



## Research paper

Effect of different seawater  $\text{Mg}^{2+}$  concentrations on calcification in two benthic foraminifersAntje Mewes<sup>a,\*</sup>, Gerald Langer<sup>b</sup>, Lennart Jan de Nooijer<sup>c</sup>, Jelle Bijma<sup>a</sup>, Gert-Jan Reichart<sup>a,c,d</sup><sup>a</sup> Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany<sup>b</sup> Department of Earth Sciences, Cambridge University, Downing St., Cambridge, CB2 3EQ, UK<sup>c</sup> Royal Netherlands Institute for Sea Research, Landsdiep 4, 't Horntje, Texel 1797 SZ, The Netherlands<sup>d</sup> Department of Earth Sciences, University of Utrecht, Budapestlaan 4, 3584 CD Utrecht, The Netherlands

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## ABSTRACT

Magnesium, incorporated in foraminiferal calcite ( $\text{Mg}/\text{Ca}_{\text{CC}}$ ), is used intensively to reconstruct past seawater temperatures but, in addition to temperature, the  $\text{Mg}/\text{Ca}_{\text{CC}}$  of foraminiferal tests also depends on the ratio of Mg and Ca in seawater ( $\text{Mg}/\text{Ca}_{\text{SW}}$ ). The physiological mechanisms responsible for these proxy relationships are still unknown. This culture study investigates the impact of different seawater  $[\text{Mg}^{2+}]$  on calcification in two benthic foraminiferal species precipitating contrasting  $\text{Mg}/\text{Ca}_{\text{CC}}$ : *Ammonia aomoriensis*, producing low-Mg calcite and *Amphistegina lessonii*, producing intermediate-Mg calcite. Foraminiferal growth and test thickness were determined and, Mg/Ca was analyzed using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). Results show that at present-day seawater  $\text{Mg}/\text{Ca}_{\text{SW}}$  of ~5, both species have highest growth rates, reflecting their adaptation to modern seawater element concentrations. Test thickness is not significantly affected by different  $\text{Mg}/\text{Ca}_{\text{SW}}$ . The relationship between  $\text{Mg}/\text{Ca}_{\text{SW}}$  and  $\text{Mg}/\text{Ca}_{\text{CC}}$  shows a distinct positive y-axis intercept, possibly reflecting at least two processes involved in foraminiferal biomineralization. The associated Mg partition ( $D_{\text{Mg}}$ ) changes non-linearly with increasing  $\text{Mg}/\text{Ca}_{\text{SW}}$ , hence suggesting that the  $D_{\text{Mg}}$  is best described by an exponential function approaching an asymptote.

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## 1. Introduction

When foraminifers build their  $\text{CaCO}_3$  shells (often also referred to as 'tests'), they also incorporate other ions than  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  from the surrounding seawater. From the incorporated elements,  $\text{Mg}^{2+}$  is of major interest because the calcite  $\text{Mg}/\text{Ca}$  ( $\text{Mg}/\text{Ca}_{\text{CC}}$ ) is known to reflect seawater temperatures (Nürnberg et al., 1996; Lea et al., 1999) and is, therefore, widely used as a paleo-proxy to estimate past sea surface (e.g. Hastings et al., 1998) and deep water (e.g. Rosenthal et al., 1997) temperatures.

A number of culture studies showed that  $\text{Mg}/\text{Ca}_{\text{CC}}$  in many marine calcifiers is influenced not only by temperature, but also by carbonate ion concentration (e.g. Lea et al., 1999; Russell et al., 2004; Dissard et al., 2010a), by salinity (e.g. Duenas-Bohorquez et al., 2009; Dissard et al., 2010b) and, moreover, by seawater  $\text{Mg}/\text{Ca}$  ( $\text{Mg}/\text{Ca}_{\text{SW}}$ ) (Stanley et al., 2002, 2005; Ries, 2006; Ries et al., 2006; Müller et al., 2011). Seawater  $\text{Mg}/\text{Ca}$  ( $\text{Mg}/\text{Ca}_{\text{SW}}$ ) varies over long time scales mainly due to geological processes. Currently, the average seawater  $\text{Mg}^{2+}$  concentration is ~50 mM, and  $\text{Ca}^{2+}$  is ~10 mM, resulting in a modern  $\text{Mg}/\text{Ca}_{\text{SW}}$  ~ 5. Since  $\text{Mg}^{2+}$  has a residence time of approximately 13 Ma and  $\text{Ca}^{2+}$  a

residence time of ~1 Ma (Hardie, 1996; Broecker and Yu, 2011), the oceanic  $\text{Mg}/\text{Ca}$  is constant over shorter time scales.

The impact of different seawater  $\text{Mg}/\text{Ca}$  ratios on test  $\text{Mg}/\text{Ca}$  ratio was quantified by Delaney et al. (1985) for planktic foraminifers and by Segev and Erez (2006) for benthic foraminifers. The culture study by Segev and Erez (2006), however, only investigated two benthic species, namely *Amphistegina lobifera* and *Amphistegina lessonii*, which both precipitate intermediate Mg-calcite. Calibrations of  $\text{Mg}/\text{Ca}_{\text{SW}}$  to  $\text{Mg}/\text{Ca}_{\text{CC}}$  for species producing low Mg-calcite are still missing. This is of particular interest because the control of Mg incorporation may be quite different between species that precipitate contrasting Mg-calcites and thus could help to elucidate physiological processes underlying calcification. Considering that different foraminiferal species precipitate calcite with highly variable amounts of Mg, it is also of great interest to investigate the isolated effect of varying  $[\text{Mg}^{2+}]$  at constant  $[\text{Ca}^{2+}]$ . In the culture study by Segev and Erez (2006)  $\text{Mg}/\text{Ca}_{\text{SW}}$  was varied at a wide range (0.5–10.1) by changing both  $[\text{Ca}^{2+}]$  and  $[\text{Mg}^{2+}]$  in the culture media. Segev and Erez (2006) also included a number of treatments with varying  $[\text{Mg}^{2+}]$  and similar  $[\text{Ca}^{2+}]$ , with a resulting  $\text{Mg}/\text{Ca}_{\text{SW}}$  range of 0.5–5.2. The isolated impact of varying  $[\text{Mg}^{2+}]$  at higher ratios is, therefore, still unknown.

The aim of this study is to investigate the effects on foraminiferal growth, test thickness and  $\text{Mg}^{2+}$  incorporation of a wider range of  $\text{Mg}/\text{Ca}_{\text{SW}}$ , varied solely by changing seawater  $[\text{Mg}^{2+}]$ . Using two

\* Corresponding author. Tel.: +49 471 4831 2291.  
E-mail address: [antje.mewes@awi.de](mailto:antje.mewes@awi.de) (A. Mewes).

benthic foraminiferal species that produce calcite with different Mg contents allows the determination of species-specific differences in response to seawater  $[Mg^{2+}]$ , potentially improving the proxy value of foraminiferal  $Mg/Ca_{CC}$  via increased understanding of the biological processes underlying these proxy relationships.

