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Incorporation of Mg and Sr in calcite of cultured benthic foraminifera: impact of calcium concentration and associated calcite saturation state

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Abstract. We investigated the effect of the calcium concentration in seawater and thereby the calcite saturation state (Ω) on the magnesium and strontium incorporation into benthic foraminiferal calcite under laboratory conditions. For this purpose individuals of the shallow-water species *Heterostegina depressa* (precipitating high-Mg calcite, symbiont-bearing) and *Ammonia tepida* (low-Mg calcite, symbiont-barren) were cultured in media under a range of [Ca^{2+}], but similar Mg/Ca ratios. Trace element/Ca ratios of newly formed calcite were analysed with Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and normalized to the seawater elemental composition using the equation $D_{TE}=(TE/Ca_{calcite})/(TE/Ca_{seawater})$. The culturing study shows that D_{Mg} of *A. tepida* significantly decreases with increasing Ω at a gradient of -4.3×10^{-5} per Ω unit. The D_{Sr} value of *A. tepida* does not change with Ω, suggesting that fossil Sr/Ca in this species may be a potential tool to reconstruct past variations in seawater Sr/Ca. Conversely, D_{Mg} of *H. depressa* shows only a minor decrease with increasing Ω, while D_{Sr} increases considerably with Ω at a gradient of 0.009 per Ω unit. The different responses to seawater chemistry of the two species may be explained by a difference in the calcification pathway that is, at the same time, responsible for the variation in the total Mg incorporation between the two species. Since the Mg/Ca ratio in *H. depressa* is 50–100 times higher than that of *A. tepida*, it is suggested that the latter exhibits a mechanism that de-creases the Mg/Ca ratio of the calcification fluid, while the high-Mg calcite forming species may not have this physiological tool. If the dependency of Mg incorporation on seawater [Ca^{2+}] is also valid for deep-sea benthic foraminifera typically used for paleostudies, the higher Ca concentrations in the past may potentially bias temperature reconstructions to a considerable degree. For instance, 25 Myr ago Mg/Ca ratios in *A. tepida* would have been 0.2 mmol/mol lower than today, due to the 1.5 times higher [Ca^{2+}] of seawater, which in turn would lead to a temperature underestimation of more than 2 °C.

1 Introduction

The ratio of magnesium to calcium (Mg/Ca) in fossil benthic foraminiferal shells is widely used to estimate past bottom water temperatures (Lear et al., 2000; Martin et al., 2002; Billups and Schrag, 2003; Katz et al., 2008; Kristjánsdóttir et al., 2007; Lear et al., 2003b; Shevenell et al., 2008; Skinner et al., 2003). The applicability of Sr/Ca ratios in foraminiferal calcite is less well defined. Whereas Sr/Ca variations in benthic foraminifera have been attributed to changes in Sr/Ca of seawater (Lear et al., 2003a), hydrostatic pressure (Elderfield et al., 1996), and temperature and/or carbonate ion concentration (Reichart et al., 2003; Rosenthal et al., 2006), those in planktic species were found to depend on pH and/or [CO_3^{2-}] (Dueñas-Bohórquez et al., 2009; Lea et al., 1999; Russell et al., 2004) and growth rate (Kιsakürek et al., 2008).

The Mg/Ca ratio of foraminiferal test carbonate has been used in combination with $\delta^{18}O$ to reconstruct past $\delta^{18}O$ of seawater (e.g., Lear et al., 2000; Billups and Schrag, 2003; Shevenell et al., 2008). However, recent studies have shown that in addition to temperature the calcite saturation state (Ω or $\Delta[CO_3^{2-}]$) potentially plays an important role in the Mg uptake into benthic foraminiferal shells (Elderfield et al., 2006; Healey et al., 2008; Rosenthal et al., 2006; Raitzsch et al., 2008). Calibrations using foraminifers from surface sediments yield a species-specific increase in Mg/Ca between 0.008 and 0.017 mmol/mol per µmol/kg $\Delta[CO_3^{2-}]$. Given the empirical exponential relationship, foraminiferal Mg/Ca changes only slightly within the generally narrow temperature range in the deep ocean of roughly 5 °C. On the other hand, potential changes in deep-sea $\Delta[CO_3^{2-}]$, for example in the North Atlantic during the Last Glacial Maximum by $\sim$25–30 µmol/kg, inferred from benthic foraminiferal B/Ca (Yu and Elderfield, 2007), would considerably bias Mg/Ca-based temperature estimations. Hence, for the ongoing development of this paleo-thermometer, it is essential to quantify the separate effects of temperature and calcite saturation state on benthic Mg/Ca ratios. Interestingly, field studies on benthic foraminifera (Elderfield et al., 2006; Healey et al., 2008; Rosenthal et al., 2006; Raitzsch et al., 2008) contradict results from culturing experiments on planktic foraminifera, which show that Mg/Ca decreases with increasing pH or $[CO_3^{2-}]$ (i.e. an increase in calcite saturation state; Kιsakürek et al., 2008; Lea et al., 1999; Russell et al., 2004).

The saturation state of seawater with respect to calcite is defined as $\Omega=[Ca^{2+}]\cdot[CO_3^{2-}]/K_{sp}$, where K_{sp} corresponds to the solubility product of calcite, that depends on ambient temperature, salinity and pressure (Zeebe and Wolf-Gladrow, 2001). Accordingly, Ω changes when $[CO_3^{2-}]$ and/or $[Ca^{2+}]$ change, but in the open ocean the calcium concentration is relatively constant with a long residence time of approximately 1.1 Ma (Broecker and Peng, 1982). Therefore, the calcite saturation state in seawater mainly varies with water depth and the carbonate ion concentration $[CO_3^{2-}]$, which is intrinsically linked to the other carbonate system parameters (i.e. DIC, alkalinity and pH; Zeebe and Wolf-Gladrow, 2001). However, a number of culturing studies have been conducted to determine the carbonate ion effect on foraminiferal Mg/Ca and Sr/Ca (Kιsakürek et al., 2008; Lea et al., 1999; Russell et al., 2004; Dissard et al., 2010a; Dueñas-Bohórquez et al., 2009), but only one on the effect of $[Ca^{2+}]$ (Bentov and Erez, 2006). Hence, it is crucial to understand whether varying $[Ca^{2+}]$ yields similar changes in the Mg and Sr incorporation as varying $[CO_3^{2-}]$ and whether the fractionations are constant between taxa.

For these reasons, we analysed Mg/Ca and Sr/Ca ratios in two species of foraminifera with contrasting calcification mechanisms, in relation to calcium concentration by means of controlled culturing experiments. For this study, we used the neritic to intertidal benthic species *Het-*

erostegina depressa (high-Mg calcite, tropical, symbiont-bearing) and *Ammonia tepida* (low-Mg calcite, temperate, symbiont-barren). Compared to deep-sea foraminifera, intertidal species are resistant to environmental fluctuations and can therefore grow at a range of experimental conditions. In our experiments, we varied the CaCO$_3$ saturation state by altering the calcium concentration $[Ca^{2+}]$ of the medium. Under natural conditions, the $[Ca^{2+}]$ covaries with salinity. By keeping salinity constant in the cultures, while varying $[Ca^{2+}]$, it is also possible to deconvolve the effect of Ω from salinity on e.g. Mg incorporation.

