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Calcium isotope fractionation in modern scleractinian corals

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Abstract

The $^{44}Ca/^{40}Ca$ ratios of cultured (*Acropora* sp.) and open ocean (*Pavona clavus*, *Porites* sp.) tropical reef corals are positively correlated with growth temperature. The slope of the temperature–fractionation relation is similar to inorganic aragonite precipitates. However, $\delta^{44/40}Ca$ of the coral aragonite is offset from inorganic and sclerosponge aragonite by about $+0.5\%$. This offset can neither be explained by the very fast, biologically controlled calcification of scleractinian corals, nor as a consequence of calcification from a partly closed volume of fluid. As corals actively transport calcium through several cell layers to the site of calcification, the most likely explanation for the offset is a biologically induced fractionation. Our results indicate a limited use of Ca isotopes in scleractinian corals as temperature proxy.
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1. Introduction

Scleractinian corals play an important role in the global calcium cycle. About 20% of modern carbonate accumulation takes place in coral reefs (Milliman and Droxler, 1996), where most calcium carbonate sediment is produced by corals (Hubbard et al., 1990; Heiss, 1994). Knowledge of Ca isotope fractionation in coral skeletons is therefore important for understanding calcium cycling in the ocean (Zhu and Macdougall, 1998; De La Rocha and DePaolo, 2000; Schmitt et al., 2003; Heuser et al., 2005; Fantle and DePaolo, 2005). Published reports on the Ca isotopic composition of coral skeletons are ambiguous (Zhu and Macdougall, 1998; Halicz et al., 1999; Chang et al., 2004; Holmden, 2005) and nothing is known about controlling factors like temperature, light, pH or growth rate.

Coral skeletons are well known for strong vital effects with respect to oxygen, carbon, and boron isotopes and trace elements (Keith and Weber, 1965; Cohen and McConnaughey, 2003; Reynaud et al., 2002; Rollion-Bard et al., 2003; Hönisch et al., 2004; Meibom et al., 2004). These vital effects are the result of a biologically controlled calcification process including cation transport and controls on CO_2 chemistry and pH (Willbur and Simkiss, 1979; Allemand et al., 2004). Scleractinian corals use Ca-ATPase to actively transport Ca^{2+} in exchange for H^+ ions from their calicoblastic cells to the calcifying fluid (Al-Horani et al., 2003; Zoccola et al., 2004). Ca^{2+} channels allow the uptake of Ca^{2+} into the calicoblastic cells and into the oral ectoderm (Zoccola et al., 1999). Intracellular Ca^{2+} transport can be performed by Ca^{2+}-binding proteins or by diffusion, but seemingly not by intracellular vesicles (Tambutté et al., 1996; Clode and Marshall, 2002a).

Despite the active Ca^{2+} transport, the calcium concentration in the calcifying fluid is only slightly (<5%) higher than in seawater. In contrast, pH can be significantly increased (>10%) in the daytime, when photosynthesis and bulk calcification are active (Al-Horani et al., 2003). From this point of view, the role of the Ca^{2+}-ATPase

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pump is to remove H^+ from the calcifying fluid and thereby increase the CO_3^{2-} concentration rather than to increase the Ca^{2+} concentration (Cohen and McConnaughey, 2003). This is a reasonable strategy as $CaCO_3$ precipitation in marine environments is usually not limited by Ca^{2+} but by the carbonate ion concentration (Lemarchand et al., 2004). Indeed, Cohen and McConnaughey (2003) suggest that much of the Ca used in calcification reaches the skeleton by passive fluid flux via pericellular pathways and through skeleton porosity, rather than by the enzymatic transport.

Experiments using the radiocative tracer ^{45}Ca (Goreau, 1959) demonstrate the presence of a large, rapidly exchangeable calcium pool in the coelenteric cavity and a much smaller pool in the coral tissue. The amount of Ca stored is sufficient to maintain calcification for about 1 h (Tambutté et al., 1995, 1996).

In general, the Ca transport from seawater to the skeleton can be summarized as follows (Fig. 1): Calcium is selectively taken up by the coral from the ambient seawater into the coelenteron for storage (Clode and Marshall, 2002b). This reservoir is usually sealed from the environment during the day when most calcification takes place (Cohen and McConnaughey, 2003), but selective uptake may still occur through Ca channels across the oral epithelium (Clode and Marshall, 2002b). From the coelenteron, calcium is taken up by the calicoblastic epithelium for active (Ca^{2+}-ATPase)

transport to the calcifying fluid in the subcalicoblastic space. There, Ca^{2+} is temporarily bound to an organic matrix and finally incorporated into the skeleton (Tambutté et al., 1996; Clode and Marshall, 2003).

This chain of reservoirs and the calcification in a confined, semiclosed system can potentially generate Rayleigh fractionation. Additionally, isotope fractionation may occur during the transport of Ca through the interior of cells and across cell membranes. Such biological fractionation has been observed in trees (Schmitt et al., 2003; Wiegand et al., 2005) and in coccolithophores (Gussone et al., in press). Further evidence for biological fractionation is provided by the strong temperature dependence of the Ca isotope fractionation in some foraminiferal populations (Nägler et al., 2000; Hippler et al., 2002; Gussone et al., 2003, 2004). In contrast, Skulan and DePaolo (1999) suggested that calcium isotope fractionation in soft tissues of vertebrates is of minor importance.

Thus, the basic questions of this study are: (1) does Ca isotope fractionation of scleractinian corals include biological ("vital") effects? and (2) how, quantitatively, does the fractionation depend on temperature? For this purpose, we measured the Ca isotopic composition of laboratory-cultured corals that grew under controlled conditions at different temperatures, and of corals grown in reefs of the Pacific Ocean and the Red Sea. The results are compared to calcium isotope values from inorganic and sclerosponge aragonites, which represent an isotope base line, potentially free of vital effects (Gussone et al., 2003, 2005).