## 2. Material and methods

### 2.1. Species selection and sampling

*Ammonia aomoriensis* (also often referred to as *Ammonia tepida*) was chosen to reveal the impact of changing  $Mg/Ca_{SW}$  on  $Mg/Ca_{CC}$  in low Mg-calcite species (0.4–4 mmol/mol, (Dissard et al., 2010b; Raitzsch et al., 2010; Duenas-Bohorquez et al., 2011)). Although *A. aomoriensis* is not commonly used for paleo-reconstructions, it serves as a good model species because the majority of planktic foraminifers, which are commonly used for paleo-reconstructions, also precipitate low-Mg calcite (e.g. Nürnberg et al., 1996; Anand et al., 2003). *A. aomoriensis* has no algal symbionts and, therefore, possible influences by symbionts on  $Mg/Ca_{CC}$  paleothermometry are excluded. In contrast to planktic foraminifers, *A. aomoriensis* is well known for its robustness under laboratory conditions, characterized by insensitivity to deprivation of natural conditions, high survival and asexual reproduction rates. *A. aomoriensis* is a cosmopolitan, shallow-water species and was sampled from an intertidal mudflat in the German Wadden Sea (near Dörumer Neufeld) in December 2009. Sediment, containing foraminifers, was collected and transported directly to the laboratory, where the material was sieved (sieve size: 630  $\mu m$ ) to remove macrofauna and then placed in incubators at 15 °C in the dark until the beginning of the experiments.

To facilitate a direct comparison between our study and the previous similar study by Segev and Erez (2006), we also collected specimens of the benthic species *A. lessonii*. This is a reef-dwelling species that has diatom symbionts, and precipitates intermediate-Mg calcite (40–60 mmol/mol, (Segev and Erez, 2006; Raja and Saraswati, 2007)). Specimens were sampled in January 2011 from a tropical reef aquarium at Burgers' Zoo (Arnhem, the Netherlands) (Ernst et al., 2011). The seawater  $Mg/Ca$  in Burgers' Zoo aquarium has a ratio of ~5 (determined with ICP-OES:  $[Mg] = 47$  mM and  $[Ca] = 9.6$  mM). Collected reef rubble containing foraminifers was maintained at 25 °C under daylight lamps with a light intensity of approximately 80  $\mu mol/m^2$  s.

### 2.2. Preparation of culture media

At present, the average  $Mg^{2+}$  concentration of seawater is ~50 mM and that of  $Ca^{2+}$  is ~10 mM, resulting in a modern  $Mg/Ca_{SW} \sim 5$ . For the culture experiment, three different treatments of artificial seawater (ASW) with different  $[Mg^{2+}]$  (10, 50 and 100 mM) and constant  $[Ca^{2+}]$  (10 mM) were prepared. Stock solutions of seawater elements were prepared and ASW was mixed in 5 L canisters according to the ASW recipe by Kester et al. (1967). Since seawater  $[Mg]$  and  $[Ca]$  varied, the three resulting seawater  $Mg/Ca$  were ~1, ~5 and ~10. All remaining

seawater elements were kept constant among the different treatments. Prior to placement of foraminifers in the culture media, pH of the ASW was determined with a pH meter (WTW pH 3110, NBS scale) and adjusted to  $pH = 8.10 (\pm 0.04)$  by adding NaOH (1 M). Salinity was measured with a salinometer (WTW, Cond 330) and adjusted to  $S = 32.6\% (\pm 0.2)$  by changing NaCl concentrations according to the amount of  $Mg^{2+}$  in each treatment. Actual  $Mg^{2+}$  and  $Ca^{2+}$  concentrations of the different treatments were verified with inductively coupled plasma-optical emission spectrometry (ICP-OES).  $Mg^{2+}$  was measured at a wavelength of 285 nm and  $Ca^{2+}$  at 316 nm. Dissolved inorganic carbon (DIC) was measured with an XY-2 Sampler (Bran and Luebbe, precision:  $\pm 7 \mu mol/kg$ ), and total alkalinity was determined by auto-titration (SI-Analytics TW alpha plus, precision:  $\pm 5 \mu mol/kg$ ) (Table 1).

### 2.3. Culture experiment

Prior to the culture experiment, living specimens of *A. aomoriensis* (>250  $\mu m$ ) and *A. lessonii* (>500  $\mu m$ ) were isolated from the sediment and reef rubble, respectively, and placed into well plates. Asexual reproduction in both species was stimulated by elevating ambient food concentration and temperature by ~5 °C. Individuals that resulted from the same asexual reproduction event will be henceforth referred to as 'broods'. After asexual reproduction, juveniles of both species were isolated from the mother cell and distributed among the different treatments. At the start of incubation, all juveniles had 3–5 chambers. Initial chambers are very tiny and thus the use of juveniles allowed us to ensure that all chambers, that we later analyzed, were grown under experimental conditions. For *A. aomoriensis*, juveniles were taken from four different broods. The juveniles were separately cultured in petri dishes, with 5–10 individuals per petri dish. For each treatment we cultured the juveniles in triplicates. For *A. lessonii*, juveniles were taken from two different broods. Again 5–10 individuals were placed in each petri dish. For each treatment we cultured the foraminifers in duplicates.

During the culture experiment, juveniles were maintained at 25 °C in 10 mL petri dishes that contained ~10 mL of the culture media. Petri dishes with juveniles of *A. aomoriensis* were kept in the dark and petri dishes with juveniles of *A. lessonii* at a light intensity of approximately 130  $\mu mol/m^2$  s. Culture media, containing *A. aomoriensis*, consisted of 100% ASW. *A. lessonii* does not grow in 100% ASW, so 10% North Sea water (NSW) was added to the culture media, slightly changing the absolute element concentrations (Table 1). We assume that a certain percentage of NSW is needed by *A. lessonii*, because this species contains algal symbionts. Algae are known to require vitamins (e.g. Provasoli and Pintner, 1953; Droop, 1957) that are present in natural seawater. It is furthermore suggested that organic substances, such as amino acids and peptides, that act as chelating agents by forming metal complexes, are important for metabolic processes taking place in the cell (Johnston, 1964; Jackson and Morgan, 1978).

Juveniles of both foraminiferal species were regularly fed with specimens of the green algae *Dunaliella salina* by adding 100  $\mu L$  of a densely concentrated algae culture (approximately  $4 \times 10^6$  cells  $\cdot mL^{-1}$ ) to each

**Table 1**  
Experimental conditions of the different treatments.

Treatment	<i>Ammonia aomoriensis</i>			<i>Amphistegina lessonii</i>		
	$Mg/Ca \sim 1$	$Mg/Ca \sim 5$	$Mg/Ca \sim 10$	$Mg/Ca \sim 1$	$Mg/Ca \sim 5$	$Mg/Ca \sim 10$
T [°C]	25	25	25	25	25	25
S [‰]	32.8	32.4	32.5	32.9	32.5	32.4
pH (NBS)	8.2	8.1	8.1	8.1	8.1	8.1
TA [ $\mu mol/kg$ ]	2530	2489	2663	2254	2297	2509
$\Omega$ (calcite)	4.82	4.52	5.06	4.80	4.50	5.08
DIC [ $\mu mol/kg$ ]	2215	2192	2236	2105	2060	2168
$Mg^{2+}$ [mM]	9.83	50.28	91.13	13.90	48.01	91.86
$Ca^{2+}$ [mM]	8.88	9.93	9.95	9.14	9.08	9.06
$Mg/Ca_{SW}$	1.11	5.07	9.16	1.52	5.29	10.14

petri dish. To maintain constant food,  $Mg^{2+}$  and  $Ca^{2+}$  concentrations, and minimize variability in pH, salinity, and oxygen content and to prevent build-up of toxic waste products, culture media were renewed every three days with ASW from the pre-prepared batch. The total duration of the culture experiment was ~4 weeks.

