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Shallow-water benthic foraminifera as proxy for natural versus human-induced environmental change

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Shallow-water benthic foraminifera as proxy for natural versus human-induced environmental change

Ondiepe benthische foraminiferen als proxy voor natuurlijke en antropogene omgevingsveranderingen

(met een samenvatting in het Nederlands)

Proefschrift

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND SUMMARY

All over the Earth, increasing human population growth and ongoing industrialization lead to deteriorating global biodiversity (e.g. Kerr and Currie, 1995; Pimm and others, 1995; Vitousek and others, 1997). It is estimated that anthropogenic activity has caused the extinction of somewhere between 20,000 and 2 million species so far (Wilson and Peter, 1988; Meyers, 1988; 1990). Most of this loss is thought to be caused by habitat fragmentation and habitat destruction (Bellwood and Hughes, 2001; Travis, 2003), while the recent global rise in temperature is likely to contribute to the current mass extinction as well (e.g. Root and others, 2003; Pounds and others, 2006). Besides extinctions, ecosystem functioning (Tilman, 1987; Duffy, 2003) and element cycling (e.g. Rast and Thornton, 1996; Exley, 2003) have been widely altered over the past centuries. Coastal areas harbor highly diverse ecosystems (Ray, 1988), but are also among the most severely affected environments. They are subjected to severe eutrophication through increased deliverance of nutrients and organic compounds by rivers, to habitat loss by trawling fishery and construction of coastal defense structures (e.g. Casey and Myers, 1998; Hutchings, 2000; Jackson and others, 2001; Lotze and Milewski, 2004).

Ecosystem composition and functioning are also subjected to natural (e.g. climate-induced) variability. To quantify human impacts on ecosystems, these natural fluctuations must be accounted for. Since long-term biological monitoring programs are rare and usually do not include the pre-human state, we must rely on traces of past ecosystems found in the geologic record. These traces come in many sorts and shapes, including fossils, minerals, stable isotopes, air bubbles in Antarctic ice and specific molecular remains of microorganisms. Each of these traces (so-called proxies) can be used to reconstruct aspects of the environment in which they originated. By combining different proxies (a multi-proxy approach), a coherent reconstruction can be made of an environment or ecosystem through time.

Foraminifera (Protista) are close relatives of the amoeba, that live predominantly in the sea and have a unique feature that makes them popular proxies: many build a shell (a so-called test) of calciumcarbonate during their life. Since they are abundant in most marine environments and their tests are often preserved in sediments, they are widely used in paleoceanography and paleoclimatology. There are two major ways in which fossil foraminifera can be used as proxies. The first is by enumerating abundances of different species in a fossil sample and to infer past habitats by the presence or absence of certain (key) species. Such reconstructions can be improved by increasing our

knowledge about the habitat preferences of modern species. In order to investigate temporal and spatial distributions of living foraminifera against an environmental background, field studies are conducted in which foraminiferal distributions and environmental parameters are recorded. In many cases, the abundance of a species is found to be positively correlated to a range of values of an environmental variable. The abundance of that same species in a fossil sample is then used to reconstruct values for that environmental parameter in those samples.
The second way in which foraminifera are used is by analyzing the chemical composition of their tests. Ratios of carbon and oxygen isotopes in foraminiferal calcite contain valuable information on, for instance, past oceanic temperature and global ice volume. Furthermore, during calcification by the foraminifer, trace elements (like Mg, Ba, Cd, Zn, Cu) can be incorporated in the $CaCO_3$-lattice by substituting Ca. Besides the concentration of trace elements in the seawater, the amount of a trace element that is incorporated in the carbonate is usually a function of several environmental parameters. In the case of magnesium, the incorporation into foraminiferal calcite is mainly determined by the temperature of the seawater. Hence, Mg concentrations in fossil calcite (commonly expressed as Mg/Ca ratios) reflect sea water temperatures at the moment when the calcite was produced. The dependency of trace element/Ca ratios on temperature, salinity, pH, as well as its dependency of cellular activity of the foraminifer is uncertain for most trace elements. Therefore they need to be quantified in order to improve their proxy-value.

The original goal of this research was to quantify human and natural influences on near-coastal Dutch ecosystems over the past 5,000 years. Ongoing population growth has increased nutrient runoff by rivers, thus enhancing primary production, thereby increasing the organic flux to the seabed where riverine input is high. In core material from the North Sea, we expected to see the effects of various stages in human history (deforestation, agriculture, use of artificial fertilizers) by analyzing foraminiferal assemblages from different ages. However, suitable core material, containing a reasonably continuous record of the past 5,000 years of North Sea sediments, was not available. Therefore, we shifted the focus of our research to develop proxies to reconstruct human influences on near-shore ecosystems by collecting living foraminifera from the North Sea and Dutch Wadden Sea. Results from these studies were used to reconstruct the history of the western Wadden Sea. In this analysis, the interplay between anthropogenic and natural influences shows that the effects of human alterations had sudden and dramatic consequences for the functioning of this ecosystem.
Our results also indicated that in this environment benthic foraminiferal species compositions may not be reliable tools to reconstruct the parameters that we were initially interested in (i.e. anthropogenic eutrophication) or environmental parameters that are of more general interest (temperature, oxygen penetration, water depth). In contrast, it appeared (see chapter 8) that foraminiferal species compositions in shallow seas are suitable to build models that can reconstruct food quality and hydrographical regimes. If core material with a substantial part of the Holocene would be available, we would argue that benthic foraminifera are primarily suitable to reconstruct the North Sea's hydrographical evolution. Whether foraminiferal community structure is (additionally)

affected by eutrophication, needs to be investigated further either by experiments or by field surveys including hydrodynamic fronts in less eutrofied environments.

In foraminiferal research, rose Bengal is commonly used as a vital staining technique to distinguish living from dead specimens. However, staining foraminifera with rose Bengal has the disadvantage that it stains all protein-bearing tests, implying that not only living specimens, but also individuals that have died recently are stained, resulting in an overestimation of foraminiferal standing stocks. In **chapter 2**, MTT is presented as a new vital staining technique. MTT is a tetrazolium salt that is transformed by enzymes from a yellow, soluble form to purple formazan crystals. Incubating living foraminifera with MTT, results in purple staining of active foraminifera. We also show that days after their death, individuals can become stained by bacteria feeding on foraminiferal cell material, but these false positives are easily recognized.

Variability in foraminiferal abundances (patchiness) is another practical issue that may lead to biased results when collecting foraminifera. In **chapter 3**, results are presented of a study on the spatial distribution of foraminifera at an intertidal mudflat in the Dutch Wadden Sea. The study comprised three different surveys: one was conducted to investigate the spatial distribution of intertidal foraminifera on a centimeter-scale, in the second, we investigated the variance of foraminiferal abundances on a larger scale (0.1 - 100 meters apart) and the third was designed to determine the relation between foraminiferal abundances and their distance from the high- and low water level. The results show that the two dominant species in the Wadden Sea (*Ammonia tepida* and *Haynesina germanica*) occur in 175-300 cm^2-patches of high abundance and that both species are positively correlated. Only at a very large distance (>50 m) there appears to be a second-order patchiness, while we found no relation of abundances with elevation at the intertidal flat. Interestingly, despite huge spatial differences in absolute abundance, the ratio between the two species was similar in space at the same sampling moment. The ratio, however, changed during the year. This suggests that seasonal variation in an environmental parameter (e.g. type of food available), causes abundances of *H. germanica* to be relatively high in spring and those of *A. tepida* relatively high in summer, while spatial variations in total standing stock at any given sampling moment may be governed by another parameter (e.g. total amount of food).

In **chapter 4**, results from a field study are presented that show foraminiferal abundances across the Frisian Front (southern North Sea). Around this tidal mixing front different hydrodynamic environments exist (mixed, frontal and stratified) that result in a variety of different benthic habitats. Stations in those habitats were sampled at four different months to quantify spatial and seasonal differences in benthic species composition. The results show that the most abundant species present show peak abundances at specific distances from the benthic front. Inter-seasonal differences in species composition were minor, while vertical (in-sediment) distributions of most species in the upper 5 cm of the sediment changed. In winter months, specimens are usually distributed evenly in the sediment, while in summer months relatively many specimens occu-

py the upper centimeter. This suggests that these foraminifera respond to the arrival of fresh organic material at the seabed in spring and early summer by moving towards the sediment-water interface or achieve shallow abundance maxima through enhanced reproduction.

Results from the sampling survey in the previous chapter are compared to distributional data of foraminifera in 1988 and 1989 across the Frisian Front (Moodley, 1990) and are discussed in **chapter 5**. Benthic macrofauna was also sampled across the Frisian Front between 1982 and 2002, during which a sudden shift in dominance was witnessed. Before 1992, the seafloor of the Frisian Front was heavily dominated by filter-feeding specimens of the brittle star *Amphiura filiformis* and after 1995, the ghost shrimp *Callianassa subterranea*, a burrowing deposit feeder, dominated the area. Despite the effects of *C. subterranea* on the physical state of the front's habitats (increased turbidity, increased bioirrigation, increased sediment oxygen uptake), the foraminiferal community remained relatively stable during the macrobenthic regime shift. This indicates that the occurrences of these foraminiferal species are not strongly influenced by these ecological and physical alterations and that they can serve as robust proxies for different benthic habitats around tidal mixing fronts.

A reconstruction of the Wadden Sea ecosystem, based on foraminiferal abundances, is presented in **chapter 6**. We discuss a record taken in Mok Bay (Dutch Wadden Sea), containing sediment from the past 180 years. The laminated core (2.8 meters long) was sliced into 1 cm thick slices and total organic carbon content and grain size distribution was analyzed in each sample. Additionally, benthic foraminifera were counted and all data were compared to historical trends on the functioning of the Wadden Sea ecosystem. The foraminifera in the core show an abrupt change in species composition: before 1930, *Elphidium excavatum* is the dominant species and after 1935, numbers decline and *Haynesina germanica* suddenly increases in abundance. The timing of the shift in dominance suggests that the construction of the Afsluitdijk in 1932 had profound effects on the Wadden Sea ecosystem. Knowing the ecological preferences of these two species (chapters 3 and 4), we hypothesize that the variability in temperature and salinity increased in Mok Bay after the construction of the Afsluitdijk and are responsible for the shift in the foraminiferal species composition.

In **chapter 7**, the incorporation of copper in foraminiferal calcite is discussed. To determine the partition coefficient of Cu (D_{Cu}) in calcite, we cultured two species of foraminifera under a range of Cu-concentrations in seawater. The Cu/Ca ratio in newly formed calcite was analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This method allowed us to analyze the chemical composition of single chambers of the cultured specimens and resulted in a calculated D_{Cu} between 0.1 and 0.3. The effect of temperature and salinity on the D_{Cu} was not found to be significant. The D_{Cu} is similar for both species cultured, despite the presence of symbionts in one species (*Heterostegina depressa*) and its absence in the other (*Ammonia tepida*). We believe that Cu/Ca ratios in fossil benthic foraminifera can be used to reconstruct human-induced, heavy metal pollution.

The conclusions of these chapters are summarized in **chapter 8**. Also, important consequences for the use of benthic foraminifera in reconstructing the history of near-coastal ecosystems are discussed. In the southern North Sea and Wadden Sea foraminiferal distributions did not appear to be limited by total food abundance or in-sediment oxygen concentrations. Additionally, foraminiferal community composition did not seem to be influenced by macrofaunal community composition (dominated by filter feeders or by burrowing species). We hypothesize that distribution of benthic foraminifera in the North Sea is mainly controlled by the type of food available (labile or refractory) and by the level of environmental variability. Different combinations of these two variables are found across habitats beneath tidal mixing fronts and therefore, benthic foraminifera in temperate, shallow seas are particularly suited to reconstruct hydrodynamic regimes.

CHAPTER 2

NOVEL APPLICATION OF MTT REDUCTION: A VIABILITY ASSAY FOR TEMPERATE SHALLOW-WATER BENTHIC FORAMINIFERA

with IAP Duijnstee and GJ van der Zwaan

ABSTRACT
Studies on living benthic foraminifera commonly involve staining samples with rose Bengal (RB) to distinguish living from dead individuals. Since RB also stains individuals that have died recently (sometimes weeks earlier) and are not fully decayed, standing stocks of foraminiferal communities are usually overestimated. To overcome this bias, we discuss a new viability assay based on the reduction of a tetrazolium salt, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide or thiazolyl blue) by living foraminifera. The tetrazolium salt MTT is actively ingested by cells and subsequently converted enzymatically from a yellow, soluble form to a reddish purple crystal. Experiments confirm that living individuals of *Ammonia beccarii* and *Globobulimina turgida* convert MTT and become stained within 24 hours. Some dead foraminifers may continue enzymatic activity for several days, but produce a different coloration than that of stained living foraminifers. With the reduced problem of false positives, this assay is an improvement over staining samples with RB whenever a higher accuracy is required (e.g., in short-term laboratory experiments).

INTRODUCTION

Benthic foraminifera are extensively used as a tool for paleoecological reconstructions. The composition of fossil communities of this abundant group of unicellular eukaryotes reflects marine paleoenvironmental conditions (e.g., van der Zwaan and others, 1999). However, in order to arrive at reliable paleoenvironmental foraminifer-based proxies, we need to improve our understanding of foraminiferal ecology. A combination of field studies (e.g., Bernhard and others, 1997; Wollenburg and Kuhnt, 2000; Gooday and others, 2001; Buzas and others, 2002; Scott and others, 2003) and laboratory experiments (e.g., Alve and Bernhard, 1995; Moodley and others, 2000; Ernst and others, 2002; Alve and Goldstein, 2003; Langezaal and others, 2004; Duijnstee and others, 2005) provide the necessary insights into the different habitat preferences of the various foraminiferal species. These studies reveal more and more the factors that are important for their ecological distribution and thereby enhance their proxy value.

In ecological studies, numbers of living specimens are enumerated at different locations and sample moments. For this it is necessary to distinguish between living and dead individuals. The widely used method of staining with rose Bengal (RB) reveals tests bearing organic material by staining them pink, while empty (dead) tests are not stained (Walton, 1953). Shells of recently dead foraminifers, however, may retain undecayed protoplasm for some time, leading to an overestimate of standing stocks, especially where decay of cell material progresses slowly (Bernhard, 1988; Murray and Bowser, 2000). Experiments are particularly vulnerable to this inaccuracy, since a vast amount of the community or population is likely to die prior to the start of the experiment because of manipulations, such as collection of sediment, transport to the lab, sieving, etc. When an experiment starts, part of the material is harvested to determine the assemblage composition at t=0, while an unknown part of the community may have died during the processes outlined above, and thus might be stained. Bernhard and others (2004) described the CellTracker Green method as a foraminiferal viability method, and a more sophisticated method is described in Bernhard and others (2003).

To overcome the widely acknowledged inaccuracy of staining with RB, alternative staining techniques have been developed, but none is as easily applicable as RB. Sudan Black B is less accurate than RB (Bernhard, 2000; Murray and Bowser, 2000), whereas ATP analysis is very accurate, but individuals have to be processed one by one (Bernhard and others, 1995; DeLaca, 1986). A good alternative to RB is CellTracker Green, which is easily used for large populations, but requires epifluorescence microscopy (Bernhard and others, 2004).

Here we present an alternative user-friendly staining technique that discriminates between living and dead foraminifers. Staining proceeds through the conversion of the soluble yellow tetrazolium salt MTT into a non-soluble purplish blue formazan by enzymes in living cells. The mechanism of MTT reduction in living cells is not fully understood, but MTT molecules are known to be taken up by endocytosis (Liu and others, 1997; Molinari and others, 2005). The MTT is then reduced in lysozymes by the activity of enzymes and the coenzyme NAD(P)H (Berridge and Tan, 1993), and finally it can be transported out of the cell by exocytosis (Bernas and Dobrucki, 2000; Molinari and others, 2005). Other contributions of MTT reduction come from membrane-bound enzymatic activity in mitochondria (Bernas and Dobrucki, 2002).

METHODS

Tetrazolium salts, such as MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide or thiazolyl blue) are frequently used as color indicators for the detection of enzymes. In the presence of enzymes, tetrazolium salts are converted to reduction equivalents, formazans. Tetrazolium salts are soluble in water, while most formazans are insoluble crystals that precipitate during reduction by enzymes. In the case of MTT, enzymes convert the yellow, soluble form into reddish blue crystals. Reduction by MTT is commonly used in medical studies to determine the enzymatic activity of cells under different conditions (Takahashi and others, 2002; Stowe and others, 1995; Bucciantini and others, 2005) or to assess the viability of cells (e.g., sperm cells, Nasr-Esfahani and others, 2002; or protozoa, Dias and others, 1999).

Staining of living and dead specimens

Living individuals of *Ammonia* cf. molecular type T6 (Hayward and others, 2004, referred to herein as *A. beccarii*) were collected from an intertidal mudflat in the Dutch Wadden Sea in June, 2004. Bulk sediment was kept in an aquarium at room temperature. Small volumes of sediment were searched for individuals >150 μm that displayed pseudopodial activity. These were returned to 2 ml of seawater (salinity 17) with 0.5 ml of sediment from their original environment, and immediately 1 ml of MTT solution (3.5 g MTT/l seawater) was added. The specimens were placed at 20° C and photographs were taken every hour to record the progress of MTT reduction. Prior to photographing, individuals were placed in transparent seawater so that the development of color in the cells was not obscured by the surrounding yellow MTT solution or by the sediment. Because it cannot be excluded that handling of specimens prior to photographing may have negatively affected the foraminifers, care was taken to avoid individuals that died during the incubation. Though none of the observations indicate that this happened, we cannot exclude that the metabolism and, thus, the staining were affected by the experiments. The photographs shown in figures 1-4 are representative of the 50 specimens observed throughout this procedure.

To extend the use of MTT as a viability staining technique, benthic foraminifers were collected from the Gullmar Fjord, Sweden in April 2005. Sediment was retrieved from the center of the fjord at a depth of 116 meters, and the material was then transported to Utrecht and kept at an ambient temperature of 10º C. Living specimens of *Globobulimina turgida* >150 μm were collected and put in MTT dissolved in seawater from the fjord (salinity 33). The individuals were kept at 10º C and photographed as described.

To track possible reduction of MTT in dead individuals, 50 living individuals of *Ammonia beccarii* were killed by transferring them for 15 minutes to seawater that was pre-heated to 50° C. Subsequently, they were placed in a solution of 1 g MTT /l seawater. Individuals were also killed by incubation for 10 minutes at 100° C, 10 minutes at -80° C and 10 minutes of incubation with 96% ethanol to investigate any development of the stain due to unforeseen alteration of the cell material during heat shocking. Per alternative treatment, 10 specimens were used and photographed, as were living individuals.

In order to investigate the effect of decay on possible post-mortem staining, other individuals of *Ammonia beccarii* were killed at 50° C and 100° C, and placed back in 0.5 ml of sediment (grain size < 50 μm) and 2 ml of seawater. The individuals were left to decay for 1, 2, 3, 4 and 7 days, respectively, at 20° C. Ten individuals were used per incubation period. At the end of each period, 1 ml of MTT solution (3.5 g /l seawater) was added and the individuals were photographed every hour.

Because dead individuals sometimes stained after incubation with MTT (see results), we developed a blind test in which people were asked to distinguish stained from non-stained specimens. Fifty four living individuals of *Ammonia beccarii* (>150 μm) were picked from a laboratory stock and killed by transferring them to seawater of 50º C for 15 minutes. They were then placed in a layer of sediment (grain size <50 μm) at 10º C. After 4 days, the 54 treated individuals and 42 living specimens were incubated with MTT for 18 hours and every individual was transferred to one of the 96 wells of a Falcon™ tissue culture plate (353072, Biosciences, San Jose, USA). Each cell of the culture plate was filled with seawater.

The 42 living and 54 dead individuals were distributed randomly over the 96 wells and on the same day, using the same microscope and same light source, ten people were asked to distinguish a 'red' and a 'yellow' category. All of the people were attached to the authors' department. Some were experienced with processing RB-stained foraminiferal samples, some with processing fossil samples only, and some were not familiar with foraminiferal research at all. To ensure that their judgement was not biased by the authors' knowledge, none of them were shown plates with MTT-stained foraminifers or even told what caused the observed difference in color.

Staining of dead and living individuals after addition of antibiotics

Bacteria also are known to reduce MTT, and their activity could cause recently dead foraminifers to appear alive. To exclude this error, specimens of *Ammonia beccarii* were killed as described above at 50 and 100° C and placed back in the sediment. After 4 days, the decaying foraminifers were incubated for 24 hours with 1 ml of the antibiotics streptomycin, neomycin and penicillin (all three combined into one mixture: P3664, Sigma-Aldrich, St Louis, USA). The concentrations were 1750 units, 1.75 mg and 3.5 mg, respectively, dissolved in 1 l seawater. One ml of MTT (3.5 g/l seawater) was added again, and photographs were taken.

Additionally, sediment that was used to isolate specimens of *Ammonia beccarii* was sieved over a 25-µm screen and the smaller fraction was plated on standard agar plates. The petridishes were incubated at 20° C, and bacterial growth was monitored for three days. At the same time, the sediment pore water was incubated with the mixture of three antibiotics and plated on the same type of plates, incubated at 20° C, and monitored for three days.

To determine the effect of antibiotics on living foraminifers, living individuals of *Ammonia beccarii* were incubated with 1 ml of the antibiotics mixture. Individuals were regularly screened for pseudopodial activity and 1 ml of MTT (3.5 g/ l seawater) was added after three days.

Preservation

In field studies, it is common practice that bulk samples are stained with rose Bengal, then dried and put aside until stained individuals can be counted under a dissection microscope. To investigate the longevity of converted MTT (formazan) in foraminiferal tests, 20 completely stained individuals were picked, air-dried and kept in a chapman slide for two months. The color of the individuals was regularly checked.

RESULTS

Staining of living and dead specimens

Living individuals of *Ammonia beccarii* that showed pseudopodial activity all had yellow colored cell material. In most individuals the last-built chambers were not filled and therefore lacked the yellow color. After MTT was added, the yellow color transformed to a purplish red, starting with the outermost filled chambers and progressing inward (fig 1A).

The speed at which the staining developed, as well as the eventual color of the stained cell material, varied among individuals. Complete staining could be accomplished with-

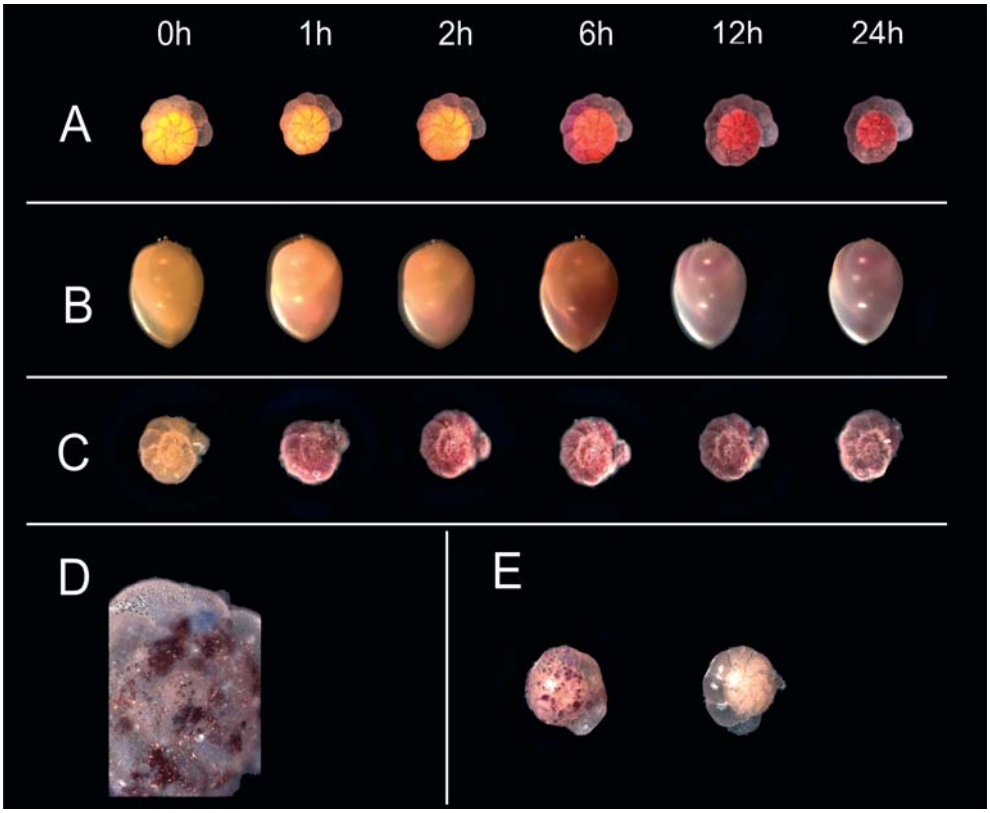


Figure 1: Reduction of MTT in living (A,B) and dead (C,D,E) individuals. A,B. true positives: staining versus incubation time in *Ammonia beccarii* (A) and *Globobulimina turgida* (B). C. false positives: development of the stain in *A. beccarii* that was dead for 4 days, killed at 50° C. D. occurrence of colored patches. E. effect of antibiotics (right) on the formation of colored patches in *A. beccarii*.

in 6 hours, and sometimes the innermost chambers of an individual were still yellow after 12 hours, though all of the 50 living individuals got completely stained within 24 hours. Incubation for longer than 24 hours resulted in progressively darker colored cell material, eventually turning the individual dark purplish to bluish brown.

The color of the cell material of living *Globobulimina turgida* was also yellow, but darker compared to that of *Ammonia beccarii*. After MTT was added, the yellow color slowly transformed to dark purple. Compared to the staining of *A. beccarii*, the stain developed slightly slower in the *Globobulimina* specimens and the final color was slightly darker (fig 1B).

Dead and decaying individuals did not stain when they were placed in MTT immediately after they were killed. However, they did change color after being left to decay, except for those killed at 100° C. The more days they remained in the sediment, the faster MTT was reduced. After more than three days in the sediment, most of the individuals were

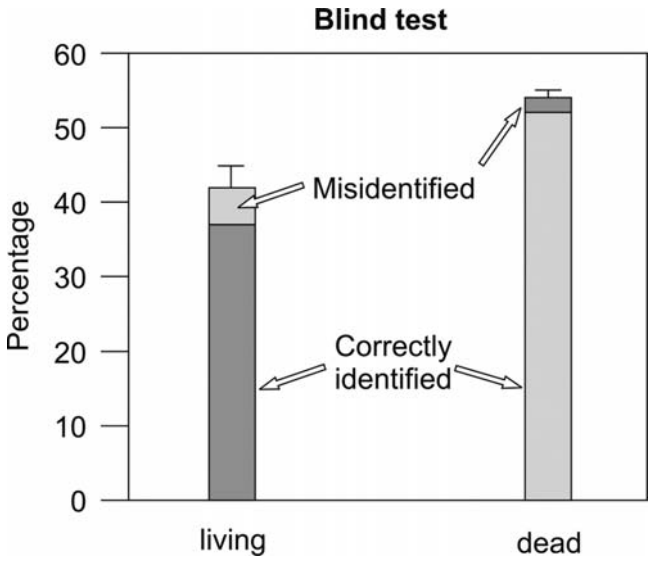


Figure 2: Identifications made in a blind test (+1 SD for misjudged numbers). Left: living individuals incubated with MTT, right: heat-shocked individuals incubated with MTT.

completely stained within one hour, though the color of these cells is more brown and less red compared to living foraminifers (fig 1A). Longer incubation with MTT caused the purple color to shift slowly to darker and browner shades (fig 1C).
Regardless of the way they were killed, individuals that were placed back in the sediment for several days and subsequently incubated with MTT regularly showed colored patches that appeared to lie at the surface of the test and that were darker than the yellow or purple color within the test (fig 1D).

Reduction of MTT in dead individuals of *Ammonia beccarii* did not occur within foraminifers that were killed at 100° C. Individuals got slowly and slightly stained after 3 or more days of decay in the sediment. However, the eventual coloration of the individuals after 24 hours of incubation with MTT is light compared to that of living individuals or of those that were killed at 50° C.
The dead individuals stained for the blind test were hardly colored, in contrast to the stained, living ones. Dead and living specimens were identified correctly 93% of the time. On average only 3% of the specimens were misidentified as false positives, and on average 11% of the specimens were misidentified as false negatives (fig 2). No relation was found between the person's experience with stained foraminiferal samples and the number of false positives or negatives scored in the blind test.

Staining of dead and living individuals after addition of antibiotics
Incubating decaying individuals of *Ammonia beccarii* with antibiotics prior to incubation

with MTT did not affect the staining of cell material in any of the treatments. It did, however, prevent the occurrence of patches forming on the outer side of the test (fig 1E).
Living individuals of *Ammonia beccarii* that were kept in a solution of 1 ml of antibiotics kept their pseudopodial activity for up to three days. Staining these individuals with MTT did not appear to be different from staining individuals that were not kept in antibiotics.

Preservation
Once cells of *Ammonia beccarii* were colored, they were air-dried and kept in chapman slides to track any changes in the color of the stained cells. The color of the cells became slightly darker, but the light purple color was preserved in all individuals after drying for at least two months. Since no change in intensity or amount of stained cell material was observed, the stained foraminifers can probably be kept for a long time between staining and picking.

DISCUSSION

Reduction by MTT stains living foraminifers. All of the 50 individuals of *Ammonia beccarii* we examined in the various experiments were stained fully after 24 hours of incubation at 20° C. Living individuals of *Ammonia* stain red to purplish blue and are easily distinguishable from individuals that are not stained. Successful incubation of this species at this temperature takes at least 6 hours, after which roughly half of the chambers are colored. Individuals of *Globobulimina turgida* stained slightly slower than the *Ammonia* specimens, and after 6 hours less than half of the chambers are brightly colored. The reduction of MTT in *A. beccarii* progressed slower at lower temperatures (results not shown here). At 5° C individuals of *A. beccarii* were hardly stained after 24 hours, while at 25° C individuals were recognizable as living after 3 hours. These observations confirm that the investigated species are mesophyllic, i.e., having enzymes that operate best under moderate temperatures.
The application of RB on deep-sea sediments in particular may lead to overestimated standing stocks because of slow decomposition rates (Heinz and others, 2001; Hemleben and Kitatzato, 1995). However, when MTT is used to stain deep-sea foraminifers, an underestimation of the standing stocks may occur, due to mortality during ascent from the seafloor. A combination of both methods may shed some light on this subject. We think that MTT is a good tool for determining the number of individuals that survive collection. This is especially important when, for instance, sediment is used in microcosm experiments.
The results show that some reduction of MTT can take place in dead individuals. Foraminifers that were killed by heat shock at 50° C displayed enzymatic activity for several days. It even appeared that this activity increased within the first 4 days. In living human cells, MTT is taken up by endocytosis, reduced mainly in lysozymes and then transported back out of the cell. This process determines the speed of the cell's staining, whereas a dead cell does not maintain this organization. Membranes break up in a dead cell, causing MTT to enter the cell passively and causing the cell's organelles to homogenize. The combination of these processes could make MTT reduce faster and be more evenly distributed throughout the cell, resulting in an overall, intense staining. This

means that dead individuals may be potentially identified as living specimens and these false positives may lead to an overestimation of standing stocks when the MTT assay is applied to field or experimental samples. However, staining is clearly different from living specimens (figs 1A and C), making the identification of false positives possible. Moreover, as opposed to staining with RB, recently dead foraminifers do not stain, and they become differently stained when dead for several days.

Since dead specimens can stain after incubation with MTT, the number of false positives and false negatives as identified by the people who did our blind test, were much lower than expected. We expect that if the same blind test was made by staining these specimens with rose Bengal, all or most of the heat-shocked specimens would have been identified as stained, hence the improvement by applying MTT is considerable. Note that the 'assemblage' used in the blind test is not comparable to 'normal' foraminiferal samples that contain live specimens, some recently deceased, and many long dead. The latter group is completely lacking in our test, in which we deliberately used an assemblage entirely composed of living specimens and potential false positives. When most of the dead specimens died long ago, as is the case in normal foraminiferal samples, obviously, the successrate for separating dead from living specimens will be much higher.

Exposing enzymes to temperatures >80° C usually denatures their three-dimensional structure. We think that this prevented staining in individuals that were given a 100° C heat shock. Killing foraminifers through freezing, drying and exposure to ethanol did not fully denature their enzymes, and these individuals stained in the same way as those killed at 50° C. The purple patches on the test of dead individuals were caused by bacterial growth, and were prevented by addition of antibiotics. The presence of patches did not depend on the killing method. The mixture and concentration of different antibiotics did stop the activity of marine bacteria. Bacteria growth was evident after 2 days on agar plates plated with pore water from the sediment in the laboratory aquariums. In contrast, no bacterial growth was evident after incubating the same extract of pore water with the antibiotic mixture. *Ammonia beccarii* was not affected by the presence of the antibiotics and showed as much pseudopodial activity after as before incubation. Finally, MTT-reduction was not visibly affected by the antibiotics.

An incubation of samples with the antibiotics stops the activity of bacteria on the test of dead individuals. We believe, however, that it is not necessary to incubate samples with antibiotics, since the activity of bacteria is easily distinguishable from active, living foraminifers. Dried, the samples can be kept for at least 2 months before being analyzed microscopically. It is not recommended that samples be stored in alcohol, as is common with rose Bengal-stained samples, because it dissolves formazan crystals.

Here we propose a new method for discriminating between living and dead foraminifers. Incubation of bulk samples with a solution of 1 g MTT/l seawater at 20° C causes living individuals to stain slowly within 24 hours. Individuals can stain rapidly if they are dead for some time before the start of incubation with MTT. However, if they do so, then they develop a stain that is distinguishable from the color of stained living cells. Before using MTT reduction as a viability assay, we recommend that the difference in developed color between living and dead specimens is checked at the temperature of incubation (i.e., the seawater temperature in which the specimens are collected) for the species relevant to the study.

CHAPTER 3

SPATIAL DISTRIBUTION OF INTERTIDAL BENTHIC FORAMINIFERA IN THE DUTCH WADDEN SEA

with IAP Duijnstee and GJ van der Zwaan

ABSTRACT
Most spatial distributions of benthic foraminifera are aggregated and the scale of the patchiness has significance for planning sampling surveys, especially for time-series. Through investigations of variation on a range of scales we demonstrate that at an intertidal flat in the Wadden Sea there is patchiness of the two dominant species (*Ammonia tepida* and *Haynesina germanica*) at a scale of decimeters and possibly additionally at a scale of > 50 meters. Despite enormous variation in standing crop, species composition at different localities at a given sample moment was remarkably constant. However, the ratio between the abundances of the two dominant species varied temporally. We conclude that for surveys to establish the general faunal composition, just a few samples would suffice. However, for time-series investigations of this area it would be necessary to adopt special sampling procedures. We argue that food availability is likely to be responsible for the variations in absolute abundances and that relative foraminiferal abundances may be caused by the ratio of the different food sources present.

INTRODUCTION
Organisms are rarely regularly dispersed in space: sometimes they have a random, but usually an aggregated distribution (see for an overview: Thrush, 1991). Such a distribution may be caused by local variations in the environment, but in turn, they themselves shape the local environment. Non-random spatial distribution of diatoms, for instance, can have profound effects on sediment stability through secreted extracellular polymeric substances (Paterson and others, 2000) and aggregated distribution of specimens may enhance biodiversity (Seuront and others, 2002).
Despite a wealth of studies on benthic foraminiferal abundances in intertidal localities (Buzas, 1970; Olsson and Eriksson, 1974; Chandler, 1989; Buzas and Severin, 1993; Alve and Murray, 1994; Buzas an Hayek 2000; Murray and Alve, 2000; Swallow, 2000; Thomas and others, 2000; Alve and Murray, 2001; Buzas and others, 2002), it is not fully understood what determines the success (and thus absolute and relative abundances) of these species. This is important for two reasons: first, in the case of low spatial sampling

resolution or small sample size, total standing stocks in field samples are easily under- or overestimated (Buzas, 1968). This makes comparison between different samples and the detection of long-term trends in foraminiferal abundances difficult. Except when specimens are evenly distributed in space, sampling procedures need to be based on observed spatial patterns in order to be accurate.
Secondly, fossil samples may be biased due to spatial heterogeneity (Edwards and others, 2004). Foraminiferal patchiness is usually claimed to be spatially dynamical and therefore, high and low abundances alternate at a location and together produce a fossil sample with average foraminiferal abundances. However, when sedimentation rates are very high or when the location of patches is stationary over time, spatial heterogeneity can still be responsible for misinterpreting paleo-abundances of foraminifera.
For different, short-term research projects bachelor students conducted various sampling surveys at an intertidal mudflat between June 2002 and May 2003. After combining these results, a consistent pattern of spatial and temporal dynamics of foraminiferal abundances was found. Here we present the combination of these three different sampling surveys and hypothesize that food availability is responsible for the spatial and temporal variations in foraminiferal abundances.

METHODS

Small scale patterns

In June 2002, we sampled an intertidal location in the south-western Wadden Sea (near Den Oever, 52° 56' N, 5° 01' E; fig 1). This location does not accommodate any sea grass and samples were taken by avoiding algal aggregates, topographical irregularities, burrows and other traces of macrofaunal activity. A metal grid consisting of 3x3 cm-squares was pushed in the sediment and 7 by 7 squares were sampled down to a depth of 1 cm, and immediately stained with rose Bengal (1 g/l ethanol). After two days, samples were sieved and the fraction >150 μm was screened for stained specimens. In May 2003, the same grid was used to sample 8 by 8 adjacent squares at the same location.

To analyze possible spatial patterns in these grids, we used the abundances to construct covariograms that summarize the relation between covariance and distance between samples. We used standardized covariograms (equation 1) to determine size and tightness of patches (Dalthorp and others, 2000). A low standardized covariance for a given distance indicates similarity between samples, while high covariances indicate dissimilarity.

$$C_s(\mathbf{h}) = 1\text{-}C(\mathbf{h})/s^2 \qquad (1)$$

Where C(**h**) is the covariance for two samples with distance **h** and s^2 is the variance between those samples. Standardized covariograms typically have low values at low distances and increase to 1 at higher distances. The starting value (commonly called the nugget) can be interpreted as the tightness of patches (lower values indicate tighter patches), whereas the size of the patches is represented by the distance where the covariance curve levels off at 1. If individuals are randomly distributed, patchiness is absent and the covariance-curve is horizontal.