2 Experimental and analytical procedures

2.1 Sample collection and preparation

Specimens of the shallow benthic species *Ammonia tepida* were isolated from sediment collected at the Dutch Wadden Sea and stored in the laboratory at 18 °C. Specimens of the symbiont-bearing species *Heterostegina depressa*, provided by Burger's Zoo (Arnhem, The Netherlands), were picked from stocks kept at 24 °C and under a 12 h light:12 h dark cycle (De Nooijer et al., 2007).

Living individuals bearing brightly coloured cytoplasm were selected and placed in vials with natural seawater admixed with calcein (fluorexon, fluorescein complex) at a final concentration of 7 mg/L. The fluorescent indicator calcein is incorporated into the calcite walls whenever new chambers are formed, whereas pre-existing chambers are not affected (Bernhard et al., 2004). Incubated specimens can therefore be scanned afterwards to determine which chambers were built during the culturing period (Fig. 1).

After being maintained in an incubator for 2–4 weeks, foraminifers were checked for new chambers under an inverted fluorescence microscope. Only specimens that had chambers clearly marked by calcein were selected for the culturing experiments. The chambers formed during the experiment could be easily identified (Fig. 1). Although it was recently established that calcein does not affect the incorporation of trace metals (Dissard et al., 2009), not admixing calcein to the culture water during the experiments avoided any possible impact.

Upon finishing the experiments, only foraminifers with new, non calcein-labelled chambers were selected for elemental analysis with laser ablation ICP-MS. The specimens were placed in sodium hypochlorite (NaOCl 5%) for 10 min to remove cytoplasm and organic material from the surface of the tests. The shells were then thoroughly washed with deionised water and dried.
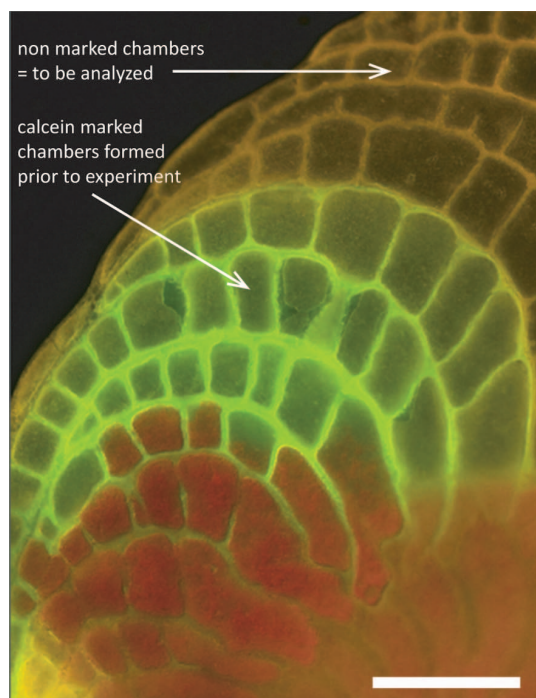
Fig. 1. *Heterostegina depressa* under a fluorescence microscope after excitation. Shell calcite marked with calcein (green) was built prior to the experiment. The younger (newly formed) non-marked chambers were formed during the experiment and were analysed with LA-ICP-MS. Scale bar is 100 μm.

2.2 Experimental setup

Four seawater solutions were prepared with different Ω values. We varied parameters based on the following relationship:

$$\Omega = \frac{[CO_3^{2-}] \cdot [Ca^{2+}]}{K^*_{sp(Cc)}} \qquad (1)$$

where Ω is the calcite saturation state of the seawater and $K^*_{sp(Cc)}$ is the solubility product of calcite.

Since we altered the chemical composition of seawater for our experiments, the solutions were composed of 50% natural seawater (NSW) from the eastern Mediterranean Sea and 50% artificial seawater (ASW). The ASW was prepared according to the methods of Kester et al. (1967) and Berges et al. (2001). Addition of the volumetric salts CaCl$_2$ (1M) and MgCl$_2$ (1M) ensured similar Mg/Ca ratios (approximately 5.2) between the different solutions (Tables 1, 2). Since Cl$^-$ is the major anion in seawater, different amounts of CaCl$_2$ and MgCl$_2$ were compensated by accordant addition of NaCl (2M). In this manner, salinity was kept constant between the media (Tables 1, 2).

All seawater solutions were filtered using a 0.2 μm filter and were air-bubbled for 24 h in order to equilibrate the seawater with atmospheric pCO$_2$. Finally, four individual solutions were obtained with the following approximate calcium concentrations: 1/2 of natural [Ca^{2+}] (group 1), natural [Ca^{2+}] (group 2), 1.5 × natural [Ca^{2+}] (group 3), 2 × natural [Ca^{2+}] (group 4); see Tables 1 and 2 for exact concentrations.

Specimens of *H. depressa* and *A. tepida* (15 and 25–30 individuals, respectively) were incubated in 30-mL containers termed culture chambers throughout this paper. These containers had a polyacetal bottom and lid, separated by a diaphanous Plexiglas ring (Fig. 2). At the bottom of the culture chamber a 0.2 μm filter (cellulose acetate) was placed, on top of which a thin layer of artificial sediment (SiO$_2$) was added. A small amount of a freeze-dried mixture of microalgae/diatom (*Dunaliella salina* and *Phaeodactylum tricornutum*) was added as food. A constant flow of seawater refreshed the culture chambers during the duration of the experiment (Fig. 2). The chambers were connected with silicon tubes to reservoirs of 1 L-glass bottles that contained the seawater with modified calcium concentrations. A peristaltic pump circulated the modified seawater through the system at a speed of approximately 1 mL/h while the media was bubbled continuously with moist air (Fig. 2). Shortly before setting up the experiments, all material was thoroughly cleaned several times with HCl (1N) and deionised water, and subsequently rinsed with the seawater used for that experimental treatment.

The complete system was placed in an incubator kept at a constant temperature (±0.5 °C). The experiment containing specimens of *A. tepida* was kept at 18 °C and in the dark since there is no need for light due to lacking symbionts and the infaunal habitat this species partly occupies in natural environments. The *H. depressa* experiment was conducted at 24 °C and exposed to an artificial light (15 W tropical reef lamp, Arcadia, FO15) cycle of 12 h light/12 h dark to allow for photosynthesis of their endosymbionts. Each experiment ran for two months. At the beginning and at the end of each experiment, seawater was subsampled for determining elemental concentrations with ICP-OES, and for alkalinity analyses using an automated titrator (702 SM Titrino, Metrohm). Salinity was checked monthly and adjusted when required. As seawater was in equilibrium with the atmosphere, a pCO$_2$ value of 365 μatm (Striegl et al., 2001) was used to calculate the other carbonate system parameters using the *co2sys* software (Pierrot et al., 2006).

