



Influence of temperature and CO₂ on the strontium and magnesium composition of coccolithophore calcite

M. N. Müller^{1,2,**}, M. Lebrato^{2,3,**}, U. Riebesell², J. Barcelos e Ramos⁴, K. G. Schulz^{2,5}, S. Blanco-Ameijeiras^{6,*}, S. Sett², A. Eisenhauer², and H. M. Stoll⁷

¹Institute for Marine and Antarctic Studies (IMAS), Private Bag 129, Hobart, TAS 7001, Australia

²GEOMAR Helmholtz Centre for Ocean Research Kiel, Wischhofstr. 1–3, 24148 Kiel, Germany

³Scripps Institution of Oceanography, UCSD, Hubbs Hall Room 2265, 8750 Biological Grade, San Diego, USA

⁴Centre of Climate, Meteorology and Global Change (CMMG), University of Azores, Rua do Capitão d'Ávila, Pico da Urze 970-0042 Angra do Heroísmo, Açores, Portugal

⁵Centre for Coastal Biogeochemistry, School of Environmental Science and Management, Southern Cross University, P.O. Box 157, Lismore, NSW 2480, Australia

⁶National Oceanography Centre, University of Southampton, European Way, SO14 3ZH Southampton, UK

⁷Department of Geology, University of Oviedo, Arias de Velasco, s/n 30005, Oviedo, Asturias, Spain

* now at: Institute F.-A. Forel, Faculty of Sciences, University of Geneva, 10 Route de Suisse, 1290 Versoix, Switzerland

** These authors contributed equally to this work.

Correspondence to: M. N. Müller (marius.muller@utas.edu.au)

Received: 11 September 2013 – Published in Biogeosciences Discuss.: 2 October 2013

Revised: 28 December 2013 – Accepted: 6 January 2014 – Published: 25 February 2014

Abstract. Marine calcareous sediments provide a fundamental basis for palaeoceanographic studies aiming to reconstruct past oceanic conditions and understand key biogeochemical element cycles. Calcifying unicellular phytoplankton (coccolithophores) are a major contributor to both carbon and calcium cycling by photosynthesis and the production of calcite (coccoliths) in the euphotic zone, and the subsequent long-term deposition and burial into marine sediments. Here we present data from controlled laboratory experiments on four coccolithophore species and elucidate the relation between the divalent cation (Sr, Mg and Ca) partitioning in coccoliths and cellular physiology (growth, calcification and photosynthesis). Coccolithophores were cultured under different seawater temperature and carbonate chemistry conditions. The partition coefficient of strontium (D_{Sr}) was positively correlated with both carbon dioxide (pCO_2) and temperature but displayed no coherent relation to particulate organic and inorganic carbon production rates. Furthermore, D_{Sr} correlated positively with cellular growth rates when driven by temperature but no correlation was present when changes in growth rates were pCO_2 -induced. Our results demonstrate the complex interaction between environmen-

tal forcing and physiological control on the strontium partitioning in coccolithophore calcite and challenge interpretations of the coccolith Sr/Ca ratio from high- pCO_2 environments (e.g. Palaeocene–Eocene thermal maximum). The partition coefficient of magnesium (D_{Mg}) displayed species-specific differences and elevated values under nutrient limitation. No conclusive correlation between coccolith D_{Mg} and temperature was observed but pCO_2 induced a rising trend in coccolith D_{Mg} . Interestingly, the best correlation was found between coccolith D_{Mg} and chlorophyll *a* production, suggesting that chlorophyll *a* and calcite associated Mg originate from the same intracellular pool. These and previous findings indicate that Mg is transported into the cell and to the site of calcification via different pathways than Ca and Sr. Consequently, the coccolith Mg/Ca ratio should be decoupled from the seawater Mg/Ca ratio. This study gives an extended insight into the driving factors influencing the coccolith Mg/Ca ratio and should be considered for future palaeoproxy calibrations.

1 Introduction

Coccolithophores, a key functional phytoplankton group, evolved about 225 Ma (million years ago), and their calcitic plates (coccoliths) that cover the cell are found in the sediment record since then (Bown et al., 2004). Over their geological history, coccolithophores have experienced various changes in Earth's climate and the geochemical composition of their intracellularly produced coccoliths has been the focus of numerous studies reconstructing past oceanographic and environmental conditions (Stoll and Ziveri, 2004; Erba, 2006; Ziveri et al., 2012). Since the industrial revolution, however, coccolithophores have been exposed to rapidly changing oceanic conditions including an increase in sea-surface temperature and shifting seawater carbonate chemistry speciation due to anthropogenically released carbon dioxide (Boyd and Hutchins, 2012). Understanding their physiological response to changing carbonate chemistry and temperature, as well as the underlying mechanisms responsible for the chemical signature of coccolith calcite, is essential to validate and apply coccolithophore-based palaeoproxies (Ziveri et al., 2012). The distribution and partitioning of divalent cations, replacing the calcium ion in the calcite lattice, are driven by a combination of thermodynamic precipitation kinetics and cellular physiology (Stoll et al., 2012; Langer et al., 2006; Müller et al., 2011). The physiological response of coccolithophores to changing carbonate chemistry speciation and temperature is relatively well described (Riebesell and Tortell, 2011; Raven and Crawford, 2012); however, associated changes in coccolith elemental partitioning and composition (e.g. Sr/Ca and Mg/Ca) are less well understood (Stoll and Ziveri, 2004; Stoll et al., 2012).

Coccolithophore productivity and growth has been hypothesised to primarily control coccolith Sr/Ca ratios with a secondary influence of temperature and the seawater Sr/Ca ratio (Stoll and Schrag, 2000; Stoll and Ziveri, 2004; Stoll et al., 2007a). Cellular concepts were developed to model and describe the Sr partitioning in coccolithophore calcite (Langer et al., 2006, 2009), based on the assumption that Ca and Sr are transported via the same pathways which is supported by experimental evidence (Müller et al., 2011). The effect of carbonate chemistry speciation on the coccolith Sr/Ca ratio is unknown as well as the associated influence of coccolithophore growth affected by seawater pH and CO₂ availability.

Coccolithophores precipitate low-Mg calcite (< 1 % mol) despite that Mg is the most abundant divalent cation in plant cells and its concentration in the cytosol is about 20 000 times higher than cytosolic-free Ca concentration (Brownlee et al., 1995; Bose et al., 2011). The transport of Mg in coccolithophores presumably differs from Sr and Ca and it is likely associated with specific ion transporters and channels (Mackinder et al., 2010; Holtz et al., 2013) as in bacteria and higher plants (Legong et al., 2001). A relation between the coccolithophore Mg/Ca ratio and temperature is still under

discussion and complicated by the nontrivial removal of organically bound Mg (Blanco-Ameijeiras et al., 2012).

Potential changes in the partitioning of Sr and Mg, and thus in the Sr/Ca and Mg/Ca ratios of calcareous sediments, may alter their biogeochemical cycling over geological timescales because biogenic calcification and the subsequent burial of calcium carbonate represents a major elemental export process from the ocean to the lithosphere. Furthermore, the dissolution dynamics of carbonate-rich sediments are, to a certain degree, controlled by their Mg/Ca ratio (Andersson et al., 2008; Morse et al., 2006).

Here we present the partitioning coefficients for Sr and Mg and the related coccolith Sr/Ca and Mg/Ca ratios from laboratory culture experiments in which temperature and carbonate chemistry of the growth medium were tightly controlled and monitored. Calcite samples were cleaned with a newly developed organic matter removal procedure (Blanco-Ameijeiras et al., 2012). Our findings should be considered when assessing possible changes in the biogeochemical cycling of Ca, Sr and Mg due to ocean warming and acidification and, furthermore, when applying palaeoproxies based on Sr/Ca and Mg/Ca coccolith ratios.

2 Methods

The present study combines results from four independent culture experiments conducted with *Emiliania huxleyi* (Exp. 1 and 2), *Coccolithus braarudii* (Exp. 1 and 3), *Gephyrocapsa oceanica* (Exp. 4) and *Calcidiscus quadriperforatus* (Exp. 1). All experiments were conducted within a temperature range from 10 to 25 °C. The carbonate system was manipulated to either keeping *p*CO₂ constant (Exp. 1) or establishing a *p*CO₂ gradient ranging from ≈ 25 to 3520 µatm (Exp. 2, 3 and 4). A summary of the seawater conditions (temperature and carbonate system) and abiotic parameters (light intensity and day length) are given in the Supplementary material. Additional information on Exp. 3 and 4 is provided in Krug et al. (2011) and Sett et al. (2014), respectively.

