Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

Biogeosciences Discussions
Open Access
EGU

Contrasting trends in element incorporation in hyaline and miliolid foraminifera

Inge van Dijk[1], Lennart J. de Nooijer[2], Gert-Jan Reichart[1,2]

[1]Department of Ocean Systems, NIOZ-Royal Netherlands Institute for Sea Research, Postbus 59, 1790 AB, Den Burg, the Netherlands, and Utrecht University.
[2]Faculty of Geosciences, Earth Sciences Department, Utrecht University, Budapestlaan 4, 3584 CD, Utrecht, the Netherlands

Abstract

We analyzed trends in element incorporation between hyaline (perforate) and miliolid (imperforate) foraminifera in order to investigate processes involved in calcification affecting element incorporation into foraminiferal carbonate. For both groups, we observed similar trends in element incorporation with pCO_2, suggesting there some mechanisms to transports ions to the site of calcification are similar for both calcification pathways, although the impact might be different across species. A previously published trans-membrane transport model assumes foraminifera utilize Ca^{2+} channels to transport calcium to the site of calcification. These channels are somewhat a-specific, leading to (accidental) transport of other free ions. By modelling the activity of free ions as a function of pCO_2, we observed that speciation of some elements (like Zn and Ba) are heavily influenced by the formation of carbonate complexes. This leads to an increase in availability of free Zn and Ba with increasing pCO_2, which leads to more transport to the site of calcification and subsequently incorporation in the foraminiferal shell. We further observed that incorporation of the trace elements studied here is positively correlated between the hyaline test building species. This could be due to dissimilar activity and/or selectivity of calcium channels between species, perhaps due to differences in size. For miliolid calcification, part of the calcium is obtained not only through channels but by also included seawater vesicles, which leads to similar element to calcium ratios between species and element partitioning which is more in line with inorganic carbonates.

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

1. Introduction

On the broadest taxonomic scale, calcareous foraminifera, cosmopolitan unicellular protists, produce tests using either one of two fundamentally different mechanisms. These calcification strategies reflect the evolutionary separation of foraminiferal groups dating back to the Cambrian diversification, from where the imperforate miliolids and perforate hyaline foraminifera, developed independently (Pawlowski et al., 2003). The calcification process of the latter group has been studied more extensively than that of the miliolids (De Nooijer et al., 2014). Although many aspects of perforate calcification remain unsolved, there is consensus that chamber formation takes place extracellularly, but within a (semi-) enclosed space, generally termed the site of calcification (SOC). The first layers of calcite precipitate on an organic matrix (the POS or primary organic sheet) that serves as a template for the calcite layer that forms the chamber wall (Hemleben et al., 1977; Erez, 2003). To promote calcification, foraminifera furthermore need to remove Mg ions and/or protons (Zeebe and Sanyal, 2002) from the seawater entering the SOC. Many larger benthic foraminifera are hyaline species although the amount of Mg in their shells is often more than 10 times higher than that of planktonic and small benthic hyaline species, hence covering a large range in Mg/Ca values.

The calcification strategy of porcelaneous foraminifera is less well studied, which may be partly explained by their limited application in paleoceanography. Porcelaneous foraminifera use a different mode of calcification (Berthold, 1976; Hemleben et al., 1986; Debenay et al., 1998; De Nooijer et al., 2009) and produce shells without pores (hence, the term imperforate) consisting of tablets or needles (Debenay et al., 1998; Erez, 2003; Bentov and Erez, 2006). These calcitic needles (2-3µm) are precipitated intracellularly (Berthold, 1976), after which they are transported out of the foraminifer to form a new chamber (Angell, 1980). At the outer and inner layers of these chambers, the needles are arranged along the same orientation so that they form an optically homogenous surface, giving it a shiny (hence the term ‘porcelaneous’) appearance. In general the Mg/Ca values of the shells of porcelaneous foraminifera are high.

Remarkably, despite this large biological control, incorporation of minor and trace elements still reflects environmental conditions, in both hyaline and porcelaneous foraminiferal shells. For instance, the Mg/Ca of foraminiferal shells is primarily determined by seawater temperature (Allen and Sanders, 1994; Nürnberg et al., 1996) and seawater Mg/Ca (Chapter 3; Segev and Erez, 2006; Evans et al., 2015; Wit et al., in review). After correcting for the effect of the latter (if necessary) the use of foraminiferal Mg/Ca has been validated by its wide application as paleothermometer (Elderfield and Ganssen, 2000; Lear et al., 2000). Insight in vital effects (Erez, 2003) and inter-specific differences in trace element incorporation (Bentov and Erez, 2006; Toyofuku et al., 2011; Wit et al., 2012) is needed for making the Mg/Ca thermometer more robust. Systematic offsets between different species, interdependence of trace elements incorporated (Langer et al., in press.) and the different response of element incorporation on

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

element speciation (Chapter 6; Keul et al., 2013; Wit et al., 2013), potentially provides useful clues for determining which processes play an important role in the biomineralization pathways.

Here we present the results from a controlled growth experiment for which we used several (intermediate- and high-Mg) hyaline and miliolid species and an inter-species comparison of trace elements. We assessed the impact of bio-calcification on element incorporation as a function of pCO_2 in order to contrast the impact of different calcification strategies. During foraminiferal calcification, incorporation of certain elements or fractionation of certain isotopes is shown to depend on the carbonate system, e.g. $U/Ca_{CALCITE}$ (Russell et al., 2004; Keul et al., 2013) and $Zn/Ca_{CALCITE}$ to $[CO_3^{2-}]$ (Marchitto et al., 2000; Chapter 6) and $\delta^{11}B$ to pH (Sanyal et al., 1996). Species-specific differences in partitioning and fractionation most likely primarily reflect differences in calcification strategy. Offsets are largest between hyaline and miliolid species, due to their fundamentally different calcification strategies (see for a summary, Toyofuku et al., 2011). Differences in chemical composition and their dependency on environmental variables can hence be used to identify key processes in miliolid and hyaline calcification. We cultured eight benthic foraminiferal species (4 hyaline and 4 porcelaneous) under four different pCO_2 conditions, analyzing incorporation of Mg, Sr, Na, Zn and Ba. Results are combined and compared with literature data, to identify processes involved in calcification.

2. Methods

2.1 Foraminiferal collection

Large samples of macroalgae (*Dictyota* sp.) were collected in November 2015 at a depth of 2-3 meters in Gallows Bay, St. Eustatius (N 17°28'31.6", W62°59'9.4"). Salinity was ~34 and temperature was ~29°C at the site of collection. The collected macroalgae were transported to the laboratory at the Caribbean Netherlands Science Institute (CNSI), where they were placed in a 5 L aquarium with aerated and unfiltered seawater. From this stock, small amounts of algae and debris were gently sieved over a 90 and 600 µm mesh to carefully dislodge foraminifera. Several species of foraminifera were picked from the resulting 90-600 µm fraction. Living specimens of *Marginopora vertebralis* (Quoy & Gaimard, 1830), *Amphistegina gibbosa* (d'Orbigny, 1839), *Laevipeneroplis bradyi* (Cushman, 1930) and *Archaias angulatus* and limited amounts (<20) of *Peneroplis pertusus* (Forskål, 1775), *Asterigerina carinata* (d'Orbigny, 1839), *Heterostegina antillarum* (d' Orbigny, 1839), and *Planorbulina acervalis* (Brady, 1884) characterized by yellow cytoplasm and pseudopodial activity, were selected for the culturing experiments.

2.2 Culture set-up

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

We used an adapted version of the culture set-up descripted in Chapter 7. In short, four barrels each containing 100 L of seawater (5µm filtered), were connected to a Li-Cor CO_2/H_2O analyzer (LI-7000), to regulate the CO_2 level in the barrels' head space. The set levels were maintained by addition of CO_2 and/ or CO_2-scrubbed air according to the monitored pCO_2. The set-points for pCO_2 were 350 (A), 450 (B), 760 (C) and 1400 (D) resulting in four batches of seawater differing only in their inorganic carbon chemistry. Salinity (34.0±0.2) was monitored with a salinometer (VWR CO310). The fluorescent compound calcein (Bis[N,N-bis(carboxymethyl)aminomethyl]-fluorescein) was added to the culture media (5 mg/L seawater) to enable determination of newly formed chambers during the culture experiment (Bernhard et al., 2004). Short-term exposure (<three weeks) to calcein has no detectable impact on the physiology of benthic foraminifera (Kurtarkar et al., 2015), and the presence of calcein has no effect on the incorporation of Mg and Sr in foraminiferal calcite (Dissard et al., 2009). Culture media was stored air-free in portions of 250 ml in Nalgene bottles with teflon lined caps at 4°C until further use.