2.3 Element analysis

Element concentrations in the test carbonate of single chambers of the cultured specimens were determined using a Geo-Las 200Q 193 nm Excimer laser (Lambda Physik) connected to a quadrupole ICP-MS (Reichart et al., 2003). Only newly formed chambers of the foraminifers were ablated, identifiable from non calcein-marked chambers on photographs taken with a fluorescence microscope. Beam diameter was set at 60 μm for *A. tepida* and 80 μm for *H. depressa*, repetition rate was 6 Hz, and energy density was set at 1 J/cm^2.

Table 1. Experimental culturing conditions, Mg/Ca and Sr/Ca ratios and partition coefficients for *H. depressa*.

Experiment I H. depressa	Group 1	Group 2	Group 3	Group 4
No. of individuals added	15	15	15	15
No. of grown individuals	6	10	5	7
No. of analysed shells[a]	6	7	5	7
Temperature (°C)	24	24	24	24
Salinity (psu)	36.2	35.8	35.6	35.6
Alkalinity (µmol/kg)	2440±123	2436±112	2400±50	2384±80
DIC (µmol/kg)[b]	2087±102	2085±74	2059±34	2046±47
$[Ca^{2+}]$ (µmol/kg)	4847±28	9534±374	13760±233	18439±242
$[CO_3^{2-}]$ (µmol/kg)[b]	246±25	243±22	237±10	234±15
Ω	2.76±0.11	5.39±0.51	7.58±0.29	10.04±0.56
Mg/Ca_{sw}	5.17±0.03	5.56±0.09	5.96±0.14	6.20±0.33
$Sr/Ca_{sw}\times1000$	17.82±0.42	9.34±0.27	6.43±0.05	4.81±0.14
Mg/Ca (mmol/mol)	143.34±11.89	145.56±8.62	151.57±8.34	155.00±11.20
$D_{Mg}\times100$	2.78±0.23	2.61±0.16	2.54±0.14	2.51±0.18
Sr/Ca (mmol/mol)	4.82±0.35	2.66±0.26	1.98±0.10	1.61±0.12
D_{Sr}	0.27±0.02	0.28±0.03	0.31±0.02	0.33±0.02

Values are given with ± standard deviations.
[a] Not all grown foraminifera were analysed because of limited space available for LA.
[b] Calculated from pCO$_2$ and alkalinity with the *co2sys* program (Pierrot et al., 2006), using constants from Mehrbach et al. (1973) and pressure corrections of the equilibrium constants from Millero (1983).
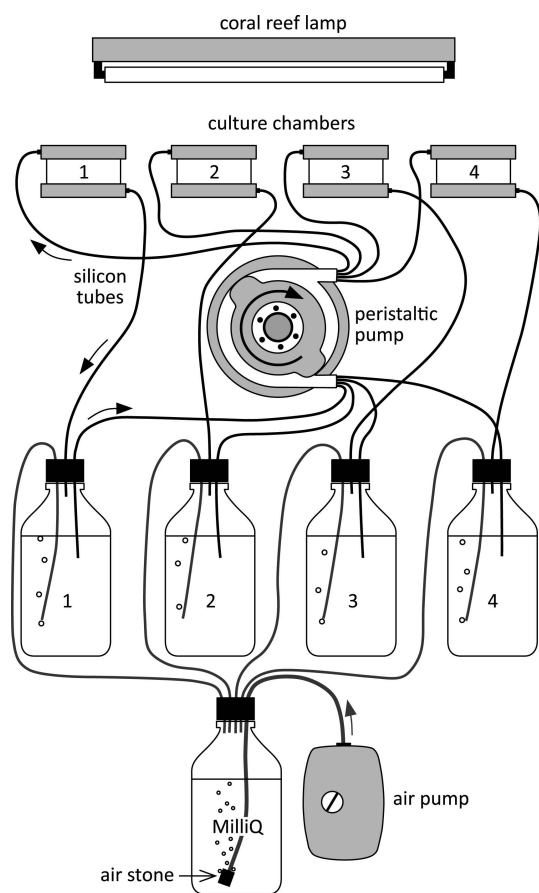
Table 2. Experimental culturing conditions, Mg/Ca and Sr/Ca ratios and partition coefficients for *A. tepida*.

Experiment II A. tepida	Group 1	Group 2	Group 3	Group 4
No. of individuals added	29	25	30	30
No. of grown individuals	8	6	8	10
No. of analysed shells[a]	6	4	8	7
Temperature (°C)	18	18	18	18
Salinity (psu)	35.2	35.3	35.2	35.3
Alkalinity (µmol/kg)	2385±29	2378±23	2363±31	2385±39
DIC (µmol/kg)[b]	2111±2	2105±16	2093±13	2111±11
$[Ca^{2+}]$ (µmol/kg)	5427±17	9786±5	14146±428	18138±539
$[CO_3^{2-}]$ (µmol/kg)[b]	201±4	200±3	198±4	200±6
Ω	2.51±0.10	4.51±0.10	6.46±0.38	8.37±0.47
Mg/Ca_{sw}	5.08±0.02	5.10±0.10	5.19±0.21	5.30±0.28
$Sr/Ca_{sw}\times1000$	15.45±0.62	8.46±0.10	5.87±0.01	4.58±0.11
Mg/Ca (mmol/mol)	2.41±0.51	1.78±0.46	1.60±0.29	3.65±1.95
$D_{Mg}\times1000$	0.47±0.10	0.37±0.08	0.31±0.06	0.70±0.40
Sr/Ca (mmol/mol)	2.56±0.68	1.35±0.19	0.91±0.05	0.78±0.12
D_{Sr}	0.17±0.04	0.16±0.02	0.16±0.01	0.17±0.03

Values are given with ± standard deviations.
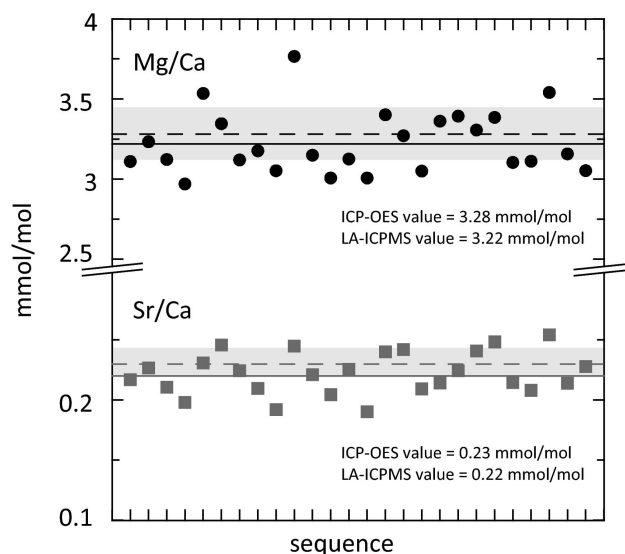[a] Not all grown foraminifera were analysed because of limited space available for LA.
[b] Calculated from pCO$_2$ and alkalinity with the *co2sys* program (Pierrot et al., 2006), using constants from Mehrbach et al. (1973) and pressure corrections of the equilibrium constants from Millero (1983).

