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Interactive comment on "Mn / Ca intra-test variability in the benthic foraminifer *Ammonia tepida*" *by* Jassin Petersen et al.

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Reply to Referee comment 1 (RC1):

Below we have copied the referees' comments one at the time and indicate how we have addressed them. Our reply contains two figures as well as an attachment with both RCs' comments and replies and a typed manuscript, which is accompanied by five figures, three tables, one appendix and one supplementary material.

"Review of "Mn/Ca intra-test variability in the benthic foraminfer Ammonia tepida" by Petersen et al., submitted to Biogeosciences, Aug., 2017. These authors present new laser ablation ICP-MS Mn/Ca (Mg/Ca and Sr/Ca) data from individual chambers of a benthic foraminer species taken from the upper sediment of a non-freshwater lake.

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These data are analyzed with respect to the potential use of Mn/Ca as a proxy for bottom water oxic conditions. The conclusion is that while there may be systematic variability, deconvoluting the three possible sources of non-ontogenetic variability (change in environment, movement of foram and timing of chamber formation) makes such data prohibitively complex. The ms. is very well written and illustrated well. Details of the methodology and results are very good. The conclusions reached are generally supported; in fact, the main criticism I have is that these data were not explored further (see my comments below). Kudos to the authors for a job well-done."

Reply: We thank the referee for the positive comments

Major Questions:

"1. why not further explore the data? The Mg/Ca and Sr/Ca are only very briefly mentioned. I understand the authors have a story about Mn/Ca and redox to discuss, and I appreciate keeping this story clear. However, their data shows a very large variability in Mg/Ca that does indeed co-vary with Mn/Ca (I calculate a r2 of 0.6). Does temperature vary this much in the lake? Can this help explain Mn/Ca variability (e.g., through a Q10 type foram response)?"

Reply: The referee raises some relevant questions about the data of Mg/Ca and possible relations to temperature variability. As we explain below, the mechanisms behind the Mg/Ca ratios are highly complex, and their discussion would indeed take the attention away from the story line in the manuscript. Regarding the bottom water temperature, we know that it varies from about 5°C in winter to about 18°C in summer at the sampling site in Lake Grevelingen (Fig. 1 of this author's response; from Hagens et al., 2015, Biogeosciences).

Average Mg/Ca is 2.4 mmol/mol for March, 3.7 mmol/mol for July and 2.2 mmol/mol for September. Only the difference in Mg/Ca between the latter two months is significant (at a 95% level). According to the calibration of De Nooijer et al. (2014a) for A. tepida, these values would correspond to high absolute temperatures (for all months

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well above the 18°C measured in summer) and a maximal temperature difference of approximately 4.3°C. However, a higher temperature in July than in September does not entirely correspond with the observations, which show high temperatures until the end of September. Summarising, at our study site, the relation between Mg/Ca and temperature appears to be complex. It is therefore unlikely that specimens which have both elevated Mn/Ca and Mg/Ca reflect a Q10 type response to increased temperature.

There is indeed a positive correlation between Mg/Ca and Mn/Ca, but only for the mean values per specimen (R^2 = 0.6). For the parameters of intra-test variability (range and % RSD) the correlation coefficients are much lower (R2 = 0.31 for the range and R2 = 0.15 for the RSD). Also the individual measurements show a much weaker correlation (R2 = 0.32), which is largely based on about 14 of the 297 measurements (the points with Mn/Ca > 0.4 mmol/mol in Fig. 2 of this author's response).

In the text we suggest that higher intra-test variability tends to lead to a higher average Mn/Ca. For Mn/Ca, maximal intra-test variability was observed in July, possibly because the foraminifera collected in July may still record the winter Mn2+ maximum in some of the chambers. However, also 3 specimens with strongly increased Mn/Ca in a single chamber, which we tentatively interpreted as transport of the foraminifera to deeper sediment layers due to macrofaunal bioturbation, substantially contribute to higher variability in Mn/Ca. It is possible that maximal bioturbation in late spring (after macrofaunal repopulation in early spring) coincides with the period of strong temperature increase, thereby explaining part of the positive correlation between Mg/Ca and Mn/Ca. Another explanation for the positive correlation is the fact that also during summer hypoxia, there is a slight increase in sediment Mn2+ concentrations. In the manuscript we have added more detail to the fact that this latter aspect has been observed in Lake Grevelingen at our sampling site (page 15 line 19-22).

