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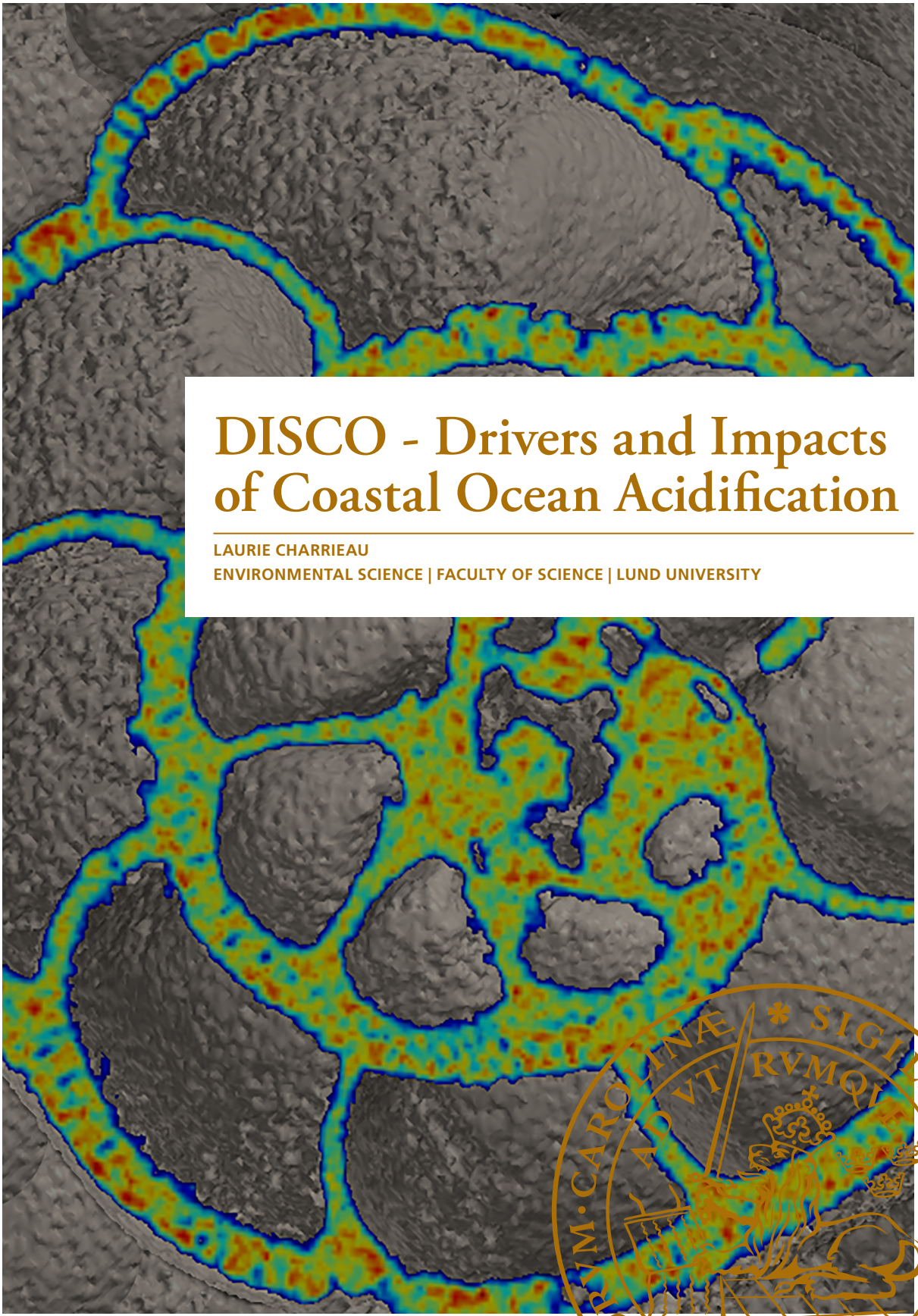

DISCO - Drivers and Impacts of Coastal Ocean Acidification

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DISCO
Drivers and Impacts of Coastal Ocean Acidification

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LUND
UNIVERSITY

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DISCO – Drivers and Impacts of Coastal Ocean Acidification

Abstract

Ocean acidification, mainly attributed to the increasing anthropogenic CO_2 in the atmosphere, is characterised by a lowering pH together with a shift in the sea water carbonate chemistry toward lower concentration of carbonate ions. On the coasts, where the environmental variability is high due to natural and human impacts, ocean acidification mainly affects the frequency, magnitude, and duration of lower pH and lower calcium carbonate saturation events. Coastal ecosystems are adapted to environmental variability such as frequent changes in salinity, temperature, pH, oxygen levels and organic matter content. However, the effects of an increase of the range of this variability on coastal species, and especially on calcifiers, are still not clear. In this context, this thesis explores the impacts of coastal ocean acidification combined with other environmental stressors on benthic foraminifera.

In the Skagerrak-Baltic Sea region, foraminifera faunas varied along a strong gradient in terms of salinity, pH, and dissolved oxygen concentration, and species were adapted to local environmental stressors. However, the specimens of *Ammonia* spp. and *Elphidium* spp. observed in the south Baltic Sea were partially to completely dissolved, probably due to a combination of different stressors affecting the required energy for biomineralisation.

In a culture study, the coastal species *Ammonia* spp. and *E. crispum* were found to be resistant to dissolution under varying salinity and pH, which reflects the environmental variations in their natural habitats. However, their resistance to lower pH is decreased when cultured in brackish water conditions, and living decalcified specimens were also observed under a salinity of 5. This underlines the importance of a high salinity in the calcification process of foraminifera.

At the entrance of the Baltic Sea, environmental changes during the last 200 years were reconstructed using foraminiferal faunas. Four periods were identified with varying oxygen levels, salinity, organic matter content, and pollution with lower pH. This highlights that foraminiferal faunas were able to adapt to multiple environmental stressors.

This thesis concludes that, even if coastal species of foraminifera can tolerate extremely varying conditions in their environment on the short term, it is likely that tolerance thresholds will be passed for benthic ecosystems under the future increase in anthropogenic impacts such as coastal ocean acidification.

Further studies of micro-organisms such as foraminifera will be necessary to improve our understanding of past environmental changes and to put present and future changes into a larger context.

Key words: coastal ocean acidification; foraminifera; environmental changes; pH; salinity

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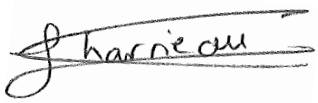
Signature  Date: 09-11-2017

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"*Tout est varié, et c'est la beauté de la nature. Pourquoi donc détruire son ouvrage ?*" - Olympe de Gouges

Contents

List of papers

This thesis is based on four papers listed below, which have been appended to the thesis. Paper I is in revision. Paper II is submitted. Paper III is a manuscript. Paper IV is in press.

Paper I. L. M. Charrieau, H. L. Filipsson, K. Ljung, M. Chierici, K. L. Knudsen and E. Kritzberg. **The effects of multiple stressors on the distribution of coastal benthic foraminifera: a case study from the Skagerrak-Baltic Sea region**. In revision. *Marine Micropaleontology*.

Paper II. L. M. Charrieau, H. L. Filipsson, Y. Nagai, S. Kawada, K. Ljung, E. Kritzberg and T. Toyofuku. **Decalcification and survival of benthic foraminifera under the combined impacts of varying pH and salinity**. Submitted to *Marine Environmental Research*.

Paper III. L. M. Charrieau, H. L. Filipsson, K. Ljung and Emma Kritzberg. **A bicentennial record of modern environmental changes in the Baltic Sea entrance**. Manuscript, to be submitted to *Estuaries and Coasts*.

Paper IV. L. M. Charrieau, L. Bryngemark, I. Hansson and H. L. Filipsson. **Improved wet splitter for micropalaeontological analysis, and assessment of uncertainty using data from splitters**. In press. *Journal of Micropalaeontology*.

Contributions

	Paper I	Paper II	Paper III	Paper IV
Concept and study design	L. M. Charrieau H. L. Filipsson	L. M. Charrieau T. Toyofuku	L. M. Charrieau H. L. Filipsson	L. M. Charrieau I. Hansson
Fieldwork	L. M. Charrieau H. L. Filipsson K. Ljung	L. M. Charrieau H. L. Filipsson T. Toyofuku Y. Nagai S. Kawada	L. M. Charrieau H. L. Filipsson K. Ljung	
Microprofiling	L. M. Charrieau			
Carbonate chemistry analysis	M. Chierici	S. Kawada		
Foraminiferal fauna analysis	L. M. Charrieau		L. M. Charrieau G. Klintvik Ahlberg	L. M. Charrieau
Taxonomy check	K. L. Knudsen			
TOC and TN analysis	L. M. Charrieau K. Ljung		L. M. Charrieau K. Ljung	
Grain-size analysis	L. M. Charrieau Y. Bokhari Friberg Å. Wallin		L. M. Charrieau Y. Bokhari Friberg Å. Wallin	
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Splitter tests				L. M. Charrieau I. Hansson
Statistics	L. M. Charrieau		L. M. Charrieau	L. Bryngemark
Data interpretation	L. M. Charrieau H. L. Filipsson K. Ljung K. L. Knudsen E. Kritzberg M. Chierici	L. M. Charrieau H. L. Filipsson T. Toyofuku Y. Nagai	L. M. Charrieau H. L. Filipsson K. Ljung E. Kritzberg	L. M. Charrieau L. Bryngemark H. L. Filipsson

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The number of people who contributed more or less consciously to this thesis is probably approaching the number of forams I've picked on the last 4 years. Consequently, it is nearly impossible to mention you all. However, if you're looking for your name on this list, you probably feel like you helped in a way or another, so thank you!

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Introduction

Coastal ocean acidification: definition and effect on marine calcifiers

Ocean acidification is globally recognised as an increasing environmental problem, threatening the marine life and the many ecosystem services provided by the oceans. The main cause of ocean acidification is attributed to the large amounts of anthropogenic carbon dioxide (CO_2) released in the atmosphere since the industrial revolution, especially through the burning of fossil fuels. Indeed, about one third of this CO_2 has already been taken up by the oceans (Sabine et al. 2004), inducing a lowering pH together with a shift in the sea water carbonate chemistry toward lower concentration of carbonate ions (CO_3^{2-}) (Box 1). Consequently, the mean surface-ocean pH, which has been remarkably stable at 8.2 during the Holocene (Zeebe and Ridgwell 2011), has already decreased by 0.1, which represents a 30% increase in acidity (Orr et al. 2005). Furthermore, model projections predict a pH decrease of 0.3 to 0.4 by the end of this century (150% increase in acidity, Feely et al. 2009). Additionally, even if CO_2 emissions were stopped now, ocean acidification will progressively reach the bottom of the oceans in the coming centuries (Caldeira and Wickett 2003).

Box 1. Carbonate equilibrium system in seawater

(1) $CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + 2H^+$
(2) $CaCO_3 \Leftrightarrow CO_3^{2-} + Ca^{2+}$

More than one third of the human population is currently living in coastal areas, and coastal ecosystems are among the most productive in the world that provide a range of social and economic services to human society (UNEP 2006). On the coasts, the pH and carbonate chemistry fluctuations are more complex compared to the open ocean (Kelly et al. 2011; review in Waldbusser and Salisbury 2014). Coastal areas are dynamic environments that display strong environmental variability such as changes in salinity, temperature, carbonate chemistry and pH, dissolved oxygen concentration, and organic matter content through river discharge (Crossland et al. 2005). These environmental parameters naturally vary according to climate, seasonal contrasts and hydrographic features. The range of this natural variability is gradually increased by anthropogenic effects, leading to global warming, eutrophication and deoxygenation. The carbonate chemistry and pH are also influenced by other factors such as fresh water inflows with lower pH and calcium carbonate ($CaCO_3$) saturation state (Salisbury et al. 2008; Chierici and Fransson 2009), upwelling of "older" CO_2-rich water with lower pH (e.g. Feely et al. 2008; Hauri et al. 2009), and eutrophication through mineralisation of organic matter by the respiration process that consume O_2 and produces CO_2 (Borges and Gypens 2010; Cai et al. 2011; Laurent et al. 2017) (Figure 1). On the coasts, where the carbonate chemistry and pH are already highly variable due to multiple factors, ocean acidification

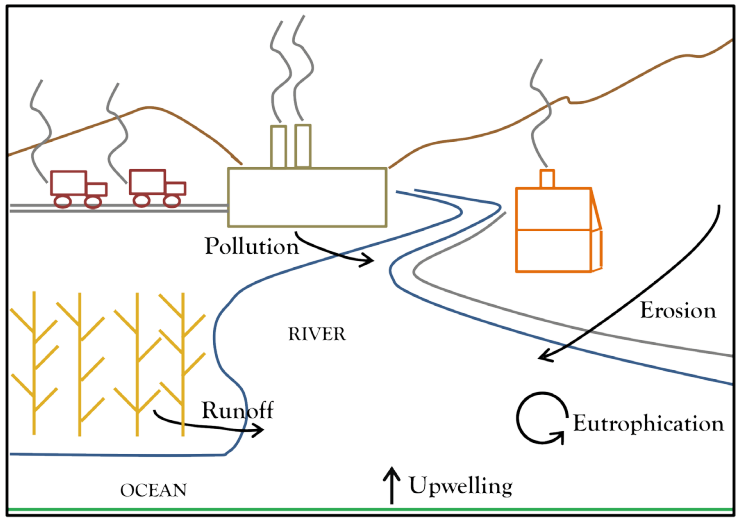


Figure 1. Main processes influencing the pH and carbonate system on coastal areas. Modified from Kelly et al. (2011).

is not only decreasing the mean pH of the water but also impacts the frequency, magnitude, and duration of lower pH events (review in Waldbusser and Salisbury 2014). These effects on pH and carbonate chemistry are referred to as coastal ocean acidification. Coastal ocean acidification alone can account for up to 50% of the decrease in pH since the pre-industrial times, for example in estuaries (Feely et al. 2010).

Changes in the pH and carbonate chemistry have an effect on the physiology of all marine organisms, but the most affected are probably the calcifiers (Kroeker et al. 2010). Indeed, the majority of the calcifiers' shells are made of $CaCO_3$, which is formed when seawater is saturated with calcium (Ca^{2+}) and CO_3^{2-} (Box 1). The increase of hydrogen ions (H^+) and the shift in the carbonate chemistry from CO_3^{2-} to HCO_3^- due to ocean acidification reduces the saturation state of CO_3^{2-}, which decreases the possibilities for the calcification process to happen. The first ecosystems that have been recognised as vulnerable to ocean acidification are coral reefs, where the combined effect of reef structure dissolution and decrease in $CaCO_3$ production is diminishing reef building (Kleypas and Yates 2009). Thus, the role of coral reefs as crucial biodiversity support and shoreline protectors against waves and erosion is strongly endangered. Other calcifiers that will be affected by ocean acidification are the mussel *Mytilus edulis* and the oyster *Crassostrea gigas*, two highly exploited species that show a strong decrease in their calcification rates when grown in culture studies at high CO_2 levels (Gazeau et al. 2007). However, the response of calcifiers to ocean acidification has also been demonstrated as species-specific, and some organisms appear unaffected by varying carbonate chemistry, with certain species even benefiting from higher CO_2 levels (Fabry 2008; Kroeker et al. 2013; Ries 2014) (Figure 2). This species-specific response to ocean acidification can be due to the

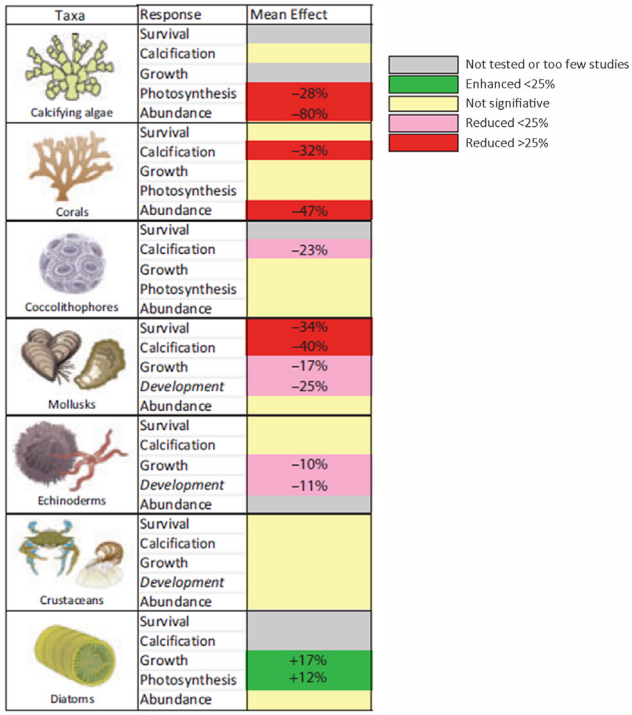


Figure 2. Species-specific response of calcifiers to a pH decrease of 0.5. Modified from Kroeker et al. (2013).

life stage of the organism, nutritional status, pH regulation possibilities at the calcification site, ability to protect the shell with organic covering, and ability to photosynthesise (Waldbusser 2010; Kroeker et al. 2013; Ries et al. 2014). The variability in the calcifiers' response makes it difficult to predict a uniform reaction of marine ecosystems to ocean acidification.

Another crucial factor that explains the capacity of some species to resist ocean acidification is the natural pH variations in their original habitat (review in Waldbusser and Salisbury 2014; Vargas et al. 2017). For example, in coastal areas, ecosystems are used to high variability in their environment and the species are adapted to frequent changes in carbonate chemistry and pH. Thus, if the pH values stay within the range that a coastal ecosystem is accustomed to, the species will not be immediately affected, and they could be considered as not concerned by ocean acidification (Duarte et al. 2013). However, as the frequency, magnitude, and duration of decreased pH events will be modified, coastal species tolerance thresholds could be passed, and coastal ocean acidification will negatively impact the ecosystems (e.g. Hauri et al. 2013; Harris et al. 2013). Interestingly, varying responses to different CO_2 concentrations have been observed within the same species, depending on where they are living (Vargas et al. 2017). For instance,

individuals of the mussel *Mytilus chilensis* collected on the coast near fresh-water inputs were more resistant to dissolution when exposed to higher CO_2 concentration than the ones collected in fully marine water (Duarte et al. 2015). Consequently, it is necessary to first study the range of the variability that coastal species can usually tolerate in their natural habitat, before concluding their reaction to lower pH and changes in carbonate chemistry (Waldbusser and Salisbury 2014; Vargas et al. 2017). Moreover, coastal species are usually under the simultaneous impact of many environmental stressors such as low salinity, low dissolved oxygen concentration, high temperature, and varying organic matter content (Solan and Whiteley 2016). The number of studies involving multiple stressors is gradually increasing. For instance, global warming and ocean acidification were demonstrated to have negative synergic effect on marine organisms (Rodolfo-Metalpa et al. 2011; Kroeker et al. 2013). More studies are still needed to fully understand the combined effect of these multiple stressors with the ongoing coastal ocean acidification problems, particularly on calcifiers.

Plate 1. Typical species of foraminifera in the Skagerrak-Baltic Sea region. 1-4. Porcelaneous: 1- *Pyrgo williamsoni*; 2- *Quinqueloculina bosciana*; 3- *Pyrgoella sphaera*; 4- *Quinqueloculina stalkeri*; 5-16. Hyalines: 5- *Bulimina marginata*; 6- *Elphidium clavatum*; 7- *Ammonia falsobeccarii*; 8- *Hyalinea balthica*; 9- *Cassidulina laevigata*; 10- *Bolivina skagerrakensis*; 11- *Nonionellina labradorica*; 12- *Nonionella* aff. *stella*; 13- *Nonionella turgida*; 14- *Nonionella iridea*; 15- *Stainforthia fusiformis*; 16- *Globobulimina turgida*; 17-24. Agglutinated: 17- soft-shell foraminifera; 18- *Cribrostomoides nitidum*; 19- *Liebusella goesi*; 20- *Eggerella scabra*; 21- *Reophax dentaliniformis*; 22- *Reophax scorpiurus*; 23- *Textularia earlandi*; 24- *Leptohalysis scotti*.

The world of Foraminifera

Benthic foraminifera are among the most diverse and abundant calcifying micro-organisms at the modern sea floor (Gooday et al. 1992; Sen Gupta 2007). Foraminifera are single-celled, they have a short life cycle - from a few weeks to a year in shallow water -, the environmental conditions where they live are usually reflected in the faunal composition, and they build a shell (called test) that may fossilise. Therefore, they are excellent indicators of past and present environmental changes, such as ocean acidification events. To build their test, some foraminiferal species cement sediment particles (agglutinated and soft-shell forms), while the majority of the species are able to produce their own calcium carbonate with Ca^{2+} and CO_3^{2-} ions from the sea water (hyalines and porcelaneous forms) (Plate 1). Like other calcifiers, benthic foraminifera are potentially threatened by a lowering pH and changes in the carbonate chemistry and similarly, the response of foraminifera to ocean acidification has been shown to be species-specific. Thus, in culture studies, some foraminiferal species displayed reduced calcification and survival rates when placed in predicted ocean acidification conditions (Green et al. 1998; Le Cadre et al. 2003; Kuroyanagi et al. 2009; Hikami et al. 2011; Khanna et al. 2013), while symbiont-bearing species that can photosynthesise do not appear dramatically affected, at least on the short term (Fujita et al. 2011; Glas et al. 2012; McIntyre-Wressnig et al. 2013, 2014).

