance and diversity of diatoms in the sediment record is associated with the cooling in the Cenozoic (65-0 Ma ago) (Bown 2005*a*). Another hypothesis suggests that the different major ion composition of seawater (in particular the Mg/Ca ratio, see 2.2) as being the primary factor causing the dominance of coccolithophores during the Cretaceous (Stanley et al. 2005; Brennan et al. 2004).

One major result of manuscript IV is that the tested two coccolithophore species do not increase their growth rate when exposed to seawater Mg/Ca ratios of the Cretaceous. One species (*E. huxleyi*) is even impaired in growth, photosynthesis and calcification rates at calcium concentrations assumed for Cretaceous seawater ($\approx 30\,\mathrm{mmol\,Ca\,L^{-1}}$; Mg/Ca of 1). This calcium concentration, on the other hand, had no effect on the measured physiological rates of *C. braarudii*. A higher sensitivity of *E. huxleyi* compared to *C. braarudii* has also been observed for a reduction/increase in seawater *p*H/*p*$\mathrm{CO_2}$ (comparing the studies of Riebesell et al. (2000) and Langer et al. (2006*a*), see 2.3).

Both species differ in size and evolutionary history. *C. braarudii* is about a magnitude bigger in volume than *E. huxleyi* and evolved 65 Ma ago, whereas *E. huxleyi* was first recorded in sediments dating back to only 250,000 thousand years. Interestingly, coccoliths from the Late Cretaceous are large in size (Bown et al. 2004) but it is questionable if the environmental conditions of the Cretaceous were favoured by large coccolithophore species or if the preservation conditions were inappropriate for smaller coccoliths (increased dissolution and diagenesis, Young et al. (2005)).

In summary, Cretaceous seawater ion composition has probably been neither an advantage nor a disadvantage for large coccolithophores such as *C. braarudii*. However, the response of non-calcifying phytoplankton taxa to elevated seawater calcium concentrations has not been investigated. Therefore, it remains an open question as to how other phytoplankton taxa will react to changing seawater calcium concentrations and Mg/Ca ratios.

Physical conditions (like warm, shallow and stratified seas) have presumably enhanced rapid accumulation and deposition of coccolithophorid calcite during the Cretaceous.

Nevertheless, extrinsic biotic factors, such as grazing and viral infection, remain unknown. Therefore, the question should be asked - why other phytoplankton competitors and grazers were not as numerous as the coccolithophores present in the sediment record? Were those taxa outcompeted by the coccolithophores or were they suppressed by the environmental conditions?

4.1.1 Sr/Ca and Mg/Ca of coccoliths

In the laboratory, coccolith Sr/Ca ratios were observed to correlate with cell division and calcification rates under nutrient limitation (Rickaby et al. 2002). In sediment samples, the Sr/Ca ratio has been proposed as an indicator for past productivity of coccolithophores (Stoll and Schrag 2000; Stoll et al. 2002). However, the seawater Sr/Ca ratio has varied over geological times (Lear et al. 2003) and it is important to verify if the strontium partition coefficient of the coccolith calcite ($\mathrm{D_{Sr}}$) is influenced by these oscillations. Recently, Langer et al. (2006*b*) reported that the $\mathrm{D_{Sr}}$ of *E. huxleyi* is unaffected by changes in seawater strontium and calcium concentrations. This is confirmed in manuscript IV and is also valid for the $\mathrm{D_{Sr}}$ of *C. braarudii*.

The percentage of $\mathrm{MgCO_3}$ in inorganically precipitated calcite is positively correlated with the Mg/Ca ratio of the mother solution (Morse and Bender 1990). A similar correlation was also reported for biogenic calcite of foraminifera, corals and echinoderms (Segev and Erez 1994; Ries 2004; Stanley 2008). The magnesium partition coefficient ($\mathrm{D_{Mg}}$) of coccolithophorid calcite is about two to three orders of magnitude lower than that of inorganic calcite, and coccolithophores are reported to precipitate low Mg-calcite ($< 4\% \, \mathrm{MgCO_3}$) (Siesser 1977; Stoll et al. 2001 2007).

In contrast are results of Stanley et al. (2005) indicating high Mg-calcite ($> 15\% \, \mathrm{MgCO_3}$) in two coccolithophore species (*Pleurochrysis carterae* and *Ochrosphaera neopolitana*) at ambient seawater conditions. In both species the mole % of $\mathrm{MgCO_3}$ is positively correlated with the seawater Mg/Ca ratio. However, in a third species investigated (*Coccolithus neohelis*) the coccolith Mg/Ca ratio remained constant over the tested seawater Mg/Ca ra-

tios and coccolith calcite displayed low $\mathrm{MgCO_3}$ content (Stanley et al. 2005).

Results of manuscript IV reveal that the coccolith Mg/Ca ratio of *E. huxleyi* and *C. braarudii* primarily depend on the physiology. In fact, the partition of magnesium ($\mathrm{D_{Mg}}$) during calcite precipitation is negatively correlated with the chlorophyll *a* production. However, even under very low $\mathrm{Chl}a_{\mathrm{prod}}$ and highest measured calcite Mg/Ca ratios the $\mathrm{MgCO_3}$ content remains under <1%.

The diverging results of Stanley et al. (2005) and manuscript IV might be explained by the genetic differences and the evolutionary origin between the species examined. On the genealogical tree (based on rDNA) *P. carterae* and *O. neopolitana* (precipitating high Mg-calcite at ambient seawater conditions) are relatively closely related to each other and originated from the same ancestors 110 million years ago. Whereas the low Mg-calcite precipitating species *C. braarudii* and *C. neohelis* descended from a distant branch (de Vargas et al. 2007), and *E. huxleyi* (low Mg-calcite) is located on a third branch apart from the others. Whether the precipitation of low or high Mg-calcite in coccolithophores has a genetic basis or not, is a question which needs to be answered by future studies.

4.1.2 $\mathrm{Ca^{2+}}$ and $\mathrm{Mg^{2+}}$ isotopic fractionation in coccoliths

Calcium isotope fractionation in calcite of several coccolithophore species is known to be influenced by temperature (Gussone et al. 2007), whereas changes in the carbonate chemistry have a negligible effect (Gussone et al. 2006).

Variations in cellular division, calcification and photosynthesis rates do not affect calcium isotope fractionation in *E. huxleyi* and *C. braarudii*, as reported in manuscript IV, neither had a changing seawater calcium concentration an effect (in agreement with results of Langer et al. (2007)). Furthermore, a doubling of the seawater magnesium concentration from 25 to $50\,\mathrm{mmol\,L^{-1}}$ did not alter the calcium isotopic composition of the coccoliths. Therefore, the calcium isotope signal in coccoliths depends primarily on the calcium isotopic composition of the seawater and secondly on temperature.

Manuscript IV presents (to the best of my knowledge) the first data set on magne-

sium isotope fractionation ($\Delta^{26}\mathrm{Mg}$) in coccolithophorid calcite. Values of $\Delta^{26}\mathrm{Mg}$ in foraminifera calcite were reported to range between -3.4 and -5.5‰ (Pogge von Strandmann 2008; Chang et al. 2004) at ambient seawater conditions. These values are by a factor of 3 to 5 lower than measured $\Delta^{26}\mathrm{Mg}$ values of *E. huxleyi* and *C. braarudii*. This difference might be an effect induced by the underlying calcification mechanisms of foraminifera and coccolithophores.