Fig. 2. Schematic illustration of the instrumental setup. See main text for details.

Fig. 3. Laser ablation derived Mg/Ca and Sr/Ca ratios of the in-house Iceland calcite spar (GJR) measured over the entire analysis period. The solid lines represent the average values and the dashed lines the ratios determined with ICP-OES. The grey shaded area delimits the 95% confidence interval.
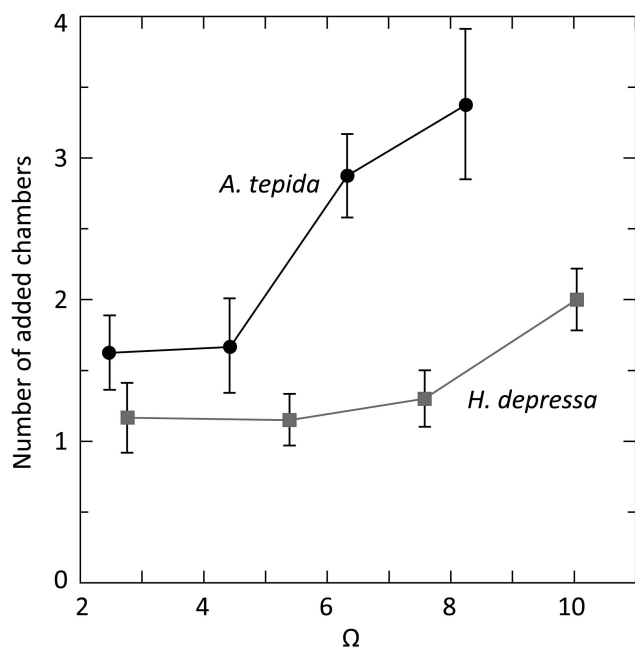
Time resolved raw data in counts per second (cps) were converted to element concentrations (ppm) using the GLIT-TER software (GEMOC/CSIRO). This data reduction software also facilitates the manual selection of intervals used for background subtraction and signal integration.

3 Results

3.1 Survival and growth rates

The survival rate for both species was very high at almost 100% in all experiments. Three specimens (out of 30) of *A. tepida* did not survive in the culture chamber that contained seawater with the highest calcite saturation state. In contrast, six juvenile foraminifers were found in the same group. The number of individuals that added new chambers is relatively constant between the different groups (Tables 1, 2). On average, 29% of the individuals of incubated *A. tepida* formed new chambers while 47% of the *H. depressa* specimens built new chambers. The reason for the low percentage of individuals grown during the incubation period may be that some of the specimens were potentially already mature from the beginning.

When counting the newly formed chambers of each foraminiferal test, we observed in both species the highest numbers of new chambers at highest calcite saturation state (Fig. 4). In the Groups 1 and 2 with the lowest Ω values, no considerable difference in chamber addition was apparent. In contrast, in Groups 3 and 4, *A. tepida* produced by up to two

Element concentrations were calculated from isotopic counts for ^{24}Mg, ^{26}Mg, ^{27}Al, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{55}Mn, and ^{88}Sr, where Al and Mn were monitored as indicators for contaminant phases. Although the foraminifers were cultured without natural sediment, some contamination from an unknown source was observed and parts of the obtained ablation profiles displaying contaminants were discarded before calculating element/Ca ratios.

Calcium was used as an internal standard assuming 40% wt, which enables to correctly calculate element to calcium ratios routinely reported in paleoceanographic studies. Before and after ~10 sample analyses, a NIST 610 silicate standard with precisely determined elemental concentrations (Pearce et al., 1997) was measured three times each as an external standard. Since the NIST was ablated with a higher energy density ($4 \, J/cm^2$) in order to enhance signal quality, an in-house Iceland spar calcite (GJR) ablated with low energy was employed as second, matrix-matched standard (Fig. 3). However, non-matrix matched calibration using a 193 nm laser was shown to be accurate for many elements (Hathorne et al., 2008).

Fig. 4. Number of new chambers and new rows of chambers for *A. tepida* and *H. depressa*, respectively, added during two months versus Ω. Error bars are standard errors of the mean.

times the number of new chambers. The amount of new rows of chambers formed by *H. depressa* was considerably higher in seawater with the highest Ω.

3.2 Partition coefficients of Mg and Sr

Since the Mg/Ca ratios of the modified seawater were not exactly the same between the different experiments (Tables 1, 2), we plotted the analysed Mg/Ca ratios of calcite (Mg/Ca$_{Cc}$) versus the ones of seawater (Mg/Ca$_{sw}$) (Fig. 5). In the *A. tepida* experiment, the variation of Mg/Ca$_{sw}$ is negligible, which indicates that this parameter is not responsible for the observed variation of Mg/Ca$_{Cc}$ (Fig. 5b). Conversely, Mg/Ca$_{Cc}$ in *H. depressa* increased slightly with increasing Mg/Ca$_{sw}$. Although the overall difference between the population means is statistically insignificant (ANOVA $p > 0.05$) at the 95% confidence level, the significant linear relationship between Mg/Ca$_{Cc}$ and Mg/Ca$_{sw}$ (F-Test $p < 0.05$) suggests a concentration effect on the Mg incorporation during this experiment. The data, however, do not follow a single through-origin linear fit but show considerable deviations from such a line (Fig. 5a). We calculated the partition coefficients of Mg according to the equation:

$$D_{TE} = \frac{TE/Ca_{Cc}}{TE/Ca_{sw}} \quad (2)$$

where TE/Ca$_{Cc}$ and TE/Ca$_{sw}$ correspond to the trace element/Ca ratio in the foraminiferal calcite and in seawater, respectively. The observed D_{Mg} values for *H. depressa* range

from 2.51×10^{-2} to 2.78×10^{-2} (Fig. 5a, Table 1), suggesting that D_{Mg} was altered by the minor variations in Mg/Ca$_{sw}$ and/or by the highly variable Ca concentrations we aimed at with our experiments.

Since the Sr/Ca ratios in seawater were not the same between the different experimental solutions due to the manipulation of the [Ca^{2+}], we calculated the partition coefficients of Sr using Eq. (2). We observe that D_{Sr} is species-specific with a value of 0.28 for *H. depressa* and 0.16 for *A. tepida* (Fig. 5c). The Sr/Ca data for both species fall close to through-origin linear relationships with Sr/Ca$_{sw}$, suggesting that D_{Sr} is not affected by seawater Sr/Ca or Mg/Ca.

However, in order to normalize the absolute trace element/Ca ratios of calcite to the seawater elemental composition, we report our data only in terms of partition coefficients D_{Mg} and D_{Sr} in the following sections.

