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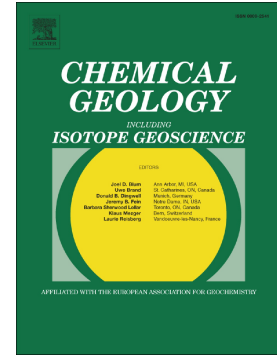
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Mn/Ca ratios of *Ammonia tepida* as a proxy for seasonal coastal hypoxia

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Abstract

Climate variability has major implications for marine geochemical cycles and biogenic carbonate production. Therefore, past climate-driven changes in marine environments are often inferred from geochemical data of the marine carbonate archive. Proxy calibration studies are essential for the reconstruction of such past environmental changes. Here, we use the geochemical composition of living specimens of the benthic foraminifer *Ammonia tepida* at three sites in a seasonally hypoxic (oxygen concentration < 63 $\mu\text{mol/L}$) marine coastal system (Lake Grevelingen, the Netherlands) to explore the use of Mn/Ca as a proxy for coastal hypoxia. The study is based on samples from three stations along a depth transect, that have contrasting seasonal cycles of Mn^{2+} concentrations in the pore water of the surface sediment. Specifically, the sediment and pore water geochemistry of the three stations in Lake Grevelingen show increased Mn^{2+} concentrations in late winter/spring, combined with increased Mn refluxing in summer, which are due to cable bacteria activity and bottom water hypoxia/anoxia, respectively.

Laser Ablation-ICP-MS (LA-ICP-MS) allowed a comparison of Mn/Ca ratios of different parts of the benthic foraminiferal test. Our results show that higher Mn/Ca ratios are registered at the deepest station, which experiences the longest and most severe seasonal periods of hypoxia/anoxia. Additionally, the signal preserved in the central part of the benthic foraminiferal tests, which is thought to reflect the entire calcification history of the analysed specimen, appears to be driven by high pore water Mn^{2+} concentrations due to cable bacteria

activity in late winter/spring. Conversely, high Mn/Ca ratios in the last chambers reflect increased Mn refluxing in the surface sediment due to summer hypoxia/anoxia. Thus, Mn/Ca ratios of *A. tepida* give insight into the complex spatial and temporal variability of pore water manganese.

Keywords: coastal hypoxia; benthic foraminifera; geochemical proxy development; redox-sensitive elements; biomineralization; micro-analytical techniques

1. Introduction

In coastal areas, the development of seasonally hypoxic zones (water bodies with O₂ concentration < 63 µmol/L or 2 mg/L) has become a wide-spread phenomenon in response to increasing riverine nutrient input and rising temperatures (e.g., Rabalais et al., 2002; Gilbert et al., 2010; reviews in Diaz and Rosenberg, 2008; Rabalais et al., 2010, 2014; Breitburg et al., 2018; Fennel and Testa, 2018). In Europe, hypoxic periods have been observed in the Baltic Sea and in the northern Adriatic Sea (e.g., Jorissen et al., 1992; Conley, 2002; Conley et al., 2007; Meier et al., 2011). Hypoxia negatively impact marine benthic fauna and alter sediment biogeochemical cycles, strongly affecting the entire ecosystem functioning (e.g., Middelburg and Levin, 2009). Direct measurements of oxygen concentrations are relatively scarce, and are generally restricted in time and space, but some tools make it possible to follow the oxygenation history of coastal ecosystems in the recent sediment record (e.g., Cooper and Brush, 1991; Barmawidjaja et al., 1995; Itoh et al., 2003; Zillén et al., 2008). Such historical studies can inform us in more detail about the onset of seasonal hypoxia in pre-anthropogenic times, and its sensitivity to climate change. Yet, it still remains a challenge for the scientific community to develop reliable proxies for seasonal hypoxia allowing the reconstruction of the historical record.

Several geochemical proxies have been proposed, based on redox-dependent elements, such as manganese, uranium, vanadium and molybdenum (Breit and Wanty, 1991; Adelson et al., 2001; Tribovillard et al., 2004; McManus et al., 2005; Algeo and Lyons, 2006; Scholz et al., 2017; van Helmond et al., 2018; review in Tribovillard et al., 2006). In environments where concentrations of bottom water oxygen (BWO) and the input of Mn bearing suspended matter are sufficient, soluble manganese (Mn²⁺) is released through reductive dissolution of Mn (oxyhydr)oxides when they are buried in (or mixed into) deeper, anoxic sediment. The Mn²⁺ can then diffuse upwards and precipitate as Mn (oxyhydr)oxides in the oxygenated surface sediment, leading to recycling of Mn in the sediment. Mn²⁺ can also diffuse downwards and precipitate as Mn carbonate (e.g., Middelburg et al., 1987; Slomp et al., 1997). Because under hypoxic conditions, Mn cycling will take place closer to the sediment

surface compared to oxygenated conditions, manganese incorporated in benthic foraminiferal calcite could be a suitable proxy for marine benthic hypoxia (Reichert et al., 2003; Munsel et al., 2010; Glock et al., 2012; Groeneveld and Filipsson, 2013; Koho et al., 2015, 2017; McKay et al., 2015; Petersen et al., 2018; Barras et al., 2018; Guo et al., 2019). The latter studies show that benthic foraminifera register environmental Mn^{2+} concentrations in their tests. A complicating factor is that in case of anoxia, Mn^{2+} oxidation takes place entirely in the bottom water (e.g., Sulu-Gambari et al., 2017), and the Mn^{2+} concentration in the surface sediments is no longer sufficient for detectable incorporation into the benthic foraminiferal test. Barras et al. (2018) have demonstrated that under hypoxic conditions, seawater Mn^{2+} linearly scales with the Mn/Ca ratio of tests of cultured *Ammonia tepida*, a coastal benthic foraminifer. Petersen et al. (2018) have additionally shown that the variability of successive single chamber Mn/Ca ratios of the same species is not only related to intrinsic variability (due to biological processes during calcification), but also reflects temporal and spatial (environmental) variability of pore water Mn^{2+} . Furthermore, Petersen et al. (2018) evoked active or passive migration of the foraminifera in the surface sediment to explain part of the observed Mn/Ca variability within single specimens. The latter observations were made in Lake Grevelingen, a coastal ecosystem in the Netherlands with seasonally hypoxic to anoxic BWO concentration, which is also the sampling area of the present study (Fig. 1).

Lake Grevelingen is a former branch of the Rhine-Meuse-Scheldt delta. This artificial saline lake was formed after the construction of dams in 1964 (connection to the river inlets were cut off) and 1971 (connection to the North Sea was cut off), and has only a minor seawater inflow through two small sluices (Bannink et al., 1984). The salty bottom waters (salinity 31-32.5, Hagens et al., 2015) of the lake are subject to seasonal hypoxia and anoxia, which strongly affects the benthic ecosystem (Seitaj et al., 2015; Sulu-Gambari et al., 2016a). In 2012, the water column was sampled monthly at three stations in the Den Osse basin in the main channel of Lake Grevelingen (water depth: 34 m, 23 m and 17 m for stations ST1, ST2 and ST3, respectively, Fig. 1). The deepest station is most affected by seasonal hypoxia/anoxia; it shows a longer duration of hypoxia and is characterized by lower BWO concentrations in summer and early autumn than the shallower stations. It has been shown that the seasonal pattern of sediment geochemical cycling is substantially influenced by the presence of cable bacteria, which increase in activity from the shallowest to the deepest station (Seitaj et al., 2015). These bacteria, which perform long-distance electron transport, are mainly active in winter and early spring (Seitaj et al., 2015, 2017). Due to the specific metabolic activity of cable bacteria, a strong enrichment of Mn^{2+} and Mn (oxyhydr)oxides occurs in the surface sediment in late winter and spring (Sulu-Gambari et al., 2016b). The electrogenic sulfur oxidation by the cable bacteria induces a chain of biogeochemical

reactions involving dissolution of iron mono-sulfides (FeS), reduction of Mn (oxyhydr)oxides by upward diffusing Fe^{2+} , leading to accumulation of Mn^{2+} in the pore water. Mn oxides are built up in the oxic layer directly below the sediment water-interface (SWI) by the upward diffusing Mn^{2+} (Seitaj et al., 2015; Sulu-Gambari et al., 2016a,b). Additional Mn oxides are supplied through deposition of Mn-bearing suspended matter (Sulu-Gambari et al., 2017).

