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

Jassin Petersen et al.

jassin.petersen@univ-nantes.fr

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Reply to Referee comment 2 (RC2):

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.

"Petersen and co-authors present laser ablation derived Mn/Ca ratios of the benthic foraminifera species Ammonia tepida and propose to use them as a proxy for bottom water oxygenation. During three different months, living specimens were collected at a lake with seasonal changes in the redox status of the upper sediment. The re-

sults show a high intra-test variability in foraminiferal Mn/Ca ratios due to ontogenetic trends, seasonal changes in pore water Mn2+ concentration and vertical migration of the foraminifera within the sediment. The authors ascribe the highest amount of the intra-test variability to variations in the Mn2+ concentration of the pore waters and that differences in calcification histories might explain observed inter-test variability. The manuscript is generally well written, logically organised and clear. The figures are mostly nice and clear. I think that this work is an interesting and important contribution and therefore suitable to be published in Biogeosciences. Nevertheless, I would like to see the points below addressed by the authors."

Reply: We thank the referee for the positive comments.

Main points

"1) I think it is hard to compare this study with the results from the culture study from Barras et al., since their study is submitted to another journal and the reader has no access to the data which makes it hard to verify the results of Petersen et al. Is there another reference that could help here that is already published?"

Reply: Indeed, there is another published culturing study of A. tepida with seawater Mn2+ as the controlling factor (Munsel et al., 2010). However, this study used relatively low concentrations of Mn2+ (11-220 nmol/L), compared to those used by Barras et al. (2-595 μmol/L) and found in the pore waters of Lake Grevelingen at our station (up to 310 μmol/L). Moreover, the study of Munsel et al. (2010) was performed under oxic conditions (whereas Barras et al. maintained hypoxic conditions), which could lead to oxide and hydroxide formation (as mentioned by Munsel et al.). For these reasons we cannot compare our Mn/Ca intra-test variability only with the data of Munsel et al. (2010). We have added a sentence explaining this in the new version of the manuscript (page 4, line 5-8). Regarding the study of Barras et al., this manuscript is still under review but following the editor's suggestions it should be accepted after revision. However, we made sure that all relevant information of this paper is given in

detail in our text. More specifically, we modified the introduction substantially to give the reader more detailed information (page 4, line 8-11).

“2) Some important information are missing or are not sufficiently explained: - It would be very helpful to have the lake pore water data for Mn2+ and O2 concentrations in actual numbers at least for the months investigated to better compare them with the measured foraminiferal Mn/Ca ratios and to assess the redox conditions in the upper sediment.”

Reply: This was partly also suggested by referee 1, so we added supplementary material including a figure of O2 concentration in Lake Grevelingen for 2012 (Fig. S2), and the Mn2+ pore water profiles for the three investigated months (Fig. S3).

“- In Section 2.3 you say that different spot sizes were used according to different chamber sizes. Did you do test measurements with different spot sizes (on standards and/or foraminifera tests) to show that the spot size does not affect the analysed Mn/Ca (and other) values? Please specify this.”

Reply: The spot sizes of 40-85 μm in diameter used in the course of this study can be compared to the depths of the laser ablation drilling holes to evaluate possible influences on results (according to Eggins et al., 1998, Applied Surface Science, it is this aspect ratio between depth and diameter that determines the fractionation at constant laser energy). In our case the depth is constrained by the thickness of the chambers (probably not exceeding 30 μm and mostly more close to 10 μm for our specimens of Ammonia tepida). Given this shallow depth in comparison to the diameter we do not expect the spot size to have a significant impact on the results.

“In Table 2, information about measured NIST 612 standard data are missing as well as measured Mg/Ca ratios for the USGS MACS-3 standard. I think this is important especially when you correct your analysed Mg/Ca ratios to the USGS MACS-3 standard considering the offset in Mg/Ca ratios for NIST 610 standard between your measurements and those of Jochum et al. 2011.”

Reply: Table 2 does not include data for NIST 612 because we used this reference material as calibration standard for all other measurements for Mn/Ca and Sr/Ca. Therefore, this material cannot be used to assess external reproducibility. Similarly, we do not report results of Mg/Ca for USGS MACS-3 in Tab. 2 because this was the calibration standard for Mg/Ca. Regarding the offset for NIST 610 between our measurements and those of Jochum et al. (2011), we have to point out that Mg seems to be subject to larger uncertainties in both carbonates and silicates compared to other elements (Jochum et al., 2012).

“3) There is a contradiction between Sections 3.3 and 4.2.2 concerning ontogenetic trends. In Section 3.3 (page 10, L14-16), you mention that there are statistical significant ontogenetic trends for the analysed data and in Section 4.2.2 (page 14, L5-7) you suddenly say, there were no systematic ontogenetic trends observed. Please specify explicitly that this is only valid if all your data were combined (like you say on page 10, L16-17).”

Reply: We added this information in section 4.2.2 (page 14, line 16/17).

“4) Your justification for analysing the standards in raster mode (page 7, L1-4) is not entirely correct. How can you keep the depth-related fractionation similar for raster and spot analyses, like you say in L2-3? It is true, that measuring in raster mode minimises the “down-hole” fractionation but the fractionation is most likely still different to spot analyses. Please rephrase this. Further, “down-hole” fractionation is probably negligible in foraminifera tests as the test walls are thin – especially when the test walls were entirely ablated within 10s at the mentioned laser settings.”