Benthic foraminifera are key-players in coastal areas. Together with the rest of the meiobenthos, they are the base of the food chains, and their predators include marine snails and small fish. They also contribute to the carbon cycle as they represent up to 5% of the annual calcium carbonate production on the coasts (Langer 2008), and to the nitrogen cycle as some species are able to denitrify (Risgaard-Petersen et al. 2006; Høgslund et al. 2008). Changes in the foraminiferal communities such as faunal shift or decrease in abundance would strongly affect the balance of coastal ecosystems. Therefore, the effects of coastal ocean acidification on benthic foraminifera need to be investigated.

The distribution of living benthic foraminifera on the surface and within the sediment typically depends on food availability and dissolved oxygen concentration (Jorissen et al. 1995; Ernst and van der Zwaan 2004). On the coasts, other environmental parameters such as temperature and salinity can influence the foraminiferal distribution (Sen Gupta 1999; Nigam et al. 2008). Thus, in order to understand to which extent coastal foraminiferal species can tolerate pH and carbonate chemistry fluctuations, it is imperative that other environmental stressors such as varying salinity, temperature, dissolved oxygen concentration and organic matter content be considered simultaneously. It will then be possible to better predict the response of benthic ecosystems to coastal ocean acidification.

Study Site

The Skagerrak-Baltic Sea area

The Baltic Sea is a region with large economic, recreational and societal values. The Baltic Sea is surrounded by 9 countries and around 85 million people live on its catchment area, making it challenging for decision makers to find a balance between social and economic uses and environmental protection. The Baltic Sea is an intra-continental sea of the North Atlantic Ocean, with restricted water exchanges with the North Sea through the Öresund and the Danish Belts (Figure 3). As a consequence, the water renewal time in the Baltic is about 30 years (Leppäranta and Myrberg 2009). The confluence of marine and fresh water makes the water circulation complex. Overall, the marine water from the Skagerrak enters the Baltic Sea through bottom currents, while the freshwater, coming from several rivers draining large areas, exit the Baltic Sea through surface currents (Figure 3). The result is the formation of strong vertical water stratification

Figure 3. Map of the studied area. Dots show the five studied stations. General water circulation: main surface currents (black arrows) and main deep currents (grey arrows). GB: Great Belt; LB: Little Belt; AW: Atlantic Water; CNSW: Central North Sea Water; JCW; Jutland Coastal Water; NCC: Norwegian Coastal Current; BW: Baltic Water.

regarding salinity in the Kattegat, Öresund and south Baltic Sea. This stratification is further amplified by the development of a thermocline during spring and summer, when freshwater input and temperatures increase.

Most of the Baltic Sea was assessed as being eutrophied (Helcom 2013), due to the increase of nutrients in urban areas, and to the restricted water exchanges. As a consequence, the Baltic Sea is more and more frequently impacted by hypoxia events (Conley et al. 2011), which are intensified by water warming (Meier et al. 2012). Ocean acidification is already measurable in the area, and significant decreases in the mean bottom water pH since 1993 have been reported, of 0.1 in the Skagerrak and 0.2 in the Baltic Sea (Andersson et al. 2008; Andersson 2010). Models applied in the Baltic Sea project a stronger pH seasonality, a decrease in the calcium carbonate saturation state, and an increase of the hypoxic area for the next approximately 100 years (Omstedt et al. 2014).

In this thesis, the focus area is a transect along the Skagerrak, Kattegat and south Baltic Sea (Figure 3). The five chosen stations show a natural gradient regarding environmental parameters, and characteristic salinity, pH, temperature and oxygen levels are observed for each region (Figure 4). Salinity and pH also have a high range of seasonal fluctuations, especially in the Öresund and south Baltic Sea (Figure 4). Moreover, it is well known which foraminiferal species are living in the Skagerrak-Kattegat (Conradsen 1993; review in Conradsen et al. 1994; Alve and Murray 1999) and in the Baltic Sea region (Lutze 1965; Brodniewicz 1965; Hermelin 1987). Thus, the area is ideal to observe the combined effects of coastal ocean acidification and multiple stressors on calcifiers that are used to varying pH and carbonates chemistry in their environment.

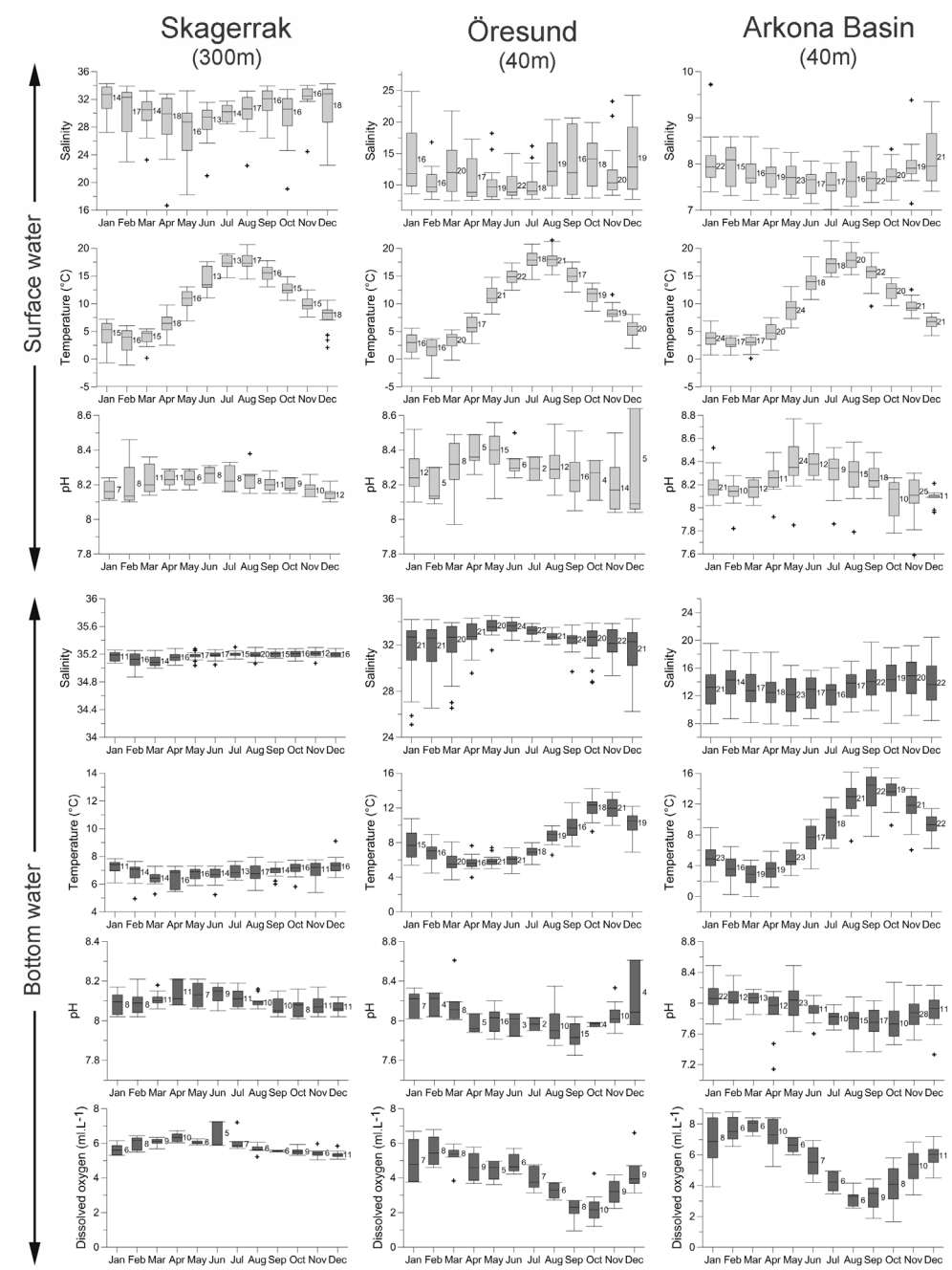


Figure 4. Seasonal variability of salinity, temperature, pH, and dissolved oxygen concentration at the surface water (light grey) and at the bottom water (dark grey) of the Skagerrak, Öresund and Arkona Basin. The data were measured between 1958 and 2016 by the SMHI (Swedish Meteorological and Hydrological Institute). The numbers of measurements is indicated for each month.

Scope of the thesis

In this thesis, the following questions are addressed:

- How do the foraminiferal faunas develop in the Skagerrak-Baltic Sea area, along a strong gradient in multiple environmental stressors?

- What are the combined effects of acidification and desalination on calcification process and survival rate of benthic foraminifera?

- What are the environmental changes that impacted the benthic foraminiferal fauna in the Öresund over the last two centuries?

- How can we reduce the analysis time of micro-organism faunas?

Material and Methods

Foraminiferal fauna analysis

Foraminiferal communities are specific for each type of environment. The density, diversity and assemblage composition of each community give information about the environment where the individuals are living (Murray 2006, 2014). For example, diversity tends to increase in open-ocean compared to the coastal areas, a high proportion of agglutinated over hyalines and porcelaneous forms is typical from intertidal marshes, and the presence of opportunistic species can indicate a polluted site. Moreover, as foraminifera's tests can easily fossilise, sediment cores constitute records of past changes in environmental conditions and allow paleoenvironmental reconstruction. Foraminiferal fauna analyses are used in this thesis to track present and past environmental conditions. Sediment cores of in average 35 cm in length are collected using a GEMAX twin barrel corer and sectioned every centimetre. Each sample is sieved through a 100 µm mesh screen and the foraminifera are picked, identified to the species level, and counted.

Living fauna: the CTG method

The CellTracker™ Green method is a staining method developed by Bernhard et al. (2006) to differentiate living from dead organisms. The CTG is a non-fluorescent component that will be cleaved by some non-specific esterases into fluorescent products, which will then be integrated in the cell's intracellular compartments. The esterases are degraded in a few hours after the death of the cell, ensuring the high precision of the method. In this thesis, coastal ecosystems face rapid environmental changes on a daily basis, which can be either beneficial or lethal for each individual. Thus, the high precision of the CTG method is required. A few µL of CTG together with seawater is added to the core top samples, which are incubated 12 hours in the dark at in situ temperature. After washing and sieving, the organisms displaying clear fluorescence under a stereomicroscope are considered alive (Figure 5).

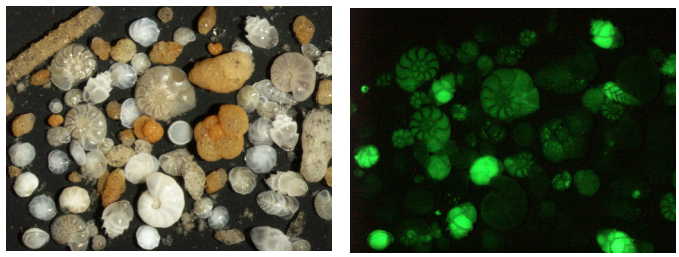


Figure 5. Foraminifera under left: normal light; right: epifluorescent light.

Splitting samples

Micro-organisms such as foraminifera are usually abundant in the sediment, which makes them an excellent tool to reconstruct environmental conditions. However, it also makes the faunal analyses very time-consuming. To split the samples into smaller subsamples, we develop our own improved wet splitter from pre-existing devices. Our wet splitter shows small sample losses and strong statistical consistency across splits. Details of the method are given in Paper IV.

Culture experiments

Culture experiments are useful to isolate the effects of one environmental parameter from the many ones affecting the ecosystems in natural environments. In this thesis, salinity and pH are the varying parameters, while temperature, oxygen concentration and food are kept at fixed values. The different salinity levels are obtained by diluting sea water with milli-Q water, and the pH is varied by CO_2 bubbling and monitored with pH-meters. The combined impact of lower salinity and pH is observed on the calcification process and survival rate of two species of benthic foraminifera: Ammonia spp. and Elphidium crispum. Living specimens are placed in aquariums with specific salinity and pH, and the experiments are conducted over one to five months. Pictures of each individual are taken every week to follow their development and life status.

Microelectrodes

Benthic foraminifera usually live at the surface or within the first centimetres of the sediment. Microelectrodes are used to measure geochemical parameters with high resolution along this water-sediment interface. In this thesis, profiles of dissolved oxygen concentration and pH are performed on the first centimetres of the sampled cores and overlaying water, using Clark-type microelectrodes (OX 100 and pH 100, Unisense). After polarisation and calibration, the microelectrodes are placed on the micromanipulator system, which is controlled by a computer. The electric signals recorded by the microelectrodes are then converted into dissolved oxygen concentration and pH values.

TOC and TN

Total Organic Carbon (TOC), Total Nitrogen (TN) contents and their ratio C/N can be used to discuss the origin of the organic matter of the sediment along the cores. High carbon contents indicates an animal origin of the organic matter such as high plankton

levels, while high nitrogen contents indicates a plants origin that is often terrestrial, owing to the proximity of land. Removal of inorganic carbon is carried out on freeze-dried samples with the in-situ acidification method based on Brodie et al. (2011), and TOC and TN are measured with a Costech ECS 4010 Elemental Analyser.

Grain size analysis

The grain size distribution of sediment gives information about the hydrodynamic conditions when the sediment was deposed. For instance, high energy environments allow higher proportions of coarse grains to sediment. The grain size also describes the type of environment where the benthic organisms are living, as large grains retain less organic matter. After several pre-treatments adapted from Murray (2002) on the freeze-dried samples, grain sizes >63 µm are separated by sieving while grain sizes <63 µm are analysed by laser diffraction using a SediGraphIII. Three size groups, sand (2000-63µm), silt (63-4µm) and clay (<4µm), are classified.

Chronology

The ^{210}Pb method is used to date sediment cores on a time-scale of 100 to 150 years. The method is based on the ^{238}U radioactive decay series and on the constant fallout of ^{210}Pb into lakes or oceans (Appleby 2001). In this thesis, the activity in the core from the Öresund is measured with an ORTEC HPGe (High-Purity Germanium) Gamma Detector. The age is deduced based on the CRS (Constant Rate of ^{210}Pb Supply) model and the sedimentation rate is calculated.

Summary of the papers

Paper I. L. M. Charrieau, H. L. Filipsson, K. Ljung, M. Chierici, K. L. Knudsen and E. Kritzberg. **The effects of multiple stressors on the distribution of coastal benthic foraminifera: a case study from the Skagerrak-Baltic Sea region**. In revision. *Marine Micropaleontology*.

This study aims to investigate the impact of multiple stressors on benthic foraminifera in their natural environment. We analysed the living foraminiferal fauna collected along a transect between the Skagerrak-Kattegat and the Baltic Sea, which follows a strong environmental gradient regarding salinity, dissolved oxygen concentration, pH, and calcium carbonate saturation.

We found that each area had typical foraminiferal density, species richness, and assemblage composition, with open ocean to coastal species adapted to each environmental condition (Figure 6). In the Baltic Sea, where the density and diversity were the lowest, the two main species displayed decalcified test, and only the inner organic linings were visible (Figure 6). The specimens were however still alive, as proved by the CTG method. This dissolution was probably due to the combined effect of multiple stressors on the foraminifera such as low salinity, low oxygen concentration, low pH and low calcium carbonate saturation, which resulted in insufficient energy left for biomineralization. The abundant organic matter in the region seems to be crucial for the survival of the foraminifera in the Baltic Sea.

We conclude that even if benthic ecosystems are used to strong variations in their environment, an increase in the range of this variability will make the species more vulnerable to coastal ocean acidification and global changes.

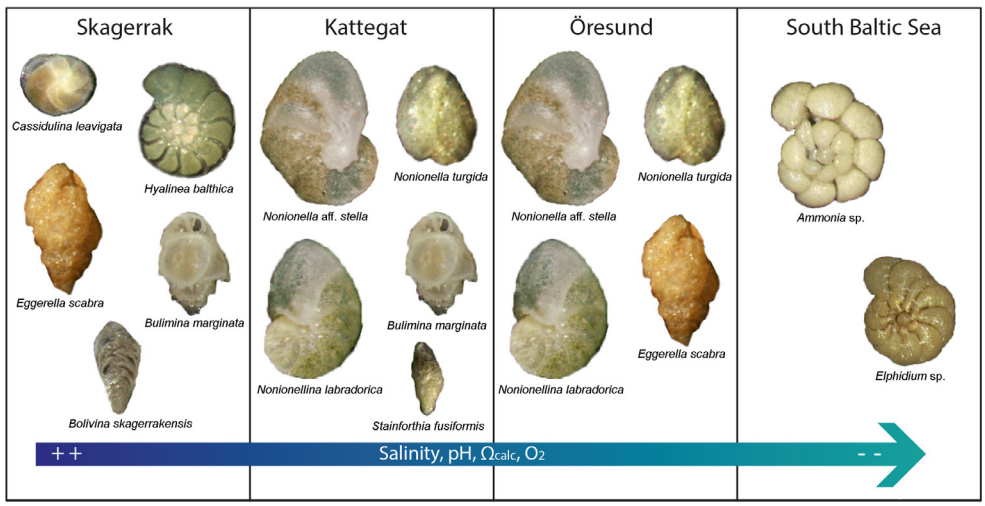


Figure 6. Characteristic foraminiferal species for each region along the environmental transect.

Paper II. L. M. Charrieau, H. L. Filipsson, Y. Nagai, S. Kawada, K. Ljung, E. Kritzberg and T. Toyofuku. **Decalcification and survival of benthic foraminifera under the combined impacts of varying pH and salinity**. Submitted to *Marine Environmental Research*.

The purpose of this study was to evaluate the combined effect of two typical environmental stressors on the coast – salinity and pH – on calcifying organisms. Two species of foraminifera (*Ammonia* sp. and *Elphidium crispum*) were collected on the Japanese coasts and cultured between one and five months under varying salinity and pH. A level of morphological state and dissolution was attributed to each individual at the end of the experiments (Plate 2).

We found that these two species could tolerate low pH and low salinity, which reflects the environmental variations in their natural habitats. However, in open ocean conditions (salinity ~35) and lower pH treatment, the species displayed resistance to test dissolution for a longer time than in brackish conditions (salinity ~5 to 20), where more peeled (L3) and fragmented (L4) specimens were observed.

As expected, the response of foraminifera to the different treatments was species-specific, and *Ammonia* sp. appeared more resistant than *E. crispum* when placed in the same conditions of pH and salinity (Figure 7). Under the combined effect of low pH and very low salinity, living dissolved specimens of juvenile *Ammonia* sp. were observed (Plate 2). However, they were not able to recalcify when returned to higher salinities (Figure 7), probably due to a sensitive balance in environmental parameters.

We conclude that coastal benthic foraminifera will not immediately be affected by ocean acidification, but rather by a combination of decreasing salinity and lowered pH.

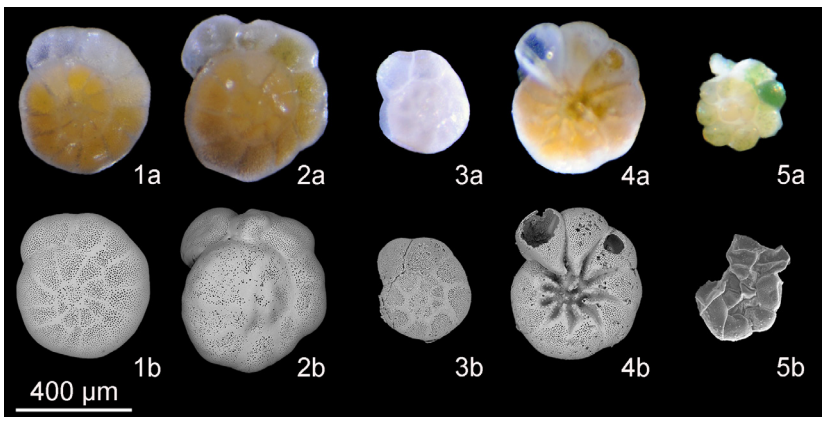


Plate 2. Digital (above) and SEM (below) pictures of the five observed morphological and dissolution levels of foraminiferal test (***Ammonia*** sp.). Level 1 – Unaffected. Level 2 – Deformed. Level 3 – Peeled. Level 4 – Fragmented. Level 5 – Dissolved.

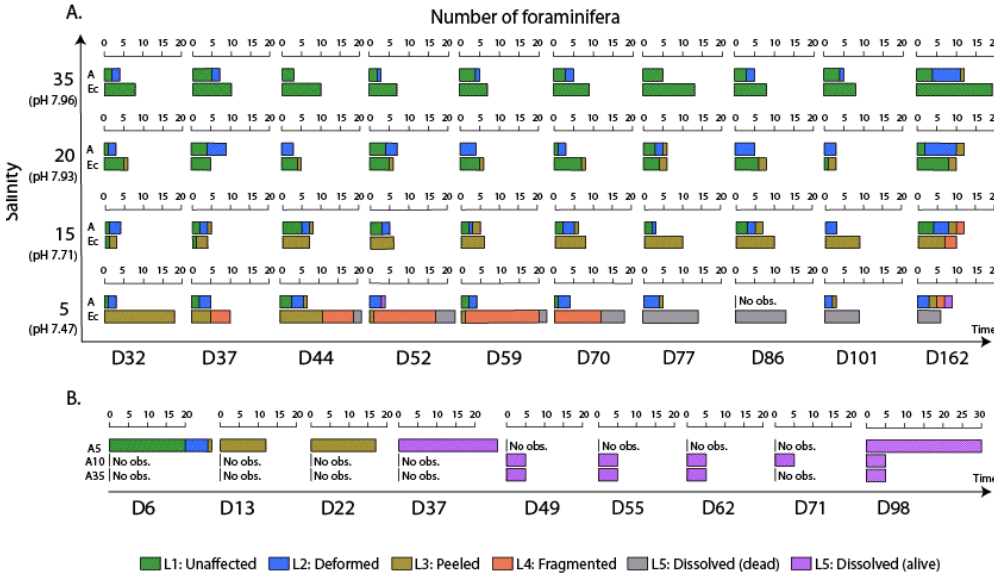


Figure 7. A. Level of test morphology and dissolution of *Ammonia* sp. (A) and *E. crispum* (Ec) at different salinities (and associated pH) and times. B. Level of test morphology and dissolution of juveniles *Ammonia* sp. at salinity 5 (A5), 10 (A10) and 35 (A35).

