

Dear Editor,

We are glad to have received two constructive sets of comments. We have addressed all of them to improve our manuscript and we would like you to consider the revised manuscript for publication in Biogeosciences. Below, we have repeated the reviews (in italics) and added point-by-point our reply.

Sincerely,

Lennart de Nooijer, also on behalf of the other authors,

Reviewer #1

Authors report new and important results of Ba/Ca and Mg/Ca measurements using LA-ICPMS of two species of benthic foraminifera. The study is technically sound and show sufficient number of important findings. I highly recommend that this manuscript should be published. The followings are my minor comments that would be better to be considered before acceptance of this manuscript.

Line 40: the mechanism why Ba can be used as a proxy of total alkalinity and salinity should be explained more (Ba shows nutrient-type distribution in the ocean; Ba is rich in terrestrial water, etc.).

The first sentences of the abstract have been re-written and now explain why foraminiferal Ba/Ca can be used to reconstruct alkalinity and salinity (lines 39-47).

Lines 133-134: I could not understand this line "foraminifera were diluted as little as possible by the solution containing the food for the foraminifera." It is seawater that can be diluted, right?

This is now changed into: "...the water in the dishes containing the foraminifera was diluted as little as possible...".

Line 143: It would be nice if an explanation regarding "the batch seawater" appears in Sec. 2.1. Also, how much seawater was prepared in each treatment?

The concentrations of elements, temperature, salinity and carbonate chemistry from the 'batch seawater' are listed in Table 1. The recipe followed to make this stock solution is described in lines 101-105 and we included the total volume of seawater prepared (5 liters for each treatment; line 102).

Line 154: It would be better to describe LA-ICPMS setups here (carrier gas, flow rate, ICPMS, laser type, etc.)

We have added some more information about the employed setup and settings used in this section (lines 168-171).

Lines 168-169: I wonder ten large specimens were randomly sampled among all the treatments?

This is true indeed: for every treatment, 2 specimens (i.e. 10 in total) were randomly selected. This is now added to line 187.

Line 176: Weren't high Mg counts used for the data screening?

No, but since surface contamination is most clearly visible in elements like Mn and Al, and the elevated peaks in Mg usually coincide with peaks in these two elements, many of the excluded parts of the ablation profiles also contain relatively much Mg. This is exactly why these parts are excluded. This is now clarified in line 194-197.

Line 184: What the Zoo's stock mean? Sediment?

This means the sediment collected originally at Burgers' Zoo and now kept in our laboratory to isolate the specimens that were cultured at each of the five treatments (beginning of section 2.2). This is now indicated at lines 205-206.

Line 185: There is no explanation what "the royal NIOS" stands for.

Royal NIOZ is the affiliation of some of the authors and the laboratory at which these analyses were performed. This is mentioned here to underline the difference of the system employed for these measurements compared to that used for the cultured specimens (which were measured at Utrecht University).

Line 191: Please add "grass standards NIST 610 and 612" here.

We have added "610" to this sentence (line 213). NIST 612 was not used, by the way.

Lines 230-231: The linear regression line of the data of H. depressa in Fig. 2 is not forced through zero. Is this intentionally or mistakenly?

This is now corrected: see adjusted figure 2.

Lines 243-245: I think these sentences need to be revised. I don't think the "field" data should be removed from the discussion, but there must be better explanation, like "the aquarium derived data is consistent with the culturing derived data, but it was not used in the regression analysis, since the conditions in which (...)".

We agree and have changed the text accordingly.

Lines 262-263: I could not understand this line. Which data, do you mean, is the outlier?

We have tried to clarify this sentence by rephrasing it so that it now reads "...lower than 0.01, indicating that the current data set does not allow rejecting the presence of a trend for *A. lessonii*." (lines 288-289).

Line 319: What the word "complete" mean?

We have changed this to "...does not account for all of the observed variability in Mg/Ca..." (lines 347-348 in the revised version of our manuscript).

Line 322 What is the significance or importance of "5% precision"?

This indicates that measuring 20 (or more) individual chambers will result in an estimate within +/- 5% of the true mean. "on average" is replaced by "at least" in this sentence.

Lines 324-325: Is this sentence implication for whole shell analysis in paleoceanography?

For example. This suggestion has been added to this sentence.

Lines 340-342: I could not read through this line. Do you mean "a small difference of environmental parameter may partly explain a slight difference in D_{Ba} between aquarium and cultured samples"?

This is indeed what we meant. This sentence is changed into: "A small impact of environmental parameters other than seawater Ba/Ca may account for the slightly higher D_{Ba} in the "field" specimens taken from the aquarium compared to the cultured ones (Figure 2)."

Fig. 4: A difference between symbols representing two species should be more distinct: For example, open and closed symbols. There seems no difference in a blurry and small figure.

We have revised this figure: in addition to using different symbols (circle versus diamond), the diamonds are now open, whereas the circles have remained filled.

Reviewer #2

*The manuscript 'Ba incorporation in benthic foraminifera' presents laboratory culture barium data for two species, *Heterostegina depressa* and *Amphistegina lessonii*. Ba/Ca ratios in foraminifera may be used to trace past changes in seawater [Ba], which in turn may be related to (e.g.) salinity or alkalinity, and the proxy is therefore of broader community interest. Whilst Ba/Ca has been successfully applied as a proxy using low-Mg foraminifera for some time, this study provides seawater-shell Ba/Ca calibrations for two high-Mg species. I was missing an explanation in the text of why the authors chose to use a range of seawater Ba/Ca ratios that are much higher than natural seawater. Nonetheless, the data are of good quality, and are suitable for publication in Biogeosciences.*

We thank the reviewer for his/her constructive comments. At the end of the introduction (lines 84-88) we have now added the rationale for using Ba/Ca exceeding the natural range. This extended range facilitates testing the mechanisms underlying Ba-incorporation in foraminiferal calcite (see section 4.5) and at the same time (given that the Ba/Ca_{cc} responds linearly to increasing Ba/Ca_{sw}),

decreases the uncertainty in the calibration over the paleoceanographically relevant range in seawater Ba/Ca.

Comments

1. *In the abstract and introduction there is no mention of upwelling, which may complicate the use of Ba/Ca as a salinity proxy, especially in benthic organisms.*

We have now added a sentence to the beginning of the introduction about the influence of upwelling to surface water Ba/Ca (lines 47-48). "These reconstructions can be complicated by upwelling affecting surface Ba/Ca (Lea et al., 1989; Hatch et al., 2013)."

2. *Lines 64-67, line 327, and lines 455-456. This is not the first time that Ba/Ca has been investigated in a high-Mg benthic species and therefore these sentences should be rephrased. Evans et al. [2015] GCA report Ba/Ca data for the highMg species *Operculina ammonoides* under variable seawater [Ba], and found a barium distribution coefficient (0.66) similar to that reported here for *H. depressa*. van Dijk et al. [2017] also report Ba/Ca data for *H. antillarum* which has a distribution coefficient of 1.2-2.2 according to that study.*

In the introduction (now lines 73-74), we added "Ba/Ca is only rarely investigated in species producing high-Mg calcite (Evans et al., 2015; Van Dijk et al., 2017)". In the discussion (lines 356-357) we have replaced "has not been observed before" by "fits previously reported (differences in) partition coefficients for barium" and in the conclusions (now lines 484-485) we have replaced "are larger than previously thought" by "can be relatively large".

3. *Section 2.2. Please state the approximate volume of seawater in which the cultures took place. As these cultures were performed in petri dishes, presumably the volume was relatively small? If so: (1) How was evaporation monitored and avoided? (2) It is likely that the foraminifera modified the carbonate chemistry of the seawater in between water exchanges (once per week). Was this monitored?*

The Petri dishes contained approximately 10 ml of culture medium, which is now added to the Method section (line 131). This volume is obviously still very large in comparison to the elemental uptake of the foraminifera. Evaporation was minimized by replacing the medium every three days (and not once a week: line 153). The carbonate chemistry was not monitored within the Petri dishes and may in theory have been slowly changing during these three days. A simple calculation shows that calcification as such cannot have had a measurable effect on the total inorganic carbon concentration. The uptake of DIC by the addition of a new chamber in juvenile foraminifera is in the order of 0.36 nmol (De Nooijer et al., 2009. Biogeosciences 6: 2669-2675). In 10 ml of our seawater (with approximately 2200 $\mu\text{mol DIC/l}$; Table 1) contained 22 μmol of DIC. Calcification of one new chamber therefore removes only $\sim 0.015\%$ of the present DIC (0.36 nmol/22000 nmol). With 20 specimens per dish, only $\sim 0.3\%$ of the DIC would have been removed if each of them produced one new chamber during those three days.

4. *The phrasing of lines 148-149 implies that the cleaning procedure has an impact on measured Ba/Ca. Either rephrase or state what this impact is.*

The impact of both cleaning with NaOCl and H₂O₂ is similar and relatively small (2-3 μmol/mol; Table 2 in Pak et al., 2004) compared to rinsing with de-ionized water. We have clarified this in the revised version of our manuscript (now lines 161-162).

5. *There is far more detail given for the laser-ablation performed at the royal NIOZ. Whilst a reference is provided for the system at Utrecht University, it may be useful to state which LA and ICPMS systems were used and the wavelength of the laser for easy comparison. What is the accuracy and precision of the system used at Utrecht University?*

As also noted by reviewer #1, we have extended the description of the platform used at the Utrecht University (lines 167-170). Relative standard deviation based on ablation of standard calcite material was 5% for Mg/Ca, as well as for Sr/Ca (lines 180-181).

6. *Lines 164-169. Here the authors state that the final chamber of A. lessonii and the final two chambers of H. depressa did not yield reliable data because the walls are thin. However, Fig. 3 shows data for F and F-1 for both species. Is this a mistake? If not, these data should not be shown if they are not reliable.*

We agree with the reviewer that this may be seen as contradictory. We have hence extended the text on this part clarifying 'unreliability' of measuring the F- and F-1 chambers. In short, shorter ablation profiles result in a higher 'within-chamber wall' Ba/Ca (as well as Mg/Ca, Sr/Ca, etc) variability. Therefore, the precision of the determined Ba/Ca is lower in thinner (i.e. built later in life) chambers. This is in line with a higher variability being observed between the F-chamber's average Ba/Ca (compare the SDs between F and F-5 in figure 3).

To minimize the variability due to shorter ablation profiles, F- and F-1 chambers were omitted from the larger dataset. Unless they were extremely short (< 5 sec; which was the case in 4 out of 10 specimens for both *Amphistegina* and *Heterostegina*), they were analyzed for the dataset focusing on Ba/Ca as a function of chamber position (figure 3). One of the reasons is that including them is the only way to show, for example, that the variability between average Ba/Ca for F-chambers is higher than for (e.g.) F-2 chambers. The text in the method section is changed to account for the above considerations (now lines 181-184).

7. *Lines 175-176. What is the possible source of Al and Mn contamination in cultured foraminifera?*

We are unfortunately not entirely sure on this, although this is commonly observed in such studies by different laboratories working on similar studies. It may be an artifact of the analytical approach in which the first few laser pulses cause the material deposited in the ablation chamber to whirl up due to the plasma plume explosion. Alternatively, it may be a remnant from the foraminiferal cell material that accumulated these metals during the culturing. The pseudopodial network is known to cover the complete outer surface, for example during chamber formation and may leave traces at the shell surface after termination of the experiment. Since this is pure speculation, and monitoring for high Al

and/or Mn counts is relatively standard procedure in laser-ablating fossil as well as recent foraminifera, we suggest to leave the text as it is on this point.

