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Calcium isotope fractionation during coccolith formation in *Emiliania huxleyi*: Independence of growth and calcification rate

Gerald Langer
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany (glanger@awi-bremerhaven.de)

Nikolaus Gussone
Research Centre Ocean Margins, University of Bremen, P.O. Box 330440, D-28334 Bremen, Germany

Gernot Nehrke
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Ulf Riebesell
Leibniz Institute for Marine Sciences, Düsternbrooker Weg 20, D-24105 Kiel, Germany

Anton Eisenhauer
Leibniz Institute for Marine Sciences, Wischhofstraße 1-3, D-24148 Kiel, Germany

Silke Thoms
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

[1] Recently, calcium isotope fractionation in the coccolithophore *Emiliania huxleyi* was shown to exhibit a significant temperature dependency. An important subsequent question in this context is whether the observed fractionation patterns are caused by temperature itself or related growth rate changes. In order to separate growth and calcification rate effects from direct temperature effects, batch culture experiments with the coccolithophore *E. huxleyi* were conducted under varying light intensities. Despite large changes in cellular growth and calcification rates, calcium isotope fractionation remained constant. Independence of calcium isotope fractionation on growth and calcification was also obtained in two additional sets of experiments in which growth rates changed in response to varying calcium concentration and seawater salinity. These experiments also showed no direct effects of calcium concentration and salinity on calcium isotope fractionation. Values for calcium isotope fractionation of *E. huxleyi* coccoliths fell within a range of -1.0 to -1.6 ($1000\ \ln\alpha$), confirming earlier results. This range is similar to that observed in several foraminiferal species and coccolith oozes, suggesting a rather homogeneous calcium isotopic composition in marine biogenic calcite. Our data further show that the calcium isotope fractionation does not change with changing isotopic composition of seawater. This is a basic requirement for reconstructing the calcium isotopic composition of the ocean over time.

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1. Introduction

[2] The sea surface temperature (SST) provides important information for modeling of climate and ocean circulation and is therefore the most wanted target in paleoceanography. A number of different proxies are used to reconstruct SST, each of which has its shortcomings. It is therefore desirable to develop new SST proxies and understand the interplay between temperature control and secondary influences, e.g., growth rate.

[3] A temperature dependent calcium isotopic fractionation in foraminiferal calcite was suggested [*Zhu and Macdougall*, 1998] and subsequently confirmed [*Nägler et al.*, 2000]. The latter study provides a temperature versus $\delta^{44/40}$Ca calibration curve of the surface dwelling planktonic foraminifer species *Globigerinoides sacculifer*. Further studies on calcium isotope fractionation revealed strong differences in the temperature response in different species. While *G. sacculifer* shows a strong temperature dependence (0.24 ‰ $°C^{-1}$), *Orbulina universa* displays a comparatively weak dependence (0.019 ‰ $°C^{-1}$) [*Gussone et al.*, 2003; *Nägler et al.*, 2000].

[4] Besides foraminifera coccolithophores are an important group of organisms playing an important role in the marine calcium cycle. They are major marine calcite producers and their calcareous plates, the coccoliths, are estimated to compose 60 to 90% of carbonaceous sediments since the Jurassic [*Berger and Roth*, 1975]. This means that it is critical to understand the controls on the isotopic composition of coccolith calcite when using calcium isotopes for reconstructing the oceanic Ca-budget [*De La Rocha and DePaolo*, 2000; *DePaolo*, 2004; *Fantle and DePaolo*, 2005; *Heuser et al.*, 2005; *Schmitt et al.*, 2003; *Skulan et al.*, 1997; *Zhu and Macdougall*, 1998]. In addition the coccolithophore sedimentary record could possibly provide useful information about long term changes in ocean chemistry [*De La Rocha and DePaolo*, 2000; *Fantle and DePaolo*, 2005].

[5] However, systematic studies on calcium isotope fractionation of pure coccolithophore calcite, obtained from controlled laboratory cultures or from field samples, are rare. Recently calcium isotope fractionation in response to temperature and carbonate chemistry in the widespread species *Emiliania huxleyi* was studied in laboratory experiments [*Gussone et al.*, 2006]. The $\delta^{44/40}$Ca values of coccoliths from cultured specimens covered a range of 0.3–0.7‰ (80–190 $^{ppm}/_{amu}$) (relative to the NIST standard SRM915a). This range is similar to that observed in several foraminiferal species and coccolith oozes [*Chang et al.*, 2004; *De La Rocha and DePaolo*, 2000; *Fantle and DePaolo*, 2005; *Gussone et al.*, 2005; *Heuser et al.*, 2005; *Sime et al.*, 2005; *Skulan et al.*, 1997], suggesting a rather homogeneous isotopic composition of marine biogenic calcite. In contrast, one study reports coccolith oozes showing considerably lighter $\delta^{44/40}$Ca values [*Zhu and Macdougall*, 1998]. The reason for this discrepancy is not clear.