Foraminifera vacuolize seawater (including the Mg^{2+} ions) and transport these specific vesicles or vacuoles to the side of calcite precipitation (Erez 2003; de Nooijer et al. 2009). Therefore, magnesium isotope fractionation in foraminifera occurs directly from the seawater solution. Coccolithophores, on the other hand, presumably transport Mg^{2+} via unique membrane channels or transporters from the seawater to the cytosol. From there, it may remain as cellular free Mg^{2+} or can be transported further to the coccolith vesicle (site of precipitation) or bound to organic molecules (like chlorophyll). The organic bound Mg^{2+} displays the biggest pool of cellular Mg^{2+}, which is about 500 times bigger than the inorganic fraction (Stoll et al. 2001). Therefore, it might be possible that the cytosol is enriched in the heavier $^{26}\mathrm{Mg}$ isotope since organic compartments preferably incorporate the lighter $^{24}\mathrm{Mg}$ isotope. This would consequently lead to a heavier magnesium isotopic composition in the cytosol from which the Mg^{2+} ions are transported to the coccolith vesicle. Thus, the coccolith vesicle would fractionate against a isotopic heavier solution compared to the isotopic signal of the seawater, what could lead to the observed offset in magnesium isotope fractionation between coccolithophores and foraminifera.

However, this theory remains hypothetical since Mg^{2+} transport mechanisms are not known for any phytoplankton taxa. Additionally, knowledge about the magnitude of the magnesium isotope fractionation inside the organic compartments is missing. Therefore, further investigations (both in the organic and inorganic material) are necessary to understand the mechanisms of magnesium isotope fractionation in coccolithophores.

4.1.3 Paleoproxy implications

Coccolithophores (together with foraminifera) are responsible for the majority of calcareous sediments since the Jurassic. For the reconstruction of the calcium cycle over time, it is necessary to evaluate and quantify the input and output fluxes of calcium (see 2.1.2). This is done by measuring the individual isotopic signatures of the involved fluxes.

One output flux of calcium is the biogenic calcification by coccolithophores and the subsequent deposition of calcite in the sediments. The isotopic signature of coccolithophorid calcite primarily depends on the isotopic compositions of the seawater and might be secondarily influenced by other seawater variables (e.g. temperature, Ca^{2+} and Mg^{2+} concentrations). Therefore, it is necessary to correct the isotopic signal of coccolithophorid calcite from the sediments against these secondary variables.

Manuscript IV shows that the calcium isotopic composition in calcite of *E. huxleyi* and *C. braarudii* is independent of the seawater concentrations of calcium and magnesium. Thus, these two seawater variables, which had changed over geological time, can be neglected in calculations to reconstruct the oceanic calcium budget (as it was done by Zhu and MacDougall (1998); De La Rocha and DePaolo (2000)).

Besides the usage of the calcium isotope fractionation in coccoliths ($\Delta^{44/40}\mathrm{Ca}$) to reconstruct the oceanic calcium budget over time, it seems promising as a paleoproxy for temperature since it is the main factor influencing the isotopic signal (besides the isotopic composition of seawater). However, since species specific differences are reported in coccolith $\Delta^{44/40}\mathrm{Ca}$ the usage of bulk carbonate sediments as a temperature proxy appears to be questionable (Gussone et al. 2007).

Magnesium is mainly located in the organic compartment of coccolithophores, what can be about 500 times higher than the magnesium content of the inorganic coccoliths. Therefore, measurements of magnesium in coccolithophorid calcite to reveal the element Mg/Ca ratio or the magnesium isotope fractionation ($\Delta^{26/24}\mathrm{Mg}$) faces two analytical problems: First, the sample has to be efficiently cleaned from organic matter and second, the sample material has to contain sufficient magnesium to allow repeated measurements.

The cleaning procedure is not critical since different methods can be verified in the

lab (Stoll et al. 2001). Sufficient sample material, on the other hand, is not a problem for elemental fractionation analysis (e.g. Mg/Ca) in culture and sediment samples (Stoll et al. 2007). At present, magnesium isotope fractionation analysis ($\Delta^{26/24}\mathrm{Mg}$) requires an amount of $\approx 1000\,\mathrm{ng\,Mg}$ what corresponds (depending on the Mg/Ca ratio) to approximately 2-4 mg of coccolithophorid $\mathrm{CaCO_3}$. This sample size will limit $\Delta^{26/24}\mathrm{Mg}$ measurements to culture experiments, where it is possible to accumulate the required amount from one coccolithophore species. Additionally, the magnesium isotope fractionation may reveal new information about the cellular process of calcification as well as ion transportation mechanisms.

The Mg/Ca ratios in biogenic calcite of foraminifera and corals are widely used as a temperature proxy (Nürnberg et al. 1996; Toyofuku et al. 2000; Mittsuguchi et al. 1996).

Recent results of sediment traps indicate that the coccolith Mg/Ca ratio of one out of three coccolithophore species is correlated to temperature (Stoll et al. 2007). Therefore, it seems that coccolith Mg/Ca ratios are less valuable as a temperature proxy than ratios revealed from coral or foraminifera calcite.

A strong negative correlation was found between coccolith $\mathrm{D_{Mg}}$ (as well as coccolith Mg/Ca) and chlorophyll *a* production in two coccolithophore species (manuscript IV). This may hint to a physiological control of the coccolith Mg/Ca ratio. However, physiological rates, like chlorophyll *a* production, are influenced by temperature and thus, abiotic factors may have a secondary control on the coccolith Mg/Ca ratio, what has to be verified in future studies (see 5.2).

4.2 Coccolithophorid calcification in the ambient ocean and the cell division cycle

The cell cycle is divided into four phases, which every cell has to go through for successful reproduction. Beginning with the G1 phase, the cell accomplishes a major part of its assimilation processes (protein and carbohydrate synthesis) and subsequently en-

ters the S phase where the genome is doubled. After DNA synthesis the cell prepares for cell division in the G2 phase and in the last sequence of the cell cycle, the M phase, the chromosomes condense along the equator of the cell, the daughter chromosomes are drawn toward the poles and the final division completes the cell cycle. The entrance to, and the exit from each phase needs to be tightly controlled in order to assure two 'healthy' daughter cells.