2. Methods and materials

2.1. Culturing setup

A single colony of the branching zooxanthellate scleractinian coral, *Acropora* sp. was maintained in the aquarium of the Centre Scientifique de Monaco. "Nubbins" (small live coral samples, Fig. 2) were obtained by cutting terminal portions of branches from the single parent colony and were then glued on glass slides (Reynaud-Vaganay et al., 1999). The coral nubbins were distributed in 5 tanks (30 L) heated to 21, 23, 25, 27 and 29 °C and fed once a week with *Artemia salina* nauplii during the course of the experiment. From each of the five different temperature experiments three samples were selected for calcium isotope analysis.

The tanks were continuously supplied with Mediterranean seawater (salinity = 38.5), heated using a temperature controller (EW, PC 902/T) and continuously mixed with a Rena® pump (6 L min^{-1}). The renewal rate was approximately 5 times d^{-1}. With that, the corals used less than 1% of the calcium in the tanks for calcifaction. The alteration of the Ca isotopic composition of the solution in the tanks by calcification was therefore negligible. Light was provided by metal halide lamps (Phillips HPIT, 400 W) on a 12:12 h photoperiod, and kept constant (400 µmol photons m^{-2} s^{-1}) during the course of the experiment.
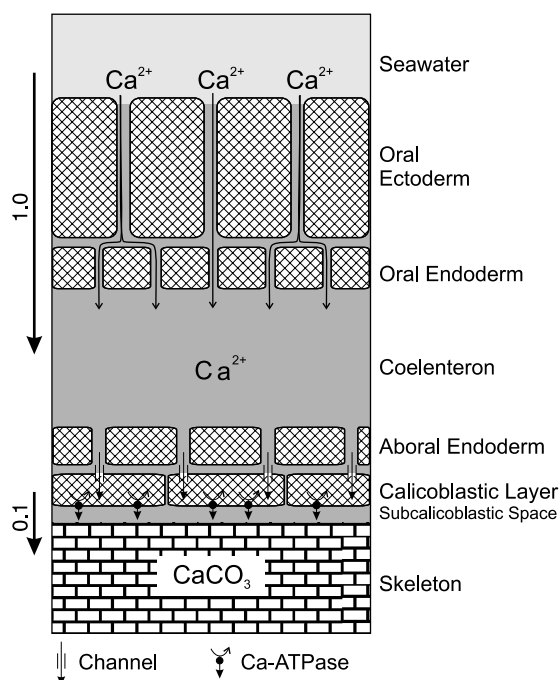

Fig. 1. Sketch of Ca^{2+} transport pathways through a scleractinian coral. Arrows on the left indicate relative Ca flux rates (dimensionless). Ca^{2+} is taken up into the coelenteron at a rate about 10 times higher than Ca is transported through the calicoblastic layer and into the skeleton. Transport through the calicoblastic layer is controlled by Ca channels and active Ca-ATPase pumps. Modified from Tambutté et al. (1996) and Bénazet-Tambutté et al. (1996).
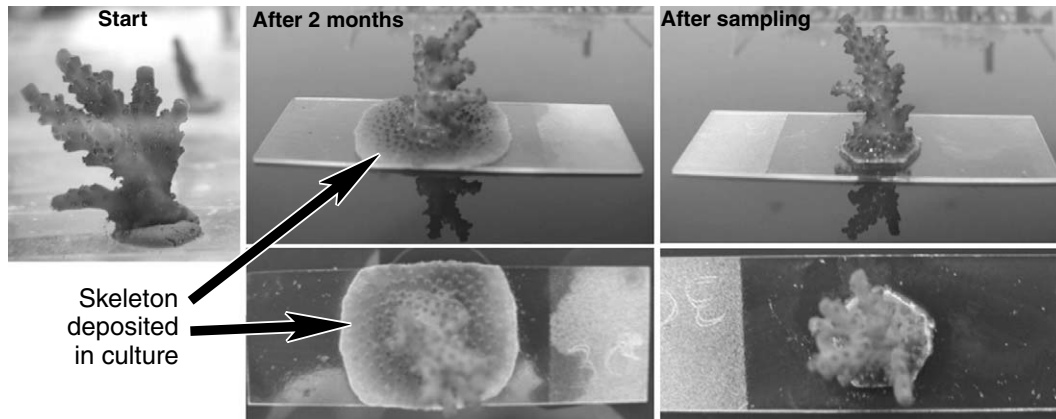
Fig. 2. Cultured *Acropora* corals. Small live nubbins are glued to glass slides where they form a sheet of skeletal aragonite attached to the slide. After two months the newly formed skeleton is sampled for analysis.

Temperature (accuracy: $\pm 0.05\,°C$) was logged at 10 min intervals using a Seamon[®] temperature recorder. Salinity and irradiance were measured using a conductivity meter (Meter LF196), and a 4π quantum sensor (Li-Cor, LI-193SA) respectively.

2.2. Galapagos coral setting

The massive zooxanthellate scleractinian coral *Pavona clavus* was collected for an oxygen isotope calibration study (Wellington et al., 1996, sample "Bart 2") in January 1995 at Bartolome, Galapagos (0.5°S, 90.5°W) at a water depth of 3 m, about 15 m off the shoreline. Before collection, the coral had been stained with Alizarin red every 6 months between January 1993 and August 1994. The linear growth rate of this coral is 1.8 mm/month in the summer and 1.2 mm/month in the winter, resulting in an average annual growth rate of about 18 mm/yr. Samples were drilled every millimeter along a 40 mm transect along the growth axis, using a Dremel[®] tool with a 1 mm drill bit, resulting in a resolution of about 17 days/sample in the warm season and 25 days/sample in the cold season.

In situ temperatures were measured hourly at the collection site. The mean temperature during the sampled period was 23.6 °C with a seasonal amplitude of 6.7 °C. Seawater $\delta^{18}O$ varied by about 0.4‰ and caused less than 10% of the variation in $\delta^{18}O$ (Wellington et al., 1996). Oxygen isotope ratios were determined from all samples. Splits of seven samples, selected for covering a maximum temperature range, were used for calcium isotope analysis. Formation temperatures were reconstructed from logging data and $\delta^{18}O$ (Wellington et al., 1996).