[6] The calcium isotope fractionation in *E. huxleyi* decreases with increasing temperature (0.027 ‰ $°C^{-1}$; 7 $^{ppm}/_{amu}$/°C), leading to a positive $\delta^{44/40}$Ca-temperature relation [*Gussone et al.*, 2006]. The slope of the temperature curve is similar to that observed in the planktonic foraminifer *Orbulina universa* (0.019 ‰ $°C^{-1}$; 5 $^{ppm}/_{amu}$/°C) and other biogenic marine carbonates [*Gussone et al.*, 2005]. On the basis of the available data it is unclear, however, whether the observed relationship solely results from a direct effect of temperature on isotope fractionation or whether it is influenced by temperature related changes in growth and calcification rate. Calcium isotope fractionation in inorganically precipitated calcite is temperature dependent and it was concluded that this dependency is due to temperature related changes in precipitation rate [*Lemarchand et al.*, 2004]. Assuming that the model of *Lemarchand et al.* [2004] is applicable to calcite precipitated by *E. huxleyi,* calcium isotope fractionation in this species would depend on calcification rate. However, in a recent study [*Gussone et al.*, 2006] it was shown that the model of *Lemarchand et al.* [2004] is not applicable to *E. huxleyi*. The main objective

of this study therefore was to test for possible independent effects of growth and calcification rate on calcium isotope fractionation in *E. huxleyi*. This was achieved by modifying growth rate through changes of environmental factors other than temperature. In autotrophic calcifiers, such as coccolithophores, light intensity strongly impacts both growth and calcification rate. In addition to variable light intensity we further investigated the effects of changing calcium concentration and seawater salinity, because both parameters are known to affect cellular growth and calcification of coccolithophores. Moreover, salinity and calcium concentration of seawater are important parameters in terms of paleoceanographic reconstructions that can vary on a regional and/or temporal scale [*Horita et al.*, 2002].

2. Material and Methods

[7] Monospecific cultures of *Emiliania huxleyi* (strain PML B92/11) were grown in sterile filtered (0.2 μm) seawater enriched with 100 μmol L^{-1} nitrate, 6.25 μmol L^{-1} phosphate and trace metals and vitamins as in F/2 media [*Guillard and Ryther*, 1962]. The calcium concentration was not elevated by these additives. Cultures were grown under a 16/8 hour light/dark cycle. Experiments were carried out at 15°C and 17°C and various light intensities from 22 to 180 μmol photons m^{-2} s^{-1} in an adjustable incubator (Rubarth Apparate GmbH, Germany). Salinities ranged from 25 to 35. Adjustment of salinity was attained by either adding ultra pure water to natural seawater or evaporating natural seawater at 65°C (for a more detailed description of salinity experiments, see *Schouten et al.* [2006]). Experiments with variable calcium concentrations were conducted using artificial seawater, a detailed composition of which is given by *Langer et al.* [2006]. Calcium concentration of the natural seawater was 10 mmol/L. Cells were pre-adapted to experimental conditions for approximately 12 generations and grown in dilute batch cultures [*Zondervan et al.*, 2002]. The duration of the main experiments ranged from 4 to 42 days depending on the growth rate of the cells. Each data point presented in the figures is the mean value of three culture experiments with the exception of the data shown in Figure 4, where each data point represents one culture experiment. Low cell densities even at the termination of the experiments resulted in the consumption of less than 5% dissolved inorganic carbon (DIC). Calcium consumption was less than 1% and hence changes of calcium isotope composition of the fluid during the course of the experiment were negligible.

[8] Samples for alkalinity measurements were filtered (approx. 0.6 μm), poisoned with 1 mL 35 g L^{-1} $HgCl_2$, and stored in 300 mL borosilicate flasks at 0°C. DIC samples were sterile filtered (0.2 μm) and stored in 13 mL borosilicate flasks free of air bubbles at 0°C. Total alkalinity was calculated from linear Gran plots [*Gran*, 1952] after potentiometric titration (in duplicate) [*Bradshaw et al.*, 1981; *Brewer et al.*, 1986]. DIC was measured photometrically [*Stoll et al.*, 2001] in triplicate. The carbonate system was calculated from temperature, salinity, and the concentrations of DIC, total alkalinity and phosphate, using the DOS-program CO_2sys [*Lewis and Wallace*, 1998]. Equilibrium constants of *Mehrbach et al.* [1973] refitted by *Dickson and Millero* [1987] were used. Samples for determination of total particulate carbon (TPC) and particulate organic carbon (POC) were filtered on precombusted (12 hours, 500°C) GF/F-filters (approx. 0.6 μm) and stored at −20°C. Prior to analysis, the POC filters were fumed for two hours with a saturated HCl solution (37%) to remove all inorganic carbon. TPC and POC were subsequently measured on a Carlo Erba NA-1500 Analyzer. Particulate inorganic carbon (PIC) was calculated as the difference between TPC and POC. For determination of cell density samples were taken at the beginning and the end of experiment and counted immediately after sampling using a Coulter ''Multisizer II''. Growth rate (μ) was calculated as

$$\mu = (\ln c_1 - \ln c_0)\Delta t^{-1} \quad (1)$$

where c_0 and c_1 are the cell concentrations at the beginning and the end of experiment, respectively, and Δt is the duration of incubation in days.

[9] Inorganic carbon production, i.e., calcification rate (P, pg PIC $cell^{-1}$ d^{-1}), was calculated according to

$$P = \mu^*(\text{cellular inorganic carbon content}) \quad (2)$$

with cellular inorganic carbon content = pg PIC per cell.

[10] Samples for calcium isotope measurements were filtered on acid rinsed polycarbonate filters (0.2 μm), dried at 60°C for 48 hours and stored in plastic petri dishes. Prior to calcium isotope analysis, coccolith samples were bleached in a sodium hypochlorite-solution (~1% active chlorine) to

