Dear Dr. Evans,

Thank you for the constructive comments we received on our manuscript. Below, all comments are listed in italics. We try to answer all specific comments raised by your review (in bold).

Trans-membrane-transport is not required to explain these results, therefore a discussion of how they would be interpreted in terms of seawater vacuolisation should be included.

We agree with the reviewer that our results are not providing conclusive evidence for either one of the current biomineralization concepts, seawater endocytosis and TMT mixing. Still, the different observed correlations between the incorporation of trace elements in hyaline and porcelaneous species are best explained by primarily vacuolization in porcelaneous and a mixed signal in hyaline species. To accommodate the reviewers concern we now included a paragraph in which we evaluate the effect of the potential contribution of vacoulized seawater using our observations. The discussion now includes a comparison of the relative low (hyaline) and high (porcelaneous) contribution of vacuolized seawater on overall El/Ca.

• *Although to my knowledge it remains to be tested, it is likely that the extent to which foraminifera raise the pH of seawater vacuoles is dependent on the ambient seawater pH (also by analogy to the ECF in corals). Therefore, seawater pH can be expected to influence (e.g.) Zn and Ba speciation in the seawater vacuole. Because a certain species is probably preferentially incorporated during crystal growth, there is no need to invoke poorly selective channels or pumps (lines 311-315).*

How (if at all) the internal pH of foraminifera dependends on seawater pH outside the foraminifer, is currently not known. If it does change in concert, the internal pH at the site of calcification (which is ≥ 9; De Nooijer et al, 2009) would vary between >9 and >8.6 in our study. In theory such a change in internal pH from >9 to >8.6 changes [CO_3^{2-}] and thus the speciation of e.g. Zn and Ba at the site of calcification. However, over this range the change in [CO_3^{2-}] will be rather limited. Hence such an effect of differential speciation within the calcifying fluid does not suffice to explain the observed sensitivity in Zn and Ba to pCO_2 in our study. This is in line with recent evidence on Zn/Ca in foraminifera, which suggests Zn incorporation is not governed by changes in seawater pH, but by carbonate ion concentration which does not change very much anymore at these high pH's (van Dijk et al., 2017, figure 5d).

• *Given that the Mg distribution coefficient for some species is greater than that of inorganic calcite, it is difficult to see how TMT helps explain the geochemistry of these foraminifera. In fact, it causes problems. If these species are still sourcing a portion of the Ca through channels or pumps (lines 309- 311 and 362-364), then presumably the Mg/Ca ratio of the calcifying fluid*

is lower than that of seawater, yet in some cases they precipitate calcite with a Mg/Ca ratio ~3 times that of inorganic calcite. There are three species shown in Figure 3 with a Mg/Ca ratio twice that of the highest Mg/Ca species of this study. A different mechanism is required here, and it is unclear how TMT could fit into this given it would require pumping Ca out of seawater to raise the Mg/Ca ratio before precipitation. In contrast to what is stated on C2 lines 357-359, the highest Mg species are equally (or even more) different from inorganic calcite as the low-Mg species.

If the D of the foraminifera would be higher, this would indeed be true. However, we apologize for erroneously plotting the wrong inorganic D for Mg, which should have been ~150-200 (Mucci and Morse, 1983; Morse et al., 2007), indicating that all known foraminiferal partition coefficients are well below the inorganic D. We refrain from replotting this based on the next comment of this reviewer. Still, to accommodate this reviewers' concerns we extended our discussion, as mentioned above, to now also include other mechanisms potentially contributing to transport of ions to the SOC.

2. Figure 3. The inorganic calcite distribution coefficients should only be displayed if they were characterised from calcite precipitated from seawater. For example, the sodium distribution coefficient is based on solutions with a chemistry very different from that of seawater, most notably the Mg concentration was much lower. It is coincidence that it is roughly the same as the miliolids and does not suggest that they precipitate shells with a similar DNa to that of inorganic calcite in seawater (lines 360-361). It is well known that Mg exerts a control on trace element distribution coefficients (see below), therefore it is not representative to compare these results to inorganic precipitation where these are carbonates precipitated from non-seawater solutions.

We have removed the inorganic D's from figure 3, since the inorganically precipitated calcites are usually not derived from (natural) seawater. Most inorganic precipitation experiments do not utilize seawater as a source for carbonate precipitation, as it complicates the design of the experiment. This makes a direct comparison with foraminiferal calcite impossible.

3. Section 4.1 and Figure 3. The reason that trace element distribution coefficients are strongly positively correlated with DMg is because the incorporation of Mg into calcite modifies the incorporation of other elements through the associated lattice distortion. For example, this has been shown in inorganic calcite in the case of DSr [Mucci & Morse, 1983] and DNa [Okumura & Kitano, 1986], and we confirmed that this is also the case in foraminifera through cultures in variable seawater Mg/Ca [Evans et al., 2015]. The point is that this effect is not a consequence of ion transport, but has a basis in crystallography, especially given that hyaline foraminifera lie on the same DX-Mg/Cacalcite line as inorganic precipitates [see Evans et al., 2015 Fig. 7]. Furthermore, the trace element distribution coefficients shown in Figure 3 would be better expressed as a function of the calcite Mg/Ca ratio rather than DMg. It will not make much difference as most of these data are from foraminifera grown in seawater with a Mg/Ca ratio

close to that of modern, but mechanistically it is the Mg concentration of calcite that is important.

We agree that Mg incorporation might distort the calcite crystal lattice, allowing for a higher incorporation of for instance Sr and Na. Some of us were actually actively involved in studies specifically targeting this (e.g. Mewes et al., 2015). In the new version of our manuscript we now emphasized this issue by add a paragraph (in 4.2), mentioning that this interdependency of element incorporation on Mg has been observed in both inorganic and culture experiments, and might partly stem from crystallography. However, although this mechanism might explain some of the observed species specific element incorporation in hyaline foraminifera, this does not explain the difference between hyaline and porcelaneous foraminifera. Porcelaneous foraminifera have in general high Mg/Ca, but we actually observe lower incorporation of Na and Sr compared to hyaline species with similar DMg (Fig 3, upper right and left panel: DMg versus DNa and DSr). When including porcelaneous species from other studies we also observe no increase in DSr over a larger range in DMg (Fig 3, upper left panel: DMg versus DSr). This indicates that the mechanisms (or mechanisms) might be very different for hyaline and porcelaneous species, which is discussed in more detail at the end of our revised discussion.

We plotted partitioning coefficients rather than element to calcium ratios since a couple of the published studies changed the Mg/Ca of the culture media, and this makes it easier for readers to evaluate their own or other data.

3. Lines 268-270. It is true that some benthic species show little response of Mg/Ca and Sr/Ca to the carbonate system, but the [CO2− 3] effect on some deep benthic foraminifera Mg/Ca is well known. These are also low-Mg so this statement is not accurate.

For deep sea species benthic foraminifera, observed response to changes in carbonation ion concentration are mainly due to calcification in undersaturated seawater, as described by 'the carbonate ion saturation hypothesis' (Elderfield et al., 2006) and also observed for Zn by Marchitto et al., (2000, 2005) and Cd and Ba by McCorkle et al. (1995). We now added this references and a few sentences on the carbonate ion effect in undersaturated water to the revised discussion (4.1). However, there is some evidence that Sr incorporation is directly influenced by the carbonate system (also shown by Keul et al., in press). Still, sensitivity of Sr/Ca to e.g. [CO_3^{2-}] is rather low (Keul et al., in press; van Dijk et al., 2017). In our experiment the change in [CO_3^{2-}] between the highest and lowest pCO_2 treatment is rather limited (90-220 µmol/kg), resulting in no observed correlation of Sr/Ca with pCO_2

4. Lines 277-279. There is no significant correlation between Mg/Ca and either DIC or alkalinity in these studies.

We removed this statement from the discussion section and state there is a correlation between Mg/Ca and the carbonate system for planktonic foraminifera.

5. Lines 283-285. It is not really the case that there is a trend between Ba/Ca and the carbonate system in the planktonic cultures of Honisch et al. [2011]. Only the lowest pH cultures suggest any trend, but there are no replicates of these. How does the difference between these results and those presented here fit into the authors preferred biomineralisation model?

If incorporation of Ba depends on speciation due to differences in [CO_3^{2-}], we expect plateauing of free Ba^{2+} when we model speciation for lower pCO2 conditions (higher [CO_3^{2-}]). This is in line with the reviewers comments.

7. Lines 334-335. I don't understand this statement. Do you mean that the selectivity of these channels depends on the amount of ions transported? Is there any evidence for this? It is more intuitive that selectivity is not changing.

We have re-written this section of our discussion. We agree with the referee that the second explanation is more logical/intuitive, with the amount of Ca^{2+} in the vicinity of the foraminifer decreasing, the relative element to calcium ratio of the seawater around the foraminifera increases. This would result in a higher transport of elements other than Ca^{2+}.

8. Figure 3. There is a plotting mistake in the DNa panel. P. acervali and H. antillarum are shown with different sodium distribution coefficients but the data in Table 3 indiate that theyit is the same in both species.

This is actually a mistake in the table. *P. acervali* has a DMg of 0.46 (aslo reflected in the higher $Mg/Ca_{CALCITE}$)

9. Figure 5. I understand the logic for plotting this as a function of pCO_2, but given that pH is what we are able to reconstruct with boron isotopes I suggest adding a second set of x-axes to enable the two to be easily related. It would also be interesting to extend this plot to include the pH at the calcification site.