Live benthic foraminifera (recognised by CellTracker Green, CTG, Bernhard et al., 2006) were sampled at three stations, and show assemblages strongly dominated by *Ammonia tepida* and *Elphidium excavatum*. The living specimens of *Ammonia tepida* in Lake Grevelingen are strongly dominated by phylotype T6, which is one of the three phylotypes occurring along the European Atlantic coast (Richirt et al., 2019). Nevertheless, since it remains very difficult to separate these phylotypes morphologically, we use the name *Ammonia tepida*, knowing that it designates a species complex rather than a single biological species.

Here, we present Mn/Ca ratios measured by laser ablation ICP-MS (LA-ICP-MS) on specimens of *Ammonia tepida* sampled at the three different stations and relate these to the geochemistry of the environment. The benefit of Lake Grevelingen is that a large dataset on water column geochemistry combined with pore water and sediment geochemistry is available for the same sites where the specimens of *A. tepida* were sampled (Hagens et al., 2015; Seitaj et al., 2015, 2017; Sulu-Gambari et al., 2016a,b, 2017a). Therefore, it is possible to compare the measured Mn/Ca ratios in the foraminiferal tests with corresponding concentrations of Mn^{2+} in the pore water. The analyses focused on the penultimate and antepenultimate chambers (n-1 and n-2), supposed to record pore water Mn^{2+} concentration prior to sampling, and the central part of the test (n-c), supposed to give an averaged signal of the life span of the specimen. We will investigate whether the incorporated manganese in the foraminiferal test has a potential to reconstruct spatial and temporal variability in pore water Mn^{2+} . The ultimate aim of this study is to develop a proxy for the extent and intensity of seasonal hypoxia in coastal ecosystems.

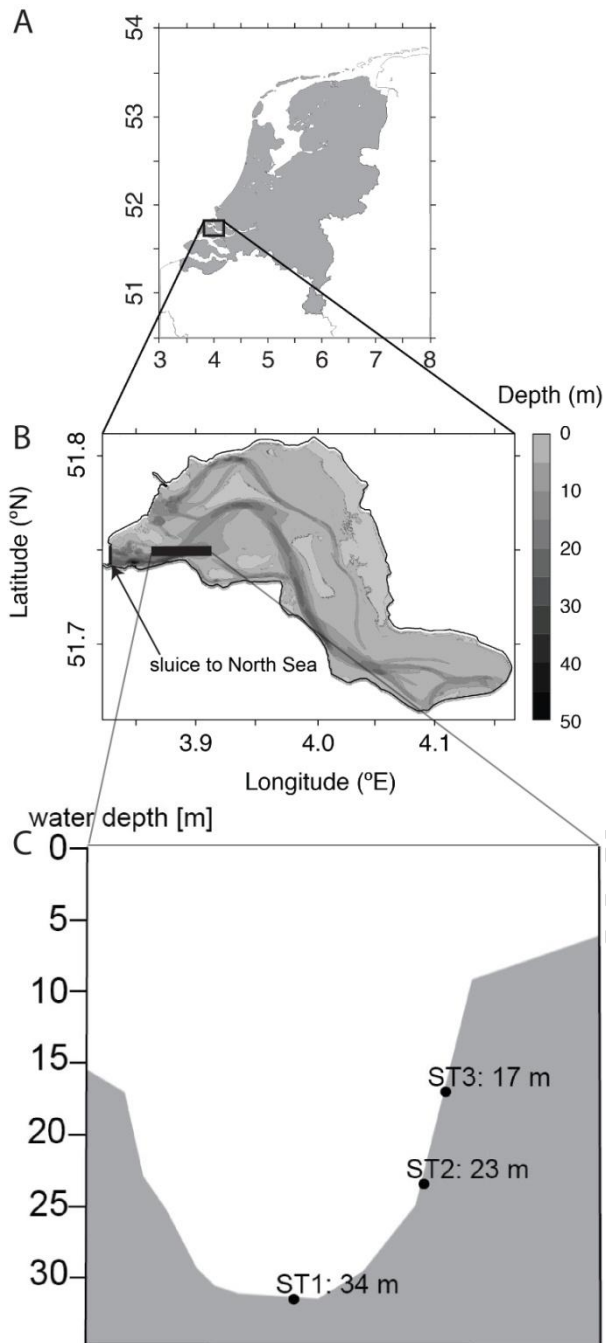


Figure 1. Location map of Lake Grevelingen and the three sampling stations in this study. A: map of the Netherlands. B: bathymetry of Lake Grevelingen, the sluce giving access to the North Sea is situated at the most western end of the lake. C: depth cross-section of the Den Osse basin (W-E) with locations of stations ST1, ST2 and ST3. Modified from Hagens et al. (2015).

2. Material and methods

2.1. Mn/Ca ratios of *Ammonia tepida* from Lake Grevelingen

The three sampling stations are located along a depth transect in the Den Osse basin, the former estuarine channel towards the North Sea (Fig. 1A,B). Water depth decreases gradually from station ST1, in the deepest part of the basin (34 m), to 23 m at ST2 and 17 m at ST3 (Fig. 1C). At ST1, ST2 and ST3, short sediment cores (~40 cm) with ~20 cm of overlying bottom water were collected with a gravity corer (UWITEC, Austria) and Plexiglas® core-liners (6 cm inner diameter; 60 cm length, Sulu-Gambari et al., 2017). For pore water measurements, one core was sliced at high resolution (0.5 cm slices over 10 cm) in a N₂-purged glovebag (Sulu-Gambari et al., 2016a). Cores for the study of live benthic foraminifera were taken bimonthly, on the same dates that cores for sediment geochemistry were sampled, starting in January 2012. Foraminiferal communities were studied in two depth intervals (0-0.5 cm and 0.5-1 cm, Richirt et al., in prep.). Live foraminifera were discriminated from dead specimens using the CTG staining method (Bernhard et al. 2006). In all the samples where living foraminifera were found, the morphospecies *Ammonia tepida* and *Elphidium excavatum* were strongly dominant. Here we measured the elemental composition of calcite tests of *A. tepida* (size fraction 150-315 µm, 0-0.5 cm interval) sampled in July 2012 at ST1, ST2 and ST3. The test size was measured on SEM images as the maximum diameter using the image processing software ImageJ (Schneider et al., 2012). The cleaning of all specimens, in order to remove sediment adherences (Barker et al., 2003), included rinsing three times with ultra-pure water, followed by one rinse in methanol and three final rinses in ultra-pure water. During each rinse, samples were slightly agitated with a vortex machine (Petersen et al., 2018). Guo et al. (2019) showed that this type of physical cleaning in combination with LA-ICP-MS yields Mn/Ca ratios of live benthic foraminifera comparable to solution analysis (by ICP-MS) of specimens which have undergone a protocol involving oxidative and reductive cleaning. In fact, the integration of contamination from the surface of the test into the calcite Mn/Ca signal is avoided by the data treatment of LA-ICP-MS analysis involving the screening of indicator elements such as Al (e.g., Koho et al., 2015; Barras et al., 2018; Petersen et al., 2018; Guo et al., 2019).

We measured the elemental composition of single chambers of the calcite tests using LA-ICP-MS, i.e., an ArF excimer laser (193 nm, Analyte G2, Teledyne Photon Machines) coupled to a quadrupole ICP-MS (Varian Bruker 820-MS). Ablations were performed in a HelEx II 2-Volume Cell with He as carrier gas, a laser energy density of 0.91 J/cm² and a 4 Hz repetition rate. Spot sizes were adapted to the chamber size in order to maximize the amount of ablated material, and they varied typically between 40 and 85 µm in diameter. Variable spot sizes have been used in other studies of foraminiferal tests and are not thought

to influence elemental fractionation (Eggins et al., 1998; Diz et al., 2012; de Nooijer et al., 2014; Petersen et al., 2018). Analyses were performed preferably in the penultimate (n-1) and antepenultimate (n-2) chambers as well as in the central part of the test (n-c, see Fig. 2 for location of ablation spots on the test). In some specimens with broken last chambers, it could not be established precisely which chamber was measured (n-u). The data were calibrated against NIST SRM 612 which was ablated in raster mode with the same repetition rate and energy density as the foraminiferal samples and a 65 μm spot size. The calibration using NIST SRM 612 was shown by Petersen et al. (2018) to yield accurate results and fractionation effects were monitored using different reference materials. External reproducibility for Mn/Ca was determined using carbonate reference materials USGS MACS-3 and NFHS (NIOZ, Netherlands Institute of Sea Research, foraminifera in-house standard, Mezger et al., 2016) and was 1.3 % and 4.5 %, respectively (calculated as $2 \times \text{RSE}$, relative standard error). Details of the LA-ICP-MS measurements, as well as of data treatment and quality control are reported in Petersen et al. (2018). Similar to the latter study, integrated intervals displaying < 10 data points (24 ablations) or a Mn signal $<$ Limit Of Quantification (LOQ, 6 ablations) were discarded from the data presented here. The final dataset includes 71 individual Mn/Ca measurements.

