Ba incorporation in benthic foraminifera

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Abstract

Barium (Ba) incorporated in the calcite of many foraminiferal species is proportional to the concentration of Ba in seawater. Since the open ocean concentration of Ba closely follows seawater alkalinity, foraminiferal Ba/Ca can be used to reconstruct the latter. Alternatively, Ba/Ca from foraminiferal shells can also be used to reconstruct salinity in coastal settings where seawater Ba concentration corresponds to salinity as rivers contain much more Ba than seawater. Incorporation of a number of minor and trace elements is known to vary (greatly) between foraminiferal species and application of element/Ca ratios thus requires the use of

species-specific calibrations. Here we show that calcite Ba/Ca correlates positively and linearly with seawater Ba/Ca in cultured specimens of two species of benthic foraminifera, *Heterostegina depressa* and *Amphistegina lessonii*. The slopes of the regression, however, vary 2-3 fold between these two species (0.33 and 0.78, respectively). This difference in Ba-partitioning resembles the difference in partitioning of other elements (Mg, Sr, B, Li and Na) in these foraminiferal taxa. A general trend across element partitioning for different species is described, which may help developing new applications of trace elements in foraminiferal calcite in reconstructing past seawater chemistry.

Keywords: foraminifera, Ba/Ca, proxies

1 Introduction

Incorporation of barium (Ba) in foraminiferal calcite is proportional to seawater barium concentrations (e.g. Lea and Boyle, 1989; 1990; Lea and Spero, 1994). Open ocean surface barium concentrations are relatively uniform (Chan et al., 1977; Broecker and Peng, 1982) and since [Ba^{2+}] is removed at the surface and regenerated at depth, its vertical concentration resembles that of alkalinity (Li and Chan, 1979; Rubin et al., 2003). For this reason, fossil foraminiferal Ba/Ca has been used to reconstruct past alkalinity (e.g. Lea, 1995). Locally, seawater [Ba^{2+}] can also reflect salinity due to the relatively high Ba/Ca of river- or meltwater input (Hanor and Chan, 1977; Guay and Falkner, 1997; 1998) and therefore Ba/Ca in foraminiferal calcite can be used to reconstruct paleosalinity (Hall and Chan, 2004b; Weldeab et al., 2007; 2014; Bahr et al., 2013). These reconstructions can be complicated by upwelling affecting surface Ba/Ca (Lea et al., 1989; Hatch et al., 2013). Moreover, Ba cycling at or close to the seafloor can affect Ba uptake in benthic foraminifera (Ni Flaithearta et al., 2010). Application of Ba/Ca critically depends on the prerequisite that temperature, salinity as such

(Lea and Spero, 1994; Hönisch et al., 2011) and photosymbiont activity (Lea and Spero, 1992; Hönisch et al., 2011) do not affect Ba incorporation in foraminiferal shell carbonate. Still, Ba/Ca ratios are known to vary within chamber walls of crust-producing planktonic foraminifera (Eggins et al., 2003; Hathorne et al., 2009). Like Mg/Ca, the values for Ba in crust carbonate are lower, which cannot be (solely) explained by migration to greater water depths during crust formation (Hathorne et al., 2009). This argues for an unknown additional imprint on Ba incorporation. On an intra-test scale, the distributions of Mg and Ba within the test wall of *Pulleniatina obliquiloculata* have been shown to co-vary to some extent, with maximum concentrations often, but not always, coinciding with the 'organic linings' (Kunioka et al., 2006). For some other elements, including Mg and Sr, incorporation has been shown to be inter-dependent (e.g. Mewes et al., 2015). Such interdependency, however, varies between pairs of elements and is explained by a combination of simultaneous fractionation by the same process (e.g. Langer et al., 2016) and by involvement of different processes during calcification (Nehrke et al., 2013). These models and experimental results may imply that also the incorporation of Ba could be influenced by these physiological processes and/ or the same fractionation process during calcite precipitation (e.g. through lattice distortion; Mucci and Morse, 1983; Mewes et al., 2015).

So far, Ba/Ca values have been reported for planktonic (Boyle, 1981; Lea and Boyle, 1991; Lea and Spero, 1992; 1994; Hönisch et al., 2011; Marr et al., 2013; Hoffmann et al., 2014) and low-Mg benthic species (Lea, 1995; Lea and Boyle, 1989; 1990; 1993; Reichart et al., 2003). Although Mg/Ca is known to vary greatly between (benthic) foraminiferal species (between ~1 and ~150 mmol/mol; Toyofuku et al., 2000; Bentov and Erez, 2006; Wit et al., 2012) Ba/Ca, which is only rarely investigated in species producing high-Mg calcite (Evans et al., 2015; Van Dijk et al., 2017). Ba/Ca in planktonic species may be used to reconstruct (changes in) open ocean alkalinity (Lea, 1995), whereas those published for benthics may be

more suitable to reconstruct salinity in coastal and shelf seas (Weldeab et al., 2007; 2014; Bahr et al., 2013). The range in Mg/Ca is known particularly for benthic foraminifera (e.g. Toyofuku et al., 2011; Sadekov et al., 2014) and inter-species variability in Ba incorporation may therefore hamper application of (benthic) foraminiferal Ba/Ca. Here we present results from a culture study using the larger benthic foraminifera, *Amphistegina lessonii* and *Heterostegina depressa*, two species with different Mg/Ca (~50 mmol/mol; Segev and Erez, 2006 and ~120 mmol/mol; Dueñas-Bohórquez et al., 2011, respectively). In these culturing experiments, the range in Ba/Ca exceeds the naturally occurring range in seawater to facilitate testing underlying controls on barium incorporation. If there is a linear increase in shell Ba/Ca (Ba/Ca_{cc}) with increasing seawater Ba/Ca (Ba/Ca_{sw}), the large range in Ba/Ca of the culturing media prepared here will furthermore decrease uncertainty of the obtained Ba/Ca_{cc}-Ba/Ca_{sw} calibration. Our results are compared to Ba/Ca in these species from field samples. Together, calibration of Ba/Ca in these species against seawater Ba/Ca and in the context of other elemental incorporation data, allows evaluation and application of incorporated Ba across a wider range of foraminiferal taxa, with contrasting element composition of their shell.

2 Methods

2.1 Culture media

To determine Ba/Ca partitioning, benthic foraminiferal culture experiments were set up with five different seawater Ba/Ca ratios (54-92 µmol/mol). Media were prepared by increasing $[Ba^{2+}]_{sw}$ while keeping the $[Ca^{2+}]_{sw}$ constant. The range of $[Ba^{2+}]$ used in these experiments exceeds the range of concentrations found naturally and allows testing the applicability of partition coefficients under conditions with artificially high seawater Ba/Ca. Seawater is only slightly undersaturated with respect to barite ($BaSO_4$) and an increase in $[Ba^{2+}]$ in the sea water will cause barite precipitation (Langer et al., 2009). To be able to increase $[Ba^{2+}]$ beyond its

natural range, artificial seawater was prepared with lower sulphate contents. All other salts were added according to the recipe of Kester et al. (1967) to produce a total of 5 litres of medium for each treatment. As *Amphistegina lessonii* and *Heterostegina depressa* do not grow well in 100% artificial seawater, the prepared media were mixed with natural seawater in a ratio 9:1 (Mewes et al., 2014). To double check concentrations and determine potential loss of elements due to precipitation, sorption and/or scavenging, element concentrations of the culture media were determined by ICP-OES at the Alfred-Wegener-Institute in Bremerhaven, except for Ba which was measured by ICP-MS at Utrecht University (Table 1).

