Volume 260, Issues 3–4 30 August 2007 ISSN 0012-821X

Available online at www.sciencedirect.com
ScienceDirect
Earth and Planetary Science Letters 260 (2007) 505–515
EPSL
www.elsevier.com/locate/epsl

Calcium isotope fractionation in coccoliths of cultured *Calcidiscus leptoporus*, *Helicosphaera carteri*, *Syracosphaera pulchra* and *Umbilicosphaera foliosa*

Nikolaus Gussone [a,b,*], Gerald Langer [c], Markus Geisen [c], Blair A. Steel [d], Ulf Riebesell [e]

[a] *Research Centre Ocean Margins, University of Bremen, P.O. Box 330440 D-28334 Bremen, Germany*
[b] *Institut für Mineralogie, Universität Münster, Corrensstr. 24, D-48149 Münster, Germany*
[c] *Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany*
[d] *Department of Palaeontology, The Natural History Museum, South Kensington, London, SW7 5BD, United Kingdom*
[e] *Leibniz Institute of Marine Sciences at the University of Kiel, Dienstgebäude Westufer, Düsternbrooker Weg 20, 24105 Kiel, Germany*

Received 22 December 2006; received in revised form 14 May 2007; accepted 1 June 2007
Available online 12 June 2007
Editor: H. Elderfield

Abstract

Four species of marine calcifying algae, the coccolithophores *Calcidiscus leptoporus*, *Helicosphaera carteri*, *Syracosphaera pulchra* and *Umbilicosphaera foliosa* were grown in laboratory cultures under temperatures varying between 14 and 23 °C, and one species, *C. leptoporus*, under varying [CO_3^{2-}], ranging from 105 to 219 μmol/kg. Calcium isotope compositions of the coccoliths resemble in both absolute fractionation and temperature sensitivity previous calibrations of marine calcifying species e.g. *Emiliania huxleyi* (coccolithophores) and *Orbulina universa* (planktonic foraminifera) as well as inorganically precipitated $CaCO_3$, but also reveal small species specific differences. In contrast to inorganically precipitated calcite, but similar to *E. huxleyi* and *O. universa*, the carbonate ion concentration of the medium has no statistically significant influence on the Ca isotope fractionation of *C. leptoporus* coccoliths; however, combined data of *E. huxleyi* and *C. leptoporus* indicate that the observed trends might be related to changes of the calcite saturation state of the medium. Since coccoliths constitute a significant portion of the global oceanic $CaCO_3$ export production, the Ca isotope fractionation in these biogenic structures is important for defining the isotopic composition of the Ca sink of the ocean, one of the key parameters for modelling changes to the marine Ca budget over time. For the present ocean our results are in general agreement with the previously postulated and applied mean value of the oceanic Ca sink (Δ_{sed}) of about −1.3‰, but the observed inter- and intra-species differences point to possible changes in Δ_{sed} through earth history, due to changing physico-chemical conditions of the ocean and shifts in floral and faunal assemblages.

Keywords: coccolithophores; calcium isotopes; *Calcidiscus leptoporus*; *Helicosphaera carteri*; *Syracosphaera pulchra*; *Umbilicosphaera foliosa*; $\delta^{44/40}$Ca; δ^{44}Ca; δ^{mu}Ca

1. Introduction

Coccolithophores are unicellular marine phytoplankton, belonging to the taxon Haptophyta and are

* Corresponding author. Institut für Mineralogie, Universität Münster, Corrensstr. 24, D-48149 Münster, Germany.
E-mail address: nikolaus.gussone@uni-muenster.de (N. Gussone).

0012-821X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.epsl.2007.06.001

characterized by an exoskeleton composed of minute calcite platelets, the 'coccoliths'. Coccolithophores are important primary producers and contribute about half the total marine $CaCO_3$ export production (Milliman, 1993). As a consequence, they are an important link between global calcium (Ca) and carbon (C) cycling. Because they act as a major Ca sink, coccoliths play an important role in determining the isotopic Ca budget of the marine realm, and consequently their isotopic composition is an important parameter for reconstructing the Ca budget of the global ocean (De La Rocha and DePaolo, 2000; Schmitt et al., 2003; Soudry et al., 2004; Heuser et al., 2005; Fantle and DePaolo, 2005). However, previous studies on coccolith oozes have reported a disparate range of seemingly conflicting Ca isotope values. De La Rocha and DePaolo (2000) and Fantle and DePaolo (2005) reported Ca isotope values of coccolith oozes similar to cultured specimens of *Emiliania huxleyi* (16 °C: $1000 \cdot \ln\alpha = -1.3‰$; with $\alpha = (^{44}Ca/^{40}Ca)_{coccolith}/(^{44}Ca/^{40}Ca)_{seawater}$) (De La Rocha and DePaolo, 2000). In contrast, Zhu and Macdougall (1998) observed considerably lighter Ca isotope values in Holocene coccolith oozes ($1000 \cdot \ln\alpha = -1.9$ to -2.6).

Detailed investigation of the Ca isotope response to changing environmental parameters such as salinity, pCO_2/pH, illumination, Ca^{2+} concentration and temperature, identified temperature, apart from the isotopic composition of the medium, as the main factor influencing the Ca isotope composition of *E. huxleyi* coccoliths (Gussone et al., 2006; Langer et al., 2007). The small but significant temperature dependence of $0.027 \pm 0.006‰$/°C (7.3 ± 1.6 ppm/amu/°C) found in *E. huxleyi* is too limited to be a possible explanation for the observed disparity in Ca isotope values between Quaternary coccolith oozes (Zhu and Macdougall, 1998; De La Rocha and DePaolo, 2000; DePaolo, 2004).

To investigate if species-specific vital effects might be responsible for the observed discrepancies in the Ca isotope composition of bulk marine carbonate oozes, we cultured four different coccolithophore species under varying temperature regimes, as well as one species under different pCO_2 levels. These parameters were chosen because previous studies revealed that temperature and carbonate chemistry can have a large effect on Ca isotope fractionation: Different species of foraminifera exhibit temperature dependent Ca isotope fractionation patterns, with temperature sensitivities differing by roughly an order of magnitude between taxa (Zhu and Macdougall, 1998; Nägler et al., 2000; Gussone et al., 2004), and Lemarchand et al. (2004) observed a large sensitivity of Ca isotope fractionation of inorganically precipitated calcite in response to changes in the carbonate ion concentration of the fluid.

