Review # 1:

Reviewer: I would like to know some basic results from ecological and micropalaeontological point of view. How about survival rates, cell colors and symbiosis of each condition?

Response: the following sentences were added to the manuscript: P17468 L 4:

Changes: "Dead specimens were identified by a change in color from brownish/greenish to pale/white, due to their loss of symbionts. Survival rates were high (ca. 95%) and not correlated with any measured parameter. Dead specimens were removed from culture".

Reviewer: The study changes calcium concentrations of culture media. Normally, too much calcium is toxic for biology. This study itself also indicates foraminiferal populations show no growth at the highest calcium condition. I wonder their survivorship at extreme calcium conditions. The information must be valuable when the adaptation possibility of recent foraminifera is considered through geologic time.

Response: This is not completely true. The study indicates that foraminifers do not grow at lowest calcium concentration. At highest calcium concentrations foraminifers still grow, however their growth rates are reduced (compare Fig. 1 and P 17470, L16-19). As mentioned before and added to the manuscript, P17468 L 4, death happened rarely and was not higher in the extreme calcium condition compared to the other treatments.

Reviewer: Further, I would like to see SEM photos of foraminiferal from each condition. The authors already indicate size normalized weight for test wall thickness. I think visual material like SEM or optical microscopic-photo with description of test morphology and test surfaces' structures bring invaluable information to an audience.

Response: We used our test material for further isotope analyzes. Therefore it is unfortunately not possible to add SEM pictures to this manuscript.

Reviewer: The latter part of section 4.2 would be one of the key feature of this study (mainly in P.17475). I support there are species specific TMT fractionation in foraminifera than coccolithophore. The discussion would be much generalized to predict specific TMT fractionation among species like solver system.

Response: We do not completely understand what the reviewer wants us to do, and the meaning of "solver system" is unclear to us. However, we take it that the reviewer alludes to the value for TMT fractionation we use. As stated in the text **(**P.17475, L. 4-8) this value is unknown, and our chosen number falls well within the range of reasonable Ca channel fractionations **(**P.17475, L. 4-

8). We do not wish to further speculate on species-specific TMT fractionations, as the reviewer seems to suggest we should do, because we feel that the high uncertainty inherently attached to such a speculation would preclude any benefit the reader might get from it.

Reviewer: P17464 L16 telative -> relative?

Response: telative was changed to relative

Reviewer: P17466 L16 No authigenic crystals are precipitated during stock?

Response: No authogenic crystals were found when culture media were observed under the microscope. In addition we determined alkalinity of the stock solution at the beginning of the experiment, once a week during the experiment and at the end of the experiment (compare P 17568 L 7-9). Alkalinity was constant during the experiment, therefore no inorganic precipitation took place.

Reviewer: P17468 L3 Why the culture dish is changed so frequently even there are risks of lost?

Response: Foraminifers were feed with *Dunaliella salina* during the culture experiment. However not all *D. salina* cells were consumed by the foraminifers. To keep foraminifers free from bacteria and putrefaction they were transferred to clean petri dishes once a week.

Changes: P 17468, L 3: "To prevent bacterial colonialization of petri dishes due to left-over food, all specimens were transferred to a clean petri dish once every week. This resulted in an occasional loss of some specimens."

Reviewer: P17469 L6 weight [ug]? Could you check the unit?

Response: yes, the unit was incorrect

Changes: weight [µm] was changed to [µg]

Review # 2:

Reviewer: I am concerned that the conclusion that an optimal Mg/Ca_{SW} and not [Ca] drives faster growth rates is reached too quickly and with not enough justification (p. 17471-2). While this may be the correct conclusion, it needs to be clearly shown and explained why increasing [Ca] (up to a point) does not drive higher growth rates (until a certain, too-high level of [Ca] is reached). This could be shown with the addition of a figure showing [Ca] vs. size, SNW or growth rate and better justification in the discussion.

Response: We agree with the reviewer and have therefore added and rephrased the text according to this concern. The answers to the specific comments (see below) list the changes that we made to clarify the distinction between the potential effect of Mg/Ca_{SW} versus that of $[Ca^{2+}]$.

Reviewer: The style of writing in the later part of the discussion section is unclear at several points, mentioning previously discussed concepts or cited sources and referring to phenomena, mechanisms and models without explaining clearly what idea is being referred to. See specific comments below for pages 17473-17475.

Response: We addressed all specific comments below and described concepts, mechanisms and models in more detail (see comments for page 17473-17475).

Reviewer: All figure captions should explain the figure content and the authors' interpretation more (the captions should summarize what the key message of each figure is), and the message(s) derived from the figures should be stated more explicitly in the text when the figures are cited (e.g. 17472, L 29, where the figure interpretation is not well-stated).

Response: We rephrased the sentence:
"The effect of Mg/Ca_{SW} on foraminiferal SNW shows the same trend (optimum curve) as the effect of Mg/Ca_{SW} on growth rates. Similar trends…" Other specific comments to this issue are addressed below.

Specific comments:

Reviewer: 17465, line 10-12: This sentence has problems. Impacts is the wrong word: variables covary in the natural environment, but impacts do not covary. The second phrase is grammatically incorrect: instead, it could be "as well as allowing seawater conditions more extreme than natural conditions."

Response: We agree and have therefore changed “impacts” to “variables” and the second phrase was changed to: “as well as allowing seawater conditions to be more extreme than naturally occurring.”

Reviewer: 17466, line 9-10: Sampling living foraminifera from a zoo aquarium instead of the natural environment seems not ideal, as the forams here are already not living in natural conditions and naturally varying seawater. Can you provide some justification for this choice?

Response: The following sentences were added to the respective chapter: (P 17466 L 9)
“The benthic foraminifer *Amphistegina lessonii* was chosen for this experiment because our experience has shown that *A. lessonii* grow and reproduce well in our laboratory. Due to its relatively large size of >1 mm it is relatively easy to observe and handle. Because of cost efficient and easy accessibility coral reef rubble with attached benthic foraminifera was sampled in April 2012 from a coral reef aquarium at Burger’s Zoo, Arnhem, The Netherlands (Ernst et al., 2011). Sampling foraminifers from the zoo aquarium instead of the natural environment seems at first view not ideal. The zoo’s aquarium is however one of the largest aquaria in the world, harboring a very rich (micro)fauna and providing spatially diverse microhabitats. In the present study we are dealing with a fairly fundamental aspect of physiology, namely with the response to concentrations and ratios of major ions in seawater. Zoo-specimens have no opportunity to adjust their physiological machinery to changing Mg and Ca, since these concentrations are the same in the aquarium as in the field.”

Reviewer: 17467, line 10-11: This sentence should make more clear that the forams used in this experiment were not the zoo-derived forams, but rather their culture-grown offspring (assuming that is the case). It is explained later, but should be made clear earlier.

Response: This sentence was changed:
“For the culture experiment we used in culture grown offspring of the zoo-derived specimens. Offspring were used to ensure……”

Reviewer: 17470, line 16-21: These described results are quite difficult to see on Fig. 1A (see technical comments below for suggested improvements to Fig. 1A).

Response: We have increased the size of the symbols in figure 1.

Reviewer: 17470, line 23: Mistake – the largest test size is in fact at SW [Ca]= 17.9 (not 7) and SW Mg/Ca 2.9, which can be seen when comparing Table 1 and Fig. 1b. However, it’s very hard to figure this out, as neither the table or the figure shows both seawater [Ca] and final test size. I

suggest including average final shell weights, SNW and growth rate for each treatment in Table 1.

Response: Yes, this is a typing error. We have changed [Ca]= 7 to [Ca]= 17.9.
We also follow the reviewer's suggestion to add final shell weights, SNW and growth rate to Table 1.

Reviewer: 17471, line 20: Please state briefly the findings of Mewes et al. 2014 so the reader can understand how this study agrees with it.

Response: This is done in page 17472, line 3ff.

Reviewer: 17471, line 20-21: The statement "suggesting that the calcium concentration itself may not be the primary driver of growth rate" strongly needs further explanation – why can you exclude [Ca] as a driver of growth rate? These data so far could also be interpreted to mean that increasing [Ca] causes faster growth until a certain toxic level of [Ca] between 18 and 34. If this is not the case, the discussion section needs to more explicitly address why this can be ruled out.