Culture media pH was adjusted to 8.0 by adding NaOH (1 M) to the prepared media. Before the start of the experiments, dissolved inorganic carbon (DIC) and total alkalinity were measured at the Alfred-Wegener-Institute. DIC was measured photometrically in triplicates with a TRAACS CS800 QuAAtro autoanalyser with an average reproducibility of ± 10 μmol L^{-1}. Alkalinity was calculated from linear Gran plots (Gran, 1952) after triplicate potentiometric titration (Bradshaw et al., 1981) using a TitroLine aplpha plus auto sampler (Schott Instruments). Parameters of the total carbonate system were calculated from temperature, salinity, DIC and alkalinity using the program CO2SYS (Lewis and Wallace, 1998) adapted to Excel by (Pierrot et al., 2006). The equilibrium constants K1 and K2 from Mehrbach et al. (1973), as reformulated by Dickson and Millero (1987) were used (Table 1).

2.2 Foraminiferal culturing

Living specimens of *A. lessonii* and *H. depressa* were isolated from sediment collected at the tropical aquarium of Burger's Zoo (Arnhem, The Netherlands) in August 2012 and transferred to the Alfred-Wegener-Institute for the culture experiments. Healthy individuals of *A. lessonii* showing pseudopodial activity, a dark brown cytoplasm and minimal signs of bleaching were handpicked with a small brush under a Zeiss Stereo microscope and transferred to well plates.

Adult specimens of *H. depressa* were picked directly from the aquarium with soft tweezers. After two weeks several individuals of both species underwent asexual reproduction. Individual *H. depressa* parent cells produced sufficient numbers of juveniles to study separate clone groups. Approximately 20 juveniles with two or three chambers from the same parent were selected for every treatment and divided over two Petri dishes (diameter 55 mm, containing approximately 10 ml of culture medium). In total, two clone groups were used in the experiments resulting in a total of at least 40 individuals per treatment. Specimens of *A. lessonii* did not produce sufficient numbers of juveniles for analysis of separate clone groups. Therefore, approximately 60 juveniles with two or three chambers from different parents were selected per treatment and distributed evenly over three Petri dishes. All experiments were carried out in an adjustable incubator (RUMED Rubarth Aparate GmbH) at a constant temperature of 25 °C. As both species are symbiont-bearing, a 12:12 light:dark cycle was applied with a constant photon flux density of approximately 250 µmol photons $m^{-2}s^{-1}$ during light hours. Pictures were taken weekly under a Zeiss Axiovert 200M inverted microscope and maximal diameters of the shells were measured with the AxioVision software to allow determining the chamber addition rates of the foraminifera in the experiments. The experiments were terminated after six weeks.

All specimens were fed *Dunaliella salina* algae every three to four days. Although *A. lessonii* hosts symbionts, this foraminiferal species does not exclusively rely on nutrients from their symbionts, but also ingests algae (Lee, 2006). To avoid changes in the barium concentration of the culture media, the water in the dishes containing foraminifera were diluted as little as possible by the solution containing the food for the foraminifera. For this purpose, foraminifera were fed 50 µl of a solution containing algae that was centrifuged at 2000 rpm for 10 minutes. Algae concentrated at the bottom of the tube were transferred to an empty tube with a pipette. To prevent changes in the culture media's carbonate chemistry by algal photosynthesis the algae were killed by heating the concentrated solution in an oven at 90 °C for 10 minutes. The cultures

were transferred to new Petri dishes every week to avoid excessive bacterial growth, potential build-up of waste products and shortage of ions or nutrients. To prevent changes in salinity by evaporation media were refreshed three days after the cultures were transferred to new dishes by pipetting approximately 5 ml of the old media out of the Petri dish and replacing it with the same volume of media from the prepared batch.

2.3 Sample preparation and analysis

At the end of the culture experiment, specimens were cleaned by placing them in a 7% NaOCl solution for approximately 30 minutes until completely bleached and organic material was removed from the tests. This cleaning method is shown to have a similar impact on average foraminiferal Ba/Ca values as cleaning with H_2O_2 and is relatively small (2-3 µmol/mol) compared to cleaning with de-ionized water only (Pak et al., 2004). Specimens were then rinsed three times for approximately 60 seconds in de-ionized water to remove the NaOCl and any residual salts from the culture solutions. Cleaned foraminifera were put in an oven at 42 °C until completely dry and mounted on sample holders using double sided adhesive tape.

Element composition of the calcite was determined using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) at Utrecht University (Reichart et al., 2003). The system consisted of a Geolas 200Q 193 nm Excimer laser (Lambda Physik) connected to a sector field-ICP-MS (Element2, Thermo Scientific). Samples were ablated in a single-volume chamber and the aerosol was carried to the ICP-MS by a Helium flow. Monitored masses included ^{23}Na, ^{24}Mg, ^{26}Mg, ^{27}Al, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba and ^{238}U and calibration was performed using a glass standard (NIST 610) that was ablated three times after every 10-12 foraminiferal samples. Diameter of the ablation crater was set to 80 µm for all specimens and pulse repetition rate was 6 Hz. The ablated calcite was measured and integrated with respect to time. Energy density for the glass was higher than for the foraminifera (5 J/cm^2 and 1 J/cm^2,

respectively). Although the resulting difference in ablation characteristics is not likely to affect obtained foraminiferal element concentrations (Hathorne et al., 2008), foraminiferal element concentrations were compared to those from an in-house made calcite standard with known element concentrations and ablated at the same energy density as the foraminifera (Dueñas-Bohórquez et al., 2009). Relative standard deviation for Mg/Ca and Sr/Ca based on repeated measurements on this material was <5% for both ratios. Due to the lamellar nature of Rotallid foraminifera, final chambers are thinnest and are therefore characterized by largest uncertainty in the estimated average element/Ca ratio. Therefore, the F chamber was not considered and instead, the F-1 chamber of *A. lessonii* was ablated for every specimen. For *H. depressa*, walls of the final two chambers were commonly too thin for reliable chemical results and, therefore, the F-2 chamber was analysed. In addition, for each species, the final 6-7 chambers of ten sufficiently large specimens (2 from each of the five treatments) were ablated to analyse intra-specimen variability in Ba/Ca, to analyse variability within chamber walls as a function of thickness and to detect potential ontogenetic trends in Ba incorporation.

Elemental concentrations were calculated from the ablation profiles with the Glitter software, using ^{43}Ca as internal standard and values from Jochum et al. (2011) for concentrations of elements in the NIST 610. This program integrates the ablation signal after subtracting the background signal to calculate the elemental concentrations. To avoid contaminated intervals of the ablation profile, sections with high ^{27}Al and ^{55}Mn counts were excluded from the analysis since these parts are often also characterized by unusually high Mg/Ca not reflecting the actual shell carbonate. Ablation profiles with a duration shorter than 5 seconds were rejected as such short profiles are unreliable due to poor counting statistics. Nine out of 188 ablation profiles were rejected for *A. lessonii* and 7 out of 140 profiles from *H. depressa* were discarded, which is less than 5%.