2. Materials and methods

2.1. Coccolithophore culturing

We cultured coccolithophore species from the CODENET culture collection maintained in the ALGOBANK laboratory at the University de Basse Normandie in Caen, France. Four species, *Syracosphaera pulchra* (strain GK7, GK17), *Calcidiscus leptoporus* (strains NS10-2, ASM31), *Umbilicosphaera foliosa* (strain ESP 6MI) and *Helicosphaera carteri* (strains NS10-8, NS8-4) were grown in monoclonal cultures covering a temperature range from 14 to 23 °C (Table 2) at The Natural History Museum (NHM), London. In addition one *C. leptoporus* strain (AS31) was grown at different CO_2 levels at the Alfred Wegener Institute (AWI), Bremerhaven. SEM illustrations of the investigated coccolithophore species are displayed in Plate I. In the past *C. leptoporus* was informally divided into three size clusters, small, intermediate, and large. Recent life cycle and morphological research resulted in a division on the subspecies level (Geisen et al., 2002). Sáez et al. (2003) however raised the subspecies to species rank based on molecular data. The cultures used for this work

Table 1
Composition of K medium

Additions	Final concentration in medium (μM)	Comments
(1) KNO_3	884	Same as *f*/2
(2) NH_4Cl	10	Addition to *f*/2
(3) Na_2 ortho-PO_4	36	Same as *f*/2 (K uses organic form)
(4) Trace metals:		
FeEDTA [a]	11.7	*f*/2 uses $FeCl_3$
$MnCl_2 \cdot 4H_2O$	0.9	Same as *f*/2
$ZnSO_4 \cdot 7H_2O$	0.03	Same as *f*/2
$CoSO_4 \cdot 7H_2O$	0.05	Same as *f*/2
$Na_2MoO_4 \cdot 2H_2O$	0.08	Same as *f*/2
$CuSO_4 \cdot 5H_2O$	0.01	One half *f*/2 level
$Na_2EDTA \cdot 2H_2O$	100	Order of magnitude higher than *f*/2
Na_2SeO_3	0.01	Addition to *f*/2
(5) Vitamins		
Thiamin–HCl	0.3	Same as *f*/2
Biotin	0.0021	Same as *f*/2
B12	0.00037	Same as *f*/2
Seawater	To 1 l	

Stock solutions are numbered 1–5. Each stock is made such that the addition of 1 ml/l yields the final concentration in the medium.
1–5 made with reagent grade chemicals and HPLC grade water.
All solutions filter-sterilised through 0.2 μm membrane filters.
1–4 stored at 4 °C.
5 stored frozen at −20 °C.
[a] Ethylenediamine tetra-acetic acid.

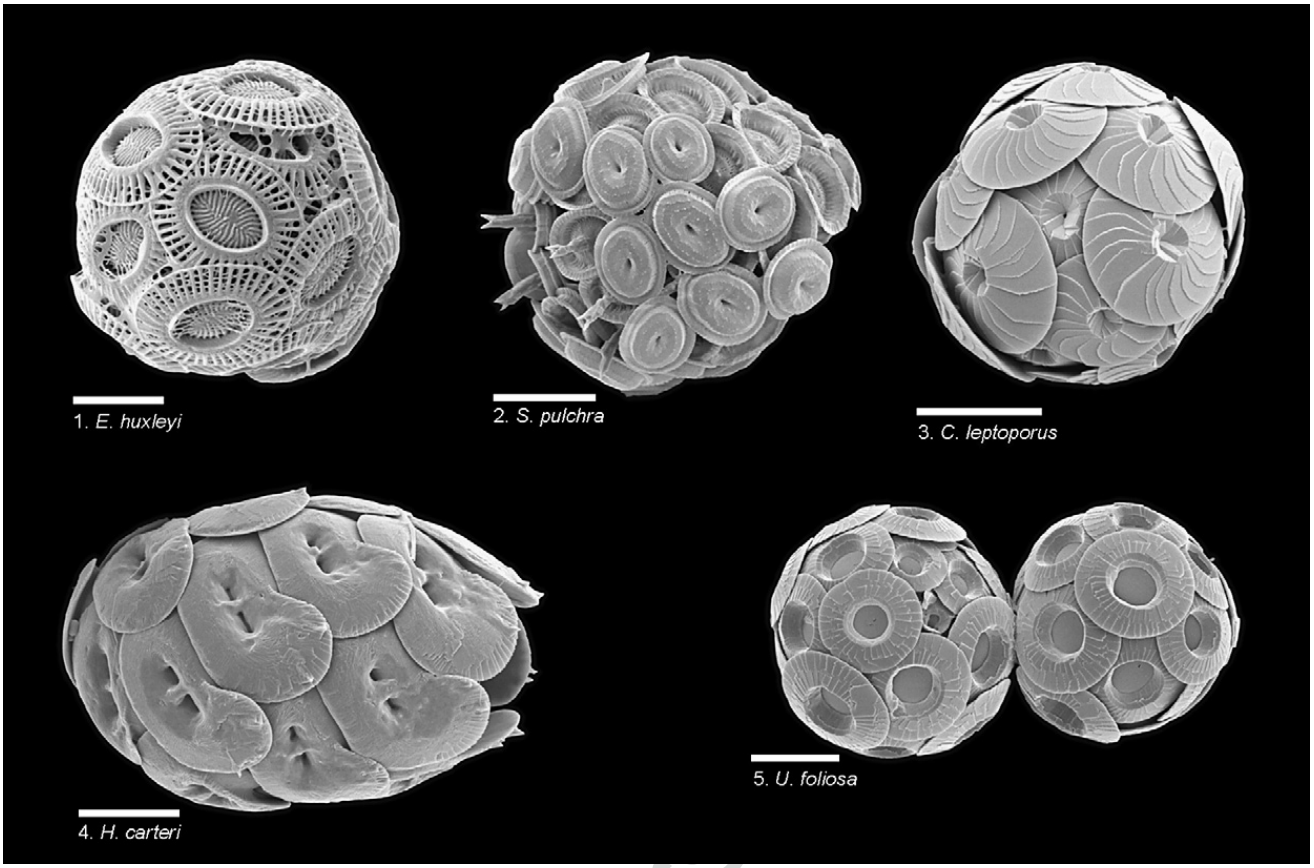

Plate I. Typical morphologies of the coccolithophore species examined in this study. 1: Scanning electron micrograph of an *E. huxleyi* coccosphere. The coccoliths exhibit the A-type morphology. Water sample, N. Atlantic, R/V Meteor cruise 42-4B, station US 1B. 2: Scanning electron micrograph of a *S. pulchra* coccosphere. This typical specimen displays endothecal and exothecal coccoliths. Coccoliths surrounding the flagellar pole are spine bearing. Water sample, N. Atlantic, off the Canary Islands, R/V Poseidon cruise P233B, station 3. 3: Scanning electron micrograph of a *C. leptoporus* coccosphere. Culture sample (NS 10-2), S. Atlantic, off South Africa, R/V Agulhas cruise MARE 2. 4: Scanning electron micrograph of a *H. carteri* coccosphere. The helicoliths show the typical spiral arrangement and possess enlarged flanges in the circumflagellar region. The central area of this specimen shows the typical morphology of two aligned slits, separated by a bar. Water sample, N. Atlantic, Portuguese shelf, R/V Andromeda cruise CODENET 2, station 6. 5: Scanning electron micrograph of two *U. foliosa* coccospheres. *U. foliosa* cells are typically found in clusters of up to four. Water sample, western Mediterranean, Alboran Sea, R/V Hesperides cruise MATER 2, station 69. Scalebar: Fig. 1 — 2 μm, Fig. 2–5 — 5 μm.

represent the former intermediate type respectively the subspecies *leptoporus*.