2.1 Culture conditions

Coccolithus braarudii RCC-1200, *Calcidiscus quadriperforatus* RCC-1135 and *Gephyrocapsa oceanica* RCC-1303 were obtained from the Roscoff Culture Collection. *Emiliania huxleyi* was isolated in 2005 during the PeECE III mesocosm study in the Raune Fjord (Norway) by M. N. Müller. Cultures were maintained in 0.20 µm filtered North Sea water (NSW) with a salinity of 34 except *G. oceanica* which was cultured in artificial seawater (ASW) with a salinity of 35. Macro- and micronutrients were added according to a modified f/2 media (Guillard, 1975) with final concentrations assuring nutrient replete conditions in all experiments (nitrate ≥ 64 µmol L⁻¹, phosphate ≥ 3.6 µmol L⁻¹, and trace metals and vitamins ≥ f/20 media

concentration). Cultures received a daily illumination between 130 and 180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ under a light : dark cycle of 14:10 h (Exp. 1 and 2) or 16:8 h (Exp. 3 and 4).

2.2 Experimental set-up

Temperature was controlled in growth cabinets (Rumed, series 1000, deviation of $\pm 0.5^\circ\text{C}$) and $p\text{CO}_2$ was adjusted in the NSW experiments (Exp. 1, 2 and 3) by addition of HCl or NaOH, keeping the dissolved inorganic carbon concentration (DIC) constant (Exp. 1: $2273 \pm 42 \mu\text{mol kg}^{-1}$, $n = 6$; Exp. 2: $2157 \pm 18 \mu\text{mol kg}^{-1}$, $n = 10$; Exp. 3: $2105 \pm 20 \mu\text{mol kg}^{-1}$, $n = 15$) while changing the total alkalinity (A_T). The carbonate system of the ASW in Exp. 4 was adjusted by keeping A_T constant ($2311 \pm 56 \mu\text{mol kg}^{-1}$, $n = 24$) and varying DIC (addition of HCl and Na_2CO_3). Precultures were kept in exponential growth and acclimated to the experimental conditions over 7–20 generations. Acclimated experimental cultures were incubated in triplicates (Exp. 1 and 3), duplicates (Exp. 2) or without replicates (Exp. 4) in acid cleaned (10% HCl) and autoclaved polycarbonate bottles at target conditions. In all experiments, cultures were inoculated to low cell densities and allowed to grow exponentially for 7–10 generations, corresponding to a maximum DIC consumption of 12% (Exp. 1 and 3) or 7% (Exp. 2 and 4). Growth media (adjusted to the target condition) was sampled for DIC, A_T and seawater elemental composition. At the termination of the experiment (2–3 h after the onset of light), samples were taken for DIC, A_T , seawater ion composition, cell number, total particulate carbon and nitrogen (TPC and TPN), particulate organic carbon (POC), chlorophyll *a* (Chl *a*) and coccolith elemental composition. Sample pellets for coccolith calcite of *G. oceanica* (Exp. 4) were sampled 2–4 days after samples for physiological parameters were taken. Thus, the coccolith geochemistry data of *G. oceanica* are not directly related to the measured seawater chemistry and physiological parameters. This issue will be considered and discussed later on (Sect. 4.1).

2.3 Carbonate chemistry

The carbonate system was monitored by DIC and A_T measurements at the start and the end of the experiments. DIC was analysed after Stoll et al. (2001), using an automated segmented-flow analyser (Quattro) equipped with an auto-sampler ($10 \mu\text{mol kg}^{-1}$ accuracy and $5 \mu\text{mol kg}^{-1}$ precision). Total alkalinity was measured by the potentiometric titration method after Dickson et al. (2003) with an accuracy and precision of 24 and $3.5 \mu\text{mol kg}^{-1}$, respectively. DIC and A_T were calibrated and measured against certified reference material supplied by A. Dickson (Scripps, La Jolla, USA). Carbonate system parameters were calculated from temperature, salinity, DIC and A_T (mean values from the start and end of experiments) using CO2SYS (version 1.05 by E. Lewis and

D. W. R. Wallace), with the stoichiometric equilibrium constants for carbonic acid given in Roy et al. (1993).

2.4 Physiological parameters

2.4.1 Cellular growth rates

Cell densities were determined with a Coulter Counter (Z Series). Samples were measured three times and the mean was used to calculate the growth rate μ (d^{-1}) as

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

where c_0 and c_1 denote the number of cells at the start (t_0) and end (t_1) of the incubation period (expressed in days).

2.4.2 Production rates of particulate organic and inorganic carbon, total nitrogen and chlorophyll *a*

Three sub-samples were taken for TPC/TPN, POC and Chl *a* from each treatment bottle (Chl *a* was not sampled in Exp. 3), filtrated onto precombusted GF/F (glass fibre) filters (500°C for 6 h) and stored frozen at -20°C . For POC analysis, filters were fumed over 37% HCl for 2 h to remove all inorganic carbon and dried overnight at 60°C . Filters for TPC/TPN and POC were measured on an Euro EA Elemental Analyser (Sharp, 1974). Particulate inorganic carbon was calculated from the difference of TPC and POC. Until analysis, Chl *a* filters were kept in the dark to avoid photo-oxidation and measurements were performed using a fluorimetric method after Welschmeyer (1994). Production rates of particulate material (PM) were calculated as

$$\text{PM}_{\text{prod}} = \text{cell quota (pg cell}^{-1}) \times \mu (\text{d}^{-1}), \quad (2)$$

where PM can be POC, PIC, TPN or Chl *a*.

2.5 Coccolith geochemistry

After samples for the physiological parameters were taken (Sect. 2.2), the remaining culture medium (replicates of each experiment were pooled; Exp. 1–3) was centrifuged at 14 000 rpm for 10 min. The supernatant was discarded and the sample pellet was dried at 60°C for 48 h and stored at room temperature. Dried pellets from Exp. 1–3 were cleaned with NaClO after Müller et al. (2011) and additionally oxidised with H_2O_2 as outlined in Blanco-Ameijeiras et al. (2012). This method removes organic material with an efficiency of above 99% and minimises the contamination of coccolith Mg/Ca analyses by organic Mg. Pellets of Exp. 4 received a reduction step with hydroxylamine-hydrochloride prior to oxidising with H_2O_2 (Blanco-Ameijeiras et al., 2012).

2.5.1 Elemental analyses

Mg/Ca and Sr/Ca ratios of the coccoliths and of the ASW (Exp. 4) were analysed using matrix-matched standards on a simultaneous dual ICP-AES (inductively coupled plasma - atomic emission spectrometry) (Thermo ICAP DUO 6300 at the University of Oviedo). Standards were prepared from single element ICP standards (CPI Corporation) diluted in the same acid matrix as the samples. The calibration standards covered the Mg/Ca and Sr/Ca range in the calcite samples. Elemental ratios are reported from measurements made in radial detection mode for Sr at 421 nm, Mg at 279.5 nm, Fe at 259 nm, Ca at 315 nm and P at 177 nm. Calibration was conducted offline using the intensity ratio method described by de Villiers et al. (2002). In this case, three standards were prepared with constant Ca concentrations and variable Sr/Ca and Mg/Ca ratios of 0.75–4 mmol mol⁻¹. Aliquots from this set of standards were uniformly diluted to provide curves for various Ca concentrations to match sample concentrations. Sr/Ca internal reproducibility was better than 0.02 mmol mol⁻¹, based on replicate analyses of the same sample dilutions. Mg/Ca internal reproducibility was better than 0.8 % of the measured ratio.

Coccolith P/Ca and Fe/Ca ratios are presented along with the Mg/Ca ratios as an indicator for Mg cleaning efficiency. Outlier detection, using the interquartile range (IQR), was performed on P/Ca and Fe/Ca ratios to determine non-sufficient Mg removal and the corresponding Mg/Ca ratios were removed from the data set. Sr/Ca ratio measurements were not excluded from the data set because Sr contamination from seawater and organic matter represents a minor issue for coccolith Sr/Ca ratio measurements (Blanco-Ameijeiras et al., 2012). Average values for P/Ca and Fe/Ca ratios (excluding outliers) were 1.36 ± 0.73 and 0.82 ± 1.19 mmol mol⁻¹ (± 1 SD; standard deviation), respectively.