Reply: We have changed the sentences accordingly (page 7, line 8-12).

Minor points

“page 1, L25 “intrinsic” – This term is explained later how it is meant in this context. So, please do not use this word in the abstract.”

Reply: The word was removed and replaced by “ontogenetic trends (i.e., size-related effects) and/or other vital effects occurring during calcification”.

“page 2, L4-6 “...thereby relating bottom water oxygenation,...” – The word “relating” sounds odd in this context. Please rephrase.”

Reply: The word has been replaced by “coupling”.

“page 2, L17-18 “...lead to hypoxic BWO conditions...” – I think it is better to say here “hypoxic bottom water conditions” otherwise it is kind of double with “hypoxic bottom water oxygenation conditions”.”

Reply: Changes have been made.

“page 4, L7-8 Did the Mn/Ca intrinsic variability only relate to changes in seawater Mn2+ concentration in the study from Barras et al.? As mentioned before, the reader has no access to the (yet) unpublished study of Barras and co-authors, hence it is difficult to see which factors contribute to the intrinsic variations in Mn/Ca ratios of A. tepida. Please give some more details on this study or state the main/controlling factors for this variability. Is it the Mn2+ concentration as briefly mentioned later?”

Reply: We added on page 4 line 8-11 that the seawater Mn2+ concentration was the controlling parameter in the study of Barras et al. and following the referee’s first main point more detail has been added to describe this culturing study.

“page 4, L9-10 Please put “in culturing experiments” in parentheses otherwise it is confusing if your study is based on culturing experiments or on field samples.”

Reply: Done (page 4, line 14/15).

“page 4, L10-12 “...species complex...” Correct word? Species group?”

Reply: Page 4 line 16: Species complex is the correct term in this context describing cryptic species. For Ammonia tepida there are several phylotypes present in Europe, with slight morphological differences, making this species complex pseudocryptic. For

our study we did not perform genetic tests on the specimens analysed so that we use the term A. tepida without specifying the phylotype. However, there is an ongoing study on the genetics of A. tepida from Lake Grevelingen.

“page 4, L17-19 “...represent a suitable context..” – better “suitable location”.”

Reply: Done (Page 4 line 22 and page 5 line 1/2).

“page 4, L19-21 The sentence reads awkward, please rephrase. Maybe: “However, one complicating factor is that it has recently been shown that the activity of cable bacteria strongly influences the seasonal pattern of sediment geochemical cycles in Lake Grevelingen.” ”

Reply: Changes have been made (page 5 line 2-5).

“page 7, L17-18 Please insert “the” between “..processed with” and “GLITTER software”.”

Reply: Done (page 8 line 5).

“page 10, L1-2 “...non-parametric test...” – Which one? Please name (again).”

Reply: Done (page 10 line 11/12).

“page 10, L15-16 “...there was a slight, significant trend...” - Please delete “slight”. If the trend is statistically significant, the word “slight” does not fit.”

Reply: We deleted “slight” before “significant trend”. To characterise the slope of this correlation we added “slightly” in front of “increasing values” (page 11 line 5/6).

“page 12, L18-20 Please reference Fig. 3 here instead of Fig. 2 as the variability is better seen in Fig. 3.”

Reply: Done (page 13 line 7).

“page 13, L10-13 “...unlike Sr, Mg is strongly discriminated against in the calcifying fluid.” – What does that mean for the analysed ratios? Please explain briefly.”

Reply: Done (page 13 line 21 and page 14 line 1).

“page 16, L11-16 As mentioned above, please give the pore water Mn2+ concentrations at least for the three months investigated, better for the entire year 2012, if the data were monitored, to better follow your interpretations.”

Reply: Pore water Mn2+ profiles for the three months investigated were added as supplementary material (Fig. S3) and a reference to it was made on page 16 line 12.

“page 17, L4-8 Please spell OPD out as this is only used twice in the entire manuscript and I really had to look for the explanation.”

Reply: Done (page 18 line 2/3).

“page 18, L7-9 “...although no systematic ontogenetic trends could be identified.” – Please insert here that this is only the case if the entire dataset is considered. Otherwise it will be a contradiction to your result section as mentioned above in the main points.”

Reply: This was added in parentheses (page 19 line 6).

“References – I am sorry but it is awful to read the reference list. Could you please use indentation “hanging” to make it easier to read?”

Reply: Done.

“Figure 2, SEM image – This is actually Fig. 1. Out of curiosity, why does this test have 11 ablation holes if 10 spots were analysed at maximum (according to Fig. 2, plots)?”

Reply: For a few specimens more than 10 spots were analyzed, however, no specimen had more than ten ablation spots fulfilling the selection criteria, this is why in Fig. 2 the x-axis is set to 10.

“Figure 2, average Mn/Ca ratios per chamber and specimen – I really like this plot but each panel is very small which makes it hard to actually read the numbers. So, is there

another way to show the data? Or at least, please lose the gray background and the grid lines and make each individual panel a bit bigger.”

Reply: We modified Figure 2 according to the referee’s suggestions.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-273/bg-2017-273-AC2-supplement.pdf

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