The cultures used at the NHM were grown under sterile but not axenic conditions on a temperature–light gradient table. The mean irradiance level was 42 (±3) μmol photons m^{-2} s^{-1} and a 16/8 h light/dark cycle was used. All the cultures were grown in 100 ml glass flasks with 75 ml medium. Medium was prepared from seawater collected from the coast of Wales. The seawater was filtered and autoclaved at 120 °C for 15 min. Nutrients (nitrate, phosphate, trace metals and vitamins) were added to the cooled seawater under sterile conditions in a laminar flow cabinet. The chemical composition of the medium resembles media used by Guillard (1975) and Keller et al. (1987). A detailed description of the medium used is given in Table 1.

The *C. leptoporus* strain AS31 used at the AWI was grown in sterile filtered (0.2 μm) seawater (collected in the North Sea near Helgoland) enriched with 100 μM nitrate and 6.25 μM phosphate and with trace metals and vitamins according to *f*/2 (Guillard and Ryther, 1962). The incident photon flux density was 350 μmol photons m^{-2} s^{-1} and a 16/8 h light/dark cycle was applied. Experiments were carried out at 15 °C. Cells were acclimated to experimental conditions for approximately 10 generations and grown in dilute batch cultures (Langer et al., 2006). Low cell density at harvest (in general less than 6000 cells per ml) resulted in less than 8% DIC (dissolved inorganic carbon) consumption (i.e. DIC consumed by the cells at the end of experiment) and a shift in pH of not more than 0.06 units. Calcium consumption was in all experiments less than 1% and therefore a significant shift in Ca isotopic composition of the seawater during the experiment due to the preferential uptake of light Ca isotopes into the precipitated $CaCO_3$ (as for instance observed for $CaCO_3$ precipitated

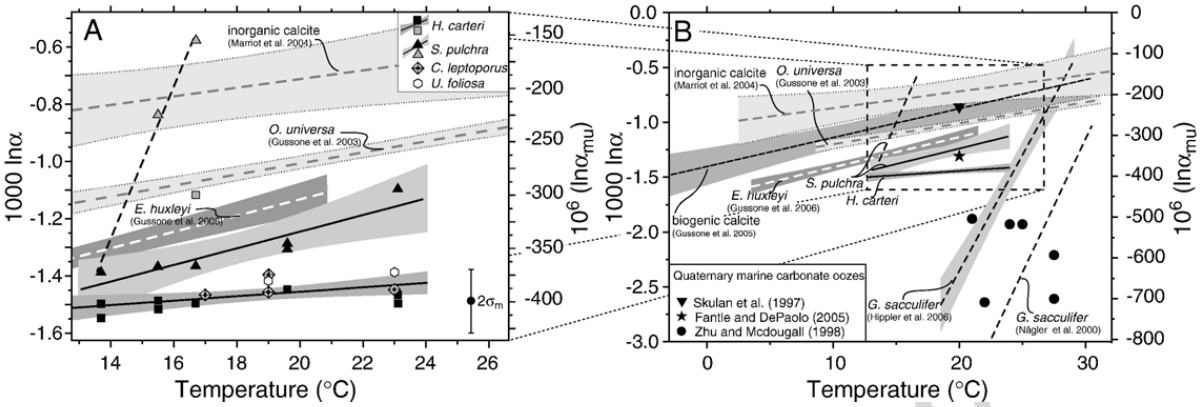

Fig. 1. Temperature dependent Ca isotope fractionation in different coccolithophore species. A: *Syracosphaera pulchra*, *Calcidiscus leptoporus*, *Umbilicosphaera foliosa* and *Helicosphaera carteri* show Ca isotope values similar to *Emiliania huxleyi* (Gussone et al., 2006). Shaded areas mark 95% confidence bands. One data point of *H. carteri* (open square) was not used for the linear regression. *S. pulchra* might exhibit a bimodal temperature response behaviour, with both a small temperature dependence (filled triangles, similar to *E. huxleyi*) and a large temperature dependence (open triangles, dashed line), similar to *G. sacculifer* cf. (Nägler et al., 2000; Chang et al., 2004; Sime et al., 2005; Hippler et al., 2006). B: Calcium isotope fractionation of previously published Quaternary marine carbonate oozes (Skulan et al., 1997; Zhu and Macdougall, 1998; Fantle and DePaolo, 2005) compared to different $CaCO_3$ calibrations. Corresponding temperatures for carbonate oozes derived from the literature were approximated by using mean annual sea surface temperatures obtained from the World Ocean Atlas (2001). The strong isotope fractionation reported for coccolith-oozes (Zhu and Macdougall, 1998) cannot be explained by the cultured coccolithophore species, but rather resembles Ca isotope fractionation of certain planktonic foraminifers (Nägler et al., 2000; Hippler et al., 2006).

in semi enclosed porewater regimes (Teichert et al., 2005)) can be excluded. CO_2 levels were adjusted by adding calculated amounts of 1 M HCl or 1 M NaOH to the medium. In order to prevent gas exchange with the atmosphere, 2.4 l borosilicate flasks were filled without headspace and closed with teflon lined screw caps. For the determination of growth rate however, regular sampling for cell counts was required. The maximum headspace created by this sampling is 6 ml and the resultant air–water CO_2 equilibration of 3% shift in the CO_2aq concentration is negligible. Samples for alkalinity measurements were filtered (approx. 0.6 μm), poisoned with 1 ml of an $HgCl_2$ solution (35 g l^{-1}) 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 duplicate potentiometric titration (Bradshaw et al., 1981; Brewer et al., 1986) and 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 program CO_2sys (Lewis and Wallace, 1998), applying the equilibrium constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

Cultured coccolithophores were harvested in the late exponential growth phase and samples were washed and centrifuged to remove salt. Pellets were dried at 60 °C for 48 h and subsequently stored at room temperature.

2.2. Sample preparation

The coccolith samples were transferred into acid cleaned PP reaction vials and bleached for 24 h in a 10% NaClO solution (~1% active chlorine). During the bleaching the samples were ultrasonicated several times. The bleach was subsequently removed and the samples were washed 6 times in distilled water, the pH of which was elevated to 8–9 by the addition of NH_4OH-solution to prevent partial dissolution of the coccolith carbonate during the cleaning process. The samples were then dissolved in 0.5 N HCl and transferred into Teflon vials. The solution was evaporated and recovered in 2.5 N HCl. Aliquots of the sample solution, corresponding to 300–400 ng Ca, were mixed with a ^{43}Ca–^{48}Ca-double spike for the correction of isotope fractionation during the data acquisition in the mass spectrometer (Heuser et al., 2002).