2.4 Aquarium samples

To compare the results from cultured specimens with Ba/Ca from specimens derived from 'natural conditions', a number of living specimens of both *A. lessonii* and *H. depressa* were isolated from the Zoo's stock (i.e. sediment collected at the zoo from which the specimens were isolated; section 2.2) and cleaned and prepared for LA-ICP-MS analyses as described in 2.3. From both species, 7 specimens were ablated twice at the Royal NIOZ using a NWR193UC (New Wave Research) laser, containing an ArF Excimer laser (Existar) with deep UV 193 nm wavelength and <4 ns pulse duration. Provided that the same reference material is used, the use of multiple laser systems (see above) is shown not to bias obtained foraminiferal element/Ca ratios (De Nooijer et al., 2014a). Laser ablation was performed with an energy density of 1 J/cm^2 at a repetition rate of 6 Hz for calcite samples and an energy density of 5 J/cm^2 for the glass (NIST610) standards. Helium was used as a carrier gas with a flow rate of 0.8 L/min for cell gas and 0.3 L/min for cup gas. From the laser chamber to the quadrupole ICP-MS (iCAP-Q, Thermo Scientific), the He flow was mixed with ~0.4 L/min nebulizer Ar. Before measuring the samples, the nebulizer gas, extraction lens, CCT focus lens and torch position were automatically tuned for the highest sensitivity of ^{25}Mg by laser ablating MACS-3. The masses measured by the ICP-MS were ^{23}Na, ^{24}Mg, ^{25}Mg, ^{27}Al, ^{43}Ca, ^{44}Ca, ^{88}Sr and ^{138}Ba. JCp-1, MACS-3 and an in-house (foraminiferal) calcite standard (NFHS) were used for quality control and measured every 10 foraminiferal samples. Internal reproducibility of the analyses was all better than 9%, based on the three different carbonate standards used. Intensity data were integrated, background subtracted, standardized internally to ^{43}Ca and calibrated against the MACS-3 signal using a custom-built MATLAB routine within the program SILLS (Guillong et al., 2008). Since ablation of the NIST SRM 610 and NIST SRM 612 could increase the sodium background, they were only ablated and analyzed at the end of every sequence and

cones were cleaned before the next sequence. Accuracy of the analyses was better than 3%, based on comparing the carbonate standards with internationally reported values (Okai et al., 2002, Wilson et al., 2008). Signals were screened for surface contamination and parts of the outside or inside of the shell with elevated Mg, Mn or Al values were eliminated from the area selected for integration.

Seawater samples from the Zoo's aquarium were measured in duplicate using a sector field-ICP-MS (Element2, Thermo Scientific). The ICP-MS was run in low resolution mode (24 cycles) for ^{138}Ba and in medium resolution (24 cycles) for ^{43}Ca. Calibration was performed through an external calibration series with increasing concentrations of Ba.

3 Results

3.1 Test diameter increase

Average shell diameters increased considerably during the experimental period (Figure 1). Overall, increase in shell diameter did not significantly differ between treatments. Treatment C (seawater Ba/Ca = 64 μmol/mol) for *A. lessonii*, however, shows somewhat reduced chamber addition rates per incubated specimen. This may be the consequence of slightly higher mortality under these conditions and a relatively high number of specimens that did not add any chambers. Although not systematically investigated, two Petri dishes from this treatment contained relatively many bleached (i.e. devoid of symbionts) specimens at the end of the 6-week period.

3.2 Barium incorporation

Calcite Ba/Ca increases linearly with seawater Ba/Ca for both species (Figure 2; Table 2). ANOVA performed on the individual data points combined with regression analyses reveals a significant increase of Ba/Ca_{cc} with Ba/Ca_{sw} for both species (Table 3). Calculated regression

slopes result in a D_{Ba} of 0.326 (±0.005) for *A. lessonii* and 0.777 (±0.007) for *H. depressa* (Figure 3, solid lines). Regression lines are forced through zero as it seems reasonable to assume that no Ba is incorporated into calcite when the Ba concentration in the seawater is zero. Without this forcing, regression slopes would be $Ba/Ca_{cc} = 0.34*Ba/C_{asw}$ -1.1 for *A. lessonii* and $Ba/Ca_{cc} = 0.92*Ba/C_{asw}$ -10 for *H. depressa*. The resulting partition coefficients ($(Ba/Ca_{cc})/(Ba/Ca_{sw})$) are constant and significantly different between the species (ANOVA) (~0.3 for *A. lessonii* and ~0.8 for *H. depressa*) over the range of seawater Ba/Ca studied here. The regression line for Ba/Ca_{cc} as a function of Ba/Ca_{sw} for *A. lessonii* corresponds well with that reported for a number of different low Mg species (Lea and Boyle, 1989).

The aquarium-derived specimens 'aquarium samples' had an diameter ranging from 550 to 1180 µm (with an average of 975 µm) for *A. lessonii* and from 1380 to 2340 µm (average: 1936 µm) for *H. depressa*. They had an average Ba/Ca of 15.4 (±2.3 SD) µmol/mol for *A. lessonii* and 35.7 (±14 SD) µmol/mol for *H. depressa*. In combination with the measured aquarium's seawater Ba/Ca of 35.7 (±3.9 SD) µmol/mol, the partition coefficients for Ba vary between 0.43 and 1.0 for *A. lessonii* and *H. depressa*, respectively. The aquarium derived data is consistent with the controlled growth derived data, but it was not used in the regression analysis (Figure 2) since the conditions (e.g. carbonate chemistry) under which the specimens from the aquarium were grown, were not determined as precisely and accurately as in our culturing experiment. Including these data in the linear regression (Figure 2) would change the sensitivity from 0.78 to 0.77 for *H. depressa* and from 0.33 to 0.32 for *A. lessonii*.

3.3 Intrachamber variability in Ba/Ca

From both species, 10 specimens were used to quantify the relation between ontogeny (i.e. size-dependent) and Ba incorporation into foraminiferal calcite. For this purpose, the final 6-7 chambers of these individuals were ablated (Figure 3). With the selected spot diameter (80 µm),

ablation of a small amount of material of adjacent chambers could not always be avoided. Some chamber walls, particularly of the youngest (i.e. built latest) chambers, were too thin for reliable measurements and were excluded from further consideration.

Since these specimens were cultured at different Ba/Ca_{sw}, the inter-chamber variability is expressed as the difference of a single-chamber Ba/Ca and the individual's average Ba/Ca. Positive single-chamber values indicate higher than average values, whereas negative values indicate single-chamber Ba/Ca below that individual's average Ba/Ca (Figure 3).

In *H. depressa*, Ba/Ca_{cc} increases significantly with subsequently new chambers added (Figure 3). Regression analysis reveals an average increase of 1.43 µmol/mol Ba/Ca_{cc} with every chamber added (Table 4). Ba/Ca_{cc} appears to decrease with chamber position in *A. lessonii,* although the ANOVA p-value shows that this is statistically not significant. Still, removing one single outlier already results in a p-value lower than 0.01, indicating that the current data set does not allow rejecting the presence of a trend for *A. lessonii*.

