Effect of different seawater Mg$^{2+}$ concentrations on calcification in two benthic foraminifers

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Magnesium, incorporated in foraminiferal calcite ([Mg/Ca]$_{CC}$), is used intensively to reconstruct past seawater temperatures but, in addition to temperature, the [Mg/Ca]$_{CC}$ of foraminiferal tests also depends on the ratio of Mg and Ca in seawater ([Mg/Ca]$_{SW}$). The physiological mechanisms responsible for these proxy relationships are still unknown. This culture study investigates the impact of different seawater [Mg$^{2+}$] on calcification in two benthic foraminiferal species precipitating contrasting Mg/Ca$_{CC}$: Armonia coarctata, 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 Mg/Ca$_{SW}$ of ~5, both species have highest growth rates, reflecting their adaptation to modern seawater element concentrations. Test thickness is not significantly affected by different Mg/Ca$_{SW}$. The relationship between Mg/Ca$_{SW}$ and Mg/Ca$_{CC}$ shows a distinct positive y-axis intercept, possibly reflecting at least two processes involved in foraminiferal biomineralization. The associated Mg partition (DMg) changes non-linearly with increasing Mg/Ca$_{SW}$, hence suggesting that the DMg is best described by an exponential function approaching an asymptote.

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1. Introduction

When foraminifers build their CaCO$_3$ shells (often also referred to as 'tests'), they also incorporate other ions than Ca$^{2+}$ and CO$_3^{2−}$ from the surrounding seawater. From the incorporated elements, Mg$^{2+}$ is of major interest because the calcite Mg/Ca ([Mg/Ca]$_{CC}$) 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 Mg/Ca$_{CC}$ 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; Diddar et al., 2010a), by salinity (e.g. Duenas-Bohorquez et al., 2009; Diddar et al., 2010b) and, moreover, by seawater Mg/Ca (Mg/Ca$_{SW}$) (Stanley et al., 2002, 2005; Ries, 2006; Ries et al., 2006; Müller et al., 2011). Seawater Mg/Ca (Mg/Ca$_{SW}$) varies over long time scales mainly due to geological processes. Currently, the average seawater Mg$^{2+}$ concentration is ~50 mM, and Ca$^{2+}$ is ~10 mM, resulting in a modern Mg/Ca$_{SW}$ ~5.

Since Mg$^{2+}$ has a residence time of ~1 Ma (Hardie, 1996; Broecker and Yu, 2011), the oceanic Mg/Ca is constant over shorter time scales.

The impact of different seawater Mg/Ca ratios on test Mg/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 Mg/Ca$_{SW}$ to Mg/Ca$_{CC}$ 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 [Mg$^{2+}$] at constant [Ca$^{2+}$]. In the culture study by Segev and Erez (2006) Mg/Ca$_{SW}$ was varied at a wide range (0.5–10.1) by changing both [Ca$^{2+}$] and [Mg$^{2+}$] in the culture media. Segev and Erez (2006) also included a number of treatments with varying [Mg$^{2+}$] and similar [Ca$^{2+}$], with a resulting Mg/Ca$_{SW}$ range of 0.5–5.2. The isolated impact of varying [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 Mg$^{2+}$ incorporation of a wider range of Mg/Ca$_{SW}$, varied solely by changing seawater Mg$^{2+}$.
benthic foraminiferal species that produce calcite with different Mg contents allows the determination of species-specific differences in response to seawater [Mg2+]i, potentially improving the proxy value of foraminiferal Mg/CaCC 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/CaSW on Mg/CaCC 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 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, also precipitate low-Mg model species because the majority of planktic foraminifers, which are 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 Mg-calcite species (0.4–4 mmol/mol, (Dissard et al., 2010b; Raitzsch et al., 2010; Duenas-Bohorquez et al., 2011)).

2.2. Preparation of culture media

At present, the average Mg2+ concentration of seawater is ~50 mM and that of Ca2+ is ~10 mM, resulting in a modern Mg/CaSW ~ 5. For the culture experiment, three different treatments of artificial seawater (ASW) with different [Mg2+] (10, 50 and 100 mM) and constant [Ca2+] (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 ±0.04 by adding NaOH (1 M). Salinity was measured with a salinometer (WTW, Cond 330) and adjusted to S = 32.6‰ ±0.2 by changing NaCl concentrations according to the amount of Mg2+ in each treatment. Actual Mg2+ and Ca2+ concentrations of the different treatments were verified with inductively coupled plasma-optical emission spectrometry (ICP-OES). Mg2+ was measured at a wavelength of 285 nm and Ca2+ at 316 nm. Dissolved inorganic carbon (DIC) was measured with an XY-2 Sampler (Bran and Luebbe, precision: ±7 μmol/kg), and total alkalinity was determined by autotitration (SI-Analytics TW alpha plus, precision: ±5 μmol/kg) (Table 1).