2.3. Red sea corals setting

Three specimens of the massive zooxanthellate scleractinian coral *Porites* sp. were collected in the northern Red Sea at Hurghada, Egypt (sample EGE2; Heiss, 1994), the central Red Sea near Port Sudan (sample

SA2) and in the Gulf of Aden near Djibouti (sample DJDC). Two samples were drilled from each specimen using a 2 mm drill bit. Calcification temperatures were calculated from oxygen isotope values, measured on splits of the drilled samples. Analyzes were carried out at the University of Erlangen Geological Institute as described in Böhm et al. (2000). The northern Red Sea temperature equation for *Porites* as given by Felis et al. (2000) was used for specimen EGE2. For the two other samples we applied the *Porites* temperature equation of Al-Rousan et al. (2003), which accounts for variable $\delta^{18}O$ of seawater ($\delta^{18}O_{sw}$). Values for $\delta^{18}O_{sw}$ were calculated from the mean annual salinities (Levitus et al., 1994) of 36.4 (location DJDC) and 39.1 (location SA2), respectively, using the equation of Andrié and Merlivat (1989). The resulting temperatures are in good agreement with atlas data of average annual SST at the collection sites (Levitus and Boyer, 1994). Linear extension rates varied for the three specimens and are 3 mm/yr (EGE2), 9 mm/yr (DJDC) and 16 mm/yr (SA2), respectively. Applying extension rate corrections to the measured $\delta^{18}O$ values (Felis et al., 2003) would result in implausible temperatures (31 °C) for specimen EGE2, but would not significantly change the results for the other two specimens. Therefore no corrections were applied.

2.4. Calcium isotopes experimental and reproducibility

Calcium isotope measurements of the coral skeletons were made on a Finnigan Triton TI thermal ionization mass spectrometer at the IFM-GEOMAR, following the method described in Heuser et al. (2002). Briefly, samples of about 300 ng Ca, dissolved in 2 N HCl were loaded with a $TaCl_5$ activator after addition of a ^{43}Ca–^{48}Ca double spike on zone-refined Re single filaments. Measurements were made in dynamic mode with $^{40}Ca/^{43}Ca$, $^{42}Ca/^{43}Ca$, and $^{44}Ca/^{43}Ca$ measured in the main cycle and $^{43}Ca/^{48}Ca$ in the second cycle. Signal intensity during acquisition was typically 4–5 V for ^{40}Ca.

The isotope values are expressed relative to NIST SRM 915a as $\delta^{44/40}Ca = ((^{44}Ca/^{40}Ca)_{sample}/(^{44}Ca/^{40}Ca)_{SRM915a} - 1) \times 1000$ (Eisenhauer et al., 2004) and as $\delta^{mu}Ca$ (ppm/amu) $= 268.3 \times \delta^{44/40}Ca$, assuming equilibrium fractionation (Gussone et al., 2005) (amu = atomic mass unit). Accordingly, fractionation is expressed as $\alpha = (^{44}Ca/^{40}Ca)_{solid}/(^{44}Ca/^{40}Ca)_{fluid}$, or in the mass independent notation as $\alpha_{mu} = \alpha^{0.2683}$.

External precision is given as two times the standard error of the mean ($2SEM = 2\sigma/n^{0.5}$) determined by sample repeat measurements. The $\delta^{44/40}Ca$ values for each session were calculated with the session mean SRM915a value. The average precision for SRM915a during a session was $\pm 0.09\permil$ ($2SEM$, $n = 4$). The long-term (2 years) mean $^{44}Ca/^{40}Ca$ of SRM915a is 0.021181 ± 0.000006 (2σ, $n = 138$). Total Ca blanks were determined as less than 2 ng, i.e., less than 0.7%.

To remove organic material, fragments of the cultured corals were bleached with ca. 10% NaClO (1% active chlorine) for 24 h, ultrasonicated and rinsed several times with ultrapure water. This treatment was repeated three to four times until the samples were white. Subsequently samples were dried, dissolved in 2 N HCl and evaporated to dryness.

Samples prepared by this technique did not produce reproducible results. Repeated measurements showed variations of up to $0.6\permil$ in $\delta^{44/40}Ca$ (Fig. 3). We therefore applied the treatment proposed by Hippler et al. (2004) to remove interfering organic molecules from sample solutions. A mixture of 5 μL concentrated HNO_3 and 5 μL concentrated H_2O_2 was added to 1 μL sample solution (300 ng Ca) and evaporated to dryness immediately before loading. With that treatment the reproducibility improved significantly and mean values shifted to higher $\delta^{44/40}Ca$ values (Fig. 3). Aliquots of the SRM915a carbonate standard showed the same $\delta^{44/40}Ca$ values with and without application of this treatment. The Ca blank of the HNO_3–H_2O_2 mixture was less than 2 ng.
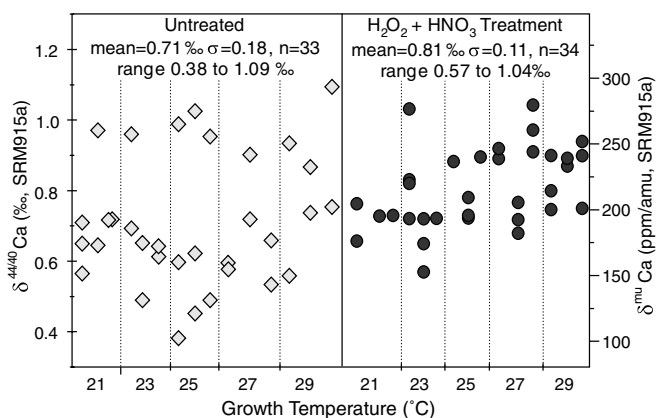
Samples of the Mediterranean seawater used in the culturing setup and IAPSO seawater standard were analyzed for $\delta^{44/40}Ca$ after a chromatographic clean-up on cation-exchange columns (BioRad) filled with MCI Gel (75–100 mesh; 0.6 mL) using 1.8 N HCl for elution. The $\delta^{44/40}Ca$ of the Mediterranean seawater was determined as $1.78 \pm 0.1\permil$ ($\pm 2SEM$, $n = 16$). This value is identical within error with the IAPSO seawater standard $\delta^{44/40}Ca$ ($1.83 \pm 0.1\permil$, $n = 29$) and the mean seawater value $\delta^{44/40}Ca = 1.88 \pm 0.04\permil$ reported by Hippler et al. (2003). Ca blanks for the column chemistry are less than 1 ng.