Foraminifera were divided over the different treatments in duplicate and placed in 70 ml Falcon ® tissue bottles with gas-tight caps in a thermostat set at 25°C (Fig. 1). The thermostat was monitored by a temperature logger (Traceable Logger Trac, Maxi Thermal), monitoring the temperature every minute. To create uniform light conditions, the thermostat was equipped with two LED shelfs, which resulted in high light conditions 12 hr/12hr. Culture media was replaced every four days, to avoid build-up of organic waste and to obtain stable seawater element concentrations and carbon chemistry. Foraminifera were fed after every water change with 0.5 ml of concentrated freeze-dried *Dunaliella salina* cells, pre-diluted with the corresponding treatment seawater. After 21 days, the experiment was terminated. Foraminifera were rinsed three times with de-ionized water, dried at 40°C and stored in micropaleontology slides until further analysis at the Royal Netherlands Institute for Sea Research (NIOZ).

2.3 Analytical methods

2.3.1 Seawater carbon parameters

At the start and termination of the experiment, 125 ml samples of the seawater at each of the different experimental conditions were collected to analyze dissolved inorganic carbon (DIC) and total alkalinity (TA) on a Versatile INstrument for the Determination of Titration Alkalinity (VINDTA) at the CNSI. Using the measured DIC and TA values and the software CO2SYS v2.1, adapted to Excel by Pierrot et al. (2006) the other carbon parameters (including $[CO_3^{2-}]$ and $\Omega_{calcite}$) were calculated. For this we used the equilibrium constants for K1 and K2 of Mehrbach et al. (1973), refitted by Dickson and Millero (1987) (Table 1).

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

128

129 **2.3.2 Seawater element concentrations**

130 At the start and end of the experiment and during replacement of the culture media, subsamples were
131 collected in duplo using 50 ml LDPE Nalgene bottles and immediately frozen at -80^{0}C. After
132 transportation to the NIOZ, melted samples were acidified with 3 times Quartz distilled HCl to pH ~1.8
133 and the seawater composition of the samples was analyzed on an Element 2 sector field double focusing
134 mass spectrometer (SF-ICP-MS) run in medium resolution mode. IAPSO Standard Seawater was used
135 as a drift monitor. Analytical precision (relative standard deviation) was 3% for Ca, 4% for Mg, 1% Na,
136 1% for Sr and 5% Ba. We obtained average values of 5.25±0.06 mol/mol for Mg/Ca, 44.6±0.6 mol/mol
137 Na/Ca, 8.63±0.05 mol/mol for Sr/Ca, and 9.04±0.47 for µmol/mol Ba/Ca.

138 A subsample was analyzed using a commercially available pre-concentration system, SeaFAST S2.
139 With the SeaFAST system elements with low concentrations can be pre-concentrated to values above
140 detection limit of the SF-ICP-MS. Accordingly, we measured Cd, Pb U, B, Ti, Mn, Fe, Co, Ni, Cu, and
141 Zn. In short, 10ml of sample was mixed with an ammonium acetate buffer to pH 6.2 and loaded on a
142 column containing NOBIAS chelating agent. After rinsing the column with a diluted ammonium acetate
143 buffer the metals were eluted in 750 µl of quartz distilled 1.5 M HNO_3 before being quantified on the
144 SF-ICP-MS. Here we use the Zn data only, as this was analyzed in the foraminifera well. Analytical
145 precision (relative standard deviation) was 5% for Zn. We obtained average values 15.3±0.5 µmol/mol
146 for Zn/Ca for all treatments. Although these values are clearly above natural open ocean values, the
147 concentrations are very uniform between treatments and when comparing start and end of the
148 experiments. The contamination with Zn might hence have occurred already when filling the culture
149 setup with the waters from the bay adjacent to the culture facility. In any concentrations are well below
150 values considered toxic (Nardelli et al., 2016).

151

152 **2.3.3 Cleaning methods**

153 After termination of the experiment, foraminiferal shells were cleaned following an adapted version of
154 Barker et al. (2003). Per treatment duplicate, all foraminifera were transferred to 10 ml PE vials. To each
155 vial, 10 mL 1% H_2O_2 solution (buffered with 0.5M NH_4OH) was added to remove organic matter. The
156 vials were heated for 10 minutes in a water bath at 95 °C, and placed in an ultrasonic bath for 30 seconds
157 (degas mode, 80kHz, 50% power), after which the oxidizing reagent was removed. These steps (organic
158 removal procedure) were repeated five times. Foraminiferal samples were rinsed five times with
159 ultrapure water, after which the vials were stored overnight in a laminar flow cabinet at room
160 temperature to dry. Dried foraminifera were placed on double sided tape on LA-ICP-MS stubs. Pictures
161 were taken of individual foraminifera with a ZEISS Axioplan 2 fluoresence microscope equipped with

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

appropriate excitation and emission optics and a ZEISS Axiocam MRc 5 camera, to assess the number of chambers added during the experiment based on the incorporation of calcein.

2.3.5. LA-ICP-MS

Element concentrations of individual fluorescent chambers were analyzed by Laser Ablation-ICP-MS (Reichart et al., 2003; Van Dijk et al., in review). To determine foraminiferal element concentrations, the laser system (NWR193UC, New Wave Research) at the Royal NIOZ was equipped with a 2-volume cell 2 (New Wave Research), characterized by a wash-out time of 1.8 seconds (1% level) and hence allowing detection of variability of obtained element to Ca ratios within chamber walls. Single chambers were ablated in a helium environment using a circular laser spot with a diameter of 80 µm (*M. vertebralis*) or 60 µm (other species). We ablated all calcein-stained chambers twice, except for the first 1-2 chambers that formed during the experiment to avoid contamination of calcite of chambers formed prior to the experiments that may be overlapped by the first labelled chambers (Fig.2).

All foraminiferal samples were ablated with an energy density of 1±0.1 J/cm^{-2} and a repetition rate of 6 Hz. The resulting aerosol was transported on a helium flow through an in house build smoothing device, being mixed with a nitrogen flow (2 L/min), before entering the quadrupole ICP-MS (iCAP-Q, Thermo Scientific). Monitored masses included ^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{27}Al, ^{43}Ca, ^{44}Ca, ^{66}Zn, ^{88}Sr and ^{137}Ba. Contrary to ^{67}Zn and ^{68}Zn, ^{66}Zn is free of interferences when measuring calcium carbonate and SRM NIST glass standards (Jochum et al., 2012). Potential contamination or diagenesis of the outer or inner layer of calcite was excluded by monitoring the Al signal. At the start of each series, we analyzed SRM NIST612 and NIST610 glass standard in triplicate (using an energy density of 5±0.1 J/cm-2), JCt-1 (coral carbonate) and two in-house standards, namely NFHS (NIOZ Foraminifera House Standard; Mezger et al., in review) and the Iceland spar NCHS (NIOZ Calcite House Standard). We further analyzed JCp-1 (Giant clam) and MACS-3 (Synthetic Calcium Carbonate) at the start of each series, and to monitor drift after every ten samples. All element to calcium ratios were calculated with an adapted version of the MATLAB based program SILLS (Guillong et al., 2008). SILLS was modified to evaluate LA-ICP-MS measurements on foraminifera, allowing import of Thermo Qtegra software sample list, laser data reduction and laser LOG files. Major adaptions include improved automated integration and evaluation of (calibration and monitor) standards, quality control report of the monitor standards and export in element to calcium ratios (mol/mol). Calibration was performed against the MACS-3 carbonate standard, with ^{43}Ca as an internal standard and we used the multiple measurements of MACS-3 for a linear drift correction. Relative analytical precision (relative standard deviation (RSD) of all MACS-3 analyses) is 3% for ^{23}Na, 3% for ^{24}Mg, 3% for ^{25}Mg, 4% for ^{66}Zn, 3% for ^{88}Sr and 3% for ^{137}Ba. In total, 961 analyses were performed on 251 specimens covering eight species cultured in four experimental conditions (see Table 2 for specifics).

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

197

198 We calculated the standard deviation (STD), RSD and standard error (STD/√n; SE) per treatment. The
199 partitioning coefficient (D) of an element (E) between seawater and foraminiferal calcite is expressed
200 as $D_E = (E/Ca_{CALCITE})/(E/Ca_{SW})$. Partition coefficients, element versus calcium ratio and growth
201 parameters were statistically compared with different experimental parameters (such as pCO_2 or $[CO_3^{2-}$
202]) using a two-sided T-test with 95% confidence levels. This also allows for the calculation of 95%
203 confidence intervals over the average per treatment. Pairwise comparisons were made for per E/Ca per
204 species and culture conditions using ANOVA. Groups that showed significant difference were assigned
205 different letters. When comparing partition coefficients to other studies, E/Ca_{SW} data was, in some
206 studies, not measured. In these cases, we used average seawater E/Ca_{SW} to calculate D_E (see also
207 supplementary Table 1).