3.3 D_{Mg} and D_{Sr} versus calcite saturation state

The experiments on *H. depressa* and *A. tepida* yield decreasing D_{Mg} with increasing Ω for both species (Fig. 6a and b). Although it is possible that an exponential function is more appropriate, we chose a linear fit through the data, where D_{Mg} decreases with 4×10^{-4} per Ω unit in *H. depressa* (Fig. 6a) and with 4×10^{-5} in *A. tepida* (Fig. 6b). Both regressions are statistically significant at the 95% confidence level ($p < 0.05$), obtained from regression analyses. For the *H. depressa* experiment, however, ANOVA (analysis of variance) tests suggests that the overall differences between the population means (i.e. between the different Ω groups) are not significant ($p > 0.05$). For *A. tepida*, in contrast, the population means are significantly different ($p = 0.005$). The higher D_{Mg} value found in Group 4 of the *A. tepida* experiment is considered an outlier and was therefore excluded from line fitting. Scanning electron microscopy suggests that the foraminiferal tests in this group are affected by inorganic overgrowths (Fig. 7). Although it is difficult to identify this phase, it possibly consists of inorganic calcite enriched in Mg, precipitated from the highly calcite-saturated seawater. The gradient through the remaining three data for *A. tepida* is similar to results from the culture experiments on the planktic foraminifer *O. universa* by Russell et al. (2004) within the same range of calcite saturation state, although D_{Mg} values for *O. universa* are by a factor of 3 higher than for *A. tepida* (Fig. 6b). In the study of Russell et al. (2004), the dependence of D_{Mg} on pH and [CO$_3^{2-}$] was stronger at lower calcite saturation states, compared to higher saturations where D_{Mg} seemed to be fairly constant (Fig. 6b). The D_{Mg} values for *G. bulloides* as well as for *G. ruber* (white) from Kısakürek et al. (2008), by comparison, decreased in a similar way with increasing Ω, but the gradients were found to be significantly steeper than those for *A. tepida* and *O. universa* (Fig. 6b).
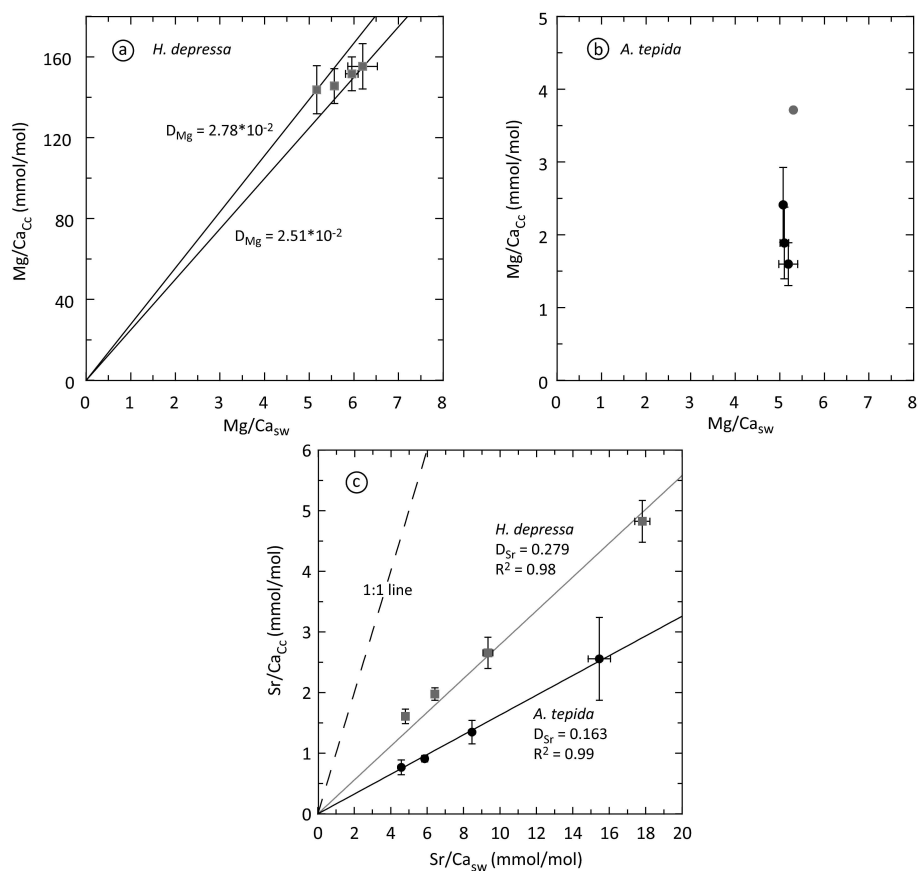
Fig. 5. Trace element to Ca ratios in calcite versus seawater for **(a, b)** magnesium and **(c)** strontium. Grey squares correspond to *H. depressa*, whereas the black dots correspond to *A. tepida*. Lines going through the origin correspond to D values indicated. Error bars (2σ) are based on multiple measurements and variability during the duration of the experiment.

The partition coefficients of strontium for *A. tepida* plotted versus Ω display no correlation (Fig. 6c). In contrast, D_{Sr} of *H. depressa* increases linearly with Ω at a gradient of $9 \times 10^{-3}/\Omega$ unit, with a high statistical significance derived from regression analysis ($p \approx 0.01$). In addition, the overall differences between population means obtained from ANOVA are highly significant ($p < 0.001$). The correlation between D_{Sr} and the calcite saturation state for *H. depressa* is considerably higher than the slight D_{Sr} increase found by Russell et al. (2004) for the planktic foraminifer *O. universa* (Fig. 6c). In our experiment, D_{Sr} for *H. depressa* increased by approximately 23%, whereas D_{Sr} for *O. universa* in the Russell et al. (2004) study increased by only 10% over the same range of Ω changes. Interestingly, the amount of Sr incorporated into tests of *H. depressa* is generally up to two times higher compared to the low-Mg foraminifer species for which the data are shown in Fig. 6c.

4 Discussion

4.1 The effect of [Ca^{2+}] on Mg and Sr incorporation

The influence of [Ca^{2+}] on the Mg incorporation in foraminiferal calcite is rarely investigated. For two benthic high-Mg species, Segev and Erez (2006) have shown that the absolute Ca concentration does not have a clear effect on foraminiferal Mg/Ca ratios. Our results show that this may be true for high-Mg foraminifera, since D_{Mg} of the four experimental solutions are not significantly different ($p > 0.05$) (Fig. 6a). In contrast, D_{Mg} of the low-Mg species *A. tepida* decreased significantly with increasing [Ca^{2+}], indicating a distinct effect of seawater [Ca^{2+}] on the Mg incorporation into shells of this species (Fig. 6b).

The various Ca^{2+} concentrations also resulted in various calcium carbonate saturations of the culturing media. A number of culturing experiments have shown that the Mg incorporation in various planktic species decreases with higher calcite saturation state (Ω), when Ω is altered by changing the [CO$_3^{2-}$] (Kısakürek et al., 2008; Lea et al., 1999; Russell et al., 2004; Fig. 6b). Our experiments show that altering Ω

by changing the $[Ca^{2+}]$ does result in a similar shift in Mg incorporation in benthic foraminifera. The D_{Mg} values in *A. tepida* decreasing with increasing Ω are in line with those reported for the planktic species (Fig. 6b), while the slight decrease in D_{Mg} for *H. depressa* is still within reproducibility of replicate measurements and thus displays no significant offset (Fig. 6a). The opposite trend (i.e. higher Mg incorporation correlated with higher Ω) has also been reported for various benthic species from field surveys (Elderfield et al., 2006; Healey et al., 2008; Raitzsch et al., 2008).

