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1                   **A HISTORICAL RECORD OF BENTHIC FORAMINIFERA IN**  
2                   **SEASONALLY ANOXIC LAKE GREVELINGEN, THE NETHERLANDS**

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32 Lake Grevelingen is a former branch of the Rhine-Meuse-Scheldt estuary, which was  
33 artificially transformed into a salt-water lake by a dam in 1971. This transformation  
34 induced profound changes in the biological community of the basin, which have been  
35 described as an ecodisaster, with seasonal hypoxia/anoxia occurring in the deepest parts  
36 of the lake. Here, we investigate a sediment core sampled in 2012 in the Den Osse Basin  
37 (34 m depth) of Lake Grevelingen, recording the last ~ 50 years and including the  
38 transition from an estuary to a salt-water lake. Sediment molybdenum (Mo)  
39 concentrations were used to refine an existing age model based on  $^{210}\text{Pb}$ , giving us an  
40 estimated precision of  $\pm 3$  years. The benthic foraminiferal succession reflects the  
41 anthropogenic modifications in Lake Grevelingen and allows four successive stages to be  
42 distinguished: 1) before 1971, when the system was estuarine; 2) from 1971 to 1978, when  
43 the system rapidly changed into an enclosed brackish water lake; 3) from 1978 to 1999,  
44 when a seaward sluice was opened during winter and 4) the period from 1999 to 2012,  
45 with a year-round opening of the sluice and doubling of water exchanges with the North  
46 Sea. The foraminiferal record, showing communities mainly dominated by *Elphidium*  
47 *selseyense*, also highlights the appearance of the putatively exotic *Ammonia confertitesta*  
48 in the mid-1980s, which thereafter progressively replaced the congeneric autochthonous  
49 *Ammonia aberdoveyensis*. Finally, we hypothesise that the activity of cable bacteria, S-  
50 oxidising prokaryotes present in the surface sediments of Den Osse Basin in winter and  
51 spring, causes dissolution of foraminiferal tests by decreasing the pore water carbonate  
52 saturation state in the sediments directly below the oxic zone. This explains the contrast  
53 between the abundant living populations and the very low numbers of foraminiferal shells  
54 preserved in the top ~ 15 cm of the sediment.

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58 **Keywords:** foraminifera, historical record, Lake Grevelingen, anoxia, cable bacteria

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# 1. INTRODUCTION

77 Foraminifera are among the most widespread groups of marine unicellular eukaryotes and  
78 constitute one of the most diverse groups of shelled organisms. They have a very rich fossil  
79 record and are widely used for palaeoenvironmental reconstructions and palaeoecological  
80 interpretations (Murray, 2006; Katz et al., 2010). Foraminifera are known to be relatively  
81 tolerant to low oxygen concentrations, anoxia and/or presence of sulphides in their habitat, on  
82 various timescales. This has been documented for many species from different types of  
83 environments in laboratory culture (e.g. Moodley and Hess, 1992; Bernhard, 1993; Alve and  
84 Bernhard, 1995; Moodley et al., 1998; Duijnsteet et al., 2003; Geslin et al., 2004; Pucci et al.,  
85 2009) as well as in field studies (e.g. Piña-Ochoa et al., 2010; Langlet et al., 2013, 2014).  
86 Unfortunately, studies on the foraminiferal response in systems affected by seasonal hypoxia–  
87 anoxia with sulphidic conditions are still very sparse. To our knowledge, all earlier studies show  
88 that the foraminiferal response to hypoxia–anoxia is species-specific (e.g. Bernhard and Alve,  
89 1996; Ernst et al., 2005; Bouchet et al., 2007; Geslin et al., 2014; Langlet et al., 2014). However,  
90 this species-specific response generally follows the same scheme (usually decrease in density,  
91 reduction of growth and/or reproduction), with different response intensities. Recently, a study  
92 investigated the temporal response of living foraminiferal communities to recent seasonal  
93 anoxia in the Den Osse Basin of Lake Grevelingen (the Netherlands, Richirt et al. 2020). The  
94 authors highlighted that the deepest part of this basin shows a severe decline in foraminiferal  
95 populations in the months following late summer–early autumn seasonal anoxia. They  
96 explained this by the presence of sulphides in the foraminiferal habitat, that would inhibit  
97 foraminiferal reproduction and growth. In the shallower parts of the basin, where the duration  
98 of anoxia is shorter, there was not such a major decline in the foraminiferal community.

99 The Den Osse Basin is located in Lake Grevelingen, a former estuary of the Scheldt-Meuse-  
100 Rhine delta, in the Netherlands. After a storm in 1953, which caused large scale flooding and  
101 more than 1800 casualties, landward (1965) and seaward (1971) dams were constructed to avoid  
102 such disasters in future (Delta Plan, Bannink et al., 1984; Figure 1). Thanks to the preservation  
103 of shelled foraminifera in the sediment record, Lake Grevelingen provides a unique opportunity  
104 to study the benthic foraminiferal response to the progressive transformation of the ecosystem  
105 from an estuary to a salt water lake, especially regarding the development of seasonal  
106 hypoxia/anoxia.

107 The living foraminiferal community of the Den Osse Basin is largely dominated by  
108 *Elphidium selseyense* (phylotype S5, Darling et al., 2016), *Elphidium magellanicum*

109 (morphospecies not yet sequenced) and *Ammonia confertitesta* (*Ammonia* sp. T6 in Richirt et  
110 al., 2020; phylotype T6 in Hayward et al., 2021). The latter species, *A. confertitesta*, is often  
111 considered as an exotic and/or invasive species, originating from East Asia (e.g. Pawlowski &  
112 Holzmann, 2008; Schweizer et al., 2011; Bird et al., 2020). *Ammonia confertitesta*, which is  
113 almost the only representative of the genus *Ammonia* in the recent living assemblages (Richirt  
114 et al., 2020), is characterised by very large pores (Richirt et al., 2019a) compared to *Ammonia*  
115 *aberdoveyensis* and to a lesser extent *Ammonia veneta* (phylotypes T2 and T1, respectively,  
116 Hayward et al., 2021). Interestingly, a preliminary study of a long core sampled in the Den Osse  
117 Basin showed a clear shift from specimens with small pores to specimens with bigger pores in  
118 recent times (Petersen et al., 2016). This indicates that in a recent past, *A. confertitesta* has  
119 progressively replaced *A. aberdoveyensis* (and/or *A. veneta*) in Lake Grevelingen. Richirt et al.  
120 (2019b), suggesting that an increase in overall porosity could be an adaptation to low oxygen  
121 concentrations. The putative replacement of *A. aberdoveyensis* (and/or *A. veneta*) by *A.*  
122 *confertitesta* could therefore be due to the larger tolerance of the latter species to hypoxia. The  
123 study of the sedimentary foraminiferal record should enable us to shed more light on this  
124 important species take-over.

125 In this study, we investigate foraminiferal assemblages of a 90 cm long sediment core  
126 sampled in the deepest part of the Den Osse Basin, representing the last ~ 50 years. We will  
127 especially address the following questions:

- 128 1) How did the foraminiferal community change in response to the closure of Lake  
129 Grevelingen in the 1970s and the subsequent management changes of the basin?
- 130 2) Has there been a substitution of the autochthonous *Ammonia* species (*A. veneta* and *A.*  
131 *aberdoveyensis*) by the putative allochthonous species *A. confertitesta*, and, if so, is it the result  
132 of an overall spreading of *A. confertitesta* across Europe, or rather of a better adaptation of this  
133 phylotype to seasonal hypoxia/anoxia?

## 134 **2. STUDY AREA**

135 In 1965, the former Grevelingen estuary was closed landward (Grevelingendam) and  
136 became a semi-enclosed embayment without river input. A second dam was constructed  
137 seaward in 1971 (Brouwersdam), closing the former Grevelingen estuary completely. This  
138 stopped the inflow of salt waters from the North Sea and increased the water residence time in  
139 the Grevelingen lake up to three to six years (instead of a few days, when the system was  
140 estuarine, Nienhuis, 1978; Bannink et al., 1984). The suppression of tidal currents and decrease

141 in salinity directly after the full closure strongly affected marine benthic animals (e.g. sponges,  
142 sea anemones, tunicates). Mass mortality of these animals contributed to increased food  
143 availability, accumulation of decomposition products and finally oxygen depletion. This  
144 “ecodisaster” induced a simplification of the ecosystem concerning both its structure and  
145 functioning (Nienhuis & Veld, 1984; Bannink et al., 1984). Within a few years after closure of  
146 the estuary, seasonal bottom water hypoxia/anoxia started to occur, followed by mass mortality  
147 of the remaining marine benthic animal populations (e.g. molluscs, worms), especially in the  
148 deeper parts of the basin (Nienhuis & Veld, 1984).

149 To remedy eutrophication phenomena and seasonal hypoxia by limiting water column  
150 stratification, the connection with the North Sea was partly reopened in 1978, by the  
151 construction of an underwater sluice in the Brouwersdam (capacity of about  $100\text{--}140\text{ m}^3\text{ s}^{-1}$ ,  
152 initially only opened in winter, Bannink et al. 1984; Muelen et al., 1984). This partial reopening  
153 improved the bottom water oxygenation in the Den Osse Basin and decreased water residence  
154 times to about half a year. Consequently, several species, which were common in the  
155 Grevelingen estuary but had disappeared when the estuary was closed, were observed again  
156 (e.g. some jellyfish and crab species; Nienhuis and Veld, 1984). A second, landward sluice  
157 (siphon) was opened in 1983 (at the Grevelingendam) to ensure a weak (compared to the natural  
158 situation without a dam) freshwater inflow (about  $100\text{ m}^3\text{ s}^{-1}$ , Bannink et al., 1984; Muelen et  
159 al., 1984).

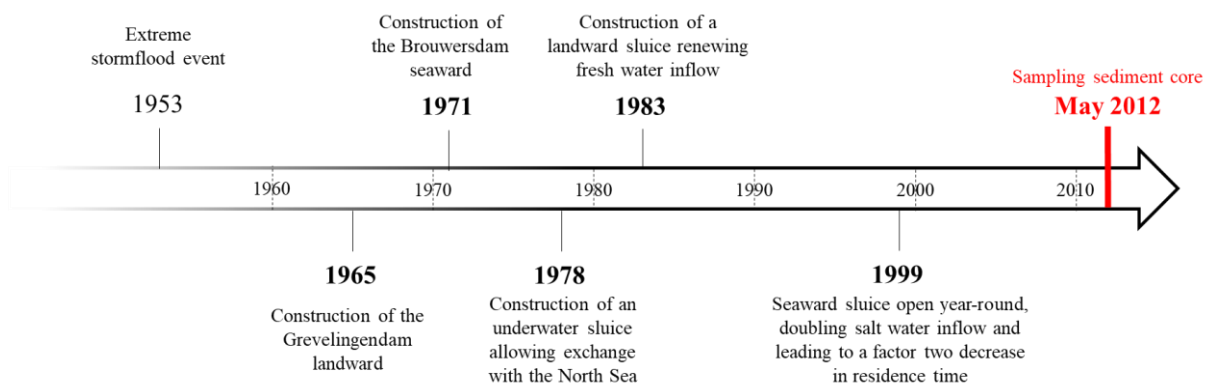
160 In 1999, the water exchanges between the Lake and the North Sea were doubled (from  
161  $1245\text{--}1255 \cdot 10^6\text{ m}^3$  to  $2688\text{--}2864 \cdot 10^6\text{ m}^3$  per year, Wetsteijn, 2011) by opening the  
162 Brouwersdam sluice the whole year, except for a few weeks in autumn and during storm events.  
163 This allowed a further decrease of the average water residence time in the Lake from 164 to 72  
164 days and limited water stratification due to high temperatures in summer (Wetsteijn, 2011).  
165 However, this situation was still very different compared to the estuarine situation before 1965,  
166 when the water residence time was estimated to be a few days (Nienhuis, 1978; Bannink et al.,  
167 1984) and average flood and ebb discharges at the location of the present Brouwersdam were  
168 about  $126000 \cdot 10^6$  and  $135000 \cdot 10^6\text{ m}^3$  per year, respectively (calculated for 1959, Louters et al.,  
169 1998), ~ 50 times higher than the current water exchanges.