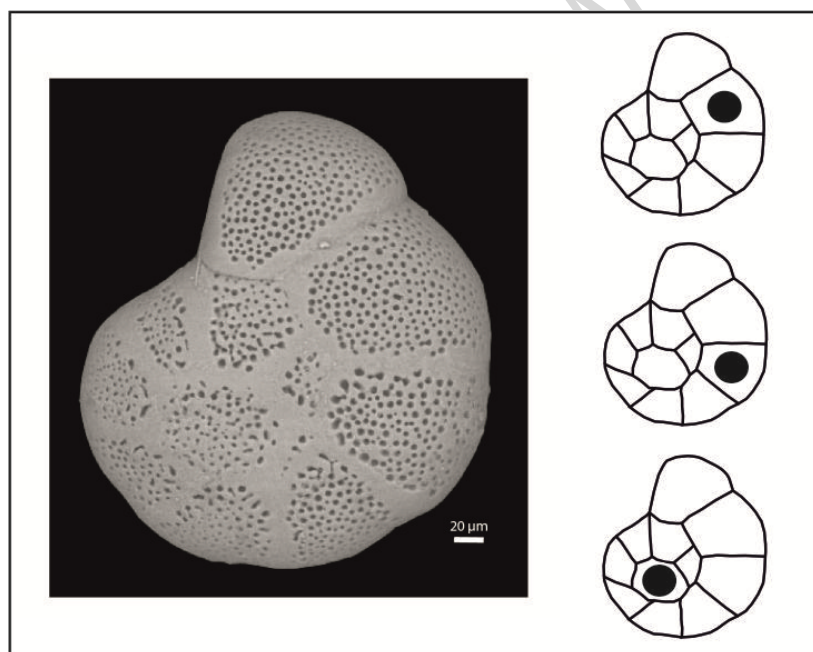


Fig 2. Left: SEM image of *A. tepida* specimen (presumably phylotype T6 based on morphological distinction as shown by Richirt et al., 2019) from Lake Grevelingen (image taken at SCIAM, University of Angers). Right: Sketches to show location of LA-ICP-MS spot analysis on penultimate chamber (top), antepenultimate chamber (middle) and central part of test (bottom).

2.2. Statistical analyses

We performed statistical analyses under R (R Core Team, 2016) and we used the package ggplot2 for graphical representation (Wickham, 2009). We used the Shapiro-Wilk test to verify if data are normally distributed. For normally distributed data, we performed ANOVA and t-tests with the Bonferroni adjustment as post-hoc test to compare datasets. For data likely not to follow a normal distribution we performed a Kruskal-Wallis test and Wilcoxon-Mann-Whitney as post-hoc test. In all cases, a p value below 0.05 was considered as significant.

3. Results: Mn/Ca ratios of *Ammonia tepida*

In order to compare the Mn/Ca ratios of specimens from the three stations, we chose samples from July 2012, for two reasons. Firstly, because at ST1, a rich population of *Ammonia tepida* was only found in May and July 2012, and secondly, because inter-station differences in BWO concentration were maximal in this month. Thirteen specimens were analysed for ST3 and ST1, respectively, and fourteen specimens for ST2. The maximum diameter of each specimen shows that specimens of ST1 were significantly smaller than specimens from ST2 and ST3 (Tab. 1, p values in Tab. 2).

All Mn/Ca ratios of individual chambers and tests are presented in Figure S2. Histograms for each station for all measurements of single chambers and of the central part of the test (Fig. 3) indicate an increased number of high Mn/Ca ratios at ST1 compared to ST2 and ST3. In fact, for this dataset which is not likely to follow a normal distribution p values for Kruskal-Wallis and Wilcoxon-Mann-Whitney as post-hoc test confirm that there is a significant difference for ST1 compared to ST2 and ST3 (not the case for ST2 and ST3, Tab. 2). Also the mean Mn/Ca per specimen is significantly higher in ST1 compared to the other two stations (0.36 ± 0.12 mmol/mol, mean \pm SD, for ST1 versus 0.18 ± 0.08 mmol/mol and 0.20 ± 0.15 mmol/mol for ST2 and ST3, respectively, Fig. 4A, p values in Tab. 3). There is no significant difference between the three stations considering the Mn/Ca ratio of the penultimate chamber, or of the combined n-1 and n-2 chambers (Fig. 4B/C, ANOVA p value = 0.404 and 0.138, respectively). The measurements made in the central part of the test (Fig. 4D) yield significantly higher Mn/Ca ratios for ST1 compared to the other two stations (p values in Tab. 3).

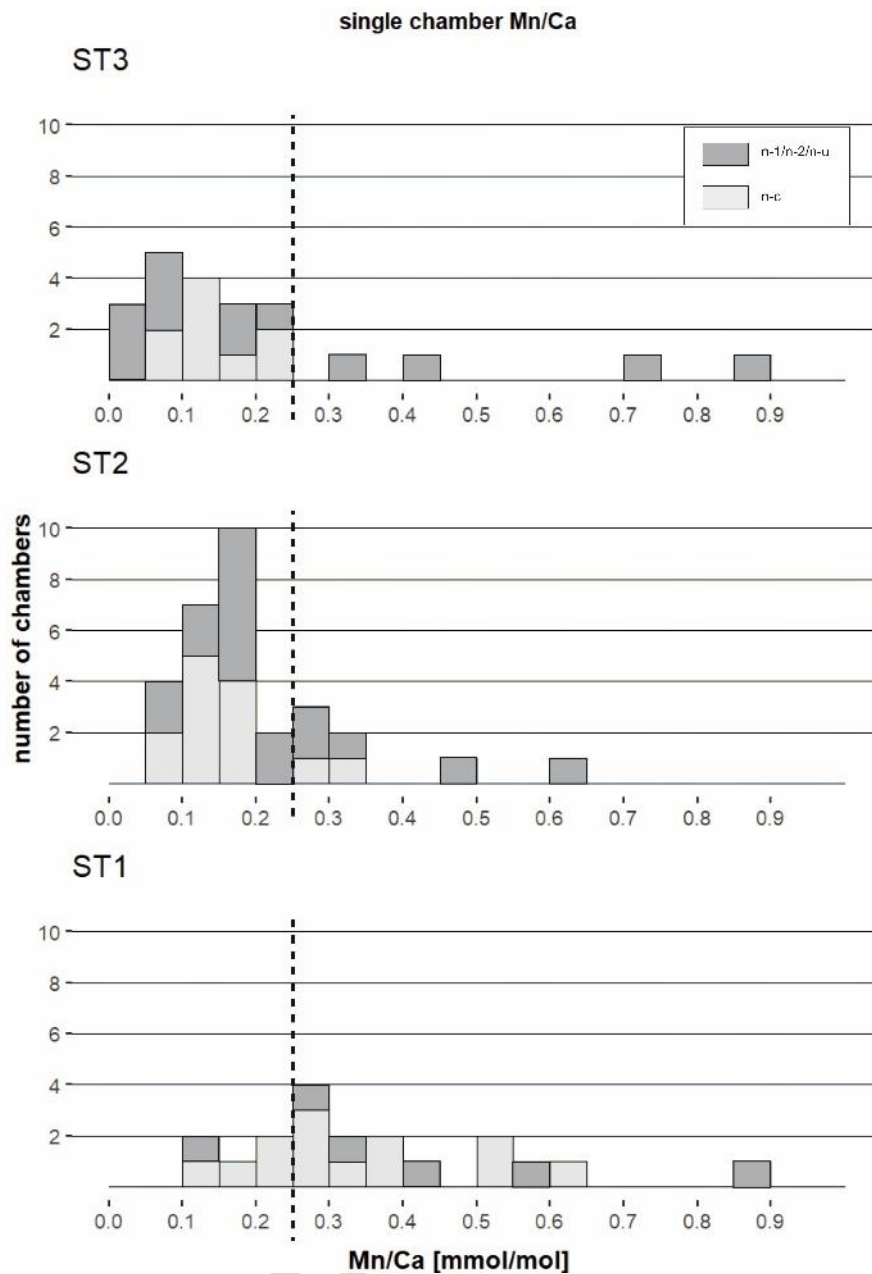


Figure 3. Histograms for all Mn/Ca measurements (individual chambers in dark grey, and central part of the test in light grey) for the three stations (bin width= 0.05 mmol/mol). Kruskal-Wallis test p value :< 0.001 (for data of n-1/n-2/n-u and n-c combined). Wilcoxon-Mann-Whitney post-hoc test p values: < 0.001 (ST1 vs ST2), < 0.001 (ST1 vs ST3) and 0.220 (ST2 vs ST3). Dashed line indicating 0.25 mmol/mol (see section 3 for explanation).