2.3. Culture experiment

Prior to the culture experiment, living specimens of A. aomoriensis (>250 μm) and A. lessonii (>500 μm) 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 μmol/m²·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 μL of a densely concentrated algal culture (approximately 4 * 10^6 cells·mL⁻¹) to each

Table 1

| Treatment | Ammonia aomoriensis | Amphistegina lessonii |
|-----------|---------------------|----------------------|
|           | Mg/Ca = 1 | Mg/Ca = 5 | Mg/Ca = 10 | Mg/Ca = 1 | Mg/Ca = 5 | Mg/Ca = 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 [μmol/kg] | 2530     | 2489     | 2663     | 2254     | 2297     | 2509     |
| Ω (calcite) | 4.82     | 4.52     | 5.06     | 4.80     | 4.50     | 5.08     |
| DIC [μmol/kg] | 2215     | 2192     | 2236     | 2105     | 2060     | 2168     |
| Mg2+ [mM] | 9.83     | 50.28    | 91.13    | 13.90    | 48.01    | 91.86    |
| Ca2+ [mM] | 8.88     | 9.93     | 9.95     | 9.14     | 9.08     | 9.06     |
| Mg/CaSW   | 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 (± 5 μm). 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 °C upon further analysis.

2.4. Weight

Mean test weight was determined for each treatment with a microbalance (Mettler Toledo UMX2, precision ± 0.1 μg). 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 (Reichart 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 μm, repetition rate to 7 Hz and energy density to approximately 1.2 J·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_{CC} 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 (~5 J·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 (~1.2 J·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 24Mg, 26Mg and 43Ca, 44Ca 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.
(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

$$D_{\text{Mg}} = \frac{\text{Mg/Calcite}}{\text{Mg/Seawater}}$$

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.
3. Results

3.1. Growth

Fig. 3a and b illustrates size (diameter; μm) 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/CaSW ~ 5 and Mg/CaSW ~ 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/CaSW ~ 5 for both species, reaching 5.01 (±0.22) μm/day for A. aomoriensis, and 7.86 (±0.75) μm/day for A. lessonii (Fig. 3 and Table 2). Lowest growth rates occurred at Mg/CaSW ~ 10 (4.74 ± 0.46 μm/day; Fig. 3 and Table 2).

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

| Treatment       | Ammonia aomoriensis | Amphistegina lessonii |
|-----------------|----------------------|------------------------|
| Slope (±std. error) [μm·d⁻¹] | Intercept (±std. error) [μm] | R² | Slope (±std. error) [μm·d⁻¹] | Intercept (±std. error) [μm] | R² |
| Mg/Ca ~ 1        | 2.84 (±0.16)         | 138.5 (±2.8)           | 0.99 | 7.26 (±0.54)         | 155.4 (±10.2)          | 0.98 |
| Mg/Ca ~ 5        | 5.03 (±0.20)         | 128.1 (±3.6)           | 1.00 | 8.35 (±0.15)         | 145.3 (±2.8)           | 1.00 |
| Mg/Ca ~ 10       | 4.04 (±0.30)         | 130.0 (±5.3)           | 0.98 | 4.73 (±0.25)         | 138.6 (±4.8)           | 0.99 |

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 CaCO₃ addition [μg/day], we included CaCO₃ 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 = 0.50 for A. lessonii) and CaCO₃ 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/Ca₀₉ is significantly positively correlated with increasing seawater Mg²⁺ concentrations and thus increasing Mg/CaSW (Fig. 5). A linear correlation and a power function can be fitted.
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 $D_{mg}$.

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 $D_{mg}$ at different Mg/CaSW. The test detected significant differences between the means of the $D_{mg}$ 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 $D_{mg}$ for both species show a decrease with increasing Mg/CaSW and for both species the $D_{mg}$ decreases towards the value of the overall calculated $D_{mg}$ 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 $D_{mg}$ of $0.64 (\pm 0.04) \times 10^{-3}$ at Mg/CaSW ~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 $D_{mg}$ at Mg/CaSW ~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 (μm/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 μm. 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 conditions, provide evidence for the suitability of both species for use as indicators of environmental conditions in the field.