170 Despite these management changes, seasonal bottom water oxygen depletion continued to  
171 occur every year, as is still the case today, especially in the deepest parts of Lake Grevelingen,  
172 such as in the Den Osse Basin that is investigated in this study (Wetsteijn, 2011; Sulu-Gambari  
173 et al. 2017). The transformation of Grevelingen from an estuary to a salt-water lake induced  
174 profound modifications at ecosystem (e.g. habitat losses, changes in trophic relationships),

175 community (e.g. decrease of biodiversity, replacement of marine by estuarine species) as well  
176 as species levels (e.g. disturbed or interrupted life cycles, change in densities, Nienhuis, 1978;  
177 Nienhuis & Veld, 1984).

178 To summarise, Lake Grevelingen, a former estuarine system, was completely closed in  
179 1971. The ensuing strong increase of water residence time, bottom water stagnation and lower  
180 salinity caused a major biotope shift in Lake Grevelingen and led to strong modifications of its  
181 biological community (Nienhuis, 1978).

182 Today, two antagonistic groups of sulphur-oxidizing bacteria showing contrasting seasonal  
183 population dynamics are present in sediments of the Den Osse Basin: cable bacteria in January-  
184 May and *Beggiatoaceae* in September–December (Seitaj et al., 2015; Sulu-Gambari et al.,  
185 2016a). Cable bacteria activity is known to strongly impact biogeochemical cycles (e.g. of  
186 sulphur, iron or calcium) in the sediment, by promoting, for example, precipitation of iron oxide  
187 in the oxic layer and dissolution of iron sulphide and calcium carbonate in the anoxic layer  
188 (Risgaard-Petersen et al., 2012; Meysman et al., 2015). In the deepest part of the Den Osse  
189 Basin, where the maximum oxygen penetration depth in the sediment is about 2 mm (Seitaj et  
190 al., 2015), this was shown to occur seasonally, as a result of cable bacteria activity (Sulu-  
191 Gambari et al., 2016a, b). This phenomenon could play a major role in foraminiferal calcium  
192 carbonate test dissolution during taphonomic processes.

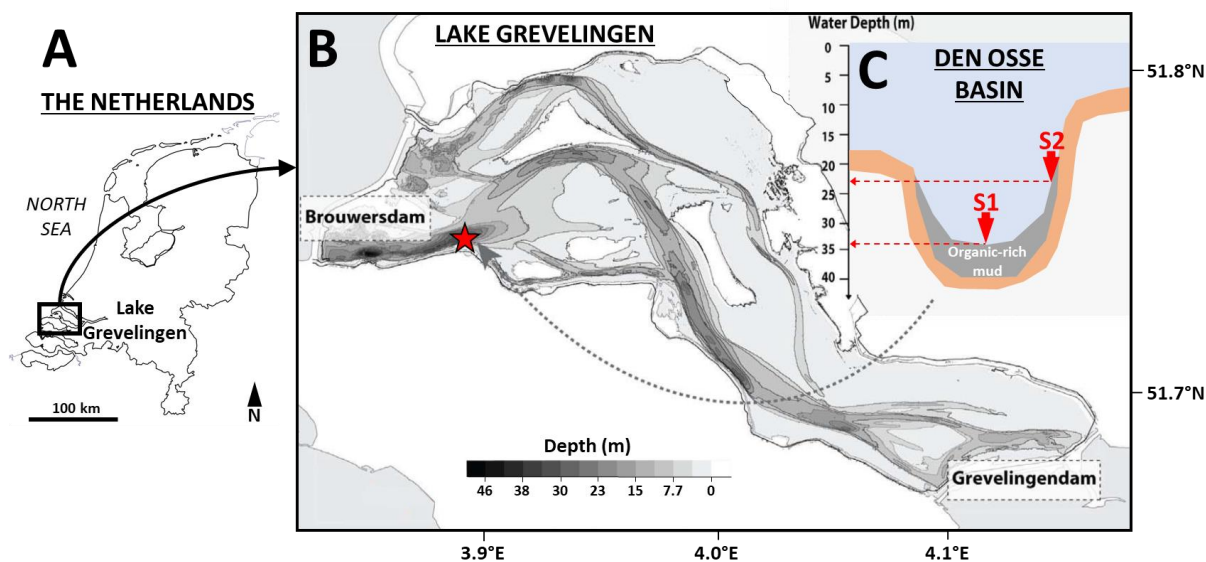


193

194 **Figure 1.** Timeline of the human-induced modifications following the Delta Plan at Lake  
195 Grevelingen. The sediment core used to investigate dead foraminiferal assemblages was  
196 sampled in May 2012 (red line).

197 **3. MATERIALS AND METHODS**

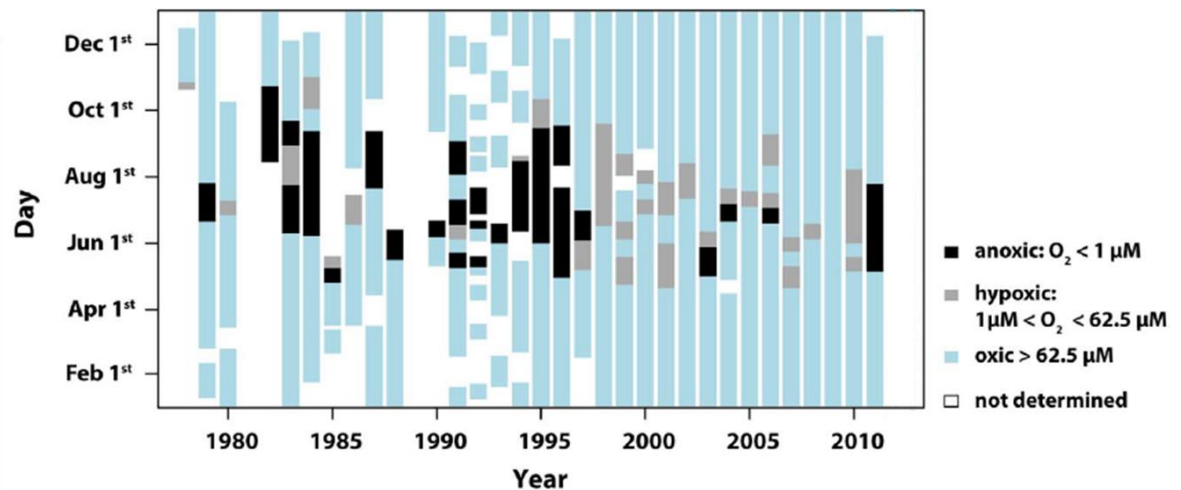
198 **3.1. SAMPLING AND LABORATORY TREATMENT**



199  
200 **Figure 2.** A. Map of The Netherlands (on the left) showing the location of Lake Grevelingen.  
201 B. Map of Lake Grevelingen (main panel) showing the location of the sediment sampling in the  
202 Den Osse Basin (red star). C. The transversal section of the Den Osse basin (top right) shows  
203 the depth at which the studied sediment core was sampled at Station 1 (S1, 34 m depth). Station  
204 2 (S2, 23 m depth) was investigated for living assemblages by Richirt et al. (2020), as noted in  
205 the discussion. This figure was modified from Sulu-Gambari et al. (2016a).

206 The study site (station 1) is in the Den Osse Basin (51° 44.834'N, 3° 53.401'E), the main  
207 former channel of the Grevelingen estuary (The Netherlands), at 34 m depth (Figure 2). Our  
208 site is located at a distance of ~ 100 m from a location where water column oxygen was  
209 intensively monitored since the late 1970s by the Dutch Water Agency (DWA; e.g. Wetsteijn,  
210 2011). Bottom water oxygen concentrations from 1978 to 2011 from the DWA database were  
211 summarised previously (Figure 3; Sulu-Gambari et al., 2017) and reveal recurring seasonal  
212 hypoxia and anoxia in bottom water. These bottom water data were collected up to about 3 m  
213 above the basin floor (and not at the sediment-water interface), which was at 37 m water depth  
214 at this location. Consequently, bottom water anoxia measured by the water column monitoring  
215 (Figure 3) is a reliable indicator of an anoxic sediment-water interface. However, oxic  
216 conditions in the water column (as frequently recorded from late 1990s to 2010, Figure 3), does  
217 not necessarily indicate that the basin floor was also oxic.





218

219 **Figure 3.** Periods of oxic (blue), hypoxic (grey) and anoxic (black) bottom water conditions  
 220 from 1978 to 2011 at the DWA site, which is located at a distance of about 100 m from station  
 221 1 in this study (figure after Sulu-Gambari et al., 2017).

222 A sediment core of 90 cm length and 6 cm diameter was collected at station 1 in May 2012  
 223 with a UWITEC gravity interface corer. In the laboratory, the core was cut into 0.5 cm thick  
 224 slices with an individual sample volume of  $\sim 14.1 \text{ cm}^3$ . All samples were sieved at 63, 125, 150  
 225 and 315  $\mu\text{m}$ , before being dried and stored in the laboratory. For this study, only foraminifera  
 226 of the fraction  $> 125 \mu\text{m}$  (including all fractions above 125  $\mu\text{m}$ ) were picked and stored in  
 227 micropalaeontological Plummer cells. Considering that picking foraminifera is very time-  
 228 consuming, we decided to look at one sample every 2.5 cm (i.e., every fifth sample). Because  
 229 the 54.5–55, 84.5–85 and 89.5–90 cm samples had a very high foraminiferal density (Suppl.  
 230 Table 1), they were split into half (125–150, 150–315 and  $>315 \mu\text{m}$  for the first two, 125–150  
 231  $\mu\text{m}$  fraction for the 89.5–90 cm sample) and complete splits were counted. Conversely, in the  
 232 top 15 cm, many samples were very poor in foraminifera (Suppl. Table 1). Consequently, we  
 233 decided to increase the resolution around these samples by picking adjacent samples. In all, the  
 234 foraminiferal content of 40 samples was studied (Suppl. Table 1). When possible, determination  
 235 was made to the species level using a stereomicroscope.

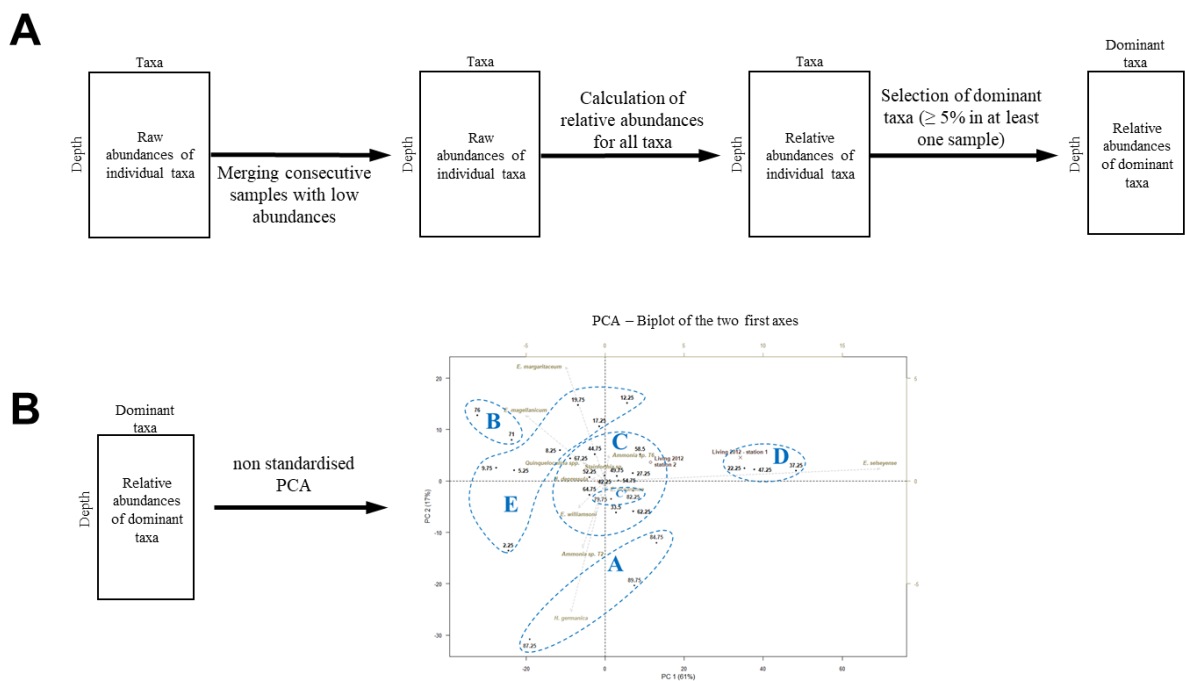
236 A major change in sediment composition was observed, from sandy deposits below 82.5  
 237 cm depth, very probably representative of the strong hydrodynamics of the former estuarine  
 238 system to muddy/silty sediments above 82.5 cm depth, indicative of the much lower  
 239 hydrodynamics of the salt lake environment.