208

209 **3. Results**

210 **3.1 Inter-species differences in element incorporation**

211 In Table 3 we present all the elemental data for the eight species investigated in this controlled pCO_2
212 culture experiment. $Mg/Ca_{CALCITE}$ of Mg in hyaline species varies between 25.9-141.3 mmol/mol
213 Mg/Ca. In contrast, $Mg/Ca_{CALCITE}$ of miliolid species ranges from 121.3-149.3 mmol/mol. This large
214 spread in foraminifera E/Ca of hyaline species is also observed for Sr (1.7-3.1 mmol/mol), Na (3.4-19.5
215 mmol/mol), Zn (9.0-97.0 µmol/mol) and Ba (2.7-20.1 µmol/mol), while miliolids only vary over a
216 narrow range (Sr = 2.0-2.2 mmol/mol; Na = 3.8-5.8 mmol/mol; Zn = 53.0-140.8 µmol/mol; Ba = 18.0-
217 29.0 µmol/mol). When comparing Mg incorporation to that of the other elements studied here (Ba, Zn,
218 Sr and Na) between species (treatment B; Table 3), we observe a positive relation between D_{Sr}
219 ($p<0.0025$), D_{Na} ($p<0.0005$), D_{Ba} ($p<0.05$) and D_{Zn} ($p<0.005$) for hyaline species (Table 4). In general
220 hyaline species are enriched similarly in all elements (Fig. 3). Compared to porcelaneous species, the
221 hyaline shell building species which incorporate the most Mg (>100 mmol/mol Mg/Ca) incorporate
222 more Na, and Sr, while incorporating less Zn and Ba. Element incorporation across miliolid species is
223 less variable then observed for hyaline species and in general partition coefficients for these species
224 seem closer to inorganic values (Fig. 3). Including data from literature (both culture and field
225 calibrations; see supplementary Table S1), preferable in which both Mg/Ca and at least one other
226 element (Na, Sr, Ba or Zn) is measured, shows that the relation based on the Caribbean species studied
227 here is also more general applicable when including more species ($D_{Sr} = p<0.005$; $D_{Na} = p<0.0005$); D_{Ba}
228 $= p<0.005$; $D_{Zn} = p<0.01$), even though this compiled data (labeled 'All studies' in Table 4) covers a
229 wide range in environmental and experiment conditions.

230

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

3.2 Element/Ca as a function of ocean acidification

In both porcelaneous and hyaline species we find an increase of $Zn/Ca_{CALCITE}$ and $Ba/Ca_{CALCITE}$ with pCO_2, while foraminiferal Sr/Ca, Mg/Ca and Na/Ca remain similar across the experimental conditions (Fig. 4 and Table 5). Sensitivity of both foraminiferal Zn/Ca and Ba/Ca to changes in seawater pCO_2 differs between the studied porcelaneous and hyaline species. When pCO_2 changes from 350 to 1200 ppm, Zn/Ca of hyaline foraminifera increase by a factor of 3.7 (*A. carinata*) or 4.5 (*A. gibbosa*) while miliolid foraminiferal Zn/Ca increases only by 1.3 (*M. vertebralis*), 1.8 (*A. angulatus*) and 2.1 (*L. bradyi*). Also sensitivity of foraminiferal Ba/Ca to the same change in pCO_2 shows a similar pattern, with Ba/Ca of hyaline species increasing by a factor of 3.6 (*A. carinata*) or 3.7 (*A. gibbosa*), while miliolid species increase Ba/Ca only with a factor of 1.8 (*M. vertebralis*), 1.6 (*A. angulatus*) or 2.1 (*L. bradyi*).

4. Discussion

4.1 Trends in element incorporation

Both miliolid and hyaline foraminifera promote calcification by increasing their internal pH (De Nooijer et al., 2009). Still, they might use different mechanisms to take up the ions (Ca^{2+} and CO_3^{2-}) necessary for chamber formation, which is reflected in the different trends observed here. Element incorporation in hyaline foraminifera is highly interdependent, i.e. species with increased Mg content also incorporate more Sr, Na, Ba and Zn (Fig. 3). This observation suggests that uptake of all these elements is controlled by the same process, which may be the transmembrane transport of calcium ions to the site of calcification. Such transport likely involves Ca^{2+} channels (Nehrke et al., 2013), capable of transferring other ions, like e.g. Mg, Sr and Na (Hess and Tsien, 1984; Allen and Sanders, 1994; Sather, 2005). This may result in an interdependence between all these elements studied such as observed here for the hyaline species if the selectivity for Ca^{2+} of these channels vary between species. In contrast, miliolid species, building porcelaneous shells show much less inter-species variation in element incorporation and ratios between incorporated elements is thus relatively similar between species (Fig. 3). This may be explained by calcification from an internal reservoir, such as intracellular vacuoles containing (modified) seawater (Hemleben et al., 1986; Erez, 2003). The fact that the Mg partitioning in this foraminiferal group is similar to the inorganic partition coefficient may indicate that the carbonate is directly precipitated from seawater, without major removal of Mg^{2+} ions. The relative similarity in partition coefficients of other elements between miliolid species are generally in line with an inorganic-like calcite precipitation, with only minor alteration of the elemental composition of the calcifying fluid by ion channels.

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

265 **4.2 Effect of ocean acidification on Element/Ca**

266 For neither miliolid nor hyaline species, foraminiferal Mg/Ca, Na/Ca and Sr/Ca systematically change
267 with pCO_2. The impact of pH (and/or $[CO_3^{2-}]$) on $Mg/Ca_{CALCITE}$ and $Sr/Ca_{CALCITE}$ in foraminifera has
268 been the subject of discussion (e.g., Elderfield et al., 1996; Dissard et al., 2010). In low-Mg benthic
269 species, both $Mg/Ca_{CALCITE}$ and $Sr/Ca_{CALCITE}$ do not seem to depend on inorganic carbon system
270 parameters, e.g. pH or $[CO_3^{2-}]$ (Allison et al., 2011; Dueñas-Bohórquez et al., 2011). However, for
271 several planktonic species pH does influence $Mg/Ca_{CALCITE}$ and $Sr/Ca_{CALCITE}$ (Lea et al., 1999; Russell
272 et al., 2004; Evans et al., 2016). The effect of pH on $Sr/Ca_{CALCITE}$ might be explained via increased
273 growth rates due to pH-associated changes in $[CO_3^{2-}]$ (Dissard et al., 2010). However, due to the limited
274 experimental set-up, we are not able to disentangle the effects of the different carbon parameters in this
275 study. Still, here we show that incorporation of Mg, Sr and Na of the selected larger benthic hyaline and
276 miliolid foraminifera are not significantly impacted when cultured over a range of pCO_2 and thus $[CO_3^{2-}]$
277 and pH values. Observed offsets in studies using acid titration (Lea et al., 1999; Russell et al., 2004;
278 Dueñas-Bohórquez et al., 2011; Evans et al., 2016) to alter the carbonate system might be related to
279 changes in alkalinity rather than pCO_2 or DIC. In the experimental setup here alkalinity was kept
280 constant between the different treatments, but pH, DIC and carbonate ion concentration varied as a
281 function of pCO_2.

282 In contrast, foraminiferal Zn/Ca and Ba/Ca are significantly impacted by pCO_2 for all species studied
283 here (Table 5; Fig. 4). Although Hönisch et al. (2011) suggested that the impact of carbonate chemistry
284 on Ba incorporation is negligible, their data does suggest a trend over the same interval in pH as studied
285 here. In hyaline foraminifera, Zn/Ca and Ba/Ca increases more as a function of pCO_2 (factor of 3.7-4.5
286 and 3.6-3.7, respectively when pCO_2 increases from 350 to 1200 ppm) compared to the miliolid species
287 (1.3-2.1 and 1.6-2.1 times, respectively). In the culture set-up used, increasing pCO_2 increases DIC,
288 reduces pH and thereby decreases seawater $[CO_3^{2-}]$. Speciation of Zn, Ba and also other elements, like
289 U (Keul et al., 2013), is primarily controlled by seawater $[CO_3^{2-}]$. Using the PHREEQC (Parkhurst and
290 Appelo, 1999) and the standard llnl database, the speciation of all elements studied here (Mg, Na, Sr,
291 Zn and Ba) for our different seawater treatments were modelled. We observed a decrease of free ions
292 (Zn^{2+} and Ba^{2+}) and an increase in Ba and Zn carbonate complexes ($BaCO_3^0$ and $ZnCO_3^0$), with
293 increasing pCO_2 (Fig. 5), while the activity of Mg^{2+}, Na^+ and Sr^{2+} remained unaffected. This suggests
294 that element incorporation in foraminiferal calcite might be depending on the bioavailability of free ions,
295 which in the case of Ba and Zn, changes with pCO_2.

296

297 **4.3 Speciation in the foraminiferal microenvironment**

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

298 During inorganic precipitation, carbonate complexes (e.g. $MgCO_3^0$) are easily incorporated into the
299 calcite crystal lattice. However, foraminifera build their test from ions available at the site of
300 calcification, which is well separated from the surrounding seawater (De Nooijer et al., 2009). During
301 calcification, Ca^{2+} is proposed to be transported from seawater to the SOC via ion channels (Nehrke et
302 al., 2013). This so-called trans-membrane transport (TMT) through Ca^{2+} channels has also been found
303 for other marine organisms, including coccolithophores (Gussone et al., 2006). These Ca^{2+} channels may
304 not discriminate perfectly between Ca ions and elements like Sr and Ba (Allen and Sanders, 1994),
305 causing accidental transport of these elements into the SOC. How much of a certain element will enter
306 the SOC in this way, depends on 1) the selectiveness of the channels and the characteristics of the
307 transported ions, 2) the element to calcium ratio in the foraminiferal microenvironment and 3) the
308 concentration gradient between seawater and the SOC. For instance, ions such as Mg^{2+} are heavily
309 fractionated against during TMT, which is reflected by the low D_{Mg} found in most species. The large
310 range in Mg/Ca values in hyaline species suggests that TMT plays an important, but also variable, role
311 in calcification of these species. The availability of some free ions, like Ba and Zn, changes as a function
312 of pCO_2 due to the formation of carbonate complexes (Fig. 5). When Zn and Ba form stable complexes
313 with carbonate ions they are no longer available for (sporadic) transport through the Ca^{2+} channels,
314 decreasing the availability at the site of calcification and subsequently, incorporation into the
315 foraminiferal calcite (Fig. 6).