Response: This statement is indeed true when not taken into account the data by Mewes et al. (2014), in which seawater [Mg] was varied (and [Ca] kept constant). Both datasets show a similar response in growth rates, therefore suggesting that [Ca] itself is not the main driver of growth.

To clearify this discussion, we have deleted the sentence the reviewer referred to and replaced it with the following sentences:
line 19: Considering the current dataset by itself one could get to the conclusion that increasing [Ca] causes faster growth until a certain toxic level at [Ca] > 18 mM. However, comparing the present dataset with the one from Mewes et al. (2014), where the absolute [Mg] was varied and [Ca] was kept constant, shows that the calcium concentration by itself is not be the primary driver of growth rate but that it is controlled by the Mg/Ca of seawater.
line 6: The varying growth rates in the Mewes et al. (2014) dataset, at constant [Ca] clearly show that not the calcium concentration itself is the primary driver of growth rates. Considering both datasets rather suggest that the Mg/CaSW ratio….

Reviewer: 17471, line 23: The choice to use a "linear regression curve fitted to the size data of the first 30 days" instead of simply the size data needs to be explained and justified better. Currently, it leaves the question why you can't just compare size or the actual calculated growth rate instead of doing a regression to size (which, lacking an explanation, seems unnecessary). Please clarify/justify.

Response: We have added the following sentence to justify the choice for a linear regression for a limited timespan:
P 17471, line 23: It is not possible to derive growth rates from a linear regression line fitted to the time span of the whole experiment (49 days), due to the saturation of growth in the present study after 30 days. As a result, the time spans of growth between the two culture studies are diferent and do not allow a simple comparison of final test size.

Reviewer: 17472, L 3-4: Please clarify what it is that reaches an optimum in the Mewes et al. 2014 study (growth rate? SNW? Something else?)

Response: It is the growth rates as explained in line 1-2 and 6-7 of page 17472.

Reviewer: 17472, L 6-7: Grammatical problem (incomplete sentence), awkwardly phrased.

Response: We rephrased the sentence: "Considering both datasets rather suggest that the seawater Mg/Ca ratio is the primary driver of growth rates and not the absolute concentrations of Ca or Mg."

Reviewer: 17472, L 6-8: This conclusion has not been adequately explained and justified in this paper – as the data is presented currently, it is not made clear that this dataset suggests that. A figure showing [Ca] vs. growth rate would be quite helpful, but lacking that, you cannot exclude the possibility that perhaps increasing [Ca] could also explain faster growth. Please justify this interpretation more.

Response: The reviewer may indeed be right: increasing [Ca] may promote foraminiferal growth rates. We argue, however, that it is not the primary driver: Not just [Ca], but also [Mg] (Mewes et al. (2014) and therefore seawater Mg/Ca determines growth rates.
We think that it makes little sense to include a graph showing [Ca] versus growth because it would be exactly the same graph as Mg/Ca versus growth, only mirrored. It would be the same since with increasing Mg/Ca_{SW}, Ca decreases. With the changes made to the previous comment (17472, L6-7), we believe that we have made our interpretation more clear.

Reviewer: 17472, L 12-13: The Segev and Erez 2006 optimal Mg/Casw value is very different from your optimal value - how do you explain this? Please propose some explanations for the difference.

Response: We agree insofar that more explanation is required. We will, however, point out that the values are not "very different". This judgment may be caused by lines 25-27, which we therefore rephrased and now read: "Together, the results of Segev and Erez (2006) and those presented here strongly suggest that growth in *Amphistegina spp.* is influenced by the Mg/Ca_{SW} ratio. With these datasets, it is currently impossible to determine the optimal Mg/Ca_{SW} ratio for foraminiferal growth, because the available datasets suggest a plateau, rather than a clearly defined peak-value. Our dataset (Fig. 4) suggests an optimum between 3 and 5 mol/mol, but may range from 2 to 5 mol/mol. The dataset of Segev and Erez (2006) locates the optimum between 1 and 2.5 mol/mol. But this is a potentially biased range, because there are no data between Mg/Ca_{SW} of 2.5 and 5 mol/mol. This implies that these two datasets combined (Fig. 4, Segev and Erez 2006) suggest an optimum between 1 and 5 mol/mol. While this might appear to be a large range, it is a reasonable interval from a physiological perspective, because physiological optima usually comprise a range of values. Well known examples are temperature, light intensity, and nutrient concentrations."

Reviewer: 17472, L 21-22: This sentence is unclear – I think what you mean is that at low Mg/Ca, lowering [Mg] produced a higher growth rate than raising [Ca].

Response: That is correct. We re-wrote the sentence. It now reads: "Interestingly, growth rates at lowest Mg/Ca is lower in the case of the Ca-variable experiment, indicating that at this particular Mg/Ca the high Ca concentration may be more detrimental to growth than the low Mg concentration."

Reviewer: 17472, L 28, 17473 L1: "foraminiferal SNW, which is correlated to the change in growth rates as a function of Mg=CaSW." This relationship is not shown in this or the other figures (or if so, it's quite obscured). If this is discussed, it needs to be shown.

Response: Probably the word "correlated" leads to a confusion here. We rephrased the sentence: "The effect of Mg/CaSW on foraminiferal SNW shows the same trend (optimum curve) as the effect of Mg/CaSW on growth rates. Similar trends…"

Reviewer: 17473 L6: What phenomenon is being discussed in this sentence and the ones before/after? This is unclear, and the paragraph is hard to make sense of for that reason.

Response: We refer to the comparison of absolute values. We have therefore deleted the word "phenomenon".

Reviewer: 17473, L19-21: This sentence is hard to understand – please clarify it.

Response: We agree and replaced lines 15-22 with the following: “This result would be in accordance with a calcification mechanism based on seawater vacuolization, if the Mg transport mechanism features a Ca fractionation independent of seawater Mg or Ca concentrations. While this is a perfectly reasonable scenario, it makes little sense when considering the assumed function of this transport, i.e. Mg homoeostasis. In other words, if the behavior of the Mg transporter is compatible with our data, it cannot perform its alleged role. Calcification based on vacuolization might exclude Mg homoeostasis as a function of this transporter and instead, the function might merely be a lowering of the Mg/Ca ratio. Hence the question whether our data are compatible a calcification mechanism based on vacuolization of seawater, depends on the precise interpretation of this mechanism.”

Reviewer: 17474, L10: Please briefly restate this mixing model (Nehrke et al. 2013) so that it’s clear what you mean when you say that your relationship agrees with it.

Response: We added a sentence, line 8:
The mixing model (Nehrke et al., 2013) predicts a linear relationship between Mg/Ca_{CC} and Mg/Ca_{SW}, intersecting with the origin.

Reviewer: 17474, L13: Please succinctly summarize the discussion in Mewes et al. 2014 that addresses this difference with the mixing model and cite it so that the point of that discussion is clear here.

The discussion of Mewes et al 2014 offers three scenarios to explain the difference between their dataset and the conceptual mixing model. In the current study we demonstrate that the conceptual mixing model agrees with the dataset. The present study furthermore presents a mathematically refined-flux based model to describe the conceptual mixing model. Therefore it would not make sense to repeat the discussion of Mewes et al. (2014) and would only confuse the reader in our opinion.

Reviewer: 17475, eqn. 3: Please define Rsw before/after equation. This term is defined only in the appendix.

Response: We agree and defined the R_{SW} in the text: 17476, Line 3: “The curve (Fig. 7a) can be fitted over the whole RSW (= Mg/Ca_{SW}) with a TMT….

Reviewer: 17475, L 16-17: Please restate the physiological mechanism you have presented – the reader is left wondering, “What was the mechanism?”

Response: We made the following changes: L 16: “We present such a physiological mechanism, which comprises transmembrane transport and seawater vacuolization.”

Reviewer: 17475, L 17-19: Please elaborate on/restate what the “promising new way of interpreting foraminiferal element to calcium ratios” you have presented is – it has gotten somewhat lost in the previous part.

