

Dear Dr Hiroshi Kitazato,

Thank you for giving me the opportunity to read the revised version of Richirt et al's MS resubmitted to Biogeosciences. I have read the revised version, the comments from the two reviewers and the response letters and I think the authors have done a good job revising the MS. However I have a few comments of more technical nature, which I hope the authors correct before publication.

The introduction and the aim are now well described, however, I lack a map of the study site. In particular, since this study is very much focused on one particular area.

As suggested by the reviewer, we added a map as figure 1 in the revised manuscript (with reference line 150).

On row 162, there should be one centimetre, to make clearer it was 0-1 cm that was studied.

We added between brackets that we considered the first centimetre (line 162):

*“For each replicate, the uppermost centimetre (0–1 cm) of the core was then transferred on board in a vial of 250 mL, and 30 mL of seawater (at the same temperature than in situ) was added in the vial.”*

row 191-195 can be excluded, it doesn't move the text forward that the authors highlight that molecular data for *E. magellanicum* is lacking. One only wonders why they didn't do the analyses then.

We would prefer to keep the statement about our attribution of *E. magellanicum* formal name to our specimens. In our opinion, this emphasizes two important things:

- 1- Contrary to *Ammonia* sp. T6 and *Elphidium selseyense*, we have no molecular data about assignation of these specimens to this formal name, thus our taxonomic attribution is less certain.
- 2- No fresh live material was collected at the time of sampling, therefore it was not possible to sequence *E. magellanicum* from Grevelingen. In addition, molecular analyses on what we usually name *E. magellanicum*, publicly unavailable yet (no

sequences published in our knowledge), are needed to better constraint what *E. magellanicum* in reality is.

rows 197-205 could be shortened, the Elphidium discussion is already in Darling et al., 2016 and no need to be repeated here.

As proposed by the reviewer we shortened this paragraph (lines 197-201).

The paragraph regarding size distribution should be shortened, in particular since the data is only reported as SI. Also figure 1 should be moved to SI1. There is no point to have the first 1 showing methods that are now moved to SI. I fully recognize the work that has been put into all those measurements.

As proposed by the reviewer we shortened as much as possible this section (section 2.5 in the manuscript, lines 207-230) without omitting information. We also moved figure 1 of the original manuscript to the supplementary material, which then became Supplementary Figure 2.

In the result section, I recommend to decrease the significant figures and avoid decimal numbers. What does 1.1 specimens/10 cc or 449.9 mean? it makes more sense to present 1 and 450 specimens /10cc. The same holds for per cent. There should be a space between the number and %.

It is evident that values such as 0.1 individual/10 cc do not make sense from a biological point of view. However, these data are statistical averages, standardised for 10 cc, which are systematically accompanied by a standard deviation, based on the comparison of two replicate samples. It is customary to give such statistical values with one decimal. The same is true for the percentage values. Next, in case of comparison of standardised data based on very different sediment volumes, the loss of the decimal value could lead to large errors. For this reason we would like to keep our numbers like they are.

We deleted % in table 2 and specified in the caption that the numbers in brackets are relative abundances in %.

Is Table 1 necessary?, the sampling date can be included in table 2.

The reviewer is right that table 1 could be merged with table 2. We did not merged them in the original manuscript for two reasons:

- 1- This would give redundant information if added to table 2 (because the two stations were sampled the same day, so two times the same day in the merged table) and this would mean to add a column where we have only double crosses (because 2 cores were sampled at all moment of sampling).
- 2- Table 1 in its current form allows the reader to quickly get the day of sampling for each month and compare the two stations very easily regarding the number of cores sampled.

For these two reasons we would like to keep these two tables separated in our manuscript.

In Table 2, including the unit in the header row and only present numbers (exclude % unit). In table 2 it is unnecessary to include 100% in the final column, I would also no call it total assemblage as it is total concentration.

We deleted the % in table 2 and specified in the caption the numbers in brackets are relative abundances in %.

We deleted the 100% in the last column and changed the header from “Total assemblage” to “Total”. We changed the caption accordingly.

We also corrected some mistakes in the values of this table after careful double-checking.

In the SI table 3, the total concentration needs to be included or the total number of counted foraminifera for each sample.

We added a column “Total” as suggested by the reviewer in the Supplementary Table 3. We changed the caption accordingly.

I'm looking forward to seeing the MS in print as it includes some very interesting data from low O<sub>2</sub> environments.

# Foraminiferal community response to seasonal anoxia in Lake Grevelingen (the Netherlands)

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**Abstract.** Over the last decades, hypoxia in marine coastal environments has become more and more widespread, prolonged and intense. Hypoxic events have large consequences for the functioning of benthic ecosystems. In severe cases, they may lead to complete anoxia and presence of toxic sulphides in the sediment and bottom-water, thereby strongly affecting biological compartments of benthic marine ecosystems. Within these ecosystems, benthic foraminifera show a high diversity of ecological responses, with a wide range of adaptive life strategies. Some species are particularly resistant to hypoxia/anoxia and consequently, it is interesting to study the whole foraminiferal community as well as species specific responses to such events. Here we investigated the temporal dynamics of living benthic foraminiferal communities (recognised by CellTracker™ Green) at two sites in the saltwater Lake Grevelingen in the Netherlands. These sites are subject to seasonal anoxia with different durations and are characterised by the presence of free sulphide (H<sub>2</sub>S) in the uppermost part of the sediment. Our results indicate that foraminiferal communities are impacted by the presence of H<sub>2</sub>S in their habitat, with a stronger response in case of longer exposure times. At the deepest site (34 m), in summer 2012, one to two months of anoxia and free H<sub>2</sub>S in the surface sediment resulted in an almost complete disappearance of the foraminiferal community. Conversely, at the shallower site (23 m), where the duration of anoxia and free H<sub>2</sub>S was shorter (one month or less), a dense foraminiferal community was found throughout the year excepted for a short period after the stressful event. Interestingly, at both sites, the foraminiferal community showed a delayed response to the onset of anoxia and free H<sub>2</sub>S, suggesting that the combination of anoxia and free H<sub>2</sub>S does not lead to increased mortality, but rather to strongly decreased reproduction rates. At the deepest site, where highly stressful conditions prevailed for one to two months, the recovery time of the community takes about half a year. In Lake Grevelingen, *Elphidium selseyense* and *Elphidium magellanicum* are much less affected by anoxia and free H<sub>2</sub>S than *Ammonia*

35 sp. T6. We hypothesise that this is not due to a higher tolerance for H<sub>2</sub>S, but rather related to the seasonal availability of food sources, which could have been less suitable for *Ammonia* sp. T6 than for the elphidiids.

## 1 Introduction

Hypoxia affects numerous marine environments, from the open ocean to coastal areas. Over the last decades, a general decline in oxygen concentration was observed in marine waters (Stramma et al., 2012), with an extent varying between the concerned  
40 regions. In coastal areas, oxygen concentrations have been estimated to decrease 10 times faster than in the open ocean, with indications of a recent acceleration, expressed by increasing frequency, intensity, extent and duration of hypoxic events (Diaz and Rosenberg, 2008; Gilbert et al., 2010). This is due to the combination of (1) global warming, which is strengthening seasonal stratification of the water column and decreasing oxygen solubility and (2) eutrophication resulting from increased anthropogenic nutrient and/or organic matter input, which is enhancing benthic oxygen consumption in response to increased  
45 primary production (Diaz and Rosenberg, 2008). Bottom water hypoxia has serious consequences for the functioning of all benthic ecosystem compartments (see Riedel et al., 2016 for a review). Benthic faunas are strongly impacted by these events (Diaz and Rosenberg, 1995) although the meiofauna, especially foraminifera, appears to be less sensitive to low dissolved oxygen (DO) concentrations than the macrofauna (e.g. Josefson and Widbom, 1988). Many foraminiferal taxa are able to withstand seasonal hypoxia/anoxia (see Koho et al., 2012 for a review), and consequently can play a major role in carbon  
50 cycling in ecosystems affected by seasonal low-oxygen concentrations (Woulds et al., 2007). Anoxia is often accompanied by free sulphide (H<sub>2</sub>S) in pore and/or bottom-waters (e.g. Jørgensen, 1982; Seitaj et al., 2015), which is considered very harmful for the benthic macrofauna (Wang and Chapman, 1999). Neutral molecular H<sub>2</sub>S can diffuse through cellular membranes and inhibits the functioning of cytochrome *c* oxydase (a mitochondrial enzyme involved in ATP production), finally inhibiting aerobic respiration (Nicholls and Kim, 1982; Khan et al., 1990; Dorman et al., 2002).

55 Lake Grevelingen (southwestern Netherlands) is a former branch of the Rhine-Meuse-Scheldt estuary, which was closed in its eastern part (riverside) by the Grevelingen Dam in 1964 and in its western part (seaside) by the Brouwers Dam in 1971. The resulting saltwater lake, with a surface of 115 km<sup>2</sup>, is one of the largest saline lakes in Western Europe. Lake Grevelingen is characterised by a strongly reduced circulation (even after the construction of a small sluice in 1978) with a strong thermal stratification occurring in the main channels in summer, leading to seasonal bottom-water hypoxia/anoxia in late summer and  
60 early autumn (Bannink et al., 1984). This situation results in to a rise of the H<sub>2</sub>S front in the uppermost part of the sediment, sometimes up to the sediment-water interface.

These observations especially concern the Den Osse Basin (i.e. one of the deeper basins, maximum depth 34 m; Hagens et al., 2015), which has been intensively monitored over the last decades, so that a large amount of environmental data is available (e.g. Wetsteijn, 2011; Donders et al., 2012). The annual net primary production in the Den Osse Basin (i.e. 225 g C m<sup>-2</sup> y<sup>-1</sup>,  
65 Hagens et al., 2015) is comparable to other estuarine systems in Europe (Cloern et al., 2014). However, there is almost no nutrient input from external sources, thus primary production is largely based on autochthonous recycling (>90 %, Hagens et

al., 2015), both in the water column and in the sediment, with a very strong pelagic/benthic coupling (de Vries and Hopstaken, 1984). The benthic environment is characterised by the presence of two antagonistic groups of bacteria, with contrasting seasonal population dynamics (i.e. cable bacteria in winter/spring and *Beggiatoaceae* in autumn/winter), which have a profound impact on all biogeochemical cycles in the sediment column (Seitaj et al., 2015; Sulu-Gambari et al., 2016a, 2016b). The combination of hypoxia/anoxia with sulphidic conditions, which is rather unusual in coastal systems without external nutrient input, and the activity of antagonistic bacterial communities make Lake Grevelingen a very peculiar environment. In the Den Osse Basin, seasonal anoxia coupled with the presence of H<sub>2</sub>S at or very close to the sediment-water interface occurs in summer (i.e. between July–September). However, euxinia (i.e. diffusion of free H<sub>2</sub>S in the water column) does not occur, because of cable bacterial activity (Seitaj et al., 2015).

Although the tolerance of foraminifera to low DO contents and long term anoxia (from weeks to 10 months) has been well documented for many species from different types of environments in laboratory culture (e.g. Moodley and Hess, 1992; Alve and Bernhard, 1995; Bernhard and Alve, 1996; Moodley et al., 1997; Duijnsteet et al., 2003; Geslin et al., 2004; Duijnsteet et al., 2005; Ernst et al., 2005; Pucci et al., 2009; Koho et al., 2011; Geslin et al., 2014) as well as in field studies (e.g. Piña-Ochoa et al., 2010b ; Langlet et al., 2013; 2014), their tolerance to free H<sub>2</sub>S is still debated. In the vast majority of previous studies, no decrease in the total abundances of living foraminifera (i.e. strongly increased mortality) was observed during anoxic events. Unfortunately, studies on foraminiferal response in systems affected by seasonal hypoxia/anoxia with sulphidic conditions are still very sparse. The few available observations are not conclusive, but suggest that H<sub>2</sub>S could be toxic for foraminifera even on fairly short time scales (Bernhard, 1993; Moodley et al., 1998b; Panieri and Sen Gupta, 2008; Langlet et al., 2014).

To our knowledge, all earlier studies show that the foraminiferal response to hypoxia/anoxia is species-specific (e.g. Bernhard and Alve, 1996; Ernst et al., 2005; Bouchet et al., 2007; Geslin et al., 2014; Langlet et al., 2014). However, this species-specific response generally follows the same scheme (usually decrease in density, reduction of growth and/or reproduction), with different response intensities. Duijnsteet et al. (2005) suggested that oxic stress leads to an increased mortality and an inhibited growth and reproduction. The suggestion of inhibited growth is supported by LeKieffre et al. (2017) who observed that the morphospecies *Ammonia tepida* (probably *Ammonia* sp. T6) showed minimal or no growth under anoxia. Conversely, Geslin et al. (2014) and Nardelli et al. (2014) suggested that, in the same morphospecies, reproduction was strongly reduced, but growth would not be affected by hypoxic and/or short anoxic events. Additionally, under low-oxygen conditions, some species are able to shift to anaerobic metabolism (i.e. denitrification, Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010a), to sequester chloroplast (i.e. kleptoplastidy, Jauffrais et al., 2018), to associate with bacterial symbionts (Bernhard et al., 2010) or to enter into a state of dormancy (Ross and Hallock, 2016; LeKieffre et al., 2017).

The highly peculiar environmental context of Lake Grevelingen offers an excellent opportunity to study this still poorly known aspect of foraminiferal ecology.

The conventional method to discriminate between live and dead foraminifera uses Rose Bengal, a compound which stains proteins (i.e. organic matter). This method was proposed for foraminifera by Walton (1952) and is based on the assumption

that “*the presence of protoplasm is positive indication of a living or very recently dead organism*”. The author already noted that this assumption implied that the rate of degradation of organic material should be relatively high. Previous studies of living benthic foraminifera in environments subjected to hypoxia/anoxia were almost all based on Rose Bengal stained samples (e.g. Gustafsson and Nordberg, 1999, 2000; Duijnsteet al., 2004; Panieri, 2006; Schönfeld and Nummerger, 2007; Polovodova et al., 2009; Papaspyrou et al., 2013). However, foraminiferal protoplasm may remain stainable from several weeks to months after their death (Corliss and Emerson, 1990), especially under low dissolved oxygen concentrations where organic matter degradation may be very slow (Bernhard, 1988; Hannah and Rogerson, 1997; Bernhard et al., 2006). The Rose Bengal staining method is therefore not suitable for studies in environments affected by hypoxia/anoxia. Consequently, the results of foraminiferal studies in low-oxygen environments based on this method have to be considered with reserve. In order to avoid this problem, we used CellTracker™ Green (CTG) to recognise living foraminifera. CTG is a fluorescent probe which marks only living individuals with cytoplasmic (i.e. enzymatic) metabolic activity (Bernhard et al., 2006). Since metabolic activity stops after the death of the organism, CTG should give a much more accurate assessment of the living assemblages at the various sampling times, and thereby avoid over-estimation of the live foraminiferal abundances.

In this study, samples were collected in August and November 2011 and then every month through the year 2012, at two different stations in the Den Osse Basin, with two replicates dedicated to foraminifera. The two stations were chosen in contrasted environments regarding water depth (34 m and 23 m, respectively) and duration of seasonal hypoxia/anoxia and sulphidic conditions. Living foraminiferal assemblages were studied in the uppermost sediment and size distributions were determined in order to get insight into the possible moment(s) of reproduction or accelerated growth in test size. The seasonal variability study of the foraminiferal community allows us (1) to better understand the foraminiferal tolerance to seasonal hypoxia/anoxia with presence of free H<sub>2</sub>S in their microhabitat and (2) to obtain information about the responses of the various species to adverse conditions. This knowledge will be useful for the development of indices assessing environmental quality (i.e. biomonitoring) and may also improve paleoecological interpretations of coastal records (e.g. Murray, 1967; Gustafsson and Nordberg, 1999).

## 2 Material and Methods

### 2.1 Studied area – environmental settings in the Den Osse Basin.

Lake Grevelingen is a part of the former Rhine-Meuse-Scheldt estuary, in the southwestern Netherlands. This former estuarine branch was turned into an artificial saltwater lake during the Delta Works project. In Lake Grevelingen, the water circulation is strongly limited by the construction of dams (in the early 1970s) and only a small sluice allows water exchanges with open sea waters (i.e. very weak hydrodynamics). In the Lake, development of bottom-water hypoxia/anoxia occurs in the deepest part of the basin in summer (i.e. July–September) to early autumn (i.e. October–December, Bannink et al., 1984; Hagens et al., 2015). In the literature, the terminology and threshold values used to describe oxygen depletion are highly variable (e.g., oxic, dysoxic, hypoxic, suboxic, microxic, postoxic; see Jorissen et al., 2007; Altenbach et al., 2012). In this study we defined

hypoxia as a concentration of oxygen  $<63 \mu\text{mol L}^{-1}$  ( $1.4 \text{ mL L}^{-1}$  or  $2 \text{ mg L}^{-1}$ ) whereas anoxia is defined as no detectable oxygen (following Rabalais et al., 2010).