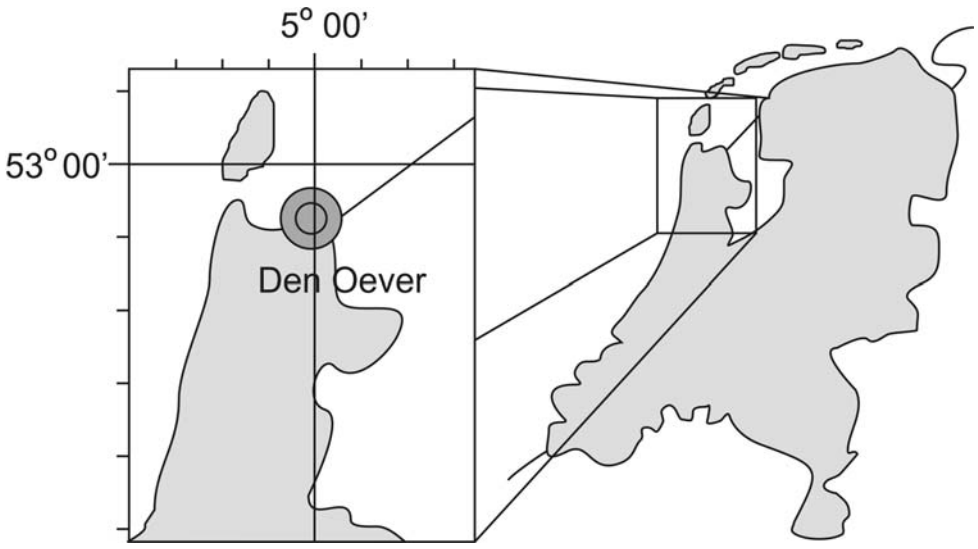


Figure 1: Location of the sampling site.

Large scale variation

In June 2002, the same intertidal location in the Wadden Sea was sampled to determine large scale spatial patterns in foraminiferal abundances. Samples were taken by using a 1 cm high ring with a diameter of 8.0 cm resulting in top-centimeter samples of 50.3 cm^3. Three pairs of samples were taken at eight locations: groups consisted of pairs at distances of 0.10, 1.0 and 10 m. Each group of three pairs were taken within 100 m^2 and all eight groups of samples were approximately 40 meters apart, located roughly parallel to the water line, in between the mean low and mean high tide lines. Samples were stained with rose Bengal (1 g/l ethanol) at the site of collection and after two days, the material was sieved over a 150 µm-screen after which the large size fraction was checked for stained foraminifera. Because samples occasionally contained many specimens, samples were split into halves, or further into one-fourths, etc. In these cases, parts were then analyzed for rose Bengal-stained specimens and numbers were multiplied to obtain abundances for the complete sample. In this way, at least 200 individuals were counted per sample.

Data were used to calculate similarity ratios (Ball, 1966) between pairs of samples for each of the three distances (equation 2).

$$SR_{ij} = \Sigma_k y_{kj} / (\Sigma_k y_{ki}^2 + \Sigma_k y_{kj}^2 - \Sigma_k y_{ki} y_{kj}) \qquad (2)$$

Where y_{ki} is the abundance of the species k at site i. This similarity index varies between 0 and 1, higher values indicating higher similarity. The 8 calculated ratios of each distance were averaged to calculate the average similarity ratio for each of the three distances.

Tidal gradient

In May 2003, at low tide, the same 1 cm high ring with a diameter of 8 cm was used to sample two parallel transects. At the same longitude (5° 01.179' E), six locations with a 0.1 minute-interval (185 meters) were sampled by taking two samples within a square meter. The two locations closest to the low water line were sampled with a distance of 0.2 minutes (370 meters) away from the nearest samples. 0.05 minutes (93 meters) west of this transect, another transect was sampled in the same way. Sampled locations were chosen so that they were evenly spaced between mean high tide and mean low tide (fig 2).
In April 2003, the same two transects were also sampled, although no replicates were taken.

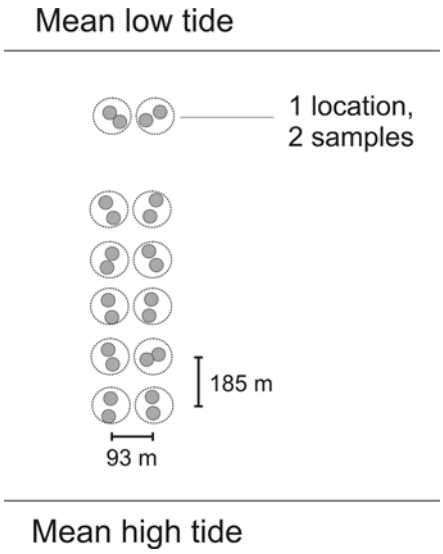


Figure 2: Samples taken along two parallel transects.

RESULTS

Small scale patterns

The grid sampled in June 2002 contained only one species in significant abundances: *Ammonia* cf. molecular type T6 (Hayward et al., 2004; here further referred to as *A. tepida*; fig 3). In chapter 2 we referred to this species as *Ammonia beccarii*, but after publication (De Nooijer and others, 2006) we agreed with others that it is more often referred to as *A. tepida*. In this and following chapters, we will use the name *tepida* for this species.
The squares containing high abundances (>300) were located in the lower right and the upper right corner of the grid. Most squares contained low abundances (<50), were located in adjacent pairs: two at the middle-lower side and two at the left side of the grid.
In May 2003, the samples of the 8 by 8 squares contained the species *Ammonia tepida* and *Haynesina germanica* (fig 4).
For both species there appeared to be two patches of higher abundances: in the upper

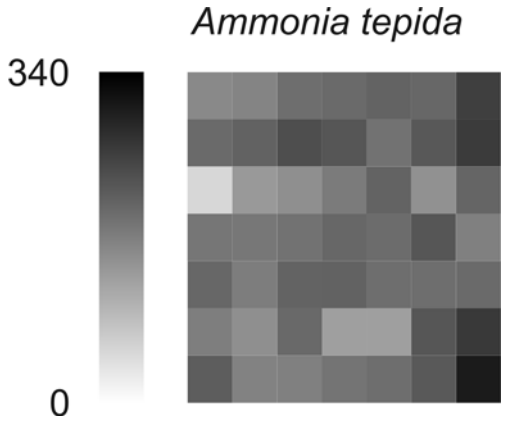


Figure 3: Small scale distribution of *Ammonia tepida* in June 2002.

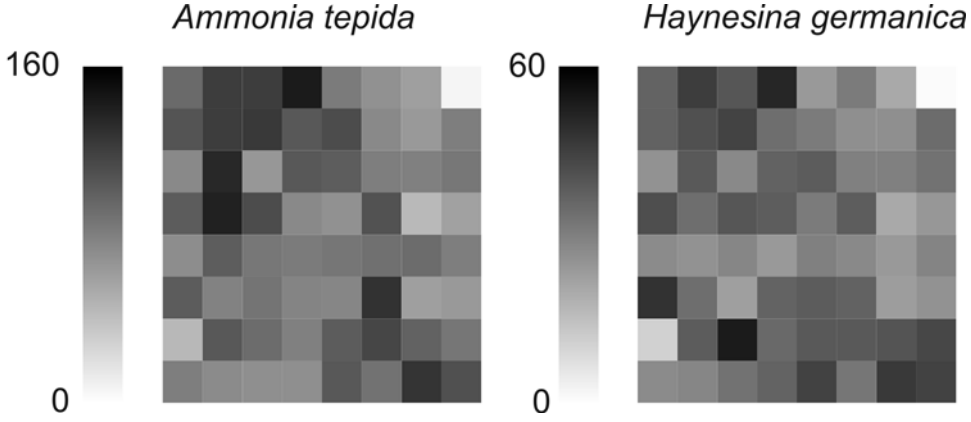


Figure 4: Small scale distribution of *Ammonia tepida* and *Haynesina germanica* in one grid in May 2003.

left and lower right corner. The relations between distance within the grids and absolute abundances in the adjacent squares (figs 3 and 4) are summarized in standardized covariograms (fig 5).

In 2002, for the smallest distance, the standardized covariance (i.e. the nugget) is 0.75, indicating that the foraminifera are distributed in diffuse patches. For 2003, the covariograms show a patchy spatial distribution for both species in the grid: *Haynesina germanica* occurs in more diffuse patches (nugget = 0.6) and *Ammonia tepida* in tighter patches (nugget = 0.4) of 15-20 and 15 cm in diameter respectively. *A. tepida* is present in much higher numbers than *H. germanica*, although the location of their patches is spatially correlated.

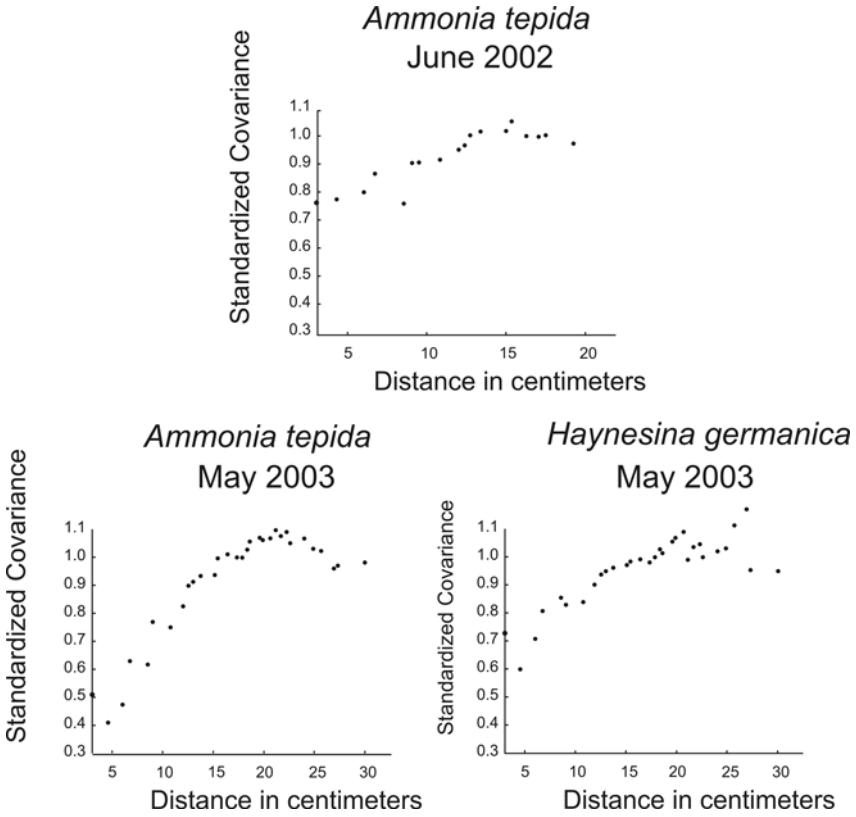


Figure 5: Standardized covariograms based on absolute numbers, top: June 2002, bottom: May 2003; left: *Ammonia tepida*, right: *Haynesina germanica*.

Large scale variation

In June 2002, the same location was sampled to investigate the distribution of benthic foraminifera at a larger scale. Again, samples contained mainly *Ammonia tepida*. The relation between the distance and similarity is expressed as the similarity ratio (fig 6).

Although standard deviations are relatively large, samples differ more when taken 10 meters apart than at smaller distances. This suggests that there may have been 2 levels at which there was spatial variability: a relatively small scale variance resulting in a similarity ratio of 0.85 and a larger scale variance with a ratio of 0.70.

With regard to the groups of sample-pairs taken within 100 m² (group 1-8), there are significant differences between average abundances of the groups (fig 7). Average abundances of *Ammonia tepida* in the samples of group 1 and 2 (located at the west side of the line on which all groups were located) is higher than that of groups 3-8. Average standing stocks of groups 1 and 2 differ significantly from all other 6 groups, but not from each other (ANOVA single factor, df = 10, $F_{1,5} > 4.96$, $p < 0.05$). Within the groups 3-8, most differences in means are significant (exceptions: 3 and 4; 5 and 7; 5 and 8; 7 and 8).

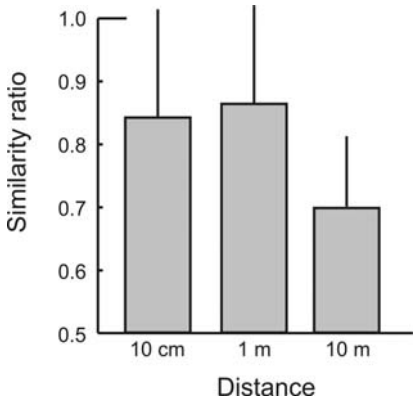


Figure 6: Relation between similarity of samples and distance (+ 1 SD).

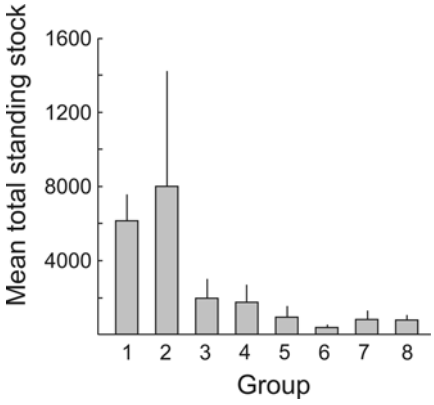


Figure 7: Mean total standing stock of groups of 6 samples taken within 100 m^2 (+ 1 SD). Groups were located approximately 40 m apart.

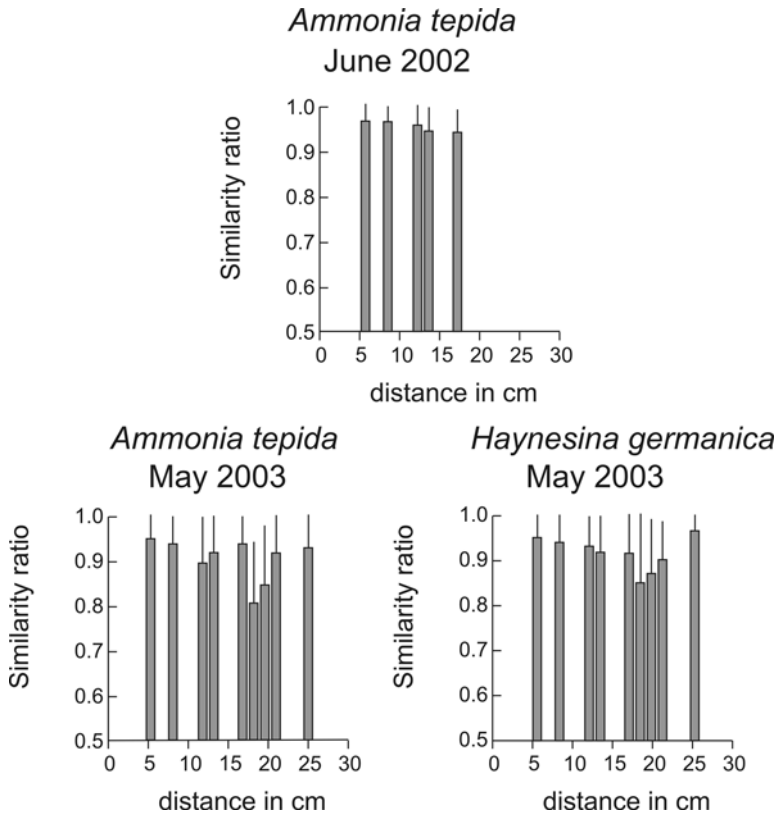


Figure 8: Similarity ratio for the upscaled grid data from figs 2 and 3 (+ 1 SD).

Relation small and large scale

To compare spatial patterns in the two discussed sets, grid samples were upscaled to match the size of the large scale samples. This was approximated by combining 4 adjacent squares into one of 6 by 6 centimeters. The centers of these new squares of 36 cm² had mutual distances ranging from 6 to 25 cm. The relation between similarity and distance was expressed similar to the large-scale samples in fig 6. Average similarity ratio between these larger squares is 0.90 - 0.95 for the grids sampled in 2002 and 2003 (fig 8).

Effect of tidal gradient

In April and May 2003, two transects were sampled at the same intertidal location in the Dutch Wadden Sea. As for the grid sampled in May that year, only *Ammonia tepida* and *Haynesina germanica* were present. Although total numbers of both species differed, the *Ammonia*/*Haynesina* ratio in each month was relatively constant among the samples (fig 9).

To compare transect samples with the other two groups of samples, their similarity ratios versus distance were calculated (table 1). Regression analysis based on all data, indicated that abundances of both species were not significantly correlated with distance to mean high or low tide.

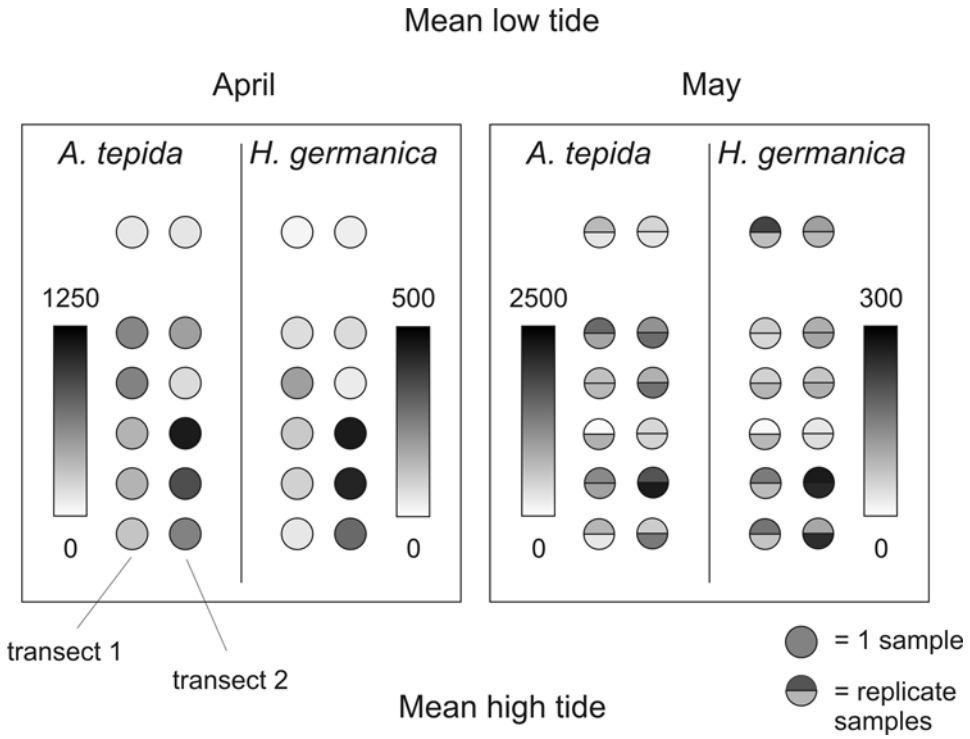


Figure 9: Numbers of *Ammonia tepida* and *Haynesina germanica* in the sampled transects in April (left) and May (right).

Similarity ratio						
	April			May		
Distance (m)	*A. tepida*	*H. germanica*	n	*A. tepida*	*H. germanica*	n
<1 (replica's)	-	-	-	0.66 +/- 0.31	0.67 +/- 0.30	12
93	0.57 +/- 0.31	0.41 +/- 0.37	6	0.64+/- 0.27	0.61 +/- 0.25	24
185-207	0.63 +/- 0.27	0.51 +/- 0.28	16	0.51 +/- 0.32	0.57 +/- 0.34	64
370-382	0.52 +/- 0.28	0.45 +/- 0.27	16	0.47 +/- 0.30	0.51 +/- 0.33	64
555-563	0.64 +/- 0.28	0.48 +/- 0.39	12	0.67 +/- 0.27	0.62 +/- 0.30	48
740-746	0.50 +/- 0.37	0.34 +/- 0.38	8	0.58 +/- 0.33	0.50 +/- 0.33	32
925-930	0.23 +/- 0.18	0.18 +/- 0.21	4	0.30 +/- 0.21	0.62 +/- 0.28	16
1110-1114	0.35 +/- 0.25	0.36 +/- 0.42	4	0.61 +/- 0.30	0.69 +/- 0.28	16

Table 1: Average similarity ratio per distance within transects for both species during April and May (+/- 1 SD).

Summary

The occurrence of the two main taxa was compared by correlating the absolute abundances of *Ammonia tepida* and *Haynesina germanica* per sample for the large scale survey (June 2002), and transects (April 2003 and May 2003: fig 10).

Before calculating the correlation coefficients between *Ammonia tepida* and *Haynesina germanica*, total numbers were log-transformed because numbers were not bivariate normally distributed. After log-transformation, this requirement was met and all correlations between *A. tepida* and *H. germanica* were positive (April 2003: $r = 0.902$, $df = 10$; May 2003: $r = 0.707$, $df = 22$; June 2002: $r = 0.835$, $df = 46$) and significant ($p < 0.0001$ for all analyses). In June 2002, average percentage of *A. tepida* in all samples was 91%, in April 2003 it was 59% and in May that year, 81% of the community consisted of *A. tepida*. The small scale data was not transformed and correlation analysis resulted in a positive ($r = 0.740$) and significant ($p< 0.001$) correlation (fig 11).

At the centimeter scale, the *Haynesina*/*Ammonia* ratio is similar to that obtained from the large scale sampling survey at the same time.

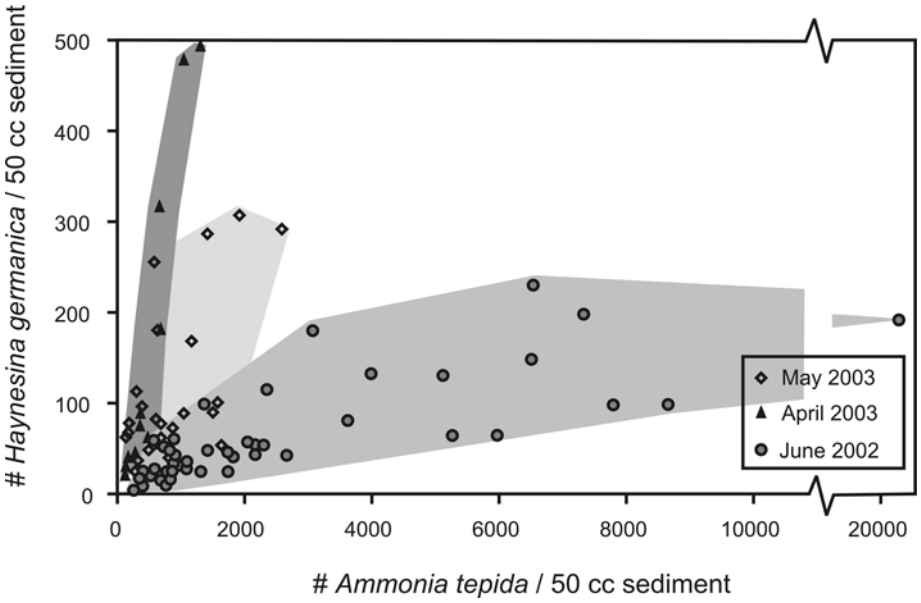


Figure 10: Relation between abundances of *Ammonia tepida* and *Haynesina germanica* in the large-scale and transect samples.

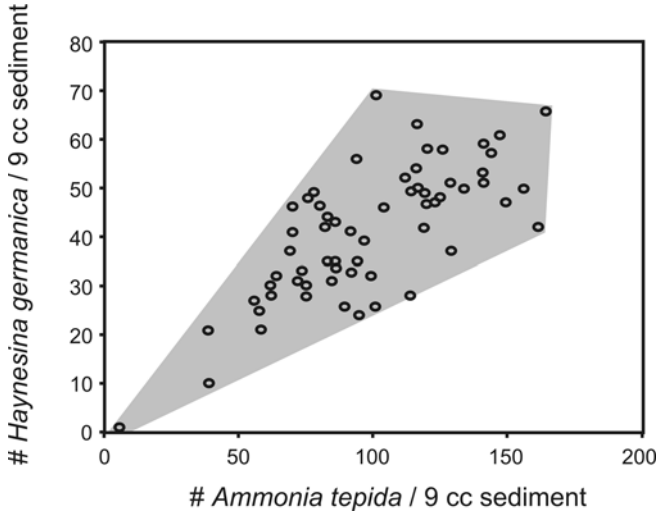


Figure 11: Relation between abundances of *Ammonia tepida* and *Haynesina germanica* in the grid samples.

DISCUSSION AND CONCLUSIONS

In this study we show that elevated foraminiferal abundances occur in patches of ~175-300 cm^2. Spatially, the abundances of *Ammonia tepida* and *Haynesina germanica* are cor-

related although the ratio of the two species varies temporally. We hypothesize that the availability of different food sources and differential food preferences of *A. tepida* and *H. germanica* are responsible for the observed spatial and temporal variability, and further explore this possibility below.

Differential food preferences

Haynesina vs *Ammonia*

Many studies suggest that *Ammonia* spp. and *Haynesina germanica* feed on different food sources. Generally, species in the genus *Ammonia* are known to feed on detritus, bacteria and refractory material (Goldstein and Corliss, 1994). *H. germanica* on the other hand, is known to prefer labile organic material such as (living) diatoms. This difference in food preference is illustrated by the fact that in our laboratory, we were able to keep *A. tepida* alive in the dark for several months, where most individuals of *H. germanica* did not survive dark conditions for a week (results not shown here).

From visual observations (Murray and Alve, 2000) and from chromatography studies (Knight and Mantoura, 1985) it is known that *A. tepida* usually does not contain algal chloroplasts. *A. tepida* is also described to be spatially positively correlated with cyanobacteria (Hohenegger, 1989). Experiments by Moodley and others (2000) show that *A. tepida* does not exclusively feed on refractory matter, but rather is capable of feeding on many food sources and perhaps utilizes refractory matter when nothing else is available or competition for labile matter is too fierce.

H. germanica on the other hand is known to contain living diatoms or their chloroplasts (Knight and Mantoura, 1985), which is also indicated by its intense green colored cytoplasm (Murray and Alve, 2000). Ward and others (2003) concluded after feeding experiments that *H. germanica* consumes living individuals of the pennate diatom *Phaeodactylum tricornutum*, and does not consume more refractory, sewage-derived organic matter. Recently, it has been shown that *H. germanica* is able to crack the frustule of the diatom *Pleurosigma*, presumably to feed on its cell material (Austin and others, 2005).

If this difference in food preference is responsible for the observed spatial and temporal patterns, three premises must be true: 1. foraminiferal food occurs in patches: 2. different types of food are correlated spatially and 3. the ratio of the food sources varies temporally.

Distribution of foraminiferal food

Microphytobenthos (the main foraminiferal food source) is reported to occur in patches of 2-100 cm^2 in muddy sediments (Blanchard, 1990; Seuront and Spilmont, 2002; Jesus and others, 2005) and in patches of 30-190 cm^2 in sandy sediments (Sandulli and Pinckney, 1999). Bacteria can also occurr in patches on a centimeter scale in near-coastal sediments (Seymour and others, 2004). Additionally, Blanchard (1990) found a correlation between the patchy distribution of microphytobenthos and meiofauna and hypothesizes that spatial and temporal variations in the abundance of meiofauna is caused by food availability. Harpacticoid copepods are also shown to be distributed spatially according to distribution of diatoms and bacteria (Decho and Castenholtz, 1986).

Spatial correlation of food sources (the second premise) is described for different

species of diatoms (Peletier, 1996; Haubois and others, 2005) and for microphytobenthos and bacteria (Hohenegger and others, 1989; Goto and others, 2001). The latter correlation can be caused by bacteria feeding on excreted polymers by diatoms (Decho, 2000).
Finally, it has been shown that intertidal microphytobenthic biomass (e.g. De Jonge and Colijn, 1994; Staats and others, 2001; Widdows and others, 2004) and species composition (e.g. Underwood, 1994; Pinckney and others, 1995) varies seasonally. Also at Dutch tidal flats these variations are recorded (e.g. Barranguet and others, 1997; Hamels and others, 1998), where diatoms dominated the sediments in spring and high amounts of cyanobacteria coexist with diatoms in summer, followed by a further decrease of diatom biomass in autumn.

Other factors
It may well be that variations in absolute and relative abundances of foraminifera in the Wadden Sea are (partly) caused by the factors determining microphytobenthic and bacterial biomass and species composition. For example, Montagna and others (1983) showed that occurrences of diatoms and other meiofauna were partly determined by physical factors (salinity, temperature and redox depth). It is also reported that microphytobenthic biofilms, formed in spring at Dutch intertidal flats, were mainly eroded by tidal waves later in the season due to increased wind stress (Staats and others, 2001; De Brouwer and others, 2000). It can not be excluded that benthic foraminiferal abundances are also determined by these factors.

Implications for sampling design
The results emphasize the need for adequate sampling procedures that cope with the observed variation in abundances. In the area described here, relative foraminiferal abundances can be determined by a low number of samples since the ratio of *Ammonia tepida* and *Haynesina germanica* is relatively constant at a given time. In contrast, the absolute numbers vary greatly, with many samples of relatively low numbers and few with high numbers. This difference manifests itself especially at the centimeter scale, which is easily accounted for by taking several replicate samples. Another major heterogeneity step occurs at the scale of >10 meters. In seasonal or multiple-year monitoring of such mudflats it is thus necessary to take samples app. 100 meters apart if one wishes to cover the full range of abundances present at the scale of the entire mudflat.
Many studies mentioned in this discussion stress the complexity of the meiofaunal-microphytobenthic-sedimentary system. Some studies reveal that biological interactions (grazing, competition), or abiotic, seasonal changes (wind stress, salinity, temperature) determine abundances and species composition in the intertidal benthic community. The role of foraminifera in the intertidal benthic food web is hardly accounted for in these studies, but as our results show, they may play an important role in the interactions between bacteria, microphytobenthos and other meiofaunal taxa.

CHAPTER 4

THE ECOLOGY OF BENTHIC FORAMINIFERA ACROSS THE FRISIAN FRONT (SOUTHERN NORTH SEA)

with IAP Duijnstee, MJN Bergman and GJ van der Zwaan

ABSTRACT
Benthic foraminifera were collected across the Frisian Front, a biologically enriched transition zone with high organic matter content below a tidal mixing front in the southern North Sea. At various seasons during cruises between 2002 and 2005, boxcores from different hydrographic regimes (i.e. tidally mixed, frontal and stratified) were subsampled. From every subsample, stained foraminifera were enumerated in the top 5 centimenter of sediment. Results indicate that standing stocks and foraminiferal diversity are higher at the central zone of the Frisian Front than further away from the frontal zone. Also, most of the abundant species occupy a specific zone relative to the front's central position. *Elphidium excavatum* is abundant at the southern edge of the Frisian Front, where input of labile organic matter is high and physical disturbance (i.e. resuspension of fine-grained material) is relatively frequent. *Ammonia tepida* and *Quinqueloculina* spp. dominate at the front's center where organic carbon input is relatively high. *Hopkinsina pacifica* has highest abundances at the deepest boundary of the front, and *Eggerella scabra* dominates the deeper, stratified Oyster Grounds north of the front. Differences in seasonal distribution patterns were minor compared to spatial distributions, although depth distributions varied between summer ('epifaunal' distribution) and winter (vertically more evenly distributed). The latter suggests that the vertical distribution of foraminifera is governed by the arrival of fresh organic matter at the seafloor in spring and summer.

INTRODUCTION

In many coastal waters, tidal mixing fronts can be found (Pingree and Griffiths, 1978; Simpson and others, 1978). These fronts are the transition zone between near-coastal waters, which are completely mixed by tidal wave action, and deeper waters that become thermally stratified in spring and summer (Jones and others, 1998; Drinkwater and Loder, 2001; Mavor and Bisagni, 2001). If the tidally mixed waters are rich in suspended matter this will sink down at such fronts where tidal currents drop below a critical velocity along a deepening slope. Enhanced settlement results in a zone of sediment

with a high mud content at the location of these fronts (Creutzberg and Postma, 1979). In the frontal zone, usually a chlorophyll maximum exists, caused by the optimal combination of light and nutrients (Holligan, 1981; Postma, 1988). At the deep boundary of a front, thermal stratification prevents upward diffusion of nutrients and at the coastal edge, turbidity prevents light penetration, both factors limiting primary production.
In the North Sea two hydrographic fronts separate the Southern Bight from the Oystergrounds: the Frisian Front off the northern Dutch coast (De Gee and others, 1991) and the Flamborough Front, which is located near the English east coast (Hill and others, 1993; Howarth and others, 1993; Tett and others, 1993). These fronts provide a variety of pelagic and benthic environments within a short bathymetrical range. Benthic studies at the Frisian Front, the zone with increased deposition of silt between the 30 and 40m isobaths, have shown enhanced biomass and diversity of the macrobenthos compared to locations outside the front (Creutzberg, 1986; Callaway and others, 2002; Dewicke and others, 2002).
Only few studies have included foraminifera in assessing the benthic community structure in the North Sea, despite their high abundances and ecological importance. Studies focusing on foraminifera across hydrodynamic fronts (e.g. Moodley, 1990; Scott, 2003) are necessary in order to reliably reconstruct Holocene shelf evolution (Moodley and Van Weering, 1993; Evans and others, 2002; Scourse and others, 2002). Another reason to monitor (meio)faunal densities and diversity results from the intention of the Dutch government to appoint the Frisian Front as a protected area in 2007 (IDON, 2005). The North Sea in general is heavily trawled and ongoing deterioration of its habitats and declining fish stocks have caused the necessity to restrict fishery in certain areas of the North Sea. The Frisian Front is one of the intended protected locations since it is acknowledged to be an ecologically unique area. Future changes in the benthic faunal diversity, community structure and densities can only be investigated by using base-line field studies that determine faunal abundances shortly before ecological intervention. Here we present results of benthic foraminiferal abundances across the Frisian Front and discuss their relation to a range of hydrodynamic and environmental conditions.

METHODS

Area description

At the transitional zone between the Southern Bight water (depth 25m) and the Oyster Grounds (50 m) the maximum tidal current velocity drops below a critical value, resulting in increased deposition of mud and organic carbon at the sea bed. This biologically enriched benthic zone between the 30 and 40m isobaths is called the Frisian Front and is located approximately between 53º 30' N, 4 00' E and 54º 00' N, 5 00' E (Creutzberg, 1986; De Gee and others, 1991). On a north-south transect along the 4º 30' E meridian, the frontal zone extends from 53º 35' N to 53º 50' N, with the highest mud content between the latitudes 53º 40' N and 53º 45' N where water depths are between 35 and 40 meters. The position of the hydrodynamic front may vary according to wind direction and speed (Hill and others, 1993), the location of the benthic front, however, remains relatively stable over the years. South of the Frisian Front, sediments consist of fine

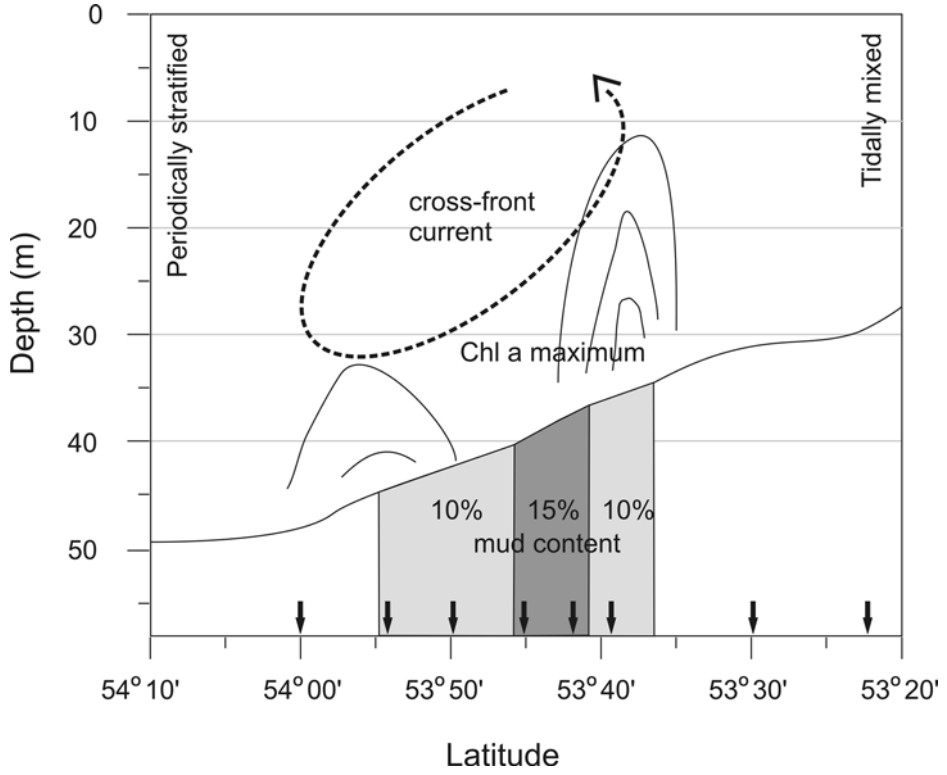


Figure 1: Position of the benthic front versus bathymetry of the seafloor. Mud content across the front, Chl-a maxima, and the cross-front current are indicated (based on Creutzberg and Postma, 1979; Creutzberg, 1986 and Van Haren and Joordens, 1990). Position of sampling stations is indicated by arrows.

sands with almost no mud and towards the front the mud content increases rapidly up to 15%, but declines somewhat towards the deeper Oyster Grounds that are characterized by spring and summer stratification (fig 1).

The hydrographic Frisian Front stretches out to the east, parallel to the Dutch and German northern coastline and to the west, where it joins the Flamborough Head Front at approximately 0º 40'E. An along-front jet flows eastwards, just south of the Frisian Front (Lwiza and others, 1991). During stratification of the water north of the Frisian Front, a colder surface layer can be distinguished just south of the stratified area. This phenomenon is ascribed to small, circular cross-frontal currents, which transfer deep and colder waters of the stratified area up to the surface (Van Haren and Joordens, 1990; fig 1). Studies on chlorophyll-a (Chl-a) content in cross-sections of the Frisian Front revealed that the Chl-a profiles are not consistent through space and time. Chl-a maxima exists regularly in summer near the sediment-water interface at the south side of the benthic front (Van Haren and Joordens, 1990) and occasionally, a weaker optimum just north of the front is observed.