Response: By adding “…,which comprises transmembrane transport and seawater vacuolization.” (see above) we hope to have clarified the issue.

Reviewer: 17486, Fig. 4: I would like to see a figure/pair of figures showing different [Ca] and [Mg] vs. shell size or growth rate (like Fig. 1a or Fig. 4). These figures alone do not rule out that higher [Ca] does not drive faster growth.

Response: As already stated before such a figure would show exactly the same as when plotted vs. Mg/Ca_{SW}.

Reviewer: 17488, Fig. 6a, b: The caption here (and in all figures) should explain the figures more. In figures 6a, 6b, is the grey line a fit to the stable [Ca] data, the stable [Mg] data, or both combined? Need to also cite the Mewes et al. 2014 data source for Mg.

Response: We added the following sentence to the figure caption 6: “Grey lines represent functions fitted to the combined dataset.”
We have added the Mewes et al. (2014) citation to the caption of figures 5, 6a and 6b.

Purely technical comments:

Reviewer: 17482, Table 1: I suggest including mean final shell weights, size normalized weight and growth rate for each treatment in this table to make it easier to compare the culture environment with the results. The table title can be modified accordingly.

Response: We agree and have added the parameters to the table (Table 1) and have changed its title accordingly to: “Details of culture media as well as morphological and chemical test parameters”.

Reviewer: 17483, Fig. 1A: the figure is very hard to read because the symbols are small, overlapping, and the error bars obscure their shape. I would recommend either using color, or

drawing lines between each type of symbols so the reader can follow each [Ca] trend. Also use error bars without end caps so the symbols are more distinguishable.

Response: We have increased the size of the symbols.

Reviewer: 17483, Fig. 1B, 1A and table 1 conflict with the text (presumably the mistake was in the text, as noted above): 1A and table 1 show the largest final tests were in the [Ca]=17.9 and [Ca]=9 sizes, but the text (pg. 17470, line 23) shows the largest final tests were in [Ca]=7 and [Ca]=9.

Response: This was indeed a mistake in the text and we have adjusted the text here.

Reviewer: 17487, Fig. 5: y-axis title is redundant – SNW already contains the word "normalized", so the axis title should not contain the word and the acronym.

Response: In this case it is not redundant, because it is referred to the normalized size normalized weight. For explanation see text of methods, P 17469, line 7-10

Reviewer: 17489: Fig. 7b is not referenced in the text.

Response: It is referenced in the text: P 17475, line 13

Impact of seawater [Ca^{2+}] on the calcification and calcite Mg/Ca of *Amphistegina lessonii*

Antje Mewes[a]*, Gerald Langer[b], Silke Thoms[a], Gernot Nehrke[a], Gert-Jan Reichart[c,d], Lennart Jan de Nooijer[c], Jelle Bijma[a]

a: Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

b: Department of Earth Sciences, Cambridge University, Downing St., Cambridge, CB2 3EQ, UK

c: Royal Netherlands Institute for Sea Research, Landsdiep 4, 1797 SZ 't Horntje, Texel, The Netherlands

d: Department of Earth Sciences, Utrecht University, Budapestlaan, 4, 3584 CD Utrecht, The Netherlands

(*correspondence to: antje.mewes@awi.de; phone: +49 (0)471 4831 2291)

Keywords: Mg/Ca; proxy; LA-ICP-MS; culture experiment; biomineralization; *Amphistegina lessonii*

Abstract

Mg/Ca ratios in foraminiferal tests are routinely used as paleo temperature proxy, but on long timescales, also hold the potential to reconstruct past seawater Mg/Ca. Impact of both temperature and seawater Mg/Ca on Mg incorporation in foraminifera have been quantified by a number of studies. The underlying mechanism responsible for Mg incorporation in foraminiferal calcite and its sensitivity to environmental conditions, however, is not fully identified. A recently published biomineralization model (Nehrke et al., 2013) proposes a combination of transmembrane transport and seawater leakage or vacuolization to link calcite Mg/Ca to seawater Mg/Ca and explains inter-species variability in Mg/Ca ratios. To test the assumptions of this model, we conducted a culture study in which seawater Mg/Ca was manipulated by varying $[Ca^{2+}]$ and keeping $[Mg^{2+}]$ constant. Foraminiferal growth rates, test thickness and calcite Mg/Ca of newly formed chambers were analyzed. Results showed optimum growth rates and test thickness at Mg/Ca closest to that of ambient seawater. Calcite Mg/Ca is positively correlated to seawater Mg/Ca, indicating that not absolute seawater $[Ca^{2+}]$ and $[Mg^{2+}]$, but their ratio controls Mg/Ca in tests. These results demonstrate that the calcification process cannot be based only on seawater vacuolization, supporting the mixing model proposed by Nehrke et al. (2013). Here we, however, suggest a transmembrane transport fractionation that is not as strong as suggested by Nehrke et al. (20013).

Introduction

Foraminiferal test Mg/Ca_{CC} is a proxy used in paleoceanography to reconstruct past seawater temperatures (e.g. Nürnberg et al., 1996; Lear et al., 2000). In addition to temperature, calcite Mg/Ca_{CC} is also controlled by seawater Mg/Ca_{SW} (Segev and Erez, 2006; Evans and Müller, 2012). Since Mg/Ca_{SW} varied over geological time due to changes in the balance between Mg and Ca input and output, paleoceanographers need to account for this ratio in seawater, when using foraminiferal Mg/Ca_{CC} to reconstruct temperatures on timescales beyond ~ 1 Ma. Due to the long residence times of Mg^{2+} (~13 Ma) and Ca^{2+} (~1 Ma), this ratio does not need to be corrected for when using foraminiferal Mg/Ca on shorter timescales (Broecker and Yu, 2011; Hardie, 1996).

Biological processes involved in calcification complicate the relationships between Mg/Ca_{CC}, temperature and Mg/Ca_{SW}, which is apparent from large inter-species differences in Mg/Ca (Bentov and Erez, 2006). To improve the reliability of proxy relationships it is hence necessary to understand the impact of cellular processes involved in calcification. Controlled culture studies allow disentanglement of ~~impacts~~ variables that often co-vary in the field, as well as allowing seawater conditions to be more extreme than naturally occurring~~exceeding naturally existing ranges in conditions~~. Studies by e.g. Erez (2003), and Bentov et al. (2009) suggested that foraminifers vacuolize seawater to acquire the ions needed for calcification. Seawater vacuolization would require the extraction of Ca^{2+} and CO_3^{2-} from the vacuoles or the removal of all unwanted ions, such as e.g. Mg^{2+}. However, studies by De Nooijer et al. (2009) and Nehrke et al. (2013) showed that the volume of vacuoles observed during calcification cannot account for the total amount of ions needed for calcification. An intracellular storage reservoir for inorganic carbon, or a "pool", was shown for the perforate foraminifer, *Amphistegina lobifera* (Ter Kuile et

al. 1989), possibly corresponding to the vacuoles described by Erez (2003) (De Nooijer et al. 2014). However, Ca^{2+} pools are absent in the benthic *Ammonia aomoriensis*, demonstrated by Nehrke et al. (2013). On the basis of their experiments these authors suggested that selective transmembrane transport (TMT) is responsible for the delivery of Ca^{2+} to the site of calcification during chamber formation. A minor portion of unfractionated elements may reach the site of calcification passively via seawater leakage or via seawater vacuolization (Nehrke et al., 2013). This model predicts a linear relationship between Mg/Ca_{SW} and Mg/Ca_{CC}, as observed for e.g. *Amphistegina lessonii* (Segev and Erez 2006, Mewes et al. 2014), *Amphistegina lobifera* (Segev and Erez 2006) and *Ammonia aomoriensis* (Mewes et al. 2014). In the experiments by Mewes et al. (2014), [Ca] was kept constant while [Mg] was varied. To verify the TMT/PT model, requires investigating the effect of varying seawater [Ca] on Mg/Ca_{CC}.