### 2.3.1. Growth

The growth of specimens was monitored once a week by measuring the maximum test diameter ( $\pm 5 \mu m$ ). This was done with the help of the computer software Axiovision and a camera (AxioCam MRc5) attached to a Zeiss microscope (Axiovert 200M). Dead specimens were identified by a different color and lack of pseudopodial activity. Upon identification, they were removed from the culture experiment and size measurements were only performed on living individuals.

### 2.3.2. Cleaning method

After termination of the culture experiments, foraminifers were rinsed with deionized water and subsequently placed in sodium hypochloride (14%) for ~45 min to remove organic matter. Afterwards they were rinsed with deionized water and dried in an oven at  $60^\circ C$  upon further analysis.

### 2.4. Weight

Mean test weight was determined for each treatment with a microbalance (Mettler Toledo UMX2, precision  $\pm 0.1 \mu g$ ). Due to their small weight it was impossible to determine the weight of each foraminifer individually, so specimens (5–10) were pooled and weight was determined for each replicate. Weight was then divided by the mean size for each of these replicates. The size-normalized weight (i.e., weight divided by diameter) removes the influence of test size on weight and,

therefore, can be used as an indication of test thickness (Beer et al., 2010).

### 2.5. LA-ICP-MS measurements

Mg/Ca ratios in calcite of the cultured foraminifers were determined with laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) at Utrecht University (Reichert et al., 2003). For this purpose, foraminifers were mounted on stubs with double-sided adhesive tape. A GeoLas 22Q Excimer laser (Lambda Physik) coupled to a sector field ICP-MS (Element 2, Thermo Scientific) was used. The laser spot size was set to  $40 \mu m$ , repetition rate to 7 Hz and energy density to approximately  $1.2 J \cdot cm^{-2}$ . The small laser spot size prevented cross-contamination between single foraminiferal chambers while four to six newly built chambers of each individual were ablated (Fig. 1). Recently, it has been shown that there is no significant difference in Mg/Ca<sub>CC</sub> when ablating different chambers within one specimen (De Nooijer et al., 2014). We can, therefore, exclude that our measurements are biased by ontogenetic trends.

Analyses were calibrated against a glass standard (NIST 610; Jochum et al., 2011) with precisely known elemental concentrations. Due to the higher ablation energy density used for glass ( $\sim 5 J \cdot cm^{-2}$ ) compared to that used to ablate calcite, a further calcite standard (in-house matrix matched Iceland spar) was ablated at the same energy density as the foraminifers ( $\sim 1.2 J \cdot cm^{-2}$ ), to account for the different ablation behavior between glass and calcite. Since no significant differences in solution measured values of the inhouse-standard were detected, no correction was applied to the foraminiferal element counts. Before and after every 10–15 ablations, three NIST and two calcite standards were measured to ensure high signal quality. Element concentrations (in ppm) were calculated from counts per second for the individual relevant isotopes and included  $^{24}Mg$ ,  $^{26}Mg$  and  $^{43}Ca$ ,  $^{44}Ca$  using GLITTER software

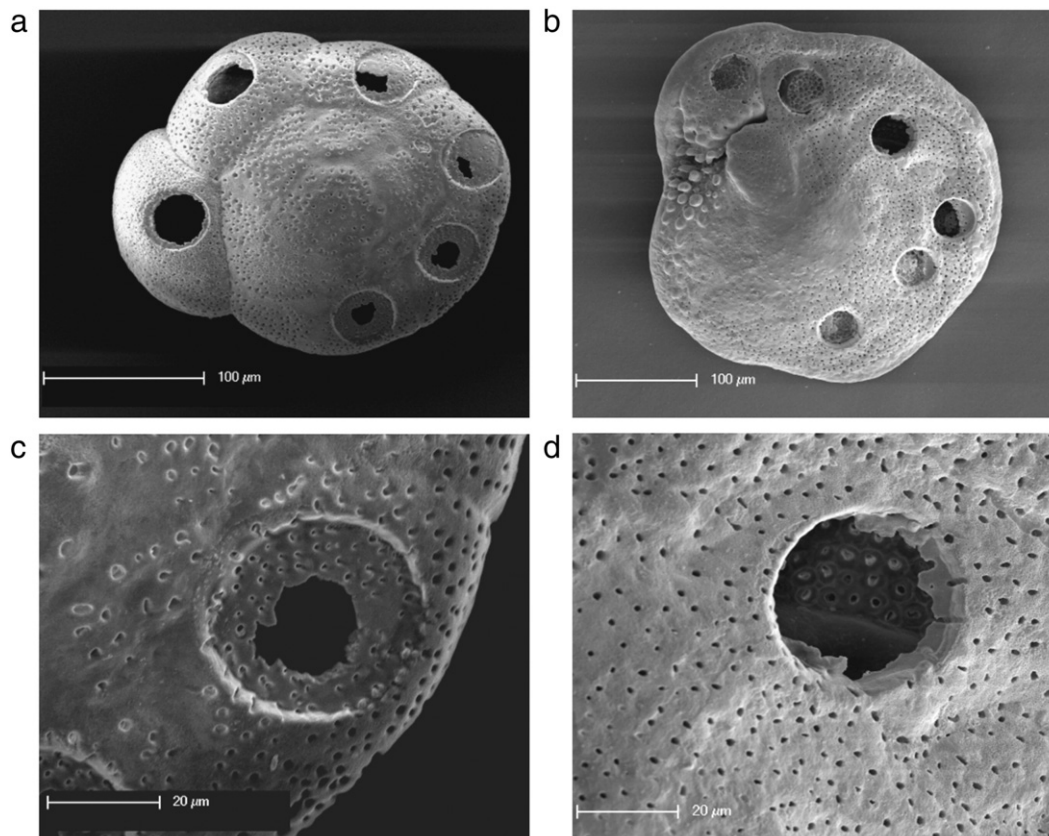


Fig. 1. SEM pictures of a) *A. aomoriensis* and b) *A. lessonii*. Single chambers were ablated. Detailed view of laser ablation crater of c) *A. aomoriensis* and d) *A. lessonii*.

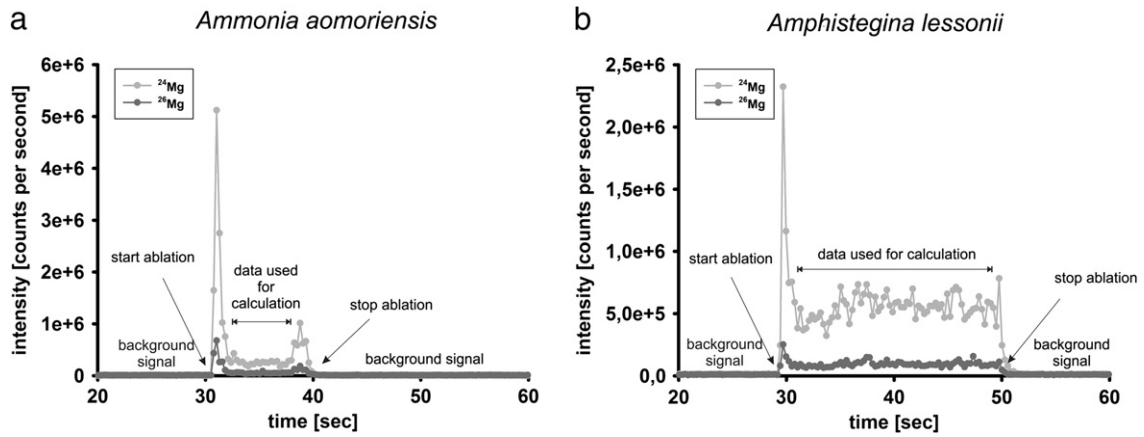


Fig. 2. Characteristic LA-ICP-MS ablation profiles of the isotopes  $^{24}\text{Mg}$  and  $^{26}\text{Mg}$  for a) *A. aomoriensis* and b) *A. lessonii*.