135 In Den Osse Basin, the nutrient input from external sources is very low and pelagic/benthic coupling is essential, as already noted by de Vries and Hopstaken (1984). In 2012, phytoplankton blooms occurred in April-May and July (Hagens et al., 2015; Fig. 10) in response to the increasing solar radiation and nutrient availability in the water column following organic matter recycling in winter. This led to an increased food availability in the benthic compartment in the same periods. In general, Chl *a* concentrations in Den Osse Basin are below  $10 \mu\text{g L}^{-1}$ , excluding very short peaks during blooms in April-May and July 140 which did not exceed  $30 \mu\text{g L}^{-1}$  in 2012 (Hagens et al., 2015). Thermal stratification of the water column and increased oxygen consumption due to organic matter input (i.e. from phytoplankton blooms) both are responsible for the development of seasonal bottom-water hypoxia/anoxia in summer (i.e. July-September). Although euxinia (i.e. the presence of free  $\text{H}_2\text{S}$  in the water column) does not occur in the Den Osse Basin due to cable bacterial activity in winter, free  $\text{H}_2\text{S}$  is present in the uppermost layer of the sediment in summer (Seitaj et al., 2015). Summarising, in the benthic ecosystem, increased food availability in 145 summer is counterbalanced by strongly decreasing oxygen contents, sometimes accompanied by the presence of free sulphides in the topmost sediment.

## 2.2 Field Sampling

The two studied sites are located along a depth gradient in the Den Osse Basin of Lake Grevelingen. Both station 1 ( $51^{\circ}44.834'$  N,  $3^{\circ}53.401'$  E) and station 2 ( $51^{\circ}44.956'$  N,  $3^{\circ}53.826'$  E) are located in the main channel, at 34 and 23 m depth, respectively 150 (see map in Hagens et al., 2015 Fig. 1).

Measurements of bottom-water oxygen (BWO) concentrations were performed at 2 m above the sediment-water interface and are from Donders et al. (2012), whereas the data for 2012 were published in Hagens et al. (2015). Sediment cores were collected monthly in 2012 using a single core gravity corer (UWITEC, Austria) using PVC core liners (6cm inner diameter, 60cm length). All cores were inspected upon retrieval and only visually undisturbed sediment cores were used for further analysis 155 (Seitaj et al., 2017). Oxygen penetration depth (OPD) and depth of free  $\text{H}_2\text{S}$  detection were determined by Seitaj et al., (2015) using profiling microsensors for station 1. The data for station 2 (Supplementary Table 1) were acquired similarly and during the same cruises but never published, for further details about the sampling method, see Seitaj et al. (2015).

Two replicate sediment cores dedicated to the foraminiferal study were sampled in August and November 2011 using the same gravity corer (UWITEC, Austria) and then monthly throughout the year 2012 at the same sampling time as for BWO 160 concentration and OPD and  $\text{H}_2\text{S}$  measurements in the sediment (see Seitaj et al., 2015). Consequently, for 2012 at station 1 and 2, OPD and  $\text{H}_2\text{S}$  were measured in the sediment column at the same time as foraminifera were sampled (Seitaj et al., 2015). For each replicate, the uppermost centimetre (0–1 cm) of the core was then transferred on board in a vial of 250 mL, and 30 mL of seawater (at the same temperature than *in situ*) was added in the vial. Then we labelled the samples with CellTracker™ Green CMFDA (CTG, 5-chloromethylfluorescein diacetate, final concentration of  $1 \mu\text{mol L}^{-1}$  following Bernhard et al., 2006)



165 and slowly agitated manually to allow the CTG diffusion in the whole sample. Samples were then fixed in 5 % sodium borate buffered formalin after 24 h of incubation in the dark.

### 2.3 Sample Treatment

All samples were sieved over 315, 150, 125 µm meshes, and foraminiferal assemblages were studied in all three size fractions. Individuals were picked wet under an epifluorescence stereomicroscope (Olympus SZX12, light fluorescent source Olympus  
170 URFL-T, excitation/emission wavelengths: 492 nm/517 nm) and placed on micropalaeontological slides. Only specimens that fluoresced brightly green were considered as living and were identified to the (morpho-)species level when possible. Since picking foraminifera under an epifluorescence stereomicroscope is particularly time-consuming, we decided to study samples only every two months for the year 2012. At a later stage, in view of the large differences in foraminiferal abundances between the samples of September and November 2012 at station 2, we decided to study the October and December 2012 samples as  
175 well for this station. The sampling dates investigated in this study are listed in Table 1.

Abundances were then standardised to a volume of 10 cm<sup>3</sup>. The abundances of living foraminifera for each sampling time and replicate are listed in Supplementary Tables 2 and 3. The mean abundance and standard deviation ( $\bar{x} \pm sd$ ) for the two replicates for each sampling date were calculated both for the total living assemblage and the individual species, as an indication of spatial patchiness.

### 180 2.4 Taxonomy of dominant species

Four dominant species (>1 % of the total assemblage) were present in our material: *Ammonia* sp. T6, *Elphidium magellanicum* (Heron-Allen and Earland, 1932), *Elphidium selseyense* (Heron-Allen and Earland, 1911) and *Trochammina inflata* (Montagu, 1808). As we identified these species on the basis of morphological criteria, we will use them as “morphospecies”.

Concerning the genus *Ammonia*, two living specimens collected at Grevelingen station 1 were molecularly identified (by DNA  
185 barcoding) as phylotype T6 by Bird et al. (2019). At the same site, we genotyped seven other living *Ammonia* specimens, which were all T6. Their sequences were deposited on GenBank (accession numbers MN190684 to MN190690) and Supplementary Figure 1 shows Scanning Electron Microscope (SEM) images of the spiral side and of the penultimate chamber at 1000x magnification for four individuals. A morphological screening based on the criteria proposed by Richirt et al. (2019) confirmed that T6 accounts for the vast majority (>98 %) of *Ammonia* individuals, whereas phylotypes T1, T2, T3 and T15  
190 are only present in very small amounts (Supplementary Table 3).

The specimens of *Elphidium magellanicum* were identified exclusively on the basis of morphological criteria, as there are no molecular data available yet. This morphospecies, although rare, is regularly recognised in Boreal and Lusitanian provinces of Europe (e.g. Gustafsson and Nordberg, 1999; Darling et al., 2016; Alve et al., 2016). However, as the type species was described from the Magellan strait (Southern Chile), the European specimens may represent a different species and further  
195 studies involving DNA sequencing of both populations are needed to confirm or infirm this taxonomic attribution (see Roberts et al., 2016).

~~In the past, *Elphidium selseyense* has often been considered as an ecophenotype of *Elphidium excavatum* (Terquem, 1875) and has been identified as *E. excavatum* forma *selseyensis* (e.g. Feyling-Hanssen, 1972; Miller et al., 1982). Recently, Darling et al. (2016) showed that the various ecophenotypes recognised in *E. excavatum* are in fact genetically separated and therefore represent different species.~~ Four living specimens ~~of the *E. excavatum* group were already~~ sampled ~~at station 1~~ for DNA analysis ~~at station 1 and~~ were all identified as the species *E. selseyense* (phylotype S5, Darling et al., 2016). We only observed minor morphological variations in our material, especially concerning the number of small bosses in the umbilical region, which we considered as intraspecific variability. Consequently, we identified all our specimens as *E. selseyense*. The specimens attributed to *Trochammina inflata* were also identified exclusively on the basis of morphological criteria, as no molecular data are available yet.

## 2.5 Size distribution measurement

In order to detect periods of increased growth and/or reproduction, size measurements were performed on all samples of 2012. The measurements were made for all species, ~~which represent together~~ (4176 individuals for station 1 and 19624 individuals for station 2. ~~Prior to measurements,)~~ and trochospiral species were all orientated ~~in the same way~~ (spiral side up). ~~prior to measurements.~~ High-resolution images (3648\*2736 pixels) of all micropalaeontological slides were taken with a stereomicroscope (Leica S9i, 10x magnification). ~~In order to obtain)~~ and individual measurements ~~for all individual specimens, images~~ were processed using ImageJ software (Schneider et al., 2012, ~~Fig. 1~~ Supplementary Figure 2). ~~The three size fractions (125–150, 150–315, >315 µm) were analysed together for the size distribution analyses.~~ Each individual was isolated ~~on the image (Fig. 1~~ (Supplementary Figure 2) and its maximum diameter was measured (i.e. Feret's diameter). We represented all size distributions using histograms with 20 µm classes (the best compromise between the total number of individuals and the size range (Supplementary Figure 2)). ~~In order to compare more easily months and species, the median and the mode (associated with the numbers of individuals) were calculated for each size distribution.~~ 3). As we only examined the size fractions >125 µm, our analysis mainly concerns adult specimens, and does not include juveniles. This limitation should be kept in mind when interpreting the results.

~~In an attempt to recognize the different cohorts for each species in each of the bimonthly samples, we assumed~~ Assuming that the size distribution was a sum of Gaussian curves, each of them representing a cohort. ~~In order, we tried~~ to identify the approximate mode for the Gaussian curves (i.e. cohorts), ~~we used)~~ using the changes in slope (i.e. inflexion points) of the second-order derivative of the total size distribution (Gammon et al., 2017). Unfortunately, this tentative to distinguish cohorts by using a deconvolution method was not conclusive. The main problem was the lack of information concerning individuals smaller than 125 µm, so that our size distributions were systematically skewed on the left side (i.e. toward small individuals). ~~An additional problem was the large number of smaller specimens which were always present.~~ Because the identification of individual cohorts was not successful, ~~parameters like reproduction rate, growth rate or lifespan were not assessable, and therefore~~ a study of population dynamics was not possible. For this reason, the data are only shown in the supplementary

material (Supplementary Figures 2 and 3). Nevertheless, the size distribution data give some clues concerning the possible moment(s) of reproduction or intensified test growth for the different species.

## 2.6 Encrusted forms of *E. magellanicum*

In our samples, we found abundant encrusted forms of *E. magellanicum* at station 1 (May 2012) and station 2 (May, July, September and December 2012, Fig. 8). -Most individuals were totally encrusted (Fig. 8a), others only partly (Fig. 8b). These crusts were hard, firmly stuck to the shell (difficult to remove with a brush), thin (Fig. 8c–e) and rather coarse. In order to determine if the crust matrix is constituted of carbonate, we placed some specimens in microtubes and exposed them to 0.1 M of EDTA (EthyleneDiamineTetraacetic Acid) diluted in 0.1 M cacodylate buffer (acting as a carbonate chelator). After an exposition of 24h, we checked under a stereomicroscope if the crust was still cohesive (no carbonate in the crust) or was disaggregated (crust contains carbonate).

## 3 Results

### 3.1 Total abundances of foraminiferal assemblages

Averaged total abundances varied between  $1.1 \pm 1.5$  and  $449.9 \pm 322.1$  ind.  $10\text{ cm}^{-3}$  for station 1, and between  $91.1 \pm 25.0$  and  $604.8 \pm 3.5$  ind.  $10\text{ cm}^{-3}$  for station 2 (Figure 2 and Table 2). For every studied month, the total density was higher at station 2 than at station 1. The seasonal succession is very different between the two sites (Figure 2). Station 1 shows very low total foraminiferal abundances for most months, contrasting with much higher densities in May and July. Conversely, station 2 shows high total foraminiferal abundances throughout the year, with somewhat lower values in November 2011, and October and November 2012 (Figure 2).

At station 1, almost no individuals were present in August ( $\bar{x} = 3.4 \pm 1.3$ ) and November 2011 ( $\bar{x} = 1.1 \pm 1.5$ ). In 2012, total abundances were very low in January ( $\bar{x} = 11.5 \pm 9.3$ ), showed a slight increase in March ( $\bar{x} = 62.1 \pm 19.3$ ) and reached a maximal abundance in May ( $\bar{x} = 449.9 \pm 322.1$ ). Total abundances then progressively decreased from May to September ( $\bar{x} = 34.0 \pm 17.0$ ) and almost no foraminifera were present in November ( $\bar{x} = 1.6 \pm 0.3$ ).

At station 2, total abundances were comparatively low in August and November 2011 ( $\bar{x} = 174.0 \pm 48.0$  and  $\bar{x} = 128.7 \pm 25.0$  ind.  $10\text{ cm}^{-3}$ , respectively). In 2012, total abundances were relatively high and stable from January to September (between  $\bar{x} = 523.6 \pm 30.7$  to  $\bar{x} = 604.8 \pm 3.5$ ), then decreased in October ( $\bar{x} = 211.5 \pm 8.0$ ) and November ( $\bar{x} = 91.1 \pm 25.3$ ) and finally increased again in December ( $\bar{x} = 377.9 \pm 38.8$ ).

### 3.2 Dominant Species

At station 1, the major species were, in order of decreasing abundances, *Elphidium selseyense* (Fig. 3a–b), *Elphidium magellanicum* (Fig. 3c–d) and *Ammonia* sp. T6 (Fig. 3e–g). In Figure 4, we added *Trochammina inflata* (Fig. 3h–j) to facilitate

comparison with station 2, where this species is among the dominant ones. The “Other species” account only for 2.2 % of the total assemblage at station 1. The fact that they are well represented in some months (e.g. 26.3 % of the assemblage in August 2011) is due to the extremely low number of individuals (see Fig. 2 and Table 2). At station 2, the dominant species, in order of decreasing abundances, were *E. selseyense*, *Ammonia* sp. T6, *E. magellanicum* and *T. inflata* (Table 2). Here, “Other species” account only for 2.6 % of the total assemblage. Whereas *E. selseyense* and *E. magellanicum* were dominant species at both stations, both *Ammonia* sp. T6 and *T. inflata* were present in much higher abundances at station 2 compared to station 1, where the latter species was almost absent (Fig. 4–5).

At station 1, only some very scarce individuals of *E. selseyense* were observed in August and November 2011 (Fig. 4 and Table 2). In 2012, *E. selseyense* abundances were very low in January started to increase in March ( $\bar{x} = 23.9 \pm 6.8$ ) to reach maximal values in May ( $\bar{x} = 336.5 \pm 275.8$ ). In July, values for *E. selseyense* were still high ( $\bar{x} = 162.0 \pm 121.5$ ) and further decreased until an almost total absence in November 2012. No specimen of *E. magellanicum* was observed in 2011 (Fig. 4 and Table 2). The abundance of *E. magellanicum* was very low in January 2012, started to increase in March ( $\bar{x} = 21.6 \pm 11.0$ ) to reach maximal values in May ( $\bar{x} = 96.4 \pm 47.3$ ), then strongly decreased in July ( $\bar{x} = 3.7 \pm 0.3$ ). The species was absent from samples in September and November 2012. *Ammonia* sp. T6 was almost absent in August and November 2011 and present with very few specimens in January 2012 ( $\bar{x} = 3.2 \pm 3.5$ ). Maximum abundances were reached between March and July 2012 (ranging between  $\bar{x} = 9.2 \pm 6.5$  and  $\bar{x} = 12.9 \pm 1.3$ ). Then abundances rapidly decreased until the species was almost absent in November. *Trochammina inflata* was absent in 2011 and was only present with very low abundances from January to May and in September 2012.

