

Dear editor,

Please find enclosed our final author comments. We thank the referees for their constructive comments which we address here and also enclose the revised manuscript as a supplement with changes tracked in red. We would like to point out that, in order to better reflect the contents, we decided to slightly change the title to "Mn/Ca intra- and inter-test variability in the benthic foraminifer *Ammonia tepida*". This is due to the fact that the manuscript includes many aspects of comparisons between specimens of different samplings.

Thank you for considering our manuscript for publication in Biogeosciences.

Kind regards and on behalf of all co-authors,

Jassin Petersen

RC1: **changes in red**

"Review of "Mn/Ca intra-test variability in the benthic foraminifer *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 foraminifer species taken from the upper sediment of a non-freshwater lake. 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 r^2 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).

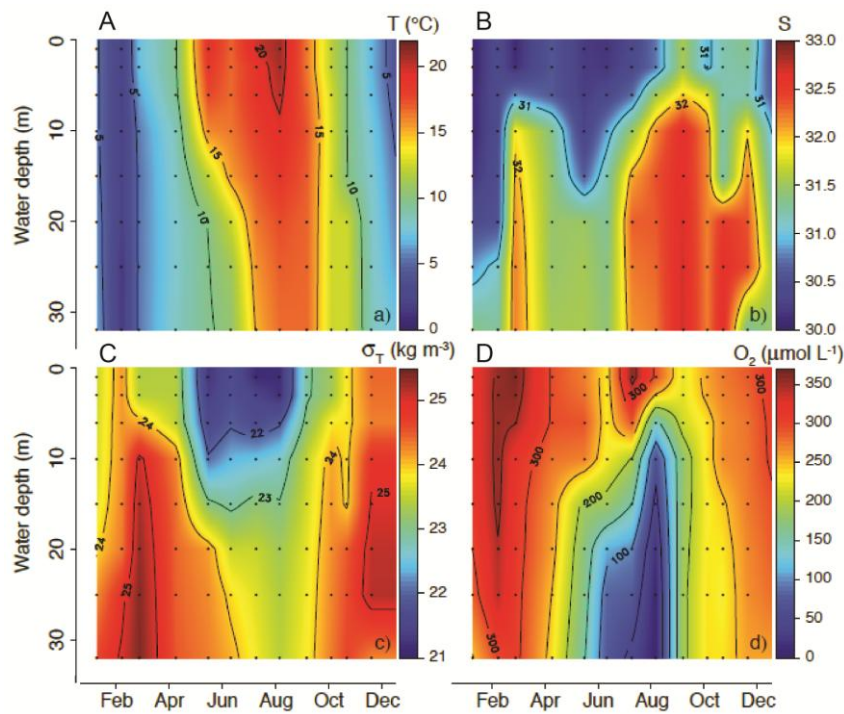


Figure 1. Water column parameters for sampling campaigns in 2012, linearly interpolated in space and time. Sampling station for this study is situated at 23 m water depth. A: temperature [°C]. B: salinity. C: density anomaly [kg m^{-3}]. D: oxygen concentration [$\mu\text{mol L}^{-1}$]. Modified from Hagens et al. (2015).

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 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 ($R^2 = 0.31$ for the range and $R^2 = 0.15$ for the RSD). Also the individual measurements show a much weaker correlation ($R^2 = 0.32$), 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).

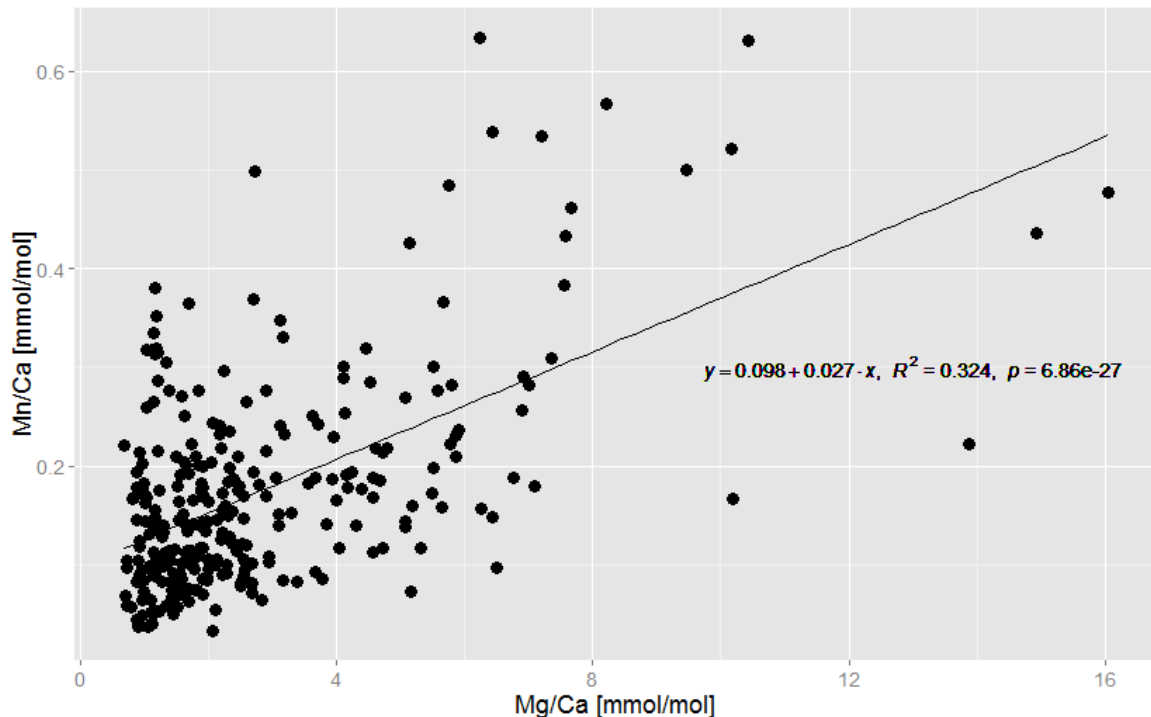


Figure 2. Mn/Ca as a function of Mg/Ca for all single chamber measurements (n=297).

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 Mn^{2+} 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 Mn^{2+} 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 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 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 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 Mn^{2+} 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 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.

RC2: changes in dark red

“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 results 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 phlotypes 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 phlotype. 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.

Mn/Ca intra- and inter-test variability in the benthic foraminifer *Ammonia tepida*

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15 **Abstract.** The adaptation of some benthic foraminiferal species to low oxygen conditions provides the prospect of using the chemical composition of their tests as proxies for bottom water oxygenation. Manganese may be particularly suitable as such a geochemical proxy, because this redox element is soluble in reduced form (Mn^{2+}), and hence can be incorporated into benthic foraminiferal tests under low oxygen conditions. Therefore, intra- and inter-test differences in foraminiferal Mn/Ca ratios may hold important information about short term variability in pore water Mn^{2+} concentrations and sediment redox conditions. Here, we studied Mn/Ca inter- and intra-test variability of living individuals of the shallow infaunal foraminifer *Ammonia tepida* sampled in Lake Grevelingen (The Netherlands) in three different months of 2012. The deeper parts of this lake are characterised by seasonal hypoxia/anoxia with associated shifts in microbial activity and sediment geochemistry, leading to seasonal Mn^{2+} accumulation in the pore water. Earlier laboratory experiments with similar seawater Mn^{2+} concentrations as encountered in the pore waters of Lake Grevelingen suggest that **intrinsic** intra-test variability **due to**
25 **ontogenetic trends (i.e., size-related effects) and/or other vital effects occurring during calcification** in *A. tepida* (11-25 % RSD) is responsible for part of the observed variability in Mn/Ca. Our present results show that the seasonally highly dynamic environmental conditions in the study area lead to a strongly increased Mn/Ca intra- and inter-test variability (average of 45 % RSD). Within single specimens, both increasing and decreasing trends in Mn/Ca ratios with size are observed. Our results suggest that the variability of successive single chamber Mn/Ca ratios reflects the temporal variability
30 of pore water Mn^{2+} . Additionally, active or passive migration of the foraminifera in the surface sediment may explain part of the observed Mn/Ca variability.

1 Introduction

In many coastal ecosystems, high summer temperatures and eutrophication lead to seasonally occurring hypoxia ($[O_2] < 63 \mu\text{M}$, Rabalais et al., 2002; Diaz & Rosenberg, 2008), linked to the emergence of water column stratification in combination with lower oxygen solubility and higher respiration rates in warmer waters (e.g., Keeling et al., 2010). On the seafloor, oxygen is consumed by respiration of marine biota, thereby ~~relating~~ ~~coupling~~ bottom water oxygenation (BWO), benthic ecosystem functioning and organic carbon cycling (Altabet et al., 1995; Levin et al., 2009; Koho et al., 2013).

Most of the organic matter (OM) deposited on the seafloor is mineralised in surface sediments by respiration processes, involving oxygen, nitrate, manganese and iron oxides or sulphate as electron acceptors (Froelich et al., 1979). If Fe and Mn (hydr)oxides are sufficiently abundant, their reduction can be relevant for the total OM decomposition in the sediment (Aller, 1990; Burdige, 1993; Canfield, 1993; Vandieken et al., 2006). When the bottom waters of coastal water bodies are oxygenated, Mn oxides are present in the oxic surface layer of the sediment, and are reduced to soluble Mn^{2+} in the deeper anoxic sediment layers. Dissolved Mn^{2+} can then diffuse upwards in the pore water across the oxic–anoxic boundary, where it precipitates again in the form of Mn oxides, leading to a continuous cycling of Mn within the upper sediment (Aller, 1994; Slomp et al., 1997). The sedimentation rate and the bioturbation intensity are important factors controlling the pore water cycling of oxidized metal oxides in sediments (van de Velde and Meysman, 2016). Macrofaunal bioturbation may introduce Mn oxides in the deeper, anoxic sediment, where these minerals are subsequently reduced (Mouret et al., 2009; Thibault de Chanvalon et al., 2016). When eutrophication and stratification of the water column lead to (seasonally) hypoxic ~~BWO~~ bottom water conditions, the oxygen penetration depth (~~OPD~~) is reduced, causing an upward movement of the Mn redox front, and diminishing the ~~chance~~ ~~possibility~~ that pore water Mn^{2+} is oxidized. In extreme cases, Mn^{2+} may diffuse from the pore water into the water column (Sundby and Silverberg, 1985; Thamdrup et al., 1994; Dellwig et al., 2007; Konovalov et al., 2007; Pakhomova et al., 2007; Kowalski et al., 2012).

Sedimentary records of manganese may reflect a variety of past environmental conditions, including bottom water redox state, continental runoff, surface water productivity and bottom water current dynamics (Reichart et al., 1997; Van der Weijden et al., 2006; Lenz et al., 2015). The use of sedimentary manganese as a proxy is complicated because of its complex biogeochemical dynamics, including remobilisation of once precipitated manganese oxides, and subsequent diagenetic overprinting (Schenau et al., 2002). Mn incorporated in the calcite of benthic foraminifera potentially represents a more reliable proxy of redox conditions, since these marine protists build their shells (tests) in the upper sediment layer, where the presence of Mn^{2+} may be a function of bottom water oxygenation, and Mn^{2+} can be permanently incorporated into the tests. However, the ecology of the various foraminiferal species is crucial and can influence manganese incorporation in the shell, since different adaptation strategies to changes in the availability of OM and of oxygen lead to different microhabitats and probably also to different calcification depths and periods (Jorissen et al., 1995; Van der Zwaan et al., 1999; Koho et al., 2015). So far, few studies have investigated the potential of using Mn/Ca ratios in benthic foraminifera as a proxy for bottom water redox state. These studies do show that benthic foraminifera **adequately** register environmental Mn^{2+} concentrations in their tests (Reichart et al., 2003; Munsel et al., 2010; Glock et al., 2012; Groeneveld & Filipsson, 2013; Koho et al., 2015, 2017; McKay et al., 2015; Barras et al., *subm.*). For example, Koho et al. (2015; 2017) demonstrated that foraminiferal species occupying a relatively deep microhabitat display higher Mn/Ca ratios than those living near the sediment surface. Furthermore, when using microanalytical techniques, capable of measuring elemental concentrations in single foraminiferal chambers, short term variability in oxygenation may be unraveled. Recent studies have shown the potential of intra-test variability in Mn/Ca to resolve vertical migration in the sediment and/or seasonal changes in oxygenation (Glock et al., 2012; McKay et al., 2015).

In order to apply Mn/Ca ratios in benthic foraminiferal tests as a quantifiable proxy of palaeo-redox conditions, it is necessary to assess the presence of ontogenetic trends (i.e., size-related effects) and/or other vital effects occurring during calcification; the variability due to such biological processes will hereafter be termed “intrinsic (intra-test) variability”. This

intrinsic part of the total variability (which can be assessed only in culturing studies) determines the threshold value above which the variability within a single specimen can be recognised successfully as a response to environmental conditions. In case the total intra-test variability is greater than the intrinsic variability, the added part can be ascribed to environmental factors, and/or active or passive migration of the foraminifera in the sediment surface layer. In such cases, single chamber measurements could provide information about the temporal variability of the Mn dynamics. A first culturing study of *Ammonia tepida* with different concentrations of seawater Mn^{2+} (in the range of naturally occurring concentrations in seawater, which is up to three orders of magnitude lower than concentrations found in pore waters of our coastal study area) was performed by Munsel et al. (2010). A more recent culturing study of *Ammonia tepida* and *Bulimina marginata* yielded the first results of intrinsic variability of Mn/Ca ratios evidences a species-specific linear relationship between Mn/Ca ratios of the tests and for a range different concentrations of dissolved Mn^{2+} in seawater concentrations commonly found in pore waters of coastal areas ($Mn/Ca_{calcite} = 0.086 \pm 0.001 Mn/Ca_{seawater}$ for *A. tepida*, $R^2 = 0.96$, $p < 0.001$, Barras et al., *subm.*). This culturing study found that Mn/Ca intrinsic variability varied between 11-25 % for *A. tepida* for a range of seawater Mn^{2+} concentrations similar to those found in the pore water of surface sediments in our study area.