2.3. Ca isotope analysis

Calcium isotope ratios were determined on a Finnigan Triton T1 thermal ionization mass spectrometer at the IFM-GEOMAR, following the method described in Heuser et al. (2002). The Ca isotope values are expressed as $\delta^{44/40}Ca$ values ($\delta^{44/40}Ca$ [‰ SRM915a]=(($^{44}Ca/^{40}Ca)_{sample}/(^{44}Ca/^{40}Ca)_{SRM\ 915a}-1)\cdot 1000$), and as $\delta^{mu}Ca$ (ppm/amu)$=268.3\cdot\delta^{44/40}Ca$ (Gussone et al., 2005), in order to increase the comparability with Ca isotope data based on Ca isotope ratios other than $^{44}Ca/^{40}Ca$ (e.g. $^{44}Ca/^{42}Ca$). The isotope fractionation between growth

Table 2
Temperature dependent Ca isotope fractionation of cultured coccolithophore species

Sample	Origin	T [°C]	$\delta^{44/40}$Ca (‰SRM915a)	$1000 \cdot \ln(\alpha)$	δ^{mu}Ca (ppm/amu)	$10^6 \cdot \ln\alpha_{mu}$
Helicosphaera carteri						
NS 10-8-1	S. Atlantic	13.7	0.38	−1.50	103	−402
NS 10-8	S. Atlantic	15.5	0.36	−1.52	96	−408
NS 10-8	S. Atlantic	16.7	0.38	−1.49	103	−401
NS 10-8	S. Atlantic	19.6	0.43	−1.44	117	−388
NS 10-8	S. Atlantic	23.1	0.41	−1.47	111	−394
NS 8-4	S. Atlantic	13.7	0.33	−1.55	90	−415
NS 8-4	S. Atlantic	15.5	0.39	−1.49	104	−401
NS 8-4	S. Atlantic	16.7	0.76	−1.12	204	−301
NS 8-4	S. Atlantic	19.6	0.43	−1.45	114	−390
NS 8-4	S. Atlantic	23.1	0.38	−1.49	103	−402
Syracosphaera pulchra						
GK 7	W. Mediterranean	13.7	0.49	−1.39	132	−373
GK 7	W. Mediterranean	15.5	1.04	−0.84	280	−224
GK 7	W. Mediterranean	16.7	0.51	−1.37	137	−368
GK 7	W. Mediterranean	19.6	0.57	−1.31	152	−352
GK 7	W. Mediterranean	23.1	0.78	−1.10	210	−294
GK 17	W. Mediterranean	15.5	0.51	−1.37	136	−369
GK 17	W. Mediterranean	16.7	1.30	−0.58	349	−156
GK 17	W. Mediterranean	19.6	0.59	−1.29	159	−345
Calcidiscus leptoporus						
NS 10-2	S. Atlantic	17	0.41	−1.47	109	−395
NS 10-2	S. Atlantic	19	0.48	−1.40	129	−376
NS 10-2	S. Atlantic	23	0.43	−1.45	115	−390
ASM 31	W. Mediterranean	19	0.42	−1.45	114	−391
Umbilicosphaera foliosa						
ESP6MI	W. Mediterranean	19	0.46	−1.42	124	−381
ESP6MI	W. Mediterranean	23	0.49	−1.38	133	−372

Column 5: fractionation factor $\alpha = ({}^{44}Ca/{}^{40}Ca)_{solid}/({}^{44}Ca/{}^{40}Ca)_{fluid}$. Fluid is seawater ($\delta^{44/40}$Ca: 1.88‰ SRM 915a).
Column 6: δ^{mu}Ca [ppm/amu SRM915a] = $(({}^{a}Ca/{}^{b}Ca)sample/({}^{a}Ca/{}^{b}Ca)SRM915a - 1) \cdot (1/(a-b)) \cdot 10^6)$ and a, b being the masses of the respective Ca isotopes (Gussone et al., 2005).
Column 7: fractionation factor $\alpha_{mu} = \alpha^{a/((b+1).(a-b))}$ (assuming equilibrium isotope fractionation) with $\alpha = ({}^{a}Ca/{}^{b}Ca_{cc})/({}^{a}Ca/{}^{b}Ca_{fluid})$ and a, b being the masses of the respective Ca isotopes (Gussone et al., 2005).

medium and carbonate is reported as $1000 \cdot \ln\alpha$ (with $\alpha = ({}^{44}Ca/{}^{40}Ca_{solid})/({}^{44}Ca/{}^{40}Ca_{fluid})$) as well as $10^6 \cdot \ln(\alpha_{mu})$, providing the isotope fractionation per 1 atomic mass unit (amu) (with $\alpha_{mu} = \alpha^{0.2683}$ (Gussone et al., 2005)). Data from previous publications were converted to NIST SRM 915a using the following relations: $\Delta^{44/40}Ca_{(Seawater-SRM\ 915a)} = 1.88‰$, $\Delta^{mu}Ca_{(Seawater-SRM\ 915a)} = 505$ ppm/amu. $\Delta^{44/40}Ca_{(CaF2-SRM\ 915a)} = 1.44‰$, $\Delta^{mu}Ca_{(CaF2-SRM\ 915a)} = 387$ ppm/amu. The average $2\sigma_m$ of our samples is 0.12‰ (30 ppm/amu) determined by repeated aliquot measurements of various sample materials.

3. Results

The Ca isotope values of the four investigated coccolithophore species (Fig. 1, Table 2) fall within the range of previous experiments on marine biogenic calcites cf. (Skulan et al., 1997; Zhu and Macdougall, 1998; De La Rocha and DePaolo, 2000; Chang et al., 2004; Gussone et al., 2005; Sime et al., 2005; Heuser et al., 2005). The relationships between temperature and Ca isotope fractionation in *S. pulchra* and *H. carteri* are given in Table 3 (Eqs. (1)–(3)). We calculated independent equations for two distinct data arrays defined by *S. pulchra*, differing in temperature sensitivity by a factor of about 10 (Table 3, Eqs. (1) and (2)). For *U. foliosa* and *C. leptoporus*, no equations are shown, because the temperature range is not large enough to define a reliable relationship.

The temperature sensitivity of *H. carteri* (Table 3, Eq. (3)) is small and does not exceed the analytical uncertainty. The temperature dependence of *S. pulchra*

Table 3
Temperature dependent Ca isotope fractionation of cultured coccolithophores

	R^2	p	n	Equation
S. pulchra (filled triangles) [a]				
$1000 \cdot \ln(\alpha) = -1.83 \pm 0.22 + (0.029 \pm 0.013) \cdot T$ (°C)	0.84	<0.001	6	(1)
$10^6 \cdot \ln(\alpha_{mu}) = -494 \pm 64 + (7.8 \pm 3.4) \cdot T$ (°C)				
S. pulchra (strong temperature dependence; open triangles) [a]				
$1000 \cdot \ln(\alpha) = -5.11 \pm 0.37 + (0.27 \pm 0.05) \cdot T$ (°C)	0.99	0.05	3	(2)
$10^6 \cdot \ln(\alpha_{mu}) = -1370 \pm 218 + (73 \pm 14) \cdot T$ (°C)				
Helicosphaera carteri (black squares) [b]				
$1000 \cdot \ln(\alpha) = -1.58 \pm 0.1 + (0.005 \pm 0.005) \cdot T$ (°C)	0.35	0.09	9	(3)
$10^6 \cdot \ln(\alpha_{mu}) = -425 \pm 25 + (1.4 \pm 1.4) \cdot T$ (°C)				

The uncertainties in the equations represent 2σ-errors.
[a] We calculated independent equations for two distinct data arrays defined by *S. pulchra*, differing in temperature sensitivity by a factor of about 10.
[b] For calculation of Eq. (3) one data point (grey square in Fig. 1) was excluded, because it strongly deviated from the other values, possibly due to contamination.