The aim of this culture study is to investigate the effect of different Mg/Ca_{SW} by varying seawater [Ca] and keeping [Mg] constant, on test growth, test wall thickness and Mg/Ca_{CC}. The results allow testing the assumptions of the calcification model by Nehrke et al. (2013) and are used to construct a refined model.

2. Materials and Methods

2.1 Sampling and Storage of Specimens

The benthic foraminifer *Amphistegina lessonii* was chosen for this experiment because our experience has shown that *A. lessonii* grow and reproduce well in our laboratory. Due to its relatively large size of >1mm it is furthermore relatively easy to observe and handle. Because of cost efficient and easy accessibility coral ~~Coral~~ reef rubble with attached benthic foraminifera was sampled in April 2012 from a coral reef aquarium at Burger's Zoo, Arnhem, The Netherlands (Ernst et al., 2011). Sampling foraminifers from the zoo aquarium instead of the natural environment seems at first view not ideal. The zoo's aquarium is however one of the largest aquaria in the world, harboring a very rich (micro)fauna and providing spatially diverse microhabitats. In the present study we are dealing with a fairly fundamental aspect of physiology, namely with the response to concentrations and ratios of major ions in seawater. Zoo-specimens have no opportunity to adjust their physiological machinery to changing Mg and Ca, since these concentrations are the same in the aquarium as in the field. Upon return to the laboratory, samples were kept in an aquarium (AQUAEL 10), containing a heating element, light source (light intensity ~80 µmol/m^2s) and a small water pump with filter to circulate the water. For the experiments, specimens of *Amphistegina lessonii* were collected from the rubble using a small brush (section 2.3).

2.2 Preparation of Culture Media

From our experience with previous culture experiments we knew that some species of foraminifera do not grow well in 100% artificial seawater (ASW). A small pilot experiment, in

which we cultured *Amphistegina lessonii* in different mixtures of artificial (ASW) and natural seawater (NSW), revealed that a mixture of 30% NSW and 70% ASW results in optimal foraminiferal growth rates. To prepare culture media with constant [Mg], but varying [Ca], elemental concentrations of the available NSW were determined. Based on this, the concentrations to be added to the ASW (based on the recipe by Kester et al. (1967)) were calculated. Six different treatments with constant [Mg] (50mM) and varying [Ca] (3, 5, 7, 10, 21, 38 mM) were prepared, resulting in media with Mg/Ca ratios of ~16.6, 10, 7.1, 5, 2.4 and 1.5. Actual concentrations in the final culture media were verified by inductively coupled plasma - optical emission spectrometry (ICP-OES) and are summarized in table 1. Since salinity varied, depending on the varying [Ca], salinity was measured for all treatments (salinometer: WTW, Cond 330) and adjusted to a constant value (S=32.4), by adding NaCl from a stock solution (5 M). pH was measured using a pH meter (WTW, pH 3110, NBS scale) and adjusted to a constant value (pH=8.01) by adding 1M NaOH. Total alkalinity (TA) and dissolved inorganic carbon (DIC) were determined using a SI-Analytics TW alpha plus and a XY-2 Sampler, Bran und Luebbe, respectively. All values are summarized in table 1.

2.3 Juvenile *Amphistegina lessonii*

For the culture experiment we used in culture grown offspring of the zoo-derived specimens. For the culture experiment~~,~~ we used in culture grown offspring of the zoo-derived specimens. ~~juvenile specimens of *A. lessonii*~~Offspring were used to ensure that most of their calcite is formed during incubation in controlled conditions. To obtain juveniles, adult specimens were picked from the stock material. Adult specimens crawled up the aquarium glass walls, facilitating selection of living specimens, and transfered to well plates. Well plates were placed in light (12h

light / 12h dark cycle) and temperature controlled incubators (RUMED, Rubarth Apparate GmbH) at 25°C. The daylight sources had a light intensity of 130 µmol/m^2/s at the level of the well plates. After a few days, about 10% of the specimens had reproduced asexually. These juveniles were selected for the culturing experiments and evenly distributed between the different treatments.

2.4 Culture Experiment

The culture protocol was the same as reported in Mewes et al. (2014), except for the manipulation of the culture media (compare 2.2). Juveniles of *A. lessonii* were incubated in petri dishes, containing ~10 ml of culturing medium. In total, juveniles of 4 different broods were used and divided equally over the treatments (each brood in duplicates containing 5-10 individuals per petri dish), resulting in 50 to 56 juveniles for every treatment. To maintain constant culture conditions, the culture media was replaced once every three days. Immediately after replacement of the media, specimens were fed 100 µl of a dense culture of the green algae *Dunaliella salina* (~4*10^6 cells*mL^{-1}). To prevent bacterial colonialization of petri dishes due to left-over food, all All specimens were transferred to a clean petri dish once every week~~.~~. This resulted ~~resulting~~ in an occasional loss of some specimens. Dead specimens were identified by a change in color from brownish/greenish to pale/white, due to their loss of symbionts. Survival rates were high (ca. 95%) and not correlated with any measured parameter. Dead specimens were removed from culture. The culture experiment ran for ~7 weeks and resulted in a final number of successfully grown juveniles between 37 and 56 per treatment.

Alkalinity was determined once every week and culture media element concentrations were measured a second time at the end of the experiment. Prior to analyses media were filtered (syringe filter 0.2 µm).

2.3. Determination of size and growth rates

The maximum test size [µm] of all specimens was measured weekly using a digital camera (AxioCam MRc5) connected to a Zeiss microscope (Axiovert 200M). Maximum test diameters were determined from pictures using the Axiovision (Zeiss) software. Foraminiferal test size increased with time and from the resulting regression, growth rates in [µm/day] were calculated. In foraminifera, biomass increases continuously, whereas chamber formation is intermittent (e.g. Signes et al., 1993). Because we did not observe the duration of actual chamber formation, reported rates refer to overall growth rates, which should not be confused with calcium carbonate precipitation rates.

2.4 Cleaning Procedure

After termination of the experiment, all specimens were rinsed with distilled water and placed in a 7% NaOCl solution for 4 hours to remove organic material. Specimens were rinsed again and dried overnight (12 h) in an oven at 60°C.

2.5 Determination of weight and size normalized weight

Test weight was determined with an ultra-microbalance (Mettler Toledo UMX2, precision: ± 0.1 µg). Due to the limited weight of individual specimens, each replicate group was weighed as a whole, resulting in n = 8 (duplicates x 4 broods) measurements. Mean weight per specimen was determined by dividing the weight of each replicate by the number of specimens in the group. Weight was normalized to the final size measured with the microscope. This size normalized weight (SNW) is an indication for test wall thickness and defined by:

$$SNW = \frac{weight\,[\mu g]}{size\,[\mu m]}$$

Size normalized weight also depends on the time spend in culture, which makes it challenging to compare SNW measured in our experiment to other experiments. Thus, we expressed size normalized weight as relative SNW [%], such that it is related to the highest SNW in each of the experiments (which equals 100%).

2.6 Element Measurements

Elemental concentrations were determined using laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). For this purpose, analyses were done on the GeoLas 22Q Excimer laser (Lambda Physik), coupled to a sector field ICP-MS (Element 2, Thermo Scientific) at Utrecht University (Reichart et al., 2003). Prior to analyses, specimens were mounted on stubs with double-sided adhesive tape. Depending on the size of the chambers, laser spot size was set to 80, 60 or 40 µm to ablate as much material as possible while at the same time avoiding contamination from adjacent chambers. From each replicate group in each of the treatments, 4-6 chambers of one to two specimens were analyzed, resulting in 50 to 65 measurements per treatment. Data from single chamber measurements were calibrated against a glass standard