Here, we investigate Mn/Ca intra- and inter-test variability in the same benthic foraminifer *A. tepida* as studied by Barras et al. (*subm.*; in culturing experiments) from field samples of Lake Grevelingen (The Netherlands). The shallow infaunal *A. tepida* represents a species complex including several pseudocryptic species (Hayward et al., 2004; Schweizer et al., 2011; Saad and Wade, 2016). In Lake Grevelingen, *A. tepida* is almost exclusively represented by the T6 genotype. *Ammonia tepida* is abundant in coastal areas of temperate climate zones, tolerating diverse biological and environmental stress factors, including low oxygen conditions (Moodley and Hess, 1992; Sen Gupta et al., 1996; Geslin et al., 2014; Nardelli et al., 2014; Thibault de Chanvalon et al., 2015; Cesbron et al., 2016). The salty bottom waters of Lake Grevelingen, an artificial lake created after the closure of a branch of the Rhine-Meuse-Scheldt estuary, are characterised by seasonal hypoxia ($[O_2] < 63 \mu M$) and anoxia ($[O_2] < \text{detection limit of } 1 \mu M$) (Hagens et al., 2015; Seitaj et al., 2017). Therefore, samples from this site

~~represent a~~ provides a suitable ~~context~~ location to study short term environmental variability (on a time scale of weeks to months) in relation to elemental incorporation into benthic foraminiferal tests. However, one complicating factor is that ~~it~~ ~~has recently been shown how the seasonal pattern of sediment geochemical cycles in Lake Grevelingen is strongly influenced by the activity of cable bacteria~~ the activity of cable bacteria strongly influences the seasonal pattern of sediment geochemical cycles in Lake Grevelingen. These electrogenic bacteria induce strong shifts in the sediment geochemistry (Seitaj et al., 2015, 2017, Sulu-Gambari et al., 2016a, 2016b), and lead to substantial Mn^{2+} mobilisation in pore waters (Rao et al., 2016; Sulu-Gambari et al., 2016b; van de Velde et al., 2016). These cable bacteria tend to have a highly patchy distribution, which hence could complicate the interpretation (Sulu-Gambari et al., 2016b; Seitaj et al., 2017). Our approach is to investigate the Mn/Ca intra- and inter-test variability of *A. tepida*, by measuring individual chambers with laser ablation ICP-MS (LA-ICP-MS) for selected living specimens sampled in three different months of 2012. We will compare the obtained Mn/Ca intra-test variability with results on strictly intrinsic intra-test variability for the same species, measured for specimens from laboratory experiments by Barras et al. (subm.), to find out whether the seasonal variability of BWO and pore water Mn^{2+} concentrations has left an imprint on benthic foraminiferal Mn/Ca ratios.

2 Material and methods

2.1 Study area

Sediment samples were recovered in Lake Grevelingen at a single location (51°44.956 N, 03°53.826 E, water depth 23.1 m, Fig. S1). The sampling site experiences seasonal hypoxia; for the year 2012, monthly recordings of temperature, salinity and water column oxygen concentrations (Hagens et al., 2015), sedimentary microbial community composition (Seitaj et al., 2015), pore water geochemistry (Sulu-Gambari et al., 2016a, 2016b) and benthic O_2 uptake rates (Seitaj et al., 2017) are available. In 2012, BWO started to decrease in April and attained a minimum of about 20 μM (~8 % saturation) in August (Fig. S2). After homogenisation of the water column in September/October, BWO quickly rose to values of ~200 μM (~80

% saturation). Pore water Mn^{2+} showed highest concentrations (up to 310 μM) in winter and early spring followed by considerably lower concentrations in summer and autumn (Sulu-Gambari et al., 2016b, Fig. S3).

2.2 Samples of living benthic foraminifera

Living specimens of *Ammonia tepida* were sampled in Lake Grevelingen in March, July and September 2012. In all three months, surface sediments of the sampling site were inhabited by dense populations of *A. tepida*. Living specimens were recognized by CellTracker Green (CTG, Bernhard et al., 2006). CTG was applied on board R/V Luctor within one hour of retrieval (Langlet, 2014). Adult specimens (size fraction 150-315 μm) from the sediment depth 0-0.5 cm were selected for LA-ICP-MS measurements. For March, July and September 10, 16 and 18 specimens were analysed, respectively. Prior to LA-ICP-MS analyses, all specimens were cleaned to remove sediment adherences (Barker et al., 2003) by rinsing them three times with ultra-pure water in 200 μL tubes, followed by one rinse in methanol and three final rinses in ultra-pure water. During each rinse, the samples were gently agitated with a vortex machine.

2.3 LA-ICP-MS operating conditions and instrument calibration

For all specimens several consecutive test chambers were measured individually by LA-ICP-MS (Fig. 1). The analyses were performed with an ArF excimer laser (193 nm, Analyte G2, Photon machines Inc.) coupled to a quadrupole ICP-MS (820-MS, Varian) at the Laboratory of Planetology and Geodynamics, Université de Nantes (France). Ablations were conducted in a HelEx 2 Volume Cell with He as a carrier gas, a laser energy density of 0.91 J/cm^2 and a repetition rate of 4 Hz. To maximise the amount of ablated material, spot sizes were adapted to the chamber size and varied typically between 40 and 85 μm in diameter. The LA-ICP-MS operating conditions are summarised in Table 1 and the isotope masses selected for analyses were ^{24}Mg , ^{27}Al , ^{43}Ca , ^{55}Mn , ^{57}Fe , ^{66}Zn , ^{88}Sr , and ^{137}Ba .

Prior to each analytical session, the ICP-MS was tuned with the NIST SRM 612 reference material to minimise oxide formation ($ThO^+/Th^+ < 0.5\%$), and elemental fractionation (U/Th close to 1), as well as to optimise the signal to noise ratio

for Mn. The typical laser ablation profile of a foraminiferal chamber includes 30 s of data acquisition of the background signal (laser turned off) followed by the ablation of the chamber wall until it was completely pierced (the laser was shut down after a visual control of the ablation), and data acquisition was stopped after another measuring interval of the background signal. The NIST SRM 612 glass reference material was analysed every 10 measurements and the NIST SRM 610 (silicate glass reference material), USGS MACS-3 (carbonate reference material) and the NFHS (NIOZ, Netherlands Institute of Sea Research, foraminifera in-house standard, Mezger et al., 2016) were analysed every 20 analytical spots. All reference materials have been analysed in raster mode, with the same laser energy as for the samples, a spot size of 65 μm and a scan speed of 10 $\mu\text{m/s}$. ~~‡The choice of raster mode for reference materials allows to keep the depth of laser ablation holes in reference materials similar as for the spot ablation of the foraminiferal chamber walls, to minimise differences in~~ elemental fractionation linked to crater depth (Eggins et al., 1998). ~~For the ablation of chambers of *A. tepida* such fractionation is probably negligible, †because data acquisition on reference materials is on average 3 times longer (30 s) compared to foraminifera data was in general short (~10 s for spot analyses).~~ All foraminiferal ablation profiles were normalised to ^{43}Ca as internal standard, and element concentrations were calculated assuming 40 % wt for the CaCO_3 . The NIST SRM 612 glass served as calibration standard for Mn/Ca and Sr/Ca of the foraminiferal samples, using the recommended values of Jochum et al. (2011). The results obtained from NIST SRM 610, USGS MACS-3 and NFHS were also normalised to the NIST SRM 612 glass to evaluate the long term reproducibility of our analyses (Table 2). For each element and analytical session we have calculated the Limit of Detection ($\text{LOD} = \text{background signal} + 3.3 \cdot \sigma$ standard deviation calculated on the background signal) and the Limit of Quantification ($\text{LOQ} = \text{background signal} + 10 \cdot \sigma$), and have discarded all data below the LOQ (Long & Winefordner, 1983; Longerich et al., 1996; R. Bettencourt da Silva & A. Williams, 2015). As a result of the low energy laser ablation conditions, ^{24}Mg profiles of NIST SRM 612 were below the LOQ for some datasets. However, the USGS MACS-3 has high Mg concentrations (1880 ± 70 ppm), resulting in signals

above the LOQ, and thus Mg/Ca ratios of foraminiferal samples, NFHS and NIST SRM 610 were normalised to USGS MACS-3 using the recommended values of Jochum et al. (2012).

2.4 Data treatment

All laser ablation profiles for each element and each sample or reference material were carefully examined and processed with the GLITTER software. Firstly, the integration interval was based on constant raw counts of ^{44}Ca as indicator of calcite ablation and on constant Sr/Ca ratios, as the intra-shell heterogeneity of Sr/Ca in foraminifera in general and of *A. tepida* in specific is known to be relatively small (e.g., Eggins et al., 2003; de Nooijer et al., 2014a). Secondly, each laser ablation profile was screened for peaks in elements that may indicate surface contamination (^{27}Al , ^{57}Fe , ^{66}Zn). Typically, high concentrations of Mn on the outer and inner shell surfaces are considered as an indicator of contamination (Marr et al., 2011; de Nooijer et al., 2014a; Leduc et al., 2014; Koho et al., 2015). In our case, where Mn is the element of interest, whenever peaks of Mn on outer and inner parts of profiles corresponded to peaks of other contaminant elements, they were discarded from our data. All integration intervals containing less than 10 data points or results with count rates below the LOQ were removed from our dataset.

The external reproducibility of our Mn/Ca analyses was 2 % (calculated as $2 \times \text{RSE}$, relative standard error) when determined on the NFHS foraminiferal carbonate standard (data for Mn/Ca, Mg/Ca and Sr/Ca in Table 2). This standard is adequate for this purpose since it has Mn concentrations comparable to our samples and the same matrix. The results for the USGS MACS-3 carbonate and NIST SRM 610 glass reference materials (Table 2) agree with the recommended values (published values have RSDs, relative standard deviations, of 4 % and 1%, respectively, Table 2). Additionally, we analysed the GJR calcite standard 4 times and the calculated concentrations for Mn and Sr (107 ± 1 ppm and 190 ± 3 ppm, respectively) compared well with published values (106 ± 7 ppm and 184 ± 15 ppm, respectively) from Wit et al. (2010).

2.5 Statistical analyses

Statistical analyses were carried out using R (R Core Team, 2016) using the package ggplot2 for graphical representation (Wickham, 2009). To verify if data were normally distributed we used the Shapiro-Wilk test. For normally distributed data we used ANOVA and t-tests with the Bonferroni adjustment as post-hoc test. In all other cases we used a Kruskal-Wallis test and Wilcoxon-Mann-Whitney as post-hoc test. When comparing Mn/Ca ratios between chambers, in order to check for ontogenetic trends, we used Spearman rank correlation. In all cases, a p-value below 0.05 was considered as significant.

3 Results

3.1 Average Mn/Ca ratios per specimen

For all specimens of *A. tepida* investigated, between four and nine chambers per specimen fulfilled our profile selection criteria for reliable Mn/Ca measurements (as defined in section 2.4). Mn/Ca ratios of individual chambers ranged from 0.03 to 0.57 mmol/mol for the 10 specimens sampled in March 2012 (Fig. 2 (03/2012), Table A.1), from 0.04 to 0.63 mmol/mol for the 16 specimens collected in July 2012 (Fig. 2 (07/2012), Table A.1), and from 0.04 to 0.48 mmol/mol for the 18 specimens sampled in September 2012 (Fig. 2 (09/2012), Table A.1). Average values for Mn/Ca per specimen were between 0.08 ± 0.02 and 0.28 ± 0.15 mmol/mol in March, between 0.08 ± 0.04 and 0.39 ± 0.19 mmol/mol in July and between 0.09 ± 0.03 and 0.22 ± 0.13 mmol/mol in September (Fig. 3A-C, Table A.1). There are no significant differences in average Mn/Ca ratios per specimen between the three sampled months (Fig. 3D; ANOVA, Table 3). However, for the standard deviations per specimen, the values from July 2012 are significantly larger than those of the two other months (ANOVA, Table 3).

3.2 Intra-test variability

The intra-test variability defined as RSD per specimen is on average 45 % (± 15 % SD for all specimens) for Mn/Ca, 49 % (± 24 %) for Mg/Ca and 9 % (± 4 %) for Sr/Ca. (Table A.1, A.2 and A.3). The specimens with the highest Mn/Ca RSD (three specimens with RSD > 70%) were sampled in July (specimens 15, 17 and 18, Fig. 2). Out of these three specimens, two had

one chamber with much higher Mn/Ca compared to the other chambers (specimens 15 and 17), whereas one specimen had an increase in Mn/Ca ratios from older to younger chambers (specimen 18).

To further investigate the variability of Mn/Ca ratios within single specimens, we calculated the range (maximal minus minimal chamber-value) for each measured individual. The so defined Mn/Ca intra-test variability shows a different pattern for all specimens from each of the three sampling campaigns (Fig. 4, data in Table A.1). For instance, in March seven out of ten specimens had a range smaller than 0.2 mmol/mol, whereas the remaining three had a maximum Mn/Ca intra-test variability up to 0.5 mmol/mol. Yet, for the specimens sampled in July, twelve out of sixteen had a range larger than 0.2 mmol/mol with one specimen showing a range of 0.5-0.6 mmol/mol. Most specimens collected in September had a Mn/Ca range within specimens of 0.1-0.2 mmol/mol, with no specimen exceeding a difference in Mn/Ca of 0.4 mmol/mol. Because the range histogram for March clearly shows a non normal distribution (p-value = 0.041) and the sample size per sampling date is small (n between 10 and 18), we investigated the differences with a non-parametric test (**Kruskal-Wallis test and Wilcoxon-Mann-Whitney as post-hoc test**) and obtained a significant difference between July and the other two months. This result confirms the higher Mn/Ca intra-test variability in July compared to March and September (Table 3).

3.3 Ontogenetic trends of Mn/Ca ratios

To investigate the presence of ontogenetic (i.e., size-related) trends, we distinguished between four types: (a) specimens with a trend towards lower values in later chambers (n=7; ID 1, 2, 5, 6, 9, 14 and 31 in Fig. 2), (b) specimens that tended to have higher Mn/Ca ratios in later chambers (n=6; ID 18, 23, 28, 30, 34 and 39 in Fig. 2), (c) specimens with only one or two values deviating from generally rather constant Mn/Ca ratios in all other chambers (n=2; ID 17 and 19 in Fig. 2), and (d) specimens with no apparent trend (the remaining 29 specimens). Most specimens of the first group were sampled in March 2012; in fact half of the specimens from March showed a tendency towards lower Mn/Ca ratios in later chambers. Most

individuals with increasing values towards later chambers were collected in September 2012. In the sample of July 2012 no dominant trend was observed.

To check for the existence of persistent ontogenetic trends, we combined for each successive chamber the values of all measured specimens and tested for statistical significant trends (Fig. 5). As expected, for March 2012 there is a significant trend towards lower values in later chambers (Fig. 5A). In July 2012 no significant trend is found (Fig. 5B) whereas in September 2012 there was a ~~slight~~, significant trend of ~~slightly~~ increasing values towards later chambers (Fig. 5C). If all specimens from the three sampled months are combined (Fig. 5D), there is no significant relation with chamber stage.