We added a second x-axis to this figure, with the corresponding pH from our culture study. We are not able to plot pH at the calcification site, since it is unknown how internal pH responses to changes in ambient pH.

10. Figure 6. Half of this figure could be cut as both panels essentially show the same thing. Or, panel B could be replaced with a schematic showing how these results would fit into a biomineralisation model wherein the ions are sourced through seawater vacuolisation.

We adapted this figure to our revised discussion, and use it to show the contribution of both mechanisms to fit our observations.

Dear Dr Fujita,

Thanks for the thorough reading of our manuscript and your constructive comments

In general, the main concern of this referee was the focus of the manuscript, which should be more towards the potential of Zn/Ca and Ba/Ca as carbonate system proxies. By changing and restructuring the introduction and discussion session, we shifted the focus of the manuscript more towards the changes in Zn and Ba incorporation as a function of pCO_2. The conclusions and abstract are changed accordingly. Another one of the suggestions by both reviewers is to broaden the discussion by including other biomineralization and ion transport models to explain the species-specific trends in element incorporation.

The comments of the referee are posted below in italics. We address these issues point by point, in bold.

General comments:

1) Authors should reconsider what is the main purpose (hypothesis), what is the main results, and what are conclusions obtained from this study. In the Introduction, you may need more elegant story to explain why you conducted pCO2 controlled experiments as well as why you used symbiotic tropical large benthic foraminifera for this study, to understand trace element incorporation in foraminifera. I think the main important results in this study were increasing some trace element incorporation (Zn, Ba) with high pCO2 environments and a chemical model to explain this phenomenon.

Based on this comment we now somewhat changed the focus of the introduction more towards carbonate system proxies (e.g. Zn/Ca) and the necessity to study this across different taxa since they are known to 1) have different calcification mechanism and 2) a different D for elements studied so far. We used tropical foraminifera since they have a higher variability in Mg incorporation and both porcelaneous and hyaline species are readily available in this region.

This also led to a reorganization of the discussion. First, we now discuss the Zn- and Ba-incorporation as a function of pCO_2 (now 4.2) and then discuss overall differences between hyaline and porcelaneous foraminifera (4.1 in the old manuscript). Then, we discuss the chemical speciation of ions as a function of pCO_2 (4.3). Finally, we evaluate existing biomineralization models based on our results.

2) Authors are mainly concerned about a correlation, but not dealt with the quantity (amount) of elemental incorporation in foraminiferal calcite. Even if both Zn and Ba ion availability increases with high pCO2 conditions, the amount incorporated in foraminiferal calcite differs between the two elements (Zn is four times more incorporated than Ba). We know that ionic radius is related to trace element incorporation in calcite from many inorganic studies. How does your chemical model explain the incorporation of elements quantitatively?

We now added these observations to the discussion. Following the TMT mixing model, selectivity of Ca^{2+} channel differs for different elements, which probably also reflects in part ionic radius (see also, Nehrke et al., 2013).

3) This paper is not discussed anywhere (only briefly mentioned in the Introduction) about effects of temperature on element incorporation in foraminifera, which are well established by many papers. In Figure 3, you compared results of your study with those of previous similar studies. However, I wonder these results are not simply comparable because of different species from different environments (water depths and latitudes, i.e. different temperature and salinity ranges) as shown in Supplementary Table S1. Temperature effects should be normalized to compare real DMg between different foraminiferal species. In addition, authors should discuss the relative sensitivity on E/Ca between temperature and carbonate chemistry when both parameters are variable.

We changed figure 3, to show the data of our study only. The previous figure 3 is now moved to the supplementary info, and we excluded data from foraminifera cultured/grown at low temperature, as they might partly have been grown under undersaturated conditions with repsect to calcite. Resulting species are all grown/cultured in a temperature range of 18-29 °C. We did not correct E/Ca for temperature, since the this effect on Na/Ca, Ba/Ca and Sr/Ca is not well constrained or unknown.

4) This study assumes only Ca-channel model as a possible ion transport mechanism. However, other possible mechanisms of ion transport have been proposed in foraminifera. I suggest that authors compare advantages and disadvantages of several transport models and justify the Ca channel model as the most appropriate to explain results in this study.

This issue was also raised by the second reviewer. The discussion is now put in a somewhat broader context, in which we also include other transport models, including seawater vacuolization (e.g. Ter Kuile and Erez, 1991; Erez, 2003, Elderfield et al., 1996; De Nooijer et al., 2014). This is added as a separated paragraphs in which we try to test/validate these models with our observations.

5) I wonder if laser abrasion (LA) method is appropriate for biocalcification study. As you know, hyaline foraminiferal shells are composed of the primary layer and the secondary layers (coating) with the organic matrix. The LA method cannot discriminate differences in element incorporation between these different layers. In addition, the spatial heterogeneity of E/Ca among calcite crystals in a chamber wall has been reported by many studies. How do authors overcome these problems by using the LA method?

Heterogeneity of elements in the chamber wall has only been observed in hyaline species, since they have (bi)laminar calcification. In this study we incubated adult foraminifera in culture media with calcein. LA-ICP-MS allows for targeting new chambers only, of which all layers of calcite were formed during the experiment. By using LA-ICP-MS we obtain an average signal of the chamber wall, averaging out any potential banding. Analysis of sufficient specimens/ chambers reduces uncertainty in average e.g. Mg/Ca values. Furthermore, we only ablated the final ~3 chambers, minimizing the potential effects of

varying number of test carbonate layers. First paragraph of the discussion now lists this as one of the potential causes for element to calcium ratio (E/Ca) variability.

6) What kinds of other trace elements except for those examined in this study are sensitive to pCO2 based on the chemical model? That is useful information to find new proxies for paleo-pCO2. In addition, I wonder what cause differences between sensitive and insensitive elements on pCO2.

In this model we only looked at elements which we also measured by LA-ICP-MS. We modelleed the elemenst which based on their know geochemical behavour are the most likely to show differences as a function of changes in carbonate chemistry. All elements have been modelled in some sort of other study previously as well (e.g. Keul et al., 2013). When considering the impact of [CO_3^{2-}] chemical speciation, the observed lack of sensitivity for Sr, Na and Mg might stem from their high concentration in seawater compared to e.g. Zn. Only a small amount of ions are hence complexed by [CO_3^{2-}]. Since there is a higher total amount of e.g. Mg ions in seawater, the amount of Mg-CO_3 complexation is relatively low. Due to their low concentrations and great affinity for carbonate ions, elements like Cu, Co, Ni, Li, may be affected in the same way. We have added the possible behavior of these elements according to changes in speciation (4.3, chemical speciation).

7) Authors are confused about the terminology of Foraminifera. Throughout the text, the authors used "hyaline" and "miliolid" as comparable terms. But the term "hyaline" indicates the quality of shell appearance and the term "miliolid" is a taxon name belonging to the Order Miliolida. I suggest authors use comparable terms of "hyaline vs. porcelaneous" as shell appearance, "perforate vs. imperforate" as shell perforation, and "rotaliid vs. miliolid" as the two main taxonomic group.

To avoid any confusion, we changed the terminology in our paper to hyaline vs. porcelaneous and perforate vs. imperforate.

L1: Title is vague and general, should be changed to include keywords and reflect the main results of this paper; for example, Calcification model of some trace element (Zn, Ba) incorporation in foraminifera under high pCO2 environments.

We changed the title to: 'Trends in element incorporation in hyaline and porcelaneous foraminifera as a function of *p*CO_2' to better cover our main results.

Introduction:

L38-40: This sentence is strictly speaking incorrect. Diverse miliolid foraminifers belonging to larger benthic foraminifera (LBF) are found particularly in the Atlantic and the Caribbean. In

addition, LBF do not cover a large Mg/Ca range, but only intermediate and high Mg/Ca ranges. The authors have to explain advantages to use LBFs for their study in more detail.

In our study we use intermediate to high $Mg/Ca_{CALCITE}$ (in our study ranging from 28.5 (*A. carinata*) to 141.3 (*H. antillarum*) mmol/mol), which is a large range in $Mg/Ca_{CALCITE}$.

New text: "A number of larger benthic foraminifera form hyaline shells, although the amount of Mg in their shells is often more than 10 times higher than that of planktonic and small benthic hyaline species, hence covering a larger range in $Mg/Ca_{CALCITE}$ values."

Methods:

L86: 90-600 µm fraction is too small for larger benthic foraminifers (almost juveniles).

We picked both directly from the macroalgae and from the 90-600 µm fraction, we now explain this in the methodology.

L86: As far as I know, Marginopora vertebralis (Quoy & Gaimard) is not distributed in the Caribbean and Atlantic (see Langer and Hottinger, 2000 Micropaleontology). Recheck if identification is correct.

We rechecked the identification of all of our species, and found that we misidentified *Sorites marginalis* as *Marginopora vertebralis.* This is now changed in the revised version of our manuscript.

L55: Is a paper in review OK to cite? If it is OK, it should be listed in the Reference.

L60: Not listed in the Reference.

L150, (Nardeli et al., 2016): not listed in the Reference.

L154, Barker et al. (2003): not listed in the Reference.

L94: Where is "Chapter 7 "? I also found other chapters in the text somewhere.

5 points above: We removed all references to chapters and manuscripts in review

L97-98: Add pCO2 unit. Explain what (A) means.