3.4 Relation between incorporation of barium and magnesium

Combining data from all five treatments, average Mg/Ca of *A. lessonii* was 64 mmol/mol, with a relative standard deviation of 47%. Within treatments, the variability in Mg/Ca is considerably lower (between 27 and 37%). Average Mg/Ca in *H. depressa* was 152 mmol/mol, with a standard deviation of 25 mmol/mol (16%). Within treatments, the relative standard deviation ranged from 4.1% (treatment E) to 17% (treatment D). The species-specific single-chamber Mg/Ca and Ba/Ca combined for all treatments are positively and significantly related (Figure 4). For *A. lessonii*, Mg/Ca = 3.1*Ba/Ca – 3.6 (t-value = 12.2, $p < 0.01$ for the slope of the regression) and for *H. depressa*, Mg/Ca = 1.1*Ba/Ca + 92 (t-value = 14.8, $p<0.01$ for the slope). The slopes of these two regressions (3.1 and 1.1) are significantly different: this is calculated

by $z = (a_{Heterostegina} - a_{Amphistegina}) / \sqrt{(SE_{a,Heterostegina}^2 + SE_{a,Heterostegina}^2)}$, where a is the value for the regression's slope and SE_a is the slope's associated standard error. For the slopes of the Mg/Ca-Ba/Ca regressions for *Amphistegina* and *Heterostegina*, the resulting z-score is higher than >7, indicating that the two slopes are significantly different.

When comparing the single-chamber D_{Ba} with D_{Mg}, of all data combined, the partition coefficient for Mg is over 30 times lower than that of for Ba (Figure 4). Over the range in Ba/Ca_{sw} studied here, the relation between D_{Ba} and D_{Mg} is linear within both species. For *A. lessonii*, $D_{Mg} = 40*D_{Ba} - 2.0$ (t-value = 7.3, $p < 0.01$ for the slope of the regression) and for *H. depressa*, $D_{Mg} = 29*D_{Ba} + 3.8$ (t-value = 6.5, $p<0.01$ for the slope). The slopes of these two regressions (40 and 29) are not significantly different (z-score 1.6). When combining the data from both species, the regression equals: $D_{Mg} = 34*D_{Ba} +0.073$ (t-value = 29.9, $p<0.01$ for the slope).

4 Discussion

4.1 Test diameter increase

The range of Ba concentrations used in the experiments did not influence the increase in shell diameter of either foraminiferal species (Figure 1). Compared to *H. depressa*, increases in shell diameter (which is proportional to the chamber addition rate) for *A. lessonii* were slightly more variable. To prevent barite precipitation it was necessary to reduce the sulphate concentration below that typically measured in natural seawater. Sulphate concentrations between 0.1 and 1 mmol/L do not affect inorganic calcite growth (Reddy and Nancollas, 1976), but a decrease in growth rates of approximately 30% was observed in coccolithophores growing in artificial seawater with a sulphate concentration 10% that of natural seawater (Langer et al., 2009). Although coccolithophores and foraminifera may respond differently to lowered sulphate concentrations, this reduction could have hampered growth of the specimens in our culturing

experiment. Chamber addition rates of *A. lessonii* in a culture set-up with a sulphate concentrations similar to that of natural seawater (Mewes et al., 2014) were approximately 20% higher than chamber addition rates observed in our experiments. Since these experiments were not performed simultaneously using specimens from the same batch, it is not straight forward to compare absolute rates and therefore the 20% difference cannot unambiguously be attributed to sulphate concentration (Hoppe et al. 2011). Unfortunately no data exist on the effect of reduced sulphate concentrations on the uptake of trace elements in foraminiferal calcite. However Langer et al. (2009) demonstrated that sulphate limitation had no discernible effect on Ba incorporation in coccolithophore calcite.

4.2 Barium incorporation

The variability in Ba/Ca between individual ablation craters is considerable, but the average foraminiferal Ba/Ca shows a consistent relation with seawater Ba/Ca. This implies that the observed variability is a reflection of the inhomogeneous distribution in the test and hence filtered out when averaging. This is similar to the behavior for Mg and Sr (Sadekov et al., 2008; Wit et al., 2012; De Nooijer et al., 2014a) and underscores the power of single-chamber analyses. If present, inhomogeneity in test wall Ba/Ca in combination with different cross section sampled during the ablation potentially account for the observed variability. This would imply that although large differences are observed within a test wall, the average still reliably reflects sea water concentration (this paper) and for Mg, still reflects seawater temperature (Hathorne et al., 2009). Comparing within-specimen and between-specimen variability, De Nooijer et al. (2014a) showed that within specimen variability does not account for all of the observed variability in Mg/Ca in *Ammonia tepida*. This seems to be similar for Ba/Ca (compare Figure 4 in this paper with Figure 5 from De Nooijer et al., 2014a), which would mean that at least 20 chambers need to be analyzed to reach a 5% relative precision (De Nooijer et al.,

2014a). This is not limited by the analytical precision, but rather due to inherent biological inter-chamber and inter-specimen variability. To reduce ontogenetic variability (in e.g. paleoceanographic applications where complete specimens are measured), a narrow size fraction should be analyzed.

Incorporation of Ba in *H. depressa* shows a partitioning which is about 2.5 times higher than in *A. lessonii*. Such a large offset of D_{Ba} between benthic species fits previously reported (differences in) partition coefficients for barium. Lea and Boyle (1989) found $D_{Ba} = 0.37 \pm 0.06$ for *Cibicidoides wuellerstorfi*, *Cibicidoides kullenbergi* and *Uvigerina* spp. for a series of core tops, comparable to the partition coefficient reported here for *A. lessonii* (0.33 ± 0.022; Figure 2). In contrast, partition coefficients for Ba in planktonic foraminifera are roughly only twice as low as these benthic foraminiferal partitioning coefficients (0.14-0.19; Hönisch et al., 2011; Lea and Boyle, 1991; Lea and Spero, 1992). Although temperature, pH, salinity and pressure were initially proposed as potential explanation for the offset between planktonic and benthic D_{Ba} (Lea and Boyle, 1991; Lea and Spero, 1992), studies by Lea and Spero (1994) and Hönisch et al. (2011) showed no significant impact of temperature, pH and salinity on Ba incorporation into planktonic foraminiferal calcite. This would leave hydrostatic pressure to explain the difference between benthic and planktonic species. Van Dijk et al. (2017) on the other hand, showed that in a number of larger benthic foraminifera, Ba/Ca is positively influenced by pCO_2. Our observations show, however, that the observed differences in D_{Ba} between *H. depressa* and *A. lessonii* and also the offset with the planktonic species are inherent to these species. A small impact of environmental parameters other than seawater Ba/Ca may account for the slightly higher D_{Ba} in the foraminifera taken from the aquarium compared to the cultured ones (Figure 2). The overall differences in partitioning seem to coincide with different taxonomic groups, which may indicate that foraminifera may differ in their controls on transporting ions from seawater to the site of calcification. For example, the contribution of transmembrane transport

versus that of seawater transport (i.e. leakage; Nehrke et al., 2013 or vacuolization; Erez, 2003) may vary between species and thereby account for differences in Mg/Ca, Ba/Ca, etc. (Nehrke et al., 2013).