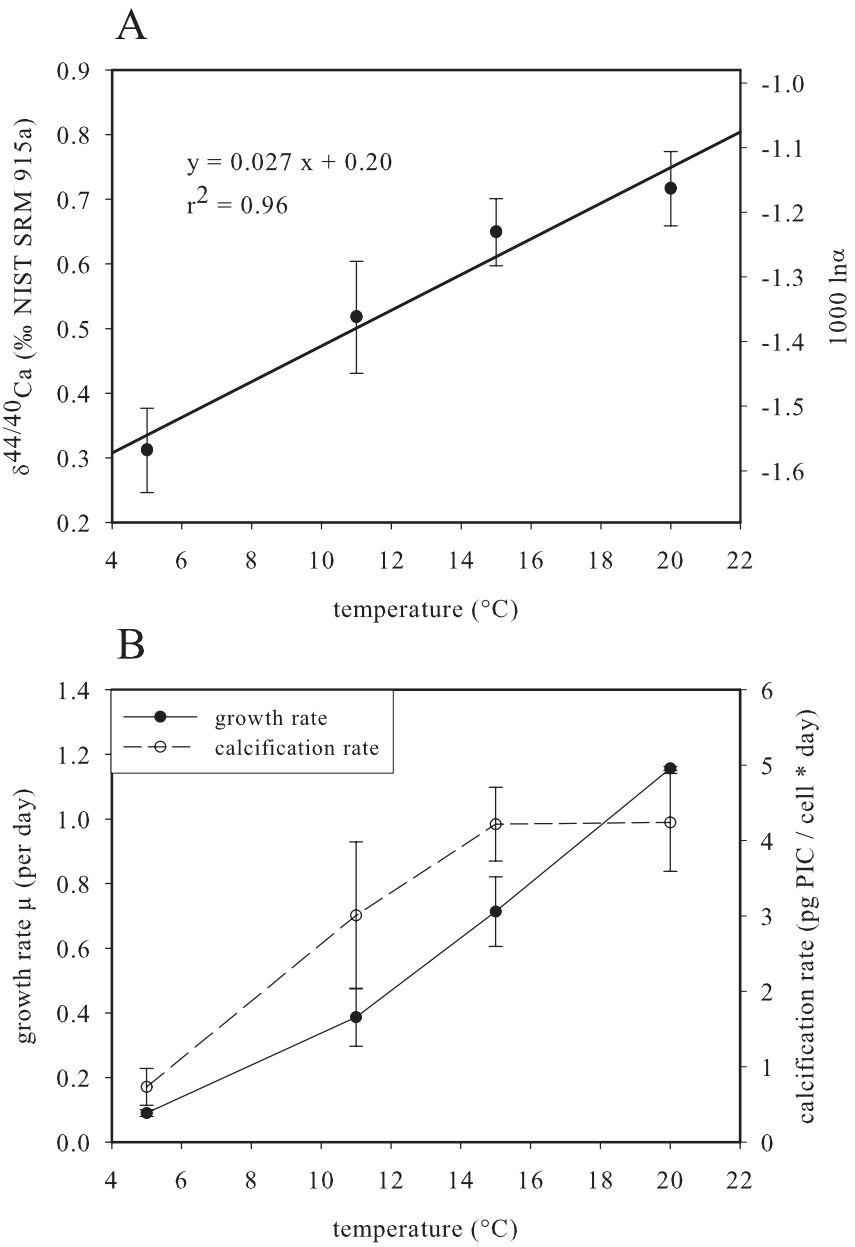

Figure 1. The dependence of (a) $\delta^{44/40}$Ca of coccolith calcite and (b) growth and calcification rate on temperature. The linear regression in Figure 1a yields a slope of 0.027 ‰ $°C^{-1}$ (calcium isotope data from *Gussone et al.* [2006]). Photon flux density was 150 μmol/m * s. Each data point is the mean value of three culture experiments.

remove organic components. Afterward, the samples were washed in distilled water, in methanol (CH_3OH), and finally six times in distilled water. The pH of the distilled water was adjusted by the addition of NH_4OH to a value of 8–9 to prevent partial dissolution of the coccoliths.

[11] The $^{44}Ca/^{40}Ca$ ratio of the coccolith $CaCO_3$ was determined on a Finnigan Triton T1 Thermal Ionization Mass Spectrometer at the Leibniz Institute for Marine Science, Kiel using a $^{43}Ca/^{48}Ca$ Double Spike technique, following the description of *Heuser et al.* [2002]. Isotope values are reported as $\delta^{44/40}$Ca (‰) relative to the SRM 915a carbonate standard provided by NIST: $\delta^{44/40}Ca\ (‰) = (((^{44}Ca/^{40}Ca)_{sample}/(^{44}Ca/^{40}Ca)_{standard}) - 1) \cdot 1000$ and as $\delta^{mu}Ca\ (^{ppm}/_{amu}) = 268.3 \cdot \delta^{44/40}Ca$, in order to improve the comparability with Ca isotope data based on Ca isotope ratios other than $^{44}Ca/^{40}Ca$ (e.g., $^{44}Ca/^{42}Ca$). Average 2 σ_m of our data is about 0.12‰ (30 $^{ppm}/_{amu}$), determined by repeated aliquot measurements of various sample materials.