The cell cycle in phytoplankton is triggered and influenced by various environmental conditions. Within a light:dark cycle cell division is synchronized to occur in the dark and a lower temperature elongates each phase of the cycle (Vaulot 1994; Chisholm 1981). The influence of nutrient limitation on the cell cycle is insufficiently understood in phytoplankton. Phosphorus limitation can prevent *Prorocentrum minimum* from entering the S phase (Antia et al. 1990) and nitrogen limitation arrests diatoms and dinoflagellates in the G1 phase (Vaulot et al. 1987; Olson and Chisholm 1986). Furthermore diatoms are highly dependent on silicate uptake during the G2+M phase (Claquin et al. 2002). Manuscript I indicates a tight correlation of the G1 phase with the process of coccolithogenesis and explains the higher calcification rate observed under phosphorus limitation compared to nitrogen limitation (Paasche 1998). Under both limitations, cells of *E. huxleyi* accumulate in the G1 phase. Nitrogen limited cells are impaired in assimilation processes, reflected by a decrease in cell size, whereas phosphorus-starved and nitrogen-replete cells increase significantly in diameter because biomass and calcite build up continues while DNA synthesis and replication are inhibited. In this regard, cell cycle analysis offers a useful tool for investigating the calcification mechanisms of coccolithophores. Additionally, it gives further evidence to the underlying processes leading to the reported responses of coccolithophorid calcification to changing environmental conditions, such as macronutrient limitation. In addition to the effect of macronutrients on the cell cycle, micronutrients should be considered. For instance, Schulz et al. (2004) reported a similar effect on calcification under zinc limitation, as seen under phosphorus starvation in manuscript I. Zinc is highly involved in the DNA transcription and acts as a cofactor for alkaline phosphatase, thus zinc is likely to act similarly to phosphorus on the cell cycle by arresting cells at the transition from the G1 to the S phase.

4.3 Ocean acidification and coccolithophores: From short to long-term response

Since it is evident that the ocean will become more acidic due to the absorption of atmospheric CO_2 emitted as a result of human activities, the research in the field of 'ocean acidification' or 'ocean carbonation' has increased significantly in the recent years. Attention has been focused on marine calcifiers because biogenic precipitation of $CaCO_3$ is likely to become more costly in energy requirements due to a decreasing seawater *p*H (Fabry et al. 2008).

Four coccolithophore species were tested to changing seawater carbonate chemistry in several laboratory experiments with species-specific results. Apart from different amplitudes in the calcification and photosynthesis responses (described in 2.3 and manuscript II+III), a general trend crystallizes for the investigated species. With experimentally increasing pCO_2 from ambient to future levels, organic carbon production per cell is elevated, whereas calcification decreases (for a detailed summary of species and strain-specific responses refer to Ridgwell et al. (2009); Zondervan (2007)). However, the examined range of pCO_2 is quite narrow and recent results indicate (Krug 2009; Langer et al. 2009), as proposed by Ridgwell et al. (2009), that under a broader pCO_2 range the physiological response follows an optimum curve with species and strain-specific optima for photosynthesis and calcification.

The majority of pCO_2 experiments on coccolithophores were conducted with cultures acclimated for ≈ 10 generations and after another similar generation time the experiments were terminated. However, experiments on the immediate response (within one generation) and the long-term response (over multiple generations) are lacking. Manuscript II discusses the short-term response of *E. huxleyi* to rising pCO_2 and reveals that after 8 hours of exposure to high pCO_2 similar trends in calcification and photosynthesis are observed as reported in previous studies for acclimated cultures. Under long-term high pCO_2 exposure (more than 60 generations) *E. huxleyi* and *C. braarudii* display a de-

creased trend in calcification. This is consistent with results obtained from shorter-term high pCO_2 exposure experiments, and it is further indicated that a gradual pCO_2 increase does not reduce pCO_2/*p*H sensitivity.

Besides the enlargement of our knowledge on the physiological responses of coccolithophores to ocean acidification, a further goal is to use the data (compiled from controlled laboratory or mesocosm studies) for the generation of global models with the aim to project future changes in the marine carbon cycle.

Coccolithophores play a multiple role in climate feedback mechanisms since decreased $CaCO_3$ production and increased organic carbon fixation would both result in a negative feedback loop. Furthermore, a reduction in the strength of the biological pump due to a diminished production of ballast matter ($CaCO_3$) is feasible and would result in a positive feedback (see also 2.1).

Data used in various models have mostly been obtained from single species experiments (Zondervan et al. 2001). However, single species react quite differently compared to natural communities. This becomes apparent by comparing two mesocosm experiments: one dominated by a bloom of *E. huxleyi* (Engel et al. 2005) and the other a mixed community of *E. huxleyi* and two diatom species (Riebesell et al. 2007; Schulz et al. 2008). In the latter study, an increase in the organic carbon production was reported, whereas no elevated organic carbon build-up per available nutrients was observed at future pCO_2 compared to ambient pCO_2 conditions in the study of Engel et al. (2005). This seems contradictory to the observed increase in organic carbon fixation of *E. huxleyi*, what is observed in laboratory studies (Zondervan et al. 2002; Feng et al. 2008). However, the reported rates from the latter studies are normalized on a 'per cell basis' and normalization to a biomass basis (e.g. per total particulate nitrogen) reveals that *E. huxleyi* builds-up the same amount of particulate organic carbon per particulate nitrogen under ambient and future pCO_2 (see manuscript III).

Several diatom species, on the other hand, were tested to increased pCO_2 under laboratory conditions and species specific changes in the carbon to nitrogen cell quota were observed (Burkhardt et al. 1999). However, we have to keep in mind, that laboratory

studies are mostly conducted under nutrient replete conditions what does not reflect the common situation in the ocean (where nitrogen is the limiting nutrient). This clearly reflects the need for 'whole community' studies under natural conditions to estimate possible changes in the marine carbon cycle.

With ongoing ocean acidification/carbonation both winners and losers will appear. Calcareous organisms will face an unavoidable dissolution of their calcium carbonate shell as soon as the calcium carbonate saturation state drops below 1. However, dissolution kinetics of calcium carbonate differ essentially with elemental composition and crystal structure (Morse et al. 2009). The highest solubility is found in high Mg-calcite at approximately $12\,\mathrm{mol}\%\ \mathrm{MgCO_3}$, followed by aragonite and low Mg-calcite (Morse et al. 2006). Up to date, very little is known about the impact of rising $p\mathrm{CO_2}$ on the coccolith trace element composition. Results of foraminifera calcite indicate no changes in the partitioning of magnesium with rising $p\mathrm{CO_2}$ (Dissard et al. 2009). However, the incorporation of $\mathrm{Mg^{2+}}$ into the calcite lattice of coccolithophores underlies different mechanisms than in foraminifera (see also 4.1.1). Therefore, it might be possible that the coccolith Mg/Ca ratio could be altered due to changes in the physiology of coccolithophores induced by ocean acidification. Recent results (see Fig. 5.2) indicate that the coccolith Mg/Ca ratio increases under elevated $p\mathrm{CO_2}$.

A future accumulation of calcite with an elevated Mg/Ca ratio at the seafloor would result in more corrodible sediments. However, dissolution of sedimentary calcium carbonate will finally neutralize much of the antrophogenic $\mathrm{CO_2}$ in the ocean on time scales of several thousands of years, whereby the carbonate compensation depth (CCD) will be shallowed (Archer et al. 1998). A comparable scenario was seen in the geological record at the PETM (Paleocene-Eocene thermal maximum), where a rapid release of $\mathrm{CO_2}$ to the atmosphere was partly neutralized by the dissolution of sea-floor carbonate, accompanied by a shallowing of the CCD within 10,000 years (Zachos et al. 2005).

5 Future Research

The collected data of this PhD-Thesis not only gives answers to scientific questions on the physiology of coccolithophores, but in addition new questions are posed by the results. In this chapter, ideas for ongoing and future research will be introduced.