(0.029±0.013‰/°C; 7.8±3.4 ppm/amu/°C; Table 3, Eq. (1)) is identical within analytical uncertainty, to that previously determined for *E. huxleyi* (0.027±0.006‰/°C; 7.3±1.6 ppm/amu/°C) (Gussone et al., 2006) and similar to the planktonic foraminifer *Orbulina universa* (0.019±0.003‰/°C; 4.8±0.8 ppm/amu/°C), inorganic aragonite (0.015±0.002‰/°C; 3.7±0.4 ppm/amu/°C) (Gussone et al., 2003), biogenic calcite (0.026±0.01‰/°C; 6.6±2.6 ppm/amu/°C), biogenic aragonite (0.017±0.006‰/°C; 4.3±1.4 ppm/amu/°C) (Gussone et al., 2005) and inorganic calcite (0.015±0.013‰/°C; 3.8±3.2 ppm/amu/°C) (Marriott et al., 2004).

The response of calcium isotope fractionation in coccoliths of *C. leptoporus* to changes in carbonate concentration is shown in Fig. 2, Tables 4 and 5 (Eq. (4)). The small and statistically insignificant dependence of Ca isotope fractionation on the $[CO_3^{2-}]$ is consistent with previous findings for *E. huxleyi* (Gussone et al., 2006) and *O. universa* (Gussone et al., 2003), but different from trends observed in inorganically precipitated calcite (Lemarchand et al., 2004), which exhibits a strong dependence of calcium isotope fractionation on $[CO_3^{2-}]$, opposite in sign, showing reduced isotope fractionation at higher carbonate ion concentrations. The lighter Ca isotope ratios (−0.3‰) of *C. leptoporus* compared to *E. huxleyi* are consistent with a small species-specific offset between both coccolithophores and the temperature difference of 5 °C between the two sets of experiments.

4. Discussion

Coccoliths of the four investigated coccolithophore species *C. leptoporus*, *U. foliosa*, *S. pulchra* and *H. carteri* show calcium isotope values in the same range as most previously published marine $CaCO_3$ samples. Together with previous results of *E. huxleyi* (De La Rocha and DePaolo, 2000; Gussone et al., 2006), the predominant part of the coccolithophores cover a range of about 0.5‰ (~130 ppm/amu), ranging from about −1.1 to −1.6‰ $1000 \cdot \ln\alpha$ (−300 to −430 ppm/amu), and the observed small temperature dependences are similar to several other marine calcifying organisms cf. (Gussone et al., 2003; Marriott et al., 2004; Gussone et al., 2005; Böhm et al., 2006). Despite this overall agreement, there are species-specific differences in coccolithophores with respect to absolute Ca isotope fractionation and temperature sensitivity. Coccoliths of *C. leptoporus*, *H. carteri* and *U. foliosa* are in general stronger fractionated compared to *E. huxleyi* and *S.*

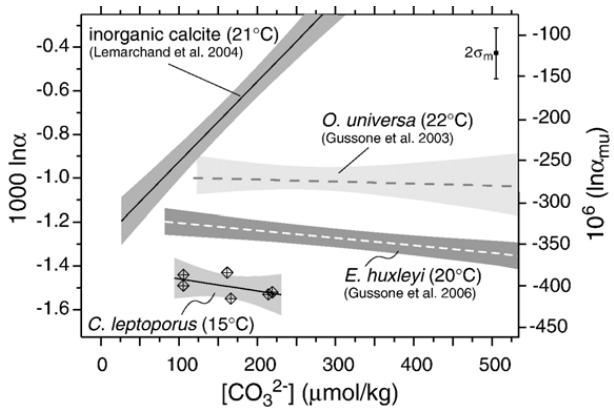

Fig. 2. Calcium isotope fractionation as a function of carbonate concentration of *C. leptoporus* (15 °C) compared to published data of inorganic calcite (21 °C) (Lemarchand et al., 2004), *O. universa* (22 °C) (Gussone et al., 2003) and *E. huxleyi* (20 °C) (Gussone et al., 2006). The response of Ca isotope fractionation on changes in $[CO_3^{2-}]$ of *C. leptoporus* is similar to *E. huxleyi* and *O. universa*, showing a minor dependence between $[CO_3^{2-}]$ and Ca isotope fractionation, in contrast to inorganic calcite (Lemarchand et al., 2004). Shaded areas represent 95% confidence bands.

Table 4
Ca isotope fractionation of *C. leptoporus* in response to $[CO_3^{2-}]$ changes

Sample	Origin	$[CO_3^{2-}]$ (μmol/kg)	$\delta^{44/40}$Ca (‰SRM915a)	1000· ln(α)	δ^{mu} Ca (ppm/amu)	$10^6 \cdot \ln\alpha_{mu}$
Calcidiscus leptoporus (15 °C)						
1 AS31 360	W. Mediterranean	214	0.33	−1.55	89	−416
2 AS31 360	W. Mediterranean	219	0.34	−1.54	91	−413
1 AS31 225	W. Mediterranean	162	0.43	−1.45	116	−389
2 AS31 225	W. Mediterranean	166	0.31	−1.57	83	−421
1 AS31 679	W. Mediterranean	106	0.42	−1.46	113	−392
2 AS31 679	W. Mediterranean	105	0.37	−1.51	99	−405

Columns 5, 6, 7: for details of calculation of α, δ^{mu}Ca and α_{mu} see captions of Table 2.

pulchra coccoliths (0.1–0.4‰; 25–100 ppm/amu) grown at identical temperatures. The difference is attenuated at lower temperatures, at which the Ca isotope fractionation of the latter species is increased due to their stronger pronounced temperature sensitivity (0.027 and 0.029‰/°C).