Paper III. L. M. Charrieau, H. L. Filipsson and K. Ljung. **A bicentennial record of modern environmental changes in the Baltic Sea entrance**. Manuscript, to be submitted to *Estuaries and Coasts*.

In this study, the aim was to reconstruct environmental changes in the Öresund thanks to foraminiferal faunas. A sediment core was collected at the entrance of the Baltic Sea (Öresund) and analysed regarding foraminiferal faunas, grain-size and organic matter content. The ^{210}Pb dating gave an age model between ~1800 and 2013. Four different zones were distinguished in the core thanks to statistical analyses on the main species (>5% in at least one of the samples), which could be linked with environmental changes along the core (Figure 8).

Among the multiple stressors affecting the foraminiferal fauna in the region, the main ones were the oxygen levels, salinity, organic matter content, and pollution with potential lower pH. The largest change in environmental conditions occurred ~1960, when the foraminiferal assemblage shifted from low diversity, dominance of the species *Stainforthia fusiformis* and muddy sediment to higher diversity, dominance of the *Elphidium* group and sandy sediment. This indicates an increased bottom water oxygenation and changes in the water circulation pattern, towards stronger currents in the area.

Moreover, inner organic linings of *Ammonia* were observed, probably linked to low pH and low calcium carbonate saturation, affecting test preservation.

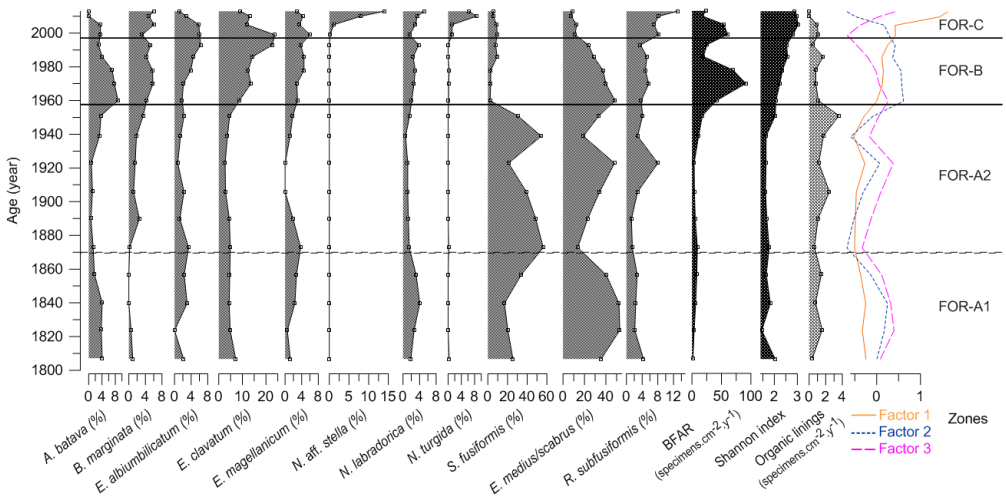


Figure 8. Relative abundances (%) of the foraminiferal major species (>5 %), benthic foraminiferal accumulation rate (BFAR, specimens.cm^{-2}.yr^{-1}), Shannon index, organic linings (specimens.cm^{-2}.yr^{-1}) and factors from the correspondence analysis. Foraminiferal zones reported. Note the different scale on the x axes.

Paper IV. L. M. Charrieau, L. Bryngemark, I. Hansson and H. L. Filipsson. **Improved wet splitter for micropalaeontological analysis, and assessment of uncertainty using data from splitters**. In press. *Journal of Micropalaeontology*.

In this study, we presented an improved device to split wet samples for micro-organism studies. In some cases, the wet picking method is preferred over the picking of dry samples, as it allows preservation of more foraminiferal forms and facilitates the picking of live foraminifera. The main improvements of our device compared to the previous ones are: a fully hermetic and symmetric splitter design with a central drainage system, and very thin, polished walls made possible by the advancement of 3D printing techniques (Figure 9).

We demonstrate small sample losses as well as statistical consistency across splits when using our splitter, and the average efficiency observed on two series of tests was 95%. We also show that the time saved picking a subset will always be larger than the relative increase in statistical uncertainty.

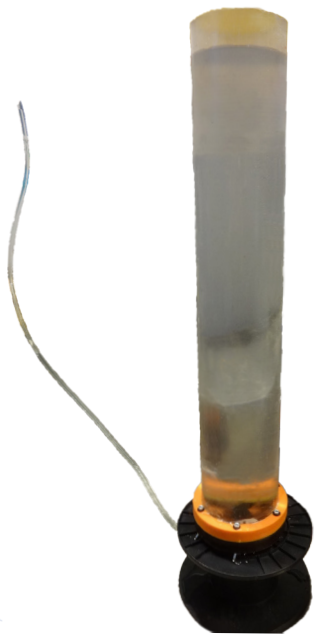


Figure 9. Picture of the wet-splitter.

Discussion

Development of decalcified foraminifera

Dissolution of foraminifera was formerly considered as a taphonomic process affecting the test of dead specimens (Martin 1999). During the remineralisation of organic matter in the sediment, while agglutinated tests are mainly affected by the oxidation of their organic cement, calcareous tests are mostly affected by changes in pore water calcium carbonate saturation state and pH, which can lead to dissolution (Berkeley et al. 2007). This is probably what happened to the tests of *Ammonia* spp. found in our core from the Öresund (Paper III). Passive dissolution of the tests after their death probably affects all the calcareous species, which explains their lower abundances compared to agglutinated species during the periods where most organic linings are found (Paper III; Hermelin 1987; Christiansen et al. 1996; Murray and Alve 1999). Furthermore, the fact that only the organic linings of the taxa *Ammonia* were observed in our core can probably be explained by them being more robust to physical stress than the ones from other species.

More surprisingly, decalcified specimens of *Ammonia* spp. and *Elphidium* spp. showing only inner organic linings were found alive at the sediment surface of the south Baltic Sea (Paper I; Figure 10). In this case, contrary to what was suggested by Cesbron et al. (2016) who also found living (CTG labeled) specimens in the Arcachon Bay, France,

Figure 10. Living decalcified foraminifera.

the low pH alone cannot explain the observed decalcification. Indeed, we showed in Paper II that foraminiferal coastal species were resistant to dissolution under low pH. Moreover, we know that foraminifera have a strong active control of their internal and

external pH during the calcification process and that they are able to mitigate the effects of a lower pH in their environment (De Nooijer et al. 2009; Glas et al. 2012; Toyofuku et al. 2017). Thus, the development of the organic linings in the Baltic Sea was attributed to a combined impact of multiple factors. It is possible that, while specimens were using their energy to subsist at low salinity, low oxygen levels, low pH and low calcium carbonate saturation state, less energy was available to maintain a fully calcified test. Among these stressors, salinity is probably an important one, as suggested by Paper II and previous culture studies, where living decalcified foraminifera developed under the combined impact of low salinity and low pH (Kurtarkar et al. 2011; Saraswat et al. 2015).

Consequently, the presence of organic linings in foraminiferal fauna can no longer only be seen as a poor preservation effect on dead specimens involving lower pH. Their presence can also be interpreted as the results of the combined impact of multiple stressors on living specimens, affecting the energy required for the calcification process. This could typically happen under coastal ocean acidification conditions.

Impact of coastal ocean acidification on benthic foraminifera

According to the results from Papers I, II, and III, benthic foraminifera will probably not be immediately affected by the ongoing coastal ocean acidification. Indeed, in the Skagerrak-Baltic Sea area, species are used to living under multiple stressor effects including varying pH, and they are adapted to the local conditions in their environments, with specific assemblages for each region. Even in the Baltic Sea, where the pH and carbonate saturation state are the lowest, specimens of *Ammonia* spp. and *Elphidium* spp. were found alive, despite being decalcified. In Paper II, we showed that the coastal species *Ammonia* spp. and *E. crispum* are resistant to low pH and low salinity. In their natural habitat, living foraminifera were previously observed at low and varying pH, around CO_2 vents in the northern Gulf of California, USA (Pettit et al. 2013), and in coastal areas such as in the Arcachon Bay, France (Cesbron et al. 2016). Thus, benthic foraminifera would probably survive coastal ocean acidification, at least on the short term.

We can wonder for how long foraminifera will be able to survive if the environmental conditions become more extreme and for a longer period. Over the geological times, some events were comparable to the current ocean acidification (Hönisch et al. 2012), and the response of foraminifera varied. During the Permian/Triassic (P/T) event (252 My ago), where life almost disappeared on Earth (Erwin 2006), calcareous foraminifera were decimated, with 91% of the species becoming extinct (Groves and Altiner 2005). On the other hand, during the Palaeocene-Eocene Thermal Maximum (PETM) event (55 My ago), which appears to be the closest event compared to the present situation (Zachos et al. 2005) and where no mass extinction was observed in living taxa, coastal foraminiferal faunas were only affected by few changes in community structure. Migration seems to have been the key mechanism for the species survival. The comparison

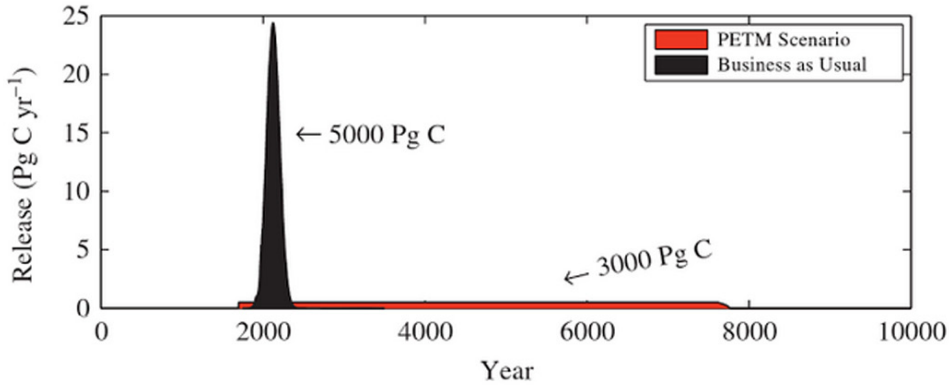


Figure 11. Carbon emissions scenarios as projected by the future and the PETM. Modified from Zeebe and Rigdwell (2011).

of organism response to previous events is however limited, mainly because the rate of the current ocean acidification appears to be greater than any ocean acidification events identified so far (Zeebe and Ridgwell 2011) (Figure 11). Defence mechanisms such as migration and faunal changes will probably be affected. Furthermore, the simultaneous shifts expected in temperature, CO_2, and hypoxia levels, will enhance species sensitivity to environmental extremes (Pörtner et al. 2005).

On the coasts, Paper I and II suggest that lower salinity will be an additional stressor for benthic ecosystems, as foraminifera were less resistant to dissolution when cultured under brackish conditions. Moreover, even if some decalcified species were able to live at low salinity in Paper I and II, it is unknown how fragile these specimens are, and for how long they can survive without their test. Evidence of test recalcification was observed on partly dissolved foraminifera in the field (Polovodova and Schönfeld 2008; Haynert et al. 2012) and in culture studies (Le Cadre et al. 2003; Kurtarkar et al. 2011). However, the recalcification process did not happen in Paper II, on our completely dissolved specimens. This suggests the existence of a threshold for foraminifera, below which the low salinity associated with the effect of multiple stressors prevents them from recalcifying, and, probably, from surviving.

In conclusion, even if coastal species of foraminifera can tolerate extremely varying conditions in their environment on the short term, it is likely that tolerance thresholds will be passed for benthic ecosystems under the future increase in anthropogenic impacts such as coastal ocean acidification.

How do we deal with ocean acidification?

Ocean acidification is emerging as a significant problem for organisms, ecosystems, and human societies. Addressing ocean acidification in a global way would require international agreements to reduce CO_2 emissions in the atmosphere. However, on the coasts, multiple drivers are affecting the pH and carbonate chemistry, and adapted response to take all the specificities of an area into account should be applied (Strong et al. 2014). Thus, regional policies and local management are recommended to mitigate the effects of coastal ocean acidification (Kelly et al. 2011).

In the Baltic Sea region, Jutterström et al. (2014) underline the necessity to use predictive models together with a good monitoring and an international cooperation to assess future environmental changes. The Baltic Marine Environment Protection Commission (HELCOM) and the OSPAR Convention are international organisations that are working on strategies and recommendations for countries in order to better protect the area. Smaller organisations such as Öresundfonden and Öresundsvattensamarbetet (Figure 12), which both aim to specifically protect the Öresund, are local initiatives that are crucial to raise public concern and influence policy makers.

Figure 12. Illustration of the bottom fauna of the Öresund. Göransson et al. (2002).

Future outlook

Coastal ecosystems live in complex environments impacted by multiple stressors. As underlined in this thesis, studying the impact of ocean acidification on coastal species in a realistic way requires preliminary knowledge about the usual variations in pH and calcium carbonate in their habitat, but also additional site-specific stressors such as salinity, temperature and oxygen levels.

Field studies on benthic ecosystems in environments with naturally low pH are still scarce. However, they will be useful to better understand the organism reactions to the ongoing acidification, as all the environmental parameters are simultaneously taken into account. In culture experiments, the implementation of mesocosms would be a way to study the impacts of multiple stressors on a larger part of the ecosystem, in order to include indirect effects of ocean acidification such as changes in species interactions and competition (Hale et al. 2011). This approach is also recommended by Haynert et al. (2014) to study ocean acidification on benthic foraminifera.

In terms of ecosystem services and economic impact, it is necessary to understand the effects of ocean acidification in coastal environments. However, open ocean ecosystems, including benthic species, will also be affected by ocean acidification. Open ocean ecosystems are not used to pH and carbonate chemistry variations in their environment, and will probably be severely impacted. For instance, during the PETM, the only major extinction occurred among deep-sea species of benthic foraminifera (Speijer et al. 2012). Describing how open ocean foraminifera will react to ocean acidification would be an interesting extension of the work described in this thesis.

Svensk sammanfattning

Marin försurning är ett allvarligt miljöproblem som huvudsakligen är orsakat av ökande halter av antropogen CO_2 / koldioxid i atmosfären. Det kännetecknas av sänkt pH i havet tillsammans med en förändring i havsvattnets karbonatkemi med resulterande lägre koncentration av karbonatjoner. Vid kuster, där variationer i miljön är stora på grund av en rad både naturliga - och antropogena orsaker, påverkar marin försurning huvudsakligen frekvensen, storleken och varaktigheten av episoder med lägsta pH och kalciumkarbonatmättnad. Kustekosystem är anpassade till stora variationer i miljön såsom frekventa förändringar i salthalt, temperatur, pH, syrgashalt och mängden organiskt material. Emellertid är konsekvenserna av en ökad variation i dessa miljövariabler, och särskilt för kalkskaliga organismer, inte klargjorda. Min avhandling behandlar effekterna och konsekvenserna av kustnära marin försurning i kombination med andra miljövariabler på marina kalkskaliga, bottenlevande mikroorganismer - foraminiferer.

I Skagerrak-Östersjöområdet varierar artsammansättningen och förekomsten av foraminiferer längs en stark gradient i salthalt, pH och syrgashalt, och arterna är anpassade till lokala miljöförhållanden. Vi noterade dock att Östersjöforaminifererna hade helt eller delvis upplösta kalkskal men trots detta levde de. Skalupplösningen orsakas förmodligen av en kombination av olika miljöstressorer som påverkar den mängd tillgänglig energi som krävs för att foraminifererna ska kunna bygga sina kalkskal.

I en annan studie i min avhandling genomförde vi experiment där vi odlade foraminiferer under olika salthalts- och pH förhållanden. Vi visade i den studien att foraminiferer som ursprungligen kom från kustområden var relativt tåliga för varierande salthalt och pH men också att toleransen för sänkt pH minskade avsevärt när vi samtidigt minskade salthalten. Foraminiferer med helt upplösta kalkskal observerades när salthalten var så låg som 5. Detta understryker betydelsen av hög/normal salthalt när foraminiferer bildar sina kalkskal.

Inom ramen för mitt avhandlingsarbete har jag även studerat miljöförändringar i Öresund, mynningen till Östersjön, under de senaste 200 åren genom att analysera sedimentkärnor och dess innehåll av foraminiferer samt kornstorleksfördelning av sedimenten. Fyra perioder identifierades och vi tolkade det som att under dessa 200 år har dels vattnets syre - och salthalt varierat men även mängden organiskt material och olika föroreningar, vilka även har resulterat i lägre pH.

Sammanfattningsvis, visar jag i min avhandling att även om foraminiferer i kustområden kan på kort sikt kan tolerera väldigt varierande miljöförhållanden, är det troligt att toleransgränser kommer att passeras för bentiska ekosystem som en konskevens av marin försurning och andra antropogena miljöförändringar.

Ytterligare studier av mikroorganismer är nödvändiga för att öka vår förståelse om dåtidens miljöförändringar och kunna sätta dagens och framtidens förändringar i ett större sammanhang.

Résumé en français

Les océans ont absorbé environ un tiers du CO_2 anthropogénique émis dans l'atmosphère depuis la révolution industrielle. Les conséquences sont une baisse du pH moyen des océans ainsi que la modification des paramètres de la chimie des carbonates, dans un phénomène que l'on appelle acidification des océans. L'acidification des océans est globalement reconnue comme une menace pour les écosystèmes, en particulier pour les organismes calcifiants. Dans les zones côtières, les fluctuations du pH et de la chimie des carbonates sont plus complexes qu'en plein océan, à cause des variations saisonnières d'une part, mais aussi à cause des apports réguliers en eau douce par les rivières et le ruissellement, des remontées d'eau profondes (upwellings), et de l'eutrophisation à travers la minéralisation de matière organique. Les écosystèmes côtiers sont donc habitués aux variations rapides et fréquentes du pH dans leur environnement. Cependant, l'acidification des océans augmente progressivement la fréquence, l'amplitude, et la durée de ces variations de pH, avec des effets mal connus sur les écosystèmes. De plus, les zones côtières sont des environnements dynamiques soumis à diverses variations d'origines naturelle et anthropique en termes de salinité, température, oxygène dissous dans l'eau, et quantité de matière organique. Dans ce contexte, cette thèse a pour but d'étudier l'impact de l'acidification des océans en milieu côtier combiné à d'autres stress environnementaux sur les organismes calcifiants, en particulier sur les foraminifères benthiques.

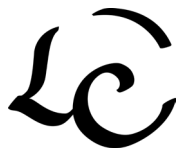
Les résultats de la 1ère étude montrent que dans la région du Skagerrak-Mer Baltique, les faunes de foraminifères varient suivant un fort gradient en termes de salinité, pH, et concentration d'oxygène dissout, et que les espèces sont adaptées aux stress environnementaux typiques pour chaque région. Cependant, les spécimens d'*Ammonia* spp. et d'*Elphidium* spp. observés dans le sud de la Mer Baltique étaient partiellement à intégralement dissouts, probablement à cause de l'effet combiné des différents stress qui affectent l'énergie nécessaire au processus de calcification.

Dans une 2ème étude, les espèces côtières *Ammonia* spp. et *E. crispum* mises en culture ont été démontrées comme résistantes à la dissolution sous différentes valeurs de salinité et de pH, ce qui reflète les variations environnementales dans leur habitat naturel. Cependant, leur résistance à un pH bas est moindre quand les spécimens sont placés en eau saumâtre, et des spécimens vivants mais décalcifiés sont aussi observés à salinité 5. Ces résultats soulignent l'importance d'une salinité élevée pour la calcification des foraminifères.

La 3ème étude se concentre sur la reconstruction des changements environnementaux sur les 200 dernières années à l'entrée de la Mer Baltique, grâce aux faunes de foraminifères. Quatre périodes ont été identifiées avec des variations de niveaux d'oxygène, de salinité, de quantité de matière organique et de niveaux de pollution avec un pH plus bas. Ces résultats montrent que les faunes de foraminifères sont capables de s'adapter à de multiples stress environnementaux.

Cette thèse conclue que, même si les espèces côtières de foraminifères peuvent tolérer des conditions extrêmement variables dans leur environnement sur le court terme, il est très probable que des seuils de tolérance vont être dépassés chez les écosystèmes benthiques côtiers sous l'influence du futur accroissement des impacts anthropogéniques, telle que l'acidification des océans.

Des études supplémentaires sur les micro-organismes comme les foraminifères seront nécessaires pour améliorer notre compréhension des changements environnementaux passés, et pour mettre les changements présents et futurs en perspective.