Sampling

A transect across the Frisian Front was sampled in different months and years to determine abundances of benthic foraminifera. Samples were taken on December 4th, 2002; June 25th, 2003; August 29th, 2004 and February 7th, 2005. Figure 2 shows the location of the front and the sampled stations.

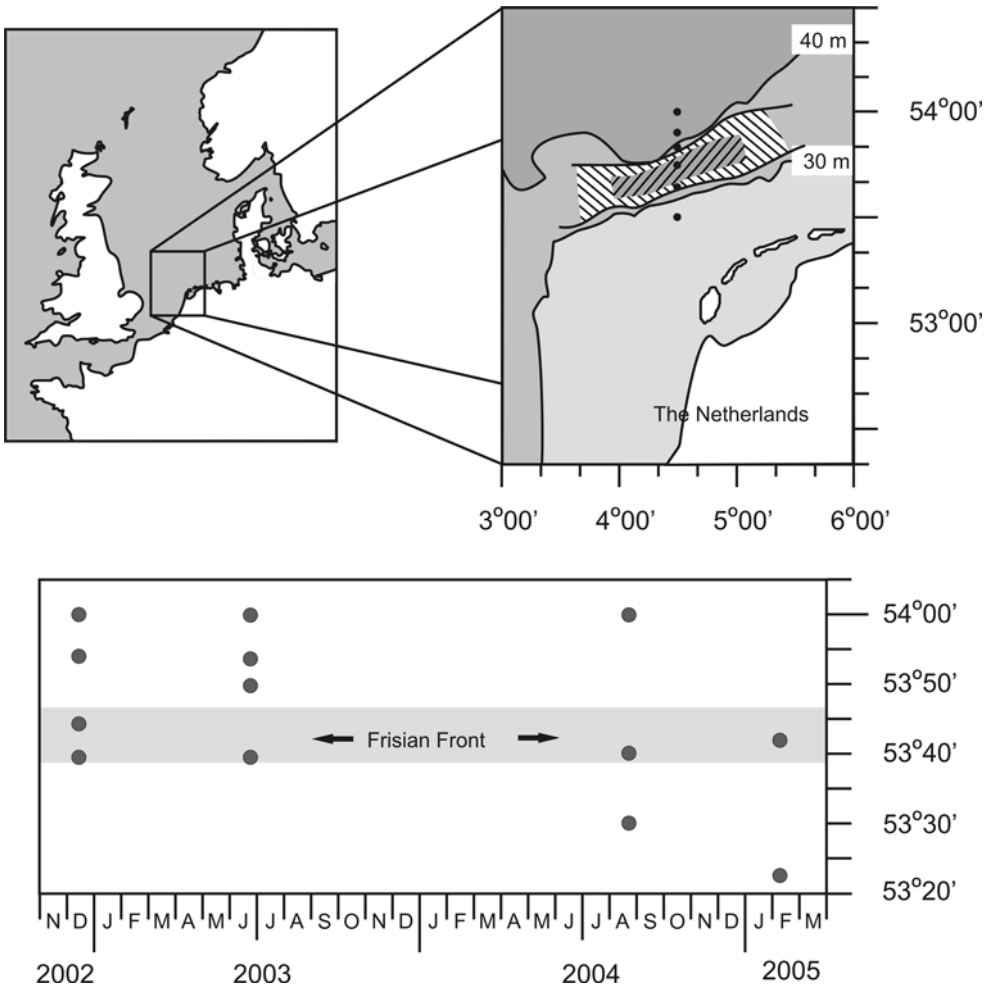


Figure 2: The Southern North Sea, the location of the enriched benthic zone in the Frisian Front (single hatched area) with the highest silt content (cross-hatched area) and the sampling scheme.

Foraminifera were subsampled from both boxcores taken at each station. Small cores (26 cm^2 in diameter, ten centimeters high) were used to slice the sediment on-board into 7 depth intervals. The top two centimeters were sliced in four layers (each 0.5 centimeter) and the lower part in three intervals of one centimeter each. All samples were stored in polyethylene jars and fixed in ethanol with rose Bengal (1 g/ l). Additionally, each boxcore was subsampled for oxygen profile measurements and the top centimeter of sediment of one boxcore per station was sampled for TOC and grain size analyses.
Measurements of the dissolved oxygen content of the pore waters were performed on board with Unisense microelectrodes (OX-10) attached to a micromanipulator and connected to a Unisense picoamperemeter. Electrodes were calibrated prior to measurements in oxygen-saturated seawater from the boxcore. At approximately 5 cm above the sediment-water interface, oxygen was measured before and after profiling the sediment to exclude any changes in the electrode's properties during the measurements.

Upon return in the laboratory, samples for TOC and grain size analysis were dried. After one week, samples for TOC were decalcified by two successive additions of 1M HCl and rinsed with demineralized water afterwards. After the samples were dried, analysis was performed on a CS-analyzer, LECO. Grain size analysis was performed using a laser particle sizer, Malvern Instruments, UK. Before analysis, material was treated with 10% H_2O_2 and with 1M HCl to remove organic material and carbonates.
A week after the samples were taken, the faunal samples were sieved over two screens to remove material smaller than 63 µm and to separate the foraminifera into two size classes that are common in micropaleontological studies: between 63 and 150 µm and larger than 150 µm. The material was screened under a dissection microscope for rose Bengal-stained (i.e. protoplasm-bearing) foraminifera.

Statistical methods

Principal Component Analysis (PCA) was used to determine the community's relation to abiotic parameters at the sampled stations and was performed in CANOCO, version 4.5 (Microcomputer Power, Ithaca, USA; Ter Braak and Šmilauer, 2002). Prior to analysis, species numbers were square root transformed and environmental parameters were plotted additionally. Also, since three samples contained very few specimens (February and August, southernmost samples) and therefore would have dominated the outcome of the PCA, they were plotted in the ordination plane as supplemental samples, thus not influencing the construction of the ordination axes. Foraminiferal abundances are partly presented by interpolating between the moments and locations of the samples. The interpolation was carried out by an Excel-embedded algorithm using third-order piecewise polynomials.
Since the samples were taken in different months of different years, the results are presented in a chronological order, (i.e. in the order in which the samples were taken). For convenience, and to facilitate the recognition of possible seasonal patterns in the data, they are also presented as if they were taken within one year. This seasonal order that is used to express the data in the following sections starts with the samples taken in December (as in the chronological order) and consequently, ends with the same samples to complete the seasonal interpretation. One should keep in mind, however, that the variability in these representations may be partly caused by interannual variability.

RESULTS

Environmental setting

The total organic carbon (TOC) in the upper centimeter, measured at the stations and at the four sample moments, was generally higher in June and December than in February and August. Along the cross section through the geographic Frisian Front, TOC was elevated between 53º 30' and 53º 45' and highest at the central front zone (fig 3).

During some of the measurements on pore-water oxygen profiles in the sediment, the electrodes were broken by large objects in the sediment. Therefore, oxygen profiles were not obtained from all sites and sample moments and we did not include oxygen as environmental variable in our statistical analyses. We noticed, however, that the obtained profiles were all relatively similar: i.e. below 0.5 cm, oxygen concentration was usually below 8 mg/l, i.e. 5% of the concentration 1 cm above sediment-water interface: data are listed in appendix I.

Total foraminiferal community

Overall, total standing stocks were relatively similar between stations and sample moments; the only exceptions were abundances in the stations at the southern border or even south of the enriched silty Frisian Front (53º 30' and 53º 22'), where densities of stained individuals were always less than 60 per sample (130 cm^3). In general, differences between the replicate samples were small (fig 4).

Abundances of all species were used to calculate Shannon's diversity index (H) and Shannon's equitability (E_H): fig 5.

Foraminiferal diversity (Shannon diversity index: H) did not differ much across the stations, although it was slightly higher at the central zone of the front than at more distant stations. Since H is a reflection of both evenness and species number, high values at the front were only partly caused by a high number of species in those samples. Shannon's equitability (E_H: value between 0 and 1) is essentially a correction of this diversity for the number of species, thus reflecting merely evenness, which increases towards the southern border of the front (fig 5, right).

To analyze spatial and temporal patterns in total foraminiferal communities, principal component analysis was performed (fig 6).

The variance in species data explained by the first and second Principal Component together is almost 75% (40.4% and 32.8%, respectively). Sample scores on the first axis are mainly dominated by the abundant *Eggerella scabra* and *Bolivina spathulata* (causing negative sample scores) and by *Elphidium excavatum*, *Bolivina pseudoplicata*, *Stainforthia fusiformis* and the rarer *Nonion depressulus* (whose abundances cause positive sample scores). This axis is negatively correlated with latitude and positively with TOC.

Sample scores on the second (vertical) axis are caused by high numbers of *Hopkinsina pacifica*, *Bulimina marginata*, *Bolivina dilatata* and *Bolivina seminuda*, and by low abundances of *Textularia* sp. and *Leptohalysis scotii*. The second axis is also positively correlated with mud content and negatively with high values for the variable 'summer'. The species composition of the three additionally plotted samples from the southern end of

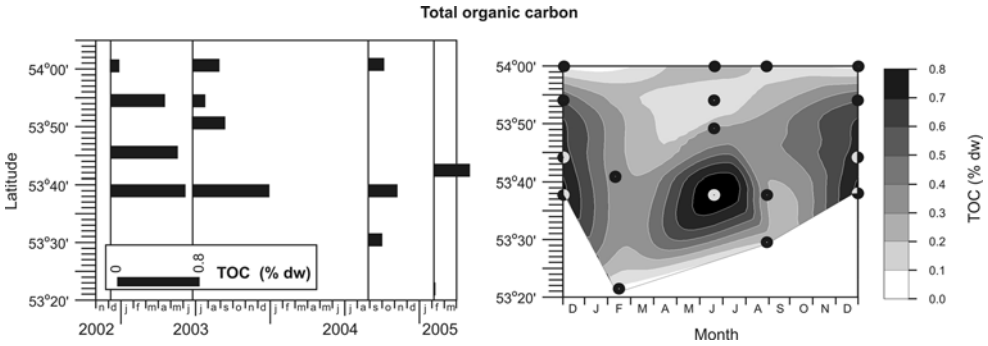


Figure 3: Total organic carbon content in the upper centimeter as a percentage of the sample's dry weight. The values are represented in the chronological order (left) and in the seasonal order with interpolated values in between the samples (right).

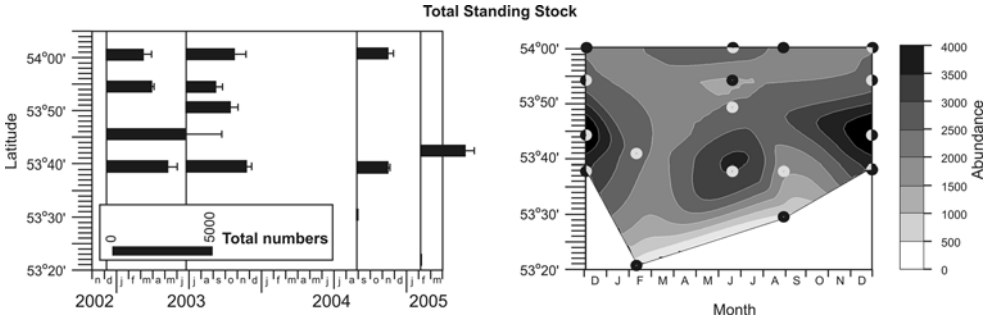


Figure 4: Total abundances per 26 cm^2 of the total community in the upper five centimeters: values are averages of duplicate samples (+1 SD in the bar chart).

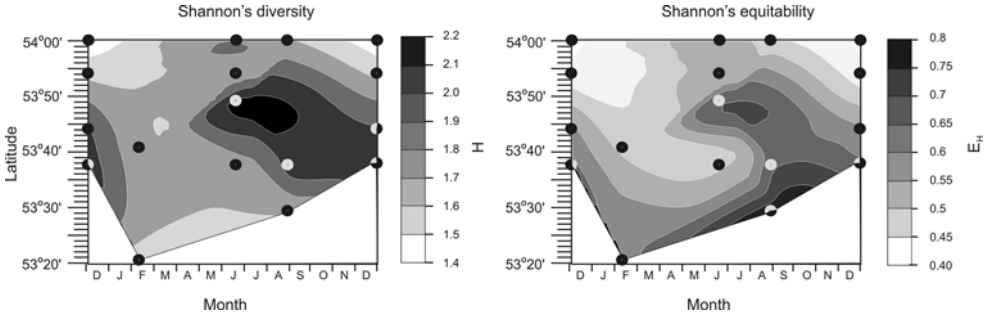


Figure 5: Shannon's diversity index (left) and Shannon's equitability (right). Data in between samples are interpolated.

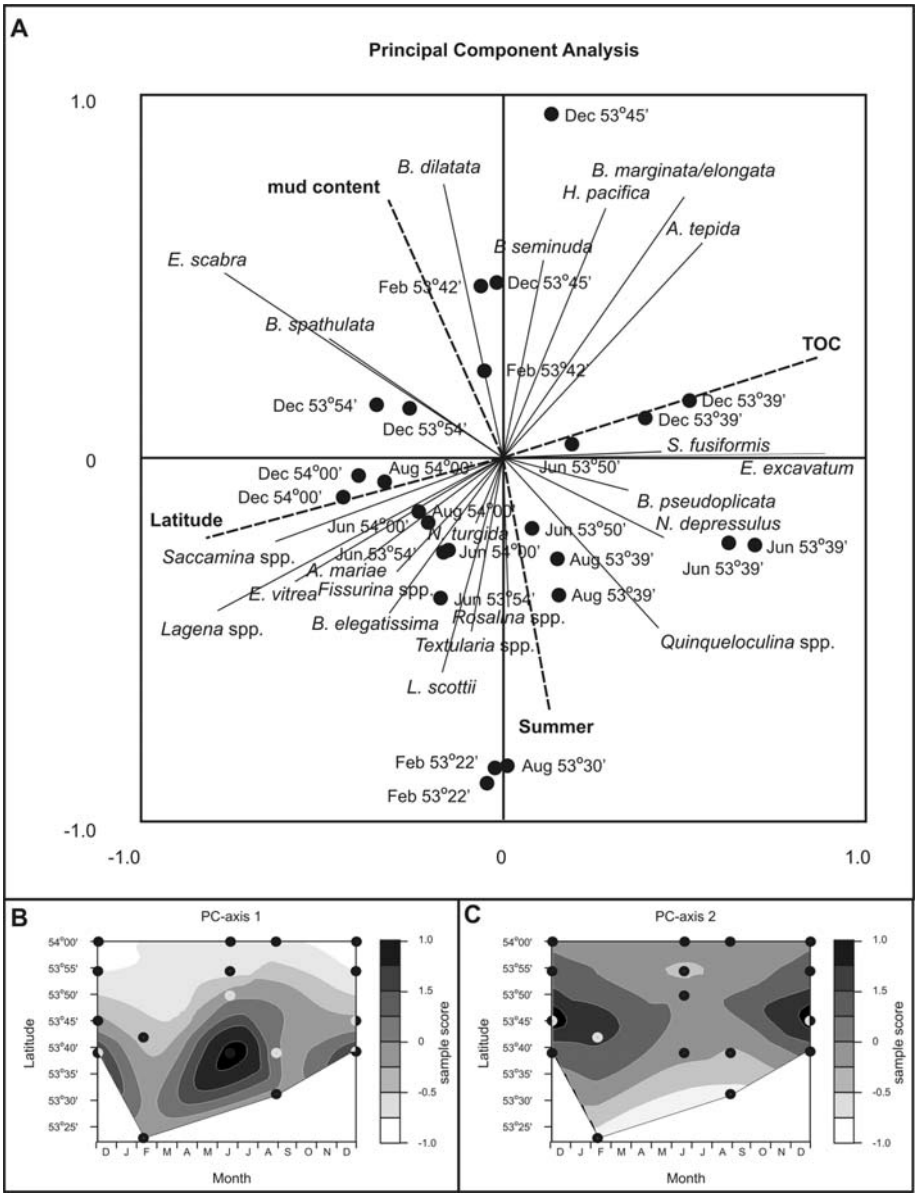


Figure 6: Principal component analysis. A: Biplot of 1st and 2nd PCA scores based on absolute abundances of all species, environmental variables are plotted additionally. Values for the variable 'summer' are based on the time lapse, calculated in months, from December (the first sample moment) onward. Although used for the analysis, species that occurred in low numbers are not shown in the ordination plane. Samples at 53°30' and 53°22' N are added as supplemental samples. B and C: spatio-temporal distribution of the sample scores on the first and second axes respectively: values in between the samples are interpolated.

the sampled transect corresponds to foraminiferal compositions of samples that have a low mud content too.
Figures 6B and C highlight the relation between the time and location of sampling and the scores of the samples in the ordination plane. The first axis reflects a transition from north (low scores on first axis) to south (high scores). The scores on the second axis, more than on the first one, represent a stronger temporal gradient.

Individual taxa
Overall, we distinguished 33 taxa, mainly determined to the species level. In this section, we will focus on the temporal and spatial distribution of the 6 most abundant species: data are listed in appendix II. Figure 7 shows that there were geographical foraminiferal zones in the stations sampled. At stations that are characterized by summer stratification (53° 50' and further north), *Eggerella scabra* was the most dominant species. Further south, at the central frontal stations (53° 40'-53° 45') *Hopkinsina pacifica*, *Ammonia tepida* and, less pronounced, *Quinqueloculina* spp. were the most abundant species. *Eggerella scabra* was the only species that displayed a clear north-front preference and *Elphidium excavatum* the only species with a south-front preference. The last of the 6 most abundant species -*Stainforthia fusiformis* - did not show a clear geographical preference, but had cross-frontal temporal peaks in abundance. Temporarily, *A. tepida* and *E. scabra* are relatively abundant in the two winter months, while *E. excavatum* and *Quinqueloculina* spp. are relatively abundant in summer months. *H. pacifica* and *S. fusiformis* do not display a seasonal preference (fig 7).

The occurrences of the 6 most abundant species are also presented as relative abundances at the four different sample moments (fig 8). These graphs emphasize the change in dominance at the stations sampled and the correlation of species with specific latitudes. *Eggerella scabra* showed increasing relative abundances towards northern stations at all sample moments. Consequently, relative abundances of *Ammonia tepida* and *Elphidium excavatum* decreased towards northern stations. *Stainforthia fusiformis* on the other hand, occurred mainly in June and December.

Depth distribution in the sediment
In the previous sections, we combined all 7 vertical depth intervals per species. In fig 9 we summarized total standing stocks versus sediment depth of 4 months combining data of various stations. Minor differences in vertical distribution patterns were observed when comparing the different stations at the same sample moment, despite significant differences in environmental conditions (e.g. organic carbon content, fig 3). However, differences are visible when comparing different sample moments. In December and February, for example, the foraminiferal community lived on average deeper than in June and August. During summer months highest foraminiferal densities were observed in the upper 0.5 cm of the sediment, while during the winter months densities were more evenly distributed throughout the sediment. This shallowing or deepening of the microhabitat occurred for all taxa rather synchronously (results not shown here) and no significant difference was noted between the more muddy and sandy stations.

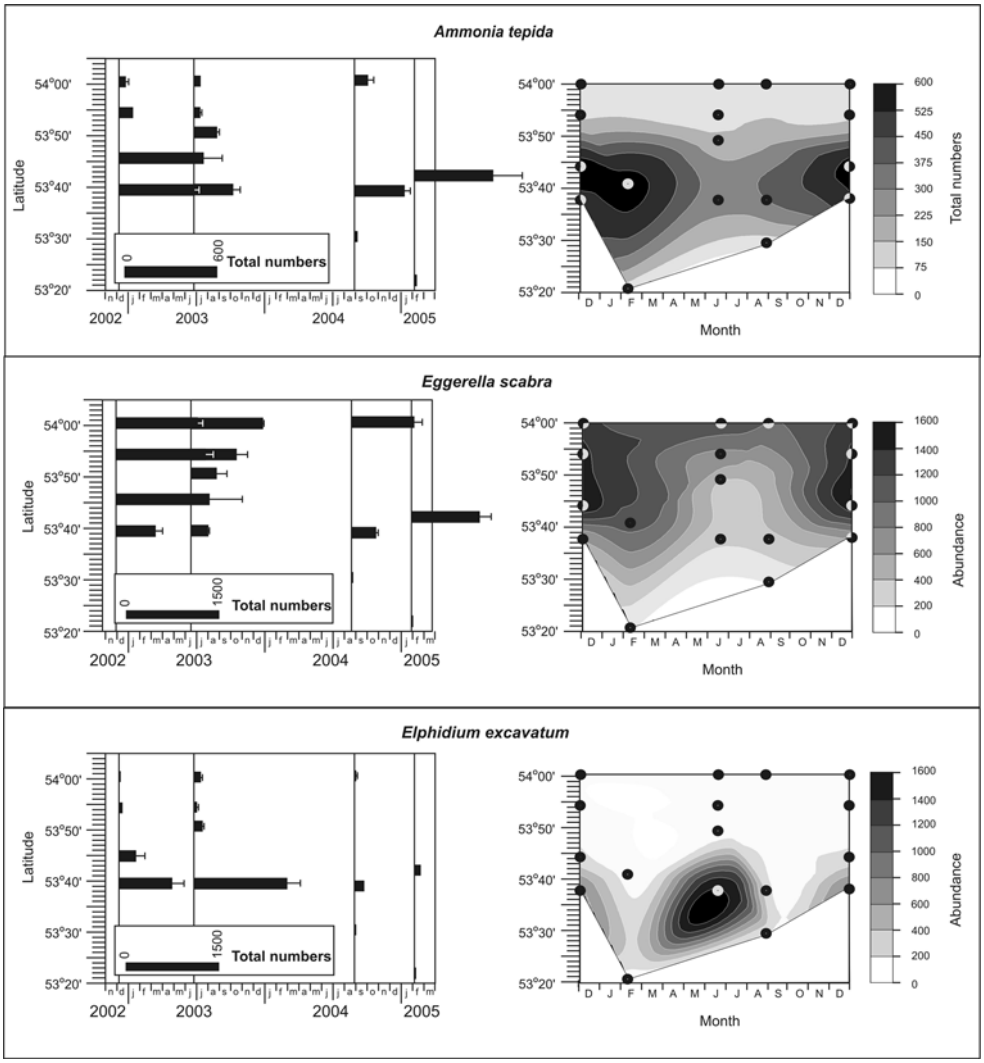
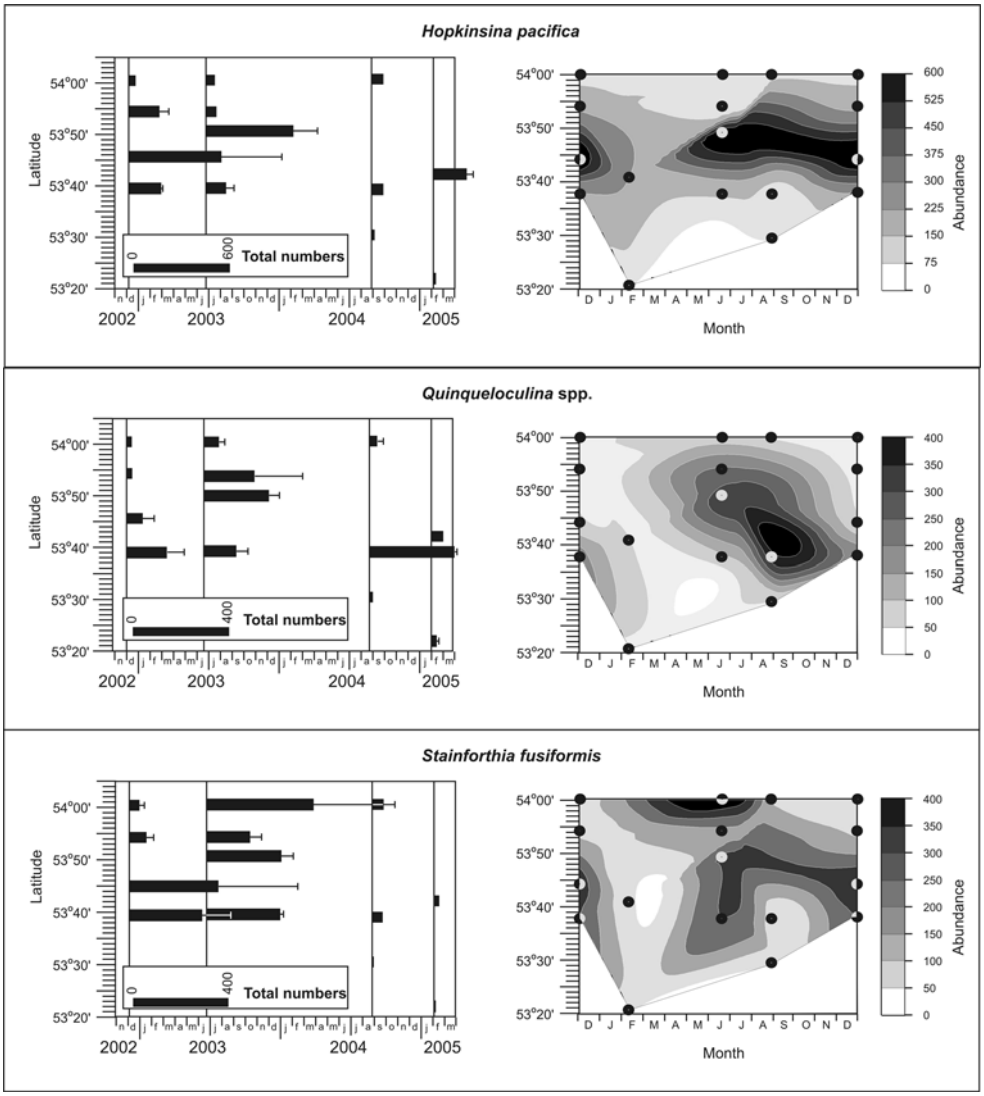


Figure 7: Total standing stocks of the six most abundant species in the upper five centimeter: values are averages of two samples (+1 SD in the bar chart).

DISCUSSION

Samples taken in this study largely consisted of dysoxic and anoxic sediment layers. The protoplasm of individuals that died at these depths will decay much slower than those in the upper, oxidized layer. Since staining samples with rose Bengal does not make it possible to distinguish between decaying and living individuals, it is argued that standing stocks in deeper, anoxic habitats are easily overestimated (Bernhard, 1988; Corliss and Emerson, 1990). Alternatives for rose Bengal are MTT (De Nooijer and others, 2006; chap-



ter 2) and CellTracker Green (Bernhard and others, 2003; 2004). Although these methods will result in more accurate determination of total standing stocks, it will hamper comparison with previous field studies. Since we found relatively low standing stocks in deeper sediment layers in summer months (fig 9), we are inclined to think that the bias caused by staining with rose Bengal is limited. Moreover, CellTracker Green and MTT do not stain those specimens that did not survive the period between sampling and incubation with the staining probe, resulting in an underestimation of the total standing stock.

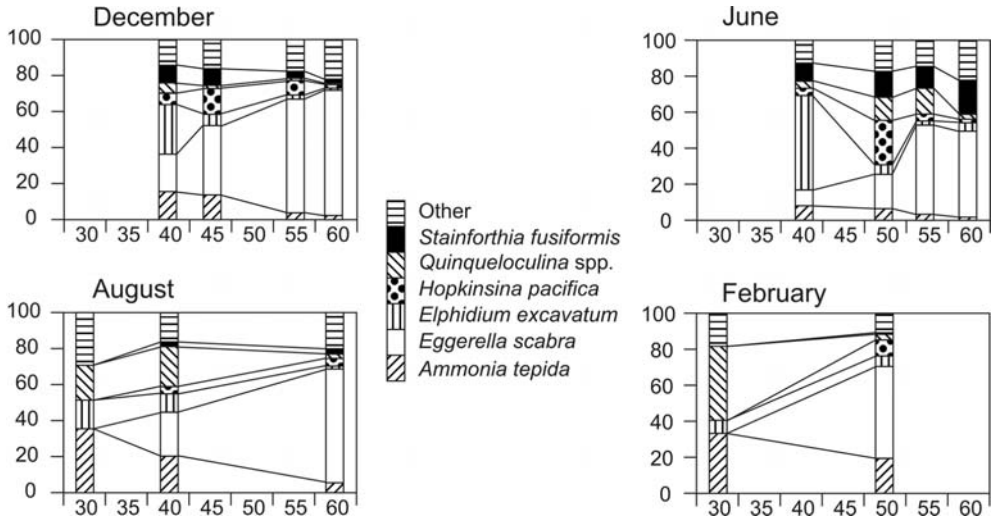


Figure 8: Relative abundances of the 6 most abundant species.

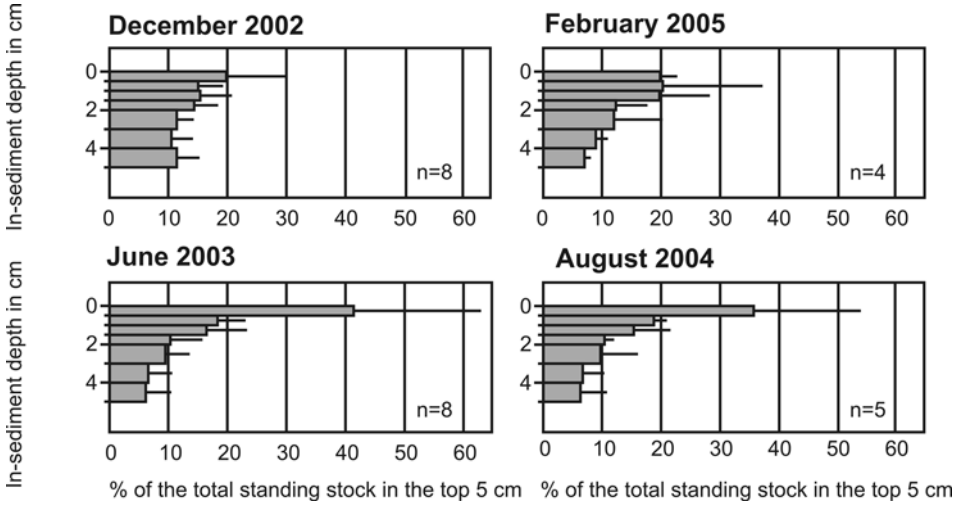


Figure 9: Average relative depth distributions in the sediment per month, stations and species combined. Values are based on averages of total numbers per depth interval (+1 SD), total number of specimens in the upper four samples are doubled to equal the volume of the other samples.

Another error in estimating abundances of benthic foraminifera can be caused by patchiness in the spatial distribution of individuals (Buzas, 1968; 1970; Murray and Alve, 2000; this thesis, chapter 3). The variability in duplicates was generally low (figs 4 and 7, left-side panels, and fig 6A), indicating that on average, abundances are representative for the stations sampled.

Environmental setting

The central position of the high concentration of total organic carbon (fig 3) fits the sedimentation of organic matter described in the literature for tidal mixing fronts (Lampitt, 1985; Cadée, 1986). Enhanced primary production at fronts (Lee and others, 2005), results in a high input of phytodetritus at the seafloor. Suspended organic carbon at the coastal side of the front sinks when current velocities drop below a critical level, somewhere along the slope of the southern North Sea. At this depth, organic matter as well as sand and clay particles are deposited (Van Haren and Joordens, 1990; Howarth, 1993; Trimmer and others, 2003). Deposition of clay is responsible for the high mud content found at the central zone of the front because the zone of sedimentation roughly coincides with the zone with primary production maxima. Organic matter strongly adsorbs to mineral surfaces (Anderson, 1988; Mayer, 1994) providing an additional explanation why mud content and total organic carbon content are strongly correlated at the Frisian Front. In our samples, the input of organic matter appeared especially high at the start of the summer (fig 3), likely a result from high phytoplankton production in spring (Lee and others, 2005). High organic carbon content at the front in December may be partly caused by the increased silt transport from the English coast, resulting from erosion of cliffs during autumn and winter (Van Raaphorst and others, 1998). As a consequence, quality of present organic carbon (i.e. labile vs. refractory) is likely to vary seasonally at the Frisian Front.

Foraminiferal community

High amounts of organic carbon at the front's center (fig 3) are positively correlated with high total foraminiferal standing stocks (fig 4) and suggest that high faunal densities are supported by elevated food availability. Phytodetritus arriving at the seafloor is mainly and rapidly consumed by bacteria (Lochte and Turley, 1988; Pfannkuche, 1993), although some studies have shown the capability of benthic foraminifera to utilize substantial amounts of this detritus too despite their relatively low biomass (Altenbach, 1992; Heinz and others, 2001; Moodley and others, 2002). It may also be that foraminifera do not feed directly on the phytodetritus, but profit from the elevated bacterial biomass.

Biodiversity is also highest at the front's center, except in February when biodiversity does not peak across the frontal zone between 53° 30' and 53° 55' (fig 5). The calculated equitability (i.e. evenness), shows that diversity at the center is partly caused by high abundances of a few species, especially in the first half year. Shannon's equitability filters out this effect, and shows that evenness is highest at, and south of the front's center, especially in the second half of the year. Summarizing, we conclude that the front's center provides favorable conditions for foraminifera, supporting high total standing stocks. Shannon's diversity of the foraminiferal community in a

similar field survey in 1988/1989 (Moodley, 1990) is also highest at the center of the Frisian Front (~2.2) compared to stations further away from the center (~1.2-2.0). In contrast to our results, diversity is reported to be higher in February than in June. Values for Shannon's equitability were similar to ours (0.43-0.91).
The reason for high diversity at the Frisian Front may be the high diversity of available organic matter, whereby different foraminiferal species feed on different food sources. Another reason could be that there are temporally separated habitats at the front's center. In winter, available food is likely to be more refractory, while in late spring labile material suddenly arrives. Later in summer, lower amounts of detritus, possibly from different sources may be deposited at the seafloor. If different species exploit different food types present and if they can survive the period between successive arrivals of their preferred food, all those species may be found at any time at these locations.
The principal component analysis further highlights the role of the position of the stations relative to the Frisian Front: spatial variations are slightly more important in explaining the variance in the foraminiferal community than temporal variability (figs 6B and C). There are however, large differences between the species' responses to different environmental variables: some species are well correlated with temporal variance, others are correlated with mud and organic carbon content. Scott and others (2003) also stressed the importance of different hydrodynamic regimes in determining the occurrences of benthic foraminiferal species and find different communities for different hydrodynamic regimes. The grain size characteristics of the stations they sampled across the Celtic Front resemble those described in this chapter. The average depth above which the Celtic Front is situated, however, is higher (50-75 meters) than that of the Frisian Front whereas organic carbon content is generally higher in the southern North Sea than in the Celtic Sea. These two differences may be responsible for the differences in species composition of the stratified assemblages: Hyalinea baltica, Bulimina marginata, Adercotryma wrighti and Nonionella turgida in the study by Scott and others (2003) versus Eggerella scabra, Bolivina spathulata and B. seminuda in our study.

Individual taxa

Temporal and spatial differences in abundances of the six most occurring species are likely to be determined by the environmental variables at work at the Frisian Front. In 1988 and 1989, a similar sampling survey was conducted by Moodley (1990): differences between these data sets are discussed in detail in chapter 6. In general, distributions of the most abundant species are similar: *Elphidium excavatum* is in both studies dominant at the stations with high input of (labile) organic matter (fig 3) and is known to be capable of consuming phytodetritus rapidly (Murray, 1991; Altenbach, 1992; chapter 8). It is also reported to withstand (temporal) anoxia (Moodley and Hess, 1992) and a combination of physical disturbance and high load of (fresh) organic material is reported to promote high densities of *E. excavatum* (Moodley, 1990; Takata and others, 2006). Our results therefore confirm that this species could be used as a proxy for eutrophic environments with relatively high physical disturbance (Filipsson and Nordberg, 2004).

Ammonia tepida is abundant in a zone around 53º 42' (fig 7) where organic carbon is relatively abundant: its abundances are also higher in winter than in summer months. It has been hypothesized repeatedly (Hohenegger, 1989; Goldstein and Corliss, 1994; Murray and Alve, 2000) that *A. tepida* is able to feed on refractory material and bacteria (see also chapter 3). In chapter 8, the distribution of *A. tepida* is hypothesized to be partly determined by its capability to withstand 'environmental variability'. At the center of the Frisian Front, conditions may be variable due to large differences in the amount of organic material arriving at the seafloor, or to different levels of physical disturbance by variability in the exact position of the hydrographic front.

Just north of the front's center, abundances of *Hopkinsina pacifica* and *Quinqueloculina* spp. are relatively high. This zone also receives occasional high amounts of fresh organic matter and it may be that peak abundances of these species are determined by the abundance of organic matter at the Frisian Front. Since high abundances of these species occur well after the arrival of phytodetritus in early summer, other factors may be equally important in determining the success of these species at the Frisian Front.
Eggerella scabra is abundant at the northernmost stations, especially in winter months, indicating that it does not depend on the availability of labile organic material. It is, however, often described to be abundant in heavily eutrofied environments (e.g. Thomas and others, 2000). Furthermore, it is described to have an infaunal microhabitat distribution (Ernst and others, 2002; 2005; Duijnstee and others, 2004), reflecting too that it does not depend on labile organic material. The absence of *E. scabra* at the southern edge of the Frisian Front may indicate that physical disturbance, or environmental variability in general, limits its distribution across the front.
The erratic abundance of *Stainforthia fusiformis* in our samples relates well to its reported opportunistic life-style. It is known to respond quickly to phytodetritus arriving at the seafloor (Gustafsson and Nordberg, 2000; 2001; Filipsson and others, 2004), to be the first recolonizer of formerly anoxic environments (Alve, 1994; 2003) and to withstand (and bloom after) prolonged periods of anoxia (Alve and Bernhard, 1995; Nordberg and others, 2000; Duijnstee and others, 2004).

Vertical distribution

Interestingly, vertical microhabitat distributions were remarkably constant over the stations and similar for most species. Since oxygen penetration in the sediment was similar in space and time (see also: Van der Zee and others, 2003) and much shallower than the depth at which most specimens lived, it once more points out the remarkable ability of these relatively large unicellular organisms to live in anoxic conditions (see also: Moodley and Hess, 1992; Bernhard and Bowser, 1999).