(version 4.4.3). Fig. 2 shows exemplarily a characteristic LA-ICP-MS profile for  $^{24}\text{Mg}$  and  $^{26}\text{Mg}$  for *A. aomoriensis* and *A. lessonii*, respectively.  $^{27}\text{Al}$  and  $^{55}\text{Mn}$  were determined in order to select the integration window and recognize contamination (for details see: Dissard et al., 2010b; Wit et al., 2010). Contamination in parts of the profiles was also evaluated for each measurement and not considered in subsequent calculations. From a total of 176 profiles, 17 had to be discarded for *A. aomoriensis*. For *A. lessonii* 5 profiles were discarded from a total of 122 profiles. Discarded profiles were mainly profiles from the final chamber. Final chambers are commonly very thin, resulting in very short ablation profiles, making it difficult to select contaminated from non-contaminated calcites resulting in relatively poor count statistics.

The obtained partition coefficient for Mg ( $D_{\text{Mg}}$ ) is defined as

$$D_{\text{Mg}} = \frac{(\text{Mg}/\text{Ca})_{\text{calcite}}}{(\text{Mg}/\text{Ca})_{\text{seawater}}}$$

and was calculated for both species at all culture conditions. The partition coefficient is a measure for the fractionation of a certain element during calcite precipitation. If  $D_{\text{Mg}} = 1$ , no net Mg fractionation occurs and Mg is incorporated into calcium carbonate with the same ratio to Ca as they occur in seawater. When  $D_{\text{Mg}} > 1$ , relatively more Mg is incorporated into calcium carbonate and when  $D_{\text{Mg}} < 1$ , less Mg is incorporated.

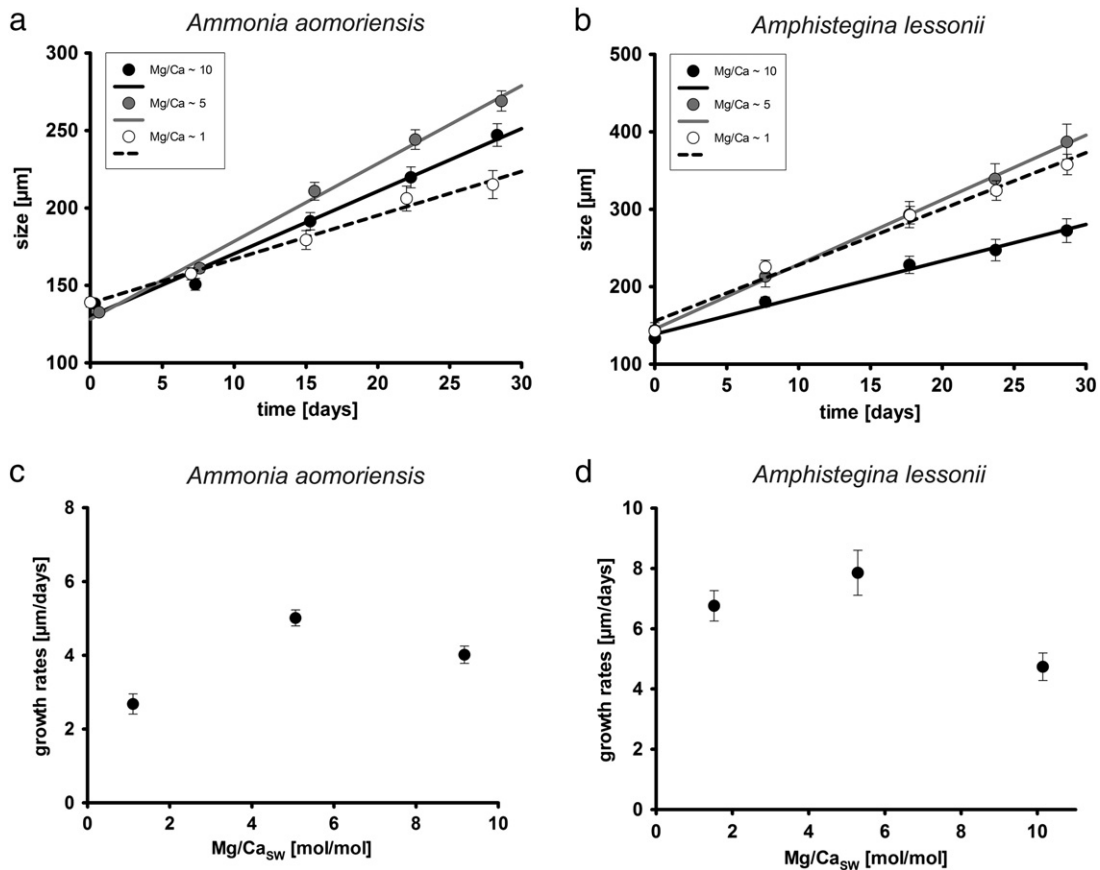


Fig. 3. Mean test size versus exposure time to different culture media for a) *A. aomoriensis* ( $n = 67\text{--}94$ ) and b) *A. lessonii* ( $n = 26\text{--}30$ ). Error bars represent standard error of the mean. Parameters of growth regression curves are shown in Table 2. Growth rates in  $\mu\text{m}/\text{day}$  versus  $\text{Mg}/\text{Ca}_{\text{sw}}$  were derived from the slopes of growth regression curves for c) *A. aomoriensis* and d) *A. lessonii*. Error bars represent standard error of the slope.



**Table 2**  
Parameters and their standard errors of growth regression curves shown in Fig. 2.

Treatment	<i>Ammonia aomoriensis</i>			<i>Amphistegina lessonii</i>		
	Slope ( $\pm$ std. error) [ $\mu\text{m}\cdot\text{d}^{-1}$ ]	Intercept ( $\pm$ std. error) [ $\mu\text{m}$ ]	R <sup>2</sup>	Slope ( $\pm$ std. error) [ $\mu\text{m}\cdot\text{d}^{-1}$ ]	Intercept ( $\pm$ std. error) [ $\mu\text{m}$ ]	R <sup>2</sup>
Mg/Ca ~ 1	2.84 ( $\pm$ 0.16)	138.5 ( $\pm$ 2.8)	0.99	7.26 ( $\pm$ 0.54)	155.4 ( $\pm$ 10.2)	0.98
Mg/Ca ~ 5	5.03 ( $\pm$ 0.20)	128.1 ( $\pm$ 3.6)	1.00	8.35 ( $\pm$ 0.15)	145.3 ( $\pm$ 2.8)	1.00
Mg/Ca ~ 10	4.04 ( $\pm$ 0.30)	130.0 ( $\pm$ 5.3)	0.98	4.73 ( $\pm$ 0.25)	138.6 ( $\pm$ 4.8)	0.99

### 3. Results

#### 3.1. Growth

Fig. 3a and b illustrates size (diameter;  $\mu\text{m}$ ) of foraminifers versus time (days) in culture. Both species showed linear growth over time, in all three treatments. Parameters of growth regression curves can be read from Table 2. Analyses of covariance (ANCOVA) reflected a significant difference ( $p = 0$ ) for the growth of *A. aomoriensis* and *A. lessonii* in the different treatments. For *A. aomoriensis* the subsequent Tukey multiple comparison test revealed significant differences ( $p < 0.01$ ) between all slopes, indicating significantly different growth rates. For *A. lessonii* the test revealed significant differences between all slopes ( $p < 0.01$ ), except for the growth in treatment Mg/Ca<sub>SW</sub> ~ 5 and Mg/Ca<sub>SW</sub> ~ 1 which are not significantly different ( $p > 0.05$ ).