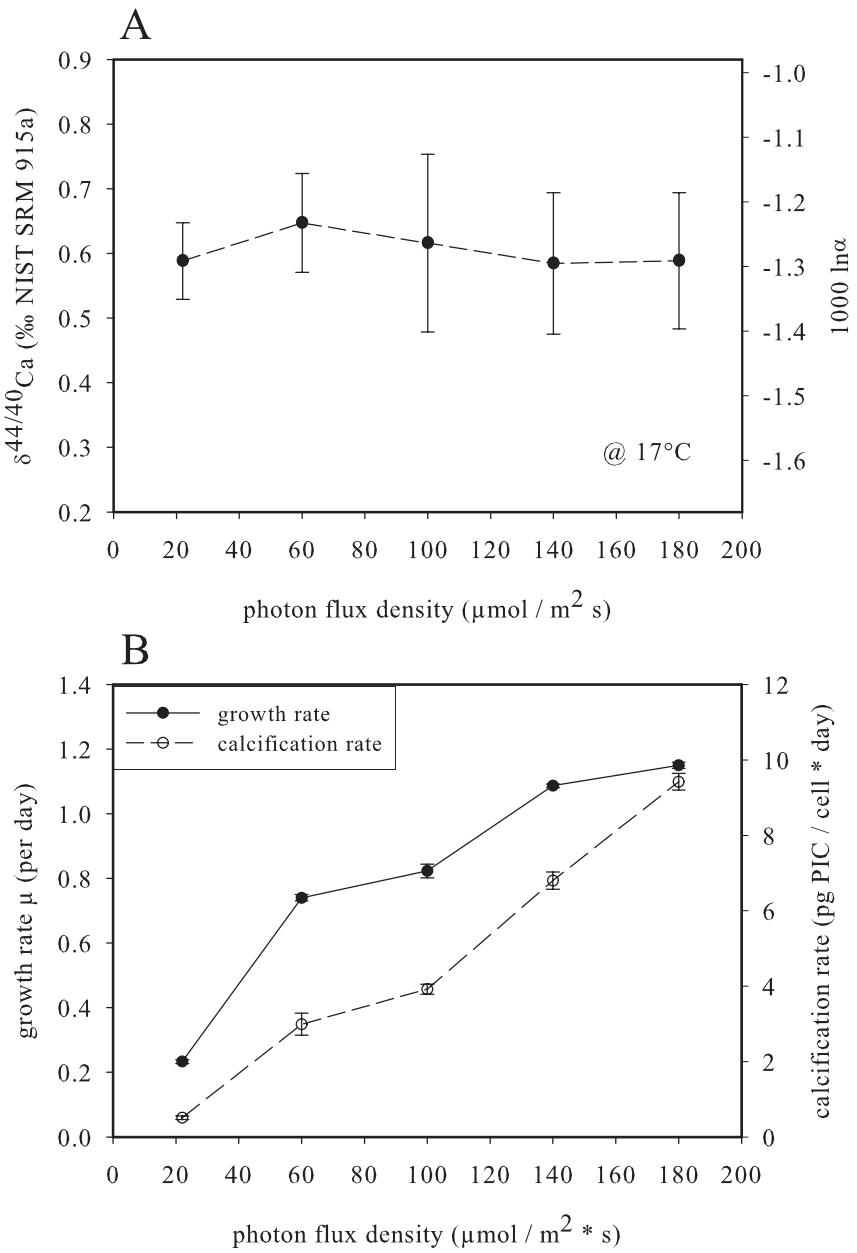

Figure 2. The dependence of (a) $\delta^{44/40}$Ca of coccolith calcite and (b) growth and calcification rate on photon flux density. Temperature was 17°C. Each data point is the mean value of three culture experiments.

The isotope fractionation between growth medium and calcite is reported as 1000 lnα with $\alpha = (^{44}Ca/^{40}Ca)_{calcite}/(^{44}Ca/^{40}Ca)_{solution}$.

3. Results

[12] The coccoliths of *Emiliania huxleyi* are depleted in ^{44}Ca relative to the seawater in which the cells were grown. This holds for all experimental conditions used in this study (Figure 1–4; Table 1). The $\delta^{44/40}$Ca value of natural seawater is +1.88‰ (505 $^{ppm}/_{amu}$), and the $\delta^{44/40}$Ca of coccolith calcite in the experiments ranged from +0.3 to +0.7‰ (80–190 $^{ppm}/_{amu}$). In Figure 1 the positive correlation of coccolith $\delta^{44/40}$Ca values and temperature [*Gussone et al.*, 2006] is shown in comparison to cell growth rates and calcification rates, which also increased with increasing temperature (Figure 1b).

[13] In contrast, no relationship was observed between calcium isotope fractionation and photon flux density (Figure 2a), despite a factor of 6 and 10 increase in cell growth and calcification,

