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# Ecosystem recovery after hypoxia: what can foraminifera indicate?

## Ecosysteem herstel na zuurstofdeficiëntie: wat kunnen foraminiferen ons vertellen?

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Utrecht, 2014

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# Ecosystem recovery after hypoxia: what can foraminifera indicate?

Ecosysteem herstel na zuurstofdeficiëntie: wat kunnen foraminiferen ons vertellen?
(met een samenvatting in het Nederlands)

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# Contents


| | | |
|---|---|---|
| **Chapter 1** | General introduction<br>Ecosystem recovery after hypoxia: what can foraminifera indicate?<br>*G.M. Brouwer* | 9 |
| **Chapter 2** | Recovery potential of benthic foraminiferal communities after re-ventilation of hypoxia-deteriorated habitats: a microcosm experiment<br>*G.M. Brouwer, M. Wolthers and I.A.P. Duijnstee* | 15 |
| **Chapter 3** | Foraminiferal recolonisation pathways and microhabitat distribution after prolonged hypoxia disturbance<br>*G.M. Brouwer, M. Wolthers, V.M. van Onselen and I.A.P. Duijnstee* | 35 |
| **Chapter 4** | Differential response of intertidal foraminifera to ecosystem recovery succeeding hypoxia<br>*G.M. Brouwer, M. Wolthers, J.H. Hazeleger, F. Rossi, L.J. Lourens, J. J. Middelburg and I.A.P. Duijnstee* | 55 |
| **Chapter 5** | Diet shifts and population dynamics of estuarine foraminifera during ecosystem recovery after hypoxia<br>*G.M. Brouwer, I.A.P. Duijnstee, J.H. Hazeleger, F. Rossi, L.J. Lourens, J. J. Middelburg and M. Wolthers* | 73 |
| **Chapter 6** | Impact of bioirrigation on the vertical distribution of benthic foraminifera from the Swedish Gullmar Fjord<br>*G.M. Brouwer, I.A.P. Duijnstee, A.B. Craun and M. Wolthers* | 93 |
| **Chapter 7** | Thesis summary & synthesis<br>Ecosystem recovery after hypoxia: what can foraminifera indicate?<br>*G.M. Brouwer* | 117 |
| | References | 127 |
| | Introductie en samenvatting in het Nederlands<br>Ecosysteem herstel na zuurstofdeficiëntie: wat kunnen foraminiferen ons vertellen?<br>*G.M. Brouwer* | 137 |
| | Dankwoord | 149 |
| | Curriculum vitae | 151 |

CHAPTER 1

# GENERAL INTRODUCTION

# ECOSYSTEM RECOVERY AFTER HYPOXIA: WHAT CAN FORAMINIFERA INDICATE?

G.M. Brouwer

## 1.1 FORAMINIFERA

The unicellular eukaryotic phylum Foraminifera comprises an ecologically and biologically very diverse group of organisms, abundantly present in nearly all marine environments. Most of these extraordinary and beautiful single-celled organisms build a calcareous or agglutinated shell to house the bulk of their cytoplasm. The shell – also called test – of some calcareous species can reach a length of 11 cm or more (Hallock, 1985), while some mud-agglutinating species within the enigmatic group of Xenophyophora even exceed dimensions of 20 cm. Nonetheless, the size of the vast majority of foraminiferal species is less than 1 mm (Loeblich and Tappan, 1964). Hard-shelled foraminifera commonly secrete calcium carbonate to build a test, while agglutinated species collect foreign particles like clastic sediment grains, sea-urchin spines, or even the shells of other foraminifera to construct their test. During growth, most shell-bearing species add new chambers of increasing size to their test. Small openings – or the group's namesake: foramina – interconnect these chambers to accommodate for the foraminiferal cytoplasm to use all chambers. The arrangement of chambers – which varies extensively among species – is of great taxonomic importance, since most foraminiferal species are defined based on test morphology. The aperture – the foramen in the youngest chamber – enables a thin threadlike net of cytoplasmic extensions to emerge through the shell. This pseudopodial network of anastomosing so-called reticulopoda is an apomorphic characteristic that all foraminifera share. These dynamic extensions of the cytoplasm can rapidly extend, split, merge and retract in a seemingly orchestrated manner. Foraminifera use their pseudopodia for several purposes, for example to gather or catch food. A diverse array of foraminiferal feeding strategies has been described. Some species graze on algae or bacteria, while others use their pseudopodia to trap detritus (Murray, 2006 – and references therein). Besides carnivorous, parasitic, kleptoplastic and cannibalistic life styles, some species have a symbiotic relation with bacteria or algae incorporated in their cytoplasm. Except for gathering food, benthic foraminifera use their pseudopodia for locomotion. Benthic foraminifera grasp sediment particles with their pseudopodia and drag themselves over and through sediments. By moving, they are not only able to collect food, it also enables them to occupy a favourable niche within the chemically stratified sediment column.

Although several species live a planktonic life style in the water column, most foraminifera find their habitat either within, or on top of marine sediments. These benthic species, the subject of this thesis, are distributed along the vertical sediment profile. Among the benthic foraminifera, epifaunal species

live on top, whereas shallow infaunal species occupy the upper part of the sediment (Linke and Lutze, 1993). Deeper-dwelling species, some of which are able to respire with nitrate instead of oxygen (Risgaard-Petersen et al., 2006), occur in high densities below the oxygen-penetration depth.
Environmental circumstances determine the taxonomic and spatial structure of the foraminiferal *assemblage* – the foraminiferal species found in association with one another. Especially the availability of oxygen and food has been considered to shape foraminiferal communities and determine the vertical and geographical distribution of the individual members of the communities (Jorissen et al., 1995; Van der Zwaan et al., 1999). In dynamic ecosystems, for instance in coastal areas, the availability of oxygen and food changes constantly, often on a combination of seasonal, diurnal and semi-diurnal time scales. Each species has its own species-specific preferences and tolerances for environmental parameters such as oxygen concentration, temperature, salinity and food availability. Some foraminiferal species are relatively stress-tolerant, and especially those that have a short life span are able to swiftly respond to environmental perturbations at the population level. Although foraminiferal life cycles are not well studied, among investigated species a high diversity in foraminiferal life span (and generation span, a foraminifers life terminates with reproduction) has been observed (Hallock, 1985). Foraminifera were noticed to postpone reproduction during unfavourable conditions and, if environmental conditions admit, to grow until improved circumstances enable them to reproduce (Hallock, 1985). Several strategies, including changeable numbers of offspring (less offspring increase the size and survival rate of the new individuals) and alternation between asexual and sexual reproduction (with small, but genetically diverse microspheric offspring after sexual reproduction and larger megalospheric offspring after asexual reproduction) may enhance the success of this community in diverse and fluctuating circumstances (Hallock, 1985). The broad diversity of niches suitable for foraminiferal occupation gives rise to a dense presence of living individuals as well as fossil specimens in almost all marine sediments.

The fossil record of foraminifera enclosed in marine sediments consists of those species that build a calcareous or agglutinated test. The high fossilisation potential of these tests and their worldwide occurrence in marine sediments provide the opportunity to use the foraminiferal remains in sedimentary records as one of the most important sources of prehistoric information covering extended periods of time. For paleo-environmental research, fossilised tests of foraminifera – occurring ever since Cambrian times, over 500 million years ago – have been extensively studied because presence of indicator species and test-chemistry can be correlated to environmental parameters. Since a strong relation exists between foraminiferal abundances and their taxon-specific required minimum level of oxygen (e.g. Bernhard and Alve, 1996; Bernhard et al., 1997; Murray, 2001), fossil and modern foraminifera are recognised as valuable proxies for, for instance, low concentrations of dissolved oxygen in marine bottom-waters. The use of selected species as indicators for ecosystem parameters or ecosystem functioning may also provide a tool to more easily monitor, manage, protect and restore present-day and future ecosystems. Studying foraminiferal dynamics in relation to environmental parameters is interesting and valuable in its own right, but additionally it holds the potential to shed light on the recovery potential of disturbed ecosystems as a whole. Living foraminifera may play an eminent role also in environmental research on extant coastal ecosystems. With their high tolerance to environmental disturbance, they are expected to be the last ones standing and among the first taxa to recolonise marine sediments following ecosystem perturbation. Moreover, compared to most other major taxa in marine ecosystems, foraminifera are tiny, have short life spans, are abundantly present throughout the marine realm and respond fast to environmental change. These characteristics enable us to study whole communities of present-day foraminifera in high temporal and spatial

resolution in field studies as well as *in situ* and laboratory experiments. These experiments contribute to our understanding of the foraminiferal ecology and, in turn, this will improve the applicability of foraminifera as indicator species for ecosystem status.

## 1.2 COASTAL ECOSYSTEMS

Coastal ecosystems provide a habitat for a diverse array of marine organisms. Within these ecosystems, especially *ecosystem engineering* species structure the composition of the benthic community (e.g. Thayer, 1979; Jones et al., 1994; Wright et al., 2004; Wright and Jones, 2006; Hastings et al., 2007; Erwin, 2008). In near coastal ecosystems like intertidal (mud)flats and fjords, the activities of several ecosystem engineering macrofaunal species enlarge the oxygen-penetration depth and transport food deeper into the sediment. Through activities such as sediment reworking (known as bioturbation) and pumping of oxygenated water into the sediment (bioirrigation), they provide a more heterogenic environment with a broader array of niches – both in number and in size. Foraminifera are among the species that may profit from the physical redistribution of resources and the concurrent increased heterogeneity in the environment created by such ecosystem engineers.

Coastal areas do not only attract benthic fauna, the many services and resources they provide are also profitable to human populations. This had led to an increased human density in near-coastal zones that is approximately three times higher compared to the global average (Small and Nicholls, 2003). These high population densities make coastal areas more prone to a rise in anthropogenic perturbations such as hypoxic events, instances of low concentrations of dissolved oxygen in (bottom-) waters. The occurrence of coastal hypoxia has strongly increased in frequency, size, and duration since human industrialisation. Hypoxia can occur due to natural processes, as it has in the geological past; nonetheless it is and will often be aggravated by human activity (Wu, 2002; Rabalais et al., 2010). With rising temperatures, the expected climatic changes will further augment the occurrence and severity of hypoxic events. Exposure to hypoxia impacts the functioning of ecosystems. When bottom-water oxygen concentrations fall below the hypoxic border of 2 mL/L, mobile fauna respond by avoiding these areas. Less mobile and sessile fauna try to maintain the oxygen delivery to their cells followed by an attempt to reduce their oxygen demand (Wu, 2002). Macrofaunal ecosystem engineering species abandon their burrows and display aberrant behaviour (Diaz and Rosenberg, 2008). When disturbance continues, or the interval between events shortens, it may affect growth and reproduction and eventually result in mass mortality of marine organisms (Wu 2002, Diaz and Rosenberg 2008). Concurrent to the hypoxia-induced loss of sensitive marine fauna, faunal activities such as bioturbation and bioirrigation diminish. This further shallows the oxygen-penetration depth and the redox gradient in the sediments, harshening the conditions for the less sensitive species (Steckbauer et al., 2011). The processes that regulate the restoration of benthic communities after hypoxia, and especially their relative contribution towards ecosystem recovery, are not well understood. In order to protect coastal communities and restore those affected by hypoxia, it is essential to thoroughly understand the functioning of both healthy and affected ecosystem and to gain insight on the impact of timing and the sequence of perturbations.

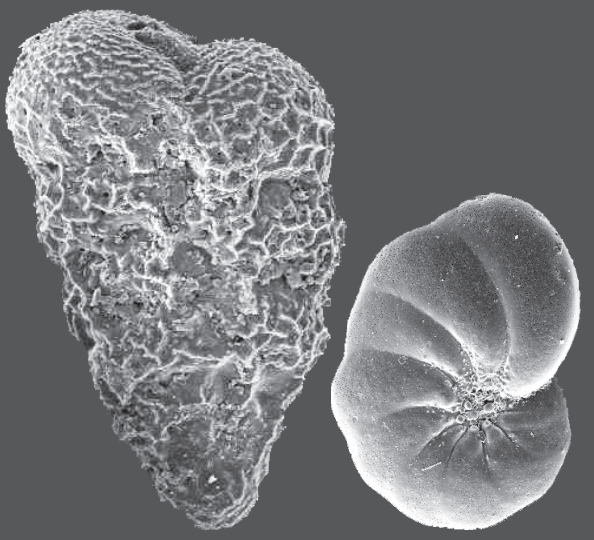
The aim of this thesis is to explore the impact of oxygen dynamics and subsequent ecosystem responses on foraminifera in marine coastal areas. Insight in the response of foraminifera to these properties will provide a better applicability of foraminifera as indicators of past, present and future ecosystem functioning, and will eventually help to monitor, manage, protect and restore coastal ecosystems.

## 1.3 THESIS CONTENT

To improve the applicability of foraminifera as indicator species for ecosystem health, experiments were conducted using sediments derived from three different areas: Lake Grevelingen – a saline lake (Chapter two and three), the Scheldt Estuary – an intertidal mudflat (Chapter four and five) and the Gullmar Fjord (Chapter six). The former two localities are located in the South-West of The Netherlands, the latter on the Swedish west coast.

The benthic community in the saline Lake Grevelingen, a closed-off estuary, is exposed to seasonal hypoxia that arises from stratification of the water column during warm summer months. Since up to now it is restricted to the narrow deeper gullies of the former estuary; the spread of hypoxia has a spatially patchy character with short distances between hypoxia-affected and unaffected areas. The biotic recovery in hypoxia-affected sediments after re-aeration of the water column may depend in part on the potential to restore the community via neighbouring communities recolonising the depleted sediments. CHAPTER TWO evaluates the species-specific dynamics over time within the foraminiferal community during short-term recovery in Lake Grevelingen. A series of laboratory microcosm experiments were carried out with sediment cores derived from anoxic and oxygenated parts of the lake bed. Insight in these dynamics will contribute to the accuracy of foraminifera to indicate restoration status during ecosystem recovery. In CHAPTER THREE we compare the vertical structure of the foraminiferal communities among several sediment types and treatments after an experimental period of 10.5 weeks to include the vertical aspect of meiofaunal resurgence and what this may tell us about ecosystem recovery. By comparing the contribution of autogenic recovery in re-ventilated but previously anoxic sediments with the foraminiferal recovery in re-ventilated anoxic sediment via horizontal migration from unaffected neighbouring areas, we evaluate the importance of the different pathways for the recovery of foraminiferal communities. The assessed relative contribution of these pathways will be used to discuss the restoration potential of the benthic community in hypoxia-affected areas of Lake Grevelingen. CHAPTER FOUR reports on the response of foraminifera to ecosystem recovery after hypoxia using an *in situ* field experiment. Hypoxia was deliberately induced during winter and late spring on an intertidal flat in the Scheldt Estuary, The Netherlands. The reassembly patterns of foraminiferal assemblages and their relation with sediment biogeochemistry, micro-organisms and other fauna are studied 2 and 5 months after the end of hypoxia. We analyse differences in the abundance and biovolume of the foraminiferal species during ecosystem recovery, and compare these with the foraminiferal dynamics in undisturbed sediments. This approach provides the opportunity to distinguish between the natural (seasonal) processes influencing the development of the foraminiferal community and those induced by processes related to ecosystem recovery after hypoxia. In CHAPTER FIVE we investigate the effect of this deliberately induced hypoxia and subsequent ecosystem recovery on foraminiferal population dynamics, foraminiferal feeding behaviour, dietary shifts and microbial carbon transfer. To study the contribution of microphytobenthos and heterotrophic bacteria to the foraminiferal diets, $^{13}$C-enriched labelled glucose and $^{13}$C-enriched labelled bicarbonate were introduced in these *in situ* experiments. Aspects of population dynamics were inferred from measuring foraminiferal test dimensions. The aim of CHAPTER SIX is to shed light on the impact of biogenic structures that enlarge the sediment-water interface and increase oxygen-diffusion into the sediment on the vertical distribution of foraminifera along the sediment profile. Permeable polyethylene tubes were vertically introduced into sieved sediments derived from the Swedish Gullmar Fjord. Sediment bordering these artificial burrows is sampled in discrete depth intervals and compared to similar samples from the surrounding material. In each sample foraminiferal abundances were enumerated and vertical species distributions assessed in order to study the impact of artificial worm holes (the permeable tubes) and the associated oxygen diffusion patterns on the

foraminiferal abundance and distribution. Additionally, the foraminiferal distribution in the cores with permeable tubes is compared to the distribution in cores without such tubes. In CHAPTER SEVEN the results presented in the separate chapters of this thesis are summarised and used in a synthesis in which we try to set our findings into the context of present-day knowledge on foraminifera and their applicability as species to indicate environmental status and ecosystem functioning.



CHAPTER 2

# RECOVERY POTENTIAL OF BENTHIC FORAMINIFERAL COMMUNITIES AFTER RE-VENTILATION OF HYPOXIA-DETERIORATED HABITATS: A MICROCOSM EXPERIMENT

G.M. Brouwer, M. Wolthers and I.A.P. Duijnstee

ABSTRACT

In Lake Grevelingen, The Netherlands, seasonal hypoxia occurs due to stratification of the water column. This spread of hypoxia in the deeper parts of the lake has a spatially patchy character, largely following the lake bottom topography. Here, we evaluate the short-term species-specific dynamics over time within the foraminiferal community during recovery of hypoxia-affected sediments from Lake Grevelingen. A series of laboratory experiments were conducted, in which hypoxia-affected and unaffected sediments were combined in microcosms and kept under ventilated conditions. The mean foraminiferal abundance (>63 µm) in the upper centimetre of the hypoxia-affected sediments increased during the experimental period of 10.5 weeks from 15% to approximately 46% of the abundance observed in unaffected control sediments. During restoration, *Ammonia beccarii* and *Elphidium excavatum,* species with relatively large test sizes, were the first to colonise the re-oxygenated sediments. They were followed by the smaller species *Hopkinsina pacifica* and *Stainforthia fusiformis*. Our results indicate that especially *E. excavatum* and *Epistominella vitrea* were able to respond opportunistically to the sudden dilution of foraminiferal density arising after foraminiferal migration from unaffected to formerly affected sediments in the microcosms, and the concurrent high availability of accumulated detrital material in these heterogeneous cores. Although the recovery rates observed in our experiment likely underestimate the recovery time needed in the field, because of the close proximity between originally affected and unaffected sediments, re-oxygenated hypoxia-affected sediments were colonised via migration by foraminifera from surrounding areas, and foraminifera clearly profited from the organic matter suddenly available from the formerly anoxic sediments.

KEYWORDS

Benthic foraminifera * Hypoxia * Recovery stages * Saline Lake Grevelingen * Foraminiferal migration

## 2.1 INTRODUCTION

Human-induced hypoxia in marine and coastal ecosystems such as in Lake Grevelingen, The Netherlands, has been recorded to increase worldwide, both in size and frequency (Diaz and Rosenberg, 2008; Zhang et al., 2010; Rabalais et al., 2010). With rising temperatures due to global warming, the occurrence and severity of anoxic events are expected to increase (Zhang et al., 2010; Steckbauer et al., 2011). Stratification of the water column due to limited water exchange between coastal waters and open seas is one of the conditions that cause hypoxia (Diaz et al., 2009; Zhang et al., 2010). The degree to which hypoxia deteriorates coastal ecosystems depends on the level of dissolved oxygen as well as on the duration, repetition and scale of the event (Zhang et al., 2010). Low-oxygen conditions and the co-occurrence of increased hydrogen sulphide concentrations strongly affect biotic compartments of the ecosystem as a lack of oxygen affects the metabolism of most organisms and sulphide is toxic and often lethal, especially to macrofauna (Gray et al., 2002). Ecosystems subjected to episodic and/or periodic hypoxia may not necessarily recover after restoration of the oxygen concentrations towards natural values (e.g. Lotze et al., 2006; De Young et al., 2008; Zhang et al., 2009; Steckbauer et al., 2011). Moreover, the time needed to restore ecosystems affected by hypoxia may exceed the time between hypoxia events. The potential for recolonisation from less-affected neighbouring areas is likely to play a role in the recovery rates. Hence also the lateral extent of the hypoxia and its spatial distribution determine the recovery potential of benthic systems after periodic hypoxia disturbance.

Lake Grevelingen is a saline (~32) lake situated in the South-West of The Netherlands. The lake is a former estuary in the Rhine-Meuse-Scheldt delta. After a flooding disaster in 1953, large-scale civil engineering measures were taken to prevent a repetition (Nienhuis, 2006). As part of this, in 1964 a dam was built on the eastern side of the Grevelingen branch of the estuary, to cut off the water inflow from the rivers Rhine and Meuse. After completion of the Brouwersdam in 1971, the connection with the North Sea was severed (Nienhuis, 2006). In 1978 a sluice was built in the Brouwersdam in order to control the water level in the lake, resulting in a slight water inflow from the North Sea (Kamermans et al., 1999). Despite the marine salt water inflow, seasonal hypoxia increased in recent years especially in the stratified deeper parts of the lake. During summer months, warming of Lake Grevelingen's surface water and restricted exchange of water with the North Sea enhance vertical stratification of the water column. This prohibits mixing of well-oxygenated surface water with oxygen-limited deeper water, allowing the development of hypoxic conditions as organic matter exported from the surface waters decays in the bottom-water and on the lake bottom. To prevent irreversible ecological degradation related to spreading hypoxic conditions, insight is needed in the impact of hypoxia on the functioning of benthic ecosystems and their recovery potential. This information will not only help to decide on managerial actions to ameliorate the deteriorating (bottom-)water conditions in the lake (for instance via larger North Sea influence in the lake), but also restoration efforts in other areas which are aimed at counter-acting human-induced hypoxia. To shed light on the functioning and the restoration potential of the benthic ecosystems in Lake Grevelingen, foraminifera were used as bioindicator species.

Foraminifera are unicellular eukaryotes in the supergroup Rhizaria that are numerously found in a wide variety of marine environments. Many of their taxa are able to withstand periodical conditions of low oxygen (e.g. Josefson and Widbom, 1988; Moodley and Hess, 1992; Bernhard, 1993, Pucci et al., 2009), but they respond fast to oxygen depletion as reflected in lowered diversity and species richness (Gooday, 2003 and references therein). As bottom-water oxygen concentrations are strongly reflected in the composition of extant and fossil benthic foraminiferal assemblages, and a strong correlation

exists between bottom-water oxygen concentration and species diversity of the benthic community, foraminifera are increasingly acknowledged as useful bioindicator to monitor the functioning of benthic marine ecosystems (Bouchet et al., 2012; Schönfeld et al., 2012).

The aim of this study is to investigate the benthic ecological development of hypoxia-affected parts of Lake Grevelingen after artificially restoring water column ventilation. In order to evaluate the dynamics of the foraminiferal community during short-term recovery, a series of laboratory microcosm experiments were carried out with sediment cores obtained from anoxic and oxygenated parts of the lake bed. With these microcosm experiments we tried to address the following questions. (1) What is the species-specific response of foraminifera during ecosystem recovery after healthy bottom-water conditions are restored? (2) Are the dynamics within the foraminiferal assemblage indicative for different recovery stages? (3) What role can migration of biota from nearby unaffected areas play in the recovery of foraminifera, and on which time-scale, after ventilation of anoxic bottom-waters? (4) What are the implications for the ecological restoration potential of benthic communities in repetitively hypoxia disturbed systems, like the deeper parts of Lake Grevelingen? In the experiments we looked at different aspects of the foraminiferal communities: in a follow-up study we will compare the vertical structure of the communities within the sediment at the very end of the experiment. By comparing the contribution of recovery from within hypoxia-affected sediments after ventilation of bottom-waters with the recovery via horizontal migration from unaffected areas, we will discuss the importance of the different recovery pathways for the foraminiferal communities. In this chapter we focus on developments over time in foraminiferal abundance and species composition. Insight in these developments will contribute to the accuracy of foraminifera to indicate restoration status during ecosystem recovery.

## 2.2 MATERIALS AND METHODS

Lake Grevelingen is a former estuary situated in the South-Western part of The Netherlands. It comprises an area of 140 $km^2$ (Wolf and Wolf, 1977). The estuary was enclosed as part of the so-called Delta Plan to prevent flooding of the Dutch coastal areas. In 1964, river water inflow on the eastern side was blocked with the completion of the Grevelingendam. The construction of the Brouwersdam in 1970 closed the connection with the saline waters of the North Sea (Kamermans et al., 1999). Over time, the enclosure of the estuary resulted in a slow decrease in salinity from 30 to 22 (Kamermans et al., 1999). When a sluice was constructed in 1978 to reconnect the water of Lake Grevelingen to the North Sea, the salinity increased to 32, but exchange of water between the North Sea and Lake Grevelingen is still very limited. Lake Grevelingen has two gullies; one in the northern part (Springersdiep) and one in the south-eastern area (Bocht van St. Jacob). Measurements of the oxygen concentrations in the water column, frequently executed by the Dutch Ministry of Infrastructure and Environment, indicate low oxygen concentrations in the gullies, especially during warm summer months.

To investigate the restoration potential of the benthic foraminiferal community in the hypoxia-affected parts of Lake Grevelingen, sediments were collected in an affected and an unaffected area. On August 23rd, 2011, sediment cores were obtained from the bottom of the lake at two stations using a boxcorer. Both stations were situated in the Southern gully. The station from which hypoxia-affected sediments were derived (GTSO 6) had a water depth of 37.0 m and a bottom-water $O_2$ concentration of 0.33 mg/L ($O_2$ saturation = 4%) at the sediment-water interface. The station that was sampled to collect unaffected sediments (GTSO 7) was, with an approximate distance of 2 km, nearby and had a water depth of 10.0 m. At the sediment-water interface, this station had an $O_2$ concentration of 6.05 mg/L

($O_2$ saturation = 78%). On board, the boxcorers were subsampled using transparent plastic corers with an internal diameter of 7 cm. The sediment cores were kept in cooling boxes and transported to the laboratory within one day.

### 2.2.1 EXPERIMENTAL SET-UP

In the laboratory, the sediment cores were split in half along the central vertical axis. Prior to this, we removed hard-shelled macrofauna visible on top of the sediment, predominantly *Crepidula fornicata* (common slipper shell present with a density of 1 ± 1 specimens per core). We did not observe living macrofauna during the sample events nor while analysing the sediment samples for foraminiferal densities. For each experimental sediment core, two half sediment cores were put together. The sediment cores that were incubated during the experiment consisted either of two halves derived from unaffected sediments (the homogeneous cores (VV)) or they comprised one half from hypoxia-affected (A(AV)) and one half from unaffected sediments (V(AV)) (the heterogeneous cores (AV); see Fig. 2.1 for experimental set-up). The cores were placed in containers filled with natural waters from Lake Grevelingen, aquarium pumps were used to aerate the water. The cores were kept at 10°C.

The experiment started on September 1st and lasted until November 14th, 2011. The experimental cores were sampled at 4 sample events (Fig. 2.1); 0, 1.5, 3 and 10.5 weeks after the onset of the experiment. Prior to each sample event (except the first sample event at the onset of the experiment), pore-water $O_2$ profiles (Appendix 2.1) were measured using a Unisense oxygen microsensor type OX50, calibrated against air-saturated water from Lake Grevelingen, and attached to a micromanipulator for accurate

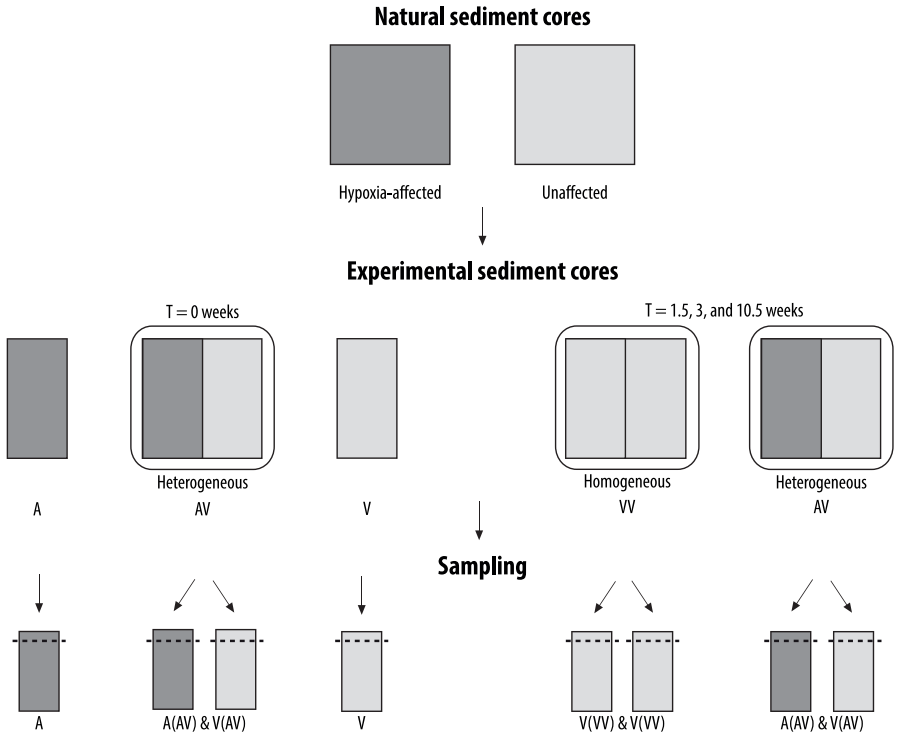



**Figure 2.1** Experimental set-up and sampling strategy.

depth-profiling. Per sample event two cores of both types (homogeneous and heterogeneous) were harvested. Only during the first sample event, at the onset of the experiment, the combined halves in two heterogeneous cores were sampled separately after re-splitting. In addition, two half cores of both the affected and unaffected sediments that had not been combined were sampled to indicate the error in foraminiferal abundance that arises from splitting and combining sediments and re-splitting them for sampling afterwards (see Fig. 2.1). The heterogeneous cores composed of combined sediments and re-split for sampling on the first day of the experiments were used as starting point (t=0) of the experiment. Cores composed of two halves of unaffected sediment (VV), were sampled as controls at each sampling moment.

For each sampling occasion, the experimental sediment cores were re-split along the same vertical plane that separated the original half sediment cores. Subsequently, the halves were sliced horizontally for foraminiferal analysis (Fig. 2.1). The upper cm of the sediment, which usually comprises the highest foraminiferal density, was used to study foraminiferal patterns. The sediment samples were stored in containers with ethanol and 1 g/L Rose Bengal to stain the foraminiferal protoplasm remains in the test. Before sieving, the sediments were dried to determine sediment dry weights. The sediment dry weights were used to correct for sampling errors arising from unevenness in the top layer of the sediment cores. The sediments were sieved using sieves with a 63 µm and a 150 µm mesh size. For foraminiferal abundance analysis, specimens with brightly stained protoplasm remains in all chambers except the last one, were picked from wet samples and enumerated unto species level. Foraminiferal abundances were corrected for the deviation between the actual dry weight of the sample and the corresponding mean dry weight.

### 2.2.2 STATISTICS

Statistical analyses were performed in PAST (PAleontological STatistics, Hammer et al., 2001). The tests were used to analyse the foraminiferal abundance in the three types of sediment over a period of 10.5 weeks (Kruskal-Wallis, Appendix 2.2). To test for differences in the assemblage of foraminifera, TWO-WAY ANOSIM (Appendix 2.3) tests and SIMPER tests were performed. The Kruskal-Wallis test and the TWO-WAY ANOSIM test are both non-parametric statistical tests used in accordance to the absence of homogeneity of the variances of the total standing stock (tested with the Levene's test). The SIMPER test (Similarity Percentage) was used to analyse overall average differences among assemblages. Moreover, the test indicates the impact of each species on these differences.

The Kruskal-Wallis test was used to indicate the significance of observed differences among the total standing stocks (Appendix 2.2). The abundances of individual species were also analysed using the Kruskal-Wallis test (Appendix 2.2). The tests were performed on the five most abundant species: *Ammonia beccarii*, *Elphidium excavatum*, *Trochammina inflata*, *Hopkinsina pacifica* and *Stainforthia fusiformis*. The TWO-WAY ANOSIM tests were used to indicate the significance of observed differences in the foraminiferal assemblage among treatments and sample moment (Appendix 2.3). The used p-values indicate sequential Bonferroni significance (to correct for multiple comparisons).

In order to explore the re-establishment of the foraminiferal community in the hypoxia-affected sediments after re-ventilation, ln-transformed foraminiferal abundance data were used in a multivariate Principal Response Curves analysis (PRC) using the software package CANOCO (Ter Braak and Šmilauer, 1998). PRC (see: Van den Brink and Ter Braak, 1998) summarised community responses during the experiment, in both sediment types of the heterogeneous cores (A(AV) and V(AV)), relative to the change in the foraminiferal community of the homogeneous control cores (VV). The resulting species weights can be used to interpret the kind of community change depicted in the PRC curves. A species weight indicates a species' affinity for the community change displayed by the PRC axis' regression

coefficients; i.e. an estimate of to what extent a taxon's abundance changed (increasing or decreasing) relative to control samples. To give an example: *Elphidium excavatum* (Fig. 2.5) had a high, positive species weight (ca. 1.7), while the t=0-community of the A(AV) treatment had the smallest, most negative regression coefficient value (ca. –1.1). Multiplying regression coefficient and species weight provides an estimate of a species' difference in abundance, relative to the control (VV)) community. In this case, for *E. excavatum* in t=0 A(AV), that would be a difference of ca. –1.9 for the ln-transformed data. On average, there were 141 individuals of *E. excavatum* in each of the t=0 V(VV) control samples. The estimate of the *E. excavatum* abundance in the concurrent A(AV) samples would therefore be: $141*e^{-1.9} = 21$, which is very close to the average of 20 actually found in the A(AV) samples.

## 2.3 RESULTS

### 2.3.1 OXYGEN FLUXES

Oxygen penetrated in all treatments approximately 4 mm into the sediment at all the measured recovery times (1.5, 3 and 10.5 weeks, Appendix 2.1).

### 2.3.2 TOTAL STANDING STOCKS

The total standing stock of foraminifera differed among the different treatments (Appendix 2.2). The non-parametric Kruskal-Wallis test (KW) indicated that the total standing stocks of foraminifera in the hypoxia-affected sediments was, over time, less similar than both types of unaffected sediment (from the heterogeneous (V(AV)) and the unaffected homogeneous (V(VV)) core halves; Appendix 2.2). In other words, the foraminiferal standing stocks in sediments derived from the hypoxia-affected part of the Grevelingen were less stable over time (KW p(same) = 0.24), whereas the unaffected sediments in the homogeneous cores showed the highest stability in foraminiferal total standing stock over time (KW p(same) = 0.52, Appendix 2.2). We will first discuss the development of the total standing stock in the hypoxia-affected sediments and secondly the concurrent development in the unaffected sediments of the heterogeneous and homogeneous cores.

During the first one-and-a-half weeks, the total standing stock of foraminifera in the hypoxia-affected sediment seemed to have increased almost threefold (Fig. 2.2). This increase in abundance was found in the >150 µm as well as in the 63-150 µm foraminiferal size fraction, but it was more pronounced in the >150 µm size fraction. At the end of the second period, after 3 weeks of foraminiferal recolonisation, the mean abundance of foraminifera in the hypoxia-affected sediments was 4 times higher compared to the start of the experiment. This increment of foraminifera in the second period has to be ascribed predominantly to an abundance increase of the smallest size fraction. During the last period of recovery, the total standing stock of foraminifera in the hypoxia affected sediments decreased. This relative decrease was highest in the >150 µm size fraction. After 10.5 weeks the abundance of foraminifera in the hypoxia-affected sediments was reduced compared to the penultimate sample moment, but still approximately three times higher compared to the start of the experiment. Despite this increase, figure 2.2 indicates that the average total standing stock of foraminifera in both unaffected sediments was more than twice as high as in the hypoxia-affected sediments.

The increase in foraminiferal total standing stock in the hypoxia-affected sediments during the first 1.5 weeks did not seem to cause a decrease in the foraminiferal abundance of the adjacent unaffected sediments (Fig. 2.2). In most cases the opposite was true. A peak in foraminiferal total standing stock is observed in unaffected sediments exposed to affected sediments for 1.5 weeks. The smallest size fractions contributed most to this increase. During the second period, from 1.5 to 3 weeks, the total

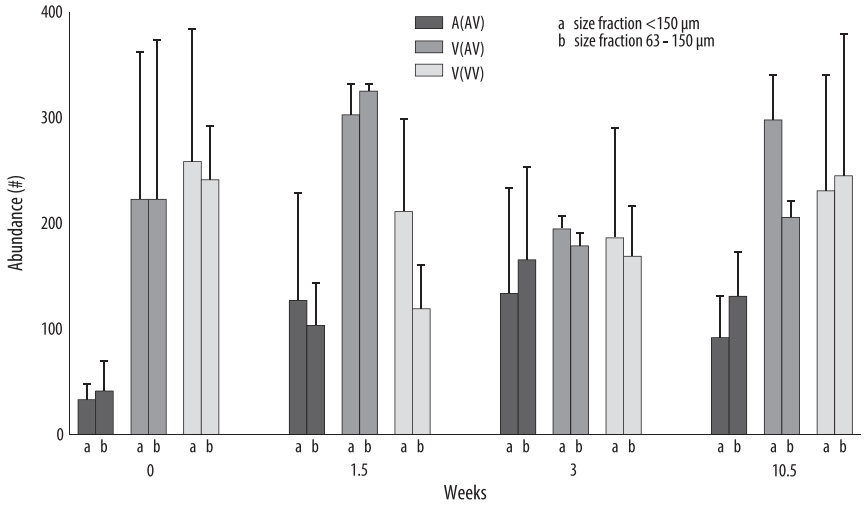



**Figure 2.2** Total standing stock of foraminifera per size class in the upper centimetre of the sediment (surface area of the sampled sediment ~19.2 $cm^2$, a. left bars >150 µm, b. right bars 63-150 µm) per sediment type and duration of the experiment with standard deviations. Dark grey bars represent average abundances in hypoxia-affected sediment (i.e. 2 A(AV) halves), grey bars indicate abundance in unaffected sediments that were combined with hypoxia-affected sediments (i.e. 2 V(AV) halves) and light grey bars show abundance in unaffected sediments (i.e. 4 V(VV) halves).

standing stock decreased in unaffected sediments that had been combined with hypoxia-affected sediment. After 3 weeks, the mean foraminiferal abundances in both types of unaffected sediments were almost equal. During prolonged recovery, from the 3rd to the 11th week, the total abundance of foraminifera in both unaffected sediment types showed an increase. At the end of the experiment, the unaffected sediments exposed to the hypoxia-affected sediments seemed to have relatively more large specimens, whereas the unaffected sediments that had not been exposed to the affected sediments seemed to have a higher abundance of small specimens.

### 2.3.3 ASSEMBLAGE DYNAMICS

Three species dominated the foraminiferal assemblage in all sediments during the whole period of recovery. The most abundant species with average densities of 50 to almost 300 individuals per sample were *Ammonia beccarii, Elphidium excavatum and Trochammina inflata* (Fig. 2.3 and see Plate 2.1). These species were found in both size fractions, as were the less numerous species *Epistominella vitrea, Quinqueloculina seminula* and *Nonion commune*. The other species in the assemblages were mainly found in the smallest size fraction. The species dominating this size interval were *Stainforthia fusiformis* and *Hopkinsina pacifica*. Less abundant smaller specimens belonged to *Bolivina dilatata, Bolivina plicatella, Bolivina seminuda, Bolivina variabilis, Bulimina aculeata, Buliminella elegantissima, Cibicides lobatulus, Elphidium lessoni, Elphidium marcellum, Elphidium williamsoni, Rosalina bradyi, Rosalina globularis*, *Textularia earlandi,* and again *Epistominella vitrea, Nonion commune and Quinqueloculina seminula*. A non-parametric Two-Way ANOSIM test was used to explore the differences in the foraminiferal assemblage composition (species composition and concurrent abundances) among the three sediment types over time (Appendix 2.3). The pooled test, comparing the assemblage

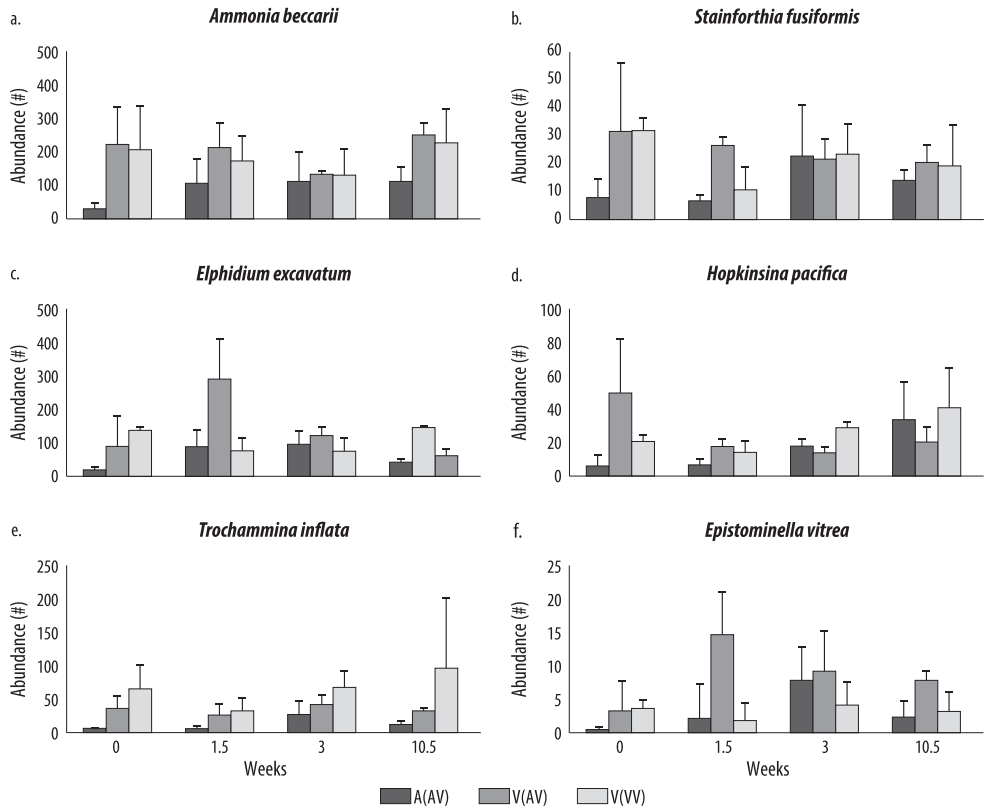



**Figure 2.3 a. - f.** a. *Ammonia beccarii*, b. *Stainforthia fusiformis,* c. *Elphidium excavatum*, d. *Hopkinsina pacifica*, e. *Trochammina inflata*, f. *Epistominella vitrea*. Average abundances and standard deviations per sediment sample (surface area ~19.2 $cm^2$) of the six species that contribute most to differences observed among assemblages.

of all sediment types over time, indicated that the treatment determined to a higher extent the assemblage than duration of the experiment. Although the differences were not significant, the pairwise comparison between all types of core halves indicated that the hypoxia-affected sediments (A(AV)) and the unaffected sediments exposed to the hypoxia affected sediments (V(AV)) differed most in foraminiferal species composition. The highest similarity between assemblages was found among both types of unaffected sediment. The SIMPER test was used to analyse the impact of each species on the observed dissimilarities among foraminiferal assemblages that had been exposed to different treatments over separate time intervals during the experimental period of 10.5 weeks. The most abundant species *Ammonia beccarii*, *Elphidium excavatum* and *Trochammina inflata* contributed most to the observed differences in the foraminiferal assemblage among the treatments and recovery times (Fig. 2.4). In the smaller size fraction, *Hopkinsina pacifica* and *Stainforthia fusiformis* had the highest impact on observed variations in foraminiferal assemblages among treatment and sample moments.
The Principal Response Curves analysis (PRC, Fig. 2.5) illustrates the development of the foraminiferal community in both sediment types of the heterogeneous core halves, relative to the dynamics in the homogeneous control cores. In the analysis, the time effect in the experiment (i.e. differences in

| a. | A(AV) | | | V(AV) | | | V(VV) | | |
|---|---|---|---|---|---|---|---|---|---|
| b. | 0 - 1.5 | 1.5 - 3 | 3 - 10.5 | 0 - 1.5 | 1.5 - 3 | 3 - 10.5 | 0 - 1.5 | 1.5 - 3 | 3 - 10.5 |
| c. | 51.4% | 31.3% | 34.7% | 37.4% | 29.8% | 21.4% | 28.8% | 30.9% | 33.4% |
| d. | *Ab* | *Ab* | *Ab* | *Ee* | *Ee* | *Ab* | *Ab* | *Ab* | *Ab* |
| | *Ee* | *Ee* | *Ee* | *Ab* | *Ab* | *Ee* | *Ee* | *Ee* | *Ti* |
| | *Sf* | *Ti* | *Hp* | *Hp* | *Ti* | *Ti* | *Ti* | *Ti* | *Ee* |
| | *Hp* | *Sf* | *Ti* | *Sf* | *Ev* | *Hp* | *Sf* | *Hp* | *Hp* |
| | *Ti* | *Hp* | *Sf* | *Ti* | *Sf* | *Sf* | *Hp* | *Sf* | *Sf* |

**Figure 2.4** SIMPER test on foraminiferal assemblages among sediment types and experimental duration.

a. sediment type

b. sampling times compared (weeks)

c. overall average dissimilarity with respect to previous sampling event

d. rank of the five species that have the highest impact on differences among assemblages

*Ab – Ammonia beccarii*, *Ee – Elphidium excavatum*, *Ev – Epistominella vitrea*, *Hp – Hopkinsina pacifica*, *Sf – Stainforthia fusiformis*, *Ti – Trochammina inflata*

assemblage deviations from the control community among the four sample moments) was 17% of the total variance. The treatment regime (i.e. the remainder of the time*treatment interaction) accounted for another 42% of all variance. The proportion of the variance covered by the treatment regime that was captured by the first PRC axis was 61% (as displayed by the PRC curves in Fig. 2.5). The three most abundant species, *A. beccarii*, *E. excavatum* and *T. inflata* also had the highest species-weights. This indicates that they most strongly corresponded to the particular type of variation in the species data (i.e. community dynamics) captured by the first PRC axis, but also that they showed the largest degree of fluctuation in their abundance. The first PRC axis demonstrates that the dominant type of community change can be summarised as a unidirectional response across the foraminiferal board, with virtually all foraminiferal taxa either increasing or decreasing in unison (depending on the specific type of treatment) relative to the control community. Not surprisingly, the unaffected sediment halves that were used in the heterogeneous cores and in the homogeneous control microcosms had generally quite similar foraminiferal communities. The exception to this rule was an initial short-lived rise in foraminiferal abundance compared to the control assemblages, as a result of the pairing to the originally anoxic A(AV) halves. This peak occurred despite the fact that migration of individuals from the V(AV) sediments to the foraminifer-depleted V(AV) core-halves had already begun. The latter was witnessed by the rapid recovery of the impoverished anoxic community (the t=0 data point on the A(AV) PRC in Fig. 2.5) to almost unaffected-control-level assemblages. In spite of this rapid initial recovery of the affected A(AV) communities, they would never quite reach the control assemblage composition within the time frame of this experiment.

### 2.3.4 SPECIES-SPECIFIC RESPONSE

The contribution of individual species to observed dissimilarities among assemblages depends on the combined effect of relative abundance and relative response to the treatment (i.e. relatively small changes in dominant taxa have a large impact on assemblage composition). Despite the generally lower contribution of low abundant species to the dissimilarity among assemblages, their changes in abundance, in response to different treatments and duration of the experiment, may provide insight into the state of the benthic ecosystem. The Kruskal-Wallis tests indicated to which

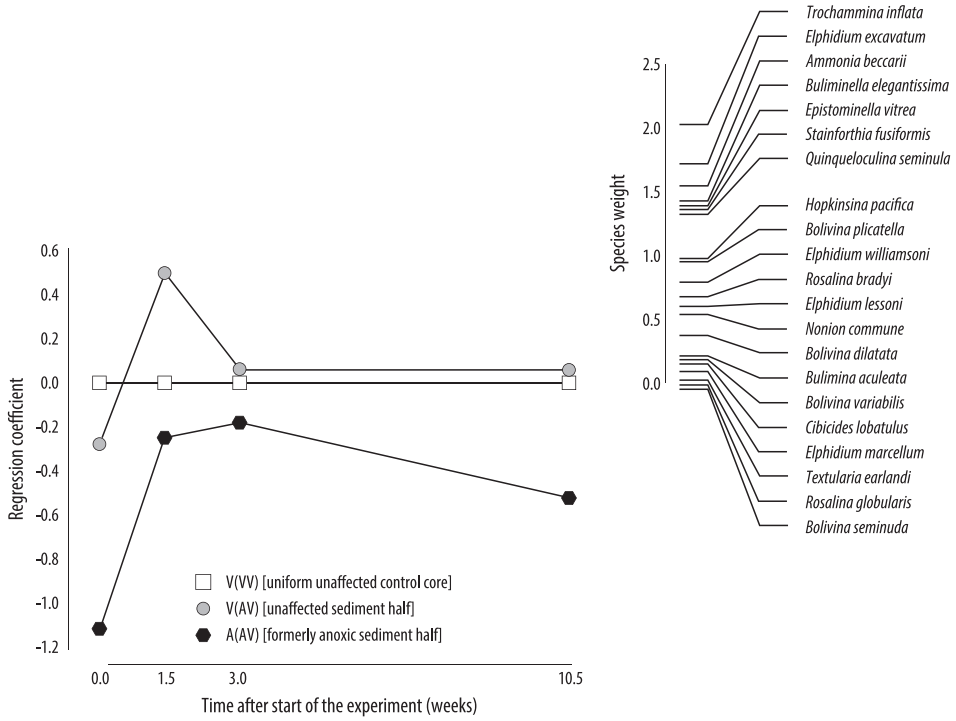



**Figure 2.5** Multivariate Principal Response Curve (PRC) analysis of the ln-transformed foraminiferal abundance data summarises the community responses during the experiment, in both sediment types (A(AV) and V(AV)) of the heterogeneous cores (AV), relative to the change in the foraminiferal community of the homogeneous control cores (VV). In the analysis, the time effect in the experiment (i.e. differences in assemblage deviations from the control community among the four sample moments) was 17% of the total variance. The treatment regime (i.e. the remainder of the time*treatment interaction) accounted for another 42% of all variance. The proportion of the variance covered by the treatment regime that was captured by the first PRC axis (as displayed by the PRC curves in Fig. 5) was 61%. The species weight indicates which species most strongly correspond to the particular type of variation in the species data (i.e. community dynamics) captured by the first PRC axis and it shows which species have the largest degree of fluctuation in their abundance.

extent the abundances per species differed among the treatments (Appendix 2.2). Species-specific foraminiferal responses were observed with respect to the treatments and the recovery period (Fig. 2.3). Among the three most abundant species *Ammonia beccarii* had relatively stable abundances in both unaffected sediment types. This species colonised the recovering hypoxia-affected sediments, although its abundance remained significantly lower compared to the unaffected sediments (Fig. 2.3). *Trochammina inflata* however, seemed to perform much better in sediments without hypoxia disturbance or contact with hypoxia-affected sediments. Similar to *A. beccarii*, *Elphidium excavatum* rapidly colonised the hypoxia-affected sediments (Fig. 2.3). Remarkably, however, *E. excavatum*, as well as *Epistominella vitrea*, showed a pronounced abundance peak in the unaffected sediments combined with the hypoxia affected sediments after 1.5 weeks from the start of the experiment. Just like the other taxa, *Stainforthia fusiformis* and *Hopkinsina pacifica* were present in all types of sediment.

However, compared to *A. beccarii*, *E. excavatum* and *E. vitrea*, these species colonised the hypoxia affected sediments in a later stage as reflected by the abundance increase in the experimental period from 1.5 to 3 weeks (Fig. 2.3).

## 2.4 DISCUSSION

The occurrence of hypoxia in the gullies of Lake Grevelingen has been suggested to arise from insufficient water exchange between the lake and the North Sea (Donders et al., 2011, 2012). Severe deterioration of foraminiferal assemblages in the hypoxia-affected parts of the lake was recently reported (Donders et al., 2011, 2012). The sub-recent foraminiferal fossil record in the sediments, used to explore the development of the foraminiferal population since 1970, revealed that locally, hypoxia has altered the foraminiferal assemblage. Since the connection between Lake Grevelingen and the North Sea was blocked (1971), the density and the composition of the foraminiferal assemblage underwent several changes that indicate an ongoing degradation of the ecosystem in hypoxia-exposed areas (Donders et al., 2012). As foraminifera are known to be among the benthic elements most tolerant to hypoxia (e.g., Josefson and Widbom, 1988; Moodley and Van Weering, 1993), the almost complete absence of living foraminifera in the gullies of Lake Grevelingen indicates the last and most severe stage in deterioration of the benthic ecosystem.

We investigated whether enhanced ventilation, which can be generated by a more open connection between Lake Grevelingen and the North Sea, can reverse the process of deterioration and will result in recovery of the benthic ecosystem in the affected areas. To this purpose, we studied the recovery potential of foraminiferal assemblages in the hypoxia-affected sediments when overlying water was ventilated and unaffected neighbouring assemblages are in close enough proximity to provide recolonising individuals. Complete recovery will be accomplished when foraminiferal assemblages in the affected sediments are indistinctive from those in unaffected sediments; both with respect to the total standing stock as to composition of the assemblage. Studying the foraminiferal development over time in these sediments contributed to our understanding of how various species respond to re-oxygenating restoration efforts.

### 2.4.1 RECOVERY DYNAMICS

Foraminiferal dynamics in a natural setting are governed by a dynamic interplay of biotic and abiotic environmental properties. Macrofaunal dynamics may for instance influence, via top-down interactions, the foraminiferal density and vertical distribution (e.g. Buzas, 1978; Bouchet et al., 2009). Since the sediments were void of macrofauna during our experiment, we presume that macrofaunal interference did not contribute to the foraminiferal dynamics observed in this study. Furthermore, the oxygen-penetration depth was similar in all experimental cores after it established at a depth of 4 mm within 1.5 week from the onset of the experiment. In the next paragraphs we will review the foraminiferal dynamics and discuss and hypothesise which processes may have instigated the observed development over the experimental period of 10.5 weeks.