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Paper I

The effects of multiple stressors on the distribution of coastal benthic foraminifera: a case study from the Skagerrak-Baltic Sea region

Laurie M. Charrieau, Helena L. Filipsson, Karl Ljung, Melissa Chierici, Karen Luise Knudsen and Emma Kritzberg.

In revision. *Marine Micropaleontology.*

The effects of multiple stressors on the distribution of coastal benthic foraminifera: a case study from the Skagerrak-Baltic Sea region

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Abstract

Coastal ecosystems are subjected to both large natural variability and increasing anthropogenic impact on environmental parameters such as changes in salinity, temperature, and pH. This study documents the distribution of living benthic foraminifera under the influence of multiple environmental stressors in the Skagerrak-Baltic Sea region. Sediment core tops were studied at five sites along a transect from the Skagerrak to the Baltic Sea, with strong environmental gradients, especially in terms of salinity, pH, calcium carbonate saturation and dissolved oxygen concentration in the bottom water and pore water. We found that living foraminiferal densities and species richness were higher at the Skagerrak station, where the general living conditions were relatively beneficial for foraminifera, with higher salinity and Ω_{calc} in the water column and higher pH and oxygen concentration in the bottom and pore water. The most common species reported at each station reflect the differences in the environmental conditions between the stations. The dominant species were *Cassidulina laevigata* and *Hyalinea balthica* in the Skagerrak, *Stainforthia fusiformis*, *Nonionella* aff. *stella* and *Nonionoides turgida* in the Kattegat and *N.* aff. *stella* and *Nonionellina labradorica* in the Öresund. The most adverse conditions, such as low salinity, low Ω_{calc}, low dissolved oxygen concentrations and low pH, were noted at the Baltic Sea stations, where the calcareous tests of the dominant living taxa *Ammonia* spp. and *Elphidium* spp. were partially to completely dissolved, probably due to a combination of different stressors affecting the required energy for biomineralization. Even though foraminifera are able to live in extremely varying environmental conditions, the present results suggest that the benthic coastal ecosystems in the studied region, which are apparently affected by an increase in the range of environmental variability, will probably be even more influenced by a future increase in anthropogenic impacts, including coastal ocean acidification and deoxygenation.

Key-words: coastal zone; benthic foraminifera; multiple stressors; salinity gradient; hypoxia; Skagerrak-Baltic Sea

1. Introduction

Coastal areas are dynamic environments highly influenced by natural climate variability. Marine coastal ecosystems are acclimatized to large natural changes such as variations in salinity, temperature, carbonate chemistry and pH, dissolved oxygen concentration, and organic matter input from river discharge (Crossland et al. 2005). However, climate change and human impact are gradually increasing the range of this natural variability and lead to effects such as global warming, deoxygenation and ocean acidification (Crossland et al. 2005). Ocean acidification is globally recognized as a threat for marine life, especially for calcifying organisms (e.g. Kroeker et al. 2013). The uptake of anthropogenic CO_2 by the oceans since the industrial revolution has lowered the pH and resulted in a shift in sea water carbonate chemistry towards lower carbonate ion concentrations, which will likely make it more difficult for calcifiers to precipitate calcium carbonate. Moreover, calcifiers become more vulnerable to the effects of a lower pH when the temperature increases (Rodolfo-Metalpa et al. 2011). Coastal areas, that annually account for ~25% of the global calcium carbonate production and ~50% of calcium carbonate accumulation in the ocean (Mackenzie et al. 2005), are especially subjected to changes in carbonate chemistry and pH variations. In coastal environments, carbonate chemistry is further influenced by a range of factors such as fresh water inputs (Salisbury et al. 2008; Chierici and Fransson 2009) and upwelling of "older" CO_2-rich water with lower pH (Feely et al. 2008). Eutrophication also affects pH and calcium carbonate saturation through production of CO_2 and oxygen consumption during intense organic matter remineralization (Borges and Gypens 2010; Cai et al. 2011). As a result, the pH fluctuations are much more complex in the coastal zones, which are regarded as "hot spots" of ocean acidification, compared to the open ocean (Cai et al. 2011; Duarte et al. 2013). How coastal ecosystems react under the combined effects of natural environmental stressors and ocean acidification is still largely unknown. In this study we focus on ecosystems in the Skagerrak-Baltic Sea area, along a strong salinity gradient ranging from 35 to 14. Recent observations have revealed that in addition to seasonal pH variations, these ecosystems have experienced a decrease in pH over the last 20 years, of 0.1 in the Kattegat and 0.2 in the Baltic Sea (Andersson et al. 2008; Andersson 2010). Moreover, it is obvious from a century long record of total alkalinity (A_T) data that the response to ocean acidification in the Baltic Sea is complex, mainly due to different mineralogy in the river drainage basins (Hjalmarsson et al. 2008). Additionally, the Baltic Sea is strongly impacted by eutrophication and hypoxia (Conley et al. 2011), which make its coastal ecosystems more susceptible to a lower pH (Cai et al. 2011; Melzner et al. 2012; Laurent et al. 2017).

In contrast to oceanic environments, the functioning of coastal ecosystems is often dominated by the benthic compartment (Middelburg et al. 2005). At the base of the food chain, benthic foraminifera are the most diverse shelled micro-organisms at the modern sea floor (Gooday et al. 1992; De Stigter 1996). These unicellular organisms are key players in coastal areas, not only as a food source, together with the rest of the meiofauna, but also as contributors to the carbon cycle, since they represent up to 5% of the annual carbonate production in coastal areas (Langer 2008). They also participate in the marine nitrogen cycle as certain species are able to denitrify (Risgaard-Petersen et al. 2006; Høgslund et al. 2008). Moreover, environmental conditions are usually reflected in the foraminiferal faunal composition, making them excellent indicators of past and present environmental changes. In general, benthic foraminiferal distribution is mainly driven by the dissolved oxygen

concentration and food availability (Van der Zwaan et al. 1999; Gross 2000; Ernst and Van der Zwaan 2004). However, closer to the coast other environmental factors such as salinity and temperature may be restrictive (Nigam et al. 2008; Saraswat et al. 2015).

To build their test (shell), some species are cementing sediment particles (agglutinated and soft-shell species), whereas a large proportion of the benthic foraminiferal species build their tests from calcium carbonate (hyaline and porcelaneous species), which potentially makes them directly threatened by a decrease in pH and carbonates saturation (Ω_{calc}). Several culture experiments using benthic foraminifera have shown a negative impact of ocean acidification conditions on their calcification process (Green et al. 1998; Le Cadre et al. 2003; Khanna et al. 2013; Haynert and Schönfeld 2014) and survival rates (Kuroyanagi et al. 2009; Saraswat et al. 2015). On the other hand, infaunal foraminifera were less sensitive to elevated CO_2 levels when the specimens were cultured with their original sediment (Haynert et al. 2014). Similarly, some symbiont-bearing species do not seem to be dramatically affected by ocean acidification conditions (Glas et al. 2012; McIntyre-Wressnig et al. 2013, 2014) and even enhanced calcification rates have been observed in short-term studies (Vogel and Uthicke 2012). These experiments underline the species-specific response of benthic foraminifera to a lowering in pH and the difficulties to predict a uniform reaction of the benthic community. Living benthic foraminiferal species have been well documented from the Baltic Sea (e.g. Lutze 1965; Brodniewicz 1965; Hermelin 1987), and from the Skagerrak-Kattegat (e.g. Corliss and van Weering 1993; Conradsen 1993; review in Conradsen et al. 1994; Alve and Murray 1995), based on samples collected between 1937 and 1993. But far less is known about their present-day distribution, which is also true for changes in the overall meiofaunal community over the more recent years. In this study, we document the present foraminiferal distribution based on the >100 µm sediment fraction in the Skagerrak-Baltic Sea region along a natural salinity gradient, which is under the impact of multiple stressors. This is a necessary step to better understand how coastal benthic ecosystems will respond to the predicted levels of future environmental change.

2. Study area

The Baltic Sea is an intra-continental sea with a restricted water exchange with the Skagerrak and the North Sea via the Öresund and the Great and Little Belt (all three known together as the Danish straits) (Fig. 1). As a consequence, the water renewal time in the Baltic is about 30 years (Leppäranta and Myrberg 2009). In the Skagerrak, the water circulation is mainly driven by North Sea currents that turn west when reaching the Swedish coasts and leave the Skagerrak through the Norwegian Coastal Current (cf. Erbs-Hansen et al. 2012). The characteristics of the bottom water in this area are stable, with a salinity around 35. Part of the marine inflow reaches the Kattegat and the Baltic Sea, where these marine waters are progressively diluted with large amounts of freshwater (about 15,000 m^3/s, Bergström and Carlsson 1994) draining into the Baltic Sea from numerous large rivers (e.g. Neva, Vistula, Daugava and Odra). The Baltic Sea can be regarded as a large estuary (Leppäranta and Myrberg 2009) with an estuarine circulation and positive water budget, with the inflow of fresh water from rivers and precipitation being considerably larger than the loss through evaporation. The low-saline Baltic Sea surface water exits the Kattegat through the Baltic Water that joins the Norwegian Coastal Current in the Skagerrak (Fig. 1). The large fresh water input and the subsequent large salinity difference between the Kattegat and Baltic Sea result in a strong vertical

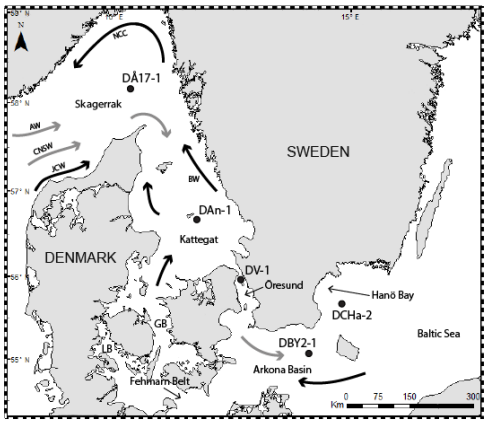

Fig. 1. Map of the studied area. Dots show the five studied stations. General water circulation: main surface currents (black arrows) and main deep currents (grey arrows). GB: Great Belt; LB: Little Belt; AW: Atlantic Water; CNSW: Central North Sea Water; JCW; Jutland Coastal Water; NCC: Norwegian Coastal Current; BW: Baltic Water.

stratification separated by a halocline, usually at 10-20 meters depth in the Kattegat, 20-30 m in the Arkona Basin and 50-60 m in Hanö Bay (Leppäranta and Myrberg 2009). The inflow from the North Sea and the limited vertical mixing lead to a salinity of the bottom waters of ~33 in the Kattegat and ~14 in the southern Baltic Sea. The vertical stratification is further strengthened by the development of a thermocline during spring and summer. Eutrophication and hypoxia events are frequent in the area, especially in the Kattegat, Öresund and Baltic Sea (Hermelin 1987; Conley et al. 2011; Wesslander et al. 2016).

3. Materials and Methods

3.1 Sampling

In November 2013, sediment cores and water samples from the whole water column were collected during a cruise with R/V *Skagerak* along a transect from the Skagerrak to the Kattegat, and the Baltic Sea (Fig. 1). Here we present data from five stations sampled along a strong environmental gradient regarding salinity, Ω_{calc}, pH and oxygen: one in the Skagerrak (DÅ17-1), one in the Kattegat (DAn-1), one in the Öresund (DV-1), and two in the Baltic Sea (DBY2-1 in the Arkona Basin and DCHa-2 in Hanö Bay, respectively). At each station, three to four cores (9-cm-inner-diameter) were collected using a GEMAX twin barrel corer (replicates resulting from different deployments of the corer, marked with capital letters in Table 3, Figs 4, 5 and Appendix A). The corer allows sampling of 80 centimeters long sediment cores, as well as the overlying water, with an undisturbed sediment-water interface. We analyzed the collected core tops for foraminiferal faunas (live and dead), carbon and nitrogen content and grain size distribution, and we performed pH and oxygen profiling of the pore water using Unisense microelectrodes.

3.2 Foraminiferal analyses

The top two centimeters from two cores per site were sliced into one centimeter intervals, except for station DÅ17-1 (Skagerrak) where only a single core was available for foraminiferal analyses. The CellTracker™ Green method was used to label living foraminifera as described by Bernhard et al. (2006). After 12 hours of incubation at 4^0C, samples were fixed in 3.8% Borax®-buffered formalin and stored in a cold room.

In the laboratory, samples were sieved through 63, 100 and 500 µm mesh screens. In the study area, the size fraction >100 µm has most commonly been used for foraminiferal analyses, although some studies applied the >125 µm fraction (see e.g. Hermelin 1987; Conradsen 1993; Conradsen et al. 1994). In order to compare the present results in the most coherent way with previous studies, we have chosen to analyze the >100 µm fraction. The majority of the 100 µm samples were split using a newly improved wet-splitter (Charrieau et al.

in press). The samples from the fractions 100-500 µm and >500 µm were counted under an epifluorescence stereomicroscope (light source Nikon Intensilight C-HGFI). In total, sixteen samples were analyzed: five core tops from the five stations, divided into 0-1 cm and 1-2 cm, and a replicate core top for three of them, also divided into 0-1 cm and 1-2 cm. The picking was divided into two parts. First, only the foraminifera displaying clear fluorescence in at least half of their total number of chambers were picked, sorted by species and counted, henceforth called "live". Second, the remaining non-labeled foraminifera were identified and counted under normal light microscope ("dead" specimens). Finally, the sum of both assemblages (live + dead) is referred to as the total fauna. As additional information, the number of living soft-shelled foraminifera was estimated. For the taxonomy at the genus level, we mainly followed Loeblich and Tappan (1964) with some updates from more recent literature, e.g. Tappan and Loeblich (1988). For the taxonomy at the species level, we mainly used Feyling-Hanssen (1964), Feyling-Hanssen et al. (1971) and Murray and Alve (2011). For original descriptions of the species, see Ellis and Messina (1940 and supplements up to 2013).

Recently, the eastern Pacific morphospecies *Nonionella stella* has been presented as an invasive species in the Skagerrak-Kattegat region (Polovodova Asteman and Schönfeld 2015). However, a comparison of *N. stella* DNA sequences from the Santa Barbara Basin (USA) (Bernhard et al. 1997) with the Swedish west coast specimens (Schweizer et al. unpublished results) demonstrates that they represent two closely related species but are not conspecific. Until this question is resolved we refer to the species found here as *N.* aff. *stella* (Plate 1). The species *Verneuilina media* (here referred to the genus *Eggerelloides*), which has often been reported in previous studies from the Skagerrak-Kattegat area, was morphologically close to

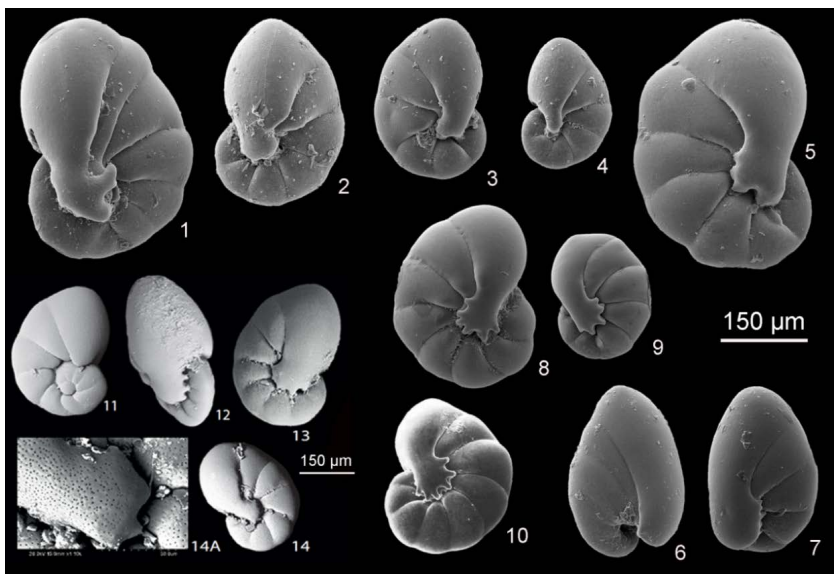

Plate 1. 1-7. Specimens collected in this study from the Kattegat-Öresund. 1-5. *N.* aff. *stella.* 6-7. *N. turgida*; 8-9. *N. stella* from the Santa Barbara Basin (Ni et al., unpublished data); 10. *N. stella* from the Santa Barbara Basin, USA (Bernhard et al. 1997); 11-14. *N. stella* from the Swedish west coast (Polovodova Asteman and Schönfeld 2015). Reproduced with permission.

Eggerelloides scabrus in the present material, and these two species have therefore been grouped as *E. medius/scabrus*. The taxon *Elp hidium excavatum* forma *clavata* (cf. Feyling-Hanssen 1972), has been referred as *Elphidium clavatum* in our study following Darling et al. (2016). A few specimens of *Elphidium selseyense* (Heron-Allen and Earland) may have been included into the species counts of *E. clavatum*, as these two taxa are difficult to separate morphologically. It is very likely that the decalcified *Elphidium* specimens we have in the Baltic Sea belong to *E. clavatum*. However, we chose to use a conservative approach and refer to the taxon as *Elphidium* spp. *Ammonia* specimens are referred to *Ammonia* spp. since only decalcified specimens were recorded in the Baltic Sea.

Total foraminiferal density was calculated for each of the replicate core for the combined top two centimeters of sediment and normalized to a volume of 50 cm^3. Detailed counting results for both depth levels are presented in Appendix A. The Shannon index H was calculated to describe the diversity of the living foraminiferal faunas.

3.3 Hydrographic and biogeochemical analyses

CTD casts were taken at each site to measure salinity, temperature and dissolved oxygen concentration in the water column. Water samples were collected at specific water depths from Niskin bottles for carbonate chemistry analyses and the samples were analyzed for total alkalinity (A_T), total dissolved inorganic carbon (DIC) and pH at the Department of Marine Sciences, University of Gothenburg, Sweden. The samples were introduced directly into 250 mL borosilicate bottles, preserved with saturated mercuric chloride ($HgCl_2$; 60 µL to 250 mL sample) and stored dark at 4°C before analysis within 6 months, following the method described in Dickson et al. (2007). DIC was determined using gas extraction of acidified samples followed by coulometric titration and photometric detection. A_T was determined in an open cell by potentiometric titration with 0.05 N hydrochloric acid as described by Haraldsson et al. (1997). pH on the total scale was determined spectrophotometrically using the sulphonephtalein dye, m-cresol purple, as indicator (Clayton and Byrne 1993). The analytical precision was estimated ±0.001 pH units, as determined by triplicate analysis of one sample every day. The magnitude of the perturbation of seawater pH caused by the addition of the indicator solution was calculated and corrected for using the method described in Chierici et al. (1999). We used a pair of A_T and pH, salinity, temperature and depth as input parameters in a CO_2-chemical speciation model (CO_2SYS program, Pierrot and Wallace 2006) to calculate pH in situ and calcium carbonate saturation for calcite (Ω_{calc}) based on the carbonate system dissociation constants (K*1 and K*2) modified by Dickson and Millero (1987) and the HSO_4^- dissociation constant from Dickson (1990). We used the DIC measurements to investigate the internal consistency and accuracy in the calculations, which gave a pH internal consistency of ±0.014 and an estimated error in calcite saturation of ±0.06.

Profiles of dissolved oxygen concentration and pH in the sediment pore water were performed on the ship, using Clark-type microelectrodes with 100 µm glass tips (OX 100 and pH 100, Unisense) connected to a multimeter (Unisense). The oxygen microelectrodes were calibrated using oxygenated bottom water and an anoxic ascorbate solution, and pH microelectrodes were calibrated using pH standard solutions. Three oxygen profiles and one to two pH profiles were measured on one core per station. A slight discrepancy can be observed between the dissolved oxygen concentration measured from the CTD and the one from the microelectrodes,

due to a short time delay between sampling and measurements with the microelectrodes, where gas exchange was possible between the water overlaying the sediment cores and the atmosphere.

Our hydrographic data were compared with data from the monitoring program, obtained from the Swedish Meteorological and Hydrological Institute (SMHI) publically available data-base SHARK (Svenskt HavsARKiv, www.smhi.se).

Total Organic Carbon (TOC) and Total Nitrogen (TN) contents were measured on the top two centimeters of two core tops for each station, and carbon and nitrogen stable isotopic ratios of organic matter ($\delta^{13}C$ and $\delta^{15}N$) were measured on the top two centimeters of one core top for each station. The samples were weighed and freeze-dried at the Department of Geology, Lund University. Approximately 8 mg of freeze-dried sediment was homogenized and used for TOC and TN analyses. Removal of inorganic carbon was carried out with the in-situ acidification method based on Brodie et al. (2011), using silver capsules and 2M HCl. TOC and TN content were analyzed with a Costech ECS 4010 Elemental Analyzer at the Department of Geology, Lund University.