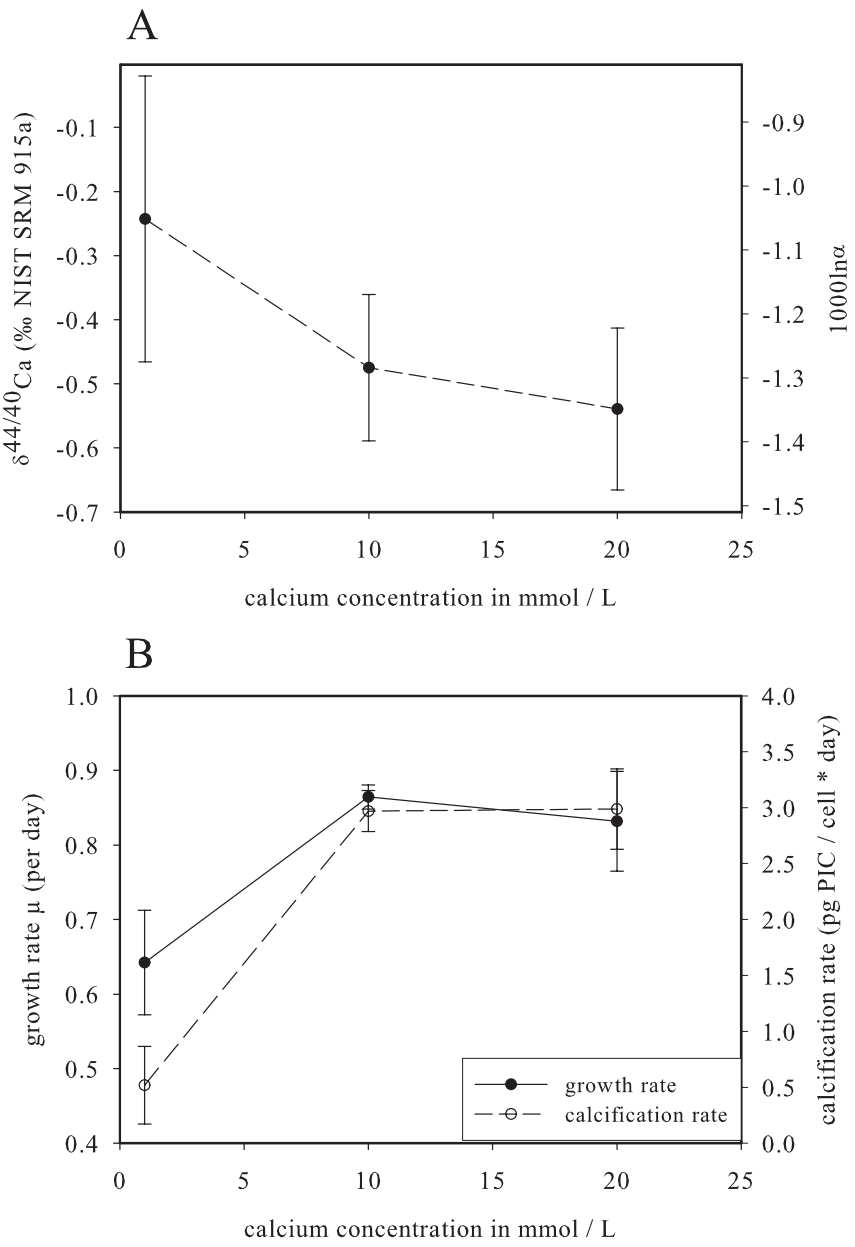

Figure 3. The dependence of (a) $\delta^{44/40}$Ca of coccolith calcite and (b) growth and calcification rate on calcium concentration of seawater. Temperature was 15°C. Note that in this experiment, artificial seawater with a $\delta^{44/40}$Ca value of +0.81‰ was used, the isotopic composition of which did not change significantly in the course of the experiment. Each data point is the mean value of three culture experiments.

respectively, with increasing photon flux density (Figure 2b).

[14] A slight increase in calcium isotope fractionation was observed with increasing calcium concentration of artificial seawater (Figure 3a). In contrast to natural seawater the artificial seawater used in this experiment had a $\delta^{44/40}$Ca value of +0.81 ± 0.15‰ (218 ± 40 $^{ppm}/_{amu}$). Cell growth rates and calcification rates were diminished by factors of 1.3 and 6 respectively at an external calcium concentration of 1 mmol/L (Figure 3b). The corresponding calcite saturation state (Ω), with $\Omega = [Ca^{2+}][CO_3^{2-}]/Ksp$ (*Ksp* = calcite solubility product) was 0.4, indicating undersaturation of seawater with respect to calcite. At an external calcium concentration of 10 mmol/L the calcite saturation state was 4.2 and at an external calcium concentration of 20 mmol/L the calcite saturation state was 9.2.