4.3 Inter-chamber variability of Ba/Ca_{cc}

In both species cultured here, Ba/Ca_{cc} decreases significantly from largest (i.e. built latest in life) towards the smaller chambers (Figure 3). Observed trends were not significantly different between *A. lessonii* and *H. depressa*, suggesting that Ba/Ca_{cc} decreases at the same rate with size, despite the overall difference in Ba/Ca_{cc} (Figure 3). Since we always analyzed chambers at the same position (F-1 for *A. lessonii* and F-2 for *H. depressa*) and since the final size of the cultured specimens was similar between treatments (Figure 1), ontogenetic trends in Ba/Ca do not influence the trends in Ba/Ca between treatments (Figure 2). Several other studies showed that element/Ca ratios can vary with chamber position. Raitzsch et al. (2011), for example, reported increasing B/Ca and decreasing Mg/Ca towards younger chambers in the benthic *Planulina wuellerstorfi*. Such patterns may be related to changes in the surface-to-volume ratio or relative changes in "vital effects" as foraminifera grow larger. For example, pH reduction in the foraminiferal microenvironment is related to the specimen's size (Glas et al., 2012) and may thereby affect the chemical speciation of minor and trace element, which in turn, may determine their uptake rates. Hönisch et al. (2011), however, showed that seawater pH has no noticeable effect on Ba incorporation in planktonic foraminiferal calcite, rendering changes in the pH of the foraminiferal microenvironment an unlikely explanation to account for the observed chamber-to-chamber variability in Ba/Ca. Alternatively, changes in the metabolic rate, the instantaneous calcification rate, or a different partitioning between the impacts of the life processes may lead to the observed ontogenetic trend.

Bentov and Erez (2006) argued that decreasing Mg/Ca with foraminifera test size could be explained by relatively high Mg-concentrations at or near the primary organic sheet (POS), which is the organic matrix on which the first layer of calcite precipitates during the formation of a new chamber. With the formation of a new chamber, a low-Mg calcite layer is deposited over all existing chambers, so that the high-Mg phase is being 'diluted' as more layers are deposited (Bentov and Erez, 2006). Future studies may indicate whether Ba/Ca is also heterogeneously distributed within chamber walls, by for example, being enriched close to the POS (Kunioka et al., 2006). If this is the case, lamellar calcification mode may also result in changing Ba/Ca with chamber position.

4.4 Coupled incorporation of barium and magnesium

If incorporation of Ba and Mg (and Na, Sr and B) are physically linked during biomineralization, inter-species differences in composition may likely be correlated across the various elements. The correlation between Mg/Ca and Ba/Ca within and between species (Figure 4) suggests that these two elements are simultaneously affected during their incorporation. The relationship between Mg/Ca and Ba/Ca is different between the two species, which may be (partly) caused by the variability in seawater chemistry between treatments (i.e. seawater Ba/Ca and Mg/Ca; Table 1). Alternatively, incorporation of Mg in *H. depressa* may be close to the maximum concentration of Mg which can be incorporated into a calcite crystal lattice at ambient conditions (Morse et al., 2007). This may result in an overall asymptotic relationship between Mg/Ca and Ba/Ca as Mg/Ca approaches ~200 mmol/mol (Figure 4).

When correcting for the different seawater Ba/Ca and Mg/Ca between treatments, incorporated Ba and Mg correlate similarly within, as well as, between the two species studied here (Figure 4). This suggests that these elements are coupled during biomineralization itself and that the ratio of Ba and Mg in seawater is preserved during calcification by these species of

foraminifera. When comparing the relation between Ba/Ca and Mg/Ca from other benthic species (e.g. Lea and Boyle, 1989; figure 2; more refs), the coupling between Ba- and Mg-incorporation is likely similar across a wide range of benthic foraminiferal species.

4.5 Biomineralization and element incorporation

Foraminiferal biomineralization determines incorporation of many elements and fractionation of many isotopes during the production of new chambers as indicated by overall large compositional differences between inorganically precipitated and foraminiferal calcite (Erez, 2003; Bentov and Erez, 2006; Nehrke et al., 2013; De Nooijer et al., 2014b). For example, Mg/Ca ratios in many species are orders of magnitude lower than what is expected from inorganic precipitation experiments. Additionally, Mg/Ca varies considerably between foraminiferal species and especially between species known to have different calcification strategies (Bentov and Erez, 2006; Toyofuku et al., 2011; Wit et al., 2012; De Nooijer et al., 2009; 2014b). Other elements such as Sr (e.g. Elderfield et al., 2000) and B/Ca (e.g. Allen et al., 2012) also vary significantly between species. Generally, concentrations for these elements correlate within taxa and hence species incorporating relatively much Mg, also have high (for example) Sr/Ca, B/Ca and Na/Ca. Miliolids and many ‘Large Benthic Foraminifera’ (LBF) produce calcite with Mg/Ca up to 100-150 mmol/mol (Toyofuku et al., 2000; Dueñas-Bohórquez et al., 2011; Sadekov et al., 2014; Evans et al., 2015), while most planktonic and symbiont-barren benthic foraminifera produce test calcite with Mg/Ca values ranging from 1-10 mmol/mol (e.g. Nürnberg et al., 1996; Elderfield et al., 2002; Lear et al., 2010; Wit et al., 2012; De Nooijer et al., 2014b). The same distinction is observed for B/Ca (compare e.g. Allen et al., 2012 and Kazcmarek et al., 2015), Li/Ca (Lear et al., 2010 versus Evans et al., 2015), Na/Ca (Wit et al., 2013 versus Evans et al., 2015) and Sr/Ca (e.g. Dueñas-Bohórquez et al., 2011). The correlation between relatively high (for example) Mg/Ca, Sr/Ca and B/Ca

corresponds to the observed trends in the data presented here for Ba/Ca and Mg/Ca in *H. depressa* and *A. lessonii* (Figure 4). The Mg/Ca in the former species is approximately 2.5 times that of the latter, which is similar to the difference observed in Ba/Ca ratios between these species and implies that Ba changes in concert with Mg, which is consistent with the single-chamber correlation between Mg/Ca and Ba/Ca (Figure 4). Such a change could potentially be caused inorganically by differences in Mg opening up the crystal lattice in such a way that it can accommodate more or less Ba. Such a mechanism is described for Mg and Sr (e.g. Morse and Bender, 1990; Mucci and Morse, 1983; Mewes et al., 2015; Langer et al., 2016) and may also apply to Ba incorporation and the influence of Mg ions that increase stress in the calcite crystal lattice. Unless the strain of incorporated Mg ions does not increase linearly with its concentration, the covariance between Mg and in this case Ba may well be interrelated during an earlier stage of the biomineralization process, e.g. during their transport from the surrounding seawater into the site of calcification (Erez, 2003; De Nooijer et al., 2014b).