In our experiment, the foraminiferal species showed species-specific responses. Temporal fluctuations in the foraminiferal density and assemblage composition were relatively small in the control sediments (V(VV)), whereas foraminiferal assemblages in the hypoxia-affected sediments (A(AV)) showed the highest rate of change in the subsequent sample occasions. The foraminiferal dynamics in the hypoxia-affected sediments suggested the occurrence of sequential foraminiferal colonisation stages within the experimental period of 10.5 weeks.

Within the first 1.5 week from the onset of the experiment, foraminifera started to recolonise the foraminifer-poor hypoxia-affected sediments. *Ammonia beccarii* and *Elphidium excavatum*, dominant species of the >150 µm size fraction, were the early recolonisers of the re-oxygenated sediments. Their abundance increased within the first 10 days by 200%. In the second experimental period, between 1.5 to 3 weeks, the abundances of *A. beccarii* and *E. excavatum* did not further increase. During this experimental period the species *Stainforthia fusiformis*, *Hopkinsina pacifica* and *Epistominella vitrea* started to colonise the hypoxia-affected sediments. Only *Hopkinsina pacifica* further increased in abundance in these sediments during the last period, from 3 to 10.5 weeks.

The relatively high proportion of large specimens (belonging to the >150 µm size fraction) observed in the hypoxia affected sediments after 1.5 weeks of re-oxygenation suggests that, in first instance, relatively large foraminifera contributed most to the recovery of these populations. This suggests that the initial recolonisation was likely caused by immigration from the unaffected neighbouring sediments, rather than recovery from within the affected sediments themselves (e.g. recruitment from small-sized propagules). Active foraminiferal migration into re-oxygenated sediments is something that has been observed in other studies (e.g. Alve and Bernhard, 1995; Duijnstee et al., 2003; Geslin et al., 2004) and for other groups of meiofauna (e.g. Steyaert et al., 2001; Wetzel et al., 2013). Nematodes, for example, have been reported to emigrate during hypoxia crises into the water column where they persist until oxic conditions re-establish and allow them to re-enter the sediment (Wetzel et al., 2013). Furthermore, like foraminifera, nematodes migrate vertically through marine sediments. Nematodes in an intertidal flat (Scheldt Estuary) were discussed to display species-specific vertical migration patterns in the sediment during tidal cycles in response to natural dynamics in several abiotic (temperature, pore-water drainage, current velocity etc.) and biotic (predation) factors (Steyaert et al., 2001). Although lateral migration of free living marine nematodes into defaunated sediments has been revealed (Schratzberger et al., 2004), most of the earlier studies on foraminiferal movement referred to vertical migration in response to fluctuating bottom-water oxygen conditions, rather than lateral migration. In their experiment, Alve and Bernhard (1995) investigated the vertical migration of foraminifera in response to lowering of the dissolved oxygen concentration. Lowering the concentration of dissolved oxygen to hypoxic condition (0.2 mL $O_2$/L) instigated foraminiferal migration within 2.5 weeks. After a four-month period of re-oxygenation of the sediment pore-water, foraminifera had migrated back downwards. Geslin et al. (2004) studied the effect of differential oxygen concentrations on foraminifera. The deep-sea foraminifera responded within 40 days to the treatment as reflected by diversification of their vertical distributions.

Through migration in response to variations in the concentration of dissolved oxygen, foraminifera may seek to maximise the overlap between their fundamental and realised niche. Foraminifera use an extended network of pseudopodia for locomotion. Pseudopodial strands attach to sediment particles and orchestrated shortening of the strands on one side of the network makes it possible for foraminifera to drag themselves through or over sediments in the desired direction. Some shallow water species have been reported to move with a speed up to a few millimetres per hour (Kitazato, 1988; Alve, 1999; Gross, 2000, 2002). Thus achieved active foraminiferal locomotion is considered to be an effective form of dispersal over short distances (Alve, 1999). Our data confirm the importance of active foraminiferal migration for short-distance dispersal. Stress tolerant species of the >150 µm size fraction were the early recolonisers of hypoxia-affected sediments, while species of the 63-150 µm size fraction appeared in these sediments in the sequential stage. This could indicate that specimens with a large test size are able to move faster than small-sized individuals and thus that the velocity of foraminiferal locomotion may be related to test-size dimensions. Kitazato (1988) observed a positive

relation between movement speed of foraminiferal species and the number of pseudopodia extruding through the aperture(s). Perhaps foraminiferal test size correlates to the number (and strength) of pseudopodia. Or, alternatively, it may imply that in this later phase of population recovery in the re-oxygenated sediments, reproduction has begun to make a significantly contribution to the recovery dynamics. In accordance with foraminifera, the (horizontal) movement speed of surface dwelling and deep-living nematodes was reported to be dissimilar; deep-living genera seem to move slower compare to their congeners living near the sediment surface (Ullberg and Olafsson, 2003). Moreover, nematode morphology has been suggested to be adapted to either oxic or anoxic conditions. A decrease in body width provides a relative increase of body surface area favourable in anoxic condition and an increasing body length improves mobility of deep-living species and moderate to migrate between anoxic and oxic sediment (Jensen, 1987; Soetaert et al., 2002) or even bridge the gap with their body (Soetaert et al., 2002).

The observed initial rapid recovery of the total foraminiferal standing stock in the hypoxia-affected sediments did not continue during the last time step in the experiment. In fact, a small abundance decrease in the hypoxia-affected sediments during this final period co-occurred with an increase in foraminiferal density in both types of unaffected sediment. The species *Ammonia beccarii*, *Hopkinsina pacifica* and *Trochammina inflata* were the main contributors to the foraminiferal increase in the control sediments (V(VV)). The concurrent abundance reduction in the hypoxia-affected sediments was also species-specific; among the dominant species, especially *Elphidium excavatum*, *T. inflata* and *Stainforthia fusiformis* had lower densities towards the end of the experiment. This observed contrast in the development of the total standing stock among the sediment types may be attributable to differences among species in their timing of reproduction (the last experimental period took place in autumn from September 22$^{nd}$ to November 8$^{th}$). When species differ in their timing of reproduction, dissimilarity in the assemblage composition may lead to contrasting developments of the total standing stock among the sediment types. In addition, the observed density decrease may also reflect a response to lowering of the concentration of organic carbon, postulated to be available as an ample food source in the former hypoxia-affected sediments during the first and second period. Moreover, competition for food with other meiofaunal groups like nematodes may have further induced the observed density decrease in the hypoxia-affected sediment during the last time step. In a field experiment in the Scheldt Estuary where patches of sediment had been induced to hypoxic condition, a nematodes abundance overshoot was reported to arise after 7-14 weeks subsequent to the start of ecosystem recovery (Van Colen et al., 2009). Despite the decrease in total standing stock during the final period, at the end of the experiment the mean total abundance in the upper centimetre of the hypoxia-affected sediments remained quite high at approximately 46% of the foraminiferal abundance in the sediments unaffected by hypoxia and 300% of the abundance in the starting material (t=0 A(AV)).

### 2.4.2 EXPOSURE TO HYPOXIA AFFECTED SEDIMENT

At the onset of the experiment the homogeneous cores with unaffected sediments contained almost twice as many foraminifera as the heterogeneous cores that combined hypoxia-affected and unaffected sediments. However, after 1.5 week this was reversed: the combined heterogeneous sediments (V(AV)+A(AV)) contained more foraminifera than the homogeneous cores (V(VV)+V(VV)). The foraminiferal recolonisation of the formerly hypoxic sediments did not diminish the total foraminiferal standing stock in the neighbouring source sediments within the same microcosm. If active foraminiferal dispersal, through self-locomotion, led to the observed increase of foraminifera

in the hypoxia-affected sediments, a decrease in the combined unaffected sediments was expected if net reproduction would have been zero. This suggests that either reproduction was higher in the heterogeneous cores, or mortality lower, or a combination of both.
Due to re-oxygenation of the formerly hypoxic sediments an underused surplus of favourable, empty sediment microhabitats became available. Additionally, the exodus of foraminifera to these sediments may have created room for expansion of the foraminiferal population that stayed behind in the sediments the others emigrated from. Furthermore, the former depletion of oxygen may have facilitated organic matter build-up – now highly available and suitable as food-source for foraminifera – by slowing down decomposition rates. Our results indicate that especially *Elphidium excavatum* responded with population growth to the sudden dilution of the foraminiferal density as a larger, previously inhabitable microhabitat volume became available during the first period of the experiment. Except for its much lower abundances, the density pattern of *Epistominella vitrea* in this experiment seemed to mimic that of *E. excavatum*. Interestingly, *E. vitrea* and its closest relative, *Epistominella exigua*, are known for their opportunistic, rapid population growth during times of sudden increased organic matter or phytodetritus availability (e.g. Ernst et al., 2005 and Gooday, 1993, respectively).

### 2.4.3 INDICATION OF ECOSYSTEM HEALTH

Jointly, the foraminiferal abundance and the assemblage composition were indicative of sediment type and the stage of recovery. Among the dominating species of the foraminiferal community in the control cores of Lake Grevelingen (VV), *Trochammina inflata* seemed to be indicative of healthy sediment – sediments that had not been exposed to hypoxia disturbance in the field or during the experiment. In the fossil foraminiferal assemblages, preserved in the sediment archive, *T. inflata* was, together with *Jadammina macrescens*, the dominant species before the tidal influence in Lake Grevelingen was blocked (Donders et al., 2012). The displayed lack of opportunity to profit from the exposure to hypoxia-affected sediments may indicate a higher sensitivity of *T. inflata* to remaining higher concentrations of reduced substances such as hydrogen sulphide at depth in the re-oxygenated core halves, or possibly this species has a preference for more refractory organic matter. Moreover, *T. inflata* may be a relatively weak competitor in these circumstances compared to other foraminiferal species in the assemblage. A restricted occurrence of *T. inflata* to oxygenated habitat was previously observed. For example, De Stigter et al. (1998) reported that along a shelf to bathyal transect in the Southern Adriatic Sea, *Trochammina inflata* exclusively occupied microhabitats near the water-sediment interface. Although many foraminiferal species are known to be relatively tolerant to oxygen depletion, differential tolerance for hydrogen sulphide in the pore-water enables some foraminifera to proliferate while others suffer (Moodley et al., 1998b).
Exposure to hypoxia was, both in the experimental set-up and in the recent history of the fossil foraminiferal assemblage in the field, reflected by a decline in foraminiferal densities. Reversely, re-aeration of hypoxia-exposed sediments stimulated the recovery of the benthic foraminiferal community. The foraminiferal density was the most apparent difference between the sediment types V(VV) and A(AV). The noticed high tolerance of *Ammonia beccarii* and *Elphidium excavatum* to fluctuation in the environment – indicated by their role as early recolonisers after the re-aeration of hypoxia-affected sediment – was also observed in the fossil assemblage from the same area (Donders et al., 2012). Together with *Haynesina germanica*, *A. beccarii*'s abundance increased in 1972 (they replaced *Trochammina inflata* and *Jademmina macrescens*), directly after Lake Grevelingen was disconnected from the North Sea. *E. excavatum* dominated the assemblage in the second period, from 1972 to 1982. In the most recent history (1990 – 2011) the contribution of *Elphidium* sp. increased

concurrently to worsening of the oxygen depletion in the bottom-waters of Lake Grevelingen (Donders et al., 2012).

### 2.4.4 FUTURE RECOVERY POTENTIAL

The results of this experimental study indicate that foraminifera recolonise hypoxia-affected benthos-depleted sediments from neighbouring unaffected assemblages after hypoxic conditions have been reversed. At the end of the experiment, the total standing stock of foraminifera in the formerly hypoxia-affected sediments had increased from 15% of that in the unaffected sediments initially, to about 46% after 10.5 weeks. Not surprisingly, recolonisation of the hypoxia-affected sediments by foraminifera was not complete within the experimental period since it was designed to observe short-term responses of the foraminiferal community to future restorative efforts in Lake Grevelingen. The observed recolonisation rates (defined by the width of our experimental cores – 7 cm, and the chosen duration of the experiment – 10.5 weeks) in our experiment underestimate the required time span of longer-distance migration (metre to kilometre scale) in a field situation and the contribution of passive dispersal of small foraminifera or propagules from foreign regions is likely to become more important for recolonisation of hypoxia-affected sediments on longer time-scales (months or even years, for instance depending on the frequency of disturbance). Nonetheless, given the observed encouragingly high rate of foraminiferal recovery in this short-term (10.5 weeks) and small-scale (7 cm) experiment, foraminiferal migration is expected to contribute to the recovery of the foraminiferal community in Lake Grevelingen. Foraminiferal migration from nearby unharmed areas may enhance the recovery rate over small distances (centimetre to metre), whereas lateral locomotion of offspring produced by early colonisers or foraminifera passively transported to formerly hypoxia-exposed areas may further increase the foraminiferal recovery rate. It seems likely that recovery of foraminiferal communities within a few years is feasible as long as there are still unaffected areas nearby, which may function as suppliers of recolonising immigrants. For proximity of unaffected areas in the field it is important that, before restorative measures are taken, the periodic hypoxic bottom-water conditions remain confined to the narrow deeper gullies in the lake. However, as the summer oxygenation-hypoxia boundary in the water column keeps shallowing, the geographic extent of the affected areas may explode once this boundary reaches the edges of the narrow gullies and unfavourable conditions can spread rapidly all over the flatter parts of the lake bed.

## 2.5 CONCLUSION

The dynamics in density and composition of the foraminiferal assemblages in our restoration experiment indicated a species-specific response and the existence of sequential stages during recovery of the foraminiferal assemblage in the re-oxygenated sediments. Species with relatively large test sizes, such as *Ammonia beccarii* and *Elphidium excavatum,* were the first to recolonise the re-oxygenated organic-rich sediments. In the second stage *Hopkinsina pacifica* and *Stainforthia fusiformis*, species with relatively smaller test sizes, entered these sediments or became more densely present due to enhanced reproduction. These observations may further indicate that foraminiferal test size is related to the velocity of locomotion, with higher movement speeds for larger specimens, or that reproduction becomes more important in the second stage. Furthermore, especially *E. excavatum* and *Epistominella vitrea* responded opportunistically to the presumably high availability of detrital organic matter in the heterogeneous sediments cores. However, *A. beccarii* and *S. fusiformis* revealed a more stable density in the unaffected sediments of the heterogeneous and homogeneous cores over the course of the experiments. Among the numerously dominant species, *Trochammina inflata* was most sensitive to disturbance. This experiment indicates that the presence of a healthy neighbouring

foraminiferal assemblage contributes to the short-term recolonisation of re-oxygenated sediments, underlining the importance that restorative efforts in Lake Grevelingen should begin before the hypoxia boundary in the water column will rise enough for hypoxic conditions to expand beyond the narrow gullies.

PLATE

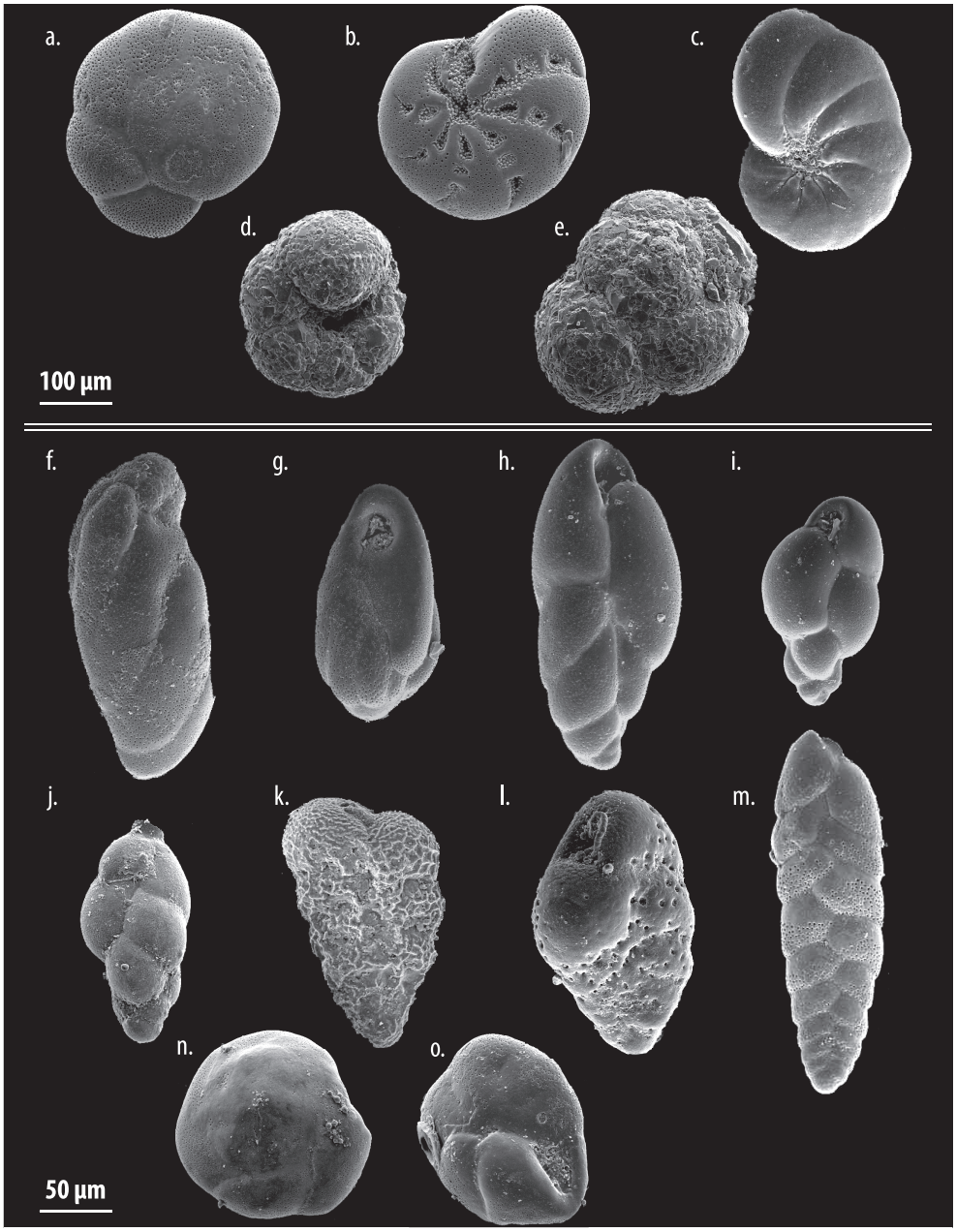
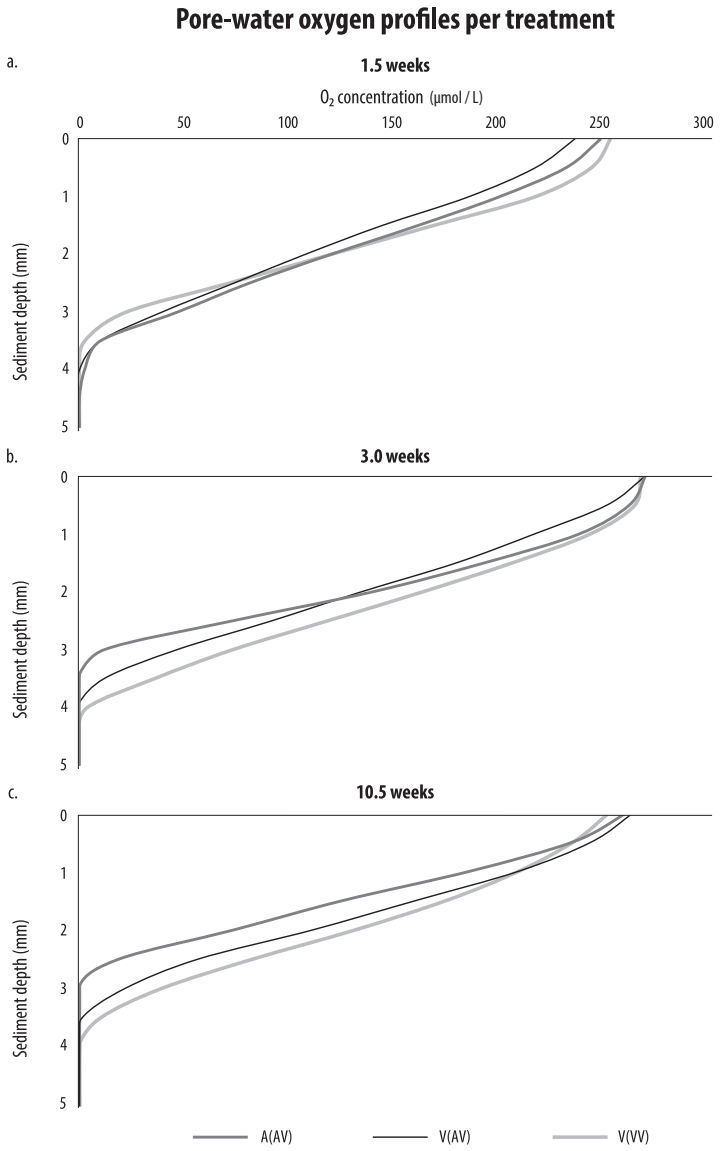



**Plate 2.1** Scanning electron microscope pictures of a selection of the foraminifera from Lake Grevelingen a. *Ammonia beccarii*, b. *Elphidium excavatum*, c. *Nonion commune*, d. *Trochammina inflata*, e. *Trochammina inflata*, f. *Buliminella elegantissima*, g. *Buliminella elegantissima*, h. *Stainforthia fusiformis*, i. *Stainforthia fusiformis*, j. *Hopkinsina pacifica*, k. *Bolivina plicatella*, l. *Bolivina dilatata*, m. *Bolivina seminuda*, n. *Epistominella vitrea*, o. *Epistominella vitrea*




**Appendix 2.1 a. - c.** Oxygen profiles per sample event a. 1.5, b. 3 and c. 10,5 weeks after the start of the experiment

| **Kruskal-Wallis** Total standing stock | |
|---|---|
| Time steps pooled | p(same) |
| A(AV) | 0.24 |
| V(AV) | 0.32 |
| V(VV) | 0.52 |

| **Kruskal-Wallis** *Ammonia beccarii* | |
|---|---|
| Time steps pooled | p(same) |
| A(AV) | 0.26 |
| V(AV) | 0.54 |
| V(VV) | 0.53 |

| **Kruskal-Wallis** *Elphidium excavatum* | |
|---|---|
| Time steps pooled | p(same) |
| A(AV) | 0.14 |
| V(AV) | 0.24 |
| V(VV) | 0.24 |

| **Kruskal-Wallis** *Trochammina inflata* | |
|---|---|
| Time steps pooled | p(same) |
| A(AV) | 0.18 |
| V(AV) | 0.76 |
| V(VV) | 0.23 |

| **Kruskal-Wallis** *Hopkinsina pacifica* | |
|---|---|
| Time steps pooled | p(same) |
| A(AV) | 0.14 |
| V(AV) | 0.30 |
| V(VV) | 0.04 |

| **Kruskal-Wallis** *Stainforthia fusiformis* | |
|---|---|
| Time steps pooled | p(same) |
| A(AV) | 0.37 |
| V(AV) | 0.98 |
| V(VV) | 0.28 |

| **Kruskal-Wallis** Total standing stock | |
|---|---|
| | p(same) |
| 0 weeks | 0.18 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 0.74 |
| 1.5 weeks | 0.11 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 0.32 |
| 3 weeks | 0.94 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 1.00 |

| **Kruskal-Wallis** *Ammonia beccarii* | |
|---|---|
| | p(same) |
| 0 weeks | 0.18 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 0.74 |
| 1.5 weeks | 0.35 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 1.00 |
| 3 weeks | 0.94 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 1.00 |

| **Kruskal-Wallis** *Elphidium excavatum* | |
|---|---|
| | p(same) |
| 0 weeks | 0.37 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 0.74 |
| 1.5 weeks | 0.13 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 0.32 |
| A(AV) - V(VV) | 1.00 |
| 3 weeks | 0.16 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 0.32 |
| A(AV) - V(VV) | 1.00 |

| **Kruskal-Wallis** *Trochammina inflata* | |
|---|---|
| | p(same) |
| 0 weeks | 0.16 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 0.74 |
| 1.5 weeks | 0.13 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 0.32 |
| 3 weeks | 0.11 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 0.74 |
| A(AV) - V(VV) | 0.32 |

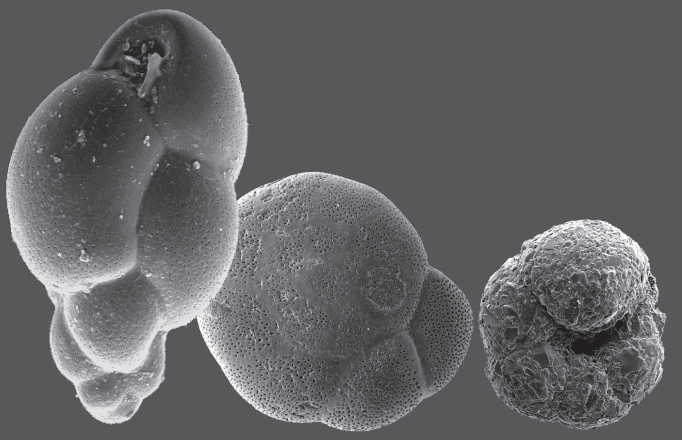
| **Kruskal-Wallis** *Hopkinsina pacifica* | |
|---|---|
| | p(same) |
| 0 weeks | 0.10 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 0.74 |
| A(AV) - V(VV) | 0.74 |
| 1.5 weeks | 0.35 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 1.00 |
| 3 weeks | 0.11 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 0.32 |
| A(AV) - V(VV) | 0.74 |

| **Kruskal-Wallis** *Stainforthia fusiformis* | |
|---|---|
| | p(same) |
| 0 weeks | 0.37 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 0.74 |
| 1.5 weeks | 0.17 |
| A(AV) - V(AV) | 0.74 |
| V(AV) - V(VV) | 0.74 |
| A(AV) - V(VV) | 1.00 |
| 3 weeks | 0.94 |
| A(AV) - V(AV) | 1.00 |
| V(AV) - V(VV) | 1.00 |
| A(AV) - V(VV) | 1.00 |

**Appendix 2.2** Kruskal-Wallis

| **TWO-WAY ANOSIM** Assemblage | **Treatment** | | **Sample moment** | |
|---|---|---|---|---|
| | R | p(same) | R | p(same) |
| A(AV) - V(AV) - V(VV) | 0.224 | 0.062 | 0.088 | 0.235 |
| A(AV) - V(AV) | 0.438 | 0.073 | 0.125 | 0.248 |
| V(AV) - V(VV) | 0.063 | 0.340 | 0.132 | 0.153 |
| A(AV) - V(VV) | 0.277 | 0.130 | 0.007 | 0.460 |

**Appendix 2.3** TWO-WAY ANOSIM



CHAPTER 3

# FORAMINIFERAL RECOLONISATION PATHWAYS AND MICROHABITAT DISTRIBUTION AFTER PROLONGED HYPOXIA DISTURBANCE

G.M. Brouwer, M. Wolthers, V.M. van Onselen and I.A.P. Duijnstee

## ABSTRACT

Short-term foraminiferal recovery and distribution patterns during and after hypoxia were investigated experimentally in order to assess the restoration potential of benthic meiofauna in sediments from frequently hypoxic parts of salt water Lake Grevelingen, the Netherlands. Both the dynamics in foraminiferal density and assemblage composition are considered in the assessment of recolonisation pathways and ecosystem health. Active migration, rather than a population increase from within the sediment seemed to dominate short-term foraminiferal recolonisation. After 10.5 weeks, hypoxia-affected sediments neighbouring unaffected sediments in recombined (heterogeneous) cores had a foraminiferal density of 38% compared to the total standing stock in (homogeneous – unaffected) control cores. The highest resemblance of assemblage composition was found between hypoxia-affected sediments in heterogeneous cores and healthy control cores. In absence of neighbouring unaffected sediments, i.e. in homogeneous affected cores kept under ventilated bottom-water conditions, the foraminiferal density was a mere 6% compared to the controls, and 5% when such cores were kept anoxic. Despite these similarly low total densities in the latter two sediment types, the assemblage composition differed substantially. In particular, the displacement of *Hopkinsina pacifica* by *Ammonia beccarii* and *Elphidium excavatum* was observed in homogeneous affected cores under re-aerated bottom-water conditions, while *H. pacifica* remained dominant in the sediments kept anoxic. At the other end of the spectrum, *Trochammina inflata* predominantly thrived in the healthy undistributed (control) sediments. In the heterogeneous cores *Ammonia beccarii*, *Hopkinsina pacifica*, *Elphidium excavatum* and *Stainforthia fusiformis* were the dominant colonisers of the affected sediments, while especially *Elphidium excavatum* had an abundance increase in the unaffected sediments. Compared to the field situation, our microcosm experiments may over-estimate the importance of active migration (because of the close proximity between hypoxia-affected and unaffected sediments) and underestimate the potential role of passive transport and activation of dormant propagules (because of the short time span) in the recovery of foraminiferal assemblages. Nonetheless, our data show the initial pathways of foraminiferal recovery at the substantial interface between healthy and frequently anoxic parts of the lake. Moreover, in comparison with literature data, our results illustrate how dispersal pathways may depend on spatial and temporal scale. Therefore, colonisation processes are likely to deviate between patchy and homogeneous hypoxia-disturbed areas.

## KEYWORDS

Benthic foraminifera * Hypoxia disturbance * Recolonisation patterns * Vertical distribution * Species-specific responses * Active migration * Lake Grevelingen

## 3.1 INTRODUCTION

The eukaryote phylum Foraminifera comprises an extensive diversity of predominantly marine, unicellular organisms. The widespread occurrence of foraminiferal species in a broad variety of marine ecosystems reflects their high tolerance to a range of environmental conditions. Despite this high resilience, foraminiferal species respond rapidly and differentially to changes in their environment, both in abundance and in assemblage composition. New habitats are quickly colonised by foraminifera (Corliss, 1991; Goldstein and Alve, 2011). Because of this and the ability of many species to tolerate a wide range of environmental conditions, foraminifera are ubiquitous throughout most marine realms and are abundant in marine sediments around the globe.

Some species of benthic foraminifera live on top of or just inside the uppermost layer of the sediment, confusingly both referred to as 'epifaunal'. Shallow infaunal species have their average life position in the top of the sediment column (0-2 cm) which is usually partly oxygenated, while the deep dwelling species occupy the lower (>2 cm), usually sub- to anoxic part of the sediment (Corliss, 1991; Linke and Lutze, 1993). The oxygen-penetration depth has a strong influence on the vertical distribution of foraminiferal populations in the sediment. Foraminifera are known to have a dynamic species-specific microhabitat preference (Corliss, 1985; Linke and Lutze, 1993; Ernst et al., 2008). The preferred microhabitat depends on the interplay between food availability and oxygen concentration in the pore-water (Jorissen et al., 1992, 1995; Van der Zwaan et al., 1999), and likely also on concentrations of other chemical species that characterise the different redox zones within the sediment (Fontanier et al., 2002; Koho et al., 2008). Additionally, the realised niche of foraminifera is influenced by biotic interactions with other organisms through competition, predation, passive transportation, chemical and structural alterations of the microhabitats, etc. (see Murray, 2006). Foraminifera migrate in response to changes in their environment. Fluctuations in food and oxygen availability stimulate or slow down locomotion (Alve and Bernhard, 1995; Duijnstee et al., 2003; Geslin et al., 2004). These migration patterns are not restricted to vertical movement through the sediment (Severin et al., 1982; Severin, 1987; Bornmalm et al., 1997). With migration speeds of up to a few millimetres per hour (Kitazato, 1988), foraminifera drag themselves through and over the sediment using an extended network of pseudopodia. Locomotion across sediments contributes to small-scale dispersal (Alve, 1999; Murray, 2006). Besides this form of active dispersal, various foraminiferal growth stages may be passively dispersed. Foraminifera themselves may be transported by water current over large distances (Alve, 1999) and over or through the sediment by bioturbating macrofauna. Especially propagules (tiny juveniles) with their small test sizes (if they have tests at all) are easily transported and deposited in new environments. It has been suggested that propagules can rest as cysts in a cryptic state for periods of up to two years until favourable conditions arise that stimulate them to become active again (Alve and Goldstein, 2003, 2010). As such, propagule banks can potentially comprise a wide variety of species, increasing the ability of whole foraminiferal communities to swiftly (re-)colonise altered environments, thus explaining their worldwide ecological success as well as the fast foraminiferal response to environmental change (Goldstein and Alve, 2011). This high potential to colonise new habitats, the ability to endure a broad range of environmental conditions, and the fast response to this variability within the sediment column, lend foraminifera a relevant role as bioindicator for current ecosystem state (Bouchet et al., 2012; Schönfeld et al., 2012). Moreover, since a strong relation exists between foraminiferal abundances and their taxon-specific required minimum level of oxygen (Murray, 2001), fossil and modern foraminifera have been recognised as valuable proxies for, for instance, frequent or persistent low-oxygen bottom-water concentrations.

A laboratory experiment was conducted using hypoxia-affected and unaffected sediments from Lake Grevelingen; an enclosed marine lake situated in the South-Western part of The Netherlands (see also

the previous chapter). Very limited exchange of water between the lake and the North Sea results in hypoxic conditions in some of the deeper areas, and further stratification of the water column in summer. Ecosystem surveys have indicated a strong deterioration of the benthic community in those deeper parts of the lake that are affected by frequent hypoxic conditions (Lengkeek et al., 2010). These sediments contain only small numbers of living foraminifera and are virtually void of benthic macrofauna (Guasti et al., 2011; Donders et al., 2012). To investigate the recovery potential of the benthic foraminiferal communities after hypoxic conditions are reversed, foraminiferal recolonisation and distribution patterns were studied. We analysed the abundance and vertical distribution of foraminiferal species in hypoxia-affected sediments that were kept under ventilated conditions while being either in or out of direct contact with unaffected sediments. The foraminiferal dynamics of those assemblages were compared to assemblages in untreated sediments and to hypoxia-affected sediments kept anoxically. By comparing the contribution of restoration from within the anoxic sediments after ventilation of bottom-water with recovery of the foraminiferal assemblage via horizontal migration from unaffected areas, we will discuss the importance of these separate recovery pathways and its implication for restoration of hypoxia-affected foraminiferal communities.

## 3.2 MATERIALS AND METHODS

The saline Lake Grevelingen is a former estuary of the North Sea along the South-West coast of The Netherlands (salinity 32, 140 $km^2$). Two dams were built to protect the hinterland from flooding; one (completed in 1964) effectively blocked outflow waters of the rivers Rhine and Meuse from entering the lake, the other (completed in 1971) closed the seaward opening of the former estuary to the North Sea. Although a sluice was constructed in the western dam in 1978 to allow for some degree of water exchange with the North Sea, the frequency and extent of seasonal hypoxic conditions increased as a consequence of stratification of the water column in absence of tidal mixing.

On August 23rd, 2011, sediments were collected in a gully in the South-Western part of Lake Grevelingen (bocht van St. Jacob) using a boxcorer. This hypoxia-affected station (GTSO 6) had a water depth of 37.0 m and an $O_2$ concentration of 0.33 mg/L at the water-sediment interface (= 4% of the saturated $O_2$ concentration). A nearby station (at approximately 2 km distance), with a water depth of 10.0 m, was sampled to collect unaffected sediments (i.e., with oxygenated bottom-water; GTSO 7). At the water-sediment interface, this station had an $O_2$ concentration of 6.05 mg/L (= 78% $O_2$ saturation). On board, the boxcorers were subsampled using plastic corers with an internal diameter of 7 cm. The sediment cores were stored in cool boxes and directly transported to the laboratory in Utrecht where they were kept at a temperature of 10°C during the experiments.

### 3.2.1 EXPERIMENTAL SET-UP

After removal of visible hard-shelled macrofauna by hand (predominantly *Crepidula fornicata* – the common slipper shell was present with a density of 1 ± 1 specimens per core), the sediment cores were split along the central vertical axis to obtain two half sediment cores. For each experimental core, two half cores were (re)combined. With this procedure, 5 different sediment types were made (see Fig. 3.1). Originally unaffected sediments were joined in cores with either hypoxia-affected ones (to form heterogeneous experimental cores) or with unaffected sediment core halves (to form homogeneous unaffected control cores); some of the originally affected sediment core halves were re-united to form homogeneous affected cores. During the experimental incubation period in the laboratory, all of these experimental composite cores were kept under oxygenated bottom-water conditions, except for some of the homogeneous affected cores (as a control group reflecting continued hypoxia in the field). At the end of the experiment, the combined core halves were re-split again along the same plane, and

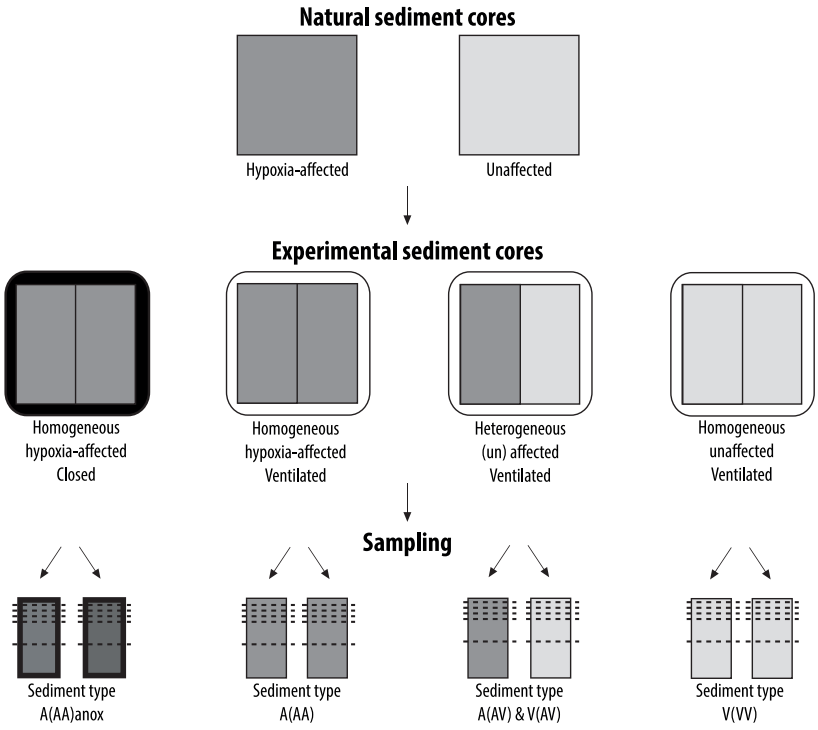



**Figure 3.1** Experimental set-up and sampling strategy.

horizontally sliced in different depth intervals (see below). Originally unaffected sediment derived from heterogeneous cores will be referred to as V(AV); the first letter indicating the provenance of the core half in the field, the two letters between brackets indicating the composition of the experimental core it came from. The affected sediments derived from these heterogeneous cores were thus labelled as A(AV), whereas the unaffected sediments in the homogeneous unaffected cores were indicated as V(VV)). Core halves from hypoxia-affected homogeneous cores were either called A(AA) or $A(AA)_{anox}$, depending on whether or not they had been exposed to anoxic bottom-water conditions during the experiment. All cores were placed in containers filled with natural waters from Lake Grevelingen, aquarium pumps were used to ventilate the water.

The cores were sampled after 10.5 weeks from the onset of the experiment. Vertical oxygen profiles were measured using a Unisense OX-50 microsensor, calibrated against air-saturated water from Lake Grevelingen and attached to a micromanipulator for accurate depth-profiling (Appendix 3.1, see also chapter two). After re-splitting the cores into the original core halves, they were sliced horizontally: the upper 2 cm in 4 slices of 0.5 cm each, followed by one interval from 2.0 to 5.0 cm sediment depth, and one last layer containing all the sediment below 5 cm sediment depth (this layer comprised on average the sediment from 5.0 to 9.7 cm sediment depth). The samples were stored in ethanol with 1g/L Rose Bengal to stain foraminiferal protoplasm. Foraminifera stained brightly pink in all but the ultimate chamber of the test were considered to be alive at or not too long before the end of the experiment. They were picked from wet samples and enumerated at the species level after the sediment had been

sieved over two mesh sizes of 63 and 150 µm. Sediment dry weights were determined to facilitate correction for sampling errors. Foraminiferal densities were expressed per sediment slice. The sediment dry weight per volume of the sediment below 5.0 centimetre sediment depth was not assessed, but assumed to be comparable to that measured for 2.0 to 5.0 centimetres sediment depth.
To assess the difference between the start and the end of the experiment, we compared the results of this chapter with the results of chapter two – in that chapter we analysed the foraminiferal dynamics in the upper centimetre of the sediment over time. We examined (for the upper centimetre – divided in two sediment layers and for both size fractions) the relative increase (or decrease) of the total standing stock and the absolute difference in the contribution of each species to the assemblage composition between the start and the end of the experiment (see previous chapter for sampling information and foraminiferal data from the onset of the experiment).

### 3.2.2 STATISTICS

Statistical analyses were performed in PAST (PAleontological STatistics, Hammer et al., 2001). The total foraminiferal abundances and the abundances per treatment of the dominant species *Ammonia beccarii, Elphidium excavatum, Trochammina inflata, Hopkinsina pacifica* and *Stainforthia fusiformis* were tested on statistical dissimilarity using the non-parametric Kruskal-Wallis test (Appendix 3.2). With a Two-Way ANOSIM test, the observed differences among foraminiferal assemblages and their vertical partitioning in the sediment were tested (Appendix 3.3). The SIMPER test was used to indicate the contribution of individual species to differences among assemblages. The SIMPER test was applied on both absolute (Appendix 3.4.1) and relative (Appendix 3.4.2) abundances of foraminifera at the end of the experiment. Using absolute abundances, the difference among assemblages is based on the occurrence of species and their abundances; when using the relative abundances, the difference among assemblages is based on differences in the contribution of each species to the assemblage composition.

## 3.3 RESULTS

### 3.3.1 $O_2$ PROFILES

At the end of the experiment, the oxygen-penetration depth was around 3.5 mm sediment depth in all sediments kept under ventilated bottom-water laboratory conditions. Obviously, the overlying water in the closed anoxic cores was void of oxygen throughout the experimental period (Appendix 3.1).

### 3.3.2 FORAMINIFERAL DENSITIES

At the end of the experimental period, a relatively high average foraminiferal density was found in the V(AV) core halves – the unaffected sediments that had been combined with hypoxia affected sediments (see Fig. 3.2). Although the average foraminiferal abundance seemed to be slightly less in the unaffected sediments that had not been exposed to hypoxia affected sediments, V(VV), both densities did not statistically differ. Figure 3.3 indicates that in this sediment type the foraminifera were most concentrated in the upper half cm of the sediment, while the first type of unaffected sediments, V(AV), seemed to have a more equal distribution of foraminifera over the upper four layers down to 2 centimetres sediment depth. The originally hypoxia-affected sediment that had been in direct contact with unaffected sediment, A(AV), showed an intermediate foraminiferal density (38% compared to the unaffected sediment in homogeneous cores). The relative distribution of foraminiferal individuals over the two size fractions and along the vertical sediment profiles resembled that of (VV). However, in the A(AV) sediments foraminifera seemed to be even more concentrated in the upper 0.5 centimetre of

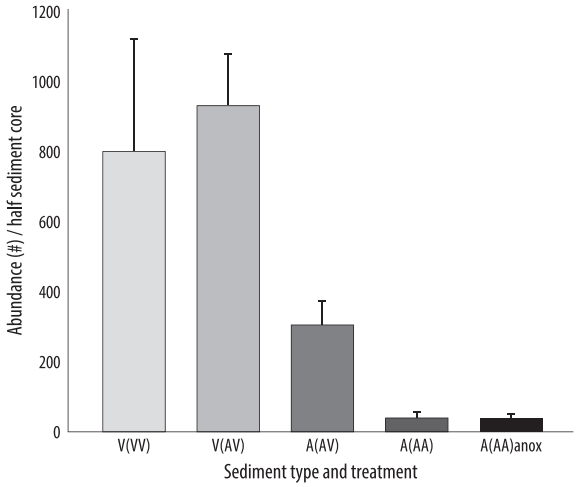



**Figure 3.2** Foraminiferal total standing stock (>63 µm) and standard deviation per half sediment core of each sediment type and treatment after 10.5 weeks. V(VV) represents abundance in unaffected ventilated sediment, V(AV) represents abundance in unaffected ventilated sediment that was combined with hypoxia-affected sediment, A(AV) represents abundance in hypoxia-affected ventilated sediment combined with unaffected sediment, A(AA) represents hypoxia-affected ventilated sediment, $A(AA)_{anox}$ represents hypoxia-affected sediment kept anoxic.

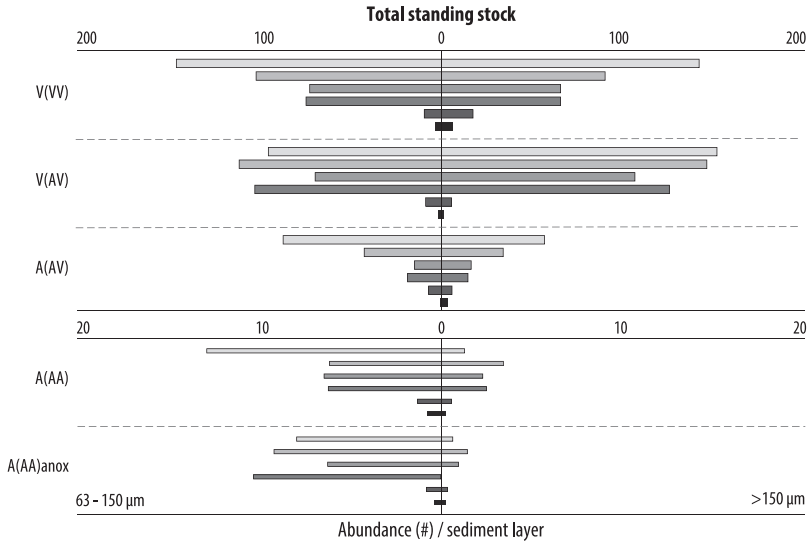



**Figure 3.3** Foraminiferal total standing stock after 10.5 weeks per half sediment core, per sediment type, sediment slice and size fraction (63-150 µm on the left side and >150 µm on the right side of the vertical axis). The foraminiferal abundances are displayed per sediment layer; 0.0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-5.0, and >5.0 cm. These depth intervals are represented by bars; the lightest bar represents the foraminiferal abundance in the upper sediment layer etc.. The abundance of the sediment types V(VV), V(AV) and A(AV) are plotted on the upper horizontal axis, while the abundances of the sediment types A(AA) and $A(AA)_{anox}$ are plotted on the lower horizontal axis.

the sediment. Low abundances were found in both types of homogeneous cores containing hypoxia-affected sediment: A(AA) and $A(AA)_{anox}$ had respectively approximately 6% and 5% of the standing stock of unaffected healthy sediments (V(VV)). In both A(AA) and $A(AA)_{anox}$ the foraminifera were rather similar distributed over the upper 2 centimetre of the sediment. In contrast to the other sediment types, the assemblage of foraminifera in these sediments consisted predominantly of relatively small specimens, i.e. smaller than 150 µm (>150 µm size fraction contributed on average 13% to the total standing stock of $A(AA)_{anox}$ sediments).

### 3.3.3 ASSEMBLAGE COMPOSITION

The foraminiferal assemblage comprised 21 species. *Ammonia beccarii, Elphidium excavatum, Trochammina inflata, Hopkinsina pacifica* and *Stainforthia fusiformis* jointly comprised 75-95% of the total standing stock in all sediment types (Table 3.1a.).

Species found in relatively low abundances are: *Bolivina dilatata, Bolivina plicatella, Bolivina seminuda, Bolivina variabilis, Buliminella elegantissima, Bulimina aculeate, Cibicides lobatulus, Elphidium excavatum, Elphidium lessoni, Elphidium marcellum, Elphidium williamsoni, Epistominella vitrea, Nonion commune, Quinqueloculina seminula, Rosalina bradyi, Rosalina globularis,* and *Textularia earlandi* (Table 3.1). The Two-Way ANOSIM test (Appendix 3.3) revealed that both depth and treatment highly contributed to the observed differences in the foraminiferal assemblages. The unaffected sediments of the homogeneous and the heterogeneous cores (V(VV) and V(AV)) had the highest similarity in foraminiferal density and assemblage composition (see Appendix 3.4.1). Despite the observed difference in density, the composition of the foraminiferal assemblages in the unaffected V(VV) sediment and those in the hypoxia affected sediments of the (AV) cores resembled each other (Appendix 3.4.2). *E. excavatum* and *H. pacifica* contributed more to dissimilarities in the assemblage composition when considering relative abundances. Still, the composition of the foraminiferal assemblage in $A(AA)_{anox}$ was most divergent to all other assemblages. *A. beccarii, E. excavatum* and *H. pacifica* were, with a contribution to the total standing stock of respectively 44.4% ± 4.4, 26.5% ± 4.6 and 12.5% ± 4.8, the most dominant colonisers of the hypoxia affected sediments of the heterogeneous cores (A(AV)). The contribution of these species to the assemblage in A(AV) resembled that of V(VV) (respectively 44.9% ± 3.6, 23.1% ± 5.9, 9.6% ± 4.4). *T. inflata* contributed on average 12.2 % ± 7.0 to the total standing stock; it was the only species that seemed to prefer V(VV) sediments. The same species trio dominated the hypoxia-affected sediments in the aerated homogeneous (AA) cores. However, with an average contribution of 38.0% ± 9.3 *E. excavatum* was most dominant, followed by *A. beccarii* that comprised 32.1% ± 11.2 of the assemblage. Although the foraminiferal density in the hypoxia-affected sediments of both types of homogeneous (AA)-cores was comparable low, the composition of the assemblage seemed to differ. *H. pacifica* was, with an average contribution of 44.8% ± 8.9, by far the most dominant species in the cores that were permanently anoxic ($(AA)_{anox}$). The density of *E. excavatum* and *A. beccarii* comprised a mere 13.9% ± 2.4 and 8.7% ± 1.1 of the $(AA)_{anox}$ assemblage composition. The contribution of *S. fusiformis* to the assemblage composition in the upper sediment layer (0.0 – 1.0 centimetre depth) decreased in all types of sediment during the experiment, but most strongly in the (AA) cores (Table 3.1b.). More rare species, such as *R. bradyi, B. elegantissima* and *Bolivina* spp., had their highest relative contribution to the total standing stock in the cores kept anoxically.

Besides driving changes in foraminiferal density through colonisation of A(AV) sediments, the exposure of V(AV) sediments to their A(AV) counterparts seemed to have altered the composition and may have increased the density of the foraminiferal community in the originally unaffected halves themselves. The V(AV) sediments showed the highest average density of foraminifera, on average even higher

**Table 3.1** Foraminiferal totals standing stock (TTS) at the start and the end of the experimental period in the upper centimetre of the sediment and the relative change in TTS (in %) compared to the onset of the experiment. Total standing stock over sediment depth (0->5 cm) at the end of the experimental period.

a. Species contribution (%) to the total assemblage (over 0->5 cm sediment depth) at the end of the experiment per sediment type and treatment.

b. Absolute change in the species contribution (%) to the assemblage in the upper centimetre per sediment type and treatment, compared between the start and the end of the experiment.

| | V(VV) | | V(AV) | | A(AV) | | A(AA) | | A(AA)anox | |
|---|---|---|---|---|---|---|---|---|---|---|
| TTS (# 0 weeks , 0 - 1 cm) | 498.68 | | 444.82 | | 73.78 | | 16.46 | | 16.46 | |
| TTS (# 10.5 weeks, 0 - 1 cm) | 479.21 | | 503.54 | | 219.38 | | 23.77 | | 19.24 | |
| TTS (# 10.5 weeks, 0 - > 5 cm) | 794.69 | | 897.53 | | 299.59 | | 44.20 | | 38.60 | |
| Relative change TTS (0 - 1 cm) | -3.90 | | 13.20 | | 197.35 | | 44.39 | | 16.86 | |
| | a. | b. | a. | b. | a. | b. | a. | b. | a. | b. |
| *Ammonia beccarii* | 44.9 | 5.8 | 42.5 | 0.5 | 46.3 | 9.5 | 31.9 | 38.6 | 8.2 | -0.1 |
| *Bolivina dilatata* | 0.1 | -0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 1.0 | 1.5 |
| *Bolivina plicatella* | 0.7 | 0.4 | 0.3 | -0.6 | 0.6 | 0.2 | 0.1 | 0.0 | 2.6 | 2.1 |
| *Bolivina seminuda* | 0.1 | -0.3 | 0.0 | -0.2 | 0.0 | -0.8 | 0.0 | 0.0 | 1.8 | 2.3 |
| *Bolivina variablilis* | 0.1 | 0.1 | 0.1 | 0.2 | 0.0 | 0.0 | 0.1 | 0.0 | 3.0 | 2.3 |
| *Bulimina aculeata* | 0.6 | 0.4 | 0.0 | 0.9 | 0.0 | -1.5 | 0.9 | -4.4 | 1.0 | -0.5 |
| *Buliminella elegantissima* | 1.7 | 0.5 | 1.5 | 0.0 | 0.4 | 0.0 | 0.5 | 1.0 | 3.6 | 1.5 |
| *Cibicides lobatulus* | 0.1 | 0.0 | 0.4 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Elphidium excavatum* | 21.7 | -14.7 | 37.7 | 8.5 | 24.6 | -8.6 | 39.6 | 6.5 | 15.8 | -7.6 |
| *Elphidium lessoni* | 0.7 | -0.2 | 0.6 | 0.0 | 0.0 | 0.0 | 0.5 | 1.0 | 0.0 | 0.0 |
| *Elphidium marcellum* | 0.3 | 0.5 | 0.4 | 0.3 | 1.1 | 0.2 | 2.3 | 1.7 | 0.0 | 0.0 |
| *Elphidium williamsoni* | 0.4 | -0.1 | 0.4 | 0.2 | 0.2 | 0.0 | 1.9 | 3.6 | 1.2 | 0.0 |
| *Epistominella vitrea* | 1.0 | -0.1 | 1.5 | 0.9 | 0.8 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Hopkinsina pacifica* | 9.0 | 4.2 | 4.0 | -7.0 | 12.9 | 6.5 | 16.7 | -25.6 | 45.9 | 9.8 |
| *Nonion commune* | 0.1 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | -3.3 | 0.0 | -3.3 |
| *Quinqueloculina seminula* | 0.1 | -0.5 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 1.2 | 1.5 |
| *Rosalina bradyi* | 0.5 | -0.9 | 0.4 | -0.5 | 1.5 | -0.7 | 2.3 | -0.4 | 5.9 | 3.4 |
| *Rosalina globularis* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 |
| *Stainforthia fusiformis* | 3.6 | -2.4 | 3.3 | -2.9 | 4.9 | -4.3 | 1.8 | -19.7 | 7.0 | -14.3 |
| *Textularia earlandi* | 0.2 | 0.2 | 0.3 | 0.5 | 0.5 | 0.7 | 0.0 | 0.0 | 0.7 | 0.0 |
| *Trochammina inflata* | 14.0 | 6.9 | 6.1 | -1.3 | 5.7 | -2.0 | 0.5 | 0.9 | 1.3 | 1.5 |

than the V(VV) sediments, despite the presumed migration to the A(AV) halves. Although *A. beccarii* was the most abundant species in both V(VV) and V(AV) sediments with an average contribution of respectively 44.9% ± 3.6 and 41.3% ± 4.1, V(AV) sediments showed a higher relative abundance and absolute density of *E. excavatum* (39.1% ± 3.6) and a lower abundance of *H. pacifica* (3.7% ± 0.5) compared to the unaffected sediments of the homogeneous cores (respectively 23.1% ± 6.0 and 9.6% ± 4.4 of the assemblage). Species with low abundances, i.e. average contribution below 5%, are not discussed further because of their low individual abundances.