Vertical distributions did vary between winter and summer months. In December and February, specimens were distributed relatively evenly throughout the upper 5 centimeters of the sediment, while in June and August, 35 - 40% of the individuals were found in the top 0.5 centimeter. This suggests that most of the species do not behave truly infaunal or epifaunal, but rather switch between both behaviors during the year. High

amounts of labile organic material in summer at the front's center, and the response of foraminifera in this study to migrate to shallower depths, or preferentially reproduce here, confirm the hypothesis by Moodley (1990) that vertical distributions around the Frisian Front are mainly caused by the settlement of phytodetritus at the seafloor. Presence of the ghost shrimp *Callianassa subterranea* at the Frisian Front may partly determine the relatively even vertical distribution of foraminifera. Bioturbation and transport of specimens and organic matter into the sediment and supplying the sediment with oxygen, may allow foraminifera to live at greater depths. Since we carefully avoided burrows in sampling for foraminifera, additional effects of burrowing organisms at the Frisian Front on the vertical distribution of foraminifera remain to be quantified (see also this thesis, chapter 6).

A series of experiments with foraminifera from the northern Adriatic Sea have shown that vertical distributions of foraminifera are determined by either food or oxygen availability (Duijnstee and others, 2003; Ernst and others, 2002; 2005). *Eggerella scabra*, *Caronia sylvestrii* and *Acostata mariae* responded to the arrival of food by migrating upwards but did not so during declining oxygen concentrations. *Nonionella turgida*, *Hopkinsina pacifica* and *Stainforthia fusiformis*, however, responded to declining oxygen levels rather than to extra food by moving to the sediment-water interface. Since in the field these two parameters are usually correlated, it is often difficult to distinguish between the two effects. Our results suggest that at the Frisian Front, it is mainly the settlement of (labile) organic material that determines the vertical distribution of benthic foraminifera in the southern North Sea.

This study reveals that within a short bathymetrical range along the frontal slope of the Frisian Front, abundances and community structure of living benthic foraminifera show sharp gradients. This indicates that shifts in fossil species composition do not necessarily reflect paleobathymetrical changes, as often inferred, but may be related to shifts in hydrodynamic regimes.

TAXONOMIC REMARKS

In this study we followed the taxonomy described in Barmawidjaja and others (1992), which is largely based on the taxonomy of Von Daniels (1970) and Jorissen (1987, 1988). Three species have been renamed: *Reophax nana* has been more appropriately described as *Acostata mariae* (Brönnimann and others, 1992). *Reophax scottii* is currently assigned to the genus of *Leptohalysis* and *Morulaeplecta bulbosa* is here described as *Caronia sylvestrii*. Also, some taxa have been lumped for practical reasons: individuals of *Textularia* and a number of scarce genera were often difficult to determine down to the species level (especially small specimens). *Quinqueloculina* spp. consisted mainly of *Q. seminulum*.

CHAPTER 5

FORAMINIFERAL STABILITY AFTER A BENTHIC MACROFAUNAL REGIME SHIFT AT THE FRISIAN FRONT (SOUTHERN NORTH SEA)

with T Amaro, IAP Duijnstee, GCA Duineveld and GJ van der Zwaan

ABSTRACT
Stations across a tidal mixing front in the southern North Sea (the Frisian Front) were sampled between 1982 and 2005. Macrofaunal and foraminiferal abundances were determined and compared with weather variables recorded at the North Sea. Results revealed that species composition of the macrobenthic community between 1992 and 1995 from heavily dominated by the suspension-feeding echinoderm *Amphiura filiformis* to a community dominated by the burrowing, deposit-feeding crustacean *Callianassa subterranea*. The ultimate cause of this benthic regime shift may be frequent and relatively long periods of increased wind stress. The resulting increased suspension of fine-grained material at the front's seabed may have hampered *A. filiformis* in its feeding behavior. After their decline, increased abundances of *C. subterranea* may have prevented the return of brittle stars by the shrimp's positive effect on resuspension of sediment and by direct competition for space. Despite effects of the benthic regime shift on biogeochemical cycling and sedimentary dynamics, and the transition from a filer-feeder dominated to a burrowing suspension-feeder dominated system, the foraminiferal community remained relatively stable during the macrofaunal shift. Vertical, in-sediment distribution of foraminifera shifted slightly towards shallower sediment layers, contrary to what may be expected by the positive effect of *C. subterranea* on oxygen penetration depths. The stability of the foraminiferal species composition during the macrofaunal shift at the Frisian Front implies that benthic foraminifera primarily reflect conditions defined by the presence of the hydrodynamic front, while the role of macrobenthic activity on their habitat (i.e. altered geochemical conditions) seems limited.

INTRODUCTION

Species compositions of communities are constantly changing. These changes occur gradually due to internal dynamics (e.g. predator-prey interactions) or gradual environmental changes. Abrupt transitions of key variables between quasi-stable states of a community also occur and are termed *regime shifts* (Mantua, 2004). Regime shifts are often caused by strong external forcings, although gradual changes in environmental variables can also

result in sudden shifts. The latter occurs when conditions reach a critical threshold and species composition and/or functioning of a community changes rapidly (Scheffer and Carpenter, 2003). Occasionally, such shifts in the state of an ecosystem or community may not simply be reversible by reversing the environmental change. This is attributed to the existence of different attractors for that ecosystem, which is then said to have alternative stable states (Noy-Meir, 1975; Petraitis and Latham, 1999; Scheffer and others, 2001).
Marine examples of regime shifts are scarce (Petraitis and Dudgeon, 2004; Scheffer and Van Nes, 2004) compared to the number described for terrestrial and lake ecosystems. Some examples from the North Sea have shown shifts on decadal scales in the community structure of decapods (Lindley and others, 1993; Beaugrand, 2003) and phytoplankton (Edwards and others, 2002; Beaugrand and Reid, 2003; Beaugrand, 2004). Although data sets for benthic biota usually span less time, changes in community structure are also reported after changes in competition and predation (Rhoads and Young, 1970; Weinberg, 1984), sediment stability (Probert, 1984), sedimentation rate (Aller and Stupakoff, 1996) and oxygen concentration (Levin and others, 1991).
In this paper, we review a number of studies that describe benthic community structures across a hydrodynamic front in the southern North Sea and compare it with our foraminiferal study of the area (chapter 4). Macrofaunal and foraminiferal abundances are available from surveys undertaken between 1982 and 2005. We hypothesize that the soft-bottom macrofaunal community in the southern North Sea is more sensitive to environmental perturbations than the foraminiferal community.

METHODS

Area description

The data we review in this paper consist of samples collected from seafloor stations in the southern North Sea, located across a tidal mixing front called the Frisian Front. This front is located at the maximum depth at which the water column is mixed by tidal wave action (Creutzberg and Postma, 1979; Van Haren and Joordens, 1990) and is thus positioned in an east-west direction, parallel to the Dutch coast, roughly between the isobaths of 30 and 40 meters (see chapter 4 for a more extensive description of this area). North of the Frisian Front, the water column is thermally stratified in spring and summer, while south of the front it is mixed by tidal wave action throughout the year. Around the front, light penetration is usually limited due to input of silt from the UK coast and resuspension of sediment. When sedimentation of silt or supply of fine-grained material decreases periodically, optimal availability of nutrients causes increased phytoplankton productivity in spring and summer. This, in turn, causes an increased flux of organic matter to the seafloor at the center of the front (Holligan, 1981; Postma, 1988).

Sampling for macrobenthos

Stations across the Frisian Front were sampled for macrofauna from 1982 until 1986 (Duineveld and others, 1987; see also for details on sampling procedures). From 1990 to 1994 and in 1997, the center of the Frisian Front (i.e. at 53° 42' N for the 4° 30' meridian) was sampled and from 1999 to 2002, a number of stations across the Frisian Front were sampled in different months (Amaro, 2005; fig 1).

Sampling for foraminifera

In June 1988 and February 1989 the area was sampled for benthic foraminifera by Moodley (1990; see also for details on sampling procedures). Stations were chosen so that a variety of hydrodynamic regimes (mixed, frontal and stratified) were included (fig 1). The second sampling survey was conducted between 2002 to 2005 and included roughly the same stations, but with a higher temporal resolution (fig 1) and is described in further detail in this thesis, chapter 4.

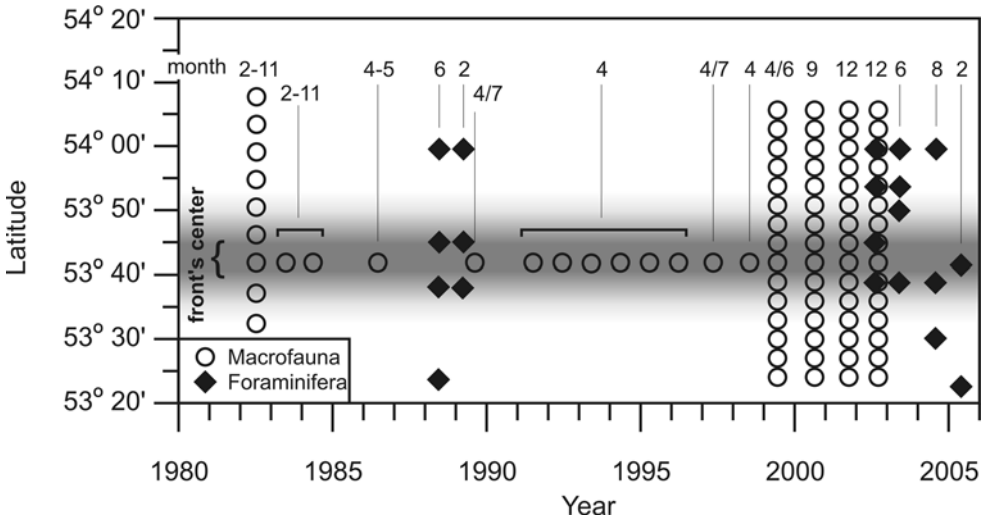


Figure 1: Sampling surveys for macrofauna and foraminifera across the Frisian Front at 4° 30', between 1982 and 2005. The central zone of the benthic front is positioned between 53° 40' and 53° 45'.

Taxonomic remarks

For foraminiferal analyses, taxonomic descriptions were based on different sources: Moodley's taxonomy (1990) is based on Gabel (1971) and Murray (1971; 1979), while de Nooijer followed Von Daniels (1970), Jorissen (1987; 1988) and Barmawidjaja (1992). The discrepancies between the taxonomic sources was overcome by renaming species and occasionally lumping species from both publications in genus groups: table 1.

Statistical analysis

To compare changes in the foraminiferal and macrofaunal data sets, Bray-Curtis similarities were calculated for all possible foraminiferal and macrofaunal sample pairs (Bray and Curtis, 1957; Clarke and others, 2006; equation 1):

$$D^{B\text{-}C} = 100 \cdot \Sigma_i \mid y_{i1} - y_{i2} \mid / \Sigma_i (y_{i1} + y_{i2}) \qquad (1)$$

this paper	de Nooijer	Moodley
Acostata mariae	*Acostata mariae*	-
Ammonia tepida	*Ammonia tepida*	*Ammonia beccarii*
Ammodiscus sp.	*Ammodiscus* sp.	-
Asterigerinata mamilla	*Asterigerinata mamilla*	*Asterigerinata mamilla*
Bolivina speudoplicata	*Bolivina pseudoplicata*	*Bolivina pseudoplicata*
Bolivina spp.	*Bolivina dilatata* + *B. seminuda* + *B. spathulata*	*Bolivina skagerrakensis* + *Brizalina pseudopunctata* + *B. variabilis*
Bulimina spp.	*Bulimina marginata/ elongata*	*Bulimina gibba/ elongata*
Buliminella elegantissima	*Buliminella elegantissima*	*Buliminella elegantissima*
Caronia sylvestrii	*Caronia sylvestrii*	-
Cassidulina spp.	*Cassidulina* sp.	*Cassidilunoides* sp.
Clavulina spp.	-	*Clavulina* sp.
Dentalina sp.	*Dentalina* sp.	-
Eggerella scabra	*Eggerella scabra*	*Eggerella scabra*
Elphidium spp.	*Elphidium advenum* + *E. excavatum*	*Elphidum articulatum* + *E. earlandi* + *E. magellanicum* + *E. excavatum* + *E.* sp.
Epistominella vitrea	*Epistominella vitrea*	*Epistominella vitrea*
Fissurina sp.	*Fissurina* sp.	*Fissurina* sp.
Haynesina germanica	*Haynesina germanica*	-
Hopkinsina pacifica	*Hopkinsina pacifica*	*Spiroloxostoma* sp.
Hyalinea baltica	*Hyalinea baltica*	-
Jadammina sp.	-	*Jadammina* sp.
Lagena sp.	*Lagena* sp.	*Lagena* sp.
Lenticulina sp.	*Lenticulina* sp.	-
Leptohalysis scotii	*Leptohalysis scotii*	-
Nonion sp.	*Nonion depressulus*	*Nonion* sp.
Nonionella sp.	*Nonionella turgida*	*Nonionella* sp.
Pyrgo spp.	*Pyrgo* sp.	*Pyrgo williamsoni*
Quinqueloculina spp.	*Quinqueloculina* sp.	*Quinqueloculina cliarensis* + *Q. oblonga* + *Q. seminulum*
Reophax monoliformis	*Reophax monoliformis*	-
Rosalina sp.	*Rosalina* sp.	*Rosalina* sp.
Saccamina spp.	*Saccamina* sp.	*Reophax fusiformis* + *Psammosphera fusca*
Stainforthia fusiformis	*Stainforthia fusiformis*	*Fursenkoina fusiformis*
Textularia sp.	*Textularia* sp.	*Textularia* sp.

where y_{i1} is the absolute abundance of species i in sample 1. Rather than using each sample individually, meio- and macrofaunal samples were grouped into four categories based on different hydrographic regimes across the front. The position of the hydrographic Frisian Front may not be static in time (Hill and others, 1993), although the position of the benthic front (i.e. the location of the sedimentary zone with a high mud content) is relatively stable. Therefore, we chose to classify the benthic samples according to the depth at which the samples were taken, representing the average hydrodynamic regimes found across the tidal mixing front: table 2.

group	depth (m)	latitude(s)	n (macrofauna)	n (foraminifera)
seasonally stratified	45	54° 00'	2	6
frontal	39-41	53° 42' - 53° 45'	4	4
south frontal	37	53° 39'	4	6
mixed	28-31	53° 22' - 53° 30'	2	3

Table 2: Four groups of samples (macro- and meiofauna) for which similarity indices are calculated.

Since in 1988/1989 stations were sampled in February and June, only samples from those months in the 2002-2005 data set are used to make a fair comparison possible.

Climate forcing

To analyze the possible influence of climate parameters on faunal community structure, daily wind speeds that are recorded at a weather station in the southern North Sea (K13; at 53° 21' N and 3° 22' E) are taken from the web site of the Royal Dutch Meteorological Institute: www.knmi.nl/samenw/hydra. Daily recorded wind speeds are used to calculate monthly average wind speeds from 1980 to 2000.

Table 1 (facing page): Taxonomic comparison between the data sets used.

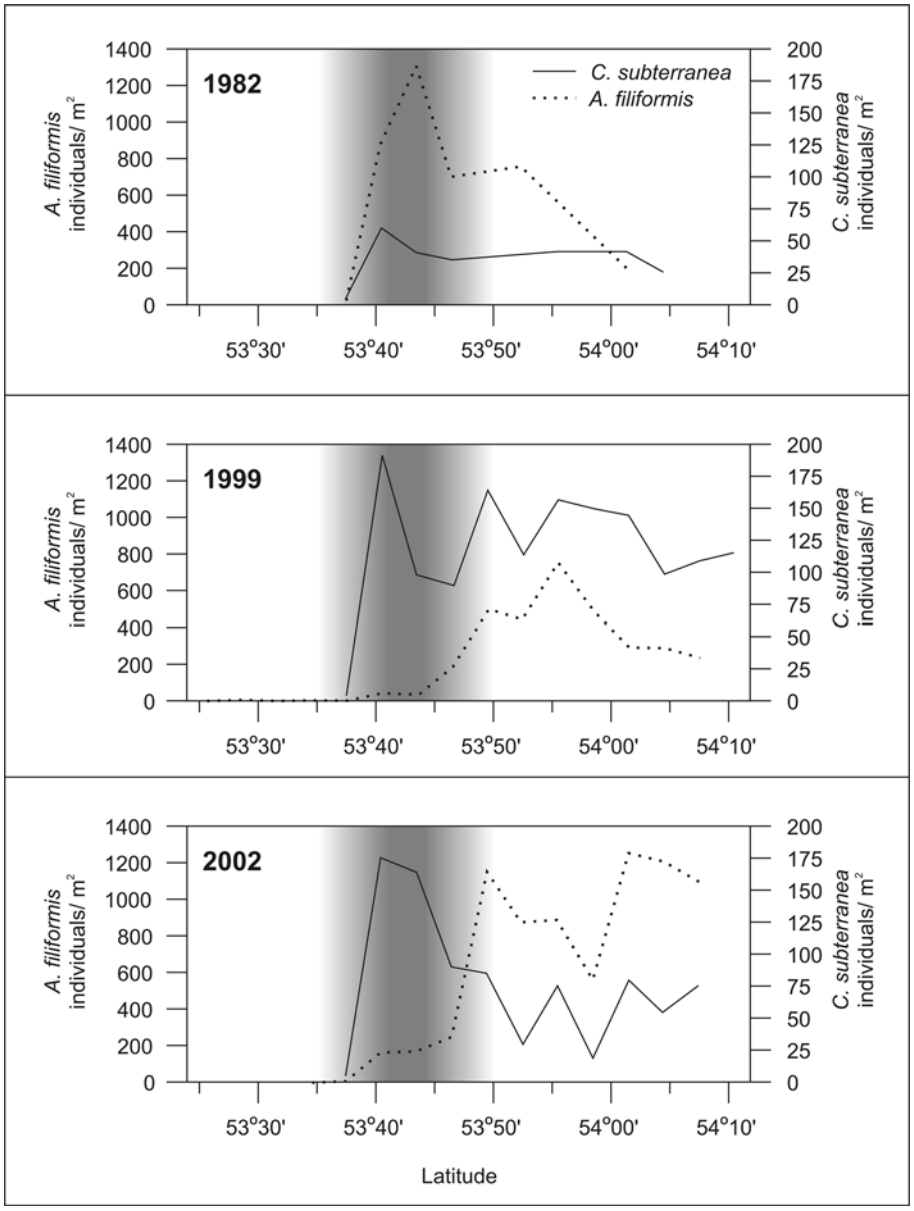


Figure 2: Abundances of *Amphiura filiformis* and *Callianassa subterranea* in 1982, 1999 and 2002 across the Frisian Front (Amaro, 2005). Shaded area: central zone of the benthic front.

RESULTS

Macrofauna

The macrofaunal community was heavily dominated by two species: the ghost shrimp *Callianassa subterranea* and the brittle star *Amphiura filiformis*. Between 1982 and 1999 the benthic fauna of the Frisian Front shifted from an *A. filiformis*-dominated to a *C. subterranea*-dominated community (fig 2).

The shift is most pronounced at the center of the benthic front and the exact timing of the shift is determined by analyzing data only from stations at the center of the front (fig 3).

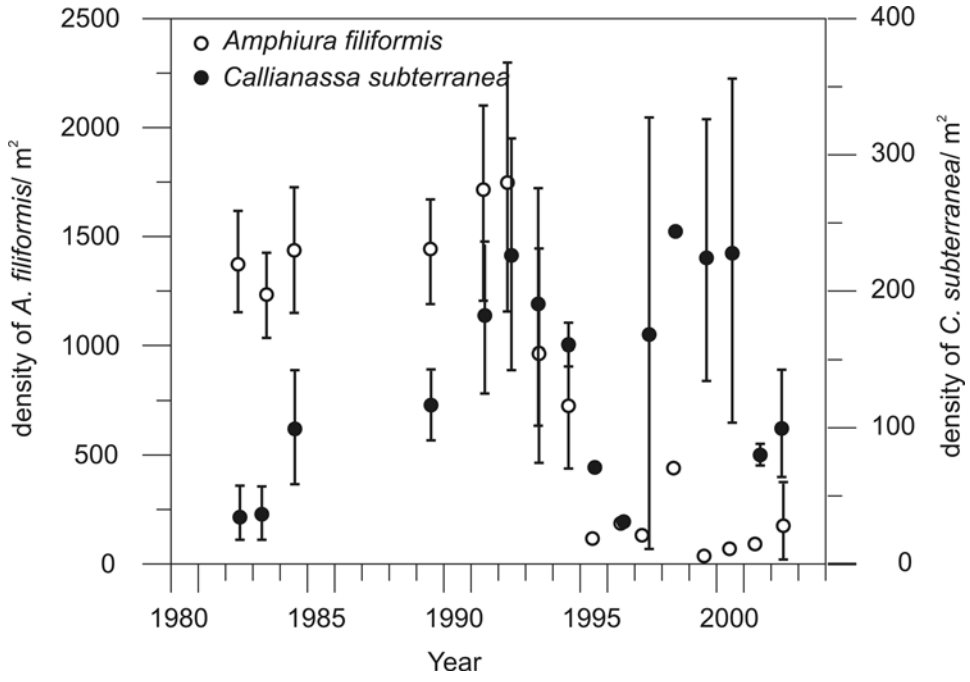


Figure 3: The two dominant macrofaunal species at the center of the benthic Frisian Front between 1982 and 2002 (Amaro, 2005).

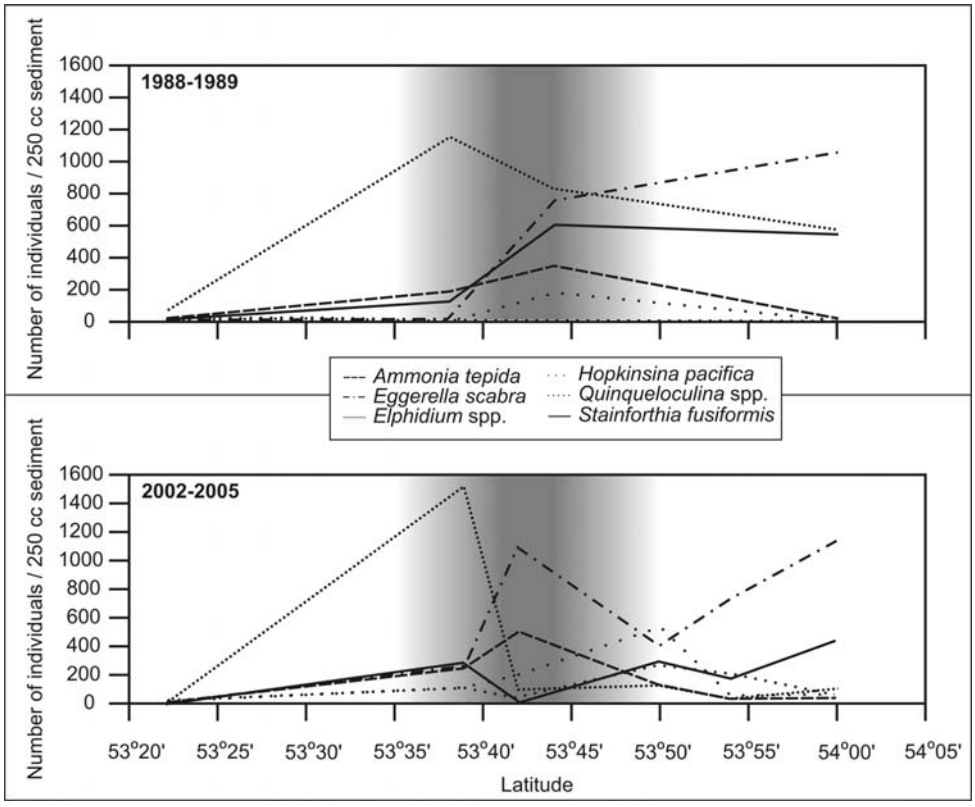


Figure 4: Average densities of the 6 most occurring species of benthic foraminifera across the Frisian Front in 1988-1989 (n=7; Moodley, 1990) and in 2002-2005 (n=11; this thesis, chapter 4).

Foraminifera

Densities in February and June 2002-2005 and in February and June 1988/1989 are similar for most foraminiferal species. Note that the spatial sampling resolution in 2002-2005 was higher than in 1988/1989, explaining the more erratic appearance of species in 2002-2005 (e.g. *Eggerella scabra*; fig 4, bottom panel). Generally, in both data sets, *Elphidium excavatum* dominates the south edge of the benthic front, *Ammonia tepida* and *E. scabra* are dominant at the center of the front, whereas in the stratified waters north of the front only *E. scabra* dominates.

For both foraminiferal data sets, abundances in the top 5 centimeters of the sediment were enumerated. Despite differences between species, specimens are generally distrib-

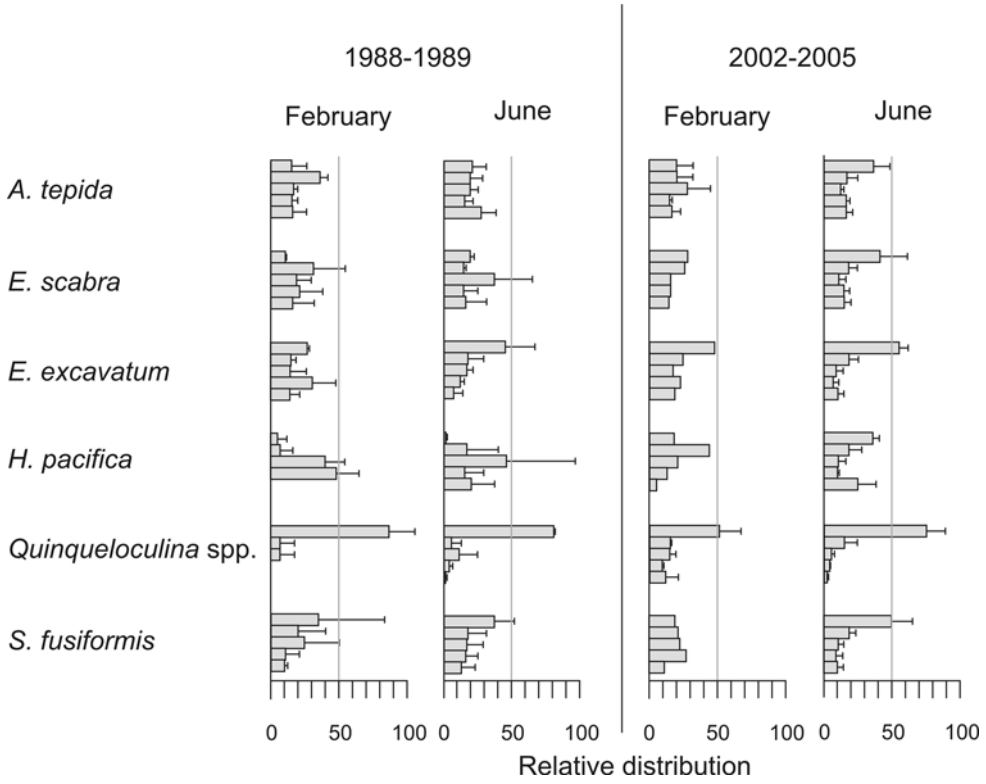


Figure 5: Relative in-sediment distribution of the key species of foraminifera in the upper 5 cm (+ 1 SD) in 1988/1989 (left) and 2002-2005 (right). Abundances of all stations at a sample moment are combined.

uted near the sediment-water interface in June and on average more evenly distributed in the sediment in February (fig 5).

Similarities within and between the data sets

Similarity indices for most sample-pairs in the foraminiferal data set were generally low and on average high for the macrofaunal samples (fig 6). In the former, exceptions were pairs from the same location, sampled at different years (fig 6: left, shaded area). In the macrofaunal data set, similarities were not higher among pairs from the same location that were sampled in different years.

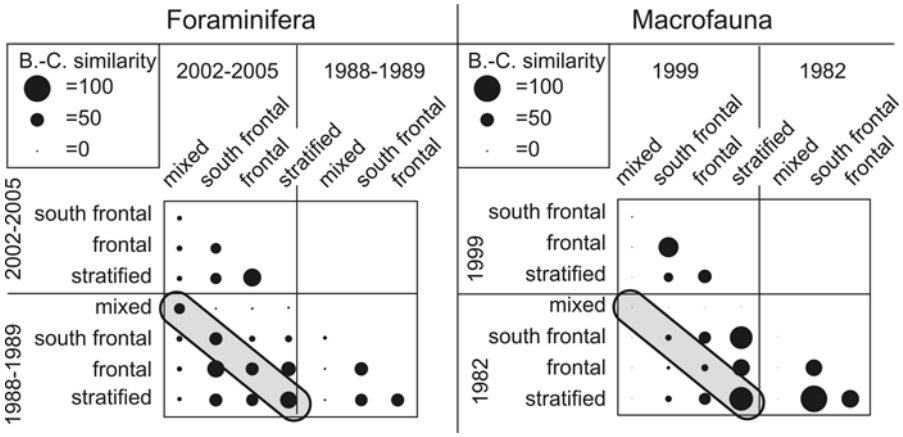


Figure 6: Similarity (Bray-Curtis) for foraminifera (left) and macrofauna (right). Shaded area in plots represents sample-pairs from the same location, and sampled at different years.

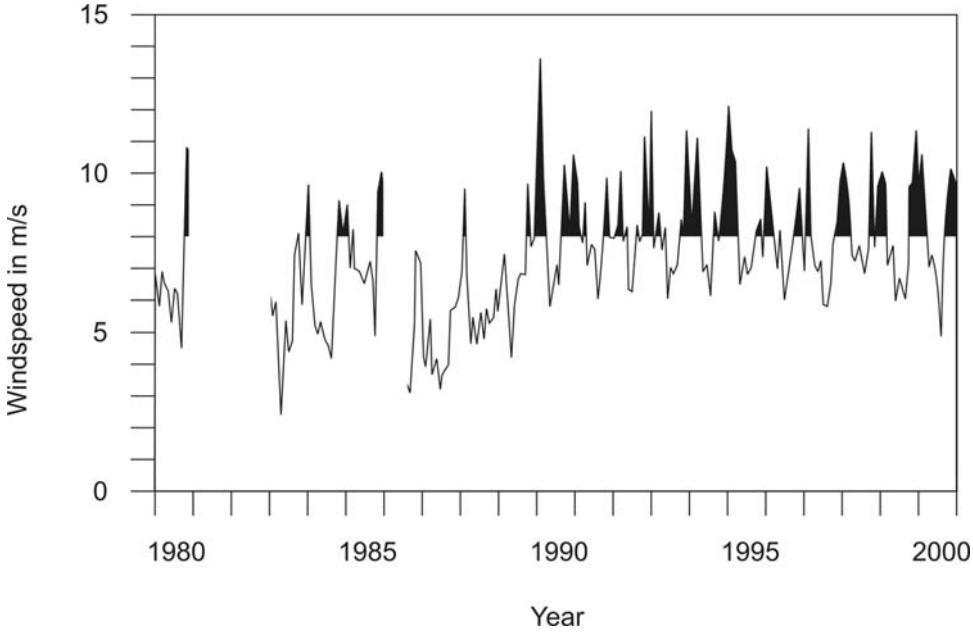


Figure 7: Monthly average of daily wind speeds measured at weather station K13 between 1980 and 2000.

Climate parameters

Monthly average wind speeds in the southern North Sea show typical high winter peaks. Before the winter of 1989/1990, wind speeds were relatively low (fig 7), while more months with relatively high wind speeds (high and broad peaks) occurred in the winters of 1990/1991, 1992/1993, 1993/1994 and 1994/1995. Until 2000, monthly average wind speeds were only slightly lower again.

DISCUSSION AND CONCLUSIONS

The macrobenthic regime shift

Although detection of regime shifts may be statistically difficult (e.g. Rudnick and Davis, 2003), the shift in dominance from *Amphiura filiformis* to *Callianassa subterranea* at the Frisian Front between 1992 and 1995 appears to be a genuine regime shift. It can be classified as such since the benthic community was stable, brittle star-dominated from at least the early 1980s until the start of the 1990s (Duineveld and others, 1991), shifted suddenly (within a few years) in its composition and functioning by becoming ghost shrimp-dominated around 1995, and remaining so until 2005. In a station at the German Bight (depth 42 meters), sampled yearly between 1980 and 2000, abundances of *A. filiformis* also dropped suddenly around 1991 (Schroeder, 2003). In addition, numbers of the bivalve *Mysella bidentata* also decreased sharply during the early 1990s. Decreasing numbers of these filter feeders in a similar environment may indicate that the regime shift at the Frisian Front is not a local phenomena, but may be caused by climate or wide-scale hydrographic forcings.

Possible forcings and consequences

Ecosystems and communities can shift rapidly in composition due to internal, stochastic processes (Ellner and Turchin, 1995; Vandermeer and Yodzis, 1999; Hsieh and others, 2005). Alternatively, the described macrobenthic regime shift can be caused by environmental forcings, of which three types will be briefly discussed (fig 8). Although in nature, many environmental variables together influence the functioning and composition of an ecosystem or community, here we treat regime shifts as if they are caused by only one variable. In the first scenario, ecosystem change simply follows a permanent change in an environmental parameter ('Direct response'). Another possibility is that a regime shift is caused by a gradual change in an environmental parameter ('Threshold response'). In such a scenario, an ecosystem suddenly changes when an environmental threshold is reached, and the old state is no longer sustainable or overtaken by another. In the last model ('Perturbation response'), an ecosystem shifts as the result of a change in an environmental parameter, but is not reversible by a return to the original environment and is therefore said to have two stable states (fig 8).

The ultimate cause of the discussed regime shift is unknown and may include contributions of many environmental and biological parameters. Also, we do not know which of the proposed mechanisms is responsible for the shift. Substrate characteristics (median grain size and organic carbon content) have remained relatively stable during the regime shift (compare Creutzberg and Postma, 1979; Van Haren and Joordens, 1990;

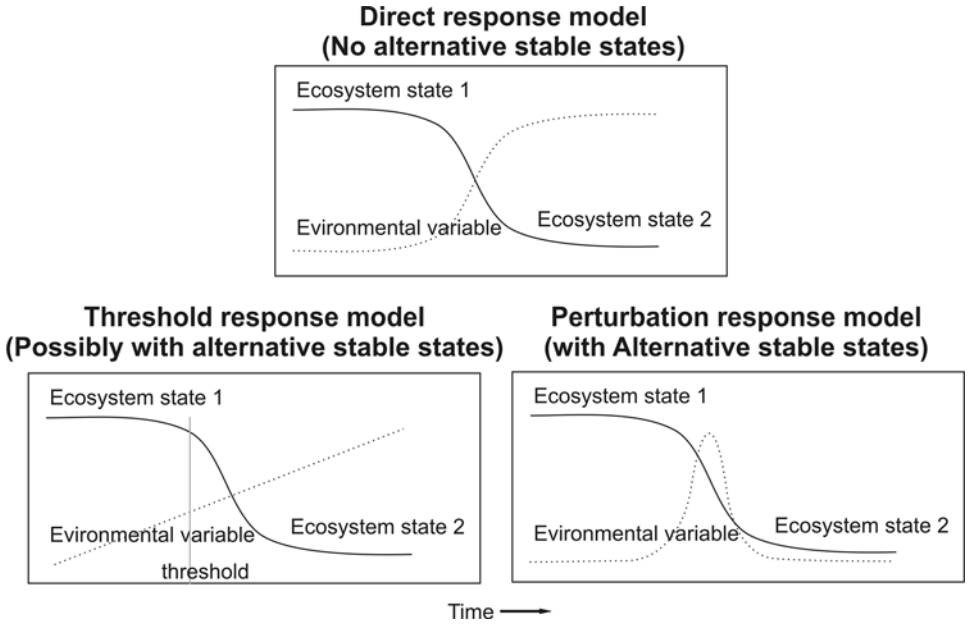


Figure 8: Models by which the macrofaunal regime shift could have occurred.

Van der Zee and others, 2003). This indicates that either these parameters do not determine to a large extent the ecosystem state or that the regime shift did not happen according to the direct response or threshold response model. Alternatively, the occurrence of a relatively short perturbation at the Frisian Front, may have caused the regime shift.
It may be that in a relatively short period, turbidity and possibly resuspension of sediment at the Frisian Front increased due to trawling or a sequence of storms. Such high amounts of suspended fine material may have hampered the filter feeding brittle stars in their food uptake. Juveniles of *A. filiformis* may be more vulnerable to high turbidity than adults, and measurements on the body size showed that juvenile numbers decreased around 1990. The long life-span of this species caused abundances to remain relatively high until 1993 (Amaro, 2005). Monthly average wind speeds were relatively high in the period before, during and after the regime shift (fig 7), possibly responsible for increased turbidity at the Frisian Front and thereby contributing to the observed benthic regime shift. If stronger winds were the main trigger for the macrofaunal regime shift, their perseverance may also be stopping *Amphiura filiformis* from regaining its dominant position in the sense of the direct response model (fig 8).
After 1992, lower abundances of *Amphiura filiformis* may have left much organic matter arriving at the Frisian Front's seafloor unconsumed, which in turn, may have positively influenced the colonization of this area by *Callianassa subterranea*. Individuals of *C. subterranea* make complex burrowing systems that cause increased total oxygen uptake by the sediment (Dobbs and Guckert, 1988; Witbaard and Duineveld, 1989; Forster and

Graf, 1995). Although individuals of *A. filiformis* can play a major role in the O_2-flux from water to sediment, in comparison with *C. subterranea*, its contribution is restricted by the relatively shallow (6-10 cm deep) burrows they produce (Ockelmann and Muus, 1978; Solan and Kennedy, 2003; Vopel and others, 2003). Although oxygen penetration was not determined consistently (since we avoided burrows during O_2-profile measurements), we believe that oxygen penetration depth and total oxygen uptake of the sediment must have increased after the macrobenthic regime shift at the Frisian Front. This, in turn, would have promoted microbial biomass and increased benthic respiration rates, thus altering the ecosystem's biogeochemical cycling.
Also, since *Callianassa subterranea* has profound effects on sediment turnover rates (Witbaard and Duineveld, 1989; Rowden and Jones, 1993), its current dominance is likely to have increased these rates and downward transportation of Chl-*a* (Boon and Duineveld, 1998), and hence of organic matter. The decreasing amounts of silt at the Frisian Front in the late 1990s (Daan and Mulder, 2005) may be (partly) caused by increasing activity of *C. subterranea*. After colonizing the Frisian Front's seafloor, this may have increased the amount of suspended matter near the seafloor at the center of the front, not only rapidly transporting organic matter and increasing benthic-pelagic exchange of particles, but possibly also preventing the return of *Amphiura filiformis* through direct competition for space (Wilson, 1990), thereby stabilizing the new macrofaunal species composition. If this is the case, the shift in macrobenthic community structure may be an example of the existence of alternative stable states in the southern North Sea (fig 8).