316 In summary, the amount of Zn and Ba available at the site of calcification is proportional to the
317 concentration of the ratio between Ca^{2+} and free Zn^{2+} and Ba^{2+} in the foraminiferal microenvironment.
318 In turn, the amount of free Zn and Ba ions in seawater is controlled by their respective concentration in
319 seawater concentration, as well as $[CO_3^{2-}]$. Foraminiferal Mg/Ca, Na/Ca and Sr/Ca is not detectably
320 affected by $[CO_3^{2-}]$, since these elements do not form carbonate complexes over the range of $[CO_3^{2-}]$
321 studied here.

322

323 **4.4 Element incorporation in hyaline species**

324 Between hyaline species, we observe simultaneous increases in all elements incorporated and this trend
325 is confirmed when including published data for other species compiled from previous studies (Fig. 4
326 and supplementary Table S1). Interestingly, the two hyaline species that are most enriched in all
327 elements studied (Mg, Na, Sr, Zn and Ba) are also the foraminiferal species with the largest average
328 adult test size (*H. antillarum* and *P. acervalis*) for which data is available (this study). The other hyaline
329 species, *A. carinata* and *A. gibbosa*, have considerably smaller maximum shell sizes and lower Mg/Ca,
330 Sr/Ca, etc. values.

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

Two processes involving these calcium channels could possibly explain the observed size trend in hyaline species. First, larger foraminifera have a smaller surface area to volume ratio and, therefore, proportionally less Ca^{2+} channels, assuming the density of these channels per surface area remains similar. This would imply that fewer channels need to transport more ions for a given volume of $CaCO_3$ precipitated, which may in turn, possibly reduce selectivity between Ca^{2+} and other divalent cations. Secondly, a larger foraminifer will need more overall Ca^{2+} compared to smaller species for the production of a single new chamber, since the volume of the chamber walls increases with the size of the individual. This increased uptake of Ca^{2+} from the microenvironment around the foraminifer, may cause a lower concentration of Ca^{2+} in the direct surroundings of the foraminifer compared to the other ions, which may subsequently translate into an increased transport of ions other than Ca^{2+} to the site of calcification.

A consequence of these hypotheses is that juvenile or smaller adults should have lower partition coefficients than fully grown adults. Although some studies have shown a size effect for several elements (e.g. Elderfield et al., 2002), other studies show no major effect of size on element partitioning (e.g. Friedrich et al., 2012; Evans and Müller, 2013). The moderate trend observed within species, in comparison to the large differences observed here between species, may indicate that species control channel density per surface area as a function of average shell size of the species. Alternatively, the maximum size of a species may be accompanied by a difference in their calcification mechanism (e.g. the relative contribution of TMT in element uptake) explaining inter-species differences in element partitioning. From an evolutionary point of view the latter explanation seems more likely.

4.5 Mechanisms for element uptake in miliolid foraminifera

In contrast to hyaline species, the miliolid species build porcelaneous shells that show much less inter-species variation in element composition (Fig. 3). While hyaline species calcify in a (semi-)enclosed space, miliolids precipitate their calcite intracellularly in vesicles in which they promote calcification by increasing pH (De Nooijer et al., 2009). This suggests that these species calcify directly from seawater (Ter Kuile and Erez, 1987). The fact that the Mg partitioning is close to the inorganic partition coefficient in this foraminiferal group (Fig. 3) reflects that the carbonate is directly precipitated from intracellular seawater, without major alteration of the original $[Mg^{2+}]$. The relative similarity in partition coefficients between different porcelaneous shell building species is in line with primarily inorganic precipitation, with only minor alteration of the elemental composition of the calcifying fluid by ion channels.

However, the observed correlation between pCO_2 and Ba and Zn (Fig. 4) suggests that Ca channels still play a (modest) role in supplying Ca^{2+} to the miliolid SOC. The contribution of Ca^{2+} through TMT is likely smaller than in hyaline species, since they already obtain calcium by including seawater in their

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

365 calcification vesicle prior to calcite precipitation. The considerably smaller flux of transmembrane Ca^{2+}
366 compared to perforate species explains the observed lower sensitivity of e.g. foraminiferal Zn/Ca and
367 Ba/Ca to changes in seawater [CO_3^{2-}] in miliolid species (Fig. 4). This approximately 2 times lower
368 sensitivity of porcelaneous foraminifera compared to hyaline species suggests that miliolid foraminifera
369 acquire half of the necessary Ca^{2+} through Ca-channels, and the other half directly from vacuolized
370 seawater. Element incorporation in miliolid foraminifera will therefore be mainly governed by their
371 respective concentrations in seawater, and to a lesser extent by the selectivity for Ca^{2+}/ permeability for
372 other ions during TMT.

373

374 **5. Conclusions**

375 Trends in element incorporation in larger benthic foraminifera can be explained by a combination of
376 differences in calcification strategy and seawater chemistry. Carbonate ion concentration in seawater
377 determines bioavailability of some ions (e.g. Zn^{2+} and Ba^{2+}), which are transported through Ca-channels
378 to the site of calcification. For hyaline foraminifera, we observed increased element incorporation for
379 larger species compared to smaller species, which can be explained by more intense activity of these
380 channels and the relative concentration in seawater during calcification. For miliolid foraminifera, only
381 half of the needed Ca is acquired through these Ca^{2+} channels, while the other half is obtained by
382 including small vesicles of seawater, leading to element partitioning to be more in line with inorganic
383 calcite.

384

385 **Acknowledgments**

386 This research is funded by the NIOZ – Royal Netherlands Institute for Sea Research and the Darwin
387 Centre for Biogeosciences project "*Double Trouble*: Consequences of Ocean Acidification – Past,
388 Present and Future –Evolutionary changes in calcification mechanisms" and the program of the
389 Netherlands Earth System Science Center (NESSC). Great thanks to all participants of the 2015
390 foraminifera culture expedition at the CNSI, St. Eustatia and Esmee Geerken for support with the salinity
391 culture experiment at the NIOZ. Furthermore, we would like to thank Kirsten Kooijman for supplying
392 *Dunaliella salina* cultures, Patrick Laan and Karel Bakker for seawater analysis and Mariëtte Wolthers
393 for providing technical support with PHREEQC. Lastly, we thank Jan-Berend Stuut (NIOZ) for the
394 usage of the Hitachi TM3000 SEM (NWO grant 822.01.008 and ERC grant 311152).

395

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

References

Allen, G.J. and Sanders, D. (1994) Two Voltage-Gated, Calcium Release Channels Coreside in the Vacuolar Membrane of Broad Bean Guard Cells. The Plant Cell 6, 685-694.

Allison, N., Austin, H., Austin, W. and Paterson, D.M. (2011) Effects of seawater pH and calcification rate on test Mg/Ca and Sr/Ca in cultured individuals of the benthic, calcitic foraminifera *Elphidium williamsoni*. Chemical Geology 289, 171-178.

Angell, R.W. (1980) Test morphogenesis (chamber formation) in the foraminifer *Spiroloculina hyalina* Schulze. Journal of Foraminiferal Research 10, 89-101.

Bentov, S. and Erez, J. (2006) Impact of biomineralization processes on the Mg content of foraminiferal shells: A biological perspective. Geochemistry, Geophysics, Geosystems 7.

Bernhard, J.M., Blanks, J.K., Hintz, C.J. and Chandler, G.T. (2004) Use of the fluorescent calcite marker calcein to label foraminiferal tests. Journal of Foraminiferal Research 34, 96-101.

Berthold, W.-U. (1976) Biomineralisation bei milioliden Foraminiferen und die Matritzen-Hypothese. Naturwissenschaften 63, 196-197.

Crocket, J.H. and Winchester, J.W. (1966) Coprecipitation of zinc with calcium carbonate. Geochimica et Cosmochimica Acta 30, 1093-1109.

De Nooijer, L.J., Spero, H.J., Erez, J., Bijma, J. and Reichart, G.J. (2014) Biomineralization in perforate foraminifera. Earth-Science Reviews 135, 48-58.

De Nooijer, L.J., Toyofuku, T. and Kitazato, H. (2009) Foraminifera promote calcification by elevating their intracellular pH. Proceedings of the National Academy of Sciences 106, 15374-15378.