### 3.3.4 CHANGE IN FORAMINIFERAL DENSITY AND ASSEMBLAGE COMPOSTITION

Compared to the onset of the experiment, the highest relative abundance increase was found in the hypoxia-affected sediments of the heterogeneous cores ((A(AV)), Table 3.1b.). Although the relative abundance increase in the homogeneous hypoxia-affected ventilated cores (AA) was far less (44% compared to 197% in A(AV)), the observed shifts in the species-specific contribution to the assemblage composition resulted in a higher resemblance to the assemblage composition found in the A(AV) sediments than to the $A(AA)_{anox}$ sediments. Especially the contribution of *Ammonia beccarii* increased when homogeneous (AA) cores were kept aerated. On the contrary, the contribution of the species *Hopkinsina pacifica* and *Stainforthia fusiformis* decreased (Table 3.1b.). In $A(AA)_{anox}$ the decrease in the contribution of *S. fusiformis* to the assemblage composition in the upper sediment layers co-occurred with an increase in the contribution of *H. pacifica*.

Figure 3.4, which displays the distribution of the foraminifera over the two size fractions in the two upper layers of sediment, indicates that overall the total standing stock in the upper half centimetre of sediment had a higher increase than in the subsequent sediment layer from 0.5 to 1.0 centimetre depth for all but the anoxic treatment. This relatively high foraminiferal density increase in the upper

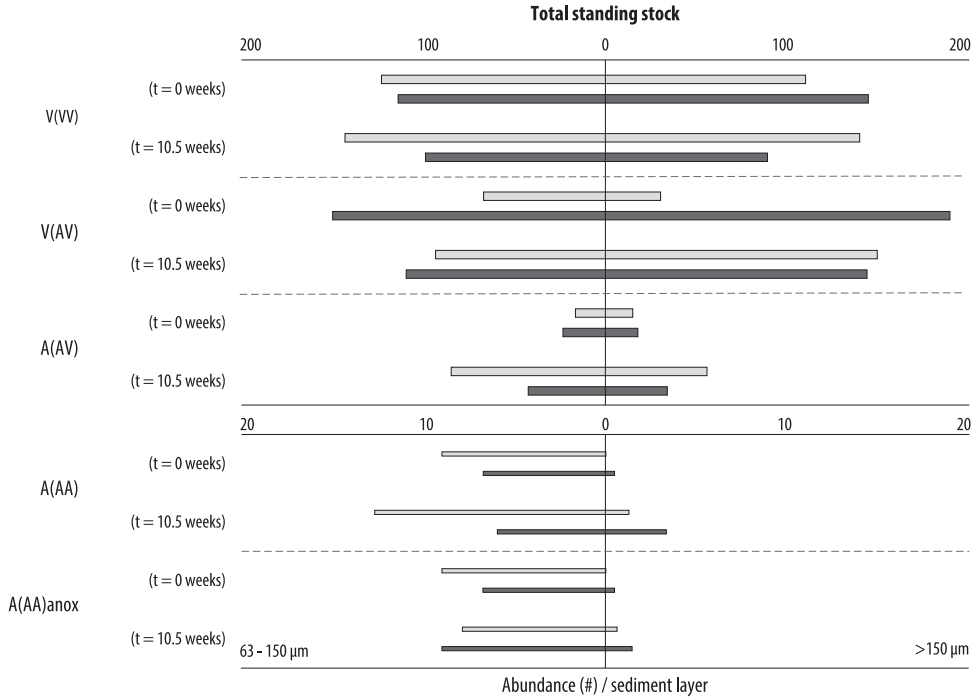
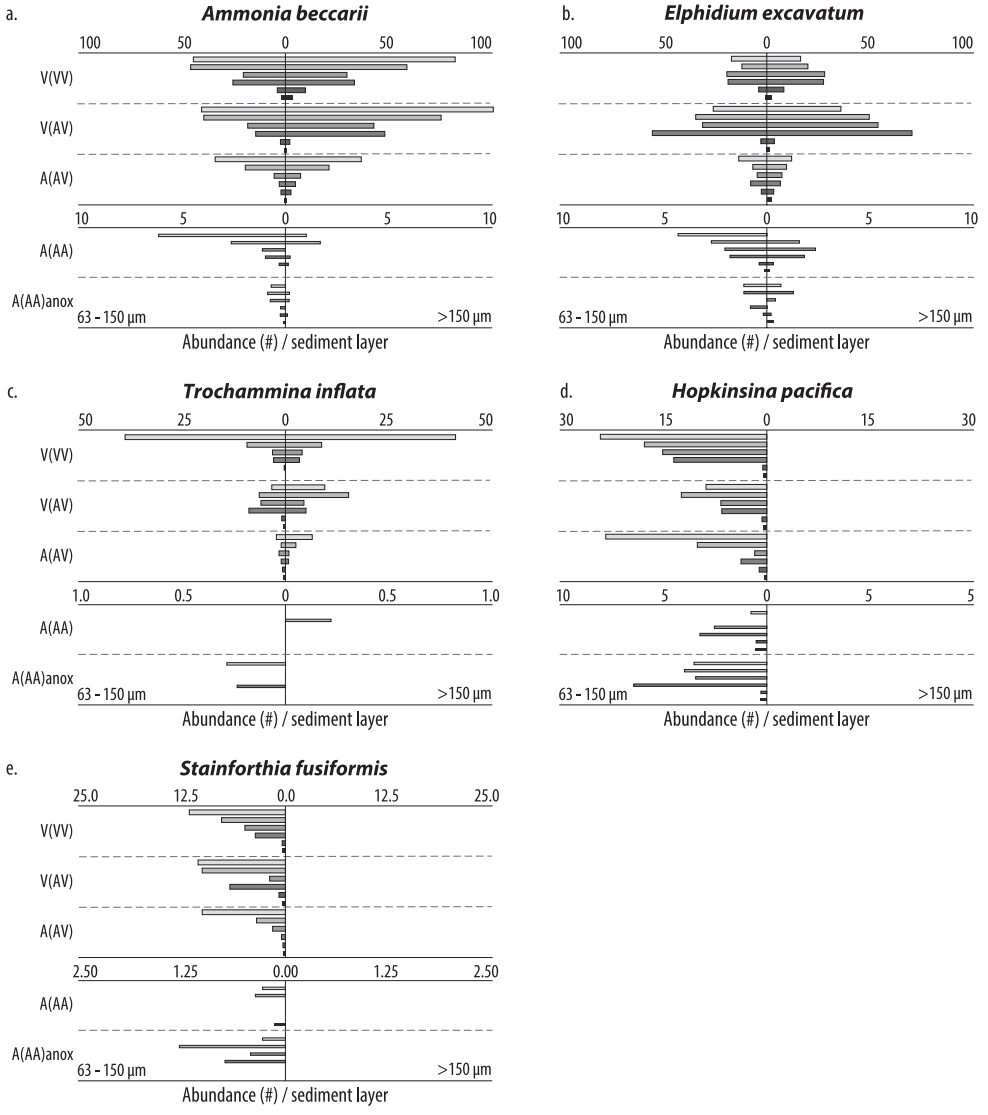



**Figure 3.4** Foraminiferal total standing stock at the start (0 weeks) and the end of the experiment (10.5 weeks) in the upper two sediment slices (0.0-0.5 cm in light grey and 0.5-1.0 cm in dark grey), per half sediment core, sediment types and size fraction (63-150 µm on the left side of the vertical axis, and >150 µm on the right side of the vertical axis). The foraminiferal abundances in V(VV), V(AV) and A(AV) sediments are plotted on the upper horizontal axis, the foraminiferal abundances in A(AA) and $A(AA)_{anox}$ sediments are plotted on the lower horizontal axis.

half centimetre was most pronounced in the (AV) cores. In the unaffected sediments of these cores this increase co-occurred with an abundance decrease in the second half centimetre of the sediment.

### 3.3.5 SPECIES DISTRIBUTION

Of the two species dominating assemblage changes in our experiments, *Ammonia beccarii* was observed to live on average closer to the sediment surface than *Elphidium excavatum* (Fig. 3.5a., b.). Pronounced is the low proportion of large specimen for both species in the hypoxia affected sediment in the homogeneous cores (A(AA) & $A(AA)_{anox}$). The similar distribution of large and small specimens in the A(AV) sediments represented an intermediate state between (AA) cores and V sediments. *Trochammina inflata* was the third most abundant species with a strong presence in both size fractions



(Fig. 3.5c.). This species was mainly found in the oxygen-rich upper half centimetre of the unaffected sediments and it was nearly absent in the hypoxia-affected sediments. *E. excavatum* had a distinctly high number of specimens in the V(AV) sediments.
*Hopkinsina pacifica,* a species with relatively small tests, was found in all sediment types, however it was the dominant species (with 44.8% ± 8.9) in permanently anoxic sediments ($A(AA)_{anox}$). Figure 3.5d. indicates that *H. pacifica* was generally rather equally distributed over the upper 2 cm In the V(AV) sediment, while it seemed to prefer to reside close to the sediment-water interface in the V(VV) and A(AV) sediments and slightly deeper in the (AA) sediments. The vertical distribution of *Stainforthia fusiformis* was comparable to that of *H. pacifica*. Both species had their lowest abundance in the A(AA) sediments.

## 3.4 DISCUSSION

Restoration of oxygen concentrations in marine benthic ecosystems does not necessarily result in recovery of the ecosystem to its pre-hypoxia state (e.g. Diaz and Rosenberg, 2008; Steckbauer et al., 2011; Riedel et al., 2013). Foraminifera, with their high tolerance to low oxygen conditions, are expected to be among the last ones standing, and among the first taxa to recolonise marine sediments following hypoxic disturbances. Studying foraminiferal colonisation patterns is interesting and valuable in its own right, but additionally it holds the potential to shed light on the recovery potential of hypoxia-affected ecosystems as a whole.
The aim of this study was to investigate the benthic ecological development of hypoxia-affected parts of Lake Grevelingen after restoration of the water column ventilation. The importance of two different foraminiferal recovery pathways was studied by comparing the recovery patterns in hypoxia-affected sediments after ventilation of bottom-waters with and without neighbouring healthy assemblages. Through this approach we were able to investigate the contribution of restoration of the foraminifera community from within the sediment and recovery via horizontal migration from unaffected areas on a short-term (10.5 weeks) and small-scale (maximum distance was defined by the core width of 7 cm). Since oxygen-penetration depth – which is related to bottom-water ventilation and oxygen demand – has a strong influence on the vertical distribution of benthic foraminifera (Jorissen et al., 1992, 1995; Van der Zwaan et al., 1999; Fontanier et al., 2002; Koho et al., 2008), insight in the development along the sediment profile will contribute to unravelling the processes that instigate foraminiferal dynamics near the sediment-water interface. Given that most surveys are restricted to the upper centimetre of the sediment (Bouchet et al., 2012; Schönfeld et al., 2012) – which in general comprises the highest abundance of living foraminifera – these insights will improve the applicability of foraminifera as bio-indicator.

### 3.4.1 RECOVERY PATHWAYS

Deterioration of the foraminiferal community due to hypoxia was observed in the sub-recent fossil foraminiferal record of Lake Grevelingen (Donders et al., 2012). Since water exchange was blocked by

⇦ **Figure 3.5 a. - e.** Vertical distribution of the foraminiferal species after 10.5 weeks (a) *Ammonia beccarii,* (b) *Elphidium excavatum*, (c) *Trochammina inflata*, (d) *Hopkinsina pacifica*, and (e) *Stainforthia fusiformis* per sediment slice (give depths), sediment type and treatment. The size fraction 63-150 µm is plotted on the left side of the vertical axis, while the size fraction >150 µm is plotted on the right side of the vertical axis. The foraminiferal abundances are displayed per sediment layer; 0.0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-5.0 and >5.0 cm. These depth intervals are represented by bars; the lightest bar represents the foraminiferal abundance in the upper sediment layer etc.. The upper x-axis is used to display the foraminiferal abundance in the sediment types V(VV), V(AV) and A(AV), whereas the lower x-axis belongs to the sediment types A(AA) and $A(AA)_{anox}$.

the Grevelingendam (1965) and the Brouwersdam (1971), the bottom-water oxygenation decreased. The sluice constructed in the Brouwersdam (1978) to permit a slight water inflow from the North Sea to Lake Grevelingen could not prevent an ongoing hypoxia-related deterioration of the benthic community in deeper parts of the lake. The fossil record did not reveal any intermittent recovery of the foraminiferal community during this period (Donders et al., 2012). Although high foraminiferal colonisation rates have been reported for sediments depleted in foraminiferal fauna – varying from a couple of days to weeks (Buzas et al., 1989; Buzas, 1993; Alve, 1999) – the foraminiferal colonisation after prolonged hypoxia in the Nordic Drammensfjord took 1 to 5 years (Alve, 1995). As the Drammensfjord, Lake Grevelingen is affected by field hypoxia. The unaffected sediment cores (VV) from Lake Grevelingen in our experiment comprised a high number of foraminifera, whereas foraminifera were almost absent in the sediments of severely hypoxia-affected areas (AA-cores). We studied the species-specific response in hypoxia-affected sediments kept with or without neighbouring unaffected sediments to explore the potential for restoration of the foraminiferal community via active migration. The foraminiferal dynamics in hypoxia-affected homogeneous cores (A(AA) and $A(AA)_{anox}$) contributed to our insights in the opportunity for recovery of foraminiferal assemblages from within the hypoxia-affected sediments after hostile conditions are reversed. The vertical distribution in these sediments was compared to those in the control cores at both ends of the spectrum (V(VV) and $A(AA)_{anox}$)).

Substantial foraminiferal colonisation of hypoxia-affected sediments in our laboratory experiments occurred within the time period of 10.5 weeks. The foraminiferal recolonisation was most pronounced in the A(AV) sediments. At the end of the experiment, A(AV) sediments had increased in foraminiferal density to 38% of the control V(VV) sediment. In contrast, in absence of a nearby healthy community (in the (AA) cores) the foraminiferal density was 6% compared to the control (VV) cores. Compared to the onset of the experiment, the relative increase in total standing stock was 197% in A(AV) and 44% in A(AA), although in (AA) cores low numbers were found both at the beginning and the end of the experiment. This suggests that, on the time (10.5 weeks) and spatial scale (defined by the width of the experimental cores, at maximum 7 cm) of this experiment, the contribution of foraminiferal migration (and reproduction) of neighbouring healthy assemblages to restoration of the assemblage outweighed that of foraminiferal population growth from within the sediment.
The progress of a community response to improved environmental circumstances depends on the species' ability to react fast, both on individual and on population scale. Although foraminiferal life cycles are not well studied, among the investigated species a high diversity has been observed in foraminiferal life span and generation span (reproduction commonly terminates the life of foraminiferal individuals, Hallock, 1985). Some foraminifera can postpone reproduction during unfavourable conditions and grow until they can reproduce as soon as circumstances improve (Hallock, 1985). Several strategies, including adjustable numbers of offspring (less offspring yields larger new individuals) and alternation between asexual and sexual reproduction (with small, but genetically diverse microspheric offspring after sexual reproduction and larger megalospheric offspring after asexual reproduction) may enhance the success of this community in diverse and fluctuating circumstances (Hallock, 1985). Species such as *Ammonia beccarii* which are found in environments with highly fluctuating environmental conditions, even on (semi)diurnal scales, were observed to have a relatively short life span, enabling populations of these species to respond fast to changing circumstances (Bradshaw 1957). *A. beccarii* kept in laboratory conditions of 20°C and fed twice a week with the microalgae *Dunaliella* sp. was able to asexually produce 50 to 200 new individuals. The majority of these new-borns grew within three weeks into the >150 µm size fraction (Toyofuku et al., 2008). Bradshaw (1957) reported a life span of two to three months for *A. beccarii*

when kept at the optimum temperature of 20°C to 30°C. Despite this demonstrated ability of *A. beccarii* to respond quickly under some circumstances, in our experiment this species did not show signs of enhanced reproduction and rapid growth in re-aerated hypoxia affected sediments without the presence of nearby healthy sediments. Multiple environmental parameters (i.e. temperature, deteriorated propagule bank, exposure to sulphide etc.), both during the experimental period or in the field situation, may have precluded a fast response in the A(AA) sediments. The density increase of foraminifera in (AV) cores, however, did demonstrate that foraminifera (including *A. beccarii*) were able to reproduce during the experimental period, but the minimal increase of foraminiferal density in the (AA) cores suggested a very small contribution of recovery from within the previously hypoxic sediments.

A high foraminiferal density was observed in the upper layer of the A(AV) sediment (Fig. 3.3). Contrastingly, in absence of an oxygen gradient ($(AA)_{anox}$ cores) the few foraminifera found were almost evenly distributed over the upper 4 layers (2 cm) of sediment. Favourable conditions arose in the upper layer of the hypoxia-affected sediments after re-aeration, which may have stimulated foraminifera to migrate specifically to these upper sediment layers of A(AV) sediments. However, given the very low foraminiferal density in $(AA)_{anox}$ cores, a vertical re-arrangement of the foraminifera along the sediment profile could have only minimally contributed to the observed high foraminiferal density in top centimetre of the A(AV) sediments. Our results may additionally support the conclusions of Kitazato (1988) that epifaunal species move faster than infaunal species. In our experiment this may have led to relatively high foraminiferal abundance in the upper sediment layer compared to the deeper layers of the A(AV) core halves. The very low increase in foraminiferal density in re-aerated homogeneous cores containing hypoxia affected sediments (AA) indicates that activation of cysts or propagules, or reproduction by surviving foraminifera minimally contributed to abundance recovery within the time span of this experiment (Fig. 3.4). Despite the small difference in foraminiferal density in both types of hypoxia-affected sediments that were not paired with unaffected sediments, the composition of these assemblages and their vertical foraminiferal distribution were different (Appendices 3.2, 3.3, 3.4.1, 3.4.2). At the start of the experiments, the assemblage in the re-ventilated A(AA) still resembled the final $A(AA)_{anox}$ assemblage, while at the end of the experiment, the A(AA) assemblage was similar to the one in V(VV). The similarity in final community composition between A(AA) and V(VV) and dissimilarity between A(AA) and $A(AA)_{anox}$, and the slightly elevated numbers of foraminifera in A(AA) sediments compared to $A(AA)_{anox}$ suggest that over longer time periods there is a potential for self-sustained recovery without the necessity of migration from unaffected areas. As for the A(AV) sediments, the relatively high concentration of foraminifera in the upper sediment layer (0.0 – 0.5 cm) of the A(AA) sediments compared to the cores kept anoxically $(AA)_{anox}$, may indicate a slight re-arrangement of the vertical distribution of foraminifera and, for certain species, a higher contribution of recovery from within the hypoxia-affected sediment or lower mortality in the upper re-oxygenated sediment layer, as will be discussed below.

The foraminiferal density in the upper centimetre of the sediment in the heterogeneous cores was half the density of the undisturbed control cores at the start of the experiment, while the densities in both core types was comparable at the end of the experiment suggesting foraminiferal reproduction and growth into the >63 µm size fraction within 10.5 weeks. Despite the observed reproduction and growth of foraminifera in the heterogeneous cores, the minor abundance increase in the A(AA) sediments implies that the time period of 10.5 weeks was too short for a full assessment of the foraminiferal community to recover in absence of neighbouring healthy assemblages. A dormant propagule bank may exist in affected sediments (as suggested by Alve and Goldstein, 2010) but it

may require more than 10.5 weeks for propagules to be re-activated and produce populations larger than 63 µm in the circumstances of our experiment. Propagules have been suggested to rest in cryptic state as cysts for up to two years (Alve and Goldstein, 2010). However, we do not know how, and to what extent the repetitive hypoxic-disturbance may have affected potential propagule banks in Lake Grevelingen. The hypoxia-affected areas in Lake Grevelingen have suffered from seasonal hypoxia over the last 20 years (since 1978 oxygen concentrations have been measured at 10-15 m water depth on a monthly basis by the Dutch Ministry of Infrastructure and Environment, Donders et al., 2012). This repeated occurrence of hypoxia may have had an impact on the potential to restore the community from within the hypoxia-affected sediment. In addition, or maybe even alternatively, the foraminifera found in the homogeneous (AA) cores may have been delivered to the sediment by lateral transport from well-oxygenated parts of the lake in the period prior to our experiment, e.g. by suspension during a storm event prior to sampling. The treatment – ventilated *versus* anoxia – then resulted in survival of different species within this pool of immigrants. Nonetheless, the repetitive character of lake hypoxia, as well as the time span in-between hypoxia events, may affect the rate and effectiveness of recovery of the foraminiferal community as it likely alters the balance between the potential to recover from within the sediment, via lateral migration or passive transport of colonisers and propagules. By the end of our 10.5 wk experimental period recovery from within the sediment (as seen in A(AA)) seemed to only just have started. Since, in the deeper parts of Lake Grevelingen, hypoxic-events increased in frequency over the last 30 years and nowadays occur almost on a yearly basis (Donders et al., 2012) and generally last for a couple of months, potential recovery from within hypoxia-affected sediment, as far as it was observable in this experiment may be too slow for the foraminiferal community to recover between two events of lake hypoxia. Our results are supported by field observations. The fossil assemblage of Lake Grevelingen revealed a decline in foraminiferal density from 1975 and onwards (Donders et al., 2012) suggesting that the foraminiferal community is not able to totally recover from harm provoked by summer hypoxia. Moreover, the summer hypoxia in 2011, during which our sediments were collected, lasted until October. In November – a couple of weeks after hypoxic conditions had ended – the foraminiferal community did not reveal any signs of recovery (Langlet et al., 2012).

The short distance foraminifera had to bridge in our experiments (defined by halve the maximum width of the experimental core – 7 cm) in order to reach former affected sediments is likely to have exaggerated the importance of active migration for recovery of the foraminiferal community in a field situation. Reversely, the short experimental time scale (10.5 weeks) likely resulted in an underestimation of the relevance of colonisation via population growth due to reproduction or growth from propagule banks. Moreover, passive dispersion of foraminifera by for instance water currents or bioturbating macrofauna was – in contrast to the natural setting – not taken into account. The discrepancy between our experimental setting and a natural field situation underlies the discrepancy between our observation and the general notion that active migration is of minimal importance for foraminiferal colonisation (reviewed by Alve, 1999). It must be noted, however, that in Lake Grevelingen the occurrence of hypoxia has a highly patchy, relatively small-scale character as hypoxia currently occurs predominantly in the long but narrow gullies. During one of the most severe recent summer hypoxia-events in July 2010, the maximum width of any hypoxia-affected area was approximately 1 km (Guasti et al., 2011). This patchy and small-scale character results in relatively short distances between affected and unaffected sediments in Lake Grevelingen. In our laboratory experiment, the distance between healthy and deteriorated foraminiferal assemblages was of course much further reduced (to a centimetre instead of a meter to kilometre scale). However, given the minimal contribution of

recovery from within the hypoxia-affected sediment after ventilation of the bottom-water and the high density increase in the hypoxia-affected sediments neighboured by healthy assemblages it is safe to assume that recovery of repeatedly hypoxia-exposed sediments depends largely on the availability of colonisers from surrounding unaffected areas. On a very small scale (with a movement speed of a couple of mm per hour a foraminifer that migrates in one direction could hypothetically bridge a distance of approximately 3.5 meter in 10.5 weeks) active migration can directly contribute. However, active migration of offspring derived from foraminifera passively dispersion to hypoxia-affected areas may enhance recolonisation on a larger spatial scale.

### 3.4.2 SPECIES-SPECIFIC RESPONSE AND COLONISATION PATTERNS

In the previous chapter we discussed the temporal dynamics of foraminiferal migration patterns in the upper centimetre of our microcosms. In this chapter we investigate the species-specific depth distribution of foraminifera at the end of the experiment due to active migration and/or recovery of foraminifera from within the sediment. With the notable exception of *Trochammina inflata*, the species assemblage in the A(AV) sediments resembled the composition in the (VV) control cores after 10.5 weeks. As mentioned in the previous chapter, the distinctive high concentration of *T. inflata* near the sediment-water interface in the control cores (VV) presumably indicates that this species is restricted to oxygenated and undisturbed habitats. Besides this small difference in the species compositions, the foraminiferal density was the most apparent difference between sediment types V(VV) and A(AV). The abundances found in the A(AV) sediment were intermediate compared to the control end-members (V(VV) and $(A(AA)_{anox})$. The increase in foraminiferal density in the A(AV) compared to $A(AA)_{anox}$ is, as mentioned above, mainly attributable to horizontal migration (and reproduction) from neighbouring unaffected sediments.

With respect to the cores kept anoxic, especially the abundance of *Ammonia beccarii* and *Elphidium excavatum* increased in the A(AV) core-halves. Both species numerously colonised these sediments. *A. beccarii* also became more prominent in the (AA) cores. The same was true, but to a smaller extent, for *E. excavatum*. The vertical distribution of both species (Fig. 3.5a., b.) showed that *A. beccarii* lived closer to the sediment-water interface than *E. excavatum*. In the healthy sediment of the homogeneous and the heterogeneous cores *A. beccarii* had twice as much specimens in the large (>150 µm) size fraction compared to the small size fraction (63–150 µm), while the opposite account the A(AA) sediments. In the A(AV) sediment the size partitioning of *A. beccarii* is intermediate with an almost evenly distribution of the specimens of *A. beccarii* over both size fractions. This may indicate that the abundance increase of *A. beccarii* in the A(AV) sediment may rely both on migration and reproduction. The specimens of *E. excavatum* are almost even distributed between both size fraction in the sediment types A(AV), V(AV) and V(VV), whereas the A(AA) sediments comprise a relatively high proportion of small specimens. Although the size partitionings of *E. excavatum* between V(VV) and V(AV) sediments resemble each other, the V(AV) sediment comprises more specimens of this species. The discussed opportunistic response of *E. excavatum* to the extra availability of food in the V(AV) sediments resulted both in growth as in reproduction. Moreover, our data suggest that small individuals of *A. beccarii* and *E. excavatum* replaced *Hopkinsina pacifica* in the (AA) cores. The replacement of foraminiferal species due to severe fluctuations in the concentration of dissolved oxygen in the bottom-water has been observed before in field studies. For instance, Polovodova Asteman and Nordberg (2013) reported that a hypoxic event in the Swedish Gullmar Fjord provoked the replacement of the typical Skagerrak-Kattegat fauna by *Stainforthia fusiformis* in the early 1980s. Eventually, after hypoxic conditions had ended, the replaced fauna re-appeared until a new severe hypoxic event in 2008 interrupted this re-establishment.

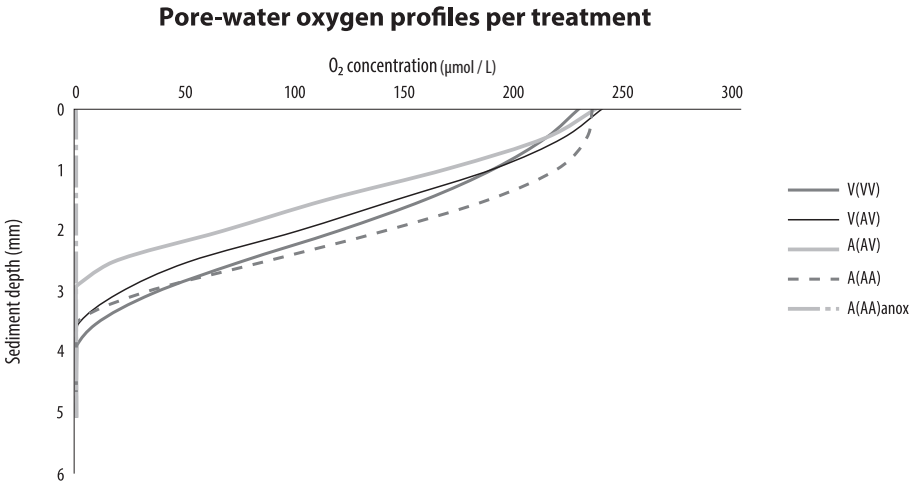
The foraminiferal density in both types of hypoxia-affected homogeneous cores ((AA) and ($AA_{anox}$) was low and nearly all specimens belonged to the smallest size fraction. *Hopkinsina pacifica* dominated these sediments, and seems therefore, despite its low abundance, indicative of prolonged anoxic conditions. Moodley et al., (1997) discussed the ability of some genera to profit from hypoxic conditions due to a decrease in biological interactions such as competition with less hypoxia-tolerant species. In sediments derived from the Adriatic Sea, *S. fusiformis* benefitted even more (or suffered less) from anoxia than *H. pacifica* (Moodley et al., 1997). Also Duijnstee et al. (2005) reported that, although both species responded opportunistically to a simulated anoxic event and organic matter pulse, the population growth of *S. fusiformis* exceeded that of *H. pacifica*. Ernst et al., (2005) reported that during hypoxia, both *S. fusiformis* and *H. pacifica* were able to increase in abundance when labile organic matter was present. In Scandinavian marine waters, *S. fusiformis* has been reported to be the first foraminiferal species to colonise hypoxia-deteriorated benthic habitats (Alve, 1995; Polovodova Asteman and Nordberg, 2013). Below the spatially and temporally dynamic Frisian Front in the North Sea, the foraminiferal assemblage was reported to comprise *A. tepida* and *E. excavatum* as well as *H. pacifica* and *S. fusiformis* (De Nooijer et al., 2008). At the centre of this hydrodynamic front, which receives a high input of labile organic matter, *E. excavatum* was the dominant species. *H. pacifica* was observed to occupy those areas of the Frisian Front that received pulses of fresh organic matter. The abundance of *S. fusiformis* was less distinctively coupled to any environmental parameter. In the present study, the relatively low contribution of *S. fusiformis* to the very modest assemblage in the $(AA)_{anox}$ sediments is not in agreement with experiments and field observations in the hypoxia-affected sediments of the Adriatic Sea and Scandinavian marine waters. However, the distribution of *S. fusiformis* confirms the dynamics found in the nearby Frisian Front.
One characteristic that enables *Stainforthia* (*Stainforthia* sp var. 1) to thrive in oxygen-pore conditions is its ability to store nitrate in the protoplasm, presumably used for respiration in absence of oxygen (Piña-Ochoa et al., 2010a). We do not know whether *H. pacifica,* the species that dominated our $(AA)_{anox}$ sediments, has a similar ability for nitrate respiration. Moreover, the hypoxic conditions of the bottom-water have supposedly led to an absence of nitrate available for respiration. In contrast, *A. beccarii,* the dominant species in all sediment types except $(AA)_{anox}$, was indicated to lack intracellular nitrate storage (Piña-Ochoa et al., 2010a). This presumably added to the observed low abundance of this species in the sediments kept under prolonged anoxia despite its excellent ability to thrive in otherwise frequently stressful environments such as the high intertidal zone.

By combining the dynamics of the total standing stock with the species responses in the various sediment types we contributed to the applicability of foraminifera as selected species for indicating ecosystem state. In summary: *Trochammina inflata* indicates undisturbed ventilated conditions and *Hopkinsina pacifica* prolonged anoxia. *Ammonia beccarii* and *Elphidium excavatum* thrived in restoring and ventilated sediments; their presence was not indicative of one specific sediment type. Nonetheless, apart from the species specific distributions, the total foraminiferal abundance was indicative of the stage of recovery (i.e. A(AV) as intermediate state in-between V(VV) and A(AA)). Since *A. beccarii* and *E. excavatum* where the dominant inhabitants of all cores kept ventilated, the abundance of both species contributed to bioindicator function of the foraminiferal community. The resemblance of assemblages in V(VV), (A(AV)) and (A(AA)) sediments, may indicated that long term recovery – long enough to accomplish and complete active migration and regeneration for within the sediment – both recovery pathways may result in the same assemblage composition. If true, the composition of the foraminiferal assemblage after re-aeration of hypoxia-affected sediments does not

intrinsically rely on the dispersal pathway but is dependent on environmental conditions such as the spatial and temporal extent and intensity of anoxia event.

## 3.5 CONCLUSION

We characterised the sediment types and treatments by their differences in foraminiferal densities and assemblage compositions. The combination of foraminiferal density and assemblage composition seemed to be indicative for the status of foraminiferal recolonisation and ecosystem health. *Trochammina inflata* was specifically found in sediments that had not been disturbed by field or experimental hypoxia. Therefore, this species seemed to be indicative of healthy, undisturbed sediments. Contrastingly, *Hopkinsina pacifica* was the dominant species during prolonged hypoxia. Within the foraminiferal assemblage of Lake Grevelingen, this species seemed to have the highest tolerance to withstand hypoxia and was indicated as most characteristic for severe hypoxia disturbance. *Ammonia beccarii* and *Elphidium excavatum*, the dominant species in the unaffected sediments, were the most abundant colonisers of hypoxia disturbed sediments after re-oxygenation. The abundance increase of *E. excavatum* in the healthy sediments of the heterogeneous cores reflected the most prominent difference with the homogeneous unaffected cores and was inferred to be an opportunistic response to a higher availability of detrital organic matter in the hypoxia-affected sediment. Although initially foraminifera dispersed rapidly into the hypoxia-affected sediments, colonisation was not complete within the experimental time span of 10.5 weeks. The large difference in foraminiferal density among hypoxia-affected sediments that were either combined with healthy or hypoxia-affected sediments suggested differential modes of foraminiferal colonisation. Within this small-scale (cm) laboratory experiment, active dispersal (migration) seemed to be of high importance compared to recovery from within the hypoxia-affected sediment (activation of propagules) after ventilation of the bottom-water. The relative contribution of dispersal pathways to the (re-)colonisation of habitats evidently depend on spatial and temporal scale. Dispersal via migration dominated the short-term (10.5 weeks) recolonisation of hypoxia-affected sediments in our small-scale (cm) experiment. In a field situation foraminifera will have to bridge longer distances (km) and may have more extended time-scales (months or even years, depending on for instance the frequency of hypoxia disturbances) for recovery. Both will influence the contribution of the studied recovery pathways for restoration of the foraminiferal community and presumably increase the importance of recovery from within the sediment compared to active dispersal. In addition, passive dispersal of foraminifera will, in contrast to our laboratory experiment, contribute to the recovery of foraminifera after hypoxic disturbances. Our results may imply that active migration of foraminifera during recolonisation and population growth (due to for instance reproduction of passively dispersed early colonisers) may enhance the spatial distribution of foraminifera in the affected area and contribute indirectly to recovery of the assemblage. This in turn affects the recovery potential and the time span involved in restoring foraminiferal communities following hypoxia.




**Appendix 3.1** Pore-water oxygen profiles measured before the sample event at 10.5 weeks after the start of the experiment.

| **Kruskal-Wallis** | | | | | |
|---|---|---|---|---|---|
| | **p(same)** | | **p(same)** | | **p(same)** |
| **Total standing stock** | 0.013 | ***Ammonia beccarii*** | 0.008 | ***Elphidium excavatum*** | 0.007 |
| V(VV) - V(AV) | 1.000 | V(VV) - V(AV) | 1.000 | V(VV) - V(AV) | 1.000 |
| V(VV) - A(AV) | 1.000 | V(VV) - A(AV) | 1.000 | V(VV) - A(AV) | 1.000 |
| V(VV) - A(AA) | 0.304 | V(VV) - A(AA) | 0.304 | V(VV) - A(AA) | 0.304 |
| V(VV) - A(AA)anox | 0.304 | V(VV) - A(AA)anox | 0.304 | V(VV) - A(AA)anox | 0.304 |
| A(AA)anox - A(AA) | 1.000 | A(AA)anox - A(AA) | 1.000 | A(AA)anox - A(AA) | 0.606 |
| A(AA)anox - A(AV) | 1.000 | A(AA)anox - A(AV) | 1.000 | A(AA)anox - A(AV) | 1.000 |
| ***Hopkinsina pacifica*** | 0.016 | ***Stainforthia fusiformis*** | 0.265 | ***Trochammina inflata*** | 0.015 |
| V(VV) - V(AV) | 1.000 | V(VV) - V(AV) | 1.000 | V(VV) - V(AV) | 1.000 |
| V(VV) - A(AV) | 1.000 | V(VV) - A(AV) | 1.000 | V(VV) - A(AV) | 1.000 |
| V(VV) - A(AA) | 0.211 | V(VV) - A(AA) | 1.000 | V(VV) - A(AA) | 0.304 |
| V(VV) - A(AA)anox | 0.211 | V(VV) - A(AA)anox | 1.000 | V(VV) - A(AA)anox | 0.304 |
| A(AA)anox - A(AA) | 1.000 | A(AA)anox - A(AA) | 1.000 | A(AA)anox - A(AA) | 1.000 |
| A(AA)anox - A(AV) | 0.552 | A(AA)anox - A(AV) | 1.000 | A(AA)anox - A(AV) | 1.000 |

**Appendix 3.2** Kruskal-Wallis test to test for statistical dissimilarity among the foraminiferal densities (total standing stock and the most prominent species) of the separate sediment types and treatments.

| TWO-WAY ANOSIM | Treatment | | Depth | |
|---|---|---|---|---|
| | R | p(same) | R | p(same) |
| **Pooled** | 0.70662 | 0.0001 | 0.6526 | 0.0001 |

**Appendix 3.3** TWO-WAY ANOSIM test to analyse the impact of sediment depth and treatment on the foraminiferal assemblages.

| *a.* | *b.* | *c.* | | | | |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | *4* | *5* |
| **Pooled (abs. #)** | 72.26 | ***Ab*** | ***Ee*** | ***Ti*** | ***Hp*** | ***Sf*** |
| **V(VV) - A(AA)anox** | 90.3 | ***Ab*** | ***Ee*** | ***Ti*** | ***Hp*** | ***Sf*** |
| **V(VV) - A(AA)** | 88.5 | ***Ab*** | ***Ee*** | ***Ti*** | ***Hp*** | ***Sf*** |
| **A(AV) - A(AA)** | 75.1 | ***Ab*** | ***Ee*** | ***Hp*** | ***Ti*** | ***Sf*** |
| **A(AA) - A(AA)anox** | 55.2 | ***Ee*** | ***Ab*** | ***Hp*** | ***Sf*** | ***Rb*** |
| **A(AV) - V(AV)** | 52.8 | ***Ee*** | ***Ab*** | ***Ti*** | ***Hp*** | ***Sf*** |
| **V(VV) - A(AV)** | 43.4 | ***Ab*** | ***Ee*** | ***Ti*** | ***Hp*** | ***Sf*** |
| **V(VV) - V(AV)** | 27.8 | ***Ee*** | ***Ab*** | ***Ti*** | ***Hp*** | ***Sf*** |

| *a.* | *b.* | *c.* | | | | |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | *4* | *5* |
| **Pooled (rel. #)** | 41.3 | ***Hp*** | ***Ab*** | ***Ee*** | ***Ti*** | ***Sf*** |
| **V(VV) - A(AA)anox** | 63.2 | ***Hp*** | ***Ab*** | ***Ti*** | ***Ee*** | ***Sf*** |
| **V(VV) - A(AA)** | 30.2 | ***Ee*** | ***Ab*** | ***Ti*** | ***Hp*** | ***Ew*** |
| **A(AV) - A(AA)** | 28.7 | ***Ee*** | ***Ab*** | ***Ti*** | ***Hp*** | ***Sf*** |
| **A(AA) - A(AA)anox** | 53.1 | ***Hp*** | ***Ee*** | ***Ab*** | ***Sf*** | ***Rb*** |
| **A(AV) - V(AV)** | 21.1 | ***Ee*** | ***Hp*** | ***Sf*** | ***Ab*** | ***Be*** |
| **V(VV) - A(AV)** | 14.5 | ***Ee*** | ***Hp*** | ***Sf*** | ***Ab*** | ***Ti*** |
| **V(VV) - V(AV)** | 27.8 | ***Ee*** | ***Hp*** | ***Ab*** | ***Ti*** | ***Be*** |

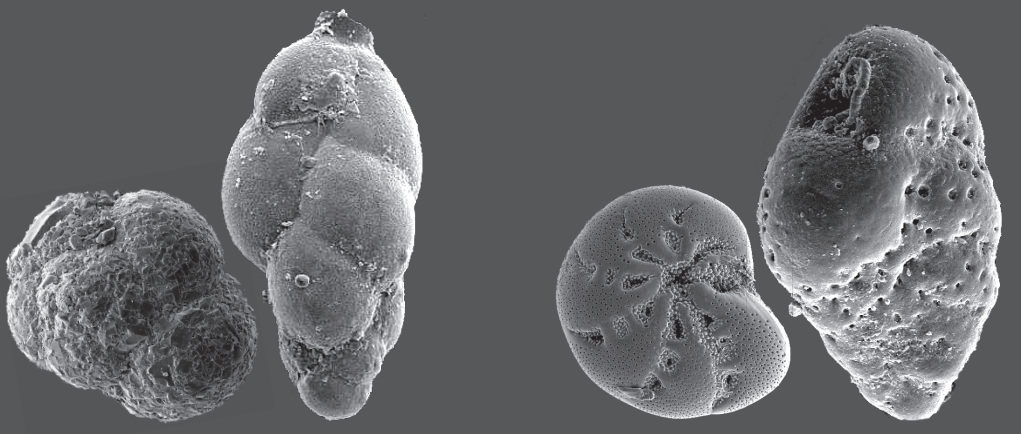
**Appendix 3.4.1 and 3.4.2** SIMPER test on foraminiferal assemblages among sediment types: 3.4.1 is based on absolute abundances and 3.4.2 is based on relative abundance.

a. sediment types

b. overall average dissimilarity between sediment types

c. rank of the five species that have the highest impact on differences among assemblages

*Ab – Ammonia beccarii, Be – Buliminella elegantissima, Ee – Elphidium excavatum, Ew – Elphidium williamsoni, Hp – Hopkinsina pacifica, Rb – Rosalina bradyi, Sf – Stainforthia fusiformis, Ti – Trochammina inflata*



CHAPTER 4

# DIFFERENTIAL RESPONSE OF INTERTIDAL FORAMINIFERA TO ECOSYSTEM RECOVERY SUCCEEDING HYPOXIA

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## ABSTRACT

Hypoxia is an important source of disturbance for marine sediments and an early detection of ecosystem status after such an event is of fundamental ecological importance and may help to address management measures. This paper investigates the reassembly patterns of foraminiferal assemblages and their relation with sediment biogeochemistry, micro-organisms and other fauna following sediment hypoxia in order to improve their use for determining ecosystem health of marine sediments. Sediment hypoxia was deliberately induced during winter and late spring on an intertidal flat in the Scheldt Estuary, The Netherlands. The foraminiferal assemblages in the upper centimetre of the sediment were studied 2 and 5 months after the end of hypoxia. The foraminiferal development was correlated to microphytobenthos, bacteria, meiofauna and macrofauna abundance which have been published in companion papers. The foraminiferal assemblage comprised, throughout the studied period, *Haynesina germanica* (Ehrenberg, 1840), *Ammonia beccarii* (Linnaeus, 1758) and *Elphidium excavatum* (Terquem, 1875). Their species-specific abundance and estimated biovolume varied depending on the timing of disturbance (winter vs. spring hypoxia) and the duration of recovery (2 vs. 5 months). These differences were correlated to the concomitant development of benthic microalgae and macrofaunal ecosystem engineers. Although all foraminiferal species were expected to benefit from recolonisation of ecosystem engineering macrofauna, *H. germanica* was negatively correlated to the abundance of macrofaunal bioturbators during ecosystem recovery. The abundance of *A. beccarii* was positively correlated to predominantly microalgal biomass. These findings revealed that foraminifera responded species-specifically after hypoxia, which makes them good candidates as ecological indicators. However, their changes following hypoxia were probably related to the concomitant recovery of other biota. To understand the species-specific response of foraminifera to environmental disturbance, environmental variability must be taken into account. To develop a new important indicator of ecosystem health, a better understanding is needed of foraminiferal responses to environmental disturbance and recovery processes.

## KEYWORDS

Intertidal * Foraminifera * Hypoxia * Biovolumes * Inverse responses * Recovery

## 4.1 INTRODUCTION

In the last few decades, coastal bottom-water hypoxia – conditions of low dissolved oxygen – has been frequently reported to become more intense, longer in duration and larger in size (Diaz and Rosenberg, 2008). In aquatic systems, natural conditions such as limited bottom-water renewal and stratification can cause low oxygen concentrations. Nevertheless, there is strong evidence that the worldwide increase of coastal dead zones is associated with human-induced eutrophication and the resulting augmented episodic export of particulate organic matter from the euphotic zone to the seafloor (Diaz and Rosenberg, 2008). The strong fluctuations in dissolved oxygen concentration are a source of environmental stress for marine organisms. Seasonal depletion of oxygen in marine environments may lead to behavioural changes and mortality of faunal species (as reviewed by e.g. Gray et al., 2002; Zhang et al., 2009; Diaz et al., 2009). The sudden lack of oxygen is often associated with loss of standing biomass and diversity before hypoxic conditions lead to a collapse of communities and their functioning (Conley et al., 2007; Diaz et al., 2009). The increased hypoxia-induced disturbance necessitates a better understanding of the effects of such perturbations on ecosystem properties and on the ecological resilience of coastal ecosystems.

Foraminifera are often among the most numerous organisms within the benthic fauna of marine ecosystems. The many species of these unicellular organisms thrive in a wide range of environmental conditions, and can be found in virtually all conceivable marine habitats. As fossils, foraminiferal shells are extensively used as tools to reconstruct paleoclimate and paleo-environmental conditions such as sea-surface temperatures, nutrient concentrations (Fischer and Wefer, 1999), or biogeochemical state of past marine environments (e.g., Emiliani, 1955). Foraminifera may also be applicable as indicator species for the functioning of modern ecosystems, but unfortunately they are generally underrepresented in present-day ecological studies of estuarine systems. The recently published recommendations on standardizing methodologies for foraminifer-based biomonitoring (Schönfeld et al., 2012; Bouchet et al., 2012) may contribute to a more central role for foraminifera as to describe the state of marine ecosystems.

Foraminifera possess several characteristics that enhance their potential usability as indicator species. Besides their small size, short life cycle, high abundance and high fossilisation potential – which facilitates collecting data covering longer time scales – foraminifera are highly resistant to local extinction due to environmental perturbations. It has been well established that various foraminiferal species are more tolerant to low-oxygen conditions than most other benthic taxa (Josefson and Widbom, 1988; Bernhard, 1993; Moodley et al., 1997; Pucci et al., 2009 and references therein). For instance Moodley et al. (1997) observed pseudopodial activity for different foraminiferal taxa after 11 weeks of anoxia. Especially hard-shelled foraminifera were highly resistant to low-oxygen conditions that continued for over a longer time period (78 d) than the hypoxia treatment in our experiment. Additionally, some genera seemed to profit from the anoxia, suggesting that hypoxia increased survival rates through decreased biological interactions such as disturbance and competition. Moreover, it has been shown that some taxa are capable of enduring $H_2S$-rich conditions for weeks (Moodley et al., 1998a). Recently, the capacity of several foraminiferal species to use nitrate instead of oxygen as an alternative electron acceptor has been described (Risgard-Petersen et al., 2006; Piña-Ochoa et al., 2010a, b). Also changes in their vertical-migratory behaviour (Duijnstee et al., 2003) and life history (Duijnstee et al., 2005) may contribute to their ability to tolerate low-oxygen concentrations. Although foraminifera have a high potential to survive disturbance, they rapidly respond to changes in environmental conditions through changes in abundance and biomass. The distribution of

living foraminifera in marine sediments is mainly governed by the interplay of their species-specific preference for, or dependence on certain levels of oxygen, nitrate and in particular food (e.g. Jorissen et al., 1992, 1995; Linke and Lutze, 1993; Van der Zwaan et al., 1999; Gross, 2000). These geochemical properties of the sediment are closely coupled to faunal activities: bioturbation, bioirrigation and feeding significantly influence seafloor geochemistry and sediment stability (Lohrer et al., 2004; Meysman et al., 2006a; Erwin, 2008; Montserrat et al., 2008; Passarelli et al., 2012). Faunal activity creates heterogeneity in biogeochemical properties of sediments and extends the sediment-water interface, resulting in an increase in micro-niches suitable for micro- and meiobenthic communities (Aller, 1983; Ray and Aller, 1985; Fenchel, 1996; Zorn et al., 2006; Bouchet et al., 2009). Consequently, foraminiferal assemblages are directly, as well as indirectly influenced through many trophic interactions that determine food and oxygen availability.

Many experimental studies on the importance of oxygen and food availability for the distribution of foraminifera have been carried out under controlled laboratory conditions (e.g. Bernhard, 1993; Moodley et al., 1997; Duijnstee et al., 2003). Although fundamental insights into the response of foraminifera to different environmental factors were gained, the full complexity of natural conditions cannot be taken into account in such studies and more *in situ* studies are needed to account for environmental complexity (e.g. Moodley and Hess, 1992; Jorissen et al., 1992; Gooday et al., 2000). This study reports on the response of foraminifera to ecosystem recovery after hypoxia using an *in situ* field experiment. Specifically, we analysed differences in the abundance and biovolume of the foraminiferal species during ecosystem recovery, and compared these with the foraminiferal dynamics in undisturbed sediments. This approach provides the opportunity to distinguish between the natural (seasonal) processes influencing the development of the foraminiferal community and those induced by processes related to ecosystem recovery after hypoxia. We tested whether the assemblage and the abundance of the foraminiferal species varied according to the duration of recovery and whether these changes were affected by timing of the disturbance. The results are set into the context of companion studies performed during the same field experiment. These parallel studies reported on the effects of hypoxia on the interplay between sediment characteristics, macrofaunal succession, abundances of nematodes, microphytobenthos, and bacteria, and ecosystem functioning (Montserrat et al., 2008, 2009; Van Colen et al., 2008, 2009, 2010 a, b, 2012; Rossi et al., 2008, 2009; Rossi and Middelburg, 2011). This allowed studying the development of foraminiferal assemblages during ecosystem recovery, taking into account the combined positive, negative, direct and indirect effects of biotic interactions, such as parallel recovery of faunal assemblages and changes in food availability. Our goal was to gain more insight into the mechanisms that govern foraminiferal communities and to improve the applicability of foraminifera as indicator species for the state and functioning of marine ecosystems recovering from hypoxia.

## 4.2 MATERIAL AND METHODS

### 4.2.1 SITE DESCRIPTION

This field experiment was carried out in the Paulina polder, an intertidal flat in the Scheldt Estuary (The Netherlands). The sediment contains an average silt content (i.e. % of particles <63 µm) of 50% (Rossi et al., 2008). This intertidal flat covers an area of approximately 1.0 $km^2$ and has a mean tidal range of 3.9 m with a semidiurnal regime. The Paulina polder tidal flat has a rich community of macrobenthos dominated by polychaetes (*Heteromastus filiformis, Arenicola marina, Pygospio elegans*) and molluscs (*Macoma balthica, Cerastoderma edule, Hydrobia ulvae*) (Rossi et al., 2007).

### 4.2.2 EXPERIMENTAL SET-UP AND SAMPLING

Hypoxic conditions covering a period of two months were deliberately induced in winter and spring on four large patches (4x4 m), 5 to 10 m apart within a 50x50 m location (51°21'23"N, 3°42'49"E). The winter hypoxia (imposed on 2 patches) was started January 30th and persisted until March 30th, 2005, whereas the spring hypoxia (imposed on 2 patches) was induced from May 9th until July 6th, 2005 (Fig. 4.1). Hypoxia was induced by covering the sediment patches with black waterproof polyethylene sheets (Van Colen et al., 2008). Two undisturbed patches were used as controls. The hypoxia resulted in a mass mortality of all macrobenthic species (Van Colen et al., 2008; Rossi et al., 2009; Van Colen et al., 2010a, b) and a sharp decline in the abundance of (meiofaunal) nematodes (Van Colen et al., 2009), but did not affect bulk organic carbon concentration or mineralisation rates (Rossi et al., 2009; Van Colen et al., 2012).

