Spatial and temporal variability of benthic foraminifera in the Grevelingenmeer, May, August and November 2011

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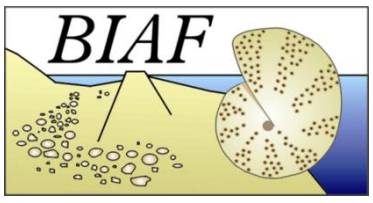

Abstract

The present report is a preliminary report on the spatial and temporal variability of benthic foraminiferal faunas in the Grevelingenmeer. Our faunal analyses, using the very critical CTG method to recognise live individuals, indicate that abundant live foraminiferal faunas occur at stations 2 and 4 (water depth 23 and 17 m, respectively) in August and November 2011. At station 1 (30-34 m), which is subject to seasonal anoxia, poor foraminiferal faunas were observed in May, August and November 2011. We suspect that these scarce living foraminifera have been transported into the deepest part of the channel, and do not represent an autochthonous viable fauna. At stations 2 and 4, the faunas are almost exclusively composed of three taxa: *Ammonia* cf. *A. batava*, *Elphidium* ex. gr. *E. excavatum* and *Trochammina* cf. *T. inflata*. No major difference in species composition is observed between the two stations, but the faunas of station 4 are about two times richer than those of station 2. Most of the species living in the Grevelingenmeer are characterised by a morphology which differs from that of the typical species. Especially in *Ammonia* cf. *A. batava*, the pore density and pore size appears to be much higher than in typical *A. batava*. Additional analyses will be performed to find out whether this is a morphological adaptation to low oxygen concentrations.

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Introduction

The Grevelingenmeer is a saline lake in the Southwest of the Netherlands. Due to the closure of this former branch of the estuary, a strong stratification of the water column is taking place seasonally, resulting in recurrent bottom water hypoxia in the deeper areas. Bottom water anoxia are developing in some of the deepest channels (Figure 1).

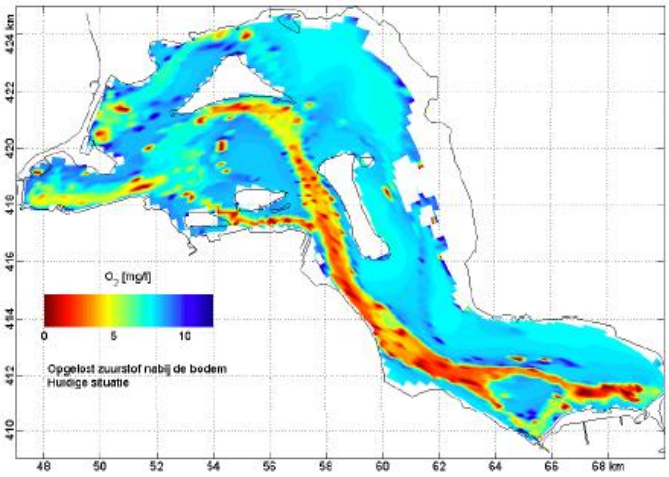

Figure 1 - Map of bottom water oxygen concentration, July 2010

In late summer, a strong bathymetrical biogeochemical zonation can be observed (Figure 2). Below 25 meters depth, bottom waters are anoxic and the sediment appears to be azoic (no macro-epibiotic specimens). Between 25 and 20 meters depth bottom waters are strongly hypoxic and bacterial mats (of *Beggiatoa sp.*) cover the sediment surface. Between 20 and 15 meters depth the sediment surface shows large amounts of the polychaete annelid worm *Ophiodromus flexuosus*. At shallower depth, above 15 m, the sediment is bioturbated. Consequently, it appears that the oxygen concentration (and perhaps other redox species) largely determines the macrofaunal and meiofaunal distribution.

The main goals of our study were to investigate:

1) whether live foraminiferal faunas are present in the Grevelingenmeer, and, if so,
2) whether the faunal assemblages show any spatial and/or seasonal variability, in response to varying oxygen concentrations.

A preliminary study of the foraminiferal assemblages, performed by TNO, which has used the Rose Bengal staining method (a method based on the staining of proteins in the foraminiferal protoplasm), suggested the absence of live foraminiferal faunas in the Grevelingenmeer.

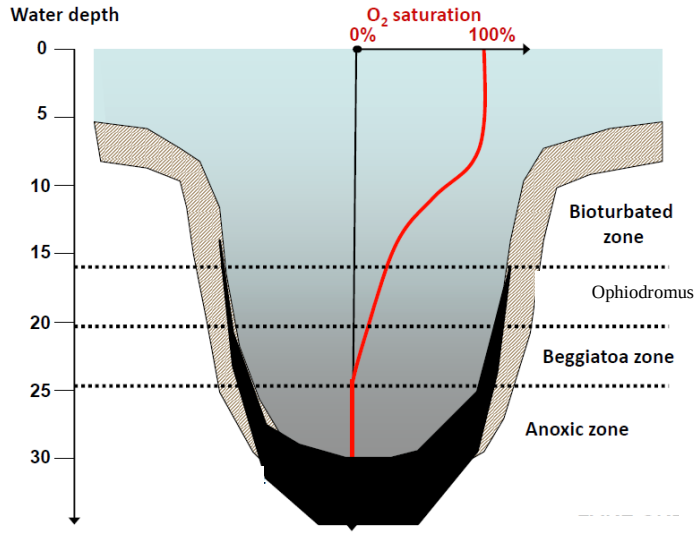

Figure 2 - Schematic representation of bathymetrical biogeochemichal zonation along the southern channel transect (after Meysman *et al.*, pers. com.)

For this study, we decided to use the Cell Tracker Green method (CTG: a method based on esterase activity), which is considered much more reliable in strongly hypoxic environments. In fact, the Rose Bengal methods is not adequate for the study of live foraminiferal faunas in low oxygen environments, because proteins may be degraded very slowly in such conditions, and dead foraminifera may still stain for several months to years after their death (Corliss & Emerson, 1990).

Consequently, we use the much more critical CTG method (CTG becomes only fluorescent in a living organism, with esterase activity, see Bernard et al., 2006) to study the spatial and seasonal variations of the foraminiferal assemblages, and to identify whether there is an effect of the spatial and temporal changes in oxygen concentration on the density, diversity and species composition of the foraminiferal faunas. In view of the large tolerance of benthic foraminifera with respect to various stress parameters (e.g. Murray, 2006), we expected to find life foraminiferal faunas at all sites, eventually with important changes in density, diversity and species composition along the oxygen gradient.