240 **3.2. FORAMINIFERAL ANALYSES**

241 In cases where samples had very low foraminiferal abundances, two to three adjacent  
 242 samples were merged (Table 1). This concerned samples in depth intervals 4.5–6, 6.5–8, 9–  
 243 10.5, 11.5–13, 32–35, 57–60, 69.5–72.5 and 74.5–77.5 cm. We compromised between (1)  
 244 maximising the number of individuals (to obtain a target value of at least 50 individuals) and  
 245 (2) maximising sampling resolution. The total thickness of merged samples never exceeded 3  
 246 cm (Table 1).

247 To document temporal changes in assemblage composition, a Principal Component  
 248 Analysis (PCA, non-standardised) was performed on the relative abundances of the dominant  
 249 species. We considered only dominant taxa (i.e. taxa representing at least 5% of the total  
 250 assemblage in at least one sample) to decrease the signal/noise ratio.

251 Data on living assemblages (0–1 cm depth interval) for our station 1 and additional station  
 252 2 (located at 23 m depth, impacted by a shorter seasonal oxygen depletion event; Figure 2) were  
 253 taken from Richirt et al. (2020) and were computed as the sum of all seasonal samples of 2012.  
 254 These samples were considered as supplementary individuals in the analysis, meaning that they  
 255 did not contribute to the construction of the principal component axes. The data pre-treatment  
 256 and multivariate analysis procedure are summarised graphically in Figure 4.

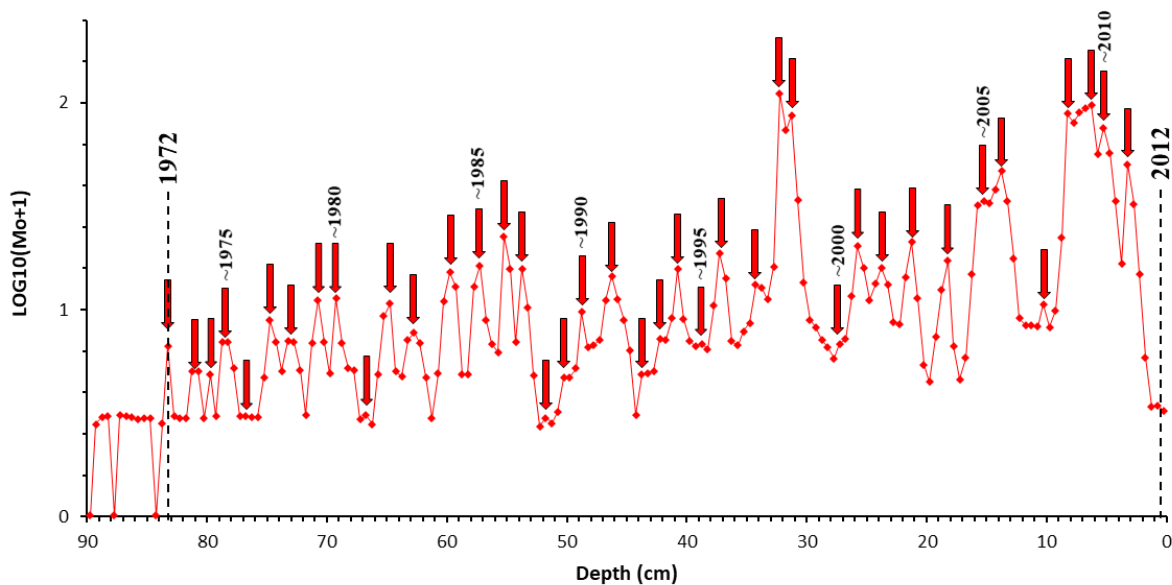


257  
 258 **Figure 4.** A: Data pre-treatment procedure performed from the initial data matrix, with data  
 259 matrix lines representing samples (depth) and data matrix columns representing taxa. B:  
 260 Principal Component Analysis (PCA) procedure.

261 **3.3. AGE MODEL**

262 For another 90 cm long core, sampled in May 2011 at station 1, an age model was developed  
263 using <sup>210</sup>Pb dating (Donders et al., 2012). This age model, which was not very well delimited,  
264 suggested an average sedimentation rate of 2.37 cm per year, meaning that the 90 cm long core  
265 covered about 37 years (1974 to 2011). This order of magnitude confirms our earlier hypothesis  
266 that the very abrupt shift in sediment composition at 82.5 cm core depth corresponds to the  
267 closure of the basin in 1971.

268 For a third core sampled at this site during the same cruise as the sediment core used in this  
269 study (in May 2012, Sulu-Gambari et al., 2017), molybdenum (Mo) concentrations were  
270 measured in the solid phase. Molybdenum is sequestered when sulphide is present in the  
271 porewater near the sediment-water interface (Crusius et al., 1996). Therefore, the successive  
272 Mo maxima in the sediment record should reflect the yearly seasonal hypoxia/anoxia coupled  
273 to the presence of sulphide (Seitaj et al., 2015), and can therefore be used to refine the age  
274 model based on <sup>210</sup>Pb levels. This principle was used earlier for a core from another site in Lake  
275 Grevelingen with an exceptionally high sediment accumulation rate (~ 13 cm y<sup>-1</sup>), in the  
276 Scharendijke Basin, slightly further west of the Den Osse Basin (Egger et al., 2016).



277 **Figure 5.** Molybdenum concentrations (ppm, detection limit = 0.06 ppm) as a function of core  
278 depth (cm), modified from Sulu-Gambari et al. (2017). All values were LOG10(x+1)  
279 transformed to better identify peaks. Each peak is highlighted with a red arrow and is assumed  
280 to represent a yearly summer hypoxia/anoxia event. Some additional yearly hypoxia/anoxia  
281 events have been added tentatively in case of broad peaks with shoulders (e.g., between 31 and  
282 33 cm depth), or broad intervals without clear peaks (e.g., between 50 and 52 cm depth). Dotted  
283 lines represent the two constraints of the age model: the first observed Mo peak corresponding  
284

285 to 1972 (first anoxic/sulphidic event assumed the year following the closure of the former  
286 estuary in 1971) and the surface layer corresponding to the sampling year in 2012.

287 In Figure 5, a very regular, apparently cyclic alternation of maximal and minimal Mo  
288 concentrations can be observed, where Mo maxima should correspond to late summer/early  
289 autumn anoxia. The first peak, at ~ 83 cm depth, which occurred after the closure of the estuary  
290 seaward in 1971, is assumed to represent a first anoxic/sulphidic event in summer/autumn 1972.  
291 Between this first peak at ~ 83 cm and the last peak, representing summer/autumn 2012, we  
292 tentatively placed 39 more annual peaks. In some years, seasonal anoxia may not have  
293 developed, or may have been very short, so that no Mo peaks developed. Another possible  
294 explanation for missing Mo peaks could be that in years with somewhat lower sedimentation  
295 rates, two seasonal Mo peaks may have merged. Consequently, our age model may be two to  
296 three years off.

297 A further potential issue is that Mo concentrations were not measured in the same core  
298 as the one used for the foraminiferal analysis. If we assume that the first Mo peak observed at  
299 ~ 83 cm is characteristic of the first anoxic/sulphidic event that occurred in 1972, the year  
300 following the transition from an estuary to a lake, the closure of the estuary in 1971 should have  
301 taken place directly before, corresponding to a core depth of ~ 84 cm. For the same core, based  
302 on a sedimentation rate of 2 cm y<sup>-1</sup> proposed in Malkin et al. (2014), the transition from an  
303 estuary to a lake was estimated to have occurred at 82 cm depth by Sulu-Gambari et al. (2017;  
304 2018). The same transition was found at 82.5 cm in the core used for foraminiferal analysis.  
305 Consequently, when applying our Mo-based age model on the foraminiferal record, there is an  
306 additional uncertainty of at most 1–2 cm, which could be the result of small differences in  
307 compaction between the cores. In conclusion, we consider that our age model is reliable with a  
308 precision of ± 3 years, which is sufficient for the aims of our study.

309 The average sediment accumulation rate calculated using the putative age model based  
310 on the Mo peaks is 2.06 cm y<sup>-1</sup>. This is consistent with the average sediment accumulation rate  
311 of 2.37 cm y<sup>-1</sup> obtained with the <sup>210</sup>Pb method by Donders et al. (2012).

### 312 **3.4. CALCIUM CARBONATE SATURATION STATE**

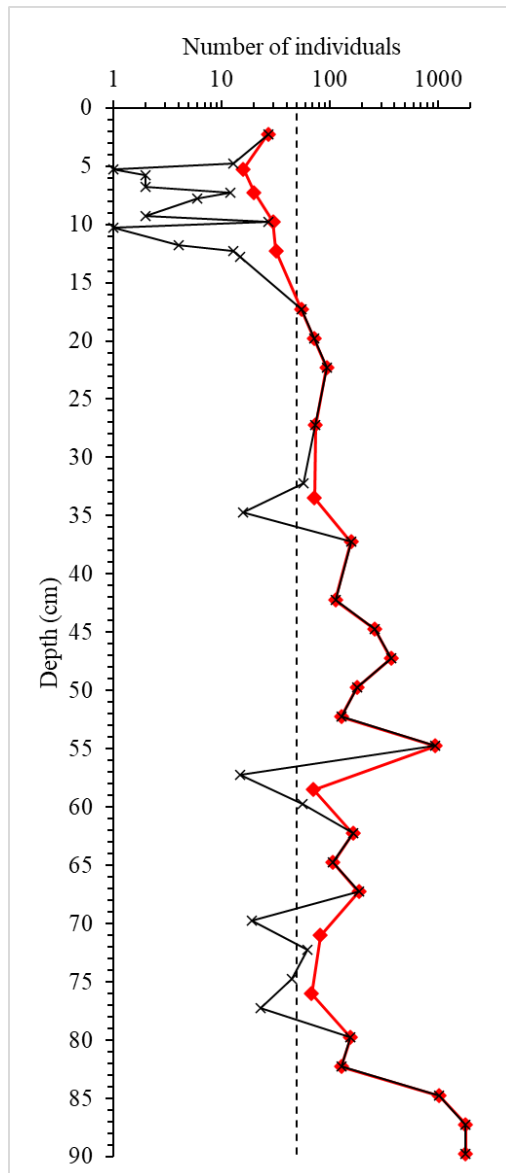
313 We used the CO2SYS software (Lewis and Wallace, 1998) to determine the variation of  
314 the calcium carbonate saturation state in the sediment at our station throughout the year.  
315 Monthly temperature and salinity data of 2012 are from Hagens et al. (2015) and were measured  
316 in the water column at 32 m depth. Monthly values for alkalinity and PO<sub>4</sub><sup>3-</sup> concentrations in

317 the pore waters are from Sulu-Gambari et al. (2016a) and were measured in 0.5 cm thick  
318 sediment slices. The values of pH were obtained by profiling the first 2.5 cm of the sediment  
319 with a microsensor in three replicate cores (data from Seitaj et al., 2015). To compute the  
320 calcium carbonate saturation state, using alkalinity and  $\text{PO}_4^{3-}$  concentrations, pH values had to  
321 be averaged for 0.5 cm layers. Following Orr et al. (2015), we used the set of constant  $K_1$  and  
322  $K_2$  from Lueker et al. (2000), the formula of Dickson (1990) for  $\text{KSO}_4$  and the total boron-to-  
323 salinity ratio from Uppström (1974) for computation. The results of the calcium carbonate  
324 saturation state calculations will be presented in the discussion section because we will only  
325 use it to corroborate a hypothesis about the role of cable bacteria in the putative dissolution of  
326 foraminiferal shells.

## 327 **4. RESULTS**

### 328 **4.1. FORAMINIFERAL COMMUNITIES**

329 The black curve in Figure 6 represents the number of individuals per sample (Suppl. Table  
330 1), whereas the red curve represents the number of individuals after merging of samples when  
331 we were below our target value of 50 individuals. However, even after this merging procedure,  
332 all samples in the top 17 cm of the core were still very poor in foraminifera, with less than 50  
333 individuals (Figure 6 and Table 1).