In contrast to the otherwise relatively limited range of Ca isotope fractionation in coccoliths from cultured specimens (−1.1 to −1.6‰; −300 to −430 ppm/amu), two samples of *S. pulchra* (Fig. 2 grey triangles) fall well outside the temperature dependent array, revealing considerably less fractionated values of up to −0.6‰ (−160 ppm/amu). It is unclear if these values represent a second array exhibiting a roughly 10-fold stronger temperature dependence (0.27‰/°C) resembling the large temperature sensitivity observed for the planktonic foraminifer *G. sacculifer* (0.24‰/°C) cf. (Nägler et al., 2000), or if they are artefacts, most likely caused by strong contamination. An analytical artefact is unlikely, because the values reproduced on replicate measurements. Though the array showing a large temperature sensitivity is poorly defined, this observation warrants further investigation, because such a behaviour would restrict the applicability of Ca isotopes in coccolithophore oozes as proxy archives, but could provide as well insights into biomineralisation-related fractionation processes. The potential occurrence of two different temperature sensitivities in one species is further indicated by considerably different values for temperature dependent Ca isotope fractionation reported for *G. sacculifer* cf. (Zhu and Macdougall, 1998; Nägler et al., 2000; Chang et al., 2004; Sime et al., 2005; Gussone et al., in revision). A potential mechanism for the establishment of two arrays in *S. pulchra* is unclear, because its physiology and biomineralisation processes are not well constrained; though it is likely that the observed variability might be associated with changes in the cellular Ca usage, including the use of internal Ca storage pools, as proposed for planktonic foraminifera (Gussone et al., 2003; Marriott et al., 2004; Gussone et al., in revision). Genetic differences seem to be unlikely to cause the observed intra-specific variability, because the two clones used for this experiment were isolated from the same water mass and data of both *S. pulchra* strains contribute to the anomalous array. An additional parameter, the ratio of endothecal to exothecal coccoliths (Plate I-2), could affect Ca isotope fractionation in coccolithophore cultures. However, we did not observe a clear shift in the amounts of cells with predominantly endothecal or exothecal coccoliths in our samples, and therewith this explanation can most likely not account

Table 5
Ca isotope fractionation of cultured coccolithophores depending on CO_3^{2-} and Ω

	R^2	p	n	Equation
CO_3^{2-}: *C. leptoporus*				
$1000 \cdot \ln(\alpha) = -1.42 \pm 0.14 - (5.5 \pm 8.2) \cdot 10^{-4}$ $[CO_3^{2-}]$ (μmol/kg)	0.31	0.25	6	(4)
$10^6 \cdot \ln(\alpha_{mu}) = -383 \pm 78 - (0.15 \pm 0.22) \cdot [CO_3^{2-}]$ (μmol/kg)				
$\Omega_{calcite}$: *E. huxleyi* (recalculated from (Gussone et al., 2006) and (Langer et al., 2007):				
$1000 \cdot \ln \alpha = -1.13 \pm 0.06 - (0.096 \pm 0.04) \ln\Omega$	0.54	<0.0001	23	(5a)
$10^6 \cdot \ln(\alpha_{mu}) = -304 \pm 18 - (26 \pm 11) \ln\Omega$				
Regression based on mean values:				
$1000 \cdot \ln \alpha = -1.13 \pm 0.01 - (0.096 \pm 0.06) \ln\Omega$;	0.996	<0.0001	6	(5b)
$10^6 \cdot \ln(\alpha_{mu}) = -304 \pm 3 - (26 \pm 18) \ln\Omega$				
$\Omega_{calcite}$: *C. leptoporus*				
$1000 \cdot \ln \alpha = -1.4 \pm 0.17 - (0.080 \pm 0.12) \ln\Omega$	0.31	0.25	6	(6a)
$10^6 \cdot \ln(\alpha_{mu}) = -377 \pm 46 - (23 \pm 34) \ln\Omega$				
Regression based on mean values:				
$1000 \cdot \ln \alpha = -1.4 \pm 0.048 - (0.080 \pm 0.03) \ln\Omega$	0.96	0.13	3	(6b)
$10^6 \cdot \ln(\alpha_{mu}) = -377 \pm 13 - (22 \pm 10) \ln\Omega$				

Uncertainties in the equations represent 2σ-errors.

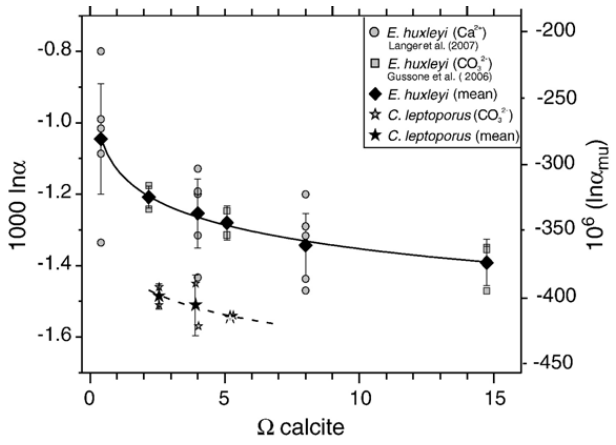

Fig. 3. Calcium isotope fractionation as a function of calcite supersaturation (Ω): Ω of *C. leptoporus* was altered by changing $[CO_3^{2-}]$, while for *E. huxleyi* Ω was modified by $[CO_3^{2-}]$ (Gussone et al., 2006) and $[Ca^{2+}]$ (Langer et al., 2007). Despite the relatively large scatter, Ω seems to influence the Ca isotope fractionation of the coccoliths. This dependence is opposite in sign to inorganic precipitation experiments (Lemarchand et al., 2004) and might rather be related to changes in the cellular Ca utilisation in response to the lowered calcite saturation in the bulk fluid, than to changes in the growth or calcification rate.

for the two temperature dependencies observed in *S. pulchra* (Fig. 1).

A further important factor besides temperature and species-specific variability which can influence Ca isotope fractionation is the carbonate system of the culturing medium. The small $[CO_3^{2-}]$ sensitivity of *C. leptoporus* (Fig. 2), in contrast to inorganic calcite (Lemarchand et al., 2004), resembles previous observations on *E. huxleyi* (Gussone et al., 2006) and *O. universa* (Gussone et al., 2003). The small negative correlation between $[CO_3^{2-}]$ and Ca isotope ratios of the three species cannot be explained as an alleviated residual rate effect, because this should lead to a reduced fractionation with increasing $[CO_3^{2-}]$. Instead, the inverse correlation between $[CO_3^{2-}]$ and Ca isotope ratios might be related to a metabolic response to changes in the ambient pH, or increased Ca utilisation from cellular Ca reservoirs at lower calcite supersaturation ($\Omega = [Ca^{2+}] \cdot [CO_3^{2-}]/K_{sp}$; with K_{sp} = seawater calcite saturation product). The latter explanation is in agreement with the observation that coccoliths of *E. huxleyi* also display slightly heavier calcium isotope values when cultured at low external Ca concentration and hence lower calcite saturation of seawater (or accordingly larger isotope fractionation when cultured at higher Ω) (Langer et al., 2007). Combining the results from the experiments with changing $[CO_3^{2-}]$ (Gussone et al., 2006) and $[Ca^{2+}]$ (Langer et al., 2007) of *E. huxleyi* and plotted as Ω, the data show a logarithmic increase of Ca isotope fractionation with increasing Ω (Fig. 3; Table 5, Eqs. (5) and (6)). The experiment using *C. leptoporus* spans a relatively small range of Ω values compared to the analytical uncertainty of the Ca isotope ratios, but the regression is almost parallel to the better defined trend of *E. huxleyi*.