5.1 Coccolithophore success during the Cretaceous

It is still under scientific discussion which environmental parameter triggered the massive deposition of coccolithophorid calcite during the Late Cretaceous. There is no doubt, that the Cretaceous ocean was a quite different environment compared to the modern ocean (see 2.2.).

A recent hypothesis is that the low seawater Mg/Ca ratio during the Late Cretaceous caused an elevation of coccolithophorid growth rate and calcite production (Stanley et al. 2005). This hypothesis is partly in contrast to findings of manuscript IV. As discussed in the previous chapter the actual success of coccolithophores could probably be an interaction of various environmental parameters. Therefore, a future experiment would be to simulate the Cretaceous ocean under laboratory conditions with regard to seawater major ion composition (Na, Mg and Ca) as well as the carbonate system (Zeebe 2001; Zeebe and Westbrook 2003; Tyrrell and Zeebe 2004).

Additionally, species-specific differences are revealed between *E. huxleyi* and *C. braarudii* in the response to elevated seawater calcium concentrations. The larger coccolithophore

C. braarudii has a higher tolerance to elevated external calcium concentrations compared to *E. huxleyi*. Verification of this presumed 'size dependence' by investigating other coccolithophore species with various cell sizes would be a next step in understanding the species-specific responses of coccolithophores.

An interesting experiment would be to study the effect of high seawater calcium concentrations on diatoms, the dominant phytoplankton group in the modern ocean. In the case that diatoms are impaired by the seawater calcium concentrations of the Cretaceous, this would give new implications regarding the rise of coccolithophores during that era. Furthermore, a protective function of calcification against toxification resulting from high cytosolic calcium concentration could be hypothesised.

5.2 Cellular calcification mechanisms

Manuscript I reveals clearly the importance of the cell division cycle for the process of biogenic calcification in *E. huxleyi* under light and macronutrient limitation. As previously mentioned, it seems likely that also micronutrient limitation (e.g. Zn) and increase/decrease of seawater $p\mathrm{CO}_2$/*p*H may have an influence on the cell division cycle since variation in cell division were observed (Schulz et al. 2004; Langer et al. 2009). Yeast cells, for example, maintain *p*H homeostasis throughout all phases of the cell cycle (Karagiannis and Young 2001), whereas in other eukaryotic systems *p*H is reported to vary with specific phases in the cell cycle (Aerts et al. 1985). Whether the cellular *p*H in phytoplankton is constant or not during the cell cycle, it could be possible that a changing external *p*H (ocean acidification) influences the regulation of the intracellular *p*H and therefore alters the progression through the cell division cycle by elongating specific phases. This could easily be verified in the laboratory by measuring the duration of each cell cycle phase under ambient and high $p\mathrm{CO}_2$.

The actual transport meachanisms and pathways of ions (e.g. Ca, Mg and Sr) to the coccolith vesicle are not well understood (Brownlee and Taylor 2004). Research on el-

ement and isotope fractionation in coccolithophorid calcite was primarily located in the field of paleoceanography in order to reveal information about past seawater conditions (Stoll and Ziveri 2004). In the recent years research has focused on the physiological basis of element and isotope fractionation in coccolithophorid calcite (Ziveri et al. 2003; Gussone et al. 2006; Langer et al. 2007; Gussone et al. 2007). Whereas the latter studies dealed with the elements Ca, Sr, O and C, manuscript IV primarily deals with Mg in biogenic calcite. Evidence is given that Mg incorporation as well as Mg isotope fractionation seems to be under a tight physiological control. The physiological control over Mg incorporation in coccolithophorid calcite is further confirmed by recent, yet unpublished, data. Fig. 5.1 displays the partition coefficient of Mg with respect to experimental tem-

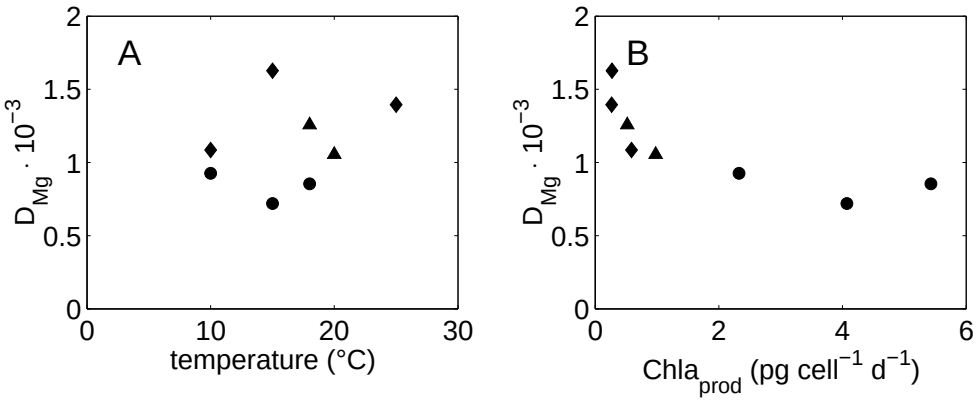

Figure 5.1: *Data from dilute batch culture experiments with E. huxleyi (diamond), C. braarudii (circle) and C. quadriperforatus (triangle).* ***A****: Partition coefficient* $\mathrm{D_{Mg}}$ *in regard to experimental temperature.* ***B****:* $\mathrm{D_{Mg}}$ *as a function of chlorophyll a production. Müller et al. unpublished data.*

perature (Fig. 5.1A) and to the chlorophyll *a* production (Fig. 5.1B). A direct temperature dependence on $\mathrm{D_{Mg}}$ was not found but strong evidence is given, that chlorophyll *a* production has a controlling factor on the partitioning of magnesium into the calcite lattice. Laboratory research on magnesium incorporation and isotope fractionation in coccolithophores, both at the inorganic and the organic matter, could probably be a very useful tool to investigate calcification pathways in coccolithophores. For example, a next step would be to verify the correlation between $\mathrm{Chl}a_{\mathrm{prod}}$ and $\mathrm{D_{Mg}}$ by altering the cellular chlorophyll quota by changing the light intensity.

5.3 Ocean acidification

Atmospheric CO_2 is an important regulator of the earth's climate and has changed the ocean's chemistry so that it is registering a decrease in *p*H (as described in 2.3.1.) since the industrial revolution. Coccolithophores, as the dominant oceanic calcifiers, are probably negatively affected due to ocean acidification (manuscript II and III) - further research in this field in urgently needed.

5.3.1 Coccolith element ratios

In manuscript IV, the influence of seawater Ca and Mg concentrations on the element ratios in coccolithophorid calcite was shown and it was highlighted that the physiology is an important regulator of Mg incorporation during calcite precipitation. How changing physiological parameters influence Sr/Ca ratios in coccolithophorid calcite was previously reported in regard to nutrient stimulated growth by Rickaby et al. (2002). Ocean acidification is known to alter physiological rates of coccolithophores such as cellular division, photosynthesis and calcification. These changes in physiology could presumably modify the elemental composition of coccolithophorid calcite. A recent study on foraminifera calcite reported no change in Mg/Ca ratios if foraminifera were cultured under high pCO_2, whereas the Sr/Ca ratios increased with raised pCO_2 (Dissard et al. 2009). First results on coccolithophores indicate an increasing trend in coccolith Sr/Ca and Mg/Ca with elevated pCO_2 (Fig. 5.2).