Stability in the foraminiferal community

Surprisingly, the shift in dominance from filter feeders to a burrowing species at the Frisian Front did not affect the foraminiferal community structure very much, nor did it influence absolute abundances (fig 4). It is mainly the densities of the well-known opportunist Stainforthia fusiformis (high in 1988/1989) that are responsible for differences between the two sample moments. Additionally, relative abundances of Elphidium excavatum decreased at the center of the front. Similarity indices for the foraminifera indicate that between-sample similarities are usually low (fig 6). Exceptions are sample-pairs that are taken at the same latitude but in different years, confirming the relative stability of the foraminiferal community during the macrobenthic regime shift at the Frisian Front between 1988 and 2005.
The increased oxygen supply to deeper sediment layers after the regime shift did not appear to have increased the average living depth of the foraminifera. In 2002-2005, specimens were distributed more evenly throughout the sediment in February, than in June, when for the six most abundant taxa more specimens were found in the top centimeter. In the 1988/1989 data set, this difference between the seasons was observed as well (fig 5). Remarkably, there is no clear evidence of microhabitat separation as often observed in deeper water and muddy stations (see discussion on TROX models: Jorissen and others, 1995; Van der Zwaan and others, 1999). In view of the macrofaunal regime shift, it is surprising that the response in species composition of foraminifera was so low. Despite apparent changes in physical disturbance, increased deep bioturbation, bioirrigation of oxygen and burial of food deep into the sediment, relative abundances

of the most occurring species remained relatively stable. The differences between the data sets, could have been partly caused by patchiness of foraminifera, that is likely to affect absolute abundances rather than relative abundances (this thesis, chapter 3). Unfortunately, the 1988/1989 data did not consist of replicate samples, making it impossible to investigate differences in small-scale, spatial distributions in benthic foraminifera at the Frisian Front.

In a recent paper, Meysman and others (2006) suggest that bioturbating macrofauna structures (subsurface) ecosystems. They convincingly show that bioturbating species act as ecosystem engineers, thereby determining to a large extent the meio- and microfaunal community composition. However, our results show that foraminifera do not seem to be affected by the changed bioturbation regime at the Frisian Front. Apparently, the changed oxygen penetration or other factors affected by bioturbation did not influence foraminiferal abundances before and after the macrobenthic regime shift.
The stability of the foraminiferal community during the macrofaunal regime shift has an important consequence for using foraminifera as paleoenvironmental proxies. Our results suggest that the apparent decoupled dynamics of macro- and meiofauna implies that foraminiferal community structure reflects the hydrodynamic environment (stratified, frontal, mixed), despite varying geochemical conditions and irrespective of the composition of the macrobenthos. On the other hand, the environmental factor that triggered (if not sustained) the macrofaunal regime shift is not reflected in the foraminiferal record.

CHAPTER 6

SUBRECENT ECOLOGICAL CHANGES IN FORAMINIFERA FROM THE WESTERN WADDEN SEA, THE NETHERLANDS

with IAP Duijnstee, HC de Stigter and GJ van der Zwaan

ABSTRACT
A 2.8 meter long core from a shallow, tide-dominated bay in the Wadden Sea (the Netherlands) was analyzed for grain size, total organic carbon content and benthic foraminiferal community structure. Sedimentation rate at this site was very high (1.56 cm/yr), allowing high-resolution reconstruction of the history of the Dutch coastal environment from 1820 to 2000. Large parts of the core consisted of alternating sand and mud laminae: the sands are likely to be of eolian origin, transported during storms from dunes surrounding the bay, while silt and clay are likely to be transported into the bay by tidal energy. The low-diversity foraminiferal community shifted around 1930 from *Elphidium excavatum*-dominated to *Haynesina germanica*-dominated. The timing of the shift in dominance suggests that the construction of a nearby coastal defense structure (the Afsluitdijk) in 1932 altered the shallow-water environment of Mok Bay by increasing variability in temperature and salinity.

INTRODUCTION

Human activity has been affecting the environment and global climate increasingly with ongoing industrial development (Levitus and others, 2001; Barnett and others, 2001; 2005; Beman, 2005). Prospects for the Earth's climate indicate that anthropogenic alterations will continue to increase levels of carbon dioxide in the atmosphere, further rising temperatures and melting of land ice, resulting in higher sea-levels (e.g. Overpeck and others, 2006). To predict the contribution of human activity on ecosystems in the near future, the relation between different kinds of pollution and natural, climatic variability in ecosystem functioning need to be quantified. The subrecent (i.e. centennial) history of human-influenced environments may provide insight in the interaction that natural variability and ongoing human activity have on ecosystem functioning.

Coastal areas are among the most heavily influenced environments: not only are they directly affected by rising sea levels, rivers also deliver sewage waste, artificial fertilizers, heavy metals and pesticides into marine ecosystems. Wherever coastal ecosys-

tems are monitored through time, severe effects of human pollution have been recorded, including local extinction of crustaceans (Wittmann, 2001), changes in phytoplankton species composition (Lotze and Milewski, 2004), and shifts from carnivore to deposit feeders' biomass (Beukema, 1991). To investigate the combined effects of eutrophication and other human interventions in coastal ecosystems on longer timescales, datasets spanning centuries should be analyzed. Since pre-1950 datasets of biological and/or geochemical records are rare, insights in interactions between human development and coastal environments on longer timescales are best based on fossil records.

The Dutch Wadden Sea is potentially a suitable study area to quantify long-term effects of anthropogenic pollution (Wolff, 2005): it is a large, tide-dominated, back-barrier system that receives input from two large rivers directly, the IJssel and Ems, and indirectly from the Rhine and Meuse. Unfortunately, as with most back-barrier and estuarine systems, sediment dynamics in the Wadden Sea are so high that a continuous sub-recent history is rarely recorded: i.e. there are hardly locations that have a net sedimentation rate for a relatively long period (Oost and De Boer, 1994; Vos and others, 2000; Andersen and others, 2006). The Mok Bay in the western Wadden Sea, however, experienced a constant net sedimentation over the last centuries and its fossil record thus provides an excellent opportunity to study centennial changes in a coastal ecosystem.

In this paper we report on high-resolution foraminiferal and sedimentological data from a laminated core taken in the Mok Bay (western Dutch Wadden Sea), covering the last 180 years in which effects of ongoing eutrophication and other human modifications of the marine environment are recorded. Foraminiferal and sedimentological patterns are discussed in relation to historical data on construction of coastal defense structures, bivalve fisheries and intensified eutrophication in the 1960s and 1970s.

METHODS

Area description

In June 2001, a gravity core (MOK2001, 53º 00'20" N and 4º 45'30" E) was taken in Mok Bay, a tide-dominated bay at the south of the island of Texel, The Netherlands (fig 1). The bay formed in the 18th century in the wake of a migrating sand shoal, which fused with the southern tip of the island of Texel. Protected from the waves and currents of the open sea it became a sink for sand and mud, resulting in a steady shallowing and narrowing of the bay. At low tide, the bay is currently 700 meters wide and 1750 meters long. At the center of the bay, where the core was taken, water depth is 2 meters at high tide and tidal amplitude is 1.4 meters. The eastern part of the Mok Bay is regularly dredged to maintain accessibility to a small navy facility: the coring site is west-northwest of the navy base and not affected by dredging (fig 1).

Average relative sea level rise in the Wadden Sea was approximately 5 cm in the 19th and 15 cm in the 20th century (Kooi and others, 1998; Vos and others, 2000; Beets and others, 2003). Since the average net sedimentation rate in the Mok Bay was 1.56 cm/yr (see 'Age model' in results section below), and the 280 centimeters of our core represent 180 years, in 1820, the Mok Bay must have been 2.6 meters deeper than at present day.

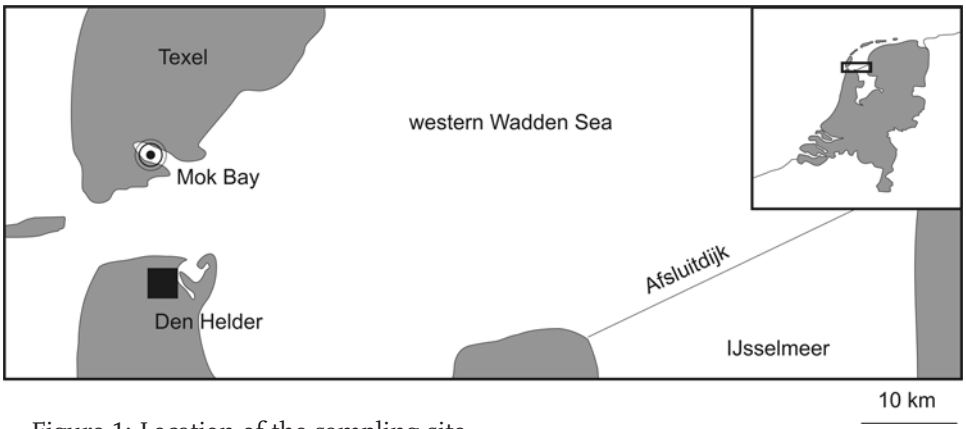


Figure 1: Location of the sampling site.

Sampling

The core consisted of 280 centimeters of sediment, was cut lengthwise and stored at -20° C. After taking X-ray photographs and 12 samples were used for Pb-210 age analysis, the core was sampled for fauna, grain size analysis and organic carbon content determination. From every centimeter, about 5 g of material was taken and stored in small glass jars. In the laboratory, material of each depth-interval was divided into three equal parts: one for foraminiferal analysis, one for total organic carbon assessment and one for grain size analysis

Foraminiferal analysis

Sediment for foraminiferal analysis was sieved over 63 and 150 µm mesh-sized screens to divide the foraminifera and sediment into size-fractions that are common in micropaleontological studies. Only specimens >150 µm were included in this study. Samples for foraminiferal analysis were taken every other centimeter in the upper 250 cm of the core, resulting in 125 samples. In these samples only 5 species were recognized: *Ammonia* cf. molecular type T6 (Hayward and others, 2004; here further referred to as *A. tepida*), *Elphidium excavatum*, *E. williamsoni*, *Haynesina germanica* and *Nonion depressulus*. Taxonomy was based on Murray (1971) and Debenay and others (2001). In most cases, foraminifer-rich samples were split up using an Otto-microsplitter until they contained at least 200 individuals. After counting, abundances were corrected for the dry weight of the sample and are represented as numbers/g dry sediment.

Organic carbon and sedimentology

Organic carbon and grain size was analyzed for all 1-cm samples down to 280 cm. Samples for total organic carbon were decalcified by two successive additions of 1M HCl and subsequently rinsed with demineralized water. After samples were dried, analysis was performed on a LECO CS-analyzer. Grain size analysis was performed with a laser particle sizer (Malvern Instruments, UK). Before analysis, material was treated with 10% H_2O_2 and with 1M HCl to remove organic material and carbonates.

^{210}Pb measurements

^{210}Pb was determined indirectly from ^{210}Po assuming secular equilibrium between ^{210}Pb and ^{210}Po. ^{210}Po was extracted from dried and homogenized sediment by total digestion of samples in a cocktail of HNO_3 and HF. After removal of the acids by evaporation, ^{210}Po was re-dissolved in weak HCl dilution and collected on silver plates by spontaneous deposition. ^{210}Po activity was then measured by alpha spectrometry, using alpha detectors of Canberra. Sedimentation rate integrated over the last ~100 years was determined from the downcore profile of ^{210}Pb, applying a conventional one-dimensional, two-layer, vertical eddy diffusion model of ^{210}Pb distribution and assuming constant ^{210}Pb flux and constant sedimentation rate (Carpenter and others, 1982; Nittrouer and others, 1984). Diffusive biological mixing was taken into account in the calculation.

Climate data

One of the permanent weather stations of the Royal Dutch Meteorological Society (KNMI) is located at the town of Den Helder (fig 1). Here, amongst others, wind speed, precipitation, and temperature have been recorded continuously since 1841. Measurements are available, free of charge at www.knmi.nl/klimatologie/maandgegevens/index.html. Average monthly air temperatures and average maximum wind speeds are calculated from these daily measurements.

Seasonal bias

Since sedimentation rate was 1.56 cm/year and faunal samples were analyzed every other centimeter, successive samples may display an artificial, cyclic pattern that does not stem from the record itself (fig 2). In this example, two different species, indicated by black and grey closed circles, produce a regular fossil record in which material is dominated by one species (grey) in the first half of every year and by another (black) in the second half of each year. The depicted sampling procedure results in a subsequent series of mixed samples and samples dominated by one of the species, regularly alternating, although there is no difference between the years. In records, subsequent years usually vary in thickness and patterns in foraminiferal abundances may not be repeated as regularly as depicted in fig 2. However, we can not exclude the possibility that seasonal cyclicity affects the foraminiferal (or other) patterns through time.

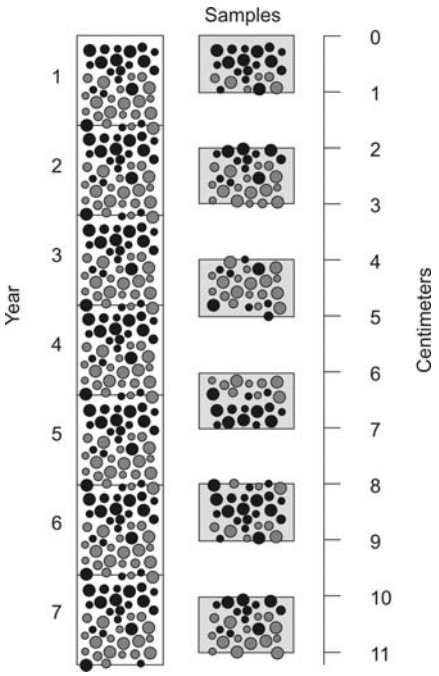


Figure 2: Hypothetical seasonal foraminiferal distribution and successive samples with alternating dominance of both species.

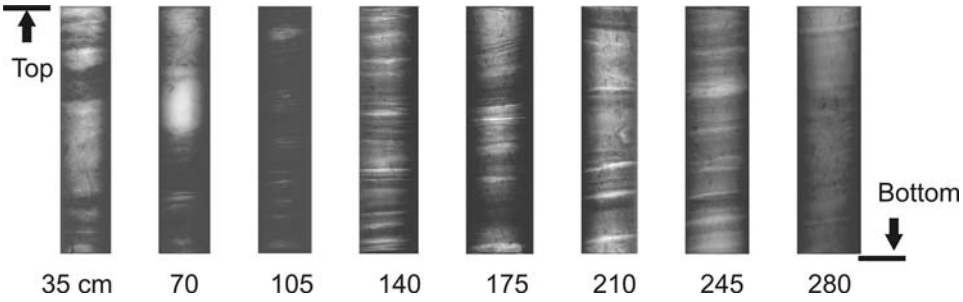


Figure 3: X-ray photograph of MOK2001.

For the Principal Component Analysis (see results), we used a moving average of three successive samples to reduce the potential effect of this seasonal bias. Furthermore, the PCA is based on relative numbers of the foraminiferal species and abundances were not transformed prior to analysis.

RESULTS

Log core

In large parts of MOK2001, laminated sediments prevail. Light and dark bands in the X-ray photograph vary in thickness: some bands are ~1 cm thick, usually with thinner laminae (1-2 mm) situated in-between. Near the bottom of the core, a number of *Mytilus* shells were present (fig 3).

Age model

Total ^{210}Pb activity in the core is highest in the upper part, varying between 72 and 76 Bq/kg between 0 and 20 cm and with a distinct subsurface maximum of 91 Bq/kg at 30 cm. Below this depth, ^{210}Pb decreases exponentially, approaching a supported level of 28

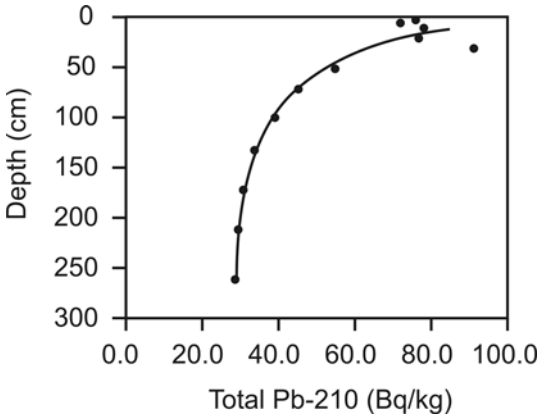


Figure 4: Pb-210 in core MOK2001.

Bq/kg toward the base of the core. Whereas the regular downcore decrease in ^{210}Pb indicates a constant sedimentation over the last century, the lack of a decreasing trend in the upper 20 cm and the subsurface maximum at 31 cm can be attributed to biological mixing (fig 4). A model fit on the observed data gives an average sedimentation rate of 1.56 cm/yr.

Foraminifera

The foraminiferal assemblage in the core exhibited large differences in total numbers/g dry weight: in 1865 and 1905, total standing stocks were high, while from 1960 onward, numbers were relatively low. Despite this variance, the benthic foraminiferal assemblage constantly consisted of 5 species throughout the core (fig 5).

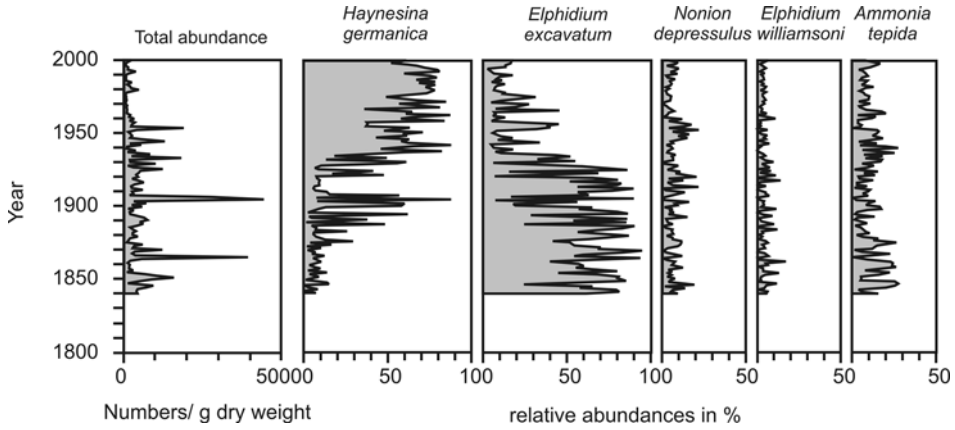


Figure 5: Total foraminiferal densities of specimens >150 µm and relative abundances of the occurring species throughout MOK2001.

Two of these species (*Elphidium excavatum* and *Haynesina germanica*) alternately dominated the assemblage. In general, *E. excavatum* did so from 1840 to 1925-1930 and *H. germanica* from 1930-2000. The other three species did not display a specific trend throughout the core, although *Ammonia tepida* had two periods of slightly higher occurrence: from 1840 to 1870 and around 1930 relative abundances reached values of almost 30%.

Sample scores from the Principal Component Analysis were plotted against time and show the change of the total assemblage through time (fig 6).

The scores of the species on the two axes (i.e. their correlation with the sample scores) show that *Elphidium excavatum* is negatively correlated and *Haynesina germanica* positively with axis 1. *Ammonia tepida* is the third most abundant species and is positively correlated with axis 2 (table 1).
The first principal component is mainly dominated by the shift from *Elphidium excavatum* to *Haynesina germanica*. Superimposed on that trend, is a more erratic, though slightly decreasing trend of the other species (mainly *Ammonia tepida*) in time.

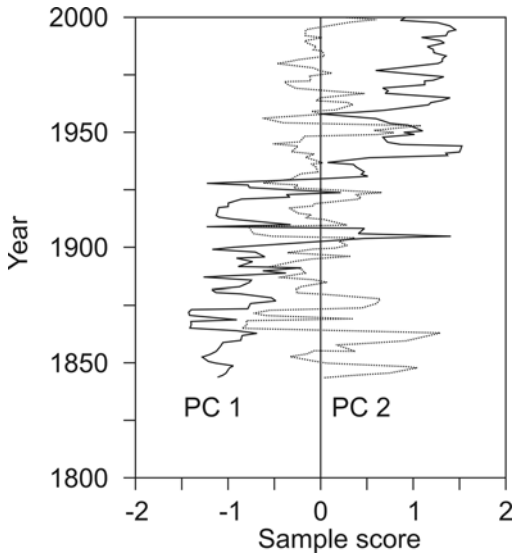


Figure 6: Sample scores in a PCA versus time. The 1st PC axis explains 95.9% of the variance in the species data, the 2nd an additional 3.4%.

	species score PC-axis 1	**species score PC-axis 2**
Ammonia tepida	-0.0255	0.582
Elphidium excavatum	-1.55	-0.505
Elphidium williamsoni	-0.0025	0.144
Haynesina germanica	1.58	-0.488
Nonion depressulus	0.0028	0.266

Table 1: Species scores on the first two PC axes.

Organic carbon content and sedimentology

Clay content in MOK2001 steadily decreased from 7% in 1820 to 4% in 1870. Until 1955, clay content fluctuated between 3% and 5%, after which it suddenly dropped to 1.5-2%. Silt fractions exhibited a similar trend, although silt levels increased after their initial drop around 1955. The two sand fractions reached peak values around 1960 and 1985 and medium and coarse sand also reached markedly high values around 1840, 1855, 1870, 1910 and 1935.

Skewness of the total grain size distribution is around 0.3 and remained constant until 1970, when skewness was suddenly high (upto 1.6) and remained relatively high after 1970 (fluctuating around 0.7).

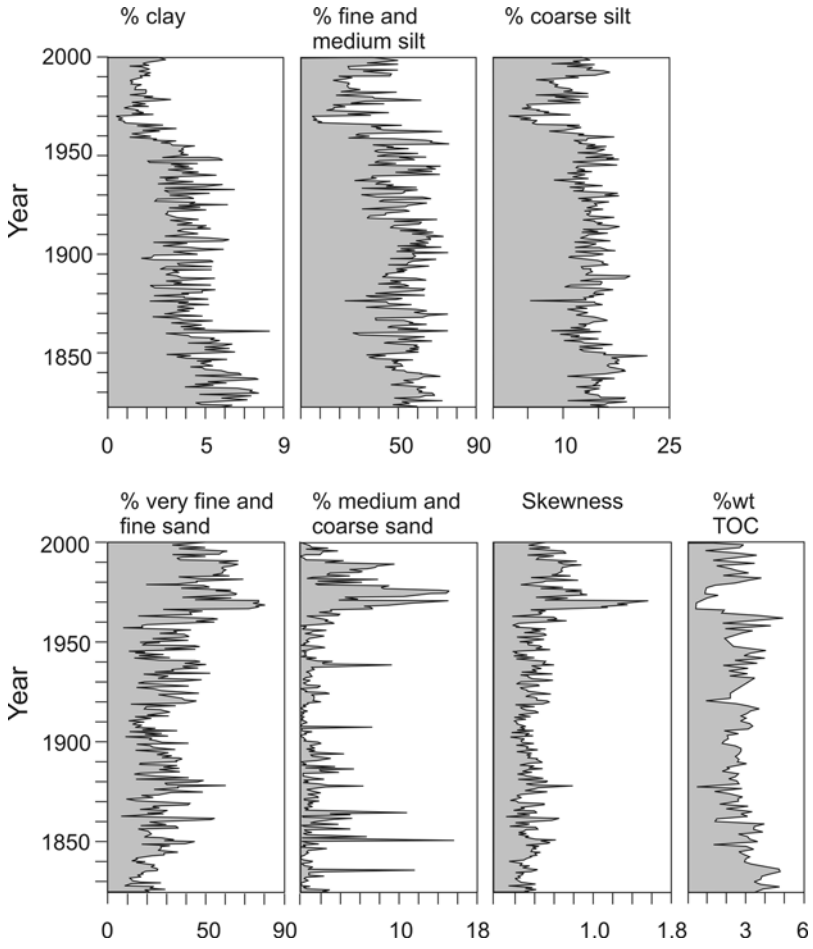


Figure 7: Distribution of different grain size classes, skewness and TOC content from 1820 to 2000.

Despite large inter-year variability, the total organic carbon content (TOC) throughout the core decreased between 1820 and 1900 and small amounts of TOC (1-2%) were preserved between 1965 and 1980 (fig 7).

Temperature and windspeed

From 1906 to 2000, air temperatures were recorded daily by the Royal Dutch Meteorological Society (KNMI) at, amongst others, a station near Mok Bay. Average monthly temperatures were used to calculate the annual average and seasonal averages through time in the Wadden Sea (fig 8).

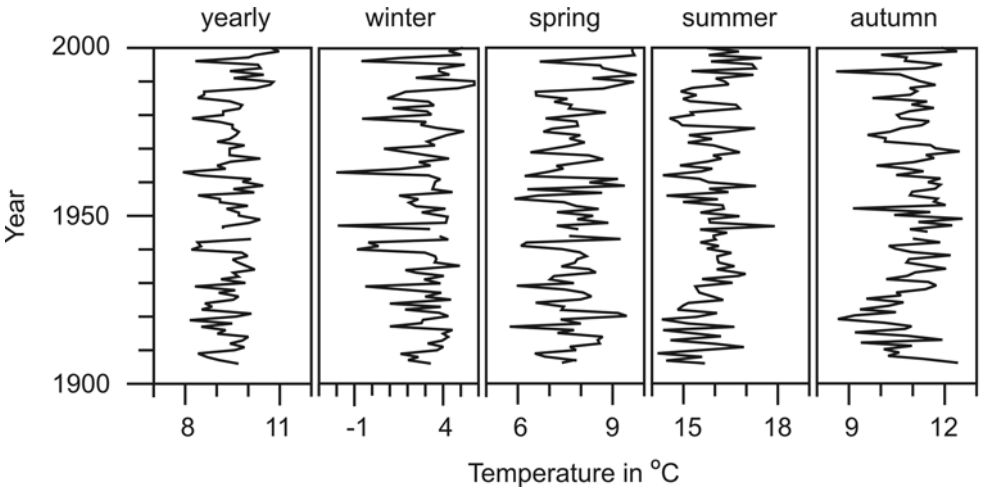


Figure 8: Average temperatures at the weather station of Den Helder from 1906-2000.

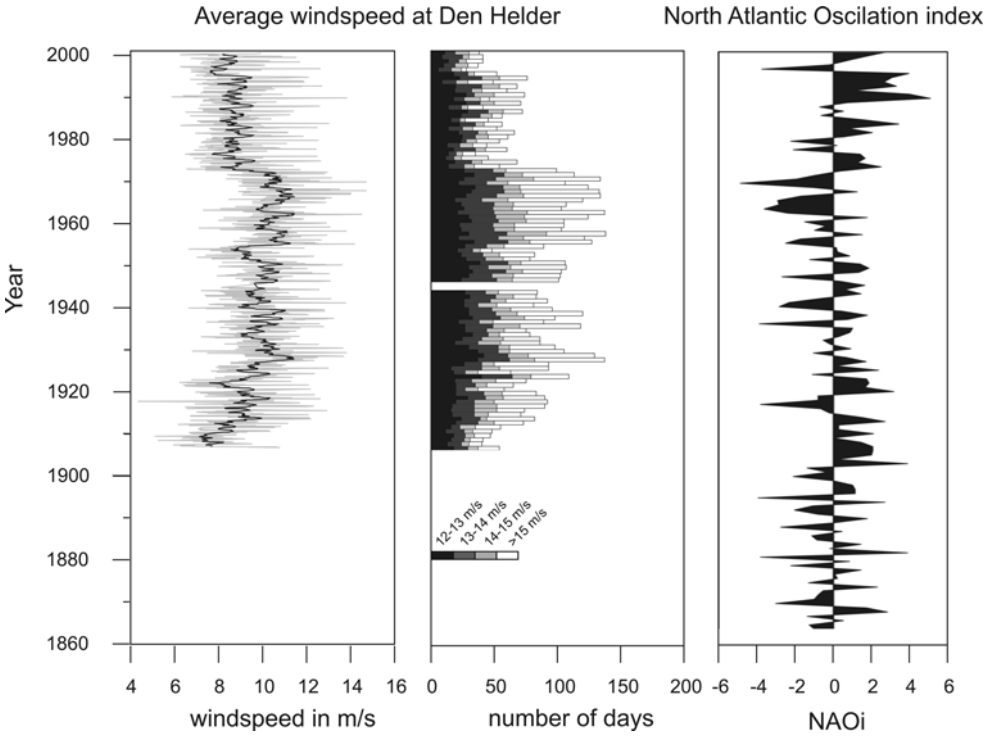


Figure 9: Monthly average wind speeds from 1906 to 2000, thick line indicates 10-point moving average (left), number of days per year in which maximum hourly wind speed is higher than 12 m/s (middle) and winter NAOindex (right).

Maximum hourly wind speeds per day from the same weather station were used to calculate monthly average wind speeds and to calculate the number of days in which maximum hourly wind speeds were higher than 12 m/s (fig 9). The North Atlantic Oscillation winter index (NAOi: Hurrell, 1995) is positively correlated with winter temperatures and average winter wind speeds (fig 9).
For the laminated part of the core (pre-1970), there is a significant positive correlation between percentage grain sizes >103 μm and yearly average wind speed. Any of the smaller grain size intervals were not correlated to average wind speeds: table 2.

grain size interval (in μm)	correlation coefficient (r)
>301	0.324
>258	0.353
>222	0.359
>190	0.352
>163	0.347
>140	0.335
>120	0.303
>103	0.250
>88	n.s

Table 2: List of grain size-classes that are positively correlated to average wind speeds. n.s. = not significant; df = 63; $p<0.05$.

DISCUSSION

Historical developments in the Wadden Sea

Construction of the Afsluitdijk

In 1932, the Zuiderzee (currently a fresh water lake called the IJsselmeer) was separated from the Wadden Sea by construction of the Afsluitdijk. This caused, amongst others, the tidal prism in the Wadden Sea to increase suddenly by 26% from 1.1 to 1.4 meter (Thijsse, 1972; Elias and Van der Spek, 2006), resulting in higher turbidity and increased light limitation (De Jonge and De Jong, 1992). Although sedimentation rates and dynamics in the western Wadden Sea have changed locally after 1932 (e.g. Berger and others, 1987), sedimentation in Mok Bay appeared not to be affected since the thickness and number of laminae is similar in the decades before and after 1932 (i.e. around a depth of 110 cm; fig 3).

Primary production

Closure of the Zuiderzee not only cause decreased algal growth by higher turbidity, but also affected primary production by enhancing decline of oysters in the Wadden Sea. The oyster population had been declining from the beginning of the 20th century, but

changed hydrographic conditions after 1932 in the western Wadden Sea, triggered oyster larvae to flush out into the North Sea. Since bivalves filter phytoplankton from the water column (Cloern, 1982; 2001; Officer and others, 1982; Peterson and Heck Jr, 1999; Jackson and others, 2001), primary production in the Wadden Sea shifted from (subtidal) microphytobenthos to pelagic production (Reise and others, 1989; Van Beusekom, 2005). The sharp drop in abundances of seagrass in 1930 (Short and others, 1988; De Jonge and De Jong, 1992; Kastler and Michaelis, 1999; Reise, 2005) and the steady decline of red and brown algae in the 19th and start of the 20th century (Lotze, 2005) were also partly caused by the loss of oyster beds. Decline of macroflora and oysters have decreased sediment stability, resulting in augmented suspension of fine particles (Piersma and others, 2001), although this trend may have been partly and locally reversed by commercial mussel farming that started in the 1950s (Van der Veer, 1989).

Eutrophication

From the 1960s to the 1990s, the Wadden Sea was subjected to intensified eutrophication (De Jonge and Postma, 1974; De Vries and others, 1998; Van Raaphorst and De Jonge, 2004). Higher levels of P and N in coastal waters trigger higher primary production and may have caused a reduction in the sediment's oxygen penetration depth (Kolbe and others, 1995). Despite this, zoobenthic biomass in the Wadden Sea is described to have increased during ongoing eutrophication in the Wadden Sea (e.g. Beukema, 1991; Beukema and others, 2002).

Climate

Although the NAO index was relatively high in the 1980s and 1990s, average wind speeds in the Netherlands were low after 1972 (fig 9). In winter months, westerlies are stronger in years with a high NAOi due to large differences in air pressure between Iceland and the Azores (Kushnir and Wallace, 1989; Hurrell, 1995). Increased westerlies also result in mild winter and spring temperatures. Apart from the influence of the NAO, global temperature increased on average with 0.6 C in the 20th century due to the increased greenhouse effect (Houghton, 2001).

Sedimentology and organic carbon content

Sedimentology

The X-ray photograph of core MOK2001 revealed laminated sediments in large parts of the core (fig 4). Laminated cores from the Wadden Sea have been described before (e.g. Berger and others, 1987) and we infer from the lamination that bioturbation or vertical mixing must have been insignificant during and after deposition of sediments in Mok Bay over the past 180 years. If horizontal transport of foraminiferal specimens into the Mok Bay is absent (see 'Foraminifera' in section below), foraminiferal tests can be regarded as autochtonously belonging to the sediment layers and corresponding time intervals. Lamination on the X-ray photograph consists of alternating dark and white bands that are commonly interpreted as intervals with high mud and sand contents, respectively. Although sample resolution for grain size analysis was too low to support this, laminae consisting of alternating sand and mud intervals was visually confirmed during subsampling of the core.

Like most tidal flats and inner margins of the Wadden Sea, Mok Bay acts as a sink for mud deposition (Davis and Hayes, 1984, Dronkers, 1986; Eisma, 1998), and therefore, constant net sedimentation has resulted in shallowing and subsequent shrinking of the Mok Bay ever since it was formed during first half of the 18th century. It is therefore likely that the muddy intervals in MOK2001 are the result of tidally transported sediments.
The sandy intervals, however, may be partly transported into the Mok Bay by wind from surrounding dunes, consisting of medium and coarse sands. Although rainfall and wind direction are likely to affect the amount of sand transported into the Mok Bay, wind speeds higher than 12 m/s are shown to transport considerable amounts of sand from Wadden Sea dunes (Arens, 1996).
From 1906 to 1970, the NAO index and wind speeds are positively correlated to sand contents in the core and negatively to mud content (table 2; figs 7 and 9). This is not true for the upper part of MOK2001, when NAOi was relatively high, and coincides with the period for which the Pb-210 datings display some scatter (fig 4) and clay content was relatively low (fig 7). This may indicate that sedimentation between 1970 and 2000 was not constant and material may have been partly reworked during this period. Although these results suggest that sands are deposited within days during storms, it remains unknown whether all of the sand is of eolian origin and, if any, how much is tidally transported into the Mok Bay.
It may be that both sands and clays are deposited (tidally and eolian) simultaneously and almost at the same rate, but that during storms material from the upper centimeters of the Mok Bay's floor is brought in suspension and re-settling with differential rates: sand first, followed by silt and clay. This process thus may lead also to a laminated record, irrespective of the mud and sand source.

Organic carbon
The organic carbon levels in Mok Bay are well correlated to the clay/fine silt and medium silt content. This has been described before for sediments in the Wadden Sea (e.g. Beukema and Cadée, 1997) and can be explained by four processes. Firstly, organic material adsorbs stronger to clay than to sand particles (Anderson, 1988; Mayer, 1994). Secondly, organic particles have the same settling dynamics as clay and other fine particles (e.g. Fries and Trowbridge, 2003). Thirdly, when the sandy intervals are deposited instantaneously and organic matter is deposited on a relatively constant rate, intervals with much sand are diluted and may thus contain low amounts of organic matter. Finally, oxygen penetration in clay and silt is lower than in sandy sediments, promoting the preservation of organic material in fine sediments. Overall, there is a decreasing trend of a preserved TOC content in core MOK2001, despite ongoing eutrophication in the Wadden Sea.

Foraminifera
Taphonomic loss due to dissolution of tests must have been low, since there was no sign of dissolution in the analyzed samples. The two peaks in total foraminiferal density (fig 5) may be caused by periods in which sedimentation rate was exceptionally low and thus accumulation rates were high. This may indicate that although sedimentation

rates in Mok Bay appear to have been constant for large parts of its history (fig 4), it is likely that during some periods, sedimentation rates varied significantly. This bias, however, is not likely to be large, since there is no correlation between foraminiferal abundances and any of the sedimentary parameters.

Individual taxa

In the record, five species of foraminifera are present, of which two dominate the community (fig 5). The shift from *Elphidium excavatum* to *Haynesina germanica* occurs around 1930, suggesting that this shift is caused by the construction of the Afsluitdijk in 1932. The main difference in the niches of these species is a result of their difference in ability to cope with variations in temperature and salinity. Although they both are cosmopolitan and often coexist in intertidal environments (e.g. Alve and Murray, 1994; 2001), *H. germanica* is reported to live in a wider range of temperatures (Murray, 1991) and is more tolerant to low salinities (Debenay and others, 2006).

The construction of the Afsluitdijk may have increased variability in temperature and salinity, thereby increasing the success of *Haynesina germanica* at the expense of *Elphidium excavatum*. In 1820, relatively great water depths (an estimated 3.5 at low tide versus 4.6 m at high tide) were likely to have buffered daily variations in water temperature and salinity. Also, this realtively large water volume may have minimized seasonal differences in these parameters. Shallowing of Mok Bay during the 19th and 20th century resulted in an estimated tidal amplitude of 1.8-2.9 m in 1930, thus increasing the potential for greater daily and seaonal differences in temperature and salinity. After construction of the Afsluitdijk in 1932, the tidal amplitude increased suddenly, resulting in a difference in a much lower water volume during low tide with a tidal range of 1.6-3.0 m. During the rest of the 20th century, the relative semidiurnal difference in water volume in Mok Bay is likely to have further increased variability in temperature and salinity. With waterlevels currently ranging from only 0.6 m to 2.0 m, the Mok Bay during low tide is prone to substantial cooling in winter, and heating and evaporation in summer.

Ammonia tepida is the third most abundant species in MOK2001. Although it is abundant in highly variable environments (see also chapter 4), it is able to feed largely on refractory matter, while *Elphidium excavatum* and *Haynesina germanica* seem to depend on labile organic matter (Knight and Mantoura, 1985; Hohenegger, 1989; Goldstein and Corliss, 1994; Moodley and others, 2000; Murray and Alve, 2000; Ward and others, 2003; Austin and others, 2005; this thesis, chapter 3). Since the ratio between *Elphidium*+*Haynesina* and *Ammonia* does not change throughout the core, it is not likely that the ratio of labile/refractory organic matter changes, despite the enhanced eutrophication and the possible, subsequent shift in the quality of the foraminiferal food.