3. Results

The results of the calcium isotope measurements are listed in Tables 1–3. The cultured coral $\delta^{44/40}Ca$ values are significantly correlated with temperature, as shown by the regression, which is significant at the 99%-level (Fig. 4):

$\delta^{44/40}Ca$ (‰ SRM915a)

$= 0.3 \pm 0.4 + (0.020 \pm 0.015) \times T(^{\circ}C); \quad R = 0.62, \; p = 0.01, \; n = 15$

$\delta^{mu}Ca$ (ppm/amu SRM915a) $= 82 \pm 101 + (5 \pm 4) \times T(^{\circ}C);$

(1)

Table 1
Calcium isotope values of cultured corals (*Acropora* sp.)

Sample	T (°C)	$\delta^{44/40}Ca$	2SEM[a]	$\delta^{mu}Ca$	2SEM[a]	n[c]
21bis	21	0.71	0.11	191	28	2
24bis	21	0.73		195		1
51bis	21	0.73		196		1
Mean[b]	21	0.72	0.01	194	3	3
11bis	23	0.85	0.13	229	35	4
15bis	23	0.65	0.09	174	23	3
45bis	23	0.72		194		1
Mean[b]	23	0.74	0.12	199	32	3
1bis	25	0.88		237		1
30bis	25	0.74	0.03	200	9	3
3bis	25	0.90		240		1
Mean[b]	25	0.84	0.10	226	26	3
16bis	27	0.90	0.03	243	8	2
19bis	27	0.76	0.08	203	20	4
40bis	27	0.98	0.08	262	20	3
Mean[b]	27	0.88	0.13	236	35	3
36bis	29	0.82	0.09	219	25	3
6bis	29	0.88	0.02	236	6	2
9bis	29	0.86	0.12	231	32	3
Mean[b]	29	0.85	0.04	229	10	3
Total mean		0.81	0.05	217	13	15

Repeated measurements were made on aliquots of the same sample solution. Calcium isotope values are given relative to standard SRM915a.
[a] $2SEM = 2\sigma/n^{0.5}$ of the calcium isotope ratios, calculated from repeated measurements.
[b] Mean of three samples grown at specified temperature.
[c] Number of repeated measurements of each sample; number of samples used for calculation of each mean value.

Fig. 3. Reproducibility and range of $\delta^{44/40}Ca$ values of the 15 cultured coral samples with and without the HNO_3–H_2O_2 treatment of Hippler et al. (2004).

Table 2
Isotope values of the Galapagos coral samples (*Pavona clavus*)

Sample	$T(°C)$	$\delta^{44/40}Ca$	$\delta^{mu}Ca$	$\delta^{18}O$
Bart2-11	22.0	0.72	193	−3.8
Bart2-7	22.5	0.82	220	−3.9
Bart2-36	23.5	0.86	231	−4.1
Bart2-23	23.5	0.64	172	−4.1
Bart2-02	25.5	0.85	228	−4.5
Bart2-15	26.5	0.83	223	−4.7
Bart2-32	27.0	0.87	233	−4.8

Calcium isotope values given relative to standard SRM915a, oxygen isotope values relative to V-PDB.

Table 3
Isotope values of the Red Sea corals (*Porites* sp.)

Sample	$T(°C)^a$	$T(°C)^b$	$\delta^{44/40}Ca$	$2SEM^c$	$\delta^{mu}Ca$	$2SEM^c$	n^d	$\delta^{18}O$
EGE2-1	25		0.83	0.05	221	13	2	−3.6
EGE2-2	27		0.86	0.17	230	46	4	−3.9
Mean^e	26	25	0.85	0.11	226	30	6	−3.8
DJDC-1	28		0.86	0.11	229	30	4	−5.0
DJDC-2	27		0.96	0.26	257	69	3	−4.8
Mean^e	28	28	0.90	0.12	243	32	7	−4.9
SA2-1	30		0.85	0.10	227	27	4	−4.5
SA2-2	28		0.88	0.14	236	38	4	−4.1
Mean^e	29	29	0.86	0.08	232	22	8	−4.3

Repeated measurements were made on aliquots of the same sample solution. Calcium isotope values given relative to standard SRM915a, oxygen isotope values relative to V-PDB.
[a] Temperature calculated from $\delta^{18}O$.
[b] Temperature from Levitus and Boyer (1994).
[c] $2SEM = 2\sigma/n^{0.5}$ of the calcium isotope ratios, calculated from repeated measurements.
[d] Number of repeated measurements of each sample.
[e] Mean values of specimens EGE2, DJDC and SA2, respectively.
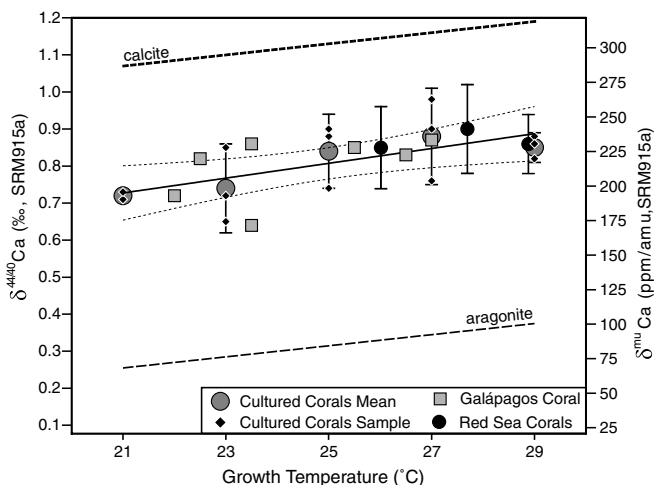