(SRM NIST 610; Jochum et al. 2011). To assure high signal quality (e.g. to correct for drift), every 10-15 measurements two NIST standards were measured. Laser repetition rate was set to 7 Hz and the energy density was set to ~1,2 J*cm^{-2} when ablating calcite and to ~5 J*cm^{-2} when ablating glas. Elemental concentrations were calculated for ^{24}Mg, ^{26}Mg and ^{43}Ca, ^{44}Ca using GLITTER (version 4.4.3). An in-house made carbonate standard with known Mg/Ca and Sr/Ca (was measured at an energy density of ~1.2 J*cm^{-2} every 10-12 foraminiferal samples and allowed to check for matrix effects that may result from switching between energy densities (Dueñas-Bohórquez et al., 2009; 2011). All profiles were evaluated individually and parts of the profiles, where ^{27}Al and/or ^{55}Mn (indicating potential contamination) was elevated, were rejected. From a total of 305 ablations, 17 had to be discarded, either because of contamination, or due to short ablation profiles, typically from the thinly calcified last chamber. Mg fractionation, expressed as the partition coefficient for Mg (D_{Mg}), was calculated by dividing the Mg/Ca of the calcite (Mg/Ca_{CC}) by the Mg/Ca of seawater (Mg/Ca_{SW}):

$$D_{Mg} = \frac{Mg/Ca_{CC}}{Mg/Ca_{SW}}$$

3. Results

3.1 Morphological Parameters

3.1.1 Size and Growth Rates

Figure 1a shows growth of foraminifers in the different treatments. At very low [Ca] (3 mM) foraminifers did not grow (Figure 1a). With increasing [Ca], growth rates progressively increased, whereas at highest seawater [Ca] (34 mM), growth rates were reduced again. At lower [Ca] (e.g. Ca = 5 mM and 7 mM), growth seemed to cease before termination of the experiment while in the treatments with higher [Ca] (e.g. Ca = 9 mM and 18 mM) growth continued throughout the experiment.

Figure 1b shows the final mean test size for the different treatments. Largest test size of 503 µm suggests that optimal growth conditions were attained at [Ca] = 17.9 mM and Mg/Ca_{SW} = 2.9, directly followed by the control treatment near ambient at [Ca] = 9 mM and Mg/Ca = 5.7 with a final test size of 428 µm (Figure 1b).

3.1.2 Size normalized weight

Figure 2 shows size normalized weight, a measure for test wall thickness, for the different treatments. Similar to growth, size normalized weights were also highest (0.21 µg/µm) at seawater [Ca] = 9 mM and Mg/Ca_{SW} = 5.7. Seawater [Ca] lower or higher than this condition resulted in reduced size normalized weight and hence test wall thicknesses.

3.2 Calcite Mg/Ca

Figure 3a shows the relationship between Mg/Ca_{CC} and Mg/Ca_{SW}. With increasing Mg/Ca_{SW} and thus decreasing seawater [Ca] (and decreasing Ω), Mg/Ca_{CC} increases. This relationship can be described by a linear regression with a positive y-intercept. The relationship between the distribution coefficient, D_{Mg}, and Mg/Ca_{SW}, is best described by an exponential decrease, approaching an asymptote (Figure 3b).

4. Discussion

4.1 Growth rates and size normalized weight

Growth rates [µm/d] varied substantially with seawater [Ca] (Figure 1). Except for the treatment with highest seawater [Ca], increased [Ca] levels correlate to increased growth rates. Considering only the current dataset by itself one could get to the conclusion that increasing [Ca] causes faster growth until a certain toxic level at [Ca] > 18 mM. However, comparing the present dataset with the one from Mewes et al. (2014), where the absolute [Mg] was varied and [Ca] was kept constant, shows ~~Except the decreased growth rates at [Ca^{2+}] of 34 mM, our results are in line with results from our earlier study (Mewes et al., 2014), suggesting~~ that the calcium concentration by itself is~~may~~ not be the primary driver of growth rate but that it is controlled by the Mg/Ca of seawater. To compare data in the present study with those from Mewes et al. (2014), growth rates (in µm/day) were derived from a linear regression curve fitted to the size data of the first 30 days (Figure 4). It is not possible to derive growth rates from a linear regression line fitted to the time span of the whole experiment (49 days), ~~This is necessary~~ due to the saturation of growth in the present study after 30 days (Figure 1a). As a result, the time spans of growth between the two culture studies are different and do not allow a simple comparison of final test size.

Mewes et al. (2014) varied seawater [Mg] and kept [Ca] constant at 10 mM, observing a similar optimum at ambient Mg/Ca_{SW}. An increase of seawater [Mg] from ~50 mM to ~ 90 mM decreased growth rates even more than lowering of [Mg] from ~50 mM to ~14 mM. The varying growth rates in the Mewes et al. (2014) dataset, at constant [Ca] clearly show that not the calcium concentration itself is the primary driver of growth rates. Considering both data-sets rather suggest that ~~the~~ seawater Mg/Ca_{SW} ratio is the primary driver of growth rates and not the absolute concentrations of Ca or Mg. ~~and not the absolute concentrations of Ca or Mg are primarily~~

~~controlling growth rates.~~ Apparently, the optimum Mg/Ca_{SW} for foraminiferal growth is between 3 and 5 mol/mol (Figure 4). In a similar study, Segev and Erez (2006) measured growth rates in *Amphistegina* spp. as a function of seawater Mg/Ca in terms of $CaCO_3$ addition, similarly concluding that the Mg/Ca ratio of seawater is the main driver of the specimens' growth rates. Their data suggest that highest growth rate is reached at Mg/Ca_{SW} of ~1, while a ratio of ~0.5 was suboptimal. Because Mg is known to inhibit inorganic calcite precipitation, they concluded that *Amphistegina spp.* is able to precipitate its test more easily from seawater with lower Mg/Ca ratios. While this argument is based on a comparison with the inorganic system, their explanation for the decline in growth rate at Mg/Ca_{SW} ~ 0.5 mol/mol is based on physiology, i.e. that a minimum of Mg is required for foraminiferal growth. This physiological explanation can in itself not fully explain our results, because the lowest Mg/Ca_{SW} in our studies (the present one and Mewes et al. 2014) was achieved through both elevating seawater [Ca] and lowering [Mg]. Interestingly, growth rates at lowest ~~at low~~ Mg/Ca_{SW}, is lower in the case of the Ca-variable experiment, indicating that at this particular Mg/Ca the high Ca concentration may be more detrimental to growth than the low Mg concentration~~elevated seawater [Ca] affected growth rate more profoundly than lowered [Mg]~~ (Figure 4). ~~-~~The latter observation can neither be explained in terms of inorganic calcite precipitation nor in terms of a minimum Mg requirement. However, it may be that high seawater [Ca] may be toxic for the cell (e.g. Martinez-Colon et al., 2009). Together, the results of Segev and Erez (2006) and those presented here strongly suggest that growth in *Amphistegina spp.* is influenced by the Mg/Ca_{SW} ratio. With these datasets, it is currently impossible to determine the optimal Mg/Ca_{SW} ratio for foraminiferal growth, because the available datasets suggest a plateau, rather than a clearly defined peak-value. Our dataset (Fig. 4) suggests an optimum between 3 and 5 mol/mol, but may range from 2 to 5 mol/mol. The dataset of Segev and Erez (2006) locates the optimum between 1 and 2.5 mol/mol. But this is a

Formatiert: Schriftart: Kursiv
Formatiert: Tiefgestellt
Formatiert: Tiefgestellt

potentially biased range, because there are no data between Mg/Ca_{SW} of 2.5 and 5 mol/mol. This implies that these two datasets combined (Fig. 4, Segev and Erez 2006) suggest an optimum between 1 and 5 mol/mol. While this might appear to be a large range, it is a reasonable interval from a physiological perspective, because physiological optima usually comprise a range of values. Well known examples are temperature, light intensity, and nutrient concentrations. ~~Together, the results of Segev and Erez (2006) and those presented here strongly suggest that growth in *Amphistegina spp.* is influenced by the Mg/Ca_{SW} ratio with an optimum close to the ratio of natural seawater.~~

The same argumentation also applies to SNW (figure 5). This is the first study showing the effect of Mg/Ca_{SW} on foraminiferal SNW. ~~,~~ The effect of Mg/Ca_{SW} on foraminiferal SNW shows the same trend (optimum curve) as the effect of Mg/Ca_{SW} on growth rates. ~~which is correlated to the change in growth rates as a function of Mg/Ca_{sw}.~~ Similar trends for growth rate and SNW in response to seawater carbonate chemistry changes were described for another benthic foraminifer, namely *Ammonia tepida* (i.e. *A. aomoriensis*) (Keul et al., 2013). It should be emphasized that comparison of absolute values for SNW or growth rate between different experiments is challenging since observed values are highly variable, even under similar culture conditions. This ~~phenomenon~~ is not confined to foraminifers, but also known from culture studies using coccolithohores (Hoppe et al., 2011). It is therefore reasonable to follow the recommendation of Hoppe et al. (2011) and base interpretations on response patterns, i.e. trends, rather than absolute values.