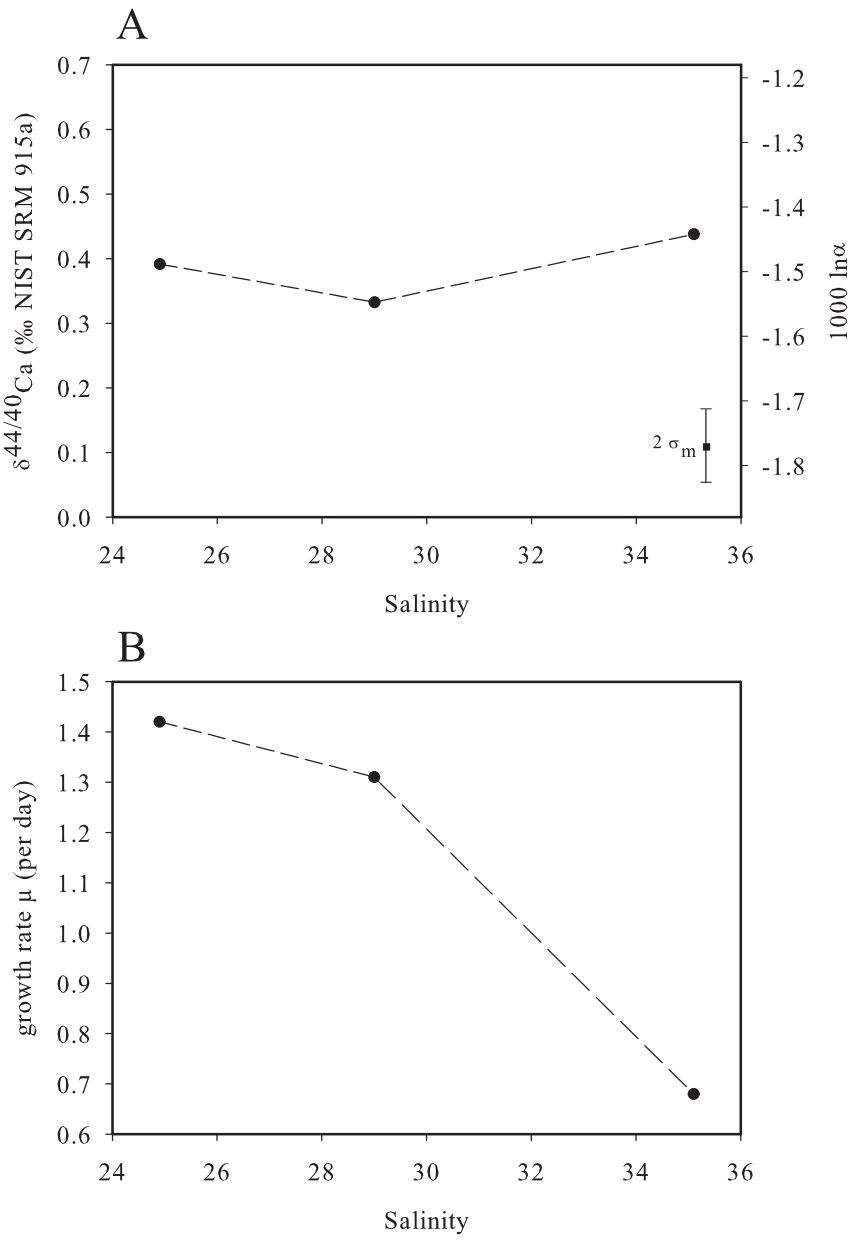

Figure 4. The dependence of (a) $\delta^{44/40}$Ca of coccolith calcite and (b) growth rate on salinity of seawater. Data in Figure 4b are from *Schouten et al.* [2006]. Temperature was 15°C. Each data point represents one culture experiment.

[15] Calcium isotope fractionation did not depend on salinity within the range tested (Figure 4a). Cell growth rate, on the other hand, was reduced by a factor of 2 at a salinity of ~35 compared to the growth rates observed for salinities up to ~30 (Figure 4b).

4. Discussion

[16] Calcium carbonate precipitation in *Emiliania huxleyi*, as in other marine organisms [cf. *Skulan et al.*, 1997] is associated with fractionation against the heavier isotopes of calcium. The $\delta^{44/40}$Ca values measured in this study are also in good agreement with previously published values of *E. huxleyi* and several carbonate oozes [*De La Rocha and DePaolo*, 2000; *DePaolo*, 2004; *Gussone et al.*, 2006]. Comparable $\delta^{44/40}$Ca values were obtained for several species of foraminifera and metazoans [*De La Rocha and DePaolo*, 2000; *DePaolo*, 2004; *Gussone et al.*, 2003; *Skulan et al.*, 1997] suggesting marine biogenic calcite to carry a relatively homogeneous calcium isotope composition.

Table 1. Data Set Derived From the Experiments

	Temperature, °C			
	5	11	15	20
Growth rate μ	0.09	0.39	0.71	1.16
Standard deviation (μ)	0.01	0.09	0.11	0.01
Calcification rate, pg PIC/cell*day	0.73	3.01	4.22	4.24
Standard deviation (calc. rate)	0.24	0.97	0.49	0.65
Cellular inorganic carbon content, pg PIC/cell	8.03	7.70	5.97	3.70
Standard deviation, pg PIC/cell	1.79	1.47	0.95	0.36
Cellular organic carbon content, pg POC/cell	13.93	13.33	11.33	7.24
Standard deviation, pg POC/cell	0.90	0.58	1.53	0.45
Cellular total carbon content, pg TPC/cell	21.97	21.03	17.33	10.94
Standard deviation, pg TPC/cell	1.06	0.95	2.52	0.09

	Photon Flux Density, μmol/m^2*s				
	22	60	100	140	180
1000 lnα	−1.29	−1.23	−1.26	−1.29	−1.29
$\delta^{44/40}$Ca (SRM915a)	0.59	0.65	0.62	0.58	0.59
Standard deviation, ‰	0.06	0.08	0.14	0.11	0.11
10^6 lnα_{mu}	−347	−331	−339	−348	−347
δ^{mu}Ca (SRM915a)	158	174	166	157	158
Standard deviation, ppm/amu	16	21	37	29	28
Growth rate μ	0.23	0.74	0.82	1.09	1.15
Standard deviation (μ)	0.01	0.01	0.02	0.01	0.01
Calcification rate, pg PIC/cell*day	0.51	2.99	3.92	6.80	9.42
Standard deviation (calc. rate)	0.05	0.29	0.13	0.23	0.22
Cellular inorganic carbon content, pg PIC/cell	2.19	4.06	4.75	6.39	8.21
Standard deviation, pg PIC/cell	0.22	0.45	0.18	0.31	0.24
Cellular organic carbon content, pg POC/cell	6.13	7.30	9.89	13.23	12.71
Standard deviation, pg POC/cell	0.09	0.45	0.09	1.07	0.47
Cellular total carbon content, pg TPC/cell	8.32	11.36	14.64	19.62	20.92
Standard deviation, pg TPC/cell	0.25	0.89	0.09	1.35	0.63

	Ca Concentration, mmol/L		
	1	10	20
1000 lnα	−1.05	−1.28	−1.35
$\delta^{44/40}$Ca (SRM915a)	−0.24	−0.47	−0.54
Standard deviation, ‰	0.22	0.11	0.13
10^6 lnα_{mu}	−283	−345	−363
δ^{mu}Ca (SRM915a)	−65	−128	−145
Standard deviation, ppm/amu	60	31	34
Growth rate μ	0.64	0.86	0.83
Standard deviation (μ)	0.07	0.02	0.07
Calcification rate, pg PIC/cell*day	0.52	2.97	2.99
Standard deviation (calc. rate)	0.35	0.18	0.36
TA, μmol/kg	2642	2614	2638
Standard deviation (TA)	59	47	9
DIC, μmol/kg	2389	2384	2384
Standard deviation (DIC)	34	34	17
pH NBS	8.21	8.17	8.21
Standard deviation (pH)	0.06	0.03	0.04
Ω	0.46	4.18	9.16
Standard deviation (Ω)	0.06	0.36	0.71
Cellular inorganic carbon content, pg PIC/cell	0.80	3.43	3.63
Standard deviation, pg PIC/cell	0.62	0.32	0.34
Cellular organic carbon content, pg POC/cell	15.71	10.01	10.43
Standard deviation, pg POC/cell	3.03	0.83	0.64
Cellular total carbon content, pg TPC/cell	16.51	13.44	14.06
Standard deviation, pg TPC/cell	3.56	1.09	0.73

Table 1. (continued)

	Salinity		
	24.9	29	35.1
1000 lnα	−1.49	−1.55	−1.44
$\delta^{44/40}$Ca (SRM915a)	0.39	0.33	0.44
10^6 lnα_{mu}	−400	−415	−387
δ^{mu}Ca (SRM915a)	105	89	118

[17] The observed temperature dependent calcium isotope fractionation in *E. huxleyi* [*Gussone et al.*, 2006] was obtained in experiments in which temperature changes were associated with simultaneous changes in growth and calcification rate (Figure 1), leaving uncertainty regarding the mechanism of this temperature dependency.