Debenay, J.-P., Guillou, J.-J., Geslin, E., Lesourd, M. and Redois, F. (1998) Processus de cristallisation de plaquettes rhomboédriques à la surface d'un test porcelané de foraminifère actuel. Geobios 31, 295-302.

Dickson, A.G. and Millero, F.J. (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers 34, 1733-1743.

Dissard, D., Nehrke, G., Reichart, G.J. and Bijma, J. (2010) Impact of seawater pCO_2 on calcification and Mg/Ca and Sr/Ca ratios in benthic foraminifera calcite: results from culturing experiments with *Ammonia tepida*. Biogeosciences 7, 81-93.

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

Dissard, D., Nehrke, G., Reichart, G.J., Nouet, J. and Bijma, J. (2009) Effect of the fluorescent indicator calcein on Mg and Sr incorporation into foraminiferal calcite. Geochemistry, Geophysics, Geosystems 10, n/a-n/a.

Dueñas-Bohórquez, A., Raitzsch, M., De Nooijer, L.J. and Reichart, G.-J. (2011) Independent impacts of calcium and carbonate ion concentration on Mg and Sr incorporation in cultured benthic foraminifera. Marine Micropaleontology 81, 122-130.

Elderfield, H., Bertram, C.J. and Erez, J. (1996) A biomineralization model for the incorporation of trace elements into foraminiferal calcium carbonate. Earth and Planetary Science Letters 142, 409-423.

Elderfield, H. and Ganssen, G. (2000) Past temperature and $\delta^{18}O$ of surface ocean waters inferred from foraminiferal Mg/Ca ratios. Nature 405, 442-445.

Elderfield, H., Vautravers, M. and Cooper, M. (2002) The relationship between shell size and Mg/Ca, Sr/Ca, $\delta^{18}O$, and $\delta^{13}C$ of species of planktonic foraminifera. Geochemistry, Geophysics, Geosystems 3, 1-13.

Erez, J. (2003) The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. Reviews in Mineralogy and Geochemistry 54, 115-149.

Evans, D., Erez, J., Oron, S. and Müller, W. (2015) Mg/Ca-temperature and seawater-test chemistry relationships in the shallow-dwelling large benthic foraminifera *Operculina ammonoides*. Geochimica et Cosmochimica Acta 148, 325-342.

Evans, D. and Müller, W. (2013) LA-ICP-MS elemental imaging of complex discontinuous carbonates: An example using large benthic foraminifera. Journal of Analytical Atomic Spectrometry 28, 1039-1044.

Evans, D., Wade, B.S., Henehan, M., Erez, J. and Müller, W. (2016) Revisiting carbonate chemistry controls on planktic foraminifera Mg/Ca: implications for sea surface temperature and hydrology shifts over the Paleocene–Eocene Thermal Maximum and Eocene–Oligocene transition. Clim. Past 12, 819-835.

Friedrich, O., Schiebel, R., Wilson, P.A., Weldeab, S., Beer, C.J., Cooper, M.J. and Fiebig, J. (2012) Influence of test size, water depth, and ecology on Mg/Ca, Sr/Ca, $\delta^{18}O$ and $\delta^{13}C$ in nine modern species of planktic foraminifers. Earth and Planetary Science Letters 319, 133-145.

Guillong, M., Meier, D.L., Allan, M.M., Heinrich, C.A. and Yardley, B.W. (2008) SILLS: A MATLAB-based program for the reduction of laser ablation ICP-MS data of homogeneous

materials and inclusions. Mineralogical Association of Canada Short Course Series 40, 328-333.

Gussone, N., Langer, G., Thoms, S., Nehrke, G., Eisenhauer, A., Riebesell, U. and Wefer, G. (2006) Cellular calcium pathways and isotope fractionation in *Emiliania huxleyi*. Geology 34, 625-628.

Hemleben, C., Be, A.W.H., Anderson, O.R. and Tuntivate, S. (1977) Test morphology, organic layers and chamber formation of the planktonic foraminifer *Globorotalia menardii* (d'Orbigny). Journal of Foraminiferal Research 7, 1-25.

Hemleben, C., Erson, O., Berthold, W. and Spindler, M. (1986) fout. Biomineralization in lower plants and animals (BSC Leadbeater, R Riding, eds) Clarendon Press, Oxford, 237-249.

Hess, P. and Tsien, R.W. (1984) Mechanism of ion permeation through calcium channels. Nature 309, 453-456.

Hönisch, B., Allen, K.A., Russell, A.D., Eggins, S.M., Bijma, J., Spero, H.J., Lea, D.W. and Yu, J. (2011) Planktic foraminifers as recorders of seawater Ba/Ca. Marine Micropaleontology 79, 52-57.

Ishikawa, M. and Ichikuni, M. (1984) Uptake of sodium and potassium by calcite. Chemical geology 42, 137-146.

Jochum, K.P., Scholz, D., Stoll, B., Weis, U., Wilson, S.A., Yang, Q., Schwalb, A., Börner, N., Jacob, D.E. and Andreae, M.O. (2012) Accurate trace element analysis of speleothems and biogenic calcium carbonates by LA-ICP-MS. Chemical Geology 318–319, 31-44.

Keul, N., Langer, G., de Nooijer, L.J., Nehrke, G., Reichart, G.-J. and Bijma, J. (2013) Incorporation of uranium in benthic foraminiferal calcite reflects seawater carbonate ion concentration. Geochemistry, Geophysics, Geosystems 14, 102-111.

Kitano, Y., Okumura, M. and Idogaki, M. (1975) Incorporation of sodium, chloride and sulfate with calcium carbonate. GEOCHEMICAL JOURNAL 9, 75-84.

Kurtarkar, S.R., Saraswat, R., Nigam, R., Banerjee, B., Mallick, R., Naik, D.K. and Singh, D.P. (2015) Assessing the effect of calcein incorporation on physiological processes of benthic foraminifera. Marine Micropaleontology 114, 36-45.

Lea, D.W., Mashiotta, T.A. and Spero, H.J. (1999) Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. Geochimica et Cosmochimica Acta 63, 2369-2379.

Lear, C.H., Elderfield, H. and Wilson, P.A. (2000) Cenozoic deep-Sea temperatures and global ice volumes from Mg/Ca in benthic foraminiferal calcite. Science 287, 269-272.

494 Marchitto, T.M., Curry, W.B. and Oppo, D.W. (2000) Zinc concentrations in benthic
495 foraminifera reflect seawater chemistry. Paleoceanography 15, 299-306.
496 Mehrbach, C., Culberson, C.H., Hawley, J.E. and Pytkowicx, R.M. (1973) Measurement of
497 the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure.
498 Limnology and Oceanography 18, 897-907.
499 Mezger, E.M., de Nooijer, L.J., Boer, W., Brummer, G.J.A. and Reichart, G.-J. (in review)
500 Salinity controls on Na incorporation in Red Sea planktonic foraminifera. Paleoceanography.
501 Mucci, A. and Morse, J.W. (1983) The incorporation of Mg^{2+} and Sr^{2+} into calcite
502 overgrowths: influences of growth rate and solution composition. Geochimica et
503 Cosmochimica Acta 47, 217-233.
504 Nehrke, G., Keul, N., Langer, G., de Nooijer, L.J., Bijma, J. and Meibom, A. (2013) A new
505 model for biomineralization and trace-element signatures of Foraminifera tests. Biogeosciences
506 10, 6759-6767.
507 Nürnberg, D., Bijma, J. and Hemleben, C. (1996) Assessing the reliability of magnesium in
508 foraminiferal calcite as a proxy for water mass temperatures. Geochimica et Cosmochimica
509 Acta 60, 803-814.
510 Parkhurst, D.L. and Appelo, C. (1999) User's guide to PHREEQC (Version 2): A computer
511 program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical
512 calculations. US Geol. Surv, Denver, Colorado.
513 Pawlowski, J., Holzmann, M., Berney, C., Fahrni, J., Gooday, A.J., Cedhagen, T., Habura,
514 A. and Bowser, S.S. (2003) The evolution of early Foraminifera. Proc Natl Acad Sci U S A
515 100, 11494-11498.
516 Pierrot, D., Lewis, E. and Wallace, D.W.R. (2006) MS Excel Program Developed for CO_2
517 System Calculations, ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak
518 Ridge National Laboratory, U.S.
519 Reichart, G.-J., Jorissen, F., Anschutz, P. and Mason, P.R. (2003) Single foraminiferal test
520 chemistry records the marine environment. Geology 31, 355-358.
521 Russell, A.D., Hönisch, B., Spero, H.J. and Lea, D.W. (2004) Effects of seawater carbonate
522 ion concentration and temperature on shell U, Mg, and Sr in cultured planktonic foraminifera.
523 Geochimica et Cosmochimica Acta 68, 4347-4361.
524 Sanyal, A., Hemming, N.G., Broecker, W.S., Lea, D.W., Spero, H.J. and Hanson, G.N.
525 (1996) Oceanic pH control on the boron isotopic composition of foraminifera: Evidence from
526 culture experiments. Paleoceanography 11, 513-517.

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

Sather, W.A. (2005) Selective Permeability of Voltage-Gated Calcium Channels, Voltage-Gated Calcium Channels. Springer US, Boston, MA, pp. 205-218.