334

335 **Figure 6.** Total number of individuals in non-merged samples (black curve) and after merging  
 336 samples to a maximum thickness of 3 cm (red curve). The dashed line represents the target  
 337 value of 50 individuals per analysed sample.

338 Eleven of the 23 observed taxa were considered as dominant ( $\geq 5\%$  in at least one  
 339 sample, Suppl. Table 1). Together, these 11 dominant taxa represent more than 90% of the total  
 340 assemblage in almost all samples (Table 1).

341 Total foraminiferal density was relatively high between 90 and 84.5 cm depth, ranging  
 342 from 1033 to 1810 individuals per sample (of 14.1 cm<sup>3</sup>; Figure 6 and Table 1). At 82.5 cm  
 343 depth, the density abruptly decreased by one order of magnitude. Total densities between 82.5  
 344 and 17 cm depth oscillated between 15 (57–57.5 cm depth) and 950 individuals (54.5–55 cm  
 345 depth) per sample, with an average of about 175 ind. per sample.

346 Finally, in the top 17.5 cm of the core, total densities were very low, between 1 and 27  
 347 individuals per sample (Figure 6 and Table 1).

348 **Table 1.** Census data for foraminifera >125 µm in the final reconstituted samples. Relative  
 349 abundances of the dominant species of foraminifera (≥ 5 % of the total assemblage in at least  
 350 one sample), total percentage of dominant taxa and total number of individuals in the  
 351 recomposed samples (consisting of a single sample of 14.1 cm<sup>3</sup>, or 2 or 3 merged samples). We  
 352 also included *A. veneta* (not considered as a dominant species), because we discuss this species  
 353 later in the manuscript. For raw data of all individual samples, see Suppl. Table 1.

Interval depth (cm)	<i>Ammonia aberdoveyensis</i>	<i>Ammonia confertifesta</i>	<i>Ammonia veneta</i>	<i>Elphidium magellanicum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium selseyense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina germanica</i>	<i>Haynesina depressula</i>	<i>Quinqueloculina spp.</i>	<i>Stainforthia sp.</i>	Total dominant taxa (%)	Total number of individuals	Number of merged samples
2–2.5	7.4	0.0	3.7	11.1	14.8	0.0	18.5	11.1	25.9	3.7	0.0	0.0	96.3	27	1
4.5–6	6.3	0.0	0.0	18.8	6.3	0.0	18.8	12.5	0.0	0.0	12.5	0.0	75.0	16	3
6.5–8	0.0	15.0	0.0	15.0	10.0	0.0	30.0	20.0	0.0	0.0	5.0	0.0	95.0	20	3
9–10.5	0.0	0.0	3.3	26.7	10.0	0.0	16.7	6.7	10.0	6.7	6.7	0.0	86.7	30	3
11.5–13	0.0	12.5	0.0	9.4	25.0	0.0	46.9	3.1	0.0	3.1	0.0	0.0	100.0	32	3
17–17.5	0.0	0.0	0.0	10.9	21.8	0.0	40.0	0.0	3.6	1.8	5.5	5.5	83.6	55	1
19.5–20	2.8	8.3	0.0	5.6	29.2	1.4	33.3	0.0	0.0	8.3	1.4	4.2	90.3	72	1
22–22.5	1.1	1.1	2.1	0.0	11.7	0.0	74.5	2.1	3.2	1.1	0.0	0.0	96.8	94	1
27–27.5	4.1	6.8	2.7	8.1	12.2	2.7	47.3	5.4	5.4	0.0	0.0	0.0	94.6	74	1
32–35	11.0	15.1	0.0	0.0	9.6	2.7	41.1	6.8	8.2	1.4	0.0	0.0	95.9	73	2
37–37.5	0.0	0.0	0.6	0.0	6.3	0.0	87.3	3.2	0.6	1.3	0.0	0.0	99.4	158	1
42–42.5	7.1	0.9	0.9	3.5	21.2	0.9	40.7	4.4	8.8	7.1	0.0	0.0	95.6	113	1
44.5–45	6.1	2.3	3.4	6.8	19.8	0.0	38.0	3.4	3.8	7.2	1.1	0.0	92.0	263	1
47–47.5	1.4	3.8	0.5	2.4	6.8	0.0	77.0	2.4	1.4	1.6	1.6	0.0	98.9	370	1
49.5–50	2.8	3.3	0.6	3.3	20.0	1.7	43.3	2.2	11.1	5.6	0.0	0.0	93.9	180	1
52–52.5	7.0	0.8	2.3	1.6	20.3	1.6	35.9	5.5	7.0	10.9	0.8	0.0	93.8	128	1
54.5–55	6.1	1.1	0.6	4.0	14.7	0.6	43.6	5.1	5.9	10.5	1.7	0.0	93.9	950	1
57–60	1.4	0.0	2.8	14.1	9.9	0.0	50.7	1.4	2.8	11.3	4.2	0.0	98.6	71	2
62–62.5	8.5	0.0	1.2	0.6	12.1	2.4	46.7	6.1	9.7	4.8	1.8	0.0	93.9	165	1
64.5–65	10.2	0.0	0.9	4.6	14.8	2.8	36.1	4.6	7.4	7.4	0.9	0.0	89.8	108	1
67–67.5	11.2	0.0	0.5	4.3	23.0	0.5	32.1	7.0	2.7	8.0	4.8	0.0	94.1	187	1
69.5–72.5	11.0	0.0	1.2	7.3	31.7	1.2	18.3	6.1	4.9	6.1	2.4	0.0	90.2	82	2
74.5–77.5	4.4	0.0	1.5	35.3	19.1	0.0	14.7	4.4	4.4	5.9	5.9	0.0	95.6	68	2
79.5–80	5.7	0.0	0.6	3.8	15.3	4.5	42.0	5.1	11.5	6.4	1.9	0.0	96.8	157	1
82–82.5	3.9	0.0	0.0	9.4	7.8	6.3	47.7	5.5	6.3	6.3	0.8	0.0	93.8	128	1
84.5–85	10.2	0.0	1.7	3.4	0.8	1.5	52.3	6.4	10.2	4.5	1.0	0.1	91.9	1033	1
87–87.5	17.3	0.0	1.5	1.9	0.8	1.4	21.0	8.8	31.3	6.2	0.3	0.0	90.6	1810	1
89.5–90	13.6	0.0	1.3	0.9	0.2	0.9	46.9	7.1	18.8	3.5	0.5	0.0	93.7	1805	1

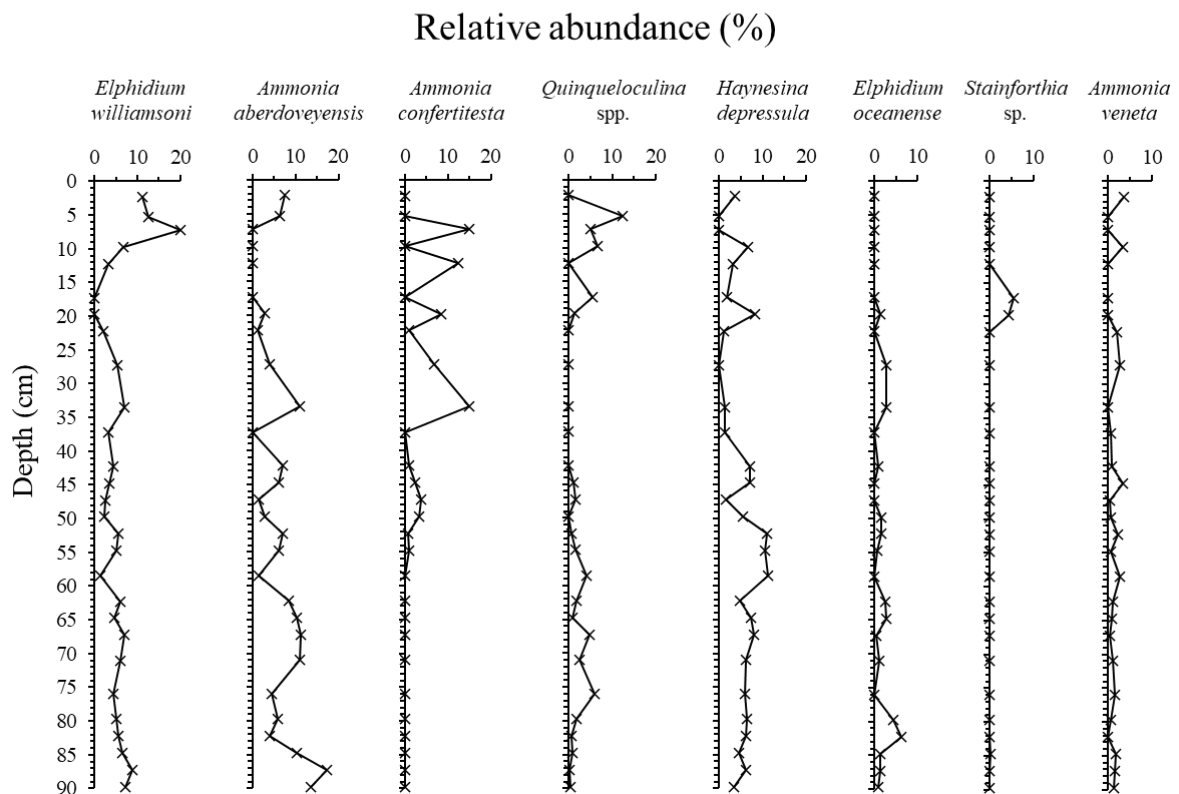
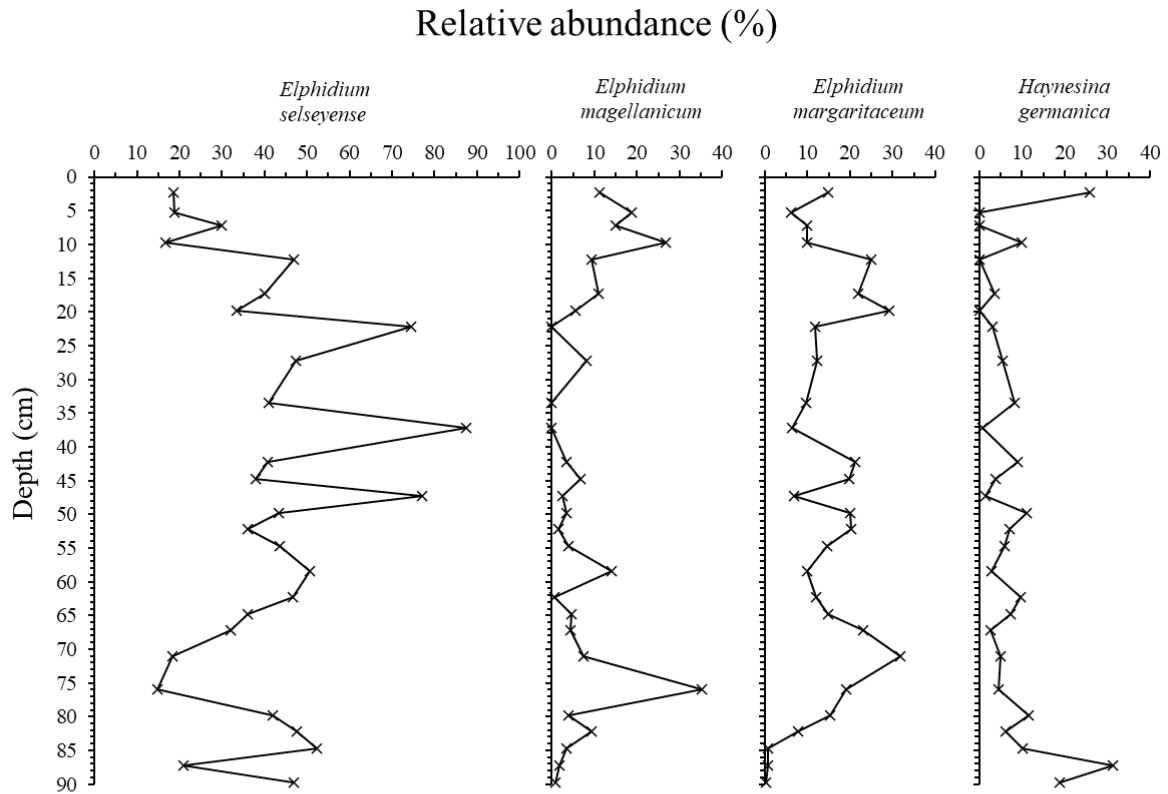
354 *Elphidium selseyense* was the dominant species in most of the samples, accounting for  
355 15 to 87 % of the total assemblage (Figure 7, Table 1). The levels 47–47.5, 37–37.5 and 22–  
356 22.5 cm presented peak values for this taxon (>70 %), whereas the assemblages of the top 10.5  
357 cm were rather poor in *E. selseyense* (between 17 and 30 %).