Interestingly, the partitioning of different elements is not the same between taxa. For example, Sr/Ca in LBFs is approximately twice as high (Dueñas-Bohorquez et al., 2011; Evans et al., 2015) as in planktonic species (Elderfield et al., 2002; Dueñas-Bohórquez et al., 2009; Hendry et al., 2009), whereas the ratio between the D_{Mg} of these groups is between 10 and 100 (see above). Comparing the offset of D between groups as a function of D itself shows an approximate logarithmic correlation (Figure 5). The distinction between the two groups on basis of their element signature coincides with known differences in biomineralization controls. Element controls in low-Mg species are thought to be determined by (highly) selective trans-membrane ion transporters, (limited) leakage of seawater into the site of calcification and/or selective Mg^{2+}-removal (Nehrke et al., 2013; De Nooijer et al., 2014b; Toyofuku et al., 2017). Miliolid foraminifera belong to the high-Mg foraminiferal group and are known to secrete their calcite within vesicles that are hypothesized to contain seawater, which may be modified after

endocytosis (Hemleben et al., 1986; Ter Kuile and Erez, 1991; De Nooijer et al., 2009). These intracellular vesicles may therefore contain relatively high concentrations of Mg^{2+}, Ba^{2+} and other ions present in seawater, although so far mainly Sr/Ca and Mg/Ca of Miliolid foraminifera have been published (supplementary information). The biomineralization of non-Miliolid, intermediate- and high-Mg benthic foraminifera may employ characteristics of both these types of calcification and therefore incorporate moderately to high concentrations of elements (cf Segev and Erez, 2006).

5 Conclusions

Results from this study indicate that differences in D_{Ba} between species of foraminifera can be relatively large. This implies that species-specific Ba partition coefficients need to be applied to reconstruct past Ba/Ca_{sw} and/or salinity (Lea and Boyle, 1989; Weldeab et al., 2007; Hoffmann et al., 2014; Evans et al., 2015). Moreover, our results underscore the necessity to account for size-related effects on Ba/Ca_{cc}. This effect may bias obtained Ba/Ca_{cc} particularly when using single chamber measurements. When determining Ba/Ca_{cc} by dissolution of whole shells, the contribution of smaller chambers (with lower Ba/Ca_{cc}) is relatively small compared to a specimen's overall Ba/Ca and thus does not affect average values. Our results also show that within species as well as between species, single-chambered Mg/Ca and Ba/Ca are linearly correlated. The difference in Ba/Ca between the two species studied here fits with previously observed variability in element/Ca ratios between foraminifera taxa and likely reflects differences in their biomineralization mechanisms.

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Tables

Table 1: measured concentrations of major and minor ions, temperature, salinity and carbonate chemistry in the five culture media (A-E).

Treatment	**A**	**B**	**C**	**D**	**E**
Ba (nmol/kg)	488.5	535.5	611.0	608.4	854.6
Ca (mmol/kg)	9.1	9.5	9.6	9.2	9.3
Ba/Ca_{sw} (mmol/mol)	53.68	56.36	63.64	66.14	91.89
Na (mmol/kg)	402	416	389	383	384
B (mmol/kg)	11	11	12	11	11
K(mmol/kg)	0.40	0.46	0.43	0.43	0.42
Mg (mmol/kg)	55	58	59	53	53
Sr (mmol/kg)	0.11	0.11	0.12	0.11	0.11
Mg/Ca_{sw} (mol/mol)	6.04	6.11	6.15	5.76	5.70
T (°C)	25	25	25	25	25
Salinity	32.4	32.4	32.4	32.4	32.4
TA (µmol/kg)	2445	2450	2662	2437	2429
DIC (µmol/kg)	2244 ± 3	2246 ± 6	2464 ± 7	2236 ± 7	2228 ± 9
$\Omega_{calcite}$	3.9	3.9	4.0	3.9	3.9

Table 2. Measured Ba/Ca and Mg/Ca for A. lessonii *and* H. depressa *for each treatment.*

Treatment	**A**	**B**	**C**	**D**	**E**
A. lessonii					
n	40	43	17	36	43
Ba/Ca (µmol/mol)	15.8	19.6	18.8	22.9	29.9
SD	3.3	3.6	3.0	4.5	5.5

Mg/Ca (mmol/mol)	37.9	49.2	70.1	89.6	80.4
SD	10	13	19	33	29
H. depressa					
n	26	27	23	25	32
Ba/Ca (µmol/mol)	41.1	41.5	46.0	50.8	74.9
SD	6.2	4.3	3.9	5.7	3.9
Mg/Ca (mmol/mol)	150	135	123	168	177
SD	12	11	6	29	7

761

762 *Table 3. Parameters of the regression analysis and ANOVA tests for significance of the*
763 *regression. Both average Ba/Ca_{cc} of each experimental condition (n=5) and all chamber-*
764 *specific Ba/Ca_{cc} (n=133/ 179) were tested versus the Ba/Ca of the 5 treatments.*

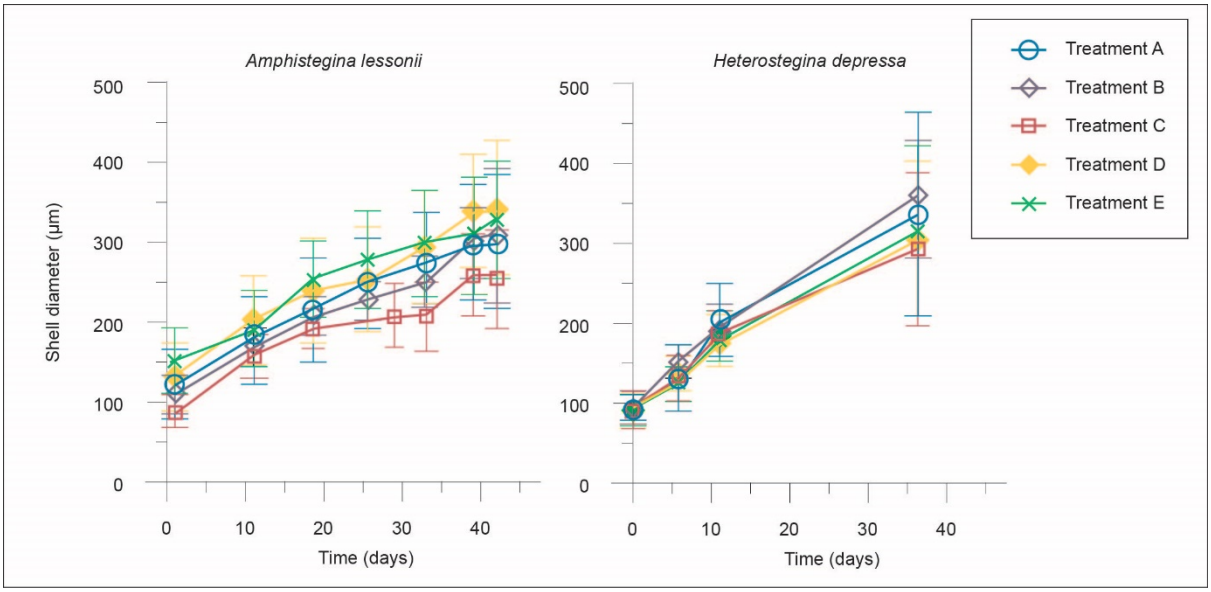
			Regression analysis	**ANOVA**	
Parameter	Species	n	R^2	F-value	p-value
Ba/Ca_{sw} vs Ba/Ca_{cc}	*H. depressa*	133	0.88	940	<0.01
	A. lessonii	179	0.56	227	<0.01
Ba/Ca_{sw} vs average Ba/Ca_{cc}	*H. depressa*	5	0.99	247	<0.01
	A. lessonii	5	0.91	32	0.011