[18] In the experiments conducted under variable light intensities changes in $\delta^{44/40}$Ca were not observed (Figure 2a), although calcification and growth rates varied by factors of 10 and 6 respectively (Figure 2b). This indicates that calcium isotope fractionation in *E. huxleyi* does not depend on growth and calcification rates, but directly on temperature. This finding is apparently in contrast to a model for calcium isotope fractionation in synthetic calcite [*Lemarchand et al.*, 2004]. In this model the authors explain the temperature dependent calcium isotope fractionation in terms of a precipitation rate effect. Growth and calcification rate of coccolithophore cells, on the other hand, are essentially physiological parameters, which cannot directly be converted to intracellular crystal growth rate. In case of *E. huxleyi* the precipitation of calcite occurs in a specialized, very small vesicle inside the cell. The data presented here support the notion of *Gussone et al.* [2006] that the calcium isotope fractionation occurs during the transport of calcium ions from the seawater into the coccolith vesicle and not during calcite precipitation inside the vesicle. The latter is the reason why the model of *Lemarchand et al.* [2004], which explains fractionation during inorganic calcite precipitation, is non-applicable to intracellular calcite precipitation as performed by *E. huxleyi* [*Gussone et al.*, 2006].

[19] The independence of calcium isotope fractionation on growth and calcification rate is confirmed by two additional experiments in which growth and calcification rate changes were induced by changes in calcium concentration and seawater salinity. Lowering the calcium concentration leads to a decrease in growth and calcification rate, and a decrease in calcium isotope fractionation (Figure 3). Although the magnitude of this decrease almost equals the magnitude of the change in fractionation in the temperature experiment (Figure 1) the implications differ substantially. While the temperature range used in the experiments represents values typical for the modern ocean (Figure 1), the range of calcium concentrations used (Figure 3) is by far greater than that experienced by the oceans in the past 100 million years [*Demicco et al.*, 2003]. Therefore this data set (Figure 3) shows that changes in calcium concentration of seawater over time do not significantly influence the isotope fractionation and the calcium isotope record based on coccoliths. It should be noted that despite partial dissolution of coccoliths a diminished production of coccolith calcite under low external calcium concentration is most likely [*Langer et al.*, 2006]. To interpret the apparent decrease in fractionation with decreasing calcium concentration two things have to be considered: firstly, the change in fractionation is opposite in sign to the change which is to be expected for a rate dependent fractionation as described by *Lemarchand et al.* [2004] (Figure 1). This also suggests that growth and calcification rate changes do not affect calcium isotope fractionation. Secondly, the standard deviation of the data points is relatively high requiring a tentative interpretation of this trend anyway. The reason for this relatively high standard deviation is not clear.

[20] Additional evidence for a growth rate independent calcium isotope fractionation is provided by the conducted salinity experiment (Figure 4). Although calcification rate was not directly determined an increase in salinity has a considerable effect on growth rate and therewith most likely also on calcification rate. Nevertheless, fractionation does not follow the change in growth rate as observed for the above discussed experiments. The observed independence of calcium isotope fractionation on salinity and calcium concentration

facilitates paleoceanographic reconstructions of the oceanic calcium isotope budget for periods in time which are characterized by altered seawater chemistry, i.e., salinity and calcium concentration [*De La Rocha and DePaolo*, 2000].

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References

Berger, A., and P. H. Roth (1975), Oceanic micropaleontology: Progress and prospect, *Rev. Geophys.*, *13*, 561–585.

Bradshaw, A. L., P. G. Brewer, D. K. Shaffer, and R. T. Williams (1981), Measurements of total carbon dioxide and alkalinity by potentiometric titration in the GEOSECS program, *Earth Planet. Sci. Lett.*, *55*, 99–115.

Brewer, P. G., A. L. Bradshaw, and R. T. Williams (1986), Measurement of total carbon dioxide and alkalinity in the North Atlantic Ocean in 1981, in *The Changing Carbon Cycle—A Global Analysis*, edited by J. R. Trabalka and D. E. Reichle, pp. 358–381, Springer, New York.

Chang, V. T.-C., R. J. P. Williams, A. Makishima, N. S. Belshawl, and R. K. O’Nions (2004), Mg and Ca isotope fractionation during $CaCO_3$ biomineralisation, *Biochem. Biophys. Res. Commun.*, *323*, 79–85.

De La Rocha, C., and D. J. DePaolo (2000), Isotopic evidence for variations in the marine calcium cycle over the Cenozoic, *Science*, *289*, 1176–1178.

Demicco, R. V., T. K. Lowenstein, and L. A. Hardie (2003), Atmospheric pCO_2 since 60 Ma from records of seawater pH, calcium, and primary carbonate mineralogy, *Geology*, *31*, 793–796.

DePaolo, D. J. (2004), Calcium isotopic variations produced by biological, kinetic, radiogenic and nucleosynthetic processes, in *Geochemistry of Non-traditional Stable Isotopes*, edited by C. M. Johnson, B. L. Beard, and F. Albarede, pp. 255–288, Mineral. Soc. of Am./Geochem. Soc., Washington, D. C.