Material and Methods

Sampling strategy

Sediment samples have been collected during 3 sampling campaigns at 6 different sites. During the May cruise on the R/V Argus, 4 sites were sampled to cover a large spatial area, with 2 sites (one oxic, one hypoxic) in the northern channel and 2 sites (one oxic, one hypoxic) in the southern channel (Table 1 and Figure 3). During the August and November cruises on the R/V Luctor, we focussed on a 3-stations bathymetric transect in the Southern channel, with a strong gradient in bottom water oxygenation in August (Figure 2).

Station	Depth (m)	Lattitude	Longitude	Sampling date	Month	Number of cores taken	O_2 saturation (%)
ST6	30	51°44'46.5341"N	3°53'22.1415"E	18/05/2011	May	2	NA
ST13	16	51°42'53.6326"N	3°59'57.5929"E	18/05/2011	May	2	NA
ST26,5	13,3	51°46'05.8762"N	3°59'11.9570"E	18/05/2011	May	2	NA
ST22	24	51°45'47.4010"N	3°52'08.5633"E	18/05/2011	May	2	NA
ST1	34	51°44.834 N	3°53.401 E	23/08/2011	August	2	4
ST2	23,1	51°44,956 N	03°53.826 E	24/08/2011	August	2	45
ST4	17,2	51°44.856 N	03°53.942 E	24/08/2011	August	2	60
ST1	34	51°44.834 N	3°53.401 E	01/11/2011	November	2	82
ST2	23,1	51°44,956 N	03°53.826 E	01/11/2011	November	2	91
ST4	17,2	51°44.856 N	03°53.942 E	01/11/2011	November	2	94

Table 1- Sampling sites, depth, coordinates, sampling dates and number of cores. O_2 saturation measured by CTD at the station 1, the value given for the stations 2 and 4 are the one at the station 1 at the same depth. NA: no data available.

Figure 3 - Location of the sampling sites (Left panel: May 2011 sampling stations; right panel: August and November 2011 sampling stations)

At all sampling stations, two cores have been taken for foraminiferal analysis. Our cores have been obtained by subsampling in a boxcorer during the May cruise and with a MiniMUC multicorer or a UWITEC corer (both interface corer) for both August and November cruises. During all 3 cruises we obtained cores with an inner diameter of 6 cm.

Foraminiferal faunal analysis

All cores were treated using the CTG method. CellTracker Green CMFDA is a non fluorescent molecule, which is hydrolyzed in the living organism by nonspecific esterase, producing a fluorescent compound (if observed with the accurate excitation and emission wavelength). When living cells are incubated in fluorogenic probes such as Cell-Tracker Green CMFDA, the probe passes through the cellular membrane, and reaches the cytoplasm where hydrolysis with nonspecific esterase causes the fluorogenic reaction.

For faunal analyses, entire sediment cores (28.3 cm^2 surface area) were sliced, every half cm between 0 and 2 cm and every cm between 2 and 5 cm. Within one hour after retrieval, sediments were stored in 200 cc bottles, which were filled with sea water and CTG-DMSO at a final CTG concentration of 1µM as described by Bernhard et al. (2006). Samples were shaken and immediately placed in a cool box at *in situ* temperature where they remained for at least 6 h in order to obtain a CTG reaction. After this reaction period, samples were taken from the incubator and fixed in 4% formalin buffered with Sodium Borax. The samples were further stored at room temperature (See also the detailed CTG protocol in Appendix C).

In the laboratory, the samples were subsequently sieved over 315, 150, 125 and 63 µm meshes. Foraminiferal counts were performed in the >125 µm fraction using an epifluorescence stereomicroscope (Olympus SZX12 with a light fluorescent source Olympus URFL-T). Samples were excited at a wavelength of 492 nm, whereas emission took place at 517 nm. Only specimens showing clear green fluorescence were picked and counted.

Results and discussion

Analysis of Foraminiferal Faunas

The analysis of the foraminiferal faunas revealed that living foraminifera are present in large numbers in the Grevelingenmeer (Figure 4). Until now, we only analysed the faunas of the transect with stations 1, 2 and 4. The average number of living individuals in the first cm of the sediment differs considerably between the 3 stations. In station 1, we only found a few individuals (a maximum of 24 specimens was found in August), 300 to 600 individuals per core were found at station 2, whereas more than thousand specimens were found in each of the cores sampled at station 4. A maximum of 2200 living foraminifera was observed at station 4 in November 2011. At station 1 (and 6, considered to represent the same conditions), the values are low for all three sampling periods, and at station 2, the foraminiferal density has the same order of magnitude in August and November. At station 3, however, the foraminiferal density was almost 2 times higher in November than in August.

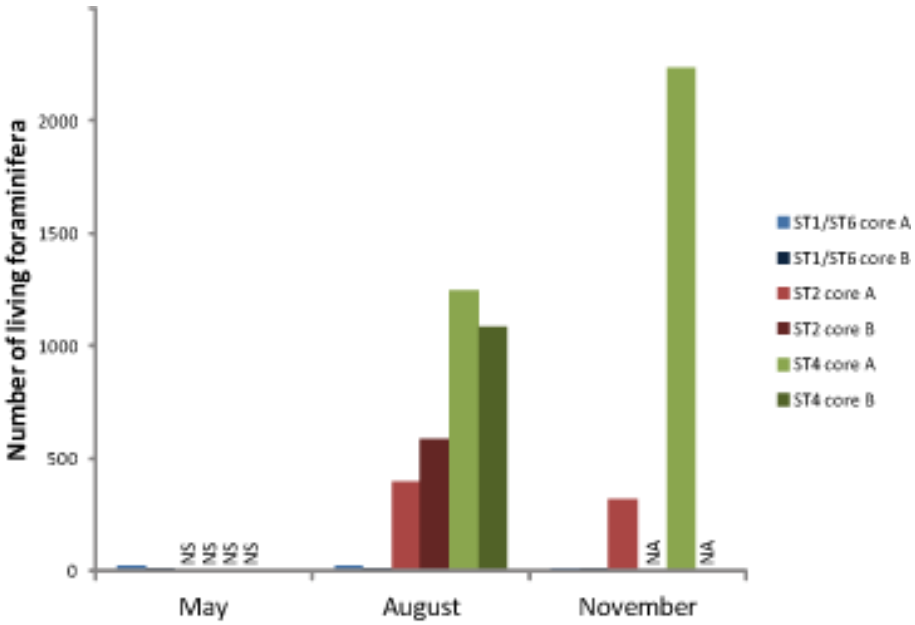

Figure 4 – Total number of living foraminifera in all studied cores (core surface 28,3 cm^2) between 0 and 1cm depth, size fraction >125µm. Please note that in May stations 2 and 4 were not sampled. For the sake of convenience, stations 6 and 1, positioned close very close to each other, are considered to represent the same conditions. NS = not sampled, NA = not yet analysed.