4.2 Calcite Mg/Ca

Our results show that Mg/Ca_{CC} increases linearly with decreasing seawater [Ca] and thus

Formatiert: Tiefgestellt

Formatiert: Tiefgestellt

Formatiert: Tiefgestellt

297 increasing Mg/Ca_{SW} (Figure 3a). Comparison to our previous study, where [Mg] was varied and
298 [Ca] was kept constant (Mewes et al., 2014) shows a strong agreement between the two data sets
299 (Figure 6a). This suggests that test Mg/Ca_{CC} is controlled by the ratio of Mg to Ca in seawater,
300 rather than by absolute concentrations. This result would be in accordance with a calcification
301 mechanism based on seawater vacuolization, if the Mg transport mechanism features a Ca
302 fractionation independent of seawater Mg or Ca concentrations. While this is a perfectly
303 reasonable scenario, it makes little sense when considering the assumed function of this transport,
304 i.e. Mg homoeostasis. In other words, if the behavior of the Mg transporter is compatible with our
305 data, it cannot perform its alleged role. Calcification based on vacuolization might exclude Mg
306 homoeostasis as a function of this transporter and instead, the function might merely be a
307 lowering of the Mg/Ca ratio. Hence the question whether our data are compatible with a
308 calcification mechanism based on vacuolization of seawater, depends on the precise
309 interpretation of this mechanism. ~~This result could be in accordance with a calcification~~
310 ~~mechanism based on seawater vacuolization, if there was not the need to fractionate strongly~~
311 ~~against Mg. Active removal of Mg by Mg^{2+} transporters has been suggested to account for the~~
312 ~~Mg fractionation (Erez 2003). For this idea to be compatible with our data, the Mg transporter~~
313 ~~would have to remove Mg, in proportion to the seawater Mg/Ca, independent of the seawater Mg~~
314 ~~concentration. A physiological basis for such a scenario is hard to envision. Therefore we~~
315 ~~conclude that our data argue against the vacuolization model.~~

316 Foraminiferal Mg/Ca_{CC} at varying Mg/Ca_{SW} can be used to test the biomineralization
317 model developed by Nehrke et al. (2013). This model assumes that foraminifers obtain the
318 majority of Ca^{2+}, needed for calcification, via highly selective transmembrane transport (TMT)
319 and that the majority of the Mg^{2+} stems from (unfractionated) seawater leakage or vacuolar
320 transport (i.e “passive transport (PT)). In contrast to the vacuole-based biomineralization model

(e.g. Bentov and Erez 2006, Bentov et al. 2009), the TMT/PT mixing model assumes that the percentage of ions transported via PT, is very small compared to those delivered by TMT. Given that elements are not fractionated during the transport of vacuoles to the site of calcification, contribution from vacuole-bound ions or seawater leakage plays a key role in determining the Mg/Ca_{CC}. The model explains the difference between low, medium and high-Mg calcite species via an increasing relative contribution of PT. As already suggested by Nehrke et al. (2013), the model predictions can be tested with culture studies such as this one.

The mixing model (Nehrke et al., 2013) predicts a linear relationship between Mg/Ca_{CC} and Mg/Ca_{SW}, intersecting with the origin. As discussed by Mewes et al. (2014), the relationship between Mg/Ca_{CC} and Mg/Ca_{SW} is best described by a linear relationship having a positive intercept (figure 6a). At high Mg/Ca_{SW} this relationship is in line with the mixing model (Nehrke et al. 2013). At very low Mg/Ca_{SW}, however, the present and our previous data have a positive y-intercept, i.e. an increased D_{Mg} (Figure 6b), which is not predicted by the model of Nehrke et al. (2013) (for discussion see Mewes et al. (2014)). Here we present a refined flux-based model, which solves this problem (for the mathematical derivation see Appendix). The model is based on the same assumptions as Nehrke et al. (2013): the total ion flux is divided into passive transport (PT) and transmembrane transport (TMT) (mixing model). The fraction of the total flux of the divalent cations transported via PT, is expressed as *x* (see equation A2). Similar to Nehrke et al. (2013), we assume no fractionation during passive transport, while we assume a strong fractionation (*frac*) during TMT (see equations A4 and A5). The Mg/Ca_{CC} ratio of the precipitated calcite represents the Mg/Ca ratio of the two different fluxes (see equation A10). A further fundamental assumption is that Mg^{2+} substitutes for Ca^{2+} in the calcite lattice, i.e. in a given volume of calcite the sum of Mg and Ca ions is constant. Based on data showing high Mg areas in conjunction with organic layers in the shell, it was traditionally assumed that Mg^{2+} may

Formatiert: Tiefgestellt

Formatiert: Tiefgestellt

345 be incorporated in the organic layers, rather than in the calcite lattice alone (Erez 2003).
346 However, by means of nano-scale synchrotron X-ray spectroscopy, Branson et al. (2013) showed
347 that most of the Mg present in foraminiferal shells substitutes for Ca in the calcite lattice.
348 Therefore the assumption that the sum of Mg and Ca is constant is justified.

349 Based on the above assumptions the refined flux-based model yields calcite Mg/Ca

350 $$\left(\frac{Mg^{2+}}{Ca^{2+}}\right)_{CC} = R_{SW}\left[\frac{frac(1-x)+x+frac\cdot R_{SW}}{1+(1-x+frac\cdot x)R_{SW}}\right]$$

351 The curve (figure 7a) can be fitted over the whole R_{SW} (= Mg/Ca_{SW}) with a TMT fractionation
352 frac = 0.005 and a contribution of PT to the total ion flux x = 0.02. The TMT fractionation, i.e.
353 0.005 (=frac) is weaker than the one assumed in the previous model (0.0001; Nehrke et al., 2013).
354 This is a reasonable modification because Mg TMT fractionation is not known in either
355 coccolithophores or foraminifera and typical Ca channels display a range of Mg fractionation
356 (e.g. White, 2000).

357 The partition coefficient for Mg is given by:

358 $$D_{Mg^{2+}} = \left(\frac{Mg^{2+}}{Ca^{2+}}\right)_{CC} \Big/ R_{SW} = \frac{frac(1-x)+x+frac\cdot R_{SW}}{1+(1-x+frac\cdot x)R_{SW}}$$

359 This refined flux-based model predicts both the trend of Mg/Ca_{CC} versus Mg/Ca_{SW} and D_{Mg}
360 versus Mg/Ca_{SW}. Especially the dependence of D_{Mg} on Mg/Ca_{SW} is interesting because the trend
361 observed here (figure. 6b and 7b) was also reported for inorganically precipitated calcite (Mucci
362 and Morse 1983). Segev and Erez (2006) already noted that curious fact. They commented: “A
363 physiological mechanism sensitive to ratio ... remains to be explored” (Segev and Erez 2006). We
364 present such a physiological mechanism, which comprises transmembrane transport and seawater

Formatiert: Tiefgestellt

vacuolization. Our refined flux-based model for major and minor element incorporation therefore represents a promising new way of interpreting foraminiferal element to calcium ratios. Future research should hence be concerned with the question whether the behavior of other elements can be reconciled with our model.

5. Summary

Our study showed optimum growth performance of *Amphistegina lessonii* at Mg/Ca_{SW} near ambient. Growth rates, test wall thickness and also test Mg/Ca_{CC} is not controlled by absolute seawater [Ca] and [Mg], but by their ratio in seawater. We provide further support for the recently developed biomineralization model by Nehrke et al. (2013) and present a refined flux-based model which predicts our experimentally determined dependence of Mg/Ca_{CC} on Mg/Ca_{SW}.