Interestingly, the measured Mg/Ca ratio in the calcite of *C. braarudii* at a pCO_2 of $3500\,\mu\mathrm{atm}$ indicates a change in the elemental composition towards high Mg-calcite. Additionally, physiological rates (like cell division and photosynthesis) were seriously reduced (-70%) at the mentioned pCO_2 condition (Krug 2009).

A shift from low to high Mg-calcite alters the dissolution kinetics towards a higher solubility (Morse et al. 2006; Andersson et al. 2008) and consequently, the maximum depth at which coccolithophores are found in the sediment will shallow.

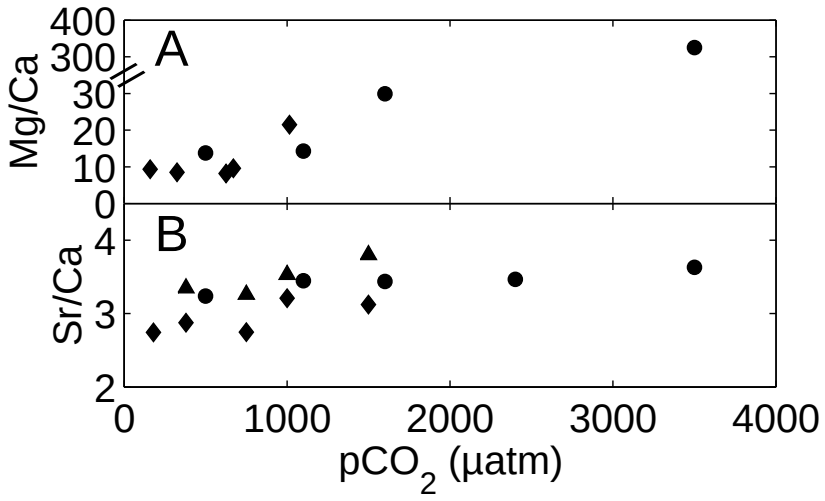

Figure 5.2: *Coccolith Mg/Ca (A) and Sr/Ca (B) ratios in mmol/mol as a function of pCO_2 in two coccolithophore species. A: E. huxleyi cultured at 10°C (diamonds) and C. braarudii cultured at 15°C (circles). B: E. huxleyi cultured at 10°C (diamonds) and 20°C (triangles) and C. braarudii cultured at 15°C (circles). Experiments were conducted by Wiebe (2008); Krug (2009).*

5.3.2 Temperature: A combined effect

Ocean acidification or carbonation is not the only factor of climate change influencing marine life. In fact, oceanic life will face a combination and an interactions of changing environmental conditions driven by climate change (temperature, ice cover, sea level, etc.). Over the past 100 years the earth's temperature has risen by 0.6 ± 0.2°C and is projected to increase to the end of the century by another 1.8 to 4.0°C (Alley et al. 2007). Increased temperature is known to accelerate the metabolic activity in phytoplankton, leading to an elevation of division rate (Eppley 1972).

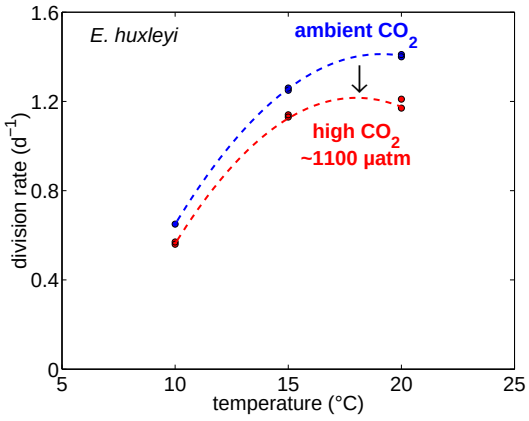

Figure 5.3: *Division rate of E. huxleyi as a function of temperature. Growth curves are displayed with regard to ambient pCO_2 (blue) and high pCO_2 (red). Data from Wiebe (2008).*

Species-specific temperature-optimum-curves of growth rate have been reported for various coccolithophores (Buitenhuis et al. 2008), but how an interaction with elevated pCO_2 may influence these optimum-curves is not known.

Manuscript II and III report a decrease of cellular growth rate due to an increased pCO_2 level. A first set of culture experiments, done with *E. huxleyi*, shows that this decrease may be amplified by rising the experimental temperature (Fig. 5.3). However, it remains an open question how physiological rates (photosynthesis, calcification, etc.) will be affected by a combination of decreasing pH and rising temperature. A first study was done by Feng et al. (2008) demonstrating that the combination of light, temperature and pCO_2 has an interacting effect on the physiological rates in *E. huxleyi*. Following up on these findings it would be a next step to verify the combined effect of temperature and ocean acidification on coccolithophores.

5.3.3 Adaptation and evolution

The eye opening work by Darwin (1859) explained the primary mechanisms of evolution and subsequently the scientific field of evolutionary biology has made incredible progress and has lead to a modern theory of evolution (see Barton et al. (2007) for a detailed summary). During the last ten years it has become plain that evolution can occur on short or ecological time scales and that the field of evolutionary biology may provide a new aspect for biologists working on global climate change (see Carroll et al. (2007) and references within). Regarding unicellular eukaryotic cells with short generation times, like coccolithophores, which are capable of sexual reproduction (Billard and Inouye 2004), the possibility of adaptation to ocean acidification seems plausible.

The process of evolution initially occurs on the molecular level by mutation or sexual recombination of the genetic material. Nevertheless, changes in the genotype have to emerge up to the phenotype to be under the process of natural selection. Therefore, experiments over multiple generations (examining the phenotype) can provide very useful information about possible adaptation mechanisms in coccolithophores. However, we

have to keep in mind that we cannot distinguish between phenotypic plasticity and genetic mutations by measuring only the physiological parameters - the use of molecular tools will probably be an additional help.

A first step in researching possible adaptation mechanisms in unicellular phytoplankton (e.g. coccolithophores) would be the set up of appropriate experiments using multiclonal populations and sexual reproduction to increase the population's heritability (mutation rate).

6 Zusammenfassung

Die vorliegende Arbeit behandelt den Einfluss variierender Umweltparameter auf die Physiologie von Coccolithophoriden, insbesondere auf den Prozess der biogenen Kalzifizierung. Im Fokus stehen Auswirkungen durch die Veränderungen der Kalzium- und Magnesiumkonzentrationen im Seewasser, sowie der Limitierung von Stickstoff, Phosphor und Licht. Des Weiteren wurden physiologische Parameter von Coccolithophoriden bezüglich einer anthropogenen Änderung der Seewasserkarbonatchemie untersucht.

Coccolithophoriden sind mitverantwortlich für die massiven Ablagerungen von kalzithaltigen Partikeln im Sediment während der Kreidezeit (145-65 Ma), wobei unterschiedliche Hypothesen zur Erklärung dieses Phänomens bestehen. Eine derzeitige These besagt, dass das auf geologischen Zeitskalen variierende Mg/Ca-Verhältnis im Seewasser einen entscheidenden Einfluss auf den evolutionären Erfolg von kalkbildenden Organismen hat. Insbesondere soll das niedrige Mg/Ca-Verhältnis von ≈ 1 während der Kreidezeit (im Vergleich zu 5.2 im heutigen Ozean) zu einer Erhöhung der Reproduktions- und Kalzifizierungsrate von Coccolithophoriden geführt haben.