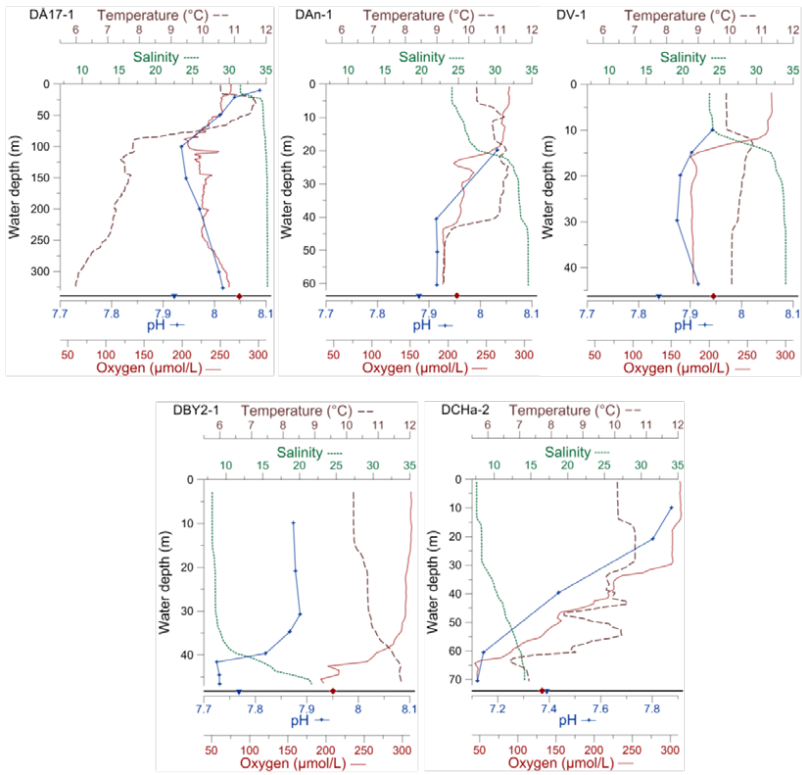

Fig. 2. CTD profiles of temperature, salinity, pH and dissolved oxygen concentration in the water column for the five studied stations. DÅ17-1: Skagerrak; DAn-1: Kattegat; DV-1: Öresund; DBY2-1: Arkona Basin; DCHa-2: Hanö Bay. The black line represents the water-sediment interface, the triangles represent the pH and the diamonds the oxygen levels values at this interface, from the microelectrodes´ measurements. Note the different pH scale for DCHa-2.

Following the same procedure, $\delta^{13}C$ and $\delta^{15}N$ were analyzed with an isotope ratio mass spectrometer Thermo Delta V at the Department of Biology, Lund University. The instruments were calibrated against in-house standards. The measurements showed a reproducibility of 0.2% and 0.03% for TOC and TN contents, respectively, and 0.07‰ and 0.06‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

3.4 Grain-size analyses

Grain-size analyses were performed on the top two centimeters at each station, using 3.5 to 5 grams of freeze-dried sediment. Organic matter was removed by adding 15 mL of 30% H_2O_2. The samples were heated for 3 to 4 minutes on a hot plate until reaction ceased, and let to cool down. Then, 10 mL of 10% HCl was added to remove carbonates. The sediment was subsequently diluted and washed until its pH was neutral. Finally, biogenic silica was removed by boiling the sediment in 100 mL solution of 8% NaOH, and then washed until neutral pH was reached again. The sand fraction (>63 µm) was subsequently separated by sieving and the mass fraction of sand in the sample was calculated. Grain sizes <63 µm were analyzed by laser diffraction using a SediGraphIII at the Department of Geology, Lund University. Three size groups, <4 µm (clay), 4–63 µm (silt) and 63-2000 µm (sand) were classified.

3.5 Statistical analysis methods

Cluster analysis was performed to investigate if the spatial variability of the foraminifera fauna between replicate cores at one station affected the spatial variability along the transect. The cluster analysis was performed with the VEGAN package in R, using the total fauna (live + dead) data, normalized to 50cm^3. To build the matrix of distance, we used the Morisita's index, which is independent of sample size, to account for the large difference in foraminiferal species density for each station. A dendrogram was constructed based on arithmetic averages with the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean) and the resulting cophenetic correlation coefficient was 0.996. Principal Component Analysis (PCA) was performed to explore which parameters explain most of the observed variations between the stations at the time of our sampling. The PCA was performed with the package ADE4 in R, using centered and standardized data from the measured environmental variables and from the major (> 2% in at least one station) living foraminiferal faunas, normalized for 50 cm^3, from the five stations and replicates.

Table 1. Environmental variables for the bottom water and water-sediment interface at the five studied stations.

Stations	Latitude (^{0}N)	Longitude (^{0}E)	Water depth (m)	Temperature (^{0}C)[1]	Salinity[1]	Oxygenation (µmol.L^{-1})[2]	Oxygen penetration depth (mm)[2]	pH[2]	Alkalinity A_T (µmol.kg^{-1})	Ω_{calc}
DÅ17-1 (Skagerrak)	58^{0}16.30'	10^{0}30.49'	328	6.0	35.18	275	9.5	7.92	2187.3	2.56
DAn-1 (Kattegat)	56^{0}40.13'	12^{0}07.00'	61	9.2	34.49	213	4.6	7.88	2188.3	3.37
DV-1 (Öresund)	55^{0}55.59'	12^{0}42.66'	45	10.1	33.98	207	3.0	7.84	2186.6	2.43
DBY2-1 (Arkona Basin)	55^{0}00.00'	14^{0}04.95'	48	11.7	21.63	210	1.7	7.77	1985.3	1.20
DCHa-2 (Hanö Bay)	55^{0}37.60'	14^{0}50.00'	71	7.3	14.13	130	2.8	7.39	2026.3	0.19

[1] From CTD
[2] From microsensors

Table 2. Average grain-size and average organic matter of the top two centimeters at the five studied stations.

Stations	Water depth (m)	cm	Sand (%)	Silt (%)	Clay (%)	TOC (%)	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
DÅ17-1	328	1	0.1	30.0	70.0	2.7	7.3	-23.7	5.7
(Skagerrak)		2	0.1	27.0	73.0	2.7	7.7	-23.4	5.7
DAn-1	61	1	1.0	26.7	72.3	2.5	7.4	-23.1	6.0
(Kattegat)		2	1.2	27.7	71.1	2.7	7.6	-23.1	6.0
DV-1	45	1	6.7	27.1	66.2	3.7	8.2	-23.5	5.7
(Öresund)		2	7.4	25.9	66.7	3.7	8.2	-23.4	5.3
DBY2-1	48	1	0.8	36.7	62.5	5.0	7.9	-32.5	3.8
(Arkona Basin)		2	0.4	37.9	61.8	5.2	7.8	-	-
DCHa-2	71	1	3.1	17.4	79.5	4.4	7.3	-25.4	3.1
(Hanö Bay)		2	3.7	17.3	79.0	3.9	7.6	-25.8	3.3

4. Results

4.1 Physical and chemical variability in the water column

The salinity profiles in the water column at the stations in the Skagerrak-Baltic Sea regions, highlighted by the CTD casts (Fig. 2), show that a brackish surface water layer covered the marine waters of the Skagerrak, and the typical halocline was observed at all stations between 10 m (DV-1, Öresund) and 40 m (DBY2-1, Baltic Sea). The temperature and the dissolved oxygen levels decreased with depth, except at DÅ17-1 (Skagerrak) where the oxygen levels were relatively higher in the bottom water, and DBY2-1 (Baltic Sea) where the temperature increased with depth. The pH values

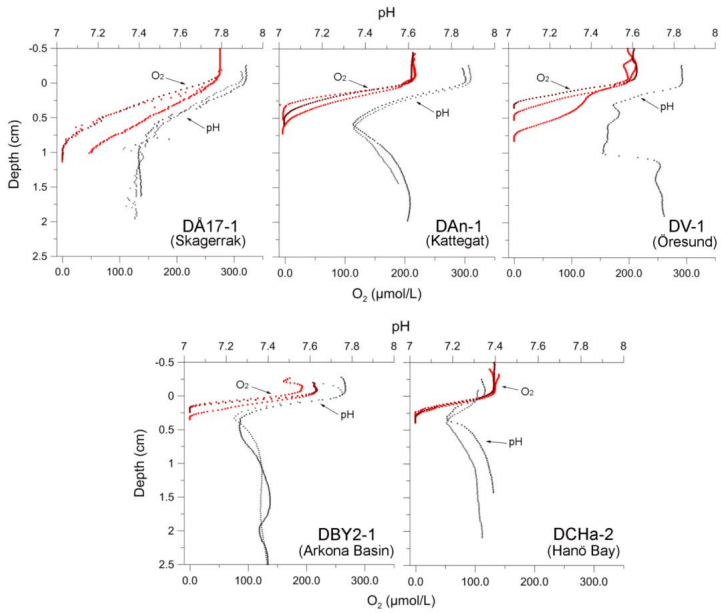

Fig. 3. Vertical distribution of dissolved oxygen concentration (in red) and pH (in black) for the five studied stations.

continuously decreased with depth, except for DÅ17-1 (Skagerrak) and DV-1 (Öresund) where they increased again when reaching the bottom water (Fig. 2).

Along the 5-stations transect, the bottom-water salinity (CTD data) decreased from 35.18 in the Skagerrak (DÅ17-1 station) to 14.14 in Hanö Bay (DCHa-2 station, Table 1). A similar pattern was observed for the water-sediment interface pH (7.92 to 7.39; microelectrodes measurements) and water-sediment interface oxygen (275 $\mu mol.L^{-1}$ to 130 $\mu mol.L^{-1}$, corresponding to 6.17 to 2.91 $ml.L^{-1}$, Table 1, Fig. 2). The bottom-water temperatures ranged from 6^0C at the deepest station DÅ17-1 (Skagerrak, 328 m) to 11.7^0C at the shallowest station DBY2-1 (Baltic Sea, 45 m). In the bottom-water, which is one meter above the sea-floor (CTD data), alkalinity values were comparable between the Skagerrak, Kattegat and Öresund stations (~2187 $\mu mol.kg^{-1}$) (Table 1), while they were lower at the Baltic stations (~2000 $\mu mol.kg^{-1}$). Ω_{calc} values were all above 1, except at the DCHa-2 station, with 0.19 (Table 1).

4.2 Sediment characteristics

On average, the top two centimeters of the sediment consisted of silty clay with ~70% of clay and ~27% of silt (Table 2). The sand content was highest at DV-1 and DCHa-2, with on average 7.0% and 3.4%, respectively (Table 2).
Pore-water oxygen profiles at all stations showed a typical decrease towards zero in the topmost cm of the sediment (Fig. 3). The oxygen penetration depth (OPD) ranged between 9.5 mm at the Skagerrak DÅ17 station and 1.7 mm at the Baltic DBY2-1 station (Table 1, Fig. 3). Pore-water pH profiles also demonstrated a rapid decrease with depth in the sediment, followed by a slow increase with depth at all the stations, except for DÅ17-1 where the pH remained stable at its lowest value (Fig. 3). The minimum pH value in the sediment was lower at the Baltic Sea stations (7.17 to 7.25) than at the three other stations (7.34 to 7.46).

The TOC values averaged over the top two centimeters ranged from 2.6% at the Kattegat DAn-1 station to 5.1% at the DBY2-1 station (Table 2). $\delta^{13}C_{TOC}$ was lowest at the Baltic Sea stations DBY2-1 and DCHa-2 with -32.5‰ and -25.6‰, respectively, and highest at the three other stations with a mean value of -23.4‰ (Table 2). A similar pattern was found for $\delta^{15}N$ with the lowest values at DBY2-1 and DCHa-2 (~3.5‰) and the highest values at the three other stations (~5.7‰). The C/N ratios ranged between 7.4 in the Baltic (DCHa-2) and 8.2 in the Öresund (DV-1) (Table 2).

4.3 Foraminiferal faunas

4.3.1 Density and diversity

Samples from the five stations could be divided into two groups with respect to their density of living (CTG-labelled) foraminifera in the >100 µm fraction (100-500 µm + >500 µm) in the top two centimeters of the cores (Table 3). Density ranged from 1284 to 3364 individuals/50 cm^3 for the stations DAn-1 (Kattegat) and DÅ17-1 (Skagerrak), while the range for the Baltic Sea stations (DBY2-1 and DCHa-2) was from 34 to 46 individuals/50 cm^3. A similar pattern was observed for the species richness, with a maximum of 54 species found in the Skagerrak (DÅ17-1) and only 2 to 4 species found in the Baltic Sea (Table 3). The DV-1 station in the Öresund showed intermediate values, with a density of 347 individuals/50 cm^3 and a species richness of 19 species. The Shannon index was highest at DÅ17-1 and DAn-1 (1.94-2.30) and lowest at DCHa-2 (0. 65-0.92) (Table 3). Soft-shelled foraminifera were more abundant at the Arkona Basin station (404 and 751 individuals/50 cm^3, for the two replicate cores) and at the Kattegat station (299 and 302 individuals/50 cm^3) than at the other stations (Table 3).

4.3.2 Major species

In the living foraminiferal faunas, twenty-three species had a relative abundance higher than 2% at least at one station. We considered these as major species (Table 3, Plate 2). The Skagerrak station (DÅ17-1) was largely dominated by the hyaline species *Cassidulina laevigata* (36%) and *Hyalinea balthica* (23%). The agglutinated species group *E. medius/scabrus* and the hyaline species *Bolivina skagerrakensis* and *Bulimina marginata* were also present (7%, 5% and 5%, respectively). The dominant species at the Kattegat station (DAn-1) were more evenly distributed, with both replicates dominated by the hyaline taxa *Stainforthia fusiformis* (31% and 22%), *N.* aff. *stella* (26% and 19%) and *Nonionoides turgida* (17% and 16%) (Table 3). *Bulimina marginata* (11% and 8%) and *Nonionellina labradorica* (11% and 6%) were also found as major species. In the Öresund (DV-1 station), *N.* aff. *stella* and *N. labradorica* represented 35% and 12% of the living foraminifera, respectively. *Nonionoides turgida* and *E. medius/scabrus* were also present with 9% and 8%, respectively. In the Baltic Sea, two taxa strongly dominated the four replicates, i.e. *Ammonia* spp. and *Elphidium* spp. Their identification to the species level was not possible due to severe decalcification of the tests. Together, they represented between 70% and

Table 3. Density, species richness, Shannon index and relative abundance of living major (>2% in at least one station) taxa in the top two centimeters in cores from the five studied stations.

	DÅ17-1C	DAn-1D	DAn-1A	DV-1I	DBY2-1E	DBY2-1G	DCHa-2E	DCHa-2D
Water depth (m)	328	61	61	45	48	48	71	71
Density (individuals.50 cm^{-3})	1284	2198	3364	347	34	40	46	35
Species richness (S)	54	44	24	19	4	3	3	2
Shannon index (H)	2.29	2.30	1.94	2.27	1.02	0.92	0.88	0.65
Species/taxa (%)								
Ammonia falsobeccarii Rouvillois	0	0.7	0	5.4	0	0	0	0
Ammonia spp.	0	0	0	0.0	55.8	52.4	46.6	64.0
Bolivina pseudopunctata Höglund	0	0	0	2.7	0	0	0	0
Bolivina skagerrakensis Qvale and Nigam	5.3	0.2	0.3	0.0	0	0	0	0
Bulimina marginata d'Orbigny	5.2	10.7	7.7	2.7	0	0	0	0
Cassidulina laevigata d'Orbigny	35.5	1.1	0.4	0.9	0	0	0	0
Elphidium clavatum Cushman	0.1	4.6	2.3	5.4	0	0	5.9	0
Elphidium spp.	0	0	0	0	15.1	38.8	47.5	36.0
Globobulimina turgida (Bailey)	2.5	0	0	0	0	0	0	0
Hyalinea balthica (Schroeter)	23.4	0.1	0	0	0	0	0	0
Haynesina depressula (Walker and Jacob)	0	0	0	2.7	0	0	0	0
Nonionella aff. *stella*	0	18.6	26.2	35.3	0	0	0	0
Nonionellina labradorica (Dawson)	0	10.6	5.7	11.8	0	0	0	0
Nonionoides turgida (Williamson)	0.1	16.1	16.9	9.1	0	0	0	0
Stainforthia fusiformis (Williamson)	2.4	21.8	30.9	2.7	0	0	0	0
Stainforthia loeblichi (Feyling-Hanssen)	0	3.4	2.2	0	0	0	0	0
Ammotium cassis (Parker)	0	0	0	0	27.9	8.7	0	0
Cribrostomoides subglobosum (G.O. Sars)	2.2	0	0	0	0	0	0	0
Eggerelloides medius (Höglund)/*Eggerelloides scabrus* (Williamson)	6.6	0.9	0.7	8.2	0	0	0	0
Leptohalysis scotti (Chaster)	0.5	2.9	2.5	1.8	0	0	0	0
Reophax subfusiformis Earland	0.2	0.2	0.2	4.5	0	0	0	0
Textularia earlandi Phleger	0.1	3.1	1.3	0.9	0	0	0	0
Trochamminopsis pusilla (Höglund)	2.0	0	0	0	0	0	0	0
Others	13.8	5.0	2.6	5.8	1.2	0	0	0
Soft-shelled (individuals.50 cm^{-3})	39	302	299	104	751	404	8	0

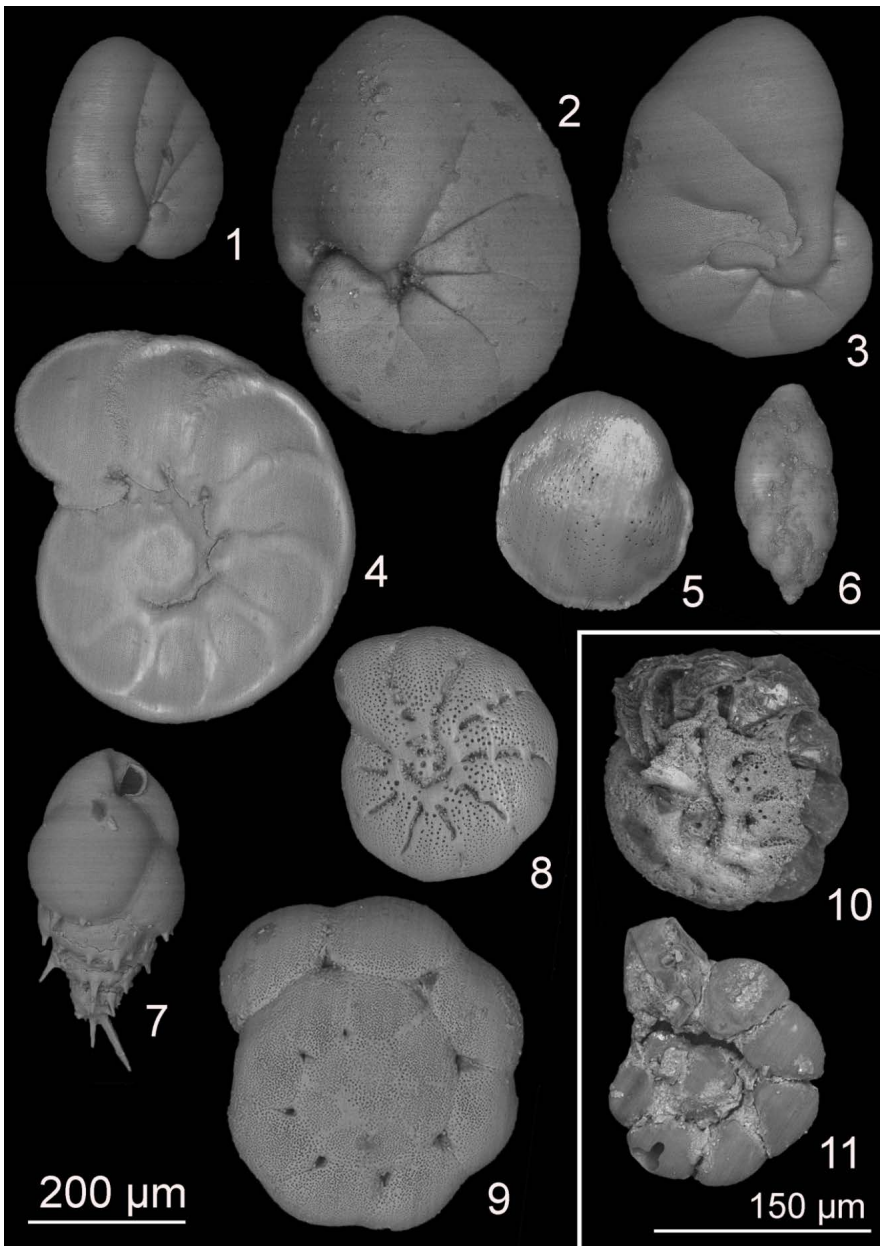

Plate 2. Main species. 1 – *Nonionoides turgida*; 2 – *Nonionellina labradorica*; 3 – *Nonionella* aff. *stella*; 4 – *Hyalinea balthica*; 5 – *Cassidulina laevigata*; 6 – *Stainforthia fusiformis*; 7 – *Bulimina marginata*; 8 – *Elphidium clavatum*; 9 – *Ammonia falsobeccarii*; 10 – *Elphidium* sp., alive and half-decalcified specimen; 11 – *Ammonia* sp., dead and decalcified specimen.