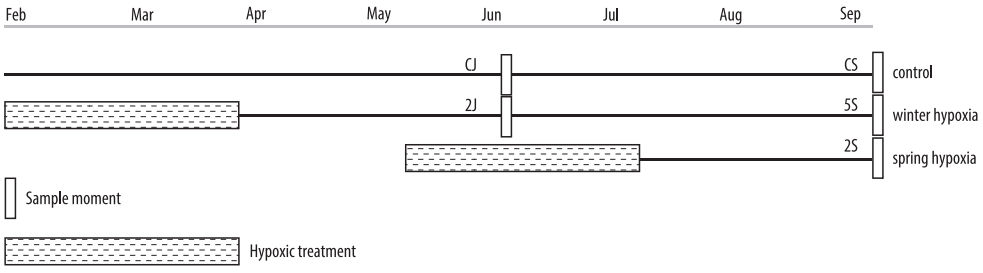



**Figure 4.1** Experimental time scale and sample set-up

Samples were taken on June 10th and September 9th, 2005. In June, samples were only taken from the patches disturbed during winter (hereafter 2J), whereas in September patches disturbed in both winter and spring were sampled. The patches disturbed in winter had been recovering for 5 months (hereafter 5S) and those disturbed in spring for 2 months (hereafter 2S; Fig. 4.1, see also Rossi et al., 2008, 2009). Sediment was also collected in the undisturbed control patches both in June (hereafter CJ) and September (CS). In each patch, four subplots of 50x50 cm were randomly chosen during each sampling occasion. Per subplot, two cores (5 cm internal diameter) were sampled to 8 cm sediment depth (Rossi et al., 2009). These cores were primarily intended to sample macrofauna (Rossi et al., 2009). In the laboratory, the cores collected in the subplots were subsampled for foraminifers or other meiofauna using a cut syringe with an internal diameter of 2 cm. In accordance with standardised biomonitoring methods recently proposed (Schönfeld et al., 2012; Bouchet et al., 2012), the top centimetre of the cores was used for foraminiferal analyses. Per sampling occasion and treatment 4 samples were analysed, except for the control patches of September for which 5 samples were analysed. The sediment samples were stored in 4% buffered formalin with Rose Bengal staining.

### 4.2.3 SAMPLE PROCESSING

Foraminiferal samples were sieved over a 63-µm mesh-size sieve. Well-stained foraminifera were picked from wet samples and enumerated at species level. Only individuals fully filled with pink stained cytoplasm (except for the ultimate chamber) were considered alive at or shortly before the time of sampling (for a discussion on the method of staining see paragraph 'limitations of our experiment').

For a small number of foraminifer-rich samples a (wet) split was used; the foraminiferal counts of these samples were recalculated to estimate the whole sample abundances and biovolumes. After picking, photos were taken with a camera mounted on a microscope with a calibrated internal scale to measure the maximum test dimension (L) of each individual specimen. Hereafter, a crude estimate of protoplasm biovolume (BV) of each specimen was calculated, based on the maximum test-size dimension, using the equation $BV = \pi L^3/16$ (this is the volume of a disk with diameter L and a height of 0.25 L). The three foraminiferal species have planspiral or very low trochospiral chamber arrangements and are thus approximately disk-shaped.

### 4.2.4 STATISTICAL ANALYSIS

Statistical tests were performed in PAST (PAleontological STatistics, Hammer et al., 2001). First, the SIMPER test (Similarity Percentage) was used to identify the dissimilarity among assemblages and the species contribution to these differences among assemblages following recovery from hypoxia (Appendix 4.1). A non-parametric Kruskal-Wallis test was used to test for pairwise differences in the abundance and biovolume of the dominant species (Appendix 4.2). The Kruskal-Wallis test is a non-parametric ANOVA, and was used because it does not assume a normal distribution. The p-values were Bonferroni corrected to conservatively correct for multiple testing. The Two-Way ANOSIM (using Bonferroni corrected p values) was done to test for differences between the composition of the foraminiferal assemblages due to timing of disturbance (CJ, CS, 2J, 2S) and recovery development (CJ, CS, 2J, 5S). To test for differences in the foraminiferal assemblage on account of dissimilar recovery stages (CS, 5S, 2S) a One-Way ANOSIM test was used (Appendix 4.3).

In order to explore the possible effect of microphytobenthos, bacteria and macrofauna on foraminiferal populations, additional data – as published by Rossi et al. (2009) – were used in a multivariate analysis using the software package CANOCO (Ter Braak and Šmilauer, 1998). Redundancy analysis (RDA, see Rao, 1964) was employed to explore relations between the assemblages of foraminifera and other biota during ecosystem recovery. Principal response curves (PRC, see Van den Brink and Ter Braak, 1998) were used to summarise time-dependent treatment effects on the community of microphytobenthos, bacteria, macrofauna and foraminifera. This technique is based on a reduced rank regression that is adjusted for temporal changes in the control treatment (Van den Brink and Ter Braak, 1998), thus allowing focus on the time-dependent treatment effects. The PRCs on the first axis are plotted against time in a PRC diagram. We used centred and standardised species abundances (mean subtracted, and divided by the standard deviation) to adjust for the various ways in which macrofauna, foraminifera, bacteria and microphytobenthos abundances are expressed. See Ter Braak and Šmilauer (1998) for a detailed description.

In order to test the explanatory potential of the co-occurring macrobenthos, bacteria and microphytobenthos as possible driving factors in the foraminiferal distributions, we used partial RDA analyses in addition to the PRC analysis. Standardised and centred abundances of the former three served as explanatory variables and foraminiferal assemblages were introduced as species data. RDA is the canonical version of Principal Component Analysis in which the sample scores on the axes are constrained to being linear combinations of environmental variables (in this case abundances of bacteria, microphytobenthos and macrobenthic taxa) that best separate species' responses along those axes. In the partial RDAs, first the potential of individual explanatory variables were tested separately (marginal effects). A second analysis tested for conditional effects: a set of variables was chosen, starting with the one with the highest marginal effect, the second variable was subsequently

added that had the highest explanatory potential in addition to the first; then a third with the highest explanatory value in addition to the first two, etcetera. The analyses were carried out using foraminiferal abundances and foraminiferal biovolume estimates.

### 4.2.5 LIMITATIONS OF OUR EXPERIMENTAL APPROACH

Before exploring the complex set of relations and their potential influence on foraminifera at different stages of recovery, we will discuss the limitations of the experiment. To distinguish recently living foraminifera from dead specimens, their protoplasm was stained with Rose Bengal. Although we have to bear in mind the drawbacks of this staining method (Murray and Bowser, 2000; Figueri et al. 2012), the well-stained specimens were assumed to represent foraminifera that survived and/or proliferated during or after the prolonged period of hypoxia. Oxygen concentrations in the top layer of the sediment re-established within a few days (Van Colen et al., 2012). Hence, all sample moments (including the field observation that took place 12 days after the treatment in the patches exposed to hypoxia) were taken from re-oxygenated sediments that, in general, have high decomposition rates for labile organic matter (such as foraminiferal protoplasm). By selecting only specimens fully filled with brightly Rose Bengal stained cytoplasm except for the ultimate chamber, the chance of false positive vital staining was further reduced.

The experimental set-up has a limited number of sampling occasions, chosen at the scale of months because the original purpose of the experiment was designed to follow macrofaunal recovery and the relation between carbon cycling and macrofauna diversity (Rossi et al., 2009; Van Colen et al., 2012). Foraminifera may respond quicker than macrofauna to some types of environmental change, often at a scale of days or weeks. Hence, the time scale of sampling may have been suboptimal for foraminiferal studies. Nonetheless, our data may be unique because they link changes in foraminifera after disturbance to those of other biota in semi-natural conditions, allowing for an evaluation of foraminifera change in a complex system.

Although the large size of the patches (16 $m^2$) limited the number of replicate-patches, the large size did permit multiple within-patch subsamples. Schönfeld et al. (2012) recommended the use of at least three replicate samples for foraminiferal biomonitoring studies in order to obtain a good estimate of population distributions. We instead used four replicates.

### 4.2.6 TAXONOMIC REMARKS

The second most abundant foraminiferal species in the assemblage was *Ammonia cf. molecular type* T6 in Hayward et al. (2004), referred to as *Ammonia tepida* in De Nooijer et al. (2006, 2008 and 2009) for the Dutch Wadden Sea, but referred to as *A. beccarii* by Moodley and Hess (1992) and Moodley and Van Weering (1993) for specimens occurring in the Dutch Scheldt Estuary. Hayward et al. (2004) made a strong case that both *Ammonia* species names should be abandoned for these specimens, but – since we lack a taxonomical revision of the genus *Ammonia* for now – we will adhere *A. beccarii* in accordance with historical local terminology.

## 4.3 RESULTS

The abundance and estimated total biovolume per species between June and September in natural sediments (control patches) did not vary significantly (Fig. 4.2a., b., c., and Appendix 4.2). However, the recolonisation stage and timing of disturbance did produce dissimilarities in assemblage composition

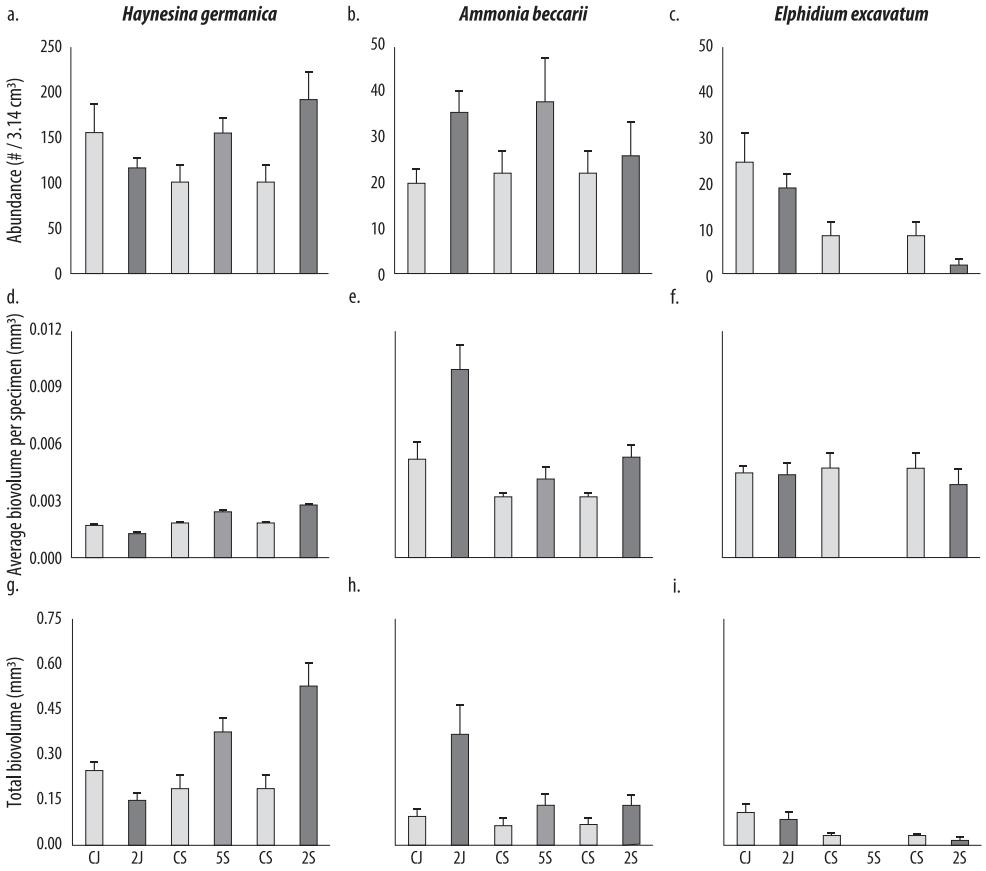



**Figure 4.2 a. - i.** a., b., c. Abundances of the three most prominent species *Haynesina germanica*, *Ammonia beccarii* and *Elphidium excavatum*, d., e., f. Average biovolumes (mm$^3$) of *Haynesina germanica*, *Ammonia beccarii* and *Elphidium excavatum*, g., h., i. Total biovolumes (mm$^3$) of *Haynesina germanica*, *Ammonia beccarii and Elphidium excavatum*.

and in the abundance and biovolume of the foraminiferal species. These differences are described below.

The foraminiferal assemblage comprised three species *Haynesina germanica*, *Ammonia beccarii* and *Elphidium excavatum* (Table 4.1). These species were responsible for dissimilarities among assemblages at different stages of recovery (Appendix 4.1, 4.2, and 4.3). The most abundant species was *H. germanica,* which was responsible for 56.7 – 84.1% of the observed differences among assemblages at different stages of recovery. Specimens of the species *A. beccarii* had relatively large test sizes resulting in a high average biovolume per individual.

**Table 4.1** Foraminiferal abundance and biovolume after hypoxia

a. Mean abundance (± 1SE) of foraminifera in the top 1 cm of the sediment surface (no x 3.14 $mm^{-3}$), b. average biovolume per specimen in the top 1 cm of the sediment surface ($mm^3$), c. mean total foraminiferal biovolume in the top 1 cm of the sediment surface ($mm^3$).

| | winter hypoxia | | | | | | spring hypoxia | | | control | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2J** | | SE | **5S** | | SE | **2S** | | SE | **CJ** | | SE | **CS** | | SE |
| a. | | | | | | | | | | | | | | | |
| *Haynesina germanica* | 116.6 | ± | 11.1 | 155.0 | ± | 16.8 | 191.9 | ± | 30.7 | 155.5 | ± | 33.2 | 100.9 | ± | 19.3 |
| *Ammonia beccarii* | 35.4 | ± | 5.0 | 36.1 | ± | 11.5 | 25.8 | ± | 7.6 | 19.8 | ± | 3.5 | 22.0 | ± | 5.0 |
| *Elphidium excavatum* | 19.0 | ± | 3.2 | | | | 2.0 | ± | 1.4 | 24.7 | ± | 6.5 | 8.5 | ± | 3.2 |
| b. | | | | | | | | | | | | | | | |
| *Haynesina germanica* | 1.27E-03 | ± | 1.31E-04 | 2.43E-03 | ± | 1.58E-04 | 2.78E-03 | ± | 8.86E-05 | 1.70E-03 | ± | 1.66E-04 | 1.84E-03 | ± | 1.08E-04 |
| *Ammonia beccarii* | 9.98E-03 | ± | 1.33E-03 | 4.19E-03 | ± | 6.70E-04 | 5.34E-03 | ± | 6.35E-04 | 5.24E-03 | ± | 9.89E-04 | 3.24E-03 | ± | 2.88E-04 |
| *Elphidium excavatum* | 4.37E-03 | ± | 6.84E-04 | | | | 3.85E-03 | ± | 9.04E-04 | 4.47E-03 | ± | 3.39E-04 | 4.71E-03 | ± | 8.25E-04 |
| c. | | | | | | | | | | | | | | | |
| *Haynesina germanica* | 1.50E-01 | ± | 2.46E-02 | 3.77E-01 | ± | 4.91E-02 | 5.29E-01 | ± | 7.65E-02 | 2.49E-01 | ± | 3.33E-02 | 1.89E-01 | ± | 4.62E-02 |
| *Ammonia beccarii* | 3.71E-01 | ± | 1.02E-01 | 1.40E-01 | ± | 4.06E-02 | 1.36E-01 | ± | 3.76E-02 | 1.04E-01 | ± | 2.36E-02 | 7.27E-02 | ± | 2.13E-02 |
| *Elphidium excavatum* | 8.62E-02 | ± | 2.66E-02 | | | | 1.72E-02 | ± | 1.13E-02 | 1.09E-01 | ± | 2.74E-02 | 3.31E-02 | ± | 1.06E-02 |

### 4.3.1 TIMING OF DISTURBANCE (CJ-CS-2J-2S)

Differences in the foraminiferal assemblages were observed between the patches that recovered for two months after hypoxia in winter or spring. The overall dissimilarity due to timing of disturbance was 26.8%. In September, a higher dissimilarity was found between the hypoxia treated and the control patches as compared to June (CJ vs. 2J and CS vs. 2S, see Appendix 4.1). In the case of the individual species *Elphidium excavatum* was significantly more abundant two months after the winter hypoxia than two months after the spring hypoxia (2J > 2S, Fig. 4.2c.). *Haynesina germanica* contributed more to the observed differences between (winter and spring) hypoxia treated patches and their corresponding control patches than *Ammonia beccarii* and *Elphidium excavatum* and in the opposite direction (Fig. 4.2a., b., c. and Appendix 4.1). *H. germanica* seemed to be more abundant two months after the spring hypoxia (2J < 2S) and also the mean total biovolume of this species was more than three times higher two months after the spring hypoxia than two months after the winter hypoxia (2J < 2S, Appendix 4.2). Although the effect of hypoxia timing on the mean total biovolume of *A. beccarii* was not significant, the trend on total biovolume of this species indicated higher abundances and per-individual biovolumes in June than in September, so in the opposite direction as for *H. germanica* (Fig. 4.2g., h.).

### 4.3.2 RECOVERY DEVELOPMENT (CJ-CS-2J-5S)

The dissimilarity between foraminiferal assemblages in the patches exposed to the winter hypoxia and the concurrent control patches was higher in September after 5 months of recovery compared to June after two months of recovery (CJ vs. 2J and CS vs. 5S, Appendix 4.1). At species level, especially the abundance and mean total biovolume of *Haynesina germanica* increased in the recovery patches whereas they decreased in the control patches (Fig. 4.2a., d., g.). *Ammonia beccarii* had a significantly higher biovolume in the recovery patches in June (2J vs. CJ and 5S vs. CS, Appendix 4.2). Although the estimated per-individual biovolume of *A. beccarii* in the recovery patches reduced by more than half

from June towards September (2J > 5S), the total biovolumes of *A. beccarii* did not differ significantly between these recovery stages (Fig. 4.2e., h.).

### 4.3.3 RECOVERY STAGES (CS-5S-2S)

In September, the dissimilarity between the patches treated with hypoxia and their concurrent control patches (CS vs. 2S and CS vs. 5S) was greater than the dissimilarity between the winter and spring hypoxia themselves (2S vs. 5S, Appendix 4.1). *Haynesina germanica* contributed most to these observed dissimilarities. The highest September density of *H. germanica* was observed in the patches that had recovered for two months after the spring hypoxia (2S > 5S > CS, Fig. 4.2a.), while the abundance of *Ammonia beccarii* showed smaller and non-significant differences ((5S > 2S > CS, Fig. 4.2b., Appendix 4.2). *Elphidium excavatum* abundances in September were very low (less than 10 individuals) in the control (CS) and the two-month recovery patches (2S). *E. excavatum* was absent in the patches that recovered for 5 months after the winter hypoxia (Fig. 4.2c.). In the patches that had been treated with a spring hypoxia, the total biovolume of *H. germanica* was significantly higher than in the control patchess (2S > CS). The total biovolumes of *A. beccarii* did not differ significantly between these patches in September (Fig. 4.2h.).

### 4.3.4 CORRELATIONS WITH MACROFAUNA, MICROPHYTOBENTHOS AND BACTERIA

The main changes in abundance within the benthic community as studied in this experiment (i.e., foraminifera, macrofauna, bacteria and microphytobenthos) were summarised in principal response curves (PRC, Fig. 4.3). The PRCs illustrate the differential community-response to the induced

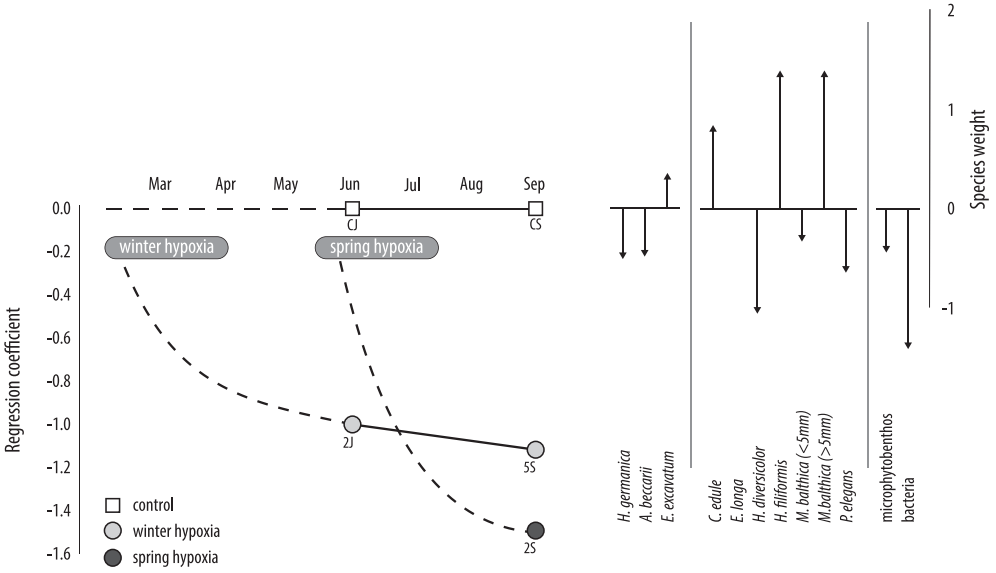



**Figure 4.3** Principle Response Curve (PRC) of Scheldt Estuary biota. Regression coefficients on the first PRC summarise the response of the benthic community to the experimental treatments across time. Species weights can be interpreted as the species' affinities with the PRC; see section 4.2.4 for further explanation. The percentage of total variance accounted for by "time" was 14%, and 57% was accounted for by "time * treatment" 82% of the variance explained by the treatment regime was captured by the first PRC axis in this figure.

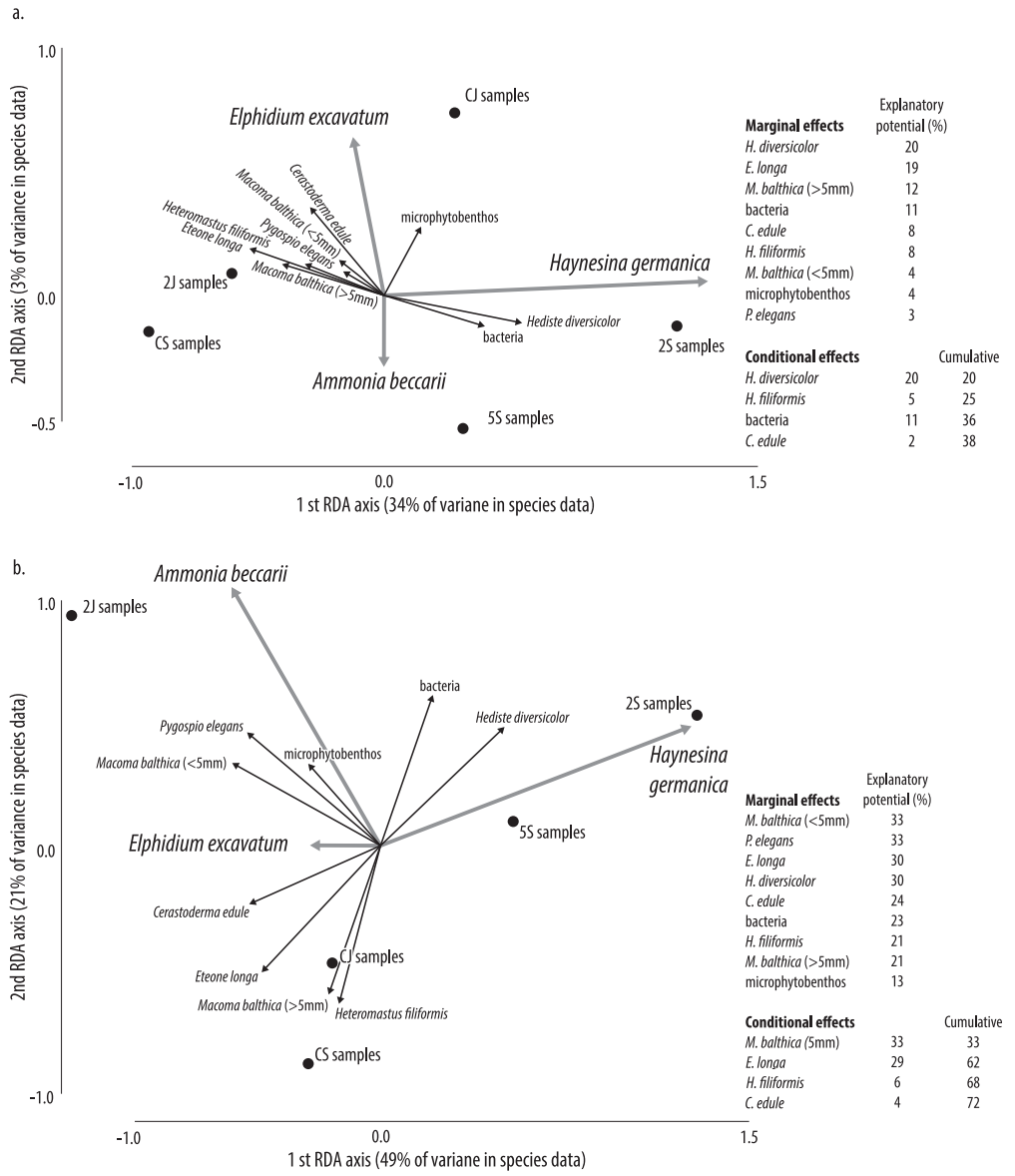



**Figure 4.4 a. - b.** Redundancy Analyses (RDA) of foraminiferal species (wide, grey arrows) and the remaining benthos (narrow, black arrows). Foraminiferal abundances were used in a., foraminiferal biovolumes were used in b. Macrofaunal, bacterial and microphytobenthos abundances were used as explanatory variables and the RDA axes are linear combinations of these abundances. First and second RDA axes are shown, as well as marginal and conditional effects (see section 4.2.4 for explanation).

disturbance, relative to changes in the control samples. The main type of community change (after temporal variability in control samples was filtered out), as highlighted by the winter hypoxia PRC,

remains almost constant 2 and 5 months after the disturbance. The initial response to the spring hypoxia displays the same pattern but deviates more from the control than the initial response after the winter hypoxia (Fig. 4.3). The abundances of the macrofaunal species *Eteone longa*, and especially *Heteromastus filiformis* and large (>5 mm) *Macoma balthica* have the highest positive species weights, indicating that their reduced abundance contributed strongly to the negative values of the presented PRC. The species weight for the two most dominant foraminiferal species *Haynesina germanica* and *Ammonia beccarii* point into the opposite direction, their response is the reverse of that of most of the community. To further explore correlations or potential causal relations between the foraminiferal assemblage and other members of the benthic community, the data were subjected to a redundancy analysis (RDA; Fig. 4.4). This allows expressing observed patterns within the foraminiferal community as a function of macrofauna, bacteria and microphytobenthos abundances. If we consider macrofauna, bacteria and microphytobenthos to be the main drivers of foraminiferal dynamics, their abundances could account for a maximum of 34% of variance in the foraminiferal abundance data (Fig. 4.4a.), and 49% of variance within the foraminiferal biovolume data (Fig. 4.4b.). Direction and length of the vectors in figure 4.4 indicate the rate of change among foraminifera along the first two RDA axes and the contribution of the other benthos to the axes. It offers a graphic summary of the degree of (anti)correlation between them. Figure 4.4a. suggests that the explanatory variables (i.e. macrofaunal abundances, bacteria and microphytobenthos) may have been to a higher extent accountable for the dynamics in the abundances of *H. germanica* than to those of *A. beccarii*. The ordination diagrams (Fig. 4.4a., b.) indicate an inverse correlation between abundances and biovolumes of *H. germanica* with the abundances of most macrofaunal species. Notably, the analyses indicate that in their potential role as explanatory variables the non-foraminiferal community members may have had a stronger impact on biovolumes of *A. beccarii* (Fig. 4.4b.), rather than the abundance of this species (Fig. 4.4a.). The second axis in the abundance-based RDA is of little relevance, explaining only 3% of total variance in species data.

## 4.4 DISCUSSION

Hypoxia can have a major impact on benthic communities, including foraminifera (Gooday et al., 2009; Levin et al., 2009). Nonetheless, our findings show that the same species dominated the foraminiferal assemblage throughout the investigated period of ecosystem recovery as in the surrounding (control) sediment. There were, however, changes in their abundances and biovolumes depending on timing of disturbance and recovery stage. The dynamics of foraminiferal communities are governed by multiple factors including bottom-up factors (e.g., availability of food), top-down forces (e.g., predation and transport by macrofaunal bioturbation), as well as sulphide toxicity and ecosystem engineering. Interplay of these factors probably played a role over the entire recovery period, but their relative contributions varied with time and between control and disturbed patches.

### 4.4.1 ECOSYSTEM RESPONSE TO HYPOXIA

At the end of hypoxia, the sediment was black and anoxic (Montserrat et al., 2008) and all macrofauna had been killed (Rossi et al., 2009). Nematodes, just like their foraminiferal companions in the meiobenthic community, were expected to have a higher resistance to hypoxic conditions (Moodley et al., 1997; Duijnstee et al., 2003, 2005) compared to macrofauna (Van Colen et al., 2008 and references therein). Although the winter hypoxia did not result in a complete mortality of the nematode assemblage, their abundance was reduced by roughly 70 percent in the treated patches (Van Colen et al., 2009). Interestingly, at the same time our field observation on foraminiferal abundances indicated that (stained) foraminifera, especially of the species *Haynesina germanica*, were numerously present.

In some samples that were taken in April – less than 2 weeks after the hypoxia (results not included here because no concurrent control samples were taken) – the total foraminiferal abundance in the disturbed patches was almost three times higher compared to two months later in June.

### 4.4.2 EARLY STAGE OF ECOSYSTEM RECOVERY

The first apparent development at the onset of ecosystem recovery in the disturbed patches was a microphytobenthos bloom that arose within the first months. After its abundance peaked at day 28 it decreased, but remained high compared to the undisturbed patches until ca. 3 months of ecosystem recovery (Montserrat et al., 2008). Although the winter hypoxia reduced the total abundance of nematodes by roughly 70%, their abundance recovered within 8 weeks towards control values (Van Colen et al., 2009). At the end of the early stage of recovery, a nematode abundance overshoot arose in the patches recovering from hypoxia. The early stage of macrofaunal recovery was characterised first by lateral colonisation by, most notably, the gastropod *Hydrobia ulvae* and subsequently (starting at 28 days following the end of the hypoxia) by a high abundance increase of predominantly the small tube-building polychaete *Pygospio elegans* (Rossi et al., 2009; Van Colen et al., 2008, 2012). The foraminiferal field observations of April and the abundance and biovolumes of the foraminifera in June, suggest a species-specific response to the previously mentioned complex set of relations that arose during ecosystem recovery. The abundances of *Haynesina germanica* and *Elphidium excavatum* sharply declined in the disturbed patches within two months after the winter hypoxia. The absence of this sharp decline in the abundance of *Ammonia beccarii* is striking, as is the concurrent high increase in average and total biovolume of the latter species. The observed species-specific foraminiferal response to hypoxia is in accordance with for example Bouchet et al. (2007). They investigated the response of foraminiferal assemblages to short-term biogeochemical disturbances in marine sediments of the Marennes-Oléron Bay (SW France), where oyster summer mortality may be involved in the occurrence of hypoxia. Species-specific responses were observed, as a relative high tolerance of *Ammonia tepida* to the temperature increase and hypoxic conditions and a higher sensitivity of *Haynesina germanica* to organic degradation and hypoxia.

A strong increase in biovolume towards the sampling occasion in June, indicated that especially the population of *Ammonia beccarii* is assumed to have profited from the benthic algal bloom in the recovery patches (Fig. 4.2e.). The PRC based on foraminiferal abundance (Fig. 4.3) and RDA analysis based on foraminiferal biovolumes (Fig. 4.4b.) respectively explore relations between foraminifera and other faunal and microfloral elements and the explanatory potential of the co-occurring macrobenthos, bacteria and microphytobenthos for fluctuations in the foraminiferal dynamics. The PRC and RDA reveal that the biovolumes of *A. beccarii* and its abundance developed in the same direction as the presence of microphytobenthos. This finding is in accordance with several studies: bacteria (Langezaal et al., 2005; Pascal et al., 2008) as well as algal material (Moodley et al., 2000) were shown to be ingested by *A. beccarii*; their microphytobenthos uptake resembled the pattern of uptake found for nematodes (Middelburg et al., 2000). Bacteria have been recorded as foraminiferal food source (e.g. Bernhard and Bowser 1992; Langezaal et al., 2005) and have also been suggested as a prerequisite for sustained reproduction (Muller and Lee, 1969). In accordance, the RDA analysis suggests a positive correlation between *Haynesina germanica* (abundances and total biovolumes) with bacterial biomass. As for the foraminifera, the microphytobenthos bloom has been suggested to also positively affect the nematode assemblage (Van Colen et al., 2009). If competition for food between *A. beccarii* and nematodes usually plays a role in their population dynamics, one could argue that food was not a likely limiting factor during the recovery period. Interestingly, a recent experimental study by Dupuy

et al. (2010) has revealed predation on live nematodes, copepods and gastropod larvae by *Ammonia* individuals identified as *Ammonia tepida* (likely the same species as our *A. beccarii*; see 'taxonomic remarks' in 'material and methods' section). This potentially reverses the hypothetical relation of competitive exclusion between *Ammonia* and nematodes if nematodes indeed turn out to be an additional food source. If nematodes were preyed upon by *A. beccarii*, the microphytobenthos bloom delivered a direct as well as an indirect food source for *A. beccarii*. In addition, our results – indicating a lack of response of the other two foraminiferal taxa to the microphytobenthos abundance – are in line with the absence of a distinct algal uptake by *Haynesina germanica* and *Elphidium excavatum* as reported by Moodley et al. (2000). Ward et al. (2003), however, demonstrated that *H. germanica* did feed actively on pennate diatoms, but not on sewage-derived degrading organic matter, whereas *A. beccarii* reaches higher abundances at sites around sewage outfalls in Long Island Sound (Thomas et al., 2000).

Besides a response to fluctuations in microphytobenthos and bacteria density, additional mechanisms might elucidate the sharp decrease in the foraminiferal density of *Haynesina germanica* and *Elphidium excavatum* in the uppermost centimetre of the sediment during the early stage of recovery. One of the mechanisms is downward migration. Although we cannot verify this with the current data, foraminifera might have migrated downwards following the re-oxygenation of the sediment. Vertical foraminiferal migration in response to fluctuations in oxygen concentrations has previously been reported. Geslin et al. (2004), for instance, showed that foraminifera can actively move to preferred habitats, and oxygen was suggested to be the main driver for this foraminiferal migration. Alve and Bernhard (1995) indicated that foraminifera actively migrated downwards to their original depth distributions after re-oxygenation of sediments that had experienced a period of anoxia. And although their results were not based on material from intertidal muds – with their steep geochemical gradients and limited vertical distribution – we cannot rule out some migratory effect on our observed distributions. Moreover, part of a decline in the abundance of the foraminifera in the top one centimetre of the sediment may be ascribed to passive transport (as for example previously reported by Moodley, 1990). Passive transport of foraminifera evolves from macrofaunal activities such as sediment reworking, or bioturbation. Although the total macrofaunal biomass was very low in the recovering patches of June (10 times lower compared to the controls), the abundance of macrofauna was actually higher in the recovery patches compared to the controls (Rossi et al., 2009). This high abundance of especially tube-building polychaete species (Van Colen et al., 2008) may have resulted in stronger reworking of the sediment surface, transporting foraminifera to layers below the top centimetre of the sediment.

### 4.4.3 SECOND STAGE OF ECOSYSTEM RECOVERY (JUNE TO SEPTEMBER)

In the subsequent stage of ecosystem recovery, from 2 to 5 months of recolonisation, macrofauna assemblages became dominated by larger species, such as the polychaete *Heteromastus filiformis,* inhabiting deeper sediment layers (Rossi et al., 2009). While the total macrofaunal biomass increased by almost 300%, its abundance decreased (roughly with 60%) in the disturbed patches. Simultaneously, the control patches revealed a small increase in macrofaunal biomass (approximately 6%) and a decrease in abundance of about 30% (Rossi et al., 2009). The replacement in the patches disturbed by winter hypoxia of numerous small tube-building polychaetes by fewer large macrofaunal elements such as the polychaete *Heteromastus filiformis* co-occurred with an increase in abundance of *Haynesina germanica*. Similar to the early stage of recovery, an inverse correlation between macrofaunal density and the abundance of *H. germanica* is observed during prolonged recovery (Fig. 4.2a., d., g. and Fig. 4.4a., b.). Contrastingly, in the undisturbed control patches both macrofaunal abundances and the density of *H. germanica* decreased, indicating a positive correlation which suggests that the observed

inverse correlation in the treated patches does not imply a linear causal relation. The dynamics in abundance of *Ammonia beccarii* (Fig. 4.4) observed in the recovery patches does not seem to be strongly affected by macrofaunal abundances, while its biovolume appears to correlate positively with macrofaunal abundances (Fig. 4.4).

Given the widely accepted concept that bioturbation and bio-irrigation promote the occurrence of oxygen-dependent meiobenthos (Aller, 1983; Ray and Aller, 1985; Fenchel, 1996; Zorn et al., 2006), we expected that high abundances of foraminifera would indicate high densities of macrofauna and *vice versa*. A high abundance of bioturbating macrofauna creates more heterogeneity in the sediment and it enlarges the sediment-water interface. Both mechanisms result in an increased amount and volume of oxygenated micro-niches that can be occupied by foraminifera and other meiobenthos. Bouchet et al. (2009) indicated that the impact of macrofaunal bioturbation on the vertical distribution of foraminifera (*Ammonia tepida, Cribroelphidium excavatum, Haynesina germanica* and *Brizalina striatula*) depends on the effect of macrofauna on bioirrigation, rather than on the constructed biogenic structure volume. Passerelli et al. (2012) described the importance of the biogenic structures for organizing benthic communities through its impact on the development of sediment stability and microphytobenthos biofilms. The contradiction between the expected and observed relation of the macrofaunal abundance with foraminiferal densities (of especially *Haynesina germanica* in the recovery patches) may be attributable to several factors. First, the expected increase in the amount of micro-niches, macrofaunal-mediated through bioturbation, may have predominantly affected the deeper sediment layers, invisible in the current dataset. As mentioned before, a higher availability of foraminiferal niches below the top 1 centimetre of the sediment may provoke lower foraminiferal densities in the top layer by stimulating downwards migration of foraminifera. Analysing the top 1 centimetre of the sediment, in correspondence with the recommendations for foraminiferal biomonitoring studies (Bouchet et al., 2012; Schönfeld et al., 2012), may lead to an unexpected outcome if processes in deeper sediment layers become increasingly important for foraminiferal dynamics. Nevertheless, biological interactions such as disturbance and competition have been suggested to influence foraminiferal survival rates (Moodley et al., 1997). Hence, in the hypoxia exposed patches, an increased physical disturbance and predation pressure due to increased macrofaunal density may have counteracted and outweighed the expected positive effects of bioturbation as an ecosystem engineering force for the establishment of additional micro-niches. Moreover, sediment reworking activities by macrofauna may injure foraminifera. For locomotion and feeding, foraminifera use reticulopodia, a network of fine extensions of the cytoplasm, emerging from the test's aperture(s). This network ventures out deep into the surrounding sediment in a sphere with a radius many times the test diameter. Higher abundances of macrofauna amplify the amount of sediment reworking and, consequentially might augment the disturbance by severing reticulopodia as this requires very little physical disturbance (Duijnstee, pers. obs.). Disturbance may thus increase the mortality rates of foraminifera leading to fewer stained specimens at higher macrofaunal abundances. In addition, macrofaunal predation pressure may negatively affect foraminiferal densities. Buzas (1978, 1982) reported that foraminifera are a food source for macrofauna. He demonstrated that predation on foraminifera plays a regulatory role in foraminiferal densities. In summary, an unexpected inverse relation between foraminiferal and macrofaunal abundances in the recovery patches may be attributable to direct biotic interactions as well as environmental factors. Hypoxia disturbed the interplay of direct and indirect interactions among macrofauna and foraminifera (Fig. 4.3), this may have resulted in the observed differential relations among the foraminiferal species and macrofauna in the disturbed versus control patches.

### 4.4.4 HYPOXIA TIMING EFFECTS OF WINTER- AND SPRING HYPOXIA

The first principle response curve for the benthic community (species-specific macrofaunal and foraminiferal abundances, bacteria and microphytobenthos, Fig. 4.3), shows a relatively consistent degree of deviation from the control situation, regardless of timing of treatment and recovery. This pattern, however, is strongly dominated by the almost complete loss, and slow recovery of the larger macrofauna (i.e. *Heteromastus filiformis* and *Macoma balthica* >5 mm) associated with the hypoxia treatment, and, to a lesser extent, with the foraminiferal response or the recovery dynamics of the entire benthic community. When individual taxa are followed throughout the experiments, a more differentiated pattern emerges. The early reassembly stages of the macrofaunal community differed in terms of species richness and abundance depending on whether hypoxia took place in winter or spring (Rossi et al., 2009). The recovering macrofaunal community comprised more individuals and species two months after the winter hypoxia (2J) compared to two months after the spring hypoxia (2S) (Rossi et al., 2009). The differences found between the macrofaunal communities were ascribed to seasonality-driven dissimilarities in the availability of larvae and juveniles in the surrounding areas for recruitment (Rossi et al., 2009). De Nooijer et al. (2007) observed that on intertidal flats in the Dutch Wadden Sea, abundances of *Haynesina germanica* peaked in spring, whereas those of *Ammonia* (*tepida*) had their optimum in summer. For our control patches, the observed pattern of the foraminiferal abundances (Fig. 4.2a., b.) seems to be consistent with De Nooijer et al. (2007) 's findings. For our recovering patches two months after winter and spring hypoxia, the patterns point into another direction: the timing aspect of the hypoxic treatment resulted for *H. germanica* in higher abundances and higher per-individual and total biovolumes (Fig. 4.2a., d., g.) two months after the spring hypoxia compared to two months after the winter hypoxia. The opposite was found for *Ammonia beccarii* that seemed to be more abundant and had higher per-individual and total biovolumes two months after the winter hypoxia. In summary; a species-specific foraminiferal response was observed for the timing of hypoxia. The differential impact, due to the timing of hypoxia, on the interplay of direct and indirect interactions within the ecosystem, and dissimilarities in the lateral availability of larvae and juveniles for repopulation, may have led to this species-specific foraminiferal response. In addition, as suggested for the macrofauna, differences among the foraminiferal species concerning their natural timing of reproduction may have contributed to the foraminiferal species-specific response to the timing of hypoxia.

## 4.5 CONCLUSION

During ecosystem recovery, the foraminiferal species showed species-specific and complex patterns of development. This development was related to interplay of variability in oxygen availability, bacteria, microphytobenthos and macrofauna abundances. During ecosystem recovery, the expected positive impact of macrofaunal ecosystem engineering through bioturbation and bioirrigation was not observed. Pending macrofaunal recolonisation, *Haynesina germanica* decreased at increasing macrofaunal abundances. Contrasting results were found among the undisturbed patches. The abundance of *H. germanica* in the upper centimetre of the sediment in the disturbed patches was inversely related to macrofaunal abundance whereas it was positively related in control patches. *Ammonia beccarii* instead seemed to be driven by the microphytobenthos surplus due to the lack of grazing after hypoxia-induced mortality of the macrofauna. Our study indicates species-specific dynamics in the abundance and biovolume of foraminifera in response to fluctuations in environmental factors.

| **SIMPER** - foraminiferal abundances | | | | |
|---|---|---|---|---|
| | dissimilarity | *H. germanica* | *A. beccarii* | *E. excavatum* |
| Time and treatment | % | % | % | % |
| CJ - CS - 2J - 5S | 25.0 | 64.4 | 18.3 | 17.3 |
| CJ - CS - 2J - 2S | 26.8 | 70.8 | 14.2 | 15.0 |
| CS - 5S - 2S | 27.2 | 76.9 | 16.8 | 6.3 |
| Time or treatment | | % | % | % |
| CJ - CS | 28.8 | 71.8 | 9.7 | 18.5 |
| 2J - CJ | 21.7 | 67.4 | 19.6 | 13.0 |
| 5S - CS | 28.6 | 70.8 | 20.5 | 8.7 |
| 2S - CS | 32.5 | 84.1 | 9.7 | 6.1 |
| 2J - 5S | 22.0 | 56.7 | 19.5 | 23.8 |
| 2J - 2S | 25.5 | 68.6 | 15.4 | 16.0 |
| 2S - 5S | 19.0 | 72.6 | 25.1 | 2.2 |

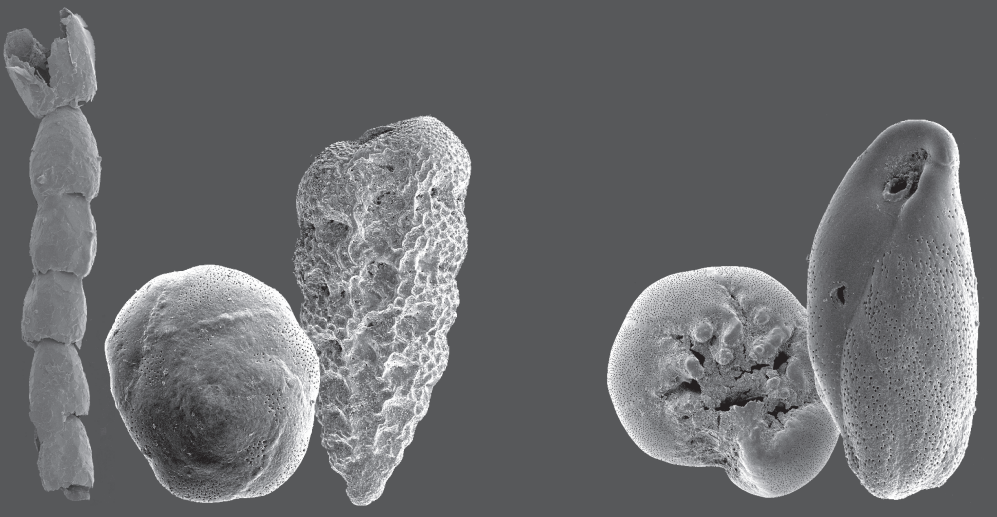
**Appendix 4.1** SIMPER analyses of the contribution per taxa to observed differences between foraminiferal assemblages.

| **Kruskal-Wallis** | | | | | |
|---|---|---|---|---|---|
| Time | Abundance | | | Total biovolumes | |
| * | *H. germanica* | *A. beccarii* | *E. excavatum* | *H. germanica* | *A. beccarii* |
| Treatment | p | p | p | p | p |
| CJ - CS | 0.18 | 1.00 | 0.07 | 0.18 | 0.71 |
| 2J - CJ | 0.47 | **0.04** | 0.56 | 0.06 | **0.03** |
| 5S - CS | 0.09 | 0.27 | **0.02** | 0.07 | 0.11 |
| 2S - CS | **0.04** | 0.71 | 0.14 | **0.04** | 0.11 |
| 2J - 5S | 0.19 | 0.67 | **0.02** | **0.03** | 0.11 |
| 2J - 2S | 0.19 | 0.19 | **0.03** | **0.03** | 0.11 |
| 2S - 5S | 0.31 | 0.47 | 0.19 | 0.19 | 0.67 |

**Appendix 4.2** Statistical analyses of the foraminiferal abundance per species (Kruskal-Wallis). Statistical analyses of the total biovolumes of *Haynesina germanica* and *Ammonia beccarii*. All p-values are Bonferroni corrected. Bold numbers represent significant differences.

| Two-Way ANOSIM | | |
|---|---|---|
| | R | p |
| 2J - CJ - 2S - CS | | |
| Time | 0.34 | **0.03** |
| Treatment | 0.20 | 0.07 |
| 2J - CJ - 5S - CS | | |
| Time | 0.27 | **0.04** |
| Treatment | 0.17 | 0.10 |
| One-Way ANOSIM | | |
| 2S - 5S - CS | 0.22 | 0.08 |

**Appendix 4.3** Statistical analyses of the foraminiferal abundance of the assemblage (ANOSIM). The Two-Way ANOSIM tests for the combined effect of time and treatment on the foraminiferal assemblage due to timing of disturbance and recovery development (taxa in).The One-Way ANOSIM tests for the effect of recovery stages on the foraminiferal assemblages (taxa in). Bold numbers represent significant differences.



CHAPTER 5

# DIET SHIFTS AND POPULATION DYNAMICS OF ESTUARINE FORAMINIFERA DURING ECOSYSTEM RECOVERY AFTER HYPOXIA

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(Under revision for Estuarine, Coastal and Shelf Science)

ABSTRACT

Estuarine ecosystems provide us with many resources and services at no charge (e.g. recreation, fisheries etc.), yet they are increasingly under attack due to human-augmented hypoxic perturbations. The use of indicator species, such as foraminifera, for ecosystem functioning can help to monitor, manage, protect and restore coastal ecosystems. For accurate use of indicator species, explicit knowledge is required on species-specific responses to fluctuations in biotic and abiotic ecosystem properties. In this study we investigate the effect of deliberately induced hypoxia and subsequent ecosystem recovery on foraminifera. $^{13}$C-labelled bicarbonate and glucose were added to the sediments to investigate foraminiferal diet shifts during restoration and test-size measurements were used to deduce population dynamics. Hypoxia-treated and undisturbed patches were compared to distinguish natural (seasonal) fluctuations from hypoxia-induced responses. The effect of timing of disturbance and duration of recovery were investigated. The foraminiferal diets and population dynamics showed higher fluctuations in the recovering patches compared to the controls. The foraminiferal diet and population structure of *Haynesina germanica* and *Ammonia beccarii* responded differentially and generally inversely to progressive stages of ecosystem recovery. Tracer inferred diet estimates in April and June and the two distinctly visible cohorts in the test-size distribution, discussed to reflect reproduction in June, strongly suggest that the ample availability of diatoms during the first month of ecosystem recovery after the winter hypoxia was likely profitable to *A. beccarii*. Enhanced reproduction itself was strongly linked to the subsequent dietary shift to bacteria. The distribution of the test dimensions of *H. germanica* indicated that this species had less fluctuation in population structure during ecosystem recovery but possibly reproduced in response to the induced winter hypoxia. Bacteria seemed to consistently contribute more to the diet of *H. germanica* than diatoms. For the diet and test-size distribution of both species, the timing of disturbance seemed to have a higher impact than the duration of the subsequent recovery period.

KEYWORDS

Intertidal * Benthic foraminifera * Hypoxia * $^{13}$C label * Diet shifts * Population dynamics

## 5.1 INTRODUCTION

Coastal areas provide many resources and services beneficial to human settlement. These favourable conditions in near-coastal zones have led to human population densities nearly three times higher than the global average (Small and Nicholls, 2003). High population densities in coastal zones make these areas more prone to a rise in anthropogenic perturbations (Diaz et al., 2009 and references therein). Consequences of such perturbations, as for example instances of low-oxygen concentrations in bottom-waters, cause a loss of ecosystem services (Diaz and Rosenberg, 2008 and references therein). Hypoxia induced stress may alter species behaviour and provoke mortality of sensitive species (as reviewed by e.g. Gray et al., 2002; Zhang et al., 2009; Diaz et al., 2009). Aberrant macrofaunal behaviour and loss of species is known to alter the functioning of estuarine communities and affect food web interactions (Conley et al., 2007; Diaz et al., 2009).

In order to be able to protect and restore complex coastal ecosystems, explicit knowledge on their functioning is required. The use of selected species as indicator for ecosystem functioning can be very helpful to more easily monitor ecosystem development. Among the benthic fauna in estuarine ecosystems, foraminiferal species are increasingly recognised as efficient bioindicators (Debenay et al., 2006; Schönfeld et al., 2012; Bouchet et al., 2012). Foraminifera are common marine heterotrophic unicellular bikont eukaryotes from the supergroup Rhizaria. These protists occupy a variety of trophic niches. Several foraminiferal feeding strategies have been described (e.g. Lipps, 1983). Some species have been reported to graze on diatoms or other algae, while others use their pseudopodia to trap detritus or even metazoans. Besides parasitism, carnivory, bactivory and cannibalism, foraminiferal symbiotic trophic relationships with bacteria and algae have been observed (Goldstein, 1999 and references therein).

Foraminifera, with their intermediate position in-between microbes and macrofauna, have several characteristics that favour their applicability as indicator species. Their numerous presences in almost all marine environments and the high fossilisation potential of the small sized foraminiferal test facilitate studies of both modern and paleo-environments. Foraminifera generally possess a high ability to survive perturbations, so they may record the higher end of the disturbance spectrum, long after macrofauna have succumbed. Notwithstanding their high potential to survive disturbance, foraminiferal populations commonly respond fast to changes in their environment (e.g. Jorissen et al., 1995; Debenay et al., 2006). The availability of oxygen and food are considered as the main parameters structuring benthic foraminiferal communities and their spatial distribution (Jorissen et al., 1995; Van der Zwaan et al., 1999).

Environmental conditions influence foraminiferal growth patterns with lower growth rates during stress (Röttger, 1972). Pulses of organic matter and oxygen depletion affect foraminiferal densities and migratory behaviour, but these responses are species-specific (Ernst et al., 2005) and variable effects may be observed in different foraminiferal size classes because of altered population dynamics and life-history strategies (Duijnstee et al., 2005). Duijnstee et al. (2005) observed that a pulse of organic matter differentially affected composition within large (>63 µm) and small (38–63 µm) benthic foraminiferal assemblages. The density of large individuals declined stronger than the density of small individuals after a simulated marine snow event. The food pulse and the provoked subsequent anoxic conditions were suggested to have inhibited growth, but enhanced fecundity and likely triggered reproduction at a very early stage.