At station 2, the two dominant species were *E. selseyense* and *Ammonia* sp. T6, which together always represented at least 70 % of the total assemblage (Fig. 5 and Table 2). These two species showed a different seasonal pattern over the considered period. Abundances of *E. selseyense* were comparable in August ( $\bar{x} = 74.8 \pm 29.8$ ) and November 2011 ( $\bar{x} = 52.3 \pm 27.0$ ) then showed a progressive increase until a maximum in September 2012 ( $\bar{x} = 365.5 \pm 70.3$ ). Abundances then showed a sharp decrease in October and November (respectively  $\bar{x} = 98.7 \pm 8.5$  and  $\bar{x} = 30.9 \pm 2.3$ ) to increase again in December ( $\bar{x} = 252.2 \pm 41.0$ ). For *Ammonia* sp. T6, abundances strongly increased between November 2011 ( $\bar{x} = 60.8 \pm 1.5$ ) and January 2012 ( $\bar{x} = 226.2 \pm 52.3$ ) and then progressively decreased until the end of 2012 ( $\bar{x} = 48.1 \pm 26.0$  in November 2012). *Trochammina inflata* showed an analogous pattern to *Ammonia* sp. T6. Abundances strongly increased between November 2011 ( $\bar{x} = 11.8 \pm 1.8$ ) and January 2012 ( $\bar{x} = 121.5 \pm 29.8$ ), and then progressively decreased until very low abundances in November ( $\bar{x} = 3.7 \pm 3.0$ ). *E. magellanicum* was completely absent in August and November 2011, almost absent in January 2012 ( $\bar{x} = 0.9 \pm 0.3$ ) and then suddenly increased until a maximum of  $\bar{x} = 116.0 \pm 6.5$  in May. Abundances stayed relatively high in July ( $\bar{x} = 37.8 \pm 2.5$ ) and September ( $\bar{x} = 72.0 \pm 35.8$ ), and then drastically decreased until minimum numbers in October and November. Finally, like all other species, *E. magellanicum* abundances increased again in December ( $\bar{x} = 25.5 \pm 13.0$ ).

### 290 3.3 Encrusted forms of *Elphidium magellanicum*

After exposition to 0.1 M of EDTA diluted in 0.1 M cacodylate buffer, the crusts remained cohesive, indicating that it does not consist of carbonate, and suggesting that it is composed of sediment particles cemented by an organic matrix.

At station 1, encrusted forms of *E. magellanicum* were present in moderate proportions in May (26.8 % of the total *E. magellanicum* population, Fig. 9) and July (47.6 %); the species disappeared thereafter. At station 2, encrusted forms strongly  
295 dominated the *E. magellanicum* population from May (72.3 %) to December (88.0 %, Fig. 9).

## 4 Discussion

### 4.1 Tolerance of foraminiferal communities to anoxia and free sulphide

At station 1, bottom-waters were hypoxic in July 2012 and became anoxic in August (Fig. 10). Both in July and August, oxygen penetration into the sediment was null, whereas it was  $0.7 \pm 0.1$  mm depth in September. In all three months (July to  
300 September 2012), sulphidic conditions were observed very close to the sediment-water interface (1 mm or less, Fig. 10 and Supplementary Table 1). In view of these results, the duration of anoxic and sulphidic conditions in the uppermost sediment layer can be estimated as one to two months (in July and August, Fig. 10).

After the strong increase of foraminiferal densities in May 2012, there was a decrease starting in July, leading to a near-absence of foraminifera at station 1 in November (Fig. 10). The most probable cause of the strong decline of the foraminiferal  
305 community appears to be a prolonged presence of sulphides in the foraminiferal microhabitat. However, the fact that foraminiferal abundances reached almost zero only in September (about two months after the first occurrence of anoxic and sulphidic conditions in the upper sediment, in July) suggests that the presence of  $H_2S$  did not cause instantaneous mortality, but that the disappearance of the foraminiferal community was a delayed response, probably caused by inhibited reproduction and, eventually, increased mortality. Inhibited reproduction has previously been suggested as a response to hypoxic/short  
310 anoxic (Geslin et al., 2014) and sulphidic conditions (Moodley et al., 1998b).

Such a time lag between a change in foraminiferal abundances and changes in environmental parameters affecting reproduction and/or growth of foraminifera has been suggested previously by Duijnsteet et al. (2004). These authors highlighted that the density patterns of some foraminiferal species showed a higher correlation with measured environmental parameters (e.g., oxygenation or temperature) when a time lag of about three months was applied.

315 For 2011, at station 1, no pore-water  $O_2$  and  $H_2S$  measurements are available. However, severe hypoxia was observed in the bottom-waters from May to August, with anoxia in June 2011 (Fig. 10). We therefore assume that like in 2012, anoxic and probably co-occurring sulphidic conditions were responsible for the very low standing stocks in August and November 2011 and January 2012.

Our observations confirm the suggestion in previous studies that the foraminiferal community is severely affected by a long-  
320 term presence of  $H_2S$  in its habitat, but does not show instant mortality. In fact, after a 66-day incubation in euxinic conditions

(a maximum of  $11.9 \pm 0.4 \mu\text{mol L}^{-1}$  of  $\text{H}_2\text{S}$  in the overlying water) of foraminiferal assemblages collected at a 19 m deep site in the Adriatic Sea, Moodley et al. (1998a) found a strong decrease of the total density of Rose Bengal stained foraminifera. After 21 days, living specimens were still observed, whereas after 42 and 66 days, the live checks (based on protoplasm movement) gave only negative results. Langlet et al. (2013, 2014), performed an *in situ* experiment with closed benthic chambers at a 24 m deep site in the Gulf of Trieste, in the Adriatic Sea. They observed a decrease of living foraminiferal density (labelled with CTG), but also found that almost all species survived after 10 months of anoxia and periodically co-occurring  $\text{H}_2\text{S}$  in the sediment and overlying water. However, the duration of sulphidic conditions, which was estimated to several weeks, could not be assessed precisely (Metzger et al., 2014). The suggestion that short-time exposure to euxinic conditions is not directly lethal for foraminifera is confirmed by the experimental results of Bernhard (1993), who found that foraminiferal activity (as determined by ATP content) was not significantly affected after 30-day exposure to euxinia ( $32.6 \pm 8.6$  % of active individuals,  $n=174$ . in control conditions versus  $29.5 \pm 6.2$  %,  $n= 173$  in sulphidic conditions).

After the 2011 hypoxia/anoxia, standing stocks at station 1 only started to increase in March 2012, indicating a very long recovery time (about 6 months) of the foraminiferal faunas after a temporary near-extinction due to anoxic and sulphidic conditions. This confirms observations of relatively long recovery times in the literature (e.g. Alve, 1995, 1999; Gustafsson and Nordberg, 2000; Hess et al., 2005). For instance, Gustafsson & Nordberg (1999) showed that in the Koljö Fjord, at comparable water depths, foraminiferal populations responded with increased densities only three months after a renewal of sea-floor oxygenation following hypoxic conditions in the bottom-waters. However, in that case, the disappearance of the foraminiferal population was only partial, and not nearly complete as in our study.

At station 2, in 2012, hypoxia was only observed in August, when the OPD was zero, and sulphidic conditions were observed in the superficial sediment (i.e. from  $0.4 \pm 0.2$  mm downwards, Fig. 11, Supplementary Table 1). Both in July and September, oxygen penetrated more than one millimetre into the sediment ( $1.3 \pm 0.4$  mm and  $1.2 \pm 0.2$  mm, respectively). However, free  $\text{H}_2\text{S}$  was still detected at about one millimetre depth in the sediment ( $1.1 \pm 0.8$  mm in July and  $0.8 \pm 0.2$  mm in September). Although the sampling plan does not allow us to be very precise about the duration of anoxic and sulphidic conditions, we can estimate their duration to be 1 month or less (Fig. 11).

Foraminiferal abundances showed a strong decrease in October and November 2012, about two months after the presence of anoxic and sulphidic conditions in the topmost part of the sediment (Fig. 11). Like at station 1, this temporal offset between the presence of anoxia/sulphidic conditions at station 2 (in August) and the strong decrease of faunal densities may be explained as a delayed response, mainly due to inhibited reproduction during the anoxic/sulphidic event. If true, the mortality of adults did not strongly increase in the months following the  $\text{H}_2\text{S}$  production in the uppermost sediment. Nevertheless, there was no replacement in the  $>125 \mu\text{m}$  fraction by growing juveniles, probably because reproduction was interrupted when  $\text{H}_2\text{S}$  was present in the foraminiferal microhabitat. A renewed recruitment after the last stage of sulphidic conditions somewhere in September would then explain why the faunal density in the  $>125 \mu\text{m}$  fraction increased again in December 2012 (Supplementary Figure 23).

355 In 2011, at station 2, bottom-waters oscillated between hypoxic and oxic conditions between May and August (Fig. 11).  
Although we have no measurements of H<sub>2</sub>S in the pore waters for this year, it seems probable that bottom-water hypoxia was  
accompanied by the presence of free H<sub>2</sub>S very close to the sediment surface, strongly affecting the foraminiferal communities.  
If we assume that, like in 2012, rich foraminiferal faunas were present in May–July 2011 at both stations, the low faunal  
densities observed in August and November 2011 could suggest that foraminifera may have also shown a delayed response to  
360 sulphidic conditions in 2011.

It is interesting to note that the foraminiferal densities observed at station 2 were lower in August 2011 than in July or  
September 2012. This may be a consequence of the repetition of short hypoxic events in the bottom-water between May and  
August 2011 (probably associated with anoxia and maybe H<sub>2</sub>S in the uppermost part of the sediment), which possibly affected  
the foraminiferal community more substantially in 2011 than in 2012, when a hypoxic event was recorded in August only.

365 The important decrease of total standing stocks at station 2 in October and November 2012 (Fig. 11) suggests that, in spite of  
the shorter duration of anoxia and sulphide conditions (compared to station 1; one month or less compared to one to two  
months), the foraminiferal faunas were still strongly affected. However, at station 2, foraminiferal abundances increased again  
in December 2012, suggesting a recovery time of about two months, which is likely much shorter than at station 1, where  
standing stocks in the >125 µm fraction only increased 6 months after the presence of anoxia and free sulphides.

370 Summarising, the foraminiferal communities of both stations 1 and 2 seem strongly impacted by the anoxic and sulphidic  
conditions developing in the uppermost part of the sediment in summer (i.e. July–September). However, at station 1, where  
anoxic and sulphidic conditions lasted for one to two months, the response is much stronger, leading ultimately (in November)  
to almost complete disappearance of the foraminiferal fauna. The delayed response at both stations shows that instantaneous  
375 mortality was limited, and suggests that the decreasing standing stocks might rather be the result of inhibited reproduction,  
and eventually, increased mortality. Recovery is much faster at station 2 (about two months) than at station 1 (about six  
months), probably because at station 1 (in contrast to station 2) the foraminiferal extinction was nearly complete, and the site  
had to be recolonised (e.g. possibly by nearby sites or by the remaining few individuals) after reoxygenation of the sediment.  
At station 2, a reduced but significant foraminiferal community remained present, explaining the faster recovery.

#### 380 4.2 Species-specific response to anoxia, sulphide and food availability in Lake Grevelingen

The comparison of the different seasonal patterns of the major species at the two investigated stations allows us to draw some  
conclusions about interspecific differences in the response to seasonal anoxic and sulphidic conditions.

First, there is a clear faunal difference between the two stations. Station 1 is dominated by *E. selseynense* and *E. magellanicum*  
while at station 2, these two taxa are accompanied by *Ammonia* sp. T6 and *T. inflata*. The latter species is almost absent at  
385 station 1, where *Ammonia* sp. T6 is present with low densities. At first view, the dominance of the two *Elphidium* species at  
station 1, would suggest that they have a greater tolerance to the seasonal anoxic and sulphidic conditions, which lasted much  
longer there. It is interesting to note that the temporal evolution of standing stocks at station 1 is different for the two *Elphidium*

species. *Elphidium magellanicum* shows a strong drop in absolute density in July 2012, at the onset of H<sub>2</sub>S presence in the uppermost part of the sediment, whereas the diminution of *E. selseyense* is more progressive and the species disappears almost completely only in November (Fig. 4). This strongly suggests that *E. magellanicum* is more affected by increased mortality than *E. selseyense* in response to the combined effects of anoxic and sulphidic conditions. This hypothesis is confirmed by the patterns observed at station 2, where the drop in standing stocks in October–November is also more drastic in *E. magellanicum* than in *E. selseyense* (Fig. 5).

As mentioned earlier, certain species of foraminifera can use an anaerobic metabolism (i.e. denitrification, Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010a), sequester chloroplasts (i.e. kleptoplastidy, Jauffrais et al., 2018), host bacterial symbiont (Bernhard et al., 2010) or enter in dormancy (Ross and Hallock, 2016; LeKieffre et al., 2017) to deal with low-oxygen conditions. Concerning the species found in this study, although the presence of intracellular nitrate was shown for *Ammonia*, denitrification tests yielded negative results (Piña-Ochoa et al., 2010a; Nomaki et al. 2014). Similarly, the presence of active symbionts was previously suggested for *Ammonia* but never confirmed (Nomaki et al., 2016; Bernhard et al., 2018). To our knowledge, denitrification or the presence of bacterial symbionts was never shown for *Elphidium* either. In conclusion, a shift to an alternative anaerobic metabolism or an association with bacterial symbionts has never been shown conclusively for the dominant foraminiferal species found in Lake Grevelingen.

The greater tolerance of *E. selseyense* to low-oxygen conditions could be explained by the fact that it is able to sequester chloroplasts from ingested diatoms, and to keep them active for several days to weeks, conversely to *Ammonia* sp. T6 (Jauffrais et al., 2018). These active chloroplasts could serve as an alternative source of oxygen and/or food through photosynthesis (Bernhard and Alve, 1996) or another metabolic pathway (Jauffrais et al., 2019), and thereby increase the capability of this species to survive anoxic events. Although sequestration of chloroplasts was never investigated for *E. magellanicum*, its abundant spinose ornamentation in the umbilical region and in the vicinity of the aperture (Fig. 3c–d) suggests that this species is capable to crush diatom frustules as some kleptoplastic species (Bernhard and Bowser, 1999; Austin et al., 2005). As Hagens et al. (2015) observed that the light penetration depth in the Den Osse Basin never exceeded 15 m in 2012, and therefore photosynthesis by kleptoplasts (Bernhard and Alve, 1996) appears unlikely for both our aphotic stations (34 and 23 m depth). However, other foraminifera from aphotic and anoxic environments such as deep fjords are kleptoplastic and use these kleptoplasts for a yet unknown purpose (Jauffrais et al. 2019).

Rather surprisingly, the drop in foraminiferal densities at station 2 in October–November, which we interpreted as a delayed response to sulphidic conditions, is less strong for *Ammonia* sp. T6 than for the two *Elphidium* species, suggesting that this species is less affected. However, this does not agree with our previous suggestion that the two *Elphidium* species would be more tolerant to anoxic and sulphidic conditions. As already proposed by LeKieffre et al. (2017), *Ammonia* seems to be able to deal with anoxia (up to 28 days, but with no sulphide) by reducing its metabolic activity, but this ability was never shown



for *Elphidium* species. If *E. selseyense* and *E. magellanicum* are indeed unable to resist to anoxia by reducing their metabolism or by entering a dormancy state, this could explain their stronger decrease in densities at station 2 compared to *Ammonia* sp. T6. Nevertheless, further studies about the ability and mechanisms of the two *Elphidium* species to resist to anoxic/sulphidic conditions are necessary.

Another remarkable observation is that *Ammonia* sp. T6 (and *T. inflata*) shows maximum densities in January–March, contrasting with the two *Elphidium* species, which have their density maxima later in the year (May–September). This temporal offset could possibly be explained by a difference in preferential food source, with food particles available in winter (January–March) being more suitable for *Ammonia* sp. T6 (and *T. inflata*), versus food particles available later in the year, resulting from phytoplankton blooms, being more favourable for *E. selseyense* and *E. magellanicum*.

In our study, for *E. selseyense* (and *E. magellanicum*), the continuous presence of a high proportion of small sized specimens and progressively increasing densities between January and September 2012 strongly suggest ongoing and continuous reproduction (Supplementary Figure 2A3A). Continuous reproduction during the year has been described earlier for different foraminiferal genera, such as *Elphidium*, *Ammonia*, *Haynesina*, *Nonion* and *Trochammina* (e.g. Jones and Ross, 1979; Murray, 1983; Cearreta, 1988; Murray, 1992; Basson and Murray, 1995; Gustafsson and Nordberg, 1999; Murray and Alve, 2000). Conversely, for *Ammonia* sp. T6, a decrease in densities coupled with a rapid increase of overall test size between March and May 2012 (small sized specimens remain present but in smaller proportions) could be indicative of a period of reduced recruitment (Supplementary Figure 2B3B).