Furthermore, the low numbers of *Ammonia tepida* in Mok Bay may indicate that horizontal transport of specimens is limited. *A. tepida* occurs in large numbers near the sediment-water interface at the intertidal flats near Mok Bay (this thesis, chapter 3). If sands would have been transported into the Mok Bay by tidal energy, it is likely to have contained substantial amounts of *A. tepida*. Since this is not the case (fig 5), we infer that foraminifera and sand found in MOK2001 are not likely to be transported horizontally by tidal energy.

Climate and foraminifera

Temperature trends (NAOi and 20th century global warming) are not reflected in the foraminiferal record of Mok Bay. This is either because abundances of intertidal species are not influenced by these rather subtle (compared to seasonal variations in temperature) trends or that the change from *Elphidium excavatum* to *Haynesina germanica* overprints any other correlation with climate forcings. The correlation with grain size, however, does suggest that sedimentation of coarse material is high during periods of relatively high average westerly wind speeds (i.e. during years with high a high NAO index).

CONCLUSIONS

The laminae in MOK2001 are likely to reflect both tidal (mud) and eolian (sand) transport of sediments into the Mok Bay. Since average winter wind speeds are governed, amongst others, by the North Atlantic Oscillation, lamination in MOK2001 may reflect the NAOi. The shift in foraminiferal dominance suggests that Mok Bay's environment has become more variable: the currently dominant *Haynesina germanica* is an indicator of highly variable environments, compared to the formerly dominant *Elphidium excavatum* (this thesis, chapter 8). This shift occurred around 1930, suggesting that the construction of the Afsluitdijk (1932) caused, or at least enhanced, variability in salinity and water temperature in Mok Bay.

CHAPTER 7

COPPER INCORPORATION IN FORAMINIFERAL CALCITE: RESULTS FROM CULTURING EXPERIMENTS

with GJ Reichart, A Dueñas-Bohòrquez, M Wolthers, SR Ernst and GJ van der Zwaan

ABSTRACT

A partition coefficient for copper (D_{Cu}) in foraminiferal calcite has been determined by culturing individuals of two benthic species under controlled laboratory conditions. The partition coefficient of a trace element (TE) is an emperically determined relation between the TE/Ca ratio in seawater and the TE/Ca ratio in foraminiferal calcite and has been established for many divalent cations. Despite its potential to act as a tracer of human-induced, heavy metal pollution, data is not yet available for copper. Since partition coefficients usually are a function of many factors (seawater temperature, pH, salinity, metabolic activity of the organism, etc.), we chose to analyze calcite from specimens cultured under controlled laboratory conditions. They were subjected to different concentrations of Cu^{2+} (i.e. different $(Cu/Ca)_{sea\ water}$) and constant temperature, salinity and pH. We monitored addition of new calcite in specimens of the temperate, shallow-water foraminifer *Ammonia tepida* and in the tropical, symbiont-bearing *Heterostegina depressa*. Newly formed chambers were analyzed for Cu/Ca ratios by laser ablation-ICP-MS. The calculated partition coefficient (0.1-0.3) was constant over a large range of $(Cu/Ca)_{sea\ water}$ and remarkably similar for both species. Neither did the presence or absence of symbionts affect the D_{Cu},nor did we find a significant effect of temperature or salinity on Cu-uptake.

INTRODUCTION

Trace elements incorporated in foraminiferal tests are widely used in paleoceanography: Mg/Ca ratios are used to reconstruct sea surface (Nürnberg et al., 1996) and deep-sea temperatures (Rathburn and DeDecker, 1997), Cd and Ba are used to estimate past seawater nutrient levels and alkalinity, respectively (Boyle, 1988; Rosenthal and others, 1997; Lea and Boyle, 1991). These proxies rely on empirically derived partition coefficients (D_{TE}) and the dependence of these coefficients on environmental variables. Temperature, salinity and pH of sea water potentially affect the D_{TE} in foraminiferal calcite (e.g. Nürnberg and others, 1996; Segev and Erez, 2006).

Although field experiments are useful to determine first order proxy relationships, reliable proxy calibrations should include the contribution of so-called vital effects and sep-

arate the effects of other possible contributing factors. The best way to unravel the contribution of separate variables is through culturing experiments, in which one variable is varied and all others are kept constant. In the case of some divalent cations, culturing experiments also allow calibration of proxies out of the range of naturally occurring environmental conditions. This is important for trace elements that are associated with anthropogenic pollution with significantly raised concentrations above natural background levels.

Anthropogenic heavy metal pollution is often characterized by, amongst others, high Cu-concentrations (Borrego and others, 2004; Sáinz and Ruiz, 2006). Foraminifera have been used in several ways to investigate pollution as high levels of heavy metals potentially deform foraminiferal chamber alignment and influence foraminiferal community stucture (Ellison and others, 1986; Samir and El-Din, 2001; Hallock and others, 2003; Armynot du Châtelet and others, 2004; Ruiz and others, 2004; Ferraro and others, 2006). However, a number of papers state that test deformations under high heavy metal concentrations occur less often than under medium pollution loads (Alve and Olsgard, 1999; Geslin and others, 2002; Le Cadre and Debenay, 2006). This suggests that reconstructions based on test deformations are not accurate.

Cu-concentrations can also be high in the proximity of hydrothermal vents (Iizasa, 1993; Douville and others, 2002; Kadar and others, 2005). Although the low pH close to acidic vent fluids dissolves foraminiferal calcite, records of benthic foraminiferal assemblages described further away from these vents may be used to reconstruct past hydrothermal activity and impact on concentrations of heavy metals in its vicinity (Molina-Cruz and Ayala-López, 1988; Jonasson and others, 1995; Panieri and others, 2005).

25 years ago, Boyle (1981) showed that Cu was one of the elements which proved to be difficult to analyze in foraminiferal calcite. However, recent analytical advances in the analyses of trace elements in foraminiferal calcite (Reichart and others, 2003) enabled us to calibrate for the first time foraminiferal Cu to seawater chemistry, using cultured benthic foraminifera. Two different intertidal to neritic species (temperate and tropical) were cultured to establish interspecific differences in the partition coefficient of Cu in foraminiferal calcite.

METHODS

Collecting and culturing foraminifera

Two similar culturing experiments were conducted. For the first experiment, sediment was collected at an intertidal flat in the Dutch Wadden Sea and was kept in the laboratory in the dark at 10° C. Large (>150 μm), living individuals of *Ammonia* cf. molecular type T6 (Hayward and others, 2004: further referred to as *A. tepida*) were transferred to custom-made flow-through culture vessels (fig 1). Vessels consist of a 32-well culture tray, sandwiched between two Plexiglas plates and cells were connected by silicon tubes, attached with luers in the upper Plexiglas lid (fig 1). Between each cell and tube, a small filter was placed to prevent specimens from moving between cells. Trays were connected individually to a 2-liter reservoir with chemically altered sea water and a peristaltic pump was used to circulate sea water through the cells with a speed of 9 ml/h: in this way, six groups of 16 cells were formed, each connected to its own sea water reservoir. In each cell, four foraminiferal specimens were placed. Sea water was enriched with Cu

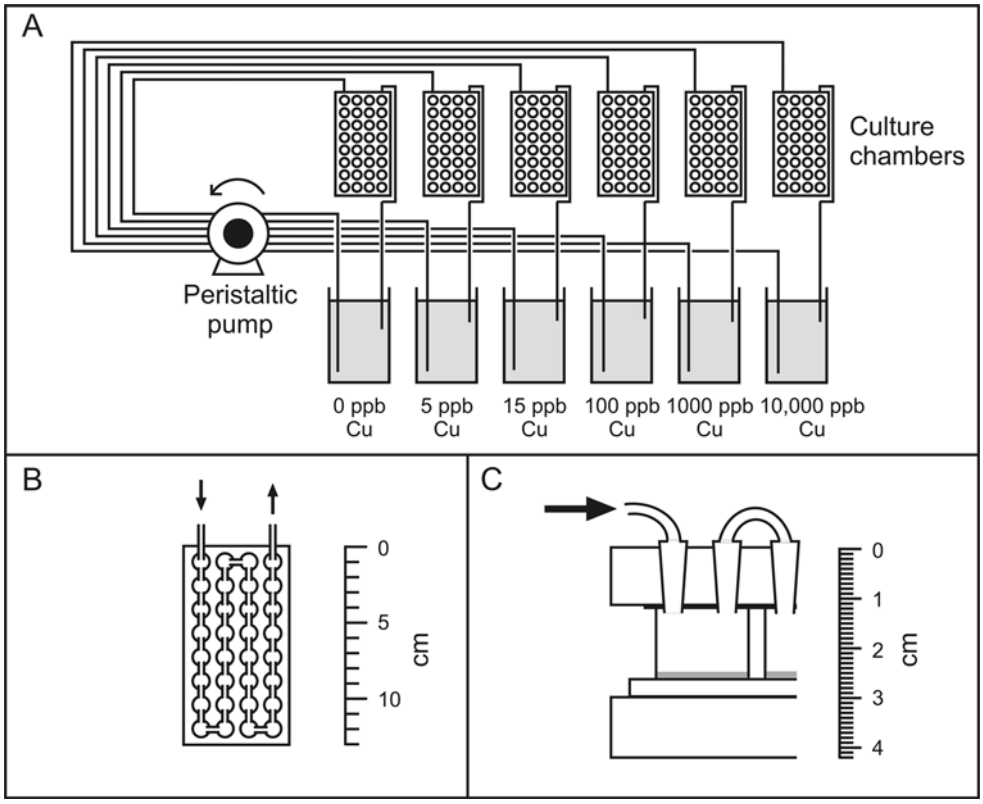


Figure 1: Design of the experimental set-up. A: overview of the system. B: culture tray with in- and outflow openings (top view). C: cross-section of culture tray with inter-cell connections, small layer of sediment and inflow opening.

from a stock solution: added concentrations of Cu in sea water were 0, 10, 20, 50, 1,000 and 2,000 ppb. Calcein (C0875, Sigma-Aldrich, St Louis, USA) was added to the Cu-enriched sea water at a concentration of 10 mg/l. Calcein is incorporated into biogenic calcite, while existing calcite (i.e. earlier formed chambers) is not affected. Since (incorporated) calcein is fluorescent, foraminiferal chambers can be recognized that have been built during the time when individuals were incubated (Bernhard and others, 2004).
Cells with specimens of *Ammonia tepida*, contained a small layer (<0.5 mm) of sieved, <37 µm, clay and silt from sediment collected at the Wadden Sea. Natural seawater from the eastern Mediterranean Sea was adjusted with MilliQ water to a salinity of 17 to mimic average Wadden Sea salinity. Salinity levels were regularly checked during the experiment with a WTW LF330 conductivity meter. All 6*16 cells were kept at a constant temperature of 10° C for two months: before and after experiments, reservoirs were sub-sampled and sea water was analyzed by ICP-MS for Cu, Mg and Ca. At the start of the incubation period, the individuals were fed ~0.5 mg of freeze-dried *Dunaliella* sp. During experiments, the set-up was subjected to the daily sunlight cycle (app. 14h light/ 10h dark).

For experiment 2, trays were replaced and lids rigorously cleaned with HCl, rinsed with MiliQ and re-used to incubate individuals of *Heterostegina depressa* in seawater with similar Cu-enrichments used in the first experiments. *H. depressa* is an epibenthic, tropical and symbiont-bearing foraminifer, that was kept in our laboratory under high light intensities (15W tropical reef lamp; Arcadia, FO15) and after transferring them into the culturing set-up, similar light conditions were maintained in a daily rhythm (14h light/ 10h dark). No sediment was added to the cells, seawater salinity was kept at 35, and the set-up was kept at 20º C. Because of their large size, only two specimens were placed in each cell.

Carbonate chemistry of culture media

For culturing *Ammonia tepida*, we diluted 35 PSU seawater with MiliQ water to mimic intertidal ambient conditions with seawater of 17 PSU The dilution decreased both $[Ca^{2+}]$ and $[CO_3^{2-}]$ and the alkalinity with approximately 50%, resulting in a pronounced reduction of the carbonate saturation state (Ω). The temperature maintained during these experiments was kept at 10° C, compared to 20° C for the *Heterostegina depressa* experiments, allowing gas exchange with the atmosphere (ambient pCO_2) in both cases. The combined effect of these changes is a reduction in saturation state from about 5.5 for the *H. depressa* experiment to about 1.0 for the *A. tepida* experiment (calculations were performed in CO2sys; Lewis and Wallace, 1998). The lower seawater saturation state for the *A. tepida* cultures was most likely responsible for the fact that newly formed chambers were thinner than the pre-experiment chambers (see Results).

Laser ablation ICP-MS

Newly formed chambers were ablated using an Excimer laser (Lambda Physik) with GeoLas 200Q optics inside a helium atmosphere flushed ablation chamber. Pulse repetition rate was set at 6 Hz, with an energy density at the sample surface of 10 mJ/cm^2. Ablation craters were 60 µm in diameter and ablated material was analyzed with respect to time (and hence depth) using a quadrupole ICP-MS instrument (Micromass Platform).

Simultaneous monitoring of Al allowed us to discard the parts of the ablation profiles contaminated by clay minerals from further calculations of elemental concentrations. Since the analytical error increases with shorter ablation time we cleaned all specimens by an incubation of 24 hours in 5% NaOCl (Gaffey and Brönniman, 1993) before analysis, maximizing the amount of data that could be used for calculating $(Cu/Ca)_{calcite}$ ratios.

Calibration strategy

The low calcite saturation state used in the experiment with *Ammonia tepida* resulted in formation of new chambers with thin walls. A similar correlation between test wall thickness and carbonate saturation state has been observed earlier for tests of cultured planktonic foraminifera (Bijma and others, 2002). Unfortunately, these thin chambers break easily during ablation when high laser energies are used. Therefore, we ablated *Ammonia tepida* with a laser energy of 1 mJ/cm^2 , ten times less as the 10 mJ/cm^2 used to ablate newly formed chambers of *Heterostegina depressa*.

Analyses were calibrated against NIST glasses 610 and 612, using concentration data of Pearce and others (1997). Calibrating calcites against glasses is possible because of the matrix independent ablation by the Excimer laser (Mason and Mank, 2001). However, when energies lower than 2 mJ/cm^2 are used, glass does not ablate properly and calibration was performed against matrix matched in-house standards (i.e. pressed calcite powder tablets). Calcium was used as an internal standard because (1) concentration is constant at 40 wt % in calcite and (2) it allows direct comparisons with trace metal to Ca ratios from wet-chemical studies. A collision and reaction cell was used to give improved results by reducing spectral interferences on the minor isotopes of Ca (^{42}Ca, ^{43}Ca and ^{44}Ca: Mason and Kraan, 2002). Both ^{63}Cu and ^{65}Cu isotopes were used to calculate Cu-concentrations.

Seawater Cu-concentration

The concentration of Cu did not vary considerably in most of our experiments during the experimental period for most of the concentrations used (table 1).

In the first experiment, all measured Cu-concentrations were lower than the target concentration and most total Cu-concentrations increased during the experiment, resulting in increased seawater Cu/Ca ratios (on average 17%). In experiment 2, most Cu-concentrations and all Cu/Ca ratios were higher at the start than at the end of the

Experiment	Target [Cu] in ppb	Measured Cu		Cu/Ca	
		at the start of experiment in ppb + 1SD	at the end of experiment in ppb + 1SD	at start (*10^{-5}) +1SD (*10^{-5})	at end (*10^{-5}) +1SD (*10^{-5})
1. *Ammonia tepida*	0	5.40	6.43 +/- 0.374	6.32 +/-5.28	2.91 +/- 0.221
	10	6.15 +/- 0.936	n.a.	2.95 +/- 0.384	n.a.
	20	12.6 +/- 0.111	16.4 +/- 0.132	6.00 +/- 0.0326	7.34 +/- 0.121
	50	21.3 +/- 0.324	n.a.	10.1 +/- 0.0143	n.a.
	1,000	804 +/- 18.8	899 +/- 55.6	378 +/- 4.62	408 +/- 6.94
	2,000	1143 +/- 31.4	1282 +/- 196	547 +/- 13.5	473 +/- 20.8
2. *Heterostegina depressa*	0	8.01 +/- 0.355	13.3 +/- 0.765	1.69 +/- 0.0236	3.62 +/- 0.343
	10	11.1 +/- 0.139	13.5 +/- 2.61	2.32 +/- 0.0670	3.74 +/- 0.500
	20	15.6 +/- 1.46	23.6	4.06 +/- 0.179	6.71
	50	82.0 +/- 2.46	80.0 +/- 3.35	20.2 +/- 0.0120	23.1 +/- 0.711
	1,000	717 +/- 0.430	665 +/- 17.8	172 +/- 0.844	212 +/- 5.88
	2,000	n.a.	n.a.	n.a.	n.a.

Table 1: Target concentrations of Cu in sea water and measured $(Cu/Ca)_{sea\ water}$ at start and end of both experiments. n.a.=not available.

experiment. Identical procedures and techniques were used during both sample moments, making it unlikely that sampling artifacts affected our measurements. Therefore, we used average solution Cu/Ca ratios to calculate the partition coefficient of Cu in foraminiferal calcite and incorporated differences between start and end concentrations for uncertainty calculations. Error bars plotted in the different graphs are based on these calculations and largely stem from these changes, which are an order of magnitude larger than the analytical uncertainties.

	Reaction	**Functional groups**	**Log K**
(1)	$L^{6-} + H^{+} = HL^{5-}$	-COOH	11.7
(2)	$HL^{5-} + H^{+} = H_2L^{4-}$	-COOH	10.8
(3)	$H_2L^{4-} + H^{+} = H_3L^{3-}$	-COOH	5.5
(4)	$H_3L^{3-} + H^{+} = H_4L^{2-}$	-OH	4.2
(5)	$H_4L^{2-} + H^{+} = H_5L^{-}$	$\equiv NH^{+}$	2.9
(6)	$H_5L^{-} + H^{+} = H_6L$	$\equiv NH^{+}$	2.1
(7)	$2Cu^{2+} + L^{6-} = Cu_2L^{2-}$	-COOH	28.9
(8)	$Cu^{2+} + H_2L^{4-} = CuH_2L^{2-}$	$\equiv NH^{+}$	8.3
(9)	$Cu^{2+} + H_4L^{2-} + H_3L^{3-} = Cu(H_4L)(H_3L)^{3-}$	-COOH + -OH	10.4[a]
(10)	$2Ca_2+ + L^{6-} = Ca_2L^{2-}$	-COOH	27.2 [b]
(11)	$Ca^{2+} + H_2L^{4-} = CaH_2L^{2-}$	$\equiv NH^{+}$	6.63
(12)	$Ca^{2+} + H_4L^{2-} + H_3L^{3-} = Ca(H_4L)(H_3L)^{3-}$	-COOH + -OH	8.73 [a b]
(13)	$2Mg^{2+} + L^{6-} = Mg\ _2L^{2-}$	-COOH	28.5 [b]
(14)	$Mg^{2+} + H_2L^{4-} = MgH_2L^{2-}$	$\equiv NH^{+}$	7.9
(15)	$Mg^{2+} + H_4L^{2-} + H_3L^{3-} = Mg(H_4L)(H_3L)^{3-}$	-COOH + -OH	10.0 [a b]

Table 2: Thermodynamic data for calcein (Ueno and others, 1992). L = calceine. [a] reactions leads to negligible metal binding; [b] values extrapolated by assuming chemical behavior similar to Cu^{2+} (see text for discussion).

Cu speciation in seawater

In the absence of organic matter, Cu in sea water forms mainly $Cu(OH)_2$ and $CuCO_3$, while small amounts of Cu^{2+} and $CuOH^-$ are also present (Zirino and Yamamoto, 1972). In natural sea water, however, usually more than 99.9% of the Cu is bound to organic compounds (Eriksen and others, 2001), mainly in the colloidal state (Mackey and Zirino, 1994). Foraminifera take up organic particles and sea water by endocytosis, likely ingesting both free Cu and Cu-ligand complexes. The internal routes that organic compounds follow are virtually uninvestigated in foraminifera and therefore, we do not know which Cu species are present at the site of calcification.

Modelling Cu speciation

The calcein present in the culturing medium is a ligand that can bind TE's and thus potentially causes concentrations of free Cu to drop. Traditionally, total calcium and TE concentrations in solution are used to calculate partition coefficients for trace elements in calcite. Ideally, activities or effective concentrations, of relevant metals are used to allow application of partition coefficients in solutions of different compositions (Morse and Bender, 1990).

To correct for Cu binding to calcein, we calculated speciation of all abundant cations (Cu, Ca and Mg) in our solution. Speciation calculations were performed in PHREEQC 2.8.03 (Parkhurst and Appelo, 1999) with the llnl database and thermodynamic data for calcein listed in Table 2. For calcein complexation of calcium and magnesium, reactions (11) and (14) are reported in the literature. These reactions will lead to competition between copper, calcium and magnesium in binding to an amine group on H_2L^{4-}, thus

Experiment	$[Cu]_t$ (ppb)	$\{Cu\}_t$ (M)	$[Cu]_t/[Ca]_t$	$[Cu]_c$ (ppb)	$\{Cu\}_c$ (M)	$[Cu]_c/[Ca]_c$
1. *Ammonia tepida*	9.94	1.33×10^{-7}	2.6×10^{-5}	9.94	1.33×10^{-7}	2.6×10^{-5}
	10.7	1.46×10^{-7}	2.9×10^{-5}	10.7	1.46×10^{-7}	2.9×10^{-5}
	24.4	3.33×10^{-7}	6.6×10^{-5}	24.4	3.33×10^{-7}	6.6×10^{-5}
	66.8	9.11×10^{-7}	18×10^{-5}	66.8	9.11×10^{-7}	18×10^{-5}
	1,182	161×10^{-7}	320×10^{-5}	1182	161×10^{-7}	320×10^{-5}
	2,417	329×10^{-7}	660×10^{-5}	2417	329×10^{-7}	660×10^{-5}
2. *Heterostegina depressa*	10.6	1.40×10^{-7}	1.6×10^{-5}	10.6	1.6×10^{-5}	1.6×10^{-5}
	12.1	1.63×10^{-7}	1.8×10^{-5}	12.1	1.8×10^{-5}	1.8×10^{-5}
	16.2	2.18×10^{-7}	2.4×10^{-5}	16.2	2.4×10^{-5}	2.4×10^{-5}
	86.5	11.6×10^{-7}	13×10^{-5}	86.5	13×10^{-5}	13×10^{-5}
	767	103×10^{-7}	110×10^{-5}	767	110×10^{-5}	110×10^{-5}

Table 3: Added and free copper concentrations and activities in the experiments. Suffix t = total copper concentration added to the experiment; suffix c = corrected ratios (total Cu or Ca minus calceine-complexed Cu or Ca).

decreasing calcein-bound Cu. It is, therefore, likely that the behavior of Ca and Mg towards calcein is similar to Cu and similar competition between the three metals occurs in binding according to reactions (7) and (9) via carboxyl groups (Lu and Allen, 2002). Composition of the solution in the model was either the Cu-enriched sea water with a salinity of 35 or 17 for the experiment with *Ammonia tepida*, and were both open to atmospheric CO_2.
Cu and Ca-concentrations used to calculate the partition coefficients were those corrected for Cu and Ca complexated with calcein (Table 3).

RESULTS

New calcite and survival rates

Specimens that grew new calcite, were recognized by fluorescent, outer chambers (fig 3).

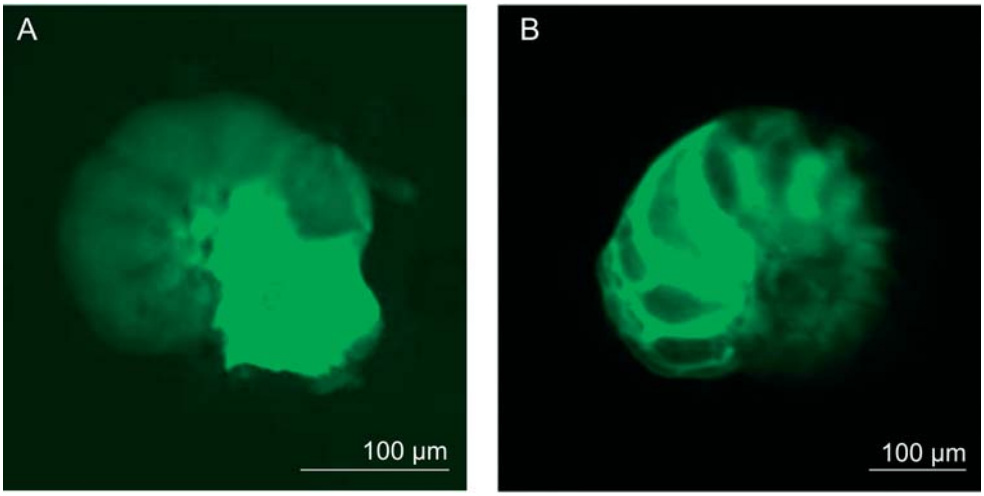


Figure 3: New chambers added by *Ammonia tepida* (A) and *Heterostegina depressa* (B), visible by fluorescence of incorporated calcein.

None of the individuals of *Heterostegina depressa* incubated at the target Cu-concentration of 2,000 ppb, survived the experimental period. At 1,000 ppb of added Cu, however, several survived of which 1 individual produced new calcite. At lower concentrations, generally more chambers were formed (Table 3). None of the added chambers (n=88) showed abnormal alignments or deformations.

For *Ammonia tepida*, the number of successful laser-ablation analyses is significantly lower (3) than the number of added chambers (34). The limited size of newly added chambers did not allow multiple analyses of a single chamber. After an unsuccessful attempt to analyze a targeted chamber it is not possible to re-do this measurement as the largest part of the carbonate has been ablated (fig 4).

Experiment	Target [Cu^{2+}] in ppb	Number of specimens at start of experiment	Number of individuals that grew new chambers	Number of new chambers added
1. *Ammonia tepida*	0	64	7	7
	10	64	11	12
	20	64	6	6
	50	64	3	3
	1,000	64	5	5
	2,000	64	1	1
2. *Heterostegina depressa*	0	32	5	8
	10	32	3	6
	20	32	0	0
	50	32	19	37
	1,000	32	1	3
	2,000	32	0	0

Table 3: Number of individuals at the start of the experiments, number of specimens that formed new calcite and total number of added chambers.

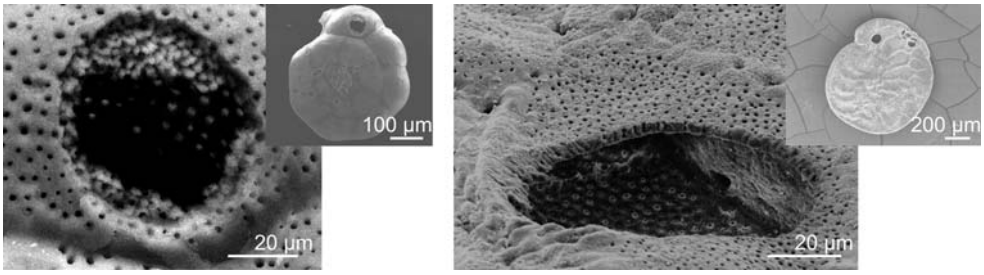


Figure 4: Scanning electron microscope image of laser ablation craters in *Ammonia* (left) and *Heterostegina* (right). Insets depict whole specimen.

Partition coefficient of Cu - *Ammonia tepida*

Two ablation profiles were obtained from two specimens of *Ammonia tepida* that grew new chambers at a low (20 ppb) Cu-concentration (fig 5). Measurements indicate that the partition coefficient lies between 0.1 and 0.3. In figure 5B, the same two measurements are depicted at the left end of the graph (note different scales). Ratios for calcite formed at higher $(Cu/Ca)_{sea\ water}$, indicated a partition coefficient between 0.1 and 0.2.

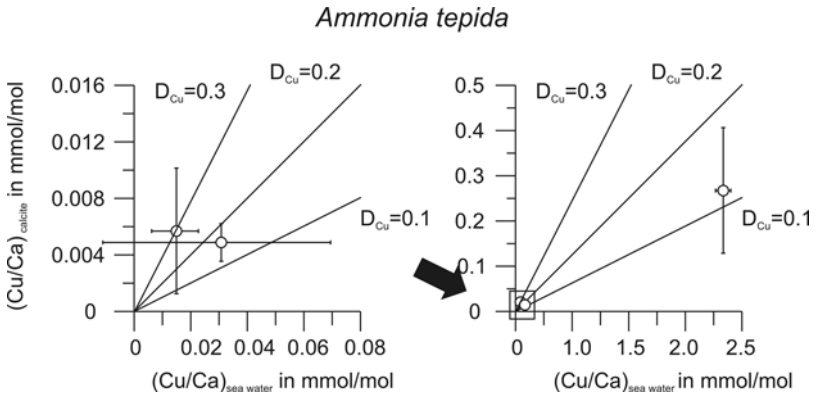


Figure 5: Cu/Ca ratios in *Ammonia tepida* test carbonate versus Cu/Ca in sea water (error bars indicate 1 SD). Left graph is an enlargement of right one: lines represent partition coefficients of 0.1, 0.2 and 0.3.

Partition coefficient of Cu - *Heterostegina depressa*

Although individuals of *Heterostegina* did not survive the highest Cu-levels, we obtained two ratios from specimens that added new chambers at a target concentration of 1,000 ppb (i.e. a realized concentration of 700 ppb Cu). From incubations with lower Cu-concentrations, more specimens were available that grew new chambers that could be analyzed for Cu-concentration (fig 6).

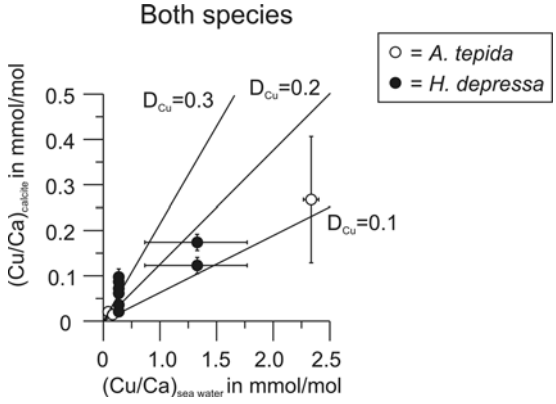


Figure 6: Plot of Cu/Ca ratios in foraminiferal calcite of the added chamber versus the Cu/Ca of the sea water in which they were incubated. Error bars indicate 1 SD. Lines indicate partition coefficients of 0.1, 0.2 and 0.3.

DISCUSSION

Within the experimental and analytical error both species show an identical $(Cu/Ca)_{sea\ water}$ to $(Cu/Ca)_{calcite}$ relation, resulting in a partition coefficient (D_{Cu}) of 0.2 +/- 0.1. No significant difference was observed in copper incorporation between *Ammonia tepida* and *Heterostegina depressa*, despite large differences in ecology and habitat. Some inter-specimen variation in $Cu/Ca_{calcite}$ was observed in *H. depressa* grown at low Cu-concentrations, in which rather large uncertainties in culture water Cu-concentration (fig 6) resulted from changes in $[Cu^{2+}]$ over time. Moreover, the alternative calibration method used for the thin-walled chambers of *A. tepida* increased the analytical uncertainty in the laser ablation-ICP-MS analyses (fig 6). Despite these errors, the calculated D_{Cu} was not significantly dependent on either temperature or salinity.

Cu in the calcite lattice

Crystalline $CuCO_3$ does not exist, because the most common coordination of Cu-carbonate complexes are distorted tetragonal pyramids or distorted octahedrons (Wells, 1984). These shapes do not allow precipitation of pure $CuCO_3$ crystals and rather $Cu_2CO_3(OH)_2$ (malachite) will form. However, sorption studies have shown that at the calcite-water interface these so-called Jahn-Teller distortions can be overcome and a solid solution $Cu_xCa_{(1-x)}CO_3$ forms (Schlosseler and others, 1999). It has been proposed that copper in calcite is present in clusters, based on studying the transformation of vaterite to calcite (Nassrallah-Aboukaïs and others, 1996; 1998). Recent XAFS work, however, has shown that this mechanism is not applicable to calcite surfaces (Elzinga and Reeder, 2002). This rather unusual complexation behavior would suggest that sorption and subsequent incorporation into the crystal lattice for copper is limited to part of the crystal surface only. This, in turn, would result in a lower partition coefficient for copper than expected based on its ionic radius only.

Contrary to these results, it has been shown that during inorganic coprecipitation experiments Cu is incorporated in calcite with a distribution coefficient (K_{Cu}) of 23 and constant under a range of Cu-concentrations (Kitano and others, 1980). In the initial stage of calcification, the K_{Cu} can be even higher (40) probably due to the strong affinity of $Cu(OH)_2$ for calcite surfaces (Franklin and Morse, 1982; Pickering 1983; Papadopoulos and Rowell, 1989). This indicates that the Jahn-Teller distortions are easily overcome during calcification.

Generally, divalent cations with an ionic radius close to Ca (=1.0 Å) have a partition coefficient in calcite close to 1. Cd has an ionic radius of 0.95 (Shannon, 1976) and is incorporated in both planktonic and benthic species with a D between 1 and 4 (Boyle, 1981; 1988; Havach and others, 2001), independent of temperature (Marchitto, 2004). Sr (ionic radius =1.31 Å) is incorporated in foraminiferal calcite with a D of 0.11-0.19, measured in several planktonic genera (Bender and others, 1975) and 0.05-0.25 in *Cibicidoides* (Elderfield and others, 1996). Coretop studies on *Cibicides* and *Uvigerina* show that Ba (1.47 Å) is incorporated with a partition coefficient of 0.3-0.4 at 3° C (Lea and Boyle, 1989). In the planktonic genera *Globorotalia* and *Globoquadrina*, Ba is incorporated with a D of 0.19 (Lea and Boyle, 1991). Cu has an ionic radius close to Mg (0.73 and 0.72 Å, respectively), but the partition coefficient of Mg is much lower ($0.1\text{-}1x10^{-3}$; Bender and others, 1975; Delaney and others, 1985) than the measured 0.1-0.3 for Cu (fig 7). The

large difference between the foraminifer-mediated Cu-incorporation and the inorganic incorporation of Cu in calcite indicates that much energy is spent on removal of Cu at the site of calcification.

Biological control on D_{Cu}

Since magnesium inhibits calcite growth (Berner, 1975; Mucci and Morse, 1983) and high levels of Mg are likely to be present in foraminiferal calcifying reservoirs, it is necessary for foraminifera to remove Mg before calcification. It has been suggested that foraminifera actively pump Mg from their calcifying reservoir in order to stimulate $CaCO_3$ precipitation. Usually, Cu is present only in very low concentrations in seawater and therefore no need exists to actively remove Cu from calcifying reservoirs, despite its ability to modify the crystalline structure of calcite. Although under high Cu-concentrations it may be beneficial to remove Cu from calcifying reservoirs, apparently the foraminifera does not do so, as the D_{Cu} is similar for high and low Cu-concentrations. Alternatively, the concentrations used in our experiments may still be too low to seriously impede $CaCO_3$ precipitation.

Another reason for active removal of trace elements from calcifying reservoirs is that these elements are necessary for cellular processes. Since Cu is known to play only minor roles in eukaryotic metabolic processes (Bruland and others, 1991; Sunda and Huntsman, 1995; Chang and Reinfelder, 2000), it is unlikely that the D_{Cu} is affected by cellular needs.

Organic compounds may increase Mg contents in foraminiferal calcite (Bentov and Erez, 2006). High concentrations of Mg in the primary organic membrane (Hemleben and others, 1986) may explain the observed intra-test variability of Mg/Ca (e.g. Toyofuku and Kitazato, 2005). Cu also has a strong affinity for organic compounds (see below), so that the D_{Cu} may be partly determined by the presence of organic compounds in the calcite. Bresler and Yanko (1995) showed that some benthic, epiphytic foraminifera have tryptofan-containing proteins that can bind Cu^{2+} and prevent intracellular Cu-concentrations from becoming harmful. When a significant part of the Cu^{2+} would have been immobilized by these Cu-binding proteins this would also have lowered the Cu activity in the solution and thus D_{Cu}. Since we have not observed such a decrease, it is unlikely that such molecules play a major role in decreasing intracellular Cu-concentrations.

Seawater pH is a potentially important modulator of trace metal uptake (Lea and others, 1999; Zeebe and Sanyal, 2002). To investigate the potential effect we compared species with and without symbionts. In the symbiont-bearing *H. depressa* the photosynthetic activity of the symbionts changes the local carbonate chemistry because CO_2 is taken up and pH lowered during light conditions. However, the lack of any systematic offset in Cu/Ca between the *H. depressa* and *A. tepida* suggests no significant effect of pH on Cu incorporation.

Test deformation and mortality

A number of studies over the last 20 years have attempted to correlate the number of deformed tests to environmental pollution (Alve, 1991; Elberling and others, 2003; Armynot du Châtelet and others, 2004). The empirical correlation between number of deformed tests and for instance heavy metal or hydrocarbon concentration levels was

interpreted to signify a causal relationship. Although in several of our experiments Cu-concentrations were well above levels found at even the most polluted sites not a single deformed chamber alignment was observed. Although the limited number of observations does not allow a statistical evaluation, our results strongly suggest that high levels of Cu do not cause test deformities. This is also shown by Alve and Olsgard (1999), who found no test deformities in foraminifera living in seawater with high Cu-concentrations. Therefore, we think that relative abundances of deformed tests in fossil samples are not suitable to reconstruct past copper concentrations. Most likely other environmental factors, co-varying with environmental trace metal levels must have been responsible for the observed increase in test deformities. The complete absence of deformations in our experiments is in high contrast to the low but still detectable levels of natural occurring test deformities under environmental pristine conditions. This suggests that the protected environment of the culture trays may actually have shielded our foraminifera from hydrodynamical turbidity, predators or other factors contributing to the test deformaties.

In seawater with the highest concentration of Cu (2,000 ppb), none of the Heterostegina's survived and only one specimen grew new chambers when cultured at 1,000 ppb. Since the growth or survival of *Ammonia tepida* did not appear to be hampered by high concentrations of Cu, we hypothesize that either the symbionts of the tropical foraminifera are vulnerable to high Cu-concentrations (Brandt and others, 1986), or that individuals of *A. tepida* are adapted to cope with (occasional) high levels of heavy metals.