When we compare the pairs of replicates cores, it appears that the foraminiferal density is fairly equal, suggesting the absence of a very strong small scale spatial patchiness in the Grevelingenmeer (Figures 5, 6 and 7).

When considering the still very incomplete data about the vertical distribution of foraminifera, it appears that foraminiferal densities decrease with depth in the sediment. This is clearly shown by core ST2-A (Figure 6).

The faunal analyses of the 12 cores, which have been studied until now, show that the foraminiferal faunas are very strongly dominated by 3 species: *Ammonia* cf. *A. batava*, *Elphidium* ex. gr. *E. excavatum* and *Trochammina* cf. *T. inflata*. Since the representatives of these three taxa systematically have a slightly different morphology than typical *A. batava*, *E. excavatum* and *T. inflata*, we prefer to put them in the open nomenclature, awaiting further (genetical and morphological) analyses.

At stations 6 and 1 (which are very close to each other), bottom waters were anoxic from early summer until October 2011. Consequently, when we consider samples from May, August and November 2011, we are looking at the foraminiferal communities before, during and a few weeks after anoxic conditions.

In May the poor faunas at this site (~15 and ~35 individuals in the two cores) are strongly dominated by *Elphidium* ex gr. *E. excavatum* (Figure 5), in August (~10 and ~35 individuals in the two cores) this species is accompanied by *Ammonia* cf. *A. batava* (Figure 6), whereas in November one core did not contain any living foraminifera, whereas the second one contained about 10 specimens of *Elphidium* ex gr. *E. Excavatum*, *Ammonia* cf. *A. batava* and *Quinqueloculina* sp. (Figure 7).

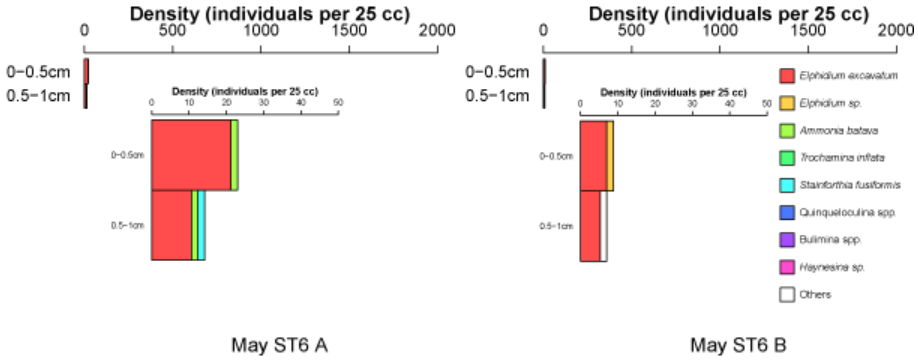

Figure 5 - Foraminiferal densities (standardised as the number of individuals per 25cc) in May 2011 at station 6. Left panel is replicate A and right panel is replicate B. Note that the plot in the center of each graph are zooms of the first centimeter.

These scarce faunas suggest that before the onset of anoxic conditions, the faunas were dominated by *Elphidium* ex gr. *E. excavatum*, whereas this taxon decreases in August and November, when the number of *Ammonia* cf. *A.batava* relatively increases.

It is surprising that at this site (stations 1 and 6); the foraminiferal faunas are very poor in all three sampling periods. It is possible that the low numbers of living foraminifera do not represent viable populations at this site, but rather foraminifera, which have been transported

to this site by bottom currents, and which survive for a certain period of time in anoxic conditions. The continuing poverty of the foraminiferal faunas in November 2011, a couple of weeks after the re-oxygenation of the bottom waters, shows that an extensive recolonisation has not taken place. Additional data are necessary to decide whether such a recolonisation does not take place at all, or whether this repopulation takes place later in the year.