In fact, foraminifera exhibit a large range of feeding strategies, with several species showing selective feeding with specific food particles (Muller, 1975; Suhr et al., 2003; Chronopoulou et al., 2019). Hagens et al. (2015) reported that in Lake Grevelingen the phytoplankton composition was different between April–May and July 2012. In April–May, the phytoplankton bloom was mainly composed of the haptophyte *Phaeocystis globosa* (Scherffel, 1899), whereas it was dominated by the dinoflagellate *Prorocentrum micans* (Ehrenberg, 1834) in July. *Elphidium* was reported to be able to feed on various food sources (e.g. diatoms, dinoflagellates, green algae; Correia and Lee, 2002; Pillet et al., 2011). However, diatoms are a major food source for kleptoplastic species (Bernhard and Bowser, 1999), such as *E. selseyense* (Jauffrais et al., 2018; Chronopoulou et al., 2019). *Ammonia* spp. seems able to feed on very diverse food sources including microalgae, diatoms, bacteria or even metazoans (Lee et al., 1969; Moodley et al., 2000; Dupuy et al., 2010; Jauffrais et al., 2016; Chronopoulou et al., 2019). Recently, Chronopoulou et al. (2019) showed different feeding preferences for *Ammonia* sp. T6 and *E. selseyense* in intertidal environments in the Dutch Wadden Sea. Although diatoms are ingested by both species (but much more by *E. selseyense*), dinoflagellates were consumed by *E. selseyense* but not by *Ammonia* sp. T6. The latter species is also capable to feed on metazoans by active predation (Dupuy et al., 2010).

These observations suggest that at station 2, the different seasonal density patterns of *Ammonia* sp. T6 and the two *Elphidium* species are not the consequence of a large difference in tolerance to anoxia/sulphides, but rather a different adjustment to the seasonal cycle of food availability. At station 1, the very low densities of *Ammonia* sp. T6 could putatively be explained by a

recolonization starting in January, when food conditions were favourable for this taxon (as testified by the strong density increase in January 2012 at station 2). However, once a more abundant pioneer population had developed (in March-May), food conditions may have been no longer favourable for *Ammonia* sp. T6, explaining why its density did not show a further increase. Conversely, the food conditions may have become optimal for the two *Elphidium* species, explaining their strong density increase between March and May 2012. If true, this would mean that the lower densities of *Ammonia* sp. T6 would not be due to a lower resistance to anoxia and free sulphides, but rather due to an unfavourable seasonal succession of food availability. Previous studies already suggested that hypoxic/anoxic conditions coupled with increased food input from autumnal phytoplankton blooms (composed of diatoms and dinoflagellates) would favour the development of *E. magellanicum* (Gustafsson and Nordberg, 1999). The fact that also at station 2, this species was mainly observed between March and September 2012 corroborates our conclusion of its dependence on a specific food regime.

Finally, encrusted forms of *E. magellanicum* were observed at both stations from May until the end of the year, but were absent in the samples of March 2012. In view of the fact that the crusts consist mainly of organic matter, the encrusted individuals appear to be specimens with preserved feeding cysts. The precise functions of cysts observed around foraminifera are not clear, and include feeding, reproduction, chamber formation, protection or resting (Cedhagen, 1996; Heinz et al., 2005). Concerning the cysts of *E. magellanicum* described here, very similar observations have been made for *Elphidium incertum* at different locations (Norwegian Greenland Sea and Baltic Sea in Linke and Lutze, 1993; Koljö Fjord in Gustafsson and Nordberg, 1999; Kiel Bight in Polovodova et al., 2009). If we assume that encrusted specimens indeed present remains of feeding cysts, the observation of abundant encrusted specimens corroborates our conclusion that the surface water phytoplankton bloom in May 2012 (i.e. probably mainly *Phaeocystis globosa*) provided a food source particularly well suited to the nutritional preferences of this species.

## 5 Conclusion

In this study we examined the foraminiferal community response to different durations of seasonal anoxia coupled with the presence of sulphide in the uppermost layer of sediment at two stations in Lake Grevelingen. In both stations investigated, foraminiferal communities are highly impacted by the combination of anoxia and H<sub>2</sub>S in their habitat. The foraminiferal response varied depending on the duration of adverse conditions, and led to a near extinction at station 1, where anoxic and sulphidic conditions were present for one to two months, compared to a drop in standing stocks at station 2, where these conditions lasted for one month or less. At both sites, foraminiferal communities showed a two-month delay in the response to anoxic and sulphidic conditions, suggesting that the presence of H<sub>2</sub>S inhibited reproduction, whereas mortality was not necessarily increased. The duration of the subsequent recovery depended on whether the foraminiferal community was almost extinct (station 1) or remained present with reduced numbers (station 2). In the former case, six months were needed for faunal recovery, whereas in the latter case, it took only two months. We hypothesize that the dominance of *E. selsejense* and *E. magellanicum* at station 1 is not due to a lower tolerance of *Ammonia* sp. T6 to anoxic and sulphidic conditions, but is rather

the consequence of a different adjustment between the two *Elphidium* species and *Ammonia* sp. T6 with respect to the seasonal cycle of food availability.

## 490 Data availability

Raw data are available in Supplementary Material.

## Author contributions

~~J.R.: generated the size distribution data. B.R. and D.L. picked the foraminifera. D.S.: provided geochemical data. All authors contributed to the writing of the manuscript.~~

495 B.R., D.L and J.R. produced the foraminiferal data. D.S., A.M. and C.S. provided and interpreted geochemical data. M.S. provided, verified and integrated all available genetic information concerning the foraminiferal taxa of Lake Grevelingen. F.M. and C.S. coordinated a much larger research project concerning Lake Grevelingen, of which this foraminiferal study makes part. They also were responsible for the foraminiferal sampling and provided environmental data. F.J. designed the foraminiferal study, and directed the postdoctoral research of B.R. and, together with A.M. and M.S., the PhD thesis of J.R. All authors contributed actively to the several successive versions of the manuscript.  
500 Competing interests

The authors declare that they have no conflict of interest.

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785 **Table 1:** Sampling dates of the samples which were investigated for living foraminifera for stations 1 and 2. x = one core investigated, o = no core investigated.

Year	Month	Day	Station 1	Station 2
2011	August	22	x x	x x
2011	November	15	x x	x x
2012	January	23	x x	x x
2012	March	12	x x	x x
2012	May	30	x x	x x
2012	July	24	x x	x x
2012	September	20	x x	x x
2012	October	18	o	x x
2012	November	2	x x	x x
2012	December	3	o	x x

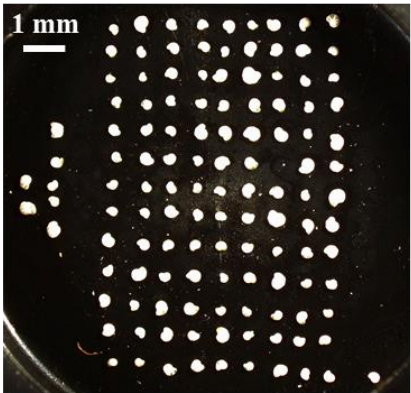
**Table 2:** Mean living foraminiferal ~~abundances~~**absolute** (ind. 10 cm<sup>-3</sup>) and relative abundances (**percentage of the total fauna**, between brackets) of the dominant species ~~and total assemblage in 2011 and 2012 for both stations 1 (top) and 2 (bottom).~~**. Last column: absolute abundance of the total fauna.**

STATION 1

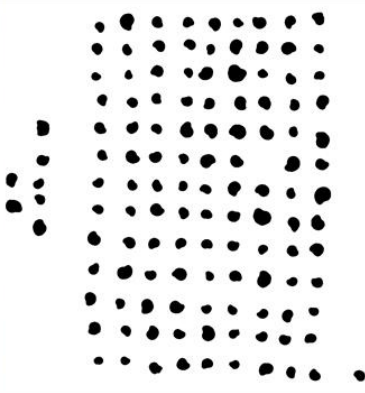
Year	Month	<i>Elphidium selseyense</i>	<i>Ammonia</i> sp. T6	<i>Elphidium magellanicum</i>	<i>Trochammina inflata</i>	Others	Total assemblage
2011	August	1.2 (36.8%) <del>)</del>	1.2 (36.8%) <del>)</del>	0 <del>(0%)</del> <del>(0.0)</del>	0 <del>(0%)</del> <del>(0.0)</del>	0.9 (26.3%) <del>)</del>	3.4 <del>(100%)</del>
2011	November	0.5 (50%) <del>.0)</del>	0.4 (33.3%) <del>)</del>	0 <del>(0%)</del> <del>(0.0)</del>	0 <del>(0%)</del> <del>(0.0)</del>	0.2 (16.7%) <del>)</del>	1.1 <del>(100%)</del>
2012	January	5.1 (44.6%) <del>)</del>	3.2 (27.7%) <del>)</del>	0.2 (1.5%) <del>)</del>	1.2 (10.8%) <del>)</del>	1.8 (15.4%) <del>)</del>	11.5 <del>(100%)</del>
2012	March	23.9 (38.5%) <del>)</del>	12.9 (20.8%) <del>)</del>	21.6 (34.8%) <del>)</del>	1.4 (2.3%) <del>)</del>	2.3 (3.7%) <del>)</del>	62.1 <del>(100%)</del>
2012	May	336.5 (74.8%) <del>)</del>	9.2 (2%) <del>.0)</del>	96.4 (21.4%) <del>)</del>	1.8 (0.4%) <del>)</del>	6.0 (1.3%) <del>)</del>	449.9 <del>(100%)</del>
2012	July	162.0 (90.2%) <del>)</del>	10.3 (5.7%) <del>)</del>	3.7 (2.1%) <del>)</del>	0 <del>(0%)</del> <del>(0.0)</del>	3.5 (2%) <del>.0)</del>	179.5 <del>(100%)</del>
2012	September	29.7 (87.5%) <del>)</del>	2.3 (6.8%) <del>)</del>	0 <del>(0%)</del> <del>(0.0)</del>	0.4 (1%) <del>.0)</del>	1.6 (4.7%) <del>)</del>	34 <del>(100%)</del> <del>.0)</del>
2012	November	1.1 (66.7%) <del>)</del>	0.4 (22.2%) <del>)</del>	0 <del>(0%)</del> <del>(0.0)</del>	0 <del>(0%)</del> <del>(0.0)</del>	0.2 (11.1%) <del>)</del>	1.6 <del>(100%)</del>
Sum		560.0 (75.4%) <del>)</del>	39.8 (5.4%) <del>)</del>	121.8 (16.4%) <del>)</del>	4.8 (0.6%) <del>)</del>	16.4 (2.2%) <del>)</del>	742.9 <del>(100%)</del>

STATION 2

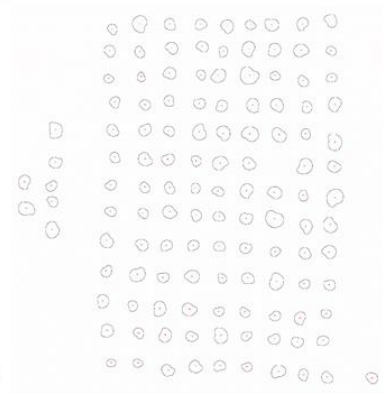
Year	Month	<i>Elphidium selseyense</i>	<i>Ammonia</i> sp. T6	<i>Elphidium magellanicum</i>	<i>Trochammina inflata</i>	Others	Total <del>assemblage</del> <del>concentration</del>
2011	August	74.8 (43% <del>),0</del> )	82.1 (47.2% <del>)</del> )	0 <del>(0%)(0.0)</del> )	14.7 (8.4% <del>)</del> )	2.5 (1.4% <del>)</del> )	174 <del>(100%),0</del>
2011	November	52.3 (40.7% <del>)</del> )	60.8 (47.3% <del>)</del> )	0 <del>(0%)(0.0)</del> )	11.8 (9.2% <del>)</del> )	3.7 (2.9% <del>)</del> )	128.7 <del>(100%)</del>
2012	January	161.8 (30.9% <del>)</del> )	226.2 (43.2% <del>)</del> )	0.9 (0.2% <del>)</del> )	121.5 (23.2% <del>)</del> )	13.3 (2.5% <del>)</del> )	523.6 <del>(100%)</del>
2012	March	214.7 (38.2% <del>)</del> )	214. <del>0</del> (38.1% <del>)</del> )	48.8 (8.7% <del>)</del> )	75. <del>0</del> (13.3% <del>)</del> )	9.9 (1.8% <del>)</del> )	562.3 <del>(100%)</del>
2012	May	288.2 (47.7% <del>)</del> )	147.1 (24.3% <del>)</del> )	116. <del>0</del> (19.2% <del>)</del> )	36.1 (6% <del>),0</del> )	17.3 (2.9% <del>)</del> )	604.8 <del>(100%)</del>
2012	July	282.6 (53.2% <del>)</del> )	158.4 (29.8% <del>)</del> )	37.8 (7.1% <del>)</del> )	31.5 (5.9% <del>)</del> )	21.2 (4% <del>),0</del> )	531.6 <del>(100%)</del>
2012	September	365.5 (64.4% <del>)</del> )	102.4 (18% <del>),0</del> )	72. <del>0</del> (12.7% <del>)</del> )	16.1 (2.8% <del>)</del> )	11.5 (2% <del>),0</del> )	567.5 <del>(100%)</del>
2012	October	98.7 (46.7% <del>)</del> )	99. <del>0</del> (46.8% <del>)</del> )	1.8 (0.8% <del>)</del> )	7.4 (3.5% <del>)</del> )	4.6 (2.2% <del>)</del> )	<del>206.9</del> <del>(100%)211.5</del>
2012	November	30.9 (34% <del>),0</del> )	48.1 (52.8% <del>)</del> )	4.1 (4.5% <del>)</del> )	3.7 (4.1% <del>)</del> )	4.2 (4.7% <del>)</del> )	91.1 <del>(100%)</del>
2012	December	252.2 (66.7% <del>)</del> )	78. <del>0</del> (20.6% <del>)</del> )	25.5 (6.7% <del>)</del> )	12.7 (3.4% <del>)</del> )	9.5 (2.5% <del>)</del> )	<del>368.4</del> <del>(100%)377.9</del>
Sum		1821.8 (48.3% <del>)</del> )	1216.1 (32.2% <del>)</del> )	306.8 (8.1% <del>)</del> )	330.5 (8.8% <del>)</del> )	<del>83.6</del> <del>97.7</del> <del>(2.6%)</del>	<del>3758.9</del> <del>(100%)3773.0</del>