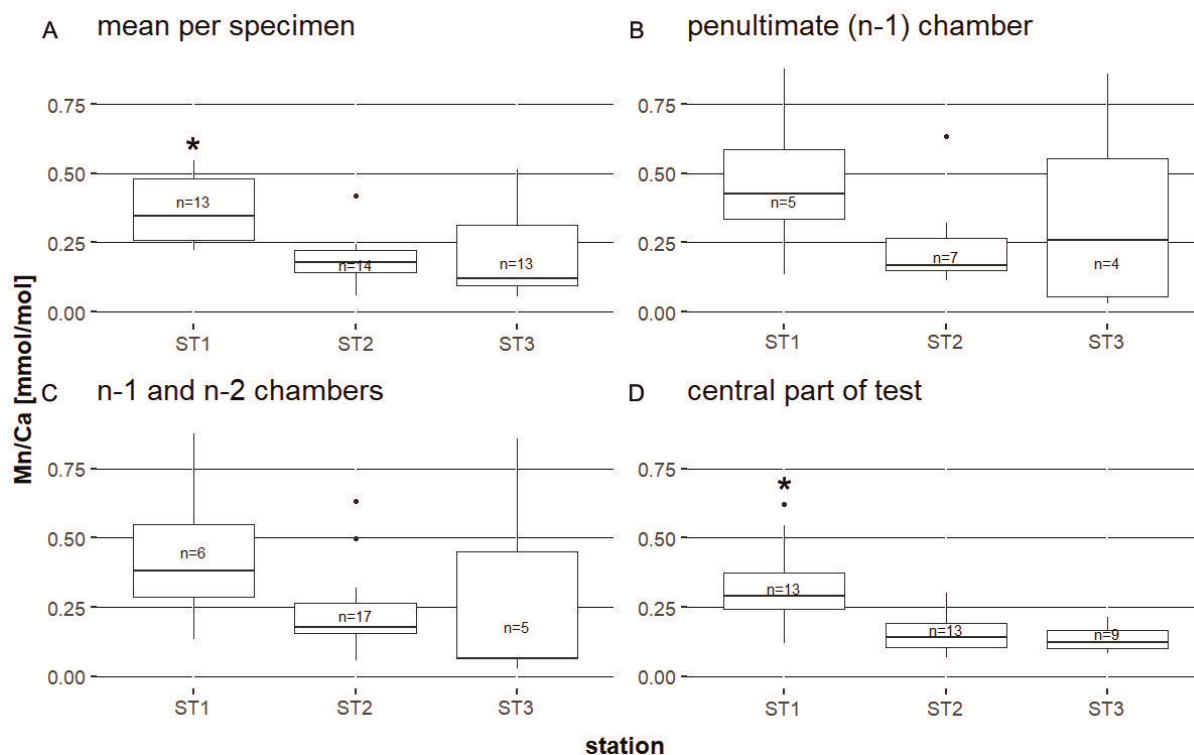


Figure 4. Boxplots of Mn/Ca ratios for all specimens of *A. tepida* from stations ST1 (34 m), ST2 (23 m) and ST3 (17 m) sampled in July 2012. A: mean per specimen (n indicates number of specimens). B: penultimate chamber (n indicates number of single chamber ablations, also in C and D). C: n-1 and n-2. D: central part of test. Data for n-u (n = 8) not represented. Significant differences for ST1 compared to ST2 and ST3 are indicated by *.

Table 1. Results (mean \pm SD) regarding test size (maximum diameter) and Mn/Ca ratios for all stations. Comparison of mean per test (including n-u), penultimate chamber (n-1), n-1 and n-2 chambers together and central part of test. In case of significant difference for value of ST1 compared to values of ST2 and ST3, value of ST1 is written in bold (p values in Tab. 2 and Tab. 3).

	Test size [μ m]	Mn/Ca mean per test [mmol/mol]	n	Mn/Ca n-1 [mmol/mol]	n	Mn/Ca n-1/n-2 [mmol/mol]	n	Mn/Ca central part [mmol/mol]	n
ST1	319 \pm58	0.36 \pm0.12	13	0.47 \pm 0.28	5	0.44 \pm 0.26	6	0.33 \pm0.15	13
ST2	376 \pm 63	0.18 \pm 0.08	14	0.25 \pm 0.18	7	0.22 \pm 0.15	17	0.16 \pm 0.07	13
ST3	407 \pm 36	0.20 \pm 0.15	13	0.35 \pm 0.39	4	0.29 \pm 0.36	5	0.14 \pm 0.05	9

Table 2. Lower left: Results for post-hoc t-test (p value, calculated with Bonferroni correction) for comparison of test size between the three stations (p value for ANOVA is 0.002). Upper right: Results for post-hoc test (p value, Wilcoxon-Mann-Whitney post-hoc test) for comparison of single chamber Mn/Ca ratios between the three stations as shown in Fig. 3 (p value for Kruskal-Wallis test is $<$ 0.001). Significant results in bold, a p value below 0.05 was considered significant.

	ST1	ST2	ST3
ST1		< 0.001	< 0.001
ST2	0.027		0.220
ST3	< 0.001	0.444	

Table 3. Results for post-hoc test (p value, Wilcoxon-Mann-Whitney post-hoc test) for comparison of Mn/Ca ratios between the three stations for mean per specimen (lower left, in black; p value for Kruskal-Wallis test is < 0.001) and central part of test (upper right, in blue; p value for Kruskal-Wallis test is < 0.001). Significant results in bold, a p value below 0.05 was considered significant.

	ST1	ST2	ST3
ST1		< 0.001	< 0.001
ST2	< 0.001		0.512
ST3	0.006	0.402	

The histograms for each station (Fig. 3) show that the majority of values of single chamber Mn/Ca ratios were between 0-0.25 mmol/mol for stations ST2 and ST3. At ST1 there were no measurements smaller than 0.1 mmol/mol and a majority between 0.1-0.4 mmol/mol (Fig. 3). High Mn/Ca ratios in benthic foraminifera are thought to reflect increased Mn^{2+} concentrations in the foraminiferal microhabitat, resulting from reductive dissolution of Mn (oxyhydr)oxides either due to hypoxic BWO conditions or intensive cable bacteria activity. As the preferred microhabitat of *A. tepida* is situated at or just below the surface of the sediment (Thibault de Chanvalon et al., 2015; Cesbron et al., 2016), it is expected that most of the calcified chambers are not subject to high pore water Mn^{2+} concentrations under oxic conditions. Consequently, only chambers calcified slightly deeper in the sediment or in the presence of increased Mn^{2+} concentrations in the surface sediment due to hypoxic conditions or cable bacteria activity would display such a high Mn/Ca. The geochemical data for the three stations (Sulu-Gambari et al., 2016a,b, 2017) show that a pore water Mn^{2+} concentration of $\sim 30 \mu\text{mol/L}$ is only reached in the bottom water in July 2012 at ST1, during strong hypoxia (Fig. S1). When using the D_{calcite} determined by Barras et al., 2018, for *A. tepida*, see supp. mat., this value should correspond to a foraminiferal Mn/Ca ratio of 0.25 mmol/mol. Consequently, we used this value as a threshold to distinguish between high (calcite formed during hypoxia or production of Mn^{2+} by cable bacteria) and low Mn/Ca (foraminiferal calcite precipitated under oxic conditions and absence of cable bacteria).

We present for each station the number of specimens with low or high Mn/Ca (following this Mn/Ca threshold of 0.25 mmol/mol) in the latest analysed chambers (n-1, n-2 and n-u), and/or in the central part of test (n-c) in Table 4 (and Figure 5). At station ST3, nine out of thirteen specimens had low Mn/Ca (≤ 0.25 mmol/mol) in all chambers whereas four out of ten specimens had high values (> 0.25 mmol/mol) in at least one of the latest calcified chambers (counting only specimens for which reliable results for one of the later chambers were obtained). No specimens with high values in the central part of test were observed. At ST2, nine out of fourteen specimens showed low Mn/Ca in all chambers. Three of eleven

specimens had high Mn/Ca in one of the latest chambers and not in n-c, whereas two out of thirteen specimens showed elevated Mn/Ca ratios in n-c and not in the latest chambers. No specimens had high Mn/Ca both in the latest chambers and in the central part of test. At ST1, only two out of thirteen specimens yielded exclusively low Mn/Ca ratios. Four out of five specimens showed high Mn/Ca in at least one of the latest chambers and nine out of thirteen specimens had high Mn/Ca in the central part of test.