Elemental composition of the NSW (Exp. 1–3) was determined via Jobin Yvon JY 170 Ultratrace series ICP-OES (optical emission spectroscopy) at GEOMAR. The internal error for each analysis is typically $< 1.2\%$ (± 2 RSD; relative SD) and the external reproducibility determined by repeat analysis of IAPSO (International Association for the Physical Sciences of Oceans) seawater standard is $\pm 1.5\%$, $\pm 0.7\%$ and $\pm 0.3\%$ (± 2 RSD, $n = 3$) for Ca, Mg and Sr, respectively. The partition coefficient of the trace metal (Sr and Mg), D_{Tr} , between calcite (c) and seawater (s) was calculated as

$$D_{Tr} = \frac{(Tr/Ca)_c}{(Tr/Ca)_s} \quad (3)$$

3 Results

At ambient pCO_2 levels, elevated temperature enhanced growth rates and PIC_{prod} in all tested coccolithophore species. The POC_{prod} was positively affected by temperature

except for *E. huxleyi*, decreasing from 10 to 20 °C in Exp. 1. Cellular ratios of PIC/POC increased or did not change with temperature.

Under changing carbonate chemistry speciation, *C. braarudii*, *G. oceanica* and *E. huxleyi* were negatively affected in growth rate, PIC_{prod} and the PIC/POC ratio when pCO_2 was increased from ambient to high levels with species-specific sensitivities depending on temperature. More detailed information regarding the physiological response of *C. braarudii* and *G. oceanica* can be found in Krug et al. (2011) and Sett et al. (2014), respectively. The POC_{prod}, however, followed an optimum-curve behaviour in response to pCO_2 peaking between 400 and 1150 μ atm. The C/N ratio in all tested coccolithophore species was insensitive to changes in pCO_2 . In general, Chl a_{prod} followed similar trends as described for POC_{prod} and the lowest Chl a_{prod} rates were observed in *E. huxleyi* (Exp. 2) at 10 °C.

Seawater carbonate chemistry speciation and physiological parameters of all experiments are listed in the Supplement Tables 1 and 2, respectively.

The coccolith Sr/Ca ratio and the strontium partition coefficient (D_{Sr}) ranged from 2.40 to 4.38 mmol mol⁻¹ and from 0.28 to 0.45, respectively. The highest values were found in calcite produced at 25 °C by *E. huxleyi* and *G. oceanica* (Fig. 1, Table 1). Elevated seawater temperature at constant pCO_2 conditions (370 ± 70 μ atm) resulted in a positive relationship between temperature and the coccolith strontium partition coefficient (when combining all species, Fig. 1a). Species-specific D_{Sr} was positively correlated to pCO_2 with the steepest slopes detected for *E. huxleyi* (Fig. 1b, data points of *G. oceanica* at 25 °C were influenced by nutrient limitation which is discussed in Sect. 4.1):

$$10^\circ\text{C} (E. huxleyi) : D_{Sr} = 6.19 \times 10^{-5} pCO_2 + 0.299 \quad (4)$$

$$(r^2 = 0.853, p = 0.025, n = 6),$$

$$17^\circ\text{C} (C. braarudii) : D_{Sr} = 1.16 \times 10^{-5} pCO_2 + 0.386 \quad (5)$$

$$(r^2 = 0.792, p = 0.043, n = 5),$$

$$20^\circ\text{C} (E. huxleyi) : D_{Sr} = 7.88 \times 10^{-5} pCO_2 + 0.355 \quad (6)$$

$$(r^2 = 0.758, p = 0.027, n = 5),$$

$$20^\circ\text{C} (G. oceanica) : D_{Sr} = 3.89 \times 10^{-5} pCO_2 + 0.347 \quad (7)$$

$$(r^2 = 0.885, p < 0.001, n = 11),$$

$$25^\circ\text{C} (G. oceanica) : D_{Sr} = -8.30 \times 10^{-7} pCO_2 + 0.437 \quad (8)$$

$$(r^2 = 0.083, p > 0.05, n = 11).$$

The Mg/Ca ratio and magnesium partition coefficient (D_{Mg}) ranged from 0.066 to 98.6 mmol mol⁻¹ and from 0.01×10^{-3} to 18.7×10^{-3} , respectively, depending on treatment and coccolithophore species (Fig. 2, Table 1). The highest D_{Mg} was measured in *E. huxleyi* (Exp. 2) at 10 °C and the lowest in *G. oceanica* (Exp. 4). No consistent correlation with temperature could be detected within the tested range

Table 1. Coccolith calcite chemistry of the four individual experiments with corresponding experimental conditions. P/Ca and Fe/Ca ratios (averages of 1.36 ± 0.73 and 0.82 ± 1.19 , respectively) were used as an indicator for Mg cleaning efficiency. Detected outliers indicated non-sufficient Mg removal (indicated with *) and corresponding Mg/Ca ratios were removed from the data set.

Species	T (°C)	pCO ₂ (µatm)	Sr/Ca (mmol mol ⁻¹)	D _{Sr}	Mg/Ca (mmol mol ⁻¹)	D _{Mg} × 10 ⁻³	P/Ca (mmol mol ⁻¹)	Fe/Ca (mmol mol ⁻¹)
Experiment 1: North Sea water, salinity = 34, light = 180 µmol photons m ⁻² s ⁻¹ , light : dark = 14 : 10 h, replicates = 3. Seawater Sr/Ca = 8.42 ± 0.30 mmol mol ⁻¹ , Mg/Ca = 5.29 ± 0.15 mol mol ⁻¹ .								
<i>C. braarudii</i>	10	417 ± 15	2.40	0.28	1.44	0.27	1.65	1.92
<i>C. braarudii</i>	15	320 ± 17	3.00	0.36	0.74	0.14	0.32	0.17
<i>C. braarudii</i>	18	501 ± 30	2.86	0.34	1.04	0.20	0.46	0.93
<i>C. quadriperforatus</i>	15	308 ± 2	2.67	0.32	1.54	0.29	2.48	2.33
<i>C. quadriperforatus</i>	18	451 ± 45	2.79	0.33	1.70	0.32	0.39	0.54
<i>C. quadriperforatus</i>	20	316 ± 46	3.10	0.37	0.62	0.12	3.41	0.22
<i>E. huxleyi</i>	10	276 ± 6	2.64	0.31	–	–	1.37	5.68*
<i>E. huxleyi</i>	15	306 ± 5	2.93	0.35	1.61	0.30	1.32	0.93
<i>E. huxleyi</i>	20	400 ± 41	3.21	0.38	1.97	0.37	1.27	0.79
<i>E. huxleyi</i>	25	427 ± 30	3.34	0.40	4.59	0.87	1.03	3.52
Experiment 2: North Sea water, salinity = 34, light = 130 µmol photons m ⁻² s ⁻¹ , light : dark = 14 : 10 h, replicates = 2. Seawater Sr/Ca = 8.42 ± 0.30 mmol mol ⁻¹ , Mg/Ca = 5.29 ± 0.15 mol mol ⁻¹ .								
<i>E. huxleyi</i>	10	193 ± 3	2.60	0.31	–	–	2.04	22.3*
<i>E. huxleyi</i>	10	467 ± 9	2.82	0.33	12.3	2.33	0.91	0.87
<i>E. huxleyi</i>	10	699 ± 5	2.75	0.33	13.1	2.50	1.90	2.61
<i>E. huxleyi</i>	10	898 ± 24	3.13	0.37	98.6	18.7	2.12	2.76
<i>E. huxleyi</i>	10	1238 ± 23	3.12	0.37	–	–	4.55*	14.8*
<i>E. huxleyi</i>	20	175 ± 1	–	–	–	–	–	–
<i>E. huxleyi</i>	20	365 ± 4	3.35	0.40	–	–	–	–
<i>E. huxleyi</i>	20	690 ± 12	3.26	0.39	–	–	–	–
<i>E. huxleyi</i>	20	812 ± 20	3.53	0.42	–	–	–	–
<i>E. huxleyi</i>	20	1106 ± 12	3.80	0.45	–	–	–	–
Experiment 3: North Sea water, salinity = 34, light = 130 µmol photons m ⁻² s ⁻¹ , light : dark = 16 : 08 h, replicates = 3. Seawater Sr/Ca = 8.42 ± 0.30 mmol mol ⁻¹ , Mg/Ca = 5.29 ± 0.15 mol mol ⁻¹ .								
<i>C. braarudii</i>	17	524 ± 27	3.26	0.39	8.49	1.61	0.43	4.66
<i>C. braarudii</i>	17	1151 ± 130	3.42	0.41	7.02	1.33	1.26	3.60
<i>C. braarudii</i>	17	1799 ± 257	3.40	0.40	15.6	2.96	0.11	0.53
<i>C. braarudii</i>	17	2311 ± 122	3.47	0.41	–	–	–	–
<i>C. braarudii</i>	17	3508 ± 172	3.63	0.43	–	–	9.71*	127*
Experiment 4: Artificial seawater, salinity = 35, light = 150 µmol photons m ⁻² s ⁻¹ , light : dark = 16 : 08 h, replicates = 1. Seawater Sr/Ca = 9.66 ± 0.22 mmol mol ⁻¹ , Mg/Ca = 5.43 ± 0.14 mol mol ⁻¹ .								
<i>G. oceanica</i>	20	25	–	–	–	–	–	–
<i>G. oceanica</i>	20	59	3.28	0.34	0.086	0.016	0.50	0.87
<i>G. oceanica</i>	20	108	3.38	0.35	0.066	0.012	0.73	0.01
<i>G. oceanica</i>	20	280	3.47	0.36	0.079	0.015	0.66	0.05
<i>G. oceanica</i>	20	413	3.51	0.36	0.080	0.015	1.01	0
<i>G. oceanica</i>	20	574	3.56	0.37	0.087	0.016	0.87	0
<i>G. oceanica</i>	20	764	3.64	0.38	0.091	0.017	1.22	0
<i>G. oceanica</i>	20	940	3.65	0.38	0.099	0.018	1.12	0
<i>G. oceanica</i>	20	1216	3.93	0.41	0.121	0.022	1.36	0
<i>G. oceanica</i>	20	1476	3.91	0.40	0.116	0.021	1.46	0
<i>G. oceanica</i>	20	1781	4.14	0.43	0.124	0.023	1.60	0
<i>G. oceanica</i>	20	2104	3.97	0.41	0.130	0.024	1.04	0
<i>G. oceanica</i>	25	26	–	–	–	–	–	–
<i>G. oceanica</i>	25	58	4.08	0.42	0.279	0.051	1.70	0.30
<i>G. oceanica</i>	25	288	4.12	0.43	0.172	0.032	1.57	0.08
<i>G. oceanica</i>	25	318	4.23	0.44	0.211	0.039	1.60	0.07
<i>G. oceanica</i>	25	447	4.27	0.44	0.330	0.061	1.95	0.08
<i>G. oceanica</i>	25	612	4.33	0.45	0.276	0.051	2.27	0.76
<i>G. oceanica</i>	25	771	4.34	0.45	0.341	0.063	2.62	0.21
<i>G. oceanica</i>	25	980	4.16	0.43	–	–	4.62*	0.29
<i>G. oceanica</i>	25	1284	4.27	0.44	–	–	6.69*	0.40
<i>G. oceanica</i>	25	1731	4.18	0.43	–	–	7.47*	0.35
<i>G. oceanica</i>	25	2067	4.28	0.44	0.946	0.174	2.01	0.15
<i>G. oceanica</i>	25	3517	4.12	0.43	–	–	12.43*	0.46