8. Section 2.4. Consider changing the phrase 'field specimens'.

We have changed this to 'aquarium samples'.

9. Lines 230-231. I agree that it is reasonable to assume no barium incorporation when there is no barium in seawater. However, forcing a linear regression through the origin also assumes that seawater and shell Ba/Ca must be linearly related across the full range of seawater Ba/Ca ratios, which may not be the case. Consider that the *H. depressa* zoo aquarium sample may be in agreement with the cultures if the regression is not forced through the origin.

When including the aquarium samples in the linear regression analysis, for both species, the R^2 decreases and the intercept with the y-axis increases. When including the aquarium samples in the linear regression and forcing the regression through (0, 0), the slopes hardly change (from 0.78 to 0.77 for *H. depressa* and from 0.33 to 0.32 for *A. lessonii*). This implies that the aquarium samples agree well with the experimental specimens when forcing the regression through the origin. Following the reviewer's suggestion, we have added this outcome to the manuscript (lines 265-270). As stated previously, the conditions under which the aquarium samples have formed their shells may have been different and since those conditions are less-well constrained than in our culturing experiment, we suggest to exclude them from the regression shown in figure 2.

10. Lines 232-233. Technically, if the regressions are not forced through the origin then there is not a single partition coefficient value, I suggest this is rephrased in terms of the seawater-shell Ba/Ca slopes.

We agree with the reviewer and have changed the text regarding the change in Ba/Ca_{cc} as a function of Ba/Ca_{sw} when the linear regression is not forced through zero (lines 254-255).

11. Lines 241-242. Why is the aquarium seawater Ba/Ca higher than most natural seawater? If this is known it would be useful to state the reason. What is the meaning of the sentence starting on line 243? Is it an analytical problem or is there reason to suspect the aquarium seawater Ba/Ca ratio was not constant?

The Ba/Ca of the aquarium's seawater is indeed higher than that of the open ocean (which is approximately 0.15 $\mu\text{mol/L Ba}$ / 10 mmol/L Ca , or 15 $\mu\text{mol Ba/mol Ca}$). In coastal waters, however, this ratio can easily be 2-3 times higher (see e.g. Shaw et al., 1998. GCA 62: 3047-3054), similar to the Ba/Ca of the zoo's water.

'Conditions' in this sentence refers to any chemical/ physical parameter that was not (accurately) measured over the life-time of the foraminifera that were used for Ba/Ca analysis. Many parameters (e.g. salinity, temperature) are regularly determined in the aquarium, but not as precise/ accurate as for our controlled growth experiment. Moreover, conditions (e.g. carbonate chemistry) within the coral rubble at the aquarium's floor (where the foraminifera were collected from) may differ from the water itself, where samples for chemical and physical monitoring were taken. To avoid the suggestion

that 'conditions' in this sentence is interpreted as 'Ba/Ca_{sw}', we have changed this sentence accordingly (lines 265-268).

12. *Section 3.3. It would aid the interpretation of these interesting data if the reader had an idea of how much of the foraminifera the final five chambers represent. Approximately how many chambers were precipitated in culture? Consider adding a representative image.*

For both species, the final ~6 chambers represent approximately half of the outer whorl. Since older (i.e. smaller) chambers are completely covered by the newly added chambers of the outer whorl, it is difficult to estimate the percentage of the final ~6 chambers of the total number of chambers. Including a picture of (ablated) specimens wouldn't allow such an estimate either.

13. *Lines 259-260. The phrasing is confusing here. Ba/Ca increases in the first sentence but decreases in the second sentence. Rephrase for consistency.*

The 'chamber position', 'chamber addition' and 'chamber number' and the directions in which these are represented, may have resulted in an apparent inconsistency here. We have rephrased the first sentence to make sure that Ba/Ca increases with subsequent new chambers added (Figure 3). The second sentence now reads: "...average increase of 1.43 $\mu\text{mol/mol}$ Ba/Ca_{cc} with every chamber added (Table 4)."

14. *Lines 273-276. If the two slopes are significantly different, why combine the data from both species?*

We agree with the reviewer and have deleted the last sentence of this section.

15. *Section 4.2 and Figure 4. I am surprised that the range in Mg/Ca is so large, both within and between experiments, and this requires further explanation. For example, compare these *H. depressa* data to those reported in Raitzsch et al. [2010]. In that paper the Mg/Ca 2SD was 17 to 24 mmol mol⁻¹ (~10-20%), which is comparable to other studies reporting laser-ablation data. Here, some experiments are in line with this while others have a far larger range, for example *H. depressa* treatment D (~110-190 mmol mol⁻¹). The *A. lessonii* data are even more surprising, treatment D has a range from 30-140 mmol mol⁻¹, and treatment C has a range of 30-120 mmol mol⁻¹. Why is there so much variation compared to other studies? Could something in the experimental design have resulted in this? Is it a result of using juvenile foraminifera?*

The total range in Mg/Ca reported here and the standard deviation in Mg/Ca reported previously (by e.g. Raitzsch et al.) are not directly comparable. In our dataset, the Mg/Ca in *Heterostegina* (average 152 mmol/mol) varies between ~110 and 190, although the SD is less than 25 mmol, or ~16% of the average Mg/Ca. A large difference between the minimum and maximum value does not necessarily result in a high relative standard deviation, particularly when datasets are relatively large (n= 133 for *H. depressa*). Therefore, the variability in Mg/Ca observed is not unusually high in this species and actually comparable to those reported earlier (e.g. Sadekov et al., 2008; De Nooijer et al., 2014a).

For *Amphistegina* (average Mg/Ca = 64 mmol/mol, RSD = 47%, n=188), the variability is indeed relatively high, but as discussed in sections 4.4 and 4.5, the increase in Ba/Ca_{sw} may have an effect on the incorporation of other elements (e.g. Mg) and therefore explain part of the variability in Mg/Ca.

Within treatments (A-E), for example, relative variability in Mg/Ca is much lower (27, 28, 28, 36 and 37%). This has been added to the text (lines 293-294).

16. *Line 363-366. Alternatively, van Dijk et al. [2017] showed that pCO₂ does impact Ba incorporation in Amphistegina, so perhaps the microenvironment carbonate chemistry can help to explain these data.*

We agree with the reviewer and have therefore added a sentence here (lines 367-368) with a reference to Van Dijk et al. (2017).

17. *Line 428-431. This argument is not valid, we would not expect a doubling in O. universa Mg/Ca to exert a resolvable impact on Ba or Sr incorporation. For example, the DSr-Mg/Ca slope for inorganic calcite is 9.1×10^{-4} [Mucci & Morse, 1983], so that a change in shell Mg/Ca of 10 mmol mol⁻¹ would result in a Sr/Ca increase of just ~0.1 mmol mol⁻¹. If the relationship between DBa and Mg/Ca is similar, we would not observe this effect in Orbulina. It is visible in high-Mg species only because the shell Mg/Ca ratios are 1-2 order of magnitude higher than low-Mg foraminifera.*

We agree and have deleted this sentence from the revised version of our manuscript.

18. *Line 446. I think repeating the assertion that miliolids calcify intracellularly should be avoided. As stated a few lines later, it is intracellular only in the sense that calcification takes place from endocytosed seawater, which may well be the case for rotaliid foraminifera as well.*

We agree that the distinction between biomineralization in rotaliids and miliolids is more subtle than stated previously. However, the contribution of ions (mainly DIC and Ca²⁺) delivered to the calcification space through cell membranes (see e.g. Nehrke et al., 2013; Toyofuku et al., 2017) suggests that the contribution of (unmodified) seawater differs greatly between these groups of foraminifera. We have altered the text in these sentences to reflect this more accurately (lines 478-481).

19. *Line 449-450. van Dijk et al. [2017] report Na, Zn and Ba data for miliolid foraminifera.*

We agree and have changed this into: “although so far mainly Sr/Ca and Mg/Ca of Miliolid foraminifera have been published”.

20. *Line 458. You could also reference Hoffmann et al. [2014] Geology 42:579 and Evans et al. [2015] G-cubed 16:2598.*

We have added these references.

21. *Figure 3. It would be more intuitive to plot the final chamber on the right hand side of the graphs, so that time goes forward from left to right.*

We have reversed the order of the chamber number in this figure.

22. *Figure 4. Please use symbols for the two species that are easier to distinguish. Consider plotting the slopes discussed in the text.*

We have changed the symbols for *H. depressa* to increase the contrast with those of *A. lessonii*. In addition, we added the regression lines and their formulas to the figure too.

Typos

1. *Line 183. 'naturel'.*
2. *Line 201. 'costume-built'.*
3. *Line 221. Write out '2'.*
4. *Line 274. Delete 'in a'.*
5. *Line 359. 'maybe'.*

All typos were corrected in the new version of our manuscript.

Ba incorporation in benthic foraminifera

Lennart J de Nooijer^{1*}, Anieke Brombacher^{2,a}, Antje Mewes³, Gerald Langer⁴, Gernot Nehrke³, Jelle Bijma³, Gert-Jan Reichart^{1,2}

¹Royal Netherlands Institute of Sea Research, Dept of Ocean Sciences, Landsdiep 4, 1797 SZ 't Horntje, The Netherlands

*Corresponding author: ldenooijer@nioz.nl

²Utrecht University, Faculty of Geosciences, Budapestlaan 4, 3584 CD Utrecht, The Netherlands

³Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Biogeosciences section, Am Handelshafen 12, 27570 Bremerhaven, Germany

⁴The Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, Devon, PL1 2PB, UK

^anow at: National Oceanography Centre, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK

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

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

34

35 Keywords: foraminifera, Ba/Ca, proxies

36

37 **1 Introduction**

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

51 (Lea and Spero, 1994; Hönisch et al., 2011) and photosymbiont activity (Lea and Spero,
52 1992; Hönisch et al., 2011) do not affect Ba incorporation in foraminiferal shell carbonate.
53 Still, Ba/Ca ratios are known to vary within chamber walls of crust-producing planktonic
54 foraminifera (Eggins et al., 2003; Hathorne et al., 2009). Like Mg/Ca, the values for Ba in
55 crust carbonate are lower, which cannot be (solely) explained by migration to greater water
56 depths during crust formation (Hathorne et al., 2009). This argues for an unknown additional
57 imprint on Ba incorporation. On an intra-test scale, the distributions of Mg and Ba within the
58 test wall of *Pulleniatina obliquiloculata* have been shown to co-vary to some extent, with
59 maximum concentrations often, but not always, coinciding with the ‘organic linings’
60 (Kunioka et al., 2006). For some other elements, including Mg and Sr, incorporation has been
61 shown to be inter-dependent (e.g. Mewes et al., 2015). Such interdependency, however, varies
62 between pairs of elements and is explained by a combination of simultaneous fractionation by
63 the same process (e.g. Langer et al., 2016) and by involvement of different processes during
64 calcification (Nehrke et al., 2013). These models and experimental results may imply that also
65 the incorporation of Ba could be influenced by these physiological processes and/ or the same
66 fractionation process during calcite precipitation (e.g. through lattice distortion; Mucci and
67 Morse, 1983; Mewes et al., 2015).