Ergebnisse aus dieser Arbeit belegen, dass diese Hypothese nicht für die zwei Coccolithophoridenarten *Emiliania huxleyi* und *Coccolithus braarudii* gültig ist. In beiden Arten sind unter Mg/Ca-Verhältnissen der Kreidezeit die gemessenen physiologischen Raten, wie Wachstums-, Chlorophyllproduktions- und Kalzifizierungsraten, um bis zu 50% verringert. Es konnte belegt werden, dass dies durch eine Erhöhung der Seewasserkalziumkonzentration ausgelöst ist und nicht durch eine Änderung der Magnesiumkonzentra-

tion oder des Mg/Ca-Verhältnisses. Des Weiteren wurde bei einer verringerten Chlorophyllproduktion ein erhöhtes Mg/Ca-Verhältnis im Kalk der untersuchten Arten gemessen, wodurch auf eine enge Verbindung der Physiologie und Kalzitchemie geschlossen wird.

Im heutigen Ozean unterliegen die Kalzium- und Magnesiumkonzentration keinen größeren Schwankungen. Jedoch können andere Umweltfaktoren, wie das Licht- und Nährstoffangebot, die physiologischen Raten von Coccolithophoriden beeinflussen.

Eine Limitation durch Stickstoff, bzw. Phosphor, hatte einen Stopp der Zellteilung mit unterschiedlichen Auswirkungen zur Folge. Während unter Phosphorlimitation die Zellen an Größe zunahmen und die Photosynthese- und Kalzifizierungsraten ungehindert weiterliefen, waren unter Stickstofflimitation beide Raten reduziert und die Zellen verringerten ihre Größe. Unter Lichtlimitation wurde die Wachstumsrate verringert, was eine Erhöhung des Kalzitgehaltes der Zelle zur Folge hatte. Diese Ergebnisse helfen, die unterschiedlichen Reaktionen in der Wachstumsrate und der Kalzifizierungsrate unter Stickstoff-, Phosphat- und Lichtlimitation im heutigen Ozean zu erklären. Des Weiteren wurde gezeigt, dass unter licht- und nährstoffgesättigten Bedingungen die Kalzifizierung in *E. huxleyi* auf eine bestimmte Phase des Zellzyklusses beschränkt ist, namentlich die G1-Phase.

Durch den anthropogenen Ausstoß von CO_2 unterläuft die Karbonatchemie im Ozean gravierende Veränderungen. Aufgenommenes CO_2 aus der Atmosphäre führt zu einer Erhöhung der aquatischen CO_2-Konzentration und einer Erniedrigung des *p*H-Wertes im Oberflächenwasser, bezeichnet als Ozeanversauerung. Die Auswirkungen der Ozeanversauerung wurde unter Laborbedingungen auf zwei Coccolithophoridenarten gestestet. Untersucht wurden die unmittelbare physiologische Reaktion (Stunden bis zu einem Tag) und die langfristige Reaktion (Tage bis Monate). Die gewonnenen Ergebnisse sind mit Daten aus der Literatur verglichen worden und ergaben zusammengefasst ein einheitliches Bild der physiologischen Antwort von Coccolithophoriden auf die prognostizierte Ozeanversauerung. Eine Verringerung der Kalzifizierungsrate tritt nach ca. acht Stunden auf und stagniert auf diesem Niveau konstant über eine langzeitige Kultivierung unter hoch-

CO_2 (bis zu 150 Generationen). Weiterhin ist keine Verminderung des beobachteten CO_2/*p*H-Effektes durch einen graduellen Anstieg der CO_2 Konzentration ersichtbar. Photosyntheseraten pro partikulärem Stickstoff sind konstant oder verringert (in *E. huxleyi* beziehungsweise in *C. braarudii*) über die Dauer der Kultivierung. Dies ist übereinstimmend mit publizierten Ergebnissen aus Experimenten, welche eine maximale Generationszahl von 20 untersuchten.

Die ozeanische Produktion von organischem Kohlenstoff und $CaCO_3$ durch marine Fauna und Flora spielen eine bedeutende Rolle in den Elementkreisläufen von Kohlenstoff und Kalzium auf kurzen und geologischen Zeitskalen. Coccolithophoriden sind in beiden Elementkreisläufen involviert und somit maßgeblich an der Regulation der marinen Kalzium-Karbonat-Sättigung seit dem Mesozoikum beteiligt.

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Conference abstracts

.1 EGU 2006, -poster-

Geophysical Research Abstracts, Vol. 8, 02218, 2006
SRef-ID: 1607-7962/gra/EGU06-A-02218

Cell cycle of *Emiliania huxleyi* under enhanced atmospheric CO_2 and its relation to calcification

M. N. Müller, A. N. Antia, J. LaRoche and U. Riebesell
Leibniz Institute of Marine Sciences, Kiel, Germany

Calcification in coccolithophores is strongly influenced by the projected decrease of future ocean pH. The physiological pathway by which ocean acidification affects the process of biogenic calcification is still not clear. Here we investigate the process of calcification on a cellular level with respect to the cell cycle of the cosmopolitan coccolithophorid *Emiliania huxleyi* in controlled lab experiments. The cell cycle can be distinguished in three phases (G1, S and G2 + M). Using DNA staining and flow cytometry it is possible to follow each phase of the cell cycle in a synchronized *E. huxleyi* population during a light:dark cycle. In parallel with cell cycle analysis we measured calcification rates in two hour intervals. This data set indicates that calcification occurs predominantly in the G1 phase of the cell cycle. Thus, changing environmental conditions, which alter the cell cycle, may also have an effect on the degree of calcification. In addition to the lab experiments, we studied the cell cycle under future CO_2 conditions during the PeECE III mesocosm field study in a Norwegian fjord. A combination of lab and mesocosm experiments provides further insight to biogenic calcification of coccolithophores.

.2 EGU 2007, -poster-

Geophysical Research Abstracts, Vol. 9, 07283, 2007
SRef-ID: 1607-7962/gra/EGU2007-A-07283

Coccolithophorid calcification and isotope fractionation in relation to seawater Mg/Ca ratios

M. N. Müller, R. Gutperlet, A. Eisenhauer and U. Riebesell
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The Mg/Ca ratio of seawater changed throughout Phanerozoic time. In the Late Cretaceous the molar Mg/Ca ratio was <1 with Ca concentrations of 25-30 mM. The modern ocean has a Mg/Ca ratio of $\sim$5, with an absolute Ca concentration of $\sim$10 mM. Recent findings (Stanley, 2005) indicate that a low Mg/Ca ratio in the Cretaceous could have caused a high reproduction rate of coccolithophores in the water column what could be a reason for the massive chalk deposition. The physiological pathway by which a changing Mg/Ca ratio affects the process of biogenic calcification is still under discussion. Here we investigate the process of calcification on a cellular level with respect to the Mg/Ca ratio of seawater in the cosmopolitan coccolithophore *Emiliania huxleyi* in controlled lab experiments. Additionally, we analysed the elemental composition (Mg/Ca) and isotopic fractionation (δ^{25}Mg, $\delta^{44/40}$Ca) of the coccolith calcium carbonate. Changing the Mg and Ca concentration has an effect on the cellular process of coccolithophorid calcification. Preliminary results based on scanning electron microscopy indicate that a structural malformation of the coccoliths appeared at Mg/Ca ratios $\leq$1 and at a high ratio of 10. The results give new insights into biogenic calcification and its response to changing seawater chemistry.