Precipitation rate could possibly be responsible for the observed variations in the Mg incorporation into biogenic carbonates. Although possibly not directly applicable to the calcitic shells of foraminifera, laboratory experiments on aragonitic corals indeed showed that an increase in $[Ca^{2+}]$ had the same effect on the precipitation rate as an increase in $[CO_3^{2-}]$ (Gattuso et al., 1998; Langdon et al., 2000). Rather than by precipitation rate, Gaetani and Cohen (2006) showed that seasonal variations of Mg and Sr incorporation in corals are best described by a combination of a temperature effect on element partitioning and variations in the "precipitation efficiency" (the mass fraction of aragonite precipitated from the calcifying fluid). This, in turn, is most likely driven by varying efficiency of the Ca-ATPase enzyme pump or ion channel transport (Gaetani and Cohen, 2006). Like corals, foraminifers possess a variety of such mechanisms to modify the chemistry of the seawater within isolated pools in order to enhance calcification. These physiological processes may be sensitive to ambient environmental conditions (Bentov and Erez, 2006).

The interpretation of benthic foraminiferal Sr fractionation and the use of Sr/Ca ratios in paleoceanography is less straightforward than that of Mg fractionation and Mg/Ca ratios as a proxy for seawater temperature. Variations in the concentration of Sr have been attributed to changes in Sr/Ca of seawater (Lear et al., 2003a), hydrostatic pressure effects (Elderfield et al., 1996), and temperature and/or carbonate ion concentration (Reichart et al., 2003; Rosenthal et al., 2006). On the other hand, Sr incorporation in planktic species was shown to increase with increasing pH or $[CO_3^{2-}]$ (Dueñas-Bohórquez et al., 2009; Lea et al., 1999; Russell et al., 2004) and increasing growth rate (Kısakürek et al., 2008). The latter is yet difficult to isolate from variations in temperature and salinity since both influence the growth rate of the foraminifer. Moreover, the growth rate dependence of Sr/Ca shown in the Kısakürek et al. (2008) study is rather small, particularly when the measurement uncertainties are considered. However, as we changed $[Ca^{2+}]$ between the different solutions, while keeping $[Sr^{2+}]$ constant, we observed nearly perfect through-origin linear correlations between the Sr/Ca ratios in the shells and the media (Fig. 5c). The accordant D_{Sr} values of *A. tepida* did not vary with changing Ω, whereas D_{Sr} of *H. depressa* increased significantly with increasing Ω (Fig. 6c).

## 4.2 Possible role of Mg/Ca$_{sw}$ on Mg and Sr incorporation

Since the Mg/Ca ratios of seawater between the different solutions displayed some variability (Table 1), we plotted Mg/Ca$_{Cc}$ versus Mg/Ca$_{sw}$ to correct for differences in the culture media and thus obtain fractionation factors for Mg in the two investigated species (Fig. 5a, b). For *A. tepida*, the negligible range of Mg/Ca$_{sw}$ from 5.1 to 5.3 (Fig. 5b) indicates that the observed variation in Mg/Ca of *A. tepida* is primarily related to the variation of seawater $[Ca^{2+}]$.

Conversely, Mg/Ca$_{Cc}$ in *H. depressa* increased with increasing Mg/Ca$_{sw}$ that had a larger range in culture media from 5.2 to 6.2 (Fig. 5a). The partition coefficient shows a clear decrease with increasing Mg/Ca$_{sw}$ (Fig. 8). This relationship is very similar to the one depicted by Segev and Erez (2006) for *Amphistegina* spp. and by Mucci and Morse (1983) for inorganically precipitated calcites (Fig. 8). Based on the observation that Mg concentrations in high-Mg foraminifera are comparable to those in inorganic calcite, it seems plausible that the Mg fractionation during calcification in *Amphistegina* spp. and *H. depressa* follows the pure inorganic fractionation.

Mucci and Morse (1983) and Ohde and Kitano (1984) showed that higher Mg/Ca$_{Cc}$ and Mg/Ca$_{sw}$ also favours Sr incorporation into $CaCO_3$. This may explain some of the variation in D_{Sr} in our *Heterostegina* experiments, which showed an increase with increasing Mg/Ca$_{Cc}$ (Fig. 6c). Such a change in D_{Sr} may be related to a very low ion activity of Mg^{2+} in solutions from which low-Mg carbonate is precipitated, and vice versa (Lorens and Bender, 1980; Morse and Bender, 1990). Alternatively, the Sr^{2+} incorporation is facilitated by the creation of cationic sites larger than Ca^{2+} due to the distortion of the calcite lattice produced by the incorporation of the smaller Mg^{2+} cations (Mucci and Morse, 1983). Both hypotheses could explain why Sr/Ca found in high-Mg calcifying organisms is generally a factor of about two higher compared to low-Mg biogenic carbonates (Morse and Bender, 1990). This is in accordance with the distinct partition coefficients of Sr determined for the high-Mg and low-Mg foraminifera in this study (Fig. 5c).