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

76 benthics may be more suitable to reconstruct salinity in coastal and shelf seas (Weldeab et al.,
77 2007; 2014; Bahr et al., 2013). The range in Mg/Ca is known particularly for benthic
78 foraminifera (e.g. Toyofuku et al., 2011; Sadekov et al., 2014) and inter-species variability in
79 Ba incorporation may therefore hamper application of (benthic) foraminiferal Ba/Ca. Here we
80 present results from a culture study using the larger benthic foraminifera, *Amphistegina*
81 *lessonii* and *Heterostegina depressa*, two species with different Mg/Ca (~50 mmol/mol;
82 Segev and Erez, 2006 and ~120 mmol/mol; Dueñas-Bohórquez et al., 2011, respectively). In
83 these culturing experiments, the range in Ba/Ca exceeds the naturally occurring range in
84 seawater to facilitate testing underlying controls on barium incorporation. **If there is a linear**
85 **increase in shell Ba/Ca (Ba/Ca_{cc}) with increasing seawater Ba/Ca (Ba/Ca_{sw}), the large range in**
86 **Ba/Ca of the culturing media prepared here will furthermore decrease uncertainty of the**
87 **obtained Ba/Ca_{cc} - Ba/Ca_{sw} calibration. Our results are compared to Ba/Ca in these species**
88 **from field samples.** Together, calibration of Ba/Ca in these species against seawater Ba/Ca
89 and in the context of other elemental incorporation data, allows evaluation and application of
90 incorporated Ba across a wider range of foraminiferal taxa, with contrasting element
91 composition of their shell.

92

93 **2 Methods**

94 *2.1 Culture media*

95 To determine Ba/Ca partitioning, benthic foraminiferal culture experiments were set up with
96 five different seawater Ba/Ca ratios (54-92 $\mu\text{mol/mol}$). Media were prepared by increasing
97 $[Ba^{2+}]_{sw}$ while keeping the $[Ca^{2+}]_{sw}$ constant. The range of $[Ba^{2+}]$ used in these experiments
98 exceeds the range of concentrations found naturally and allows testing the applicability of
99 partition coefficients under conditions with artificially high seawater Ba/Ca. Seawater is only
100 slightly undersaturated with respect to barite ($BaSO_4$) and an increase in $[Ba^{2+}]$ in the sea

101 water will cause barite precipitation (Langer et al., 2009). To be able to increase $[Ba^{2+}]$
102 beyond its natural range, artificial seawater was prepared with lower sulphate contents. All
103 other salts were added according to the recipe of Kester et al. (1967) to produce a total of 5
104 litres of medium for each treatment. As *Amphistegina lessonii* and *Heterostegina depressa* do
105 not grow well in 100% artificial seawater, the prepared media were mixed with natural
106 seawater in a ratio 9:1 (Mewes et al., 2014). To double check concentrations and determine
107 potential loss of elements due to precipitation, sorption and/or scavenging, element
108 concentrations of the culture media were determined by ICP-OES at the Alfred-Wegener-
109 Institute in Bremerhaven, except for Ba which was measured by ICP-MS at Utrecht
110 University (Table 1).

111 Culture media pH was adjusted to 8.0 by adding NaOH (1 M) to the prepared media. Before
112 the start of the experiments, dissolved inorganic carbon (DIC) and total alkalinity were
113 measured at the Alfred-Wegener-Institute. DIC was measured photometrically in triplicates
114 with a TRAACS CS800 QuAAtro autoanalyser with an average reproducibility of $\pm 10 \mu\text{mol}$
115 L^{-1} . Alkalinity was calculated from linear Gran plots (Gran, 1952) after triplicate
116 potentiometric titration (Bradshaw et al., 1981) using a TitroLine alpha plus auto sampler
117 (Schott Instruments). Parameters of the total carbonate system were calculated from
118 temperature, salinity, DIC and alkalinity using the program CO2SYS (Lewis and Wallace,
119 1998) adapted to Excel by (Pierrot et al., 2006). The equilibrium constants K1 and K2 from
120 Mehrbach et al. (1973), as reformulated by Dickson and Millero (1987) were used (Table 1).

121

122 2.2 Foraminiferal culturing

123 Living specimens of *A. lessonii* and *H. depressa* were isolated from sediment collected at the
124 tropical aquarium of Burger's Zoo (Arnhem, The Netherlands) in August 2012 and transferred
125 to the Alfred-Wegener-Institute for the culture experiments. Healthy individuals of *A. lessonii*

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

145 All specimens were fed *Dunaliella salina* algae every three to four days. Although *A. lessonii*
146 hosts symbionts, this foraminiferal species does not exclusively rely on nutrients from their
147 symbionts, but also ingests algae (Lee, 2006). To avoid changes in the barium concentration
148 of the culture media, the water in the dishes containing foraminifera were diluted as little as
149 possible by the solution containing the food for the foraminifera. For this purpose,
150 foraminifera were fed 50 μl of a solution containing algae that was centrifuged at 2000 rpm

151 for 10 minutes. Algae concentrated at the bottom of the tube were transferred to an empty
152 tube with a pipette. To prevent changes in the culture media's carbonate chemistry by algal
153 photosynthesis the algae were killed by heating the concentrated solution in an oven at 90 °C
154 for 10 minutes. The cultures were transferred to new Petri dishes every week to avoid
155 excessive bacterial growth, potential build-up of waste products and shortage of ions or
156 nutrients. To prevent changes in salinity by evaporation media were refreshed **three days** after
157 the cultures were transferred to new dishes by pipetting approximately 5 ml of the old media
158 out of the Petri dish and replacing it with the same volume of media from the prepared batch.

159

160 *2.3 Sample preparation and analysis*

161 At the end of the culture experiment, specimens were cleaned by placing them in a 7% NaOCl
162 solution for approximately 30 minutes until completely bleached and organic material was
163 removed from the tests. This cleaning method is shown to have a similar impact on average
164 foraminiferal Ba/Ca values as cleaning with H₂O₂ **and is relatively small (2-3 μmol/mol)**
165 **compared to cleaning with de-ionized water only (Pak et al., 2004)**. Specimens were then
166 rinsed three times for approximately 60 seconds in de-ionized water to remove the NaOCl and
167 any residual salts from the culture solutions. Cleaned foraminifera were put in an oven at 42
168 °C until completely dry and mounted on sample holders using double sided adhesive tape.

169 Element composition of the calcite was determined using Laser Ablation-Inductively Coupled
170 Plasma-Mass Spectrometry (LA-ICP-MS) at Utrecht University (Reichert et al., 2003). **The**
171 **system consisted of a Geolas 200Q 193 nm Excimer laser (Lambda Physik) connected to a**
172 **sector field-ICP-MS (Element2, Thermo Scientific). Samples were ablated in a single-volume**
173 **chamber and the aerosol was carried to the ICP-MS by a Helium flow. Monitored masses**
174 **included ²³Na, ²⁴Mg, ²⁶Mg, ²⁷Al, ⁴³Ca, ⁴⁴Ca, ⁵⁵Mn, ⁸⁸Sr, ¹³⁸Ba and ²³⁸U** and calibration was
175 performed using a glass standard (NIST 610) that was ablated three times after every 10-12

176 foraminiferal samples. Diameter of the ablation crater was set to 80 μm for all specimens and
177 pulse repetition rate was 6 Hz. The ablated calcite was measured and integrated with respect
178 to time. Energy density for the glass was higher than for the foraminifera (5 J/cm^2 and 1
179 J/cm^2 , respectively). Although the resulting difference in ablation characteristics is not likely
180 to affect obtained foraminiferal element concentrations (Hathorne et al., 2008), foraminiferal
181 element concentrations were compared to those from an in-house made calcite standard with
182 known element concentrations and ablated at the same energy density as the foraminifera
183 (Dueñas-Bohórquez et al., 2009). Relative standard deviation for Mg/Ca and Sr/Ca based on
184 repeated measurements on this material was <5% for both ratios. Due to the lamellar nature of
185 Rotallid foraminifera, final chambers are thinnest and are therefore characterized by largest
186 uncertainty in the estimated average element/Ca ratio. Therefore, the F chamber was not
187 considered and instead, the F-1 chamber of *A. lessonii* was ablated for every specimen. For *H.*
188 *depressa*, walls of the final two chambers were commonly too thin for reliable chemical
189 results and, therefore, the F-2 chamber was analysed. In addition, for each species, the final 6-
190 7 chambers of ten sufficiently large specimens (2 from each of the five treatments) were
191 ablated to analyse intra-specimen variability in Ba/Ca, to analyse variability within chamber
192 walls as a function of thickness and to detect potential ontogenetic trends in Ba incorporation.
193
194 Elemental concentrations were calculated from the ablation profiles with the Glitter software,
195 using ^{43}Ca as internal standard and values from Jochum et al. (2011) for concentrations of
196 elements in the NIST 610. This program integrates the ablation signal after subtracting the
197 background signal to calculate the elemental concentrations. To avoid contaminated intervals
198 of the ablation profile, sections with high ^{27}Al and ^{55}Mn counts were excluded from the
199 analysis since these parts are often also characterized by unusually high Mg/Ca not reflecting
200 the actual shell carbonate. Ablation profiles with a duration shorter than 5 seconds were

201 rejected as such short profiles are unreliable due to poor counting statistics. Nine out of 188
202 ablation profiles were rejected for *A. lessonii* and 7 out of 140 profiles from *H. depressa* were
203 discarded, which is less than 5%.

204

205 2.4 *Aquarium samples*

206 To compare the results from cultured specimens with Ba/Ca from specimens derived from
207 'natural conditions', a number of living specimens of both *A. lessonii* and *H. depressa* were
208 isolated from the Zoo's stock (i.e. sediment collected at the zoo from which the specimens
209 were isolated; section 2.2) and cleaned and prepared for LA-ICP-MS analyses as described in
210 2.3. From both species, 7 specimens were ablated twice at the Royal NIOZ using a
211 NWR193UC (New Wave Research) laser, containing an ArF Excimer laser (Existar) with
212 deep UV 193 nm wavelength and <4 ns pulse duration. Provided that the same reference
213 material is used, the use of multiple laser systems (see above) is shown not to bias obtained
214 foraminiferal element/Ca ratios (De Nooijer et al., 2014a). Laser ablation was performed with
215 an energy density of 1 J/cm² at a repetition rate of 6 Hz for calcite samples and an energy
216 density of 5 J/cm² for the glass (NIST610) standards. Helium was used as a carrier gas with a
217 flow rate of 0.8 L/min for cell gas and 0.3 L/min for cup gas. From the laser chamber to the
218 quadrupole ICP-MS (iCAP-Q, Thermo Scientific), the He flow was mixed with ~0.4 L/min
219 nebulizer Ar. Before measuring the samples, the nebulizer gas, extraction lens, CCT focus
220 lens and torch position were automatically tuned for the highest sensitivity of ²⁵Mg by laser
221 ablating MACS-3. The masses measured by the ICP-MS were ²³Na, ²⁴Mg, ²⁵Mg, ²⁷Al, ⁴³Ca,
222 ⁴⁴Ca, ⁸⁸Sr and ¹³⁸Ba. JcP-1, MACS-3 and an in-house (foraminiferal) calcite standard (NFHS)
223 were used for quality control and measured every 10 foraminiferal samples. Internal
224 reproducibility of the analyses was all better than 9%, based on the three different carbonate
225 standards used. Intensity data were integrated, background subtracted, standardized internally

226 to ^{43}Ca and calibrated against the MACS-3 signal using a custom-built MATLAB routine
227 within the program SILLS (Guillong et al., 2008). Since ablation of the NIST SRM 610 and
228 NIST SRM 612 could increase the sodium background, they were only ablated and analyzed
229 at the end of every sequence and cones were cleaned before the next sequence. Accuracy of
230 the analyses was better than 3%, based on comparing the carbonate standards with
231 internationally reported values (Okai et al., 2002, Wilson et al., 2008). Signals were screened
232 for surface contamination and parts of the outside or inside of the shell with elevated Mg, Mn
233 or Al values were eliminated from the area selected for integration.