The foraminiferal test size and the foraminiferal fecundity are related to the type and availability of food sources. Muller and Lee (1969) reported that some foraminifera require bacteria to sustain reproduction. Parfrey and Katz (2010) discovered that the diet of the foraminifer *Allogromia laticollaris* influenced the amount of DNA in the foraminiferal protoplasm. Specimen that foraged on a mixture of

algae and bacteria had a 2-fold increase in DNA compared to specimens feeding on bacteria only. This higher DNA content has been linked to increased fecundity for this particular monothalamid resulting in a higher number of offspring (Parfrey and Katz, 2010). Hallock (1985) compared the adult test diameter, the fecundity and longevity of eight foraminiferal species. Among these species, *Ammonia beccarii* (Bradshaw, 1957) had the shortest lifespan (2 to 3 months) and relatively few offspring (17 to 44 individuals). The short-living species have been discussed to be able to respond faster to newly available resources than the long-living species (Hallock, 1985).
Foraminiferal diets depend on a dynamic interplay between biotic and abiotic interactions within the food web. Hypoxic disturbance may alter the availability and composition of food resources and influence foraminiferal carbon utilisation (e.g. Gustafsson and Nordberg, 2000). In order to accurately use foraminifera as bioindicator for the recovery potential of ecosystems a thorough understanding is needed on the impact of ecosystem properties, such as food availability and macro- and meiofaunal presence, on foraminiferal feeding and population dynamics, especially in disturbed ecosystems.

The main question addressed in this study is how hypoxia and ecosystem recovery influence the dynamics of foraminiferal species; i.e. their population structures (e.g. size distribution, survival, growth and reproduction) and feeding strategies. To address these objectives, sediments of an intertidal flat in the Scheldt Estuary on the Dutch coast were exposed to human-induced hypoxia in winter or late spring. To study the carbon flow from micro-organisms at the base of the food web – such as diatoms and heterotrophic bacteria – to the dominant foraminiferal species *Ammonia beccarii* and *Haynesina germanica*, $^{13}$C-labelled glucose and bicarbonate were introduced in these *in situ* experiments to enrich heterotrophic bacteria and benthic algae, respectively. To investigate the effect of hypoxia on foraminiferal population dynamics, we studied the distribution of test-sizes combined with foraminiferal food consumption patterns at different times in a 5 month period of ecosystem recovery. Previous work on the same set of field experiments has shown that the timing of experimentally-induced disturbance lead to differential responses in ecosystem properties that are subsequently assumed to directly or indirectly influence foraminifera – e.g. via differential alterations of food availability (chapter four), or differences in predation pressure and disturbance effects of recovering nematodes and macrofauna (see Montserrat et al., 2008, 2009; Van Colen et al., 2008, 2009, 2010a, b, 2012; Rossi et al., 2008, 2009; Rossi and Middelburg 2011). These hypoxia-related changes in ecosystem properties and the direct impact of hypoxia on foraminifera determine the net impact of hypoxia on foraminifera. Hence, our results will be discussed in the context of findings published in these parallel studies.

## 5.2 MATERIAL AND METHODS

### 5.2.1 SITE

The field experiments took place on a tidal flat bordering the Paulina Polder on the Southern bank of the Scheldt Estuary in The Netherlands. The mudflat covers an area of around 1.0 km$^2$ and has a mean tidal range of 3.9 m with a semidiurnal regime. Under natural conditions, the macrofaunal community is dominated by polychaetes (*Heteromastus filiformis, Arenicola marina, Pygospio elegans*) and molluscs (*Macoma balthica, Cerastoderma edule, Hydrobia ulvae*) (Rossi et al., 2009). For food supply, this community of macrofaunal invertebrates relies on the carbon flow that starts at the bottom of the food web with microphytobenthos and chemo-autotrophic bacteria and subsequently passes through the meiofaunal community of (predominantly) nematodes and foraminifera and macrofauna,

heterotrophic bacteria utilise carbon from each trophic level and may return carbon to the system as food source especially for the meiofaunal part of the system.

### 5.2.2 EXPERIMENTAL SET-UP: LABELLING AND SAMPLING

Hypoxic conditions were created for two months in winter and late spring. To this end, four large patches (4x4 m) within a 50x50 m location (51°21'23"N, 3°42'49"E) were covered with black waterproof polyethylene sheets – both stopping oxygenic photosynthesis and effectively preventing the replenishment of oxygen that was consumed during decomposition of the ample organic matter content of the sediment, and thus rendering the sediment hypoxic. The winter hypoxia (two patches) lasted from January 30th until March 30th, 2005 and a spring hypoxia (also two patches) from May 9th until July 6th, 2005. Two undisturbed patches were used as controls. In the same area multiple patches were similarly treated and have been used in parallel studies to investigate macrofaunal recolonisation, sediment characteristics and carbon cycling from microbes to macrofauna (Van Colen et al., 2008, 2009, 2010a, b, 2012; Rossi et al., 2009 and Rossi and Middelburg 2011).

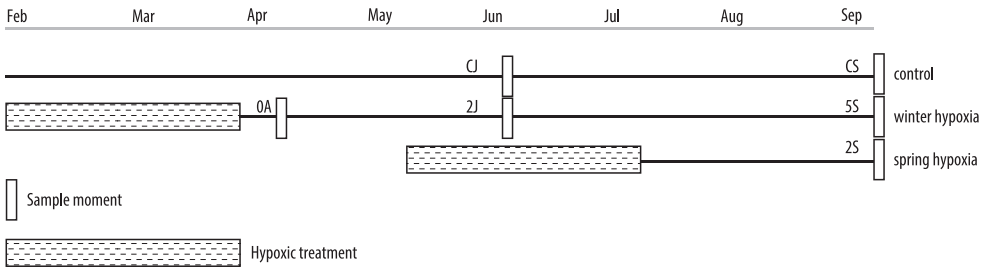



**Figure 5.1** Experimental time scale and sample moments.

Samples were taken for foraminiferal and microbial analyses in June (2J) and September (5S) in patches exposed to a winter hypoxia. The two-character treatment coding (Fig. 5.1) refers to control (C) or months of recovery (0, 2, 5) and month of sampling (A, J, S). In April, two weeks after the winter hypoxia samples were only taken in the recovering patches (0A). The patches that endured a spring hypoxia were sampled in September (2S). In June (CJ) and September (CS) samples were taken in the control patches (see sampling scheme, Fig. 5.1). During low tide, 96 hours preceding each sample occasion the hypoxia-affected and the control patches were treated with the addition of $^{13}$C-labelled glucose or $^{13}$C-labelled bicarbonate (see Rossi et al., 2009 and Rossi and Middelburg, 2011). Subplots of 50x50 cm were supplied with either 250 mg of $^{13}$C-labelled bicarbonate (99% $^{13}$C), or with 114.75 mg $^{13}$C-labelled glucose (99% $^{13}$C); both dissolved in 250 mL of filtered seawater. The timing and amount of added label was chosen based on previous studies (Middelburg et al., 2000; Van Oevelen et al., 2006). The $^{13}$C-labelled bicarbonate was intended to be used by microphytobenthos in photosynthesis, and therefore sprayed on top of the sediment. The addition of $^{13}$C-labelled glucose was aimed to label heterotrophic bacteria. The labelled glucose was injected into the subplots and spread equally over the sediment column up to a sediment depth of 8 cm. For the injection, the subplots were divided into squares of 2.5x2.5 cm. Each square was injected with the $^{13}$C-labelled glucose solution. In each glucose and bicarbonate subplot, two cores (5 cm internal diameter) were taken to 8 cm sediment depth (see Rossi et al., 2009). In the laboratory the cores were subsampled, and the top one centimetre

was used for biomass and $^{13}C$ incorporation of microbes or foraminifera using a cut syringe with an internal diameter of 2 cm. For microbial analyses, polar-lipid-derived fatty acid (PLFA) biomarkers – the lipids composing organisms cell membrane are group specific – were extracted from 3 gram of dry sediment using Bligh and Dyer extraction (details in Rossi et al., 2009). The bacterial-specific PLFA biomarkers 14:0 iso, 15:0 iso, 15:0 anteiso and 16:0 iso as well as the diatom PLFA 20:5w3 were used to trace label incorporation and diatom and bacterial biomass (Rossi and Middelburg, 2011).

### 5.2.3 SAMPLE PROCESSING

The sediment samples used for foraminiferal analyses were stored in 4% buffered formalin with Rose Bengal staining. Although formalin may slightly alter the isotopic composition, this effect can be ignored in tracer applications (Rossi and Middelburg, 2011). After foraminiferal cytoplasm was stained, the sediment samples were sieved over a mesh size of 63 µm. Well-stained foraminifera were picked from the sieved (wet) samples and enumerated at species level. Only individuals fully filled with vividly pink stained cytoplasm (except for the ultimate chamber) were considered alive close to the time of sampling. After picking, photos were taken with a Hitachi Camera, type Hv-c20A mounted on a (Leica MZ12(5)) microscope with a calibrated internal scale to measure the maximum test dimension of each individual specimen. In order to analyse the distribution of test dimensions, foraminiferal individuals were binned in 10 size classes of 39 µm for *Haynesina germanica* and 56 µm for *Ammonia beccarii*. The smallest size class starts with 63-102 µm for *Haynesina germanica* and 63-119 µm for *Ammonia beccarii*; species-specific bin sizes were used for optimal distribution of the test-size dimension of both species. The test-size frequency was expressed as a percentage of the total population (per species, sample moment and treatment) found in a size bin. Using test-size dimension to provide insight in the population structure of foraminiferal species, one has to bear in mind that sediments are generally sieved over a 63, 125 or 150 µm mesh size. Small, juvenile specimen will be lost. It is not suitable for following the growth of specific cohorts (Murray and Alve, 2000; Debenay et al., 2006). In this study we do not calculate foraminiferal production rates. By measuring test sizes at time intervals of respectively 2 and 3 months, results were obtained on the population structure which is discussed in the context of population dynamics.

In addition, the size measurements were used to calculate individual foraminiferal biomasses. These individual biomasses were required to semi-quantitatively derive the transfer of carbon from microbes to foraminifera. The maximum test-size dimension (L) was used to estimate the foraminiferal biovolume of each individual (BV), using the equation $BV = \pi L^3/16$ (this is the volume of a cylinder with diameter L and a height of 0.25 L). Subsequently, the individual biovolumes were used to obtain the individual biomasses. Moodley et al. (2000) reported for the species *Ammonia beccarii* an average individual biomass of 1.10 µgC/individual to co-occur with an average length of 325 µm and for *Haynesina germanica* an average biomass of 1.48 µgC/individual to co-occur with an average length of 381 µm. These values were used to convert the individual biovolumes of both species to individual biomasses.

To analyse the uptake of labelled carbon by both foraminiferal species, stained specimens were randomly selected, rinsed with Milli-Q to remove debris and placed in silver boats (see Moodley et al., 2000 for processing details). To remove the foraminiferal tests 50 µL of 2.5% HCl was added to dissolve the carbonate. The completeness of decalcification of the foraminiferal test was visually checked and an extra (50µL 2.5%) HCL was added in case of incomplete decalcification. The samples were dried and concentrations of carbon isotopes were measured using a Carlo Erba 1106 Elemental Analyzer coupled online with a Finnigan Delta S isotope ratio mass spectrometer (after Moodley et al., 2000).

The incorporation of label in microbes and foraminifera was used to determine foraminiferal diets as well as the contribution of foraminifera to the transfer of microbial carbon through the food web.

### 5.2.4 LABEL INCORPORATION – FORAMINIFERAL DIETS (Δ-RATIOS)

The relative importance of bacteria and diatoms as food source for foraminifera was quantified by the Δ-ratio, the fraction of the total carbon in the consumer that is derived from either bacteria or diatoms (see Van Oevelen et al., 2006; Rossi and Middelburg, 2011). The diets, i.e. Δ-ratios, were for both foraminiferal species determined using the equations:

$$\Delta\text{-ratio}_{\text{foraminifera/microbe}} = (\Delta\delta^{13}C_{foraminifera}/\Delta\delta^{13}C_{microbe})$$

The incorporation of $^{13}C$ label in foraminifera and microbes is expressed as $\Delta\delta^{13}C$:

$$\Delta\delta^{13}C = \delta^{13}C_{sample} - \delta^{13}C_{background}$$

The $\Delta\delta^{13}C$ of the foraminifera and the microbes is the difference in label enrichments between samples derived from the experimental patches (all treated with either the addition of $^{13}C$-labelled glucose or $^{13}C$-labelled bicarbonate) and natural enrichment in $^{13}C$ in the background. The $\delta^{13}C$ of the foraminifera and the microbes from treated patches and background values are both expressed as relative deviation ($\delta^{13}C$ in ‰) from the ratio ($^{13}C/^{12}C$) in the Vienna Pee Dee Belemnite standard (VPDB). The Δ-ratios were estimated to infer diet shift during ecosystem recovery.

The bacterial $\Delta\delta^{13}C$ was calculated as weighted average based on the label incorporation and the concentration of each of the bacterial-specific PLFA's biomarkers extracted from dry sediment and the diatom $\Delta\delta^{13}C$ was determined using the label incorporation and concentration of the diatom-specific PLFA 20:5w3 (for details see: Middelburg et al., 2000). The $\Delta\delta^{13}C$ of the foraminiferal species were established directly from the foraminiferal flesh (see above). Background values of -16.6‰ and -12.9‰ were respectively used for *Ammonia beccarii* and *Haynesina germanica* (after Moodley et al., 2000). By using these equations, we assumed a steady state between $\Delta\delta^{13}C_{consumer}$ and $\Delta\delta^{13}C_{resource}$. In correspondence with Van Oevelen et al. (2006) we used this equation because label incorporation was not measured in time series necessary to estimate carbon flow via the (more accurate) isotope model.

### 5.2.5 LABEL INCORPORATION – CARBON TRANSFER

The measured incorporation of label in the foraminifers and the microbes was also used to semi-quantitatively estimate the transfer of carbon from microbes to foraminifera. To determine this transfer of carbon, the relative uptake of label (*I*) by foraminifera ($I_{foraminifera}$) was expressed as a percentage of the label uptake by bacteria ($I_{bacteria}$) and diatoms ($I_{diatom}$).

Label uptake by foraminifera and microbes was calculated as the product of excess (*E*) and foraminiferal biomass or microbial PLFA carbon. Total incorporation of $^{13}C$ is excess (*E*) multiplied by the total biomass per standardised sample volume. *E* can be calculated by taking the difference between the $^{13}C$ fractions (*F*) of biota (e.g. foraminifera) from sediments treated with label and those from non-labelled background sediments (or: $E = F_{treated} - F_{background}$). In turn, *F* is defined as $R_{sample}/(R_{sample} + 1)$, where $R_{sample}$ is the isotope ratio $(^{13}C/^{12}C)_{sample}$ which can be derived from our $\delta^{13}C$ values as follows: $R_{sample} = R_{VPDB}([\delta^{13}C/1000] + 1)$. Combining the above with an estimate for $R_{VPDB}$ of 0.0112372 (after Middelburg et al., 2000; Moodley et al., 2002) yields the following equation for *E*:

$$E = \frac{(\delta^{13} C_{sample}+1000)}{(\delta^{13} C_{sample}+91909)} - \frac{(\delta^{13} C_{control}+1000)}{(\delta^{13} C_{control}+91909)}$$

$I_{foraminifera}$ can now be obtained by multiplying $E$ with the estimated biomass of the foraminiferal cytoplasm. Label uptake into bacteria and diatoms was analysed using PLFA's biomarkers extracted from dry sediments samples. $I_{bacteria}$ was estimated as:

$$I_{bacteria} = \Sigma I_{PLFAbacteria}/a * b$$

Where $a$ is the estimated contribution of the measured bacterial PLFA biomarkers to the total bacterial PLFA content (≈0.14, after Moodley et al., 2002), and $b$ is the contribution of carbon in bacterial PLFAs to the total bacterial carbon content (≈0.056 $gC_{PLFA}/gC_{bacteria}$, after Middelburg et al., 2000).
Similarly, label uptake by algae ($I_{diatom}$) was estimated as:

$$I_{diatom} = I_{PLFA20:5w3}/c * d$$

Where $c$ is the estimated contribution of carbon in PLFAs to the total microphytobenthic carbon content (≈0.035 $gC_{PLFA}/gC_{microphytobenthos}$, see Middelburg et al., 2002), and $d$ is the estimated contribution of the used diatom PLFA biomarker (20:5w3) to the total microphytobenthos PLFA population ([PLFA] 20:5w3/(total [PLFA] minus bacterial [PLFA])), ≈0.11. In order to compare $I_{foraminifera}$ with $I_{bacteria}$ and $I_{diatom}$ a dry density of 2.5 g sediment per $cm^3$ was used and a sediment porosity of 0.75% was assumed.

### 5.2.6 STATISTICS

The Chi-square test for Independence (Pearson) was performed to analyse the difference in species-specific size-class distribution, calculated as percentages of the total population, between treatments and recovery stages. The calculated p-values were used to designate the similarity between the observed frequency distributions. When frequencies of the test sizes are similarly distributed the p-value is 1. Size classes were grouped in those cases where frequency occurred below 1%.

### 5.2.7 LIMITATIONS OF THE EXPERIMENT

The experimental set-up has a limited number of sampling occasions. These sample occasions were first and foremost chosen to follow macrofaunal recovery and the relation between sediment biogeochemistry and macrofauna diversity and density (Rossi et al., 2009; Van Colen et al., 2012). In general, foraminifera respond faster than macrofauna. Hence, smaller time intervals between sample occasions would have been preferable to study foraminiferal dynamics. The chosen time intervals do reflect the different stages of ecosystem restoration.
There are no data available on the control patches of April and on glucose derived $^{13}C$ label incorporation in the recovering patches of April. The data collected in April were used to interpret the foraminiferal bicarbonate derived $^{13}C$ label incorporation and population dynamics succeeding hypoxia.

## 5.3 RESULTS

### 5.3.1 CONTROL PATCHES

Diatoms incorporated more bicarbonate and glucose derived $^{13}C$ label in the control patches of September than of June (Fig. 5.2a.). Bacteria showed a similar pattern for the incorporation of

bicarbonate derived $^{13}C$ label, whereas that for glucose was almost the same in both control patches (Fig. 5.2b.). The $^{13}C$ enrichment of (autotrophic) diatoms in the bicarbonate-treated sediments was interpreted as direct uptake of the labelled bicarbonate and, similarly $^{13}C$-enrichment in (heterotrophic) bacteria in glucose-treated sediments as direct uptake of labelled glucose. However, diatoms in all patches treated with labelled glucose also revealed enrichment in $^{13}C$. This unintended enrichment was discussed by Rossi et al. (2009) and interpreted as a direct uptake of glucose by diatoms some

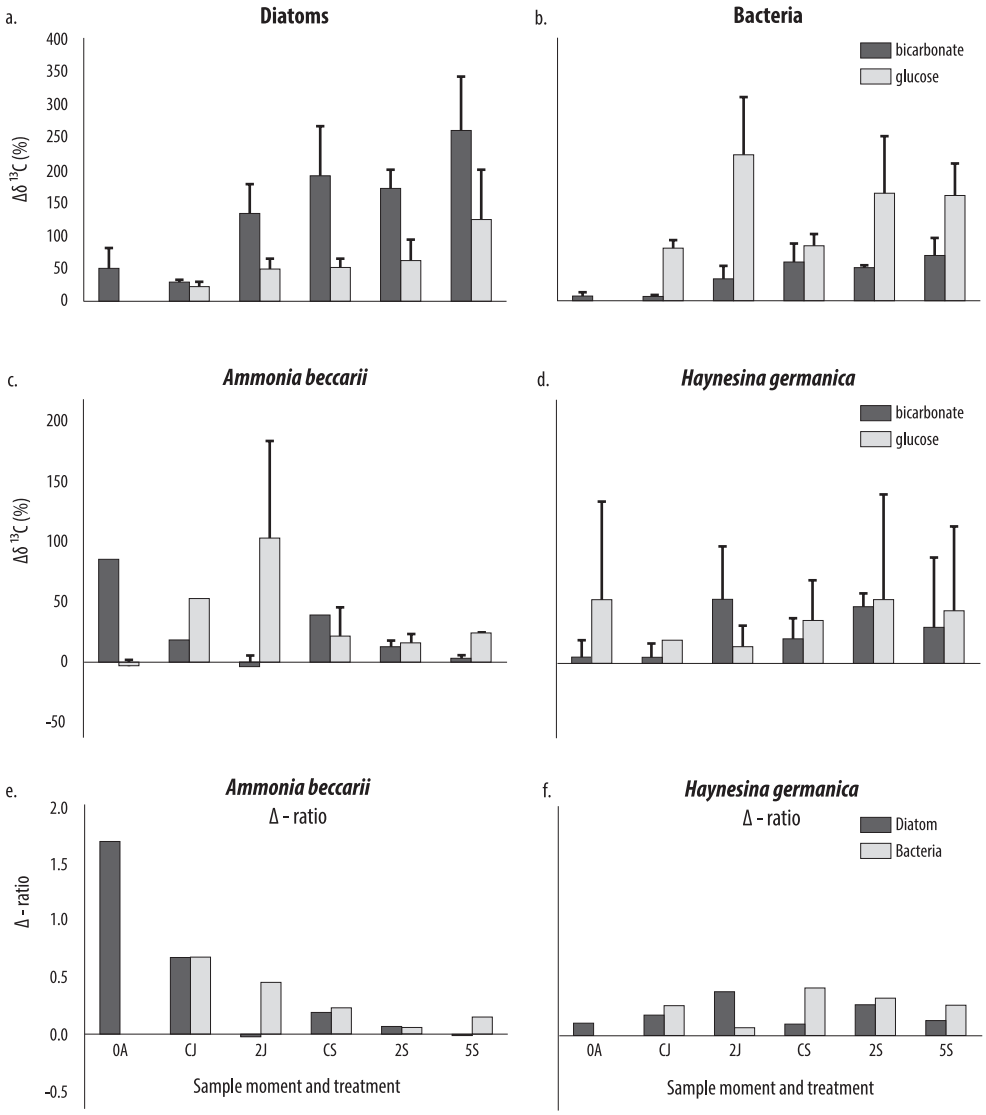



**Figure 5.2 a. - f.** $^{13}C$ Label incorporation; a.: in diatom specific PLFA; b.: in bacteria specific PLFAs; c.: in *Ammonia beccarii*; d.: in *Haynesina germanica*; e.: Δ-ratio i.e. the contribution of bacteria and diatoms to the diet of *Ammonia beccarii*; f.: Δ-ratio in *Haynesina germanica*. All $\Delta\delta^{13}C$ values are shown plus one standard deviation.

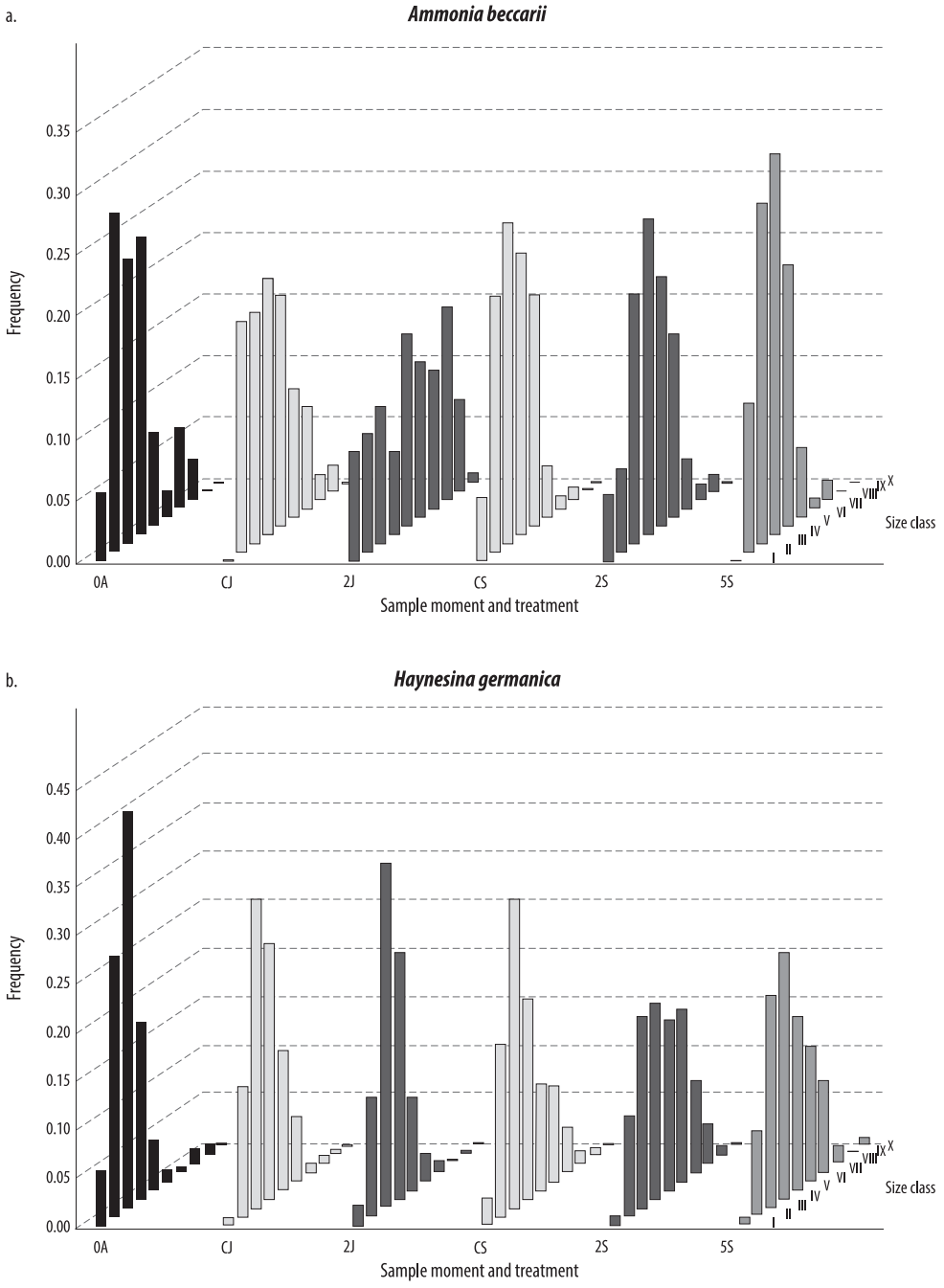



**Figure 5.3 a. - b.** Frequency distribution of the test size dimensions of a) *Ammonia beccarii* (56 µm per size class, starting at 63 µm) and b) *Haynesina germanica* (39 µm per size class, starting at 63 µm).

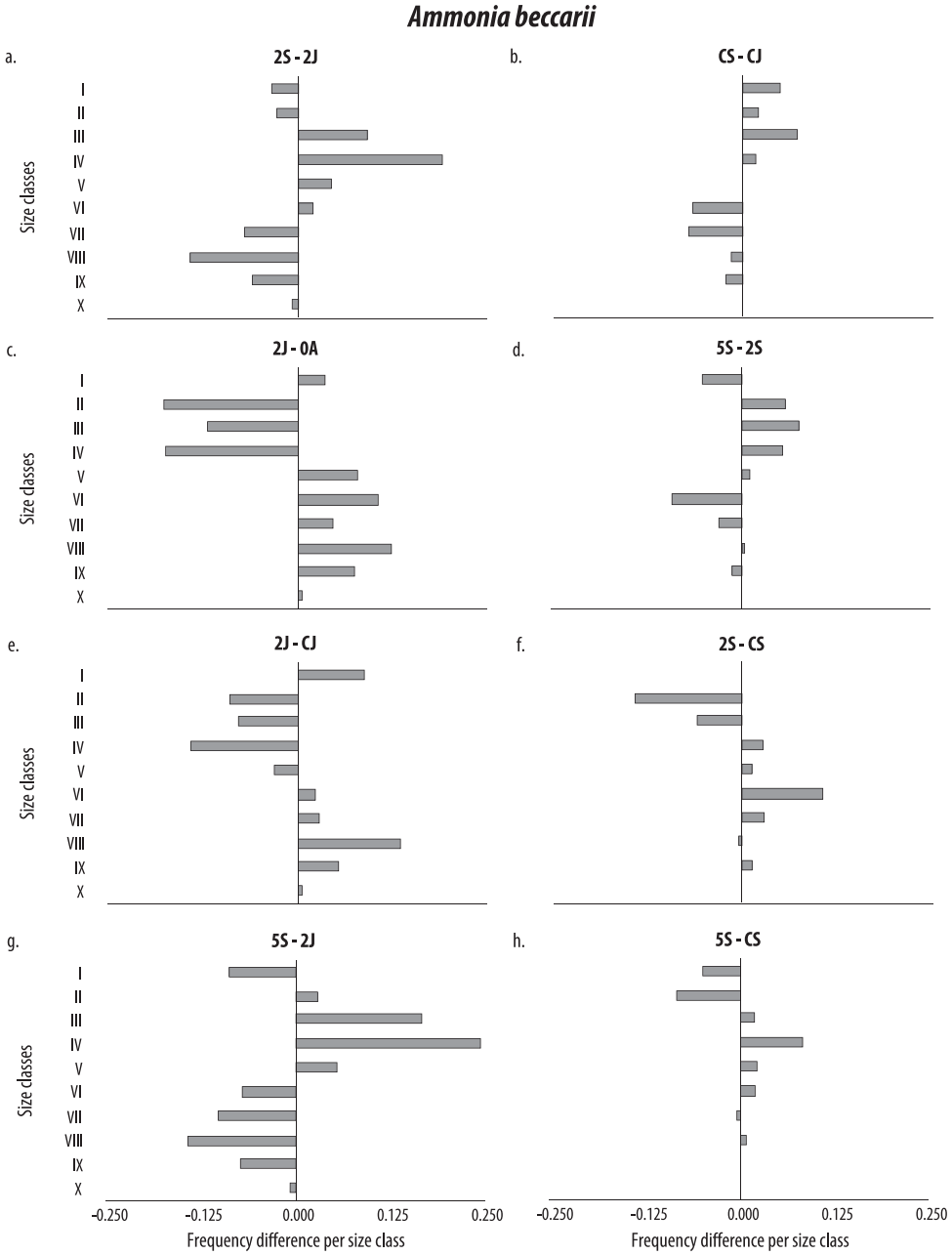



**Figure 5.4 a. - h.** Difference in frequency per size class (56 µm per class starting at 63 µm) of the test sizes of *Ammonia beccarii*; a., b., c. and d.: time effects (respectively 2S – 2J, CS – CJ, 2J – OA, and 5S – 2S); e., f., g. and h.: hypoxia treatment and timing/duration of system recovery effects (respectively 2J – CJ, 2S – CS, 5S – 2J and 5S – CS)

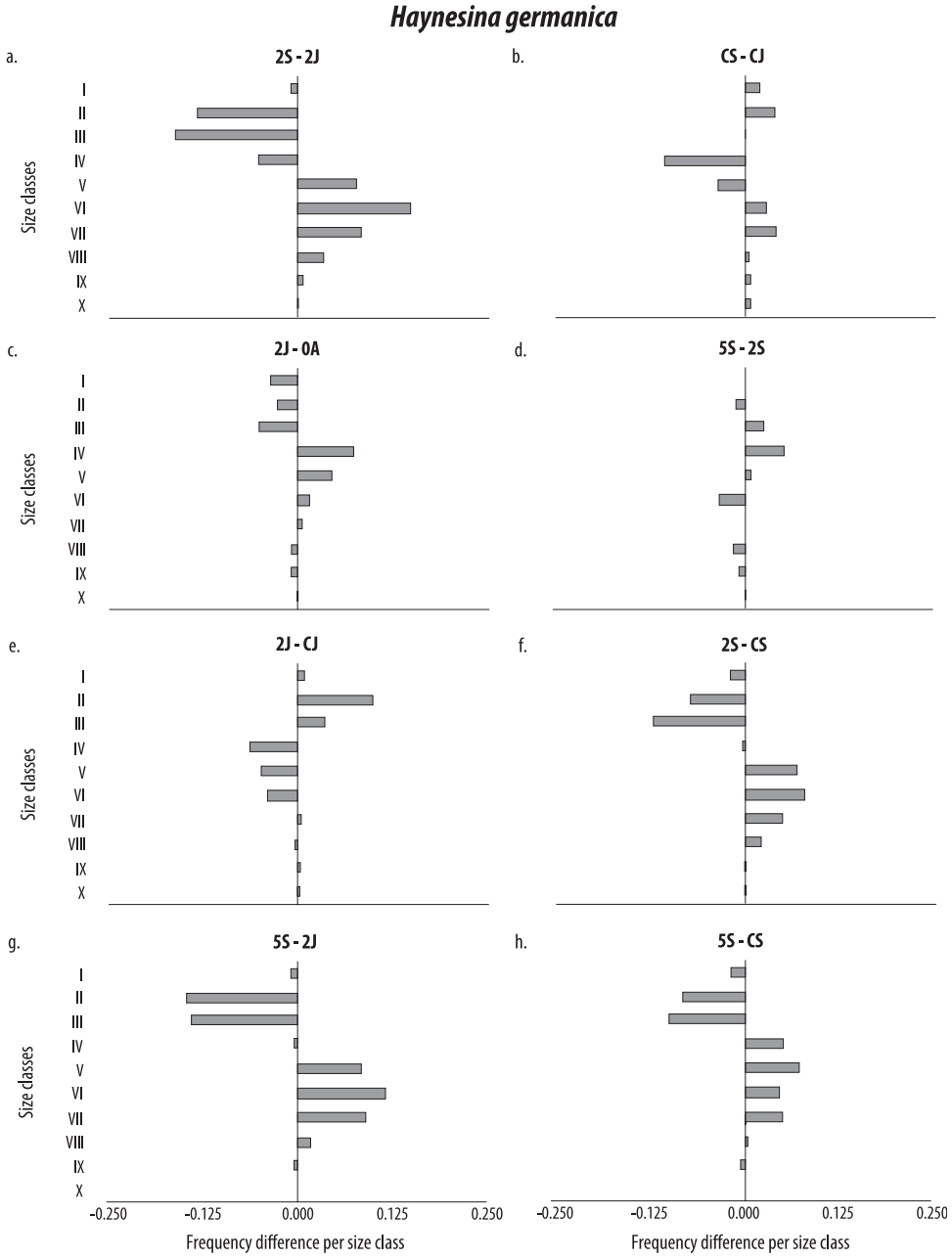



**Figure 5.5 a. - h.** Difference in frequency per size class (39 µm per class starting at 63 µm) of the test sizes of *Haynesina germanica*; a., b., c. and d.: time effects (respectively 2S – 2J, CS – CJ, 2J – OA, and 5S – 2S); e., f., g. and h.: hypoxia treatment and timing/duration of system recovery effects (respectively 2J – CJ, 2S – CS, 5S – 2J and 5S – CS)

of which are known for their capability to live heterotrophically in the absence of light. Also bacteria revealed an unexpected enrichment of $^{13}C$ in the patches treated with $^{13}C$ labelled bicarbonate. This enrichment is thought to reflect rapid bacterial consumption of alga-derived carbon enriched in $^{13}C$ label (Middelburg et al., 2000; Oakes et al., 2012). Both types of unintended, but unavoidable $^{13}C$ enrichments were considerably lower compared to the intended labelling.

The uptake of $^{13}C$-label by *Ammonia beccarii* in the control patches indicated a decrease in glucose derived $^{13}C$-label and an increase in bicarbonate derived $^{13}C$-label from June to September (Fig. 5.2c.). *Haynesina germanica* incorporated more bicarbonate and glucose derived $^{13}C$-label in September than in June (Fig. 5.2d.). The calculated Δ-ratios of *A. beccarii* and *H. germanica* in the control patches, based on label incorporation in foraminifers and microbes, indicated for *A. beccarii* a very similar food-uptake pattern in June and September (CJ vs. CS, Fig. 5.2e.). In the control patches of September the relative contribution of bacteria to the diet of *H. germanica* had increased compared to June (Fig. 5.2f.). During both sample occasions, the size distribution in the control patches was relatively similar for both species (CJ vs. CS, Fig. 5.3a., b. and Fig. 5.4b. and 5.5b.).
The next paragraphs present the development of the foraminiferal diets as well as size-distribution following from their population dynamics in relation to the timing of the hypoxic treatments and the duration of recovery. The last paragraph reports on the estimates of the amount of carbon transferred from microbes to foraminifera.

### 5.3.2 TIMING OF DISTURBANCE (2J vs. 2S)

The bacteria incorporated more glucose derived $^{13}C$-label in the patches that had recovered for two months after the winter- or spring hypoxia compared to the concurrent control patches (Fig 5.2b.). Analogously, for diatoms a distinct difference in the bicarbonate derived $^{13}C$-label was observed between the hypoxia-treated and the control patches of June (Fig. 5.2a). The timing of disturbance seemed to have differentially affected the diet of *Haynesina germanica* and *Ammonia beccarii* (Fig. 5.2c., d., e., f.). The Δ-ratio, for instance, suggested that *A. beccarii* fed predominantly on bacteria and *H. germanica* on diatoms, two months after the winter hypoxia, whereas the diet of both species was comparable two months after the spring hypoxia (Fig. 5.2e. and f.). In particular, both species consumed slightly more bacteria than diatoms two months after the spring hypoxia, though *A. beccarii* had a very low incorporation of label, implying reduced food uptake. In short, diets of both species differed more from the controls after the winter than after the spring hypoxia (Fig. 5.2a., b.).
The distribution of test sizes of *A. beccarii*, displayed in figure 5.3a. and 5.4, revealed that the disturbed patches contained a relatively high proportion of large specimens compared to the control; this difference is most pronounced after the winter hypoxia. This observation is confirmed by the Chi-square test; the p-value of 2J vs. CJ (2.24E-02) is smaller than the p-value of 2S vs. CS (4.96E-02) which in turn smaller than CJ vs. CS (7.80E-02, see Appendix 5.1). In contrast, the specimens of *H. germanica* were on average smaller in the disturbed patches of June compared to those in the control and the disturbed patches of September. These latter patches contained the highest proportion of large individuals (Fig 5.3b. and 5.5).

### 5.3.3 RECOVERY DEVELOPMENT (0A vs. 2J vs. 5S)

In April, *Ammonia beccarii* had a peak uptake of bicarbonate-derived label in the disturbed patches (Fig. 5.2c.); the bicarbonate-derived $\Delta\delta^{13}C$ value of *A. beccarii* exceeded bicarbonate-derived the $\Delta\delta^{13}C$ of the diatoms (Fig. 5.2a.). Two months later, *A. beccarii* fed predominantly on bacteria (Fig. 5.2e.). In September, *A. beccarii* revealed a low uptake of both glucose and bicarbonate-derived $^{13}C$-label in

all patches (Fig. 5.2c., e.), despite high concurrent levels of label incorporation in both diatoms and bacteria (Fig. 5.2a., b.). Contrastingly, after the winter hypoxia, *Haynesina germanica* fed on bacteria in April, and predominantly on diatoms in June (Fig. 5.2f.). Interestingly, the foraminiferal incorporation of label in the control patches did not indicate major diet shifts.
*A. beccarii* had, in the recovery patches after winter hypoxia, a relatively high proportion of large test sizes in June compared to April and September, whereas the specimens of *H. germanica* increased in size during ecosystem recovery (Fig. 5.3a., 5.4 and Fig 5.3b., 5.5). The distribution of the test size of both species in the control patches of June and September differed less than those between the hypoxia-treated patches in June and September (Appendix 5.1).

### 5.3.4 RECOVERY STAGES (2S vs. 5S)

Although label uptake by bacteria and diatoms was high in all patches sampled in September (Fig. 5.2a., b.), *Ammonia beccarii* showed a low uptake of label suggesting lower feeding activity (Fig. 5.2c., e.). In contrast, *Haynesina germanica* showed a more pronounced uptake of label in at least a part of the replicate samples (i.e., standard deviations were high) in all September patches (Fig. 5.2d., f.). On average in this time of year, bacteria seemed to contribute slightly more to the diet of *H. germanica* than diatoms, although a relatively high incorporation of likely alga-derived $^{13}C$ label was found in the patches treated with a spring hypoxia.
The Chi-square test indicated that the test-size distribution of *A. beccarii* differed among the September samples; the pairwise comparison of 2S versus CS gave a p-value of 0.05 and that of 5S vs. Cs and 2S vs. 5S a p-value of respectively 0.19 and 0.17 (see Appendix 5.1). Although the dissimilarity was relatively small, the species from sediments that suffered spring hypoxia were proportionately larger than the winter hypoxia ones, and the control samples contained a higher proportion of smaller specimens than the hypoxia-treated sediments. September populations of *H. germanica* displayed relatively large specimens in the hypoxia-disturbed patches and a high similarity in the size distribution among the patches treated with winter or spring hypoxia (Fig. 5.5d., see Appendix 5.1).

### 5.3.5 CARBON TRANSFER FROM MICROBES TO FORAMINIFERA

*Ammonia beccarii* was estimated to have consumed on average 3.0% of the total bacterial biomass and *Haynesina germanica* 2.9% (Table 5.1). The consumption of diatoms by *A. beccarii* and *H. germanica* was estimated at respectively 0.6% and 0.8% of the total diatom biomass (Table 5.1). Interestingly, despite the high similarity between the mean bacterial and diatom uptake of *A. beccarii* and *H. germanica*, large differences were found among treatments and sample moments within and between populations of both foraminiferal species. The estimated carbon transfer from bacteria to *A. beccarii* was much higher

**Table 5.1** Carbon transfer in % of diatom-specific PLFA and bacterial-specific PLFA's to *Ammonia beccarii* and *Haynesina germanica*

| Sample moment * Treatment | *Ammonia beccarii* / Diatom | *Ammonia beccarii* / Bacteria | *Haynesina germanica* / Diatom | *Haynesina germanica* / Bacteria |
|---|---|---|---|---|
| CJ | 3.13 | 5.01 | 1.89 | 3.20 |
| 2J | -0.20 | 7.43 | 0.84 | 0.39 |
| CS | 0.13 | 0.84 | 0.15 | 3.14 |
| 2S | 0.05 | 0.46 | 0.69 | 4.65 |
| 5S | 0.01 | 1.16 | 0.22 | 3.33 |
| Average | 0.63 | 2.98 | 0.76 | 2.94 |

in June compared to September (Table 5.1). There was a distinct deviation between its contribution to the transfer of the diatom- and bacterial-derived carbon among the recovery and disturbed patches of June; no significant amount of diatom-derived carbon seemed to have been used up by this species in the recovery patches. *H. germanica* predominantly contributed to the transfer of bacterial-derived carbon, except for the control patches of June where it also took up a substantial amount of the diatom-derived carbon (Table 5.1).

## 5.4 DISCUSSION

Label incorporation and test-size distributions of *Ammonia beccarii* and *Haynesina germanica* differed among recovery stages and between hypoxia-affected and unaffected control sediments. In the following paragraphs foraminiferal diet shifts and population dynamics will be interpreted and set in the context of ecosystem recovery and timing of disturbance.

### 5.4.1 FORAMINIFERAL DIET SHIFTS AND POPULATION DYNAMICS

Similarly to the earlier mentioned unintended labelling of diatoms, direct uptake of $^{13}$C-labelled glucose by foraminifera may have occurred in our experiments since least some foraminifera are probably capable to directly use dissolved organic matter. In an *in situ* experiment with large arborescent agglutinated foraminifera from Antarctica, DeLaca et al. (1981) could demonstrate the uptake of glucose. The two species that have been shown to utilise dissolved organic carbon (DOC) live in an exceptionally oligotrophic environment in shallow waters below sea ice in which primary productivity is very low and restricted to one or two months a year (DeLaca et al., 1981). It is likely that the capability of using DOC is a specialised adaption to survive the long yearly periods lacking production of particulate organic matter. Moreover, uptake was a function of cell surface, requiring a large membrane surface area, in this particular case the 'root system' of these 2-cm long dendriform foraminifera. Although we cannot rule out some direct foraminiferal uptake of labelled glucose in our experiments – potentially leading to overestimated importance of bacteria as food source – our variable label uptake results do not suggest a strong influence of direct glucose consumption. The observed relatively high dissimilarity in label uptake by foraminifera among replicate samples was previously noticed by Moodley et al. (2002); it is presumably attributable to dissimilarities in individual feeding activity among the specimens.

Notwithstanding these uncertainties, the Δ-ratio revealed foraminiferal diet shifts and the distribution of the maximum test-size dimension indicated shifts in the population structure of *Ammonia beccarii* and *Haynesina germanica*, especially during ecosystem recovery. In April, a high enrichment in $^{13}$C was measured in *A. beccarii* suggesting that this species profited from the development of a dense mat of benthic diatoms during the first period of ecosystem recovery. The Δ-ratio was above 100%; suggesting selective feeding by *A. beccarii* on autotrophs with an above average enrichment in $^{13}$C within the group of algae (i.e. with a higher growth rate). Comparing label incorporation in the dinoflagellate PLFA 22:6w3 with that in diatom PLFA 20:5w3 could not corroborate this hypothesis. The clear uptake of algal carbon by *A. beccarii* is in agreements with previous reports (e.g. Moodley et al., 2000; Pascal et al., 2008). Pascal et al. (2008) suggested that *Ammonia tepida* (likely the same species as *A. beccarii* in this paper) was mainly dependent on algal resources. As their experiment was conducted in March (2006), these results are in agreement with ours. In our experiment, the high proportion of large test sizes in the recovery patches of June may reflect individual growth of *A. beccarii* in response this amply available food source, leaving relatively low numbers of intermediately-sized individuals. Concurrently, high numbers of small specimens may represent stable reproduction rates, similar to

those that sustained the relatively high numbers of smaller foraminifera in the control samples. By June, *A. beccarii* had shifted from grazing on diatoms towards consumption of bacteria. *A. beccarii* ingesting bacteria has been previously reported by e.g. Chandler (1989); Langezaal et al., (2005); Pascal et al., (2008); Mojtahid et al., (2011). Muller and Lee (1969) discussed that the consumption of bacteria may stimulate foraminiferal fecundity and reproduction. The observed diet shift of *A. beccarii* towards bacteria during reproduction could corroborate a possible importance of bacteria or bacterial DNA for reproduction. In the winter hypoxia-disturbed patches of September (5S, Fig. 5.3a.), the distribution of the test-size dimension indicated a relatively high proportion as well as absolute abundance of medium-sized specimens of *A. beccarii*. The low $^{13}C$ enrichment of these September foraminifera may indicate that *A. beccarii* became relatively inactive.

Stress, induced by the hypoxic treatment possibly triggered reproduction of *Haynesina germanica*. Two weeks after the hypoxic treatment we observed high numbers of relatively small sizes specimens in comparison to subsequent sample moments. Towards June, after two months of ecosystem recovery, the density of *H. germanica* had declined by approximately 70% (chapter 4). The distribution of the test-size dimensions of this species revealed slightly larger test sizes in the recovery patches of June compared to April, although they were small in contrast to the specimens in the control patches. The strong decline in abundance may be related to for instance high mortality rates, downwards migration induced after re-establishment of oxygen pore-water concentrations and increased predation pressure by nematodes and small sized macrofauna that abundantly colonised the disturbed patches (van Colen et al., 2012). Despite the high fluctuations in abundance, the enrichment in $^{13}C$ measured in *H. germanica* indicated smaller fluctuations in food uptake and diets shifts compared to *A. beccarii*. Although the amply available diatoms (Rossi et al., 2009) contributed more to the diet of *H. germanica* in the disturbed patches of June, bacteria seemed to dominate the diet of this species in all other patches. In contrast to *A. beccarii*, this species did not seem to profit from the high availability of benthic algae during the first months of ecosystem recovery in terms of reproduction or growth reflected in the test-size distribution of the populations. The absence of a clear response of *H. germanica* to high algal densities has previously been reported (Moodley et al., 2000). Feeding on bacteria may be preferential for *H. germanica*; however – although diatom biomasses were high in April – perchance (the larger) *A. beccarii* was better enabled to respond fast to the diatom bloom arising in the period directly after the winter-hypoxia had ended. Moodley et al. (2000) suggested that differential responses between *A. beccarii* and *H. germanica* might indicate resource partitioning. The observed opposite shift in diet between *A. beccarii* and *H. germanica* is in line with this hypothesis, but it would require that preferred food availability was low enough to be limiting population sizes. Given the wealth of high quality food due to a lack of grazers in the early recovery phase after the hypoxia (Van Colen et al., 2008) this does not seem to be a very likely scenario. However, if it were true, the in recovery patches of September observed growth of the test sizes may indicate that *H. germanica* profited from reduced competition for food with the in September less active *A. beccarii*. Notably, in both recovery and control patches, the specimens of *H. germanica* increased in size predominantly in the period from June to September whereas specimens of *A. beccarii* became larger in the period from April to June.

### 5.4.2 IMPACT OF BIOLOGICAL INTERACTIONS ON FORAMINIFERA

*Haynesina germanica* responded differently – often even inversely compared to *Ammonia beccarii* – to the hypoxic treatment with respect to abundance, test-size distribution and food-consumption patterns. Competition between these species as well as a differential impact of ecosystem properties

presumably provoked these opposite responses. As suggested by Duijnstee et al. (2005) some foraminiferal taxa change their life-history strategy during times of stress and disturbance, whereas other may not. This may produce differential population-level responses to the same set of changing environmental conditions.

The hypoxic treatments and the succeeding recovery impacted various aspects of the estuarine ecosystem. The exposure to hypoxic conditions resulted in a mass mortality of all macrobenthic species (Van Colen et al., 2008, 2012; Rossi et al. 2009). The absence of macrofaunal grazers during the first period of ecosystem recovery facilitated the development of a benthic algal mat exploited by especially *Ammonia beccarii*. During ecosystem recovery, macrofaunal recolonisation developed via stages that differed in species composition, total abundance and biomass of the assemblage. The first stage (April to June) was characterised by an abundance increase of predominantly small-sized macrofaunal species and in the sequel stage (June to September) these numerous small-sized specimens were replaced by fewer, but larger individuals (Rossi et al., 2009).

In contrast to foraminifera, nematode abundance sharply declined due to the hypoxic perturbation (Van Colen et al., 2009, 2012). As suggested by Moodley et al. (1997), nematodes and foraminifera may compete for food. Therefore, the hypoxia-induced lowering of the nematode abundance may have been beneficial to the stress tolerant foraminiferal species *A. beccarii*. Succeeding the hypoxia-induced decline in abundance, nematode numbers recovered fast to control levels (within 56 days) and peaked three months after the onset of recovery (Van Colen et al., 2009). The high increase in nematode abundance and the concurrent arrival of numerous small-sized macrofaunal specimens during the first period of recovery, may have contributed to the sharp decline observed in densities of *H. germanica* (chapter 4). The difference between both foraminiferal species in maximum and mean test sizes, with smaller test sizes for *H. germanica* than for *A. beccarii*, may have resulted in a higher predation pressure of, for instance, (meiofaunal) nematodes and (small-sized) macrofauna on *H. germanica* compared to that on *A. beccarii*.

### 5.4.3 SEASONAL EFFECT OF HYPOXIA

As mentioned earlier, the foraminiferal population structure and food consumption patterns of both foraminiferal species indicated that the populations in the patches that recovered for 2 months after the spring hypoxia (2S) were more similar to those left to recover for 5 months (5S, i.e. those sampled in September) than to those that recovered for 2 months after the winter hypoxia and sampled in June (2J). As reported for macrofaunal abundance, biomass, and species diversity (Rossi et al., 2009) our results suggest that for the foraminiferal diet and population structure timing of sampling during recovery after disturbance (i.e. when in the seasonal cycle) might be of greater importance than being allowed to recover for a period of two or five months. Besides biotic interactions as food web dynamics (e.g. algal spring bloom, a dissimilar response of macrofauna to the winter- and spring-hypoxia due to for instance larval availability, predation pressure and competition for food), also seasonal dynamics in abiotic ecosystem properties may have influenced the foraminifera.

Temperature has been reported to impact in foraminiferal growth, feeding and reproduction (Bradshaw 1957, Pascal et al., 2008). The optimum temperature for growth and reproduction of *Ammonia* is 25 to 30 ˚C and the optimum of bacterial uptake by *Ammonia* is recorded at 30 ˚C (Bradshaw 1957). The generally low temperature during winter (below 10˚C *Ammonia* fails to grow, Bradshaw 1957, and below 5˚C bacteria uptake by *Ammonia* stops, Pascal et al. 2008) has been suggested to limit growth and reproduction, while high summer temperatures may provoke foraminiferal mortality (Pascal et al. 2008). The effect of hypoxia disturbance may thus depend on its timing, and may therefore cause seasonal temperature stress, affecting growth and reproduction of *Ammonia* (and possibly other

foraminiferal species), and which may impact the resilience of foraminiferal populations to disturbance. Generally, however, hypoxia occurs in summer, when foraminifera (and other organisms) reproduce.

### 5.4.4 CARBON FLOW

Foraminiferal grazing on bacteria was estimated at 3.0% for *Ammonia beccarii* and 2.9% for *Haynesina germanica*. We estimated that on average 0.6% of the diatom biomass was eaten by *A. beccarii* and 0.8% by *H. germanica*. Despite the similarity in the mean contribution of *A. beccarii* and *H. germanica* to the microbial transfer, grazing on microbes differed per sample moment and treatment. The contribution of foraminifers to the transfer of carbon rests on several properties as, for instance, the microbial biomass and the percentage of microbes enriched in $^{13}C$, the total foraminiferal biomass, interspecific competition for food among foraminifera and other faunal species, etc.. The combined effect of these separate properties determines the estimated importance of foraminifera in the transfer of microbial carbon. These properties fluctuate not only seasonally in the same patches (as in our experiment), but they are also highly variable among environmental settings. Moodley et al. (2000) estimated that *Ammonia* ingested 1 to 7% of the green algae that were added in their study within 3 to 53 hours. If the amount of available algal material per foraminifer in their study does not exceed the available algal biomass per foraminifer in our experiment, and if there is no difference in food preference between Moodley et al. 's green algae and our microphytobenthos then this might imply that the role of foraminifera in using up microphytobenthos resources was somewhat greater in our experiment than in that of Moodley et al.. What further complicates this comparison, though, is that Moodley et al. (2000) added pre-cultured, already labelled, freeze-dried green algae whereas we labelled the ambient environment of the community of benthic algae *in situ*. Moreover, selective feeding of *Ammonia* within the benthic algal community was indicated by Δ-ratio above 100%.