Appendix: refined TMT+PT mixing model

The transport of Mg^{2+} and Ca^{2+} in our flux-based model is described in terms of the total flux of the bivalent cations

$$F_{CAT} = F_{Ca^{2+}} + F_{Mg^{2+}} \quad . \tag{A1}$$

The total ion flux is sub-divided into passive transport (PT) and transmembrane transport (TMT). Assuming that a fraction x of the total flux is transported via PT, the fluxes of bivalent cations for both transport pathways are expressed as

$$F_{PT} = F_{PT,Ca^{2+}} + F_{PT,Mg^{2+}} = xF_{CAT} \tag{A2}$$

$$F_{TMT} = F_{TMT,Ca^{2+}} + F_{TMT,Mg^{2+}} = (1-x)F_{CAT} \tag{A3}$$

The contribution of Ca^{2+} and Mg^{2+} to PT and TMP is controlled by the fractionation during transport. It is assumed that no fractionation takes place during passive transport, but a strong fractionation (*frac*) during TMT. Based on this assumption the ratios of Ca^{2+} and Mg^{2+} fluxes are given by

$$\frac{F_{PT,Mg^{2+}}}{F_{PT,Ca^{2+}}} = R_{SW}\,, \tag{A4}$$

$$\frac{F_{TMT,Mg^{2+}}}{F_{TMT,Ca^{2+}}} = frac \cdot R_{SW}\,, \tag{A5}$$

where R_{SW} is the seawater Mg/Ca. Combination of equations (A2)-(A5) yields the Ca^{2+} and Mg^{2+} fluxes for the PT and TMT pathways

$$F_{PT,Mg^{2+}} = \frac{R_{SW}}{1+R_{SW}} x \cdot F_{CAT}\,, \tag{A6}$$

$$F_{PT,Ca^{2+}} = \frac{1}{1+R_{SW}} x \cdot F_{CAT}\,, \tag{A7}$$

$$F_{TMT,Mg^{2+}} = \frac{frac \cdot R_{SW}}{1+\ frac \cdot R_{SW}} (1-x) F_{CAT}\,, \tag{A8}$$

$$F_{TMT,Ca^{2+}} = \frac{1}{1+\ frac \cdot R_{SW}} (1-x) F_{CAT}\,. \tag{A9}$$

The Mg/Ca_{CC} ratio of the precipitated calcite represents the Mg/Ca ratio of the ion fluxes:

$$\left(\frac{Mg^{2+}}{Ca^{2+}}\right)_{CC} = \frac{F_{TMT,Mg^{2+}} + F_{PT,Mg^{2+}}}{F_{TMT,Ca^{2+}} + F_{PT,Ca^{2+}}} = \frac{\frac{frac \cdot R_{SW}}{1+\ frac \cdot R_{SW}} (1-x) F_{CAT} + \frac{R_{SW}}{1+R_{SW}} x \cdot F_{CAT}}{\frac{1}{1+\ frac \cdot R_{SW}} (1-x) F_{CAT} + \frac{1}{1+R_{SW}} x \cdot F_{CAT}}, \tag{A10}$$

which can be written as:

$$\left(\frac{Mg^{2+}}{Ca^{2+}}\right)_{CC} = R_{SW}\left[\frac{frac(1-x)+x+\ frac \cdot R_{SW}}{1+(1-x+\ frac \cdot x)R_{SW}}\right]. \tag{A11}$$

Equation (A11) indicates that the calcite Mg/Ca depends on the seawater Mg/Ca, but not on the total flux of the bivalent cations (F_{CAT}). This explains why test Mg/Ca is controlled by the ratio of Mg and Ca, but not by their absolute concentrations in seawater. The partition coefficient for Mg (D_{Mg2+}) is defined with respect to seawater Mg/Ca, thus

$$D_{Mg^{2+}} = \left(\frac{Mg^{2+}}{Ca^{2+}}\right)_{CC} \Big/ R_{SW} = \frac{frac(1-x)+x+\ frac \cdot R_{SW}}{1+(1-x+\ frac \cdot x)R_{SW}}. \tag{A12}$$

Formatiert: Englisch (USA)

406 **References**

407 Branson, O., Redfern, S. A., Tyliszczak, T., Sadekov, A., Langer, G., Kimoto, K., and Elderfield,
408 H.: The coordination of Mg in foraminiferal calcite, Earth and Planetary Science Letters 383,
409 134-141, 2013.

410 Broecker, W.,Yu, J.: What do we know about the evolution of Mg to Ca ratios in seawater?,
411 Paleoceanography 26, PA3203, 2006.

412 Bentov, S. and Erez, J.: Impact of biomineralization processes on the Mg content of foraminiferal
413 shells: A biological perspective, Geochemistry Geophysics Geosystems 7, 1-11, 2006.

414 Bentov, S., Brownlee, C. and Erez, J.: The role of seawater endocytosis in the biomineralization
415 process in calcareous foraminifera, Proceedings of the National Academy of Sciences 106,
416 21500-21504, 2009.

417 De Nooijer, L. J., Langer, G., Nehrke, G., and Bijma, J.: Physiological controls on seawater
418 uptake and calcification in the benthic foraminifer *Ammonia tepida*, Biogeosciences, 6, 2669-
419 2675, 2009.

420 Dueñas-Bohórquez, A., da Rocha, R. E., Kuroyanagi, A., Bijma, J., Reichart, G. J.: Effect of
421 salinity and seawater calcite saturation state on Mg and Sr incorporation in cultured planktonic
422 foraminifera, Marine Micropaleontology, 73(3), 178-189, 2009.

423 Dueñas-Bohórquez, A., Da Rocha, R. E., Kuroyanagi, A., De Nooijer, L. J., Bijma, J., Reichart,
424 G. J.: Interindividual variability and ontogenetic effects on Mg and Sr incorporation in the
425 planktonic foraminifer *Globigerinoides sacculifer*, Geochimica et Cosmochimica Acta, 75(2),
426 520-532, 2011.

427 Erez, J: The Source of Ions for Biomineralization in Foraminifera and Their Implications for
428 Paleoceanographic Proxies, Reviews in Mineralogy and Geochemistry 54, 115-149, 2003.

429 Ernst, S., Janse, M., Renema, W., Kouwenhoven, T., Goudeau, M. L., & Reichart, G. J.: Benthic
430 foraminifera in a large Indo-Pacific coral reef aquarium, The Journal of Foraminiferal Research,
431 41(2), 101-113, 2011.

432 Evans, D., & Müller, W.: Deep time foraminifera Mg/Ca paleothermometry: Nonlinear
433 correction for secular change in seawater Mg/Ca, Paleoceanography, 27(4), 2012.

434 Glas, M. S., Langer, G., and Keul, N.: Calcification acidifies the microenvironment of a benthic
435 foraminifer (*Ammonia sp.*), Journal of Experimental Marine Biology and Ecology, 424, 53-58,
436 2012.

Hardie, L.A.: Secular variation in seawater chemistry: An explanation for the coupled secular variation in the mineralogies of marine limestones and potash evaporites over the past 600 my, Geology 24, 279-283, 1996.

Hoppe, C. J. M., Langer, G. and Rost, B.: *Emiliania huxleyi* shows identical responses to elevated pCO_2 in TA and DIC manipulations, Journal of Experimental Marine Biology and Ecology, 406, 54-62, 2011.

Jochum, K.P., Weis, U., Stoll, B., Kuzmin, D., Yang, Q.C., Raczek, I., Jacob, D.E., Stracke, A., Birbaum, K., Frick, D.A., Gunther, D. and Enzweiler, J.: Determination of reference values for NIST SRM 610-617 glasses following ISO guidelines, Geostandards and Geoanalytical Research 35, 397-429, 2011.

Kester, D.R., Duedall, I.W., Connors, D.N. and Pytkowicz, R.M.: Preparation of artificial seawater, Limnology and Oceanography 12, 176-179, 1967.

Keul, N., Langer, G., de Nooijer, L. J. and Bijma, J.: Effect of ocean acidification on the benthic foraminifera *Ammonia sp.* is caused by a decrease in carbonate ion concentration, *Biogeosciences*, *10*, 6185-6198, 2013.