358 The relative abundance of *E. magellanicum* oscillated between 0 and 35 %. A major  
359 peak was present at 74.5–77.5 cm (35 %). This species showed relatively high percentages (9–  
360 27 %) in the low-density samples of the topmost 17.5 cm (Figure 7, Table 1).

361 *Elphidium margaritaceum* was nearly absent between 89.5–90 and 84.5–85 cm. Above  
362 these levels, relative abundances varied between 6 and 32 % with maxima around 70 cm depth,  
363 from 52–52.5 to 42–42.5 cm depth, and from 19.5–20 to 11.5–13 cm depth (Figure 7, Table 1).

364 The relative proportions of *H. germanica* fluctuated generally between 1 and 10 %. This  
365 species was abundant (19 and 31 %) in the first two samples (89.5–90 cm and 87–87.5 cm  
366 depth), whereas it was present in low numbers, or even absent, in the 20–4.5 cm interval.  
367 *Haynesina germanica* showed again an elevated percentage (26 %) in the most recent sample  
368 (2–2.5 cm depth, Figure 7, Table 1).





369

370 **Figure 7.** Relative abundances (%) of the dominant species as a function of depth (cm). We  
 371 also included *A. veneta* (not considered as a dominant species), because we discuss this species  
 372 later in the manuscript.

373 All other taxa only rarely contributed more than 10 % in the total assemblage (Figure 7,  
 374 Table 1). *Elphidium williamsoni* showed maximum values (11–20 %) in the top 10 cm of the  
 375 core, whereas *Elphidium oceanense* always accounted for only low percentages (0–6 %),  
 376 slightly decreasing upward. This taxon was absent in the top 17.5 cm. *Ammonia aberdoveyensis*  
 377 showed a general decrease from about 10–17 % at the bottom of the core to a near absence at  
 378 about 20 cm depth. However, it showed higher percentages again (6–7 %) in the topmost two  
 379 samples (4.5–6 and 2–2.5 cm depth). *Ammonia confertitesta* was totally absent until 55 cm core  
 380 depth and fluctuated between absence and 15 % thereafter. *Ammonia veneta* was always present  
 381 in low percentages, never exceeding 4 %, without a clear pattern. *Quinqueloculina* spp. showed  
 382 two main presence intervals, between 80 and 44.5 cm (up to 6 %) and from 20 to 4.5 cm depth  
 383 (up to 13 %). *Haynesina depressula* (up to 11 %) tended to be more abundant in the lower 40  
 384 cm of the core. Finally, *Stainforthia* sp. was absent in most samples, but was present at 19.5–  
 385 20 and 17–17.5 cm depth (with 4 and 6 %, respectively).

## 386 4.2. MULTIVARIATE ANALYSIS

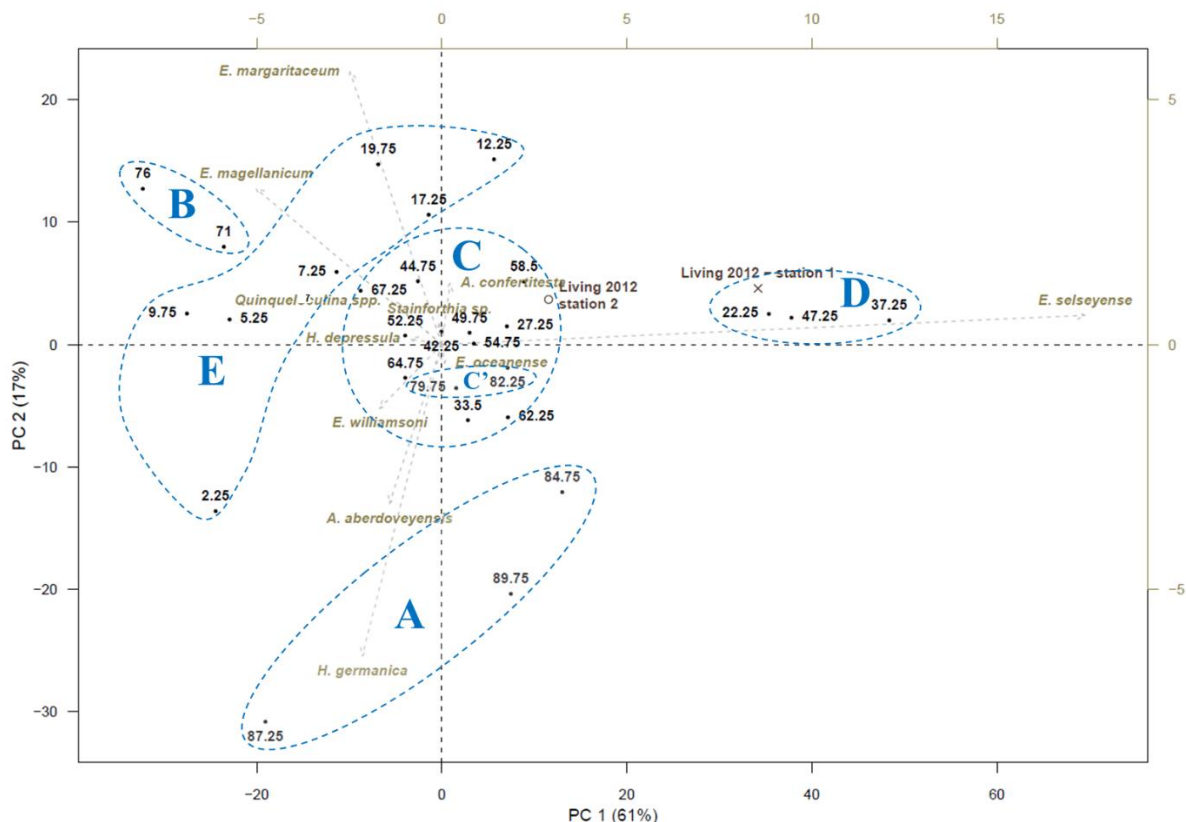
387 The first two axes of the non-standardised PCA based on relative abundance data account  
 388 for 61 % and 17 % of the total variability of the dataset, respectively (Suppl. Table 2).

389 Table 2 presents the contributions of the dominant taxa to the first two PCs. *Elphidium*  
 390 *selseyense* has a strong positive contribution to PC1, whereas *E. magellanicum* has a slight  
 391 negative contribution to this axis. *Elphidium margaritaceum* and *E. magellanicum* have a  
 392 positive contribution to PC2, whereas *H. germanica* and *A. aberdoveyensis* load negatively on  
 393 this axis. Figure 8 presents a scatter plot for PC1 and PC2, in which we added the living  
 394 assemblages of station 1 (same station as the long core) and of the nearby, shallower station 2  
 395 (water depth 23 m) which is less severely affected by seasonal hypoxia/anoxia (Richirt et al.,  
 396 2020), as supplementary individuals.

397 **Table 2.** Taxa contributions to the first two PCs from the PCA. Values in bold are superior to  
 398 the average contribution (|9.09|, if all species would contribute equally).

Species / PC contribution	PC 1	PC 2
<i>Ammonia aberdoveyensis</i>	-0.6	<b>-10.9</b>
<i>Ammonia confertitesta</i>	0.0	1.6
<i>Elphidium selseyense</i>	<b>87.5</b>	0.4
<i>Elphidium magellanicum</i>	-7.3	<b>10.4</b>
<i>Elphidium margaritaceum</i>	-1.8	<b>32.0</b>

<i>Elphidium williamsoni</i>	-0.8	-1.8
<i>Elphidium oceanense</i>	0.0	-0.2
<i>Haynesina germanica</i>	-1.3	<b>-41.8</b>
<i>Haynesina depressula</i>	-0.2	0.0
<i>Quinqueloculina</i> spp.	-0.5	0.8
<i>Stainforthia</i> sp.	0.0	0.2



399

400 **Figure 8.** Scatter plot showing the taxa loadings and sample scores on the first two PCA axis.  
 401 Core depth (cm) is indicated in black. Dominant taxa are represented by arrows. Supplementary  
 402 individuals (living assemblages observed in 2012 from stations 1 and 2; Richirt et al., 2020)  
 403 are indicated by a cross and a circle for stations 1 and 2, respectively. Blue dotted lines show the  
 404 different, arbitrarily defined, groups of samples.

405 In Figure 8, five groups of samples have been recognised arbitrarily based on their  
 406 position on the PCA scatter plot and their proximity in the core:

407 **Group A** is constituted of samples with high negative scores on PC2, reflecting high  
 408 proportions of *A. aberdoveyensis* and *H. germanica*. This concerns the samples from 90 to 84.5  
 409 cm core depth.

410 **Group B** contains samples with strongly negative scores on PC1 and positive scores on  
 411 PC2, characterised by high relative abundances of *E. magellanicum* and *E. margaritaceum* and  
 412 relatively low percentages of *E. selseyense*. This concerns the 74.5–77.5 and 69.5–72.5 cm

413 samples, which are both merged samples because the number of individuals in the separate  
414 samples was very low.

415 **Group C** is composed of samples with low absolute scores on both PCs (i.e. centred on  
416 the scatter plot, Figure 8), indicative of average assemblages. These “typical” assemblages are  
417 dominated by *E. selseyense*, have a high percentage of *E. margaritaceum*, a lower percentage  
418 of *A. aberdoveyensis* and *H. germanica* (compared to group A), and contain *A. confertitesta*  
419 from 55 cm onward. This concerns most samples from 67.5 to 27 cm depth as well as the 82–  
420 82.5 and 79.5–80 cm samples (subgroup C'). The living community of station 2 (Den Osse  
421 Basin, 23 m water depth) plots together with this group.

422 **Group D** is composed of three samples with very high scores on PC1, reflecting  
423 uncommonly high percentages of *E. selseyense*. This concerns the 22–22.5 cm, 37–37.5 cm and  
424 47–47.5 cm samples. The living assemblage of station 1 is very similar, as shown by its  
425 proximity on the scatter plot (Figure 8).

426 **Group E** groups samples intermediate between groups B and C, mostly with negative  
427 scores on PC1, and positive scores on PC2. Assemblages in these samples are characterised by  
428 high relative abundances of *E. magellanicum* and *E. margaritaceum*, although lower than the  
429 samples of group B. Group E contains all samples between 20 and 2 cm core depth. The 2–2.5  
430 cm sample is rather far away from the other samples of this group due to its high percentage of  
431 *H. germanica* (Table 1). However, most samples in group E show a low absolute total  
432 abundance, so that minor changes in the counts may lead to substantial changes in relative  
433 proportions.

## 434 **5. DISCUSSION**

### 435 **5.1. TEMPORAL EVOLUTION OF THE FORAMINIFERAL COMMUNITY**

436 Here we will focus on the temporal changes in foraminiferal densities and assemblage  
437 composition through time, and we will investigate whether it is possible to link the main  
438 assemblage changes to the anthropogenic modifications of Lake Grevelingen during the last 50  
439 years (Figure 9). As we look at 0.5 cm thick layers in a context where the average sediment  
440 accumulation rate is about 2 cm y<sup>-1</sup>, individual layers may represent a single season and not a  
441 complete year. Consequently, samples with high foraminiferal densities could be deposited  
442 during spring/early summer seasons, whereas samples with low foraminiferal densities could  
443 be representative of the late summer/autumn/winter seasons.

444 Additionally, we judge that vertical displacements of foraminiferal shells, either caused by  
445 the vertical migration of infaunal species (when alive) or by macrofaunal bioturbation (living  
446 as well as dead individuals), are strongly limited in the sedimentary environment studied here.  
447 The severe anoxic and sulphidic conditions at this site (sulphide was present in the pore water  
448 below 2 cm depth during the whole year in 2012, Seitaj et al., 2015) largely restrict the presence  
449 of macrofauna and probably limit their bioturbation to the top 2 cm at most (Seitaj et al., 2017;  
450 Hermans et al., 2020). For the same reason, living foraminifera are not expected to dwell deeper  
451 than the superficial oxic layer, being limited by the sulphidic conditions immediately below.