4 Discussion

4.1 Comparison of Mn/Ca ratios between benthic foraminiferal species from coastal and deep-sea ecosystems

Recently, Mn/Ca ratios in benthic foraminiferal tests have been proposed as a potential palaeo-proxy for BWO. This suggestion is based on several observations made in recent deep-sea ecosystems (Reichart et al., 2003; Glock et al., 2012; Groeneveld and Filipsson, 2013; Koho et al., 2015, 2017). Furthermore, the application of foraminiferal Mn/Ca ratios as a proxy for dissolved Mn has been tested in laboratory conditions, where calibration studies show a linear relation between seawater dissolved Mn²⁺ concentrations and foraminiferal Mn/Ca ratios, though with species-dependent partition coefficients (Munsel et al., 2010; Barras et al., *subm.*). The use of foraminiferal Mn/Ca ratios as a palaeo-proxy for BWO has further been explored in several studies of deep-sea sediment records (Klinkhammer et al., 2009; Ní Fhlaithearta et al., 2010; McKay et al., 2015).

Our study is the first to investigate benthic foraminiferal Mn/Ca ratios in a coastal ecosystem. The results for *A. tepida* show an average Mn/Ca ratio of 0.17 ± 0.08 mmol/mol and a range of 0.08 ± 0.04 to 0.39 ± 0.19 mmol/mol for the average Mn/Ca per specimen (Table A.1). This range is comparable to that found in living specimens of some deep-sea infaunal species from the NE Japan margin (*E. batialis*, *B. spissa*, *U. cf. graciliformis*, *U. akitaensis*, *N. labradorica*; 0.0020 to 0.277

mmol/mol; Koho et al., 2017), but is elevated compared to Mn/Ca ratios measured in single tests of living benthic foraminifera from the Peruvian OMZ (*B. spissa*; 0.0021 to 0.010 mmol/mol; Glock et al., 2012). This latter difference can be explained by the much higher pore water Mn²⁺ concentrations in our study (< 310 µmol/L) and in the study of Koho et al. (2017; <5 µmol/L) compared to the study of Glock et al. (2012; <0.1 µmol/L). Despite the different pore water Mn²⁺ concentrations in our study compared to Koho et al. (2017), we found rather similar foraminiferal Mn/Ca ratios, and this contrast could be partly resolved by higher partition coefficients in deep-sea species compared to coastal species (Barras et al., subm.). For *A. tepida*, the range measured in specimens from Lake Grevelingen in our study compares well with measured Mn/Ca ratios in the study of Barras et al. (subm.; 0.13 and 0.86 mmol/mol) for a similar range of pore water Mn²⁺ concentrations compared to the concentration of dissolved Mn in the seawater of the culturing experiments (10 to 100 µmol/L).

4.2 Intra-test variability of elemental ratios in benthic foraminiferal tests

Intra-test variability is less well documented for Mn/Ca ratios than for other elemental ratios (el/Ca; e.g., Mg/Ca, Sr/Ca) and it has not yet been established what portion of the total variability can be attributed to either intrinsic (i.e., intra-test variability due to ontogenetic trends or other vital effects) or environmental factors (e.g., seasonality of Mn cycling in the surface sediments, microhabitat effects). In fact, it is essential to know what degree of intrinsic variability can be expected in a population having experienced the same environmental conditions, and above what threshold changes in Mn/Ca ratios can be ascribed to environmental factors. When using LA-ICP-MS, or other microanalytical techniques, Mn/Ca ratios are measured on small parts of the foraminiferal test, making knowledge of intra-test variability even more crucial for the interpretation of the measurements. In case of a high intrinsic variability, independent of environmental parameters, more spot measurements will be necessary to obtain a reliable mean value for one specimen or several specimens from the same stable environment (Sadekov et al., 2005; de Nooijer et al., 2014a). Although contamination is an important issue in Mn/Ca measurements (Boyle, 1983; Barker et al., 2003; Pena et al., 2005, 2008), pre-treatment cleaning and a precise targeting of

the measurement interval (when using secondary ion mass spectrometry, SIMS, or LA-ICP-MS), should largely eliminate the potential influence of contaminant phases and/or diagenetic overgrowths (Glock et al., 2012; Koho et al., 2015; McKay et al., 2015). Therefore, the Mn/Ca intra-test variability should ideally not have a diagenetic contribution.

The results for all specimens measured in this study showed an average Mn/Ca intra-test variability for *A. tepida* of 45 ±15 % (RSD average for all chambers measured on a single specimen, Table A.1), which is comparable to that reported in some previous studies (30-50 %; Glock et al., 2012; McKay et al., 2015; Koho et al., 2017). Our results from the different sampling campaigns showed more specimens with higher variability in July compared to March and September (Fig. 23 and Fig. 4). In order to investigate if this significant difference can be attributed to environmental factors, our results will be compared to variability reported in other elements.

10 4.2.1 Approach for estimating (intrinsic) intra-test variability of Mn/Ca, Mg/Ca and Sr/Ca

The average Mn/Ca intra-test variability observed for *A. tepida* (45 %) is comparable to that measured for Mg/Ca (49 %, Table A.2) but is larger than for Sr/Ca (9 %, Table A.3). Similarly, in other field studies of benthic foraminifera, average intra-test variability was ~20-50 % RSD for Mg/Ca (Allison & Austin, 2003; Curry & Marchitto, 2008; Raitzsch et al., 2011), and 5 % RSD for Sr/Ca (Allison & Austin, 2003). In these studies it could not be determined how much of the observed variability was due to intrinsic and environmental factors, respectively. In contrast to field studies, the use of cultured foraminifera offers the advantage that specimens have grown under exactly the same stable physico-chemical conditions (no environmental variability), thus intra-test variability of elemental ratios is entirely due to biological processes. These culture studies suggest that when only intrinsic intra-test variability is considered, the RSD is ~30 % for Mg/Ca, compared to ~8 % for Sr/Ca (values for all chambers analysed by LA-ICP-MS, de Nooijer et al., 2014a). This difference between intrinsic intra-test variability of Mg/Ca and Sr/Ca was explained by the fact that during biomineralisation processes, unlike Sr, Mg is strongly discriminated against in the calcifying fluid, and that these processes may vary over time and/or

position in the test (Bentov and Erez, 2005, 2006; Nehrke et al., 2013; de Nooijer et al., 2014b). In some field studies (Allison and Austin, 2003; Curry and Marchitto, 2008), values for Mg/Ca intra-test variability were similar to those observed in the culturing experiment, whereas only a single field study (Raitzsch et al., 2011) reported substantially higher Mg/Ca intra-test variability, with an RSD of 51%. In the case of our *A. tepida* specimens, the intra-test variability of Mg/Ca was 5 similarly high (49 %). Contrastingly, the Sr/Ca RSD of *A. tepida* was 9 %, comparable to variability in Sr/Ca from culturing studies.

4.2.2 Intrinsic intra-test variability of Mn/Ca and ontogenetic trends

Similar as for Mg/Ca ratios it is possible to estimate the amount of the intrinsic intra-test variability of Mn/Ca based on results from cultured benthic foraminifera. Culture experiments performed with *A. tepida* in controlled and stable conditions 10 show 85 % of variability (RSD) for very low Mn²⁺ concentrations of 2 µmol/L (all chambers calcified under laboratory conditions, measured by LA-ICP-MS, Barras et al., subm.). However, for seawater Mn²⁺ concentrations of 12 and 101 µmol/L, comparable to the pore water concentration at our site, the variability (RSD) in Mn/Ca was 25 and 11 %, respectively (Barras et al., subm.). For even higher seawater Mn²⁺ concentrations of 595 µmol/L the Mn/Ca RSD was 17 % (Barras et al., subm.). Despite analytical considerations, the large RSD observed for the lowest Mn²⁺ concentration was 15 partly due to the presence of a clear decrease of Mn/Ca during the ontogeny. At higher concentrations, no such ontogenetic tendencies were found (Barras et al., subm.). In our material there were no systematic ontogenetic trends either (for all specimens of all investigated months combined, Fig. 2 and 5D), so that the 11-25 % RSD range measured for Mn²⁺ concentrations of ~10-100 µM, respectively, should be representative for the intrinsic intra-test variability of our specimens. This range is much lower than the average total intra-test variability of 45 % found for living *A. tepida* in our study of Lake 20 Grevelingen.

Consequently, it appears that at most, about half of the total variability in Mn/Ca can be attributed to intrinsic factors. The remaining part of the variability in Mn/Ca should be due to changing pore water Mn^{2+} concentrations in the calcification environment of the foraminifera. This may be due to environmental changes during the lifespan of the individuals, or, alternatively, to active or passive vertical foraminiferal migration through different biogeochemical micro-environments.

5 4.2.3 Seasonality of environmental factors as explanation for Mn/Ca intra-test variability

As explained in the previous paragraph, it appears that half or more of the measured Mn/Ca intra-test variability of *A. tepida* (total intra-test variability was 45 % RSD compared to 11-25 % RSD intrinsic variability) can be attributed to environmental parameters. Here, we will consider which specific factors may be responsible for this variability. First of all, the sampling site of *A. tepida* in Lake Grevelingen at 23.1 m water depth shows strong seasonal fluctuations in bottom water oxygenation (section 2.1, Fig. S2, Hagens et al., 2015). In theory, under oxic conditions the zone of manganese reduction should lie below the microhabitat of *A. tepida*, which is situated close to the sediment-water interface (SWI) (Thibault de Chanvalon et al., 2015; Cesbron et al., 2016), and chambers calcified in this condition should show low Mn/Ca ratios. When bottom water oxygen concentrations decrease (for instance due to enhanced fluxes of organic matter to the seafloor, or stagnation of bottom waters), the zone of manganese reduction will migrate upward; under hypoxic conditions the Mn redox front will be situated closer to the SWI and more Mn^{2+} should be incorporated into chambers of *A. tepida*. However, in extreme cases, the upward migration of the Mn redox front can lead to high amounts of Mn^{2+} diffusing into the water column (Sundby and Silverberg, 1985; Konovalov et al., 2007; Pakhomova et al., 2007), and in case of seafloor anoxia, Mn^{2+} may almost totally seep out of the sediment into the bottom water (Slomp et al., 1997). Thus, foraminiferal Mn/Ca ratios from chambers calcified under anoxic conditions should be nominal. At our station in Lake Grevelingen, which is experiencing hypoxic but not anoxic events in summer, these events lead to dissolution of Mn oxides in surface sediments, and suspended matter Mn in the water column (sampled ~500 away from our station) shows maximum values in July 2012 (Sulu-Gambari et al., 2016b, 2017). Thus, we would expect higher Mn^{2+} concentrations below the SWI in summer than in winter coinciding with

higher Mn/Ca ratios in chambers of *A. tepida* calcified in summer. However, contrary to these theoretical expectations, observations showed maximum pore water Mn^{2+} concentrations in the topmost cm from February to April 2012 whereas Mn^{2+} concentrations remained relatively low during the rest of 2012 (Sulu-Gambari et al., 2016a, 2016b). Recently, similar seasonal Mn^{2+} pore water patterns at a nearby, but slightly deeper station (~500 m away, depth 34 m) in Lake Grevelingen were explained by the presence of cable bacteria in winter and early spring, which are capable of performing electrogenic sulfur oxidation resulting in the dissolution of FeS (Seitaj et al., 2015; Sulu-Gambari et al., 2016a, 2016b). Consequently, upward diffusing Fe^{2+} is oxidized by manganese oxides which produces an accumulation of Mn^{2+} in the pore water in winter (Sulu-Gambari et al., 2016a, 2016b). This process would be responsible for a temporal offset of ~4-6 months between minimum BWO and maximum Mn^{2+} concentrations. Although cable bacteria were not detected at our sampling station with fluorescence *in situ* hybridization (FISH), which may be due to their patchy distribution (Sulu-Gambari et al., 2016b; Seitaj et al., 2017), the similarity in pore water data between the two stations strongly suggests that, also at our sampling station, cable bacteria activity is responsible for the observed strong seasonality in pore water Mn^{2+} (Fig. S3). Therefore, it seems probable that *A. tepida*, sampled in March, July and September 2012, respectively, were confronted with strongly different pore water Mn^{2+} concentrations, and may also have experienced important fluctuations in Mn^{2+} in their microenvironment during their lifespan.

The foraminiferal life-span generally varies from 3 months to about 2 years (Boltovskoy and Lena, 1969; Murray, 1983). No precise data are available for *A. tepida*, although on the basis of field evidence, Goldstein & Moodley (1993) and Morvan et al. (2006) concluded that their longevity should be at least one year. However, during the lifespan, chamber formation is probably not a continuous process, and could be faster in juvenile stages (on the basis of lower metabolic rates in later ontogenetic stages, inferred from increasing $\delta^{13}C$ ratios, e.g., Schmiedl et al., 2004; Mackensen, 2008; Schumacher et al., 2010; Raitzsch et al., 2011). Under experimental conditions, growth rates of *A. tepida* showed a decrease with ontogeny from 1 chamber per day to 1 chamber per week during a 3 weeks period (de Nooijer et al., 2014a). It has been suggested

(e.g., Jorissen, 1988) that at later ontogenetic stages, calcification could be limited to short periods with favorable conditions, such as abundant food supply. In view of the rather scarce information about the timing and duration of calcification, we assume that *A. tepida* from Lake Grevelingen calcifies during several successive seasons, and that the specimens of our three samples (March, July and September 2012) have each recorded a different part of the yearly pore water Mn^{2+} cycle. The different degrees of intra-test variability between sampled months (Fig. 3 and 4) could be explained by the fact that some specimens mainly calcify during periods with stable sediment Mn^{2+} concentrations (i.e., a large part of specimens sampled in March and September), whereas others have calcified during periods with rapid changes, such as in winter, when cable bacteria activity and Mn^{2+} concentrations rapidly increase, or in spring, when these two parameters decrease again (i.e., a large part of specimens sampled in July). **This temporal aspect of Mn/Ca variability in relation to environmental changes can be further studied by considering all penultimate (and n-2, etc.) chambers from the same sampling (Fig. 5). However, the comparison of Mn/Ca ratios in the successive chambers of different individuals of the same sample shows important differences (Fig. 2 and Fig. 5). This suggests that even within the same sample, all individuals have slightly different calcification histories, and have not registered exactly the same time window.**

4.2.4 Foraminiferal vertical migration as explanation for Mn/Ca intra-test variability

Ammonia tepida is a shallow infaunal taxon, preferring a microhabitat close to the SWI, implying that the chemical composition of its test in principle reflects conditions in the superficial sediment layer (Thibault de Chanvalon et al., 2015; Cesbron et al., 2016). Observations made in laboratory experiments show that specimens of this species introduced in deeper anoxic sediments rapidly decrease their activity (Langlet et al., 2013; Maire et al., 2016) and, according to a field study from the Loire estuary, only specimens from the top 2 cm appear to be capable to regain their preferred niche at the sediment surface (Thibault de Chanvalon et al., 2015). However, Geslin et al. (2014) and Barras et al. (subm.) have shown that several foraminiferal species (among which *A. tepida*) repeatedly calcify new chambers in hypoxic conditions, whereas Nardelli et al. (2014) showed that even in anoxic conditions, *A. tepida* is capable of producing at least one new chamber. These data

suggest that individuals which have actively (through vertical migration) or passively (being transported by burrowing macrofauna) moved to deeper sediment layers, with higher Mn^{2+} levels (such as in February and March 2012, when the ~~OPD~~ oxygen penetration depth was ~0.2-0.3 cm below the SWI, Seitaj et al., 2016) could incidentally calcify a single chamber with a much higher Mn/Ca ratio. This could be the explanation for the patterns observed, for instance, in specimens 15, 17 and 19 sampled in July 2012 (Fig. 2). This phenomenon could be responsible for a small contribution to increased Mn/Ca intra-test variability (specimens 15 and 17 have high RSD values, Table A.1). In conclusion, we propose that a large part of the overall Mn/Ca intra-test variability can be explained by a different timing of calcification events with respect to the seasonal cycle of pore water Mn^{2+} concentrations, whereas extremely high values in single chambers could be due to occasional chamber formation in deeper sediment layers.