We added the pCO_2 unit (ppm) and explain the treatment names A-D

L108-109: Add the precision of temperature control.

The average temperature over the whole experiment was 25±0.2°C, which is now added to the revised manuscript.

L110-111: Note the light intensity level. In addition, I wonder if LEDs and yellow culture bottles (Fig. 1) affect wave length and hence the growth of symbiotic foraminifers?

The culture bottles themselves are not yellow, it's the calcein added to the culture media. Almost all incubated foraminifera grew new chambers.

L113: Does food affect water quality and chemical composition?

The descripted *dunaliella* feeding solutions do not change the chemical composition, since these dunaliella were rinsed, centrifuged, freeze-dried and subsequently diluted in the culture media. Water was replaced every four days to keep organic waste buildup at a minimum

L155: Could the organic matrix in a shell be removed by this method? Does data not include any elemental incorporation in the organic matrix?

In this cleaning step we remove the organic matrix from the foraminiferal shells. Although, in theory, it might be possible there are small amounts of matrix remaining in the carbonates, the amount of organic matter in foraminiferal shells is low, even before cleaning (e.g. 0.1-0.2 wt% of the total shell of *Heterostigina depressa*; wenier and Erez, 1984). However, for future work it might be interesting to analyze the organic matrix, to evaluate its potential contribution to the total E/Ca.

L167: What is the main difference between this paper and Van Dijk et al. (in review)?

We removed this reference, since this article is still in review. The differences with this study and Reichart et al. (2003) are summarized in this paragraph. For instance, we use different ICP-MS's, different cells, and used additional standards.

Results:

L217-218: Explain the rationale (hypothesis) why the authors compare Mg/Ca with other TE/Ca?

We included this figure in our manuscript because of the obseverd link between Mg and other TE published earlier (like e.g. Evans et al,., 2015).

L218-219: not only significant values, but also R2 values should be noted.

The R^2 values are presented in Table 3

Discussion:

L249-251: Compare advantages and disadvantages of several ion transport models and justify a Ca-channel model as the most appropriate to explain results in this study.

L260: I think miliolid foraminifera still need the major removal of Mg ions even if carbonate is directly precipitated from seawater.

We broaden the discussion to include also other transport models and how these might differ for porcelaneous and hyaline species, when comparing them to our observations.

L289: PHREEQC needs explanation

L290: llnl database?

PHREEQC and the llnl database are now explained in more detail in the new paragraph 4.3, which focusses on the modelling of chemical speciation.

Section 4.3: this section is mostly a review of previous studies. I suggest that authors explain an incorporation model shown in Fig. 6 in detail.

Due to a reorganization in our discussion, we now spent two paragraphs on different transport models.

Section 4.4: Does size matter? authors mentioned that they measured only small size (L86). Calcarinids (Neorotalia in #15 in Fig. 3) are similar in size to Amphistegina, but have high Mg contents similar to a bigger Heterostegina. You may need another interpretation to explain the difference between two taxa. In addition, I think larger benthic foraminifers (in particular some taxa dwelling at a lower euphotic depth) have a strategy to attain a high surface area to volume ratio by flatting to get light for algal C6 symbionts. Please show the surface area to volume ratio between comparing taxa to justify your interpretations. Less Ca channels in the membrane of LBFs are also unlikely, because LBFs are bigger thus have much more membranes and channels than smaller foraminifers if channel density are the same. I think the second process is more feasible than the first process.

L347-350: I think this explanation is more plausible. Hyaline foraminifers are highly diverse and may have similar but slightly different calcification strategy acquired during evolution. I guess the relative contributions of primary and secondary layers and organic matrix may depend on hyaline foraminiferal taxa, which may cause interspecific variability of E/Ca compositions.

We removed this part of our discussion, since we agree it is the less likely explanation of our observations for hyaline species. We end our discussion with a paragraph on the contribution of different mechanisms, which might explain our observations for both hyaline and porcelaneous species.

Section 4.5. L359: I think the major removal of Mg is necessary because your results show that DMg is much lower than 1.

With the seawater vacuolization model (Erez, 2003) it is indeed necessary to remove Mg ions from the calcification fluid. But the removal of Mg is not necessary when ions are transported by TMT, since these channels mainly import Ca to the site of calcification. Only very few Mg ions would be transported (1 for every 10.000 Ca ions).

L366-369: Is this correct? lower? I think higher or similar based on slope inclinations in Fig. 4. I do not understand how to estimate the relative contribution of seawater endocytosis and transmembrane transport. I guess some trans-membrane ion exchanges (Mg removal) occur between seawater vesicles and intrashell cytoplasm. High pCO2 seawater contains relatively large amounts of Zn and Ba ions, which are incorporated into foraminiferal cytoplasm via seawater vacuolization. Calcite needles are then precipitated from seawater vesicles with modifications by trans-membrane ion exchanges between seawater vesicles and intrashell cytoplasm.

No, high pCO_2 contains the same amount of Zn and Ba ions, only speciation differs. Seawater vacuolized at different pCO_2 will have the same Zn and Ba concentration. The calcite needles which are precipitated from these vacuoles will have the same Zn/Ca and Ba/Ca, unrelated to ambient seawater carbonate chemistry. However, by combining TMT and seawater vacuolization, calcification fluid starts with ambient seawater, which is diluted with Ca^{2+} by TMT. During this processes, the amount of ions other than Ca^{2+} transported to the site of calcification depends on the chemical speciation (amount of free ions), the relative abundance compared to Ca^{2+} and the selectivity of and thus discrimination by the Ca^{2+} channels. This is now described in more detail in the discussion section.

Trends in element incorporation in hyaline and porcelaneous foraminifera as a function of pCO_2

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Abstract

In this study we analyzed the impact of seawater carbonate chemistry on the incorporation of elements in both hyaline and porcelaneous larger benthic foraminifera. We observed a higher incorporation of Zn and Ba when pCO_2 increases from 350 to 1200 ppm. Modelling the activity of free ions as a function of pCO_2, shows that speciation of some elements (like Zn and Ba) are mainly influenced by the formation of carbonate complexes in seawater. Hence, differences in foraminiferal uptake of these might be related primarily by the speciation of these elements in seawater. We investigated differences in trends in element incorporation between hyaline (perforate) and porcelaneous (imperforate) foraminifera in order to unravel processes involved in element uptake and subsequent foraminiferal calcification. In hyaline foraminifera we observed a correlation of element incorporation of different elements between species, reflected by a general higher built-in of elements in species with higher Mg content. Between porcelaneous species inter-element differences are much smaller. Besides these contrasting trends in element incorporation, however, similar trends are observed in element incorporation as a function of seawater carbonate chemistry in both hyaline and porcelaneous species. This hints at similar mechanisms responsible for the transportation of ions to the site of calcification for these groups of foraminifera, although the contribution of these processes might differ across species.

1. Introduction

Calcareous foraminifera, cosmopolitan unicellular protists, are widely used to reconstruct past environmental conditions, since the chemical composition of the carbonate shells reflect a wide variety of environmental parameters. For instance, the Mg/Ca of foraminiferal shells is primarily determined by seawater temperature (Nürnberg et al., 1996; Allen and Sanders, 1994) and seawater Mg/Ca (Segev and Erez, 2006; Evans et al., 2015) and has been widely applied as a paleothermometer (Elderfield and Ganssen, 2000; Lear et al., 2000). The use of foraminifera as proxies for the inorganic carbon system in the past (seawater pH, alkalinity, saturation state, etc.) has more recently been added to the foraminiferal proxy tool-box. For example, the concentrations of trace elements in foraminiferal shells, including U (Keul et al., 2013; Russell et al., 2004), Zn (Marchitto et al., 2000; van Dijk et al., 2017) and B (Yu and Elderfield, 2007) correlates to seawater carbonate ion concentration ($[CO_3^{2-}]$), while the boron isotopic composition of foraminiferal calcite used as a proxy for pH (Sanyal et al., 1996). However, insight in vital effects (Erez, 2003) and inter-specific differences in trace element incorporation (Bentov and Erez, 2006; Toyofuku et al., 2011; Wit et al., 2012) is needed to increase robustness of these proxies.

On the broadest taxonomic scale foraminifera produce tests using either one of two fundamentally different mechanisms. These calcification strategies reflect the evolutionary separation of foraminiferal groups dating back to the Cambrian diversification, from where the imperforate porcelaneous species and perforate hyaline foraminifera, developed independently (Pawlowski et al., 2003). Previously observed species-specific differences in partitioning and fractionation of elements most likely primarily reflect these differences in calcification strategy, since these offsets are largest between hyaline and porcelaneous species (see for a summary, Toyofuku et al., 2011). The calcification process of the latter group has been studied more extensively than that of the porcelaneous species (De Nooijer et al., 2014). Although many aspects of perforate calcification remain unsolved, there is consensus that chamber formation takes place extracellularly, but within a (semi-) enclosed space, generally termed the site of calcification (SOC). The first layers of calcite precipitate on an organic matrix (the POS or primary organic sheet) that serves as a template for the calcite layer that forms the chamber wall (Erez, 2003; Hemleben et al., 1977). To promote calcification, foraminifera furthermore need to remove Mg ions

436 and/or protons (Zeebe and Sanyal, 2002) from the seawater entering the SOC. A number of larger
437 benthic foraminifera form hyaline shells, although the amount of Mg in their shells is often more than
438 10 times higher than that of planktonic and small benthic hyaline species, hence covering a larger range
439 in $Mg/Ca_{CALCITE}$ values. The calcification strategy of porcelaneous foraminifera is less well studied,
440 which may be partly explained by their limited application in paleoceanography. Porcelaneous
441 foraminifera use a different mode of calcification (Debenay et al., 1998; De Nooijer et al., 2009;
442 Berthold, 1976; Hemleben et al., 1986) and produce shells without pores (hence, the term imperforate)
443 consisting of tablets or needles (Erez, 2003; Bentov and Erez, 2006; Debenay et al., 1998). These calcitic
444 needles (2-3µm) are precipitated intracellularly (Berthold, 1976), after which they are transported out
445 of the foraminifer to form a new chamber (Angell, 1980). At the outer and inner layers of these
446 chambers, the needles are arranged along the same orientation so that they form an optically
447 homogenous surface, giving it a shiny (hence the term 'porcelaneous') appearance. In general the Mg/Ca
448 values of the shells of porcelaneous foraminifera are high.