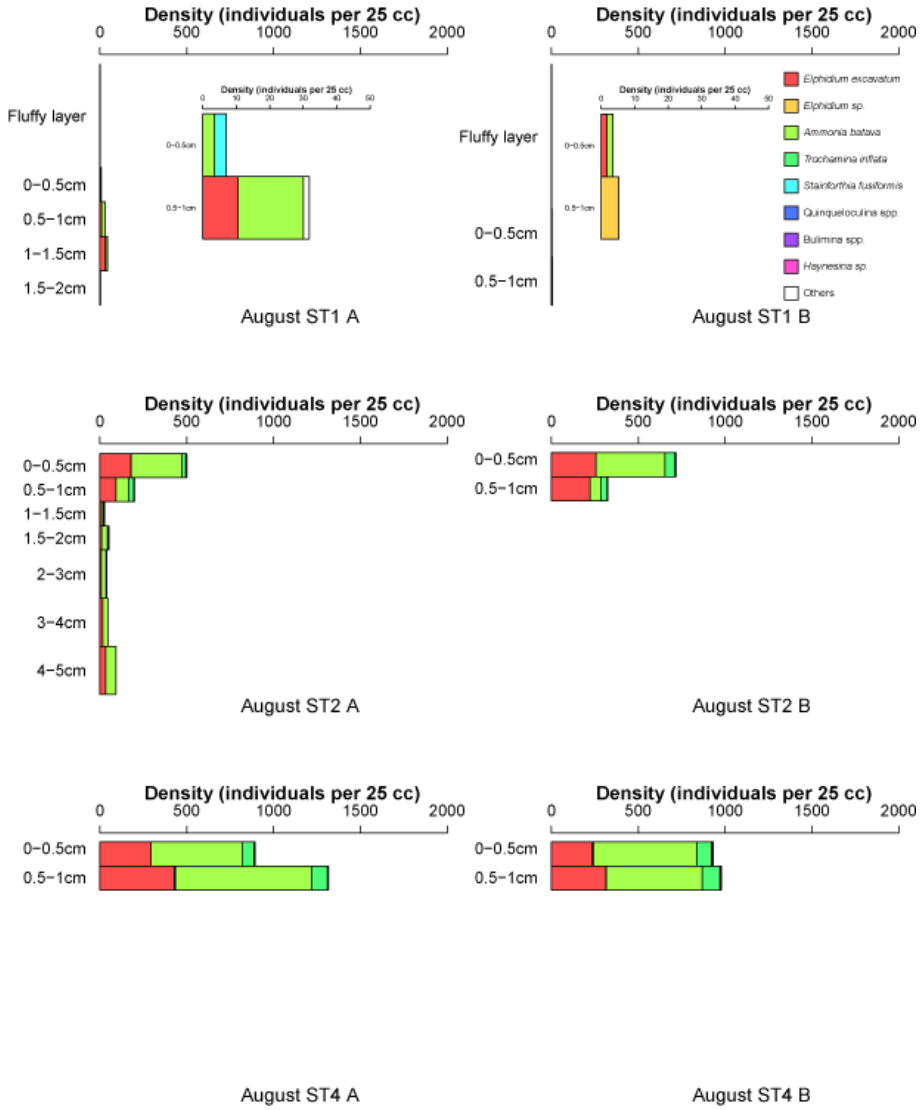

Figure 6 - Foraminiferal densities (standardised as the number of individuals per 25cc) in August 2011 at stations 1, 2 and 4. Left panel is replicate A and right panel is replicate B. Note that the plots in the center of the upper 2 graphs are zooms of the first centimeter.

At stations 2 and 4 we observe a very similar species composition, and very similar patterns in the replicate cores. The foraminiferal faunas are strongly dominated by only three taxa: *Ammonia* cf. *A. batava*, *Elphidium* ex. gr. *E. excavatum* and *Trochammina* cf. *T. inflata*. Both in August and November, foraminiferal densities are about 2 times higher at station 4 than at station 2. At station 2, the faunas tend to be slightly richer in August than in November. Conversely, at station 4, the single core sampled in November studied until now, shows a faunal density which is about two times higher than that of both cores sampled in August (Figure 7).

The higher standing stocks observed in November 2011 at station 4 could be due to an earlier re-oxygenation of this site in comparison to the deeper stations 2 and 1. This hypothesis needs to be verified with a more detailed temporal sampling scheme, accompanied by accurate oxygen measurements at the sediment-water interface.

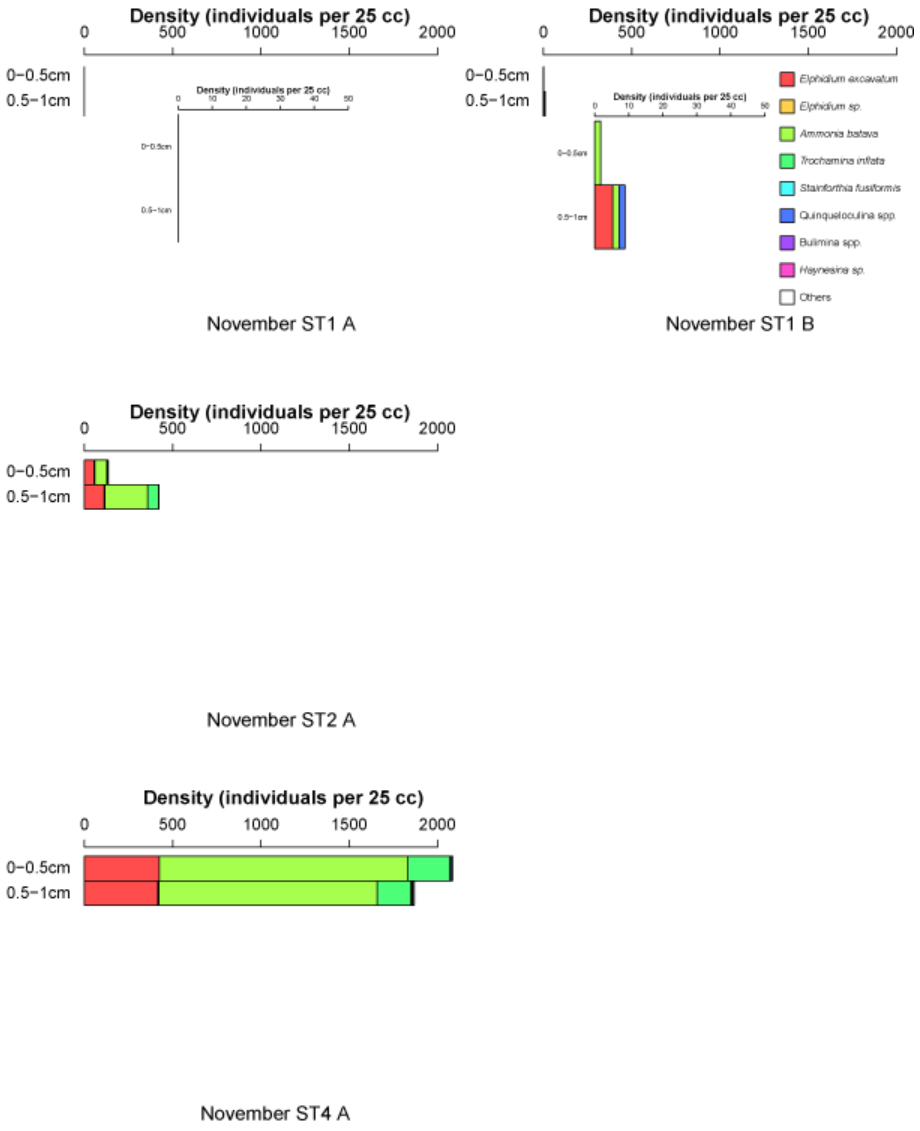

Figure 7 - Foraminiferal densities (standardised as the number of individuals per 25cc) in November 2011 at stations 1, 2 and 4. Left panel is replicate A and right panel is replicate B. Note that the plots in the center of the 2 upper graphs are zooms of the first centimeter.