10 **4.3 Interest of benthic foraminiferal Mn/Ca ratios in coastal environments and implications for palaeoceanographic studies**

Concerning the reliability of single chamber measurements, in view of the large intra-test variability, in the very dynamic environment of Lake Grevelingen it is necessary to measure several chambers to obtain reliable average Mn/Ca ratios for a single specimen. In this specific context, bulk measurements could be a more practical solution to study the long term evolution of BWO. However, the large intra-test variability may include very useful information about the seasonal variability of pore water dynamics driven by redox conditions and sedimentation rates but also microbial activity. Such information can only be obtained by spot measurements of individual chambers. In fact, a comparison of Mn/Ca ratios of successive individual chambers can potentially inform us about the extent of these seasonal changes, and implicitly, about cable bacteria activity. If the calcification season of the foraminifera could be determined more precisely (for instance by using single specimen stable isotope analyses, e.g., Diz et al., 2009), it would potentially even be possible to reconstruct the annual cycle of pore water dynamics.

5 Conclusion

Tests of the coastal benthic foraminifer *A. tepida*, sampled in three different months at 23.1 m depth in the seasonally hypoxic/anoxic Lake Grevelingen show Mn/Ca ratios with a range of 0.08 ± 0.04 to 0.39 ± 0.19 mmol/mol (for average values per specimen), associated with a very large intra-test variability (average RSD = 45 %). This high intra-test variability may partly represent intrinsic factors (due to biological processes), although no systematic ontogenetic trends could be identified (for all specimens from the three sampled months combined). However, we ascribed the larger part of the Mn/Ca intra-test variability to the large temporal variability of environmental parameters and to different timing of calcification of the analysed specimens. We suggest that the strong seasonal dynamics of pore water Mn induced by seasonal hypoxia and cable bacteria activity, leading to variations in absolute Mn^{2+} concentrations and/or migration of the redox front in the sediment, is the main factor responsible for this large intra-test variability. Differences in the timing of calcification could explain the different degrees of intra-test variability observed for the three sampled months, whereas differences in individual life history (between the individuals found in a single sample) could even explain inter-test differences observed in each of our three samples. Some individual chambers with exceptionally high Mn/Ca ratios could be due to active or passive migration to, and calcification in, slightly deeper sediment layers. In conclusion, in environments with strong seasonal changes in redox conditions and microbial activity, Mn/Ca measurements of successive chambers of individual tests may provide a powerful proxy to reconstruct the seasonal variability of these parameters.

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References

- Aller, R. C.: Bioturbation and Manganese Cycling in Hemipelagic Sediments, *Philos. Trans. R. Soc. London A Math. Phys. Eng. Sci.*, 331(1616), 51–68 [online] Available from: <http://rsta.royalsocietypublishing.org/content/331/1616/51.abstract>, 1990.
- 5
- Aller, R. C.: The sedimentary Mn cycle in Long Island Sound: Its role as intermediate oxidant and the influence of bioturbation, O₂, and C_{org} flux on diagenetic reaction balances, *J. Mar. Res.*, 52(2), 259–295, doi:10.1357/0022240943077091, 1994.
- Allison, N. and Austin, W. E. N.: The potential of ion microprobe analysis in detecting geochemical variations across individual foraminifera tests, *Geochemistry, Geophys. Geosystems*, 4(2), 1–9, doi:10.1029/2002GC000430, 2003.
- 10
- Altabet, M. A., Francois, R., Murray, D. W. and Prell, W. L.: Climate-related variations in denitrification in the Arabian Sea from sediment ¹⁵N/¹⁴N ratios, *Nature*, 373, 506–509, 1995.
- Barker, S., Greaves, M. and Elderfield, H.: A study of cleaning procedures used for foraminiferal Mg/Ca paleothermometry, *Geochemistry, Geophys. Geosystems*, 4(9), 1–20, doi:10.1029/2003GC000559, 2003.
- 15
- Barras, C., Mouret, A., Nardelli, M. P., Metzger, E., Petersen, J., La, C., Filipsson, H. L. and Jorissen, F. J.: Experimental calibration of Mn incorporation in foraminiferal calcite, *Geochim. Cosmochim. Acta*, submitted.
- Bentov, S. and Erez, J.: Novel observations on biomineralization processes in foraminifera and implications for Mg/Ca ratio in the shells, *Geology*, 33(11), 841–844, doi:10.1130/G21800.1, 2005.
- Bentov, S. and Erez, J.: Impact of biomineralization processes on the Mg content of foraminiferal shells: A biological perspective, *Geochemistry, Geophys. Geosystems*, 7(1), doi:10.1029/2005GC001015, 2006.
- 20
- Bernhard, J. M., Ostermann, D. R., Williams, D. S. and Blanks, J. K.: Comparison of two methods to identify live benthic foraminifera: A test between Rose Bengal and CellTracker Green with implications for stable isotope paleoreconstructions, *Paleoceanography*, 21(4), 1–8, doi:10.1029/2006PA001290, 2006.
- Bettencourt da Silva, R. and Williams, A., Eds.: *Eurachem/CITAC Guide: Setting and Using Target Uncertainty in Chemical Measurement*, 1st ed. [online] Available from: www.eurachem.org, 2015.
- 25
- Boltovskoy, E. and Lena, H.: Seasonal occurrences, standing crop and production in benthic foraminifera of Puerto Deseado, *Contrib. from Cushman Found. Foraminifer. Res.*, 20, Part 3, 87–95, 1969.
- Boyle, E. A.: Manganese carbonate overgrowths on foraminifera tests, *Geochim. Cosmochim. Acta*, 47, 1815–1819, 1983.
- Burdige, D. J.: The biogeochemistry of manganese and iron reduction in marine sediments, *Earth-Science Rev.*, 35, 249–284, 1993.
- 30
- Canfield, D. E.: Organic matter oxidation in marine sediments, in *Interactions of C, N, P and S Biogeochemical Cycles and*

- Global Change, edited by R. Wollast, F. T. Mackenzie, and L. Chou, pp. 333–363, Springer Berlin Heidelberg, 1993.
- Cesbron, F., Geslin, E., Jorissen, F. J., Delgard, M. L., Charrieau, L., Deflandre, B., Jézéquel, D., Anschutz, P. and Metzger, E.: Vertical distribution and respiration rates of benthic foraminifera: contribution to aerobic remineralization in intertidal mudflats covered by *Zostera noltei* meadows, *Estuar. Coast. Shelf Sci.*, 179, 23–38, doi:10.1016/j.ecss.2015.12.005, 2016.
- 5 Curry, W. B. and Marchitto, T. M.: A secondary ionization mass spectrometry calibration of *Cibicidoides pachyderma* Mg/Ca with temperature, *Geochemistry, Geophys. Geosystems*, 9(4), 1–13, doi:10.1029/2007GC001620, 2008.
- Dellwig, O., Bosselmann, K., Kölsch, S., Hentscher, M. and Hinrichs, J.: Sources and fate of manganese in a tidal basin of the German Wadden Sea, *J. Sea Res.*, 57, 1–18, doi:10.1016/j.seares.2006.07.006, 2007.
- 10 Diaz, R. J. and Rosenberg, R.: Spreading dead zones and consequences for marine ecosystems., *Science*, 321(5891), 926–929, doi:10.1126/science.1156401, 2008.
- Diz, P., Jorissen, F. J., Reichart, G. J., Poulain, C., Dehairs, F., Leorri, E. and Paulet, Y.-M.: Interpretation of benthic foraminiferal stable isotopes in subtidal estuarine environments, *Biogeosciences*, 6, 2549–2560, doi:10.5194/bgd-6-7453-2009, 2009.
- 15 Eggins, S. M., Kinsley, L. P. J. and Shelley, J. M. G.: Deposition and element fractionation processes during atmospheric pressure laser sampling for analysis by ICP-MS, *Appl. Surf. Sci.*, 127–129, 278–286, doi:10.1016/S0169-4332(97)00643-0, 1998.
- Eggins, S. M., De Deckker, P. and Marshall, J.: Mg/Ca variation in planktonic foraminifera tests: Implications for reconstructing palaeo-seawater temperature and habitat migration, *Earth Planet. Sci. Lett.*, 212(3–4), 291–306, doi:10.1016/S0012-821X(03)00283-8, 2003.
- 20 Froelich, P. N., Klinkhammer, G. P., Bender, M. L., Luedtke, N. A., Heath, G. R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B. and Maynard, V.: Early Oxidation of Organic-Matter in Pelagic Sediments of the Eastern Equatorial Atlantic - Suboxic Diagenesis, *Geochim. Cosmochim. Acta*, 43(7), 1075–1090, doi:10.1016/0016-7037(79)90095-4, 1979.
- 25 Geslin, E., Barras, C., Langlet, D., Nardelli, M. P., Kim, J.-H., Bonnin, J., Metzger, E. and Jorissen, F. J.: Survival, reproduction and calcification of three benthic foraminiferal species in response to experimentally induced hypoxia, in *Approaches to study living foraminifera: collection, maintenance and experimentation*, edited by H. Kitazato and J. M. Bernhard, p. 238, Springer, Japan, 2014.
- Glock, N., Eisenhauer, A., Liebetrau, V., Wiedenbeck, M., Hensen, C. and Nehrke, G.: EMP and SIMS studies on Mn/Ca and Fe/Ca systematics in benthic foraminifera from the Peruvian OMZ: A contribution to the identification of potential redox proxies and the impact of cleaning protocols, *Biogeosciences*, 9(1), 341–359, doi:10.5194/bg-9-341-2012, 2012.
- 30 Goldstein, S. T. and Moodley, L.: Gametogenesis and the life cycle of the foraminifer *Ammonia beccarii* (Linné) forma *tepida* (Cushman), *J. Foraminifer. Res.*, 23(4), 213–220, 1993.
- Groeneveld, J. and Filipsson, H. L.: Mg/Ca and Mn/Ca ratios in benthic foraminifera: the potential to reconstruct past

- variations in temperature and hypoxia in shelf regions, *Biogeosciences*, 10(7), 5125–5138, doi:DOI 10.5194/bg-10-5125-2013, 2013.
- Sen Gupta, B. K., Turner, R. E. and Rabalais, N. N.: Seasonal oxygen depletion in continental-shelf waters of Louisiana : Historical record of benthic foraminifers, *Geology*, 24(3), 227–230, 1996.
- 5 Hagens, M., Slomp, C. P., Meysman, F. J. R., Seitaj, D., Harlay, J., Borges, A. V. and Middelburg, J. J.: Biogeochemical processes and buffering capacity concurrently affect acidification in a seasonally hypoxic coastal marine basin, *Biogeosciences*, 12(5), 1561–1583, doi:10.5194/bg-12-1561-2015, 2015.
- Hayward, B. W., Holzmann, M., Grenfell, H. R., Pawlowski, J. and Triggs, C. M.: Morphological distinction of molecular types in *Ammonia* – towards a taxonomic revision of the world’s most commonly misidentified foraminifera, *Mar. Micropaleontol.*, 50(3–4), 237–271, doi:10.1016/S0377-8398(03)00074-4, 2004.
- 10 Jochum, K. P., Weis, U., Stoll, B., Kuzmin, D., Yang, Q., Raczek, I., Jacob, D. E., Stracke, A., Birbaum, K., Frick, D. A., Günther, D. and Enzweiler, J.: Determination of reference values for NIST SRM 610-617 glasses following ISO guidelines, *Geostand. Geoanalytical Res.*, 35(4), 397–429, doi:10.1111/j.1751-908X.2011.00120.x, 2011.
- Jochum, K. P., Scholz, D., Stoll, B., Weis, U., Wilson, S. A., Yang, Q., Schwalb, A., Börner, N., Jacob, D. E. and Andreae, M. O.: Accurate trace element analysis of speleothems and biogenic calcium carbonates by LA-ICP-MS, *Chem. Geol.*, 15 318–319, 31–44, doi:10.1016/j.chemgeo.2012.05.009, 2012.
- Jorissen, F. J.: Benthic foraminifera from the Adriatic Sea; Principles of phenotypic variation, *Utr. Micropaleontologac Bull.*, 37, 174pp, 1988.
- Jorissen, F. J., de Stigter, H. and Widmark, J. G. V: A conceptual model explaining benthic foraminiferal microhabitats, 20 *Mar. Micropaleontol.*, 26, 3–15, 1995.
- Keeling, R. F., Körtzinger, A. and Gruber, N.: Ocean Deoxygenation in a Warming World, *Ann. Rev. Mar. Sci.*, 2(1), 199–229, doi:10.1146/annurev.marine.010908.163855, 2010.
- Klinkhammer, G. P., Mix, A. C. and Haley, B. A.: Increased dissolved terrestrial input to the coastal ocean during the last deglaciation, *Geochemistry, Geophys. Geosystems*, 10(3), doi:10.1029/2008GC002219, 2009.
- 25 Koho, K. A., Nierop, K. G. J., Moodley, L., Middelburg, J. J., Pozzato, L., Soetaert, K., Van Der Plicht, J. and Reichart, G. J.: Microbial bioavailability regulates organic matter preservation in marine sediments, *Biogeosciences*, 10(2), 1131–1141, doi:10.5194/bg-10-1131-2013, 2013.
- Koho, K. A., de Nooijer, L. J. and Reichart, G. J.: Combining benthic foraminiferal ecology and shell Mn/Ca to deconvolve past bottom water oxygenation and paleoproductivity, *Geochim. Cosmochim. Acta*, 165, 294–306, 30 doi:10.1016/j.gca.2015.06.003, 2015.
- Koho, K. A., de Nooijer, L. J., Fontanier, C., Toyofuku, T., Kazumasa, O., Kitazato, H. and Reichart, G.-J.: Benthic foraminiferal Mn/Ca ratios reflect microhabitat preferences, *Biogeosciences*, 14, 3067–3082, doi:10.5194/bg-2016-547, 2017.
- Konovalov, S. K., Luther, G. W. and Yücel, M.: Porewater redox species and processes in the Black Sea sediments, *Chem.*