449 Remarkably, despite this large biological control, incorporation of minor and trace elements still reflects
450 environmental conditions, in both hyaline and porcelaneous foraminiferal shells. Systematic offsets
451 between different species, interdependence of trace elements incorporated (Langer et al., 2016) and the
452 different response of element incorporation on element speciation (Wit et al., 2013; Keul et al., 2013;
453 van Dijk et al., 2017), potentially provides useful clues for determining which processes play an
454 important role in the biomineralization pathways. Here we present the results from a controlled growth
455 experiment for which we used several (intermediate- and high-Mg) hyaline and porcelaneous species
456 combined with an inter-species comparison of trace element incorporation. We assessed the impact of
457 bio-calcification on element incorporation as a function of pCO_2 in order to explore the proposed
458 inorganic carbonate proxies (e.g. Zn/Ca; van Dijk et al., 2017) and the impact of different calcification
459 strategies (hyaline versus porcelaneous) on element partitioning. We cultured eight benthic
460 foraminiferal species (4 hyaline and 4 porcelaneous) under four different pCO_2 conditions, analyzing
461 incorporation of Mg, Sr, Na, Zn and Ba.

462

2. Methods

2.1. Foraminiferal collection

Large samples of macroalgae (*Dictyota* sp.) were collected in November 2015 at a depth of 2-3 meters in Gallows Bay, St. Eustatius (N 17°28'31.6", W62°59'9.4"). Salinity was ~34 and temperature was ~29°C at the site of collection. The collected macroalgae were transported to the laboratory at the Caribbean Netherlands Science Institute (CNSI), where they were placed in a 5 L aquarium with aerated and unfiltered seawater. From this stock, small amounts of algae and debris were gently sieved over a 90 and 600 µm mesh to carefully dislodge foraminifera. Several species of foraminifera were picked from the resulting 90-600 µm fraction and directly from the macroalgae. Living specimens of *Sorites marginalis* (Lamarck, 1816), *Amphistegina gibbosa* (d'Orbigny, 1839), *Laevipeneroplis bradyi* (Cushman, 1930) and *Archaias angulatus* and limited amounts (<20) of *Peneroplis pertusus* (Forskål, 1775), *Asterigerina carinata* (d'Orbigny, 1839), *Heterostegina antillarum* (d' Orbigny, 1839), and *Planorbulina acervalis* (Brady, 1884) characterized by yellow cytoplasm and pseudopodial activity, were selected for the culturing experiments.

2.2. Culture set-up

Four barrels, each filled with 100 L of seawater (5µm filtered), were connected to a Li-Cor CO_2/H_2O analyzer (LI-7000), to regulate the CO_2 level in the barrels' head space. The set-points were maintained by addition of CO_2 and/ or CO_2-scrubbed air according to the monitored pCO_2. The set-points for pCO_2 were 350, 450, 760 and 1400 ppm resulting in four batches of seawater (treatment A-D) differing only in their inorganic carbon chemistry. Salinity (34.0±0.2) was monitored with a salinometer (VWR CO310). The fluorescent compound calcein (Bis[N,N-bis(carboxymethyl)aminomethyl]-fluorescein) was added to the culture media (5 mg/L seawater) to enable determination of newly formed chambers during the culture experiment (Bernhard et al., 2004). Short-term exposure (<three weeks) to calcein has no detectable impact on the physiology of benthic foraminifera (Kurtarkar et al., 2015), and the presence of calcein has no effect on the incorporation of Mg and Sr in foraminiferal calcite (Dissard et

al., 2009). Culture media was stored air-free in portions of 250 ml in Nalgene bottles with teflon lined caps at 4°C until further use.

Foraminifera were divided over the different treatments in duplicate and placed in 70 ml Falcon® tissue bottles with gas-tight caps in a thermostat set at 25°C (Fig. 1). The thermostat was monitored by a temperature logger (Traceable Logger Trac, Maxi Thermal), recording the temperature every minute. The average temperature over the whole experiment was 25 ±0.2°C. To create uniform light conditions, the thermostat was equipped with two LED shelfs, which resulted in high light conditions 12 hr/12hr. Culture media was replaced every four days, to avoid build-up of organic waste and to obtain stable seawater element concentrations and carbon chemistry. Foraminifera were fed after every water change with 0.5 ml of concentrated freeze-dried *Dunaliella salina* cells, pre-diluted with the corresponding treatment seawater. After 21 days, the experiment was terminated. Foraminifera were rinsed three times with de-ionized water, dried at 40°C and stored in micropaleontology slides until further analysis at the Royal Netherlands Institute for Sea Research (NIOZ).

2.3. Analytical methods

2.3.1. Seawater carbon parameters

At the start and termination of the experiment, 125 ml samples of the seawater at each of the different experimental conditions were collected to analyze dissolved inorganic carbon (DIC) and total alkalinity (TA) on a Versatile INstrument for the Determination of Titration Alkalinity (VINDTA) at the CNSI. Using the measured DIC and TA values and the software CO2SYS v2.1, adapted to Excel by Pierrot et al. (2006) the other carbon parameters (including $[CO_3^{2-}]$ and $\Omega_{CALCITE}$) were calculated. For this we used the equilibrium constants for K1 and K2 from Lueker et al. (2000) and $KHSO_4$ from (Dickson, 1990) (Table 1).

2.3.2. Seawater element concentrations

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

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

2.3.3. Cleaning methods

After termination of the experiment, foraminiferal shells were cleaned following an adapted version of Barker et al. (2003). Per treatment duplicate, all foraminifera were transferred to 10 ml polyethylene

vials. To each vial, 10 mL 1% H_2O_2 solution (buffered with 0.5M NH_4OH) was added to remove organic matter. The vials were heated for 10 minutes in a water bath at 95 °C, and placed in an ultrasonic bath for 30 seconds (degas mode, 80kHz, 50% power), after which the oxidizing reagent was removed. These steps (organic removal procedure) were repeated five times. Foraminiferal samples were rinsed five times with ultrapure water, after which the vials were stored overnight in a laminar flow cabinet at room temperature to dry. Dried foraminifera were placed on double sided tape on LA-ICP-MS stubs. Pictures were taken of individual foraminifera with a ZEISS Axioplan 2 fluoresence microscope equipped with appropriate excitation and emission optics and a ZEISS Axiocam MRc 5 camera, to assess the number of chambers added during the experiment based on the incorporation of calcein.

2.3.4. LA-ICP-MS

Element concentrations of individual fluorescent chambers were analyzed by Laser Ablation-ICP-MS (Reichart et al., 2003; van Dijk et al., 2017). To determine foraminiferal element concentrations, the laser system (NWR193UC, New Wave Research) at the Royal NIOZ was equipped with a Two Volume 2 cell (New Wave Research), characterized by a wash-out time of 1.8 seconds (1% level) and hence allowing detection of variability of obtained element to Ca ratios within chamber walls. Single chambers were ablated in a helium environment using a circular laser spot with a diameter of 80 µm (*S. marginalis*) or 60 µm (other species). We ablated all calcein-stained chambers twice, except for the first 1-2 chambers that formed during the experiment to avoid contamination of calcite of chambers formed prior to the experiments and overlapped by the labelled chambers (Fig.2).

All foraminiferal samples were ablated with an energy density of 1±0.1 J/cm^2 and a repetition rate of 6 Hz. The resulting aerosol was transported on a helium flow through an in house build smoothing device, being mixed with a nitrogen flow (5 ml/min), before entering the quadrupole ICP-MS (iCAP-Q, Thermo Scientific). Monitored masses included ^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{27}Al, ^{43}Ca, ^{44}Ca, ^{66}Zn, ^{88}Sr and ^{137}Ba. Contrary to ^{67}Zn and ^{68}Zn, ^{66}Zn is free of interferences when measuring calcium carbonate and SRM NIST (National Institute of Standard and Technology, U.S., Standard Reference Material) glass

standards (Jochum et al., 2012). Potential contamination or diagenesis of the outer or inner layer of calcite was excluded by monitoring the Al signal. At the start of each series, we analyzed SRM NIST612 and NIST610 glass standard in triplicate (using an energy density of 5±0.1 J/cm^2), JCt-1 (coral carbonate) and two in-house standards, namely NFHS (NIOZ Foraminifera House Standard; Mezger et al., 2016) and the Iceland spar NCHS (NIOZ Calcite House Standard). We further analyzed JCp-1 (coral, Porites sp.; Okai et al., 2002) and MACS-3 (Synthetic Calcium Carbonate) at the start of each series, and to monitor drift after every ten samples. All element to calcium ratios were calculated with an adapted version of the MATLAB based program SILLS (Signal Integration for Laboratory Laser Systems; Guillong et al., 2008). SILLS was modified by NIOZ to evaluate LA-ICP-MS measurements on foraminifera, allowing import of Thermo Qtegra software sample list, laser data reduction and laser LOG files. Major adaptions include improved automated integration and evaluation of (calibration and monitor) standards, quality control report of the monitor standards and export in element to calcium ratios (mmol/mol). Calibration was performed against the MACS-3 carbonate standard, with ^{43}Ca as an internal standard and we used the multiple measurements of MACS-3 for a linear drift correction. Relative analytical precision (relative standard deviation (RSD) of all MACS-3 analyses) is 3% for ^{23}Na, 3% for ^{24}Mg, 3% for ^{25}Mg, 4% for ^{66}Zn, 3% for ^{88}Sr and 3% for ^{137}Ba. In total, 961 analyses were performed on 251 specimens covering eight species cultured in four experimental conditions (see Table 2 for details).