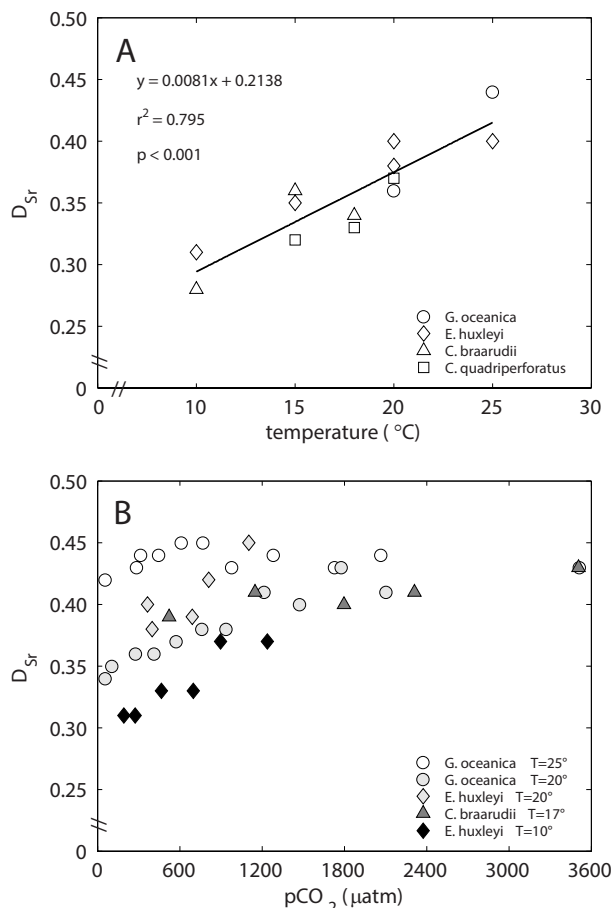


Fig. 1. Strontium partition coefficient (D_{Sr}) as a function of (A) temperature ($pCO_2 = 370 \pm 70 \mu atm$) and (B) pCO_2 (temperature as indicated). Black line in (A) represents a linear regression through all data points. Please note that *G. oceanica* experienced nutrient limitation at 25 $^{\circ}C$ (see text for details).

(when combining all species, Fig. 2a). Within the applied pCO_2 range, an increasing trend was apparent in D_{Mg} of *G. oceanica*, *C. braarudii* and *E. huxleyi* (Fig. 2b), with a significant correlation present in *G. oceanica*:

$$10^{\circ}C \text{ (} E. huxleyi \text{)} : D_{Mg} = 0.037 pCO_2 - 17.585 \quad (9)$$

$$(r^2 = 0.719, p = 0.356, n = 3),$$

$$17^{\circ}C \text{ (} C. braarudii \text{)} : D_{Mg} = 1.07 \times 10^{-3} pCO_2 + 0.731 \quad (10)$$

$$(r^2 = 0.609, p = 0.43, n = 3),$$

$$20^{\circ}C \text{ (} G. oceanica \text{)} : D_{Mg} = 5.58 \times 10^{-6} pCO_2 + 0.013 \quad (11)$$

$$(r^2 = 0.898, p < 0.001, n = 11),$$

$$25^{\circ}C \text{ (} G. oceanica \text{)} : D_{Mg} = 5.49 \times 10^{-5} pCO_2 + 0.028 \quad (12)$$

$$(r^2 = 0.913, p \leq 0.001, n = 7).$$

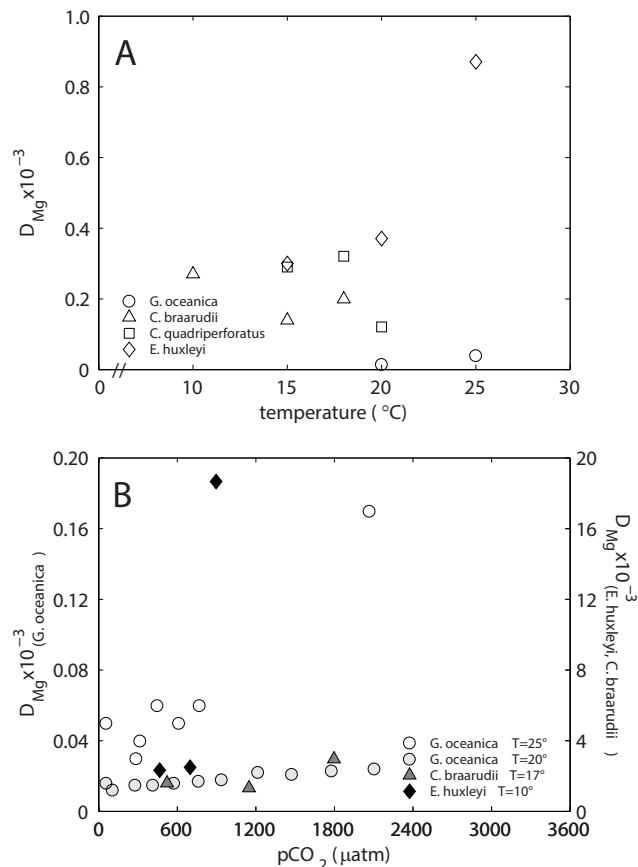


Fig. 2. Magnesium partition coefficient (D_{Mg}) as a function of (A) temperature ($pCO_2 = 370 \pm 70 \mu atm$) and (B) pCO_2 (temperature as indicated). In (B) markers of *E. huxleyi* and *C. braarudii* are related to the y axis on the right. Please note that *G. oceanica* experienced nutrient limitation at 25 $^{\circ}C$ (see text for details).