- Geol., 245(3–4), 254–274, doi:10.1016/j.chemgeo.2007.08.010, 2007.
- Kowalski, N., Dellwig, O., Beck, M., Grunwald, M., Dürselen, C., Badewien, T. H., Brumsack, H., Beusekom, J. E. E. Van and Böttcher, M. E.: A comparative study of manganese dynamics in the water column and sediments of intertidal systems of the North Sea, *Estuar. Coast. Shelf Sci.*, 100, 3–17, doi:10.1016/j.ecss.2011.03.011, 2012.
- 5 Langlet, D.: Réponse des foraminifères benthiques à l’anoxie dans les milieux côtiers : études in situ de l’écologie des foraminifères benthiques, expériences en laboratoire et analyse du métabolisme anaérobie, Ph.D. thesis, Université d’Angers; Université Nantes Angers Le Mans (UNAM), France. [online] Available from: <https://hal-lirmm.ccsd.cnrs.fr/UNIV-ANGERS-THESE/tel-01144631>, 2014.
- Langlet, D., Geslin, E., Baal, C., Metzger, E., Lejzerowicz, F., Riedel, B., Zuschin, M., Pawlowski, J., Stachowitsch, M. and
10 Jorissen, F. J.: Foraminiferal survival after long-term in situ experimentally induced anoxia, *Biogeosciences*, 10(11), 7463–7480, doi:10.5194/bg-10-7463-2013, 2013.
- Leduc, G., Garbe-Schönberg, D., Regenberg, M., Contoux, C., Etourneau, J. and Schneider, R.: The late Pliocene Benguela upwelling status revisited by means of multiple temperature proxies, *Geochemistry Geophys. Geosystems*, 15(2), 475–491, doi:10.1002/2013GC004940, 2014.
- 15 Lenz, C., Jilbert, T., Conley, D. J. and Slomp, C. P.: Hypoxia-driven variations in iron and manganese shuttling in the Baltic Sea over the past 8 kyr, *Geochemistry Geophys. Geosystems*, 16, 3754–3766, doi:10.1002/2015GC005960, 2015.
- Levin, L. A., Whitcraft, C. R., Mendoza, G. F., Gonzalez, J. P. and Cowie, G.: Oxygen and organic matter thresholds for benthic faunal activity on the Pakistan margin oxygen minimum zone (700–1100 m), *Deep. Res. Part II Top. Stud. Oceanogr.*, 56(6–7), 449–471, doi:10.1016/j.dsr2.2008.05.032, 2009.
- 20 Long, G. L. and Winefordner, J. D.: Limit of detection: a closer look at the IUPAC definition, *Anal. Chem.*, 55(7), 712A–724A, 1983.
- Longerich, H. P., Jackson, S. E. and Günther, D.: Inter-laboratory note. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation, *J. Anal. At. Spectrom.*, 11(9), 899–904, doi:10.1039/JA9961100899, 1996.
- 25 Mackensen, A.: On the use of benthic foraminiferal $\delta^{13}\text{C}$ in palaeoceanography: constraints from primary proxy relationships, *Geol. Soc. London, Spec. Publ.*, 303(1), 121–133, doi:10.1144/SP303.9, 2008.
- Maire, O., Barras, C., Gestin, T., Nardelli, M.-P., Romero-Ramirez, A., Duchêne, J.-C. and Geslin, E.: How does macrofaunal bioturbation influence the vertical distribution of living benthic foraminifera?, *Mar. Ecol. Prog. Ser.*, 561, 83–97, 2016.
- 30 Marr, J. P., Baker, J. A., Carter, L., Allan, A. S. R., Dunbar, G. B. and Bostock, H. C.: Ecological and temperature controls on Mg/Ca ratios of *Globigerina bulloides* from the southwest Pacific Ocean, *Paleoceanography*, 26(2), 1–15, doi:10.1029/2010PA002059, 2011.
- McKay, C. L., Groeneveld, J., Filipsson, H. L., Gallego-Torres, D., Whitehouse, M. J., Toyofuku, T. and Romero, O. E.: A comparison of benthic foraminiferal Mn / Ca and sedimentary Mn / Al as proxies of relative bottom-water oxygenation

- in the low-latitude NE Atlantic upwelling system, *Biogeosciences*, 12(18), 5415–5428, doi:10.5194/bg-12-5415-2015, 2015.
- Mezger, E. M., Nooijer, L. J., Boer, W., Brummer, G. J. A. and Reichart, G. J.: Salinity controls on Na incorporation in Red Sea planktonic foraminifera, *Paleoceanography*, 31, 1562–1582, doi:10.1002/2016PA003052, 2016.
- 5 Moodley, L. and Hess, C.: Tolerance of infaunal benthic foraminifera for low and high oxygen concentrations, *Biol. Bull.*, 183, 94–98, doi:10.2307/1542410, 1992.
- Morvan, J., Debenay, J. P., Jorissen, F., Redois, F., Bénéteau, E., Delplancke, M. and Amato, A. S.: Patchiness and life cycle of intertidal foraminifera: Implication for environmental and paleoenvironmental interpretation, *Mar. Micropaleontol.*, 61(1–3), 131–154, doi:10.1016/j.marmicro.2006.05.009, 2006.
- 10 Mouret, A., Anschutz, P., Lecroart, P., Chaillou, G., Hyacinthe, C., Deborde, J., Jorissen, F. J., Deflandre, B., Schmidt, S. and Jouanneau, J. M.: Benthic geochemistry of manganese in the Bay of Biscay, and sediment mass accumulation rate, *Geo-Marine Lett.*, 29(3), 133–149, doi:10.1007/s00367-008-0130-6, 2009.
- Munsel, D., Kramar, U., Dissard, D., Nehrke, G., Berner, Z., Bijma, J., Reichart, G. J. and Neumann, T.: Heavy metal incorporation in foraminiferal calcite: Results from multi-element enrichment culture experiments with *Ammonia tepida*, *Biogeosciences*, 7(8), 2339–2350, doi:10.5194/bg-7-2339-2010, 2010.
- 15 Murray, J. W.: Population dynamics of benthic foraminifera; results from the Exe Estuary, England, *J. Foraminifer. Res.*, 13(1), 1–12, doi:10.2113/gsjfr.13.1.1, 1983.
- Nardelli, M. P., Barras, C., Metzger, E., Mouret, A., Filipsson, H. L., Jorissen, F. and Geslin, E.: Experimental evidence for foraminiferal calcification under anoxia, *Biogeosciences*, 11(14), 4029–4038, doi:10.5194/bg-11-4029-2014, 2014.
- 20 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(10), 6759–6767, doi:10.5194/bg-10-6759-2013, 2013.
- Ní Fhlaithearta, S., Reichart, G. J., Jorissen, F. J., Fontanier, C., Rohling, E. J., Thomson, J. and De Lange, G. J.: Reconstructing the seafloor environment during sapropel formation using benthic foraminiferal trace metals, stable isotopes, and sediment composition, *Paleoceanography*, 25(4), 1–17, doi:10.1029/2009PA001869, 2010.
- 25 de Nooijer, L. J., Hathorne, E. C., Reichart, G. J., Langer, G. and Bijma, J.: Variability in calcitic Mg/Ca and Sr/Ca ratios in clones of the benthic foraminifer *Ammonia tepida*, *Mar. Micropaleontol.*, 107, 32–43, doi:10.1016/j.marmicro.2014.02.002, 2014a.
- de Nooijer, L. J., Spero, H. J., Erez, J., Bijma, J. and Reichart, G. J.: Biomineralization in perforate foraminifera, *Earth-Science Rev.*, 135, 48–58, doi:10.1016/j.earscirev.2014.03.013, 2014b.
- 30 Pakhomova, S. V., Hall, P. O. J., Yu, M., Rozanov, A. G., Tengberg, A. and Vershinin, A. V.: Fluxes of iron and manganese across the sediment – water interface under various redox conditions, *Mar. Chem.*, 107, 319–331, doi:10.1016/j.marchem.2007.06.001, 2007.
- Pearce, N. J. G., Perkins, W. T., Westgate, J. A., Gorton, M. P., Jackson, S. E., Neal, C. R. and Chenery, S. P.: A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass

- reference materials, *Geostand. Newsl.*, 21(1), 115–144, doi:10.1111/j.1751-908X.1997.tb00538.x, 1997.
- Pena, L. D., Calvo, E., Cacho, I., Eggins, S. and Pelejero, C.: Identification and removal of Mn-Mg-rich contaminant phases on foraminiferal tests: Implications for Mg/Ca past temperature reconstructions, *Geochemistry, Geophys. Geosystems*, 6(9), 25 pp, doi:10.1029/2005GC000930, 2005.
- 5 Pena, L. D., Cacho, I., Calvo, E., Pelejero, C., Eggins, S. and Sadekov, A.: Characterization of contaminant phases in foraminifera carbonates by electron microprobe mapping, *Geochemistry, Geophys. Geosystems*, 9(7), 1–12, doi:10.1029/2008GC002018, 2008.
- Rabalais, N. N., Turner, R. E. and Wiseman, W. J.: Gulf of Mexico Hypoxia, a.k.a. “The Dead Zone,” *Annu. Rev. Ecol. Syst.*, 33(1), 235–263, doi:10.1146/annurev.ecolsys.33.010802.150513, 2002.
- 10 R Core Team: R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria., 2016.
- Raitzsch, M., Hathorne, E. C., Kuhnert, H., Groeneveld, J. and Bickert, T.: Modern and late pleistocene B/Ca ratios of the benthic foraminifer *Planulina wuellerstorfi* determined with laser ablation ICP-MS, *Geology*, 39(11), 1039–1042, doi:10.1130/G32009.1, 2011.
- 15 Rao, A. M. F., Malkin, S. Y., Hidalgo-Martinez, S. and Meysman, F. J. R.: The impact of electrogenic sulfide oxidation on elemental cycling and solute fluxes in coastal sediment, *Geochim. Cosmochim. Acta*, 172, 265–286, doi:10.1016/j.gca.2015.09.014, 2016.
- Reichart, G. J., Den Dulk, M., Visser, H. J., Van Der Weijden, C. H. and Zachariasse, W. J.: A 225 kyr record of dust supply, paleoproductivity and the oxygen minimum zone from the Murray Ridge (Northern Arabian Sea),
- 20 *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 134(1–4), 149–169, doi:10.1016/S0031-0182(97)00071-0, 1997.
- Reichart, G. J., Jorissen, F., Anschutz, P. and Mason, P. R. D.: Single foraminiferal test chemistry records the marine environment, *Geology*, 31(4), 355–358, doi:10.1130/0091-7613(2003)031<0355:SFTCRT>2.0.CO;2, 2003.
- Saad, S. A. and Wade, C. M.: Biogeographic distribution and habitat association of *Ammonia* genetic variants around the coastline of Great Britain, *Mar. Micropaleontol.*, 124, 54–62, doi:10.1016/j.marmicro.2016.01.004, 2016.
- 25 Sadekov, A. Y., Eggins, S. M. and De Deckker, P.: Characterization of Mg/Ca distributions in planktonic foraminifera species by electron microprobe mapping, *Geochemistry, Geophys. Geosystems*, 6(12), doi:10.1029/2005GC000973, 2005.
- Schenau, S. J., Reichart, G. J. and de Lange, G. J.: Oxygen minimum zone controlled Mn redistribution in Arabian Sea sediments during the late Quaternary, *Paleoceanography*, 17(4), 1–10, doi:10.1029/2000, 2002.
- 30 Schmiedl, G., Pfeilsticker, M., Hemleben, C. and Mackensen, A.: Environmental and biological effects on the stable isotope composition of recent deep-sea benthic foraminifera from the western Mediterranean Sea, *Mar. Micropaleontol.*, 51(1–2), 129–152, doi:10.1016/j.marmicro.2003.10.001, 2004.
- Schumacher, S., Jorissen, F. J., Mackensen, A., Gooday, A. J. and Pays, O.: Ontogenetic effects on stable carbon and oxygen isotopes in tests of live (Rose Bengal stained) benthic foraminifera from the Pakistan continental margin, *Mar.*