Fig. 4. Measured $\delta^{44/40}Ca$ data of cultured and open ocean scleractinian corals plotted as a function of temperature and compared to values for marine biogenic aragonite (sclerosponges, pteropods) and calcite (brachiopods, sclerosponges, red alga) (Gussone et al., 2005). Coral regression line was calculated from cultured corals only and is shown with 95%-confidence bounds. Error bars are ±2SEM.

or cast in terms of isotope fractionation, using $\delta^{44/40}Ca_{seawater} = 1.88‰$ (Hippler et al., 2003):

$$1000 \times \ln(\alpha) = -1.6 \pm 0.4 + (0.020 \pm 0.015) \times T(°C)$$

$$10^6 \times \ln(\alpha_{mu}) = -422 \pm 101 + (5 \pm 4) \times T(°C); \qquad (2)$$

A regression of the open ocean coral data results in a similar equation, however significant only at the 95%-level:

$\delta^{44/40}Ca$ (‰ SRM915a)
$$= 0.3 \pm 0.5 + (0.022 \pm 0.021) \times T(°C); \quad R = 0.64, \; p = 0.05, \; n = 10$$
$$\delta^{mu}Ca \text{ (ppm/amu SRM915a)} = 74 \pm 146 + (6 \pm 6) \times T(°C);$$
$$\qquad (3)$$

The mean $\delta^{44/40}Ca$ values of the cultured ($0.81 \pm 0.05‰$, $T_{mean} = 25.0\,°C$) and open ocean corals ($0.82 \pm 0.05‰$, $T_{mean} = 25.3\,°C$) are identical within errors, but are significantly different from the mean $\delta^{44/40}Ca$ of marine biogenic aragonites of the same temperature range ($0.44 \pm 0.02‰$, $T_{mean}=26.2\,°C$; Gussone et al., 2005) and inorganic aragonites precipitated at $25\,°C$ ($0.32 \pm 0.06‰$; recalculated from Gussone et al. (2003) to precipitation from a fluid with $\delta^{44/40}Ca$ of seawater).

The regression line slopes are identical within uncertainty to the values of *O. universa* calcite ($0.019 \pm 0.003‰/K$), calcite of brachiopods, algae and sclerosponges ($0.014 \pm 0.01‰/K$), aragonite of sclerosponges and pteropods ($0.017 \pm 0.006‰/K$), and inorganic aragonite ($0.015 \pm 0.002‰/K$) (Gussone et al., 2003, 2005).

4. Discussion

4.1. Reproducibility, temperature dependence, and intraspecific variability

Our coral $\delta^{44/40}Ca$ data agree with the published values of Chang et al. (2004) and Holmden (2005). The data of Halicz et al. (1999) and of Zhu and Macdougall (1998), however, differ significantly (Fig. 5). This may partly be due to laboratory offsets, but also points to methodological problems like matrix effects or isobaric interferences, as indicated by the differences between treated and untreated samples in this study (Fig. 3).

As a result of the observed temperature dependence of $0.02‰/K$ and the currently possible precision of $0.1‰$ for $\delta^{44/40}Ca$ (95% confidence level, Table 1) only temperature variations exceeding 5 K can be recorded by coral $\delta^{44/40}Ca$. Therefore, unless the measurement precision can be improved significantly, calcium isotopes in scleractinian corals provide a poor temperature proxy. This is further illustrated by the fact that even with a temperature range of $8\,°C$ and controlled culturing conditions only a regression analysis of our data proves with high statistical confidence that coral $\delta^{44/40}Ca$ values depend on growth temperature (Fig. 4).

The $\delta^{44/40}Ca$ variance of specimens that were cultured at a specific temperature (Table 1, Fig. 4) is equal to the reproducibility of the repeated measurements (Fig. 3).
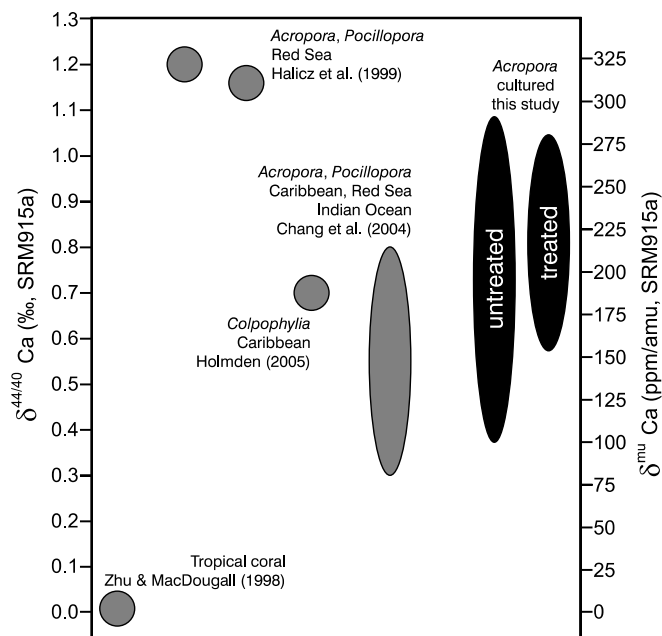
Fig. 5. Ca isotopes of scleractinian corals from different locations, measured in different laboratories show a wide range of values. This may partly be an artefact of measurement techniques as indicated by the range of untreated samples measured in this study. Rescaling from $\delta^{44/42}$Ca to $\delta^{44/40}$Ca values was carried out by simple multiplication. For conversion from seawater to the SRM915a standard an offset of $-1.88‰$ was assumed (Hippler et al., 2003).

The average standard deviation is 0.07‰ in both cases. This indicates that intraspecific $\delta^{44/40}$Ca variability is probably low. The $\delta^{44/40}$Ca means of individual samples cultured at a specific temperature agree within analytical uncertainty (Student's *t*-test, 95% confidence level; Table 1). Only one of the samples cultured at 27 °C differs significantly from the other two (98% confidence level) (Table 1). It must however be noted that all cultured specimens were derived from a single colony. Therefore, our data can not provide conclusive evidence for or against intraspecific variability of calcium isotope fractionation in corals. The good agreement between $\delta^{44/40}$Ca values measured in different species (Fig. 4), on the other hand, shows that interspecific variability among scleractinian corals is probably small.