4 Discussion

4.1 Experimental approaches and procedures

The pCO_2 conditions in Exp. 1 varied from ≈ 280 to 500 μatm (average of $370 \pm 70 \mu atm$). Within this pCO_2 range, however, expected changes in coccolithophore physiology are below the detection limit of the applied measurements (Krug et al., 2011; Bach et al., 2011). Therefore, all changes in physiological rates of Exp. 1 can be attributed to variations in seawater temperature because all other experimental parameters were constant.

In Exp. 2, 3 and 4 the seawater carbonate system was manipulated by applying two different methods. First, seawater A_T was changed while keeping DIC concentration constant by the addition of HCl or NaOH (Exp. 2 and 3) and, second, adjusting DIC and A_T by the addition of Na_2CO_3 and HCl to artificial seawater (Exp. 4). The advantages and disadvantages of these two methods have been presented and discussed in Riebesell et al. (2010) and Schulz et al. (2009).

The second method correctly mimics ocean acidification but both methods are comparable when applying $p\text{CO}_2$ levels within a range from ≈ 180 to $1100 \mu\text{atm}$ (Schulz et al., 2009). Therefore, physiological and geochemical measurements derived from $p\text{CO}_2$ conditions above $1100 \mu\text{atm}$ (Exp. 2 and 3) or below $180 \mu\text{atm}$ (Exp. 4) should be considered with caution when relating to the process of ocean acidification. From a cellular perspective, however, the changes in the individual carbonate species (CO_2 , HCO_3^- and CO_3^{2-}) and H^+ concentrations, rather than DIC and A_T , are driving changes in physiology and biomineralisation. Consequently, both methods are comparable and, indeed, result in similar physiological responses (Hoppe et al., 2011).

Calcite pellets of *G. oceanica* were obtained 2–4 days after samples were taken for physiological determinations and seawater carbonate chemistry. This approach was preferred to ensure sufficient material for elemental Mg analyses. However, the exponential growth of *G. oceanica* and increasing cell density induced a fast consumption of available nutrients (including DIC). Depending on growth rate and time of calcite sampling, this might have induced nutrient limitation, major changes in the seawater carbonate chemistry speciation and thus in cell physiology. In this regard, the theoretical total drawdown of DIC and dissolved inorganic nitrogen was calculated from growth rate, carbon and nitrogen cell quotas, and the time of calcite pellet sampling. For the experiment with *G. oceanica* conducted at 20°C , this resulted in an average drawdown of DIC and nitrogen of 18 and 33 %, respectively, indicating no nutrient limitation at the time of calcite pellet sampling. At 25°C , however, calcite pellets were sampled for when cells had already consumed all available nitrogen, probably being in limitation for more than 2 days. Nitrogen and phosphorus were initially added to the growth media in a Redfield ratio of 16/1 and thereby a combined limitation of nitrogen and phosphorus is plausible for *G. oceanica* at 25°C . Consequently, the calcite geochemistry data of *G. oceanica* at 25°C cannot be related to physiological parameters or the seawater carbonate chemistry speciation but rather indicates a response to nutrient limitation.

4.2 Calcite geochemistry and physiological influence

The partition coefficient of strontium (D_{Sr}) for each tested coccolithophore species increased with seawater temperature (Fig. 1a) and was well within the range of reported values from other culture experiments (Rickaby et al., 2002; Stoll et al., 2002; Langer et al., 2006). Species-specific D_{Sr} was significantly correlated to $p\text{CO}_2$ at 10, 17 and 20°C (Fig. 1b) but a correlation was not present at 25°C . The correlation between D_{Sr} and $p\text{CO}_2$ in *G. oceanica* at 25°C is presumably biased by the effect of nutrient limitation (discussed above) and therefore not comparable to the regressions found at 10, 17 and 20°C .

No general relation was found between D_{Sr} and a specific physiological parameter (growth, POC_{prod} , PIC_{prod} , TPN_{prod}

and $\text{Chl } a_{\text{prod}}$) when using combined data from temperature and carbonate chemistry manipulations. A significant correlation for all tested coccolithophore species was found between D_{Sr} and temperature induced variations in growth rate at ambient $p\text{CO}_2$ ($370 \pm 70 \mu\text{atm}$) levels (Fig. 3a). This correlation is likely induced by physiological control rather than temperature itself because Sr incorporation during inorganic calcite precipitation is reported to be negatively correlated with temperature (Tang et al., 2008). It seems that coccolith D_{Sr} is an appropriate indicator for coccolithophore productivity (in terms of growth rate) and a similar relation between D_{Sr} and growth rate would be expected when changes in growth rate are induced by $p\text{CO}_2$ (or carbonate chemistry speciation). Whereas $p\text{CO}_2$ was positively correlated with D_{Sr} in all tested coccolithophore species (see Fig. 1b and equations in Sect. 4), no species-specific correlation was found between $p\text{CO}_2$ induced variations in growth rate and D_{Sr} (Fig. 3b). Coccolithophore growth rate and POC_{prod} follow a species-specific optimum-curve behaviour peaking at $p\text{CO}_2$ values between ≈ 400 and $1100 \mu\text{atm}$ (Krug et al., 2011; Sett et al., 2014; Bach et al., 2011). Here, we demonstrate that over an extended range of $p\text{CO}_2$ from 25 to $3500 \mu\text{atm}$ the D_{Sr} follows a linear regression depending on temperature and species (Fig. 1b). This suggests that seawater carbonate chemistry speciation has a significant influence on coccolithophore D_{Sr} independent of cellular productivity (growth and POC_{prod}). The Palaeocene–Eocene thermal maximum (PETM), for example, was marked by a massive input of carbon which resulted in elevated oceanic and atmospheric CO_2 concentrations (Zachos et al., 2005). A theoretical increase in $p\text{CO}_2$ from 1000 to $1700 \mu\text{atm}$ during the PETM (Zeebe et al., 2009) would translate to an increase of 0.27 in the coccolith Sr/Ca ratio of *G. oceanica* at 20°C . This CO_2 induced rise in coccolith Sr/Ca can partly explain the observed increase in fossil coccolith Sr/Ca ratios related to the PETM (Bolton et al., 2012; Stoll et al., 2007b). Therefore, fossil coccolith Sr/Ca ratios from times that experienced changing carbonate chemistry speciation have to receive corrections based on an independent $p\text{CO}_2$ proxy, similar to changes in temperature and sea level (Stoll and Schrag, 2001; Bolton et al., 2012). It remains open to debate which carbonate chemistry parameter is responsible for the observed change in coccolith D_{Sr} , and thus the Sr/Ca ratio. This study was not set up to disentangle the effects of single carbonate parameters on coccolith elemental ratios but future laboratory experiments can accommodate this missing gap (see Bach et al., 2013). Besides temperature and $p\text{CO}_2$, nutrient limitation has a considerable influence on coccolith D_{Sr} and Sr/Ca ratios. The coccolith Sr/Ca ratio correlates well with nitrogen limited growth of coccolithophores in laboratory experiments (Rickaby et al., 2002; Stoll et al., 2007a), and therefore the application as a productivity proxy seems feasible. However, all studies to date focus solely on the effect of nitrogen as limiting factor, even though the diverging effects on coccolithophore growth and

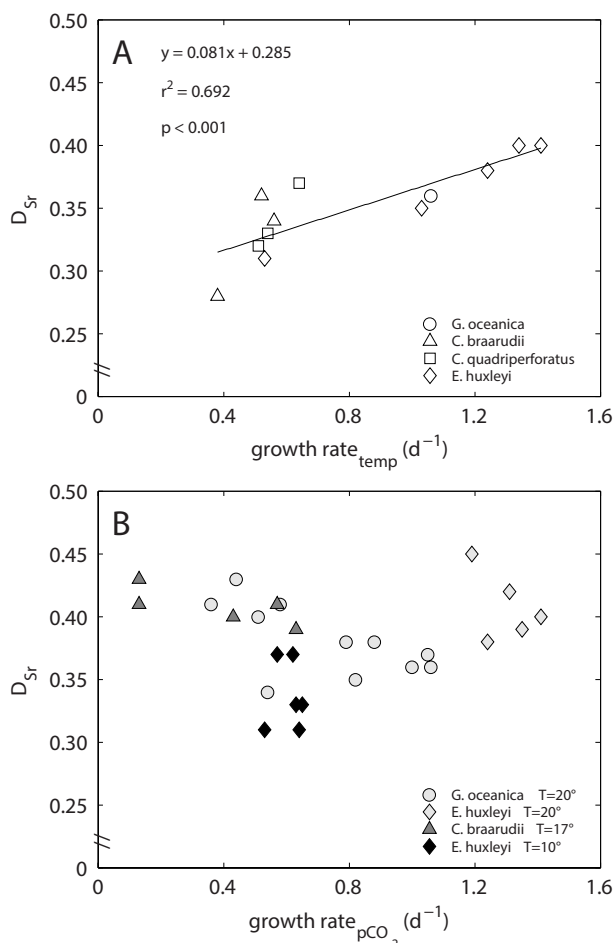


Fig. 3. Strontium partition coefficient (D_{Sr}) as a function of growth rate (excluding the data from *G. oceanica* at 25°C). **(A)** Growth rate driven by changes in temperature ranging from 10 to 25°C ($p\text{CO}_2 = 370 \pm 70 \mu\text{atm}$); black line indicates linear regression through all points. **(B)** Growth rate driven by $p\text{CO}_2$ ranging from ≈ 25 to $3520 \mu\text{atm}$ (temperatures as indicated).