Oakes et al. (2012) investigated microphytobenthos-derived carbon transfer in Australian subtropical subtidal sandy sediments by adding $NaH^{13}CO_3$ to label the DIC pool in the water column. Sediments were sampled at 6 times in a period from 3 to 33 days after the addition of label. At their study site, foraminifera dominated the meiobenthic community and macrofaunal species were scarce. Three species dominated the foraminiferal community, *Cellanthus craticulatis*, *Ammonia beccarii* (potentially a different, yet closely related species from ours) and *Elphidium advenum*. Especially *C. craticulatis*, the biomass of which peaked at 3.8% of the total organic carbon biomass, accounted for up to 31% of the $^{13}C$ within the sediment. *Ammonia beccarii* and *Elphidium advenum* represented respectively 0.2% and 0.1% of the total organic carbon biomass; their contribution to the $^{13}C$ in the sediment corresponded to their contribution to the organic carbon biomass. The high dissimilarity among the contribution of the benthic foraminiferal species to the transfer of carbon (relative to their biomass) was suggested to be attributable to functional chloroplasts in *C. craticulatis* and the absence of these chloroplasts in the other foraminifera. The relative role of *Ammonia beccarii* and *Elphidium advenum* in the consumption of the total microphytobenthos standing stock roughly corresponded to our estimates of the contribution of *Ammonia beccarii* (consumed on average 0.6% of the available diatoms) and *Haynesina germanica* (consumed on average 0.8% of the available diatoms) to the diatom derived carbon transfer by foraminifera.

## 5.5 CONCLUSION

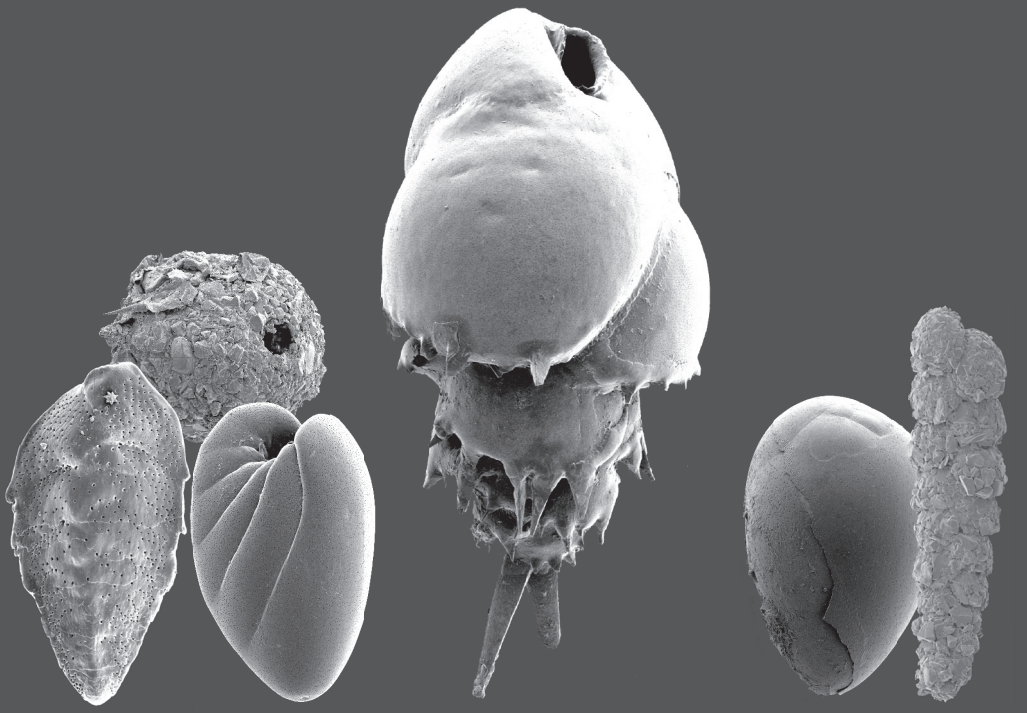
With regard to their diet and population dynamics, the dominant foraminiferal species *Ammonia beccarii* and *Haynesina germanica* responded differentially and generally inversely to progressive stages of ecosystem recovery succeeding hypoxia. The Δ-ratio values strongly suggest that the development of a dense mat of benthic algae during the first month of ecosystem recovery after the

winter hypoxia was likely profitable to *A. beccarii*. This food pulse may have stimulated reproduction as well as growth as indicated by two distinctly visible cohorts in the test-size distribution after two months: a relative high proportion of small and large sized specimens in the recovery patches of June compared to controls. Enhanced reproduction itself was strongly linked to the subsequent dietary shift to bacteria. The distribution of the test dimensions of *H. germanica* indicated that this species had less fluctuation in population structure during ecosystem recovery but possibly reproduced in response to the induced winter hypoxia. Also its inferred dietary composition fluctuated markedly less than that of *A. beccarii*. Bacteria seemed to contribute slightly, but almost consistently more to the diet of *H. germanica* than diatoms. The timing of sampling after disturbance seemed to be a more important factor to the foraminiferal dietary and population structure patterns than whether the duration of recovery from the hypoxia had been 2 or 5 months.

## APPENDIX

| Sample moment * Treatment | *Ammonia beccarii* | | | *Haynesina germanica* | | |
|---|---|---|---|---|---|---|
| | Chi2 | df | p | Chi2 | df | p |
| 0A vs 2J | 53.6 | 7 | 2.83 E-09 | 34 | 6 | 6.81 E-06 |
| 2J vs 5S | 70.8 | 5 | 6.86 E-14 | 129.2 | 5 | 3.54 E-26 |
| 2J vs CJ | 16.3 | 7 | 2.24 E-02 | 30.5 | 5 | 1.17 E-05 |
| 5S vs CS | 7.4 | 5 | 1.93 E-01 | 50.4 | 6 | 3.92 E-09 |
| 2S vs CS | 14.1 | 7 | 4.96 E-02 | 61.1 | 6 | 2.73 E-11 |
| 2J vs 2S | 33.4 | 8 | 5.22 E-05 | 161.1 | 6 | 3.55 E-32 |
| CJ vs CS | 9.9 | 5 | 7.80 E-02 | 37.8 | 5 | 4.08 E-07 |
| 2S vs 5S | 7.8 | 5 | 1.65 E-01 | 12.9 | 6 | 4.42 E-02 |

**Appendix 5.1** Chi-Square test-derived p-values comparing pairwise test size distributions of *Ammonia beccarii* and *Haynesina germanica* among treatments and duration of recovery



CHAPTER 6

# IMPACT OF BIOIRRIGATION ON THE VERTICAL DISTRIBUTION OF BENTHIC FORAMINIFERA FROM THE SWEDISH GULLMAR FJORD

G.M. Brouwer, I.A.P. Duijnstee, A.B. Craun and M. Wolthers

ABSTRACT

Marine benthic ecosystem engineers modify their environment by particle reworking and ventilating the sediment. Besides physical mixing of particles, these activities enrich the usual anoxic deeper sediment layers in both oxygen and food. This increased sediment heterogeneity has been reported to extend habitats or even provide new niches for benthic meiofauna such as foraminifera. Apart from these positive effects of ecosystem engineers on meiofauna, negative effects such as physical disturbance and increased predation pressure have been described. Ruling out these negative effects, we conducted a laboratory microcosm experiment using artificial gas-permeable vertical burrows. Density, assemblage composition and the vertical distribution of benthic foraminifera in these microcosms were studied. By mimicking only the geochemical aspects of a bioturbation-derived increase in sediment heterogeneity, we aimed to contribute to disentangling the effects of various processes that instigate the dynamic relation between macrofauna, local environmental conditions and foraminifera. The lack of differences in food availability, $O_2$ penetration depth and dissolved nitrate between the experiment and control core was reflected by the absence of an overall strong positive effect of the artificial burrows on foraminiferal abundances and species diversity. Despite the fact that the dissimilarity in pore-water chemistry was restricted to the visually observed reddish-brown colour of the sediment surrounding the burrows, and the co-occurrence of elevated dissolved $Fe^{2+}$ concentrations – interpreted as Fe micro-cycling observed in pore-water near the burrows – the foraminifera derived from the Swedish Gullmar Fjord responded species-specifically and differential in the experimental cores with and without burrows. Interestingly, the presumed impact of the burrows and the observed foraminiferal dynamics corresponded to the reported patterns of the dominant species during specific field conditions.

KEYWORDS

Benthic foraminifera * Ecosystem engineering * Sediment heterogeneity * Artificial burrows * Bioirrigation * Pore-water chemistry * Swedish Gullmar Fjord

## 6.1 INTRODUCTION

Ecosystem engineers are organisms that physically or chemically modify their environment thus creating and maintaining habitats suitable for a specific suit of associated organisms (e.g. Jones et al., 1994; Wright and Jones, 2006). Ecosystem engineers play a prominent role in structuring communities as they determine important aspects of the functioning of ecosystems. Large burrowing animals act as benthic ecosystem engineers in marine coastal areas, in lakes and in soils (Jones et al., 1994; Meysman et al., 2006a, b). In a meta-analysis, Olafsson (2003) compared the results of 77 studies on the structuring effect of 43 macrofaunal species on meiofauna. In general, the studies indicated that biogenic (macrofaunal) structures enhance species diversity. By constructing tubes (bioturbation) and ventilating burrows (bioirrigation), macrofaunal species create heterogeneity in their environment and enlarge the sediment-water interface; oxygen and organic matter are transported deeper into the sediment (e.g. Aller, 1983; Meysman et al., 2005). This increased sediment heterogeneity has been reported to extend niches or even generate extra niches suitable for other organisms (Reise, 1981), such as foraminifera (Bouchet et al., 2009).

Macrofaunal activities that enlarge the sediment-water interface and deepen the oxygen-penetration depth may deepen the average and maximum living depth of benthic foraminifera (Bouchet et al., 2009). Although foraminifera are in general most densely present in the upper sediment layer (review in Alve and Murray, 2001), the vertical distribution of benthic foraminifera in the sediment is far from static and differences between taxa are great. Oxygen concentration and food availability have been acknowledged as main resources structuring this vertical distribution (e.g. Linke and Lutze, 1993; Jorissen et al., 1995; Van der Zwaan et al., 1999). Foraminifera actively migrate in response to changes in the concentration of dissolved oxygen (Duijnstee et al., 2003; Geslin et al., 2004) and also migration to macrofaunally mediated habitats has been observed (Linke and Lutze, 1993; Alve and Bernhard, 1995). In contrast to this reported positive influence, other studies revealed an overall negative impact of macrofaunal presence on meiofaunal densities (e.g. Buzas 1978; Jones et al., 1997; Olafsson, 2003). Mechanisms that instigate this negative influence are for example macrofaunal predation of meiofauna, competition for food among macro- and meiofauna, or physical disturbance of meiofaunal habitats due to macrofaunal sediment reworking (Olafsson, 2003). Buzas (1978) reported higher foraminiferal densities inside a cage excluding macrofauna compared to surrounding sediment. Various macrofaunal species outside these cages had foraminifera in their gut, leading to the conclusion that the lower foraminiferal densities outside the cage were the result of macrofaunal predation on foraminifera. Obviously, the net impact of macrofauna on the density and vertical distribution of foraminifera depends on the magnitude of both positive and negative interactions (e.g. Lohrer et al., 2004).

Compared to meiofauna, marine benthic macrofaunal species are relatively sensitive to disturbance such as hypoxia (e.g. Josefson and Widbom, 1988). Hypoxia – low oxygen concentration in the bottom-water – is reported to increasingly occur in coastal areas (Diaz et al., 2009). When the concentration of dissolved-oxygen in the overlaying-water falls below the hypoxic border of 2 mL/L, mobile fauna tries to avoid these harmful conditions by migrating to other areas. Less mobile and sessile fauna are not able to escape hypoxic conditions. In first instance they respond by lowering their oxygen demand (Wu, 2002), i.e., they become less active. Macrofaunal ecosystem engineering species abandon their burrows and display aberrant behaviour (Diaz and Rosenberg, 2008). When disturbance continues or intensifies, or when the interval between disturbance events shortens, hypoxia may affect growth and reproduction and eventually result in mass mortality of macrobenthic marine organisms (Wu, 2002;

Diaz and Rosenberg, 2008). Concurrent with the disappearance of ecosystem engineers, the niches they provide are no longer maintained (Coleman and Wiliams, 2002).

Since the abundance, vertical distribution and species diversity of the foraminiferal community depends on a dynamic interplay of biotic and abiotic factors within coastal ecosystems, foraminifera may be useful as bioindicators for ecosystem functioning. Studying foraminiferal dynamics to indicate ecosystem status may help to monitor and protect coastal areas for instance in the case of hypoxic disturbance, and may contribute to decide on managerial actions to restore affected systems. The value of foraminifera as proxies for ecosystem status is increasingly acknowledged (e.g. Bouchet et al., 2012; Schönfeld et al., 2012). Several characteristics contribute to this applicability of foraminifera as bioindicators: foraminifera occur worldwide, are densely present in most marine sediments and occupy an intermediate ecological position inbetween macrofauna and microbes. A general short life span enables foraminifera to respond rapidly to environmental change, reflected in alterations in both density and assemblage composition, preserved in the record of decay-resistant shells (tests) that they produce. The high fossilisation potential of their tests enables us to collect extensive data covering even long time-scales. Nonetheless, to improve the accuracy of foraminiferal species as indicators for ecosystem status in both healthy and disturbed areas, a thorough understanding is needed of the species-specific response of foraminifera to changes in the sediment that arises from the dynamic interaction among biotic and abiotic factors within the ecosystem.

The main aim of the experiment was to study the impact of geochemical aspects of biogenic structures (i.e., vertical burrows) on foraminiferal species, their vertical distribution in the sediment and contribution to the assemblage composition. In order to do so, we isolated effects of enhanced oxygen-penetration and bioirrigation by excluding the physical and biological interactions with burrowing macrofauna (such as sediment reworking, predation and competition for food). To investigate the effect of extending excursions of the sediment-water interface into the sediment – via oxygen diffusion and transport within biogenic structures – artificial burrows were introduced in microcosms containing sediments collected from the Swedish Gullmar Fjord. Subsequent changes in the vertical distribution of the foraminiferal assemblage were analysed.

## 6.2 MATERIALS AND METHODS

### 6.2.1 GULLMAR FJORD

The sediments used in this experiment were collected in the Swedish Gullmar Fjord. This fjord is a narrow, 1 to 2 km wide and 28 km long, silled basin with a maximum basin depth of 120 m located on the Swedish West coast. With high sedimentation rates and marginal tidal movement, the fjord is ideally suited to study foraminiferal assemblages recorded in the sediment archive (e.g. Josefson and Widbom, 1988; Nordberg et al., 2000; Gustafsson and Nordberg, 2001; Filipsson and Nordberg 2004; Polovodova Asteman and Nordberg, 2013). The local foraminiferal assemblage underwent several changes during the last century, mainly due to fluctuations in the bottom-water oxygenation beyond the seasonal variations (e.g. Polovodova Asteman and Nordberg, 2013).

### 6.2.2 PREPARATION OF EXPERIMENTAL MICROCOSM CORES

On May 28$^{th}$, 2009 sediments were taken from several locations in the Gullmar Fjord using a boxcorer. The collected sediment cores were transported in coolboxes to The Netherlands within 1 day after collection, where they were stored in a dark climate room at a constant temperature of 11˚C. Algae

suspended in saline (Gullmar Fjord) water were added to all cores on the following dates: June 25th, July 9th and 23rd and October 15th, 2009. At the end of October 2009, 10 sediment cores (Ø 7 cm) were divided in 5 horizontal layers (0-1 cm, 1-2 cm, 2-4 cm, 4-8 cm and 8-12 cm sediment depth) which were sieved over a 0.5 mm screen to remove macrofauna. Despite this procedure, a very small juvenile specimen of the macrofaunal species *Scalibregma inflata* must have passed the screen. This juvenile polychaete grew alongside the wall of the experimental core (sampled as B4) and lived at 6 – 7 cm sediment depth (see Appendix 6.1). Following sieving, the 5 sediment layers were stratigraphically reassembled by transferring sediment into microcosm cores using a large syringe, starting with 4 cm of the deepest sediment type, followed by 4 cm of the second deepest, and respectively 2, 1 and 1 cm of the 3 consecutively shallower sediment types, thus returning sediments to their original depth interval (respectively 8-12, 4-8, 2-4, 2-1 and 0-1 cm sediment depth). To minimise dissimilarities among experimental cores, the combined material from each of the initial sediment layers was homogenised prior to restoring the vertical order in the microcosms. The cores were left to settle and compact for a month. The final vertical structure was somewhat compressed compared to that in the field. After this month, the continuous vertical distribution of foraminiferal populations – in contrast to the discontinuous distributions associated with the discrete reassembled sediment intervals – was assumed to have re-established.

Two treatments were induced after the incubation period; each on 5 experimental sediment cores (Ø 7 cm per core, see Fig. 6.1). Half of the experimental cores were used as control; they did not receive any further treatment (cores are referred to as C). The other 5 experimental cores (cores are referred to as B) were used to mimic the effect of ventilated macrofaunal burrows, thus focusing on the geochemical

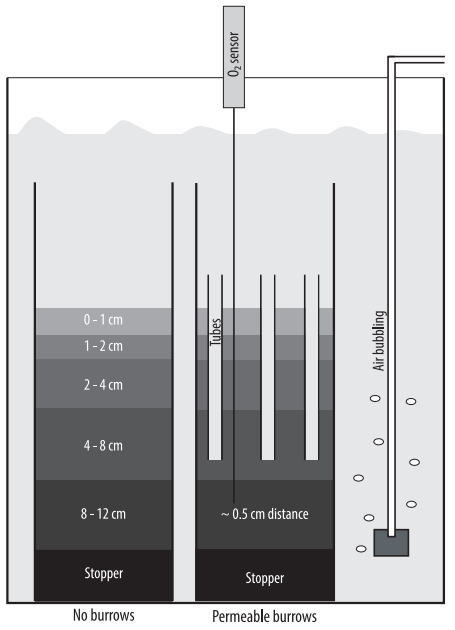



**Figure 6.1** Experimental set-up with two types of experimental cores (with permeable burrows (B) and without permeable (C)) in an aquarium filled with natural Gullmar Fjord water. Oxygen profiles were measured at an approximate distance of ~0.5 cm from the burrows, and in the centre of the cores. Aquarium pumps were used to ventilate the water.

aeration effects of burrows, rather than the physical mixing aspects of bioturbation. In each of these cores we vertically inserted three artificial burrows (corresponding to a density of ~ 800 burrows per $m^2$). These burrows were permeable tubes made of partly fused polyethylene particles (10 to 50 µm grain size). These tubes had an external diameter of 10 mm and an internal diameter of 6 mm. The artificial permeable burrows where 8 cm long and vertically inserted in the sediment: the lower end of the burrow reached into the deepest sampled sediment layer while the upper end was positioned slightly above the sediment-water interface. During insertion a stainless steel rod inside the tube prevented infill of sediment. The rod was removed very slowly after the burrow was positioned in the sediment. All cores were placed in an aquarium filled with natural waters derived from the Gullmar Fjord. Aquarium pumps were used to ventilate the water. The experiment started on December 8th, 2009.

### 6.2.3 SEDIMENT SAMPLING

After one and a half month (20th of January, 2010), we analysed the chemical composition of the sediment pore-water. We measured the concentration of $O_2$, $NO_3^-$, $NH_4^+$, $Mn^{2+}$ and $Fe^{2+}$ in the pore-water with depth in both types of sediment cores. For the oxygen measurements we used a calibrated OX-50 Unisense micro-electrode attached to a micromanipulator to obtain measurements at half a millimetre intervals. After oxygen measurements, the core was inserted into an Ar-filled glove box and the remaining bottom-water was removed. The core was then sliced at a resolution of 0.5 cm (0–2 cm depth) and 1 cm (2–8 cm depth). An aliquot of each wet sediment sample was transferred to a 50 mL plastic centrifuge tube, which was capped, removed from the glove box and centrifuged at 2500 g for 10–30 minutes. The centrifuged tubes were then returned to the glove box. The supernatant water from each sample, including the pore-water sample, was dispensed via a 20 mL plastic syringe through a 0.45 µm filter and collected in a 15 mL plastic centrifuge tube. Subsamples were taken for analysis of dissolved iron and manganese (acidified with 10 µL of 37% HCl per mL of subsample and stored at 5°C) and ammonium and nitrate (stored at −20°C). $NO_3^-$ and $NH_4^+$ pore-water concentrations were analysed colorimetrically with a Nutrient Auto-analyzer 3 (Bram and Luebbe) using standard procedures. Mn and Fe concentrations were measured using ICP OES (Perkin Elmer Optima 3000; precision and accuracy < 5%, based on calibration to standard solutions; all dissolved iron was assumed to be present as $Fe^{2+}$, and similarly all dissolved manganese as $Mn^{2+}$ (e.g. Stumm and Morgan, 1996).

The remaining cores (4 per treatment) were harvested for foraminiferal analysis in the period from March 26th to June 16th, 2010 (according to the sampling scheme presented in Appendix 6.1). The experimental cores were sampled during separate sample events; all cores had been in the experimental set-up for at least three months before they were harvested. These cores are not true replicates due to the temporal separation of sampling moments and while it would have been preferable to sample all cores at the same time, this was not practically feasible. Nevertheless, there did not seem to be a trend of declining or increasing numbers over time (Appendix 6.1). Moreover, the difference in foraminiferal density (total standing stock and five most prominent species) among samples harvested on the same date or with a relatively short time interval was in general not smaller compared to the difference among samples harvested over a relatively long time interval (Appendix 6.1). We assume that the treatment period of 3 to 5 months was long enough to reach a steady state in all experimental cores. We therefore regard the cores as replicates and report average distributions with standard deviations over the different cores. At the start of each sample event, vertical oxygen profiles were made near the artificial burrows (at approximately 5 mm distance from the outside of the tube) and in the centre of each core (at maximum distance of about 2 cm from the artificial burrows).

The experimental cores with artificial burrows were subsampled to separately investigate the foraminiferal vertical distribution close to permeable burrows (from now on referred to as B-near) and in the sediment further away from the artificial burrows (afterwards referred to as B-far). The experimental cores without burrows are referred to as C. Prior to subsampling, glass tubes (internal diameter of 18 mm) were inserted in the sediment over each artificial burrow so that they enclosed the sediment directly surrounding the artificial burrows. The sediment in-between the glass sampling tube (Ø 18 mm) and the artificial burrow (Ø 10 mm) was sampled separately from the sediment in the rest of the core, but in the same discrete depth intervals (the upper 2 cm in slices of 0.5 cm each, from 2 to 8 cm sediment depth in slices of 1 cm). Because the subsampling design hindered the conventional method of sampling sediment cores by slicing the sediment in separate layers we subsampled the experimental cores by carefully siphoning off thin layers of sediment, using a controlled under-water vacuuming system that ensured a subtle but steady sediment-eroding flow of bottom-water into a carefully manipulated long sharp nozzle with a 1-mm opening. The sediment samples were stored in ethanol with 1 g/L Rose Bengal to stain the foraminiferal protoplasm remains. Stained specimens, i.e. individuals with brightly pink-coloured chambers except for the very last one, were assumed to have been alive at or close to the time of sampling. Specimens larger than 63 µm were enumerated at species level. For comparison, all foraminiferal densities were standardised to a volume of 100 $cm^3$ sediment per layer.

### 6.2.4 STATISTICAL ANALYSES

To analyse the effect of the treatment on the foraminiferal assemblages along a vertical gradient in the sediment directly surrounding the burrows (B-near), the sediment further from the artificial burrows in these cores (B-far) and the cores without burrows (C) SIMPER tests were used in the software package PAST (PAleontological Statistics, Hammer et al., 1999). Using the SIMPER method one can assess which taxa are primarily responsible for dissimilarities among the assemblages. We used both absolute and relative abundances of the assemblage per core.

## 6.3 RESULTS

### 6.3.1 PORE-WATER CHEMISTRY

The vertical profiles of the $O_2$, $NO_3^-$, $NH_4^+$, and $Mn^{2+}$ concentrations in the pore-water did not reveal any clear differences between the core with and without burrows (see Fig. 6.2a., b.) and in the case of $O_2$ also with core incubation time (Fig. 6.2c., d.). The main difference between the pore-water profiles was the relatively high concentration of $Fe^{2+}$ in the sediment layers from 4 to 6 centimetre sediment depth in the cores with permeable burrows (B) compared to those without burrows (C, Fig. 6.2a., b.).

### 6.3.2 TOTAL DENSITIES

The highest average foraminiferal density was found in the experimental cores that did not contain permeable tubes (C, Fig. 6.3a.). The standardised average entire-core foraminiferal density for the treatment without burrows (C) was 13781 ± 2127 per 1000 $cm^3$. The standardised average total standing stock in the sediment surrounding the burrows (B-near) was almost identical to the remainder of the sediment in the same cores (B-far) with 10355 ± 2369 and 10914 ± 931 individuals per 1000 $cm^3$ respectively. All sediment types had their highest foraminiferal density in the uppermost (0.5 cm) sediment layer (Fig. 6.3a., b.). Compared to the sediment further from the artificial burrows, the average foraminiferal density was slightly lower in the upper layer of the sediment directly surrounding

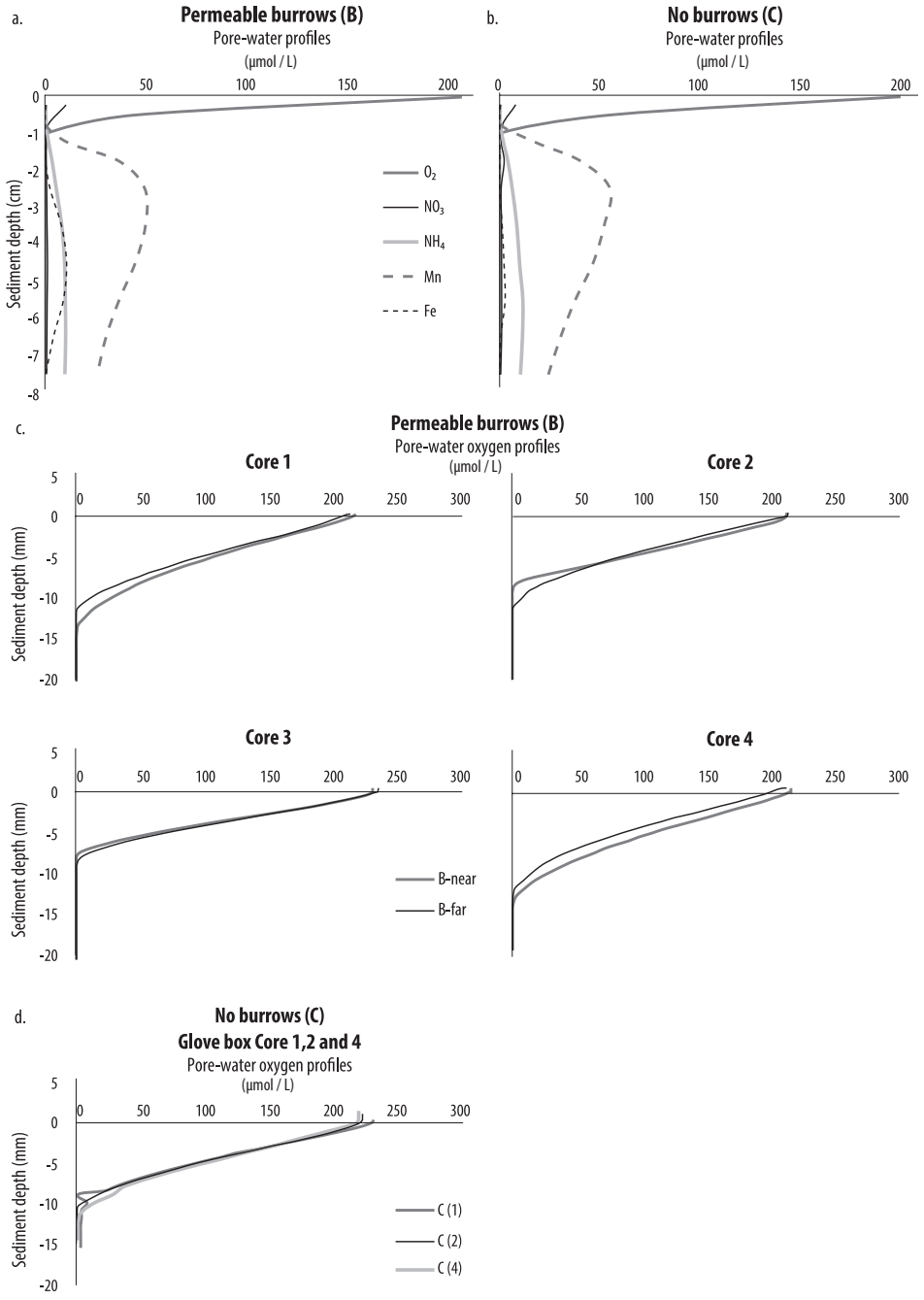



**Figure 6.2 a. - d.** a. Vertical profile of the concentration of $O_2$, $NO_3$, $NH_4$, $Mn^{2+}$ and $Fe^{2+}$ (µmol/L) in pore-water of the core with permeable burrows. (B) and b. of the core without burrows (C). c. Vertical profile of the oxygen concentration in the pore-water nearby the burrows (B-near) and at maximum distance in the centre of the core (B-far). d. Vertical profile of the oxygen concentration in the pore-water of the cores without burrows (C).

the burrows (B-near), whereas in the deepest sediment layer (7-8 cm sediment depth) the opposite was observed (Fig. 6.3c.).

### 6.3.3 ASSEMBLAGE COMPOSITION AND VERTICAL DISTRIBUTION

The foraminiferal assemblages in the experimental cores comprised the following species: *Adercotryma glomerata*, *Ammonia beccarii*, *Bolivina dilatata*, *Bolivina plicatella*, *Bolivina variabilis*, *Bulimina marginata*, *Buliminella elegantissima*, *Cibicides lobatulus*, *Eggerella advena*, *Eggerella scabra*, *Elphidium* spp., *Epistominella vitrea*, *Globobulimina turgida, Hopkinsina pacifica*, *Hyalinea balthica*, *Leptohalysis scottii*, *Nonionella* spp., *Quinqueloculina seminula*, *Reophax fusiformis*, *Reophax scorpiorus*, *Rosalina bradyi*, *Saccammina sphaerica, Stainforthia fusiformis*, *Textularia earlandi and Trochammina inflata* (Plate 6.1, 6.2). The same species were found in all sediment types e.g. control (C), further away from (B-far) and close to the burrows (B-near). As mentioned above, the foraminiferal total standing stock in the cores with burrows (B) revealed an almost identical foraminiferal density in B-near and B-far sediment. However, the contribution of the individual species to the total foraminiferal abundance per sediment type differed slightly among the sediment types (Table 6.1). Moreover, the vertical distribution of the separate species revealed more distinct dissimilarities among the treatments (Fig. 6.3d. – 6.3r.).

**Table 6.1** a. Species abundances and b. relative abundances per treatment as the sum of the foraminiferal counts per layer, standardised to a volume of 1000 $cm^3$. B-near is the sediment surrounding the burrows; B-far is the sediment further from the burrows; C is the sediment from the cores without burrows.

| Species | B-near | | | | B-far | | | | C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a. | std. dev. | b. | std. dev. | a. | std. dev. | b. | std. dev. | a. | std. dev. | b. | std. dev. |
| ***Stainforthia fusiformis*** | 2853.0 | 520.9 | 27.8 | 2.5 | 2994.4 | 729.3 | 27.4 | 6.3 | 4357.2 | 202.2 | 32.0 | 3.9 |
| ***Bulimina marginata*** | 2478.6 | 127.0 | 24.9 | 5.9 | 2383.0 | 454.0 | 21.8 | 3.7 | 2812.9 | 420.3 | 20.5 | 2.4 |
| ***Adercotryma glomerata*** | 1649.2 | 248.1 | 16.3 | 2.6 | 1446.4 | 379.8 | 13.2 | 2.9 | 1845.8 | 478.0 | 13.3 | 2.1 |
| ***Nonionella spp.*** | 597.1 | 253.6 | 5.7 | 2.1 | 992.0 | 131.4 | 9.1 | 1.0 | 1107.5 | 97.0 | 8.1 | 1.1 |
| ***Trochammina inflata*** | 511.8 | 168.1 | 4.9 | 0.9 | 508.0 | 157.7 | 4.6 | 1.0 | 592.7 | 109.4 | 4.4 | 1.0 |
| ***Ammonia beccarii*** | 350.7 | 701.4 | 2.7 | 5.5 | 301.1 | 478.5 | 2.9 | 4.7 | 23.4 | 27.3 | 0.2 | 0.2 |
| ***Elphidium spp.*** | 383.9 | 339.2 | 3.3 | 2.4 | 321.4 | 63.4 | 2.9 | 0.3 | 377.0 | 166.7 | 2.7 | 1.1 |
| ***Cibicides lobatulus*** | 52.1 | 32.4 | 0.6 | 0.5 | 12.9 | 12.6 | 0.1 | 0.1 | 57.2 | 60.3 | 0.4 | 0.4 |
| ***Textularia earlandi*** | 488.1 | 523.6 | 4.3 | 4.4 | 735.2 | 379.9 | 6.7 | 3.3 | 1125.7 | 1112.4 | 7.6 | 6.4 |
| ***Eggerella scabra*** | 255.9 | 173.4 | 2.3 | 1.3 | 303.0 | 168.3 | 2.7 | 1.2 | 590.1 | 227.4 | 4.2 | 1.2 |
| ***Eggerella advena*** | 222.7 | 62.6 | 2.2 | 0.7 | 206.9 | 118.8 | 2.0 | 1.2 | 210.6 | 115.3 | 1.5 | 0.8 |
| ***Bolivina plicatella*** | 23.7 | 35.9 | 0.2 | 0.3 | 11.1 | 14.1 | 0.1 | 0.1 | 5.2 | 6.0 | 0.0 | 0.0 |
| ***Bolivina dilatata*** | 56.9 | 88.9 | 0.6 | 0.9 | 83.1 | 27.9 | 0.8 | 0.3 | 85.8 | 60.9 | 0.6 | 0.5 |
| ***Bolivina variabilis*** | 156.4 | 90.7 | 1.5 | 0.8 | 201.4 | 223.3 | 1.9 | 2.1 | 137.8 | 67.6 | 1.0 | 0.6 |
| ***Hyalinea balthica*** | 19.0 | 21.9 | 0.2 | 0.3 | 9.2 | 14.0 | 0.1 | 0.1 | 18.2 | 10.0 | 0.1 | 0.1 |
| ***Buliminella elegantissima*** | 37.9 | 43.8 | 0.4 | 0.4 | 25.9 | 22.2 | 0.2 | 0.2 | 41.6 | 30.6 | 0.3 | 0.2 |
| ***Rosalina bradyi*** | 37.9 | 46.4 | 0.3 | 0.4 | 40.6 | 25.2 | 0.4 | 0.2 | 150.8 | 56.6 | 1.1 | 0.3 |
| ***Quinqueloculina seminula*** | 28.4 | 19.0 | 0.3 | 0.2 | 29.6 | 24.9 | 0.3 | 0.2 | 31.2 | 22.5 | 0.2 | 0.2 |
| ***Hopkinsina pacifica*** | 4.7 | 9.5 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***Leptohalysis scottii*** | 28.4 | 45.1 | 0.3 | 0.5 | 25.9 | 25.2 | 0.2 | 0.2 | 26.0 | 6.0 | 0.2 | 0.0 |
| ***Epistominella vitrea*** | 9.5 | 19.0 | 0.1 | 0.2 | 9.2 | 7.1 | 0.1 | 0.1 | 62.4 | 14.7 | 0.5 | 0.1 |
| ***Reophax fusiformis*** | 37.9 | 53.6 | 0.3 | 0.4 | 12.9 | 12.6 | 0.1 | 0.1 | 10.4 | 12.0 | 0.1 | 0.1 |
| ***Reophax scorpiorus*** | 19.0 | 21.9 | 0.2 | 0.2 | 64.7 | 16.4 | 0.6 | 0.2 | 36.4 | 35.5 | 0.3 | 0.3 |
| ***Globobulimina turgida*** | 14.2 | 18.1 | 0.1 | 0.2 | 59.1 | 24.1 | 0.5 | 0.2 | 18.2 | 24.6 | 0.1 | 0.2 |
| ***Saccammina sphaerica*** | 0.0 | 0.0 | 0.0 | 0.0 | 96.1 | 153.3 | 0.9 | 1.5 | 52.0 | 12.0 | 0.4 | 0.1 |
| ***Unidentifiable*** | 14.2 | 18.1 | 0.1 | 0.2 | 33.3 | 38.9 | 0.3 | 0.3 | 5.2 | 6.0 | 0.0 | 0.0 |

*Stainforthia fusiformis* was the most abundant species. The contribution of this species to the total standing stock was 27.8% ± 2.5 in B-near sediment, 27.4% ± 6.3 in B-far sediment and 32.0% ± 3.9 in the cores without burrows (C, Table 6.1). The SIMPER test based on absolute abundances per core as well as on relative abundances per core elucidated that in general this species also made the highest contribution to dissimilarities in assemblage composition among the sediment types (Appendix 6.2). *Bulimina marginata* was slightly less prominent with a contribution the total foraminiferal abundance of 24.9% ± 5.9 in B-near sediment, 21.8% ± 3.7 in B-far sediment and 20.5% ± 2.4 in C sediment (Table 6.1). The SIMPER test also confirmed the prominent role of *B. marginata* in assemblage variability; the relative abundance of this species contributed most to the dissimilarity between the assemblages in B-near sediments. *Adercotryma glomerata* and *Nonionella spp.* were the third and fourth most abundant species. Their contributions to the total standing stock were respectively 16.3% ± 2.6 and 5.7% ± 2.1 in B-near sediment, 13.2% ± 2.9 and 9.1% ± 1.0 in B-far sediment and 13.2% ±2.1 and 8.1% ± 1.1 in the control sediment (C, Table 6.1). The relative abundances of *Textularia earlandi* were 4.3% ± 4.4 B-near, 6.7% ± 3.3 in B-far and 7.6% ± 6.4 in the control sediment (C, Table 6.1). The species *T. earlandi* and *Nonionella spp.* had a lower relative abundance in B-near, while the species *B. marginata* and *A. glomerata* had a higher relative abundance in B-near (Table 6.1). Accordingly, the SIMPER test based on relative abundances indicated that overall the contribution of *T. earlandi* to dissimilarities in the assemblage compositions among the sediment types was higher than the contribution of the species *Nonionella spp.* and *A. glomerata* (Appendix 6.2).

The impact of the artificial burrows on the vertical distribution of the foraminifera seemed to differ between species. *Stainforthia fusiformis* had the most pronounced dissimilarity in the vertical distribution among the sediment types (Fig. 6.3d.). *S. fusiformis*, a deep infaunal species, had higher densities in the deep layers surrounding the burrows (B-near) compared to the sediment further from the burrows (B-far), while the opposite was true near the sediment surface (Fig. 6.3d). This dissimilarity in the vertical distribution of *S. fusiformis* was even more distinct (Fig. 6.3e., f.). The shallow infaunal species *Bulimina marginata* did not have a distinct dissimilarity in the absolute and relative vertical distribution of its abundance among the sediment types, nor in its relative depth distribution (Fig. 6.3g., h., and i.). *Nonionella spp.* is the species within the assemblage that lived at intermediate sediment depth, this species did not reveal any clear cut differences in the vertical distribution among the sediment types (Fig. 6.3m., n.). *Adercotryma glomerata and Textularia earlandi* were almost restricted to the upper sediment layer (Fig. 6.3j., k., p., q.). The difference in the relative depth distribution between sediment near and further from the burrows was very similar for both species (Fig. 6.3l., r.). These two agglutinant species seemed to live slightly lower in the sediment near the burrow compared to that further from the burrow.

In order to explore the signal of burrow-related changes in vertical distribution as it may transfer to the fossil record, figure 6.4 displays the difference in the relative abundance of the five most prominent species in the depth-specific foraminiferal assemblage per sediment layer when comparing the sediment directly surrounding the burrows (B-near) compared to that further from the burrows (B-far). A negative value indicates that the relative abundance of the foraminiferal species was – in that specific layer – smaller in the sediment surrounding the burrows (B-near) compared to the further from the burrows (B-far). When interpreting the figure, one has to bear in mind that, since the total foraminiferal standing stock was not evenly distributed over depth, a decrease or increase in the contribution of a species among sediment layers does not automatically imply an abundance decrease or increase of that species with depth. The depth distribution of the relative abundances of *Stainforthia fusiformis* (Fig. 6.4a.) revealed that the contribution of this species to the assemblage was in the upper part of the core (to 2 cm sediment depth) smaller in the sediment surrounding the burrows (B-near) and higher

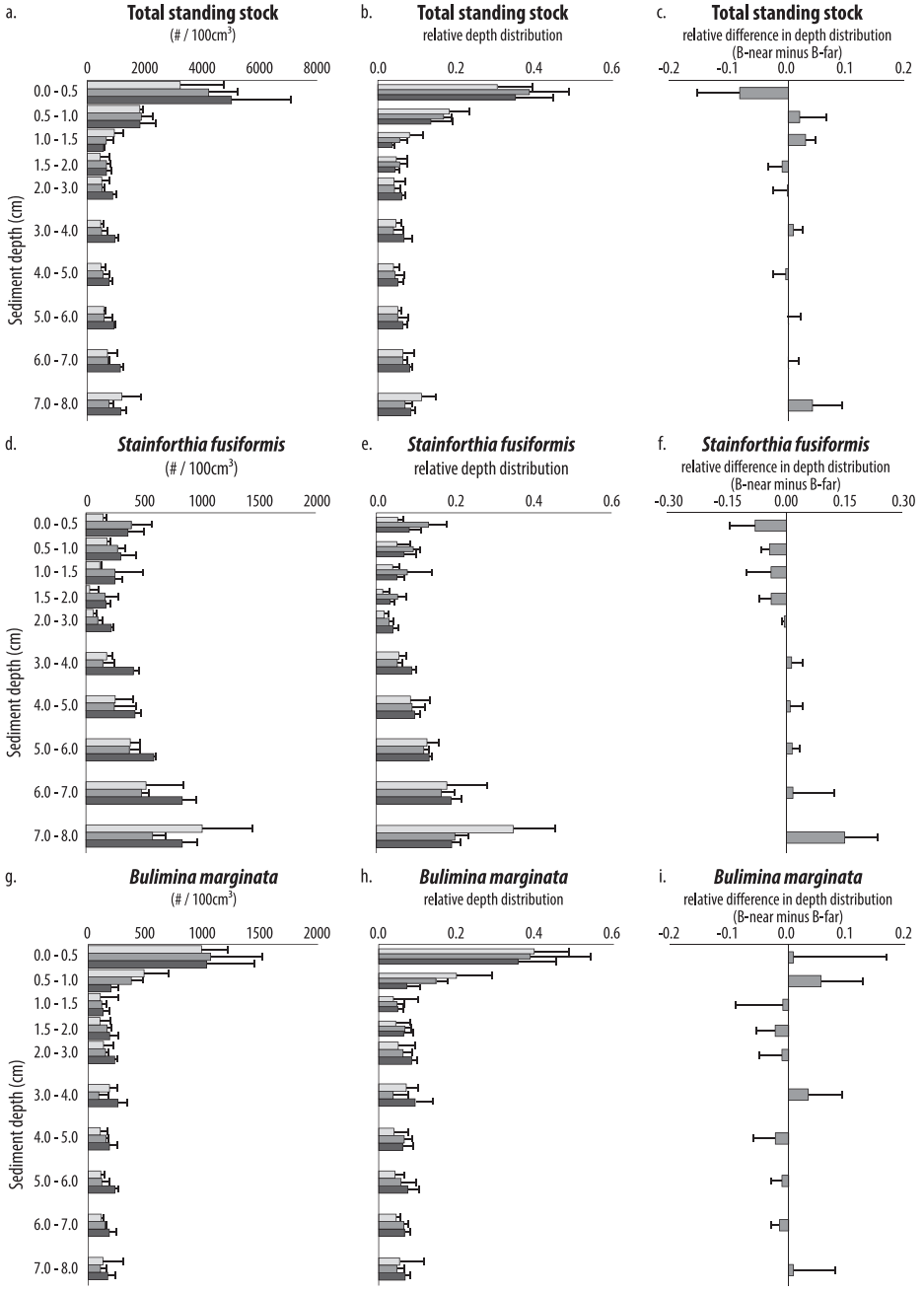
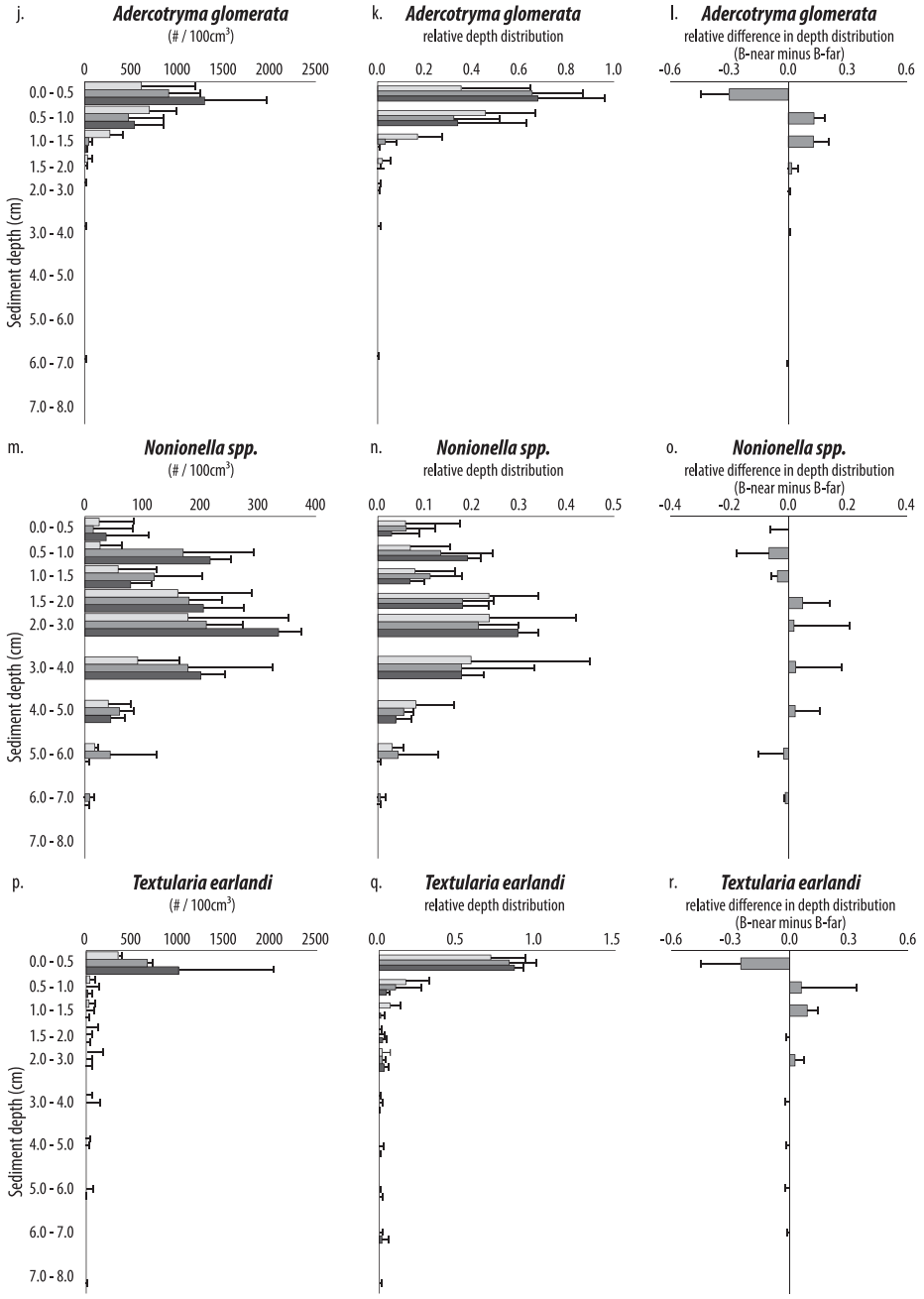



**Figure 6.3 a. - r.** a., d., g., j., m., p.: Vertical distribution of the total standing stock and the most prominent foraminifera standardised to abundance per 100 $cm^3$ per sediment layer. Light grey bars refer to the sediment directly surrounding the burrows (B-near), medium grey bars refers to the sediment further from the burrows (B-far) and dark grey bar refers to the sediment in the cores without burrows (C). ⇨




⇦ b., e., h., k., n. q.: Relative depth distribution of the relative foraminiferal abundances per sediment type. Light grey bars refer to the sediment directly surrounding the burrows (B-near), medium grey bars refers to the sediment further from the burrows (B-far) and dark grey bars refers to the sediment in the cores without burrows (C).

c., f. i., l., o. r.: Difference in the relative depth distribution of the total standing stock and the five most prominent species between sediment near the burrow (B-near) and that further from the burrow (B-far).

in the deeper sediment layers (from 2 to 8 cm sediment depth) compared to the sediment further from the burrow (B-far). Despite the displayed highest relative abundance of *S. fusiformis* in the deepest sediment layer (Fig. 6.3d., e.), the highest positive difference in the contribution to the assemblage was found at a sediment depth of 4 to 5 centimetres (Fig. 6.4a.). The relative abundance of *Bulimina marginata* per sediment layer did not reveal a clear-cut pattern in its difference between B-far and B-near sediment (Fig. 6.4b.). The vertical distribution of the difference in the relative contribution to the

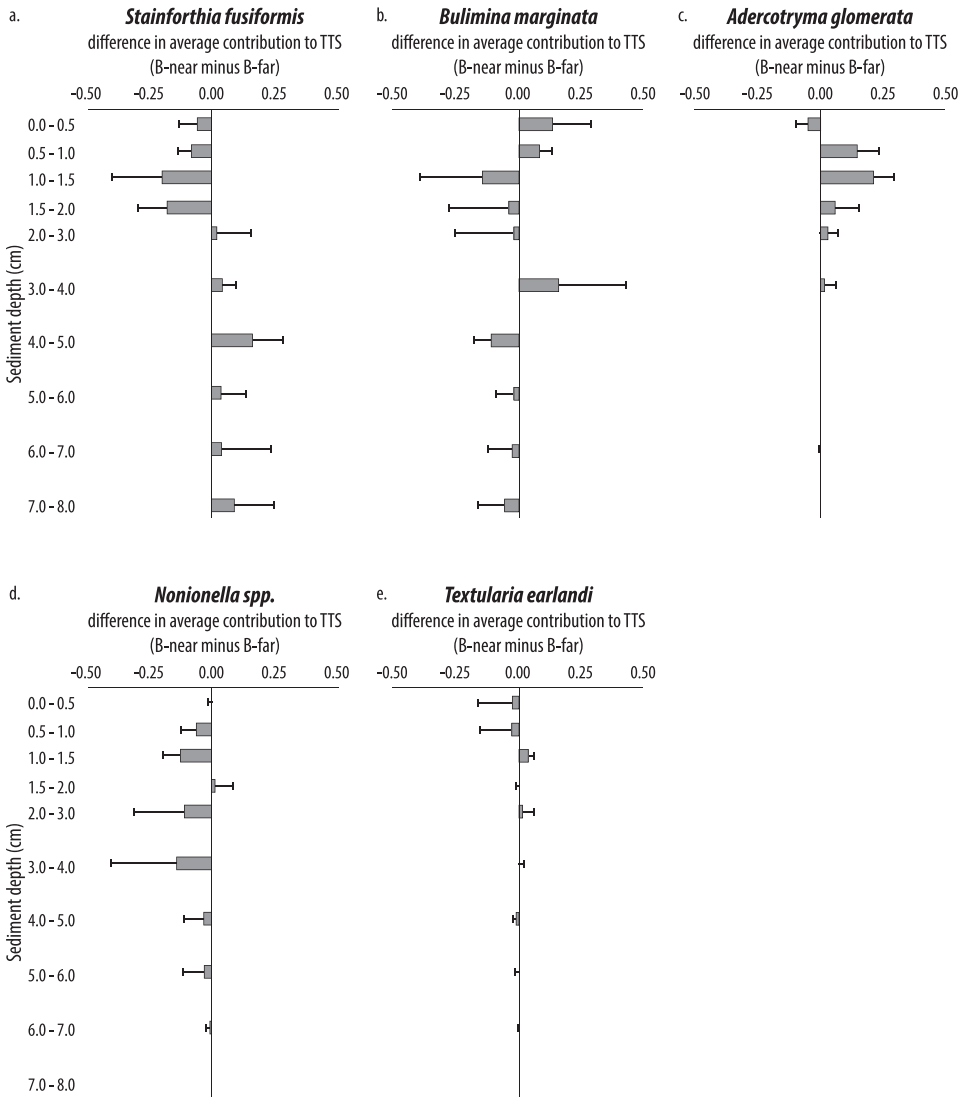



**Figure 6.4 a. - e.** The graphs displays per sediment layer the difference in the relative abundance of the foraminiferal species within the assemblage. a. *Stainforthia fusiformis*, b. *Bulimina marginata*, c. *Adercotryma glomerata*, d. *Nonionella spp.* and e. *Textularia earlandi.*

assemblage was opposite for the species *Adercotryma glomerata* (Fig. 6.4c.) and *Nonionella spp.* (Fig. 6.4d.). Apart from the upper sediment layer, *A. glomerata* had a relative higher abundance in the B-near sediment, while *Nonionella spp.* contributed more to the assemblage in almost all sediment layers (except layer 4, at 1.5 to 2.0 cm sediment depth) of B-far sediments. Among the dominant foraminiferal species, *Textularia earlandi* (Fig. 6.4e.) had the smallest dissimilarity in the relative abundance per sediment layer.