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

we used average seawater E/Ca_{SW} to calculate D_E (see also supplementary Table 1), allowing comnparing partitioning coefficients.

3. Results

3.1. Element/Ca as a function of ocean acidification

In hyaline species $Mg/Ca_{CALCITE}$ varies between 25.9-141.3 mmol/mol Mg/Ca. In contrast, $Mg/Ca_{CALCITE}$ of porcelaneous species ranges from 121.3-149.3 mmol/mol (Table 3). This large spread in foraminifera E/Ca of hyaline species is also observed for Sr (1.7-3.1 mmol/mol), Na (3.4-19.5 mmol/mol), Zn (9.0-97.0 µmol/mol) and Ba (2.7-20.1 µmol/mol), while porcelaneous species only vary over a narrow range (Sr = 2.0-2.2 mmol/mol; Na = 3.8-5.8 mmol/mol; Zn = 53.0-140.8 µmol/mol; Ba = 18.0-29.0 µmol/mol).

In both porcelaneous and hyaline species we find an increase of $Zn/Ca_{CALCITE}$ and $Ba/Ca_{CALCITE}$ with pCO_2, while foraminiferal Sr/Ca, Mg/Ca and Na/Ca remain similar across the experimental conditions (Fig. 3 and Table 4). Sensitivity of both foraminiferal Zn/Ca and Ba/Ca to changes in seawater pCO_2 differs between the studied porcelaneous and hyaline species. When pCO_2 changes from 350 to 1200 ppm, Zn/Ca of hyaline foraminifera increase by a factor of 3.7 (*A. carinata*) or 4.5 (*A. gibbosa*) while porcelaneous foraminiferal Zn/Ca increases only by 1.3 (*S. marginalis*), 1.8 (*A. angulatus*) and 2.1 (*L. bradyi*). Also sensitivity of foraminiferal Ba/Ca to the same change in pCO_2 shows a similar pattern, with Ba/Ca of hyaline species increasing by a factor of 3.6 (*A. carinata*) or 3.7 (*A. gibbosa*), while porcelaneous species increase Ba/Ca only with a factor of 1.8 (*S. marginalis*), 1.6 (*A. angulatus*) or 2.1 (*L. bradyi*).

3.2. Inter-species differences in element incorporation

When comparing Mg incorporation to that of the other elements studied here (Ba, Zn, Sr and Na) between hyaline species (treatment B; Table 3), we observe a positive relation between D_{Mg} with D_{Sr} ($p<0.0025$), D_{Na} ($p<0.0005$), D_{Ba} ($p<0.05$) and D_{Zn} ($p<0.005$). In general hyaline species are enriched

similarly in all elements (Fig. 4). Compared to porcelaneous species, the hyaline shell building species which incorporate most Mg (>100 mmol/mol Mg/Ca) incorporate more Na, and Sr, while incorporating less Zn and Ba. Element incorporation across porcelaneous species is less variable than observed for hyaline species. Including data from literature (both culture and field calibrations; see supplementary Table S1), preferable in which both Mg/Ca and at least one other element (Na, Sr, Ba or Zn) is measured, shows that the relation based on the Caribbean species studied here is also more general applicable when including more species (D_{Sr} = $p<0.005$; D_{Na} = $p<0.0005$); D_{Ba} = $p<0.005$; D_{Zn} = $p<0.01$), even though this compiled data (labeled 'All studies' in Tabel S2) covers a somewhat wider range in environmental and experiment conditions.

4. Discussion

4.1. Effect of ocean acidification on Element/Ca

For neither porcelaneous nor hyaline species, foraminiferal Mg/Ca, Na/Ca and Sr/Ca systematically change with pCO_2. The impact of pH (and/or $[CO_3^{2-}]$) on $Mg/Ca_{CALCITE}$ and $Sr/Ca_{CALCITE}$ in foraminifera has been the subject of discussion (e.g., Dissard et al., 2010; Elderfield et al., 1996). In deep-sea benthic species, incorporation of certain elements is governed by carbonate system as observed for Zn by Marchitto et al. (2005) and Cd and Ba by McCorkle et al. (1995). Observed response to changes in carbonation ion concentration are in these studies mainly due to calcification in undersaturated seawater, as described by 'the carbonate ion saturation hypothesis' (Elderfield et al., 2006). In some low-Mg benthic species, both $Mg/Ca_{CALCITE}$ and $Sr/Ca_{CALCITE}$ do not seem to depend on inorganic carbon system parameters, e.g. pH or $[CO_3^{2-}]$ (Allison et al., 2011; Dueñas-Bohórquez et al., 2011). However, for several planktonic species pH does influence $Mg/Ca_{CALCITE}$ and $Sr/Ca_{CALCITE}$ (Russell et al., 2004; Lea et al., 1999; Evans et al., 2016). The effect of pH on $Sr/Ca_{CALCITE}$ might be explained via increased growth rates due to pH-associated changes in $[CO_3^{2-}]$ (Dissard et al., 2010). However, due to the limited experimental set-up, we are not able to disentangle the effects of the different carbon parameters in this study. Still, here we show that incorporation of Mg, Sr and Na of the selected larger benthic hyaline and

643 porcelaneous foraminifera are not significantly impacted when cultured over a limited range of pCO_2
644 and thus [CO_3^{2-}] and pH values.

645 In contrast, foraminiferal Zn/Ca and Ba/Ca are significantly impacted by pCO_2 for all species studied
646 here (Table 4; Fig. 3). Although Hönisch et al. (2011) suggested that the impact of carbonate chemistry
647 on Ba incorporation is negligible, their data does suggest a trend over the same interval in pH as studied
648 here. In hyaline foraminifera, Zn/Ca and Ba/Ca increases more as a function of pCO_2 (factor of 3.7-4.5
649 and 3.6-3.7, respectively when pCO_2 increases from 350 to 1200 ppm) compared to the porcelaneous
650 species (1.3-2.1 and 1.6-2.1 times, respectively). This observation suggests that the mechanisms
651 involved in uptake of Ba and Zn are similar for porcelaneous and hyaline species, although the
652 contribution of this process is different, which leads to differences in the sensitivity of Zn and Ba
653 incorporation with pCO_2

654

655 **4.2. Speciation in the microenvironment**

656 In the culture set-up used, increasing pCO_2 increases DIC, reduces pH and thereby decreases seawater
657 [CO_3^{2-}]. Speciation of Zn, Ba and also other elements, like U (Keul et al., 2013; Russell et al., 2004; van
658 Dijk et al., 2017), is primarily controlled by seawater [CO_3^{2-}]. The speciation of all elements studied
659 here (Mg, Na, Sr, Zn and Ba) for our different seawater treatments were modelled using PHREEQC, a
660 computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical
661 calculations (Parkhurst and Appelo, 1999). For this we used the standard in-software llnl database, a
662 PHREEQC database that implements most of the inorganic aqueous species and minerals in the
663 thermodynamic data and includes many elements not available in any other PHREEQC database. We
664 observed a decrease of free ions (Zn^{2+} and Ba^{2+}) and an increase in Ba and Zn carbonate complexes
665 ($BaCO_3^0$ and $ZnCO_3^0$), with increasing pCO_2 (Fig. 5), while the activity of Mg^{2+}, Na^+ and Sr^{2+} remained
666 similar. This suggests that element incorporation in foraminiferal calcite might be depending on the
667 availability of free ions, which in the case of Ba and Zn, changes with pCO_2. This is contrary to inorganic

668 precipitation, were carbonate complexes (e.g. $MgCO_3^0$) are easily incorporated into the calcite crystal
669 lattice.