Lear, C. H., Elderfield, H., & Wilson, P. A.: Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca in benthic foraminiferal calcite, Science, 287(5451), 269-272, 2000.

Lee, A. and East, J.: What the structure of a calcium pump tells us about its mechanism, Biochemical Journal, 356, 665-683, 2001.

Martínez-Colón, M., Hallock, P. and Green-Ruíz, C.: Strategies for using shallow-water benthic foraminifers as bioindicators of potentially toxic elements: a review, The Journal of Foraminiferal Research, 39, 278-299, 2009.

Mewes, A., Langer, G., de Nooijer, L.J., Bijma, J. and Reichart, J.G.: Effect of different seawater Mg^{2+} concentrations on calcification in two benthic foraminifers, Marine Micropaleontology, 2014.

Mucci, A. and Morse, J. W.: The incorporation of Mg^{2+} and Sr^{2+} into calcite overgrowths: influences of growth rate and solution composition, Geochimica et Cosmochimica Acta, 47(2), 217-233, 1983.

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

Nelson D. L. and M. M. Cox: Lehninger Principles of Biochemistry, W. H. Freeman, 6th revised international edition 2013

Nürnberg, D., Bijma, J., & Hemleben, C.: Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures, Geochimica et Cosmochimica Acta, 60(5), 803-814, 1996.

Raitzsch, M., Dueñas-Bohórquez, A., Reichart, G. J., de Nooijer, L. J., and Bickert, T.: Incorporation of Mg and Sr in calcite of cultured benthic foraminifera: impact of calcium concentration and associated calcite saturation state, Biogeosciences, 7(3), 869-881, 2010.

Segev, E. and Erez, J.: Effect of Mg/Ca ratio in seawater on shell composition in shallow benthic foraminifera, Geochemistry Geophysics Geosystems 7 (2), 1-8, 2006.

Spero, H. J.: Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*, Marine Biology, 99, 9-20, 1988.

Signes, M., Bijma, J., Hemleben, C., and Ott, R.: A model for planktic foraminiferal shell growth, Paleobiology, 71-91, 1993.

Ter Kuile, B., Erez, J. and Padan, E.: Mechanisms for the uptake of inorganic carbon by two species of symbiont-bearing foraminifera, Marine Biology, 103(2), 241-251, 1989.

White, P. J.: Calcium channels in higher plants, Biochimica et Biophysica Acta (BBA) - Biomembranes, 1465(1), 171-189, 2000.

Acknowledgement

Max Janse and his team are acknowledged for providing specimens from a coral reef tank at Burger's Zoo, in Arnhem, The Netherlands. Laura Wischnewski is acknowledged for her assistance with culturing foraminifers. We furthermore like to thank the technical assistance of Ulrike Richter, who prepared algae cultures, Jana Hölscher for doing DIC measurements, Ilsetraut Stölting for doing ICP-OES analyses and Helen de Waard for assisting during LA-ICP-MS analyzes at Utrecht University. This work was funded in part by The European Research Council (ERC grant 2010-NEWLOG ADG-267931 HE).

Tables

Table 1: ~~Details of culture media~~ Details of culture media as well as morphological and chemical test parameters

	Amphistegina lessonii					
	treat. 1	treat. 2	treat. 3	treat. 4	treat. 5	treat. 6
SW Mg^{2+} [mM]	51.64	52.56	52.75	52.66	52.05	52.40
SW Ca^{2+} [mM]	34.19	17.86	9.22	6.63	4.77	3.18
Mg/Ca_{SW} [mol/mol]	1.51	2.94	5.72	7.95	10.91	16.47
± st. error	±0.00	±0.03	± 0.02	± 0.05	± 0.07	± 0.09
Mg/Ca_{CC} [mmol/mol]	22.95	40.79	52.08	67.50	83.35	-
± st. error	±0.81	±1.38	±1.72	±2.37	±1.96	
T [°C]	25	25	25	25	25	25
S ‰	32.4	32.4	32.4	32.4	32.4	32.4
pH (NBS)	8.1	8.1	8.1	8.1	8.1	8.1
TA [µmol/kg]	2615	2545	2504	2504	2492	2479
Ω (calcite)	16.75	8.74	4.49	3.24	2.33	1.55
DIC [µmol/kg]	2302	2298	2286	2295	2294	2290
final test size [µm]	362.7	503.4	427.5	341.1	240.6	138.4
± st. error	±9.3	±9.1	±6.4	±5.9	±7.9	±7.8
growth rate [µm/day]	4.21	8.15	6.91	5.89	2.95	-0.53
± st. error	±0.36	±0.46	±0.84	±0.13	±0.91	±0.21
mean SNW*1000	124.64	190.90	206.22	163.34	78.94	24.60
[µg/µm] ± st. dev.	±26.13	±29.40	±23.61	±26.51	±40.56	±16.43
	~~*Amphistegina lessonii*~~					
	~~treat. 1~~	~~treat. 2~~	~~treat. 3~~	~~treat. 4~~	~~treat. 5~~	~~treat. 6~~
~~SW Mg^{2+} [mM]~~	~~51.64~~	~~52.56~~	~~52.75~~	~~52.66~~	~~52.05~~	~~52.40~~
~~SW Ca^{2+} [mM]~~	~~34.19~~	~~17.86~~	~~9.22~~	~~6.63~~	~~4.77~~	~~3.18~~
~~Mg/Ca_{SW} [mol/mol] ± st. error~~	~~1.51~~ ~~±0.00~~	~~2.94~~ ~~±0.03~~	~~5.72~~ ~~± 0.02~~	~~7.95~~ ~~± 0.05~~	~~10.91~~ ~~± 0.07~~	~~16.47~~ ~~± 0.09~~
~~Mg/Ca_{CC} [mmol/mol] ± st. error~~	~~22.95~~ ~~±0.81~~	~~40.79~~ ~~±1.38~~	~~52.08~~ ~~±1.72~~	~~67.50~~ ~~±2.37~~	~~83.35~~ ~~±1.96~~	~~-~~
~~T [°C]~~	~~25~~	~~25~~	~~25~~	~~25~~	~~25~~	~~25~~
~~S ‰~~	~~32.4~~	~~32.4~~	~~32.4~~	~~32.4~~	~~32.4~~	~~32.4~~
~~pH (NBS)~~	~~8.1~~	~~8.1~~	~~8.1~~	~~8.1~~	~~8.1~~	~~8.1~~
~~TA [µmol/kg]~~	~~2615~~	~~2545~~	~~2504~~	~~2504~~	~~2492~~	~~2479~~
~~Ω (calcite)~~	~~16.75~~	~~8.74~~	~~4.49~~	~~3.24~~	~~2.33~~	~~1.55~~
~~DIC [µmol/kg]~~	~~2302~~	~~2298~~	~~2286~~	~~2295~~	~~2294~~	~~2290~~

Figure captions

Figure 1: a) Mean test size ± st. error for all treatments versus time in culture (n = 37-56). b) Mean test size ± st. dev. at the end of the experiment versus seawater Mg/Ca.

Figure 2: Size normalized weight versus seawater Mg/Ca (n = 8).

Figure 3: a) Mg/Ca_{CC} versus Mg/Ca_{SW} ([Ca] and thus Ω decreases with increasing Mg/Ca_{SW}) and b) D_{Mg} x 1000 versus Mg/Ca_{SW} (n=50-65 ablations per treatment).

Figure 4: Growth rates [µm/day], derived from linear regression curves fitted to size data of the first 30 days in culture, versus Mg/Ca_{SW}.

Figure 5: % mean size normalized weight [µg/µm] versus Mg/Ca_{SW}.

Figure 6: a) Mg/Ca_{CC} versus Mg/Ca_{SW}. b) D_{Mg} x 1000 versus Mg/Ca_{SW}. Grey lines represent functions fitted to the combined dataset.

Figure 7: Model fit to the data of our present and previous study (Mewes et al., 2014) for a) Mg/Ca_{CC} versus Mg/Ca_{SW}. b) D_{Mg} x 1000 versus Mg/Ca_{SW}.