calcification of nitrogen, phosphorus and micronutrient limitation are known (Müller et al., 2008; Schulz et al., 2004). Controlled chemostat experiments, where it is possible to vary the source of limitation while keeping the carbonate system constant (Müller et al., 2012), provide a suitable but labour intensive tool to investigate coccolith elemental partitioning in regard to nutrient limitations (e.g. C, P, Fe and Zn).

The Mg/Ca ratio of biogenic carbonates of foraminifera and corals has been positively correlated with sea surface temperature (Mitsuguchi et al., 1996; Marr et al., 2011). This relationship, however, was not found in calcite samples in this study (Fig. 2a) and neither in the majority of coccolithophore field samples (Stoll et al., 2007a). Values for D_{Mg} of *G. oceanica* were about 1–2 orders of magnitude lower than values measured of *C. braarudii*, *C. quadripforatus* and *E. huxleyi* (Table 1). An offset due to the additional re-

ductive cleaning step, used for calcite pellets of *G. oceanica* (see Sect. 3.5), can be ruled out because no similar offset was detected in the P/Ca and Fe/Ca ratios (used as indicators for the cleaning efficiency, Table 1). *Gephyrocapsa oceanica* was cultured in artificial seawater media whereas all other species were grown in natural seawater. An effect of the different culture media is unlikely as all other physiological and coccolith chemistry parameters are comparable (Table 1 and Supplement Table 2). Therefore, we assume the observed offset in D_{Mg} of *G. oceanica* is species-specific. Interspecies differences in the coccolith Mg/Ca signature might complicate the analysis of bulk fossil coccolith material due to an inherited species composition bias. The time intensive procedure to clean coccolith material from organic Mg aggravates a practical application of Mg/Ca ratios as palaeoproxy. A new and time saving method, based on the removal of labile organic bound Mg by active heavy metals, shows promising results and the potential to simplify this cleaning process (manuscript in preparation).

A consistent increase in D_{Mg} with elevated $p\text{CO}_2$ was detected in *C. braarudii*, *G. oceanica* and *E. huxleyi*. This rising trend was statistically significant for *G. oceanica* at 20°C but the small sample size for *C. braarudii* and *E. huxleyi* precluded a statistical interpretation for the latter two species. At 25°C , values for D_{Mg} of *G. oceanica* were up to 7 times higher and displayed a greater variability than at 20°C which was presumably triggered by the experienced nutrient limitation before the calcite pellets were sampled. Nutrient limitation is commonly experienced by coccolithophores in the open ocean and during bloom events (Van Oostende et al., 2012) and our data indicate that this effect has the potential to greatly influence the Mg/Ca signature of fossil coccoliths.

The relatively high intraspecific variability in D_{Mg} values from the presented culture experiments and from field samples (Stoll et al., 2007a) compared to D_{Sr} lets us suggest that the control mechanisms and pathways for Mg and Sr partitioning in coccoliths are likely to be different. Magnesium plays a crucial role in all energy demanding processes by activating ATP in a number of enzymatic reactions as well as in DNA and RNA synthesis. In plants, Mg is furthermore essential for photosynthesis and the production of chlorophyll. The influx of Mg into the cell is presumably managed through unique membrane channels (Legong et al., 2001). Regarding the numerous processes involving Mg, the distribution of free Mg in the cytosol is likely to be under tight physiological control involving Mg-binding proteins. A disturbance or change in the physiological control/utilisation of the cytosolic-free Mg might have a secondary effect on the calcite magnesium partitioning. Indeed, a relationship between coccolith Mg chemistry and chlorophyll *a* was suggested earlier (Müller et al., 2011; Ra et al., 2010). This is supported by plotting the D_{Mg} as a function of Chl a_{prod} rate (Fig. 4a), resulting in a significant negative correlation for *G. oceanica*. The values for Chl a_{prod} and D_{Mg} of *E. huxleyi*, *C. quadripforatus* and *C. braarudii* displayed a wide

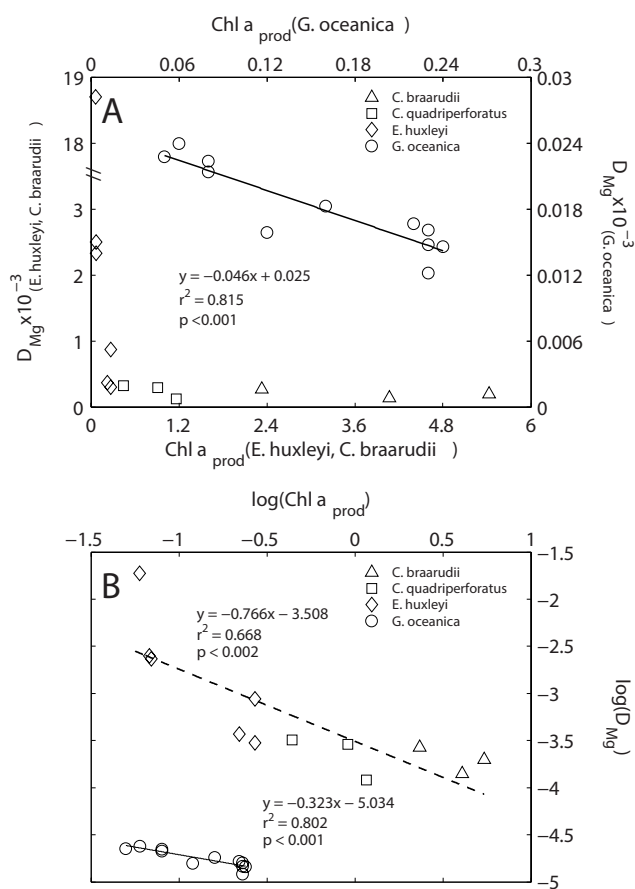


Fig. 4. Magnesium partition coefficient (D_{Mg}) as a function of chlorophyll *a* production rates (A), black line indicates linear regression through data points of *G. oceanica* at 20 °C. (B) The log-transformed data of (A) to illustrate the linear correlation in the combined data of *E. huxleyi*, *C. braarudii* and *C. quadriperforatus* (dashed line). The solid line in (B) indicates the linear correlation through data points of *G. oceanica* at 20 °C.

range from 0.06 to 5.43 pg Chl *a* cell⁻¹ day⁻¹ (Supplement Table 2) and from 0.12 to 18.7×10^{-3} (Table 1), respectively. A correlation, combining the latter three species, became visible when analysing log-transformed data whereby the correlation for *G. oceanica* remained significant (Fig. 4b).