.3 EMBS 2007, -poster-

42nd European Marine Biology Symposium - Kiel, 2007

Coccolithophorid adaptation to rising atmospheric CO_2: a running long-term experiment

Müller, M. N.[1] & U. Riebesell[1]

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Abstract

The predicted ocean acidification due to rising atmospheric CO_2 has a severe impact on calcifying organisms like coccolithophores. Lab and field experiments clearly showed an adverse effect of decreasing seawater pH on the cellular process of biogenic calcification in coccolithophores. In all these experiments the organisms were exposed to an abrupt CO_2 enrichment. In a natural environment phytoplankton could adapt over decades to centuries to rising atmospheric CO_2. Considering the short generation time of coccolithophores of 1 to 2 days it could be possible for these unicellular algae to adapt genetically and/or physiological to a changing environment like decreasing ocean pH. We are running a long-term experiment with three coccolithophore species in which we slowly raise the atmospheric CO_2 level to allow physiological adaptation. Physiological status of the coccolithophore species is monitored by growth and calcification rate as well as photosynthesis. Additionally, the carbonate system is observed by alkalinity and dissolved inorganic carbon measurements. Presently, the first five months and the feasibility of a long-term growth experiment with coccolithophores are presented. With the collected data and future genetic analyses we will get new insights into the potential for physiological and/or evolutionary adaptation.

.4 Geologische Vereinigung e.V., 2007 -oral-

Room 2890 (GW2 building)

Session B5: Biomineralization and implications for paleoceanography

Conveners: N. Gussone (Münster), J. Bijma (Bremerhaven), E. Hathorne (Bremen)

16:00 – 16:30	B5-1	Meibom, A. (*Keynote talk)* Coral biomineralization: A sharper optic reveals ultra-structural properties
16:30 – 16:45	B5-2	Cuif, J.P., Y. Dauphin, A. Ball, B. Farre, J. Nouet, M. Salomé, C.T. Williams Diachronic superposition of distinct crystallisation domains: a parallel between coral and mollusc skeletogenesis
16:45 – 17:00	B5-3	Hiebenthal, C., M. Wahl & A. Eisenhauer Ca isotope fractionation ($\delta^{44/40}$Ca) in bivalves shells in response to environmental conditions (temperature and salinity) and bivalve fitness
17:00 – 17:15	B5-4	Kisakürek, B., A. Eisenhauer, J. Erez, F. Böhm & D. Garbe-Schönberg Calcium isotope fractionation in cultured *Globigerinoides ruber*
17:15 – 17:30	B5-5	Müller, M.N., A. Kolevica, R. Gutperlet, A. Eisenhauer & U. Riebesell Coccolithophorid calcification and isotope fractionation in relation to seawater Mg/Ca ratios
17:30 – 17:45	B5-6	Schmidt, D.N., S.A. Kasemann & B. Donner Biology of boron isotopes in planktic foraminifers: implications for palaeo-pH predictions
17:45 – 18:00	B5-7	Zonneveld, K., A. Mackensen & K.-H. Baumann Stable isotopes of the calcareous dinoflagellate *Thoracosphaera heimii* – a culture experiment

Coccolithophorid calcification and isotope fractionation in relation to seawater Mg/Ca ratios

M. N. Müller, A. Kolevica, R. Gutperlet, A. Eisenhauer and U. Riebesell

The Mg/Ca ratio of seawater changed throughout Phanerozoic time. In the Late Cretaceous the molar Mg/Ca ratio was <1 with Ca concentrations of 25-30 mM. The modern ocean has a Mg/Ca ratio of ~5, with an absolute Ca concentration of ~10 mM. Recent findings (Stanley, 2005) indicate that a low Mg/Ca ratio in the Cretaceous could have caused a high reproduction rate of coccolithophores in the water column what could be a reason for the massive chalk deposition. The physiological pathway by which a changing Mg/Ca ratio affects the process of biogenic calcification is still under discussion. Here we investigate the process of calcification on a cellular level with respect to the Mg/Ca ratio of seawater in the cosmopolitan coccolithophore *Emiliania huxleyi* and in the evolutionary older species *Coccolithus pelagicus* in controlled lab experiments. Additionally, we analysed the elemental composition (Mg/Ca) and isotopic fractionation (δ^{26}Mg, δ^{44}Ca) of the coccolith calcium carbonate. Changing the Mg and Ca concentration has an effect on the cellular process of coccolithophorid calcification. Preliminary results indicate a major effect on cellular calcification by changing the Ca concentration rather than varying the Mg concentration. Additionally, the two coccolithophore species are responding in different ways on a changing seawater Mg/Ca ratio. The results will give new insights into biogenic calcification of coccolithophores.

.5 The ocean in a high-CO_2 world II, 2008 -oral-

DAY 3: WEDNESDAY, 8 OCTOBER

ADAPTATION AND MICROEVOLUTION

A BRIEF HISTORY OF SKELETONS IN THE OCEANS [I]
Knoll, Andrew H.

Eukaryotes existed for more than a billion years before the biomineralized skeletons entered the fossil record. Consistent with phylogenetic evidence for the widespread use of silica by protists, the earliest mineralized elements are chromalveolate and rhizarian scales in 750-800 Ma rocks. Carbonate and silica skeletons occur as part of Ediacaran animal diversification, but a relatively late and taxonomically limited part. In contrast, carbonate, phosphatic and siliceous skeletons all diversified markedly as part of the greater Cambrian radiation of animal life. By the mid-Ordovician Period, animal and benthic algal skeletons had come to play much the same biomechanical, ecological and biogeochemical roles they do today. But the course of skeletal evolution never did run smooth. A remarkable decline in skeletal abundance and diversity occurred at the end of the Permian Period (252 Ma), when mass extinction devastated marine ecosystems. Patterns of extinction versus survival match predictions informed by physiological research on hypercapnia; rapid imposition high P_{CO2} at a time when deep ocean water masses already tended toward dysoxia may explain this greatest of all extinction events. Other mass extinctions show differing biological patterns, suggesting that Permo-Triassic scenarios cannot be applied to all such events. Episodic crashes of hypercalcifiers (including reef builders), however, may reflect transient but repeated intervals of high P_{CO2} and deep water anoxia in the world's oceans. Deep ocean oxygen levels have rarely been higher than they are at present. In this respect, at least, the present day challenges of ocean acidification depart from some deep time analogs.