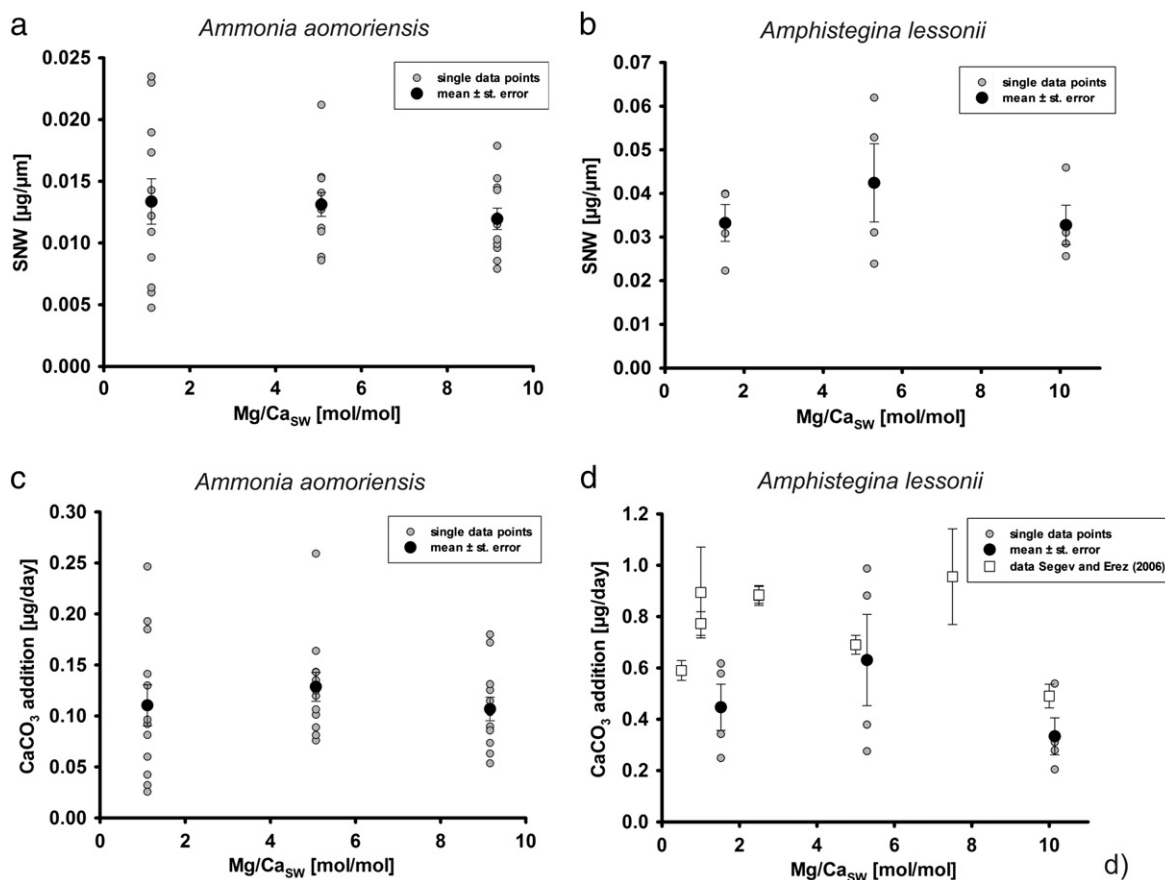
Slopes of the growth regression curves are plotted against the different treatments in Fig. 3c and d. Highest growth rates occurred at Mg/Ca<sub>SW</sub> ~ 5 for both species, reaching  $5.01 (\pm 0.22) \mu\text{m}/\text{day}$  for *A. aomoriensis*, and  $7.86 (\pm 0.75) \mu\text{m}/\text{day}$  for *A. lessonii* (Fig. 3 and Table 2). Lowest growth rates occurred at Mg/Ca<sub>SW</sub> ~ 1 for *A. aomoriensis* ( $2.68 \pm 0.27 \mu\text{m}/\text{day}$ ), while *A. lessonii* had the lowest growth rates at Mg/Ca<sub>SW</sub> ~ 10 ( $4.74 \pm 0.46 \mu\text{m}/\text{day}$ ; Fig. 3 and Table 2).

#### 3.2. Weight

Size normalized weights (SNW) of foraminifers, grown in the different treatments, are shown in Fig. 4 and Table 3. To facilitate comparison with previously reported results of the study by Segev and Erez (2006), that measured weight in terms of CaCO<sub>3</sub> addition [ $\mu\text{g}/\text{day}$ ], we included CaCO<sub>3</sub> addition to our data. As mentioned in the Material and methods section (compare Section 2.4) the low weight of foraminifers forced us to determine the weight for each replicate, resulting in a small sample size ( $n = 12$  for *A. aomoriensis*,  $n = 4$  for *A. lessonii*). Due to this small sample size and relatively high standard deviations, the performed analysis of variances (ANOVA) at 95% confidence interval could not detect significant differences in SNW ( $p = 0.72$  for *A. aomoriensis* and  $p = 0.5$  for *A. lessonii*) and CaCO<sub>3</sub> addition ( $p = 0.58$  for *A. aomoriensis* and  $p = 0.27$  for *A. lessonii*) between treatments.

#### 3.3. LA-ICP-MS measurements

For both species, Mg/Ca<sub>CC</sub> is significantly positively correlated with increasing seawater Mg<sup>2+</sup> concentrations and thus increasing Mg/Ca<sub>SW</sub> (Fig. 5). A linear correlation and a power function can be fitted



**Fig. 4.** Size normalized weight for a) *A. aomoriensis* ( $n = 12$ ) and b) *A. lessonii* ( $n = 4$ ) and CaCO<sub>3</sub> addition versus Mg/Ca<sub>SW</sub> for c) *A. aomoriensis* ( $n = 12$ ) and d) *A. lessonii* ( $n = 4$ ). Single data points result from single weight measurements.

**Table 3**Mean SNW and CaCO<sub>3</sub> addition and their standard errors for *A. aomoriensis* and *A. lessonii* in the different treatments.