Application of Cu/Ca ratios in foraminiferal calcite

In order to quantify pollution levels, heavy metal concentrations are often analyzed using strong acid extractions and subsequent ICP-MS analyses of bulk sediment. Since heavily polluted sites are frequently characterized by high concentrations of (labile) organic matter, polluted sediments are often anoxic with high levels of sulphate reduction and associated production of free sulfide. These high sulfide-levels result in immobilization of heavy metals such as Zn, Cu, Cd and Pb, which are precipitated as the highly insoluble minerals PbS, CuS and ZnS, or as co-precipitates in pyrite. Because these metals are no longer bioavailable they do not reflect toxicity of the overlying water to, for instance, benthic biota. Actual analyses of the overlying water itself or organisms living in these waters would give a much more applicable concentration to assess pollution. This becomes even more important when at a later stage organic loads decrease and/or the oxygen level increases, (e.g. after improved wastewater treatment). Under these conditions lower sedimentary trace metal levels could result in higher actual toxicity as these metals are remobilized by progressive reoxidation of the sediment and escape to the overlying water. Monitoring foraminiferal test Cu/Ca ratios could be used to establish the bioavailable fraction of Cu and potentially also could record relatively short episodes with elevated bottom water Cu-levels.

Sludge dump sites and associated elevated concentration levels of heavy metals are mostly limited to coastal and estuarine environments. These settings experience considerably varying seasonal and even daily temperatures and salinity levels. A significant impact of either temperature or salinity on partition coefficients would, therefore, ren-

der foraminiferal trace metal records useless for any reliable reconstruction and/or monitoring of such dump sites. Since the obtained D_{Cu} is not markedly dependent on either temperature or salinity, foraminiferal Cu/Ca ratios are a powerful proxy for the quantitative reconstruction of past heavy metal pollution, even in highly variable environments.

CONCLUSIONS

Copper is incorporated into foraminiferal calcite with a partitioning coefficient of about 0.2 +/- 0.1 with respect to sea water Cu/Ca values. No additional effects could be observed with the experimental setup used here. Additional experiments are needed to better constrain D_{Cu} and unravel the effects of other likely important environmental factors such as temperature, salinity and seawater carbonate chemistry.

CHAPTER 8

CONCLUSIONS

Anthropogenic influences on ecosystems

Most chapters of this thesis deal with benthic foraminifera in environments that are subjected to human-induced pollution. Some types of pollution can be directly reconstructed with results that are presented here (like heavy metal pollution; see chapter 7 and below), while other types (e.g. eutrophication) may not be so easily inferred from our results, as the samples across the Frisian Front (and their environmental differences) do not provide a solid analogue for the different stages of eutrophication. In this chapter, we will summarize the conclusions of this thesis and discuss implications for reconstructing the history of shallow water environments.

Heavy metal pollution

Heavy metal pollution (HMP) in marine ecosystems affects survival, growth and reproduction of organisms in harbors, near heavy industry and mines, and in environments where riverine input is high. Although HMP has declined in many coastal areas over the last decades, past HMP may still affect modern and future ecosystems. Sludge that is dumped in the past may have contained high concentrations of heavy metals (HMs) as well as large amounts of organic material, the latter causing anoxia of the sediment thus immobilizing HMs. When these sites are re-oxygenated due to decreased organic pollution, the HMs may become bio-available again. To investigate the long-term (decadal) effects of HMP on ecosystems, we need proxies to reconstruct bio-available concentrations of HMs.

We have shown that foraminiferal Cu/Ca ratios can serve as a proxy for paleo-$Cu_{sea\ water}$ concentrations. The low partition coefficient for Cu (0.1-0.3) indicates that this method can only be successfully applied if seawater of paleoenvironments contained high levels of bio-available Cu. These levels may be reached in near-shore, heavy polluted areas, near mines or near hydrothermal vents. A lack of dependence of the derived partition coefficient on temperature and salinity, together with minor inter-species differences, indicate that $(Cu/Ca)_{foraminiferal\ calcite}$ may serve as a proxy under a wide range of environmental settings and that potentially many foraminiferal species can be used to reconstruct paleo Cu-concentrations.

Eutrophication

Organic carbon supply and oxygen availability are suggested to be the most important factors in controlling foraminiferal niche partitioning (Jorissen and others, 1995; Van der Zwaan and others, 1999), foraminifera in turn are therefore thought to be good indi-

cators for the magnitude of organic pollution. Many authors have shown the effect of ongoing eutrophication (and usually subsequent anoxia) on foraminiferal community structure in the subrecent history (Alve, 1995a; Barmawidjaja and others, 1995; Thomas and others, 2000; Platon and others, 2005; Tsujimoto and others, 2006). Commonly, eutrophication leads to a loss of foraminiferal diversity and increasing dominance of one or a few species.
The southern North Sea and the Wadden Sea are both naturally rich in organic matter and therefore, the additional effects of human-induced eutrophication might be limiting (see Alve, 1995b for comparable analysis of seasonal flux of organic matter). Below, we will argue that in systems where environmental variability is high, the effects of eutrophication on the foraminiferal community structure could be limited. Of course, in oligotrophic or stable environments foraminifera are more likely to be influenced by eutrophication and/ or bottom water anoxia.

Implications for transfer functions
Murray (1991; 2001) has pointed out that (a combination of) many factors can limit foraminiferal distributions. This implies that in some cases the distribution of a species is limited by one environmental variable (e.g. low salinity), while elsewhere it is limited by another (e.g. abundance of a predator). This in turn, may explain why sometimes a species' distribution is correlated to one environmental parameter and sometimes to another. Therefore, transfer functions are potentially unreliable and should only be used to reconstruct the environmental parameters that are likely to be limiting foraminiferal abundances in that particular paleoenvironment.
Many foraminifera-based transfer functions are developed to reconstruct bottom water oxygen concentrations and organic carbon flux (Sen Gupta and others, 1996; Kaiho, 1999; Kuhnt and others, 1999; Wollenburg and Kuhnt, 2000; Osterman and others, 2005). Organic flux probably is the most important variable controlling foraminiferal abundances in deep sea environments, since most other factors are relatively stable or do not limit foraminferal growth or reproduction. Also in some fjords it has been shown that temporal anoxia of bottom waters are among the most important variables structuring the foraminiferal community. Therefore, foraminiferal records from such environments may indeed be used to reconstruct organic flux and/or oxygen concentrations (e.g. Gustafsson and Nordberg, 1999).
Our results, however, do not show a clear response of foraminiferal species composition to oxygen concentrations or total organic carbon content. Rather, we suggest that foraminifera from the Wadden Sea and North Sea can be used to reconstruct the type of food available and environmental variability.

Type of food
Lee (1974) has summarized a number of tracer studies that have shown that foraminifera are very selective in their feeding habit. Of the 28 algal species offered to different species of foraminifera (including *Ammonia beccarii*) only 4-5 were consumed in significant quantities. Some bacterial species are also ingested by foraminifera, although the consumed biomass is much lower than that of the ingested algae. It is also shown that the concentration of the food source is important for the growth rate of indi-

vidual foraminifera (Lee, 1974 and references therein).
In chapter 3, we have suggested that the composition of available food may be very important in structuring foraminiferal species composition. Several intertidal species are reported to have specific food preferences (Murray and Alve, 2000) or even test adaptations to feed on specific food sources (Austin and others, 2005). Various species of the genus *Ammonia* are regularly described to be able to feed on detritus and bacteria (*A. tepida*: Hohenegger and others, 1989: *A. beccarii*: Goldstein and Corliss, 1994) or are shown not to contain algal symbionts (*A. tepida*: Knight and Mantoura, 1985). On the other hand, *Haynesina germanica* and *Elphidium excavatum* have both been reported to feed primarily on fresh (living) phytodetritus or labile organic matter in general (Lopez, 1979; Alexander and Banner, 1984; Knight and Mantoura, 1985; Bernhard and Bowser; 1999; Gustafsson and Nordberg, 1999; Ward and others, 2003; Austin and others, 2005) and not on refractory organic matter (Ward and others, 2003; Topping and others, 2006). Abundances of these taxa could be used to develop simple transfer functions of food quality, given enough data on seasonal variation in the composition of organic carbon.

Environmental variability

Both surveyed environments are characterized by a high variability of environmental parameters. In the southern North Sea there are large seasonal differences in sea water temperature, bottom chlorophyll a content and nitrate availability (e.g. Gieskes and Kraay, 1977; Radach and others, 1990; Howarth and others, 1993; Hydes and others, 1999). In the Wadden Sea, these seasonal fluctuations are accompanied by large semidiurnal variations in temperature, salinity and physical disturbance. It has been suggested that foraminiferal diversity is usually low at intertidal flats due to low salinity (Hayward and Hollis, 1994) or high variability in temperature and salinity, despite high abundance of food (e.g. Alve and Murray, 1994). Only few species are able to withstand these fluctuations. Presence of such species at the Frisian Front (i.e. *Elphidium excavatum* and *Ammonia tepida*) may therefore indicate that habitats across the Frisian Front are characterized by high environmental variability.
The habitat of *Eggerella scabra* represents the area that is least affected by seasonal differences in temperature and food supply. Although variability in food supply, temperature, etc. also exists at the north edge of the front, they are likely to be less extreme than at or at the southern edge of the benthic front. This suggests that the realized niche of *E. scabra* is determined by a moderate influence of environmental variability and that the presence of this species is indicative for relatively stable conditions in shallow seas.

Wadden Sea

Three species are often described to live in temperate, intertidal mudflats: *Ammonia tepida* (sometimes referred to as *A. beccarii* forma *tepida*), *Elphidium excavatum* and *Haynesina germanica* (e.g. Murray 1991; Debenay and others, 2006). In our sampling surveys we have found mainly *A. tepida* and *H. germanica*, although others have described other combinations (e.g. *A. tepida* and *E. excavatum*; Hohenegger and others, 1989). Sen Gupta (1996) has suggested that the ratio between *Ammonia parkinsoniana* and *Elphidium excavatum* (i.e. the Ammonia-Elphidium index) can be used to reconstruct hypoxia. In our field surveys, however, relative numbers of *Ammonia* and

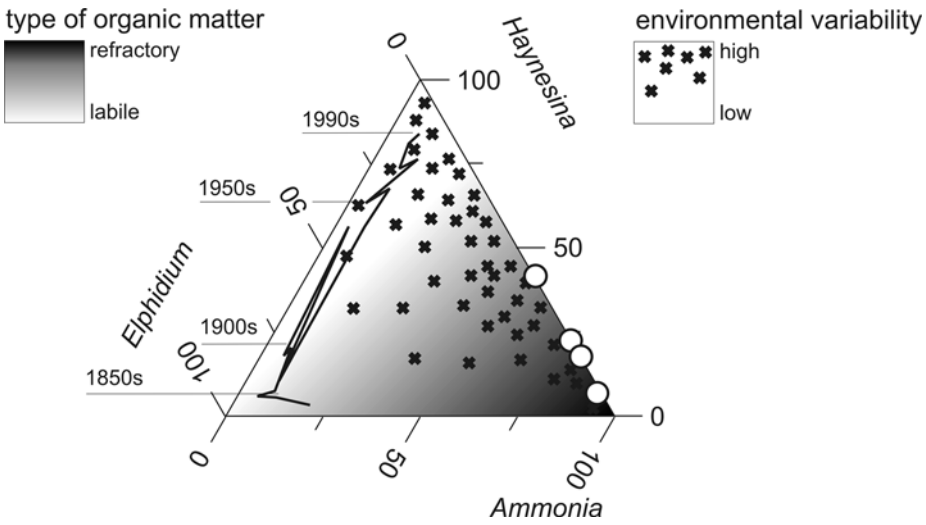


Figure 1: The troika-model: hypothetical relation between the relative abundances of *Ammonia*, *Elphidium* and *Haynesina* and their relation to environmental variability and quality of organic matter. Historical changes in Mok Bay is indicated by a black line. Average seasonal values near Den Oever are shown in open circles: from top to bottom circles represent samples taken in April, May, May, and June (number of samples: 12, 64, 24, and 48 respectively). See text and chapters 3 and 6 for more details.

Elphidium do not reflect bottom water hypoxia, but other sources of stress (or even disturbance) caused by a great amplitude of physico-chemical extremes. Furthermore, *Ammonia* may be replaced by *H. germanica*, depending on the type of food supplied. Therefore, we believe that the relative abundances of *Ammonia*, *Elphidium* and *Haynesina* in intertidal areas are determined by two other factors: type of food available and environmental variability, and their relative abundances can thus be used to semi-quantitatively characterize the environment in these terms. We will further refer to the relation between these environmental variables and the three foraminiferal species as the troika-model (fig 1).

In chapter 6, we described the development of foraminiferal species composition in Mok Bay in the past two centuries. The community composition shows a marked shift in dominance from *Elphidium excavatum* to *Haynesina germanica*. According to our troika-model, the shift in foraminiferal dominance in Mok Bay reflects primarily a change in environmental variability (fig 1). Besides the human-induced environmental changes in the Dutch Wadden Sea (completion of the Afsluitdijk in 1932, sealing of the former Zuiderzee; see Chapter 6) one of the reasons for this increase of local environmental amplitude may well be the progressive shallowing of the Mok Bay, reducing the buffer capacity of the water column against seasonal variations. Since the sampling location has always been subtidal, however, a constant supply of relatively fresh phytoplankton is assured, explaining the lack of excursions towards the *Ammonia* corner in fig 1. In con-

trast, the living assemblages from the Den Oever mud flat (chapter 3) reveals another type of variability. This intertidal area is permanently exposed to semidiurnal/seasonal temperature and salinity changes. Hence, all samples plot at the high-variability side of the graph. No change in the environmental variability signal was to be expected, and accordingly none was found in fig 1. Instead, we see that the seasonal development in food quality dominates.

Southern North Sea

Our study of the Frisian Front (chapter 5) shows that within a short bathymetrical range, completely different foraminiferal communities can exist. High abundances of *Ammonia tepida* and *Elphidium excavatum* indicate that, like at intertidal flats, across the Frisian Front there is relatively much environmental variability. Especially at the south edge of the benthic front, where abundances of these species are highest, there may be much variation in the arrival of organic matter and there may be occasional physical disturbance by tidal wave action. The location and timing of peak abundances of *E. excavatum* coincide with the organically enriched zone where input of fresh matter is temporarily high (fig 2). The distribution of *A. tepida* (higher in winter and at the center of

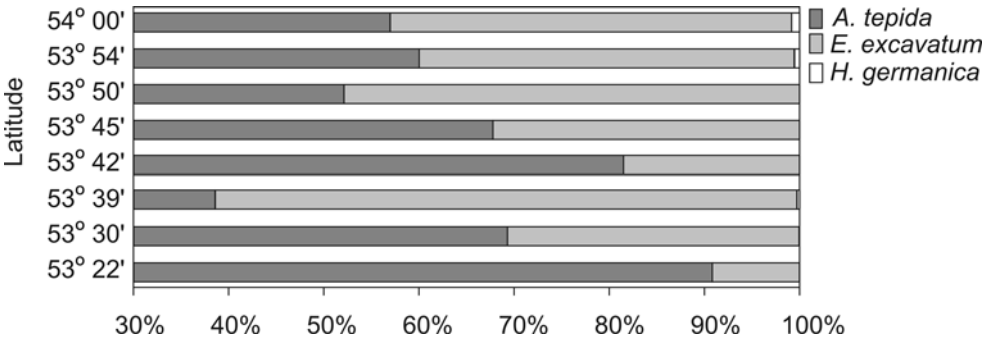


Figure 2: Relative distribution of *Ammonia tepida*, *Elphidium excavatum* and *Haynesina germanica* accross the Frisian Front. Center of the benthic front is located at 53° 42'.

the front), fits its common description of being able to feed primarily on refractory organic material.

Across the Frisian Front, *Eggerella scabra* is dominant in the northernmost stations, characterized by stratification and likely to experience relatively low environmental variability. The erratic occurrence of *Stainforthia fusiformis* confirms its opportunistic lifestyle (Alve, 1994; 1995b). The net result of the distribution of these species (see chapter 5 for details) is that the foraminiferal community structure correlates well with different hydrodynamic regimes. In the southern North Sea, high relative abundances of *Elphidium excavatum* indicate an environment with a high input of labile organic matter. *Eggerella scabra* on the other hand, represents an environment that is relatively stable: i.e. the area characterized by spring and summer stratification of the water layer, not influenced by tidal wave action, limited activity of bioturbators and a relative stable sup-

ply of food. Obviously, we do not know whether such patterns are found in the rest of the North Sea too. It could well be that in deeper, northern parts there is a correlation between organic flux and community structure since inter-seasonal differences are less pronounced and food is less abundant.

Perhaps, our most remarkable observation is that microhabitat separation apparently does not occur in our shallow water associations. this is in marked contrast with deeper water muddy environments. We suggest that this is due to the dynamics of the environment. Bioturbation and sediment movement cause regular disturbance of microhabitats and individual specimens are brought in regularly into the aerated surface layer, thus escaping the anoxic zone which is kept in place by the abundant organic matter.

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SAMENVATTING

Overal op aarde zorgt bevolkingsgroei en de daarmee gepaard gaande bouw van infrastructuur, industrialisatie en een toenemende energieconsumptie voor een achteruitgang van de biodiversiteit (o.a. Kerr en Currie, 1995; Pimm et al., 1995; Vitousek et al., 1997). Volgens schattingen heeft anthropogene activiteit tot op heden voor het uitsterven van ongeveer 20.000 á 2 miljoen soorten geleid (Wilson en Peter, 1998; Meyers, 1998; 1990). Dit verlies is vooral te wijten aan habitat-fragmentatie en habitat-destructie (Bellwood en Hughes, 2001; Travis, 2003), terwijl de recente stijging in temperatuur ook bijdraagt aan deze massa-extinctie (o.a. Root et al., 2003; Pounds et al., 2006). Afgezien van het uitsterven van soorten, zijn ook het functioneren van ecosystemen (Tilman, 1987; Duffy, 2003) en de kringloop van sommige elementen (o.a. Rast en Thornton, 1996; Exley, 2003) in de afgelopen eeuwen sterk aan verandering onderhevig. Kustgebieden bestaan uit zeer diverse ecosystemen (Ray, 1988), maar behoren tegelijkertijd tot de meest bedreigde ecosystemen die er bestaan. Enerzijds worden deze gebieden bedreigd door sterke eutrofiëring doordat hoge concentraties nutrienten en organisch materiaal worden aangevoerd door rivieren, anderzijds worden de habitats van ondiepe zeeën vernield door visserij en de bouw van dijken, inpoldering, etc. (o.a. Casey en Myers, 1998; Hutchings, 2000; Jackson et al., 2001; Lotze en Milewski, 2004).
De samenstelling en het functioneren van een ecosysteem is echter ook onderhevig aan veranderingen die natuurlijke oorzaken hebben, bijvoorbeeld door schommelingen in het klimaat. Om de effecten van de menselijke bijdrage aan ecosysteem-veranderingen goed te kunnen schatten, is inzicht in deze natuurlijke 'achtergrondvariatie' essentieel. Helaas zijn lange-termijn biologische monitoringsprogramma's zeldzaam en bevatten zij nooit de situatie van vóór het menselijk ingrijpen. Daarom kunnen we veranderingen in ecosystemen op lange termijn alleen onderzoeken met behulp van geologisch materiaal dat de resten bevat van vroegere omgevingen. Zulke resten zijn er in veel soorten en maten, waaronder fossielen, mineralen, stabiele isotopen, luchtbellen in Antarctisch ijs en specifieke moleculen van micro-organismen. Ieder van deze resten (zogenaamde proxies) kan worden gebruikt om een bepaald aspect van een vroeger ecosysteem of van het klimaat uit die tijd te reconstrueren. Door verschillende proxies te combineren (een multi-proxy benadering), kan een volledige reconstructie gemaakt worden van het klimaat, of een gebied of ecosysteem door de tijd heen.
Foraminiferen (Protisten) zijn nauwe verwanten van de amoeben, die vooral in de zee leven en een bijzondere eigenschap hebben dat de meeste soorten tot populaire proxies maakt: veelal maken deze organismen tijdens hun leven een schaaltje van van calciumcarbonaat (kalk). Omdat deze organismen veelvuldig voorkomen in de wereldzeeën en hun schaaltjes relatief makkelijk bewaard worden in de zeebodem, worden ze vaak gebruikt om het klimaat van vroeger te reconstrueren. Foraminiferen kunnen op twee manieren als proxy gebruikt worden. De eerste manier is door het tellen van verschil-

lende soorten in fossiel materiaal, waardoor de omgeving uit die periode aflezen kan worden aan de hand van het voorkomen van specifieke soorten. Zo'n aanpak is vooral succesvol als we goed weten welke soorten foraminiferen tegenwoordig indicator soorten zijn van bepaalde omgevingen. Om het voorkomen van levende foraminiferen in de ruimte en in de tijd te onderzoeken, worden er veldstudies gedaan tijdens welke er allerlei omgevingsvariabelen worden vergeleken met het voorkomen van verschillende foraminiferen. Positieve correlaties die gevonden zijn, kunnen vervolgens gebruikt worden om van fossiele monsters die omgevingsvariabelen te reconstrueren.
De tweede manier waarop foraminiferen gebruikt kunnen worden om het paleomilieu te reconstrueren, is door het analyseren van de chemische samenstelling van hun schaaltje. De verhouding van zuurstof-isotopen in foraminiferen-kalk reflecteert bijvoorbeeld de temperatuur van het zeewater en de totale hoeveelheid poolijs in de periode waarin het schaaltje groeide. Tijdens het produceren van het kalk (calcificatie) worden ook allerlei spore-elementen (zoals Mg, Ba, Cd, Zn, Cu) ingebouwd in het $CaCO_3$-rooster door te substitueren voor Ca. Naast de concentratie van deze spore-elementen of -metalen in het zeewater, zijn veel omgevingsparameters bepalend voor de hoeveelheid hiervan die wordt ingebouwd in het calciumcarbonaat. De inbouw van magnesium bijvoorbeeld wordt in grote mate bepaald door de temperatuur van het zeewater waarin calcificatie plaatsvindt. Zodoende wordt de concentratie Mg in fossiel kalk van foraminiferen (uitgedrukt als de Mg/Ca ratio) gebruikt als een afspiegeling van de vroegere zeewater temperatuur. De afhankelijkheid van een spore-element/ Ca ratio op temperatuur, saliniteit, pH en haar afhankelijkheid van de cellulaire activiteit van de foraminifeer is onbekend voor de meeste spore-elementen. Daarom is er biologisch onderzoek naar calcificatie nodig om de proxy-waarde van spore-elementen in kalk nader te bepalen.

Het oorspronkelijke doel van dit promotieonderzoek was te onderzoeken wat de relatie was tussen natuurlijke en menselijke invloeden op het functioneren van Nederlandse kustecosystemen gedurende de afgelopen 5.000 jaar. De groei van de menselijke populatie in Europa heeft er in de afgelopen eeuwen voor gezorgd dat steeds meer nutriënten werden afgevoerd door rivieren, waardoor de primaire productie toenam in kustwateren en de hoeveelheid organisch materiaal dat op de bodem terechtkwam, steeg. Via boorkernen genomen in de Noordzee, hoopten we te zien wat de effecten zijn geweest van de verschillende stadia van groei van de menselijke druk op op het functioneren van het mariene ecosysteem. Helaas bleek geschikt kernmateriaal, met daarin de geschiedenis van de afgelopen duizenden jaren in voldoende detail gedocumenteerd, niet voorhanden. In plaats daarvan richtte het onderzoek zich op het ontwikkelen van proxies om vervuiling te kunnen reconstrueren in ondiep water door foraminiferen te bestuderen uit de Noord- en Waddenzee. Resultaten uit deze studies zijn gebruikt om de geschiedenis te analyseren van de westelijke Waddenzee in de afgelopen twee eeuwen. In deze analyse wordt duidelijk dat ondanks natuurlijke variaties, de menselijke invloeden dramatische en plotselinge effecten kunnen hebben op de dynamiek van dit ecosysteem.
Onze resultaten laten ook zien dat in zulke omgevingen de soortensamenstelling van totale gemeenschap van benthische foraminiferen wellicht niet geschikt is als proxy om eutrofiëring te reconstrueren. Wat de soortensamenstelling van foraminiferen in ondiepe zeeën blijkbaar wel goed laat zien, zijn de kwaliteit van het aanwezige organisch

materiaal en verschillende hydrografische regimes. Als boormateriaal met een substantieel deel van het Holoceen beschikbaar zou zijn, denken we dat benthische foraminiferen bij uitstek geschikt zijn om de hydrografische evolutie van de Noordzee te reconstrueren. Of de soortensamenstelling van de foraminiferen-gemeenschap daarnaast ook nog beïnvloed wordt door eutrofiëring moet nog onderzocht worden door middel van experimenten of door veldstudies rondom hydrografische fronten in minder eutrofe gebieden.

In foraminiferen-onderzoek, wordt Bengaals Rood vaak gebruikt als zogenaamde *vital staining technique* om levende individuen te kunnen onderscheiden van lege schaaltjes of dode individuen. Helaas kleurt Bengaals Rood alle eiwit-bevattende schaaltjes, zodat individuen die net zijn doodgegaan ook worden gekleurd en dus als levend worden geteld, wat op zijn beurt resulteert in een overschatting van de het aantal levende individuen in een genomen monster. In **hoofdstuk 2**, wordt MTT gepresenteerd als een vital staining technique als alternatief voor het vaak gebruikte Bengaals Rood. MTT is een zogenaamd tetrazoliumzout dat wordt getransformeerd door enzymen van een gele, opgeloste stof in paarse formazan kristallen. Wanneer levende foraminiferen worden geïncubeerd met MTT, resulteert dat in een paarse kleuring van actieve individuen. We laten in dit hoofdstuk ook zien dat dagen na hun dood, individuen kunnen kleuren door aanwezige bacteriën die zich voeden met celmateriaal van de foraminiferen. Deze false positives worden zijn echter makkelijk te onderscheiden van levende, gekleurde individuen.

Ruimtelijke variatie in het voorkomen van foraminiferen (*patchiness*) zorgt voor een ander probleem bij het doen van veldonderzoek. In **hoofdstuk 3** presenteren we resultaten van een aantal kleine studies naar de ruimtelijke verspreiding van foraminiferen op een getijdeplaat in de Waddenzee. Deze studie bestaat uit drie verschillende onderdelen: één is uitgevoerd om de variatie op kleine schaal (centimeters) in kaart te brengen, de tweede is gedaan om de variatie op een grotere schaal (0,1-100 meter) te onderzoeken en de derde is uitgevoerd om de relatie in kaart te brengen tussen het voorkomen van foraminiferen en hun afstand tot de hoog- en laagwaterlijn. De resultaten laten zien dat de twee dominante soorten die in de Waddenzee leven (*Ammonia tepida* en *Haynesina germanica*) voorkomen in clusters met hoge aantallen van 175-300 cm^2 en dat de locatie van de clusters van de twee soorten sterk positief gecorreleerd zijn aan elkaar. Alleen op de grootste schaal (>50 meter) lijkt er ook een niet-willekeurige verspreiding te zijn van foraminiferen, terwijl er geen relatie is gevonden tussen aantallen foraminiferen en het hoog- of laagwater niveau. Het bleek ook dat ondanks grote verschillen in totale aantallen, de verhouding tussen de twee soorten overal opmerkelijk constant was. Deze verhouding echter, veranderde wel gedurende het jaar. Het lijkt erop alsof een seizonale parameter (bijvoorbeeld het type voedsel dat aanwezig is in de Waddenzee) ervoor zorgt dat de relatieve aantallen van *H. germanica* hoog zijn in het voorjaar en dat de aantallen *A. tepida* hoog zijn in de zomermaanden. Ruimtelijke variatie in het voorkomen van beide soorten lijkt daarentegen bepaald te worden door een andere parameter (bijvoorbeeld de totale hoeveelheid voedsel op een plek kunnen zijn).

In **hoofdstuk 4** presenteren we resultaten van een veldstudie waarin aantallen foraminiferen rondom het Friese Front (zuidelijke Noordzee) zijn onderzocht. Rondom dit *tidal mixing front* zijn verschillende hydrodynamische omgevingen aanwezig (constant gemengde waterlaag, een zone met een verhoogde productie en een gebied dat een gelaagde waterkolom heeft in de zomermaanden), die resulteren in een verscheidenheid aan bodemhabitats. Stations in deze verschillende habitats zijn bemonsterd in vier verschillende seizoenen om ruimtelijke en seizoensgebonden variaties in het voorkomen van soorten te kwantificeren. De resultaten laten zien dat de aanwezige soorten, meestal in zones op specifieke afstanden van het centrum van het front voorkomen. Verschillen tussen de seizoenen qua voorkomen van soorten waren relatief klein, terwijl seizoensgebonden verschillen in de dieptedistributie in de bovenste 5 centimeter van het sediment wel veranderde door de tijd. In de wintermaanden zijn individuen overwegend gelijk verdeeld in het sediment, terwijl in de zomer relatief veel individuen zich in de bovenste centimeter van de bodem ophouden. Deze resultaten suggereren dat deze soorten reageren op de komst van vers organisch materiaal op de zeebodem in de lente en in de vroege zomer door te migreren naar het sediment-water oppervlak of door een verhoogde reproductie in het ondiepe sediment.

De resultaten van de veldstudie uit hoofdstuk 4 zijn vergeleken met eenzelfde soort studie naar foraminiferen rondom het Friese Front in 1988 en 1989 (Moodley, 1990) en dit wordt gepresenteerd in **hoofdstuk 5**. De macrofauna in de bodem rondom het Friese Front is ook onderzocht in de periode 1982-2002, waarbij een plotselinge verandering werd gesignaleerd in de samenstelling van de fauna. Vóór 1992 werd de bodem van het Friese Front gedomineerd door de slangster *Amphiura filiformis*, een organisme dat zijn voedsel vergaart door het zeewater te filteren. Na 1995 domineerde de kreeft *Calianassa subterranea* de bodem rondom het Friese Front, die uitgebreide gangenstelsels graaft en zijn voedsel niet primair uit het water, maar uit de bodem haalt. Ondanks de effecten van deze *regime shift* op de fysieke staat van het Friese Front (er komt meer fijn materiaal in de waterkolom, de bodem wordt intensiever omgewoeld), is de soortensamenstelling van de foraminiferen relatief constant gebleven. Dit betekent dat het voorkomen van de foraminiferen rondom het Friese Front niet sterk wordt beïnvloed door deze ecologische en fysische veranderingen en dat zij kunnen dienen als robuuste proxies voor de verschillende habitats die worden bepaald door de aanwezigheid van het Friese Front.

Een reconstructie van bodem-ecosystemen in de Waddenzee, gebaseerd op het voorkomen van foraminiferen, wordt gepresenteerd in **hoofdstuk 6**. Dit wordt gedaan aan de hand van een boorkern die genomen is in de Mokbaai (westelijke Waddenzee) en die bestaat uit materiaal van de afgelopen 180 jaar. Het sediment in deze kern (2,8 meter lang) is gelamineerd, wat betekend dat het materiaal bestaat uit afwisselende, dunne laagjes van verschillende sediment-typen. De kern is in plakjes van 1 centimeter gesneden, waarna van elk van de monsters de totale hoeveelheid organisch materiaal is gemeten. Tegelijkertijd is de korrelgrootte van het sediment in elk van de 280 monsters bepaald, en zijn van elke tweede centimeter de benthische foraminiferen geteld. De resultaten van de verschillende bronnen worden vergeleken met historische data om tot een nauwkeurige reconstructie te komen van het Waddengebied. De foraminiferen

laten een plotselinge omslag in dominantie zien: vóór 1930 is *Elphidium excavatum* de dominante soort en na 1935 dalen deze aantallen en gaat *Haynesina germanica* domineren. De timing van deze verschuiving suggereert dat de bouw van de Afsluitdijk in 1932 een groot effect heeft gehad op het ecosysteem van de Waddenzee. Met de ecologische kennis van de twee besproken soorten (hoofdstukken 3 en 4), denken we dat de toegenomen variabiliteit in temperatuur en saliniteit in de Mokbaai na de constructie van de Afsluitdijk verantwoordelijk is voor de verschuiving in dominantie van de foraminiferen.

In **hoofdstuk 7** worden de resultaten gepresenteerd van experimenten die als doel hadden om te analyseren hoe foraminiferen koper (Cu) in hun kalk ($CaCO_3$) inbouwen. Deze relatie tussen de hoeveelheid koper in zeewater en de hoeveelheid koper die terechtkomt in het calciet van foraminiferen, kan worden uitgedrukt als de partitiecoëfficient van koper (D_{Cu}). Om de D_{Cu} te bepalen, hebben we individuen van twee soorten foraminiferen in cultuur gebracht en ze aan verschillende concentraties Cu blootgesteld. De Cu/Ca ratio in calciet die werd gevormd tijdens de experimenten werd gemeten met een combinatie van laser-ablatie en massa-spectrometrie. Door deze combinatie van methodes is het mogelijk om de chemische samenstelling te analyseren van een enkele kamer (ongeveer 80 µm of 0,08 mm in doorsnede) van de individuen die tijdens het experiment nieuw kalk hadden gemaakt. Na metingen bleek dat foraminiferen koper inbouwden met een D_{Cu} van 0,1-0,3. De effecten van temperatuur en saliniteit op de D_{Cu} was niet significant, terwijl de D_{Cu} voor beide soorten gelijk was ondanks de aanwezigheid (in *Heterostegina depressa*) of afwezigheid (in *Ammonia tepida*) van fotosynthetiserende symbionten. We denken dat met deze resultaten de Cu/Ca ratios in fossiele benthische foraminiferen gebruikt kunnen gaan worden om zware metalen-vervuiling te reconstrueren.

De conclusies van al deze hoofdstukken zijn samengevat in **hoofdstuk 8**. Ook de consequenties van de besproken resultaten voor het gebruik van foraminiferen om ecosystemen van kustwateren te reconstrueren worden hierin vermeld. In het zuiden van de Noordzee en in de Waddenzee lijkt het er niet op dat het voorkomen van foraminiferen beperkt of gereguleerd wordt door de hoeveelheid voedsel die aanwezig is of door de beschikbare hoeveelheid zuurstof (iets dat regelmatig wordt geclaimd). Ook lijkt het voorkomen van foraminiferen niet bepaald te worden door de aanwezige macrofauna, maar we denken dat de distributie van benthische foraminiferen vooral bepaald wordt door het type organisch materiaal dat aanwezig is en door de mate van omgevingsvariabiliteit in het systeem. Verschillende combinaties van deze twee parameters zijn aanwezig rondom hydrodynamische fronten en daarom zijn foraminiferen in ondiepe wateren vooral geschikt om hydrodynamische regimes te reconstrueren.

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This is it. My thesis is finished, but -needless to say- I haven't done it all by myself. It all started with Bert van der Zwaan asking me to join the strat/pal group in 2002 to work on Holocene foraminifera from the North Sea. Although the focus of my project changed a number of times over the years, he and my daily supervisor Ivo Duijnstee stimulated and supported me both scientifically and personally. In particular, Bert's talent for 'selling' scientific results and Ivo's honesty and enthusiasm for multivariate statistics (!) were truly inspirational.
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CURRICULUM VITAE

Lennart Jan de Nooijer was born on December 4th, 1978 in the city of Middelburg, the Netherlands. After secondary school, he started in 1996 with the higher laboratory education in Nijmegen. In 1997, he switched to study biology at the Radboud University (formerly called the Catholic University of Nijmegen). During his masters, he did a research project on population dynamics in benthic foraminifera at the Utrecht University and a project on the modelling of mating behavior at the University of Essex (UK). After his graduation in 2002, he started a PhD at the Utrecht University under the supervision of Prof Dr van der Zwaan and Dr Duijnstee, working on living and subrecent benthic foraminifera from the Dutch Wadden Sea and southern North Sea.

APPENDIX I

In-sediment oxygen profiles measured at stations across the Frisian Front. Values are in %, relative to completely saturated seawater as measured before profiling.