4.2. Factors controlling calcium isotope fractionation

Scleractinian corals build an aragonitic skeleton. The temperature dependence of Ca isotope fractionation in the studied corals is similar to that of aragonite and calcite samples used in previous studies. However, as shown above, the observed calcium isotope fractionation in open ocean and cultured corals is significantly weaker than in the inorganic and biogenic aragonite studied by Gussone et al. (2003, 2005). The corals are enriched in $\delta^{44/40}$Ca by about 0.5‰ with respect to these materials. How can this difference be explained?

Two major factors control the Ca isotope fractionation during inorganic and simple extracellular biogenic $CaCO_3$

precipitation: mineralogy and precipitation rate. Gussone et al. (2005) showed that slowly precipitated aragonite ($<10^3$ μmol/m^2/h) is depleted in $\delta^{44/40}$Ca by about 0.6‰ with respect to calcite. Our coral data seem to contradict this result.

Scleractinian corals calcify at rates much higher than 10^3 μmol/m^2/h (e.g., *Porites* typically calcifies at $10^{4.3\pm0.2}$ μmol/m^2/h (Lough and Barnes, 1997)). The calcification rates of *Acropora* used in the culturing experiments are in the same range, about $10^{4.4}$ μmol/m^2/h (Reynaud-Vaganay, 2000; Reynaud et al., 2004). Accordingly, based on both theoretical and experimental data (see discussion below) one would expect higher $\delta^{44/40}$Ca with increased calcification rate. So, can the higher calcification rate explain the offset of coral $\delta^{44/40}$Ca from the aragonite fractionation value?

4.3. Rate dependence of calcium isotope fractionation

Lemarchand et al. (2004) observed a strong rate dependence of Ca isotope fractionation in calcite precipitation experiments. They explained this observation with an increasing incorporation of unfractionated Ca into the crystal with increasing precipitation rate. Calcium is in isotopic equilibrium with the crystal surface only within a very thin interface layer between the fluid and crystal. With increasing precipitation rate unequilibrated Ca from outside this layer is incorporated into the growing crystal.

Apparent fractionation in this model can be quantified by simple two-end-member mixing. The first end-member is Ca in isotopic equilibrium with the crystal, aragonite or calcite ($\delta^{44/40}Ca_{eq,sp}$), the second is Ca in the bulk fluid ($\delta^{44/40}Ca_{fl}$):

$$\delta^{44/40}Ca = x \times \delta^{44/40}Ca_{eq,sp} + (1 - x) \times \delta^{44/40}Ca_{fl} \quad (4)$$

where x is the fraction of equilibrated Ca incorporated into the crystal. This fraction depends on the time (t) available for Ca approaching the fluid–solid interface to equilibrate before it is incorporated into the crystal lattice. This isotopic equilibration is an exchange process, where ions randomly diffuse in and out of the interface layer with slightly different probabilities depending on the isotope's mass. It can be described by a common reaction kinetics equation (Zeebe and Wolf-Gladrow, 2001, p. 90):

$$x = 1 - e^{-t/\tau} \quad (5)$$

where x is the fraction of equilibrated Ca, t is the time available for equilibration and τ is the equilibration time constant. We can calculate t from the precipitation rate (R) and the thickness of the interface layer (h):

$$t(s) = h/(R/\rho_{sp} \times M_{CaCO_3} \times 10^{-6}/3.6) \quad (6)$$

where ρ_{sp} is the density of aragonite (2.95 g/cm^3) or calcite (2.71 g/cm^3), M_{CaCO_3} is the molar weight of $CaCO_3$ (100.0869 g/mol), and the final term is a factor for unit conversion. The denominator of Eq. (6) represents the speed of

the advancing crystal surface. At a precipitation rate, R, of 500 μmol/m^2/h the surface of a calcite crystal advances by 0.005 nm/s. With an interface layer thickness, h, of 0.5 nm (Watson, 2004) the time available for equilibration, t, is 100 s. Combining Eqs. (4 and 5) gives a rate dependence for Ca isotope fractionation:

$$\delta^{44/40}Ca = (1 - e^{-t/\tau}) \times \delta^{44/40}Ca_{eq,sp} + e^{-t/\tau}$$
$$\times \delta^{44/40}Ca_{fl} \tag{7}$$

The unknown parameters τ, $\delta^{44/40}Ca_{eq,cc}$, $\delta^{44/40}Ca_{eq,ar}$ and $\delta^{44/40}Ca_{fl}$ are estimated by fitting Eq. (7) to the data of Lemarchand et al. (2004) and Gussone et al. (2005) as shown in Fig. 6 and listed in Table 4. For this, it is assumed that h/τ is identical for aragonite and calcite. The fit to both the calcite and aragonite data is reasonable for precipitation rates between $10^{1.5}$ and 10^3 μmol/m^2/h. However, calcite that precipitated at rates between $10^{3.5}$ and $10^{4.3}$ μmol/m^2/h deviated significantly from the fitted curve (Fig. 6a). The fit can be improved when a two-step fractionation process is assumed (Fig. 6b, Table 4). In this case, Ca dissolved in the fluid (Ca$_{fl}$) is first fractionated by forming an intermediate phase (e.g., a CaCO$_{3(aq)}$ complex, Ca$_{comp}$; Lin

Table 4
Parameters used for the data fit of Eq. (7) shown in Fig. 6

Polymorph	h/τ (pm/s)[a]	$\delta^{44/40}Ca_{fl}$[b]	$\delta^{44/40}Ca_{eq,sp}$[c]	RMS[d]
Fig. 6a				
Calcite	6.3	1.88	0.7	0.29
Aragonite	6.3	1.88	0.2	0.10
Fig. 6b				
Calcite	3.6	1.3 to1.88	0.7	0.12
Aragonite	3.6	1.3 to1.88	0.1	0.08
Ca complex	250	1.88	1.3	—

Calcium isotope values given relative to standard SRM915a.
[a] Ratio of interface layer thickness (h) to equilibration time constant (τ).
[b] Calcium isotope composition of dissolved or complex end-member.
[c] Calcium isotope composition of solid or complex end-member.
[d] Root mean square of modelled and measured value residuals.

and Singer, 2005; Nilsson and Sternbeck, 1999) with a much larger h/τ quotient than for the crystallization reaction (Table 4). The precipitation of solid CaCO$_3$ uses Ca$_{comp}$ with an additional fractionation during incorporation into the crystal lattice.