Summarising, it is highly unlikely that average elemental ratios of the measured specimens are representative of the time of sample collection; this makes it challenging to directly relate environmental parameters with shell chemistry. Therefore, we suggest

keeping the focus of our manuscript only on the Mn/Ca variability and the linkage with redox conditions.

“2. Following from point 1, it would be useful to have better context for the life environment of these forams. At least there should be a location map where the samples were taken, and some idea of salinity and temperature. Other details, such as organic loading and bioactivity (even human activity) would be useful too. The authors suggest all these data exist - can they plot some of them over the time of interest? Best of all, of course, would be some record of bottom water redox condition over the time of interest. Does any such record exist?”

Reply: For the location map a supplementary figure has been added to the manuscript (Figure S1). As mentioned before, temperature and salinity are published in Hagens et al. (2015) which is now explicitly mentioned in the manuscript (page 5 line 17/18). Information about organic loading can be obtained from Seitaj et al. (2017). The record of bottom water oxygenation for the sampled station is now added as a figure to the supplementary material (Figure S2).

“3. More on data comparisons: Can the authors plot the variability of chambers that might be considered time-equivalent? That is, the ultimate chamber of each (presumably live) specimen should be time-equivalent. Can all these be compared? Then similarly for all the second chambers (assuming all the individuals grew similarly, which may be a false assumption, I know.) I did not see such a plot, and could not find the necessary data to generate one myself. This would seem to me to be instructive about variability between individuals that are living in the same chemical environment.”

Reply: The plot that the referee asks for was already included in the manuscript as Fig. 5. For all specimens from one sample (and for all specimens combined) all the penultimate, antepenultimate, etc. chambers are plotted together. However, as already suggested by the referee, it is not certain that all penultimate chambers from different specimens of the same sample calcified at the same time (and hence, under

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the same conditions). Nevertheless, we have added some discussion (page 17, line 9-13), explaining that this figure indeed reflects temporal variability of Mn/Ca in relation to environmental changes, but that the different calcification histories of individual specimens add uncertainty to this interpretation.

In fact, we performed such a comparison on the basis of a substantially larger data set than the one presented in this manuscript, to test whether specimens from the same sample produced their chambers simultaneously (which did not appear to be the case). In order to keep our manuscript focused, we did not add this analysis to our manuscript. Instead, the relation between Mn/Ca, environmental conditions and timing of calcification will be discussed in detail in a future manuscript.

Minor Issues:

“Cite Froelich et al., 1979, and even add a comment regarding "remnant Mn peaks" and moving fronts of redox state. These would certainly pertain in this time-sensitive data.”

Reply: Froelich et al. (1979) is cited on page 2 line 7/8. To address the moving fronts of redox states changes have been made on page 2 line 17-19. Regarding remnant Mn peaks, this was observed by Froelich et al., however, in the Lake Grevelingen data the Mn2+ (and also solid phase Mn) show no such pattern, so we refrain from adding too much detail in the introduction.

“What does "adequately" mean on L.13 pg. 2?”

Reply: This comment refers to page 3 line 12. Indeed, the word does not add further information here, so it was deleted.

“Could there be any problem with Mn or Ca in CellTracker Green?”

Reply: Samples treated with CellTracker Green (5-chloromethylfluorescein diacetate) were incubated for at least 6 h before they were fixed with formalin. Although possible, it is unlikely that foraminifera calcified during the reaction period, and it is highly unlikely

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that more than one chamber would be added during this time interval. Since we did not analyse the geochemical composition of the last chamber, we are sure not to have sampled a chamber that was calcified during incubation with CTG.

Another possible influence of CTG on the test could be adsorption effects on the outside of chamber walls. However, Bernhard et al. (2006) point out that CTG will not leak out of the cell via ion channels in the cell membrane once it is incorporated inside the cell. Therefore, it is unlikely that there is an interference with the calcite from this organic compound (long) after the forams are stained. Moreover, the laser ablation signal from the outside of chamber walls is omitted from further data treatment.

“If these are living (stained) forams, I am confused how high values on the inner and outer shell can be contamination. Of what?”