Segev, E. and Erez, J. (2006) Effect of Mg/Ca ratio in seawater on shell composition in shallow benthic foraminifera. Geochemistry, Geophysics, Geosystems 7, n/a-n/a.

Ter Kuile, B. and Erez, J. (1987) Uptake of inorganic carbon and internal carbon cycling in symbiont-bearing benthonic foraminifera. Marine Biology 94, 499-509.

Toyofuku, T., Suzuki, M., Suga, H., Sakai, S., Suzuki, A., Ishikawa, T., de Nooijer, L.J., Schiebel, R., Kawahata, H. and Kitazato, H. (2011) Mg/Ca and $\delta^{18}O$ in the brackish shallow-water benthic foraminifer *Ammonia 'beccarii'*. Marine Micropaleontology 78, 113-120.

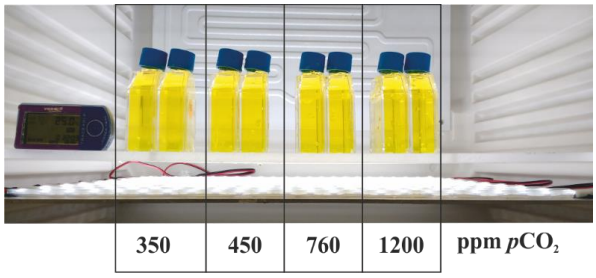
Van Dijk, I., de Nooijer, L.J., Wolthers, M. and Reichart, G.-J. (in review) Impacts of pH and $[CO_3^{2-}]$ on the incorporation of Zn in foraminiferal calcite Geochimica et Cosmochimica Acta).

Wit, J.C., de Nooijer, L.J., Barras, C., Jorissen, F.J. and Reichart, G.J. (2012) A reappraisal of the vital effect in cultured benthic foraminifer *Bulimina marginata* on Mg/Ca values: assessing temperature uncertainty relationships. Biogeosciences 9, 3693-3704.

Wit, J.C., de Nooijer, L.J., Wolthers, M. and Reichart, G.J. (2013) A novel salinity proxy based on Na incorporation into foraminiferal calcite. Biogeosciences 10, 6375-6387.

Zeebe, R.E. and Sanyal, A. (2002) Comparison of two potential strategies of planktonic foraminifera for house building: Mg^{2+} or H^+ removal? Geochimica et Cosmochimica Acta 66, 1159-1169.

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016



548

549 **Figure 1. Photograph of the culture set-up. Treatment with corresponding set-points are A=350,**
550 **B=450 ppm, C=760 ppm, D=1200 ppm.**

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

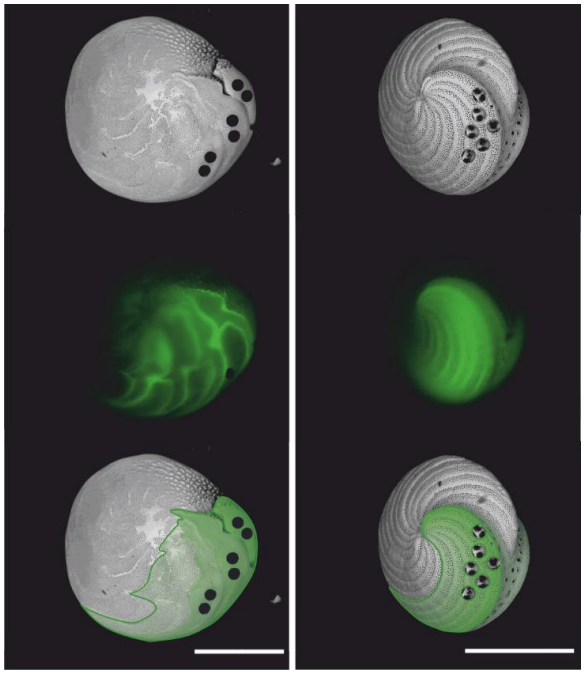
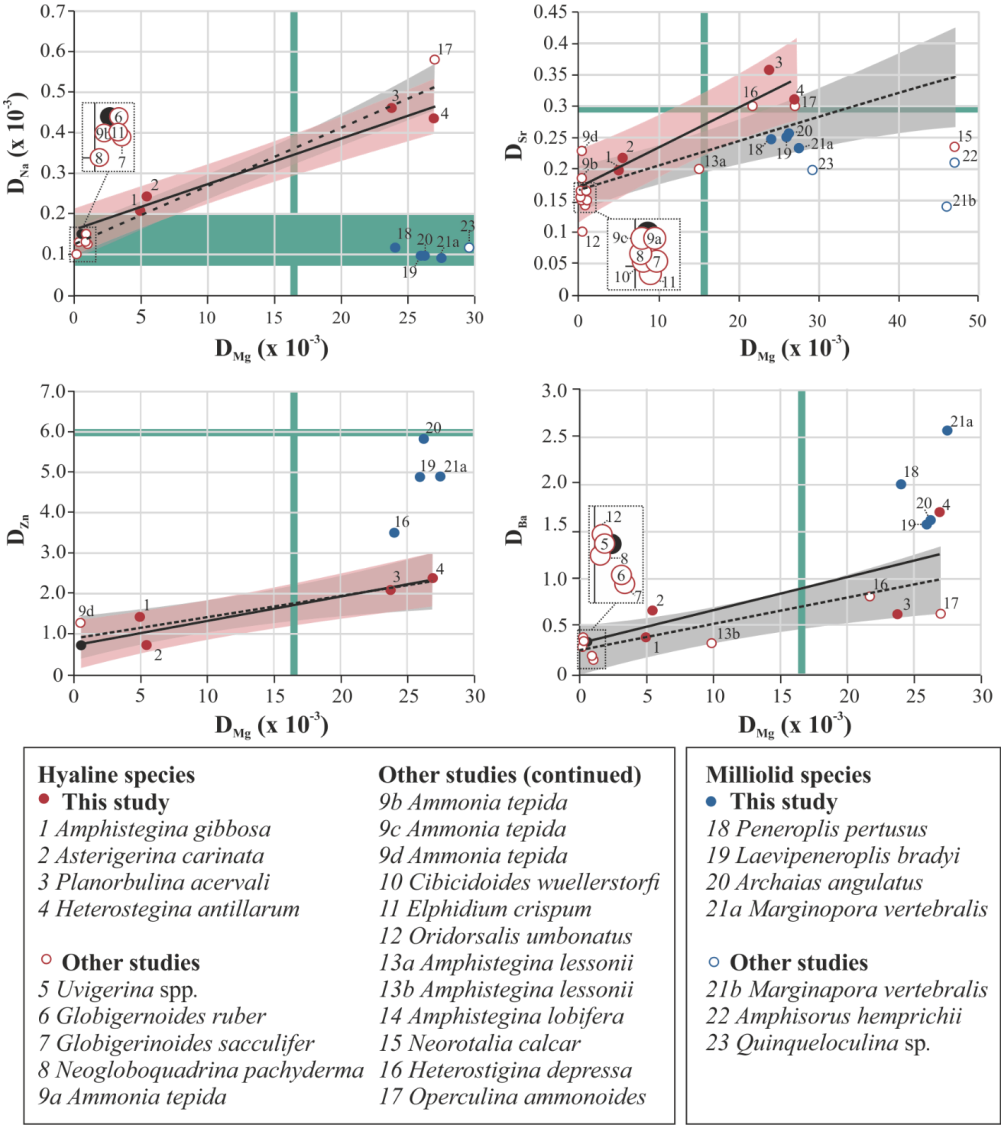


Figure 2. SEM (top panels) and fluorescent microscope (middle panels) photographs of *A. gibbosa* (left) and *A. angulatus* (right) to assess newly formed chambers for laser ablation (lower panels). Scalebars = 500 µm.