The apparent relation between Chl *a*_{prod} and the coccolith D_{Mg} under both temperature and pCO_2 variations suggests a physiological control over the cytosolic-free Mg available for incorporation into the calcite lattice. We suggest that Mg bound to the calcite lattice originates from the same pool as organically associated Mg, resulting in the observed correlation of coccolith D_{Mg} and Chl *a*_{prod}. As Ca antagonist, Mg can alter the morphological structure and dissolution kinetics of calcium carbonate precipitates (Loste et al., 2003) and, indeed, laboratory studies indicate drastic malformation of coccolith at high Mg concentration ($> 80\text{ mmol L}^{-1}$) in the external growth media (Herfort et al., 2004). The pathway of Mg to the site of calcification (coccolith vesicle) is

unknown for coccolithophores but previous results (Langer et al., 2006; Müller et al., 2011) and the current study indicate that Mg is transported into the cell and to the site of calcification on a distinctly different pathway than Ca and Sr. Therefore, the Mg/Ca ratio of extant and fossil coccoliths should be influenced by the individual concentrations of Mg and Ca in seawater rather than the seawater Mg/Ca ratio. The Mg signature of foraminifera tests, however, can reflect changes in seawater Mg/Ca ratios (Segev and Erez, 20013). This obvious difference between foraminifera and coccolithophores is explained by their diverging calcification mechanisms. Coccolithophores presumably transport Mg and Ca over the cellular transmembrane system to the site of calcification whereas in foraminifera an additional source of Mg and Ca is derived from a passive transport, such as seawater vacuolisation or leaking gaps in the pseudopodial network (Nehrke et al., 2013). Nehrke et al. (2013) introduced a conceptual model to explain the trace-elemental composition of foraminifera tests, and our study on coccolithophore calcite gives supportive information to further develop this model.

5 Conclusions

The D_{Sr} and Sr/Ca ratio of coccolithophore calcite has been subject to numerous palaeoceanographic studies and the application as proxy for coccolithophore productivity (in terms of growth rate) is widely applied. Our laboratory results indicate that this relationship is not valid when coccolithophore growth is influenced by variations in the seawater carbonate chemistry speciation as opposed to temperature. Furthermore, we found that carbonate chemistry speciation has a distinct effect on the D_{Sr} independent of coccolithophore physiology. Strontium and calcium share similar transport pathways from seawater to the site of calcification but magnesium seems to follow distinctly different cellular control mechanisms, indicating that coccolith Mg partitioning is independent from the seawater Mg/Ca ratio. Whereas no overall correlation was present between D_{Sr} and one of the physiological parameters measured, coccolith D_{Mg} was correlated to chlorophyll *a* production (both under temperature and carbonate chemistry variations), suggesting a tight link between coccolith Mg partitioning and Mg-associated physiological processes. These findings provide a useful contribution for the calibration of palaeoproxies based on coccolith Sr/Ca and Mg/Ca ratios, and give further insights into the underlying cellular mechanisms that lead to coccolith Sr and Mg partitioning.

Supplementary material related to this article is available online at <http://www.biogeosciences.net/11/1065/2014/bg-11-1065-2014-supplement.pdf>.

Acknowledgements. We thank P. Wiebe and S. Krug for providing calcite sample pellets and conducting experiment 2 and 3, respectively. We thank M. Meyerhöfer for DIC analysis, Ian Probert for providing us *Coccolithus braarudii*, *Calcidiscus quadriperforatus* and *Gephyrocapsa oceanica*. This work was funded by the “Deutsche Forschungsgemeinschaft” as part of the ESF project (DFG Ei272/24-1) CASIOPEIA, by the BMBF project BIOACID (BMBF, FKZ 03F0608A), by “Fundo Regional da Ciência dos Açores” and by the Australian Research Council (ARC, DP 1093801). This work was also funded by the European Project on Ocean Acidification (EPOCA) (which received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 211384), the Abbey–Santander Internationalisation Fund and the European Research Council (ERC-STG-240222PACE).

Edited by: D. Gillikin

The service charges for this open access publication have been covered by a Research Centre of the Helmholtz Association.

References

- Andersson, A. J., Mackenzie, F. T., and Bates, N. R.: Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers, *Mar. Ecol.-Prog. Ser.*, 373, 265–273, 2008.
- Bach, L. T., Schulz, K. G., and Riebesell, U.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, *Limnol. Oceanogr.*, 55, 2040–2050, 2011.
- Bach, L. T., Mackinder, L. C. M., Schulz, K. G., Wheeler, G., Schroeder, D. C., Brownlee, C., and Riebesell, U.: Dissecting the impact of CO₂ and pH on the mechanisms of photosynthesis and calcification in the coccolithophore *Emiliania huxleyi*, *New Phytol.*, 199, 121–134, 2013.
- Blanco-Ameijeiras, S., Lebrato, M., Stoll, H. M., Iglesias-Rodriguez, M. D., Mendez-Vicente, A., Sett, S., Müller, M. N., Oschlies, A., and Schulz, K. G.: Removal of organic magnesium in coccolithophore carbonates, *Geochim. Cosmochim. Ac.*, 89, 226–239, 2012.
- Bolton, C. T., Stoll, H. M., and Mendez-Vicente, A.: Vital effects in coccolith calcite: Cenozoic climate pCO₂ drove the diversity of carbon acquisition strategies in coccolithophores?, *Paleoceanography*, 27, PA4204, doi:10.1029/2012PA002339, 2012.
- Bose, J., Babourina, O., and Rengel, Z.: Role of magnesium in alleviation of aluminium toxicity in plants, *J. Exp. Biol.*, 62, 2251–2264, 2011.
- Bown, P. R., Lees, J. A., and Young, J. R.: Calcareous nannoplankton evolution and diversity through time, in: *Coccolithophores – from Molecular Processes to Global Impact*, edited by: Thierstein, H. R. and Young, J. R., Springer, New York, 2004.
- Boyd, P. W. and Hutchins, D. A.: Understanding the response of ocean biota to a complex matrix of cumulative anthropogenic change, *Mar. Ecol.-Prog. Ser.*, 470, 125–135, 2012.
- Brownlee, C., Davies, M., Nimer, N., Dong, L. F., and Merrett, M. J.: Calcification, photosynthesis and intracellular regulation in *Emiliania huxleyi*, *Bull. Inst. Oceanogr. Monaco*, 14, 19–35, 1995.
- de Villiers, S., Greaves, M., and Elderfield, H.: An intensity ratio calibration method for the accurate determination of Mg/Ca and Sr/Ca of marine carbonates by ICP-AES, *Geochem. Geophys. Geos.*, 3, doi:10.1029/2001GC000169, 2002.
- Dickson, A. G., Afghan, J. D., and Anderson, G. C.: Reference materials for oceanic CO₂ analysis: a method for the certification of total alkalinity, *Mar. Chem.*, 80, 185–197, 2003.
- Erba, E.: The first 150 million years history of calcareous nannoplankton: biosphere–geosphere interactions, *Palaeogeogr. Palaeoclimatol.*, 232, 237–250, 2006.
- Guillard, R. R.: Culture of phytoplankton for feeding marine invertebrates, in: *Culture of Marine Invertebrates*, edited by: Smith, W. and Chanley, M., Plenum, 1975.
- Herfort, L., Lose, E., Meldrum, F., and Thake, B.: Structural and physiological effects of calcium and magnesium in *Emiliania huxleyi* Hay and Mohler, *J. Struct. Biol.*, 148, 307–314, 2004.
- Holtz, L.-M., Thoms, S., Langer, G., and Wolf-Gladrow, D. A.: Substrate supply for calcite precipitation in *Emiliania huxleyi*: assessment of different model-approaches, *J. Phycol.*, 49, 417–426, 2013.
- Hoppe, C. J. M., Langer, G., and Rost, B.: *Emiliania huxleyi* shows identical responses to elevated pCO₂ in TA and DIC manipulations, *J. Exp. Mar. Biol. Ecol.*, 406, 54–62, 2011.
- Krug, S. A., Schulz, K. G., and Riebesell, U.: Effects of changes in carbonate chemistry speciation on *Coccolithus braarudii*: a discussion of coccolithophorid sensitivities, *Biogeosciences*, 8, 771–777, doi:10.5194/bg-8-771-2011, 2011.
- Langer, G., Nehrke, G., Riebesell, U., Eisenhauer, A., Kuhnert, H., Rost, B., Trimborn, S., and Thoms, S.: Coccolith strontium to calcium ratios in *Emiliania huxleyi*: the dependence on seawater strontium and calcium concentrations, *Limnol. Oceanogr.*, 51, 310–320, 2006.
- Langer, G., Nehrke, G., Thoms, S., and Stoll, H. M.: Barium partitioning in coccoliths of *Emiliania huxleyi*, *Geochim. Cosmochim. Ac.*, 73, 2899–2906, 2009.
- Legong, L., Tutone, A. F., Drummond, R. S. M., Gardner, R. C., and Luan, S.: A novel family of magnesium transport genes in *Arabidopsis*, *Plant Cell*, 13, 2761–2775, 2001.
- Lose, E., Wilson, R. M., Seshadri, R., and Meldrum, F. C.: The role of magnesium in stabilising amorphous calcium carbonate and controlling calcite morphologies, *J. Cryst. Growth*, 254, 206–218, 2003.
- Mackinder, L., Wheeler, G., Schroeder, D., Riebesell, U., and Brownlee, C.: Molecular mechanisms underlying calcification in coccolithophores, *Geomicrobiol. J.*, 27, 585–595, 2010.
- Marr, J. P., Baker, J. A., Carter, L., Allan, A. S. R., Dunbar, G. B., and Bostock, H. C.: Ecological and temperature controls on Mg/Ca ratios of *Globigerina bulloides* from the southwest Pacific Ocean, *Paleoceanography*, 26, PA2209, doi:10.1029/2010PA002059, 2011.
- Mitsuguchi, T., Matsumoto, E., Abe, O., Uchida, T., and Isdale, P. J.: Mg/Ca thermometry in coral skeletons, *Science*, 274, 961–963, 1996.
- Morse, J. W., Andersson, A. J., and Mackenzie, F. T.: Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO₂ and “ocean acidification”: role of high Mg-calcites, *Geochim. Cosmochim. Ac.*, 70, 5814–5830, 2006.