Table 4. Specimens with high Mn/Ca (> 0.25 mmol/mol) in comparison to total number of specimens, counting only specimens for which reliable results for the concerned chambers or part of the test were obtained.

	No high values	High values in n-1/n-2/n-u	High values in n-c
ST1	2/13	4/5	9/13
ST2	9/14	3/11	2/13
ST3	9/13	4/10	0/9
total	20/40	11/26	11/35

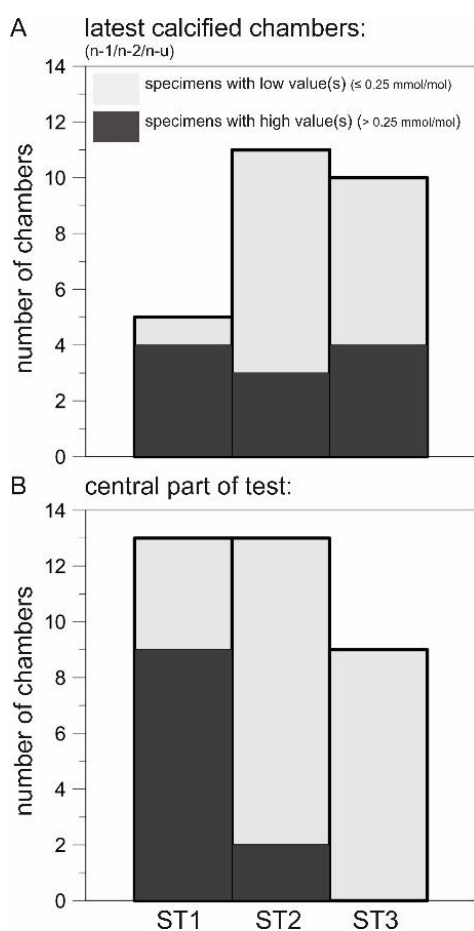


Figure 5. Specimens with high Mn/Ca ratios in comparison to total number of specimens measured for each station (data in Tab. 4). A: Latest calcified chambers (n-1, n-2, n-u). B: Central part of test.

4. Discussion

4.1. Environmental conditions in the study area

4.1.1. Oxygen variations in the bottom and pore waters

In order to present our approach to disentangle the different processes behind Mn/Ca variability of *Ammonia tepida* from Lake Grevelingen, we need first to explain the variations of the environmental parameters in this study area. Results for the 2012 monthly campaigns show that the water column was stratified from late spring (May) and during summer in response to atmospheric temperature changes and a density gradient caused by the rapid warming of surface waters (Hagens et al., 2015; Seitaj et al., 2017). The water column remained stratified until September when stormy conditions induced a mixing of surface and deeper waters (Hagens et al., 2015). The oxygen concentrations of the bottom waters changed in phase with this stratification pattern; in winter BWO concentrations were maximal, whereas bottom waters became oxygen depleted in summer (Fig. 6A). Sediment oxygen uptake and the degradation of autochthonous and allochthonous organic matter (OM) contributed to the decrease of oxygen concentration in the water column (Hagens et al., 2015; Seitaj et al., 2017), leading to a decrease in the oxygen penetration depth (OPD; the depth in the sediment below which O₂ concentration is < 1 µmol/L, Fig. 6B; Seitaj et al., 2017). At the deepest station ST1 there was a hypoxic season of 2 to 2.5 months with approximately two weeks of anoxia (Fig. 6A) during which the OPD decreased to zero, meaning that there was an oxygen-free sediment in late summer at this station. At the intermediate station ST2 hypoxia lasted for 1 to 1.5 months, and oxygen was absent in the sediment only in August. The hypoxic period started later at ST2 than at ST1. Finally, at the shallowest station ST3, oxygen levels remained above the hypoxia threshold throughout the summer of 2012. The OPD was, at all three stations, slightly deeper in winter than in summer (Fig. 6B). In conclusion, BWO concentration in the basin showed seasonal hypoxia to anoxia which varied in intensity and duration as a function of water depth (Seitaj et al., 2017).

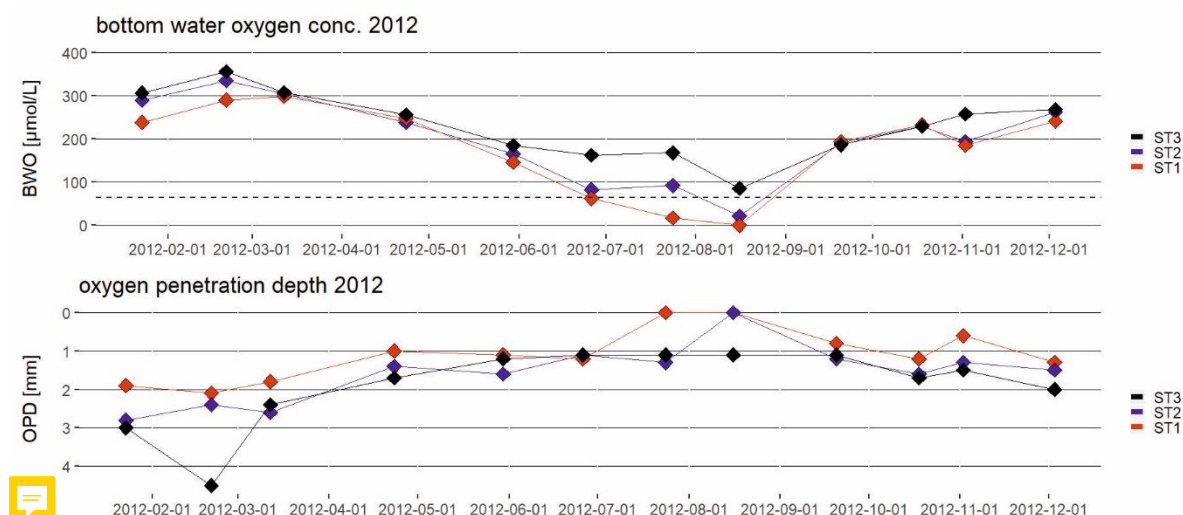


Figure 6. A: BWO concentration for stations ST1 (34 m), ST2 (23 m) and ST3 (17 m) with indicated sampling dates in 2012. The dashed line indicates the threshold for hypoxic conditions (63 $\mu\text{mol/L}$). B: Oxygen Penetration Depth for ST1, ST2 and ST3 measured from sediment cores sampled on the same dates in 2012 as water column parameters. Data from Hagens et al. (2015) and Seitaj et al. (2017).

4.1.2 Sediment geochemistry

During the same monthly sampling campaigns in 2012 the pore water and solid-phase sediment geochemistry was also analysed. This large data set has been presented previously by Seitaj et al. (2015, 2017) and Sulu-Gambari et al. (2016a,b, 2017a). Despite the strong seasonal oxygen depletion in Lake Grevelingen, the occurrence of free sulfide in the bottom water (euxinia) is likely prevented or at least delayed, due to the presence of cable bacteria (Seitaj et al., 2015). The metabolic activity of these filamentous bacteria involves an electrical coupling between oxygen reduction at the sediment surface and sulfide oxidation in the deeper suboxic sediment via long-distance electron transport (Malkin et al., 2014; Meysman et al., 2015; Nielsen et al., 2010; Pfeffer et al., 2012). The sulfide oxidation induces both carbonate and FeS dissolution, which releases Fe^{2+} into the pore water as initially only demonstrated in laboratory experiments (Rao et al., 2016; Risgaard-Petersen et al., 2012). Similar processes occur in Lake Grevelingen; Sulu-Gambari et al. (2016a,b) demonstrated that the Fe^{2+} is released and diffuses upwards, and that a major proportion is oxidised by manganese oxides (input of Mn from the water column, Sulu-Gambari et al., 2016b). Mn oxides are supplied to the sediment surface through deposition of suspended matter from the water column. This supply is an important prerequisite for the impact of cable bacteria activity on the Mn cycle in winter (Sulu-Gambari et al., 2016b). In fact, Sulu-Gambari et al. (2016b, 2017) show a strong enrichment of solid-phase (total) Mn in the sediment surface in winter/early spring compared to summer months at all three stations. In