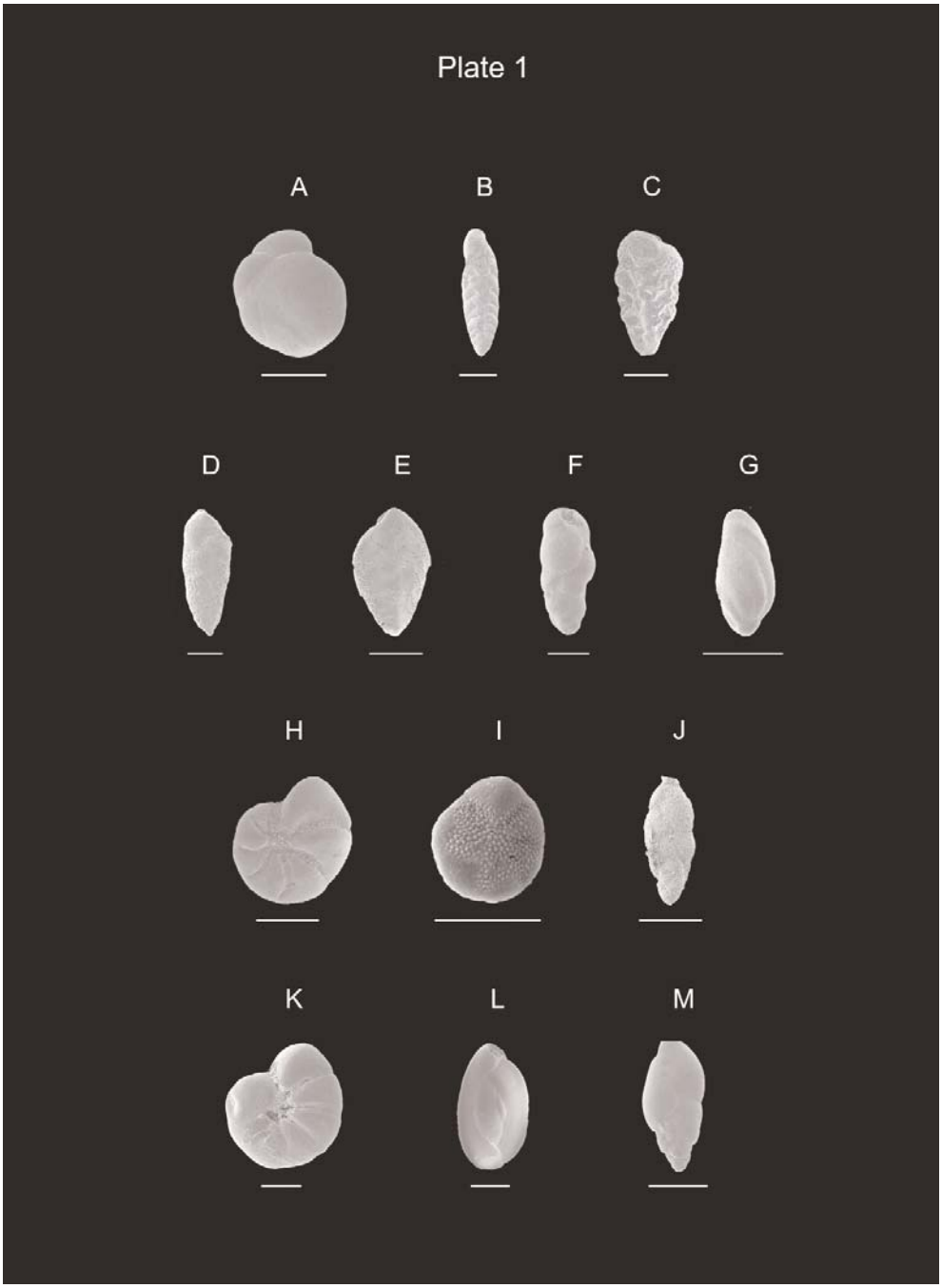
Depth in sediment (cm)	53° 39'		53° 45'		53°54'		54° 00'
4.5	98	93					
1.8			64	63			98
0.95					84	73	
0.45							92
0.3	88	85					89
0.15			59	61	73	63	88
0 (sediment-water interface)	50	83	25	36	61	49	62
-0.15	24	38	9.1	11	32	28	15
-0.3	9.2	13	5.3	6.0	20	20	5.9
-0.45	4.1	8.2	3.8	3.7	8.1	13	4.4
-0.6	2.0	5.7	2.3	1.5	8.1	3.8	3.7
-0.75	1.0	3.3	1.5	0.75	2.7	3.8	2.9
-0.9	0	1.6	0.76	0.75	1.4	1.3	1.5
-1.05	0	0.82			1.4	1.3	1.5
-1.2	0	0.82					0.74
-1.3	0	0.82					
-1.8	0	0.82	0	0	1.4	1.3	
-2.2							0.74
-2.8	0	0.82	0	0	1.35	0	
-3.2							0
-3.8					0	0	
after profiling:							
1.25					70	64	
1.8			59	74			90
4.5	65	73					

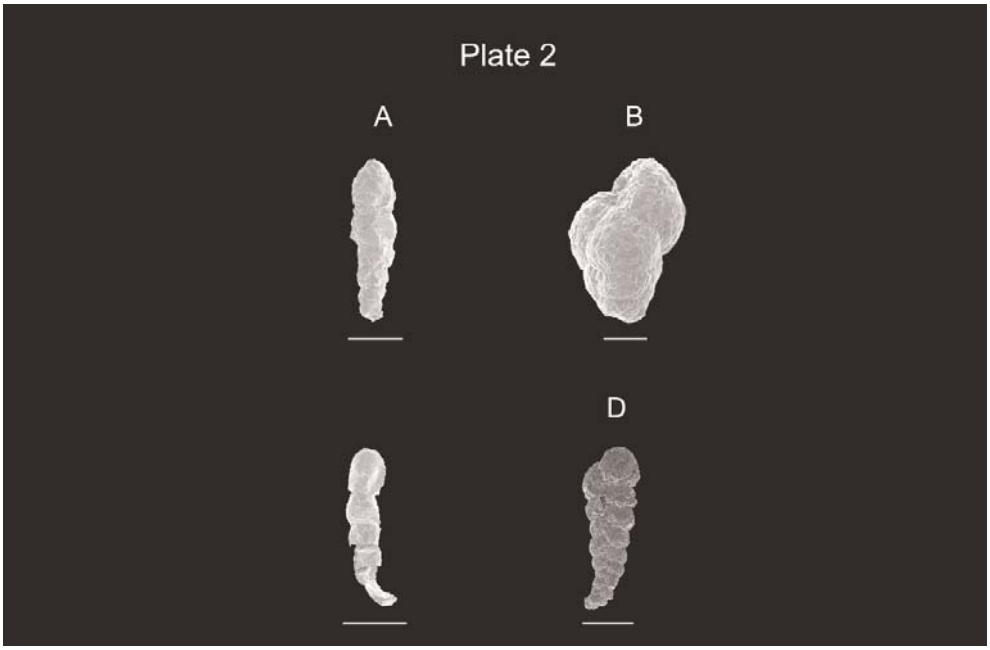
Table 1: Oxygen profiles measured in December.

Depth in sediment (cm)	53° 22'		53° 42'	
0.2	66	78	43	51
0.1	67	66	43	43
0 (sediment-water interface)	65	34	42	22
-0.1	48	15	32	10
-0.2	32	7.7	21	5.0
-0.3	11	2.8	6.9	1.8
-0.4	3.1	1.4	2.0	0.9
-0.5	2.8	1.2	1.8	0.8
-0.6	1.5	1.2	1.0	0.8
-0.7	1.4	1.2	0.9	0.8
-0.8	1.4	0	0.9	0

Table 2: Oxygen profiles measured in February.

APPENDIX II





In all figures the scale bar represents 100 μm.

Plate 1

A	*Ammonia tepida*
B	*Bolivina dilatata*
C	*Bolivina pseudoplicata*
D	*Bolivina seminuda*
E	*Bolivina spathulata*
F	*Bulimina elongata*
G	*Buliminella elegantissima*
H	*Elphidium excavatum*
I	*Epistominella vitrea*
J	*Hopkinsina pacifica*
K	*Nonion depressulus*
L	*Quinqueloculina* sp.
M	*Stainforthia fusiformis*

Plate 2

A	*Acostata mariae*
B	*Eggerella scabra*
C	*Leptohalysis scottii*
D	*Textularia* sp.

APPENDIX III

Foraminiferal abundances at various sample moments and stations. Replicate samples are indicated by A and B. n.a. = not available.

December 2002, 53° 39' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	4	0	3	4	2	4	4
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	81	64	47	57	99	89	68
Asterigerinata mamilla	0	0	0	1	1	2	2
Bolivina dilatata	4	0	3	3	4	7	4
Bolivina pseudoplicata	0	0	1	2	5	6	6
Bolivina seminuda	1	9	7	10	4	8	4
Bolivina spathulata	0	0	0	0	0	0	0
Bulimina marginata/ elongata	7	4	10	12	37	20	33
Buliminella elegantissima	2	2	9	7	9	26	40
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	47	71	64	59	159	193	126
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	55	18	14	33	114	253	508
Epistominella exigua	0	0	0	0	0	0	3
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	2	0	0	0	0	2	2
Haynesina germanica	7	0	0	0	0	0	0
Hopkinsina pacifica	4	14	59	38	44	38	21
Hyalinea baltica	0	0	0	0	0	1	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	1	0	0	0	0	1	0
Nonion depresslus	1	10	5	6	9	9	14
Nonionella turgida	0	1	1	0	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	35	36	19	39	42	29	22
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	9	35	70	39	75	70	95
Textularia sp.	2	0	2	5	8	11	6
indeterminable	2	0	0	1	2	0	2
total	**264**	**264**	**314**	**316**	**614**	**769**	**960**

December 2002, 53° 39' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	12	9	1	8	18	15	14
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	89	93	75	47	65	41	52
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	5	11	8	1	4	8	3
Bolivina pseudoplicata	9	9	4	5	7	4	5
Bolivina seminuda	0	1	0	2	0	0	0
Bolivina spathulata	2	0	4	0	6	2	0
Bulimina marginata/ elongata	27	12	6	14	18	14	5
Buliminella elegantissima	0	7	2	2	9	15	10
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	44	54	54	57	98	139	113
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	136	49	56	33	74	129	222
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	3	3	0	0	2	2	1
Fissurina sp.	5	0	0	0	5	0	0
Haynesina germanica	1	1	0	1	0	0	0
Hopkinsina pacifica	0	13	13	17	63	53	27
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	0	0	0	0
Nonion depresslus	2	5	0	3	0	2	0
Nonionella turgida	3	3	0	0	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	37	23	20	8	13	15	8
Rosalina sp.	2	0	0	0	2	2	2
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	10	14	19	43	82	31	21
Textularia sp.	0	0	2	2	26	15	11
indeterminable	0	0	0	0	0	0	0
total	**387**	**308**	**264**	**244**	**491**	**487**	**494**

December 2002, 53° 45' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	13	5	5	4	2	17	14
Ammodiscus sp.	0	0	1	0	0	0	0
Ammonia tepida	104	104	66	47	78	95	129
Asterigerinata mamilla	0	1	0	0	0	3	0
Bolivina dilatata	6	24	32	18	35	29	15
Bolivina pseudoplicata	4	6	9	5	2	16	3
Bolivina seminuda	5	13	28	30	11	4	6
Bolivina spathulata	12	0	3	3	8	13	21
Bulimina marginata/ elongata	42	38	35	9	14	46	35
Buliminella elegantissima	8	4	3	7	19	24	21
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	1
Dentalina sp.	1	0	0	0	0	0	0
Eggerella scabra	266	285	219	169	238	353	368
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	62	47	34	32	38	102	53
Epistominella exigua	0	0	1	1	0	0	0
Epistominella vitrea	0	0	0	0	0	2	3
Fissurina sp.	1	0	5	3	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	39	56	106	117	156	196	173
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	2	0	0	0
Lenticulina sp.	2	2	2	4	0	0	0
Leptohalysis scottii	0	0	2	0	1	0	0
Nonion depresslus	0	8	1	1	0	3	0
Nonionella turgida	4	0	15	4	6	0	1
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	24	15	14	9	8	16	10
Rosalina sp.	2	0	0	0	0	0	2
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	22	54	111	127	150	66	75
Textularia sp.	0	3	4	2	0	6	2
indeterminable	2	0	3	1	2	0	1
total	**619**	**665**	**699**	**595**	**768**	**991**	**933**

December 2002, 53° 45' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	3	1	1	4	4	5	1
Ammodiscus sp.	0	0	0	1	0	0	0
Ammonia tepida	107	86	54	49	56	53	54
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	8	5	18	6	19	10	4
Bolivina pseudoplicata	8	0	1	4	11	7	4
Bolivina seminuda	1	0	4	2	2	0	2
Bolivina spathulata	23	6	25	4	6	0	2
Bulimina marginata/ elongata	22	14	25	9	23	19	13
Buliminella elegantissima	5	2	3	5	3	17	7
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	1	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	238	117	170	109	177	180	154
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	31	11	21	25	23	26	32
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	3	1	4	2	1	2	0
Fissurina sp.	0	0	5	0	5	0	5
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	23	15	61	46	55	46	61
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	1	2	2	0
Leptohalysis scottii	0	0	0	0	0	0	0
Nonion depresslus	0	0	0	0	2	0	0
Nonionella turgida	1	0	0	1	3	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	10	6	4	4	4	0	2
Rosalina sp.	2	0	2	0	5	2	0
Saccamina sp.	3	1	4	2	1	0	7
Stainforthia fusiformis	19	3	28	9	38	17	19
Textularia sp.	0	2	3	2	5	3	0
indeterminable	0	0	0	0	0	0	0
total	**506**	**270**	**434**	**284**	**445**	**390**	**367**

December 2002, 53° 54' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	1	1	5	7	6	7	15
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	23	11	9	7	15	13	10
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	5	6	7	6	7	5	0
Bolivina pseudoplicata	7	0	1	0	0	2	3
Bolivina seminuda	11	3	1	1	1	0	1
Bolivina spathulata	7	0	3	3	0	3	2
Bulimina marginata/ elongata	13	4	1	0	4	5	1
Buliminella elegantissima	10	3	6	6	25	20	3
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	2	0	1	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	89	94	83	139	337	293	315
Elphidium advenum	0	2	0	0	0	0	0
Elphidium excavatum	5	8	6	0	12	10	16
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	4	3
Fissurina sp.	2	0	0	0	0	1	0
Haynesina germanica	0	0	1	0	1	0	0
Hopkinsina pacifica	8	16	39	29	76	53	8
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	1	2	3	4	6	5	0
Lenticulina sp.	0	0	0	0	0	1	0
Leptohalysis scottii	8	1	0	2	4	11	4
Nonion depresslus	0	0	0	0	0	1	0
Nonionella turgida	9	3	6	6	7	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	3	5	1	2	4	8	4
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	5
Stainforthia fusiformis	8	6	15	14	27	36	5
Textularia sp.	2	2	2	0	2	0	8
indeterminable	3	2	2	0	0	0	1
total	**217**	**169**	**192**	**226**	**534**	**478**	**405**

December 2002, 53° 54' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	31	5	0	1	13	5	8
Ammodiscus sp.	0	0	0	0	0	0	1
Ammonia tepida	38	10	3	6	13	6	10
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	14	8	3	1	8	0	5
Bolivina pseudoplicata	15	1	0	1	0	1	5
Bolivina seminuda	0	1	1	0	1	0	0
Bolivina spathulata	8	0	0	0	0	0	0
Bulimina marginata/ elongata	26	4	4	5	3	2	4
Buliminella elegantissima	16	7	2	2	7	3	17
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	1	0	0	0	0	0	0
Eggerella scabra	491	196	53	94	194	168	333
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	27	4	1	1	7	1	11
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	3	2	0	0	1	0	0
Fissurina sp.	0	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	15	13	6	25	21	32	30
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	1	0	1
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	2	0	0	0
Nonion depresslus	0	0	0	0	0	1	0
Nonionella turgida	13	1	7	0	0	0	0
Pyrgo williamsoni	1	0	0	0	0	0	0
Quinqueloculina spp.	6	1	3	2	1	0	6
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	70	20	2	9	25	7	31
Stainforthia fusiformis	9	10	5	3	17	5	12
Textularia sp.	3	0	0	0	2	3	0
indeterminable	0	0	0	0	0	0	1
total	**785**	**285**	**90**	**153**	**313**	**236**	**476**

December 2002, 54° 00' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	0	4	1	6	7	2	n.a.
Ammodiscus sp.	0	0	0	0	0	0	n.a.
Ammonia tepida	21	18	5	5	3	3	n.a.
Asterigerinata mamilla	0	0	0	0	0	0	n.a.
Bolivina dilatata	2	6	4	3	2	4	n.a.
Bolivina pseudoplicata	1	1	0	0	0	0	n.a.
Bolivina seminuda	0	3	0	0	0	0	n.a.
Bolivina spathulata	3	6	2	0	0	2	n.a.
Bulimina marginata/ elongata	4	6	3	4	2	2	n.a.
Buliminella elegantissima	2	2	10	4	3	11	n.a.
Caronia sylvestrii	0	0	1	0	0	0	n.a.
Cassidulina sp.	0	0	0	0	0	0	n.a.
Dentalina sp.	0	0	0	0	0	0	n.a.
Eggerella scabra	133	215	172	115	189	222	n.a.
Elphidium advenum	0	0	0	0	0	0	n.a.
Elphidium excavatum	0	3	1	0	1	1	n.a.
Epistominella exigua	0	0	0	0	0	0	n.a.
Epistominella vitrea	2	0	0	1	0	1	n.a.
Fissurina sp.	0	0	0	0	0	0	n.a.
Haynesina germanica	0	0	0	0	0	0	n.a.
Hopkinsina pacifica	4	4	7	0	3	4	n.a.
Hyalinea baltica	0	0	0	0	0	0	n.a.
Lagena sp.	0	2	3	2	0	2	n.a.
Lenticulina sp.	0	0	0	0	0	0	n.a.
Leptohalysis scottii	0	0	0	1	1	1	n.a.
Nonion depresslus	0	0	0	0	1	0	n.a.
Nonionella turgida	0	3	3	0	0	0	n.a.
Pyrgo williamsoni	0	0	0	0	0	0	n.a.
Quinqueloculina spp.	2	4	4	3	0	1	n.a.
Rosalina sp.	0	0	0	0	0	0	n.a.
Saccamina sp.	0	0	0	7	3	3	n.a.
Stainforthia fusiformis	0	3	6	0	4	4	n.a.
Textularia sp.	0	1	1	3	3	0	n.a.
indeterminable	0	0	0	0	0	0	n.a.
total	**174**	**281**	**223**	**154**	**222**	**263**	**n.a.**

December 2002, 54° 00' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	5	1	1	9	4	9	6
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	10	5	3	4	0	2	4
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	3	6	3	1	3	1	3
Bolivina pseudoplicata	3	1	1	1	3	1	1
Bolivina seminuda	1	1	0	1	0	0	2
Bolivina spathulata	6	0	4	2	2	0	2
Bulimina marginata/ elongata	8	6	0	8	6	3	4
Buliminella elegantissima	2	3	9	14	40	3	23
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	162	102	223	249	251	182	201
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	1	3	1	1	14	2	4
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	2	2	4	2	0	1	2
Fissurina sp.	0	0	0	0	5	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	2	4	6	15	9	4	6
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	1	1	1	0	1
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	0	0	2	0
Nonion depresslus	0	0	0	0	0	0	0
Nonionella turgida	0	0	3	0	1	1	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	5	2	2	0	6	0	2
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	51	43	72	42	44	45	25
Stainforthia fusiformis	2	3	7	21	19	0	5
Textularia sp.	3	0	6	2	8	3	1
indeterminable	0	0	0	0	0	0	0
total	**265**	**184**	**345**	**375**	**415**	**260**	**293**

June 2003, 53° 39' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	27	5	0	5	5	2	2
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	131	14	6	4	26	48	47
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	11	2	1	0	2	2	7
Bolivina pseudoplicata	4	1	0	4	1	17	5
Bolivina seminuda	5	4	0	0	0	0	4
Bolivina spathulata	1	0	0	0	0	0	1
Bulimina marginata/ elongata	35	28	1	1	4	9	3
Buliminella elegantissima	37	0	9	12	14	7	10
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	138	15	8	18	25	34	15
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	704	171	74	38	102	139	153
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	1	0	0	1	0
Fissurina sp.	1	0	0	0	0	1	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	29	3	3	2	0	12	43
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	1
Leptohalysis scottii	5	4	0	0	0	0	0
Nonion depresslus	11	0	0	0	0	0	0
Nonionella turgida	7	9	0	0	2	0	5
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	65	5	2	0	0	3	2
Reophax monoliformis	0	0	0	1	0	0	0
Rosalina sp.	0	0	0	0	2	0	1
Saccamina sp.	1	0	1	0	0	0	1
Stainforthia fusiformis	76	56	30	19	37	21	43
Textularia sp.	18	4	0	0	0	7	0
indeterminable	6	0	1	0	4	2	0
total	**1312**	**321**	**137**	**104**	**224**	**305**	**343**

June 2003, 53° 39' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	28	7	1	1	0	3	3
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	98	14	8	9	23	31	25
Asterigerinata mamilla	0	0	1	0	0	0	0
Bolivina dilatata	21	4	1	0	1	7	6
Bolivina pseudoplicata	10	5	2	2	0	14	5
Bolivina seminuda	8	0	0	1	0	0	0
Bolivina spathulata	2	0	0	1	0	0	0
Bulimina marginata/ elongata	32	8	2	0	7	7	5
Buliminella elegantissima	49	19	7	0	4	3	3
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	163	22	12	5	11	21	35
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	1098	228	78	47	34	112	89
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	1	0	0	0	0
Fissurina sp.	0	0	1	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	41	27	2	2	7	14	70
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	8	2	0	0	0	0	2
Nonion depresslus	3	0	0	0	0	0	0
Nonionella turgida	19	0	0	0	1	13	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	119	16	4	4	2	3	1
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	1	0	0	1	0
Saccamina sp.	6	0	0	0	0	0	0
Stainforthia fusiformis	128	55	25	12	20	21	43
Textularia sp.	10	2	0	0	0	2	0
indeterminable	5	3	1	1	0	2	1
total	**1848**	**413**	**147**	**85**	**109**	**253**	**287**

June 2003, 53° 50' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	10	0	0	1	1	0	0
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	27	11	1	20	15	23	27
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	16	7	0	0	0	4	0
Bolivina pseudoplicata	18	3	0	1	0	1	3
Bolivina seminuda	5	0	2	0	0	1	1
Bolivina spathulata	18	6	0	0	6	12	6
Bulimina marginata/ elongata	11	0	1	3	0	5	12
Buliminella elegantissima	21	36	13	3	3	13	10
Caronia sylvestrii	1	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	302	27	9	16	31	16	57
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	56	13	8	3	5	3	20
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	4	0	0	1	0	2	0
Fissurina sp.	0	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	41	68	18	11	21	27	103
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	2	0	0	0	0	1	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	2	2	2	0	5	0	0
Nonion depresslus	7	1	0	0	1	0	0
Nonionella turgida	13	11	4	0	0	0	2
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	266	15	7	6	0	8	3
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	5	0	0	0	0	0	0
Saccamina sp.	2	0	1	0	1	1	1
Stainforthia fusiformis	87	91	24	7	7	35	30
Textularia sp.	15	2	0	0	0	0	2
indeterminable	1	1	0	0	0	1	0
total	**930**	**294**	**90**	**72**	**96**	**153**	**277**

June 2003, 53° 50' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	5	0	0	1	0	1	0
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	43	8	26	14	25	16	16
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	5	4	8	4	4	5	10
Bolivina pseudoplicata	4	3	5	1	7	4	0
Bolivina seminuda	6	4	4	1	2	1	1
Bolivina spathulata	4	10	8	0	2	4	10
Bulimina marginata/ elongata	12	5	12	10	6	3	11
Buliminella elegantissima	28	5	7	12	5	7	24
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	1	0	0	0	0	0	0
Eggerella scabra	94	27	74	47	17	46	57
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	52	9	26	21	13	4	16
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	3	0	0	0	0	0	0
Fissurina sp.	0	0	0	0	5	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	67	279	194	110	63	27	53
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	1	0	1	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	2	4	0	2	4	0	0
Nonion depresslus	2	1	1	0	0	0	1
Nonionella turgida	17	13	10	16	18	6	18
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	153	24	13	8	25	11	11
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	2	2	0	2	0
Saccamina sp.	0	0	0	0	0	1	0
Stainforthia fusiformis	38	71	47	57	59	42	33
Textularia sp.	8	0	0	2	0	2	0
indeterminable	1	0	1	0	0	0	0
total	**546**	**467**	**439**	**308**	**255**	**182**	**261**

June 2003, 53° 54' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	5	1	1	9	1	8	0
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	8	7	4	7	11	11	3
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	9	4	0	0	3	4	3
Bolivina pseudoplicata	0	1	1	1	0	1	0
Bolivina seminuda	9	0	0	0	0	1	0
Bolivina spathulata	1	0	2	0	0	0	0
Bulimina marginata/ elongata	8	1	0	0	0	1	2
Buliminella elegantissima	13	5	9	7	16	3	10
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	69	35	42	84	119	140	128
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	2	4	3	1	4	1	3
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	1	0	0	0	1	0	2
Fissurina sp.	1	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	7	13	6	15	8	2	15
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	4	0	0	0	1	1	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	1	0	0	0	0	0	0
Nonion depresslus	4	0	2	0	0	0	0
Nonionella turgida	2	3	6	6	1	6	3
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	43	9	5	2	2	3	2
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	1	1	1	1	2	1	1
Stainforthia fusiformis	52	17	24	26	43	10	38
Textularia sp.	2	2	0	2	0	0	0
indeterminable	8	0	0	1	0	0	0
total	**267**	**103**	**106**	**162**	**212**	**193**	**210**

June 2003, 53° 54' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	6	8	6	3	9	10	6
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	3	8	6	2	3	6	8
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	3	0	3	5	3	0	1
Bolivina pseudoplicata	1	1	4	1	0	5	0
Bolivina seminuda	0	0	0	0	0	0	0
Bolivina spathulata	0	0	2	2	0	0	2
Bulimina marginata/ elongata	0	2	1	0	2	5	0
Buliminella elegantissima	7	7	16	7	7	24	5
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	133	128	97	65	131	168	143
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	12	9	6	3	3	7	5
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	2	3	5	1	1	1	1
Fissurina sp.	0	5	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	9	6	6	4	13	9	4
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	3	1	0	2	0	1	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	0	0	4	0
Nonion depresslus	0	0	1	0	0	1	0
Nonionella turgida	3	0	6	3	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	81	75	80	35	58	21	7
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	2	0
Saccamina sp.	0	6	1	0	11	2	1
Stainforthia fusiformis	24	23	26	10	21	31	12
Textularia sp.	5	2	3	0	0	0	0
indeterminable	0	1	2	0	0	0	0
total	**292**	**285**	**271**	**143**	**262**	**297**	**195**

June 2003, 54° 00' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	5	3	10	5	3	4	9
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	16	3	3	5	3	6	6
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	30	4	3	1	0	0	5
Bolivina pseudoplicata	15	0	3	4	1	5	4
Bolivina seminuda	5	0	0	0	0	0	0
Bolivina spathulata	2	0	0	0	0	0	6
Bulimina marginata/ elongata	8	1	3	1	1	1	3
Buliminella elegantissima	144	45	36	30	23	14	24
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	2	1	0	0	0	0	0
Eggerella scabra	269	113	158	157	124	174	178
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	74	6	1	10	7	11	6
Epistominella exigua	1	0	0	0	0	0	0
Epistominella vitrea	5	1	1	2	2	1	0
Fissurina sp.	20	0	0	5	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	23	6	4	6	4	9	4
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	10	1	2	2	1	0	2
Lenticulina sp.	1	0	0	0	0	0	0
Leptohalysis scottii	41	4	10	6	0	0	6
Nonion depresslus	0	0	0	0	0	0	0
Nonionella turgida	21	4	0	0	3	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	56	2	5	12	2	0	2
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	5	0	0	0	0	0	0
Saccamina sp.	0	0	1	0	10	0	0
Stainforthia fusiformis	480	78	35	42	21	23	19
Textularia sp.	23	0	4	2	3	3	0
indeterminable	11	0	0	0	0	0	0
total	**1267**	**272**	**279**	**290**	**208**	**251**	**274**

June 2003, 54° 00' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	6	6	8	5	12	4	8
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	6	2	2	3	3	8	11
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	10	4	3	1	3	4	1
Bolivina pseudoplicata	12	3	4	1	1	4	3
Bolivina seminuda	0	0	2	0	1	0	1
Bolivina spathulata	0	0	2	0	0	0	0
Bulimina marginata/ elongata	0	4	2	4	4	0	4
Buliminella elegantissima	31	30	26	26	28	17	5
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	129	94	153	124	223	160	258
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	20	19	22	12	16	2	2
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	5	11	12	6	6	2	2
Fissurina sp.	0	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	8	2	8	2	11	4	15
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	1	0	3	4	4	1	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	14	0	0	0	0	0	0
Nonion depresslus	1	0	0	0	0	1	0
Nonionella turgida	0	0	0	0	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	24	5	5	7	2	4	1
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	5	0	5	0
Stainforthia fusiformis	45	71	31	30	19	5	7
Textularia sp.	8	3	3	2	8	0	0
indeterminable	0	0	0	0	0	0	0
total	**321**	**253**	**286**	**232**	**340**	**222**	**318**

August 2004, 53° 30' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	0	0	0	0	0	0	0
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	2	1	5	4	0	4	2
Asterigerinata mamilla	0	1	1	0	1	0	0
Bolivina dilatata	1	0	0	0	0	0	0
Bolivina pseudoplicata	0	0	0	0	0	0	0
Bolivina seminuda	0	0	0	0	0	0	0
Bolivina spathulata	0	0	0	0	0	0	0
Bulimina marginata/ elongata	2	0	0	0	0	0	0
Buliminella elegantissima	0	2	1	0	0	0	0
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	0	0	0	0	0	0	0
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	2	2	1	0	3	0	0
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	0	0	1	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	0	0	0	0	0	0	0
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	0	0	0	0
Nonion depresslus	0	1	0	1	2	1	0
Nonionella turgida	0	0	0	0	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	5	2	1	0	2	0	0
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	0	0	0	0	0	0	0
Textularia sp.	0	0	0	0	0	0	0
indeterminable	0	0	0	0	0	0	0
total	**12**	**9**	**10**	**5**	**8**	**5**	**2**

August 2004, 53° 39' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	14	0	4	1	1	3	3
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	171	53	26	16	3	11	16
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	1	0	0	0	1	0	1
Bolivina pseudoplicata	5	4	1	0	0	0	1
Bolivina seminuda	7	2	1	0	0	0	0
Bolivina spathulata	6	0	0	0	0	0	0
Bulimina marginata/ elongata	18	3	2	1	0	0	3
Buliminella elegantissima	3	3	7	9	14	0	3
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	165	39	27	33	31	27	34
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	42	13	20	29	35	9	17
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	5	0	5	0	0	0	0
Haynesina germanica	1	0	1	0	0	0	0
Hopkinsina pacifica	27	9	4	2	8	0	9
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	36	44	4	4	2	1	0
Nonion depresslus	3	0	3	0	0	1	2
Nonionella turgida	3	0	0	0	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	261	38	22	9	10	9	13
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	1	0	0	2	2
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	9	5	2	3	10	7	7
Textularia sp.	6	5	2	0	0	0	0
indeterminable	0	0	0	0	0	0	0
total	**783**	**219**	**133**	**108**	**115**	**69**	**112**

August 2004, 53° 39' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	9	4	3	1	0	1	4
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	187	52	10	36	15	12	38
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	9	3	3	1	0	4	0
Bolivina pseudoplicata	7	0	0	4	0	0	1
Bolivina seminuda	2	1	0	1	0	0	0
Bolivina spathulata	0	0	0	0	0	0	0
Bulimina marginata/ elongata	27	3	1	5	0	2	1
Buliminella elegantissima	2	3	3	5	0	2	3
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	150	56	39	33	33	33	69
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	24	35	18	30	14	20	17
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	0	0	0	5	5	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	40	2	8	6	4	11	6
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	55	24	6	4	2	8	0
Nonion depresslus	0	0	3	3	0	0	0
Nonionella turgida	9	3	0	0	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	262	30	19	11	10	6	11
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	10	5	9	5	6	5	2
Textularia sp.	3	3	3	0	2	2	2
indeterminable	0	0	0	0	0	0	0
total	**796**	**224**	**124**	**150**	**91**	**106**	**154**

August 2004, 54° 00' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	4	10	3	5	14	9	12
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	26	34	6	9	13	17	13
Asterigerinata mamilla	0	0	1	0	0	0	1
Bolivina dilatata	6	1	1	3	1	1	3
Bolivina pseudoplicata	1	1	1	1	1	1	1
Bolivina seminuda	1	0	0	1	0	1	1
Bolivina spathulata	4	0	2	0	2	6	8
Bulimina marginata/ elongata	3	0	4	0	8	4	7
Buliminella elegantissima	16	10	14	7	24	7	21
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	124	130	87	78	291	173	209
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	4	0	0	0	0	2	1
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	1	0	0
Fissurina sp.	11	0	0	5	5	0	0
Haynesina germanica	1	0	0	0	0	0	0
Hopkinsina pacifica	11	8	6	2	30	6	11
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	1	0	1	1	1	0	1
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	11	2	0	0	0	8	8
Nonion depresslus	0	0	0	0	0	0	0
Nonionella turgida	3	0	0	0	2	3	3
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	14	11	7	3	6	1	3
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	4	1	8	0	6	1	7
Stainforthia fusiformis	0	3	2	5	26	7	9
Textularia sp.	5	3	0	7	3	1	0
indeterminable	0	0	0	0	0	0	0
total	**250**	**215**	**142**	**127**	**435**	**248**	**318**

August 2004, 54° 00' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	4	1	10	5	9	3	9
Ammodiscus sp.	1	0	0	0	0	0	0
Ammonia tepida	16	9	8	3	11	11	4
Asterigerinata mamilla	0	0	0	1	1	1	1
Bolivina dilatata	11	3	1	3	0	1	1
Bolivina pseudoplicata	0	0	0	3	1	0	1
Bolivina seminuda	1	0	0	0	0	2	1
Bolivina spathulata	0	2	2	2	0	0	2
Bulimina marginata/ elongata	3	1	0	1	1	6	1
Buliminella elegantissima	10	16	10	3	24	10	17
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	111	95	103	79	204	162	146
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	6	4	4	5	6	7	6
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	5	0	5	0	0	0	5
Haynesina germanica	0	3	1	0	0	0	0
Hopkinsina pacifica	17	6	9	6	15	6	8
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	1	0	0	0	1	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	14	4	4	2	2	0	0
Nonion depresslus	0	0	0	0	0	0	0
Nonionella turgida	0	3	0	0	6	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	1	9	6	1	1	2	1
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	1	0	0	0	0	0	0
Stainforthia fusiformis	5	3	10	5	14	2	10
Textularia sp.	8	6	4	3	0	0	3
indeterminable	1	0	0	0	0	0	0
total	**216**	**165**	**179**	**122**	**296**	**214**	**217**

February 2005, 53° 22' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	0	0	0	0	0	0	0
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	0	0	0	0	4	0	3
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	0	0	0	0	0	0	0
Bolivina pseudoplicata	0	0	0	0	0	0	0
Bolivina seminuda	0	0	0	0	0	0	0
Bolivina spathulata	0	0	0	0	0	0	0
Bulimina marginata/ elongata	0	0	0	0	0	0	0
Buliminella elegantissima	0	0	0	0	0	0	0
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	0	0	0	0	0	0	0
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	0	0	0	0	0	0	0
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	0	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	0	0	0	0	0	0	0
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	0	0	0	0
Nonion depresslus	0	0	0	0	0	0	0
Nonionella turgida	0	0	0	3	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	5	0	7	0	7	3	0
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	0	0	0	0	0	0	0
Textularia sp.	0	0	0	0	0	0	0
indeterminable	0	0	0	0	0	0	0
total	**5**	**0**	**7**	**3**	**11**	**3**	**3**

February 2005, 53° 22' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	0	0	0	0	0	0	0
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	0	2	2	0	2	3	0
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	0	0	0	1	0	1	0
Bolivina pseudoplicata	1	1	0	0	0	0	0
Bolivina seminuda	0	0	0	0	0	0	0
Bolivina spathulata	0	0	0	0	0	0	0
Bulimina marginata/ elongata	1	0	0	0	0	0	0
Buliminella elegantissima	0	0	0	0	0	0	0
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	0	0	0	0	0	0	0
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	1	1	0	0	0	0	0
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	0	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	0	0	0	0	0	0	0
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	0	0	0	0
Nonion depresslus	0	0	0	0	0	0	0
Nonionella turgida	0	0	0	0	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	2	5	0	0	0	1	3
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	0	0	0	0	0	0	0
Textularia sp.	0	0	0	0	0	0	0
indeterminable	0	0	0	0	0	0	0
total	**5**	**9**	**2**	**1**	**2**	**5**	**3**

February 2005, 53° 42' A

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	6	4	4	0	1	3	1
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	90	85	114	65	109	90	95
Asterigerinata mamilla	1	1	1	1	0	1	0
Bolivina dilatata	0	14	4	5	4	5	0
Bolivina pseudoplicata	3	3	3	0	0	0	1
Bolivina seminuda	0	5	4	0	0	0	0
Bolivina spathulata	2	2	2	2	0	2	0
Bulimina marginata/ elongata	7	7	8	4	6	7	4
Buliminella elegantissima	2	3	10	7	5	7	3
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	182	199	164	134	191	156	204
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	26	13	16	13	10	19	10
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	0	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	21	32	34	51	46	34	11
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	1	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	2	2	6	0
Nonion depresslus	0	2	0	0	0	0	0
Nonionella turgida	3	6	3	3	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	15	5	3	7	5	7	3
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	2	3	2	2	0	7	2
Textularia sp.	3	2	0	0	3	0	0
indeterminable	0	0	0	0	0	0	0
total	**363**	**385**	**371**	**295**	**381**	**343**	**336**

February 2005, 53° 42' B

	depth-interval (cm)						
	0-0.5	**0.5-1.0**	**1.0-1.5**	**1.5-2.0**	**2.0-3.0**	**3.0-4.0**	**4.0-5.0**
Acostata mariae	4	6	5	4	0	4	1
Ammodiscus sp.	0	0	0	0	0	0	0
Ammonia tepida	52	61	74	43	58	48	35
Asterigerinata mamilla	0	0	0	0	0	0	0
Bolivina dilatata	5	4	10	5	3	0	1
Bolivina pseudoplicata	1	3	3	3	4	1	0
Bolivina seminuda	0	1	3	1	0	0	0
Bolivina spathulata	0	2	6	0	0	0	0
Bulimina marginata/ elongata	9	7	3	2	4	4	2
Buliminella elegantissima	5	7	10	3	9	2	10
Caronia sylvestrii	0	0	0	0	0	0	0
Cassidulina sp.	0	0	0	0	0	0	0
Dentalina sp.	0	0	0	0	0	0	0
Eggerella scabra	75	157	130	128	159	179	114
Elphidium advenum	0	0	0	0	0	0	0
Elphidium excavatum	40	24	8	14	7	6	10
Epistominella exigua	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0
Fissurina sp.	5	0	0	0	0	0	0
Haynesina germanica	0	0	0	0	0	0	0
Hopkinsina pacifica	8	13	53	28	36	19	9
Hyalinea baltica	0	0	0	0	0	0	0
Lagena sp.	0	0	0	0	0	0	0
Lenticulina sp.	0	0	0	0	0	0	0
Leptohalysis scottii	0	0	0	0	0	0	0
Nonion depresslus	0	0	0	0	0	0	0
Nonionella turgida	3	9	3	3	0	0	0
Pyrgo williamsoni	0	0	0	0	0	0	0
Quinqueloculina spp.	34	10	3	0	5	0	3
Reophax monoliformis	0	0	0	0	0	0	0
Rosalina sp.	0	0	0	0	0	0	0
Saccamina sp.	0	0	0	0	0	0	0
Stainforthia fusiformis	0	2	2	2	9	3	2
Textularia sp.	4	3	0	3	5	2	3
indeterminable	0	0	0	0	0	0	0
total	**245**	**309**	**313**	**241**	**297**	**269**	**191**