- Micropaleontol., 76(3–4), 92–103, doi:10.1016/j.marmicro.2010.06.002, 2010.
- Schweizer, M., Polovodova, I., Nikulina, A. and Schönfeld, J.: Molecular identification of *Ammonia* and *Elphidium* species (Foraminifera, Rotaliida) from the Kiel Fjord (SW Baltic Sea) with rDNA sequences, *Helgol. Mar. Res.*, 65(1), 1–10, doi:10.1007/s10152-010-0194-3, 2011.
- 5 Seitaj, D., Schauer, R., Sulu-Gambari, F., Hidalgo-Martinez, S., Malkin, S. Y., Burdorf, L. D. W., Slomp, C. P. and Meysman, F. J. R.: Cable bacteria generate a firewall against euxinia in seasonally hypoxic basins., *Proc. Natl. Acad. Sci. U. S. A.*, 112(43), 13278–13283, doi:10.1073/pnas.1510152112, 2015.
- Seitaj, D., Sulu-Gambari, F., Burdorf, L. D. W., Romero-Ramirez, A., Maire, O., Malkin, S. Y., Slomp, C. P. and Meysman, F. J. R.: Sedimentary oxygen dynamics in a seasonally hypoxic basin, *Limnol. Oceanogr.*, 62, 452–473, doi:10.1002/lno.10434, 2017.
- 10 Slomp, C. P., Malschaert, J. F. P., Lohse, L. and van Raaphorst, W.: Iron and manganese cycling in different sedimentary environments on the North Sea continental margin, *Cont. Shelf Res.*, 17(9), 1083–1117, 1997.
- Sulu-Gambari, F., Seitaj, D., Meysman, F. J. R. and Schauer, R.: Cable bacteria control iron-phosphorus dynamics in sediments of a coastal hypoxic basin, *Environ. Sci. Technol.*, 50(3), 1227–1233, doi:10.1021/acs.est.5b04369, 2016a.
- 15 Sulu-Gambari, F., Seitaj, D., Behrends, T., Banerjee, D., Meysman, F. J. R. and Slomp, C. P.: Impact of cable bacteria on sedimentary iron and manganese dynamics in a seasonally-hypoxic marine basin, *Geochim. Cosmochim. Acta*, 192, 49–69, doi:10.1016/j.gca.2016.07.028, 2016b.
- Sulu-Gambari, F., Roepert, A., Jilbert, T., Hagens, M., Meysman, F. J. R. and Slomp, C. P.: Molybdenum dynamics in sediments of a seasonally-hypoxic coastal marine basin, *Chem. Geol.*, 466(July), 627–640, doi:10.1016/j.chemgeo.2017.07.015, 2017.
- 20 Sundby, B. and Silverberg, N.: Manganese fluxes in the benthic boundary layer, *Limnol. Oceanogr.*, 30(2), 372–381, doi:10.4319/lo.1985.30.2.0372, 1985.
- Thamdrup, B., Fossing, H. and Jørgensen, B. B.: Manganese, iron, and sulfur cycling in a coastal marine sediment. Aarhus Bay, Denmark, *Geochim. Cosmochim. Acta*, 58(23), 5115–5129, 1994.
- 25 Thibault de Chanvalon, A., Metzger, E., Mouret, A., Cesbron, F., Knoery, J., Rozuel, E., Launeau, P., Nardelli, M. P., Jorissen, F. J. and Geslin, E.: Two-dimensional distribution of living benthic foraminifera in anoxic sediment layers of an estuarine mudflat (Loire estuary, France), *Biogeosciences*, 12(20), 6219–6234, doi:10.5194/bg-12-6219-2015, 2015.
- Thibault de Chanvalon, A., Mouret, A., Knoery, J., Geslin, E., Péron, O. and Metzger, E.: Manganese, iron and phosphorus cycling in an estuarine mudflat, Loire, France, *J. Sea Res.*, 118, 92–102, doi:10.1016/j.seares.2016.10.004, 2016.
- 30 Vandieken, V., Nickel, M. and Jørgensen, B. B.: Carbon mineralization in Arctic sediments northeast of Svalbard: Mn(IV) and Fe(III) reduction as principal anaerobic respiratory pathways, *Mar. Ecol. Prog. Ser.*, 322, 15–27, doi:10.3354/meps322015, 2006.

- van de Velde, S. and Meysman, F. J. R.: The influence of bioturbation on iron and sulphur cycling in marine sediments: a model analysis, *Aquat. Geochemistry*, 22, 469–504, doi:10.1007/s10498-016-9301-7, 2016.
- van de Velde, S., Lesven, L., Burdorf, L. D. W., Hidalgo-Martinez, S., Geelhoed, J. S., Van Rijswijk, P., Gao, Y. and Meysman, F. J. R.: The impact of electrogenic sulfur oxidation on the biogeochemistry of coastal sediments: A field study, *Geochim. Cosmochim. Acta*, 194, 211–232, doi:10.1016/j.gca.2016.08.038, 2016.
- 5 Van der Weijden, C. H., Reichart, G.-J. and van Os, B. J. H.: Sedimentary trace element records over the last 200 kyr from within and below the northern Arabian Sea oxygen minimum zone, *Mar. Geol.*, 231, 69–88, doi:10.1016/j.margeo.2006.05.013, 2006.
- Wickham, H.: *ggplot2: elegant graphics for data analysis*, Springer New York, New York., 2009.
- 10 Wit, J. C., Reichart, G. J., A Jung, S. J. and Kroon, D.: Approaches to unravel seasonality in sea surface temperatures using paired single-specimen foraminiferal $\delta^{18}\text{O}$ and Mg/Ca analyses, *Paleoceanography*, 25(4), 1–15, doi:10.1029/2009PA001857, 2010.
- Van der Zwaan, G. J., Duijnste, I. A. P., den Dulk, M., Ernst, S. R., Jannink, N. T. and Kouwenhoven, T. J.: Benthic foraminifers : proxies or problems ? A review of paleocological concepts, *Earth-Science Rev.*, 46, 213–236, 1999.

15

Table 1: Summary of LA-ICP-MS operating conditions.

Analyte G2 laser ablation system (193 nm)	
Laser fluence	0.91 J/cm ²
Laser pulse repetition rate	4 Hz
He flow rates for the HelEx 2 Volume cell	0.7 and 0.3 L/min
Projected spot size	40-85 μm
Quadrupole Varian 820-MS	
Dwell time	20 ms
RF power	1.15 kW
Sheath gas flow	0.8-0.9 L/min
²³² Th ¹⁶ O ⁺ / ²³² Th ⁺	< 0.5%
U/Th	~1

Table 2: Evaluation of external reproducibility of laser ablation ICP-MS analyses for Mg/Ca, Mn/Ca and Sr/Ca determined on NFHS in-house foraminiferal standard, USGS MACS-3 and NIST SRM 610. For Mn/Ca and Sr/Ca all values are calibrated against NIST SRM 612 and are given as average with standard deviations from all sessions (in bold). For Mg/Ca all values are calibrated against USGS MACS-3. Reference values with standard deviation are given according to Jochum et al. (2011), Jochum et al. (2012), USGS (S. Wilson, USGS, unpubl.) and Pearce et al. (1997). Values for NFHS are in mmol/mol for better comparison with results from samples (standard deviation of standard applied as error bar on samples) as well as in ppm because reference values are given in this unit (NIOZ, personal communication) and for comparison to concentrations of other reference materials. Values for USGS MACS-3 and NIST SRM 610 are in ppm for comparison with published reference values.

		NFHS			USGS MACS-3			NIST SRM 610		
		this study	this study	NIOZ (p. com.)	this study	USGS	Jochum et al. (2012)	this study	Jochum et al. (2011)	Pearce et al. (1997)
		[mmol/mol]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]
Mg/Ca	2.8 ±0.2 (n=44)	Mg	688 ±40	660	-	1756 ±136	1880 ±70 (n=36)	520 ±12 (n=19)	432 ±29	465 ±27
Mn/Ca	0.15 ±0.02 (n=115)	Mn	85 ±9	88	520 ±26 (n=115)	536 ±28	532 ±23 (n=36)	445 ±5 (n=35)	444 ±6	433 ±32
Sr/Ca	1.38 ±0.04 (n=115)	Sr	1204 ±37	1300	6590 ±383 (n=115)	6760 ±350	6570 ±170 (n=36)	513 ±16 (n=35)	515.5 ±0.5	497 ±18

Table 3: Statistical results for comparison of Mn/Ca measurements of *A. tepida* for different sampling months. Significant results (p-value < 0.05) are indicated in bold.

	Average Mn/Ca	SD Mn/Ca	Range Mn/Ca
p-value ANOVA	0.118	0.016	-
p-value Kruskal-Wallis	-	-	0.044
p-value post-hoc test:			
03/2012 vs 07/2012	0.720	0.045	0.041
03/2012 vs 09/2012	1.000	1.000	0.621
07/2012 vs 09/2012	0.120	0.036	0.033

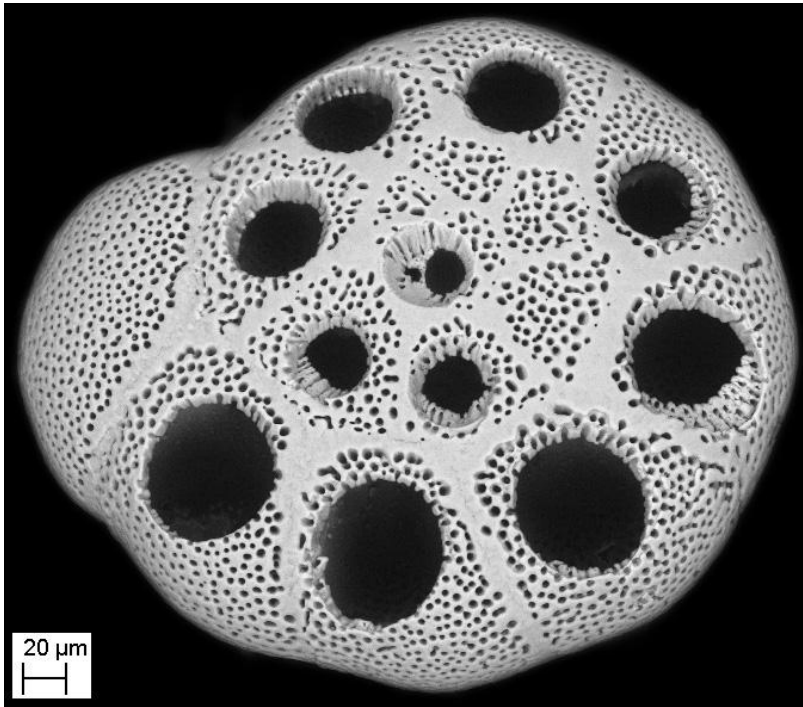


Figure 1: SEM image of *A. tepida* specimen after laser ablation analysis of multiple chambers. Image taken at SCIAM, University of Angers.

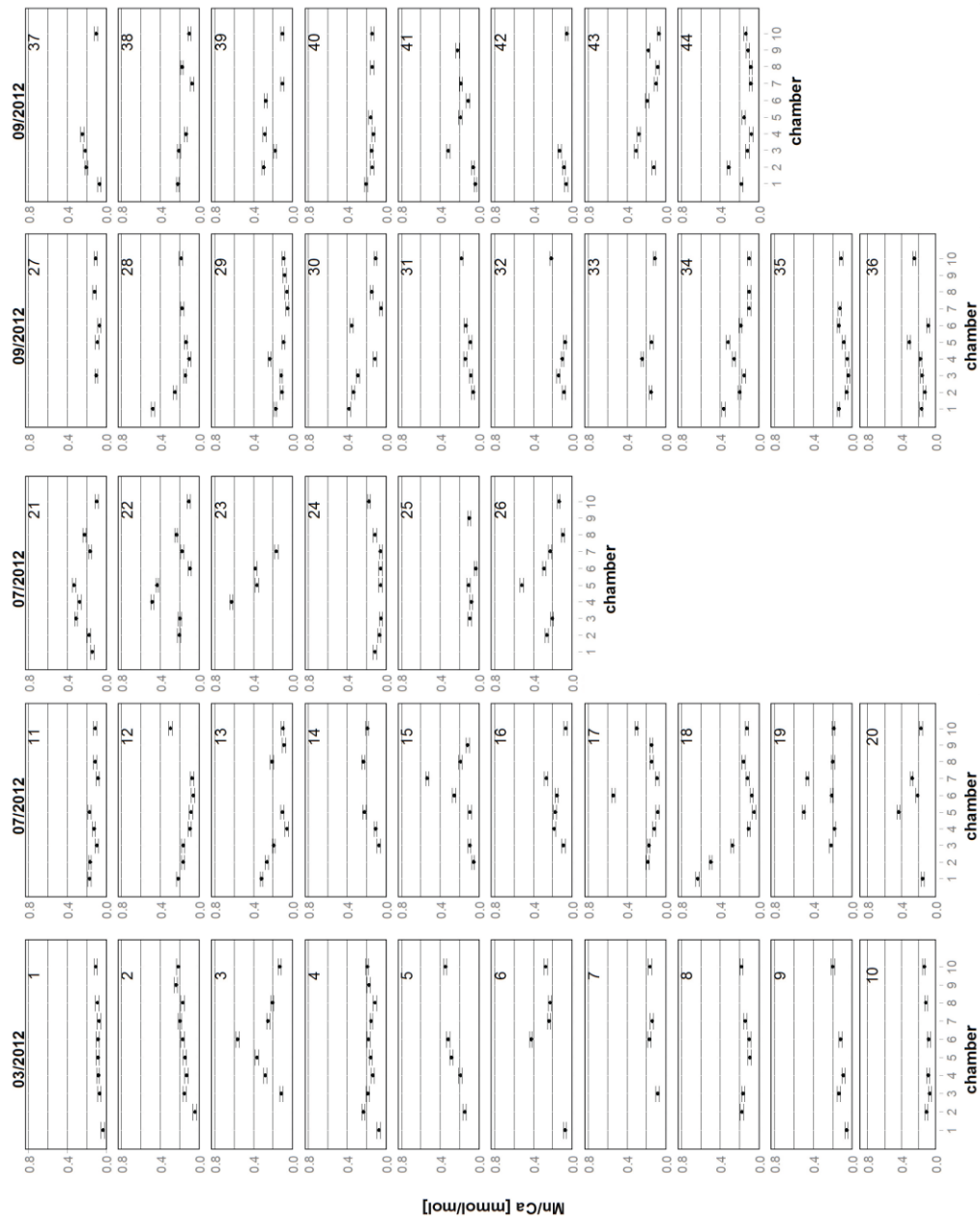


Figure 2: Mn/Ca for each chamber in living specimens of *A. tepida* from Lake Grevelingen, collected in March (03/2012), July (07/2012) and September (09/2012) 2012 (specimens labelled from 1 to 44 as in Table A.1). One plot represents one specimen. Numbers on x-axis indicate chambers: 1 = penultimate chamber 2 = antepenultimate chamber, etc., 10= central part of test. Error bars represent the Mn/Ca standard deviation of multiple analyses of a foraminiferal carbonate standard NFHS (see section 2.3).