The calcium isotope fractionation of coccolithophores investigated so far (−1.1 to −1.6‰; −300 to −430 ppm/amu) falls within the range of previously determined marine biogenic carbonates (−0.7 to −1.7‰; −190 to −460 ppm/amu cf. (Skulan et al., 1997; De La Rocha and DePaolo, 2000; Gussone et al., 2003; DePaolo, 2004; Chang et al., 2004; Marriott et al., 2004; Gussone et al., 2005; Sime et al., 2005), and is in general agreement with the previously postulated mean Δ_{sed} of −1.3‰ (−350 ppm/amu) cf. (De La Rocha and DePaolo, 2000). But our results show as well significant inter- and intra-specific differences in Ca isotope fractionation of coccolithophores of up to 0.5‰ (135 ppm/amu) (considering the anomalous data of *S. pulchra* up to 1.0‰; 270 ppm/amu) as well as differences between coccolithophores and other marine calcifiers, indicating that coccolithophores exhibit slightly more fractionated Ca isotope values compared to most foraminifera cf. (Skulan et al., 1997; Chang et al., 2004; Gussone et al., 2005; Sime et al., 2005) and corals cf. (Böhm et al., 2006). The observed variability of coccolithophore Ca isotope fractionation has the potential to significantly affect the global isotopic Ca budget, by introducing a temporal and spatial variability of the Ca isotope composition of marine carbonate sediments. While short term climate variability affects mainly the Ca isotope fractionation for the local sediments (and having less effect on the mean isotopic composition of the oceanic Ca-sink (Δ_{sed}) (Fantle and DePaolo, 2005)), long term shifts in ocean chemistry and major shifts in floral and faunal assemblages and the regional distribution of $CaCO_3$ export production might alter the mean global Δ_{sed} through earth history. These factors become more important for oceanic Ca budget reconstruction when extended further back in earth history cf. (Farkaš et al., 2007; Soudry et al., 2004) to eras in which the chemical composition of the ocean and assemblages of calcifying biota significantly differed from today's conditions.

To interpret the comparatively small variabilities of paleo $\delta^{44/40}Ca_{sw}$ values reliably when modelling the oceanic Ca budget over time, it is of particular importance to use well constrained values of the global mean Δ_{sed} and $1000 \cdot \ln\alpha$ of the respective proxy archives, which are to a considerable extent influenced by the Ca isotopic composition of coccolithophore calcite. For instance, the carbonate fraction of surface sediments in the oligotrophic gyres of the South Atlantic is dominated by

coccolithophores (up to 70 wt.%), while coccolith calcite makes up 20 wt.% of the carbonate fraction of sediments on the continental margins, where foraminifera are more important (Baumann et al., 2004). In mid-Atlantic Ridge sediments of the central South Atlantic *C. leptoporus* contributes more than 50 wt.% to the total carbonate, while *S. pulchra* coccoliths constitute up to 4 wt.%. In the equatorial and central South Atlantic *Helicosphaera* spp. contribute up to 10 wt.%, which makes them more important carbonate producers than *E. huxleyi* and *U. foliosa* (about 3 wt.% each) in this region. In view of the significant contribution to the oceanic $CaCO_3$ export, the determined Ca isotope fractionation of the investigated coccolithophore species help to better define the isotopic composition of the Ca sink in the marine realm.

Despite the variability shown by the cultured coccolithophores with respect to species-differences, temperature or carbonate chemistry our data cannot explain the strong fractionation reported for coccolithophore oozes by Zhu and Macdougall (1998). Judging from the five cultured species it appears unlikely that the observed differences in calcium isotope values between different Quaternary coccolith oozes (Zhu and Macdougall, 1998; De La Rocha and DePaolo, 2000; DePaolo, 2004; Fantle and DePaolo, 2005) are caused by species-specific calcium isotope fractionation of coccolithophores. It is more likely that components other than coccoliths are responsible for the light Ca isotopic signature of these oozes; these might include other biogenic carbonates (e.g. certain planktonic foraminifera) or products of early diagenesis. Since the origin and the spatial or temporal distribution of these oozes is still unclear it cannot be decided whether these oozes bear a meaning for the global mean Δ_{sed}. However, also on local scales such sediments can be problematic, if bulk sediment samples are used as Ca isotope proxy archives.

5. Summary and conclusions

The calcium isotopic composition of coccoliths of the five coccolithophore species analysed so far, *C. leptoporus*, *H. carteri*, *S. pulchra*, *U. foliosa* and *E. huxleyi*, resembles previously reported marine carbonates with respect to absolute fractionation and temperature sensitivity, but shows some significant species-specific differences, which are however relatively small compared to the variability of reported coccoliths oozes cf. (Zhu and Macdougall, 1998; De La Rocha and DePaolo, 2000). Changes in floral composition are therefore unlikely to explain the large Ca isotopic differences observed in the different coccolith oozes. The origin of these light $\delta^{44/40}$Ca values is unclear, but might be related to other biogenic $CaCO_3$ components or be the result of early diagenesis. Unlike inorganic calcite, *C. leptoporus* shows only a small and statistically insignificant response of Ca isotope fractionation to $[CO_3^{2-}]$, similar to results of *E. huxleyi* and *O. universa*; nevertheless, Ca isotope fractionation in coccolithophores might be influenced by a metabolic response to calcite supersaturation of the medium, leading to larger isotope fractionation at high Ω and less fractionation at low Ω, possibly due to increased cellular Ca utilisation at decreasing Ω. Calcium isotope fractionation of cultured coccolithophores is in agreement with a present mean Δ_{sed} of about −1.3‰, but also indicate that Δ_{sed} may not be constant over time, being affected by long- and short-term shifts in faunal and floral assemblages and variation of physical and chemical conditions of the ocean (e.g. temperature and Ω). The use of bulk carbonate sediment as a proxy archive appears to be problematic, because the fractionation factor for the locally deposited $CaCO_3$ sediments is not constant, being affected by the processes discussed above with respect to Δ_{sed}.

Acknowledgements

The authors would like to thank I. Probert (Algobank, Universite de Basse Normandie, Caen, France), who has provided cultures of the investigated species as well as J. Young and K. Henriksen for providing valuable insight into coccolith calcification. We are grateful to A. Terbrüggen, C. Lorenzen and A. Kolevica for assistance in the laboratory and A. Eisenhauer for providing funding and making the mass spectrometer at the IFM-GEOMAR available. We thank three anonymous reviewers for constructive and helpful comments and H. Elderfield for the editorial handling. This work was funded by the Deutsche Forschungsgemeinschaft as part of the DFG-Research Center "Ocean Margins" of the University of Bremen (no. RCOM0481), and by DFG grant EI 272/12-1, as well as NEBROC (Netherlands Bremen Oceanography Science Co-Operation in Marine Research).