## 6.4 DISCUSSION

The fossilised foraminiferal assemblage that is stored in the sediment archive of the Gullmar Fjord contains a relatively broad array of species that has been extensively studied. These studies indicated that, during the last century, the foraminiferal dynamics in the Gullmar Fjord were mainly governed by fluctuations in the bottom-water oxygen concentration, resulting in fluctuations in the geochemical conditions of the benthic realm (e.g. Josefson and Widbom, 1988; Nordberg et al., 2000; Gustafsson and Nordberg, 2001; Filipsson and Nordberg, 2004; Polovodova Asteman and Nordberg, 2013). Over the last decade the foraminiferal assemblage in the fjord comprised both species sensitive to oxygen depletion and oxygen-stress tolerant species. This makes the foraminiferal assemblage from this particular region very suitable for our study.

In the next paragraphs we will relate our results to previous reports on the foraminiferal dynamics in the Gullmar Fjord and we will set our findings in the context of other studies that investigated the impact of macrofauna or macrofaunal (biogenic) structures on the presence, the vertical distribution and the assemblage composition of benthic foraminifera, but first we will briefly discuss the sediment biogeochemistry.

### 6.4.1 SEDIMENT BIOGEOCHEMISTRY

The pore-water profiles of $O_2$, $NO_3^-$, $NH_4^+$ and $Mn^{2+}$ are generally very similar among the various sediment types, and in the case of $O_2$ also over the course of the experiments (Fig. 6.2). The control on depth resolution for $O_2$ measurements, using a micromanipulator, was much more accurate than the sediment-slicing method used to obtain $NO_3^-$, $NH_4^+$, $Mn^{2+}$ and $Fe^{2+}$ pore-water profiles. Our methods used to assess the vertical pore-water profiles of $O_2$, $NO_3^-$, $NH_4^+$ and $Mn^{2+}$ lacked the spatial resolution necessary to reveal subtle dissimilarities in the pore-water composition among the sediment types. The colouring of the sediment alongside the burrows – reddish-brow instead of dark grey – below the horizontal oxygen-penetration depth, was indicative of diffusion of oxygen through the burrow wall. The burrow radius (5 mm, including the 2 mm thick burrow wall) was smaller than the oxygen-penetration depth at the sediment-water interface (7 – 11 mm). Our $O_2$ pore-water profiles measured near and further from the burrows confirm that the burrow cannot be treated as a simple extension of the sediment surface (Meysman et al., 2010); we were not able to measure elevated oxygen concentrations at an approximate distance of 5 mm from the artificial burrow. Deeper in the sediment, a centimetre away from the artificial burrows, very reducing redox conditions prevailed. It seems likely that this provided a continuous, relatively high lateral supply of reduced compounds towards the artificial burrows, thus limiting the extent of the free oxygen diffusion halo surrounding the burrows to a diameter smaller than 5 mm.

The high resemblance of the vertical pore-water profiles of $O_2$, $NO_3^-$, $NH_4^+$ and $Mn^{2+}$ between the C and B-core can be explained by the control of organic matter content on the sediment biogeochemistry of these compounds. The amount and quality of organic matter, and therefore the overall organic matter remineralisation, was similar in the different cores because the sediment was homogenised prior to

core construction. Without a substantial supply of oxygen alongside the burrow and similar oxygen consumption, nitrate reduction and ammonia production in all core-types, comparable pore-water profiles were measured for different treatments. The main biogeochemical difference between the control and burrowed sediment is the higher concentration of dissolved $Fe^{2+}$ in the sediment layers from 4 to 6 centimetre sediment depth in the B-core compared to C-core. This $Fe^{2+}$ accumulation suggests intensive micro-cycling of iron as described by Sobolev and Roden (2001). Such Fe micro-cycling has been observed before in cores where $O_2$ diffusion into the sediment was enhanced by artificial burrows (Ferro et al., 2003). In the pore-waters of our B-cores, $Fe^{2+}$ accumulation was lower than observed by Ferro et al. (2003; ~ 10 µM versus >200 µM) and $Mn^{2+}$ did not increase relative to our C-cores, in contrast to those of Ferro et al. (2003). Perhaps the shape of the burrows (U-shaped versus linear) caused a difference in the overall $O_2$ diffusive flux and therefore micro-cycling intensity of both Fe and Mn. However, a more likely explanation is a difference in substrate sediments. Our sediment from the hypoxic Gullmar Fjord with a maximum basin depth of 120 m likely has a smaller pool of reactive Fe and Mn than the intertidal flat sediment from the Katsplaat, Oosterschelde, The Netherlands. Therefore, the diffusive $O_2$ flux will result in less Fe and Mn micro-cycling in our burrowed cores. Still, the $Fe^{2+}$ accumulation in the pore-waters of our B-cores and the observed reddish-brown colouring of the sediment alongside burrows strongly suggest iron micro-cycling and therefore local/small-scale differences in the sediment biogeochemistry that may be related to foraminiferal microhabitat redistribution in the vicinity of the burrows. The observed dissimilarities in the assemblage composition and the vertical distribution of the species are hypothesised to be instigated by the visually confirmed, yet laterally limited zone of oxygen diffusion along the artificial burrows.

### 6.4.2 FORAMINIFERA OF THE GULLMAR FJORD

The dynamics, both in density and composition of the benthic (fossilised) foraminiferal community over the last 100 years has been well-studied and discussed in the context of seasonal depletion of oxygen in the bottom-waters and associated alteration of pore-water chemistry (e.g. Josefson and Widbom, 1988; Nordberg et al., 2000; Gustafsson and Nordberg, 2001; Filipsson and Nordberg, 2004; Polovodova Asteman and Nordberg, 2013). The small impact of tidal movement and the concurrent high sedimentation rate in the Gullmar Fjord has resulted in a high-resolution sediment-archive containing numerous fossilised foraminifera. Filipsson and Nordberg (2004) reported that, in the period from 1930 to 1980 *Bulimina marginata*, *Hyalinea balthica* and *Textularia earlandi* were the most common species – they reflected the relatively stable fjord environment. A major shift in the foraminiferal assemblage was observed in the late 1970s and early 1980s (Josefson and Widbom, 1988; Nordberg et al., 2000; Gustafsson and Nordberg, 2001; Filipsson and Nordberg, 2004). A decrease in dissolved oxygen in the bottom-water during the late 1970s provoked a strong abundance increase of predominantly *Stainforthia fusiformis* and to a lesser extent it instigated higher densities of species such as *Bolivinellina pseudopunctata*, *Quinqueloculina stalkeri*, *Cassidulina reniforme* and *Epistominella vitrea* (Nordberg et al., 2000). In the period preceding this shift, the species *S. fusiformis* comprised 10% of the total density, whereas afterwards it dominated with 50 to 60% of the slightly decreased total foraminiferal standing stock. This overall decline of the total standing stock was attributable to a density decrease of foraminiferal species sensitive to oxygen depletion: *H. balthica*, *Nonionella labradorica* and *B. marginata* (so-called Skagerrak – Kattegat species, Nordberg et al., 2000). Concurrently to the loss of oxygen-sensitive foraminifera, the macrofaunal species experienced a much stronger hypoxia related decline in abundance (Josefson and Widbom, 1988). By the end of the century, *S. fusiformis* was noticed to have further increased in abundance (Nordberg et al., 2000). Apart from S. *fusiformis*, also the species *B. pseudopunctata*, *B. marginata*, *Nonionella turgida* and *T. earlandi* had become more densely

present. This small faunal shift was explained by regular bottom-water renewal (Polovodova Asteman and Nordberg, 2013). Recently, the foraminiferal dynamics over the last decade were added to the historical record (Polovodova Asteman and Nordberg, 2013; Polovodova Asteman et al., 2013). From 2001 to 2007 a new decline in the foraminiferal density was observed, however now related to the less frequent occurrence of hypoxic conditions enabling the macrofaunal community to re-establish. In 2008 a new severe hypoxic event stopped the further restoration of the macrobenthic community and, as in the late 1970s, the stress-tolerant foraminiferal species replaced the Skaggerak-Kattegat communities (Polovodova Asteman and Nordberg, 2013). Polovodova Asteman and Nordberg (2013) indicated that in the recent decade the assemblage was dominated by the (relatively hypoxia tolerant) foraminiferal species *B. pseudopunctata*, *S. fusiformis* and *T. earlandi*. Several other species were found in lower abundances e.g. *B. marginata*, *N. turgida*, *Q. stalkeri*, and *Adercotryma glomerata*.

The assemblage in our experiment comprised both oxygen-stress tolerant and sensitive species. *Stainforthia fusiformis*, *Bulimina marginata* and *Adercotryma glomerata* – with an average contribution of 27-31%, 20-24% and 13-16% to the total standing stock – were the most abundant species. *Nonionella spp.* and *Textularia earlandi* followed with an average contribution between 5 and 9%. As summarised above, *S. fusiformis* has been indicated to be strongly related to periods of oxygen depletion in the bottom-waters of the Gullmar Ford, while *B. marginata* and *A. glomerata* dominated from 1950 to 1980 when stable oxygenated conditions prevailed. In our experiment, the contribution of *S. fusiformis* to the total foraminiferal abundance was slightly higher in the cores without burrows (C) compared to those with burrows (B). In contrast, *B. marginata* and *A. glomerata* had a higher contribution to the total standing stock in the cores with burrows (B). Moreover, in the B-cores, these two species were generally more densely present in the surrounding the burrows (B-near) compared to the sediment further from the burrows (B-far). *Nonionella spp.* had its highest contribution to the assemblage in the sediment outside the direct influence of burrows, potentially indicating an intermediate ecological strategy between *S. fusiformis* on the one hand and *B. marginata* and *A. glomerata* on the other hand. Interestingly, these findings are in line with the reported relation between the occurrences of those species either during hypoxic, intermediate or stable fjord conditions (e.g. Josefson and Widbom, 1988; Nordberg et al., 2000; Gustafsson and Nordberg, 2001; Filipsson and Nordberg, 2004; Polovodova Asteman and Nordberg, 2013).

The similarity described above between foraminiferal response to artificial irrigation in this experiment and bottom-water oxygenation in field studies was somewhat surprising, because the decadal patterns in the foraminiferal communities, as observed in the sedimentary record, are manifestations of foraminiferal ecology on very different temporal, spatial and population dynamics scales, compared to our laboratory experiment. For instance, while in the experiment microhabitat preference may play a key role, the dominance of species such as *Stainforthia fusiformis* over stability-requiring species in habitats with frequent oxygen disturbance, is not so much because this species *prefers* the disturbed conditions. It seems that they are better equipped for surviving the disturbance than typical equilibrium species, but most importantly, they excel in population recovery after disturbance (e.g., Nordberg et al., 2000; Duijnstee et al., 2005). Therefore, part of disturbance-taxa's success is that they are less impacted by the disturbance event and have the population dynamics to profit from the underused resources in largely emptied habitats after the disturbance. Hence, they get to outnumber equilibrium species, although the latter may well be the stronger competitors. Even if the disturbance is ephemeral, the incumbency of the new disturbance taxa is not easily broken, and recovering equilibrium species have difficulties to reclaim their dominance (Duijnstee et al., 2004). The field observations in the Gullmar Fjord – with its long-lasting *Stainforthia*-dominated fauna – corroborate

this general pattern. This is very different from the short-term microcosm situation. However, an explanation for the observed similarity in foraminiferal patterns may be that in some sense the vertical and lateral distribution of microhabitats in the core represents the spatial equivalent of a temporal dysoxia event, and the spatial distribution of foraminifera to some degree reflects temporal changes in the foraminiferal community. The analogue of the event itself would be the deep, anoxic habitat away from the artificial burrows. The oxygenation sediment surface and its inhabitants mimic the long-lasting stable oxygenated benthic environment, and the oxygenated halo around the burrow – deep in the sediment – resembles the recovering benthic environment after the hypoxia event. Finally, foraminiferal capacity for migration of individuals from one microhabitat to another provides an analogue for the temporal dimension of a disturbance event. In this model – if we incorporate the ecological strategies of disturbance and equilibrium taxa – it would be expected (1) to find *Bulimina marginata*, *Adercotryma glomerata* and *Textularia earlandi* inhabiting the surface sediments, regardless of the treatment, (2) to find *Stainforthia fusiformis* as the most dominant taxon and deep anoxic parts of the sediment column, (3) to find *Stainforthia* relatively enriched in oxygenated deep sediments around the lower parts of the burrows, and (4) to find *Nonionella spp.* in a role consistent with its intermediate ecological strategy.

On a more subtle level, also among equilibrium species we could observe some degree of response to the burrow treatment in our experiment. *Adercotryma glomerata,* for instance – almost exclusively found in the upper sediment layers – seemed to have a slightly deeper average living depth in the presence of burrows. Apart from its vertical distribution, this species contributed more to the assemblage in the sediment surrounding the burrows compared to that further from the burrows in the same cores. These results confirm the reported preference of *A. glomerata* (Polovodova et al., 2011) to occupy oxygenated sediments of the Gullmar Fjord. *Bulimina marginata* was less restricted to the sediment-water interface (suggesting relatively frequent excursions by individuals into the deep), nonetheless, also this species was most abundant in the upper sediment layers. Although the contribution of this species to the assemblage in each sediment layer seemed to differ between B-near and B-far, these dynamics were not as straight forward as those of *A. glomerata*. This might indicate that within the group of equilibrium species, *B. marginata* is slightly less sensitive to oxygen depletion than *A. glomerata*. This sort of behaviour of *B. marginata* is supported by observations of the turnover in foraminiferal assemblages in the subfossil record of the gradually eutrophicating orthern Adriatic Sea over the last 150-200 years (Barmawidjaja et al., 1995). Barely into the eutrophication sequence, which is correlated with the onset of episodic but still infrequent and moderate hypoxia events, *B. marginata* gained importance in the foraminiferal community. However, as the consequences of eutrophication increased in severity they declined somewhat while species such as *Nonionella turgida* and *Stainforthia fusiformis* peaked much later at maximum eutrophic (i.e. high disturbance frequency) conditions.

Within the assemblages in our experimental microcosms, *Nonionella spp.* lived mainly at intermediate depths; above *Stainforthia fusiformis* but below *Adercotryma glomerata* and *Bulimina marginata*. *Nonionella spp.* had its highest contribution to the standing stock in the cores with burrows but outside their direct influence. In earlier experiments and in field studies it could be shown that *Nonionella turgida*, although it has a demonstrated capacity to withstand disturbance relatively, seemed to recover slower after disturbance events than *S. fusiformis* (Duijnstee et al., 2005). This underlying difference in the biology of *Nonionella* and *Stainforthia* might also be responsible for lack of enrichment in *Nonionella* close to the burrow, especially since – as pointed out earlier – the sediments directly surrounding the burrows are to some extent similar to benthic environments after a hypoxia event. The difference in *Nonionella*'s contribution to the assemblage per sediment layer – with also a higher contribution to the assemblage outside the direct influence of the burrows – confirmed its position in

the group of foraminiferal species that can thrive in relatively oxygen-poor conditions. In line with *B. marginata* also the average living depth of *T. earlandi* (most densely present near the sediment surface) seemed to have slightly deepened reflected in a lower contribution to the assemblage in the two upper layers of the sediment surrounding the burrows and a higher contribution in third sediment layer. The almost restricted occurrence of this species in the upper sediment layers suggested that this species belonged to the group that lives in oxygen-rich conditions, while in contrast its contribution to the total assemblage indicated the opposite.

### 6.4.3 ECOSYSTEM ENGINEERING

The net impact of ecosystem engineers on the abundance and species diversity of other species within an ecosystem depends on the magnitude of both positive and negative ecological interactions (Jones et al., 1997). Within the ecosystem, some species may benefit from newly formed niches constructed by ecosystem engineers, while others do not profit or are even harmed by activities of ecosystem engineers due to, for instance, destruction of their habitat (Jones et al., 1997). Moreover, the net impact may differ on temporal and spatial scales (e.g. Jones et al., 1997, Wright et al., 2004; Hastings et al., 2007). Marine tube-building macrofauna modify the sediment through mixing of particles and solutes (e.g. Ferro et al., 2003). The impact of large tube-building organisms on biogeochemical processes in marine sediments has been reported to be species-specific and dependent on their feeding mode and sediment characteristics (Reise, 1981; Meyers et al., 1988; Widdicombe et al., 2000; Meysman et al., 2005, 2006a; Mermillod-Blondin and Rosenberg, 2006). In this study we investigated the effect of artificial permeable burrows – and the diffusion of solutes – without particle mixing and direct interaction between macro- and meiofauna. The lack of differences in food availability, $O_2$ penetration depth and dissolved nitrate between the experiment and control may elucidate the absence of an overall strong positive effect of the artificial burrows on the total foraminiferal stock and the species diversity. While others have found a positive impact of ecosystem engineers on foraminifera (e.g. Reise, 1981; Bouchet et al., 2009) we did not find such an overall positive effect of artificial burrows on foraminiferal abundances and the species diversity. Physical disturbance of the foraminifers' through the insertion of the burrows may have caused slightly lower average foraminiferal abundances in the cores with burrows compared to those without burrows. Interestingly, despite the lack of an overall positive effect on the foraminifera and the almost absence of measured dissimilarities in the pore-water composition, species-specific responses as well as dissimilarity in the assemblage composition were observed suggesting dissimilarity in the microhabitat distribution among the sediment types (as discussed previously). In the next paragraph we will set our findings gained from a laboratory microcosm experiment with simplified ecosystem interactions in the context of other (*in situ*) studies reporting on the impact of tube-building macrofauna or biogenic structures on meiofaunal dynamics. In addition to the impact of (artificial) burrows we will discuss other aspects of the dynamic relation between tube-building macrofaunal species and meiofauna that did not play a (prominent) role in our experiment.

Food web interactions affect the impact of macrofaunal bioturbation and bioirrigation on foraminifera and other meiofaunal species. Passerelli et al. (2012) placed artificial structures *in situ* in low and high densities to mimic biogenic structures. The highest increase in foraminiferal density (over a period of 56 days) was reported for those sediments that had received the highest density of structures. These results were discussed to be not directly related to the presence of artificial tubes, but to be instigated by a high availability of food (the microphytobenthos biomass was positively related to the number of tubes) and the lowered pressure of macrofaunal predation on foraminifera (macrofaunal abundance was negatively related to tube density). The outcome of this study was related to direct and indirect food web interactions. Despite similarities in the outcome, our findings were dominated by other

processes i.e. physical disturbance and increased sediment heterogeneity through insertion of the burrows. Food web effects may also be related to the material used for constructing burrows. Thomsen and Altenbach (1993) observed up to three times as many foraminifera in the near presence of burrows inhabited by *Echiurus echiurus* (spoonworm) compared to the surrounding sediment. The assemblage investigated by Thomsen and Altenbach (1993) comprised high numbers of the foraminiferal species *Elphidium excavatum*, *Eggerelloides scarbus* and *Ammonia beccarii*. The distinctively high dissimilarity in the foraminiferal density between ambient sediment and sediment neighbouring tubes was discussed to be related to the high abundance of bacteria present on the tubes available as a potential food for foraminifera. The artificial burrows used in our experiment were made of polyethylene instead of organic material. Differences in burrow wall structure and composition can affect the microbial communities living within the burrow micro-environment (Zorn et al., 2006; Papaspyrou et al., 2006). While our artificial burrows themselves are supposedly not an extensive food source for bacteria, it is likely that some bacterial growth occurred close to the artificial burrows. This might have been involved in the intense Fe micro-cycling observed in our pore-water chemistry data. Still, the impact of our artificial burrows on bacterial growth will not be as strong as the impact of the burrows constructed by the spoonworm *Echiurus echiurus*. In addition to the extra food source supplied as organic material used to construct burrows, burrow-ventilation by tube-building macrofauna may further enhance bacterial growth.

Braeckman et al. (2010) reported that stimulation of mineralisation – i.e. increased nutrient turnover – depends on the mode of activity displayed by macrofauna. Moreover, the structuring effect of ecosystem engineers on a nematode community was reported to be dependent on species-specific macrofaunal activities (Braeckman et al., 2011a, b). In turn, these activities are strongly related to the permeability of the sediment (Meysman et al., 2005). The mode of bioirrigation depends among others on the permeability of the sediment (Meysman et al., 2005). Where macrofauna in sandy sediments pump water into the blind end of their burrow to ventilate it (causing advective transport of solutes), U-shaped burrows (with two openings) are required for bioirrigation in muddy sediment (Meysman et al., 2005). In contrast to sandy-sediment where burrow-water and dissolved solutes are directly pumped into the sediment, transport of solutes in muddy sediments – as in our experiment – occurs via diffusion from the water inside the burrow to the sediment pore-water. These differences in diffusion-dominated and advective-dominated systems were reported to even differentially impact microbial processes (Mermillod-Blondin and Rosenberg 2006). In consequence – as discussed by Braeckman et al. (2011a) – the structuring effect of ecosystem engineers is habitat-dependent.

## 6.5 CONCLUSION

The lack of differences in food availability, $O_2$ penetration depth and dissolved nitrate between the experiment and control core was reflected by the absence of an overall strong positive effect of the artificial burrows on foraminiferal abundances and species diversity. The visually observed reddish-brown colour of the sediment surrounding the burrows, and the co-occurrence of elevated $Fe^{2+}$ concentrations – interpreted as Fe micro-cycling observed in pore-water near the burrows – might have caused the observed dissimilarities in the species-specific contribution to the total foraminiferal stock and the vertical distribution of foraminifera in the sediment. Interestingly, the presumed impact of the burrows and the observed foraminiferal dynamics corresponded to the reported patterns of the dominant species during hypoxic, intermediate or stable oxygenated fjord conditions. *Stainforthia fusiformis* dominated over stability-requiring species in habitats with frequent oxygen disturbance, this species excels in population recovery after disturbance. *Bulimina marginata*, *Adercotryma glomerata* and *Textularia earlandi* inhabited the surface sediments, regardless of the treatment. Within the group

of equilibrium species, *B. marginata* is slightly less sensitive to oxygen depletion than *A. glomerata* In-between the oxygen-stress tolerant and sensitive species *Nonionella spp.* displayed an intermediate ecological strategy. The impact of artificial burrows on foraminiferal dynamics – with simplified ecosystem interactions – were set in the context of other (*in situ*) studies reporting on the impact of tube-building macrofauna or biogenic structures on meiofaunal dynamics. Dissimilarities were discussed to be related to food web interactions and habitat-dependent differences.

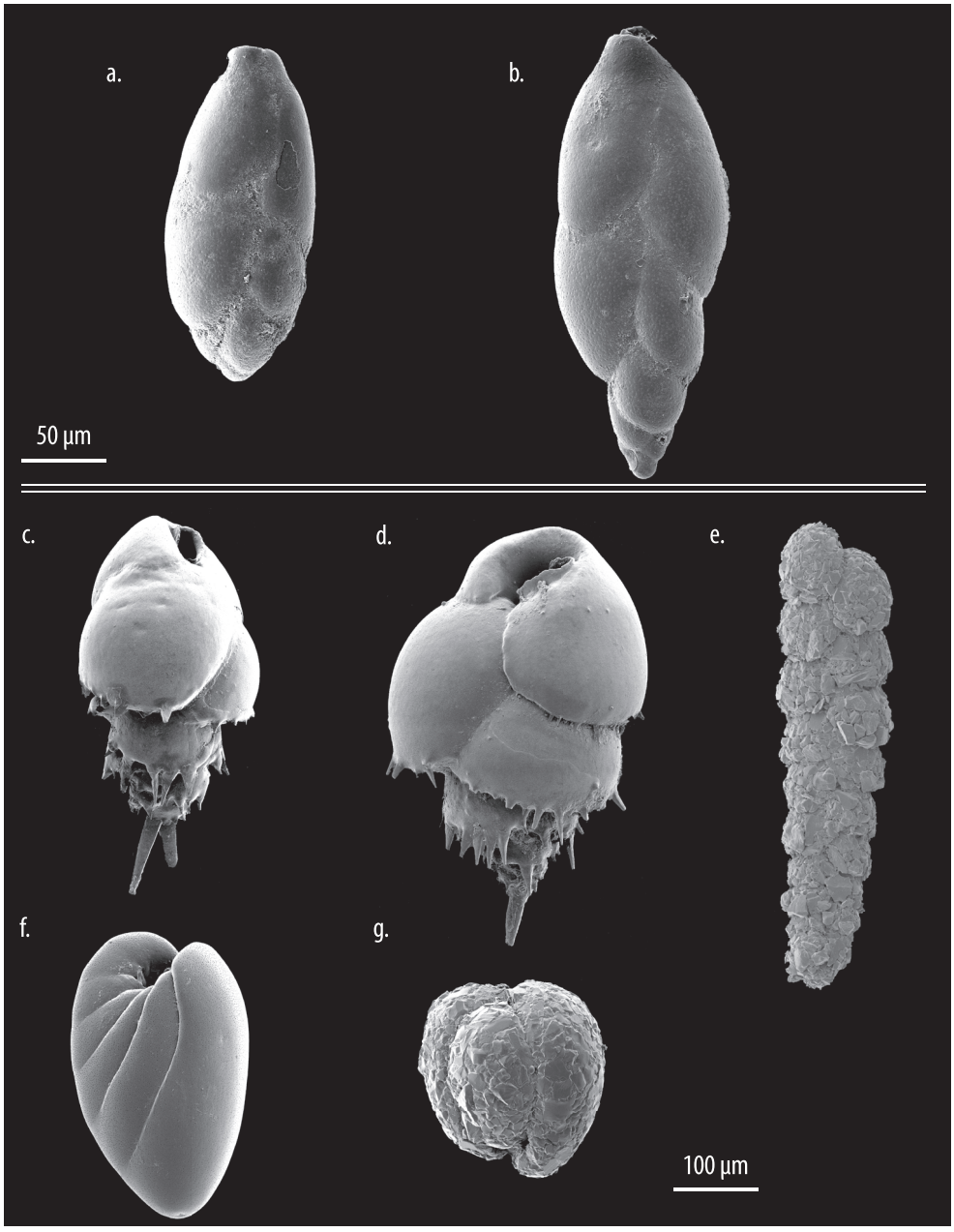



**Plate 6.1** a. *Stainforthia fusiformis*, b. *Stainforthia fusiformis* c. *Bulimina marginata,* d. *Bulimina marginata*, e. *Textularia earlandi*, f. *Nonionella* spp., g. *Adercotryma glomerata*

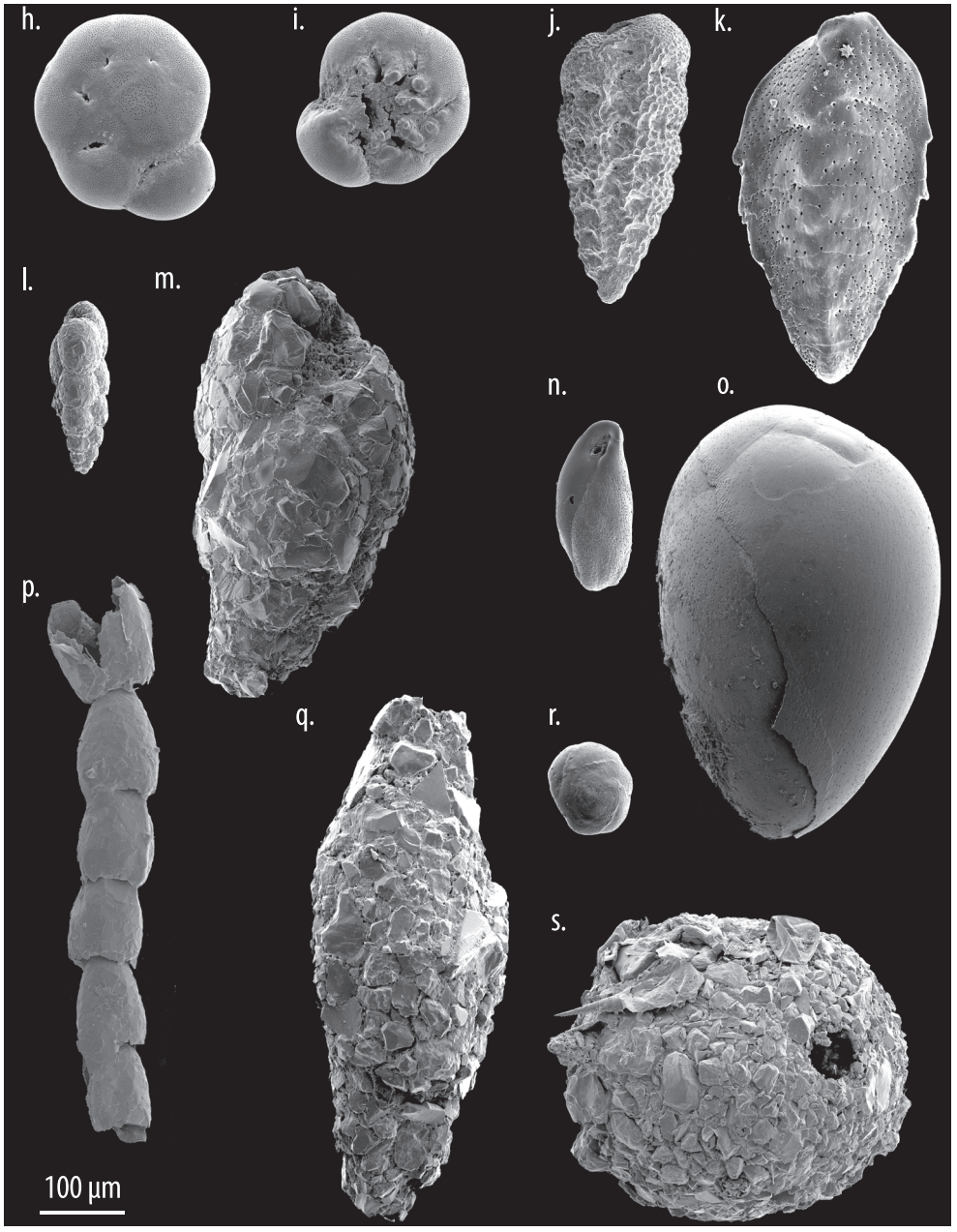


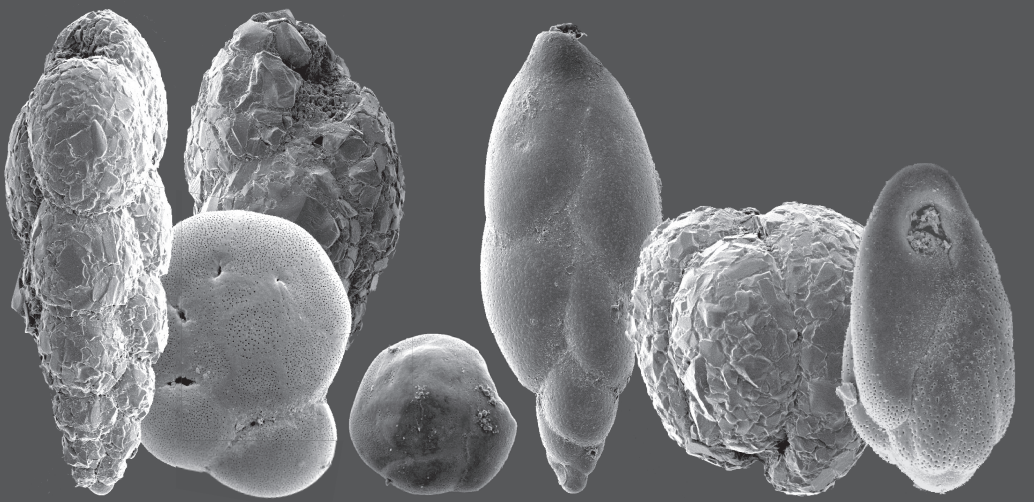

**Plate 6.2** h. *Ammonia beccarii* (spiral side), i. *Ammonia beccarii* (umbilical side), j. *Bolivina plicatella*, k. *Bolivina dilatata*, l. *Eggerella advena*, m. *Eggerella scabra*, n. *Buliminella elegantissima*, o. *Globobulimina turgida*, p. *Leptohalysis scottii*, q. *Reophax fusiformis*, r. *Rosalina bradyi*, s. *Saccammina sphaerica*

| Experimental core | Date of harvest | Abundance | Abundance | Abundance | Abundance | Abundance | Abundance |
|---|---|---|---|---|---|---|---|
| | | **total** | *S. fusiformis* | *B. marginata* | *A. glomerata* | *Nonionella spp.* | *T. earlandi* |
| C (1) | 16-4-2010 | 11792.5 | 4357.2 | 2298.2 | 1227.1 | 987.9 | 727.9 |
| C (2) | 16-4-2010 | 16409.7 | 4544.4 | 2890.9 | 2391.8 | 1081.5 | 2766.2 |
| C (3) | 4-6-2010 | 14579.5 | 4450.8 | 3317.3 | 1913.4 | 1216.7 | 717.5 |
| C (4) | 13-7-2010 | 12343.7 | 4076.4 | 2745.4 | 1851.0 | 1143.9 | 291.2 |
| B-near (1) | 26-3-2010 | 11601.5 | 3071.0 | 2350.6 | 1611.3 | 853.0 | 1251.1 |
| B-near (2) | 31-5-2010 | 9724.7 | 3014.1 | 2578.1 | 1516.5 | 758.3 | 246.4 |
| B-near (3) | 4-6-2010 | 12757.8 | 3241.6 | 2597.1 | 2009.4 | 473.9 | 379.1 |
| B-near (4) | 10-7-2010 | 7336.2 | 2085.2 | 2388.5 | 1459.7 | 303.3 | 75.8 |
| B-far (1) | 26-3-2010 | 10507.3 | 3450.7 | 1736.4 | 923.6 | 960.6 | 1123.1 |
| B-far (2) | 31-5-2010 | 12295.5 | 3354.7 | 2734.0 | 1832.5 | 1137.9 | 990.1 |
| B-far (3) | 4-6-2010 | 10270.9 | 1906.4 | 2401.5 | 1492.6 | 1041.9 | 339.9 |
| B-far (4) | 10-7-2010 | 10581.2 | 3266.0 | 2660.1 | 1536.9 | 827.6 | 487.7 |

**Appendix 6.1** Sampling scheme and foraminiferal densities (#/1000 $cm^3$) per core: total standing stock, *Stainforthia fusiformis*, *Bulimina marginata*, *Adercotryma glomerata*, *Nonionella spp.* and *Textularia earlandi*

| | | Pooled groups | | B-far vs B-near | | B-near vs C | | B-far vs C | |
|---|---|---|---|---|---|---|---|---|---|
| | | abundancies | rel. abundancies | abundancies | rel. abundancies | abundancies | rel. abundancies | abundancies | rel. abundancies |
| a. Average dissimilarity (%) | | 16.73 | 17.77 | 14.58 | 19.03 | 19.48 | 18.26 | 16.12 | 16.03 |
| b. Species | 1 | *S. fusiformis* | *S. fusiformis* | *S. fusiformis* | *B. marginata* | *S. fusiformis* | *T. earlandi* | *S. fusiformis* | *S. fusiformis* |
| | 2 | *B. marginata* | *T. earlandi* | *B. marginata* | *S. fusiformis* | *B. marginata* | *B. marginata* | *B. marginata* | *T. earlandi* |
| | 3 | *E. scabra* | *B. Marginata* | *E. scabra* | *T. earlandi* | *E. scabra* | *S. fusiformis* | *E. scabra* | *B. marginata* |
| | 4 | *Nonionella spp.* | *Ammonia spp.* | *Elphidium spp.* | *Ammonia spp.* | *T. Inflata* | *A. glomerata* | *A. glomerata* | *Ammonia spp.* |
| | 5 | *A. glomerata* | *A. glomerata* | *T. Inflata* | *A. glomerata* | *Nonionella spp.* | *Ammonia spp.* | *Nonionella spp.* | *A. glomerata* |

**Appendix 6.2** SIMPER analyses of the contribution per taxa to observed differences between foraminiferal assemblages.



CHAPTER 7

# THESIS SUMMARY & SYNTHESIS

# ECOSYSTEM RECOVERY AFTER HYPOXIA: WHAT CAN FORAMINIFERA INDICATE?

G.M. Brouwer

## 7.1 HYPOXIC DISTURBANCE AND ECOSYSTEM FUNCTIONING

The many resources and services provided by coastal ecosystems (e.g. food, fertile soils), make these areas valuable habitats for marine life and human occupation. Expanding human population sizes and the associated increase of human exploitation of coastal zones has made these areas prone to perturbations. Human settlement has affected coastal marine ecosystems by overexploitation, habitat fragmentation and pollution (e.g. Small and Nicholls, 2003; Lotze et al., 2006). In recent decades (human-induced) hypoxia became one of the prominent phenomena to cause destabilisation of marine communities. The occurrence of low oxygen availability in marine bottom-waters spreads worldwide in frequency, severity and size; it increasingly affects the functioning of, and the services provided by marine ecosystems (e.g. Wu, 2002; Diaz and Rosenberg, 2008; Zhang et al., 2010; Rabalais et al., 2010; Steckbauer et al., 2011; Riedel et al., 2013). Positive feedback mechanisms often amplify the impact of hypoxia and induce a nonlinear response of ecosystem functioning during and after disturbance (e.g. Conley et al., 2009; Zhang et al., 2010).

A dynamic interplay of global processes – for instance rising temperatures – and local conditions – such as elevated nutrient loads and stratification of water masses – regulate the impact of hypoxia and the restoration potential of hypoxia-affected ecosystems (e.g. Lotze et al., 2006; deYoung et al., 2008; Rabalais et al., 2010; Steckbauer et al., 2011). These processes are driven by natural or anthropogenic forcing, but most commonly a combination of the two; the relative contribution of each is often difficult to separate (deYoung et al., 2008). Only very few marine ecosystems that were impacted by hypoxia in the recent decades have shown signs of recovery (Diaz and Rosenberg, 2008). Restoring the oxygen concentrations towards a more natural situation does not necessarily culminate in the return of lost species (e.g. Suding et al., 2004; Byers et al., 2006). Recovery of harmed ecosystems to the status preceding the disturbance may never be reached, and the patterns of recovery are generally different from the reverse patterns of deterioration (Lotze et al., 2006; deYoung et al., 2008; Steckbauer et al., 2011). A more hostile exposure to hypoxia results in a higher dissimilarity between the pathway of hypoxia-induced ecosystem deterioration and the recovery pattern after the oxygen concentration has been restored to pre-hypoxia conditions: hysteresis-like responses have been reported after severe hypoxia (Diaz and Rosenberg, 2008; Van Colen et al., 2012). The predictability of the effect of management and restoration effort is hindered by this nonlinearity in response and recovery pathways (Scheffer and Carpenter, 2003; Suding et al., 2004), thus posing an extra hurdle to convince policy makers to take appropriate action. In order to prevent the occurrence of hypoxic disturbance and to

stimulate recovery of affected ecosystems, insight needs to be gained on the recovery pathways and the coupling of abiotic and biotic mechanisms within the ecosystems.
We investigated hypoxia-induced foraminiferal dynamics during ecosystem recovery to gain better insight in the use of foraminifera as indicator species for ecosystem status and to improve our understanding of ecosystem development. Living foraminifera are increasingly acknowledged for their value as indicators for ecosystem health. Lowering of the oxygen concentration in marine bottom-water instigates a more shallow oxygen-penetration depth in marine sediments, thus compressing or even eliminating tiers of within-sediment microhabitats. Benthic foraminifera that thrive in oxygenated sediment (the vast majority) respond to this by migration towards more beneficial (oxygenated) habitats such as the sediment surface (Alve and Bernhard 1995; Geslin et al., 2004) or biogenic structures made by tube building ecosystem engineers (Linke and Lutze, 1993; Alve and Bernhard, 1995). Apart from migration of individual foraminifera, the diversity in hypoxia tolerance among foraminiferal species, combined with their generally short generation time, induces a fast response of the foraminiferal assemblage to oxygen depletion, reflected in both the total foraminiferal density and the assemblage composition.
In addition to the oxygenation of marine bottom-waters, macrofaunal ecosystem engineers influence the biogeochemical composition of the sediment pore-water, and thus the composition of the foraminiferal assemblage. Through activities such as bioturbation (reworking) and bio-irrigation (ventilation), macrofauna increase the biogeochemical heterogeneity of marine sediments (e.g. Aller, 1983; Meysman et al., 2005). Macrofaunal mediated deepening of the oxygen-penetration depth and the transport of food to subsurface sediment layers has been discussed to enlarge the availability of niches within the sediment – both in numbers and size – suitable for foraminiferal occupation (Reise, 1981; Bouchet et al., 2009). Since macrofaunal species are among the benthic organisms most sensitive to hypoxic disturbance (Josefson and Widbom, 1999), the occurrence of hypoxia almost always alters the macrofaunal community (Gray et al., 2002; Wu, 2002). Apart from reduced growth and feeding activities, hypoxic conditions stimulate the replacement of sensitive species by more tolerant macrofaunal species (Wu, 2002; Vaquer-Sunyer and Duarte, 2008). Eventually, prolonged and severe hypoxia induces macrofaunal mass mortality. After a hypoxia-induced collapse of the macrofaunal community, the macrofauna-mediated increase of sediment heterogeneity is no longer maintained. Therefore, in addition to the direct influence of a reduced oxygen availability in marine bottom-waters it also indirectly (via macrofauna) affects the distribution of benthic foraminifera and their microhabitats. With field and laboratory experiments we aimed to improve our understanding of the contribution of direct and indirect effects of hypoxia – and the combination of both – on foraminiferal communities in coastal ecosystems. These insights will help to improve their role as indicator species for ecosystem health in hypoxia-affected marine coastal areas.

## 7.2 THESIS SUMMARY

We conducted several studies to improve the usability of foraminifera as indicator species for ecosystem health, with a strong emphasis on ecosystem recovery after hypoxia. The next paragraphs comprise a summary of the results and conclusions presented in the separate chapters of this thesis.

In chapter two and three we reported on the hypoxia-induced dynamics of the foraminiferal community from Lake Grevelingen, an enclosed salt water lake in the South-Western part of The Netherlands. Lake Grevelingen is exposed to seasonal, spatially patchy hypoxia; the influence of riverine water outflow and North Sea water inflow is blocked by dams and this inhibits mixing of water especially during warm summer months. The arising stratification of the water column

instigates the occurrence of hypoxia and that affects particularly the benthic community in the deep gullies. The nowadays nearly annual repetition of seasonal hypoxia has resulted in a local absence of macrofauna and a strong decrease in the number of foraminifera in the deep gullies indicating the most severe state of decay. In CHAPTER TWO we evaluated the species-specific short-term dynamics over time within the foraminiferal community during recovery of hypoxia-affected sediments. To this purpose, hypoxia-affected and unaffected sediments were combined in microcosms and kept under ventilated laboratory conditions. In this microcosm experiment, we observed that re-oxygenated hypoxia-affected sediments were colonised by foraminifera migrating from neighbouring unaffected sediments. The mean foraminiferal abundance (>63 µm) in the upper centimetre of the hypoxia-affected sediments increased during the experimental period of 10.5 weeks from 15% to approximately 46% of the abundance observed in unaffected control sediments. The dynamics in density and composition of the foraminiferal assemblages indicated a species-specific response and the existence of sequential stages during recovery of the foraminiferal assemblage in the re-oxygenated sediments. Species with relatively large test-sizes, *Ammonia beccarii* and *Elphidium excavatum*, were the first to recolonise the hypoxia-affected sediments. In the second stage, the small-sized species *Hopkinsina pacifica* and *Stainforthia fusiformis* increasingly contributed to the colonisation of hypoxia-affected sediments. Our observations suggest that foraminiferal test size is related to the velocity of locomotion, with higher movement speeds for larger specimens. Furthermore, our results confirmed species-specific relations with abundances and environmental circumstances: especially *E. excavatum* seemed able to respond opportunistically to the sudden dilution of foraminiferal density arising after foraminiferal migration from unaffected to formerly affected sediments in the microcosms, and the concurrent high availability of accumulated detrital material in these heterogeneous cores, while in contrast *A. beccarii* and *S. fusiformis* revealed a more stable density in the unaffected sediments of the heterogeneous and homogeneous cores over the course of the experiments. Among the numerously dominant species, *Trochammina inflata* was most sensitive to disturbance and seemed therefore to be indicative of healthy, undisturbed sediments. In this chapter we revealed that a healthy neighbouring foraminiferal assemblage facilitates short-term recolonisation of re-oxygenated sediments. Expanding on this, in CHAPTER THREE we discussed the contribution of separate foraminiferal recolonisation pathways for short-term (10.5 weeks) recovery and we investigated the vertical microhabitat distribution after prolonged hypoxic disturbance. The vertical dynamics in foraminiferal density and assemblage composition were considered in the assessment of ecosystem health. As in the previous chapter, hypoxia-affected sediments were neighboured by unaffected sediments and homogeneous control cores comprising unaffected sediments were used. In addition to the sediment types studied in chapter two, in chapter three we analysed the foraminiferal dynamics with sediment depth in homogeneous cores containing hypoxia-affected sediments kept either with or without oxygen in the overlaying water. At the end of the experimental period, the hypoxia-affected core-halves neighboured by unaffected sediments comprised 38% of the foraminiferal total standing stock in (homogeneous, unaffected) control cores. Without neighbouring unaffected sediments this foraminiferal density in hypoxia-affected sediments was a mere 6% of control densities when kept aerated, and only 5% when anoxia persisted. Active migration rather than recovery from within the originally affected sediment seemed to have dominated short-term foraminiferal recolonisation. Despite comparably low densities in both types of homogeneous hypoxia-affected sediment cores (with and without oxygenated bottom-water), the assemblage composition was quite dissimilar. Although recovery of foraminifera from within the homogeneous hypoxia-affected cores did not result in a notable abundance increase, it seemed to contribute to a shift of the assemblage composition. The composition of the foraminiferal assemblage in the aerated homogeneous hypoxia-affected cores resembled those of the unaffected

control cores, whereas foraminiferal composition in the cores that were kept anoxic was very dissimilar. Aeration of hypoxia-affected sediment seemed to cause replacement of the species *Hopkinsina pacifica* by the species *Ammonia beccarii* and *Elphidium excavatum*. The replacement of species in absence of an overall abundance increase suggests that recovery from within the sediment needs a longer time-scale to significantly contribute to foraminiferal recovery. Compared to the field situation, our microcosm experiments over-estimate the importance of active migration (because of the close proximity between hypoxia-affected and unaffected sediments in our experiment, defined by the maximum width of 7 cm of the experiment cores) and underestimate the potential role of passive, waterborne transport of propagules and activation of already available dormant ones (because of the short time span and the absence of passive water transported in our experiments).

In the next chapters (four and five) we studied the effects of sediment hypoxia, deliberately induced during winter or late spring, on an intertidal flat in the Scheldt Estuary, The Netherlands. This field experiment enabled us to set the observed foraminiferal dynamics in the context of companion studies that reported on the development of several different ecosystem properties (e.g. macrofaunal and nematode recolonisation, microbial and microalgal abundances) during recovery after hypoxia crises. In CHAPTER FOUR we discussed the differential response of intertidal foraminifera to ecosystem recovery succeeding hypoxia. The foraminiferal assemblage of the Scheldt Estuary comprised mainly three benthic species: *Ammonia beccarii*, *Haynesina germanica* and *Elphidium excavatum*. Reassembly patterns of foraminiferal assemblages and their relation with sediment biogeochemistry, micro-organisms and faunal elements were investigated in the upper centimetre of the sediment 2 and 5 months after the end of hypoxia. The foraminiferal species showed species-specific and complex patterns of development during ecosystem recovery. Their species-specific abundance and estimated biovolume – a measure combining numerical abundance and observed test sizes – varied depending on the timing of disturbance (winter vs. spring hypoxia) and the duration of recovery (2 vs. 5 months). The expected positive impact of macrofaunal ecosystem engineering, explained at the beginning of this synthesis, was not observed in all patches. The abundance of *H. germanica* in the disturbed patches was inversely related to macrofaunal abundance whereas it was positively related in control patches. Instead, *Ammonia beccarii* seemed to be driven by the microphytobenthos surplus due to the lack of grazing after hypoxia-induced mortality of the macrofauna. In CHAPTER FIVE we investigated the effect of the deliberately induced hypoxia and subsequent ecosystem recovery on foraminiferal diet shifts and foraminiferal population dynamics. To this end, $^{13}C$-labelled bicarbonate and glucose had been added to the sediments at certain times during the ongoing field experiment. Subsequently, label concentrations in different trophic compartments of this benthic ecosystem (including foraminifera) were traced over time, and foraminiferal test-sizes were measured to infer aspects of population dynamics. As in chapter four, hypoxia-treated and undisturbed patches were compared to distinguish natural (seasonal) fluctuations from hypoxia-induced responses, and the effect of timing of disturbance and duration of recovery was investigated. With regard to their diet and population dynamics, the dominant foraminiferal species *Ammonia beccarii* and *Haynesina germanica* responded differentially and generally inversely to progressive stages of ecosystem recovery succeeding hypoxia; higher fluctuations in the foraminiferal diets and population dynamics were found in the recovering patches compared to the controls. Label-inferred diet estimates confirmed that the development of a dense mat of benthic algae in April, during the first month of ecosystem recovery after the winter hypoxia, was likely profitable to *A. beccarii*. This food pulse may have stimulated reproduction as well as growth – as indicated by two distinctly visible cohorts in the test-size distribution after two months: a relative high proportion of small- and large-sized specimens in the recovery patches of June compared to

controls. Enhanced reproduction itself was strongly linked to the subsequent dietary shift to bacteria. The distribution of test dimensions of *H. germanica* indicated that this species had less fluctuation in population structure during ecosystem recovery but possibly reproduced in response to the induced winter hypoxia. Bacteria seemed to consistently contribute more to the diet of *H. germanica* than diatoms. Based on chapters four and five, we concluded that timing of disturbance seemed to have a higher impact on the abundance, biovolume, feeding behaviour and population structure of both *Ammonia beccarii* and *Haynesina germanica*, than whether the sediment had been recovering for 2 or 5 months.

In CHAPTER SIX we evaluated the impact of artificial burrows in a laboratory microcosm experiment using gas-permeable vertical burrows. Density, assemblage composition and the vertical distribution of benthic foraminifera from the Swedish Gullmar Fjord were studied. By mimicking only the geochemical aspects of a bioturbation-derived increase in sediment heterogeneity, we aimed to contribute to disentangling the effects of various processes that instigate the dynamic relation between macrofaunal ecosystem engineering and foraminifera. The lack of differences in food availability, $O_2$ penetration depth and dissolved nitrate between the experiment and control core was reflected by the absence of an overall strong positive effect of the artificial burrows on foraminiferal abundances and species diversity. Despite the fact that the dissimilarity in pore-water chemistry was restricted to the visually observed reddish-brown colour of the sediment surrounding the burrows, and the co-occurrence of elevated $Fe^{2+}$ concentrations – interpreted as Fe micro-cycling observed in pore-water near the burrows – the foraminifera derived from the Swedish Gullmar Fjord responded species-specifically, and differentially in the experimental cores with and without burrows. Interestingly, the presumed impact of the burrows and the observed foraminiferal dynamics corresponded to the reported patterns of the dominant species during hypoxic, intermediate or stable oxygenated fjord conditions. *Stainforthia fusiformis* dominated over stability-requiring species in habitats with frequent oxygen disturbance, this species excels in population recovery after disturbance. *Bulimina marginata*, *Adercotryma glomerata* and *Textularia earlandi* inhabited the surface sediments, regardless of the treatment. Within the group of equilibrium species, *B. marginata* was slightly less sensitive to oxygen depletion than *A. glomerata*. In-between the oxygen-stress tolerant and sensitive species *Nonionella spp.* displayed an intermediate ecological strategy. The impact of artificial burrows on foraminiferal dynamics – with simplified ecosystem interactions – were set in the context of other (*in situ*) studies reporting on the impact of tube-building macrofauna or biogenic structures on meiofaunal dynamics. Dissimilarities were discussed to be related to food web interactions and habitat-dependent differences.

## 7.3 FORAMINIFERA AS INDICATOR SPECIES GAINED INSIGHTS

The aim of the research presented in this thesis was to validate and improve the applicability of foraminifera to indicate ecosystem health and development during and after hypoxic disturbance in coastal ecosystems. The following sections include an integration of the reported conclusions; the implications for the role of foraminifera as bioindicator species will be discussed.

### 7.3.1 GENERAL SPECIES TOLERANCE AND MICROHABITAT DISTRIBUTION

The use of sediments and foraminiferal assemblages derived from three different habitats (and separated geographical locations) provided the opportunity to compare the impact of oxygen fluctuations on foraminiferal dynamics among different environments comprising assemblages dissimilar in density and species composition. We examined an enclosed salt-water lake (Lake

Grevelingen, The Netherlands), an intertidal mudflat in an estuarine system (Scheldt Estuary, The Netherlands) and a Swedish saline fjord (Gullmar Fjord) connected via the Skagerrak with the North Sea and the Atlantic Ocean. The foraminiferal community in the hypoxia-affected (deep) parts of Lake Grevelingen contained hardly any living foraminifera. In contrast, sediments outside the direct influence of (seasonal) hypoxia in Lake Grevelingen had a relative high species diversity compared to the geographically nearby, but typically intertidal Scheldt Estuary mudflats, where the foraminiferal community had its lowest species diversity. At the latter intertidal locality, only few species seemed to be able to withstand the natural, but – even on a (semi)diurnal scale – severe fluctuations in environmental properties, such as temperatures and salinity. The Swedish Gullmar Fjord had a foraminiferal assemblage that comprised a diverse array of species, despite the occurrence of field hypoxia in the year before we collected the sediment (Polovodova Asteman and Nordberg, 2013). Lake Grevelingen and the Gullmar Fjord have in common that alternations between hypoxic and oxygenated conditions occurred in the recent past; these alternations have dominated the foraminiferal dynamics locally over the past 50 to 100 years.