670

671 **4.3. Trends in element incorporation**

672 Element incorporation in hyaline foraminifera is highly interdependent, i.e. species with increased Mg
673 content also incorporate more Sr, Na, Ba and Zn (Fig. 3). The linear increase in Sr incorporation with
674 increasing Mg/Ca has also been observed for other hyaline species, like *Operculina ammonoides* (Evans
675 et al., 2015), *Amphistegina lessonii* and *Ammonia aomoriensis* (Mewes et al., 2015), which both fall on
676 the same trend as that for inorganic calcite (Mucci and Morse, 1983). Evans et al. (2015) hypothesize
677 that the incorporation of other alkali elements, like Na is also related, due to lattice distortion by the
678 incorporation of Mg, which has been found in inorganic calcite (Okumura and Kitano, 1986). However,
679 although this mechanism might explain some of the observed species specific element incorporation in
680 hyaline foraminifera, this does not explain the observed difference between hyaline and porcelaneous
681 foraminifera (Figure 4). Porcelaneous foraminifera have generally high Mg/Ca, but we actually observe
682 lower incorporation of Na and Sr compared to hyaline species with similar D_{Mg} (Fig 4, upper right and
683 left panel: D_{Mg} versus D_{Na} and D_{Sr}). When including porcelaneous species from other studies we also
684 observe no increase in D_{Sr} over a larger range in D_{Mg} (Fig S1, upper left panel: D_{Mg} versus D_{Sr}).
685 Therefore, even though this interdependence might partly stem from mechanisms associated with
686 crystallography, differences between hyaline and porcelaneous foraminifera suggest it could also be
687 caused by mechanisms involved in take up the ions (Ca^{2+} and CO_3^{2-}) necessary for chamber formation,
688 which are different for hyaline and porcelaneous species, reflected in the different trends observed here.

689

690 **4.4. Ion transport models**

691 Both porcelaneous and hyaline foraminifera promote calcification by increasing their internal pH (De
692 Nooijer et al., 2009). Still, they might use different mechanisms to take up the ions (Ca^{2+} and CO_3^{2-})

693 necessary for chamber formation, which is reflected in the different trends observed here. In both
694 porcelaneous and hyaline foraminifera, E/Ca respond similarly to changes in pCO_2 (Fig 3), suggesting
695 uptake of all these elements is controlled by the same process. However, we observed different inter-
696 element relations between hyaline and porcelaneous foraminifera (Fig. 4), indicating the mechanisms
697 for ion transport might be different for these two groups. Two of the main concepts of ion transport in
698 foraminifera are transmembrane transport (Nehrke et al., 2013) and the inclusion of seawater by
699 seawater endocytosis (Bentov et al., 2009). Here we try to validate these two concept by comparing
700 them with our observations.

701

702 **4.4.1. Transmembrane transport**

703 During calcification, Ca^{2+} is proposed to be transported from seawater to the SOC via ion channels
704 (Nehrke et al., 2013), likely in exchange for protons (Toyofuku et al., accepted). This so-called trans-
705 membrane transport (TMT) through Ca^{2+} channels has also been found for other marine organisms,
706 including coccolithophores (Gussone et al., 2006). These Ca^{2+} channels may not discriminate perfectly
707 between Ca ions and elements like Mg, Sr, Ba, Na (Sather, 2005; Allen and Sanders, 1994; Hess and
708 Tsien, 1984), causing accidental transport of these elements into the SOC. How much of a certain
709 element will enter the SOC in this way, depends on 1) the selectiveness of the channels and the
710 characteristics of the transported ions (like atomic radius), 2) the element to calcium ratio in the
711 foraminiferal microenvironment and 3) the concentration gradient between seawater and the SOC. The
712 availability of some free ions, like Ba and Zn, changes as a function of pCO_2 due to the formation of
713 carbonate complexes (Fig. 5). When Zn and Ba form stable complexes with carbonate ions they are no
714 longer available for (sporadic) transport through the Ca^{2+} channels, decreasing the availability at the site
715 of calcification and subsequently, incorporation into the foraminiferal calcite. Thus, transmembrane
716 transport of ions by Ca^{2+} to the SOC is in agreement with our results (Fig. 3). The amount of Zn and Ba
717 available at the site of calcification is proportional to the concentration of the ratio between Ca^{2+} and
718 free Zn^{2+} and Ba^{2+} in the foraminiferal microenvironment. In turn, the amount of free Zn and Ba ions in
719 seawater is controlled by their respective concentration in seawater concentration, as well as carbonate

chemistry (Fig 5). Foraminiferal Mg/Ca, Na/Ca and Sr/Ca is not detectably affected, since the availability of Mg^{2+}, Na^{+} and Sr^{2+} does not change over the range of $[CO_3^{2-}]$ studied here. However, the large range in Mg/Ca values in hyaline species suggests that TMT might play a variable role in the calcification process of these species. This may result in an interdependence between all these elements studied such as observed here for the hyaline species if the selectivity for Ca^{2+} of these channels varies between species.

4.4.2. Seawater endocytosis

Another proposed ion transport mechanism is seawater endocytosis (Bentov et al., 2009; Erez, 2003), in which seawater is vacuolized, altered and then used as a calcifying fluid. The chemistry or elemental composition of the vacuolized seawater or the calcification fluid is in this case depending on the seawater concentration. Inter-species differences would therefore be minimized, as is observed for porcelaneous foraminifera in our study (Fig. 4). However, this concept cannot explain the overserved changes in Zn/Ca and Ba/Ca as a function of pCO_2, which are caused by the speciation of elements in seawater due to changes in the carbonate chemistry (Fig. 5). The concentration of total e.g. Zn in the seawater vacuoles does not change for the different treatments, only the species of Zn present. Only if the internal pH in the vacuole depends on ambient seawater pH, which is currently unknown, there is a potential for changes in speciation. In theory, if pH will change in concert with ambient seawater, such a change in internal pH from >9 (De Nooijer et al., 2009) to >8.6 (ΔpH = 0.4 in our treatment) changes $[CO_3^{2-}]$ and thus the speciation of e.g. Zn (and Ba) at the site of calcification. However, over this range the change in $[CO_3^{2-}]$ will be rather limited and hence such an effect of differential speciation within the calcifying fluid does not suffice to explain the observed sensitivity of Zn and Ba to pCO_2 in our study. This is in line with recent evidence on Zn/Ca in foraminifera, which suggests Zn incorporation is not primarily governed by changes in seawater pH, but by carbonate ion concentration, which does not change much at these high pH's (van Dijk et al., 2017).

4.5. Consequences for calcification in hyaline and porcelaneous species

Both ion transport mechanisms, and their consequences for Zn and Ba incorporation, are summarized in Fig. 6. The observed species-specific element incorporation in hyaline foraminifera (Fig. 4) is compatible with the transmembrane transport mixing model proposed by Nehrke et al. (2013), where species specific differences in E/Ca are explain by the relative contribution of transmembrane transport and so-called passive transport. In contrast to hyaline species, the porcelaneous species show much less inter-species variation in element composition (Fig. 3), suggesting that this group of foraminifera calcify from a fluid comparable to ambient seawater (Ter Kuile and Erez, 1987), by e.g. seawater endocytosis (Fig. 6, panel B) with only minor alteration of the elemental composition of the calcifying fluid by ion channels. However, the observed correlation between pCO_2 and Ba and Zn (Fig. 3) suggests that Ca channels still play a (modest) role in supplying Ca^{2+} to the porcelaneous SOC, since possible speciation of minor and trace elements in the SOC caused by a change in the internal pH is probably not sufficient to explain observed patterns (4.4.2). However since porcelaneous species already obtain calcium by including seawater in their calcification vesicle prior to calcite precipitation, contribution of Ca^{2+} through TMT is likely smaller than in hyaline species, which may explain the observed lower sensitivity of e.g. foraminiferal Zn/Ca and Ba/Ca to changes in seawater [CO_3^{2-}] in porcelaneous species (Fig. 3). This approximately 2 times lower sensitivity of porcelaneous foraminifera compared to hyaline species suggests that porcelaneous foraminifera acquire half of the necessary Ca^{2+} through Ca-channels compared to hyaline species. Element incorporation in porcelaneous foraminifera will therefore be mainly governed by their respective concentrations in seawater, and to a lesser extent by the selectivity for Ca^{2+}/permeability for other ions during TMT.

5. Conclusions

Trends in element incorporation in larger benthic foraminifera can be explained by a combination of differences in calcification strategy and seawater chemistry. Carbonate chemistry of seawater determines speciation and therefore availability of some ions (e.g. Zn^{2+} and Ba^{2+}), which are available

for ion transport to the site of calcification. For hyaline foraminifera, we observed species-specific interdependence of element incorporation, which can be explained by a previously proposed transmembrane transport model and the bioavailability of ions in seawater during calcification. For porcelaneous foraminifera, species specific difference are small, hinting at a higher contribution of another ion source, like e.g. seawater endocytosis.

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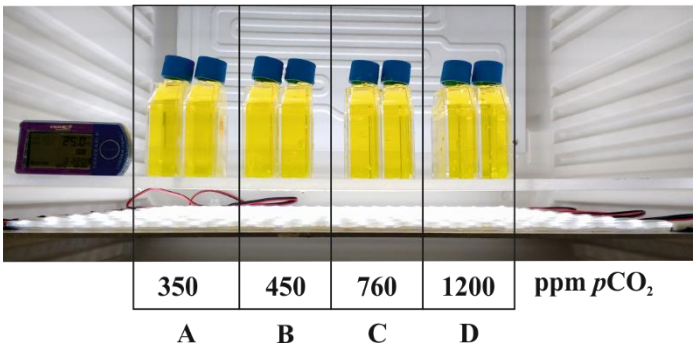


Figure 1. Photograph of the culture set-up. Four duplicate bottles with culture media (with calcein added) and adult specimens of foraminifera. Treatment with corresponding set-points are A=350 ppm, B=450 ppm, C=760 ppm, D=1200 ppm CO_2.