Raw picture

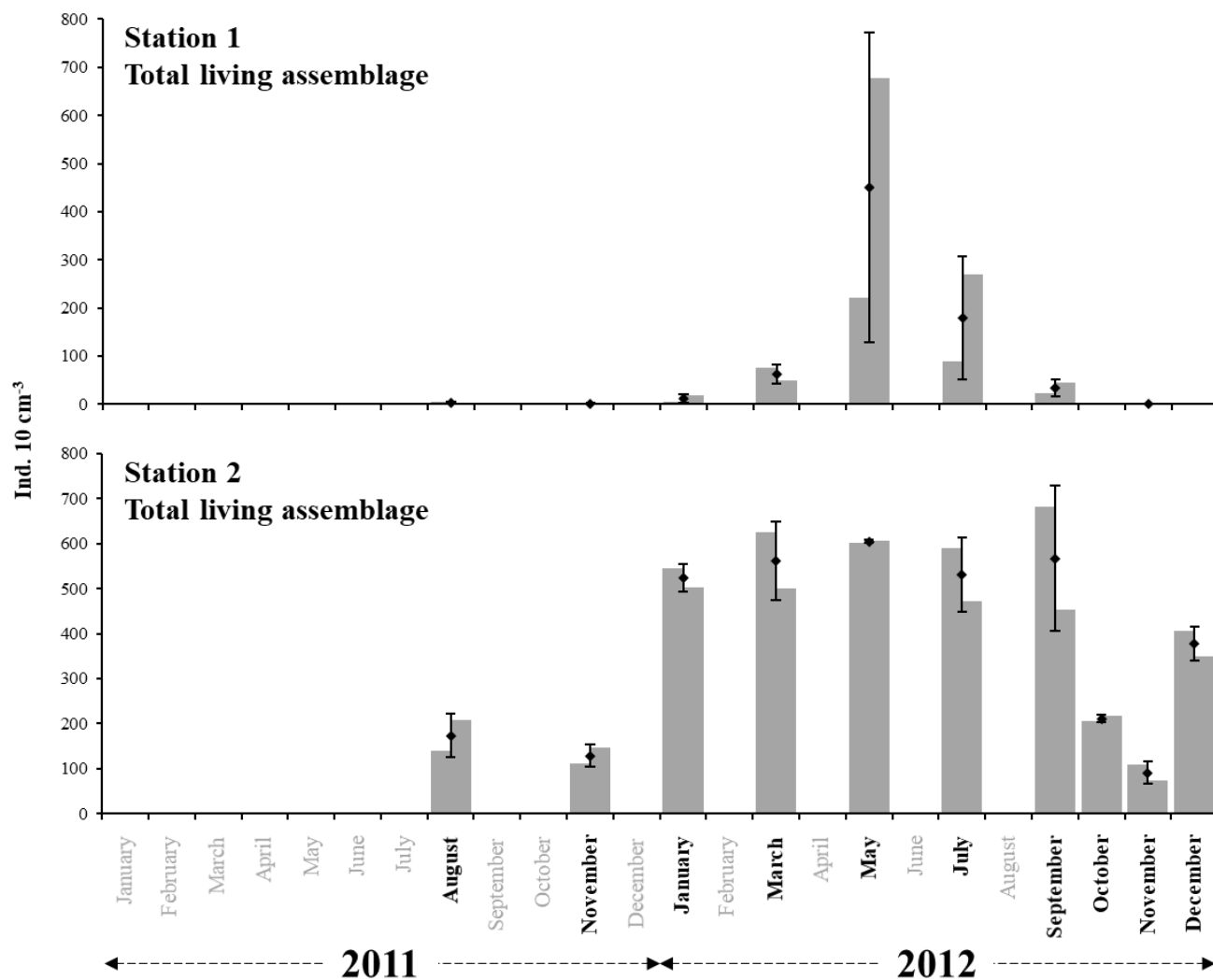


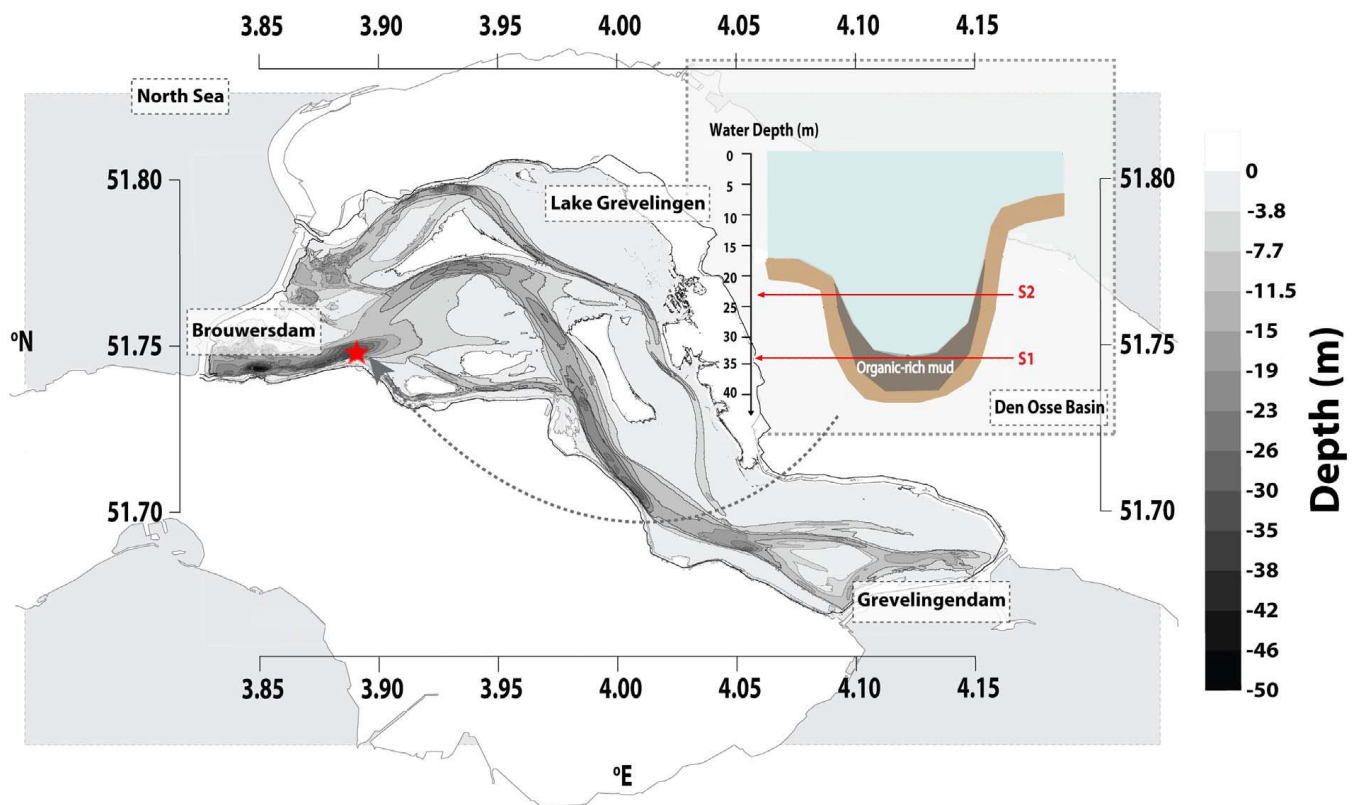
Foraminifera isolation



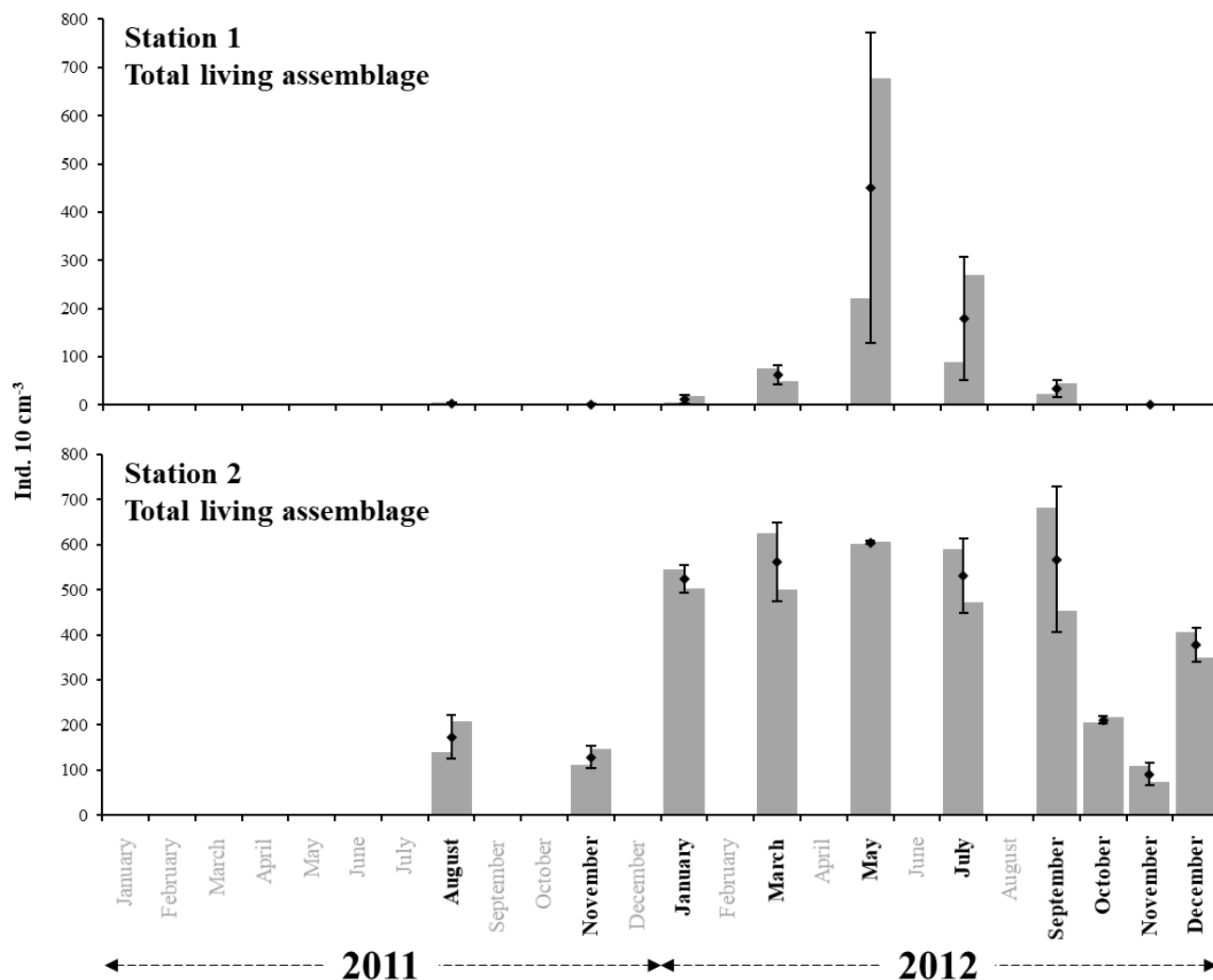
Individual measurement

**Figure 1:** Numerical treatment used for the size measurement for each image performed with ImageJ software. The left figure shows the untreated image, the middle figure presents the next step, when all individual foraminifera are depicted. Finally, the figure on the right shows the individual foraminiferal outlines which were measured.



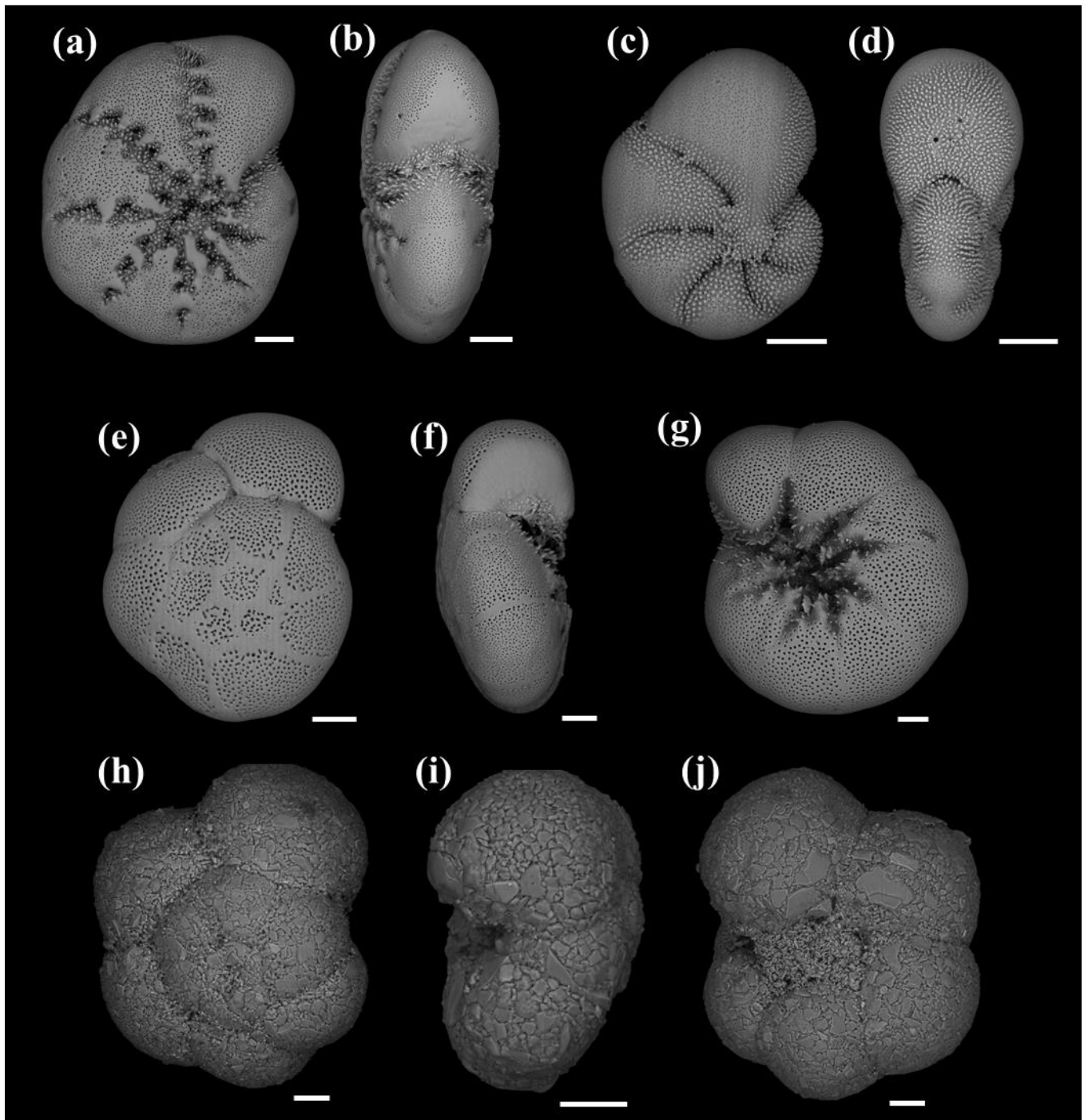


**Figure 1: Map of Lake Grevelingen showing the location of the two sampled stations in the Den Osse basin (red star). The transversal section of the Den Osse basin (top right) shows the depth at which station 1 (S1) and station 2 (S2) were sampled (34 m and 23 m depth, respectively). This figure was modified from Sulu-Gambari et al. 2016b.**



800 **Figure 2:** The grey bars represent the living foraminiferal abundances for the two replicates. The mean abundances (diamonds) and standard deviations (black error bars) were calculated for the two replicates for stations 1 (34 m depth, top panel) and 2 (23 m depth, bottom panel). All abundance values are for the 0–1 cm layer and were standardised to 10 cm<sup>3</sup>. Months where foraminiferal communities were investigated are indicated in bold (excluding October and December at station 1).