452 Finally, the high sedimentation rate (about 2 cm.y<sup>-1</sup>) further decreases the possibility of  
453 mixing of sediment layers with different ages, suggesting that most samples contain  
454 contemporaneous assemblages, representing short periods of time (a single season to a year at  
455 most).

456 As explained before, the Principal Component Analysis allows us to recognise five groups  
457 of samples, each representing different vertical intervals from the sediment core, and therefore,  
458 successive stages of the evolution of the Lake Grevelingen.

459 **Group A** includes samples from 90 to 84.5 cm depth (estimated period ~ 1968 to 1971).  
460 They contain a sandy sediment, and are situated immediately below the shift to a much finer  
461 sediment at 82.5 cm. These samples represent the estuarine phase of the basin, before its  
462 seaward closure in 1971. The foraminiferal assemblages are characterised by high density  
463 (between 1000 and 2000 ind. per 14.1 cm<sup>3</sup> sample), and by high percentages of typical  
464 estuarine/mudflat species such as *H. germanica* and *A. aberdoveyensis* (e.g. Alve & Murray,  
465 1994; 2001; Thibault de Chanvalon et al., 2015; Saad & Wade, 2016; 2017). In view of the  
466 location of station 1, in the middle of the Den Osse Basin at 34 m depth (the main former  
467 channel of the Grevelingen estuary), the sandy sediment reflects the strong hydrodynamics of  
468 this site, when it was still an estuary. Currents were too strong to allow deposits of clays and  
469 silts, so that the former channel floor was covered by a sandy lag deposit, with a very high  
470 foraminiferal density. The strong currents undoubtedly facilitated the transport of allochthonous  
471 foraminifera, such as the taxa cited above, which are typical of estuarine mudflats. However,  
472 *H. depressula*, a species typical of marine influence (Murray 1983; Darling et al., 2016) is  
473 present with substantial proportions as well, showing that foraminifera were not only  
474 transported from the inner to the outer estuary, but also from the North Sea into the outer  
475 estuary.

476 **Subgroup C'** of group C includes the next two samples, from 82.5 to 79.5 cm depth  
477 (estimated period ~ 1972 to 1975). The sediment is mainly composed of clay/silt, confirming

478 its deposition after the seaward closure of the basin, when hydrodynamics became much  
479 weaker. The strong drop of foraminiferal densities (from higher than 1000 to less than 100 ind.  
480 per sample) shows that these samples no longer constitute a lag deposit, but rather that the  
481 foraminiferal populations are diluted by supplies of fine-grained sediment (about 2 cm y<sup>-1</sup>). The  
482 weaker hydrodynamics, together with the suppression of tides explain why the foraminiferal  
483 assemblage is much less affected by transport, and probably represents an autochthonous  
484 community.

485         The assemblages of these two samples show a strongly increased percentage of the more  
486 marine species *E. margaritaceum* and the euryhaline species *E. oceanense* (Darling et al.,  
487 2016), contrasting with a strong decrease of the percentages of the more estuarine species *H.*  
488 *germanica* and *A. aberdoveyensis*. This suggests that in the first years after the closure of the  
489 former estuary, the basin floor was still inhabited by species typical of marine influence (high  
490 salinity), whereas inner estuarine species were no longer living, or transported to the study area.  
491 Salinity, measured on surface waters, was consistently high (~ 30) in the years immediately  
492 following the closure of the basin (1972–1974, Bannink et al., 1984), allowing marine taxa to  
493 inhabit the former main channel.

494         **Group B**, which represents the 77.5 to 69.5 cm interval (estimated period ~ 1975–1980)  
495 contains only two merged samples (i.e. 74.5–77.5 and 69.5–72.5 cm). Their assemblage  
496 composition is somewhat peculiar, because of the high relative proportions of *E. magellanicum*  
497 and *E. margaritaceum*, contrasting with the relatively low, but still substantial contribution of  
498 *E. selseyense*.

499         In this period, surface water salinity showed a progressive decrease, from ~ 30 in 1974  
500 to a minimum of ~ 23 in 1978, due to the input of rainwater (Bannink et al., 1984). In case these  
501 salinity values partly reflect conditions on the basin floor, this could suggest that *E.*  
502 *magellanicum* and *E. margaritaceum* are more tolerant to lower salinities than *E. selseyense*.  
503 Unfortunately, the ecology of these three species is still imperfectly known. In the literature,  
504 the European representatives of *E. magellanicum* are generally considered as euryhaline (20–  
505 30 in Fjords, Gustafsson & Nordberg, 1999; 2000; 10–20 in the western Baltic Sea, Schönfeld  
506 et al., 2018; shallow marine at Dunkirk beach, Lévy et al., 1969). *Elphidium selseyense* appears  
507 to be opportunistic and tolerant to a large range of salinity (Darling et al., 2016). Conversely,  
508 *E. margaritaceum* has been reported as a more marine species which tolerates only slight  
509 salinity variations (Alve & Murray, 1999; Darling et al., 2016).

510         The transition to a lacustrine system also strongly increased the average water residence  
511 in the lake, up to several years, compared to a few days when Grevelingen was an estuary. This

512 strongly modified the ecosystem structure and functioning of Lake Grevelingen (Nienhuis,  
513 1978; Saeijs & Stortelder, 1982). The strengthened water column stratification and the much  
514 longer residence times led to a progressive increase of nutrient concentrations between 1971  
515 and 1978 (Bannink et al., 1984). The opening of the Brouwersdam in late autumn 1978,  
516 resulting in limited salt water inflow, induced a further strengthening of the salinity  
517 stratification of the water column, which led to hypoxia/anoxia and mass mortality in the  
518 deepest parts of the basin in summer 1979 (Bannink et al., 1984).

519 The three *Elphidium* species that dominated the assemblages during this period were  
520 apparently more resistant to these rapidly changing conditions than other taxa. Their tolerance  
521 regarding low oxygen conditions is shown by their presence in the recent assemblages of this  
522 site (Richirt et al., 2020), but also by their presence in fjords affected by seasonal anoxia  
523 (Gustafsson & Nordberg, 1999; 2000).

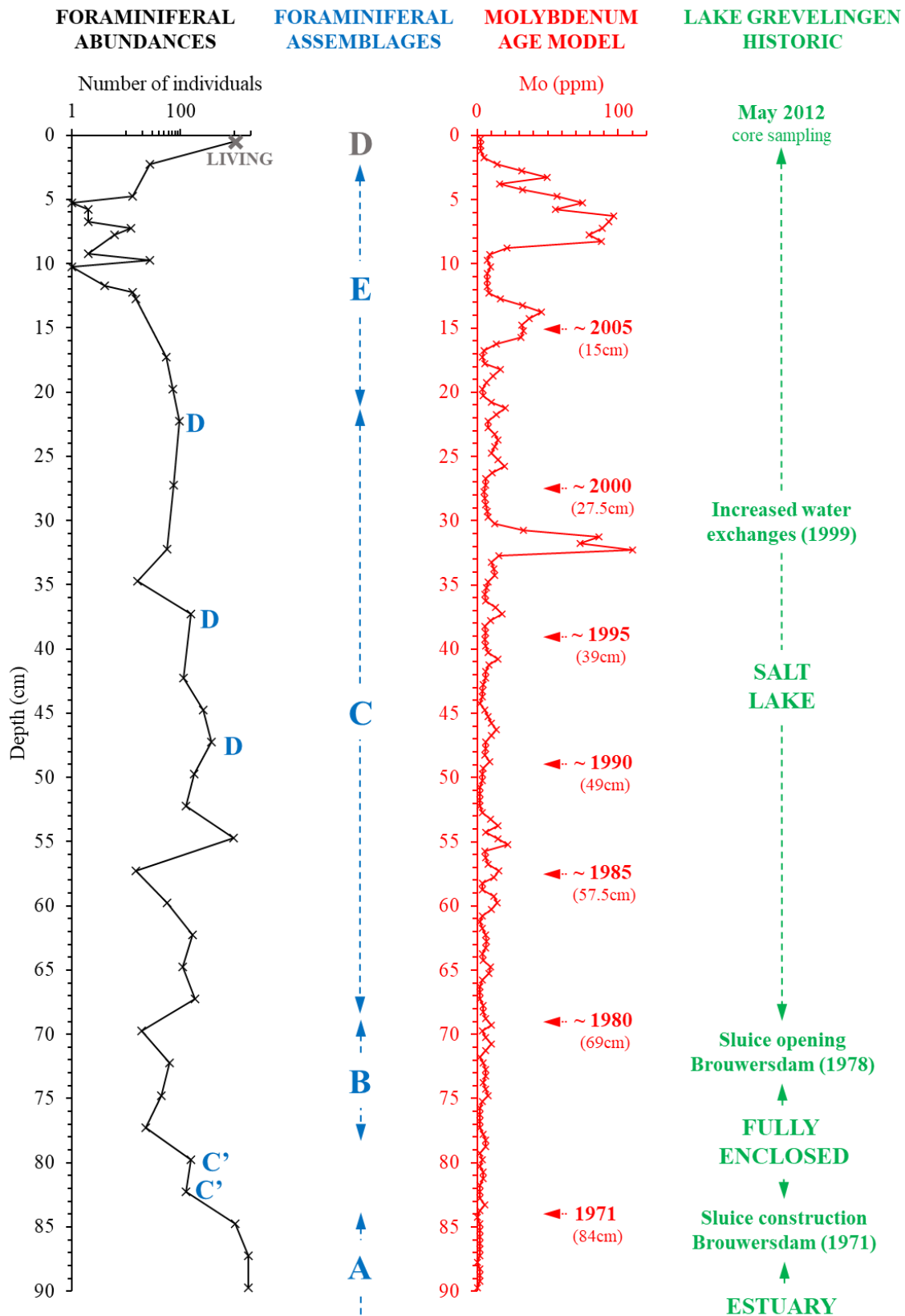
524 The remaining samples of **Group C** represent the 67.5 to 27 cm interval (estimated  
525 period ~ 1980 to 2000). To remedy the increase of eutrophication phenomena observed in the  
526 1970s, the basin was partly reopened by an underground sluice in 1978. This sluice was initially  
527 opened only in winter. Consequently, surface water salinity almost immediately rose to ~ 30  
528 and nutrient concentrations showed a major decrease (Bannink et al., 1984). However, this did  
529 not solve the problem of bottom water oxygen deficiency, and in the next 20 years, an  
530 intermittent succession of major later summer/early autumn hypoxic/anoxic periods was  
531 observed, especially in the deepest parts of Lake Grevelingen (Figure 3). This is confirmed by  
532 the results of Lindeboom & Sandee (1984), who found anoxic conditions coupled with the  
533 presence of sulphide at a few mm depth in the sediment in May and June 1982, at much  
534 shallower stations, with depths < 10 m. In 1999, the sluice was opened year-round, to further  
535 increase water exchanges.

536 During this period (~ 1980 to 2000), the total foraminiferal density showed strong  
537 variations, between ~ 70 and 950 individuals per sample. This alternance of high and lower  
538 foraminiferal density could be due to differences between successive years regarding bottom  
539 water oxygen content (Figure 3; Seitaj et al., 2015; 2017), more specifically due to inter-annual  
540 variability in the intensity and duration of the summer hypoxia/anoxia (with or without sulphide  
541 in the topmost sediment). Richirt et al. (2020) showed that in case of prolonged summer anoxia  
542 combined with the presence of sulphides in the topmost sediment, the foraminiferal community  
543 almost completely disappears, and the site is recolonised the following winter, leading to low  
544 standing stocks the next spring. The strong Mo peak at ~ 32 cm (estimated age ~ 1998, Figure  
545 9), could indicate particularly severe anoxic and sulphidic conditions in the sediment (anoxia

546 with presence of sulphides). This could explain why we observe a very low foraminiferal  
547 density (16 ind. per sample) at ~ 35 cm, immediately after this period. This apparent depth shift  
548 of ~ 3 cm between these two events could be explained by the fact that Mo concentrations and  
549 foraminiferal densities were measured on two different cores (see Materials & Methods  
550 section). Conversely, layers with high foraminiferal densities (such as sample at 54.5–55 cm,  
551 estimated age ~ 1986) could correspond to periods following weaker and/or shorter anoxic  
552 events, eventually without sulphides in the foraminiferal habitat.