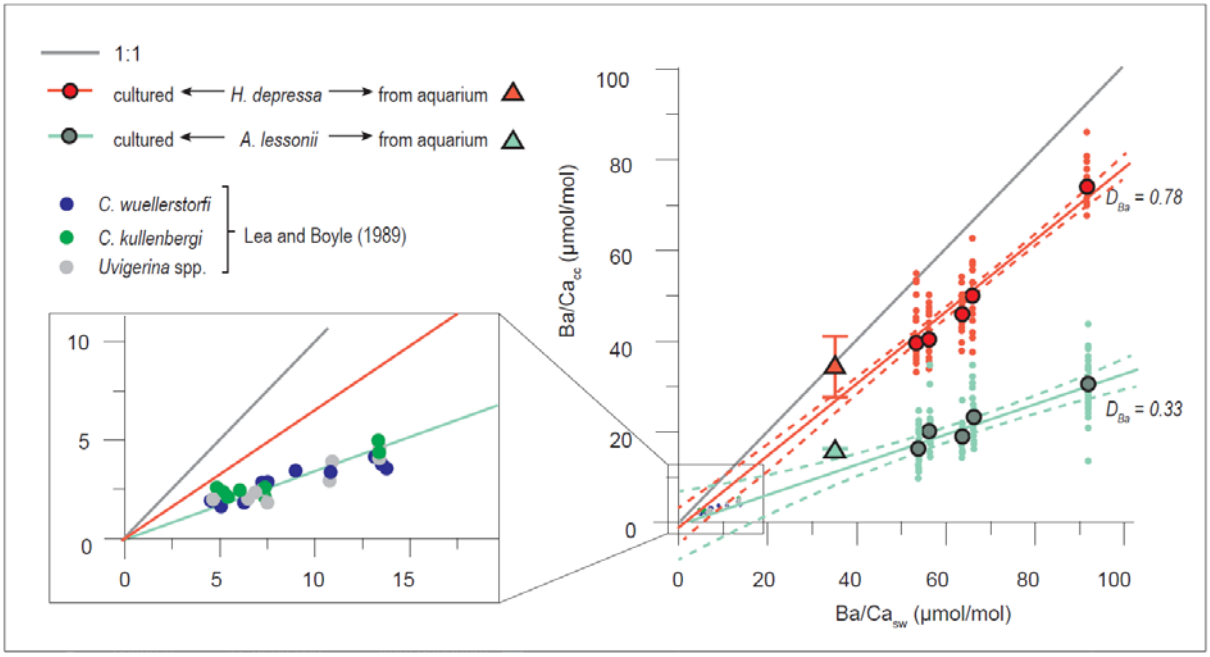
765

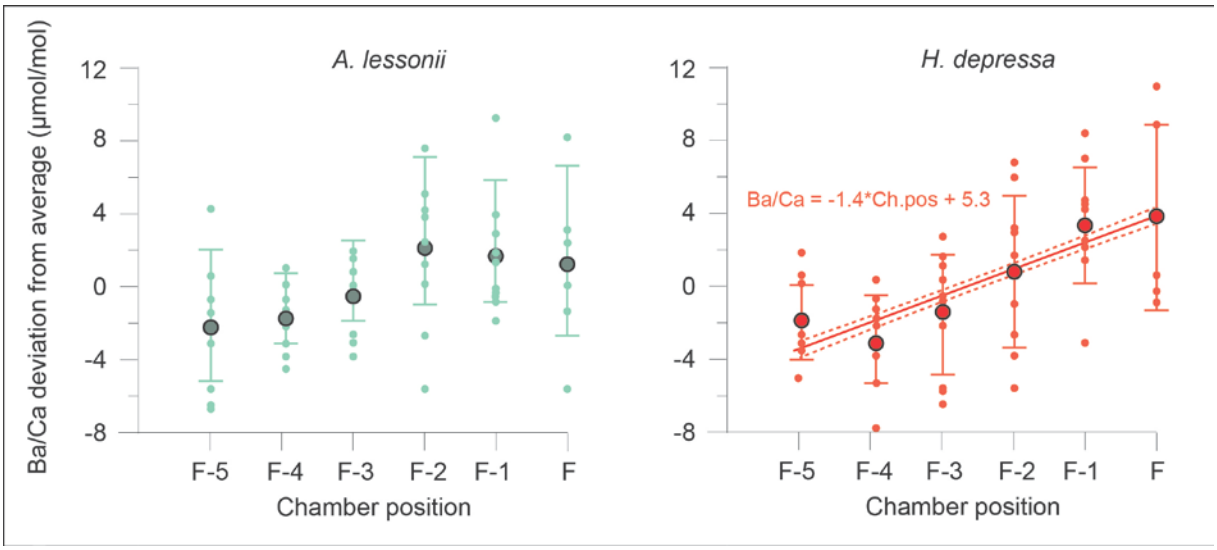
766 *Table 4. ANOVA parameters of single-chamber measurements*

ANOVA	**Species**	**F**	**p**
	A. lessonii	2.47	0.06
	A. lessonii (f-1 and f-2)	0.11	0.744
	H. depressa	6.09	< 0.01

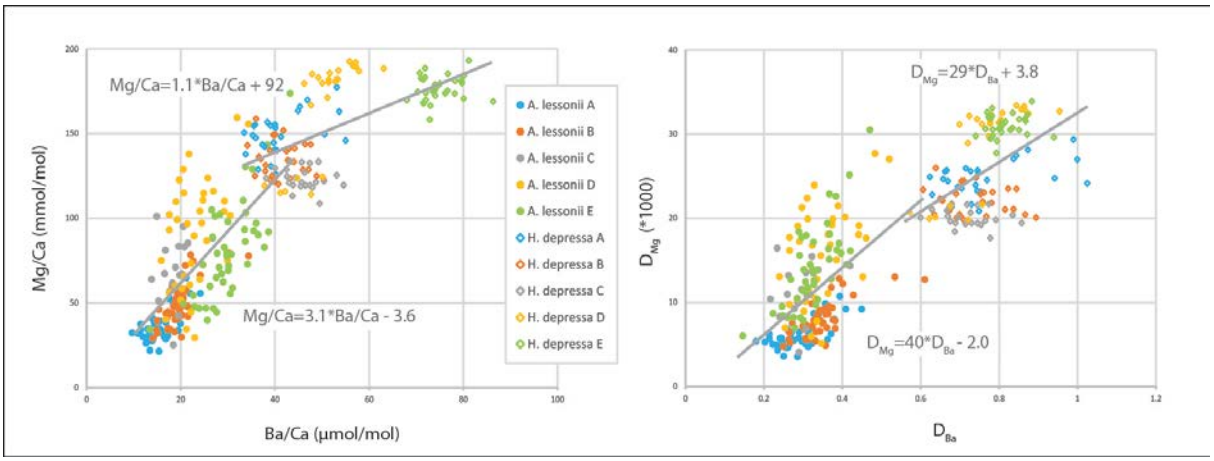
Figures


Amphistegina lessonii
Heterostegina depressa
Shell diameter (µm)
Time (days)
Time (days)
Treatment A
Treatment B
Treatment C
Treatment D
Treatment E