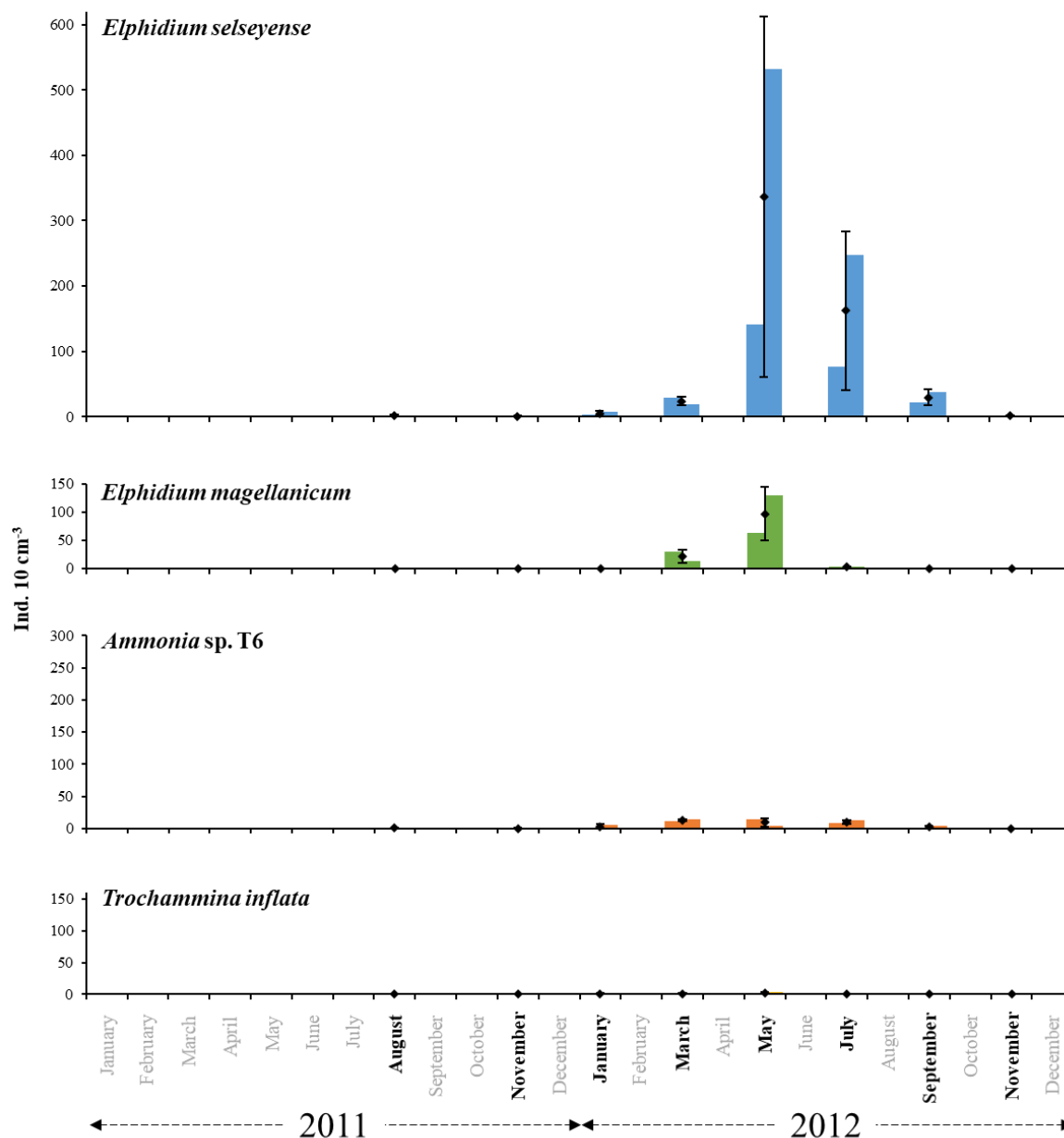




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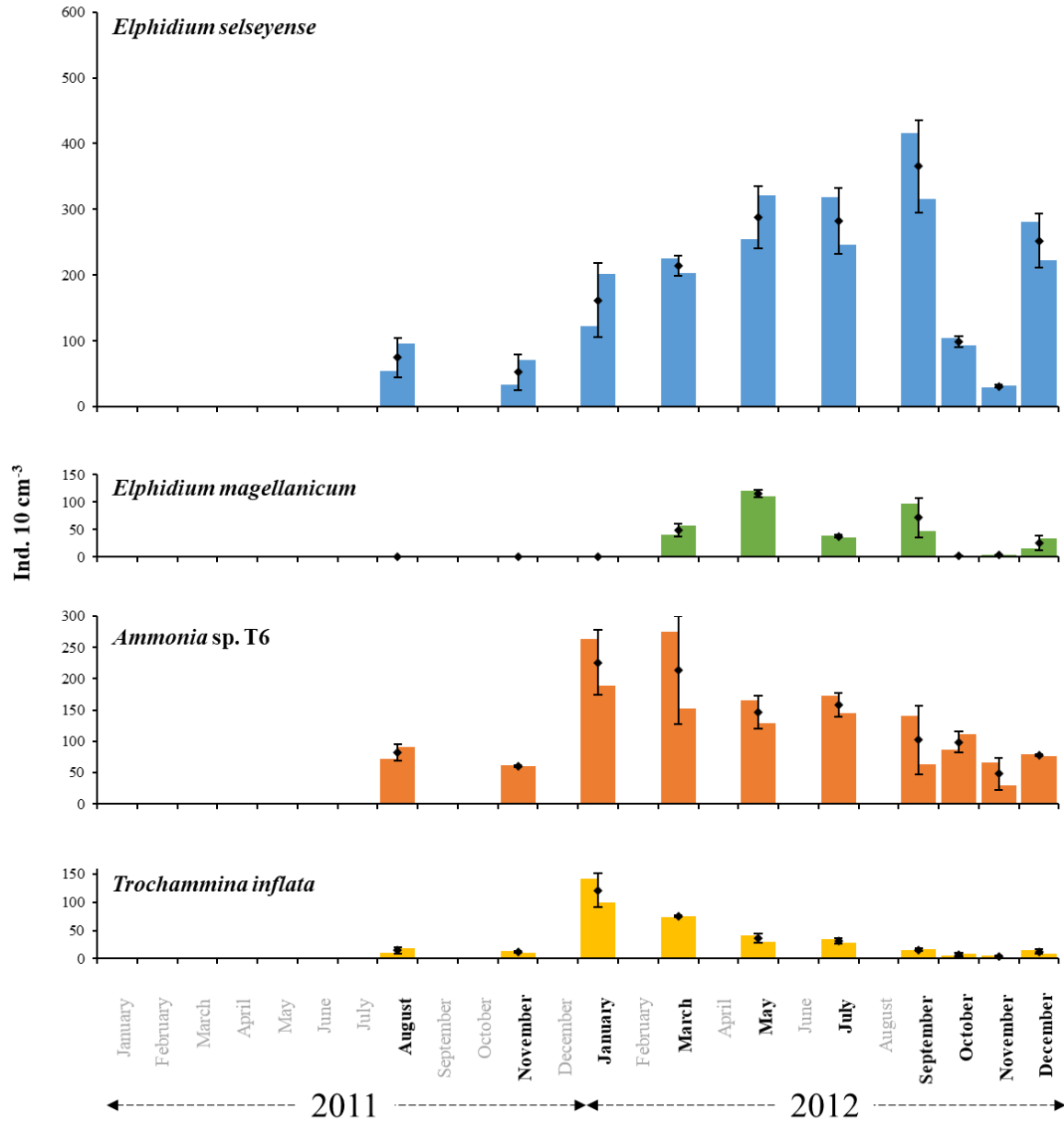
**Figure 3:** SEM images of *Elphidium selseyense* in lateral (a) and peripheral (b) view, *Elphidium magellanicum* in lateral (c) and peripheral (d) view, *Ammonia* sp. T6 in spiral (e), peripheral (f) and umbilical (g) view, and *Trochammina inflata* in spiral (h), peripheral (i) and umbilical (j) view. All scale bars are 50 μm.

# Station 1

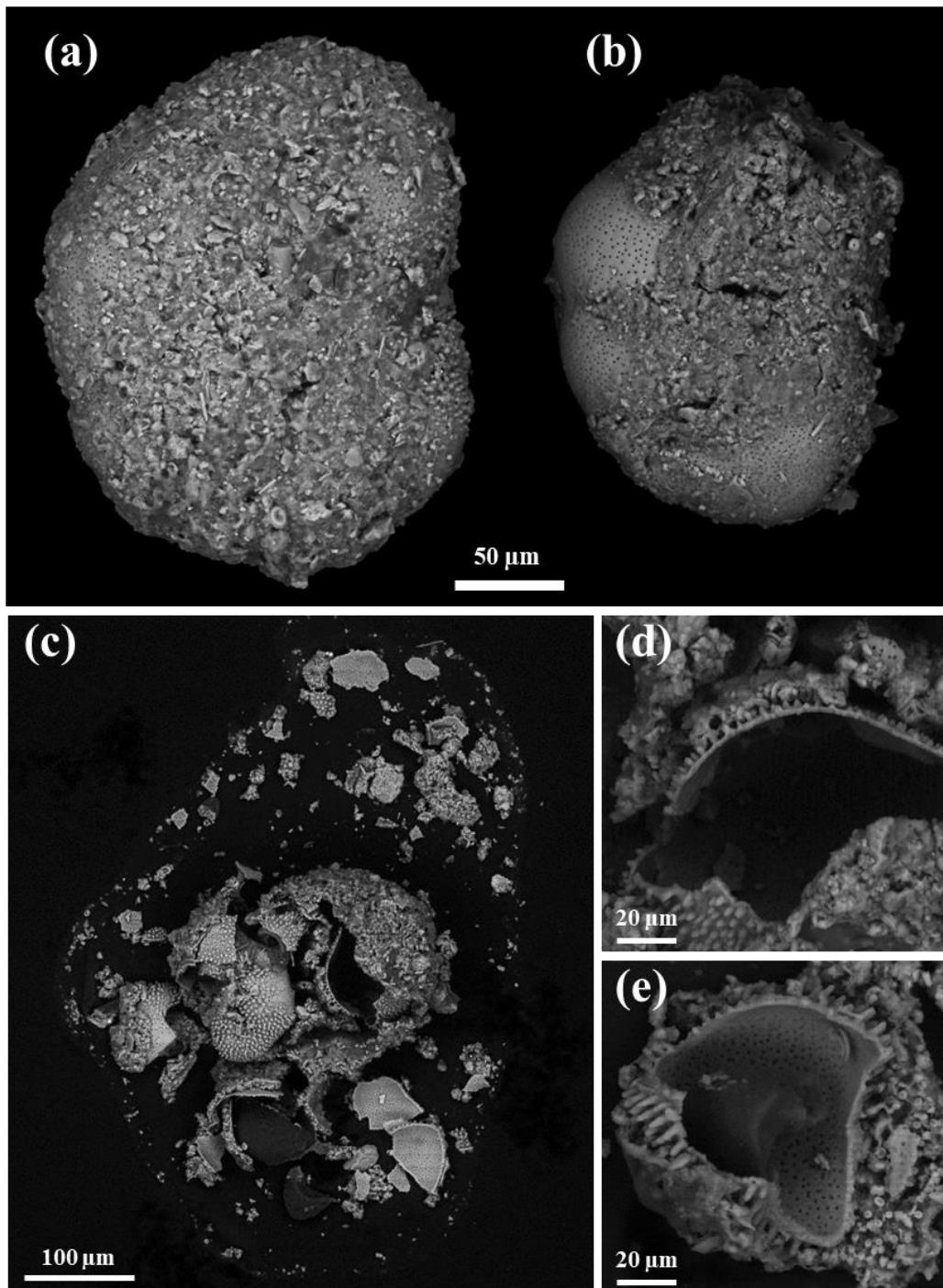


**Figure 4:** The bars represent the living foraminiferal abundances for the two replicates for *Elphidium selseyense* (blue), *Elphidium magellanicum* (green), *Ammonia* sp. T6 (orange) and *Trochammina inflata* (yellow) at station 1 in 2011 and 2012. The mean abundances (diamonds) and standard deviations (black error bars) were calculated for the two replicates. All abundances values are for 0–1cm layer and were standardised to 10 cm<sup>3</sup>. Months where foraminiferal communities were investigated are indicated in bold. Scales were chosen in order to facilitate comparison with station 2.

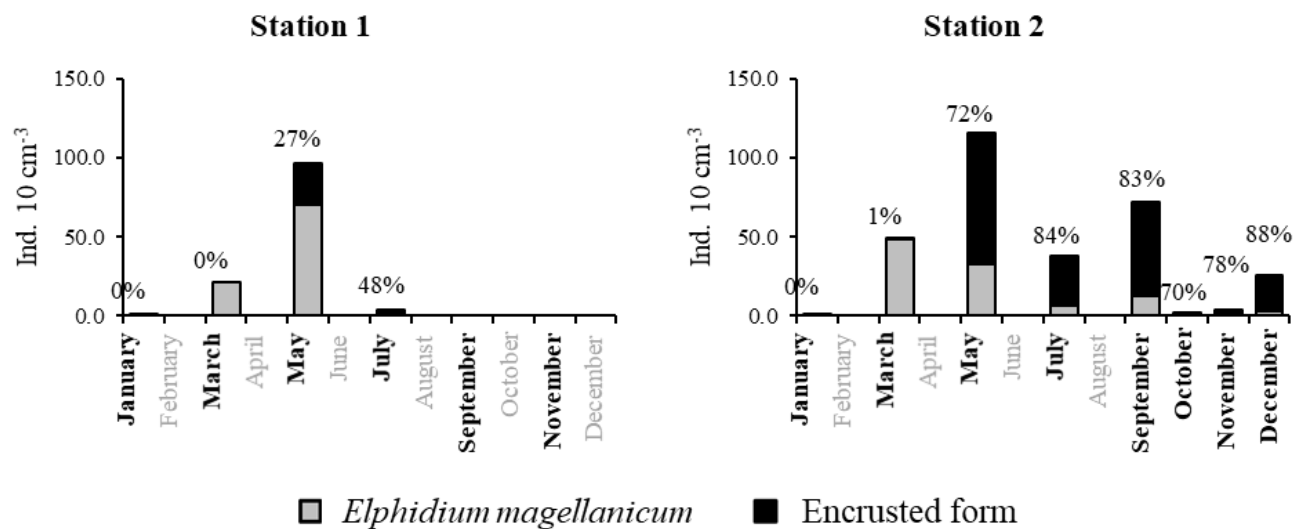
## Station 2



815 **Figure 5:** The bars represent the living foraminiferal abundances for the two replicates for *Elphidium selseyense* (blue), *Elphidium magellanicum* (green), *Ammonia* sp. T6 (orange) and *Trochammina inflata* (yellow) at station 2 in 2011 and 2012. The mean abundances (diamonds) and standard deviations (black error bars) were calculated for the two replicates. All abundances values are for 0–1cm layer and were standardised to 10 cm<sup>3</sup>. Months where foraminiferal communities were investigated are indicated in bold. Scales were chosen in order to facilitate comparison with station 1.



**Figure 8:** SEM images of (a) fully encrusted specimen, (b) partially encrusted specimen, (c) crushed encrusted specimen of *Elphidium magellanicum*. Note the thinness of the crust and the spinose structures on (d) and (e).



**Figure 9:** Mean abundances (ind. 10 cm<sup>-3</sup>) of non-encrusted (grey) and encrusted forms (black) of *Elphidium magellanicum* in 2012, at station, 1 (left) and 2 (right), with proportion of encrusted forms above each bar (in %). Investigated months are indicated in bold.