### Table 3

Mean SNW and CaCO$_3$ addition and their standard errors for $A. aomoriensis$ and $A. lessonii$ in the different treatments.

| Treatment | $A. aomoriensis$ | $A. lessonii$ |
|-----------|-----------------|---------------|
|           | SNW (±std. error) [μg/μm] | CaCO$_3$ addition (±std. error) [μg/day] | SNW (±std. error) [μg/μm] | CaCO$_3$ addition (±std. error) [μg/day] |
| Mg/Ca ~ 1 | $1.34 \times 10^{-2} (\pm 0.19 \times 10^{-2})$ | $0.11 (\pm 0.02)$ | $3.32 \times 10^{-2} (\pm 0.42 \times 10^{-2})$ | $0.45 (\pm 0.09)$ |
| Mg/Ca ~ 5 | $1.31 \times 10^{-2} (\pm 0.10 \times 10^{-2})$ | $0.13 (\pm 0.01)$ | $4.24 \times 10^{-2} (\pm 0.90 \times 10^{-2})$ | $0.63 (\pm 0.18)$ |
| Mg/Ca ~ 10 | $1.20 \times 10^{-2} (\pm 0.05 \times 10^{-2})$ | $0.11 (\pm 0.01)$ | $3.08 \times 10^{-2} (\pm 0.45 \times 10^{-2})$ | $0.33 (\pm 0.07)$ |

Fig. 5. Mg/CaCc and mean partition coefficient $D_{mg}$ versus Mg/CaSW 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/CaSW, albeit that for *A. lessonii* growth rates at low Mg/CaSW are not significantly different from those at intermediate values (Fig. 3). Impaired growth at higher Mg/CaSW is observed in other marine calcifiers and may be explained by increased energy spent on calcification by overcoming inhibition by Mg2⁺ on CaCO3 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 Mg2⁺ at the site of calcification. Still, both species show optimal growth rates at Mg/CaSW values similar to present day levels (Fig. 3c and d) and a clear tendency to impaired growth at elevated and low Mg/CaSW values. Since Mg/CaCC correlates almost linearly to Mg/CaSW (compare Section 4.3) the observed differences in foraminiferal growth rates do not affect incorporation of Mg2⁺.

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. CaCO3 addition [μg/d] 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 CaCO3 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/CaSW, CaCO3 addition [μg/d] is lower in our data set. The small offset might be due to the fact that Segev and Erez (2006) partially varied Ca2⁺ instead of Mg2⁺. Our new data does not support the hypothesis of Segev and Erez (2006), that lower Mg/CaSW is always advantageous for foraminiferal calcite addition.

In summary, our experiment in which Mg/CaSW was varied exclusively through [Mg2⁺], suggests no influence on size-normalized weight or CaCO3 addition, with optimal growth rates at Mg/CaSW ~ 5, reflecting a good adaptation to modern Mg/CaSW. This seems counter-intuitive, because seawater Mg/CaSW was close to 1 during the Cretaceous (Hardie, 1996; Stanley and Hardie, 1998), when the superfamilies Rotalidae and Aspergerinoidae (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

### Table 4

| Ammonia aomoriensis | Amphistegina lessonii |
|---------------------|----------------------|
| Mg/CaSW [mol/mol]   | Mg/CaCC (± std. error) [mmol/mol] | Dm6 ± 1000 (± std. error) | Mg/CaSW [mol/mol] | Mg/CaCC (± std. error) [mmol/mol] | Dm6 ± 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 coccolithophors such different phases have not been observed, however, they also show increasing values for DMg at lower Mg/CaSW 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 DMg (relative change in Mg/CaCC as a function of Mg/CaSW, respectively) for 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/CaCC on Mg/CaSW, where the slope is determined by the relative contribution of passive transport. At higher Mg/CaSW (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/CaSW, 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/CaSW-Mg/CaCC relationship, by causing a relatively high DMg at low Mg/CaSW. 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/CaSW, based on a shift in Mg/CaSW 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/CaSW close to the crystal surface (Fig. 6). Such an effect of locally altered Mg/CaSW would have a stronger impact at low Mg/CaSW, 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/CaSW. 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/CaSW and Mg/CaCC 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/CaSW and Mg/CaCC can be represented by a power function at low ratios that turns into a linear function at high ratios. Since this involves a varying DMg at low and a constant DMg at high ratios, an exponential function that approaches an asymptote is appropriate to describe the relationship between DMg and Mg/CaSW:

\[
 f(x) = a e^{-bx} + A
 \]

\(a\) and \(b\) being species specific constants, and \(A\) the asymptote equaling the slope of a linear regression line between Mg/CaSW and Mg/CaCC. This function provides a new suggestion for paleo-oceanographers, by describing the relationship between DMg and Mg/CaSW 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/CaCC-Mg/CaSW relationship between foraminiferal species with contrasting Mg/CaCC (e.g. Ammonia and Amphistegina), different marine calcifying organisms, and even inorganic CaCO3 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.

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