winter/early spring the reductive dissolution of Mn oxides by Fe^{2+} leads to the mobilisation of Mn^{2+} (Reed et al., 2011; Thamdrup et al., 1994), and to distinct Mn^{2+} pore water peaks below the OPD (Fig. S1, Sulu-Gambari et al., 2016a,b). Rao et al. (2016) additionally proposed that substantial amounts of Mn^{2+} are released during CaCO_3 dissolution (induced by the cable bacteria). In our study area, cable bacteria activity was assessed with microscopic observation and evidenced with fluorescence *in situ* hybridisation (FISH; Seitaj et al., 2015). Cores from all stations were examined with FISH in March, May, August and November, and cable bacteria were detected with this technique at ST1 and ST3 in March and May (Seitaj et al., 2015). Conversely, FISH did not give any indication of cable bacteria at ST2. However, there is evidence that the presence of cable bacteria in the basin may be very patchy (Seitaj et al., 2015, 2017). Based on the pore water Mn^{2+} and Fe^{2+} depth profiles, the presence of cable bacteria is likely in January at ST1 and in February and March at ST2 (Fig. S1) in addition to their confirmed presence at ST1 and ST3 in March and May (Sulu-Gambari et al., 2016b). The signature of pH, OPD and hydrogen sulfide profiles confirms the presence of cable bacteria at ST2 in March of another sampling year (Seitaj et al., 2015). It is therefore likely that cable bacteria were active throughout winter and spring in 2012 at all stations, although with an important spatial patchiness. This patchiness of the pore water profiles indicates that despite the supposedly spatially similar settling flux of Mn from the water column in winter, the activity of the cable bacteria determines what part of this flux is transformed to Mn^{2+} . In summary, in a context of ample input of solid-phase Mn through the water column and an oxygenated overlying water column, cable bacteria activity is responsible for the intensification of Mn cycling in the surface sediment in winter and early spring.

Cable bacteria were not observed in summer and autumn in Lake Grevelingen, and with the beginning of hypoxia, Mn oxides are removed from the sediment, whereas Fe (oxyhydr)oxides are transformed back to FeS (Sulu-Gambari et al., 2016b). In fact, Sulu-Gambari et al. (2017a) presented data of strongly increased total Mn in the suspended matter in the water column in July 2012, suggesting that during summer hypoxia, due to the release of Mn^{2+} from the pore water into the bottom water, accelerated Mn refluxing took place. Mn refluxing has been described for the Chesapeake Bay as settling of Mn oxides leading to further reductive dissolution in the surface sediment (Adelson et al., 2001), and is with one exception (the depth where oxygen is met in the water column) probably similar in Lake Grevelingen (Sulu-Gambari et al., 2017a).

Contrary to the classic model, in Lake Grevelingen, seasonal hypoxia (and variability of Mn input) is not the major control on the seasonal variability of Mn^{2+} concentrations in the pore waters. Nevertheless, although not necessarily during summer hypoxia, deeper in the basin,

where oxygen concentrations in summer are lower, pore water Mn^{2+} peaks reached higher values. The metabolic activity of cable bacteria explains the strongly increased Mn^{2+} accumulation in the pore waters in some of the cores sampled in winter and early spring. Concerning the gradient of increasing intensity and vertical extent of the pore water Mn^{2+} and Fe^{2+} peaks from ST3 to ST1, Sulu-Gambari et al. (2016a) and Seitaj et al. (2017) ascribe this to the activity of cable bacteria related to the increasing organic matter mineralisation rates from ST3 to ST1 as well as the interference with more abundant macrofauna, competition with other micro-organisms and/or higher oxygen concentrations at the two shallower stations (Sulu-Gambari et al., 2016b). Here, we will investigate to what extent Mn/Ca ratios of benthic foraminifera such as *Ammonia tepida* enable us to reconstruct this variability of pore water Mn^{2+} in the past by first understanding how living foraminifera are recording the complex present conditions.

4.2. General aspects regarding Mn/Ca and biomineralization of benthic foraminifera

The average Mn/Ca ratio of *Ammonia tepida* shows a significantly higher value at ST1 compared to ST2 and ST3. In Petersen et al. (2018), it is argued that the Mn/Ca intra-test (and implicitly also inter-test) variability may be due to three different factors: 1) intrinsic factors such as ontogenetic trends or vital effects, 2) microhabitat effects (active or passive vertical migration), and 3) temporal variability of the environment. The influence of diagenetic coating on the foraminiferal test with Mn carbonates is not considered here because we analysed only live specimens. Moreover, specimens were cleaned according to a specific protocol followed by LA-ICP-MS data treatment resulting in a Mn/Ca signal of the (biogenic) calcite (Guo et al., 2019). The same three factors have to be evoked here, in addition to spatial variability of the environment, i.e., differences between the three stations. Concerning intrinsic Mn/Ca intra-test variability for *A. tepida*, directly related to the biomineralization process, it was shown that 11-25 % variability occurs in newly calcified chambers under constant laboratory conditions for the range of dissolved Mn concentrations found in our study area (10-100 $\mu\text{mol/L}$; based on data of Barras et al., 2018; further explanation in Petersen et al., 2018). The higher average intra-test variability of 45 % RSD in comparison to this intrinsic variability led Petersen et al. (2018) to the conclusion that it is possible to trace elemental variability based on intra- and inter-specimen Mn/Ca comparisons of *A. tepida*.

Since *A. tepida* has an estimated longevity of one to two years (Goldstein and Moodley, 1993; Morvan et al., 2006), we estimate that it calcifies during several successive seasons. However, during the foraminiferal lifespan, chamber addition is supposedly not regular, and

could be accelerated during short periods of favorable conditions, for example in response to increased food supply (e.g., Jorissen, 1988). Conversely, when conditions are less favorable, e.g., in winter, when primary production is minimal, and less organic matter arrives at the sediment surface, it is possible that construction of new chambers is occurring less often, or may even not take place at all. At ST1, adult living *A. tepida* specimens were only encountered in larger numbers from March to July 2012 (Richirt et al., in prep.). This observation strongly suggests that at ST1, the *A. tepida* population disappears in late summer, due to anoxia (and possibly due to free sulfide at shallow depth in the sediment, Seitaj et al., 2015), and repopulates the site in (late) winter. Therefore, we suggest that these foraminifera started calcifying in (late) winter, and have progressively added chambers over a ~6 month period. In fact, the faunal data collected by Richirt et al. (in prep.) show an ongoing size increase of the population from March to July. It appears therefore that the later calcified parts of the test will be representative for the summer conditions, whereas the earliest parts of the tests may have registered the late winter and/or early spring conditions. Conversely, the year-round presence (with high standing stocks) of *A. tepida* at ST2 and ST3 (Richirt et al., in prep.), and their significantly larger size (Tab. 1) suggest that these specimens have calcified over longer periods of time. This implies that the specimens collected at stations ST2 and ST3 would have registered a (much) larger part of the (inter)annual cycle. In summary, although the last chamber(s) of the foraminifer is (are) supposed to have registered the conditions prevailing in the weeks (to months) prior to sampling, the test as a whole may have integrated environmental conditions over a (much) longer period, of 3 to 6 months (ST1) possibly even up to 12 months or more (ST2 and ST3). Because of the trochospiral coiling and the lamellar character of the tests of *A. tepida*, the LA-ICP-MS measurement made at the central part of the test represents an average of a large number of chambers, representative of the total life history of the individual.

As a shallow infaunal taxon, *A. tepida* prefers a microhabitat close to the sediment-water interface (SWI), where most of its chamber addition probably takes place (e.g., Thibault de Chanvalon et al., 2015; Cesbron et al., 2016). Active or passive transport processes can lead to the presence of living individuals in deeper layers (see section 4.4). Considering the Mn^{2+} pore water profiles (Fig. S1), it appears that at all three stations, throughout the year, Mn^{2+} concentrations are mostly zero at the SWI and rapidly increase immediately below the sediment surface during certain periods. In fact, maximum Mn^{2+} values are often found between 1 and 3 cm depth during winter, but are situated between 0-0.5 cm in hypoxic summer months. Even if a steep gradient of pore water Mn^{2+} is present in the first cm below the sediment surface, if *A. tepida* calcifies in the well oxygenated niches very close to the sediment surface, it should normally not be confronted with high concentrations of Mn^{2+} , and

only chambers calcified slightly deeper should show increased Mn/Ca ratios. However, on the basis of the strongly increased total Mn in water column suspended matter in July, Sulu-Gambari et al. (2017a) concluded that during summer hypoxia Mn^{2+} is released from the surface sediment into the water column (see section 4.1). Evidently, this process of Mn-refluxing must have been strongest at the most hypoxic station ST1, but the data show that also at the other two stations total Mn in water column suspended matter was increased by two orders of magnitude in summer compared to winter months (Fig. 3 and Tab. S2 in Sulu-Gambari et al., 2017a). It appears therefore that at the three stations, abundant Mn^{2+} must have been present in the foraminiferal niches close to the SWI during summer hypoxia (immediately prior to our sampling).