Morphology of benthic foraminifera in the Grevelingenmeer: an adaptative response to low-oxygen conditions?

The living foraminiferal faunas of the Grevelingenmeer are strongly dominated by the taxa *Ammonia* cf. *A. batava*, *Elphidium* ex. gr. *E. excavatum* and *Trochammina* cf. *T. inflata.* For all three taxa, their morphology is different from typical representatives of the species, and for this reason we placed them in the open nomenclature, for the time being. This anomalous morphology is most striking in *Ammonia* cf. *A. batava.* At all three stations this taxon shows a high density of very large pores (Figure 8 and 9), whereas the marginal parts of the chambers are very often imperforate (Figure 9, 500 x magnification). These aspects differ from typical

A. batava, in which the pores are at least two times smaller, the number of pores per surface area is lower, and no imperforate zones are observed.

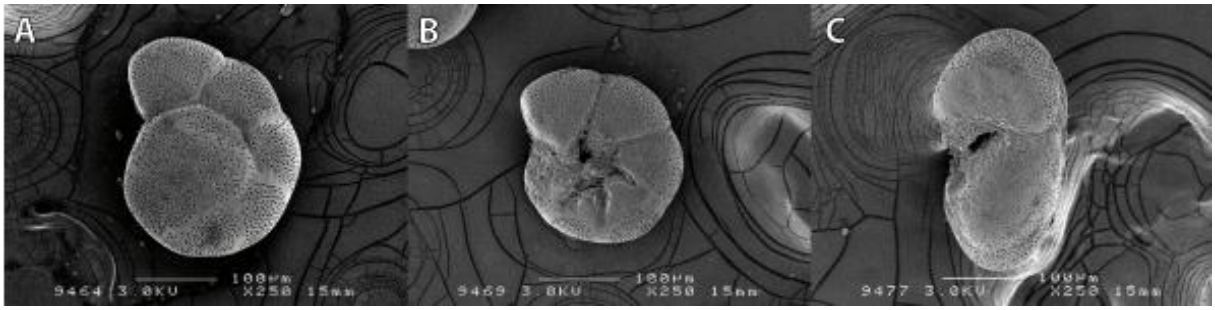

Figure 8 - SEM micrographs of 3 living *Ammonia* cf. *A. batava* (ST3 - August 2011). Panel A: dorsal view, panel B: ventral view, panel C: apertural view

Several studies have tried to link foraminiferal pore size and pore density to bottom water oxygen concentration (e.g. Leutenegger and Hansen, 1979; Moodley and Hess, 1992; Bernhard et al., 2010). Consequently, we paid special attention to these parameters. Figure 9 shows typical representatives from the live faunas sampled at stations 1, 2 and 4.

On these pictures, it can be seen that at all stations the pore density is very high. The pore diameter varies between 2 and 3 µm, which is very high for this taxon. At first view, there are no major differences between the three sites. However, the specimen of station 2 shows a large number of cases where two or three pores are connected. Additional studies will be carried out to find out whether this particular aspect is due to partial dissolution of the tests, or a specific treat at this station, which could be interpreted as an adaptative response to low oxygen conditions.

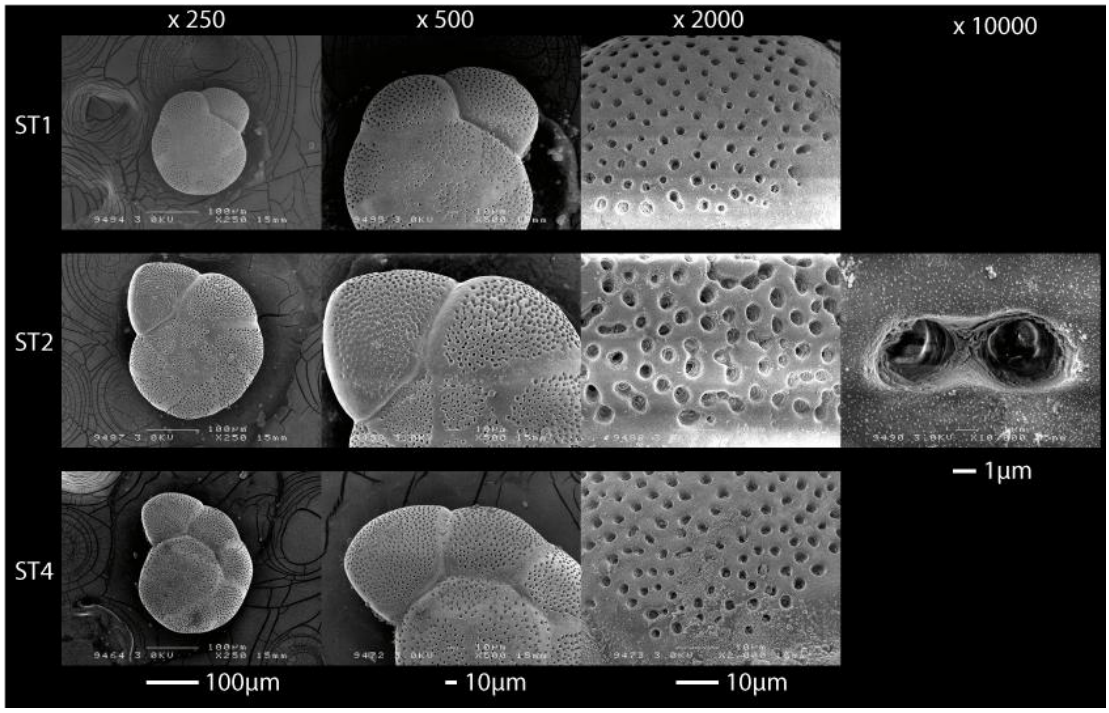

Figure 9 - SEM micrographs of 3 living Ammonia cf. *A.* batava from 3 different stations (ST1, ST2 and ST4) in August 2011 with close-ups of the pore distribution in the penultimate chamber, at a magnification of 250, 500, 2000 and 10000 times.