- Müller, M. N., Antia, A. N., and LaRoche, J.: Influence of cell cycle phase on calcification in the coccolithophore *Emiliana huxleyi*, *Limnol. Oceanogr.*, 53, 506–512, 2008.
- Müller, M. N., Kısakürek, B., Buhl, D., Gutperlet, R., Kolevica, A., Riebesell, U., Stoll, H., and Eisenhauer, A.: Response of the coccolithophores *Emiliana huxleyi* and *Coccolithus braarudii* to changing seawater Mg²⁺ and Ca²⁺ concentrations: Mg²⁺, Sr²⁺ incorporation and $\delta^{44/40}\text{Ca}$, $\delta^{26/24}\text{Mg}$ in calcite, *Geochim. Cosmochim. Ac.*, 75, 2088–2102, 2011.
- Müller, M. N., Beaufort, L., Bernard, O., Pedrotti, M. L., Talec, A., and Sciandra, A.: Influence of CO₂ and nitrogen limitation on the coccolith volume of *Emiliana huxleyi* (Haptophyta), *Biogeosciences*, 9, 4155–4167, doi:10.5194/bg-9-4155-2012, 2012.
- Nehrke, G., Keul, N., Langer, G., de Nooijer, L. J., Bijma, J., and Meibom, A.: A new model for biomineralization and trace element signatures of Foraminifera tests, *Biogeosciences*, 10, 6759–6767, doi:10.5194/bg-10-6759-2013, 2013.
- Ra, K., Kitagawa, H., and Shiraiwa, Y.: Mg isotopes in chlorophyll *a* and coccoliths of cultured coccolithophores (*Emiliana huxleyi*) by MC-ICP-MS, *Mar. Chem.*, 122, 130–137, 2010.
- Raven, J. and Crawford, K.: Environmental controls on coccolithophore calcification, *Mar. Ecol.-Prog. Ser.*, 470, 137–166, 2012.
- Rickaby, R. E. M., Schrag, D. P., Zondervan, I., and Riebesell, U.: Growth rate dependence of Sr incorporation during calcification of *Emiliana huxleyi*, *Global Biogeochem. Cy.*, 16, 1006, doi:10.1029/2001GB001408, 2002.
- Riebesell, U. and Tortell, P. D.: Effects of ocean acidification on pelagic organisms and ecosystems, in: *Ocean Acidification*, edited by: Gattuso, J.-P. and Hansson, L., Oxford University Press, New York, 2011.
- Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P.: Guide to Best Practices for Ocean Acidification Research and Data Reporting, Publications Office of the European Union, Luxembourg, 2010.
- Roy, R., Roy, L., Vogel, K., Porter-Moore, C., Pearson, T., Good, C., Millero, F., and Campbell, D.: The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 °C, *Mar. Chem.*, 44, 249–267, 1993.
- Schulz, K. G., Zondervan, I., Gerringa, L. J. A., Timmermans, K. R., Veldhuis, M. J. W., and Riebesell, U.: Effect of trace metal availability on coccolithophorid calcification, *Nature*, 430, 6730–676, 2004.
- Schulz, K. G., Barcelos e Ramos, J., Zeebe, R. E., and Riebesell, U.: CO₂ perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations, *Biogeosciences*, 6, 2145–2153, doi:10.5194/bg-6-2145-2009, 2009.
- Segev, E. and Erez, J.: Effect of Mg/Ca ratio in seawater on shell composition in shallow benthic foraminifera, *G cubic*, 7, Q02P09, doi:10.1029/2005GC000969, 2013.
- Sett, S., Bach, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., and Riebesell, U.: Temperature Modulates Coccolithophorid Sensitivity of Growth, Photosynthesis and Calcification to Increasing Seawater *p*CO₂, *PLoS ONE*, 9, e88308, doi:10.1371/journal.pone.0088308, 2014.
- Stoll, H. M. and Schrag, D. P.: Coccolith Sr/Ca as a new indicator of coccolithophorid calcification and growth rate, *Geochim. Geophys. Geosy.*, 1, 1–24, 2000.
- Stoll, H. M. and Ziveri, P.: Coccolithophorid-based geochemical paleoproxies, in: *Coccolithophores – from Molecular Processes to Global Impact*, edited by: Thierstein, H. R. and Young, J. R., Springer, New York, 2004.
- Stoll, H. M. and Schrag, D. P.: Sr/Ca variations in Cretaceous carbonates: relation to productivity and sea level changes, *Palaeogeogr. Palaeoclimatol., Palaeoecol.*, 168, 311–336, 2001.
- Stoll, M. H. C., Bakker, K., Nobbe, G. H., and Haese, R. R.: Continuous-flow analysis of dissolved inorganic carbon content in seawater, *Anal. Chem.*, 73, 4111–4116, 2001.
- Stoll, H. M., Rosenthal, Y., and Falkowski, P.: Climate proxies from Sr/Ca of coccolith calcite: calibrations from continuous culture of *Emiliana huxleyi*, *Geochim. Cosmochim. Ac.*, 66, 927–936, 2002.
- Stoll, H. M., Ziveri, P., Shimizu, N., Conte, M., and Theroux, S.: Relationship between coccolith Sr/Ca ratios and coccolithophore production and export in the Arabian Sea and Sargasso Sea, *Deep-Sea Res. Pt. II*, 54, 581–600, 2007a.
- Stoll, H. M., Shimizu, N., Archer, D., and Ziveri, P.: Coccolithophore productivity response to greenhouse event of the Paleocene-Eocene Thermal Maximum, *Earth Planet. Sci. Lett.*, 258, 192–206, 2007b.
- Stoll, H. M., Langer, G., Shimizu, N., and Kanamaru, K.: B/Ca in coccoliths and relationship to calcification vesicle pH and dissolved inorganic carbon concentrations, *Geochim. Cosmochim. Ac.*, 80, 143–157, 2012.
- Tang, J., Köhler, S. J., and Dietzel, M.: Sr²⁺/Ca²⁺ and ⁴⁴Ca/⁴⁰Ca fractionation during inorganic calcite formation: I. Sr incorporation, *Geochim. Cosmochim. Ac.*, 72, 3718–3732, 2008.
- Van Oostende, N., Harlay, J., Vanelslander, B., Chou, L., Vyverman, W., and Sabbe, K.: Phytoplankton community dynamics during late spring coccolithophore blooms at the continental margin of the Celtic Sea (North East Atlantic, 2006–2008), *Progr. Oceanogr.*, 104, 1–16, 2012.
- Welschmeyer, N. A.: Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments, *Limnol. Oceanogr.*, 39, 1985–1992, 1994.
- Zachos, J. C., Roehl, U., Schellenberg, S. A., Sluijs, A., Hodell, D. A., Kelly, D. C., Thomas, E., Nicolo, M., Raffi, I., Lourens, L. J., McCarren, H., and Kroon, D.: Rapid Acidification of the Ocean During the Paleocene-Eocene Thermal Maximum, *Science*, 308, 1611–1615, 2005.
- Zeebe, R. E., Zachos, J. C., and Dickens, G. R.: Carbon dioxide forcing alone insufficient to explain Palaeocene-Eocene Thermal Maximum warming, *Nat. Geosci.*, 2, 576–580, 2009.
- Ziveri, P., Thoms, S., Probert, I., Geisen, M., and Langer, G.: A universal carbonate ion effect on stable oxygen isotope ratios in unicellular planktonic calcifying organisms, *Biogeosciences*, 9, 1025–1032, doi:10.5194/bg-9-1025-2012, 2012.