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

238

239 **3 Results**

240 *3.1 Test diameter increase*

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

249

250 *3.2 Barium incorporation*

251 Calcite Ba/Ca increases linearly with seawater Ba/Ca for both species (Figure 2; Table 2).
252 ANOVA performed on the individual data points combined with regression analyses reveals a
253 significant increase of Ba/Ca_{cc} with Ba/Ca_{sw} for both species (Table 3). Calculated regression
254 slopes result in a D_{Ba} of 0.326 (±0.005) for *A. lessonii* and 0.777 (±0.007) for *H. depressa*
255 (Figure 3, solid lines). Regression lines are forced through zero as it seems reasonable to
256 assume that no Ba is incorporated into calcite when the Ba concentration in the seawater is
257 zero. Without this forcing, regression slopes would be $Ba/Ca_{cc} = 0.34 * Ba/C_{asw} - 1.1$ for *A.*
258 *lessonii* and $Ba/Ca_{cc} = 0.92 * Ba/C_{asw} - 10$ for *H. depressa*. The resulting partition coefficients
259 ((Ba/Ca_{cc})/(Ba/Ca_{sw})) are constant and significantly different between the species (ANOVA)
260 (~0.3 for *A. lessonii* and ~0.8 for *H. depressa*) over the range of seawater Ba/Ca studied here.
261 The regression line for Ba/Ca_{cc} as a function of Ba/Ca_{sw} for *A. lessonii* corresponds well with
262 that reported for a number of different low Mg species (Lea and Boyle, 1989).
263 The aquarium-derived specimens 'aquarium samples' had an diameter ranging from 550 to
264 1180 µm (with an average of 975 µm) for *A. lessonii* and from 1380 to 2340 µm (average:
265 1936 µm) for *H. depressa*. They had an average Ba/Ca of 15.4 (±2.3 SD) µmol/mol for *A.*
266 *lessonii* and 35.7 (±14 SD) µmol/mol for *H. depressa*. In combination with the measured
267 aquarium's seawater Ba/Ca of 35.7 (±3.9 SD) µmol/mol, the partition coefficients for Ba vary
268 between 0.43 and 1.0 for *A. lessonii* and *H. depressa*, respectively. The aquarium derived data
269 is consistent with the controlled growth derived data, but it was not used in the regression
270 analysis (Figure 2) since the conditions (e.g. carbonate chemistry) under which the specimens
271 from the aquarium were grown, were not determined as precisely and accurately as in our
272 culturing experiment. Including these data in the linear regression (Figure 2) would change the
273 sensitivity from 0.78 to 0.77 for *H. depressa* and from 0.33 to 0.32 for *A. lessonii*.

274

275 *3.3 Intrachamber variability in Ba/Ca*

276 From both species, 10 specimens were used to quantify the relation between ontogeny (i.e.
277 size-dependent) and Ba incorporation into foraminiferal calcite. For this purpose, the final 6-7
278 chambers of these individuals were ablated (Figure 3). With the selected spot diameter (80
279 μm), ablation of a small amount of material of adjacent chambers could not always be
280 avoided. Some chamber walls, particularly of the youngest (i.e. built latest) chambers, were
281 too thin for reliable measurements and were excluded from further consideration.

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

286

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

293

294 3.4 Relation between incorporation of barium and magnesium

295 Combining data from all five treatments, average Mg/Ca of *A. lessonii* was 64 mmol/mol ,
296 with a relative standard deviation of 47%. Within treatments, the variability in Mg/Ca is
297 considerably lower (between 27 and 37%). Average Mg/Ca in *H. depressa* was 152
298 mmol/mol , with a standard deviation of 25 mmol/mol (16%). Within treatments, the relative
299 standard deviation ranged from 4.1% (treatment E) to 17% (treatment D). The species-
300 specific single-chamber Mg/Ca and Ba/Ca combined for all treatments are positively and

301 significantly related (Figure 4). For *A. lessonii*, $Mg/Ca = 3.1 * Ba/Ca - 3.6$ (t-value = 12.2, $p <$
302 0.01 for the slope of the regression) and for *H. depressa*, $Mg/Ca = 1.1 * Ba/Ca + 92$ (t-value =
303 14.8 , $p < 0.01$ for the slope). The slopes of these two regressions (3.1 and 1.1) are significantly
304 different: this is calculated by $z = (a_{Heterostegina} - a_{Amphistegina}) / \sqrt{(SE_{a,Heterostegina}^2 +$
305 $SE_{a,Heterostegina}^2)}$, where a is the value for the regression's slope and SE_a is the slope's
306 associated standard error. For the slopes of the Mg/Ca-Ba/Ca regressions for *Amphistegina*
307 and *Heterostegina*, the resulting z-score is higher than >7 , indicating that the two slopes are
308 significantly different.

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

317

318 **4 Discussion**

319 *4.1 Test diameter increase*

320 The range of Ba concentrations used in the experiments did not influence the increase in shell
321 diameter of either foraminiferal species (Figure 1). Compared to *H. depressa*, increases in
322 shell diameter (which is proportional to the chamber addition rate) for *A. lessonii* were
323 slightly more variable. To prevent barite precipitation it was necessary to reduce the sulphate
324 concentration below that typically measured in natural seawater. Sulphate concentrations
325 between 0.1 and 1 mmol/L do not affect inorganic calcite growth (Reddy and Nancollas,

326 1976), but a decrease in growth rates of approximately 30% was observed in coccolithophores
327 growing in artificial seawater with a sulphate concentration 10% that of natural seawater
328 (Langer et al., 2009). Although coccolithophores and foraminifera may respond differently to
329 lowered sulphate concentrations, this reduction could have hampered growth of the specimens
330 in our culturing experiment. Chamber addition rates of *A. lessonii* in a culture set-up with a
331 sulphate concentrations similar to that of natural seawater (Mewes et al., 2014) were
332 approximately 20% higher than chamber addition rates observed in our experiments. Since
333 these experiments were not performed simultaneously using specimens from the same batch,
334 it is not straight forward to compare absolute rates and therefore the 20% difference cannot
335 unambiguously be attributed to sulphate concentration (Hoppe et al. 2011). Unfortunately no
336 data exist on the effect of reduced sulphate concentrations on the uptake of trace elements in
337 foraminiferal calcite. However Langer et al. (2009) demonstrated that sulphate limitation had
338 no discernible effect on Ba incorporation in coccolithophore calcite.

339

340 *4.2 Barium incorporation*

341 The variability in Ba/Ca between individual ablation craters is considerable, but the average
342 foraminiferal Ba/Ca shows a consistent relation with seawater Ba/Ca. This implies that the
343 observed variability is a reflection of the inhomogeneous distribution in the test and hence
344 filtered out when averaging. This is similar to the behavior for Mg and Sr (Sadekov et al.,
345 2008; Wit et al., 2012; De Nooijer et al., 2014a) and underscores the power of single-chamber
346 analyses. If present, inhomogeneity in test wall Ba/Ca in combination with different cross
347 section sampled during the ablation potentially account for the observed variability. This
348 would imply that although large differences are observed within a test wall, the average still
349 reliably reflects sea water concentration (this paper) and for Mg, still reflects seawater
350 temperature (Hathorne et al., 2009). Comparing within-specimen and between-specimen

351 variability, De Nooijer et al. (2014a) showed that within specimen variability **does not**
352 **account for all of the observed variability in Mg/Ca** in *Ammonia tepida*. This seems to be
353 similar for Ba/Ca (compare Figure 4 in this paper with Figure 5 from De Nooijer et al.,
354 2014a), which would mean that **at least** 20 chambers need to be analyzed to reach a 5%
355 relative precision (De Nooijer et al., 2014a). This is not limited by the analytical precision, but
356 rather due to inherent biological inter-chamber and inter-specimen variability. **To reduce**
357 **ontogenetic variability (in e.g. paleoceanographic applications where complete specimens are**
358 **measured), a narrow size fraction should be analyzed.**

359 Incorporation of Ba in *H. depressa* shows a partitioning which is about 2.5 times higher than
360 in *A. lessonii*. Such a large offset of D_{Ba} between benthic species **fits previously reported**
361 **(differences in) partition coefficients** for barium. Lea and Boyle (1989) found $D_{Ba} = 0.37 \pm$
362 0.06 for *Cibicidoides wuellerstorfi*, *Cibicidoides kullenbergi* and *Uvigerina* spp. for a series of
363 core tops, comparable to the partition coefficient reported here for *A. lessonii* (0.33 ± 0.022 ;
364 Figure 2). In contrast, partition coefficients for Ba in planktonic foraminifera are roughly only
365 twice as low as these benthic foraminiferal partitioning coefficients (0.14-0.19; Hönisch et al.,
366 2011; Lea and Boyle, 1991; Lea and Spero, 1992). Although temperature, pH, salinity and
367 pressure were initially proposed as potential explanation for the offset between planktonic and
368 benthic D_{Ba} (Lea and Boyle, 1991; Lea and Spero, 1992), studies by Lea and Spero (1994)
369 and Hönisch et al. (2011) showed no significant impact of temperature, pH and salinity on Ba
370 incorporation into planktonic foraminiferal calcite. This would leave hydrostatic pressure to
371 explain the difference between benthic and planktonic species. **Van Dijk et al. (2017) on the**
372 **other hand, showed that in a number of larger benthic foraminifera, Ba/Ca is positively**
373 **influenced by pCO_2 .** Our observations show, however, that the observed differences in D_{Ba}
374 between *H. depressa* and *A. lessonii* and also the offset with the planktonic species are
375 inherent to these species. **A small impact of environmental parameters other than seawater**

376 Ba/Ca may account for the slightly higher D_{Ba} in the foraminifera taken from the aquarium
377 compared to the cultured ones (Figure 2). The overall differences in partitioning seem to
378 coincide with different taxonomic groups, which may indicate that foraminifera may differ in
379 their controls on transporting ions from seawater to the site of calcification. For example, the
380 contribution of transmembrane transport versus that of seawater transport (i.e. leakage;
381 Nehrke et al., 2013 or vacuolization; Erez, 2003) may vary between species and thereby
382 account for differences in Mg/Ca, Ba/Ca, etc. (Nehrke et al., 2013).