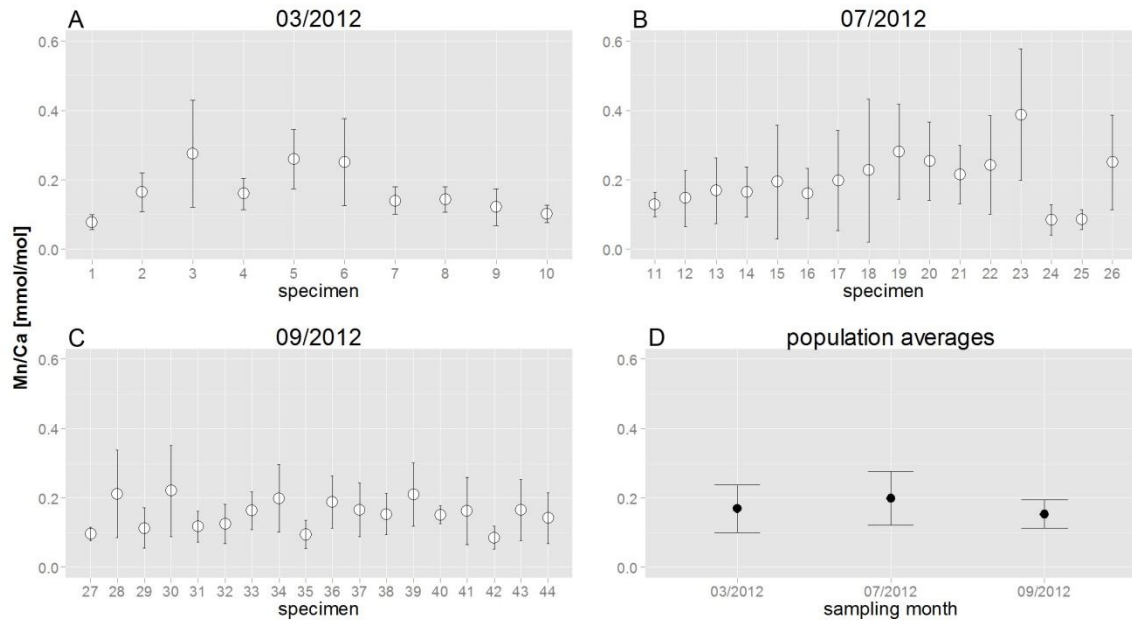


Figure 3: Average Mn/Ca values and standard deviation for all measured chambers of each analysed specimen of *A. tepida* for the three sampling campaigns (A: March, B: July, C: September). D: Population averages with standard deviation calculated on the basis of mean values per specimen of all analysed specimens for each of the three sampling months.

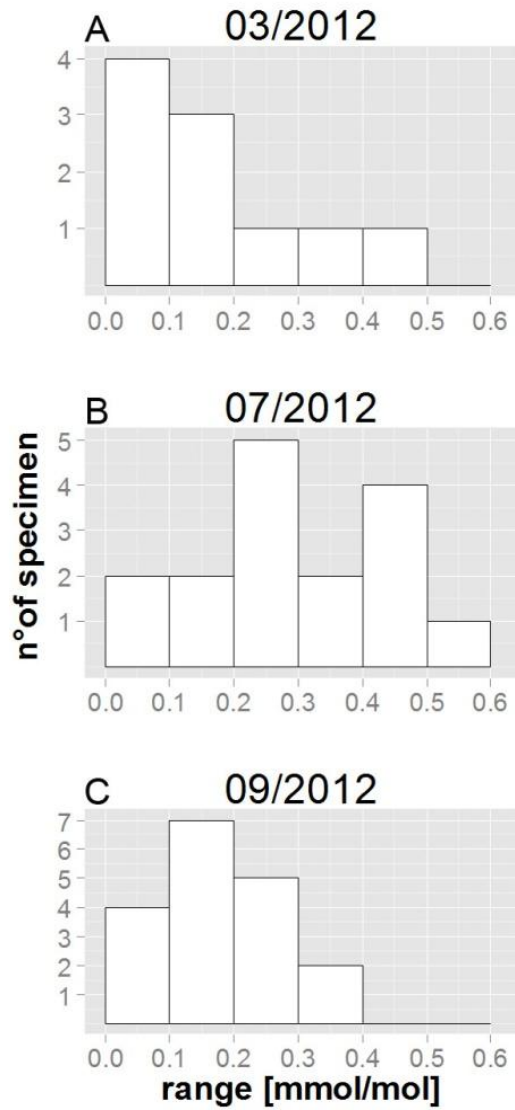


Figure 4: Histograms representing Mn/Ca intra-test variability as range (difference between maximum and minimum Mn/Ca per specimen of *A. tepida*). A: March, 10 specimens; B: July, 16 specimens; C: September, 18 specimens.

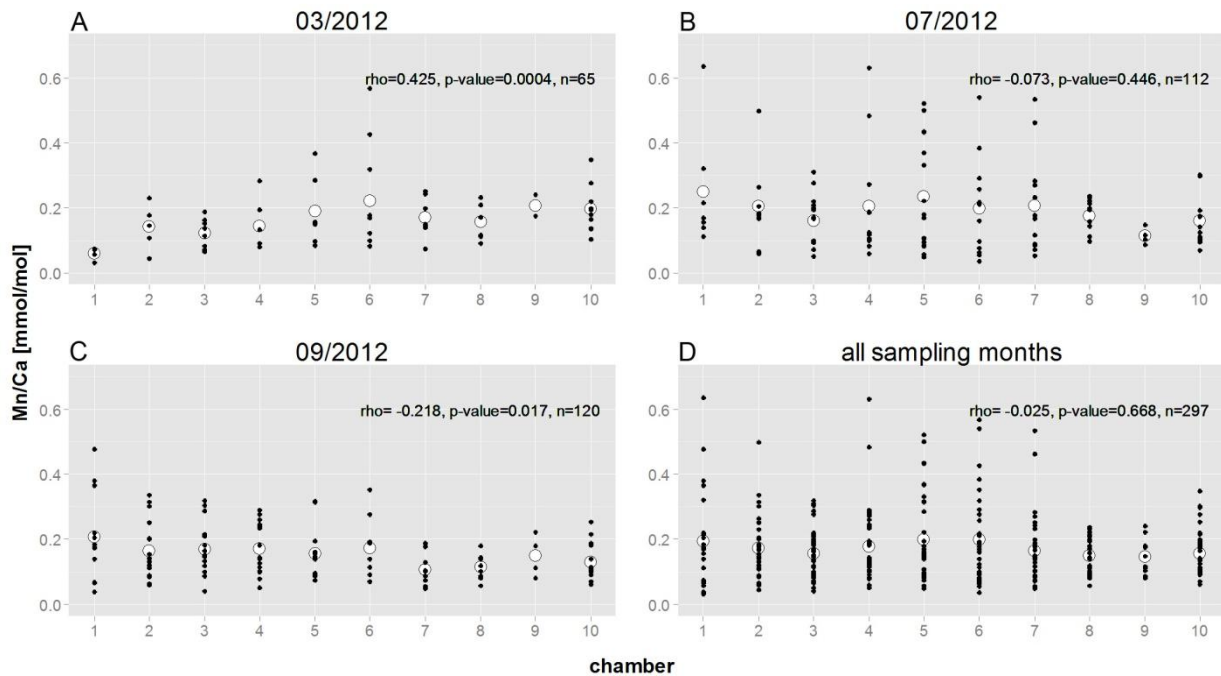


Figure 5. Mn/Ca of all measured specimens as a function of chamber succession. A: March, B: July, C: September, D: all combined. Numbers on x-axis indicate chambers: 1 = penultimate chamber 2 = antepenultimate chamber, etc., 10= central part of test. Small black dots represent Mn/Ca of single measurements; larger white circles represent average values per chamber, when all specimens are combined. Trends were tested on statistical significance with Spearman rank correlation (correlation coefficient rho, p-value and n (total number of data points) are indicated).

5

Table A.1. Results of Mn/Ca measurements for different samplings of living specimens of *A. tepida* from Lake Grevelingen, station ST2. All data are normalised to SRM NIST 612. Values are calculated per specimen (ID corresponds to numbers attributed to specimens in Fig. 2). The number of chambers included is indicated in column “n”. Calculations were performed using all decimals.

sampling date	ID	n	Mn/Ca			Mn/Ca		
			average [mmol/mol]	SD [mmol/mol]	RSD [%]	max [mmol/mol]	min [mmol/mol]	range [mmol/mol]
03/2012	1	8	0.08	0.02	27.4	0.10	0.03	0.07
03/2012	2	9	0.16	0.06	34.4	0.24	0.04	0.20
03/2012	3	7	0.28	0.15	56.3	0.57	0.11	0.45
03/2012	4	10	0.16	0.04	28.4	0.23	0.07	0.16
03/2012	5	5	0.26	0.09	32.9	0.35	0.15	0.20
03/2012	6	5	0.25	0.13	50.3	0.43	0.07	0.35
03/2012	7	4	0.14	0.04	28.3	0.17	0.08	0.09
03/2012	8	6	0.14	0.04	26.2	0.18	0.10	0.08
03/2012	9	5	0.12	0.05	44.3	0.20	0.06	0.14
03/2012	10	6	0.10	0.02	23.6	0.14	0.07	0.06
07/2012	11	8	0.13	0.03	27.1	0.17	0.08	0.08
07/2012	12	8	0.15	0.08	56.1	0.30	0.06	0.23
07/2012	13	8	0.17	0.09	56.1	0.32	0.06	0.26
07/2012	14	5	0.16	0.07	44.2	0.23	0.07	0.16
07/2012	15	7	0.19	0.16	85.0	0.53	0.06	0.48
07/2012	16	6	0.16	0.07	45.1	0.27	0.07	0.20
07/2012	17	9	0.20	0.14	72.7	0.54	0.08	0.46
07/2012	18	9	0.23	0.21	90.8	0.63	0.05	0.58
07/2012	19	7	0.28	0.14	49.0	0.50	0.19	0.31
07/2012	20	5	0.25	0.11	44.3	0.44	0.16	0.28
07/2012	21	8	0.21	0.08	39.2	0.33	0.10	0.24
07/2012	22	8	0.24	0.14	58.8	0.48	0.10	0.39
07/2012	23	4	0.39	0.19	49.1	0.63	0.17	0.46
07/2012	24	8	0.08	0.04	51.6	0.17	0.05	0.12
07/2012	25	5	0.09	0.03	33.9	0.11	0.04	0.07
07/2012	26	7	0.25	0.14	54.7	0.52	0.10	0.42
09/2012	27	5	0.10	0.02	19.3	0.12	0.07	0.05
09/2012	28	7	0.21	0.13	59.9	0.48	0.10	0.38
09/2012	29	9	0.11	0.06	51.2	0.23	0.05	0.18
09/2012	30	8	0.22	0.13	59.5	0.38	0.05	0.33
09/2012	31	6	0.12	0.04	37.9	0.18	0.06	0.12
09/2012	32	5	0.13	0.06	45.7	0.22	0.07	0.14
09/2012	33	4	0.16	0.05	33.3	0.24	0.11	0.13
09/2012	34	9	0.20	0.10	48.9	0.37	0.10	0.26
09/2012	35	8	0.09	0.04	43.4	0.14	0.04	0.10
09/2012	36	7	0.19	0.08	40.2	0.32	0.09	0.22
09/2012	37	5	0.17	0.08	46.3	0.24	0.07	0.18
09/2012	38	6	0.15	0.06	38.8	0.22	0.07	0.15
09/2012	39	6	0.21	0.09	43.7	0.30	0.10	0.20
09/2012	40	7	0.15	0.03	16.7	0.20	0.13	0.08
09/2012	41	7	0.16	0.10	60.3	0.32	0.04	0.28
09/2012	42	4	0.09	0.03	38.4	0.13	0.06	0.07
09/2012	43	8	0.17	0.09	53.4	0.30	0.07	0.24
09/2012	44	9	0.14	0.07	51.9	0.31	0.08	0.24
average		6.8	0.17	0.08	45 (±15)	0.31	0.08	0.22

Table A.2. Results of Mg/Ca measurements for different samplings of living specimens of *A. tepida* from Lake Grevelingen, station ST2. All data are normalised to USGS MACS-3. Values are calculated per specimen (ID corresponds to numbers attributed to specimens in Fig. 2). The number of chambers included is indicated in column “n”. Calculations were performed using all decimals.

sampling date	ID	n	Mg/Ca			Mg/Ca		
			average [mmol/mol]	SD [mmol/mol]	RSD [%]	max [mmol/mol]	min [mmol/mol]	range [mmol/mol]
03/2012	1	8	1.62	0.20	12.2	2.06	1.43	0.63
03/2012	2	9	2.51	0.74	29.5	3.30	0.87	2.43
03/2012	3	7	4.16	2.48	59.6	8.21	1.67	6.54
03/2012	4	10	2.39	0.90	37.7	3.96	1.35	2.62
03/2012	5	5	3.78	0.89	23.5	4.52	2.55	1.97
03/2012	6	5	2.77	1.62	58.6	5.14	1.40	3.74
03/2012	7	4	2.47	1.42	57.4	4.56	1.41	3.16
03/2012	8	6	1.31	0.35	27.0	1.86	0.89	0.97
03/2012	9	5	1.83	0.66	36.1	2.52	0.78	1.73
03/2012	10	6	1.26	0.35	27.9	1.92	0.94	0.99
07/2012	11	8	1.19	0.42	35.5	1.97	0.82	1.15
07/2012	12	8	2.46	1.29	52.5	5.17	1.22	3.95
07/2012	13	8	1.99	1.55	78.3	4.73	0.73	3.99
07/2012	14	5	1.77	0.85	48.2	3.19	0.91	2.28
07/2012	15	7	3.12	2.75	88.3	7.21	0.74	6.47
07/2012	16	6	3.81	1.42	37.2	5.18	1.59	3.59
07/2012	17	9	4.92	1.29	26.2	6.44	2.55	3.89
07/2012	18	9	3.11	1.78	57.2	6.24	1.45	4.79
07/2012	19	7	5.69	2.32	40.7	9.47	2.40	7.07
07/2012	20	5	6.81	5.05	74.2	14.93	1.24	13.69
07/2012	21	8	3.34	2.24	67.0	7.36	1.12	6.23
07/2012	22	8	4.03	2.20	54.6	7.57	1.63	5.94
07/2012	23	4	7.71	3.58	46.4	10.42	2.71	7.71
07/2012	24	8	1.56	0.42	26.8	2.21	1.11	1.10
07/2012	25	5	1.71	1.13	65.9	2.95	0.73	2.22
07/2012	26	7	5.34	2.94	55.1	10.17	1.50	8.67
09/2012	27	5	2.24	1.74	77.6	5.32	1.09	4.23
09/2012	28	7	4.28	5.33	124.4	16.05	0.89	15.16
09/2012	29	9	1.84	0.60	32.7	2.51	0.93	1.58
09/2012	30	8	1.26	0.33	25.7	2.00	0.97	1.03
09/2012	31	6	1.64	0.31	19.1	1.99	1.11	0.88
09/2012	32	5	1.80	0.65	36.2	2.90	1.17	1.72
09/2012	33	4	2.29	0.14	6.3	2.45	2.14	0.30
09/2012	34	9	1.64	0.62	37.7	3.05	1.04	2.01
09/2012	35	8	1.73	0.69	40.0	3.10	1.12	1.98
09/2012	36	7	1.58	1.14	72.1	4.13	0.98	3.15
09/2012	37	5	1.29	0.59	45.8	2.08	0.72	1.36
09/2012	38	6	1.68	0.65	38.9	2.49	0.68	1.80
09/2012	39	6	3.46	1.53	44.2	5.58	1.40	4.18
09/2012	40	7	2.18	1.33	61.1	5.07	1.17	3.90
09/2012	41	7	4.32	4.71	109.0	13.88	0.91	12.98
09/2012	42	4	1.17	0.23	19.5	1.44	0.96	0.47
09/2012	43	8	2.69	1.86	68.8	7.08	1.33	5.75
09/2012	44	9	2.45	1.25	51.0	4.58	1.16	3.41
average		6.8	2.78	1.47	49 (±24)	5.20	1.26	3.94