Reply: It is considered that sediment particles are attached to the surface of tests of living benthic foraminifera even after the cleaning procedure (Koho et al., 2015, 2017).

“Does their LOD and LOQ not preclude them from measuring low Mn? e.g., what you expect to find in more oxic conditions?”

Reply: We expect calcification to normally occur at the sediment-water interface where oxic conditions prevail throughout most of the year. Therefore, we consider the Mn/Ca measured for most of the chambers, with a large majority of values (207 out of 298 measurements) between 0.05 and 0.2 mmol/mol, Fig. 2, to be representative for oxic conditions.

However, we agree with the referee that we cannot exclude that some of the measurements below the LOQ correspond to very low Mn/Ca values calcified in oxic conditions. Fortunately, only 4 of the 44 analysed specimens presented more than 2 chambers with values below the LOQ. Since on average, 7.7 chambers were measured per specimen, the impact on the average value and on the intra-test variability for the individual would be small, even more so, because a large majority of the measurements below

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the LOQ is found in specimens with systematically low Mn/Ca values (and a low Mn/Ca intra-test variability).

“The range of data in Fig. 4 is perhaps most interesting; is the minimum the same in all cases? i.e., is there a minimum Mn incorporation in shells regardless of environment/ ontogeny?”

Reply: Minimum Mn/Ca values are listed in Tab. A.1. On average, this was 0.08 mmol/mol, but depending on the specimen it was as low as 0.03 mmol/mol or as high as 0.19 mmol/mol. Concerning minimum Mn incorporation regardless of environment, it is probably best to refer to culturing studies because it is the only way to keep Mn2+ concentrations in the solution constantly low.

“The section on ontogeny on p.16 should come earlier, and provide information on how fast are chambers grown, what kind of time range does each chamber represents, and if the chambers grow at all times of the year. At least to the ability that the authors can provide this information.”

Reply: Although we understand the referee’s suggestion that this important information should be stated earlier in the text, we think that it is at its right place here in the discussion, where temporal factors are discussed. Unfortunately there is not much more information available to our knowledge, so that we cannot provide more details.

“Section 4.2.2. - The use of % variability might be a poor option here, as it depends on the Mn/Ca measured.”

Reply: We think that the use of % RSD (i.e., the standard deviation normalized to the average value) is correct here because it is exactly our intention to compare the extent of variability between different absolute values. The % RSD is the most accepted measure which allows to do so.

“Conclusion presented (e.g., L. 15, conclusions): the intra-test variability may be caused by environmental change (Mn front shifting), but wouldn’t this be recorded more

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consistently in all the samples?"

Reply: We think it is not consistently recorded because of the "different timing of calcification" of individual specimens, as stated in the conclusion, page 19 line 6-8.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-273/bg-2017-273-AC1-supplement.pdf