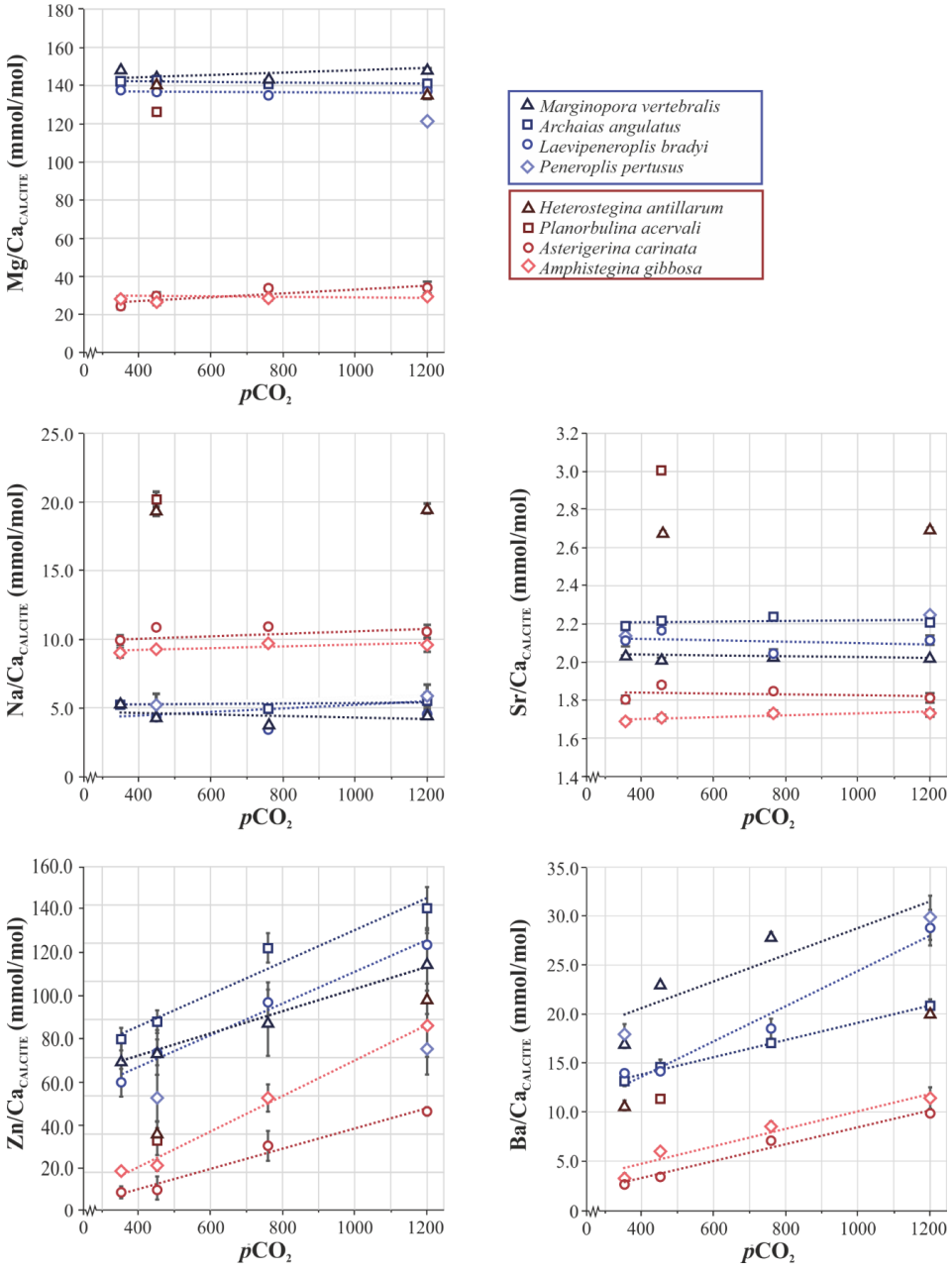
Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016



555

556 **Figure 3. Partition coefficient of Na, Sr, Zn and Ba versus D_{Mg} of hyaline (red symbols) and**
557 **miliolid (blue symbols) species in this study (closed symbols) and other studies (open symbols).**
558 **Black lines represent trendlines (solid = this study; dashed = all studies). The 95% confidence**
559 **intervals are indicated in pink (this study) and grey (all studies), which sometimes overlap. Black**
560 **dots represent the NFHS, in-house carbonate standard, consisting of planktonic foraminifera. In**
561 **green, inorganic partition coefficient from Mucci and Morse (1983), Ishikawa and Ichikuni (1984),**
562 **Kitano et al. (1975) and Crocket and Winchester (1966). Numbers correspond to foraminiferal**
563 **species analyzed (See supplementary Table S1)**

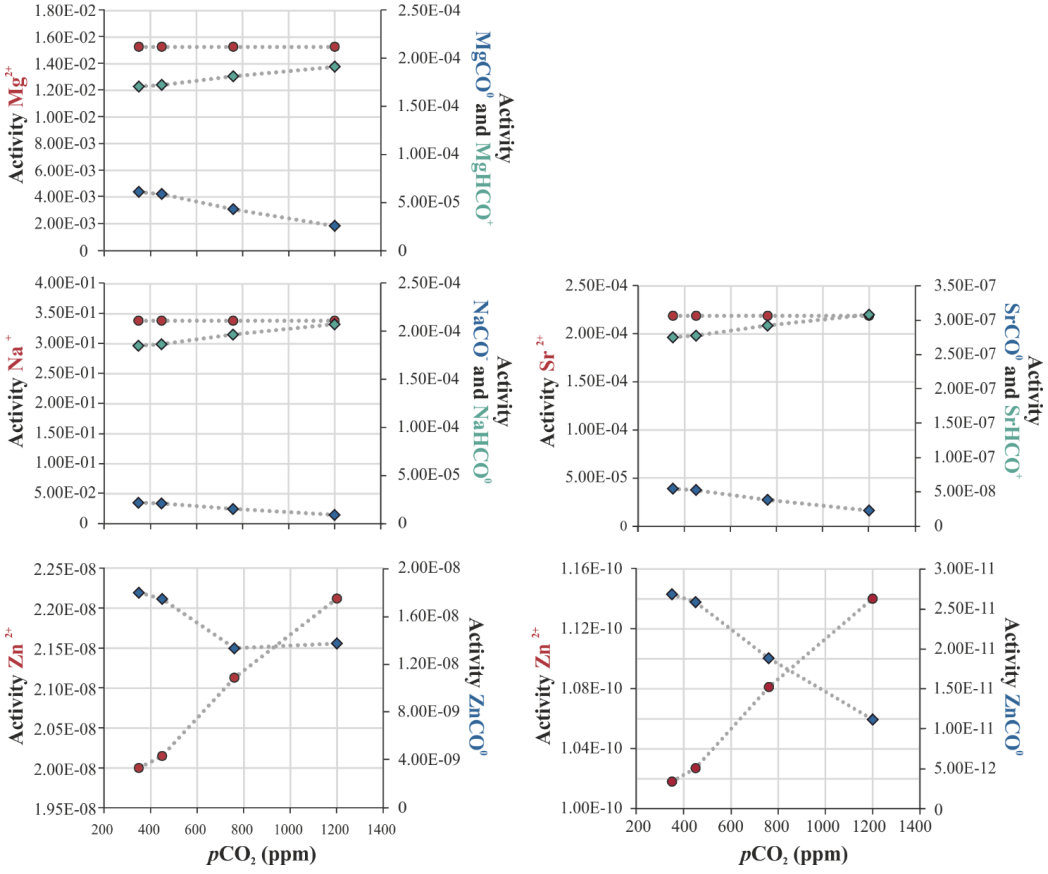
Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016



564

565 **Figure 4. Element to Ca ratios (±SE) of different species of foraminifera over a range of pCO_2**
566 **values. In some cases, the error bar is smaller than the symbol. Miliolid species in blue (triangles**
567 **= *M. vertebralis*; squares = *A. angulatus*; circles = *L. bradyi*; squares = *P. pertusus*) and hyaline**
568 **species in red (triangles = *H. antillarum*; squares = *P. acervalis*; circles = A. *carinata;* diamonds =**
569 ***A. gibbosa*).**

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016



570

571 **Figure 5. Speciation of Mg, Na, Sr, Zn and Ba in the different seawater treatments modelled in**
572 **PHREEQC (Parkhurst and Appelo, 1999). Activities of free ions (red) and element (E)-carbonate**
573 **complexes (ECO_3 = blue diamonds and $EHCO_3$ = green diamonds).**

574

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

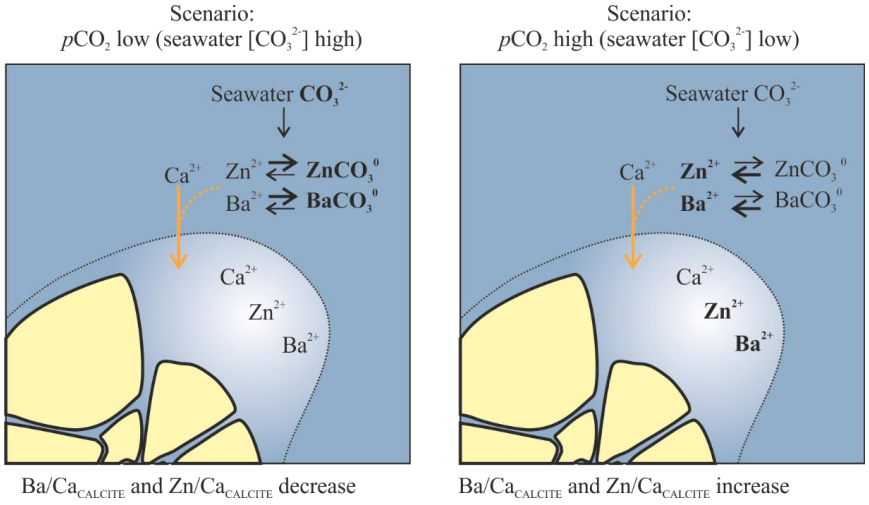


Figure 6. Schematic of incorporation of Zn and Ba during foraminiferal calcification under low (left panel) and high (right panel) pCO_2 conditions. Amount of free ions (e.g. Zn^{2+} and Ba^{2+}) is influenced by speciation due to changing $[CO_3^{2-}]$. Orange arrow indicates transport of Ca^{2+} through channel, with the associated accidental transport of Zn^{2+} and Ba^{2+}.