Treatment	<i>Ammonia aomoriensis</i>		<i>Amphistegina lessonii</i>	
	SNW ( $\pm$ std. error) [ $\mu\text{g}/\mu\text{m}$ ]	CaCO <sub>3</sub> addition ( $\pm$ std. error) [ $\mu\text{g}/\text{day}$ ]	SNW ( $\pm$ std. error) [ $\mu\text{g}/\mu\text{m}$ ]	CaCO <sub>3</sub> addition ( $\pm$ std. error) [ $\mu\text{g}/\text{day}$ ]
Mg/Ca ~ 1	$1.34 \cdot 10^{-2}$ ( $\pm 0.19 \cdot 10^{-2}$ )	0.11 ( $\pm 0.02$ )	$3.32 \cdot 10^{-2}$ ( $\pm 0.42 \cdot 10^{-2}$ )	0.45 ( $\pm 0.09$ )
Mg/Ca ~ 5	$1.31 \cdot 10^{-2}$ ( $\pm 0.10 \cdot 10^{-2}$ )	0.13 ( $\pm 0.01$ )	$4.24 \cdot 10^{-2}$ ( $\pm 0.90 \cdot 10^{-2}$ )	0.63 ( $\pm 0.18$ )
Mg/Ca ~ 10	$1.20 \cdot 10^{-2}$ ( $\pm 0.09 \cdot 10^{-2}$ )	0.11 ( $\pm 0.01$ )	$3.28 \cdot 10^{-2}$ ( $\pm 0.45 \cdot 10^{-2}$ )	0.33 ( $\pm 0.07$ )

to the data, yielding similar values of  $R^2$ . The linear correlation shows a distinct positive y-axis intercept, leading to the observed decreasing partition coefficient  $D_{\text{Mg}}$ .

Due to the inhomogeneity of variances and different sample sizes the non-parametric Kruskal–Wallis Test was performed to detect significant differences between the means of the  $D_{\text{Mg}}$  at different Mg/Ca<sub>SW</sub>. The test detected significant differences between the means of the  $D_{\text{Mg}}$  for both species at a 99.9% confidence interval ( $H = 62.9$  for *A. aomoriensis* and  $H = 41.8$  for *A. lessonii*).

Means of the  $D_{\text{Mg}}$  for both species show a decrease with increasing Mg/Ca<sub>SW</sub> and for both species the  $D_{\text{Mg}}$  decreases towards the value of the overall calculated  $D_{\text{Mg}}$  that is derived from the slope of the regression line: For *A. aomoriensis* the slope of the regression line has a value of  $0.53 (\pm 0.04) \times 10^{-3}$  (Fig. 5a). This value should equal the asymptotic value and is close to the  $D_{\text{Mg}}$  of  $0.64 (\pm 0.04) \times 10^{-3}$  at Mg/Ca<sub>SW</sub> ~ 10 (Fig. 5b). Accordingly, for *A. lessonii*, the slope of the regression line and thus the asymptotic value is  $5.52 (\pm 0.21) \times 10^{-3}$  (Fig. 5b). This value is also very close to the  $D_{\text{Mg}}$  at Mg/Ca<sub>SW</sub> ~ 10 with a value of  $5.90 (\pm 0.21) \times 10^{-3}$  (Fig. 5d).

## 4. Discussion

### 4.1. Growth

The results demonstrated higher absolute growth rates ( $\mu\text{m}/\text{day}$ ) for *A. lessonii* than *A. aomoriensis* (Table 2, Fig. 3). This may be explained by the fact that *A. lessonii* is a larger benthic foraminifer that can reach sizes of more than 1 mm, while adult *A. aomoriensis* commonly do not exceed diameters larger than ~600  $\mu\text{m}$ . To reach their maximum size individuals of *A. aomoriensis* would need on average 94 days and *A. lessonii* 102 days, as can be inferred from the growth regression curves (Fig. 3). This indicates that, relative to their respective maximum test sizes, growth was similar between the two species. Even though *A. aomoriensis* was cultured in 100% artificial seawater and *A. lessonii* required an addition of 10% NSW in order to calcify, their relatively similar growth rates suggest a similar performance of the two species in the laboratory. Furthermore, growth rates of *A. lessonii* compare very well with previous laboratory and field studies (Hallock, 1981; Hallock et al., 1986). These consistent growth rates, even under rather different

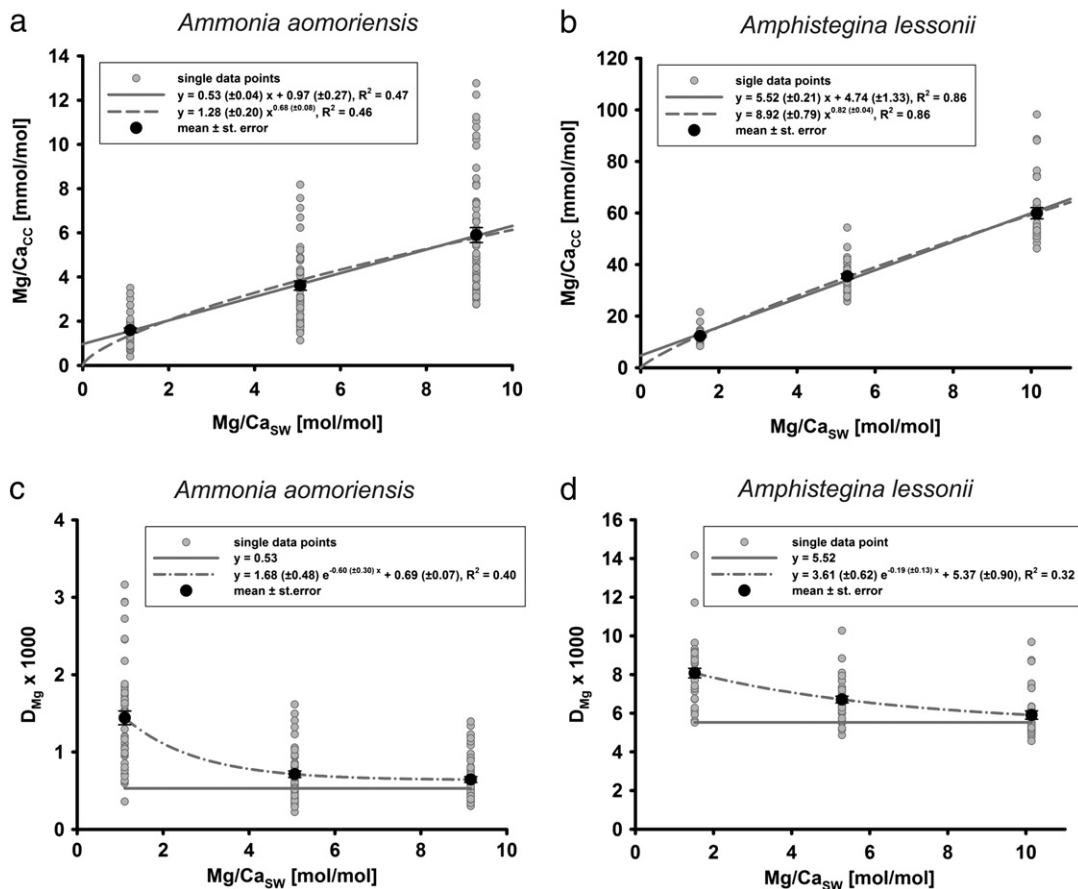


Fig. 5. Mg/Ca<sub>cc</sub> and mean partition coefficient  $D_{\text{Mg}}$  versus Mg/Ca<sub>sw</sub> for a + c) *A. aomoriensis* (n = 51–60) and b + d) *A. lessonii* (n = 36–42).

culture conditions, imply that *A. lessonii* is well-suited for controlled growth studies.