Calculating the theoretical change of D_{Sr} for *H. depressa* using the observations by Mucci and Morse (1983) and Carpenter and Lohmann (1992) from inorganic and various biogenic carbonates, D_{Sr} should have increased by merely 1% as a result of the total Mg/Ca$_{Cc}$ increase by 8% (Table 1). We observed a D_{Sr} increase of 23% (Fig. 6c), suggesting that only a small portion of the D_{Sr} variation can be attributed to the Mg/Ca$_{Cc}$ increase. Consequently, the obtained increase in D_{Sr} of *H. depressa* is largely related to the variable Ca concentrations among the media.
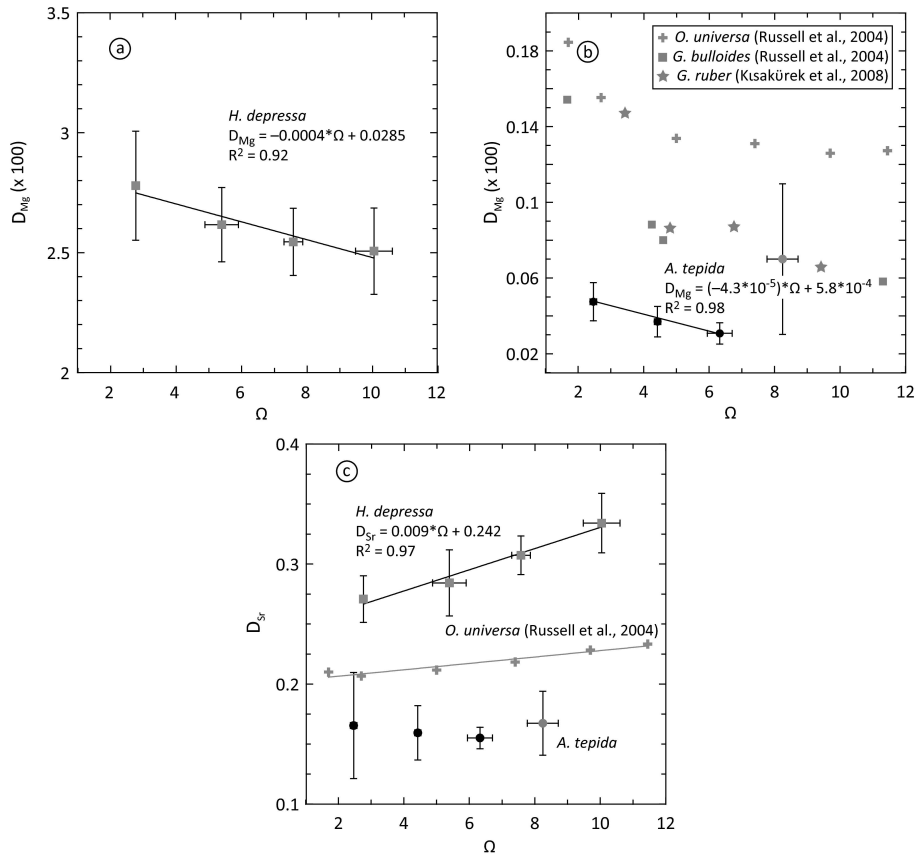
Fig. 6. Relation between D_{Mg} of cultured foraminifers and calcite saturation state of the media for **(a)** *H. depressa* and **(b)** *A. tepida*. Error bars represent standard deviations (2σ). The partition coefficients D_{Mg} of both species decrease with increasing Ω. The grey data point in (b) is considered an outlier (see text for details). Additionally plotted are the data of Russell at al. (2004) for the planktic species *Orbulina universa* (crosses) and *G. bulloides* (squares), as well as the *G. ruber* (stars) data from Kısakürek et al. (2008). **(c)** Partition coefficient for Sr (D_{Sr}) plotted versus Ω. Black circles correspond to *A. tepida*, grey squares correspond to *H. depressa*. Data are plotted along with strontium data for *O. universa* (crosses) from Russell et al. (2004).
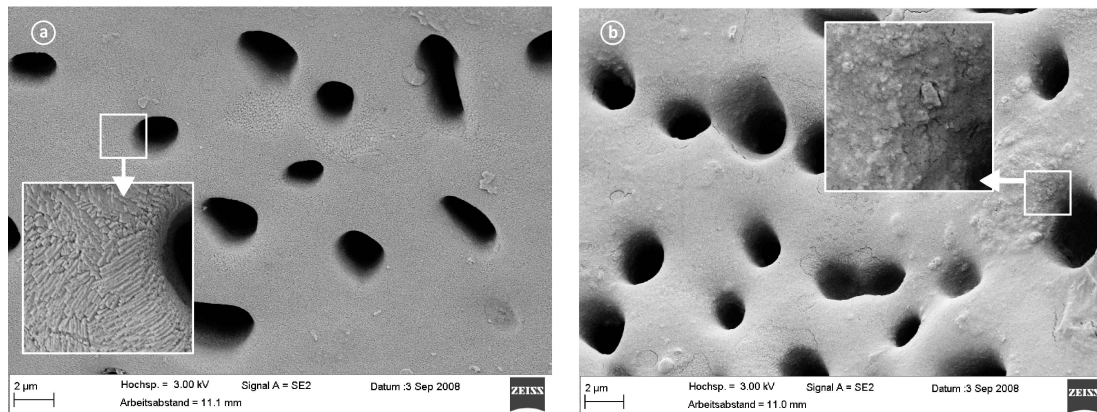
Fig. 7. SEM pictures showing the shell surface of *A. tepida* grown in the solutions with **(a)** natural $[Ca^{2+}]$ and **(b)** the highest $[Ca^{2+}]$. The surface in (a) shows a detailed structure of tiny calcite needles, whereas the shell in (b) is covered with a crusted veneer of an unidentified phase.
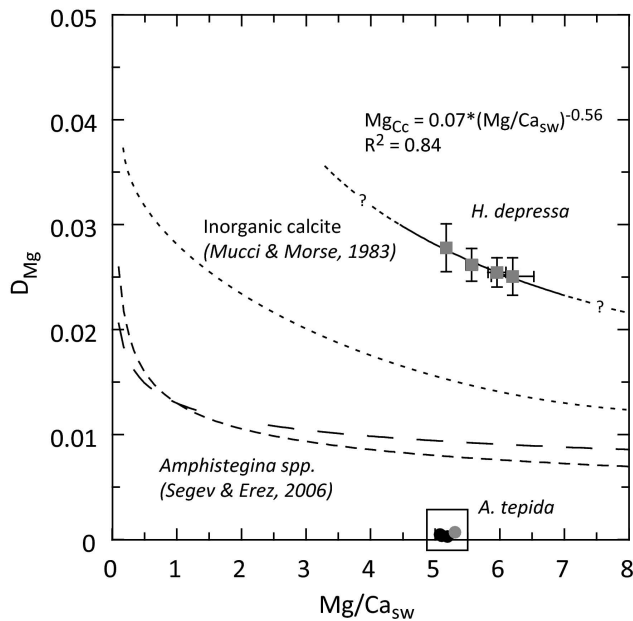
Fig. 8. Shell D_{Mg} in relation to the Mg/Ca ratio of seawater. The extrapolated potential fit through the data for *H. depressa* exhibits a similar shape as found by Segev and Erez (2006) for *Amphistegina lobifera* (widely dashed line) and *A. lessonii* (closely dashed line) and by Mucci and Morse (1983) for inorganic calcite overgrowths (dotted line).

4.3 Biomineralisation mechanisms

Based on field studies and culturing experiments showing the impact of amongst others temperature, salinity, Ω and seawater elemental concentrations on trace element incorporation and stable isotope fractionation, several conceptual models have been developed to explain these relationships. These models are commonly based on a foraminiferal calcification pathway starting with seawater vacuolisation (De Nooijer et al., 2009a; Erez, 2003) and subsequent modifications resulting in the separate production of intracellular pools containing calcium and carbonate (Bentov and Erez, 2006; Erez, 2003). The low Mg/Ca ratios in calcite of most species imply that during formation of the internal Ca pool, these species actively discriminate against Mg so that the Ca pool has a Mg/Ca ratio considerably lower than that of seawater. Although not identified directly, this could be caused by selective removal of Mg^{2+} from the Ca reservoir or by Ca-pumps actively pumping Ca^{2+} from vacuolised seawater into the Ca reservoir. Both of these mechanisms can explain how foraminifers produce a Ca pool with a low Mg/Ca ratio. It has been shown that the Mg isotopes in low-Mg foraminiferal calcite are strongly depleted in comparison to those of inorganically precipitated calcite (Pogge von Strandmann, 2008). This implies that the physiological mechanism reducing Mg/Ca ratios in the intracellular Ca pool selectively let in isotopically lighter Mg ions. This, in turn, suggests

that these species transport Ca using seawater vacuolisation, since active Mg^{2+} removal would more likely result in an isotopically enriched Mg signature in the intracellular Ca pool. In any version of a calcification model, selective sequestration of Mg^{2+} by organic compounds may be involved in reducing the Mg/Ca ratio of the internal calcification fluid (Bentov and Erez, 2006).