Table A.3. Results of Sr/Ca measurements for different samplings of living specimens of *A. tepida* from Lake Grevelingen, station ST2. All data are normalised to SRM NIST 612. Values are calculated per specimen (ID corresponds to numbers attributed to specimens in Fig. 2). The number of chambers included is indicated in column “n”. Calculations were performed using all decimals.

sampling date	ID	n	Sr/Ca			Sr/Ca		
			average [mmol/mol]	SD [mmol/mol]	RSD [%]	max [mmol/mol]	min [mmol/mol]	range [mmol/mol]
03/2012	1	8	1.36	0.17	12.6	1.59	1.00	0.59
03/2012	2	9	1.45	0.07	4.6	1.51	1.35	0.16
03/2012	3	7	1.43	0.08	5.5	1.55	1.33	0.22
03/2012	4	10	1.42	0.15	10.6	1.61	1.12	0.50
03/2012	5	5	1.43	0.08	5.9	1.55	1.32	0.23
03/2012	6	5	1.28	0.30	23.4	1.73	0.93	0.81
03/2012	7	4	1.37	0.14	10.1	1.46	1.16	0.30
03/2012	8	6	1.31	0.11	8.4	1.42	1.18	0.24
03/2012	9	5	1.43	0.10	6.9	1.51	1.26	0.25
03/2012	10	6	1.23	0.07	5.9	1.37	1.15	0.22
07/2012	11	8	1.19	0.04	3.7	1.25	1.12	0.12
07/2012	12	8	1.42	0.14	10.2	1.64	1.20	0.44
07/2012	13	8	1.42	0.17	12.3	1.64	1.17	0.46
07/2012	14	5	1.52	0.08	5.4	1.62	1.40	0.22
07/2012	15	7	1.44	0.09	6.4	1.59	1.34	0.26
07/2012	16	6	1.48	0.14	9.8	1.66	1.31	0.36
07/2012	17	9	1.52	0.10	6.5	1.64	1.38	0.27
07/2012	18	9	1.29	0.09	6.7	1.41	1.18	0.23
07/2012	19	7	1.31	0.14	10.5	1.51	1.16	0.35
07/2012	20	5	1.29	0.13	9.8	1.44	1.09	0.34
07/2012	21	8	1.38	0.13	9.1	1.63	1.23	0.40
07/2012	22	8	1.44	0.12	8.6	1.58	1.26	0.32
07/2012	23	4	1.31	0.23	17.4	1.65	1.18	0.47
07/2012	24	8	1.43	0.15	10.4	1.74	1.29	0.45
07/2012	25	5	1.20	0.17	13.8	1.43	1.01	0.42
07/2012	26	7	1.32	0.22	16.4	1.73	1.07	0.66
09/2012	27	5	1.21	0.15	12.2	1.44	1.06	0.37
09/2012	28	7	1.38	0.16	11.8	1.63	1.09	0.55
09/2012	29	9	1.31	0.10	7.4	1.52	1.19	0.33
09/2012	30	8	1.23	0.08	6.8	1.38	1.10	0.28
09/2012	31	6	1.45	0.06	4.0	1.54	1.39	0.16
09/2012	32	5	1.47	0.13	8.8	1.70	1.40	0.30
09/2012	33	4	1.25	0.12	9.5	1.36	1.09	0.27
09/2012	34	9	1.42	0.10	7.0	1.60	1.29	0.31
09/2012	35	8	1.39	0.08	5.4	1.54	1.27	0.27
09/2012	36	7	1.39	0.15	11.2	1.64	1.14	0.50
09/2012	37	5	1.27	0.12	9.1	1.38	1.10	0.29
09/2012	38	6	1.33	0.12	9.3	1.46	1.10	0.35
09/2012	39	6	1.37	0.06	4.6	1.42	1.26	0.17
09/2012	40	7	1.40	0.09	6.6	1.52	1.25	0.27
09/2012	41	7	1.59	0.29	18.3	2.09	1.30	0.79
09/2012	42	4	1.42	0.07	4.9	1.50	1.34	0.16
09/2012	43	8	1.21	0.13	10.5	1.38	1.06	0.33
09/2012	44	9	1.30	0.16	12.2	1.56	1.10	0.46
average		6.8	1.36	0.13	9 (±4)	1.55	1.20	0.35

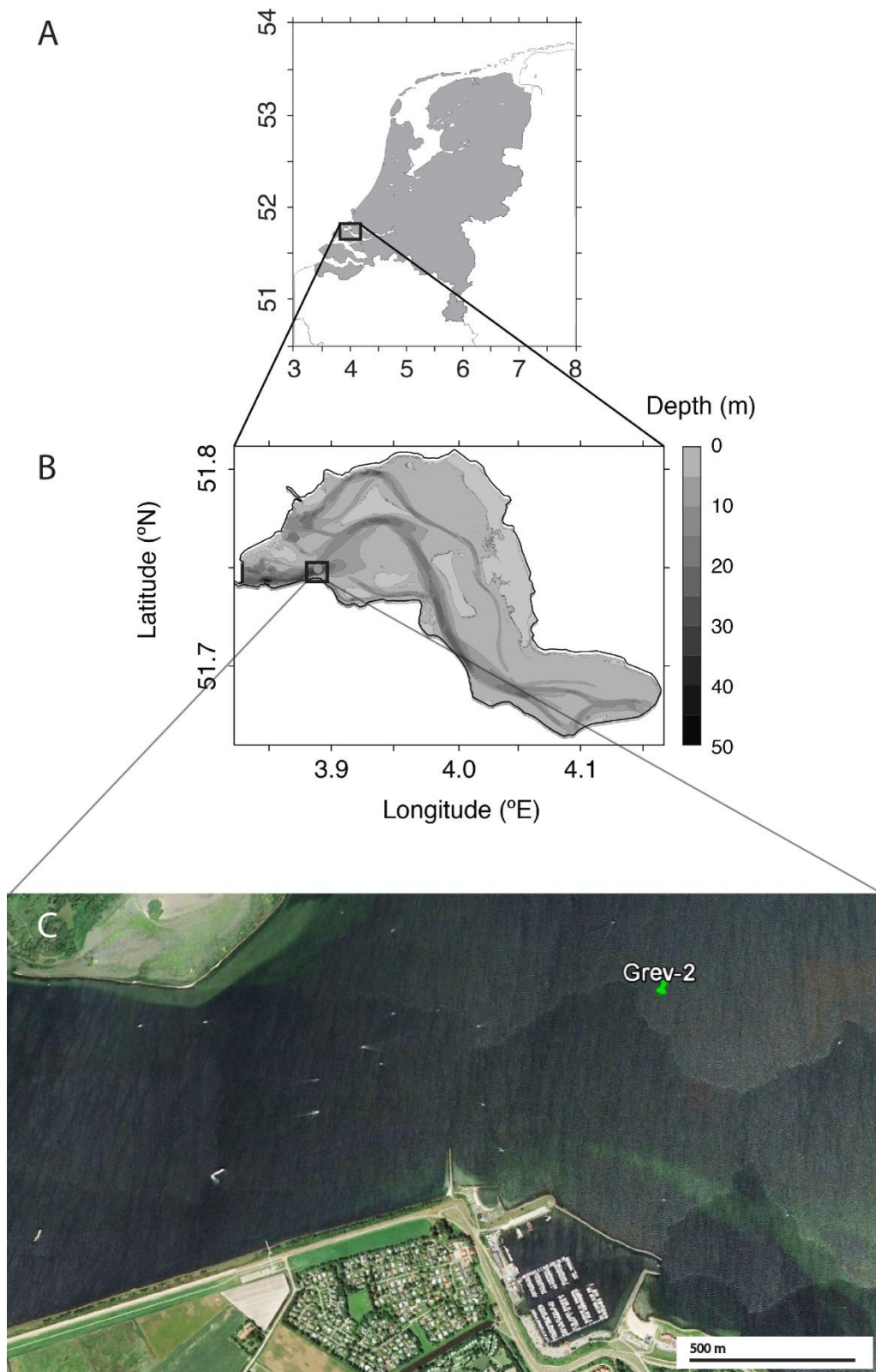


Figure S1. Location map of Lake Grevelingen and sampling station for the material used in this study. **A:** map of the Netherlands. **B:** bathymetry of Lake Grevelingen, the sluice giving access to the North Sea is situated at the most western end of the lake. **A** and **B** modified from Hagens et al. (2015). **C:** satellite image of sampling station Grev-2 (image from Google Earth, date of image 8/7/2013).

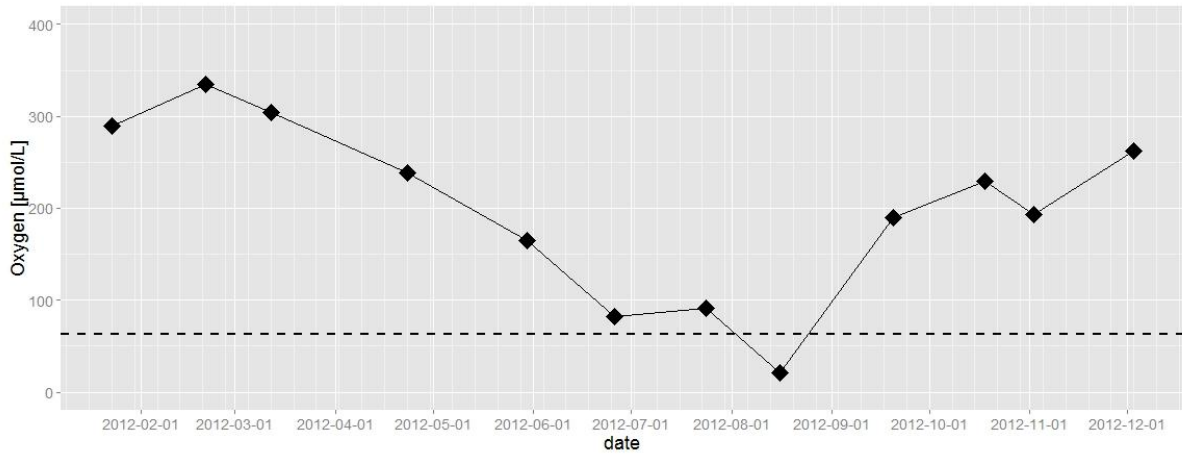


Figure S2. Oxygen concentration in 2012 for sampled station in Lake Grevelingen (depth=23.1 m, for location see Fig. S1). Dashed line indicates threshold for hypoxic conditions (63 µmol/L). Data from Hagens et al. (2015) and Seitaj et al. (2017).

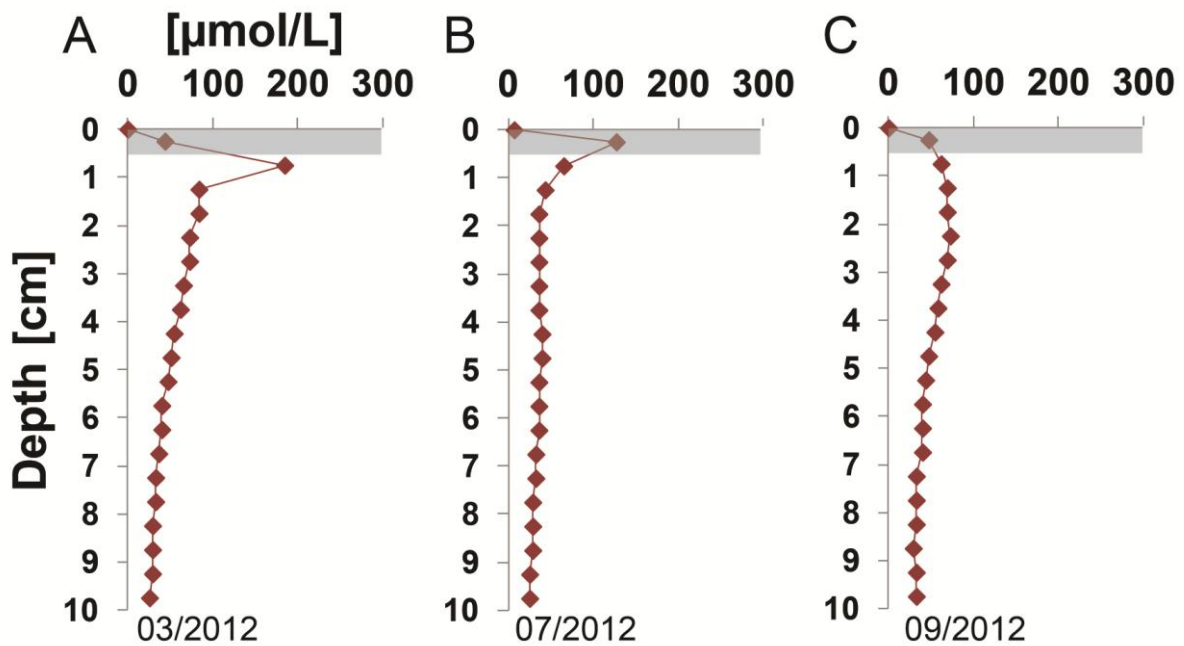


Figure S3. Mn²⁺ pore water concentrations for March (A), July (B) and September (C) 2012 at sampled station in Lake Grevelingen. Grey area indicates sampling interval for specimens of *Ammonia tepida* (0-0.5 cm). Data from Sulu-Gambari et al. (2016a, 2016b).