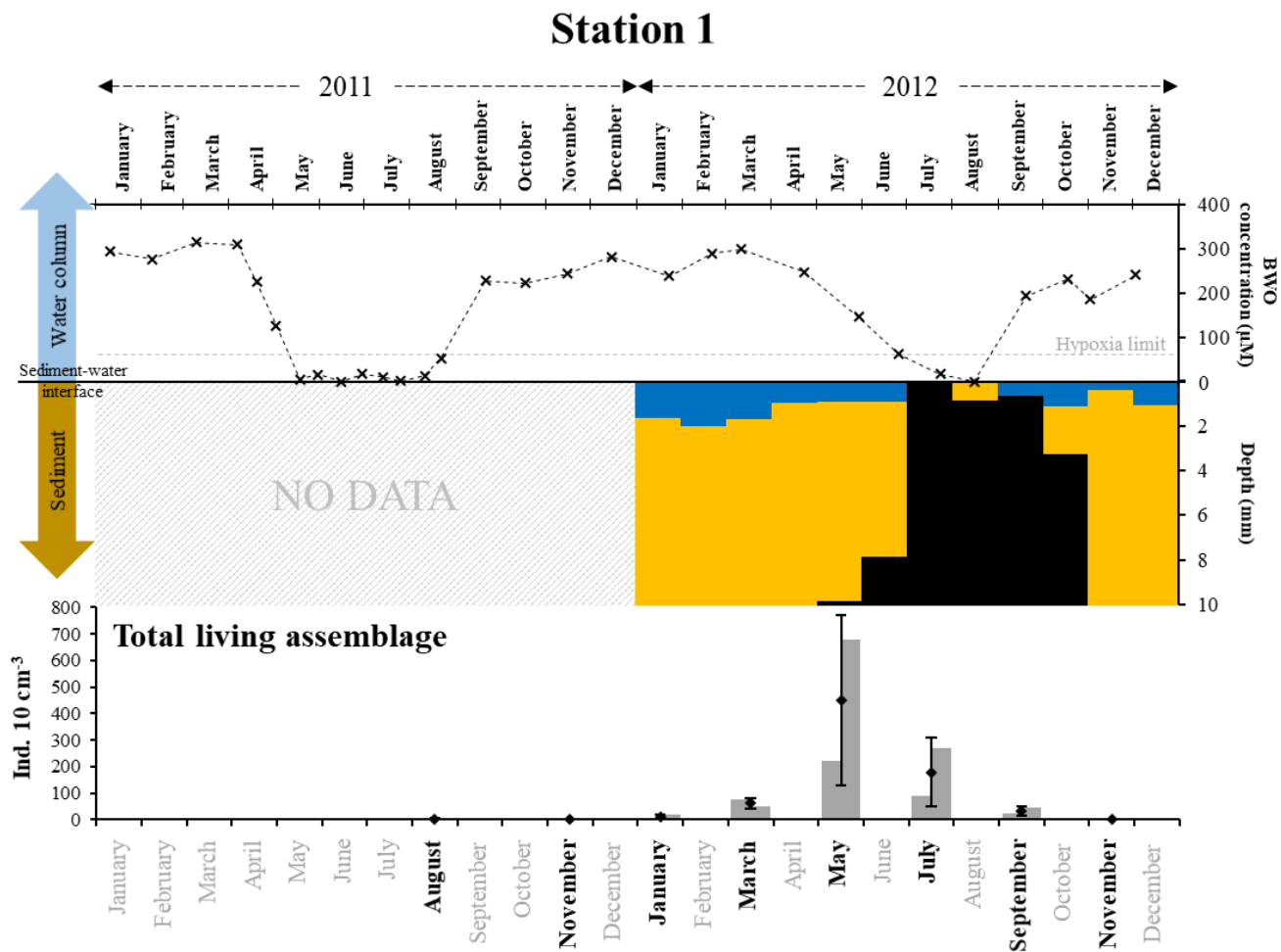
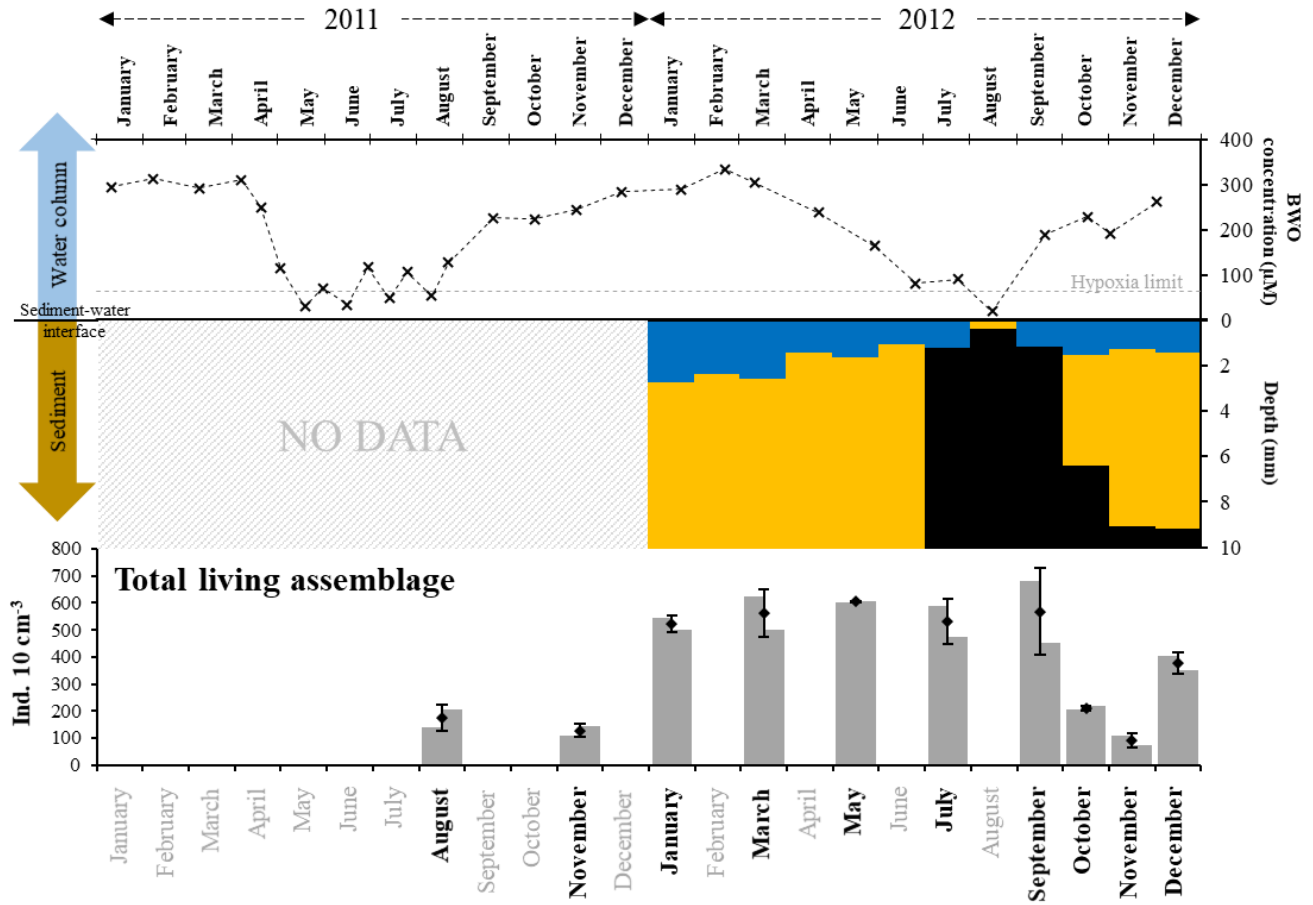


Figure 10: The top panel represents bottom-water oxygen concentrations ( $\mu\text{mol L}^{-1}$ ) in 2011 and 2012 at station 1, from Donders et al. (2012) and Seitaj et al. (2017). The grey horizontal dotted line indicates the hypoxia limit ( $63 \mu\text{mol L}^{-1}$ ). The middle panel represents the depth (in mm) distribution of the oxic (blue), absence of oxygen and sulphides (orange,) and sulphidic (black) zones within the sediment in 2012, from Seitaj et al. (2015). The bottom panel shows the total living foraminiferal abundances for both replicates (grey bars), mean abundances (diamonds) and standard deviations (black error bars) calculated for the two replicates, for all investigated months (in bold) in 2011 and 2012.

## Station 2



835 Figure 11: The top panel represents bottom-water oxygen concentrations ( $\mu\text{mol L}^{-1}$ ) in 2011 and 2012 at station 2, from Donders et  
 al. (2012) and Seitaj et al. (2017). The grey horizontal dotted line indicates the hypoxia limit ( $63 \mu\text{mol L}^{-1}$ ). The middle panel  
 represents the depth (in mm) distribution of the oxic (blue), suboxic (orange, absence of oxygen and sulphides) and sulphidic (black)  
 zones within the sediment in 2012. The bottom panel shows the total living foraminiferal abundances for both replicates (grey bars),  
 mean abundances (diamonds) and standard deviations (black error bars) calculated for the two replicates, for all investigated  
 840 months (in bold) in 2011 and 2012.

## SUPPLEMENTARY MATERIAL

*Associated with the manuscript:*

### **Foraminiferal community response to seasonal anoxia in Lake Grevelingen (the Netherlands)**

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**Supplementary Table 1. Oxygen Penetration Depth  $\pm$  sd and free H<sub>2</sub>S detection depth  $\pm$  sd for each month in 2012 for both stations 1 and 2 (in mm).**

Station	Month	OPD (mm)	H <sub>2</sub> S depth (mm)
Station 1	January	1.7 $\pm$ 0.3	16.5 $\pm$ 3.2
	February	2 $\pm$ 0.4	17.1 $\pm$ 2.8
	March	1.7 $\pm$ 0.3	17.5 $\pm$ 0.7
	April	1 $\pm$ 0.2	18.6 $\pm$ 4.8
	May	1 $\pm$ 0.1	9.9 $\pm$ 2.2
	June	0.9 $\pm$ 0.1	7.9 $\pm$ 5.3
	July	0 $\pm$ 0	0.1 $\pm$ 0.1
	August	0 $\pm$ 0	0.9 $\pm$ 1.1
	September	0.7 $\pm$ 0.1	0.3 $\pm$ 0.2
	October	1.1 $\pm$ 0.1	3.3 $\pm$ 1.1
	November	0.4 $\pm$ 0	10.3 $\pm$ 1.9
	December	1.1 $\pm$ 0.2	13.4 $\pm$ 1.8
Station 2	January	2.8 $\pm$ 0	19.6 $\pm$ 2
	February	2.4 $\pm$ 0.2	15.8 $\pm$ 1.2
	March	2.6 $\pm$ 0.6	20.3 $\pm$ 3.3
	April	1.4 $\pm$ 0.2	23.3 $\pm$ 0.3
	May	1.6 $\pm$ 0	26.4 $\pm$ 1
	June	1.1 $\pm$ 0.4	17.1 $\pm$ 0.4
	July	1.3 $\pm$ 0.4	1.1 $\pm$ 0.8
	August	0 $\pm$ 0	0.4 $\pm$ 0.2
	September	1.2 $\pm$ 0.2	0.8 $\pm$ 0.2
	October	1.6 $\pm$ 0.3	6.4 $\pm$ 2.9
	November	1.3 $\pm$ 0.2	9.1 $\pm$ 3.3
	December	1.5 $\pm$ 0.2	9.2 $\pm$ 0.7

Supplementary Table 2. Living foraminiferal abundances for each replicate for the dominant species and total assemblage (ind./10cm<sup>3</sup>, 10cm<sup>3</sup>).

STATION 1											
Species		<i>Elphidium selseyense</i>		<i>Ammonia</i> sp. <i>T6</i>		<i>Elphidium magellanicum</i>		<i>Trochammina inflata</i>		Total assemblage	
Year	Month	A	B	A	B	A	B	A	B	A	B
2011	August	2.1	0.4	1.4	1.1	0.0	0.0	0.0	0.0	4.2	2.5
2011	November	0.0	1.1	0.0	0.7	0.0	0.0	0.0	0.0	0.0	2.1
2012	January	2.8	7.4	0.7	5.7	0.0	0.4	0.4	2.1	5.0	18.0
2012	March	28.6	19.1	12.0	13.8	29.4	13.8	2.1	0.7	75.7	48.5
2012	May	141.5	531.6	13.8	4.6	63.0	129.8	0.4	3.2	222.1	677.6
2012	July	76.0	247.9	8.1	12.4	3.9	3.5	0.0	0.0	88.4	270.6
2012	September	21.2	38.2	0.7	3.9	0.0	0.0	0.0	0.7	21.9	46.0
2012	November	0.7	1.4	0.4	0.4	0.0	0.0	0.0	0.0	1.4	1.8
STATION 2											
Species		<i>Elphidium selseyense</i>		<i>Ammonia</i> sp. <i>T6</i>		<i>Elphidium magellanicum</i>		<i>Trochammina inflata</i>		Total assemblage	
Year	Month	A	B	A	B	A	B	A	B	A	B
2011	August	53.8	95.8	72.5	91.6	0.0	0.0	10.6	18.7	140.1	208.0
2011	November	33.2	71.4	61.9	59.8	0.0	0.0	13.1	10.6	111.1	146.4
2012	January	122.0	201.6	263.1	189.2	1.1	0.7	142.5	100.4	545.4	501.9
2012	March	225.6	203.7	275.2	152.8	41.0	56.6	73.9	76.0	624.2	500.5
2012	May	254.6	321.8	165.9	128.4	120.6	111.4	42.1	30.1	602.3	607.3
2012	July	318.3	246.9	172.2	144.7	39.6	36.1	35.4	27.6	589.9	473.2
2012	September	415.2	315.8	141.1	63.7	97.3	46.7	14.9	17.3	681.2	453.8
2012	October	104.7	92.7	87.0	111.1	2.1	1.4	5.3	9.5	205.8	217.2
2012	November	29.4	32.5	66.5	29.7	3.9	4.2	5.0	2.5	108.9	73.2
2012	December	281.2	223.2	78.9	77.1	16.3	34.7	15.9	9.5	405.3	350.5

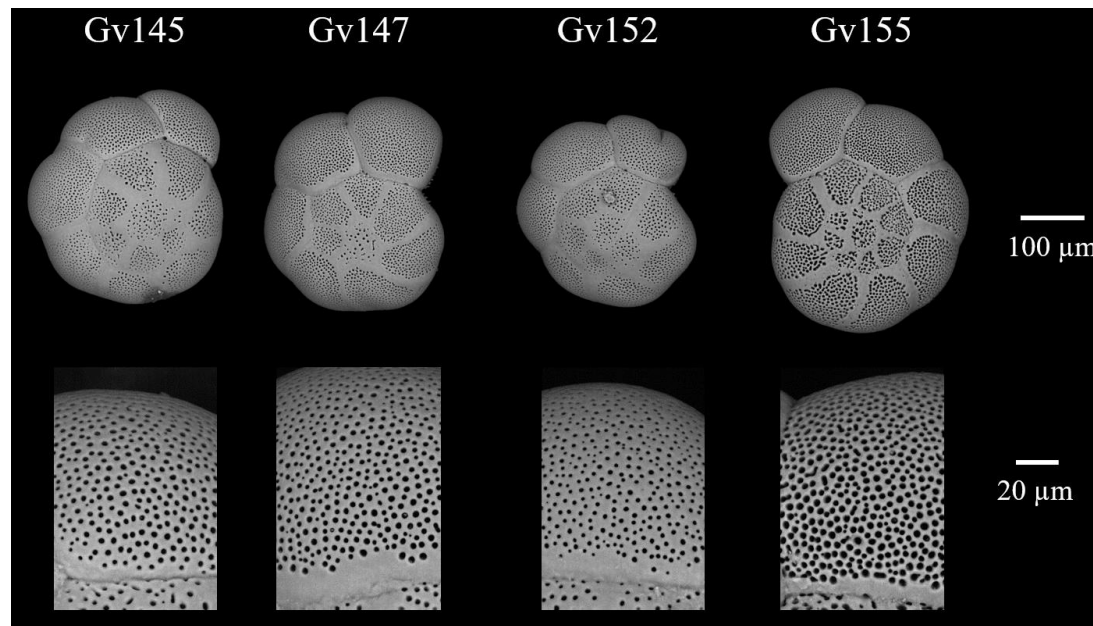
Supplementary Table 3. Living foraminiferal abundances for each replicate, year and month for all the species of the assemblage (ind./10cm<sup>3</sup>, 10cm<sup>-3</sup>). Empty cases represent the absence in the sample.  
Last column: absolute abundance of the total fauna.

Year	Station	Replicate	Month	<i>Ammonia falsobaccarii</i> (T15)	<i>Ammonia</i> sp. T1	<i>Ammonia</i> sp. T2	<i>Ammonia</i> sp. T3	<i>Ammonia</i> sp. T6	<i>Bulinina denudata</i>	<i>Bulinina elongata</i>	<i>Bulinina marginata</i>	<i>Bulinina</i> sp.	<i>Cassidulina</i> sp.	<i>Elphidium selseyense</i>	<i>Elphidium magellanicum</i>	<i>Elphidium magellanicum</i> (encrusted)	<i>Elphidium margaritaceum</i>	<i>Elphidium</i> sp.	<i>Episominella</i> sp.	<i>Haynesina depressula</i>	<i>Haynesina germanica</i>	<i>Hopkinsina</i> sp.	<i>Leptohalysis</i> sp.	Non determined	<i>Nonion</i> sp.	<i>Nonionella</i> sp.	<i>Quinqueloculina levigata</i>	<i>Quinqueloculina</i> sp.	<i>Stainforthia</i> sp.	<i>Textularia</i> sp.	<i>Trochammina inflata</i>	Total
2011	1	A	August	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.4	0.0	0.0	0.0	4.2
2011	1	A	November	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
2012	1	A	January	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	5.0	
2012	1	A	March	0.4	0.0	1.1	0.0	12.0	0.4	0.0	0.0	0.0	0.0	28.6	29.4	0.0	0.0	0.4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.4	2.1	75.7
2012	1	A	May	0.0	0.0	0.0	0.0	13.8	1.1	0.0	0.4	0.0	0.0	141.5	47.7	15.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.4	1.1	0.0	0.4	0.0	0.4	222.1
2012	1	A	July	0.0	0.0	0.0	0.0	8.1	0.0	0.0	0.0	0.0	0.0	76.0	1.8	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	88.4
2012	1	A	September	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	21.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.9
2012	1	A	November	0.0	0.0	0.4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
2011	1	B	August	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5
2011	1	B	November	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	2.1
2012	1	B	January	0.0	0.0	0.7	0.0	5.7	0.0	0.0	0.0	0.0	0.0	7.4	0.4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.4	0.0	0.0	2.1	18.0
2012	1	B	March	0.0	0.0	0.0	0.0	13.8	0.0	0.0	0.0	0.0	0.0	19.1	13.8	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0	0.0	0.7	48.5
2012	1	B	May	0.0	0.0	0.0	0.0	4.6	0.4	0.0	0.0	0.0	0.0	531.6	93.4	36.4	0.4	0.0	0.7	0.4	0.0	0.0	0.0	2.1	0.0	0.4	0.4	1.1	2.5	0.4	3.2	677.6
2012	1	B	July	0.0	0.0	0.4	0.0	12.4	0.4	0.0	0.7	0.0	0.0	247.9	2.1	1.4	1.4	0.4	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.7	0.4	1.8	0.0	0.0	270.6
2012	1	B	September	0.0	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	38.2	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.4	0.7	46.0
2012	1	B	November	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8
2011	2	A	August	0.0	0.0	0.0	0.0	72.5	0.0	0.0	0.0	0.0	0.0	53.8	0.0	0.0	0.7	0.0	0.0	0.0	0.4	0.0	1.1	0.0	0.0	0.4	0.0	0.4	0.0	0.4	10.6	140.1
2011	2	A	November	0.0	0.0	0.0	0.0	61.9	0.0	0.0	0.0	0.0	0.0	33.2	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	1.1	0.0	0.0	13.1	111.1
2012	2	A	January	0.7	0.0	2.5	8.8	263.1	0.0	1.1	0.0	0.0	0.0	122.0	1.1	0.0	0.7	0.4	1.1	0.0	0.0	0.0	0.0	0.7	0.4	0.0	0.0	0.0	0.4	142.5	545.4	
2012	2	A	March	0.0	0.0	1.4	0.0	275.2	0.0	0.0	0.0	1.8	0.0	225.6	40.0	1.1	0.4	0.0	0.4	0.0	0.0	0.0	0.0	0.7	0.7	0.0	1.4	0.0	1.8	73.9	624.2	
2012	2	A	May	0.0	0.0	1.1	0.0	165.9	0.0	0.0	0.4	3.9	0.0	254.6	38.6	82.1	0.4	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	3.2	0.4	2.1	1.4	5.0	42.1	602.3
2012	2	A	July	0.0	0.0	1.8	0.0	172.2	6.0	2.1	0.4	0.4	0.0	318.3	3.9	35.7	1.4	0.0	0.4	0.7	0.0	0.0	0.0	0.0	0.4	0.0	7.1	1.8	2.1	35.4	589.9	