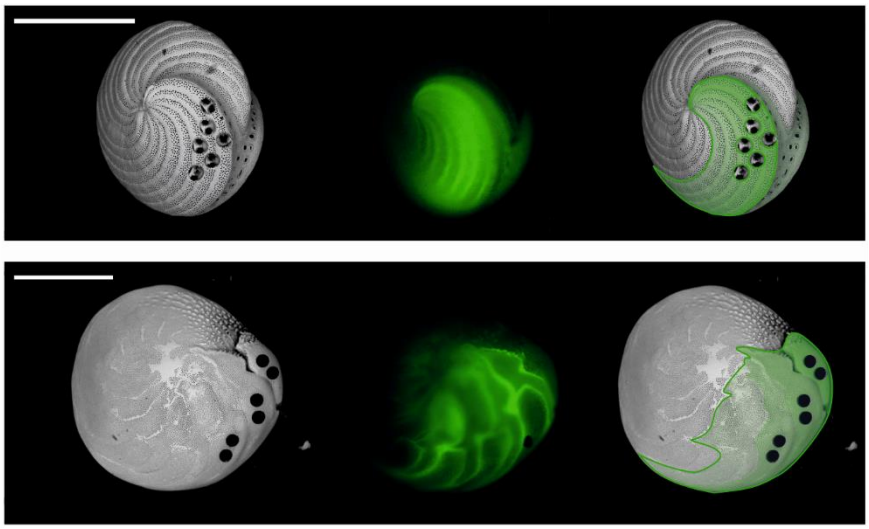
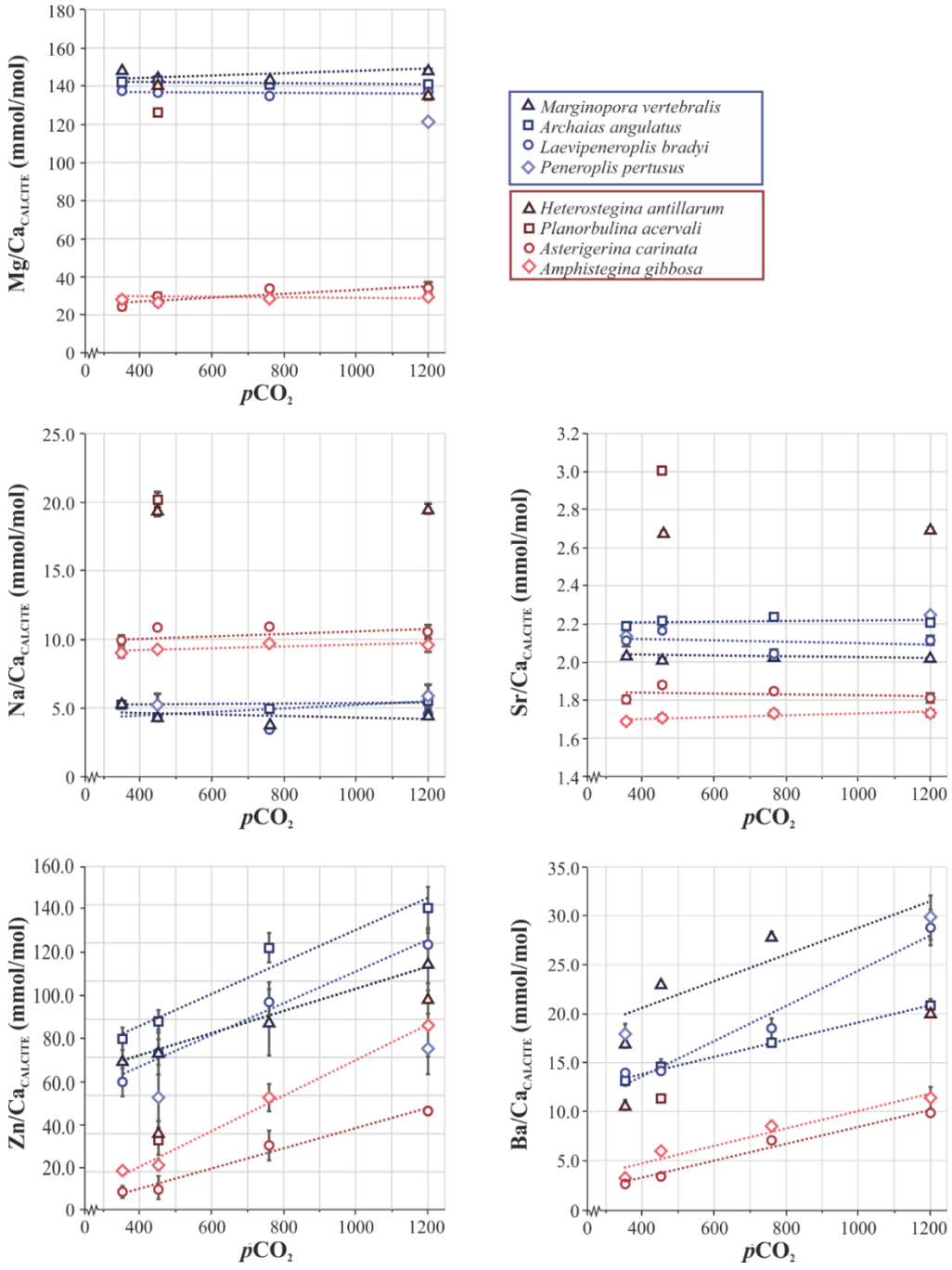
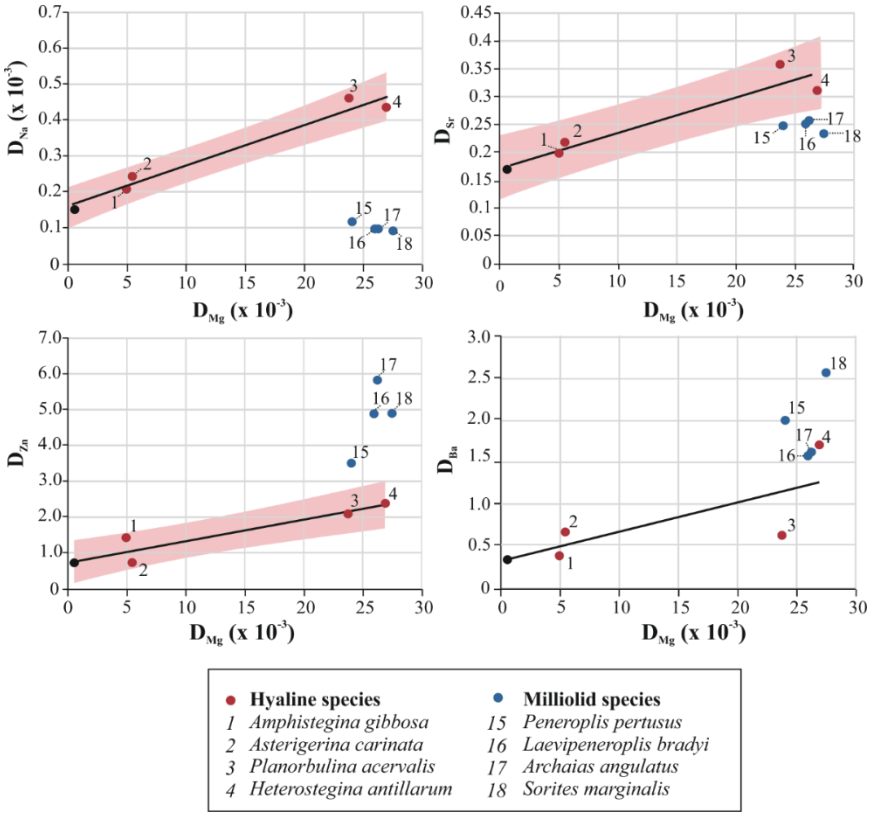


Figure 2. SEM (left) and fluorescence microscope (middle) photographs of *A. angulatus* (top series) and *A. gibbosa* (bottom series) to assess newly formed chambers for laser ablation (right). Scale bar = 500 µm.



989

990 **Figure 3. Element to Ca ratios (±SE) of different species of foraminifera over a range of pCO_2**
991 **values. In some cases, the error bar is smaller than the symbol. Porcelaneous species in blue**
992 **(triangles = *S. marginalis*; squares = *A. angulatus*; circles = *L. bradyi*; squares = *P. pertusus*) and**
993 **hyaline species in red (triangles = *H. antillarum*; squares = *P. acervalis*; circles = A. *carinata;***
994 **diamonds = *A. gibbosa*).**



995

996 **Figure 4. Partition coefficient of Na, Sr, Zn and Ba versus D_{Mg} of hyaline (red symbols) and**
997 **porcelaneous (blue symbols) species. Black lines represent linear trendlines. The 95% confidence**
998 **intervals of signification trends ($p<0.025$) are indicated in red. Black dots represent the NFHS, in-**
999 **house carbonate standard, consisting of planktonic foraminifera. Numbers correspond to**
1000 **foraminiferal species analyzed, numbers 5-14 and 18-21 represent values from previously**
1001 **published species and are included in Figure S1 (supplementary information).**