Conclusions

- Our faunal analyses using the very critical CTG method indicate that abundant live foraminiferal faunas occur at stations 2 and 4 (water depth respectively 23 and 17 m) in August and November 2011.
- At station 1 (30-34 m), which is subject to seasonal anoxia, poor foraminiferal faunas were observed in May, August and November 2011. We suspect that these scarce foraminifera have been transported by currents into the deepest part of the channel, and do not represent autochthonous viable faunas. Additional samplings are necessary to find out whether this station is repopulated in winter or early spring, when oxygen contents are maximal.
- Both at stations 2 and 4, the faunas are almost exclusively composed of three species: *Ammonia* cf. *A. batava*, *Elphidium* ex. gr. *E. excavatum* and *Trochammina* cf. *T. inflata.* No major difference in species composition is observed between the two stations, although the faunas of station 4 are about two times richer than those of station 2.
- The three dominant taxa are characterised by a morphology which differs from that of the typical species; for this reason we placed them in the open nomenclature for the moment. Especially in *Ammonia* cf. *A. batava*, the pore density and pore size appears to be much higher than in typical *A. batava*. Additional analyses will be performed to find out whether this is a morphological adaptation to low oxygen concentrations.

Perspectives

The present report presents a still incomplete data set. In the next four months, we will produce:

1) A complete faunal inventories for the top cm of 9 pairs of replicate cores.
2) A study of the faunas down to 5 cm for 1 core of each of the pairs of replicate cores.
3) A detailed taxonomical analysis of the three dominant taxa, on the basis of the morphological characteristics. We will ask our colleagues at Edinburgh University to study the genetics of these very uncommon morphotypes.
4) A more detailed analysis of the pore size and pore distribution of *Ammonia* cf. *A. batava*, in order to verify that the pore patterns are indeed different from typical *A. batava*, and to investigate whether there are significant differences between the 3 investigated stations.

A final report will be transmitted in May 2012.

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Appendix A: SEM micrographs of the main species living in the Grevelingenmeer

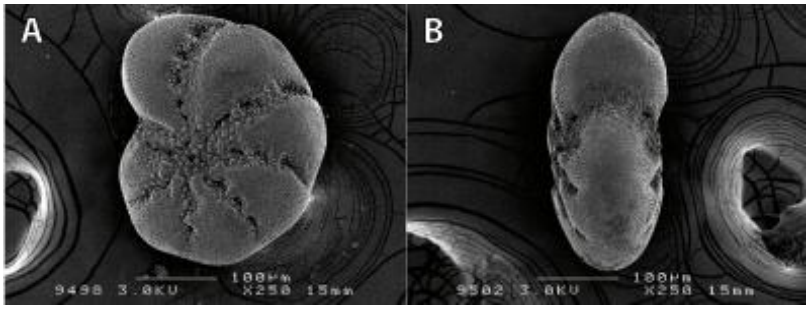

Figure 10 - SEM micrographs of 2 living specimens of *Elphidium* ex. gr. *E. excavatum*. Panel A: side view, panel B: apertural view.

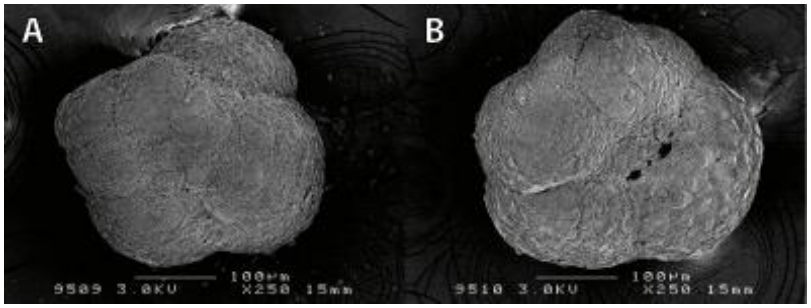

Figure 11 - SEM micrographs of 2 living specimens of *Trochammina* cf. *T. inflata*. Panel A: side view, panel B: apertural view.

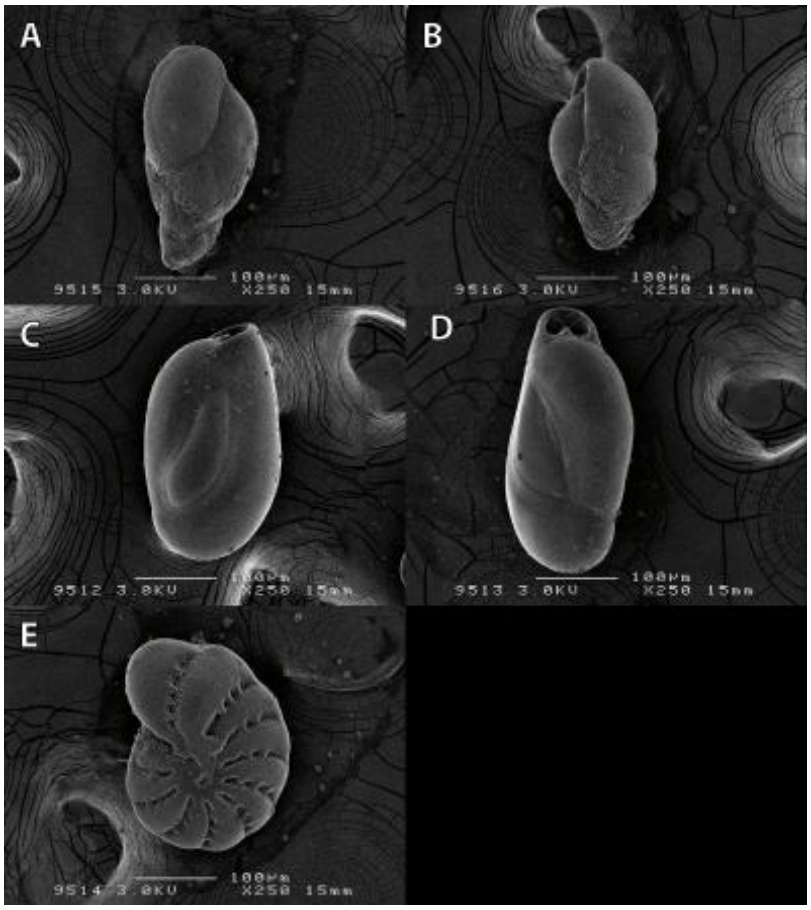

Figure 12 - SEM micrographs of 3 species occurring in low numbers in the living faunas. Panels A and B: side view and apertural view of living *Stainforthia fusiformis*. Panels C and D: side view and apertural view of living *Quinqueloculina sp.*. Panel E: side view of living *Elphidium incertum*.