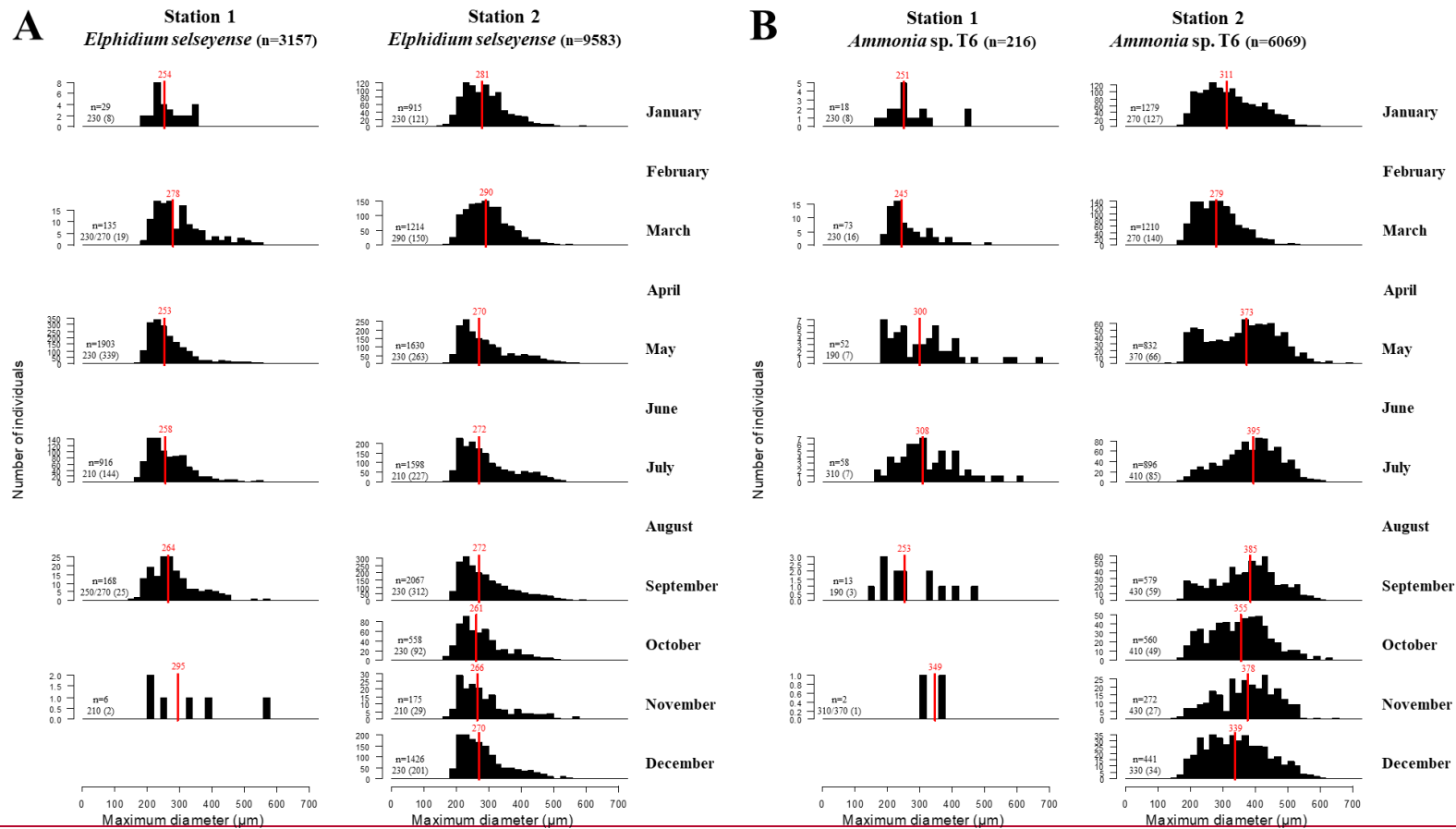
Cellules insérées

Mis en forme : Police :Italique

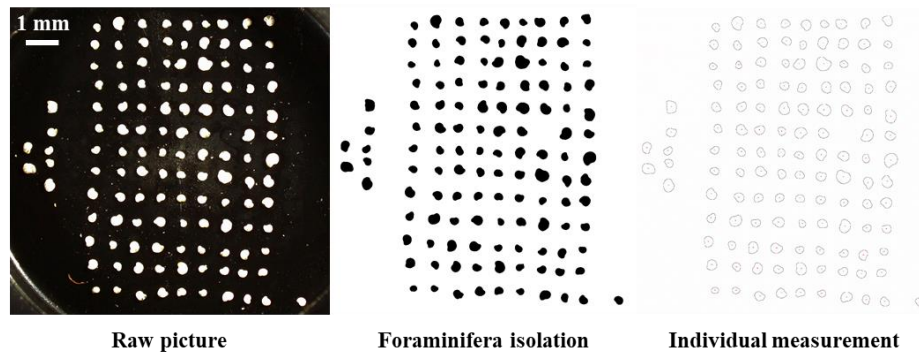
2012	2	A	September	0.0	0.7	0.0	0.0	141.1	0.0	1.4	0.4	0.0	0.0	415.2	16.3	81.0	0.4	0.4	3.2	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.4	1.4	3.2	14.9	<u>681.2</u>	
2012	2	A	October	0.0	0.4	0.7	0.0	87.0	1.1	2.5	0.4	0.0	0.0	104.7	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	1.4	5.3	<u>205.8</u>		
2012	2	A	November	0.0	0.0	0.0	0.0	66.5	0.7	0.0	0.4	0.0	0.0	29.4	0.0	3.9	0.4	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.7	0.0	5.0	<u>108.9</u>	
2012	2	A	December	0.7	0.0	1.8	0.0	78.9	1.1	0.7	1.4	0.0	0.0	281.2	0.4	15.9	0.0	0.0	0.7	0.4	0.0	1.8	0.0	0.4	0.0	0.4	0.4	3.2	15.9	<u>405.3</u>		
2011	2	B	August	0.0	0.0	0.0	0.0	91.6	0.0	0.0	0.0	0.4	0.0	95.8	0.0	0.0	0.0	0.0	0.0	0.7	0.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	18.7	<u>208.0</u>	
2011	2	B	November	0.0	0.0	0.0	0.0	59.8	0.0	0.0	0.0	0.4	0.0	71.4	0.0	0.0	1.1	0.0	0.0	1.1	0.0	0.0	0.0	1.1	0.0	0.0	1.1	0.0	0.0	10.6	<u>146.4</u>	
2012	2	B	January	0.0	0.4	2.1	0.0	189.2	0.0	0.4	0.0	0.0	0.0	201.6	0.7	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0	0.4	100.4	<u>501.9</u>	
2012	2	B	March	0.0	0.0	1.1	0.0	152.8	0.4	0.0	0.0	2.1	0.0	203.7	56.2	0.4	1.1	0.7	1.4	0.0	0.0	0.0	0.0	0.0	1.1	0.4	0.0	1.8	0.7	0.7	76.0	<u>500.5</u>
2012	2	B	May	0.0	0.0	1.4	0.0	128.4	2.1	0.0	0.7	0.0	0.4	321.8	25.8	85.6	0.0	0.0	0.4	0.4	0.0	0.0	0.0	1.8	0.0	2.8	1.1	0.7	1.1	2.8	30.1	<u>607.3</u>
2012	2	B	July	0.0	1.1	1.4	0.0	144.7	0.4	1.8	1.8	2.1	0.0	246.9	8.1	27.9	0.7	0.0	1.1	1.1	0.0	0.0	0.0	0.0	0.0	0.4	2.1	1.1	0.7	2.5	27.6	<u>473.2</u>
2012	2	B	September	0.0	0.0	0.4	0.0	63.7	1.8	0.7	0.0	0.0	0.0	315.8	8.1	38.6	1.4	0.4	2.1	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.4	1.4	1.4	17.3	<u>453.8</u>	
2012	2	B	October	0.0	0.7	1.1	0.0	111.1	0.4	0.0	0.0	0.0	0.0	92.7	1.1	0.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.5	<u>217.2</u>	
2012	2	B	November	0.0	0.0	0.4	0.0	29.7	1.1	0.0	0.4	0.0	0.0	32.5	1.8	2.5	0.4	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.7	2.5	<u>73.2</u>	
2012	2	B	December	0.0	0.0	0.0	0.0	77.1	1.4	0.7	0.0	0.0	0.0	223.2	5.7	29.0	1.1	0.0	1.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0	9.5	<u>350.5</u>	



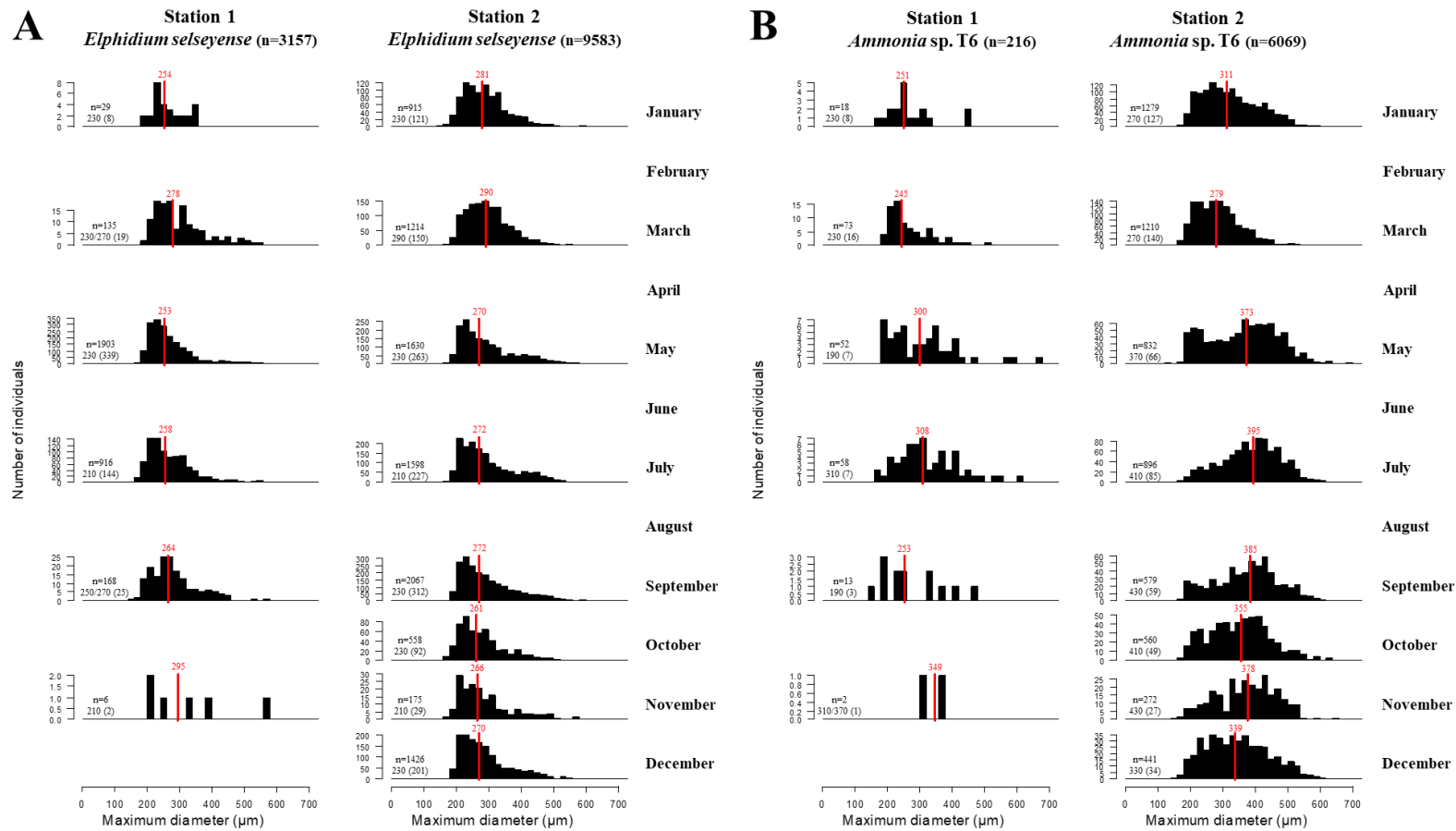
Supplementary **Figure 1**. SEM images of spiral side and a 1000x magnification of the penultimate chamber for four individuals from Grevelingen station 1 identified T6 by molecular identification.



**Supplementary Figure 2**



Supplementary Figure 2: Numerical treatment used for the size measurement for each image performed with ImageJ software. The three size fractions (125–150, 150–315, >315  $\mu\text{m}$ ) were analysed together for the size distribution analyses. The left figure shows the untreated image, the middle figure presents the next step, when all individual foraminifera are depicted. Finally, the figure on the right shows the individual foraminiferal outlines which were measured.



**Supplementary Figure 3:** A: size distribution (maximum diameter for each individual in μm) of *Elphidium selseyense* for stations 1 (left) and 2 (right) in 2012. B: size distribution (maximum diameter for each individual in μm) of *Ammonia* sp. T6 for stations 1 (left) and 2 (right) in 2012. For each month, the number of individuals (n), the mode and the number of individuals associated to the mode (between brackets) are indicated in black. The medians are indicated by the red bars in each panel. In order to base our analysis on a sufficiently high number of specimens, we focused on *E. selseyense* and *Ammonia* sp. T6. As explained before, we only considered specimens retained on a 125 μm mesh meaning that juvenile specimens are not represented. Only the samples taken in 2012 were considered. The size distribution of *E. selseyense* was relatively similar between the two stations regarding the median, ranging from 253 μm (in May) to 295 μm (in November) at station 1 and from 261 μm (in October) to 290 μm (in March) at station 2. At both stations, we observed the presence of an abundant group of smaller specimens, with a mode that never exceeded 250 μm, except in March at station 2, when it is difficult to separate this subpopulation from the larger specimens. The main difference between the two stations was the higher proportion of larger individuals (>400 μm) at station 2, which



was visible through the better-developed tails at the right side of the distribution graphs. The low number of *Ammonia* sp. T6 individuals at station 1 did not allow us to draw any firm conclusion concerning the size distribution at this station (Supplementary Figure 3). At station 2, a group of individuals with smaller diameters ( $< 300\text{ }\mu\text{m}$ ) was always present. The overall size distribution showed a clear shift to higher diameters between March (median =  $279\text{ }\mu\text{m}$ ) and May (median =  $373\text{ }\mu\text{m}$ , Fig. 7), which is also evidenced by the much higher proportion of larger individuals. Specimens larger than  $400\text{ }\mu\text{m}$  were abundantly found until November (median =  $378\text{ }\mu\text{m}$ ), but started to diminish in December, as is also shown by the decrease of the median to  $339\text{ }\mu\text{m}$ . Our tentative to distinguish cohorts by using a deconvolution method to separate the total size distributions into a sum of Gaussian curves was not conclusive. The main problem was the fact that we did not have any information concerning individuals smaller than  $125\text{ }\mu\text{m}$ , so that our size distributions were systematically skewed on the left side (i.e. toward small individuals). An additional problem was the large number of smaller specimens which were always present. Because the identification of individual cohorts was not successful, parameters like reproduction rate, growth rate or lifespan were not assessable. Nevertheless, the size distribution data give some clues concerning the population dynamics of the two dominant species.