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

Table 1. Carbon parameters (TA= Total alkalinity, n=2, DIC=Dissolved Inorganic Carbon, n=2) with (relative) standard deviation of the culture water per treatment of the pCO_2 experiment. CO2SYS was used to calculate seawater carbonate ion concentration, calcite saturation state and pH from measured TA and DIC.

Treatment	Set-point	Measured		Calculated CO2SYS		
	pCO_2 ppm	TA µmol/kg	DIC µmol/kg	$[CO_3^{2-}]$ µmol/kg	pH (total scale)	$\Omega_{CALCITE}$
A	350	2302.8±8.2	2007.5±10.7	211.2	8.04	5.1
B	450	2305.2±5.8	2021.3±12.5	204.1	8.02	4.9
C	760	2304.4±0.9	2100.8±13.4	153.3	7.86	3.7
D	1200	2300.3±0.7	2201.4±4.1	92.7	7.61	2.2

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

586 **Table 2. Total number of LA-ICP-MS measurements per species, per treatment (A-D).**

Species	n LA(n specimens)			
	A: 350 ppm	**B: 450ppm**	**C:760 ppm**	**D:1200 ppm**
A. angulatus	62(19)	72(21)	76(21)	51(14)
M. vertebralis	48(14)	49(15)	57(18)	33(11)
A. gibbosa	106(28)	126(32)	75(18)	59(15)
L. bradyi	21(5)	38(13)	27(5)	16(4)
A. carinata	12(2)	14(1)	19(4)	5(1)
P. pertusus		12 (2)		11 (2)
H. antillarum		12 (1)		14 (2)
P. acervalis		8 (2)		
Total	187(49)	331(87)	254(66)	189(49)

587

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

588 **Table 3. Overview of element to Ca ratios in foraminiferal calcite (Avg=average; SE=standard**
589 **error) and partition coefficients D_E, with D_E of ambient conditions (treatment B) in bold. Letters**
590 **([a] to [d]) indicate (per species per E/Ca) groups that are statistical different (one-way ANOVA).**

Species	pCO_2	Mg/Ca mmol/mol		Na/Ca mmol/mol		Sr/Ca mmol/mol		Zn/Ca µmol/mol		Ba/Ca µmol/mol	
		Avg±SE	D_{Mg} $*10^{-3}$	Avg±SE	D_{Na} $*10^{-3}$	Avg±SE	D_{Sr}	Avg±SE	D_{Zn}	Avg±SE	D_{Ba}
A. angulatus	350	139.4±0.6$_a$	26.6	5.2±0.1[a]	0.12	2.2±0.02[a]	0.25	80.0±5.1[a]	5.3	13.2±0.5[a]	1.5
	450	137.7±0.5$_b$	**26.3**	4.3±0.1[b]	**0.10**	2.2±0.01[a]	**0.26**	88.1±5.2[b]	**5.8**	14.6±0.5[b]	**1.6**
	760	137.4±0.7$_b$	26.2	4.9±0.1[c]	0.11	2.2±0.01[a]	0.26	122.6±7.0[c]	8.1	17.0±0.6[b]	1.9
	1200	138.6±1.1[a]	26.4	5.4±0.2[a]	0.12	2.2±0.02[a]	0.26	140.8±9.9[d]	9.3	20.9±0.2[c]	2.3
M. vertebralis	350	147.7±0.6[a]	28.2	4.8±0.1[a]	0.11	2.0±0.01[a]	0.24	70.0±10.1[a]	4.6	17.0±0.5[a]	1.9
	450	144.2±0.8[b]	**27.5**	4.1±0.1[b]	**0.09**	2.0±0.01[a]	**0.23**	74.0±10.6[b]	**4.9**	23.1±0.5[b]	**2.6**
	760	143.0±0.6[a]	27.3	3.8±0.1[a]	0.09	2.0±0.01[a]	0.23	87.7±15.5[c]	5.8	27.9±0.6[c]	3.1
	1200	148.3±0.5[b]	28.3	4.5±0.2[c]	0.10	2.0±0.01[a]	0.23	115.6±15.3[d]	7.6	30.1±0.2[d]	3.3
L. bradyi	350	137.8±1.3[a]	26.3	5.2±0.2[c]	0.12	2.1±0.03[a]	0.24	60.0±6.5[a]	4.0	14.0±0.5[a]	1.5
	450	136.2±0.7[a]	**26.0**	4.3±0.1[b]	**0.10**	2.2±0.01[b]	**0.25**	73.8±6.0[b]	**4.9**	14.2±0.5[a]	**1.6**
	760	134.4±1.2[b]	25.6	3.4±0.1[a]	0.08	2.0±0.02[c]	0.24	97.5±9.4[c]	6.4	18.5±0.6[b]	2.1
	1200	136.9±1.1[a]	26.1	6.2±0.2[d]	0.14	2.1±0.02[a]	0.24	124.2±7.8[d]	8.2	28.8±0.2[c]	3.2
P. pertusus	350										
	450	126.1±1.8[a]	**24.0**	5.2±0.3[a]	**0.12**	2.1±0.07[a]	**0.25**	53.0±10.8[a]	**3.5**	18.0±0.5[a]	**2.0**
	760										
	1200	121.3±1.0[a]	23.1	5.8±0.2[a]	0.13	2.2±0.02[a]	0.26	75.5±11.9[b]	5.0	29.8±0.2[b]	3.3
H. antillarum	350										
	450	141.3±0.3[a]	**26.9**	19.4±0.5[a]	**0.44**	2.7±0.02[a]	**0.31**	36.0±14.7[a]	**2.4**	10.7±0.5[a]	**1.2**
	760										
	1200	136.9±16[a]	26.1	19.5±0.4[a]	0.44	2.7±0.02[a]	0.31	97.0±18.3[b]	6.4	20.1±0.2[b]	2.2
P. acervalis	350										
	450	139.1±1.2	**26.5**	19.5±0.7	**0.44**	3.1±0.02	**0.36**	31.6±6.6	**2.1**	11.3±0.5	**1.3**
	760										
	1200										
A.	350	23.6±1.5[a]	4.5	9.9±0.4[a]	0.22	1.8±0.02[a]	0.21	9.0±2.6[a]	0.6	3.2±0.5[a]	0.4

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

	450	28.5±2.4[b]	**5.4**	10.8±0.1[a]	**0.24**	1.9±0.01[a]	**0.22**	10.9±5.5[a]	**0.7**	6.0±0.5[b]	**0.7**
	760	33.1±1.2[b]	6.3	10.9±0.2[a]	0.24	1.8±0.01[a]	0.21	30.7±7.0[b]	2.0	8.5±0.6[c]	0.9
	1200	33.5±3.1[b]	6.4	10.6±0.5[a]	0.24	1.8±0.03[a]	0.21	46.4±2.1[b]	3.1	11.4±0.2[d]	1.3
A. gibbosa	350	27.8±0.5[a]	5.3	9.0±0.1[a]	0.20	1.7±0.01[a]	0.20	19.0±1.8[a]	1.3	2.7±0.5[a]	0.3
	450	25.9±0.6[b]	**4.9**	9.2±0.1[a]	**0.21**	1.7±0.02[a]	**0.20**	21.5±2.5[b]	**1.4**	3.4±0.5[a]	**0.4**
	760	28.2±0.7[a]	5.4	9.7±0.1[b]	0.22	1.7±0.02[a]	0.20	52.8±6.1[c]	3.5	7.1±0.6[b]	0.8
	1200	28.7±0.6[a]	5.5	9.6±0.1[b]	0.21	1.7±0.02[a]	0.20	85.8±11.3[d]	5.7	9.9±0.2[c]	1.1

591

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

592 **Table 4. R^2 and p-values of linear trendline of D_E versus D_{Mg} of all hyaline species of this studies**
593 **and compilated literature studies (all studies).**

D_E versus D_{Mg}		R^2	p-value
D_{Na}	This study	0.97	<0.0005
	All studies	0.95	<0.0005
D_{Sr}	This study	0.90	<0.0025
	All studies	0.53	<0.005
D_{Zn}	This study	0.88	<0.005
	All studies	0.80	<0.01
D_{Ba}	This study	0.58	<0.05
	All studies	0.56	<0.005

594

Biogeosciences Discuss., doi:10.5194/bg-2016-402, 2016
Manuscript under review for journal Biogeosciences
Published: 27 September 2016

595 **Table 5. Regression and p-values of foraminiferal Zn/Ca and Ba/Ca versus pCO_2 values of**
596 **different species (Fig. 4).**

Species	Zn/Ca		Ba/Ca	
	R^2	p-value	R^2	p-value
M. vertebralis	0.99	<0.0005	0.81	<0.025
A. angulatus	0.95	<0.0025	0.99	<0.0005
L. bradyi	0.98	<0.0005	0.97	<0.0025
A. carinata	0.98	<0.001	0.94	<0.005
A. gibbosa	0.99	<0.0005	0.98	<0.001

597