Appendix B: Census data (raw, non normalised counting values) for benthic foraminifera for all 4 stations

See next page.

Date	Station	Core	Depth level	Fraction	Elphidium ex gr E. excavatum	Elphidium sp1	Elphidium sp2	Ammonia cf. A. batavus	Trochammina sp.	Stainforthia fusiformis	Quinqueloculina bosciana	Quinqueloculina stalkeri	Quinqueloculina seminula	Triloculina oblonga	Quinqueloculina sp	Uvigerina sp.	Bulimina sp	Bulimina marginata	Bulimina accueleata	Haynesina germanica	Nonion sp.	Rosalina sp.	Buccella sp.	Buccella sp	Bolivina sp.	Textularia sp	Leptohalysis scottii	Indéterminé blanc	Indeterminé agglut	Indéterminé
18/05/2011	ST6	A	0-0.5cm	125	2																									
18/05/2011	ST6	A	0-0.5cm	150	10			1																						
18/05/2011	ST6	A	0-0.5cm	315																										
18/05/2011	ST6	A	0.5-1cm	125						1																				
18/05/2011	ST6	A	0.5-1cm	150	6			1																						
18/05/2011	ST6	A	0.5-1cm	315																										
18/05/2011	ST6	B	0-0.5cm	125	3	1																								
18/05/2011	ST6	B	0-0.5cm	150	1																									
18/05/2011	ST6	B	0-0.5cm	315																										
18/05/2011	ST6	B	0.5-1cm	125	1																							1		
18/05/2011	ST6	B	0.5-1cm	150	2																									
18/05/2011	ST6	B	0.5-1cm	315																										
23/08/2011	ST1	A	Fluffy layer	100				3		1						2														
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23/08/2011	ST1	A	1-1.5cm	315	1																									
23/08/2011	ST1	A	1.5-2cm	125	2																									
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23/08/2011	ST1	B	Fluffy layer	125				2																						
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23/08/2011	ST1	B	0-0.5cm	315																										
23/08/2011	ST1	B	0.5-1cm	125																										
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01/11/2011	ST1	A	0.5-1cm	125																										
01/11/2011	ST1	A	0.5-1cm	150																										
01/11/2011	ST1	A	0.5-1cm	315																										
01/11/2011	ST1	B	0-0.5cm	125				1																						
01/11/2011	ST1	B	0-0.5cm	150																										
01/11/2011	ST1	B	0-0.5cm	315																										
01/11/2011	ST1	B	0.5-1cm	125	1			1			1																			
01/11/2011	ST1	B	0.5-1cm	150	2																									
01/11/2011	ST1	B	0.5-1cm	315																										

Date	Station	Core	Depth level	Fraction	Elphidium ex gr E. excavatum	Elphidium sp1	Elphidium sp2	Ammonia cf. A. batavus	Trochammina sp.	Stainforthia fusiformis	Quinqueloculina bosciana	Quinqueloculina stalkeri	Quinqueloculina seminula	Triloculina oblonga	Quinqueloculina sp	Uvigerina sp.	Bulimina sp	Bulimina marginata	Bulimina accueleata	Haynesina germanica	Nonion sp.	Rosalina sp.	Buccella sp.	Buccella sp	Bolivina sp.	Textularia sp	Leptohalysis scottii	Indéterminé blanc	Indéterminé agglut	Indéterminé
24/08/2011	ST2	A	0-0.5cm	125	43			44														1					2			
24/08/2011	ST2	A	0-0.5cm	150	56	2		114	13				1																	
24/08/2011	ST2	A	0-0.5cm	315	1			6																						
24/08/2011	ST2	A	0.5-1cm	125	28			11	3											1						1	1			
24/08/2011	ST2	A	0.5-1cm	150	22			29	14																					
24/08/2011	ST2	A	0.5-1cm	315	2			1																						
24/08/2011	ST2	A	1-1.5cm	125	1			1	1																					
24/08/2011	ST2	A	1-1.5cm	150	2			6	2																					
24/08/2011	ST2	A	1-1.5cm	315	2																									
24/08/2011	ST2	A	1.5-2cm	125	1			4		2							2					1								
24/08/2011	ST2	A	1.5-2cm	150	2			14																						
24/08/2011	ST2	A	1.5-2cm	315	2			2																						
24/08/2011	ST2	A	2-3cm	125	4			10																	1					
24/08/2011	ST2	A	2-3cm	150	5			20	1												1		1							
24/08/2011	ST2	A	2-3cm	315				1																						
24/08/2011	ST2	A	3-4cm	125	10			3		1																				
24/08/2011	ST2	A	3-4cm	150	8			27												1										
24/08/2011	ST2	A	3-4cm	315	1			2																						
24/08/2011	ST2	A	4-5cm	125	14			18																						
24/08/2011	ST2	A	4-5cm	150	24			46																						
24/08/2011	ST2	A	4-5cm	315				3																						
24/08/2011	ST2	B	0-0.5cm	125	65			78	6										1											
24/08/2011	ST2	B	0-0.5cm	150	80			143	26											1	1									
24/08/2011	ST2	B	0-0.5cm	315				4																						
24/08/2011	ST2	B	0.5-1cm	125	37			11	1																					
24/08/2011	ST2	B	0.5-1cm	150	89			21	20												1									1
24/08/2011	ST2	B	0.5-1cm	315				2																						
01/11/2011	ST2	A	0-0.5cm	125	10			12	2			1																		
01/11/2011	ST2	A	0-0.5cm	150	21		2	26	1					1																
01/11/2011	ST2	A	0-0.5cm	315																										
01/11/2011	ST2	A	0.5-1cm	125	16	1	1	34	9						1															
01/11/2011	ST2	A	0.5-1cm	150	45	1		97	24																					
01/11/2011	ST2	A	0.5-1cm	315	2			6	1																					
24/08/2011	ST3	A	0-0.5cm	125	24	1		75	2			1										1								
24/08/2011	ST3	A	0-0.5cm	150	139			215	33				1																	
24/08/2011	ST3	A	0-0.5cm	315	3			7	3																					
24/08/2011	ST3	A	0.5-1cm	125	51	1		116	6																					
24/08/2011	ST3	A	0.5-1cm	150	187	3		315	44						1					2										
24/08/2011	ST3	A	0.5-1cm	315	4			12	2																					
24/08/2011	ST3	B	0-0.5cm	125	54	1		90	11	1					2				1											
24/08/2011	ST3	B	0-0.5cm	150	77		2	238	36			1						1												
24/08/2011	ST3	B	0-0.5cm	315	2			9	1																					
24/08/2011	ST3	B	0.5-1cm	125	53	1		60	8														1							
24/08/2011	ST3	B	0.5-1cm	150	122	1		242	48			1							1		1		1							
24/08/2011	ST3	B	0.5-1cm	315	2			10	1																					
01/11/2011	ST3	A	0-0.5cm	125	58			139	22	1						1														
01/11/2011	ST3	A	0-0.5cm	150	178			586	113			3	1		1														1	
01/11/2011	ST3	A	0-0.5cm	315	4			71																						
01/11/2011	ST3	A	0.5-1cm	125	62	1		160	13	1									4					1						2
01/11/2011	ST3	A	0.5-1cm	150	173	3		517	94			1											1							
01/11/2011	ST3	A	0.5-1cm	315				21	2																					