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INFLUENCE OF HIGH CO_2 ON COCCOLITHOPHORES UNDER LONG-TERM CULTIVATION
Müller, Marius N., Kai G. Schulz, Peter Wiebe, and Ulf Riebesell

Studies on the sensitivity of coccolithophores to elevated CO_2 have generally relied on relatively short-term incubations over <20 and in some cases <5 generations. A critical issue in the assessment of ocean acidification impacts on marine biota is the ability of CO_2/pH sensitive organisms to adapt to changing environmental conditions. To approach this question we have exposed three coccolithophore species to gradually increasing CO_2 concentrations over more than 140 generations, namely *Emiliania huxleyi* (max. pCO_2 1150 µatm), *Coccolithus pelagicus braarudii* (max. 930 µatm) and *Calcidiscus leptoporus quadriperforatus* (max. 740 µatm). All three species showed significant decreases in cellular division rates ranging from 10 to 30%, whereas organic matter production rates increased. Surprisingly, the increase of cellular organic matter was not reflected in an increase in cell diameter. In fact, *C. pelagicus braarudii* even shrank in size. Responses in cellular calcification rates were found to be species-specific as shown in previous studies. Whereas *C. pelagicus braarudii* reduced the calcification rate by 25 % and was covered with partly malformed coccoliths, an effect on *E. huxleyi* could not be detected due to high standard deviations. Additionally, we tested the effect of temperature in combination with high CO_2 on the performance of *Emiliania huxleyi*. Increased temperature amplified the decrease in cell division rate under high CO_2, but partly compensated the observed effect on calcification. These results are generally consistent with published data, but indicate a higher sensitivity of coccolithophores to ocean change than previously reported.

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First acknowledgements are directed to my advisor Ulf Riebesell for giving me the opportunity to realize a PhD-thesis in his newly raised group at the IFM-GEOMAR in Kiel, for fruitfull discussions and comments as well as an open door at nearly any time.

Prof. Anton Eisenhauer for starting the ESF project 'Casiopeia' and giving my the possibilities to travel to various international conferences.

Special thanks are given to Avan N. Antia, who guided my first steps in science and is still interested in my recent work.

Thanks to Prof. Julie LaRoche for a nice and very cooperative work.

Kai G. Schulz for fruitful and sometimes difficult discussions about biogeochemical processes, calcification, the carbonate system and especially for not believing everything (thanks!).

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My lunch group and Phillipe, the chef of the art school. Both helped to have a relaxing and tasting break during the day. Additionally, thanks to my Capoeira group and 'the boys' for a lot of things.

Last but not least, I would like to thank my family for some understanding in the last month of this thesis. Additionally, for supporting me with encouraging words and keeping my finances balanced.

Curriculum vitae

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Education

Ph.D. position under supervision of Prof. U. Riebesell.
Scientific researcher in the ESF project 'Casiopeia' (Evaluation of **Ca** Isotope **S**ystem (δ^{44}Ca) **i**n Carbonate Polym**o**rphs as a new **P**roxy for Seawater Temperature and Secular Var**i**ations of Ca Concentration and Fractionation throughout E**a**rth History), since 2005.

Diploma in Biologies at the CAU Kiel. Major subjects: Marine Biogeochemistry, Oceanography, Botany, Marine Chemistry. Grade: 1,7. 2005-1998.

Alternative service as paramedic at the Red Cross in Giessen (Germany), 1998-1997.

Higher education entrance qualification at the 'Weidiggymnasium' in Butzbach (Germany), 1997.

Research experience

Guest researcher — Mai 2006
Jonathan Erez — Hebrew University, Israel
Intracellular pH measurements via confocal microscopy.

Scientific Researcher — Mai-June 2005
Ulf Riebesell — IFM-GEOMAR
PeECE (**Pe**lagic **E**cosystem **C**O2 **E**nrichment Study) III Mesocosmn Study in Norway.

Student assistant — October 2002
Avan N. Antia / Karin Lochte — FS Meteor
Research cruise in the project SOLAS (**S**urface **O**cean **L**ower **A**tmosphere **S**tudy).

Student assistant — October 2001
Norbert Rohlf — FS Alkor
Research cruise in the project ICES (**I**nternational **C**ouncil for the **E**xploration of the **S**ea).

Student assistant — January 2001
Norbert Rohlf — FS Alkor
Research cruise in the project ICES (**I**nternational **C**ouncil for the **E**xploration of the **S**ea).

Teaching activities

Advisor in the frame work of 'NaT-Working Meeresforschung', 2006

Advisor for Semester- and Master theses, since 2005

Advisor in the practical course for students of Marine Sciences, since 2004

Instructor for first aid, since 2003

Tourist guide at the Marine Biology Station in Laboe (Germany), since 2002

APPENDIX . CURRICULUM VITAE

Research interests

Evolution and adaptation of coccolithophores to changing environmental conditions.

Marine carbon cycle.

Coccolithophores as paleoproxy.

Regulation of the cell cycle in marine phytoplankton and linked processes.

Publications

M. N. Müller, H. Stoll, P. Ziveri, A. Kolevica, R. Gutperlet, A. Eisenhauer and U. Riebesell. 2009. Response of coccolithophores to changing magnesium and calcium concentrations: Mg, Sr incorporation and $\delta^{44/40}\mathrm{Ca}$, $\delta^{26/24}\mathrm{Mg}$ in calcite. In prep.

M. N. Müller, K. G. Schulz and U. Riebesell. 2009. Influence of high CO_2 on two coccolithophore key species under long-term cultivation. In prep.

J.Barcelos e Ramos, **M. N. Müller** and U. Riebesell. 2009. Short-term response of the coccolithophore *Emiliania huxleyi* to abrupt changes in seawater carbon dioxide concentrations. BGD 6: 4739-4763.

M. N. Müller, A. N. Antia and J. LaRoche. 2008. Influence of cell cycle phase on calcification in the coccolithophore *Emiliania huxleyi*. Limnol. Oceanogr. 53(2): 506-512.

K. G. Schulz, U. Riebesell, R. G. J. Bellerby, H. Biswas, M. Meyerhöfer, **M. N. Müller**, J. K. Egge, J. C. Nejstgaard, C. Neill, J. Wohlers, and E. Zöllner. 2008. Build-up and decline of organic matter during PeECE III. BG 5: 707-718.

Selected oral and poster presentations

Influence of high CO_2 on coccolithophores under long-term cultivation, oral, The ocean in a high CO_2 world. 2nd international Symposium (Monaco) 2008.

Coccolithophorid calcification and isotope fractionation in relation to seawater Mg/Ca ratios, oral, Geologische Vereinigung (Bremen) 2007.

Coccolithophorid adaptation to rising atmospheric CO_2? -preliminary data-, invited oral, ESF Euroclimate Workshop (Barcelona) 2007.

Coccolithophorid adaptation to rising atmospheric CO_2: a running long-term experiment, poster, EMBS (Kiel) 2007.

Coccolithophorid calcification in relation to seawater Mg/Ca ratios, poster, EGU (Wien) 2007.

Calcification in relation to the cell cycle of Emiliania huxleyi, invited oral, FB-Meeting (AWI, Bremerhaven) 2007.

Cell cycle of Emiliania huxleyi under enhanced atmospheric CO_2 and its relation to calcification, poster, EGU (Wien) 2006.

Key factors, influencing the ecological development of the Baltic Sea, oral, HELCOM Youth Forum (Rostock) 2005.

Awards and grants

Travel grant of the 'Prof. Dr. Werner Petersen - Stiftung', 2006.

Young Scientists' Outstanding Poster Paper (YSOPP) Award at the EGU General Assembly 2006.