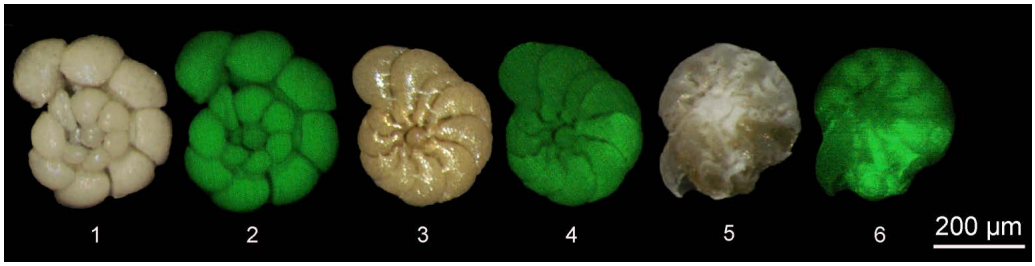

Plate 3. Living decalcified foraminifera in DCHa-2, the same specimens are shown under normal light and epifluorescent light. 1-2: *Ammonia* sp. 3-4: *Elphidium* sp. 5-6: half decalcified *Elphidium* sp.

100% of the foraminiferal community at these stations (Table 3). At the Arkona station DBY2-1, the agglutinated species *Ammotium cassis* was also common, accounting for 28% and 9% of the living foraminifera, whereas it was absent in Hanö Bay (DCHa-2 station).

The cluster analysis of the total foraminiferal fauna (Fig. 4) shows that both replicate cores for all the stations are very similar, suggesting that the spatial variability between two replicate cores did not significantly affect the large scale spatial variability.

4.3.3 Test dissolution

The two hyaline foraminiferal taxa found at the Baltic Sea stations DBY2-1 and DCHa-2 displayed considerable test dissolution (Plates 2, 3). The CTG labelling attests that the specimens were alive, despite dissolution. At DCHa-2 (Hanö Bay), the dissolution was sometimes only partial and some pieces of the test were still visible (Plate 2, Plate 3), whereas at the Arkona station, the dissolution was complete. The remaining inner organic lining kept the form of the original tests, allowing the identification to the genus level of the two hyaline taxa *Ammonia* spp. and *Elphidium* spp.

A similar phenomenon was observed for the dead foraminiferal fauna, where most of the *Ammonia* spp. and *Elphidium* spp. showed partially to completely dissolved tests at both stations (Plate 2). Moreover most of the dead specimens, hyaline or agglutinated, had particularly fragile tests.

4.4 PCA

The PCA analysis shows that the first four axes together explain 97% of the variance in our

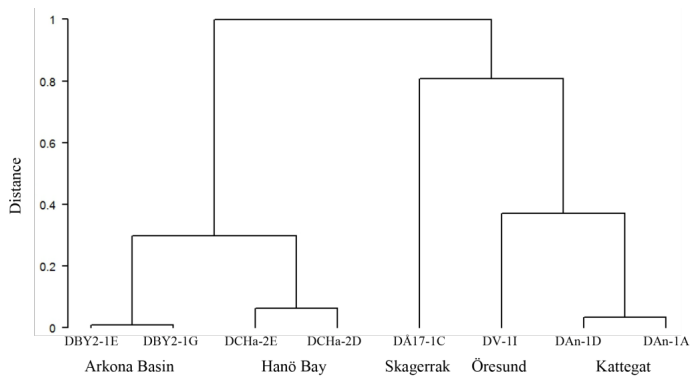

Fig. 4. Dendrogram produced by the cluster analysis based on the Morisita index and the UPGMA clustering method.

dataset. Four groups of samples can be distinguished in the bi-plot of the first two axes: one group for the Skagerrak station DÅ17-1, one for the Kattegat station DAn-1 and replicate, one for the Öresund station DV-1 and one for the two Baltic Sea stations DBY2-1 and DCHa-2 with replicates (Fig. 5). The first axis accounts for 43% of the variance and is correlated to salinity, alkalinity and Ω_{calc} in the water, $\delta^{15}N$, pH and organic matter contents in the sediment, and thus represents the main salinity gradient between the Skagerrak-Kattegat stations and the Baltic Sea stations (Fig. 5). The second axis accounts for 26% of the variance and is related to temperature, which is negatively correlated with the water depth. The deepest station DÅ17-1 (Skagerrak) is opposed to the shallowest ones such as DAn-1 (Kattegat) and DV-1 (Öresund). The third axis accounts for 16% of the variance and shows variance in C/N, separating the more coastal DV-1 station (Öresund) from the other stations, which are more marine. The fourth axis accounts for 13% of the variance and describes the variance in clay and sand content. The sandy stations DCHa-2 (Hanö Bay) and DV-1 (Öresund) are opposed to the DBY2-1 station (Arkona Basin). Regarding the major foraminiferal species (>2%), the first axis separates the Baltic Sea taxa *Elphidium* spp., *Ammonia* spp. and *A. cassis* from all other species (Fig. 5). The second axis separates the group of foraminiferal species that are typical for the Skagerrak (e.g. *C. laevigata*, *H. balthica*, *B. skagerrakensis* and *Globobulimina turgida*) from those typical for the Kattegat and Öresund (e.g. *N.* aff. *stella*, *N. turgida* and *N. labradorica*). The third axis separated the species typical of the Öresund station (DV-1) (typically *Haynesina depressula* and *Bolivina pseudopunctata*) from the species composition of the rest of the stations, and the fourth axis separated the species composition in the Öresund (DV-1) from those typical from the Arkona Basin (DBY2-1).

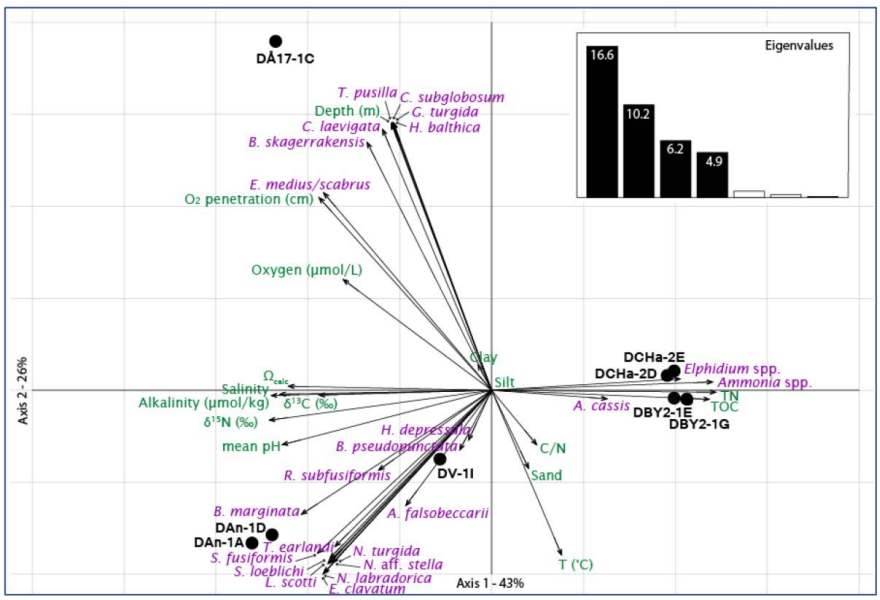

Fig. 5. Biplot of the first two axes of PCA on environmental variables and major foraminiferal taxa (>2% in at least one sample). The eigenvalues are shown on the screeplot.

5. Discussion

5.1 Modern distribution of the living foraminiferal fauna

The density and species richness of living foraminifera clearly reflect the strong environmental gradient in the area. Relatively high density and species richness were found in the Skagerrak-Kattegat stations, where the salinity and Ω_{calc} in the bottom water, mean pH in the sediment and oxygen levels at the sediment-water interface were high, and the organic matter content was relatively low (Table 3, Fig. 5). In contrast, the density and species richness were lower at the Baltic Sea stations, where salinity, Ω_{calc}, pH and oxygen levels were low. In the Baltic Sea, the land-locked location, large fresh-water inputs and remineralization of large amounts of organic matter – due to eutrophication and land run-off – explain the low oxygen levels and penetration depths, low Ω_{calc} and low pH values at these stations (Table 3, Fig. 5). Thus, the composition of the living foraminiferal fauna was specific for each area, with typical species composition associated with certain environmental conditions.

The two dominant species found at our Skagerrak station (*C. laevigata* and *H. balthica*) were representative of this well-oxygenated and food rich region. *Cassidulina laevigata* is reported from areas with high organic matter levels, well-oxygenated b ottom-water and a water depth between 200 and 400 meters (Conradsen et al. 1994; De Stigter et al. 1998). *Hyalinea balthica* is often considered as an opportunistic species, with high reproductive rates as soon as food is available (Hess and Jorissen 2009). By considering the total (living + dead) foraminifera, it is possible to compare the present species distribution with the study of Conradsen et al. (1994). These authors identified five major assemblages in the Skagerrak-Kattegat region based on total faunas of 177 surface samples collected between 1947 and 1990. The relative abundance ranges of the characteristic foraminiferal species explaining most of the variance of each assemblage were given. The relative abundances of our dominant species at the Skagerrak station were in the range of the ones observed in the "*C. laevigata* assemblage" of Conradsen et al. (1994) (Table 4). By comparing more specifically our station DÅ17-1 with the nearby station, denoted 9011 in Conradsen et al. (1994), we found that the same main species were observed (Table 4). *Cassidulina laevigata* was also largely dominant at station 9011, representing one third of the foraminiferal fauna. The differences in the relative abundances are probably due to spatial patchiness (Table 4). We noticed that *Pullenia osloensis* contributed 10% in our assemblage at DÅ17-1, while it was absent from the characteristic species of the "*C. laevigata* assemblage" and at the Conradsen et al.'s station 9011. Conradsen et al. (1994) found that *P. osloensis* was only sporadically occurring in their 177 Skagerrak-Kattegat samples, with a maximum of 7% at about 200 meters depth. This could indicate either that *P. osloensis* is not continuously distributed in the area, but rather characterized by sporadic occurrences, or that the distribution of the species has changed since 1994. This "*C. laevigata* assemblage" of Conradsen et al. (1994) was found in the transitional zone between the shallow coastal zones and the stable Skagerrak deep-water.

In the Kattegat, where the salinity, oxygen levels and pH were lower and the temperature higher than in the Skagerrak, the highly opportunistic species *S. fusiformis* was dominant in our living faunas. This species is known to be tolerant to low-oxygen conditions (Alve 1994; Filipsson and Nordberg 2004) and capable of denitrification (Piña-Ochoa et al. 2010). *Nonionella* aff. *stella*, *N. turgida* and *N. labradorica*, major species in our assemblages, have also been observed in oxygen-depleted environments (Bernhard and Bowser 1999;

Cedhagen 1991), as well as *B. marginata*, even though this latter species is less tolerant to hypoxia than *S. fusiformis* (cf. Bernhard and Alve 1996). *Bulimina marginata* has also been found in organic-rich sediments (Conradsen et al. 1994), at salinities between 25 and 35, and in environments where seasonal stratification was observed (Eichler et al. 2014). The species *B. marginata* and *N. labradorica* were characteristic of the "*B. marginata* assemblage" of Conradsen et al. (1994), and the relative abundances of these two species in the total fauna from our Kattegat station were in the same range (Table 4). Conradsen et al. (1994) described the distribution of *S. fusiformis* in the region as patchy, with locally high densities at specific stations of the Skagerrak-Kattegat area, which can explain the absence of this species in the characteristic species of the "*B. marginata* assemblage". We compared our DAn-1 station with the closest station of Conradsen et al. (1994), denoted 41 (Table 4). The same main species were observed at both stations, even though the percentages were very different, with *B. marginata* being strongly dominant (50%), probably because of spatial patchiness. This "*B. marginata* assemblage" was found along the Swedish coast of the Skagerrak and in the deepest Kattegat (Conradsen et al. 1994).

The fact that *N.* aff. *stella*, which was common at DAn-1, was absent from the Conradsen's stations has to be emphasized. Polovodova Asteman and Schönfeld (2015) suggested that *N. stella* arrived to the Skagerrak region around 1985, probably brought by ship ballast tanks. As discussed by the authors, this species differs from *N. turgida* in the extension of the last chamber. *N. stella* exhibits a lobate and hand-shaped extension with clear finger-like processes over the suture, while *N. turgida* has a straight, rounded or drop-shaped extension (Polovodova Asteman and Schönfeld 2015). Our *N.* aff. *stella* specimens appear to be morphologically similar to the *N. stella* as described by Polovodova Asteman and Schönfeld (2015), and the straight and rounded extension typical from *N. turgida* was not observed (Plate 1). As also illustrated by Polovodova Asteman and Schönfeld (2015), our material shows a pronounced variability in the development of the finger-like processes of the final chamber of *N.* aff. *stella*. This character is not always clearly visible, and sometimes the fingers are even missing (Plates 1, 2). This is, however, not the case for the *N. stella* specimens coming from the Santa Barbara Basin, where the finger structures are well-developed (Plate 1). The increasingly low oxygen levels and the ability of

Table 4. Relative abundance of dominant species in the total foraminiferal faunas: comparison of the species characterizing the assemblages defined in Conradsen et al. (1994) and the present study data. The details of one station are given as an example.

	This study: DÅ17-1	Conradsen et al. 1994:	Conradsen et al. 1994: 9011
Coordinates	*58 ^{0}16'30''N - 10 ^{0}30'49''E*	The *C. laevigata* assemblage	*58 ^{0}15'33''N - 10 ^{0}30'28''E*
Main species (%)	*C. laevigata* – 28.3%	*C. laevigata* – 8 to 55%	*C. laevigata* – 35.1%
	H. balthica – 10.4%	*H. balthica* – 0 to 46%	*H. balthica* – 6.2%
	E. medius/scabrus – 7.5%		*E. medius/scabrus* – 4.8%
	B. skagerrakensis – 6.0%	*B. skagerrakensis* – 0 to 11%	*B. skagerrakensis* – 11.0%
	G. turgida – 5.1%	*G. turgida* – 0 to 47%	*G. turgida* – 11.0%
	B. marginata – 4.5%		*B. marginata* – 8.4%
	Melonis barleeanus – 1.0%	*Melonis barleeanus* – 0 to 27%	*L. goesi* – 6.5%
	P. osloensis – 9.7%		
	This study: DAn-1	Conradsen et al. 1994:	Conradsen et al. 1994: 41
Coordinates	*56 ^{0}40'13''N - 12 ^{0}07'00''E*	The *B. marginata* assemblage	*56 ^{0}40'26''N - 12 ^{0}09'14''E*
Main species (%)	*S. fusiformis* – 28.6%		*S. fusiformis* – 7.3%
	N. turgida – 16.0%		*N. turgida* – 3.0%
	B. marginata – 13.9%	*B. marginata* – 15 to 60%	*B. marginata* – 49.5%
	N. labradorica – 6.5%	*N. labradorica* – 1 to 29%	*N. labradorica* – 11.2%
	N. aff. *stella* – 14.3%		*E. clavatum* – 13.4%

N. stella to denitrify (Piña-Ochoa et al. 2010) may further explain why *N.* aff. *stella* has expanded in the Kattegat region. In this study, we can confirm that *N.* aff. *stella* is a recent addition to the foraminiferal assemblages in the Kattegat-Skagerrak region, but so far the genetic characterization has shown that it is not the same species as the eastern Pacific one (see section 3.2). Further taxonomic and genetic investigations, as well as studies of the distribution patters of these two different species, are needed.

Nonionella aff. *stella* made up most of the living foraminiferal fauna at the Öresund station (DV-1) (35%, Table 3). The foraminiferal faunal distribution in the Öresund was similar to that in the Kattegat, the main difference being the foraminiferal density, which was ten times lower in the Öresund. This could possibly be linked with the lower salinity (Table 1), but also with the difference in substrate, as the sediment at DV-1 was coarser than the other stations (Table 2). Representative species from the Öresund were *B. pseudopunctata* and *H. depressula* (Fig. 5). In addition, typical species from estuarine environment are present at the Öresund station, such as *Elphidium clavatum* and the agglutinated taxa *E. medius/scabrus* and *R. subfusiformis* (cf. Sen Gupta 1999). A study by Hansen (1965) of living foraminiferal distribution in a shallow area of the northern part of the Öresund reveals similar low foraminiferal densities (11 to 189 individuals/50 cm^3), as found in our data from the Öresund. The faunal composition was quite different, with a group of three *Elphidium* species as strongly dominant taxa, and *Buliminella elegantissa*, *S. fusiformis* (referred as *Virgulina fusiformis* in the study), *Buccella frigida*, *B. marginata* and *E. medius/scabrus* (Hansen 1965). This is presumably mainly due to the difference in water depth, which ranges between 7 and 25 m in Hansen's study against 45 m in our DV-1 station (Table 3). It is interesting to note that Hansen (1965) did not register *N.* aff. *stella* in his material which was collected in 1964.

The three main taxa able to survive in the Baltic Sea are the hyaline *Ammonia* spp. and *Elphidium* spp., and the agglutinated *A. cassis*. Our results are compared with those of Hermelin (1987), who published a study of living (Rose Bengal stained) foraminiferal fauna on 69 surface samples in the Baltic Sea. *Elphidium* spp. and *A. cassis* were both reported in his study, but *Ammonia* spp. was not mentioned. *Ammonia beccarii* (Linné) was, however, reported by Lutze (1965) to occur the western part of the Baltic Sea, but only as far east as the Fehmarn Belt and not in the Arkona Basin. Although decalcified specimens were noticed, the *Elphidium* specimens in Hermelin's study mostly had calcified tests, allowing the identification of *E. excavatum* (presumably including *E. clavatum* and/or *E. selseyense*). In the Arkona Basin, Hermelin (1987) reported comparable foraminiferal density in the summer compared to our densities in the autumn (27 individuals/50 cm^3 and 34 to 40 individuals/50 cm^3, respectively). Our results show that the present environmental conditions allow *A. cassis* to survive in the Arkona Basin, and it is also the area where the highest densities of soft-shelled foraminifera were found (Table 3). All the above mentioned taxa are known to withstand oxygen depletion and high variability in environmental parameters such as salinity, temperature and organic matter content, as found in that area (cf. Sen Gupta 1999; Sabbatini et al. 2013). However, the conditions with respect to salinity, pH, organic matter and oxygen concentration are probably too extreme for them to survive in Hanö Bay, as no *A. cassis* specimens were found and only very few soft-shelled foraminifera (Table 3; see also section 5.2).Conversely, the taxon *Elphidium* spp. was found to be more common in Hanö Bay than in the Arkona Basin, presumably because of the difference in grain size, as this taxon is often found in sandy

sediment such as in Hanö Bay (cf. Sen Gupta 1999).

Hermelin (1987) reported six hard-shelled species as alive in his material, while we only reported three taxa. This may be explained by his use of the Rose Bengal method which often overestimates the living fauna (Bernhard et al. 2006). For example, *E. medius/scabrus*, *Miliammina fusca* and *Reophax scorpiurus* (referred as *Reophax dentaliniformis* in the study) which were reported as living in Hermelin's study from the Baltic, were found only in the dead assemblage in our study. This could, however, be a seasonal effect. Depending on time of the year, and the precise reproductive period of each species, the ratio between living and dead specimens may vary.

5.2 Decalcified foraminifera

In our study, which is based on samples collected in November 2013, between 70 and 100% of the living foraminiferal fauna at the Baltic Sea stations was composed of decalcified specimens (Plate 3). Dissolution of foraminiferal tests was formerly considered as a taphonomic process mostly concerning dead specimens (Martin 1999). In the Skagerrak-Baltic region, dead specimens with only organic linings have often been observed (Jarke 1961; Hermelin 1987: Baltic Sea; Christiansen et al. 1996: Kattegat; Murray and Alve 1999: Skagerrak-Kattegat, Filipsson and Nordberg 2004: Koljö Fjord, Swedish west coast). However, in other areas, evidence of test dissolution has more recently also been reported for living foraminifera. For example in Nueces Bay, Texas, shell loss occurred in living (Rose Bengal stained) *Ammonia parkinsoniana* (cf. Buzas-Stephens and Buzas 2005), and decalcified living (CTG labeled) *Ammonia tepida* have been reported in a mudflat of the Arcachon Bay, France (Cesbron et al. 2016). In western Baltic Sea studies, Polovodova and Schönfeld (2008) and Haynert et al. (2012) found dissolution and recalcification signs on living (Rose Bengal stained) *Ammonia beccarii* and *Ammonia aomoriensis*, respectively.

The most commonly suggested cause of foraminiferal test dissolution in these studies was the low pH (Buzas-Stephens and Buzas 2005; Polovodova and Schönfeld 2008; Cesbron et al. 2016). Le Cadre et al. (2003) clearly demonstrated the link between pH and test dissolution in foraminiferal culture experiments. The Ω_{calc} seasonality in the sediment pore water was also mentioned as a cause of test dissolution (Haynert et al. 2012). In the current study, the minimum pH in the pore water and the Ω_{calc} in the bottom-water at the Baltic Sea stations could not directly explain the observed dissolution. In fact, half-decalcified tests were observed in Hanö Bay (DCHa-2) (Plate 2, Plate 3), where the minimum pH was 7.17 and the Ω_{calc} was 0.19, whereas only completely decalcified tests were found in the Arkona Basin (DBY2-1), where the minimum pH was 7.25 and the Ω_{calc} 1.2 (Fig. 3, Table 1). Several studies have showed that foraminifera have a strong active control of their internal and external pH during calcification (De Nooijer et al. 2009; Glas et al. 2012; Toyofuku et al. 2017). Together with the fact that the pH measured at the water-sediment interface at the Baltic Sea stations was low but still typical for November conditions (SMHI data), this suggests that it is probably not the pH that limits the calcification process. Other carbonate system values recorded in the water column were evaluated to explain the difference between the two Baltic stations. For example, the alkalinity (and correlated carbonate chemistry) data would suggest more difficult conditions to precipitate calcium carbonate at the Arkona Basin (DBY2-1) than at Hanö Bay (DCHa-2) (1985.3 and 2026.3 $\mu mol.kg^{-1}$, respectively, Table 1). However, these numbers reflect bottom water conditions and it is possible that conditions in the sediment where the specimens lived, were different.