383

384 4.3 Inter-chamber variability of Ba/Ca_{cc}

385 In both species cultured here, Ba/Ca_{cc} decreases significantly from largest (i.e. built latest in
386 life) towards the smaller chambers (Figure 3). Observed trends were not significantly different
387 between *A. lessonii* and *H. depressa*, suggesting that Ba/Ca_{cc} decreases at the same rate with
388 size, despite the overall difference in Ba/Ca_{cc} (Figure 3). Since we always analyzed chambers
389 at the same position (F-1 for *A. lessonii* and F-2 for *H. depressa*) and since the final size of the
390 cultured specimens was similar between treatments (Figure 1), ontogenetic trends in Ba/Ca do
391 not influence the trends in Ba/Ca between treatments (Figure 2). Several other studies showed
392 that element/Ca ratios can vary with chamber position. Raitzsch et al. (2011), for example,
393 reported increasing B/Ca and decreasing Mg/Ca towards younger chambers in the benthic
394 *Planulina wuellerstorfi*. Such patterns may be related to changes in the surface-to-volume
395 ratio or relative changes in “vital effects” as foraminifera grow larger. For example, pH
396 reduction in the foraminiferal microenvironment is related to the specimen’s size (Glas et al.,
397 2012) and may thereby affect the chemical speciation of minor and trace element, which in
398 turn, may determine their uptake rates. Hönisch et al. (2011), however, showed that seawater
399 pH has no noticeable effect on Ba incorporation in planktonic foraminiferal calcite, rendering
400 changes in the pH of the foraminiferal microenvironment an unlikely explanation to account

401 for the observed chamber-to-chamber variability in Ba/Ca. Alternatively, changes in the
402 metabolic rate, the instantaneous calcification rate, or a different partitioning between the
403 impacts of the life processes may lead to the observed ontogenetic trend.

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

413

414 *4.4 Coupled incorporation of barium and magnesium*

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

426 When correcting for the different seawater Ba/Ca and Mg/Ca between treatments,
427 incorporated Ba and Mg correlate similarly within, as well as, between the two species
428 studied here (Figure 4). This suggests that these elements are coupled during
429 biomineralization itself and that the ratio of Ba and Mg in seawater is preserved during
430 calcification by these species of foraminifera. When comparing the relation between Ba/Ca
431 and Mg/Ca from other benthic species (e.g. Lea and Boyle, 1989; figure 2; more refs), the
432 coupling between Ba- and Mg-incorporation is likely similar across a wide range of benthic
433 foraminiferal species.

434

435 *4.5 Biomineralization and element incorporation*

436 Foraminiferal biomineralization determines incorporation of many elements and fractionation
437 of many isotopes during the production of new chambers as indicated by overall large
438 compositional differences between inorganically precipitated and foraminiferal calcite (Erez,
439 2003; Bentov and Erez, 2006; Nehrke et al., 2013; De Nooijer et al., 2014b). For example,
440 Mg/Ca ratios in many species are orders of magnitude lower than what is expected from
441 inorganic precipitation experiments. Additionally, Mg/Ca varies considerably between
442 foraminiferal species and especially between species known to have different calcification
443 strategies (Bentov and Erez, 2006; Toyofuku et al., 2011; Wit et al., 2012; De Nooijer et al.,
444 2009; 2014b). Other elements such as Sr (e.g. Elderfield et al., 2000) and B/Ca (e.g. Allen et
445 al., 2012) also vary significantly between species. Generally, concentrations for these
446 elements correlate within taxa and hence species incorporating relatively much Mg, also have
447 high (for example) Sr/Ca, B/Ca and Na/Ca. Miliolids and many 'Large Benthic Foraminifera'
448 (LBF) produce calcite with Mg/Ca up to 100-150 mmol/mol (Toyofuku et al., 2000; Dueñas-
449 Bohórquez et al., 2011; Sadekov et al., 2014; Evans et al., 2015), while most planktonic and
450 symbiont-barren benthic foraminifera produce test calcite with Mg/Ca values ranging from 1-

451 10 mmol/mol (e.g. Nürnberg et al., 1996; Elderfield et al., 2002; Lear et al., 2010; Wit et al.,
452 2012; De Nooijer et al., 2014b). The same distinction is observed for B/Ca (compare e.g.
453 Allen et al., 2012 and Kazcmarek et al., 2015), Li/Ca (Lear et al., 2010 versus Evans et al.,
454 2015), Na/Ca (Wit et al., 2013 versus Evans et al., 2015) and Sr/Ca (e.g. Dueñas-Bohórquez
455 et al., 2011). The correlation between relatively high (for example) Mg/Ca, Sr/Ca and B/Ca
456 corresponds to the observed trends in the data presented here for Ba/Ca and Mg/Ca in *H.*
457 *depressa* and *A. lessonii* (Figure 4). The Mg/Ca in the former species is approximately 2.5
458 times that of the latter, which is similar to the difference observed in Ba/Ca ratios between
459 these species and implies that Ba changes in concert with Mg, which is consistent with the
460 single-chamber correlation between Mg/Ca and Ba/Ca (Figure 4). Such a change could
461 potentially be caused inorganically by differences in Mg opening up the crystal lattice in such
462 a way that it can accommodate more or less Ba. Such a mechanism is described for Mg and Sr
463 (e.g. Morse and Bender, 1990; Mucci and Morse, 1983; Mewes et al., 2015; Langer et al.,
464 2016) and may also apply to Ba incorporation and the influence of Mg ions that increase
465 stress in the calcite crystal lattice. Unless the strain of incorporated Mg ions does not increase
466 linearly with its concentration, the covariance between Mg and in this case Ba may well be
467 interrelated during an earlier stage of the biomineralization process, e.g. during their transport
468 from the surrounding seawater into the site of calcification (Erez, 2003; De Nooijer et al.,
469 2014b).

470 Interestingly, the partitioning of different elements is not the same between taxa. For example,
471 Sr/Ca in LBFs is approximately twice as high (Dueñas-Bohorquez et al., 2011; Evans et al.,
472 2015) as in planktonic species (Elderfield et al., 2002; Dueñas-Bohórquez et al., 2009; Hendry
473 et al., 2009), whereas the ratio between the D_{Mg} of these groups is between 10 and 100 (see
474 above). Comparing the offset of D between groups as a function of D itself shows an
475 approximate logarithmic correlation (Figure 5). The distinction between the two groups on

476 basis of their element signature coincides with known differences in biomineralization
477 controls. Element controls in low-Mg species are thought to be determined by (highly)
478 selective trans-membrane ion transporters, (limited) leakage of seawater into the site of
479 calcification and/or selective Mg^{2+} -removal (Nehrke et al., 2013; De Nooijer et al., 2014b;
480 Toyofuku et al., 2017). Miliolid foraminifera belong to the high-Mg foraminiferal group and
481 are known to secrete their calcite within vesicles that are hypothesized to contain seawater,
482 which may be modified after endocytosis (Hemleben et al., 1986; Ter Kuile and Erez, 1991;
483 De Nooijer et al., 2009). These intracellular vesicles may therefore contain relatively high
484 concentrations of Mg^{2+} , Ba^{2+} and other ions present in seawater, although so far mainly Sr/Ca
485 and Mg/Ca of Miliolid foraminifera have been published (supplementary information). The
486 biomineralization of non-Miliolid, intermediate- and high-Mg benthic foraminifera may
487 employ characteristics of both these types of calcification and therefore incorporate
488 moderately to high concentrations of elements (cf Segev and Erez, 2006).

489

490 5 Conclusions

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

501 previously observed variability in element/Ca ratios between foraminifera taxa and likely
502 reflects differences in their biomineralization mechanisms.

503

504 **References**

505 Allen, K.A., Hönisch, B., Eggins, S.M., Yu, J., Spero, H.J., Elderfield, H., 2011. Controls on
506 Boron incorporation in cultured tests of the planktic foraminifer *Orbulina universa*.
507 Earth Planet. Sci. Lett. 309, 291-301.

508 Allen, K.A., Hönisch, B., Eggins, S.M., Rosenthal, Y., 2012. Environmental controls on B/Ca
509 in calcite tests of the tropical planktic foraminifer species *Globigerinoides ruber* and
510 *Globigerinoides sacculifer*. Earth Planet. Sci. Lett. 351-352, 270-280.

511 Babila, T.L., Rosenthal, Y., Conte, M.H., 2014. Evaluation of the biogeochemical controls on
512 B/Ca of *Globigerinoides ruber* from the Ocean Flux Program, Bermuda. Earth
513 Planet. Sci. Lett. 404, 67-76.

514 Bahr, A., Schönfeld, J., Hoffmann, J., Voigt, S., Aurahs, R., Kucera, M., Slögel, S., Jentzen,
515 A., Gerdes, A., 2013. Comparison of Ba/Ca and $\delta^{18}\text{O}_{\text{water}}$ as freshwater proxies: A
516 multi-species core-top study on planktonic foraminifera from the vicinity of the
517 Orinoco River mouth. Earth Planet. Sci. Lett. 383, 45-57.

518 Bentov, S., Erez, J., 2006. Impact of biomineralization processes on the Mg content of
519 foraminiferal shells: A biological perspective. *Geochem. Geophys. Geosyst.* 7,
520 Q01P08.

521 Bian, N., Martin, P.A., 2010. Investigating the fidelity of Mg/Ca and other element data from
522 reductively cleaned planktonic foraminifera. *Paleoceanography* 25, PA2215.

523 Boyle, E.A., 1981. Cadmium, zinc, copper and barium in foraminifera tests. Earth Planet. Sci.
524 Lett. 53, 11-35.

525 Bradshaw, A.L., Brewer, P.G., Shafer, D.K., Williams, R.T., 1981. Measurements of total
526 carbon dioxide and alkalinity by potentiometric titration in the GEOSECS program.
527 Earth Planet. Sci. Lett. 55, 99-115.

528 Broecker W.S., Peng T.-H., 1982. Tracers in the Sea, Eldigo Press, Lamont-Doherty
529 Geological Observatory, Palisades, New York, USA, pp. 690.

530 Bryan, S.P., Marchitto, T.M., 2008. Mg/Ca-temperature proxy in benthic foraminifera: New
531 calibrations from the Florida Straits and a hypothesis regarding Mg/Li.
532 Paleocronography 23, PA2220.

533 Chan, L.H., Drummond, D., Edmond, J.M., Grant, B., 1977. On the barium data from the
534 Atlantic GEOSECS expedition. Deep-Sea Res. 24, 613-649.

535 Dawber, C.F., Tripathi, A., 2012. Relationships between bottom water carbonate saturation and
536 element/Ca ratios in coretop samples of the benthic foraminifera *Oridorsalis*
537 *umbonatus*. Biogeosciences 9, 3029-3045.

538 De Nooijer, L.J., Toyofuku, T., Kitazato, H., 2009. Foraminifera promote calcification by
539 elevating their intracellular pH. Proc. Natl. Acad. Sci. USA 106, 15374-15378.

540 De Nooijer, L.J., Hathorne, E.C., Reichart, G.J., Langer, G., Bijma, J., 2014a. Variability in
541 calcitic Mg/Ca and Sr/Ca ratios in clones of the benthic foraminifer *Ammonia tepida*.
542 Marine Micropaleontology 107, 33-43.

543 De Nooijer, L.J., Spero, H.J., Erez, J., Bijma, J., Reichart, G.J., 2014b. Biomineralization in
544 perforate foraminifera. Earth-Sci. Rev. 135, 48-58.

545 Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for dissociation
546 constants of carbonic acid in seawater media. Deep Sea Res. 34, 1733-1743.