The high Mg/Ca ratios in *Heterostegina depressa* may indicate that the calcification strategy in this species is fundamentally different from that of *A. tepida*, which may in turn explain the atypical response of the former group to altered Ω. The low partition coefficient for Mg of *A. tepida* (as in most foraminiferal species; Blackmon and Todd, 1959; Erez et al., 2003) compared to those for most other trace elements, suggests that foraminifers adopt a physiological mechanism to discriminate between Ca^{2+} and Mg^{2+} during production of the internal Ca pool. Some species, however, produce calcite that has a Mg concentration comparable to calcium carbonates inorganically precipitated from media with Mg/Ca ratios comparable to seawater (Katz, 1973; Oomori et al., 1987). This suggests that these species do not have this discriminating mechanism and that they solely rely on increasing the pH at the site of calcification (Erez, 2003; Zeebe and Sanyal, 2002), which is also found in high-Mg species (De Nooijer et al., 2009b; Zeebe and Sanyal, 2002). This also implies that Mg/Ca ratios in high-Mg taxa are directly related to the Mg/Ca ratio in seawater, rather than to absolute concentrations of Ca and Mg, which explains the similar Mg/Ca ratios in *H. depressa* in our experiment.

5 Conclusions and implications for paleoreconstructions

Culturing experiments show that the Mg incorporation into shells of *Heterostegina depressa* and *Ammonia tepida* decreased with increasing calcium concentration and thereby calcite saturation state of the seawater, Ω. The decrease in D_{Mg} of *A. tepida* at a gradient of 4.3×10^{-5} per Ω unit is statistically significant, whereas the one of *H. depressa* is insignificant. The slight decrease in D_{Mg} with increasing Ω observed in the *H. depressa* experiment, however, may rather be related to minor variations in the Mg/Ca ratio between the different groups.

The observed strong variability of Mg/Ca in *A. tepida* suggests a mechanism actively depleting Mg and/or enriching Ca in the intracellular calcification pool. The efficiency of this mechanism may depend on the ambient seawater conditions. The insignificant change in D_{Mg} of *H. depressa* between the different groups suggests that this high-Mg species possibly lacks such a mechanism to fundamentally alter the internal Ca and/or Mg concentrations, using a different calcification pathway (e.g., increasing pH by endosymbiotic activity).

Due to the influence of seawater [Ca^{2+}] on Mg/Ca in the shallow-water foraminifer *A. tepida* observed in this study, the question arises whether this might also bias paleotemperature estimations based on deep-sea benthic species for time periods when the calcium concentration was considerably different from today. Reconstructions of past [Ca^{2+}] of seawater using fossil echinoderms (Dickson, 2002) and fluid inclusions in halite (Horita et al., 2002) suggest that the calcium concentration of seawater decreased from ∼23 mmol/kg during the Cretaceous to 10 mmol/kg at present. For instance, 25 Myr ago seawater [Ca^{2+}] was about 1.5 times higher than in the modern ocean. In our culture experiments, Mg/Ca ratios in *A. tepida* were found to be 1.6 mmol/mol at this Ca concentration, whereas Mg/Ca was 1.8 mmol/mol at modern [Ca^{2+}]. Using a general Mg/Ca-temperature relationship, this change in Mg/Ca would imply a temperature increase since 25 Ma of more than 2 °C. However, it is not only [Ca^{2+}] that has changed over time, but also the Mg/Ca of seawater. The secular variation of seawater Mg/Ca is well constrained through different approaches, which include Mg/Ca analyses of fluid inclusions in halite (Horita et al., 2002; Lowenstein et al., 2001; Zimmermann, 2000), fossil echinoderms (Dickson, 2002), and calcium carbonate veins from mid-ocean ridge flanks (Coggon et al., 2010). All these studies revealed remarkably similar results, showing that Mg/Ca$_{sw}$ increased since the Cretaceous from slightly more than 1 to 5.2 at present. The culturing study by Segev and Erez (2006) shows that Mg/Ca in foraminifera decreases rapidly when Mg/Ca of seawater becomes lower than 5.2, whereas at higher ratios the effect on shell Mg/Ca is smaller. The problem of the lower Mg/Ca$_{sw}$ in the past is well known and usually considered in estimations of Mg/Ca temperatures covering longer time periods (e.g., Lear et al, 2000), but the potential additional effect of [Ca^{2+}]$_{sw}$ on foraminiferal Mg/Ca is a new issue that might be addressed in these paleoreconstructions. However, since this conclusion is drawn from experiments on *A. tepida*, which is never used for temperature reconstructions, future culturing studies on deep-sea benthic species are required to test whether the effect of the Ca concentration on shell Mg/Ca is also valid for foraminifera typically used for paleostudies.

The influence of Ω on the partition coefficient of strontium was different between both species, displaying no effect in *A. tepida* and a highly significant effect in *H. depressa* with a sensitivity of 0.009/Ω unit. The D_{Sr} values of both species are shown to be largely independent from the Sr/Ca ratio of seawater, suggesting that Sr/Ca ratios in fossil shells of these shallow-water benthic foraminifera may be used to reconstruct past variations in Sr/Ca of seawater. The reconstruction by Lear et al. (2003a) using deep-sea benthic foraminifera suggested that Sr/Ca of seawater has increased from ∼7.5 mmol/mol at 40 Ma BP to the present day value of ∼8.5 mmol/mol. In a very recent study, Coggon et al. (2010) who investigated the chemical composition of carbonate veins from mid-ocean ridge flanks come

to the conclusion that 40 Myr ago the Sr/Ca ratio of seawater was much lower (∼2 mmol/mol) than suggested by the foraminiferal study of Lear et al. (2003a). They attributed the discrepancy between these two studies to the largely unknown effects of physiological processes on the elemental partition coefficients. According to the partition coefficient determined in this study, the Sr/Ca$_{sw}$ ratio of 2 mmol/mol at 40 Ma BP depicted by Coggon et al. (2010) would have resulted in Sr/Ca ratios in *A. tepida* that are by approximately 1 mmol/mol lower than today. This species seems to be adequate for testing this hypothesis since our study shows that the Sr incorporation is not affected by the Ca concentration, and the effects of salinity and pH on Sr/Ca seem to be comparatively small, with sensitivities of roughly 0.1 mmol/mol per 9‰ and 0.1 mmol/mol per 0.8 pH unit, respectively (Dissard et al., 2010a, b).

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