553 All samples of group C are strongly dominated by *E. selseyense*, with *E. margaritaceum*  
554 being the second most dominant species. This seems to reflect increased salinity (more marine  
555 influence) and development of seasonal hypoxia favouring opportunistic species such as *E.*  
556 *selseyense*. The arrival of *A. confertitesta*, at about 55 cm core depth (~ 1986) corresponds to a  
557 progressive decrease of *H. depressula*, *Quinqueloculina* spp. and *A. aberdoveyensis*. It is  
558 interesting to note that the assemblage of group C is comparable to the composition of the living  
559 assemblage at the shallower station 2 (depth 23 m), as shown by the PCA scatter plot (Figure  
560 8), suggesting that the environmental conditions may have been comparable, with a rather short  
561 anoxic event in summer (maximum of 1 month, Richirt et al. 2020).





562

563 **Figure 9.** Timeline of the human-induced modifications following the Delta Plan at Lake  
 564 Grevelingen. The sediment core used to investigate dead foraminiferal assemblage density  
 565 record (black curve, excluding the top layer 0.5 cm showing living assemblage in grey) and the  
 566 Mo record (red curve). Both long cores were sampled in May 2022. Blue letters emphasise the  
 567 different groups of layers highlighted in the PCA scatter plot.

568           **Group D** contains only three samples (47–47.5, 37–37.5 and 22–22.5 cm core depth,  
569 deposited in ~ 1991, ~ 1996 and ~ 2003, respectively). The foraminiferal densities in these  
570 samples are comparable to densities from group C and range from 94 to 370 ind. per sample.  
571 The composition of these samples stands out by exceptionally high proportions of *E. selseyense*  
572 (> ~ 75 %). This is very similar to the composition of the living assemblage in 2012 at the same  
573 station (Richirt et al., 2020), as shown by their proximity on the PCA scatter plot. This suggests  
574 that the three samples of group D reflect similar conditions, which could be characterised by  
575 rather severe anoxic conditions with the presence of sulphides in the topmost sediment, as  
576 recorded in 2012 (Seitaj et al., 2015). It appears that *E. selseyense* could be particularly tolerant  
577 to such low oxygen conditions; this is confirmed by its strong dominance in the living  
578 community sampled at this site in 2012 (Richirt et al., 2020).

579           Finally, **Group E** comprises all samples in the top 20 cm of the core (estimated period  
580 ~ 2003 to 2011). This group of samples has been deposited in the years after the decision had  
581 been taken to open the seaward sluice year-round (in 1999), leading to a further decrease in  
582 water residence times. The total foraminiferal densities are extremely low in this interval (rarely  
583 exceeding 15 ind. per sample). Because of the low foraminiferal densities, several samples had  
584 to be merged. In spite of the merging procedure, relative proportions are still based on low  
585 numbers (from 16 to 72 specimens) and should be considered with caution. Even when taking  
586 into account the high sedimentation accumulation rate at this location, the unexpected very low  
587 densities strongly contrast with the elevated densities of living communities, which attained a  
588 maximum of 634 individuals per 14.1 cm<sup>3</sup> sample in May 2012 when the long core was sampled  
589 (recalculated from Richirt et al., 2020; Figure 9).

590           The foraminiferal assemblages of the samples from group E are relatively poor in *E.*  
591 *selseyense*, whereas the percentages of *E. margaritaceum*, *E. magellanicum* and *E. williamsoni*  
592 show a strong increase. *Quinqueloculina* spp. is also somewhat more frequent (Table 1). It is  
593 surprising that the taxonomic composition of the group E samples is very different from the  
594 year-averaged living assemblage from this site (Figure 8).

595           In view of the important density differences between the rich living populations and  
596 poor taphocoenoses, with a very different species composition, we suspect that the dead  
597 assemblages have suffered from important taphonomic losses during early diagenesis,  
598 specifically due to carbonate dissolution. In fact, in the deepest parts of Lake Grevelingen, two  
599 antagonistic bacterial populations occur: cable bacteria develop mainly between January and  
600 May, whereas *Beggiatoceae* mats occupy the sea floor from September to December (Seitaj et  
601 al., 2015). The reasons why these two filamentous S-oxidising bacteria show such an

602 antagonistic seasonal succession is not well understood yet (Seitaj et al. 2015), but it is clear  
 603 that they strongly affect the geochemistry of the topmost sediment layer.

604 **5.2. CABLE BACTERIA ACTIVITY RESPONSIBLE FOR FORAMINIFERAL TEST**  
 605 **DISSOLUTION**

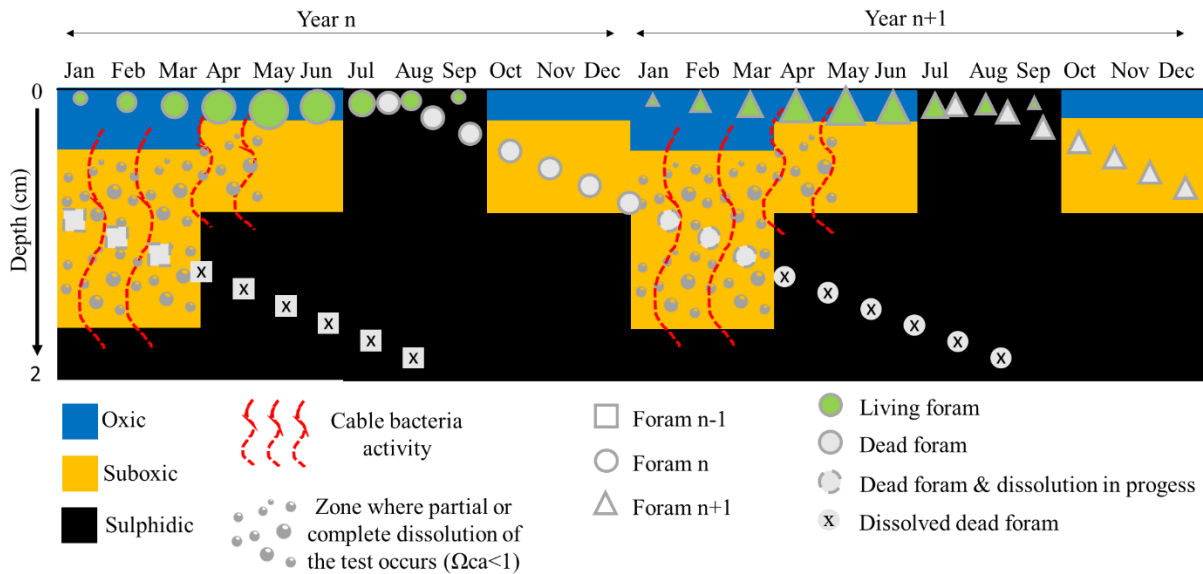
606 Cable bacteria activity promotes carbonate precipitation in the oxic zone (maximum oxygen  
 607 penetration depth about 2 mm at this site, even in winter, see Seitaj et al., 2015), and carbonate  
 608 dissolution immediately below, where oxygen is absent (Risgaard-Petersen et al., 2012;  
 609 Meysman et al., 2015). Consequently, the carbonate saturation state ( $\Omega_{Ca}$ ) is low in the first  
 610 centimetres of the sediment ( $\Omega_{Ca} < 1$  at least down to 2 cm, see Table 3), which could result in  
 611 the partial or complete dissolution of calcareous foraminiferal tests. In fact, for 2012, calculated  
 612  $\Omega_{Ca}$  are  $< 1$  from January (excluding in the top 0.5 cm) to April, when cable bacteria were  
 613 active, indicating strongly increased calcium carbonate ( $CaCO_3$ ) dissolution (Table 3). This is  
 614 corroborated by a release of  $Ca^{2+}$  measured in the first centimetres in pore waters at the same  
 615 station in January and March (Sulu-Gambari et al. 2016a).

616 **Table 3.** Values of calcium carbonate saturation state at station 1 at different depths in the  
 617 sediment for each month of 2012. Red:  $\Omega_{Ca} < 1$ , indicating increased carbonate dissolution.  
 618 Blue:  $\Omega_{Ca} > 1$ , indicating no carbonate dissolution. NA: data not available.

Depth (cm)	January	February	March	April	May	June	July	August	September	October	November	December
0.25	2.30	NA	NA	0.91	NA	1.93	3.08	NA	1.86	NA	2.81	3.06
0.75	0.54	0.90	0.68	0.70	NA	2.39	2.67	NA	2.30	7.46	9.62	14.68
1.25	0.29	0.41	NA	0.55	NA	2.41	2.87	NA	2.90	NA	14.76	22.66
1.75	0.20	0.27	0.24	0.51	NA	2.13	2.87	NA	NA	4.60	NA	21.11
2.25	NA	0.22	0.23	0.67	NA	1.99	2.75	NA	3.95	5.03	5.90	13.49
2.75	NA	0.21	NA	0.61	NA	2.40	2.78	NA	NA	NA	NA	7.75
3.25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.29	6.98
3.75	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.68	NA

619 Living foraminifera have mechanisms to deal with a lower carbonate saturation state  
 620 (e.g. active control of their internal and external pH during the calcification process,  
 621 intracellular storage of  $Ca^{2+}$  ions, Toyofuku et al., 2008; de Nooijer et al., 2009; Toyofuku et  
 622 al., 2017). When dwelling in environments with a lower carbonate saturation state, living  
 623 specimens only rarely show serious signs of test dissolution (e.g. Charrieau et al., 2018). Hence,  
 624 most dissolution is thought to occur after the death of the foraminifera. Furthermore, we note  
 625 that the dense living populations, which are not much affected by carbonate dissolution,

626 predominantly live in the thin oxic zone (only a few mm), which is not affected by the strongly  
 627 decreased  $\Omega_{Ca}$  (Seitaj et al. 2015). Only when the tests of dead foraminifera passively cross  
 628 this low  $\Omega_{Ca}$  zone (due to sediment deposition at the sediment water interface), assemblages  
 629 are probably affected by important losses due to carbonate dissolution. The very low  
 630 foraminiferal densities in the top 20 cm of the core suggest that this situation has existed since  
 631 about 2003, some years after the intensification of water exchanges. In the cartoon presented in  
 632 Figure 10, the seasonal succession of events is illustrated.



633 **Figure 10.** Conceptual scheme showing two consecutive years to explain the low density of  
 634 dead foraminifera (in grey) in the first 20 cm of the core. The seasonal succession of oxic (blue),  
 635 suboxic (orange) and sulphidic (black) zones and presence of cable bacteria (red dotted curves)  
 636 are from Seitaj et al. (2015). Presence and densities (size of the signs) of living foraminifera (in  
 637 green) are from Richirt et al. (2020). Squares, circles and triangles represent the yearly  
 638 successive foraminifera generations.  
 639

640 If our hypothesis that the low density of foraminifera in the top 20 cm is due to  
 641 dissolution resulting from cable bacteria activity is correct, this implies that cable bacteria  
 642 activity in the Den Osse Basin started, or substantially increased between 2000 and 2005. This  
 643 fits remarkably well with the year-round opening of the Brouwersdam sluice in 1999.

644 The increased inflow of saline, warm and well oxygenated water induced by the opening  
 645 of the Brouwersdam sluice in 1999 should have improved the oxygenation and led to a  
 646 reduction of the duration of anoxic events in the bottom waters compared to the situation before  
 647 1999 (Figure 3; Wetsteijn, 2011; Sulu-Gambari et al., 2017). However, the increased  
 648 concentration and large peaks in the Mo record in the last ~ 30 cm of the core suggest the  
 649 presence of increased sulphide concentrations in the pore waters during the same period. Sulu-  
 650 Gambari et al. (2017) explained this contradiction (i.e. the better oxygenation of bottom waters

651 but development of more sulphidic conditions in sediment) by the introduction of large amounts  
652 of phytoplankton (mainly *Phaeocystis globosa*) into Lake Grevelingen from the North Sea  
653 (Hagens et al. 2015) since ~ 2000. The intensified remineralisation of organic matter induced  
654 by this extra input of dead algal material enhanced the sulphate reduction rate resulting in an  
655 increase of the porewater sulphide concentration. This process may have contributed to the  
656 exceptionally long hypoxia/anoxia in 2011 and 2012, compared to the period 1999–2010 (Sulu-  
657 Gambari et al. 2017).

658 The suggested increased cable bacteria activity resulting from the increased water flow  
659 in 1999 is corroborated by the fact that the presence of an oxygen-sulphide interface is a  
660 prerequisite for the development of S-oxidising bacteria, such as cable bacteria and *Beggiatocea*  
661 (Jørgensen, 1982).