4.3. Foraminiferal Mn/Ca response to summer hypoxia and cable bacteria activity

Specimens collected in July 2012 showed significant differences in Mn/Ca between the three sampling stations. Compared to ST2 and ST3, much more specimens of station ST1 showed elevated Mn/Ca ratios. Mn/Ca ratios were significantly higher in the central part of the test and also for all measurements on separate individuals taken together (later chambers and central part, Fig. 3 and 4A,D). In fact, the mean Mn/Ca value of 0.36 mmol/mol for ST1 is about two times higher than those of ST2 (0.18 mmol/mol) and ST3 (0.20 mmol/mol). The difference is much larger than could be accounted for by intrinsic (intra-test) variability (11-25 %, observed in the study of Barras et al., 2018, under laboratory conditions). This suggests that the difference in Mn/Ca of *A. tepida* between ST1 and the other two stations is reflecting higher Mn^{2+} concentrations in the surface sediment.

In Fig. 7, we plotted for each station and for each sampling campaign (i.e., each month), the maximum pore water Mn^{2+} concentration in the topmost centimeter of the sediment against the BWO concentration (data from Hagens et al., 2015; Sulu-Gambari et al., 2016a,b, 2017b,c; Seitaj et al., 2017). The plot shows that increased Mn^{2+} concentration is mainly caused by two different processes. First, the data in the upper left part of the plot show a combination of high pore water Mn^{2+} and high BWO concentration. These data correspond to the winter and (early) spring months when cable bacteria activity caused strongly increased Mn^{2+} concentrations. Second, in the right part of the diagram, some data points show a combination of low BWO conc. and relatively increasing Mn^{2+} contents at ST1. In our opinion, these data represent summer hypoxia/anoxia (see previous sections for further explanation which is based on total Mn in water column suspended matter). The diagram also shows that Mn^{2+} concentrations may reach much higher levels in winter (due to cable bacteria activity

and increased input of Mn from the water column) than in summer (due to seasonal hypoxia). Nevertheless, although somewhat masked by the much higher winter/ spring Mn^{2+} values related to cable bacteria activity, a trend of increasing pore water Mn^{2+} with decreasing BWO conc. can be observed at ST1 for the summer months (inset in Fig. 7).

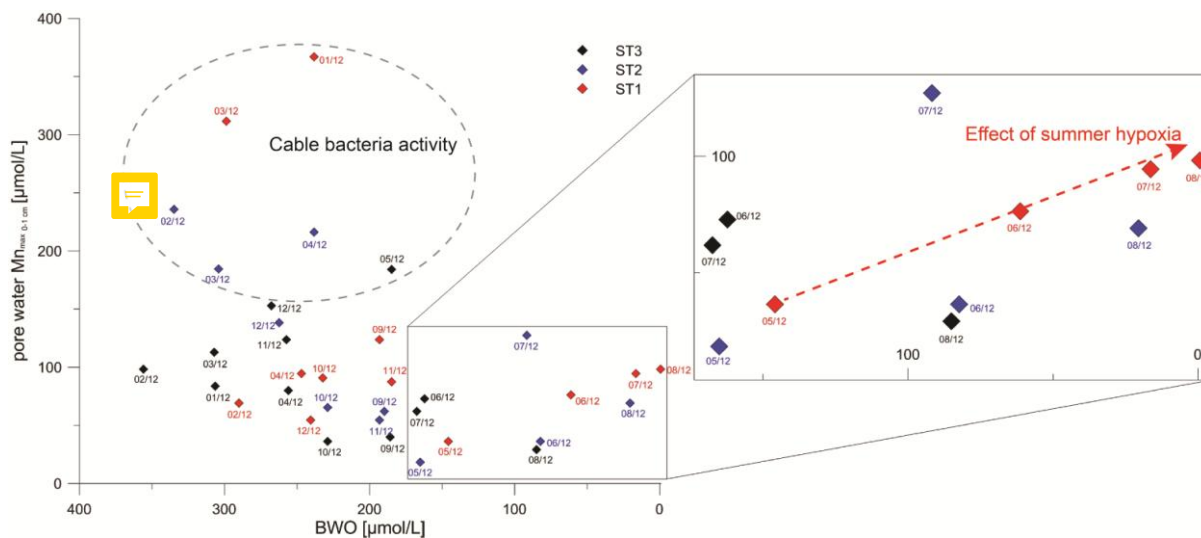


Figure 7. Pore water Mn^{2+} (maximum for depth 0-1 cm) in function of BWO concentration for the three stations. High pore water Mn^{2+} values associated with cable bacteria activity are indicated by the dashed ellipse. The inset on the right presents values measured in summer months for which at ST1 an increasing trend is observed. Data from Hagens et al. (2015), Sulu-Gambari et al. (2016a,b, 2017b,c) and Seitaj et al. (2017). The entire pore water Mn^{2+} profiles are displayed in Figure S1.

It is interesting to notice that the strongly increased Mn^{2+} concentrations in winter/spring were mostly situated slightly deeper in the sediment, in the 0.5-1.0 cm level, as opposed to the summer Mn^{2+} maxima, which were always measured immediately below the sediment surface, in the 0-0.5 cm sample layer (Fig. S1). The more superficial character of the Mn^{2+} peaks related to summer hypoxia increases the probability that they are recorded in the tests of foraminifera calcifying close to the SWI, in spite of the fact that the maximum values are much lower than in winter. Since a substantial part of the specimens (11 out of 26 specimens for which the last chambers were measured; Tab. 4 and Fig. 5) showed increased Mn/Ca ratios in the last chambers, it appears that the Mn^{2+} maxima due to summer hypoxia have indeed been registered. The lack of this signal in the remaining 15 specimens may be due to the fact that these specimens did not calcify any new chambers after the onset of hypoxia, or (at stations ST2 and ST3) calcified the last chambers at the SWI (corresponding to bottom water sediment samples), where Mn^{2+} concentrations were still much lower than at some mm's depth in the sediment.

Regarding the winter/early spring Mn^{2+} maximum, related to cable bacteria activity, we suggest that this has not been registered in one of the last chambers, since several months had passed when the foraminifera were sampled in July 2012. However, increased Mn^{2+} related to cable bacteria activity may have caused increased values in earlier chambers, which should cause an increase of the Mn/Ca ratio in the central part of the test, which represent an average value for the entire growth period(s) of the individual. In fact, at ST1 nine out of the 13 specimens had increased values in the central part of test, compared to two out of 13 specimens at ST2 and zero out of nine specimens at ST3 (Tab. 4 and Fig. 5). At ST1, the Mn^{2+} concentration in March, when cable bacteria were actually observed, was more than twice as high as the peaks of ST2 and ST3, and concentrations exceeded 250 $\mu\text{mol/L}$ in the 0-1 cm depth interval, which was never reached at the other stations (Fig. 7). The decreasing trend in maximum pore water Mn^{2+} from ST1 to ST2 and ST3 has been attributed to decreasing cable bacteria activity towards shallower waters for reasons that could be indirectly attributed to contrasting BWO concentrations (see section 4.1; Sulu-Gambari et al., 2016a,b; Seitaj et al., 2017). At ST2, there are some indications for the influence of cable bacteria activity on the pore water Mn^{2+} peak for February, March and April 2012 (Fig. S1). Except for April 2012, the Mn^{2+} maxima were positioned slightly deeper in the sediment, and the 0-0.5 cm layer showed only a moderate increase of Mn^{2+} , suggesting that high Mn/Ca ratios could only have been registered in some of the chambers. At ST3, finally, we have indications for the presence of cable bacteria in March and May 2012, but Mn^{2+} concentrations never attained the levels observed at ST1 or ST2. Regarding average Mn/Ca ratios, the statistically significantly higher values from specimens of ST1 in comparison to ST2 and ST3 can be explained by the fact that for some specimens the average is mostly reflecting n-c measurements (as later chambers did not yield results, see Tab.1). Summarizing, it appears that in our samples the Mn/Ca ratios in the central part of the foraminiferal test accurately reflect the bathymetrical trend of increasing Mn^{2+} concentrations due to cable bacteria activity.