Another factor, which is possibly contributes to the tests decalcification, is the low salinity

recorded at the Baltic Sea stations (Table 1). Kurtarkar et al. (2011) and Saraswat et al. (2015) demonstrated in benthic foraminiferal culture experiments that the salinity-induced pH changes reduced the foraminiferal calcification and reproduction ability. They observed test dissolution at salinity below 23 and 15, respectively, values which are comparable to those at stations DBY2-1 (Arkona Basin, 21.63) and the DCHa-2 (Hanö Bay, 14.13) stations (Table 1). Moreover, foraminiferal species typically reveal an optimal set of salinity-temperature conditions that is most suitable for their calcification process (Nigam et al. 2008). However, foraminifera at the Baltic Sea stations are probably adapted to these salinity and temperature conditions, as these hydrographic conditions typically occur in the south Baltic Sea in November (SMHI data).

In our area, it is presumably a combined effect of different environmental variables, rather than any single factor, that affected the foraminiferal tests. It is possible that, while specimens were using their energy to subsist at low salinity, low oxygen levels, low pH and low Ω_{calc}, less energy was available to maintain a fully calcified test. Permanently having a calcitic test is apparently not essential to the foraminiferal survival. Even if a dissolved test does not immediately affect the foraminifer's life, it must be disadvantageous, and it is not known how long they are able to survive in this state. As soon as the living conditions are improved, the foraminifera can probably rebuild their test as observed in the western Baltic Sea (Polovodova and Schönfeld 2008; Haynert et al. 2012). It is also interesting to note that we did not observe any decalcified living foraminifera in Hanö Bay (DCHa-2) during a sampling cruise in June 2015, 1.5 years after the sampling of the material used in our study. This was six months after a major Baltic inflow in December 2014 (Mohrholz et al. 2015) that brought saline and more oxygenated water into the Baltic Sea (in the Arkona Basin, a salinity of 23 compared to 21.63 in November 2013 resulting in an oxygen concentration of 322 compared to 210 $\mu mol.L^{-1}$).

The high TOC content at the Baltic Sea stations was probably crucial for the survival and the potential recalcification of the benthic foraminifera (3.9 to 5.2%, Table 2). The importance of the food availability, allowing the organisms to better resist to ocean acidifications conditions has been suggested by Pettit et al. (2013), in a study on foraminiferal faunas along a natural pH gradient on a CO_2 vent in the Gulf of California, and by Thomsen et al. (2013) in a culture experiment on the mussel *Mytilus edulis*. However, the overall low density of foraminifera at the Baltic Sea stations (34 to 46 individuals/50 cm^3, Table 3) strongly suggested that the environmental conditions were stressful and that the specimens with only organic linings were in less good shape than those with a calcified test, and may have been less resistant to additional physical stress such as predation and abrasion.

6. Conclusion

The distribution of benthic foraminifera in the Skagerrak-Baltic Sea area reflects the strong environmental gradients in terms of species density, richness and faunal composition. The species density and richness at each station were gradually lower as the salinity and Ω_{calc} in the water column, the average pH in the sediment and the oxygen concentration at the sediment-water interface decreased. Each station was dominated by specific major (>2%) foraminiferal species adapted to the local environmental conditions. The dominant species were *C. laevigata* and *H. balthica* in the Skagerrak, *S. fusiformis*, *N.* aff. *stella* and *N. turgida* in the Kattegat and *N.* aff. *stella* and *N. labradorica* in the Öresund. In the Skagerrak-Kattegat, the present foraminiferal distribution could be included in the assemblages reported in previous studies, and the recently reported

modern expansion of *N.* aff. *stella* has been confirmed for the area. In the Baltic Sea, where very low standing stocks suggest strongly adverse conditions for foraminifera, the taxa *Ammonia* spp. and *Elphidium* spp. were dominant. These two taxa tolerate a high rate of environmental changes and are typical for coastal areas. However, in the present area, the foraminiferal density was low, and abundant organic matter seems to be important for their survival. In fact, these taxa may be close to a critical point for their existence in our studied area of the Baltic Sea. They were found alive, but with partially to completely dissolved tests, with only the inner organic linings left. This dissolution was probably due to the combined effect of multiple stressors on the foraminifera such as low salinity, low oxygen concentration, low pH and low Ω_{calc}, which resulted in insufficient energy left for biomineralization. It is apparently not essential for the individuals to permanently keep a calcified test for their survival, even though we cannot presently determine the effect of shell loss on foraminiferal life expectancy.

The present results suggest that there is a delicate balance between the foraminiferal fauna and coastal environmental conditions. This implies that future pH related changes in coastal areas, caused by increasing ocean acidification, eutrophication, and other environmental stressors, may have large negative effects on calcareous organisms as the balance may be shifted and critical ecological thresholds could potentially be passed.

Supplementary data

Appendix A with the raw counts of living and dead foraminifera in the first and the second centimeter of the cores is available in the online version of the article.

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Paper II

Decalcification and survival of benthic foraminifera under the combined impacts of varying pH and salinity

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Decalcification and survival of benthic foraminifera under the combined impacts of varying pH and salinity

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ABSTRACT: Coastal areas display natural environmental variability such as frequent changes in salinity, pH, and carbonate chemistry. Anthropogenic impacts – especially ocean acidification – increase this variability, which may affect the living conditions of coastal species in particular calcifiers. We performed culture experiments on living benthic foraminifera to study the combined effects of lowered pH and salinity on the calcification abilities and survival of the coastal, calcitic species *Ammonia* sp. and *Elphidium crispum*. We found that in open ocean conditions (salinity ~35) and lower pH, these species displayed resistance to test dissolution for a longer time than in brackish conditions (salinity ~5 to 20). However, the response was species-specific as when placed in the same conditions of salinity and pH, *Ammonia* sp. specimens survived longer than *E. crispum* specimens. Living, decalcified juveniles of *Ammonia* sp. were observed and we show that desalination is one cause for the decalcification. Finally, we highlight the ability of foraminifera to survive under Ω_{calc} <1, and that high salinity and $[Ca^{2+}]$ as building blocks are crucial for the foraminiferal calcification process.

KEY-WORDS: coastal ocean acidification; desalination; culture experiment; benthic foraminifera; multiple stressors; calcification process

1. Introduction

The oceans have absorbed around one third of the anthropogenic CO_2 released into the atmosphere since the Industrial Revolution (Sabine et al. 2004). As a consequence, the mean surface pH of the open oceans has decreased from 8.2 to 8.1, and models predict a further decrease of 0.7 units by the year 2200 (Caldeira and Wickett 2003). The decrease in mean pH in the ocean is concomitant with a shift in carbonate chemistry towards lower carbonate ion concentrations, which could potentially impair the production of calcium carbonate by marine calcifying organisms (Orr et al. 2005). However, the response of calcifiers to ocean acidification is not uniform, and experimental studies demonstrate that calcification/ dissolution rates under varying CO_2 concentrations differ between and within taxa (Fabry 2008; Doney et al. 2009; Fabricius et al. 2011; Kroeker et al. 2013; Ries et al. 2014).

In coastal areas, the pH and carbonate chemistry display larger variability due to climate, seasonal contrasts, and hydrographic features. Various coastal

environmental parameters vary under natural and anthropogenic impacts such as salinity, temperature, dissolved oxygen concentration, and organic matter inputs (Crossland et al. 2005). Additionally, coastal carbonate chemistry is also influenced by eutrophication through organic matter mineralization (Borges et Gypens 2010; Cai et al. 2011; Laurent et al. 2017), input of fresh water with lower pH and alkalinity (Salisbury et al. 2008), and regional by upwelling of CO_2-rich water (Feely et al. 2008; Hauri et al. 2009). Thus, pH is lower and more variable along the coasts compared with the open ocean – a fact that needs to be taken into account when studying the effects and consequences of coastal ocean acidification (review in Waldbusser and Salisbury 2014; Vargas et al. 2017). The impacts of ocean acidification in combination with other environmental variables are likely to affect coastal ecosystems, by increasing the range of variability of coastal species habitats. For example, the combined effect of low pH and high temperature reduces mollusks' and corals' abundance, diversity and resistance to dissolution (Hale et al. 2011; Rodolfo-Metalpa et al. 2011). The response of coastal ecosystems to ocean acidification in low salinity conditions is still unclear, however. As brackish water conditions are common in coastal areas, in this study we investigate the combined effect of desalination and lower pH on calcifying benthic foraminifera. The results will improve our knowledge about the response of costal ecosystems to pH changes.

Benthic foraminifera are among the most diverse and abundant meiobenthos living on and in the sea floor. They have a relatively short life cycle – from a few weeks to a year in shallow water (Sen Gupta 2007) – and many of them build a shell (test) of calcium carbonate, which makes them excellent indicators of environmental change and ocean acidification. Moreover, benthic foraminifera contribute up to 5% of the annual carbonate production in coastal areas (Langer 2008). Salinity is an abiotic factor limiting the distribution of foraminiferal species, and characteristic coastal species are found in brackish water environments (Sen Gupta 1999). Calcareous foraminifera have also been found alive at low pH in their natural environment, for instance around CO_2 vents in the northern Gulf of California (Pettit et al. 2013), in the Arcachon Bay in France (pH 6.2 to 6.7: (Cesbron et al. 2016) and in the southern Baltic Sea (pH 7.39: Charrieau et al. in revision). For all these sites, however, foraminiferal abundance and diversity were lower than at sites with a higher pH, and the specimens demonstrated signs of test dissolution. Notably, the calcite tests of the majority of the foraminifera in the Arcachon Bay and the Baltic Sea were entirely dissolved and only the inner organic linings were present (Cesbron et al. 2016, Charrieau et al. in revision). The CellTracker™ Green labeling attested that the individuals were indeed alive, despite dissolution. The low pH and the low salinity in the southern Baltic Sea were suggested to be the main factors causing the observed decalcification (Charrieau et al. in revision). Indeed, the saturation state of the calcium carbonate (Ω_{calc}) is mainly dominated by the pH and the salinity in natural marine environments (Zeebe and Wolf-Gladrow 2001). Furthermore, recalcification of dissolved foraminiferal tests have been observed in the field (Haynert et al. 2012) and in culture studies (Le Cadre et al. 2003; Kurtarkar et al. 2011) suggesting a potential resilience of benthic foraminifera in low pH conditions. Culture studies permit isolating the effects of pH and salinity from the multiple environmental parameters that influence the foraminiferal populations.

Here, we analyzed the effects of lowering pH in open ocean - and brackish conditions, as well as the effects of salinity-induced pH change on the two foraminiferal species *Ammonia* sp. and *Elphidium crispum.* Both species typically live in coastal areas, and *Ammonia* sp. is known to be more tolerant to a wider range of salinity conditions than *E. crispum* (Murray 2014). Moreover, some species of the genus *Ammonia* can also be tolerant to low pH, as demonstrated in a culture experiment by Le Cadre et al. (2003). Our aim was to study what environmental conditions create living decalcified foraminifera, and also to observe if they can recalcify when pH and salinity return to usual values for these species. The results will aid our understanding of the response of the benthic compartment to ongoing coastal ocean acidification.

2. Materials and Methods

2.1 Sampling

Benthic foraminifera were collected in June 2016 along the Pacific coast of Japan. The top-most millimeters of sediment containing *Ammonia* sp. were collected with a spatula at the Nojima tidal flat (Tokyo Bay, 35.324 °N, 139.636 °E). In the area, the annual mean pH in the bottom water had varied between 7.65 and 7.95 (Total Scale) on the last 20 years (Yokosuka City data), and the salinity is 25.8±4.0 (Koshikawa et al. 2000). The *Ammonia* population on the Japanese coasts is composed of a mix of the phylotypes T1 and T6 which are morphologically difficult to separate (Schweizer M. pers. comm.). Therefore, in this study we use a conservative approach and refer to the species as *Ammonia* sp. Coralline algae, which *Elphidium crispum* spends its life attached to, were collected in tidal pools at Cape Tomyozaki (Tokyo Bay, 35.228°N, 139.729°E). In the area, the annual mean pH in the bottom water had varied between 7.95 and 8.25 (Total Scale) on the last 20 years (Yokosuka city data). The foraminifera were transported to JAMSTEC (Japanese Agency for Marine-Earth Science and Technology), stored at in situ temperature (8°C) in sea water that has been previously filtered with GF/F filter (salinity 35), and fed with *Dunaliella* sp. Before the experiments, the living state of foraminifera was attested by pseudopodial activity, feeding and signs of movement.

2.2 Culture experiments

We conducted three sets of experiments. One, termed the pH experiment, where the salinity was either brackish or open ocean conditions, and there were low (~7.25 to ~7.34) and higher (~7.53 to ~7.93) pH treatments. In the second experiment - salinity experiment A - the salinity was gradually lowered by dilution from 35 to 5, and the pH was measured. In the third experiment – salinity experiment B – salinity was also gradually lowered and the specimens were then returned to starting levels of salinity. Salinity experiment A included *Ammonia* sp. and *E. crispum*, whereas salinity experiment B included only juvenile *Ammonia* sp.

2.2.1 The pH experiment

Six tanks of 9 liters were divided into two groups with respect to salinity: three tanks (Tanks 1, 3 and 5) had brackish water conditions (~20), obtained by diluting sea water with milli-Q water; three tanks (Tanks 2, 4 and 6) had salinity close to open ocean conditions (~34 to ~37) (**Table 1**). We altered the pH in the tanks by CO_2 bubbling. pH was continuously controlled by pH meter (Thermo Scientific Orion 5-star Plus) equipped with a glass electrode (Thermo Scientific, PrpHecT® ROSS® Micro Combination pH electrode 8220BNWP), and the CO_2 bubbling was adjusted to keep a stable pH. The low pH treatments were ~7.34, ~7.28, and ~7.25 (tanks 1, 3 and 6, respectively) and the higher pH treatments were ~7.67, ~7.93, and ~7.53 (tanks 2, 4 and 5, respectively)

Table 1. Geochemical parameters in each tank for the pH experiment

	T (°C)	Salinity	pH	Total alkalinity (at 25°C)	Ω_{calc}	CO_3^{2} (µmol.kg-sw^{-1})	Ca^{2+} (mmol.kg-sw^{-1})
Tank 1	8	19.71 ±0.45	7.34 ±0.04	2454 ±45	0.54	20.95	5.79
Tank 2	8	37.43 ±1.05	7.67 ±0.06	2494 ±121	1.58	67.23	11.00
Tank 3	14	20.75 ±1.30	7.28 ±0.20	1499 ±25	0.38	14.44	6.10
Tank 4	14	34.00 ±0.87	7.93 ±0.06	2182 ±45	2.75	114.65	10.00
Tank 5	14	20.13 ±0.33	7.53 ±0.09	1450 ±19	0.62	23.98	5.92
Tank 6	14	34.13 ±0.60	7.25 ±0.19	2215 ±33	0.65	27.25	10.03

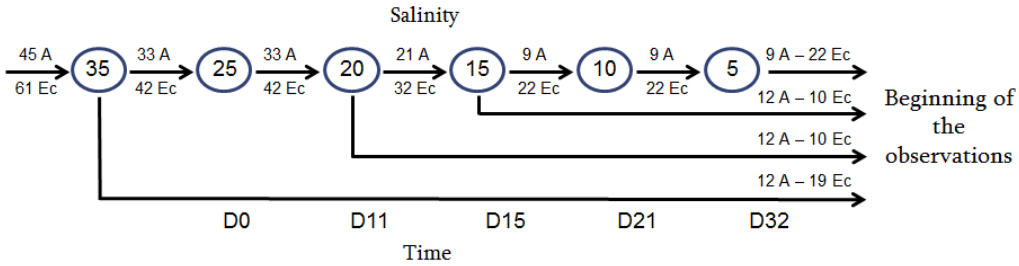

Fig. 1. Time frame of salinity experiment A and number of moved *Ammonia* sp. (A) and *E. crispum* (Ec) at each step.

(**Table 1**). The culture cups contained five living specimens each: two culture cups with specimens of *Ammonia* sp. and one to two culture cups with specimens of *E. crispum* per treatment. To insure pH homogeneity, the culture cups were not placed directly in the tanks but in separate plastic containers, and the water circulated from the tanks to the containers through Tygon tubes, controlled by peristaltic pumps TP-1973R, Asone. The tanks, linked to the containers, were placed at either 8°C or 14°C (**Table 1**). In total, 110 individuals were monitored and the experiment lasted between 28 and 57 days. Every week, the foraminifera were fed with *Dunaliella* sp., and digital pictures of each individual were taken to observe test morphology and dissolution state. Between one and two foraminifera were removed from the culture cups every week and/or at the termination of the experiment to be imaged using a Scanning Electron Microscope (SEM).

2.2.2 Salinity experiments A and B

Sea water bottles with salinities of 35, 25, 20, 15, 10 and 5 obtained by diluting sea water with milli-Q water were prepared, and petri dishes (40 mm in diameter) were filled with these different sea waters. Living foraminifera were exposed to gradually declining salinities, with 4 to 11 days in between individual steps (**Fig. 1**). Approximately 40 specimens of *Ammonia* sp. and 60 specimens of *E. crispum* were placed in petri dishes with salinity 35. After approximately six hours, around two thirds were moved to salinity 25. A fraction of them were then moved to salinity 20, then 15, then 10, and finally some specimens were moved to the lowest salinity 5, which was reached after 32 days. Foraminifera were fed with *Dunaliella* sp. after every dish change. Specimens were kept at the experimental salinities of 35, 20, 15 and 5 over a total of 162 days (**Fig. 1**). In this experiment, the pH was monitored at the

Table 2. Geochemical parameters in the dishes at the beginning of salinity experiments A and B.

	T (°C)	Salinity	pH		Total alkalinity (at 25°C)	Ω_{calc}	CO_3^{2-} ($\mu mol.kg\text{-}sw^{-1}$)	Ca^{2+} ($mmol.kg\text{-}sw^{-1}$)
			Start	End				
Dishes 35	9.20	35	7.96	7.96	2407	2.793	117.25	10.29
Dishes 20	9.20	20	7.98	7.93	1333	1.252	48.56	5.88
Dishes 15	9.20	15	7.91	7.71	1251	0.901	33.94	4.41
Dishes 10	9.20	10	7.77	-	572	0.249	9.09	2.94
Dishes 5	9.20	5	7.21	7.47	53	0.004	0.16	1.47
Dishes 10b	9.20	10	-	7.69	-	-	-	-
Dishes 35b	9.20	35	-	7.97	-	-	-	-

beginning and at the end of treatment (**Table 2**). The temperature was kept at 9.2^{0}C. Once the experimental batch had reached the final salinity, the foraminifera were fed every week and at the same time digital pictures of random individuals per petri dish were collected to observe test morphology and dissolution state. In the Results section, this is referred to as salinity experiment A.

Approximately 40 juvenile specimens of *Ammonia* sp. were subjected to the same steps of gradually decreasing salinity treatments, but with shorter intervals, reaching the salinity of 5 after six days. After that, the juvenile specimens were fed and monitored as described above. After 49 days, 20 juvenile foraminifera with dissolved tests were returned to higher salinities: 5 specimens to salinity 10 and 5 specimens to salinity 35. The experiment was concluded after 98 days in total. In the Results section this is referred to as salinity experiment B.

In salinity experiments A and B, SEM images of all the specimens, as well as pictures using a Zeiss Axio Observer Z1 inverted microscope for some selected specimens, were taken at the end of the experiment.

2.3 Geochemistry

Salinity, pH (on the Total Scale) and total alkalinity in the water were monitored in the experiments. In pH experiment, the water was sampled in the tanks, while in salinity experiments A and B the water was sampled in the preparation bottles. Total alkalinity was determined by the pH method (Culberson et al. 1970; Dickson and Goyet 1994). The CO_2calc software program (Robbins et al. 2010) was used to estimate other carbonate system parameters such as calcite saturation (Ω_{calc}), [CO_3^{2-}] and [Ca^{2+}] (**Tables 1 and 2**). The sea-water scale and the equilibrium constants for K1 and K2 of (Millero 2010) were used.

2.4 SEM pictures

The foraminifera selected for SEM imaging were mounted on aluminum stubs and placed in a Hitachi Miniscope TM3000, JAMSTEC. Decalcified foraminifera obtained during the salinity experiment were fixed before imaging so as to avoid shrinkage during the drying process. The specimens for fixation were placed in a bath of 3% ASW (Artificial Sea Water), 2.5% glutaraldehyde (GA) and 3% paraformaldehyde (PFA). When needed for the SEM imaging session, the decalcified foraminifera were rinsed several times with milli-Q water, mounted on stubs and imaged with a Tescan Mira3 High Resolution Schottky FE-SEM at Lund University.