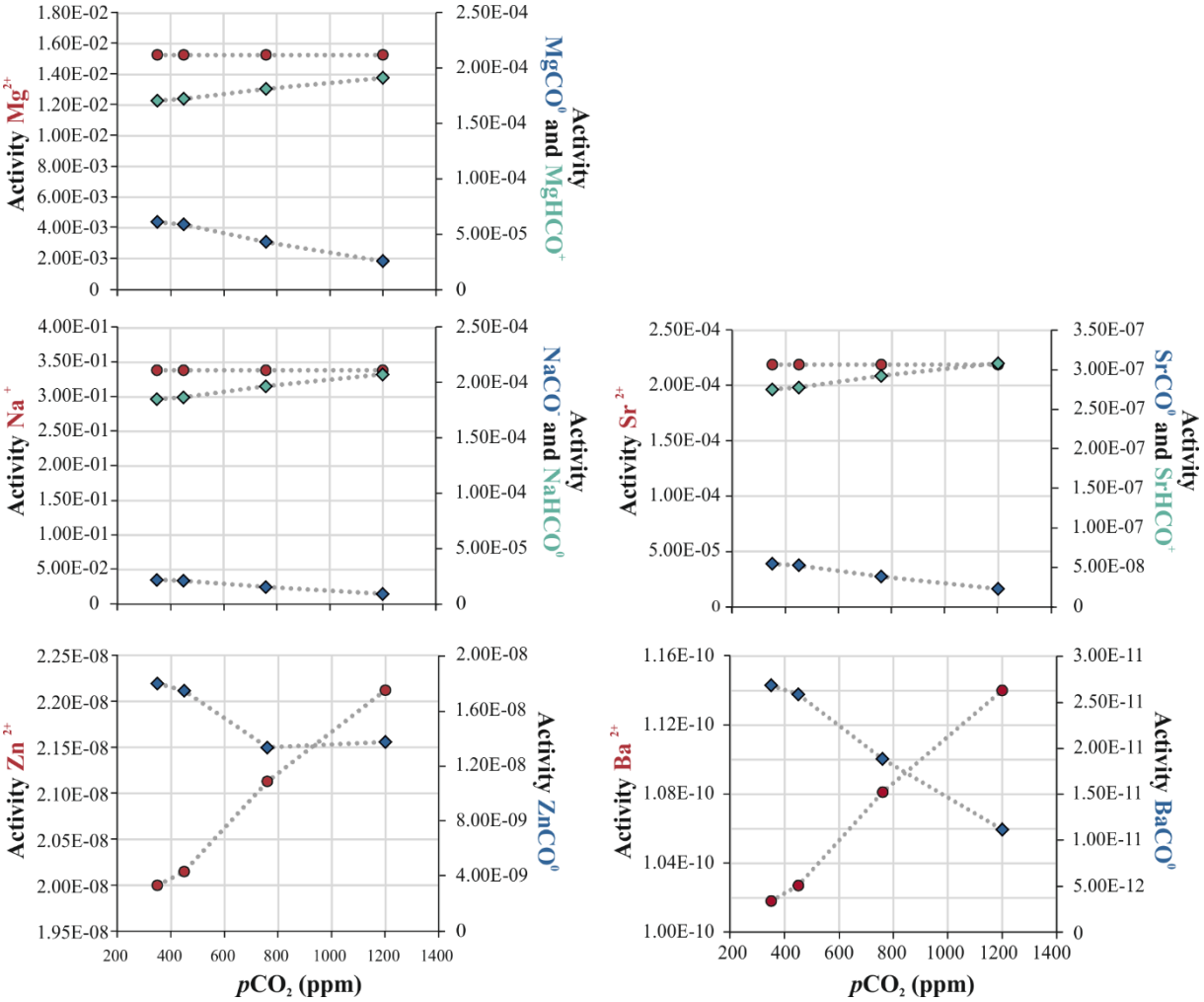


Figure 5. Speciation of Mg, Na, Sr, Zn and Ba in the different seawater treatments modelled in PHREEQC (Parkhurst and Appelo, 1999). Activities of free ions (red) and element (E)-carbonate complexes (ECO_3 = blue diamonds and $EHCO_3$ = green diamonds).

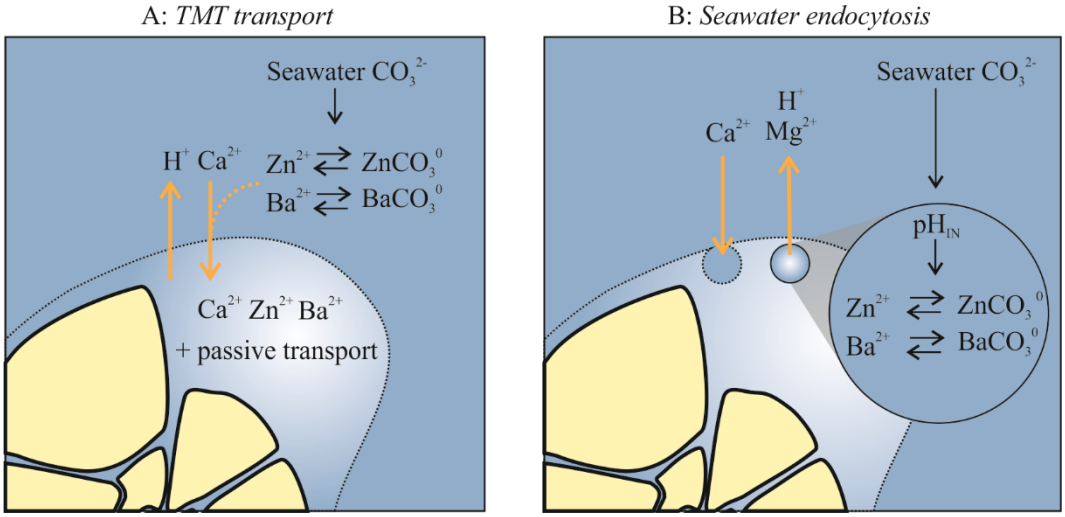


Figure 6. Schematic overview of different mechanisms for ion transport during foraminiferal calcification. Orange arrows indicate transport of ions. A) The transmembrane transport (TMT) mixing model as proposed by Nehrke et al. (2013). The amount of free ions, e.g. Zn^{2+} and Ba^{2+}, available for transport by Ca^{2+} in exchange for protons (Toyofuku et al., accepted) is influenced by speciation due to changes in seawater [CO_3^{2-}]. The composition of the seawater at the site of calcification is determined by both TMT and passive transport B) Simplified overview of seawater endocytosis (Bentov et al., 2009; Erez, 2003), were speciation of Zn^{2+} and Ba^{2+} is determined by changes in internal pH (pH_{IN}), which changes with ambient seawater carbonate chemistry. Based on our observations, panel A likely applies to hyaline species, whereas panel B represents porcelaneous calcification.

Treatment	Set-point	Measured		Calculated CO2SYS		
	pCO_2 ppm	TA µmol/kg	DIC µmol/kg	$[CO_3^{2-}]$ µmol/kg	pH (total scale)	$\Omega_{CALCITE}$
A	350	2302.8±8.2	2007.5±10.7	220.7	8.06	5.4
B	450	2305.2±5.8	2021.3±12.5	200.0	8.01	4.9
C	760	2304.4±0.9	2100.8±13.4	153.7	7.87	3.7
D	1200	2300.3±0.7	2201.4±4.1	92.2	7.61	2.2

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

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

1021 **Table 2. Total number of LA-ICP-MS measurements per species, per treatment (A-D).**

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

Hyaline species											
H. antillarum	350										
	450	141.3±0.3[a]	26.9	19.4±0.5[a]	0.44	2.7±0.02[a]	0.31	36.0±14.7[a]	2.4	10.7±0.5[a]	1.2
	760										
	1200	136.9±1.6[a]	26.1	19.5±0.4[a]	0.44	2.7±0.02[a]	0.31	97.0±18.3[b]	6.4	20.1±0.2[b]	2.2
P. acervalis	350										
	450	139.1±1.2	26.5	19.5±0.7	0.46	3.1±0.02	0.36	31.6±6.6	2.1	11.3±0.5	1.3
	760										
	1200										
A. carinata	350	23.6±1.5[a]	4.5	9.9±0.4[a]	0.22	1.8±0.02[a]	0.21	9.0±2.6[a]	0.6	3.2±0.5[a]	0.4
	450	28.5±2.4[b]	5.4	10.8±0.1[a]	0.24	1.9±0.01[a]	0.22	10.9±5.5[a]	0.7	6.0±0.5[b]	0.7
	760	33.1±1.2[b]	6.3	10.9±0.2[a]	0.24	1.8±0.01[a]	0.21	30.7±7.0[b]	2.0	8.5±0.6[c]	0.9
	1200	33.5±3.1[b]	6.4	10.6±0.5[a]	0.24	1.8±0.03[a]	0.21	46.4±2.1[b]	3.1	11.4±0.2[d]	1.3
A. gibbosa	350	27.8±0.5[a]	5.3	9.0±0.1[a]	0.20	1.7±0.01[a]	0.20	19.0±1.8[a]	1.3	2.7±0.5[a]	0.3
	450	25.9±0.6[b]	4.9	9.2±0.1[a]	0.21	1.7±0.02[a]	0.20	21.5±2.5[b]	1.4	3.4±0.5[a]	0.4
	760	28.2±0.7[a]	5.4	9.7±0.1[b]	0.22	1.7±0.02[a]	0.20	52.8±6.1[c]	3.5	7.1±0.6[b]	0.8
	1200	28.7±0.6[a]	5.5	9.6±0.1[b]	0.21	1.7±0.02[a]	0.20	85.8±11.3[d]	5.7	9.9±0.2[c]	1.1

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

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

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