547 Dueñas-Bohórquez, A., Da Rocha, R., Kuroyanagi, A., Bijma, J., Reichart, G.J., 2009. Effect
548 of salinity and seawater calcite saturation state on Mg and Sr incorporation in
549 cultured planktonic foraminifera. Marine Micropaleontology 73, 178-189.

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

553 Eggins, S., De Deckker, P., Marshall, J., 2003. Mg/Ca variation in planktonic foraminifera
554 tests: implications for reconstructing palaeo-seawater temperature and habitat
555 migration. *Earth Planet. Sci. Lett.* 212, 291-306.

556 Elderfield, H., Cooper, M., Ganssen, G., 2000. Sr/Ca in multiple species of planktonic
557 foraminifera: Implications for reconstructions of seawater Sr/Ca. *Geochem.*
558 *Geophys. Geosyst.* 1, GC000031.

559 Elderfield, H., Vautravers, M., Cooper, M., 2002. The relationship between shell size and
560 Mg/Ca, Sr/Ca, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$ of species of planktonic foraminifera. *Geochem.*
561 *Geophys. Geosyst.* 3, GC000194.

562 Elderfield, H., Yu, J., Anand, P., Kiefer, T., Nyland, B., 2006. Calibrations for benthic
563 foraminiferal Mg/Ca paleothermometry and the carbonate ion hypothesis. *Earth*
564 *Planet. Sci. Lett.* 250, 633-649.

565 Erez, J., 2003. The source of ions for biomineralization in foraminifera and their implications
566 for paleoceanographic proxies. *Rev. Mineral. Geochem.* 54, 115-149.

567 Evans, D., Erez, J., Oron, S., Müller, W., 2015. Mg/Ca-temperature and seawater-test
568 chemistry relationships in the shallow-dwelling large benthic foraminifera
569 *Operculina ammonoides*. *Geochim. Cosmochim. Acta* 148, 325-342.

570 Foster, G.L., 2008. Seawater pH, $p\text{CO}_2$ and $[\text{CO}_3^{2-}]$ variations in the Caribbean Sea over the
571 last 130 kyr: A boron isotope and B/Ca study of planktonic foraminifera. *Earth and*
572 *Planetary Science Letters* 271, 254-266.

573 Glas, M.S., Langer, G., Keul, N., 2012. Calcification acidifies the microenvironment of a
574 benthic foraminifer (*Ammonia* sp.). *J. Exp. Mar. Biol. Ecol.* 424-425, 53-58.

575 Gran, G., 1952. Determination of the equivalence point in potentiometric titrations-- Part II.
576 The Analyst 77, 661-671.

577 Guay, C.K., Falkner, K.K., 1997. Barium as a tracer of Arctic halocline and river waters,
578 Deep Sea Res. Part II 44, 1543– 1569.

579 Guay, C.K., Falkner, K.K., 1998. A survey of dissolved barium in the estuaries of major
580 Arctic rivers and adjacent seas. Cont. Shelf Res. 18, 859– 882.

581 Guillong, M., Meier, D.L., Allan, M.M., Heinrich, C.A., Yardley, B.W.D., 2008. SILLS: A
582 MATLAB-based program for the reduction of laser ablation ICP-MS data of
583 homogeneous materials and inclusions. Mineralogical Association of Canada Short
584 Course 40, 328-333.

585 Hall, J.M., Chan, L.-H., 2004a. Li/Ca in multiple species of benthic and planktonic
586 foraminifera: Thermocline, latitudinal, and glacial-interglacial variation. Geochim.
587 Cosmochim. Acta 68, 529-545.

588 Hall, J.M., Chan, L.-H., 2004b. Ba/Ca in *Neogloboquadrina pachyderma* as an indicator of
589 deglacial meltwater discharge into the western Arctic Ocean. Paleoceanography 19,
590 PA000910.

591 Hanor, J.S., Chan, L.-H., 1977. Non-conservative behavior of barium during mixing of
592 Mississippi River and Gulf of Mexico waters. Earth Planet. Sci. Lett. 37, 242–250.

593 Hathorne, E.C., James, R.H., Savage, P., Alard, O., 2008. Physical and chemical
594 characteristics of particles produced by laser ablation of biogenic calcium carbonate.
595 J. Anal. Atom. Spectrom. 23, 240-243.

596 Hathorne, E.C., James, R.H., Lampitt, R.S., 2009. Environmental versus biomineralization
597 controls on the intratest variation in the trace element composition of the planktonic
598 foraminifera *G. inflata* and *G. scitula*. Paleoceanography 24, PA001742.

599 Hemleben, C., Anderson, O.R., Berthold, W., Spindler, M., 1986. Calcification and chamber
600 formation in foraminifera - an overview. pp 237-249. In: Biomineralization in lower
601 plants and animals, Leadbeater, B.S.C. and Riding, R. (eds) The Systematics
602 Society, London.

603 Hendry, K.R., Rickaby, R.E.M., Meredith, M.P., Elderfield, H., 2009. Controls on stable
604 isotope and trace metal uptake in *Neogloboquadrina pachyderma* (sinistral) from an
605 Antarctic sea-ice environment. *Earth Planet. Sci. Lett.* 278, 67-77.

606 Hatch, M.B.A., Schellenberg, S.A., Carter, M.L., 2013. Ba/Ca variations in the modern
607 intertidal bean clam *Donax gouldii*: An upwelling proxy? *Palaeogeogr. Palaeoclim.*
608 *Palaeoecol* 373, 98-107.

609 Hoffmann, J., Bahr, A., Voigt, S., Schönfeld, J., Nürnberg, D., Rethemeyer, J., 2014.
610 Disentangling abrupt deglacial hydrological changes in northern South America:
611 Insolation versus oceanic forcing. *Geology* 42, 579-582.

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

615 Hoppe, C.J.M., Langer, G., Rost, B., 2011. *Emiliana huxleyi* shows identical responses to
616 elevated pCO₂ in TA and DIC manipulations. *J. Exp. Mar. Biol. Ecol.* 406, 54–62.

617 Jochum, K.P., Weis, U., Stoll, B., Kuzmin, D., Yang, Q., Raczek, I., Jacob, D.E., Stracke, A.,
618 Birbaum, K., Frick, D.A., Günther, D., Enzweiler, J., 2011. Determination of
619 reference values for NIST SRM 610-617 glasses following ISO guidelines.
620 *Geostand. Geoanal. Res.* 35, 397-429.

621 Kaczmarek, K., Langer, G., Nehrke, G., Horn, I., Misra, S., Janse, M., Bijma, J., 2015. Boron
622 incorporation in the foraminifer *Amphistegina lessonii* under a decoupled carbon
623 chemistry. *Biogeosciences* 12, 1753-1763.

624 Kester, D.R., Duedall, I.W., Connors, D.N., 1967. Preparation of artificial seawater. *Limnol.*
625 *Oceanogr.* 12, 176-179.

626 Kunioka, D., Shirai, K., Takahata, N., Sano, Y., Toyofuku, T., Ujiie, Y., 2006.
627 Microdistribution of Mg/Ca, Sr/Ca, and Ba/Ca ratios in *Pulleniatina obliquiloculata*
628 test by using a NanoSIMS: Implication for the vital effect mechanism. *Geochem.*
629 *Geophys. Geosyst.* 7, GC001280.

630 Langer, G., Nehrke, G., Thoms, S., Stoll, H., 2009. Barium partitioning in coccoliths of
631 *Emiliania huxleyi*. *Geochim. Cosmochim. Acta* 73, 2899-2906.

632 Langer G., Sadekov, A., Thoms, S., Keul, N., Nehrke, G., Mewes, A., Greaves, M., Misra, S.,
633 Reichart, G.J., De Nooijer, L.J., Bijma, J., Elderfield, H., 2016. Sr partitioning in the
634 benthic foraminifera *Ammonia aomoriensis* and *Amphistegina lessonii*. *Chemical*
635 *Geology* 440: 306-312.

636 Lea, D.W., 1995. A trace metal perspective on the evolution of Antarctic circumpolar
637 deepwater chemistry. *Paleoceanography* 10, 733-747.

638 Lea, D., Boyle, E., 1989, Barium content of benthic foraminifera controlled by bottom-water
639 composition. *Nature* 338, 751-753.

640 Lea, D.W., Shen, G.T, Boyle, E.A., 1989. Coralline barium records temporal variability in
641 equatorial Pacific upwelling. *Nature* 340, 373-376.

642 Lea, D.W., Boyle, E.A., 1990. A 210,000-year record of barium variability in the deep
643 northwest Atlantic Ocean. *Nature* 347, 269-272.

644 Lea, D.W., Boyle, E.A. 1991. Barium in planktonic foraminifera. *Geochim. Cosmochim. Acta*
645 55, 3321-3331.

646 Lea, D.W., Boyle, E.A., 1993. Determination of carbonate-bound barium in foraminifera and
647 corals by isotope dilution plasma-mass spectrometry. *Chem. Geol.* 103, 73-84.

648 Lea, D.W., Spero, H.J., 1992. Experimental determination of barium uptake in shells of the
649 planktonic foraminifera *Orbulina universa* at 22° C. *Geochim. Cosmochim. Acta* 56,
650 2673-2680.

651 Lea, D.W., Spero, H.J., 1994. Assessing the reliability of paleochemical tracers: Barium
652 uptake in the shells of planktonic foraminifera. *Paleoceanography* 9, 445-452.

653 Lea, D.W., Mashiotta, T.A., Spero, H.J., 1999. Controls on magnesium and strontium uptake
654 in planktonic foraminifera determined by live culturing. *Geochim. Cosmochim. Acta*
655 63, 2369-2379.

656 Lear, C.H., Mawbey, E.M., Rosenthal, Y., 2010. Cenozoic benthic foraminiferal Mg/Ca and
657 Li/Ca records: Toward unlocking temperatures and saturation states.
658 *Paleoceanography* 25, PA001880.

659 Lee, J.J., 2006. Algal symbiosis in larger foraminifera. *Symbiosis* 42, 63-75.

660 Lewis, E., Wallace, D., 1998. Program developed for CO₂ system calculations, p 38.

661 Li, Y.-H., Chan, L.-H., 1979. Desorption of Ba and ²²⁶Ra from river-borne sediments in the
662 Hudson Estuary. *Earth Planet. Sci. Lett.* 37: 242-250.

663 Marr, J.P., Carter, L., Bostock, H.C., Bolton, A., Smith, E., 2013. Southwest Pacific Ocean
664 response to a warming world: Using Mg/Ca, Zn/Ca, and Mn/Ca in foraminifera to
665 track surface ocean water masses during the last deglaciation. *Paleoceanography* 28,
666 347-362.

667 Marriott, C.S., Henderson, G.M., Crompton, R., Staubwasser, M., Shaw, S., 2004. Effect of
668 mineralogy, salinity, and temperature on Li/Ca and Li isotope composition of
669 calcium carbonate. *Chemical Geology* 212, 5-15.

670 Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.N., 1973. Measurement of the
671 apparent dissociation constants of carbonic acid in seawater at atmospheric pressure.
672 *Limnol. Oceanogr.* 18, 897-907.

673 Mewes, A., Langer, G., De Nooijer, L.J., Bijma, J., Reichart, G.J., 2014. Effect of different
674 seawater Mg^{2+} concentrations on calcification in two benthic foraminifers. *Marine*
675 *Micropaleontology* 113, 56-64.