For both *A. aomoriensis* and *A. lessonii*, growth rates are highest at intermediate Mg/Ca<sub>SW</sub>, albeit that for *A. lessonii* growth rates at low Mg/Ca<sub>SW</sub> are not significantly different from those at intermediate values (Fig. 3). Impaired growth at higher Mg/Ca<sub>SW</sub> is observed in other marine calcifiers and may be explained by increased energy spent on calcification by overcoming inhibition by Mg<sup>2+</sup> on CaCO<sub>3</sub> crystal nucleation and growth (e.g. Ries, 2010). In comparison to *A. aomoriensis*, intermediate/high-Mg calcifiers like *Amphistegina*, precipitate their calcite with a weaker fractionation of Mg<sup>2+</sup> at the site of calcification. Still, both species show optimal growth rates at Mg/Ca<sub>SW</sub> values similar to present day levels (Fig. 3c and d) and a clear tendency to impaired growth at elevated and low Mg/Ca<sub>SW</sub> values. Since Mg/Ca<sub>CC</sub> correlates almost linearly to Mg/Ca<sub>SW</sub> (compare Section 4.3) the observed differences in foraminiferal growth rates do not affect incorporation of Mg<sup>2+</sup>.

#### 4.2. Weight

Size normalized weight is an indication of test wall thickness (Barker and Elderfield, 2002; De Moel et al., 2009). Results showed no significant difference in SNW (test wall thickness) between different treatments for both species. CaCO<sub>3</sub> addition [μg/day] also does not differ between treatments as this is calculated based on dividing total test weight by the length of the growth period. Although this seems to be in contrast to the earlier study by Segev and Erez (2006), showing highest CaCO<sub>3</sub> addition [μg/d] for two foraminiferal species within the genus *Amphistegina* at Mg/Ca ~ 1, plotting our data together with that of Segev and Erez (2006, Table 1) actually shows a close correspondence (Fig. 4d). Only at very low Mg/Ca<sub>SW</sub>, CaCO<sub>3</sub> addition [μg/day] is lower in our data set. The small offset might be due to the fact that Segev and Erez (2006) partially varied Ca<sup>2+</sup> instead of Mg<sup>2+</sup>. Our new data does not support the hypothesis of Segev and Erez (2006), that lower Mg/Ca<sub>SW</sub> is always advantageous for foraminiferal calcite addition.

In summary, our experiment in which Mg/Ca<sub>SW</sub> was varied exclusively through [Mg<sup>2+</sup>], suggests no influence on size-normalized weight or CaCO<sub>3</sub> addition, with optimal growth rates at Mg/Ca<sub>SW</sub> ~ 5, reflecting a good adaptation to modern Mg/Ca<sub>SW</sub>. This seems counter-intuitive, because seawater Mg/Ca<sub>SW</sub> was close to 1 during the Cretaceous (Hardie, 1996; Stanley and Hardie, 1998), when the super-families Rotalidae and Asterigerinoidea (to which *A. aomoriensis* and *A. lessonii*, respectively, belong) evolved (Boudagher-Fadel, 2008). During the Cretaceous, seawater [Mg] was lower compared to modern concentrations (Hardie, 1996). However, it should be acknowledged that evolution takes place over such a long time span of >100 Ma, making it reasonable that foraminifers are well adapted to modern conditions.

#### 4.3. LA-ICP-MS measurements

##### 4.3.1. Calcite Mg/Ca

Mg/Ca<sub>CC</sub> values measured here (Table 4, Fig. 5) are comparable to Mg/Ca<sub>CC</sub> measured for *A. aomoriensis* (*A. tepida*, respectively) and *A. lessonii* in previous studies (e.g. Toler et al., 2001; Segev and Erez, 2006; Raitzsch et al., 2010; Duenas-Bohorquez et al., 2011). Minor

differences for *A. lessonii* between our data and that of Segev and Erez (2006) might be due to different experimental procedures such as variations in the composition of the artificial seawater used. Here we varied Mg/Ca<sub>SW</sub> solely by changing [Mg<sup>2+</sup>], in contrast to Segev and Erez (2006) who varied both [Mg<sup>2+</sup>] and [Ca<sup>2+</sup>]. Although the range of Mg/Ca<sub>CC</sub> values of individual measurements is considerable, the large number of analyses means that the standard error is relatively small. The standard error never exceeds 7% of the mean value for *A. aomoriensis* and 4% of the mean value for *A. lessonii*.

At both intermediate and high Mg/Ca<sub>SW</sub> values, *A. lessonii* shows 10-times higher Mg/Ca<sub>CC</sub> compared to *A. aomoriensis*. At the lowest Mg/Ca<sub>SW</sub>, however, *A. lessonii* shows only 6-times higher Mg/Ca<sub>CC</sub> compared to *A. aomoriensis* (compare Fig. 5a and b). Nonetheless, both species show a linear correlation between Mg/Ca<sub>CC</sub> and Mg/Ca<sub>SW</sub> with an apparent positive intercept. For *A. aomoriensis* the y-axis intercept is 0.97 mmol/mol whereas *A. lessonii* has an intercept at 4.74 mmol/mol (Fig. 5a and b). Using this linear regression, the calculated values for D<sub>Mg</sub> result in an increasingly higher partition coefficient for Mg at low Mg/Ca<sub>SW</sub> (Fig. 5c and d). Clearly at very low Mg/Ca<sub>SW</sub> also Mg/Ca<sub>CC</sub> should approach 0. Hence, Evans and Mueller (2012), Hasiuk and Lohmann (2010) and Mucci and Morse (1983) proposed to fit a power function to such similar data sets. Since the D<sub>Mg</sub> decreases asymptotically towards the value of the slope of the regression line we speculate that the best way to describe the relationship between Mg/Ca<sub>SW</sub> and Mg/Ca<sub>CC</sub> is a power function at very low Mg/Ca<sub>SW</sub>, turning into a linear function at higher Mg/Ca<sub>SW</sub>. This is consistent with both previous analyses on foraminifera grown under different Mg/Ca<sub>SW</sub> conditions (Delaney et al., 1985; Segev and Erez, 2006; Raitzsch et al., 2010) and inorganic calcite precipitation data (Mucci and Morse, 1983). The rapid change in D<sub>Mg</sub> at very low Mg/Ca<sub>SW</sub> values (<1 mmol/mol), followed by the asymptotic approach to a constant D<sub>Mg</sub> at high Mg/Ca<sub>SW</sub> values, suggests that Mg/Ca<sub>CC</sub> is controlled by (at least) two processes: one causing a relatively strong increase in Mg/Ca<sub>CC</sub> at low Mg/Ca<sub>SW</sub>, which can be described by a power function and a second process which is responsible for a linear increase in Mg/Ca<sub>CC</sub> with increasing Mg/Ca<sub>SW</sub>. There may be several explanations for the two processes which could be responsible for the observed relationship between Mg/Ca<sub>SW</sub> and Mg/Ca<sub>CC</sub>. These are (i) presence of two different CaCO<sub>3</sub> layers, (ii) involvement of two different biomineralization pathways or (iii) a change in the “effective” [Mg] at low Mg/Ca<sub>SW</sub>.