More experimental data are necessary to decide whether one-step or two-step fractionation better describes CaCO$_3$ precipitation. Nevertheless, using Eq. (7) to calculate the expected calcium isotopic composition of coral aragonite in both cases clearly shows that high precipitation rates cannot explain the observed Ca isotopic composition of corals. For precipitation rates on the order of 10^4 μmol/m^2/h the one-step and two-step models predict $\delta^{44/40}Ca$ values of about 1.8‰ and 1.3‰, respectively. This is significantly higher than the measured mean coral $\delta^{44/40}Ca$ of 0.8 ± 0.05‰. Even if it is assumed that the fractionation mechanism for aragonite differs from that of calcite in a way that produces a constant isotope offset between the two polymorphs, the coral data would still be significantly depleted in ^{44}Ca with respect to inorganic aragonite (Fig. 6).

Rayleigh fractionation in a semi-closed system would lead to an even higher $\delta^{44/40}Ca$, because precipitation preferentially removes ^{40}Ca from the fluid and consequently increases $\delta^{44/40}Ca_{fl}$ (Teichert et al., 2005). Therefore, high precipitation rates, with or without invoking a semi-enclosed system, can not explain the observed Ca isotope composition of scleractinian corals.

4.4. Biological fractionation in cellular tissues

The calcium used for skeleton formation in scleractinian corals is transported from the coelenteron through the calicoblastic cell layer (Fig. 1). To pass this layer Ca is dehydrated, passes through ion channels into the cells and is subsequently actively pumped out of the cells by Ca-ATPase. Extreme Ca^{2+} concentration gradients exist between the coelenteron (about 10 mM), the calicoblastic cell plasma (about 0.1 μM), and the calcifying fluid (about 11 mM) (Al-Horani et al., 2003; Allemand et al., 2004). To allow the transport of large amounts of calcium through
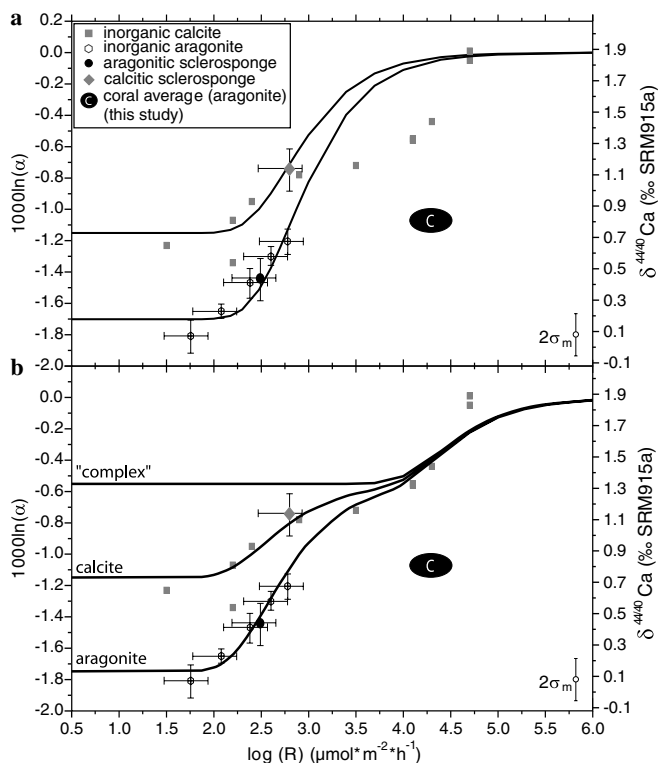

Fig. 6. Calcium isotope fractionation as a function of precipitation rate. Inorganic calcite data are from Lemarchand et al. (2004), inorganic aragonite and sclerosponge data (*C. nicholsoni, A. wellsi*) from Gussone et al. (2003, 2005). Average scleractinian coral value is plotted with average *Porites* precipitation rate from Lough and Barnes (1997). The fit of Eq. (7) to the data is calculated with the parameters given in Table 4. (a) Assuming a one-step fractionation process between fluid and solid. (b) Assuming a two-step fractionation process with fractionation between Ca$_{fl}$ and Ca$_{comp}$ in the first step and further fractionation between Ca$_{comp}$ and CaCO$_3$ in a second step.

the calicoblastic cells, while maintaining a low intracellular free calcium concentration, an effective transport mechanism must be at work, probably involving Ca-binding proteins (Allemand et al., 2004). We suggest that a "biological" Ca isotope fractionation occurs during the passage through the calicoblastic layer, either at the cell membranes or within the cells.

Calcium isotope fractionation during the transport of Ca through cells or cell membranes was recently found in coccolithophores (Gussone et al., in press). Gussone et al. suggested that fractionation occurs during dehydration of the dissolved Ca^{2+} ions at the cell membranes. It was found that biological fractionation in the coccolithophore *Emiliania huxleyi* leads to a $\delta^{44/40}Ca$ of $0.7 \pm 0.1\%$ at 20 °C. This value is in good agreement with the observed coral $\delta^{44/40}Ca$ of $0.72 \pm 0.01\%$ at 21 °C (Table 1).

If indeed the biological fractionation observed for *E. huxleyi* is representative also of corals, the observed coral isotope ratios do not allow additional Ca isotope fractionation during the calcification process itself. This lack of significant fractionation during calcification could best be explained by aragonite precipitation within a confined steady state system, where a steady flux of Ca^{2+} to the calcifying fluid is balanced by an equivalent calcium removal into the skeleton. This is in good agreement with the setup of the culturing experiments, where the coral skeleton formed in a closed space between a glass plate and the coral tissue (see Section 4.5).