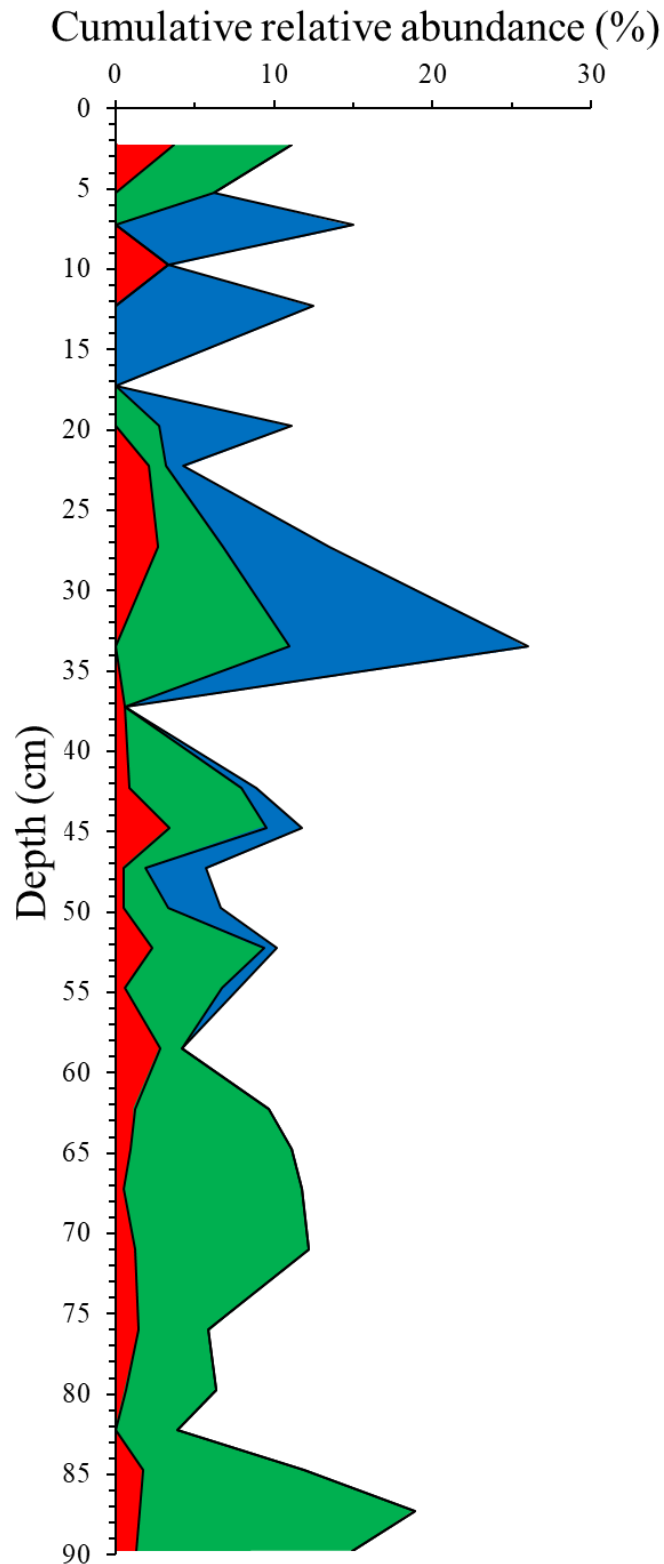
### 662 **5.3. TEMPORAL SUCCESSION OF AMMONIA SPECIES**

663 The historical record for Lake Grevelingen presented here shows the arrival of *A.*  
664 *confertitesta* at 54.5–55 cm core depth (estimated year ~ 1986), and its subsequent progressive  
665 replacement of *A. aberdoveyensis* (Figure 11). It has already been suggested that *A. confertitesta*  
666 could be an exotic and/or invasive species originating from east Asia and introduced in the  
667 eastern Atlantic Ocean by anthropogenic activities (Pawlowski & Holzmann, 2008; Schweizer  
668 et al, 2011; Bird et al., 2020). Concerning the other two representatives of the *Ammonia* genus  
669 observed in our sediment core, *A. veneta* is considered cosmopolitan (Holzmann & Pawlowski,  
670 2000; Hayward et al., 2021) whereas *A. aberdoveyensis* seems to be restricted to the north  
671 Atlantic (Hayward et al., 2004; Hayward et al., 2021). The latter two species are both assumed  
672 autochthonous in Europe and could at present be progressively replaced by the seemingly exotic  
673 *A. confertitesta* (Pawlowski & Holzmann, 2008; Schweizer et al. 2011).

674 This species replacement suggests that *A. confertitesta* is more resistant than *A.*  
675 *aberdoveyensis* to the seasonal hypoxia/anoxia found in Lake Grevelingen after the closure in  
676 1971. This hypothesis is corroborated by the much higher test porosity measured in *A.*  
677 *confertitesta* compared to *A. aberdoveyensis* ( $22.45 \pm 3.85$  % versus  $12.18 \pm 3.8$  %,   
678 respectively, Richirt et al. 2019a), suggesting that this phylotype could resist oxygen deficiency  
679 by increasing its gas exchanges with the surrounding environment. The fact that *A. confertitesta*  
680 is rather poorly represented (about 5 % of the total assemblage) in the living assemblages in  
681 2012 was explained by a phase offset between: 1) the time of repopulation of the site in winter

682 2011–2012 after its decimation due to anoxic and sulphidic conditions in summer 2011, and 2)  
683 the availability of its preferred food sources, in spring 2012 (Richirt et al. 2020).

684 Alternatively, the rather poor density of *A. confertitesta* could also mean that this taxon  
685 tolerates hypoxia and short phases of anoxia but is not able to withstand prolonged periods of  
686 oxygen depletion, together with a presence of sulphides in its microhabitat. This could explain  
687 the much higher percentage of this taxon in the living assemblages of station 2 (shallower  
688 station in Den Osse Basin), where the duration of seasonal hypoxia/anoxia is shorter than at  
689 station 1 (Richirt et al. 2020).



690

691 **Figure 11.** Cumulative relative abundances (in % of the total assemblage) of the three different  
 692 *Ammonia* species *A. veneta* (red), *A. aberdoveyensis* (green) and *A. confertitesta* (blue) as a  
 693 function of core depth (cm). Please keep in mind that total densities are relatively low in the top  
 694 15 cm of the core (~15-30 ind. per sample), meaning that small variations in absolute  
 695 abundances induce large variations in relative abundances.

696 Finally, *A. confertitesta* is virtually the only *Ammonia* species found in recent living  
697 communities (~ 5 % of the 1044 individuals, total foraminiferal assemblage, Richirt et al.,  
698 2020). In view of the strong anoxic events with co-occurring sulphidic conditions in 2011 and  
699 2012 at this site (Seitaj et al. 2015; Richirt et al. 2020), this underlines the higher resistance of  
700 *A. confertitesta*, compared to *A. veneta* and *A. aberdoveyensis*, to such adverse conditions.

701 Today, *A. confertitesta* is found in all (closed or open) branches of the Rhine-Meuse-Scheldt  
702 estuary (Jorissen, unpublished data). We propose two hypotheses for the arrival of *A.*  
703 *confertitesta* in Lake Grevelingen (i.e. Den Osse Basin), around 1986:

- 704 1) *Ammonia confertitesta* is indeed an exotic species, and its arrival in Lake Grevelingen  
705 was synchronous with its arrival on the eastern Atlantic coast.
- 706 2) *Ammonia confertitesta* was already present in the North Sea, either as an autochthonous  
707 species, or after a first anthropogenic introduction from eastern Asia, and its arrival in  
708 Lake Grevelingen reflects a secondary, local migration event.

709 We note that *A. confertitesta* was not observed in the assemblages of the lower part of the  
710 core (90–85 cm) when Grevelingen was open to the North Sea. This strongly suggests that this  
711 species reached this part of the North Sea between the closure of the lake in 1971 and its arrival  
712 in the basin circa 1986. This would rather argue in favour of its exotic nature, as suggested by  
713 Pawlowski & Holzmann (2008), who hypothesised an introduction of *A. confertitesta* in the  
714 North Sea in the late 19<sup>th</sup> century by means of ship ballast water. Massive imports of Japanese  
715 oysters, from 1964 to about 1980 could also represent a possible vector for *A. confertitesta*, as  
716 this has been reported for a large number of different metazoa (e.g. Polychaeta, Amphipoda,  
717 Tunicata, Cirripedia, etc., Wolff & Reise, 2002).

718 Unfortunately, this study does not allow us to definitely settle whether *A. confertitesta* is an  
719 exotic and/or invasive species in Europe. Even today, *A. confertitesta* is not present in all  
720 estuaries of the mid latitude East Atlantic coast. For instance, it is absent in the French Auray  
721 estuary (Fouet et al., in prep.). Therefore, the absence of *A. confertitesta* in the Grevelingen  
722 estuary before its closure in 1971 could also be due to the fact that this species was not present  
723 in this particular estuary. Finally, the transformation of the Grevelingen estuary to a marine lake  
724 with occurrence of seasonal anoxia may have facilitated the colonisation of the Den Osse Basin  
725 by *A. confertitesta*, putatively more resistant to hypoxia/anoxia than its congeneric species.



726

## 6. CONCLUSION

727 Our investigation of the sediment record of one of the deepest basins in Lake Grevelingen  
728 highlights the important changes undergone by the foraminiferal community over the last 50  
729 years. These changes reflect the major anthropogenic modifications of Lake Grevelingen,  
730 which was artificially turned from an estuary into a marine lake during this period. The Mo  
731 concentrations in the sediment record mark the yearly late summer–early autumn anoxic events  
732 with co-occurring presence of free sulphides in the sediment pore water. This record provides  
733 a very useful tool to detail the more conventional age model based on  $^{210}\text{Pb}$ , allowing us to  
734 obtain a precision of about  $\pm 3$  years.

735 The seaward closure of the basin in 1971 induced an important change in foraminiferal  
736 communities, from high proportions of *H. germanica* and *A. aberdoveyensis*, which are typical  
737 estuarine mudflat species, to assemblages dominated by *E. margaritaceum*, typical for marine  
738 influence, *E. oceanense*, considered as an opportunistic species, and *E. magellanicum*, which  
739 seems to be tolerant to oxygen depletion. This taxonomic change could be explained by the  
740 disappearance of tides, much weaker hydrodynamics, increased eutrophication and increased  
741 salinity immediately after the closure of the basin. The opening of the seaward sluice (in the  
742 Brouwersdam) in autumn 1978, to counterbalance the eutrophication, increased the water  
743 renewal, but did not solve the problem of seasonally occurring hypoxia/anoxia. At first, the  
744 sluice was opened only in winter and *E. selseyense* strongly dominated the foraminiferal  
745 assemblages, suggesting that this species benefited of more marine influence and development  
746 of seasonal hypoxia. Around 1986, the emergence of *A. confertitesta* was observed coupled  
747 with a progressive diminution of the proportion of *A. aberdoveyensis*, *H. depressula* and  
748 *Quinqueloculina* spp. in the assemblages. After the sluice was opened almost year-round in  
749 1999 and a further reduction of the water residence time by doubling the exchange with the  
750 North Sea, the foraminiferal assemblages became very poor, so that the assemblage  
751 composition should be interpreted very carefully. The extreme scarcity of foraminiferal tests in  
752 the top 15 cm of the core, which strongly contrasts with the high abundance of living  
753 foraminiferal communities, is ascribed to post-mortem dissolution, resulting from the strongly  
754 diminished carbonate saturation state due to cable bacteria activity in the anoxic part of the  
755 sediment.

756 Our results indicate that *A. confertitesta* arrived in Lake Grevelingen around 1986 and has  
757 progressively supplanted other *Ammonia* species (*A. veneta* and especially *A. aberdoveyensis*)  
758 in the record. We hypothesise that this progressive takeover is the result of a greater tolerance

759 to seasonal hypoxia/anoxia. However, our results do not definitely confirm the exotic and/or  
760 invasive nature of *A. confertitesta*. The absence of this taxon in the lower part of the core could  
761 also indicate its absence along the Dutch coast in 1971 or that the human-induced  
762 transformation of Lake Grevelingen created more favourable environmental conditions for its  
763 settlement.

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