In order to test whether the Mn/Ca values measured in our *A. tepida* specimens are realistic, we applied the partitioning coefficient of $D= 0.086$ determined for calcite Mn/Ca of *Ammonia tepida* by Barras et al. (2018) to calculate the maximum possible Mn/Ca ratios of *A. tepida* based on the pore water Mn and Ca profiles, for specimens calcifying in the topmost cm of the sediment (see supplementary material for further explanation and data). It turned out that the maximum values of Mn/Ca observed in the last chambers were close to the maximum theoretical values expected for June/July. Conversely, the maximum values measured in the central part of the test were a half to almost one order of magnitude lower than the theoretical maximum values. This corroborates our conclusion that the sediment Mn^{2+}

maximum was largely coinciding with the foraminiferal microhabitat in summer, whereas it was situated deeper in the sediment in winter.

4.4 Potential effect of vertical migration of *Ammonia tepida* on the Mn/Ca signal

In the previous section we explained that the differences in Mn/Ca ratios between the three stations show a response to two different processes. High Mn/Ca ratios in the central part of the test appear to reflect increased sediment Mn^{2+} levels due to cable bacteria activity in late winter/spring, whereas high Mn/Ca ratios in the last chambers would rather reflect increased Mn^{2+} levels due to summer hypoxia. An important question is to what degree foraminiferal migration through the upper sediment, either active or passive (by macrofaunal burrowing), may contribute to this pattern. In fact, a number of experimental studies have observed (coastal) benthic foraminifera actively migrating in the surface sediment layer, often in response to gradients in oxygen concentration (Alve and Bernhard, 1995; Duijnsteet al., 2003; Ernst et al., 2005; Ernst and Van Der Zwaan, 2004; Geslin et al., 2004; Gross, 2000; Langezaal et al., 2003; Moodley et al., 1998). Additionally, for *A. tepida*, Maire et al. (2016) observed in the laboratory a lower proportion of active individuals under anoxic than under oxic conditions. Thibault de Chanvalon et al. (2015) suggested that hypoxic BWO conditions induced an upward active migration of *A. tepida* in the surface sediment.

According to Petersen et al. (2018), vertical migration of the foraminifera within the surface sediment layer may lead to substantial variability in measured Mn/Ca ratios in time-equivalent chambers of different specimens of *A. tepida* in the same sample. In fact, for some individuals, Petersen et al. (2018) observed strongly varying Mn/Ca ratios in successive chambers of the same individual, which they ascribed to the calcification of the chambers with high Mn/Ca in deeper sediment layers, following active or passive migration of the individual. In the present study, where only two or three measurements were made per specimen, such patterns cannot be evidenced. Nevertheless, our data, which show increased Mn/Ca ratios in response to two different phenomena, suggest that the foraminiferal Mn/Ca signal is to some extent modified by migration of the individuals within the topmost sediment.

Concerning the winter/spring Mn^{2+} maxima due to cable bacteria activity, since these maxima where present more profoundly in the sediment, it appears that only foraminifera calcifying slightly deeper in the sediment, following active or passive migration, have registered this phenomenon. This would explain the rather low percentage of foraminifera (11 out of 35 specimens, Tab. 4 and Fig. 5) showing increased Mn/Ca in the central part of the test.

Conversely, in case of the increased Mn/Ca in the later chambers, related to summer hypoxia, since in this case the increased Mn^{2+} levels also affected the topmost sediment layer, it is not necessary to invoke bioturbation to explain the raised Mn/Ca ratios. This is coherent with the fact that macrofauna was absent at ST1 in July 2012 (Seitaj et al., 2017), excluding the possibility of passive migration. For this study live specimens have been sampled from the topmost cm of the surface sediment, thus excluding interpretations about active or passive migration based on depth distribution.

4.5 Paleocological consequences of the observations of Mn/Ca ratios in benthic foraminifera from Lake Grevelingen

The present data show that the registration of the pore water Mn dynamics in the coastal foraminifer *A. tepida* is not straightforward, which is to a large degree due to the high complexity of the Mn cycling itself. The two phenomena leading to strong increases of Mn^{2+} concentration in the upper sediment layers, cable bacteria activity and summer hypoxia, are registered for our specimens sampled in summer in the central part and in the last chambers of the test, respectively. However, since at least at ST2 and ST3, where *A. tepida* is present year-round, specimens would have continued to add chambers after the summer hypoxia, in fossil specimens the response to summer hypoxia would not systematically be found in the last added chambers. This restricts the use of foraminiferal Mn/Ca ratios in coastal foraminifera measured by LA-ICP-MS to measurements of the central part of the test, which represents an average value for the whole life history of the individual, including periods with high Mn^{2+} due to cable bacteria activity and due to summer hypoxia.

Nevertheless, micro-analytical measurements of successive individual chambers may be highly informative. First of all, we have shown that in spite of the much lower Mn^{2+} levels, the summer hypoxia events are maybe even better inscribed in the foraminiferal calcite as the cable bacteria related Mn^{2+} maxima, the latter being positioned slightly deeper in the sediment. Furthermore, the intensity of cable bacteria activity seems to be strongly related (inversely) with the extent of summer hypoxia. Sulu-Gambari et al. (2016a,b) and Seitaj et al. (2017) describe a gradient of decreasing cable bacteria activity from ST1 to ST3 which they explain by the decrease in anaerobic organic matter remineralisation and associated sulphate reduction with decreasing depth in the basin. In fact, in spite of the high complexity of the Lake Grevelingen ecosystem, we found for our three stations a clear inverse relationship between Mn/Ca ratios in the central part of the test and the extent of bottom water hypoxia (which lasted longer and was more intense at ST1 compared to ST2 and ST3, see Fig. 6). This suggests that Mn/Ca ratios in coastal benthic foraminifera, measured by LA-

ICP-MS in the central part of the test, may be an important tool to gain insight into the redox processes of complex coastal ecosystems. For future studies it could be envisaged to disentangle different processes by using a multi-proxy geochemical approach and to analyse other elemental ratios reflecting variability of the S cycle which is also influenced by cable bacteria activity.

5. Conclusion

We studied Mn/Ca ratios in living specimens of the benthic foraminiferal species *A. tepida* in the complex environmental setting of Lake Grevelingen. It appears that increased Mn/Ca ratios in some of the specimens reflect mainly two factors:

- 1) seasonal increases of pore water Mn^{2+} due to cable bacteria activity in late winter/spring, when the bottom water is well oxygenated and Mn-bearing suspended matter is supplied through the water column, lead to higher foraminiferal Mn/Ca in the central part of the test, which represent an average of the specimen's calcification history.
- 2) increased Mn-refluxing in surface sediments due to bottom water hypoxia/anoxia in summer leads to increased Mn/Ca ratios in the last formed of our specimens collected in July.

Although the maximum pore water Mn^{2+} concentrations generated by cable bacteria activity are higher than those directly related to hypoxia/anoxia and the duration of the cable bacteria activity in winter is longer than the hypoxia period in summer, the Mn^{2+} peaks related to summer hypoxia are positioned closer to the sediment-water interface. Consequently, there is a higher chance that the latter peaks are registered in the foraminiferal shells.

It appears that cable bacteria activity in late winter/spring is more intense at sites which are more affected by the summer hypoxia/anoxia, and it seems that these two factors are at least to some extent related. Therefore, in spite of the complex nature of the formation of the Mn/Ca signal, the inter-site differences in seasonal bottom water oxygenation history are well reflected by the foraminiferal Mn/Ca ratios, with higher Mn/Ca average values at ST1 than at ST2 and ST3.

Considering the proxy potential of benthic foraminiferal Mn/Ca ratios, in spite of the extremely complex environmental context of Lake Grevelingen (as in comparable coastal ecosystems), the signature measured in the central part of the test could be a potential proxy for the overall redox state of manganese. Although the Mn/Ca ratios of *A. tepida* cannot be

explained as a direct response to cable bacteria activity, or to bottom water hypoxia, the close linkage between these two processes makes it clear that foraminiferal Mn/Ca could nevertheless be a powerful indirect proxy for the overall redox state of the basin. Furthermore, the analysis of successive individual chambers may provide more precise insight into seasonal cycles of redox elements. In this respect a multi-proxy approach may be helpful to further disentangle the different processes leading to the Mn/Ca signal as well as calcification periods of individual coastal benthic foraminifera.

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Highlights for manuscript **Mn/Ca ratios of *Ammonia tepida* as a proxy for seasonal coastal hypoxia** submitted by Petersen et al.

- Inter-site differences in Mn/Ca reflect contrasts in seasonal bottom water hypoxia
- Main processes causing high Mn/Ca are cable bacteria activity and seasonal hypoxia
- Mn/Ca of the central part reliably displays inter-site environmental differences

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