The uptake of seawater Ca into the coral coelenteron is an order of magnitude faster than the uptake of calcium into the skeleton during calcification (Fig. 1; Tambutté et al., 1996). Therefore, the biological fractionation of Ca isotopes in corals most likely occurs when Ca is taken up from the coelenteron into the aboral tissue layers, probably during the passage of Ca through the calicoblastic layer. If this is the case, Ca concentration changes in the coelenteric cavity may potentially lead to isotopic variability of the skeleton, depending on the frequency of Ca exchange between coelenteric fluid and seawater.

Gussone et al. (in press) found that the biological fractionation of *E. huxleyi* is temperature dependent, with a sensitivity of $0.027 \pm 0.006\%/K$. This value is in good agreement with the temperature sensitivity observed in the corals of $0.020 \pm 0.015\%/K$. We therefore conclude that biological fractionation can explain the observed $\delta^{44/40}Ca$ values of scleractinian corals as well as their temperature dependence.

4.5. Calcium transport pathways in scleractinian corals

Calcification in scleractinian corals takes place in the subcalicoblastic space, which is a closed system with respect to calcium. Ca uptake experiments with ^{45}Ca and channel inhibitors show that there is no diffusive exchange between the coelenteric cavity and the calcifying fluid. All Ca used for calcification has to pass through ion channels and the calicoblastic cells. Ca transport through pericellular pathways is insignificant (Tambutté et al., 1996).

Diffusion of Ca from seawater through the skeleton into the subcalicoblastic space can also be excluded. It was shown by Al-Horani et al. (2003) that the Ca concentration in the calcifying fluid is slightly higher than in seawater. Consequently, excess Ca ions can diffuse out from the calcifying fluid into seawater, but not the other way round.

In the setup used for the coral culturing experiments the skeleton is closely attached to a glass plate and thus sealed from below (Fig. 2), while the upper surface is completely covered by coral tissue. Therefore no direct exchange with seawater is possible. Conservation of mass in this closed system strongly restricts fractionation in the calcifying fluid, regardless of the precipitation rate. Consequently, the $\delta^{44/40}Ca$ measured in the cultured coral aragonite accurately records the $\delta^{44/40}Ca$ of the calcifying fluid without any significant fractionation.

If, for instance, 20% of the calcium transported into the subcalicoblastic space would escape through pericellular channels, and if fractionation during precipitation of coral aragonite follows the model shown in Fig. 6b, the skeletal $\delta^{44/40}Ca$ would only be 0.1% lighter than in a completely closed system. For a rate dependent fractionation model as shown in Fig. 6a no significant isotope effect would occur, even in a fully open system. This can be calculated by a steady state mass balance (Eq. (8)), where x is the fraction of Ca removed by calcification (e.g., $x = 0$ in an open system), δ is the isotopic composition and Δ is the fractionation between fluid and solid. The subscripts are: "ar" precipitated aragonite, "sub" fluid in the subcalicoblastic space, "in" input to the subcalicoblastic space.

$$\delta_{in} - x*(\delta_{sub} + \Delta_{ar}) - (1-x) \times \delta_{sub} = 0; \text{ with } \delta_{ar}$$
$$= \delta_{sub} + \Delta_{ar}; \tag{8}$$

With the limited volume of the subcalicoblastic space it is reasonable to assume a steady state of the Ca concentration in the calcifying fluid on the time scales relevant for skeleton formation (hours to days). The subcalicoblastic space is a few micrometers thick (Johnston, 1980). With the Ca concentrations measured by Al-Horani et al. (2003) the amount of calcium stored in this volume is sufficient to maintain coral calcification for only a few seconds, i.e., for less than one nanometer of linear extension. Therefore, calcium in the subcalicoblastic space must continuously be restored to maintain skeletal growth. Transient changes in the Ca concentration of the calcifying fluid would cause only very limited isotopic effects. These would hardly be expressed in the skeleton due to the long Ca isotope equilibration time constant of aragonite, which is on the order of 10^2 s (Table 4).

The good agreement between the coral and coccolith $\delta^{44/40}Ca$ values corroborates the view of the subcalicoblastic space as a confined system. The good accordance between the $\delta^{44/40}Ca$ values of the cultured and open ocean corals of different species shows that calcification in a

closed system with respect to Ca is not limited to the culturing setup, but is the rule for scleractinian coral calcification in general.

5. Conclusions

Scleractinian corals are enriched in $\delta^{44/40}$Ca by 0.4 to 0.5‰ compared to slowly precipitated sclerosponge and inorganic aragonite. Considering their extremely fast calcification rate, they are significantly depleted in $\delta^{44/40}$Ca compared to what is expected from fast rate inorganic precipitates.

Scleractinian corals form their skeleton in a closed, biologically controlled system with respect to calcium and all of the Ca in the subcalicoblastic space is quantitatively removed for calcification. Therefore the specific aragonite crystallization step involves no significant fractionation effect. Accordingly, neither precipitation rate nor mineralogy have any influence on the Ca isotopic composition of the coral skeleton. The skeletal aragonite accurately records the Ca isotopic composition of the calcifying fluid in the subcalicoblastic space. Calcium in this fluid however is fractionated during the transport through the calicoblastic cell layer. This latter biological Ca isotope fractionation is very similar in corals and coccoliths, which points to a common mechanism. The biological fractionation depletes $\delta^{44/40}$Ca of the calcifying fluid by about $-1.2‰$ at 21 °C and $-1.0‰$ at 29 °C relative to the external seawater Ca source.

The closed system calcification may be common in many other biota, especially those with intracellular biomineralization. In these cases, the skeletal Ca isotope composition is not controlled by factors during calcification such as precipitation rate (Lemarchand et al., 2004) and mineralogy (Gussone et al., 2005), but rather by biological fractionation during transport into the cell (Gussone et al., in press).

Cultured and open ocean corals show a temperature dependence of Ca isotope fractionation that is similar to the temperature dependence of inorganic aragonite (Gussone et al., 2003). This weak temperature sensitivity and possible interferences from biological fractionation effects severely limit the use of Ca isotopes in scleractinian corals as temperature proxy.

Acknowledgments

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