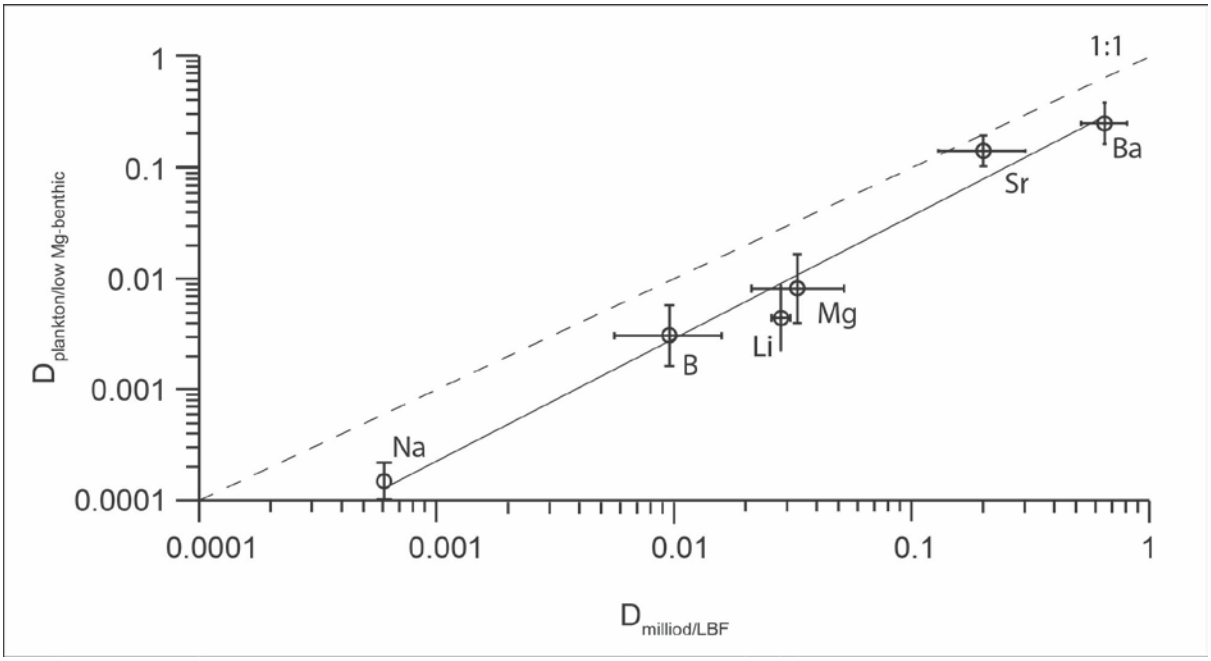

1:1
cultured ← H. depressa → from aquarium
cultured ← A. lessonii → from aquarium
C. wuellerstorfi
C. kullenbergi
Uvigerina spp.
Lea and Boyle (1989)
Ba/Ca_cc (µmol/mol)
Ba/Ca_sw (µmol/mol)
D_Ba = 0.78
D_Ba = 0.33


A. lessonii
H. depressa
Ba/Ca deviation from average (µmol/mol)
Ba/Ca = -1.4*Ch.pos + 5.3
F-5
F-4
F-3
F-2
F-1
F
Chamber position

770


Mg/Ca=1.1*Ba/Ca + 92
Mg/Ca=3.1*Ba/Ca - 3.6
Mg/Ca (mmol/mol)
Ba/Ca (µmol/mol)
A. lessonii A
A. lessonii B
A. lessonii C
A. lessonii D
A. lessonii E
H. depressa A
H. depressa B
H. depressa C
H. depressa D
H. depressa E
D_Mg=29*D_Ba + 3.8
D_Mg=40*D_Ba - 2.0
D_Mg (*1000)
D_Ba

771


1:1
Ba
Sr
Mg
Li
B
Na
D plankton/low Mg-benthic
D milliod/LBF

772

Figure Captions

Figure 1. Average increase in shell diameter for A. lessonii *(left panel) and* H. depressa *(right panel). Dots represent the average of all analysed individuals from one treatment. Error bars represent the standard deviation of the mean.*

Figure 2. Foraminiferal Ba/Ca as a function of seawater Ba/Ca. Light circles indicate individual laser ablation measurements, larger, darker shaded circles represent the average Ba/Ca_{cc} *for one treatment. Relative standard deviation varies between 16 and 20% for* Ba/Ca_{cc} *in* A. lessonii *and between 5 and 15% for* H. depressa*. Average Ba/Ca for the two species collected from the aquarium are indicated by triangles (+/- 1 SD) and were not taken into account when calculating the regression. Calculated regressions are accompanied by their 95% confidence intervals (dashed lines) over the* Ba/Ca_{sw} *range from 50 to 90 µmol/mol. Data from Lea and Boyle (1989) is plotted additionally for comparison.*

Figure 3. Average (large, darker shaded circles) and single chamber measurements (lighter circles) Ba/Ca_{cc}*, expressed as their deviation from the mean shell* Ba/Ca_{cc} *for* A. lessonii *(left) and* H. depressa*. Error bars represent the standard deviation of the mean, the dashed lines in the right panel indicate the 95% confidence intervals for the linear regression.*

Figure 4. Relation between the Ba/Ca and Mg/Ca (left panel) and the partition coefficients for Ba and Mg (right panel). Every dot represents one single-chamber measurement. The data for A. lessonii *are indicated by circles, those for* H. depressa *are represented by open diamonds. Every treatment (A-E; Table 1) is indicated by a separate color.*

Figure 5: Partition coefficients for Li, B, Na, Mg, Sr and Ba for two groups of foraminifera (Large Benthic Foraminifera+Miliolids and the low-Mg species). Data on which the average partition coefficients are based, are listed in the online supplement, the ranges indicate the maximum range in published partition coefficients. The linear regression between the partition coefficients for these two groups is described by: $D_{plankton/low\ Mg\text{-}benthic} = 0.3992 * D_{miliolid/LBF} + 0.0081$. *Elemental results for Milliolid species are confined to Mg/Ca and Sr/Ca. Li/Ca ratios were taken from Delaney et al. (1985), Hall and Chan (2004a), Marriott et al. (2004), Yu et al. (2005), Ni et al. (2007), Bryan and Marchitto (2008), Hathorne et al. (2009), Dawber and Tripati (2012) and Evans et al. (2015); B/Ca ratios are from Yu et al. (2005), Yu and Elderfield (2007), Foster (2008), Hendry et al. (2009), Allen et al. (2011; 2012), Dawber and Tripati (2012), Babila et al. (2014) and Kaczmarek et al. (2015); Na/Ca are from Delaney et al. (1985), Ni et al. (2007), Bian et al. (2009), Wit et al. (2013) and Evans et al. (2015); Mg/Ca are from Toyofuku et al. (2000), Raja et al. (2005), Yu et al. (2005), Elderfield et al. (2006), Segev and Erez (2006) , Hendry et al. (2009), Dueñas-Bohórquez et al. (2009; 2011), Dawber and Tripati (2012), Wit et al. (2012; 2013), Babila et al. (2014), De Nooijer et al. (2014a), Sadekov et al. (2014) and Evans et al. (2015). Foraminiferal Sr/Ca are taken from Raja et al. (2005), Yu et al. (2005), Hendry et al. (2009), Dueñas-Bohórquez et al. (2009; 2011), Dawber and Tripati (2012), Wit et al. (2013), De Nooijer et al. (2014a) and Evans et al. (2015). Ba/Ca are from this study, Lea and Boyle (1989), Lea and Boyle (1991), Lea and Spero (1994), Hall and Chan (2004b), Ni et al. (2007), Hönisch et al. (2011) and Evans et al. (2015).*