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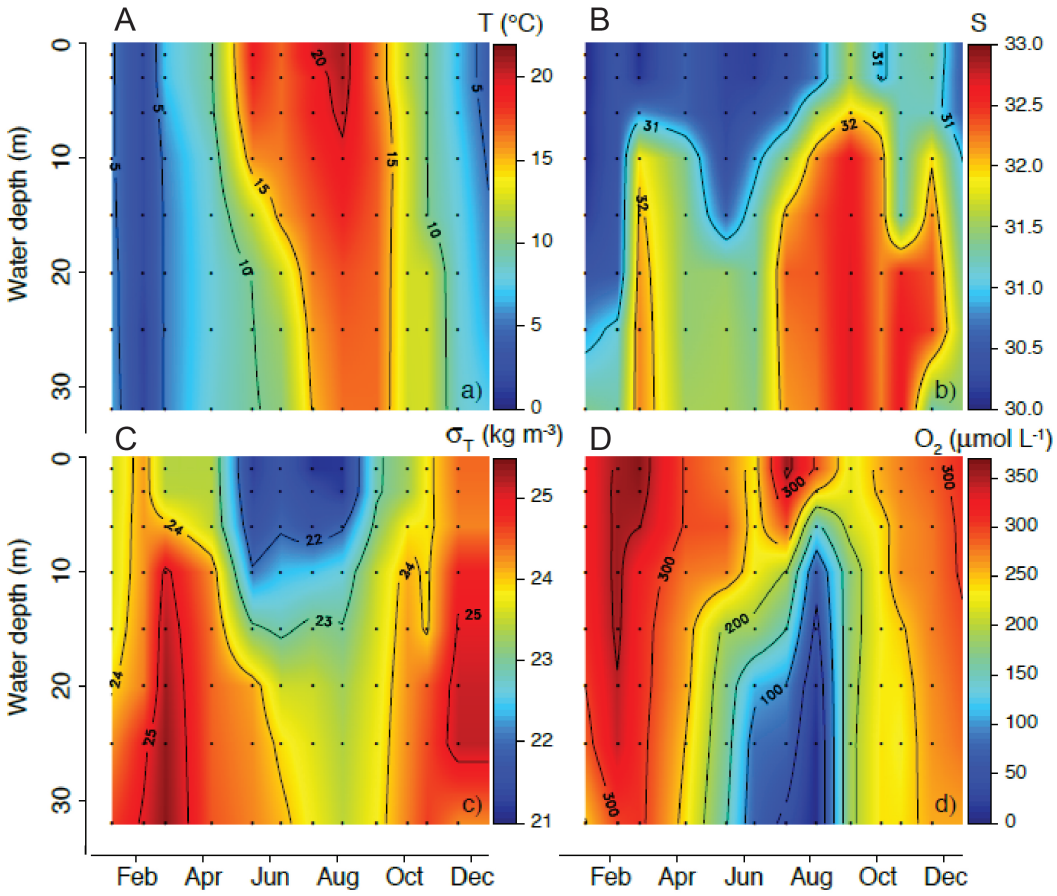

Fig. 1. Water column parameters (sampling station for this study at 23 m water depth). A: temperature [°C]. B: salinity. C: density anomaly [kg/m3]. D: oxygen conc. [μmol/L]. From Hagens et al. (2015).

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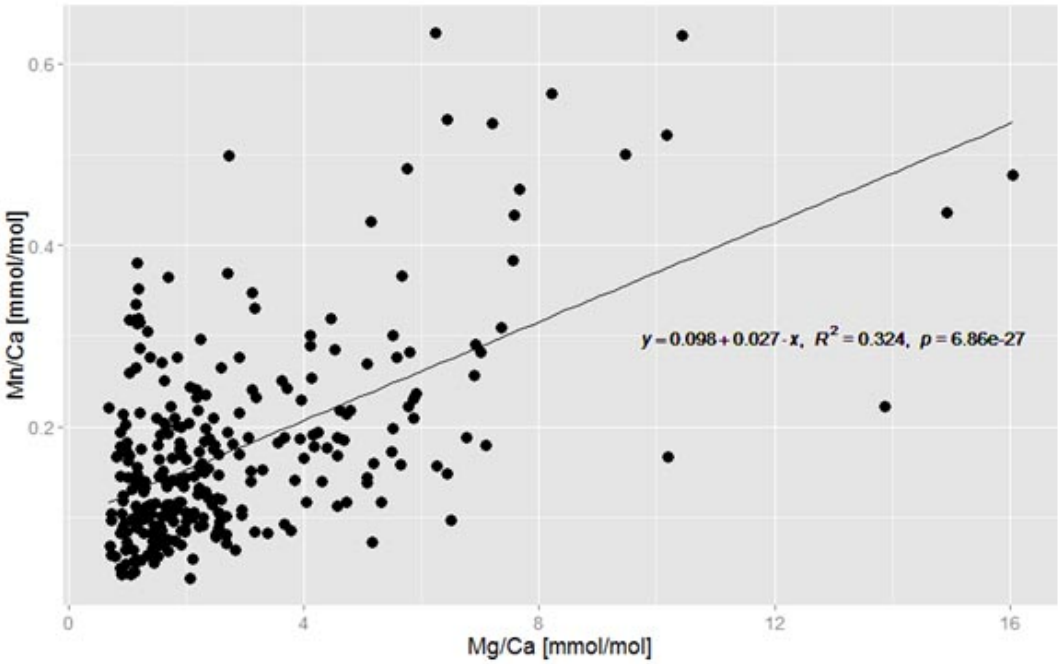

Fig. 2. Mn/Ca as a function of Mg/Ca for all single chamber measurements (n=297).

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