The first explanation is based on results from Bentov and Erez (2005) and Branson et al. (2013), showing that *A. lobifera* produces calcite crystals with two distinct Mg/Ca<sub>CC</sub>. These two, different calcite phases are not a result of migration through the water column (and thus different environmental parameters), but are always precipitated. The ratio between these two phases, however, may vary as a function of physical parameters, e.g. temperature (Bentov and Erez, 2006). With varying Mg/Ca<sub>SW</sub>, the ratio between high- and low-Mg calcite layers could also vary, thus explaining the observed change in D<sub>Mg</sub> with Mg/Ca<sub>SW</sub>. This is in line with high and low Mg/Ca<sub>CC</sub> being observed in both planktic (Eggins et al., 2004; Sadekov et al., 2005, 2008) and benthic foraminifera (Bentov and Erez, 2005). Such different Mg phases have, to the best of our knowledge, never been observed in species of the genus *Ammonia*. Furthermore they do, however, not explain the similar relationship observed for inorganic calcite, for which, to the best of our knowledge, no bands of alternating high- and low-Mg/Ca<sub>CC</sub>

**Table 4**  
Mean Mg/Ca<sub>CC</sub>, D<sub>Mg</sub> and their standard errors for *A. aomoriensis* and *A. lessonii*.

<i>Ammonia aomoriensis</i>			<i>Amphistegina lessonii</i>		
Mg/Ca <sub>SW</sub> [mol/mol]	Mg/Ca <sub>CC</sub> (± std. error) [mmol/mol]	D <sub>Mg</sub> × 1000 (± std. error)	Mg/Ca <sub>SW</sub> [mol/mol]	Mg/Ca <sub>CC</sub> (± std. error) [mmol/mol]	D <sub>Mg</sub> × 1000 (± std. error)
1.11	1.60 ± 0.10	1.44 ± 0.09	1.52	12.28 ± 0.37	8.08 ± 0.24
5.07	3.61 ± 0.21	0.71 ± 0.04	5.29	35.45 ± 0.90	6.70 ± 0.17
9.16	5.90 ± 0.34	0.64 ± 0.04	10.14	59.89 ± 2.17	5.90 ± 0.21

have been reported. Similarly, for coccolithophorids such different phases have not been observed, however, they also show increasing values for  $D_{Mg}$  at lower  $Mg/Ca_{SW}$  values (Stanley et al., 2005; Müller et al., 2011).

The second explanation involves two different pathways for Mg during calcification. Nehrke et al. (2013) suggested that differences observed in  $D_{Mg}$  (relative change in  $Mg/Ca_{CC}$  as a function of  $Mg/Ca_{SW}$ , respectively) between foraminiferal species may be the result of the relative contribution of transmembrane (TMT) versus passive transport (PT) of ions (i.e., the TMT/PT ratio). For a given TMT/PT ratio, this model predicts a linear dependence of  $Mg/Ca_{CC}$  on  $Mg/Ca_{SW}$ , where the slope is determined by the relative contribution of passive transport. At higher  $Mg/Ca_{SW}$  (5–10 in our experiment; Fig. 5), this model explains the linear trend reported here and thus supports the idea that the different Mg concentrations observed in the tests of the two different species, *A. aomoriensis* and *A. lessonii*, are caused by a different contribution of passive ion transport. At lower  $Mg/Ca_{SW}$ , however, our data are not completely in agreement with the biomineralization model by Nehrke et al. (2013). This suggests an additional (physiological) process, which influences the  $Mg/Ca_{SW}$ – $Mg/Ca_{CC}$  relationship, by causing a relatively high  $D_{Mg}$  at low  $Mg/Ca_{SW}$ . So far, this process remains unknown and highlights the complexity of foraminiferal biomineralization.

A possible third mechanism is based on inorganic precipitation experiments by Mucci and Morse (1983). This could explain the observed positive y-axis intercept as well as the largely linear slope at higher  $Mg/Ca_{SW}$ , based on a shift in  $Mg/Ca_{SW}$  at the atomic scale due to differential sorption of  $Mg^{2+}$  and  $Ca^{2+}$  to mineral surfaces. Mucci and Morse (1983) suggested  $Mg^{2+}$  being more strongly adsorbed to crystal surfaces, increased actual  $Mg/Ca_{SW}$  close to the crystal surface (Fig. 6). Such an effect of locally altered  $Mg/Ca_{SW}$  would have a stronger impact at low  $Mg/Ca_{SW}$ , resulting in the observed positive intercept. In foraminifera sorption of  $Mg^{2+}$  to organic phases (Hemleben et al., 1977; Debenay et al., 2000) might add to such an offset.

## 5. Implications

Foraminifera, irrespective of precipitating low- or intermediate-Mg calcite, are well adapted to current  $Mg/Ca_{SW}$ . Existing literature (compare Mucci and Morse, 1983; Stanley et al., 2005; Segev and Erez, 2006; Hasiuk and Lohmann, 2010; Ries, 2010; Müller et al., 2011; Evans and Mueller, 2012) describes the relationship between  $Mg/Ca_{SW}$  and  $Mg/Ca_{CC}$  either as a linear or a power function. On the basis of this existing literature and our new data we suggest, however, that the relationship between  $Mg/Ca_{SW}$  and  $Mg/Ca_{CC}$  can be represented by a power function at low ratios that turns into a linear function at high ratios. Since this involves a varying  $D_{Mg}$  at low and a constant  $D_{Mg}$  at high ratios, an exponential function that approaches an asymptote is appropriate to describe the relationship between  $D_{Mg}$  and  $Mg/Ca_{SW}$ :

$$f(x) = a e^{-bx} + A$$

$a$  and  $b$  being species specific constants, and  $A$  the asymptote equaling the slope of a linear regression line between  $Mg/Ca_{SW}$  and  $Mg/Ca_{CC}$ . This function provides a new suggestion for paleo-oceanographers, by describing the relationship between  $D_{Mg}$  and  $Mg/Ca_{SW}$  in a more reliable and robust way.

Although there are numerous physiological processes (potentially) affecting Mg-incorporation in foraminifera, there is a striking similarity of the  $Mg/Ca_{CC}$ – $Mg/Ca_{SW}$  relationship between foraminiferal species with contrasting  $Mg/Ca_{CC}$  (e.g. *Ammonia* and *Amphistegina*), different marine calcifying organisms, and even inorganic  $CaCO_3$  precipitation. This suggests that Mg-incorporation in biogenic and abiotic calcium carbonate is governed by a basic, common underlying principle, and modulated to different extents by species-specific, additional controls.

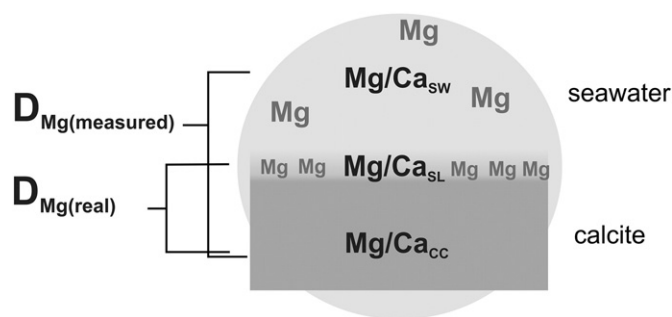


Fig. 6. Differential sorption of  $Mg^{2+}$  involves alteration of  $Mg/Ca_{SW}$  at the crystal surface.

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