676 Mewes, A., Langer, G., Reichart, G.J., De Nooijer, L.J., Nehrke, G., Bijma, J., 2015. The
677 impact of Mg contents on Sr partitioning in benthic foraminifers. *Chem. Geol.* 412,
678 92-98.

679 Morse, J.W., Bender, M.L., 1990. Partition coefficients in calcite: Examination of factors
680 influencing the validity of experimental results and their application to natural
681 systems. *Chem. Geol.* 82, 265-277.

682 Morse, J.W., Arvidson, R.S., Lüttge, A., 2007. Calcium carbonate formation and dissolution.
683 *Chem. Rev.* 107: 342-381.

684 Mucci, A., Morse, J.W., 1983. The incorporation of Mg^{2+} and Sr^{2+} into calcite overgrowths:
685 influences of growth rate and solution composition. *Geochim. Cosmochim. Acta* 47,
686 217-233.

687 Nehrke, G., Keul, N., Langer, G., De Nooijer, L.J., Bijma, J., Meibom, A., 2013. A new
688 model for biomineralization and trace-element signatures of foraminifera tests.
689 *Biogeosciences* 10, 6759-6767.

690 Ni, Y., Foster, G.L., Bailey, T., Elliott, T., Schmidt, D.N., Pearson, P., Haley, B., Coath, C.,
691 2007. A core top assessment of proxies for the ocean carbonate system in surface-
692 dwelling foraminifera. *Paleoceanography* 22, PA3212.

693 Ni Flaithearta, S., Reichart, G.J., Jorissen, F.J., Fontanier, C., Rohling, E.J., Thomson, J., De
694 Lange, G., 2010. Reconstructing the seafloor environment during sapropel formation
695 using benthic foraminiferal trace metals, stable isotopes, and sediment composition.
696 *Paleoceanography* 25, PA4225.

697 Nürnberg, D., Bijma, J., Hemleben, C., 1996. Assessing the reliability of magnesium in
698 foraminiferal calcite as a proxy for water mass temperatures. *Geochim. Cosmochim.*
699 *Acta* 80, 803-814.

700 Okai, T., Suzuki, A., Kawahata, H., Terashima, S., Imai, N., 2002. Preparation of a New
701 Geological Survey of Japan Geochemical Reference Material: Coral JCp-1,
702 *Geostandards Newsletter* 26, 95-99.

703 Pak, D.K., Lea, D.W., Kennett, J.P., 2004. Seasonal and interannual variation in Santa
704 Barbara Basin water temperatures observed in sediment trap foraminiferal Mg/Ca.
705 *Geochem. Geophys. Geosyst.* 5, Q12008.

706 Pierrot, D., Lewis, E., Wallace, D.W.R., 2006. MS Excel program developed for CO₂ system
707 calculations. ORNL/CDIAC-105. Carbon dioxide Information Analysis Center, Oak
708 Ridge National Laboratory, US department of Energy, Oak Ridge, Tennessee.

709 Raitzsch, M., Hathorne, E.C., Kuhnert H., Groeneveld, J., Bickert, T., 2011. Modern and late
710 Pleistocene B/Ca ratios of the benthic foraminifer *Planulina wuellerstorfi* determined
711 with laser ablation ICP-MS. *Geology* 39, 1039-1042.

712 Raja, R., Saraswati, P.K., Rogers, K., Iwao, K., 2005. Magnesium and strontium compositions
713 of recent symbiont-bearing benthic foraminifera. *Marine Micropaleontology* 58, 31-
714 44.

715 Reddy, M.M., Nancollas, G.H., 1976. The crystallization of calcium carbonate: IV. The effect
716 of magnesium, strontium and sulfate ions. *J. Crystal Growth* 35, 33-38.

717 Reichart, G.J., Jorissen, F., Anschutz, P., Mason, P.R.D., 2003. Single foraminiferal test
718 chemistry records the marine environment. *Geology* 31, 355-358.

719 Rubin, S.I., King, S.L., Jahnke, R.A., Froelich, P.N., 2003. Benthic barium and alkalinity
720 fluxes: Is Ba an oceanic paleo-alkalinity proxy for glacial atmosphere CO₂? *Geophy.*
721 *Res. Lett.* Vol. 30, 1885.

722 Sadekov, A., Eggins, S.M., De Deckker, P., Kroon, D., 2008. Uncertainties in seawater
723 thermometry deriving from intratest and intertest Mg/Ca variability in
724 *Globigerinoides ruber*. *Paleoceanography* 23, PA0014502.

725 Sadekov, A., Bush, F., Kerr, J., Ganeshram, R., Elderfield, H., 2014. Mg/Ca composition of
726 benthic foraminifera *Miliolacea* as a new tool of paleoceanography.
727 *Paleoceanography* 29, 990-1001.

728 Segev, E., Erez, J., 2006. Effect of Mg/Ca ratio in seawater on shell composition in shallow
729 benthic foraminifera. *Geochem. Geophys. Geosyst.* 7, GC000969.

730 Ter Kuile B.H., Erez, J., 1991. Carbon budgets for two species of benthonic symbiont-bearing
731 foraminifera. *Biological Bulletin* 180, 489-495.

732 Toyofuku, T., Kitazato, H., Kawahata, H., 2000. Evaluation of Mg/Ca thermometry in
733 foraminifera: Comparison of experimental results and measurements in nature.
734 *Paleoceanography* 15, 456-464.

735 Toyofuku, T., Suzuki, M., Suga, H., Sakai, S., Suzuki, A., Ishikawa, T., De Nooijer, L.J.,
736 Schiebel, R., Kawahata, H., Kitazato, H., 2011. Mg/Ca and $\delta^{18}\text{O}$ in the brackish
737 shallow-water benthic foraminifer *Ammonia 'beccarii'*. *Marine Micropaleontology*
738 78, 113-120.

739 Toyofuku, T., Matsuo, M.Y., De Nooijer, L.J., Nagai, Y., Kawada, S., Fujita, K., Reichart,
740 G.-J., Nomaki, H., Tsuchiya, M., Sakaguchi, H., Kitazato, H., 2017. Proton pumping
741 accompanies calcification in foraminifera. *Nature Communications* 8, 14145.

742 Van Dijk, I., De Nooijer, L.J., Reichart, G.-J., 2017. Trends in element incorporation in
743 hyaline and porcelaneous foraminifera as a function of $p\text{CO}_2$. *Biogeosciences* 14,
744 497-510.

745 Weldeab, S., Lea, D.W., Schneider, R.R., Andersen, N., 2007. 155,000 years of west African
746 monsoon and ocean thermal evolution. *Science* 316, 1303-1307.

747 Weldeab, S., Lea, D.W., Oberhänsli, H., Schneider, R.R., 2014. Links between southwestern
748 tropical Indian Ocean SST and precipitation over southeastern Africa over the last 17
749 kyr. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 410, 200-212.

750 Wilson, S. A., Koenig, A. E., Orklid, R., 2008. Development of microanalytical reference
751 material (MACS-3) for LA-ICP-MS analysis of carbonate samples, *Geochimica et*
752 *Cosmochimica Acta Supplement* 72, 1025.

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

756 Wit, J.C., De Nooijer, L.J., Wolthers, M., Reichart, G.J., 2013. A novel salinity proxy based
757 on Na incorporation into foraminiferal calcite. *Biogeosciences* 10, 6375-6387.

758

759 Yu, J., Day, J., Greaves, M., Elderfield, H., 2005. Determination of multiple element/ calcium
760 ratios in foraminiferal calcite by quadrupole ICP-MS. *Geochemistry, Geophysics,*
761 *Geosystems* 6, Q08P01.

762 Yu, J., Elderfield, H., 2007. Benthic foraminiferal B/Ca ratios reflect deep water carbonate
763 saturation state. *Earth and Planetary Science Letters* 258, 73-86.

764

765 **Tables**

766 *Table 1: measured concentrations of major and minor ions, temperature, salinity and*
 767 *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
Ω _{calcite}	3.9	3.9	4.0	3.9	3.9

768

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

Treatment	A	B	C	D	E
<i>A. lessonii</i>					
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
<i>H. depressa</i>					
n	26	27	23	25	32
Ba/Ca ($\mu\text{mol/mol}$)	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

770

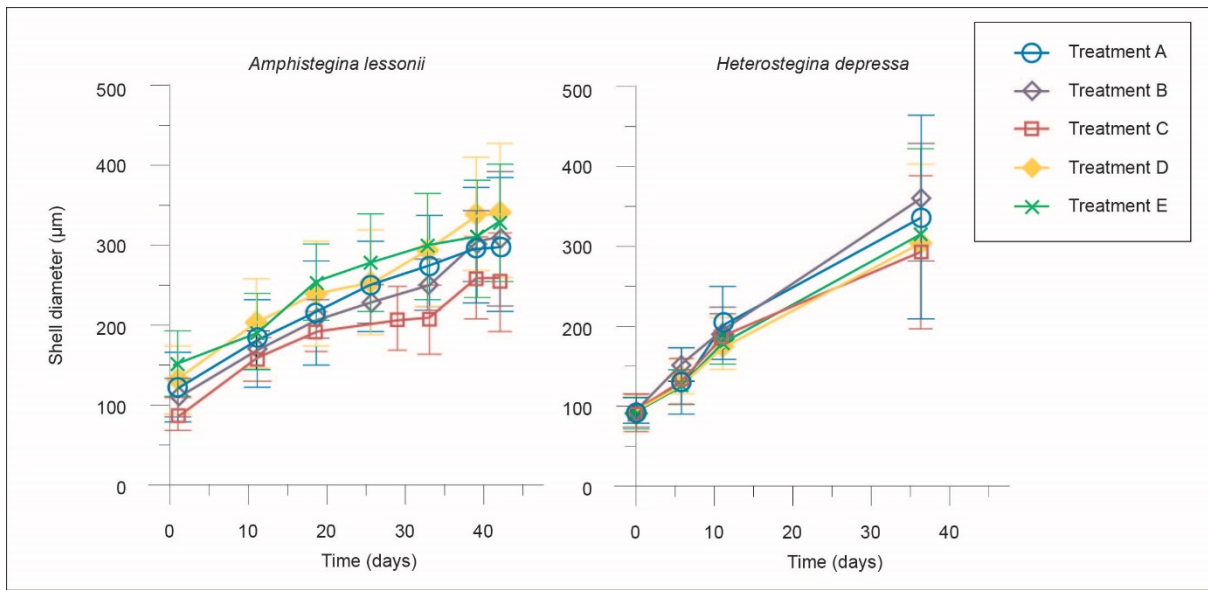
771 *Table 3. Parameters of the regression analysis and ANOVA tests for significance of the*
772 *regression. Both average Ba/Ca_{cc} of each experimental condition (n=5) and all chamber-*
773 *specific Ba/Ca_{cc} (n=133/ 179) were tested versus the Ba/Ca of the 5 treatments.*