Dickson, A. G., and F. J. Millero (1987), A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, *Deep Sea Res., Part A*, *34*, 1733–1743.

Fantle, M. S., and D. J. DePaolo (2005), Variations in the marine Ca cycle over the past 20 million years, *Earth Planet. Sci. Lett.*, *237*, 102–117.

Gran, G. (1952), Determination of the equivalence point in potentiometric titrations of seawater with hydrochloric acid, *Oceanol. Acta*, *5*, 209–218.

Guillard, R. R. L., and J. H. Ryther (1962), Studies of marine planktonic diatoms, I, *Cyclotella nanna* (Hustedt) and *Detonula convervacea* (Cleve), *Can. J. Microbiol.*, *8*, 229–239.

Gussone, N., A. Eisenhauer, A. Heuser, M. Dietzel, B. Bock, F. Bohm, H. J. Spero, D. W. Lea, J. Bijma, and T. F. Nagler (2003), Model for kinetic effects on calcium isotope fractionation (δ^{44}Ca) in inorganic aragonite and cultured planktonic foraminifera, *Geochim. Cosmochim. Acta*, *67*, 1375–1382.

Gussone, N., F. Bohm, A. Eisenhauer, M. Dietzel, A. Heuser, B. M. A. Teichert, J. Reitner, G. Worheide, and W.-C. Dullo (2005), Calcium isotope fractionation in calcite and aragonite, *Geochim. Cosmochim. Acta*, *69*, 4485–4494.

Gussone, N., G. Langer, S. Thoms, G. Nehrke, A. Eisenhauer, U. Riebesell, and G. Wefer (2006), Cellular calcium pathways and isotope fractionation in *Emiliania huxleyi*, *Geology*, *34*, 625–628.

Heuser, A., A. Eisenhauer, N. Gussone, B. Bock, B. T. Hansen, and T. F. Nagler (2002), Measurement of calcium isotopes (d^{44}Ca) using a multicollector TIMS technique, *Int. J. Mass Spectrsc.*, *220*, 385–397.

Heuser, A., A. Eisenhauer, F. Böhm, K. Wallmann, N. Gussone, P. N. Pearson, T. F. Nägler, and W.-C. Dullo (2005), Calcium isotope ($\delta^{44/40}$Ca) variations of Neogene planktonic foraminifera, *Paleoceanography*, *20*, PA2013, doi:10.1029/2004PA001048.

Horita, J., H. Zimmermann, and H. D. Holland (2002), Chemical evolution of seawater during the Phanerozoic: Implications from the record of marine evaporites, *Geochim. Cosmochim. Acta*, *66*, 3733–3756.

Langer, G., N. Gussone, G. Nehrke, U. Riebesell, A. Eisenhauer, H. Kuhnert, B. Rost, S. Trimborn, and S. Thoms (2006), Coccolith strontium to calcium ratios in *Emiliania huxleyi*: The dependence on seawater strontium and calcium concentrations, *Limnol. Oceanogr.*, *51*, 310–320.

Lemarchand, D., G. J. Wasserburg, and D. A. Papanastassiou (2004), Rate-controlled calcium isotope fractionation in synthetic calcite, *Geochim. Cosmochim. Acta*, *68*, 4665–4678.

Lewis, E., and D. W. R. Wallace (1998), Program developed for CO_2 system calculations, *Rep. ORNL/CDIAC-105*, Carbon Dioxide Inf. and Anal. Cent., Oak Ridge Natl. Lab., U.S. Dep. of Energy, Oak Ridge, Tenn.

Mehrbach, C., C. H. Culberson, J. E. Hawley, and R. M. Pytkovicz (1973), Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, *Limnol. Oceanogr.*, *18*, 897–907.

Nägler, T. F., A. Eisenhauer, A. Müller, C. Hemleben, and J. Kramers (2000), The d^{44}Ca-temperature calibration on fossil and cultured *Globigerinoides sacculifer*: New tool for reconstruction of past sea surface temperatures, *Geochem. Geophys. Geosyst.*, *1*, doi:10.1029/2000GC000091.

Schmitt, A.-D., F. Chabaux, and P. Stille (2003), The calcium riverine and hydrothermal isotopic fluxes and the oceanic calcium mass balance, *Earth Planet. Sci. Lett.*, *213*, 503–518.

Schouten, S., J. Ossebaar, K. Schreiber, M. V. M. Kienhuis, G. Langer, A. Benthien, and J. Bijma (2006), The effect of temperature, salinity and growth rate on the stable hydrogen isotopic composition of long chain alkenones produced by *Emiliania huxleyi* and *Gephyrocapsa oceanica*, *Biogeosciences*, *3*, 113–119.

Sime, N. G., C. L. De La Rocha, and A. Galy (2005), Negligible temperature dependence of calcium isotope fractiona-

tion in 12 species of planktonic foraminifera, *Earth Planet. Sci. Lett.*, *232*, 51–66.

Skulan, J., D. J. DePaolo, and T. L. Owens (1997), Biological control of calcium isotopic abundances in the global calcium cycle, *Geochim. Cosmochim. Acta*, *61*, 2505–2510.

Stoll, M. H. C., K. Bakker, G. H. Nobbe, and R. R. Haese (2001), Continuous-flow analysis of dissolved inorganic carbon content in seawater, *Anal. Chem.*, *73*, 4111–4116.

Zhu, P., and J. D. Macdougall (1998), Calcium isotopes in the marine environment and the oceanic calcium cycle, *Geochim. Cosmochim. Acta*, *62*, 1691–1698.

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