Apart from general conclusions, such as the observation that foraminifera are able to respond fast to even small oxygen fluctuations, more specific conclusions can be drawn based on our results. Despite the obvious difference between the marine environments, several species were found in more than one or all three of the geographical locations enabling a comparison of their dynamics among the environmental settings. For other species a distinct dependence on specific environmental conditions was found. By combining results derived from the various experiments, the prevalent species could be associated with environmental conditions and, or stages of ecosystem development. Not surprisingly, a high tolerance for oxygen depletion was observed for species such as *Stainforthia fusiformis*, *Hopkinsina pacifica* and to a smaller extent *Nonionella* spp.. Species such as *Ammonia beccarii, Haynesina germanica* and *Elphidium excavatum* seemed to form a group of early recolonisers of sediment that had been exposed to hypoxic conditions and were especially able to withstand high fluctuation in environmental conditions on very short (e.g. seasonal, diurnal) time scales. *Elphidium excavatum* was not only an early coloniser, this species showed the ability to respond opportunistically to an increased availability of labile organic matter in absence of the main consumers of this food source. Species that revealed to be relatively stress-sensitive were e.g. *Trochammina inflata*, *Adercotryma glomerata* and, surprisingly, *Bulimina marginata*.

In chapters three and six we examined the effect of oxygen fluctuations on the vertical distribution of benthic foraminifera. Commonly, investigation of benthic foraminiferal dynamics for ecosystem surveys is based on fluctuations in the upper centimetre of the sediment (Bouchet et al., 2012; Schönfeld et al., 2012). Studying the vertical structure of the foraminiferal community may help to understand foraminiferal patterns observed in studies limited to that upper centimetre of the sediment. In chapter two – where we observed the foraminiferal dynamics in the upper centimetre – the hypoxia-affected assemblages neighboured by healthy assemblages recovered to 46% of the foraminiferal density observed in healthy control assemblages. Whereas in chapter three – where we investigated the foraminiferal dynamics over on average 10 cm sediment depth – the foraminiferal density in this sediment type was 38% of the density in the unaffected control sediments. The upper centimetre of the hypoxia-affected sediments of the heterogeneous cores (A(AV)) comprised 73% of the total foraminiferal standing stock in these core halves. This relatively higher increase in abundance in the upper centimetre of the sediment indicates that the dynamics were most prevalent near the sediment surface. Nonetheless, one has to bear in mind that an important stage in ecosystem recovery after hypoxia crises is the restoration of the macrofaunal community. Recolonizing macrofauna will

– in addition to the deepening of the oxygen-penetration depth after restoration of bottom-water ventilation – change the vertical oxygen distribution pattern of the sediment pore-water. In chapter six – where we examined the geochemical aspects of a bioturbation-derived increase in sediment heterogeneity – the vertical distribution of the foraminiferal microhabitats over 8 cm sediment depth differed mainly in the deeper sediment layers. The legitimacy to restrict the investigation of the foraminiferal dynamics in an ecosystem survey to the upper centimetre of the sediment seems to depend on the status of ecosystem recovery, the extent of oxygen fluctuations and the required accuracy.

### 7.3.2 ECOSYSTEM INTERACTIONS

In marine sediments, ecosystem engineers modify their environment by mixing particles and solutes; they redistribute resources as food and oxygen. This directly and indirectly structures communities. Ecosystem engineers create and maintain habitats suitable for a specific suite of associated organisms (Jones et al., 1994, Wright and Jones, 2006). The net impact of ecosystem engineers depends on the magnitude of both positive and negative ecological interactions (Jones et al., 1997). On small temporal and spatial scales some species may benefit from newly formed niches constructed by ecosystem engineers, while others do not profit or are even harmed by their activities (Jones et al., 1997; Wright et al., 2004; Hastings et al., 2007). In our experiments the potential contribution of macrofauna to the foraminiferal dynamics was limited to the role they played in the system. This ranged from no macrofauna (Lake Grevelingen, Chapters two and three), via mimicking the geochemical aspects of a bioturbation-derived increase in sediment heterogeneity (Gullmar Fjord, Chapter six), to the concurrent restoration of macrofauna (and meiofauna and microbes) after hypoxia (Scheldt Estuary, Chapter four and five). Macrofaunal recolonisation in hypoxia-disturbed patches in the Scheldt Estuary did not positively impact foraminiferal abundances and species diversity (Chapter four). Nor did the insertion of vertical artificial burrows enhance foraminiferal densities in the sediment from the Gullmar Fjord. Interestingly, the presumed impact of oxygen diffusion along the artificial burrows and the observed spatial foraminiferal dynamics corresponded to the reported patterns of the dominant species on a temporal scale during specific field conditions (Chapter six). Several environmental aspects – e.g. food web dynamics and habitat-dependent parameters such as sediment permeability – were discussed to elucidate dissimilarities between the results gained from our experiments and those reported in literature. Our results suggest that the net effect of macrofaunal species on foraminiferal densities and microhabitat distribution differs between unaffected and recovering ecosystems. The balance between negative (predation on foraminifera, physical disturbance, competition for food, etc.) and positive interference of macrofauna (replenishment of oxygen and food in deeper sediment layers, extra microbial biomass available as food source for foraminifera around macrofaunal burrows, etc.) on foraminiferal dynamics may depend on the composition of the macrofaunal community in successive stages of recovery from the hypoxic disturbance. This conclusion is in line with for example the observation of Widdicombe et al. (2000) that the variability in density and species composition of macrofaunal bioturbating organisms largely determines the structuring effect of ecosystem engineers on infaunal communities. Experimental studies on the influence of specific parameters and complex *in situ* experiments in diverse habitats are both needed to improve the reliability of foraminifera as proxy for ecosystem status.

### 7.3.3 TIMING AND SPATIAL SCALE OF HYPOXIA

In coastal marine ecosystems, seasonal fluctuations – for instance in temperature, light and larval availability, water motion and currents – govern ecosystem state. Since ecosystem state is far from

static, the timing of disturbance and its spatial distribution, together with severity and duration determine the harmfulness of hypoxia on ecosystem functioning and the potential for recovery after the perturbation has ended. The experiments conducted in the Scheldt Estuary revealed that timing of disturbance – in winter or late spring – produced more foraminiferal variability between the experiments than duration of recovery – 2 or 5 months – with respect to the foraminiferal abundances, biovolumes, population structure and food intake at the final sample moment in September. In the same experimental set-up, a similar observation was made for macrofaunal species and discussed in the context of seasonal dissimilarity in larval availability (Rossi et al., 2009). Seasonal variability in foraminiferal abundance and species composition in field settings has been observed (as for example reviewed by Gooday and Rathburn, 1999). In contrast to many macrofaunal elements, foraminiferal dynamics have been related to seasonal variability in oxygen and food availability rather than to a fixed reproduction period. Nonetheless, foraminifera appear to have a specific range of environmental circumstances in which they proliferate and are able to compete with other species (Bradshaw, 1957). If seasonality in the environmental circumstances influences growth and reproduction, timing of disturbance may also impact the recovery pattern of foraminifera. Yet, just as *Streblus beccarii* var. *tepida* (Bradshaw, 1957 – potentially the same species as our *Ammonia beccarii*), presumably also other foraminiferal species have the ability to adjust growth, fecundity and generation time to environmental circumstances contributing to its capacity to withstand and recover more swiftly after perturbations than macrofaunal species. Nonetheless, the results from Lake Grevelingen (Chapters two and three) show that the amount of time available for affected ecosystems to recover from summer hypoxia before the growing season ends may be (too) limited for full recovery.

Our experiments indicate that besides timing of hypoxic disturbance, also its spatial distribution influences the opportunity to recover affected ecosystems. Short-term (weeks) recovery of foraminifera in re-aerated hypoxia-affected sediments from Lake Grevelingen was more successful in the presence of a healthy neighbouring assemblage compared to those without. Since we observed that the recovery of a foraminiferal community from within affected sediments is very minimal, the availability of nearby healthy ecosystems providing colonisers – and thus the spatial distribution of hypoxia – may determine the opportunity for (short-term) community recovery. If this proposition is correct, colonisation processes may deviate between patchy and homogeneous hypoxia-disturbed areas. This will affect the recovery potential and, with the progressing frequency, duration and geographic extent of hypoxia (for instance in Lake Grevelingen), the timing of restoration efforts becomes a major factor for the rate of recovery and its level of success.

### 7.3.4 PAST, PRESENT AND FUTURE

Next to the role of living foraminifera as selected species to indicate present-day ecosystem state, their fossilised congeners have been used since Emiliani (1955) by earth scientists to study paleoclimates. Studying the response of living foraminifera to fluctuations in environmental conditions, and comparing these responses among diverse habitats from several geographical areas does not only improve the accuracy of foraminifera as bioindicators for modern ecosystem functioning, it may also help to more accurately interpret foraminiferal dynamics reflected by fossilised communities. The proxy value of foraminifera to reflect (sub-recent, past and paleo-) environmental conditions does not only rest on the distribution pattern and species composition of the assemblage. Information on several (recent, past and paleo) environmental conditions can be derived from analysing the chemical composition of the foraminiferal test: the composition of the test depends on the chemical environment during foraminiferal growth.

In this thesis we studied foraminiferal dynamics instigated by abiotic and biotic properties. We tried to unravel the single and combined importance of biotic and abiotic processes on foraminiferal dynamics that jointly govern ecosystem functioning during and after hypoxic disturbance. Foraminifera fill an intermediate position in-between microbes and macrofauna, but seemingly have a minimal influence on both (Chapter five). We observed that foraminiferal dynamics in sediments where biota were recovering from hypoxic disturbance developed in an opposite direction compared to untreated controls where natural (seasonal) fluctuations governed ecosystem status. There was a (large) deviation between the time needed to re-establish the pore-water chemistry and that needed to restore the faunal assemblage towards the controls in the Scheldt Estuary (Chapter four and five).

A gradual change in the oxygenation of marine bottom-waters (acting as a driver of ecosystem development) has been reported to induce a non-linear response of ecosystem functioning (e.g. Conley et al., 2009; Zhang et al., 2010). Moreover, severe hypoxia may even provoke a hysteresis-like response (Diaz and Rosenberg, 2008). In these situations a small change in the driver's state can instigate a large shift in ecosystem status. At the point of transition, a relatively small decrease of the oxygen concentration of the bottom-water (or the frequency or duration of recurring hypoxia on longer timescales) induces an ecosystem to abruptly shift towards a contrasting state. Characteristic of hysteresis is the irreversibility of the catastrophic state shift: the earlier state is not recovered by an equally small change of the driver in the opposite direction (here re-oxygenation of the bottom-water, or decreased frequency or duration of hypoxia; Scheffer and Nes, 2004). Consequently, one specific state of bottom-water oxygenation regime may therefore belong to either a healthy or a deteriorated ecosystem status – depending on whether the development follows a degradation or recovery pathway towards the threshold. Moreover, as the system comes closer to this point of transition, ecosystem resilience lowers and recovery from a perturbation becomes slower (e.g. Veraart et al., 2011). Even although the exact oxygen concentration, hypoxia frequency, etc., at the tipping point might not be known until the system has collapsed, this slowdown in the rate of recovery after perturbations gives valuable information on whether the system is approaching a tipping point.

In the light of the anthropogenic global change problems our planet is currently facing, and against the background of the gradual and continuous environmental change we thus produce, there is a rapidly increasing demand for methods to predict when we are approaching catastrophic tipping points in natural systems (e.g. Barnosky et al., 2012). Since fossil foraminifera provide an unparalleled source of paleo-environmental information (e.g. because of their test chemistry proxy value) and at the same time their fossil assemblages provide a record of community dynamics (i.e. biological system state), they potentially provide an excellent model to develop hysteresis detection methods. If we carefully study foraminiferal fossil records that cover catastrophic community state shifts and their aftermath (e.g. the sub-recent Gullmar Fjord fossil record) and if we assume a good foraminifer-based proxy for the physical or chemical environmental parameter that may bring about those catastrophic shifts, then in theory we can quantify the gradual environmental change in the very same material that gives us information about the variations in the biological part of the system prior to the catastrophic state shift and the long-term developments afterwards. By studying the response and recovery rate of the ecosystem (reflected in the foraminiferal assemblage composition) to fluctuations of the oxygenation of the bottom-water (reflected in the test chemistry) we may become able to more precisely deduce the distance of a given ecosystem status towards the point where a catastrophic shift will occur. This would be useful to interpret changes in both in modern and paleo-ecosystems. Furthermore, this information is crucial for policy makers to be able to intervene with managerial action before a tipping point will be passed and the ecosystem collapses.

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# INTRODUCTIE & SAMENVATTING IN HET NEDERLANDS

# ECOSYSTEEM HERSTEL NA ZUURSTOFDEFICIËNTIE; WAT KUNNEN FORAMINIFEREN ONS VERTELLEN?

G.M. Brouwer

## ALGEMENE INLEIDING

### FORAMINIFEREN

Het phylum der foraminiferen bestaat uit een zeer diverse groep eukaryote organismen. Het merendeel van deze eencellige organismen, die in bijna alle mariene milieus voorkomen, bouwt een schelpje rondom de cel. Hoewel dit schelpje bij enkele soorten kan groeien tot een grootte van meer dan 11 centimeter, bereikt het bij de overgrote meerderheid maximaal een lengte van 1 millimeter (Loeblich and Tappan, 1964). Waar sommige soorten – de zogenaamde 'agglutinanten' – sediment korrels, stekels van zee-egels of zelfs schelpjes van andere foraminiferen gebruiken als bouwmateriaal, construeren weer andere foraminiferen hun schelpje door het uitscheiden van kalk. Door de aanbouw van nieuwe, steeds grotere kamers breidt de foraminifeer zijn huisvesting uit. De vorm en rangschikking van de kamers vertoont een grote variatie tussen soorten. Dit is goed te zien op de foto's (plate 2.1 op pagina 31 en plate 6.1 & 6.2 op de pagina's 112 & 113) gemaakt met behulp van elektronen microscopie. De diversiteit in vorm en rangschikking van de kamertjes wordt binnen het onderzoek naar foraminiferen gebruikt – het onderscheid tussen soorten wordt gebaseerd op de morfologie van de schelp.

De opeenvolgende kamers staan door middel van een kleine opening – de foramen – in verbinding met elkaar. Hierdoor kan de foraminifeer zijn gehele schelp gebruiken als onderkomen. Via de opening die de laatste kamer met de buitenwereld verbindt (de apertuur) kunnen uitstulpingen van het cytoplasma naar buiten. Deze zogenaamde pseudopodiën of 'schijnvoetjes' komen bij alle soorten voor. Dit netwerk van cytoplasmadraden kan zeer snel van vorm veranderen; pseudopodiën kunnen zich op een ogenschijnlijk gecoördineerde manier splitsen, samenvoegen, uit- en intrekken. De foraminifeer gebruikt deze pseudopodiën voor verschillende doeleinden, waaronder het verzamelen van voedsel. Binnen de groep der foraminiferen bestaat een grote verscheidenheid aan voedingsstrategieën. Sommige soorten begrazen algen of bacteriën, andere soorten leven van het opvangen van detritus. Ook het jagen op andere foraminiferen, het houden van algen als symbiont en een parasitaire levensstijl komen binnen de groep voor als aanpak om in de voedselbehoefte te voorzien. Pseudopodiën zijn ook onmisbaar voor voortbeweging. Door sedimentdeeltjes vast te grijpen kunnen foraminiferen zich door en over het sediment slepen. Dit stelt de foraminifeer niet alleen in staat voedsel te verzamelen, maar ook om een geschikte verblijfplaats te vinden in het, in chemisch opzicht, gestratificeerde sediment.

Hoewel er soorten zijn die planktonisch (in de waterkolom) leven, vinden de meeste foraminiferensoorten hun onderkomen op of in het mariene sediment. Deze benthische soorten, waarover het in dit proefschrift gaat, zijn verticaal verspreid over het sediment profiel. Op het sediment leven de epifaunale soorten en in de sedimentlagen bevinden zich de infaunale soorten (Linke en Lutze, 1993). Diep-levende infaunale soorten vinden hun habitat in sedimentlagen die afgesloten zijn van opgelost zuurstof: sommige van hen zijn in staat om voor hun respiratie nitraat in plaatst van zuurstof te gebruiken (Risgaard-Peterson en anderen, 2006).

Verschillende omgevingskarakteristieken bepalen zowel de taxonomische als ruimtelijke verspreiding van de foraminiferen *assemblage* – de soorten die in een associatie met elkaar gevonden worden. Met name de aanwezigheid van zuurstof en voedsel lijkt de verticale en geografische verspreiding te bepalen (Jorissen en anderen, 1995; Van der Zwaan en anderen, 1999). In een dynamisch ecosysteem, zoals mariene kustnabije gebieden, verschilt het aanbod van voedsel en zuurstof sterk op zowel seizoenale, wekelijke als dagelijkse schaal. Elke soort heeft hierbij een eigen voorkeur of tolerantie voor bepaalde omgevingsparameters zoals, naast voedsel en zuurstof, het zoutgehalte en de temperatuur. De gevoeligheid van elke individuele soort verschilt en met name die soorten waarbij de generaties elkaar in hoog tempo kunnen opvolgen lijken in staat om op populatie niveau snel te reageren op veranderingen in de omgeving. Hoewel er tot op heden relatief weinig bekend is over de levenscyclus van de meeste foraminiferen, is er tussen de soorten waarvan dit wel bestudeerd is een grote variatie aangetroffen (Hallock, 1985). De reproductie lijkt bovendien door de foraminifeer vervroegd dan wel uitgesteld te kunnen worden – al naar gelang de omstandigheden gunstig of juist ongunstig zijn. Daarnaast bezitten foraminiferen het vermogen om zich seksueel dan wel aseksueel voort te planten. De seksuele reproductie levert in vergelijking tot de aseksuele reproductie kleinere, maar in genetische opzicht meer diverse nakomelingen op. De afwisseling tussen seksuele en aseksuele reproductie vergroot mogelijk de overlevingskansen van de foraminiferengemeenschap in zeer verschillende omstandigheden. Dit, te samen met de grote verscheidenheid aan de soorten, maakt dat zowel levende als fossiele foraminiferen in grote aantallen gevonden worden in bijna alle sedimenten van mariene oorsprong.

Het schelpje dat door de foraminifeer wordt gebouwd heeft een hoge potentie om als fossiel bewaard te blijven in het sediment. Al sinds het geologische tijdvak dat we kennen als het Cambrium (dat meer dan 500 miljoen jaar geleden begon) worden schelpjes van foraminiferen als fossiel in het sediment opgeslagen. Deze fossiele schelpjes kunnen goed gebruikt worden als informatiebron binnen het onderzoek naar prehistorische ecosystemen. Zowel in het voorkomen van soorten – aantallen en dichtheden – als in de chemische samenstelling van de schelp ligt informatie besloten over (prehistorische) omgevingsparameters. Zo bestaat er een nauw verband tussen het voorkomen van soorten en hun soort-specifieke minimum eisen voor de aanwezigheid van zuurstof. Deze relatie maakt dat foraminiferen binnen de aardwetenschappen al geruime tijd beschouwd worden als goede indicator voor het weerspiegelen van de concentratie van het opgelost zuurstof in het bodemwater (o.a. Bernhard en anderen, 1997; Murray, 2000). Naast het gebruik van foraminiferen voor paleoreconstructies wordt hun waarde als indicatoren voor het functioneren van moderne mariene ecosystemen in toenemende mate onderkend. Het bestuderen van het dynamische verband tussen foraminiferen en hun omgeving is door hun verscheidenheid en veelzijdigheid *an sich* zeer interessant. Maar wellicht van nog grotere waarde is de toepasbaarheid van foraminiferen als mogelijke indicatoren van verstoring van het ecosysteem in bredere zin. Levende foraminiferen kunnen mogelijk

een grote bijdrage leveren aan het onderzoek naar de impact van verstoring en het verloop van het herstel in kustgebieden. Door de hoge stresstolerantie van de groep als geheel behoren foraminiferen vaak tot de organismen die ten tijde van verstoring als laatste verdwijnen en bovendien verschijnen ze vaak als een van de eersten zodra het herstel is ingezet. Doordat de gemeenschap van foraminiferen bestaat uit kleine organismen die snel reageren op veranderingen in het milieu, kan onderzoek met behulp van foraminiferen op zowel kleine ruimtelijke, als korte tijdschaal worden toegepast in zowel de veldsituatie als ook in laboratorium experimenten.

### ZUURSTOFDEFICIËNTIE IN KUSTNABIJE ECOSYSTEMEN

Voor veel faunale groepen zijn mariene kustnabije ecosystemen zeer aantrekkelijke leefgebieden. In de benthische (op en in de bodem levende) gemeenschap bevinden zich zogenaamde *ecosysteem ingenieurs*. Deze ecosysteem ingenieurs hebben een sleutelrol en bepalen in hoge mate de structuur en de samenstelling van de gemeenschap. Deze (macrofaunale) organismen zorgen door het omwoelen (bioturbatie) en het ventileren (bioirrigatie) van het sediment voor aanvoer van zuurstof en voedsel naar de diepere lagen van het sediment. De activiteiten van deze ecosysteem ingenieurs vergroten de heterogeniteit van het sediment: er ontstaat een breder scala aan *niches* (standplaatsen) – zowel in omvang als in aantal. Foraminiferen behoren tot de soorten die profijt kunnen hebben van het ontstaan van deze extra niches.

Niet alleen voor benthische fauna zijn kustgebieden attractief als plek om te vestigen. De bevolkingsdichtheid in kuststreken is drie keer hoger dan het mondiale gemiddelde (Small en anderen, 2003). De hoge bevolkingsdichtheid maakt mariene kustnabije ecosystemen kwetsbaar voor antropogene verstoring. Overexploitatie, habitat fragmentatie en vervuiling komen in toenemende mate voor (Small en anderen, 2003; Lotze en anderen, 2006). In de afgelopen decennia is de verstoring door zuurstofloosheid in mariene wateren wereldwijd sterk toegenomen in zowel aantal, omvang, als duur (Diaz en Rosenberg, 2008). Zuurstofloosheid in het bodemwater en het poriewater van het sediment verstoort mariene gemeenschappen en daarmee het functioneren van het ecosysteem. Wanneer de zuurstofconcentratie in het zeewater daalt tot beneden de 2 mL/L, wordt het bodemleven aangetast. Sommige soorten trekken weg, andere (minder mobiele) soorten proberen hun behoefte aan zuurstof te verlagen (Wu, 2002). Als de zuurstofconcentratie verder daalt, of de verstoring langdurig aanhoudt, kan er een massale sterfte onder met name macrofauna optreden. De door macrofauna geïnitieerde heterogeniteit in het sediment gaat met deze massale sterfte verloren. De afstand waarover zuurstof doordringt in het sediment (de zuurstof penetratie diepte) wordt ondieper. Dit verslechtert de omstandigheden voor soorten die minder gevoelig zijn voor de initiële verstoring. Een dynamisch samenspel tussen mondiale processen – zoals bijvoorbeeld een stijgende temperatuur – en lokale omstandigheden – zoals vervuiling en stratificatie van de waterkolom – reguleert de mate waarin zuurstofloosheid een ecosysteem aantast en vervolgens de mogelijkheid om na het opheffen van de verstoring het ecosysteem te herstellen. Deze processen worden zowel door natuurlijke als antropogene invloeden gestuurd: vaak is het moeilijk een onderscheid te maken tussen de bijdrage van beide.

Een zeer gering aantal ecosystemen toonde in de afgelopen decennia herstel na het optreden van zuurstofdeficiëntie (Diaz en Rosenberg, 2008). Het herstellen van de zuurstofconcentratie leidt niet noodzakelijkerwijs tot een terugkeer van het ecosysteem naar de status van voor de verstoring (Suding en anderen, 2004; Byers en anderen, 2006). Terugkoppelingsmechanismen kunnen het effect van de verstoring versterken. Hierdoor ontwikkelt het ecosysteem zich niet continu met het verloop van de zuurstofconcentratie (Conley en anderen, 2009; Zhang en anderen, 2009). Hoe sterker de verstoring, hoe groter de afstand tussen de ontwikkeling van een ecosysteem tijdens aantasting en herstel;

hysterese-achtige reacties lijken voor te komen. De niet-lineaire respons van het ecosysteem op dalende en stijgende zuurstofconcentraties in het bodemwater bemoeilijkt het doorgronden van de processen die invloed hebben op het verloop van de verstoring en het mogelijke herstel nadat de zuurstofconcentratie weer terug is op een gezond niveau. Om aantasting van mariene ecosystemen door zuurstofloosheid te kunnen voorkomen en herstel van verstoorde ecosystemen te bespoedigen is inzicht nodig in de biotische en abiotische processen en de terugkoppelingsmechanismen die een rol spelen in het functioneren van het ecosysteem.
Met verschillenden experimenten hebben we geprobeerd een bijdrage te leveren aan het inzicht in de directe en indirecte invloed van zuurstofdeficiëntie op de foraminiferengemeenschap in mariene kustnabije ecosystemen. Hiermee willen we de toepasbaarheid van foraminiferen als indicatoren voor het functioneren van voorbije, huidige en toekomstige mariene ecosystemen vergroten. Verkregen inzicht kan helpen bij het verbeteren van de monitoring, het beheren en de bescherming van kunstnabije mariene ecosystemen.

## SAMENVATTING VAN DE EXPERIMENTEN

Voor hoofdstuk twee en drie onderzochten we de soort-specifieke ontwikkeling binnen de foraminiferengemeenschap uit het Grevelingenmeer ten tijde van herstel na aantasting door zuurstofdeficiëntie. Het ecosysteem in het Grevelingenmeer is sterk veranderd na de bouw van de Zeeuwse deltawerken. Om een herhaling van de overstroming zoals die in 1953 in Zeeland plaatsvond te voorkomen, is aan de oostzijde van het Grevelingenmeer de Grevelingendam opgetrokken. Deze dam blokkeert de uitwisseling van water tussen het meer en de rivieren Rijn en Maas. Aan de westzijde sluit de Brouwersdam de verbinding met de Noordzee af. De afsluiting van het Grevelingenmeer zorgt in toenemende mate voor zuurstofdeficiëntie in het bodemwater. Met name tijdens zomerse maanden ontstaat er stratificatie in de waterkolom als de bovenste waterlaag opwarmt. De opwarming van de bovenste waterlaag veroorzaakt een verschil in dichtheid tussen het zuurstofrijke, relatief warme oppervlakte water en het ondergelegen (koude) water. Dit verschil in dichtheid tussen de waterlagen verhindert menging van water binnen de waterkolom. Hierdoor stokt de aanvoer van zuurstof naar de diepere waterlagen. Afbraak van organisch materiaal dat naar de bodem van het meer zinkt verbruikt vervolgens de nog aanwezige zuurstof. Met name in het bodemwater van de diepe geulen treedt hierdoor zuurstofdeficiëntie op. De aantasting van het benthische ecosysteem in die geulen is dusdanig dat er geen macrofauna meer wordt waargenomen. Foraminiferen komen in deze gebieden nog maar voor in heel lage aantallen. De verstoring door zuurstofdeficiëntie bevindt zich in een ver gevorderd stadium. Zolang de zuurstofloosheid beperkt blijft tot de diepe geulen is de ruimtelijke verspreiding fragmentarisch met kleine afstanden tussen aangetaste en onaangetaste gebieden. De aanwezigheid van een naburige gezonde foraminiferengemeenschappen in onaangetaste sedimenten kan mogelijk het herstel van de foraminiferen in aangetaste delen bespoedigen zodra de zuurstofloosheid wordt opgeheven. Om dit te onderzoeken brachten we voor HOOFDSTUK TWEE sedimenten uit de door zuurstofdeficiëntie aangetaste gebieden in microcosmossen samen met sedimenten uit onaangetaste delen van het Grevelingenmeer – deze microcosmossen werden onder zuurstofrijke condities in het laboratorium geplaatst. Gedurende de onderzoeksperiode van 10.5 weken zagen we dat de sedimenten die aan het begin nagenoeg geen foraminiferen bevatten, gedurende het experiment in toenemende mate bevolkt werden door foraminiferen (>63 µm) afkomstig uit de aangrenzende gezonde sedimenten. De gemiddelde dichtheid van de foraminiferen in de bovenste sedimentlaag (0–1 cm) steeg ten opzichte van de dichtheid in de gezonde (homogene) controle sedimenten van 15% aan het begin, tot 46% aan het eind van de

experimentele periode. Er leken verschillenden stadia doorlopen te worden tijdens het herstel. De eerste foraminiferen die de aangetaste sedimenten herkoloniseerden waren de relatief grote individuen van *Ammonia beccarii* en *Elphidium excavatum*. In het tweede stadium (1.5 tot 3 weken na aanvang van het experiment) volgden de kleinere soorten: *Hopkinsina pacifica* en *Stainforthia fusiformis*. Hiermee lijkt de snelheid waarmee foraminiferen voortbewegen zich positief te verhouden tot de grootte van de schelp. *Elphidium excavatum* bleek bovendien in staat opportunistisch te reageren op de migratie van foraminiferen vanuit de gezonde naar aangetaste sedimenten. Deze opportunistische respons – toename in aantal – werd mogelijk gestimuleerd door het hoge aanbod aan voedsel dat opgeslagen lag in het aangetaste sediment en nu beschikbaar kwam als voedsel in de samengestelde microcosmossen. In het daaropvolgende hoofdstuk (HOOFDSTUK DRIE) werd gebruik gemaakt van dezelfde typen microcosmossen als in hoofdstuk twee, maar het experiment werd uitgebreid met typen die waren samengesteld uit twee halve sedimentkernen met door zuurstofdeficiëntie aangetast sediment. Van deze homogene aangetaste microcosmossen werd de helft onder zuurstofrijke en de andere helft onder zuurstofloze omstandigheden gehouden. Na 10.5 week vanaf de start van het experiment werd de samenstelling en de verticale verspreiding (tot ongeveer 10 cm diepte) van de foraminiferengemeenschap in het sediment onderzocht. Hieruit konden we afleiden hoe groot de bijdrage van de verschillenden herkolonisatie trajecten was aan de toename van de foraminiferendichtheid in voorheen aangetast sediment. In aanwezigheid van een gezonde foraminiferengemeenschap in naburig sediment steeg de foraminiferendichtheid in 10.5 week tot 38% van die in de homogene onaangetaste microcosmossen. Zonder aangrenzende gezonde populatie was de dichtheid niet meer dan 6% in de microcosmossen die onder zuurstofrijke omstandigheden waren gehouden en 5% als de zuurstoflоosheid uit de veldsituatie was voortgezet. De resultaten toonden aan dat op de ruimtelijke en temporele schaal van dit experiment, actieve migratie van foraminiferen uit naburige sedimenten een hogere bijdrage leverde aan het herstel van de foraminiferengemeenschap dan aanwas van foraminiferen vanuit de sedimenten zelf. Ondanks dat de dichtheid aan foraminiferen vergelijkbaar laag was tussen beide typen microcosmossen met homogeen aangetast sediment, verschilde de samenstelling van de gemeenschap tussen beide sedimenttypen na afloop van de experimentele periode. De onder zuurstofrijke condities gehouden foraminiferengemeenschap ging meer lijken op die in de homogene microcosmossen met onaangetast sediment. Opheffing van de zuurstofdeficiëntie in het bodemwater resulteerde in een afname van de bijdrage van *Hopkinsina pacifica* en een toename in de bijdrage van de soorten *Ammonia beccarii* en *Elphidium excavatum* aan de totale populatie. Het herstel van de foraminiferengemeenschap heeft meer tijd nodig dan de in dit experiment beschikbare 10.5 week. In een veldsituatie zal bovendien de bijdrage van actieve migratie voor het herstel kleiner zijn – de afstand tussen gezond en aangetast sediment is in het veld groter dan in onze microcosmossen. Daarnaast zal door stroming van het water ook passief transport van foraminiferen een (belangrijkere) bijdrage leveren aan het herstel.

In hoofdstuk vier en vijf bestudeerden we het effect van ecosysteem herstel na zuurstofdeficiëntie op de foraminiferengemeenschap in het estuarium van de Westerschelde. De zuurstofdeficiëntie werd kunstmatig toegebracht door het sediment *in situ* met zeil af te dekken in de winter of in het late voorjaar. De ontwikkeling van de foraminiferengemeenschap in verstoorde en niet verstoorde sedimenten van het estuarium van de Westerschelde werd met elkaar vergeleken om onderscheid te maken tussen natuurlijke variatie en verschil ontstaan door de behandeling. De dynamiek in de foraminiferengemeenschap in dit veldexperiment werd geplaatst in het kader van de ontwikkeling van verschillenden biotische en abiotische factoren (bijvoorbeeld sedimentchemie en de herkolonisatie van macrofauna, nematoden en microben). Deze aspecten werden onderzocht

binnen aan ons onderzoek gerelateerde studies. In HOOFDSTUK VIER zagen we dat zowel de abundantie als het geschatte biovolume (totale volume per soort, gebaseerd op abundantie en schelpgroottes) van de drie voorkomende foraminiferensoorten – *Ammonia beccarii*, *Haynesina germanica* en *Elphidium excavatum* – afhankelijk was van het moment van verstoring (winter of late voorjaar) en van de tijdsspanne (2 of 5 maanden) tussen het beëindigen van de verstoring en de monstername. Het veronderstelde positieve effect van de (her)koloniserende macrofauna op de foraminiferengemeenschap werd in het verstoorde sediment niet waargenomen. De abundantie van *Haynesina germanica* was in dit sediment negatief en in de onverstoorde sedimenten positief gecorreleerd aan de abundantie van macrofauna. *Ammonia beccarii* daarentegen leek vooral beïnvloed door de aanwezigheid van een mat van benthische algen die ontstond op (en in) de verstoorde sedimenten in de afwezigheid van grazende macrofauna. Voor HOOFDSTUK VIJF bestudeerden we het effect dat herstel van het ecosysteem na de zuurstofloosheid had op de soort-specifieke dieetkeuze en de populatie dynamiek van de foraminiferen. Aan het sediment in het estuarium van de Westerschelde werd $^{13}$C-gelabeld bicarbonaat en $^{13}$C-gelabeld glucose toegevoegd om de bijdrage van algen en bacteriën aan het dieet van de foraminiferen te meten. De autotrofe algen werden verondersteld het gelabelde bicarbonaat te consumeren en de heterotrofe bacteriën de glucose. De populatiedynamiek binnen de foraminiferengemeenschap werd per soort afgeleid van de verdeling van de individuele schelpgroottes. De dieetkeuze van de twee meest voorkomende foraminiferen soorten, *Ammonia beccarii* en *Haynesina germanica*, liet een soort-specifiek en veelal tegengesteld patroon zien. De fluctuatie in zowel de dieetkeuze als de populatieopbouw was groter in de verstoorde dan in de controle sedimenten. De dieetkeuze bevestigde de positieve invloed van de overvloed aan benthische algen op *Ammonia beccarii*. Deze overdaad aan voedsel stimuleerde mogelijk zowel de groei als reproductie van *Ammonia beccarii*. In juni, twee maanden na de zuurstofloosheid in de winter, leek de verdeling van de schelpgroottes van *Ammonia beccarii* twee cohorten aan te tonen binnen de populatie. De veronderstelde toename in de reproductie leek samen te gaan met een verschuiving van de voedselvoorkeur – van algen naar bacteriën. De verdeling van de schelpgroottes binnen de populatie van *Haynesina germanica* liet minder fluctuaties zien tijdens het herstel van het ecosysteem. Voor voedselopname leek deze soort meer afhankelijk te zijn van bacteriën dan van algen.

Voor HOOFDSTUK ZES onderzochten we in een laboratorium experiment het effect van verticaal in het sediment aangebrachte permeabele kunstmatige graafgangen. De foraminiferendichtheid, de samenstelling van de assemblage en de verticaal verspreiding van de foraminiferen in sediment afkomstig uit het Zweedse Gullmar Fjord werd bestudeerd. Door alleen het geochemische effect van macrofauna op mariene sedimenten na te bootsen probeerden we een bijdrage te leveren aan het ontrafelen van de invloed van de afzonderlijke processen die een rol spelen in de dynamische relatie tussen ecosysteem ingenieurs en foraminiferen. Met de chemische analyse van het poriewaterprofiel konden we niet een duidelijk effect aantonen van de kunstmatige graafgangen op zowel de zuurstof penetratie diepte, de nitraat concentratie als ook op het voedselaanbod in het sediment. De dichtheid en de diversiteit van foraminiferen gemeenschap was dan ook niet hoger in microcosmossen met graafgangen ten opzichte van die zonder graafgangen. De analyse van het poriewaterprofiel toonde wel een verschil aan in de concentratie van opgelost ijzer. Bovendien zagen we een roodbruine verkleuring van het sediment rondom de graafgangen – dit werd geïnterpreteerd als een microkringloop van ijzer in het poriewater dichtbij de graafgangen. De foraminiferen reageerden soort-specifiek op de graafgangen. Opvallend was dat de (veronderstelde) invloed van de graafgangen op de verspreiding van de verschillende soorten binnen de foraminiferen assemblage overeen kwam met de beschreven patronen tijdens zuurstofverstoring, dan wel onverstoorde omstandigheden in het veld. In de veld situatie domineert *Stainforthia fusiformis* wanneer zuurstofgebrek regelmatig

optreedt – met name tijdens een pril herstel van de zuurstofconditie is deze soort in staat om snel de overhand te nemen. In ons experiment nam *Stainforthia fusiformis* in de diepe sedimentlagen rondom de graafgangen in aantal toe. De soorten die stabiele zuurstofrijke omstandigheden nodig hebben in de veldsituatie, *Bulimina marginata*, *Adercotryma glomerata* en *Textularia earlandi* bleken in ons experiment met name de bovenste sedimentlagen te bevolken – ongeacht de aan- of afwezigheid van kunstmatige graafgangen. Binnen deze groep was *Bulimina marginata* net wat minder gevoelig voor zuurstofstress dan *Adercotryma glomerata*. Tussen de stresstolerante en de stressgevoelige soorten bevond zich *Nonionella spp*. De respons van foraminiferen op de permeabele kunstmatige graafgangen in ons experiment werd vergeleken met de uitkomsten uit andere, soortgelijke studies: met name interacties binnen het voedselweb en habitat specifieke verschillen (zoals de permeabiliteit van het sediment) lijken ten grondslag te liggen aan tegengestelde uitkomsten.

### FORAMINIFEREN ALS INDICATOR  VERKREGEN INZICHTEN

In HOOFDSTUK ZEVEN werden de afzonderlijke hoofdstukken samengevat en de resultaten gecombineerd om te komen tot een overkoepelende interpretatie. Door gebruik te maken van sediment afkomstig uit verschillenden typen ecosystemen – met assemblages die verschilden in dichtheid en soortensamenstelling – konden we het effect van de zuurstofdynamiek op de foraminiferengemeenschap vergelijken tussen ecosystemen. In de door zuurstofgebrek aangetaste geulen van het Grevelingenmeer was de dichtheid aan foraminiferen veel lager dan in de gebieden daarbuiten. Het naburige onaangetaste sediment herbergde bovendien een relatief grote soortenrijkdom. Deze soortenrijkdom in het ecosysteem van de geografisch dichtbij gelegen riviermonding van de Westerschelde was in vergelijking tot het Grevelingenmeer vele malen lager. Weinig soorten lijken in staat te overleven in dit ecosysteem waar fluctuaties in de omgevingsvariabelen (saliniteit, temperatuur etc.) zo groot zijn. Opvallend was dat ondanks het zuurstofgebrek dat optrad in het jaar voorafgaand aan het moment waarop wij sediment verzamelden in het Zweedse Gullmar Fjord de diversiteit aan soorten daar toch hoog was. Zowel voor het Grevelingenmeer als voor het Gullmar Fjord geldt dat de afwisseling tussen wel en geen zuurstofdeficiëntie in hoge mate bepalend is geweest voor de dynamiek binnen de foraminiferengemeenschap in de afgelopen 50 tot 100 jaar.

### MICROHABITAT VERDELING

Naast algemene bevindingen, zoals de observatie dat foraminiferen snel kunnen reageren op fluctuaties in de zuurstofconcentratie, zijn er meer specifieke conclusies te trekken op basis van de verzamelde resultaten. Hoewel de ecosystemen zeer van elkaar verschilden waren er soorten die in meer dan één van de gebieden voorkwamen. De respons van deze soorten kan daardoor vergeleken worden tussen de ecosystemen. Voor andere soorten vonden we een sterk verband tussen hun voorkomen en specifieke omstandigheden. Door de studies met elkaar te vergelijken kunnen we de aanwezigheid van bepaalde soorten relateren aan omgevingsvariabelen en, of het ontwikkelingsstadium van het ecosysteem.

Een hoge bestendigheid tegen zuurstofdeficiëntie werd gevonden voor *Stainforthia fusiformis*, *Hopkinsina pacifica* en in mindere mate ook voor *Nonionella spp*. *Ammonia beccarii*, *Elphidium excavatum* en *Haynesina germanica* behoorden tot de groep van vroege (her)kolonisten. Zij waren in staat om op korte tijdsschaal zeer grote fluctuaties in hun omgeving te doorstaan. Een hoge

gevoeligheid voor zuurstofloosheid werd gevonden voor onder andere de soorten *Trochammina inflata*, *Adercotryma glomerata* en *Bulimina marginata*.

De mate waarin foraminiferen bestand zijn tegen zuurstofdeficiëntie wordt niet alleen gereflecteerd door de ruimtelijke, maar ook door de verticale verspreiding van de assemblage in het sediment. Veelal wordt voor het onderzoek naar de dynamiek binnen de benthische foraminiferen slechts dat deel van de gemeenschap bestudeerd dat zich in de bovenste centimeter van het sediment bevindt. In hoofdstuk drie en zes onderzochten wij deze dynamiek tot op grotere diepte – we vergeleken de verticale verspreiding van de foraminiferen tussen de verschillende behandelingen. Hiermee wilden we het inzicht in hoe de dynamiek in de bovenste centimeter van het sediment tot stand komt verbeteren. In hoofdstuk twee, waar we alleen de bovenste centimeter van het sediment bestudeerden, zagen we dat de foraminiferengemeenschap in de door zuurstofloosheid aangetaste sedimenten in de aanwezigheid van gezonde assemblages toenam tot 46% van die in de onaangetaste controle sedimenten. Vergeleken we de foraminiferendichtheid tussen beide sediment typen over (ongeveer) 10 centimeter sedimentdiepte, dan nam de dichtheid toe tot 38%. Verder bleek 73% van de foraminiferen in de heterogene kernen zich te bevinden in de bovenste sedimentlaag, net onder het sediment oppervlak. Ook de grootste toename van de foraminiferendichtheid vond plaats in de bovenste centimeter van het sediment. Hoewel het herstel van de foraminiferengemeenschap in de door zuurstofloosheid aangetaste sedimenten van het Grevelingenmeer met name in de bovenste centimeter van het sediment plaats vond, speelt (her)kolonisatie van onder andere macrofauna een belangrijke rol tijdens een het verdere herstel van het ecosysteem. Deze (her) koloniserende macrofauna zal de zuurstof penetratiediepte in het sediment, na het herstel van de zuurstofconcentratie in het bodemwater, verder vergroten. In hoofdstuk zes onderzochten we het effect van kunstmatige graafgangen op de verticale verspreiding van de foraminiferengemeenschap: de grootste verschillen tussen sedimenttypen werden gevonden in de diepste sedimentlagen. De juistheid van de keuze om het bestuderen van de foraminiferengemeenschap te beperken tot de bovenste centimeter van het sediment hangt onder meer af van de status van het ecosysteemherstel en de benodigde nauwkeurigheid.

### ECOSYSTEEM INTERACTIES

In mariene sedimenten zorgen ecosysteem ingenieurs voor het mengen van deeltjes en opgeloste stoffen: ze herverdelen zuurstof en voedsel. Dit heeft zowel directe als indirecte gevolgen voor de structuur van de benthische gemeenschap. De uiteindelijke invloed van macrofauna op de foraminiferen is afhankelijk van de balans tussen positieve en negatieve interacties tussen beide. Bovendien kunnen bepaalde foraminiferensoorten wellicht profiteren waar andere vooral hinder ondervinden van de aanwezigheid van ecosysteem ingenieurs. De terugkeer van macrofauna in de door zuurstofloosheid aangetaste sedimenten in het estuarium van de Westerschelde had geen positieve invloed op de dichtheid van, en de soortenrijkdom binnen de foraminiferengemeenschap. Ditzelfde gold voor de foraminiferengemeenschap afkomstig uit het Zweedse Gullmar Fjord die gebruikt werd om het effect van de kunstmatige graafgangen te bestuderen (hoofdstuk zes). De resultaten gepresenteerd in dit proefschrift geven aan dat de invloed van macrofauna op de foraminiferengemeenschap lijkt te verschillen tussen gezonde en verstoorde situaties. Verschillende factoren zoals interacties binnen het voedselweb en habitat specifieke parameters zoals de permeabiliteit van het sediment werden besproken om de verschillen tussen onze en in literatuur beschreven resultaten te duiden.

### TEMPORELE EN RUIMTELIJKE SCHAAL VAN ZUURSTOFLOOSHEID

In mariene kustnabije ecosystemen bepalen fluctuaties in bijvoorbeeld temperatuur, licht, de aanwezigheid van larven, water stroming etc. de status waarin het ecosysteem zich bevindt. Een ecosysteem is verre van statisch: hierdoor kan het moment waarop een verstoring plaatsvindt en de ruimtelijke verspreiding ervan bepalend zijn voor de gevolgen en het mogelijke herstel van het ecosysteem nadat bijvoorbeeld zuurstofloosheid is opgeheven. Het experiment met de geïnduceerde zuurstofloosheid in het estuarium van de Westerschelde liet zien dat het moment waarop de verstoring plaats had gevonden – winter of voorjaar – meer invloed had op de dichtheid, de biovolumes, de opbouw van de populatie en de voedselvoorkeur van de foraminiferengemeenschap in september dan de periode tussen de verstoring en het monstername – 2 of 5 maanden. Dezelfde uitkomst werd gevonden voor macrofauna en gerelateerd aan seizoenale verschillen in de aanwezigheid van larven die kunnen bijdragen aan het herstel van de macrofaunagemeenschap (Rossi en anderen, 2009). Hoewel seizoenale verschillen beschreven zijn voor de foraminiferengemeenschap, worden deze veelal verklaard aan de hand van de beschikbaarheid van zuurstof en voedsel en niet gekoppeld aan een vaststaande reproductie periode. Desalniettemin stellen foraminiferen wel bepaalde eisen aan hun omgeving voordat zij overgaan tot reproductie (Bradshaw, 1957). Als seizoenale fluctuaties in omgevingsvariabelen groei en reproductie beïnvloeden, dan kan het moment waarop de verstoring plaatsvindt en de tijdspanne tussen opeenvolgende verstoringen, een directe rol spelen in de mate waarin de verstoring de gemeenschap schaadt. Ook de ruimtelijke verspreiding van zuurstofloosheid lijkt invloed te hebben op het herstel nadat de verstoring is opgeheven. Het herstel van de foraminiferengemeenschap in door zuurstofloosheid aangetaste sedimenten uit het Grevelingenmeer verliep vele malen voorspoediger in de aanwezigheid van naburige gezonde assemblages. Het minimale herstel dat plaatsvond in de afwezigheid van naburige gezonde assemblages suggereert dat de afstand tussen verstoorde en onverstoorde sedimenten mede bepalend is voor de snelheid waarmee de foraminiferengemeenschap zich kan herstellen.

### VERLEDEN, HEDEN EN TOEKOMST

Naast de toepasbaarheid van levende foraminiferen als indicatoren voor het functioneren van mariene ecosystemen, worden hun gefossiliseerde soortgenoten al decennia gebruikt voor het bestuderen van paleomilieus. Studies zoals die van ons – naar de respons van de foraminiferengemeenschap op fluctuaties in omgevingsfactoren – kunnen mogelijk ook bijdragen aan het verbeteren van de toepasbaarheid van fossiele foraminiferen voor paleoecologische reconstructies. De waarde van foraminiferen voor dergelijk onderzoek ligt zowel besloten in het voorkomen van soorten in bepaalde dichtheden als in de chemische samenstelling van de schelpjes – deze is afhankelijk van de omgeving waarin de foraminifeer leeft, of heeft geleefd.

Voor dit proefschrift bestudeerden we zowel gezonde als door zuurstofdeficiëntie aangetaste ecosystemen. We onderzochten hoe abiotische (levenloze) en biotische (levende) factoren de dynamiek in de foraminiferengemeenschap bepalen. Foraminiferen bevinden zich binnen een ecosysteem tussen enerzijds de microben en anderzijds de macrofauna, maar foraminiferen lijken beide niet sterk te beïnvloeden. Wel zagen we dat de relatie tussen foraminiferen en hun omgeving tegenovergesteld kan zijn in gezonde en van zuurstofdeficiëntie herstellende ecosystemen. Bovendien was er een groot verschil in tijd nodig voor herstel van de zuurstof penetratie diepte en die nodig voor het herstel van de faunale assemblages.

Een geleidelijke veranderingen in de zuurstofconcentratie van het bodemwater (fungerend als een aansturing van de ontwikkeling van een ecosysteem) kan een niet-lineaire respons veroorzaken in het functioneren van het ecosysteem. Zuurstofloosheid kan zelfs een hysterese-achtige respons veroorzaken. In die situatie zorgt een kleine verandering in de zuurstofconcentratie voor een grote verschuiving in het functioneren van het ecosysteem. Rond het zogenaamde kantelpunt verandert de status van het ecosysteem zeer abrupt. Karakteristiek voor hysterese is de onomkeerbaarheid van de verandering: het ecosysteem herstelt niet nadat de zuurstofconcentratie verhoogd wordt met eenzelfde kleine stap als de verlaging die de status van het ecosysteem deed omklappen (Scheffer en Nes, 2004). Dit heeft tot gevolg dat een bepaalde zuurstofconcentratie in het bodemwater zowel kan horen bij een gezond als bij een aangetast ecosysteem. De status van het ecosysteem bij de betreffende zuurstofconcentratie in het bodemwater wordt dan bepaald door de voorafgaande toestand. Daarnaast is de veerkracht van een ecosysteem om zich te herstellen na een verstoring afhankelijk van de afstand tot het kantelpunt – deze is laag rondom het kantelpunt (Veraart en anderen, 2011). Doordat de veerkracht van het ecosysteem afhankelijk is van de afstand tot het kantelpunt, kan de respons van het ecosysteem op relatief kleine zuurstoffluctuaties informatie geven over hoever het ecosysteem verwijderd is van het kantelpunt.

De door de mens geïnduceerde klimaatverandering waar onze planeet vandaag de dag mee te maken heeft en de daarmee gepaard gaande veranderingen die we veroorzaken in het milieu, zorgen in toenemende mate voor een noodzaak de afstand tot kantelpunten te kunnen voorspellen (bijvoorbeeld Barnosky en anderen, 2012). Doordat foraminiferen met hun aanwezigheid in moderne en fossiele assemblages ons van informatie kunnen voorzien over het functioneren van het ecosystemen en tegelijkertijd de chemische samenstelling van de schelp in de toekomst mogelijk gebruikt kan worden om de zuurstofconcentratie van het bodemwater te bepalen, zijn deze organismen wellicht buitengewoon geschikt voor het afleiden van de afstand waarop een gegeven ecosysteem zich bevindt ten opzichte van een kantelpunt. Met deze cruciale informatie zouden beleidsmakers kunnen ingrijpen voordat het kantelpunt bereikt wordt en het ecosysteem (bijna) onomkeerbaar verslechtert.

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# CURRICULUM VITAE

Margreet Brouwer werd op 14 augustus 1979 geboren en groeide op in Franeker. Nadat ze in 1998 haar atheneum diploma had behaald, begon ze aan de studie Biologie aan de Rijks*universiteit* Groningen. Ofschoon ze met name vakken volgde bij de vakgroepen Plantenecologie en Mariene Biologie, maakte ze ook uitstapjes richting de archeologie en culturele antropologie. Voor het behalen van haar doctoraal diploma verrichtte Margreet twee afstudeeronderzoeken, twee literatuurstudies en een educatieve stage. Bij prof. dr. Grootjans, verbonden aan de vakgroep Plantenecologie, onderzocht ze of de samenstelling van het water de niche-separatie van veenmossoorten (sphagnum) langs de gradiënt van bult naar slenk in de hoogvenen van het Drentse Dwingelderveld zou kunnen verklaren. Ze vervolgde haar studie met een archeozoölogisch afstudeeronderzoek naar het gebruik van schapen en geiten door vroeg-Neolithische mensen in het Syrische Bouqras. Tegelijkertijd werd de methodologie om de molaren van schapen en geiten te onderscheiden op basis van een aantal geselecteerde karakteristieken getest. Dit onderzoek werd uitgevoerd onder leiding van dr. Buitenhuis, verbonden aan het 'Archaeological Research & Consultancy BV'. Na twee literatuuronderzoeken (vakgroepen Mariene Biologie en Dierecologie) sloot Margreet haar opleiding af met een educatieve stage bij het Drents Museum te Assen waar ze onder meer lesmateriaal ontwikkelde.

Na het behalen van haar doctoraaldiploma verhuisde Margreet naar Wageningen. Daar vervulde ze verschillende functies; zo werkte ze als gastmedewerken bij de Wageningen Universiteit aan een literatuuronderzoek over vegetatie in overstromingsgebieden en was ze betrokken bij WILDzoekers. In 2008 begon ze bij de vakgroep Paleontologie en Stratigrafie van de Universiteit Utrecht aan het interdisciplinaire promotieonderzoek dat heeft geresulteerd in dit proefschrift getiteld 'Ecosystem recovery after hypoxia: what can foraminifera indicate'.