Appendix C: CTG staining protocol

CTG protocol established on November the 21st ,2011

Sampling strategy

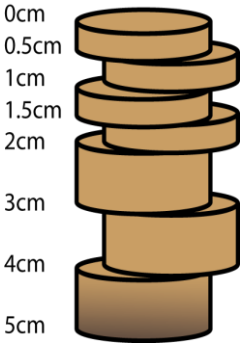
"Small campaign"

- ST1, ST2 and ST4
- 3 cores per station (**6 cm** inner diameter core A, B and C)
- Core slicing: 0-0.5cm and 0.5-1cm

"Bigger campaign"

- ST1, ST2 and ST4
- 3 cores per station (**6 cm** inner diameter core A, B and C)
- Core slicing from 0 to 5 cm (0-0.5cm, 0.5-1cm, 1-1.5cm, 1.5-2cm, 2-3cm, 3-4cm and 4-5cm)

Core slicing



For both types of campaigns, cores interface should ideally be undisturbed and as flat as possible. (If the surface is tilted please set the 0 at the center of the inclined surface).

For the small campaigns, slice from 0 to 1cm with a 0.5cm resolution (i.e. 2 samples per core). For the big campaigns slice for 0 to 5cm depth with a 0.5cm resolution from 0 to 2cm and a 1cm resolution from 2 to 5 cm (i.e. 7 samples per core).

At ST1, the sediment interface can be very fluffy. If so, syringe out all the fluffy sediment until you can see that the sediment is not liquid anymore. Add all the fluffy sediment syringed out to an extra (8th) sampling pot labeled "fluffy layer". This extra

sample will be treated with the same protocol as the other ones. Then, the "actual" interface (0cm) will be considered as the part where the sediment starts to be "consequent".

Before to start slicing the cores please collect **the overlaying water** in a wash bottle (see part III – Sample treatment).

CTG protocol

CTG (Cell Tracker Green) is a non fluorescent molecule, which is hydrolyzed by nonspecific esterase, producing a fluorescent compound (if observed with the accurate excitation and emission wavelength). When living cells are incubated in fluorogenic probes such as CellTracker Green CMFDA, the probe passes through the cellular membrane, and reaches the cytoplasm where hydrolysis with nonspecific esterase causes the fluorogenic reaction (Bernhard et al., 2006 ; Pucci et al., 2009)

Preparation of the CTG/DMSO solution:

CTG has to be stocked at -20°C and has to stay as much as possible in the shadow. CTG has to be diluted in DMSO before being added to the samples. To prepare the CTG reagent, add 1mL of DMSO to the 1 mg CTG tube and make sure that the small flake of CTG is well dissolved into the DMSO. The DMSO/CTG solution can be prepared after slicing the first set of cores. This solution should be stored in the fridge or in the freezer with aluminium foil around because CTG is sensitive to light.

Preparation of the Borax-buffered formalin:

The CTG reaction is fixed in formalin. To control the pH of the solution, we add Borate to the formalin. To prepare 3.8%Borax-buffered formalin, add **20g** Sodium Borate to **5L** Formalin solution 10% (approx 4% formaldehyde).

Sample treatment:

The quantity of CTG added to the sample depends on the final volume of sediment and seawater in the vial. The quantity of CTG added depends then on the slicing resolution:

For a 0.5 cm sediment layer

- Put 0.5 cm layer of sediment (meaning 14 cm^3 of sediment for a 6cm core diameter) in a 200mL plastic vial with 14 ml of *in situ* temperature seawater (if possible the overlying seawater in the core);
- Add **17.5 µl** of DMSO/CTG solution
- Shake gently the 200mL vial
- Incubate it at *in situ* temperature in the dark for 10 to 19 hours
- After this time, put roughly **60** ml of Borax-buffered formalin in the vial to fix the CTG reaction

For a 1 cm sediment layer ("Bigger campaign")

- Put 1 cm layer of sediment (meaning 28 cm^3 of sediment for a 6cm core diameter) in a 200mL plastic vial with 28 ml of *in situ* temperature seawater (if possible the overlying seawater in the core);
- Add **35 µl** of DMSO/CTG solution

- Shake gently the 200mL vial
- Incubate it at *in situ* temperature in the dark for 10 to 19 hours
- After this time, put roughly **120** ml of Borax-buffered formalin in the vial to fix the CTG reaction

Material needed

- Nice and beautiful cores;
- Core extruder;
- Spatulas to slice the core;
- Plastic pipe (to pump out the overlaying sea water → keep it in a "wash bottle");
- A syringe (to suck out the liquid sediment);
- 200mL sampling pots (vials);
- CTG tube(s);
- DMSO;
- Formalin;
- Sodium Borate;
- 1mL pipette;
- 100µL pipette;
- ...

	small campaign	big campaign
# stations	3	3
# cores	3	3
# 0,5cm slice/core	2	4
# 1cm slice/core	0	3
CTG add/0,5cm slice	17,5	17,5
CTG add/1cm slice	35	35
TOTAL CTG (µL)	315	1575
# of CTG Tubes (1 SAFETY included)	2	3
Formalin add/0,5cm slice	60	60
Formalin add/1cm slice	120	120
TOTAL Formalin (mL)	1080	5400
# sampling pots	18	63
# sampling pots (safety)	12	17
# sampling pots (TOTAL)	30	80