References

Baumann, K-H., Böckel, B., Frenz, M., 2004. Coccolith contribution to South Atlantic carbonate sedimentation. In: Thierstein, H.R., Young, J.R. (Eds.), Coccolithophores — From Molecular Processes to Global Impact. Springer-Verlag, Berlin, pp. 367–402.

Böhm, F., Gussone, N., Eisenhauer, A., Dullo, W.-C., Reynaud, S., Paytan, A., 2006. Calcium isotope fractionation in modern scleractinian corals. Geochim. Cosmochim. Acta 70, 4452–4462.

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

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

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

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

DePaolo, D.J., 2004. Calcium isotopic variations produced by biological, kinetic, radiogenic and nucleosynthetic processes. Rev. Mineral. Geochem. 55, 255–288.

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

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

Farkaš, J., Buhl, D., Blenkinsop, J., Veizer, J., 2007. Evolution of the oceanic calcium cycle during the late Mesozoic: evidence from $\delta^{44/40}$Ca of marine skeletal carbonates. Earth Planet. Sci. Lett. 253, 96–111.

Geisen, M., Billard, C., Broerse, A.T.C., Cros, L., Probert, I., Young, J.R., 2002. Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation? Eur. J. Phycol. 37, 531–550.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum, New York, pp. 26–60.

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

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

Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H.J., Lea, D.W., Bijma, J., Nägler, T.F., 2003. Model for kinetic effects on calcium isotope fractionation (δ^{44}Ca) in inorganic aragonite and cultured planktonic foraminifera. Geochim. Cosmochim. Acta 67 (7), 1375–1382.

Gussone, N., Eisenhauer, A., Tiedemann, R., Haug, G.H., Heuser, A., Bock, B., Nägler, T.F., Müller, A., 2004. δ^{44}Ca, δ^{18}O and Mg/Ca reveal Caribbean Sea surface temperature and salinity fluctuations during the Pliocene closure of the Central-American gateway. Earth Planet. Sci. Lett. 227, 201–214.

Gussone, N., Böhm, F., Eisenhauer, A., Dietzel, M., Heuser, A., Teichert, B.M.A., Reitner, J., Wörheide, G., Dullo, W.-C., 2005. Calcium isotope fractionation in calcite and aragonite. Geochim. Cosmochim. Acta 69 (18), 4485–4494.

Gussone, N., Langer, G., Thoms, S., Nehrke, G., Eisenhauer, A., Riebesell, U., Wefer, G., 2006. Cellular calcium pathways and isotope fractionation in *Emiliania huxleyi*. Geology 34 (8), 625–628.

Gussone, N., Hönisch, B., Heuser A., Hemleben, C. Controls of Ca isotope ratios in planktonic foraminifera. Geochim. Cosmochim. Acta (in revision).

Heuser, A., Eisenhauer, A., Gussone, N., Bock, B., Hansen, B.T., Nägler, T.F., 2002. Measurement of calcium isotopes (δ^{44}Ca) using a multicollector TIMS technique. Int. J. Mass Spectrom. 220, 385–399.

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

Hippler, D., Eisenhauer, A., Nägler, T.F., 2006. Tropical Atlantic SST history inferred from Ca isotope thermometry over the last 140 ka. Geochim. Cosmochim. Acta 70, 90–100.

Keller, M.D., Selvin, R.C., Claus, W., Guillard, R.R.L., 1987. Media for the culture of oceanic ultraphytoplankton. Journal of Phycology 23, 633–637.

Langer, G., Geisen, M., Baumann, K-H., Kläs, J., Riebesell, U., Thoms, S., Young, J.R., 2006. Species-specific responses of calcifying algae to changing seawater carbonate chemistry. Geophys. Geosyst. 7. doi:10.1029/2005GC001227.

Langer, G., Gussone, N., Nehrke, G., Kläs, J., Riebesell, U., Eisenhauer, A., Thoms, S., 2007. Calcium isotope fractionation during coccolith formation in *Emiliania huxleyi*: independence of growth and calcification rate. Geochem. Geophys. Geosyst. 232 (1/2), 51–66.

Lemarchand, D., Wasserburg, G.J., Papanastassiou, D.A., 2004. Rate-controlled calcium isotope fractionation in synthetic calcite. Geochim. Cosmochim. Acta 68 (22), 4665–4678.

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

Marriott, C.S., Henderson, G.M., Belshaw, N.S., Tudhope, A.W., 2004. Temperature dependence of δ^{7}Li, δ^{44}Ca and Li/Ca during growth of calcium carbonate. Earth Planet. Sci. Lett. 222, 615–624.

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

Milliman, J.D., 1993. Production and accumulation of calcium carbonate in the ocean: budget of a nonsteady state. Glob. Biogeochem. Cycles 7 (4), 927–957.

Nägler, T.F., Eisenhauer, A., Müller, A., Hemleben, C., Kramers, J., 2000. δ^{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.

Sáez, A.G., Probert, I., Geisen, M., Quinn, P., Young, J.R., Medlin, L.K., 2003. Pseudo-cryptic speciation in coccolithophores. Proc. Natl. Acad. Sci. U. S. A. 100, 7163–7168.

Schmitt, A.D., Stille, P., Vennemann, T., 2003. Variations of the ^{44}Ca/^{40}Ca ratio in seawater during the past 24 million years: evidence from δ^{44}Ca and δ^{18}O values of Miocene phosphates. Geochim. Cosmochim. Acta 67 (14), 2607–2614.

Sime, N.G., De La Rocha, C.L., Galy, A., 2005. Negligible temperature dependence of calcium isotope fractionation in 12 species of planktonic foraminifera. Earth Planet. Sci. Lett.

Skulan, J., DePaolo, D.J., Owens, T.L., 1997. Biological control of calcium isotopic abundances in the global calcium cycle. Geochim. Cosmochim. Acta 61 (12), 2505–2510.

Soudry, D., Segal, I., Nathan, Y., Glenn, C.R., Halicz, L., Lewy, Z., VonderHaar, D.L., 2004. ^{44}Ca/^{42}Ca and ^{143}Nd/^{144}Nd isotope variations in Cretaceous–Eocene Tethyan francolites and their bearing on phosphogenesis in the southern Tethys. Geology 32 (5), 389–392.

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

Teichert, B.M.A., Gussone, N., Eisenhauer, A., Bohrmann, G., 2005. Clathrites: archives of near-seafloor pore-fluid evolution ($\delta^{44/40}$Ca, δ^{13}C, δ^{18}O) in gashydrate environments. Geology 33 (3), 213–216.

World Ocean Atlas 2001: http://www.nodc.noaa.gov/OC5/WOA01F/prwoa01f.html, (27.04.2007).

Zhu, P., Macdougall, D., 1998. Calcium isotopes in the marine environment and the oceanic calcium cycle. Geochim. Cosmochim. Acta 62 (10), 1691–1698.