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

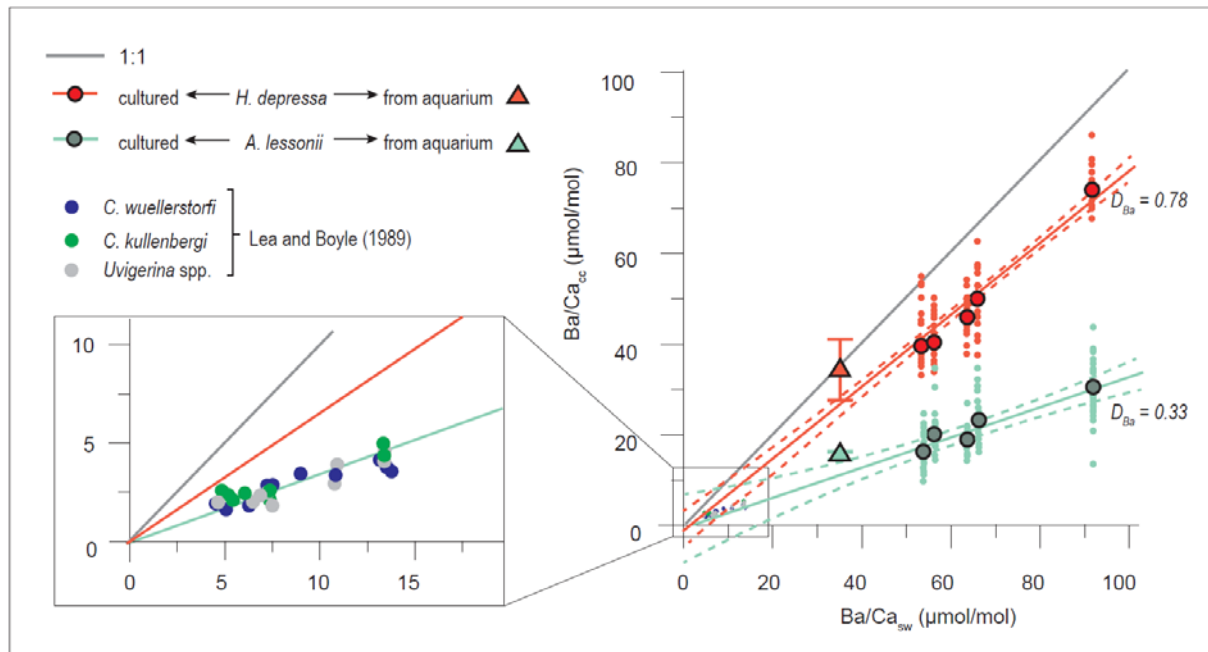
774

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

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

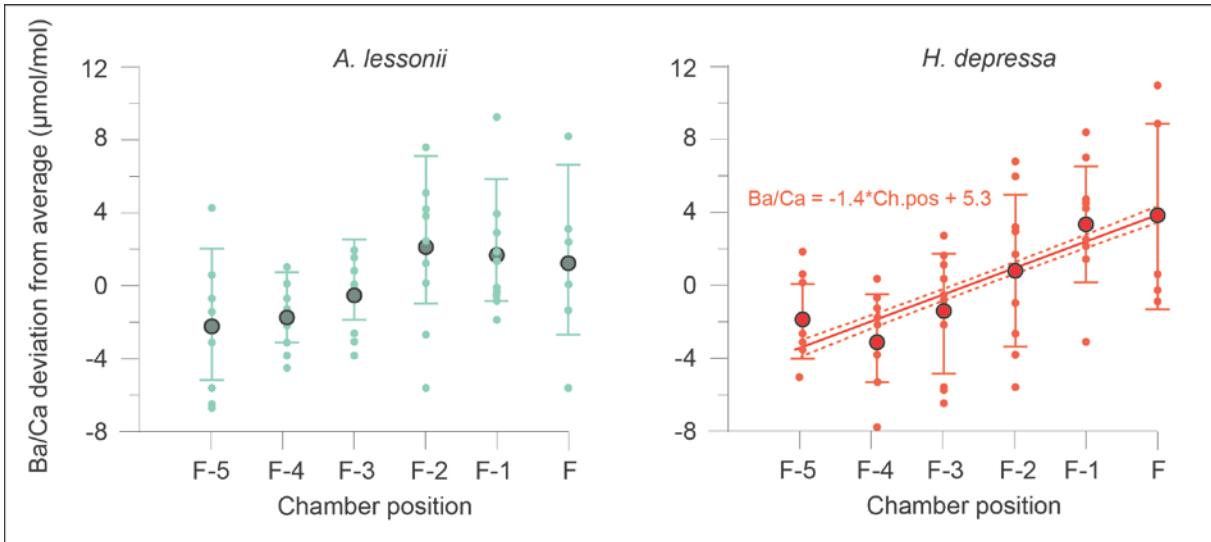


777

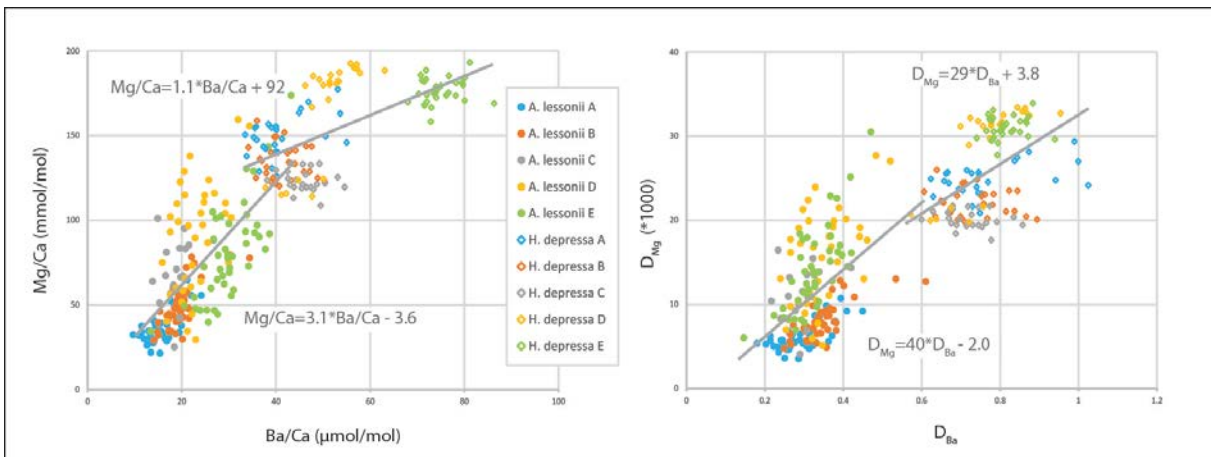


778

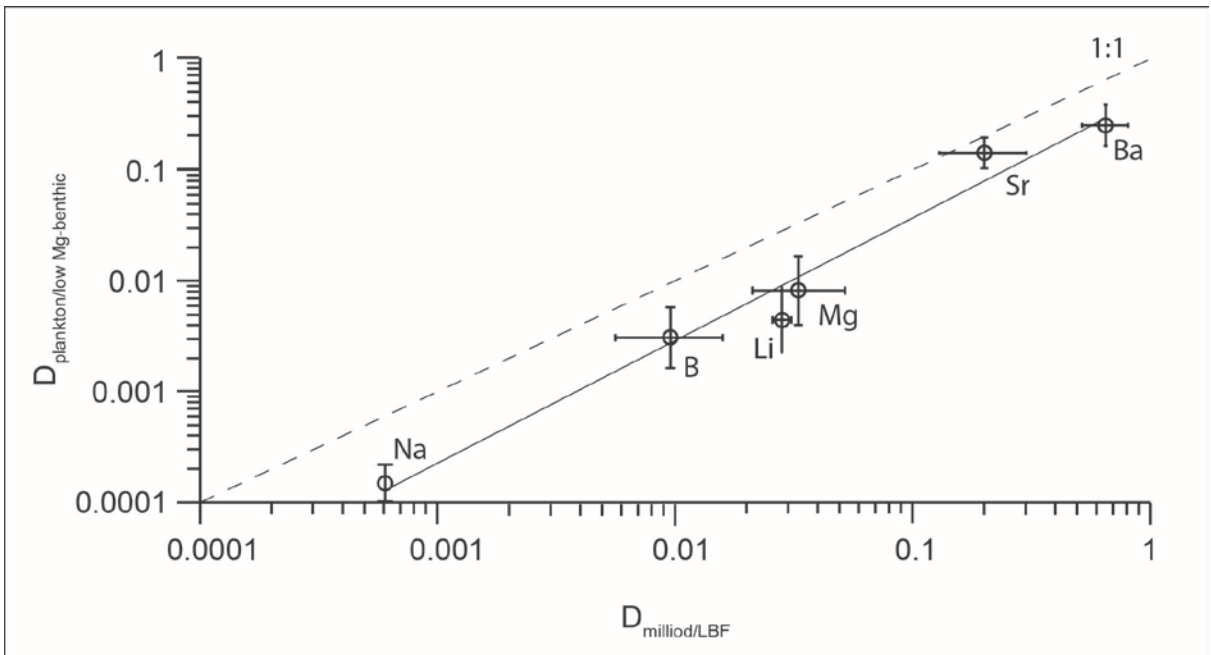
779



780



781



782 **Figure Captions**

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

786

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

795

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

800

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

805

806 *Figure 5: Partition coefficients for Li, B, Na, Mg, Sr and Ba for two groups of foraminifera*
807 *(Large Benthic Foraminifera+Miliolids and the low-Mg species). Data on which the average*
808 *partition coefficients are based, are listed in the online supplement, the ranges indicate the*
809 *maximum range in published partition coefficients. The linear regression between the*
810 *partition coefficients for these two groups is described by: $D_{\text{plankton/low Mg-}}$*
811 *$D_{\text{benthic}}=0.3992*D_{\text{miliolid/LBF}} + 0.0081$. Elemental results for Miliolid species are confined to*
812 *Mg/Ca and Sr/Ca. Li/Ca ratios were taken from Delaney et al. (1985), Hall and Chan*
813 *(2004a), Marriott et al. (2004), Yu et al. (2005), Ni et al. (2007), Bryan and Marchitto (2008),*
814 *Hathorne et al. (2009), Dawber and Tripathi (2012) and Evans et al. (2015); B/Ca ratios are*
815 *from Yu et al. (2005), Yu and Elderfield (2007), Foster (2008), Hendry et al. (2009), Allen et*
816 *al. (2011; 2012), Dawber and Tripathi (2012), Babila et al. (2014) and Kaczmarek et al.*
817 *(2015); Na/Ca are from Delaney et al. (1985), Ni et al. (2007), Bian et al. (2009), Wit et al.*
818 *(2013) and Evans et al. (2015); Mg/Ca are from Toyofuku et al. (2000), Raja et al. (2005), Yu*
819 *et al. (2005), Elderfield et al. (2006), Segev and Erez (2006) , Hendry et al. (2009), Dueñas-*
820 *Bohórquez et al. (2009; 2011), Dawber and Tripathi (2012), Wit et al. (2012; 2013), Babila et*
821 *al. (2014), De Nooijer et al. (2014a), Sadekov et al. (2014) and Evans et al. (2015).*
822 *Foraminiferal Sr/Ca are taken from Raja et al. (2005), Yu et al. (2005), Hendry et al. (2009),*
823 *Dueñas-Bohórquez et al. (2009; 2011), Dawber and Tripathi (2012), Wit et al. (2013), De*
824 *Nooijer et al. (2014a) and Evans et al. (2015). Ba/Ca are from this study, Lea and Boyle*
825 *(1989), Lea and Boyle (1991), Lea and Spero (1994), Hall and Chan (2004b), Ni et al.*
826 *(2007), Hönisch et al. (2011) and Evans et al. (2015).*

827