Review # 2:

Reviewer: I am concerned that the conclusion that an optimal Mg/CaSW 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