2.5 Image analysis

The digital pictures and SEM images were used to describe foraminiferal test morphology and test dissolution in the three experiments. A total of approximately 1400 pictures were analyzed. A level was attributed to each specimen (**Plate 1**): level 1 (L1) for specimens with healthy tests (unaffected), L2 for specimens with at least one deformed chamber (deformed), L3 for specimens with signs of dissolution such as decalcification of the superficial calcite layers (peeled), L4 for partially to fully fragmented specimens, showing severe dissolution signs such as holes (fragmented), and L5 for partially to fully decalcified specimens, with the inner organic lining visible (dissolved). The number of observed specimens at each specific level was noted for each observation time, for every tank or petri dish. For salinity experiment A, this number corresponds to the number of foraminifera observed at a given time; it is not the absolute number of specimens present in the tank or petri dish. The number of observed foraminifera in the three experiments (initially 110, 100 and 40 specimens, respectively) was determined to be too low to justify advanced statistical analysis. Therefore, the results are presented in a qualitative way.

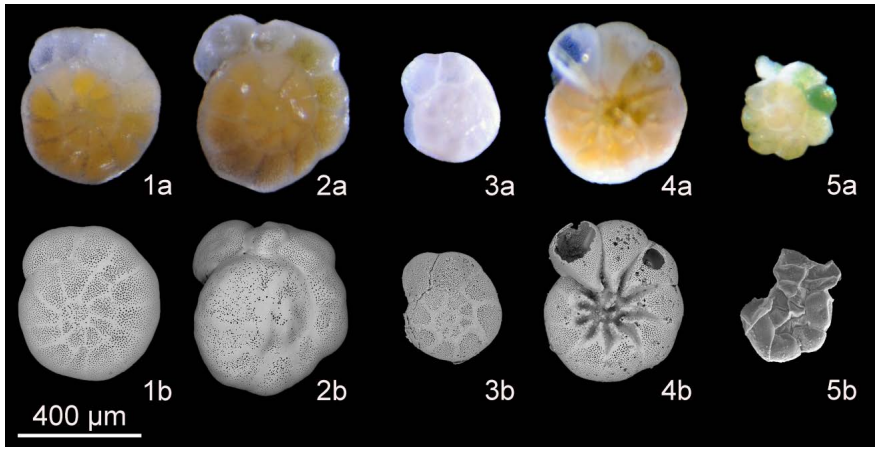

Plate 1. Digital (above) and SEM (below) pictures of the five observed morphology and dissolution levels of foraminiferal test (*Ammonia* sp.). Level 1 – Unaffected: specimen showing a healthy test. Level 2 – Deformed: specimen that shows at least one deformed chamber. Level 3 – Peeled: specimen with a test that often looks white in normal light and showing decalcified layers on the SEM picture. Level 4 – Fragmented: specimen with a test showing holes or broken parts. Level 5 – Dissolved: specimen partially of fully dissolved, with the inner organic lining visible.

3. Results

3.1 Dissolution levels

At the beginning of the experiments the specimens were a mix of unaffected and deformed tests (L1 and L2, **Fig. 2**). From the unaffected stage (L1), some specimens progressed to the deformed stage (L2), while some specimens progressed directly to the peeled stage (L3). From L3, some specimens started to fragment (L4) while others dissolved even further (L5) (**Fig. 2**). At the fully fragmented stage (L4), the majority of the specimens showed no signs of activity and were considered dead. At the dissolved stage (L5), however, the specimens were still moving in some cases and thus considered alive.

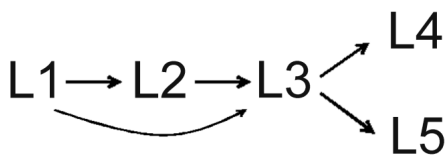

Fig. 2. Possible ways of development of the foraminifera regarding test morphology and dissolution levels.

3.2 pH experiment

At both brackish salinity and open ocean salinity, the morphology and dissolution of the foraminiferal tests (summarized in the levels L1-L5) varied over time (**Fig. 3**), depending on the pH during the experiment, the species, and the individual response.

At open ocean salinity (~34 and ~37) and highest pH (~7.93 and ~7.67), none of the *Ammonia* sp. specimens displayed any signs of dissolution at the end of the experiments, even though one or two specimens showed deformed chambers (L2) throughout the experiment (tanks 2 and 4, **Fig. 3**). At high salinity and the lowest pH (~7.25, tank 6), two individuals reached the peeled stage (L3) after ~30 days in the tank. Most of the *E. crispum* specimens followed the same trend for the open ocean conditions tanks, although specimens with deformed chambers (L2) were not observed (**Fig. 3**). At the lowest pH, however, *E. crispum* responded earlier than *Ammonia* sp. Two L3 specimens of *E. crispum* appeared after only ~9 days; after 17 days the majority of the specimens were peeled (tank 6, Fig 3). After ~30 days, a set of *E. crispum* specimens appeared fragmented (L4) and were considered dead (tank 6, **Fig. 3**).

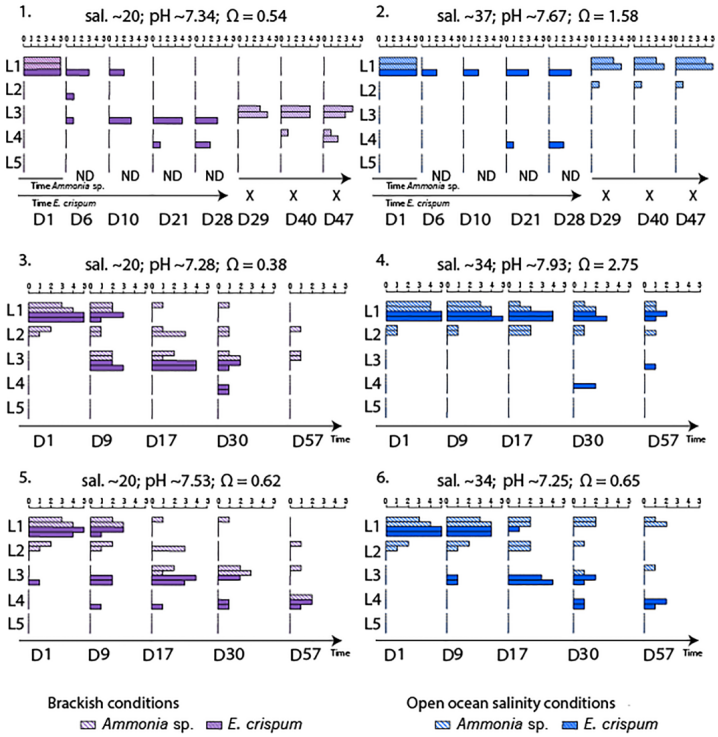

Fig. 3. Levels of test morphology and dissolution as a function of time, under two different salinities and six different pH. Each bar corresponds to one culture cup. ND: no observations for *Ammonia* sp. X: No observations for *E. crispum*.

At brackish salinity (~20), both species showed signs of increasing dissolution over time, with no visible difference between the three pH treatments (tanks 1, 3 and 5; **Fig. 3**). For *Ammonia* sp., the development was comparably slower. Four *Ammonia* sp. specimens at the unaffected (L1) and deformed (L2) stages showed signs of dissolution (L3) after only ~9 days (tank 3), with the majority of *Ammonia* sp. reaching that point after ~30 days (**Fig. 3**). One to two specimens started to fragment (L4) after ~40 and ~57 days (tanks 1 and 5). For *E. crispum*, the development appeared earlier (**Fig. 3**). After ~9 days, half of the observed specimens were at the peeled stage (L3); after ~17 days all the specimens had reached L3. *Elphidium crispum* also fragmented more quickly; L4 was observed for one specimen after only ~9 days (tank 5).

Despite Ω_{calc} being below 1 in four of the tanks, the foraminifera were able to maintain tests and survive approximately 30 days for *E. crispum*, and at least 57 days for *Ammonia* sp., even though both species demonstrated signs of dissolution (**Fig. 3**). No living dissolved specimens (L5) were observed. Moreover, some specimens of *Ammonia* sp. displayed unaffected (L1) tests after treatment and did not seem to be affected by the low pH, salinity, and Ω_{calc} during the first 30 days of the experiment.

3.3 Salinity experiments A and B

The salinity modifications in every preparation bottle – and thus every petri dish – caused changes in pH and Ω_{calc}. The largest decrease occurred between salinity 10 and 5, where the pH dropped from 7.77 to 7.21 and Ω_{calc} from 0.249 to 0.004 (**Table 2**). At the end of the experiment, the pH was similar in the

dishes with salinities 35 and 20, but lower at salinity 15 (7.91 against 7.71) and higher at salinity 5 (7.21 against 7.47).

In salinity experiment A, the two species of foraminifera responded differently to the salinity treatments. In the dishes with salinity 35, the observed specimens of *Ammonia* sp. showed unaffected (L1) or deformed (L2) tests throughout the experiment (**Fig. 4A**). At salinity 20, a larger fraction of the foraminifera observed had deformed tests, and a few L3 specimens occurred after ~77 days. At salinity 15, specimens of *Ammonia* sp. displayed a mix of unaffected (L1) and deformed (L2) tests during the first days. Peeled specimens (L3) appeared and became common after ~37 days. Fragmented specimens (L4) were also observed at the end of the experiment (~162 days). Finally at salinity 5, a majority of the observed *Ammonia* sp. was deformed at each observation time, and a few fragmented specimens appeared at the end of the experiment (**Fig. 4A**). In the dishes with salinity 5, one juvenile individual with dissolved test (L5) was observed after ~44 days and another two after ~162 days. Only the inner organic linings were visible, with some remaining pieces of calcite around the chamber junctions (**Plate 1**). The specimens still demonstrated signs of feeding and movement and were considered alive.

In salinity experiment A at salinity 35, the *E. crispum* observed showed unaffected tests (L1) until termination of the experiment (**Fig. 4A**). At salinity 20, a small fraction of the specimens started to peel (L3) from day 32. At salinity 15, the vast majority of the observed *E. crispum* were at the peeled stage (L3), and two fragmented specimens (L4) were noticed at the end of the experiment (162 days). Finally at salinity 5, the specimens displaying peeling (L3) on day 32 became fragmented (L4) between days 37 and 70. *Elphidium crispum* with dissolved tests (L5) appeared from day 44 until the end (**Fig. 4A**). Neither

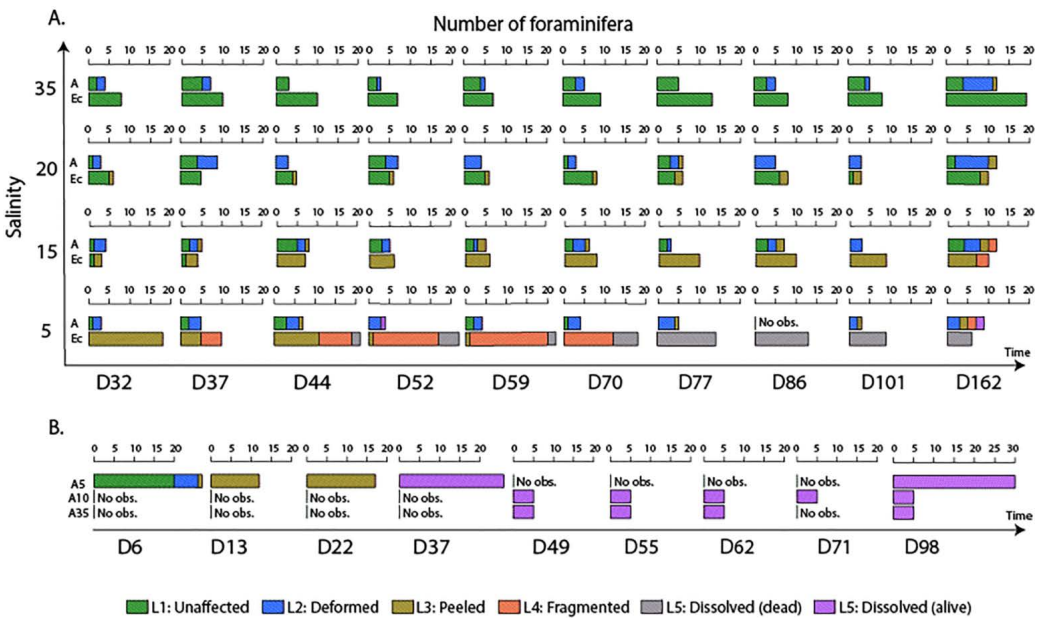

Fig. 4. A. Level of test morphology and dissolution of *Ammonia* sp. (A) and *E. crispum* (Ec) at different salinities and times. Note that, except at day 162, the number of foraminifera does not refer to the total amount of foraminifera present but to the number of observed specimens at a given time. **B.** Level of test morphology and dissolution of juveniles *Ammonia* sp. at salinity 5 (A5), 10 (A10) and 35 (A35).

the fragmented nor the dissolved specimens were observed to have moved and were considered dead.

In salinity experiment B at salinity 5, the majority of the juvenile *Ammonia* sp. displayed unaffected tests (L1) on day 6 (**Fig. 4B**). On days 13 and 22, all the observed specimens showed signs of dissolution (L3). From day 37 until the end of the experiment, the foraminifera were dissolved (L5), with only the organic lining visible. As they still demonstrated signs of activity they were considered alive; this activity was documented in a movie (**S1 movie**). The specimens returned to the higher salinities 10 and 35 did not display any signs of test recalcification by the end of the experiment (**Fig. 4B**). After 98 days (and the termination of the experiment), all juvenile specimens of *Ammonia* sp. observed in the three salinity conditions were considered alive.

As also observed in the pH experiment, foraminiferal calcite was present even at very low Ω_{calc} values such as 0.004 when salinity was 5. Despite the very low Ω_{calc}, calcified tests were observed until ~70 days for *E. crispum* and until ~162 days for *Ammonia* sp. (**Fig. 4A**).

4. Discussion

4.1 pH effects under open ocean salinity conditions

At open ocean salinity, *Ammonia* sp. specimens were weakly affected by lower pH. The resistance of the genus *Ammonia* to low pH has previously been reported in a study by Le Cadre et al. (2003), where no dissolution signs were observed on specimens (identified as *Ammonia beccarii* in the study) after 70 days at salinity 38 and pH 7.5. In the study by Le Cadre et al. (2003), peeled tests (L3) and test dissolution (L5) were observed when the specimens were kept at pH 7 for 5 and 7 days, respectively. The specimens were considered alive as intense pseudopod emission was observed when returned to higher pH (Le Cadre et al. 2003). Together with our results, it shows that some species of the genus *Ammonia* are tolerant of low pH at open ocean salinity, even if test dissolution occurs. This ability probably contributes to the common occurrence of the species in variable coastal areas. However, the specimens will almost certainly dissolve and die if the pH stays low for a longer time, or if the pH reaches a certain lower threshold.

Elphidium crispum also demonstrated some resistance to lower pH at open ocean salinity, but the higher number of specimens showing signs of dissolution indicates that the species is less tolerant than *Ammonia* sp. both in terms of time at lower pH and pH lower limit (**Fig. 3**; tanks 2, 4, and 6). The majority of *E. crispum* were fragmented and died after ~30 days at the lowest pH. Similarly, the intertidal species *Elphidium williamsoni* has previously been shown to be affected by a lower pH, as demonstrated by significantly thinner tests and with rougher chambers when cultured at salinity 33 and pH 7.7 over 45 days (Allison et al. 2010). Both *Elphidium* species appear to be less adapted than *Ammonia* sp. to natural pH changes in coastal areas and will probably be more sensitive to an increase in pH variability. Species-specific response to low pH in open ocean salinity conditions have also been observed in a range of culture studies on planktonic foraminifera (34, 35) and on large benthic foraminifera (Hikami et al. 2011; Fujita et al. 2011; Vogel and Uthicke 2012).

Deformed chambers often occur on benthic foraminifera living under environmental stress (39, 40). This implies that even though all the specimens of *Ammonia* sp. were alive at the end of the experiment, the living conditions were probably not optimal. The specimens of *E. crispum* did not display such test deformation, which may be because the individuals were able to calcify normally also under culture conditions, or, on the contrary, because the individuals were alive but did not construct any new chambers during the experiment.

4.2 Effects of pH in brackish conditions

At brackish salinity, *Ammonia* sp. was affected by the lower pH throughout the experiment; six individuals even died at termination. In comparison with the experiment at open ocean conditions, these results suggest that *Ammonia* sp. is more vulnerable to low pH when the salinity is lower. In a previous culture study, *Ammonia aomoriensis* specimens were placed at salinity 17 to 19.5 and pH 7.7 over 42 days (Haynert et al. 2011). Despite the relatively high pH

for this species, peeled specimens were observed among living individuals by the end of the experiment. The dissolution is probably linked to the water used in the experiment to mimic the low salinity of the natural environment for *A. aomoriensis* (Haynert et al. 2011). Similarly, *A. tepida* specimens displayed lower shell weight when cultured at pH 7.5 and salinity 24 as compared to salinity 33 (over ~45 days), although no signs of dissolution were observed (Dissard et al. 2010). Even if *A. tepida* is a coastal species adapted to large salinity variations (Sen Gupta 2007), its tolerance for lower pH also appears to benefit from open ocean salinity.

As in the experiment at open ocean conditions, *E. crispum* appears less tolerant to low pH than *Ammonia* sp. in brackish conditions, and half of the specimens were dead after ~30 days at all pH treatments. The results suggest that the combination of low salinity and low pH would quickly become lethal for *E. crispum*. Our results demonstrate that, as regards open ocean salinity conditions, the response of foraminifera to lower pH in brackish conditions is species-specific.

4.3 Effects of salinity-induced pH changes

Both *Ammonia* sp. and *E. crispum* are coastal species used to variations in salinity - and pH - (Murray 2014), which can explain the high number of individuals that survived throughout the entire experiment. However, their response to lower salinity-induced pH changes differs. Deformed specimens were again observed for *Ammonia* sp., which further supports the hypothesis that it is a result of stress caused by culture conditions. Test abnormalities were previously observed on the benthic foraminifera *Rosalina globularis* under lowered salinity (Saraswat et al. 2015). Moreover, *E. crispum* displayed almost solely peeled (L3) individuals at salinity 15, whereas the *Ammonia* sp. specimens were mostly healthy (L1 and L2). This underlines the lower resistance of *E. crispum* to conditions of lower salinity - and lower pH - compared to *Ammonia* sp. The species-specific response to lower salinity-induced pH changes could also be observed at the lowest salinity treatment (5), where *E. crispum* specimens progressively died whereas some *Ammonia* sp. specimens remained alive until the end of the experiment. Signs of dissolution due to lower salinity-induced pH changes have previously been observed in culture experiments on other foraminiferal coastal species such as *Rosalina leei* (Kurtarkar et al. 2011) and *R. globularis* (Saraswat et al. 2015). Similarly, the test dissolution noted in both species was probably caused by the combined effects of low salinity and low pH.

Partial to full test decalcification (L5) in living foraminifera has been observed in other culture studies involving salinity-induced pH changes (27, 43). *Rosalina leei* and *R. globularis* tests dissolved after 15 days at salinity <17 (pH <7.5) and after 39 days at salinity 15 (pH 7.54), respectively (27, 43). Feeding and other signs of activity were used as the criteria for assessing a specimen was alive (27, 43). The fact that living specimens with dissolved tests appeared at lower salinity (5) in our experiment can be explained by the more robust tests of juvenile *Ammonia* sp. compared to *R. globularis* (Saraswat et al. 2015). We observed no living dissolved foraminifera when varying the pH in the pH experiment even in brackish conditions (salinity ~20), and such specimens only appeared at the lowest salinity in salinity experiment B. Moreover in field studies, where living dissolved foraminifera were found in their natural environment, lower salinity was also a contributing factor. Indeed, in the Arcachon Bay the salinity can vary between 22 and 32 over the year (Plus et al. 2010), and in the south Baltic Sea the salinity was 14 and 22 at the two stations studied during the 2013 sampling period (Charrieau et al. submitted). Therefore, we can conclude that the combined effects of low salinity and low pH are most probably the cause of living dissolved foraminifera in our experiments.

However, even if the specimens were alive as indicated by cytoplasm activity (**S1 movie**), the juvenile *Ammonia* sp. did not recalcify when returned to higher salinity and pH. Recalcification has previously been observed for artificially dissolved foraminiferal tests, e.g. for *A. beccarii*, which rebuild their chambers once returned to normal salinity (Le Cadre et al. 2003), and for *R. leei*, which started to rebuild their tests as soon as the salinity was above 10 (Kurtarkar et al. 2011). In both cases, the new tests displayed abnormalities and deformed chambers. In

our case, the culture conditions could have been too extreme to rebuild a test. For example, the availability of nutrients and food appears to be a crucial parameter for counteracting the impact of lower pH conditions (Pettit et al. 2013). Providing more food to the foraminifera could have helped them with the recalcification process. The resilience of foraminifera after test dissolution appears to be dependent upon a sensitive balance of environmental parameters.

4.4 Living with calcite undersaturation

In all the experiments (except salinity experiment B), calcite was present until the termination of treatment despite the Ω_{calc} being below 1 (**Tables 1 and 2**). Calcified tests were previously observed under low Ω_{calc} in a culture study on *A. tepida* (Dissard et al. 2010). Several organic layers usually coat the foraminiferal tests (Banner et Williams 1973), and pseudopodia strands are able to wrap around the surface of the tests. The layers and the pseudopodia probably form a barrier between the calcite and sea water, which probably helps the foraminifera resist dissolution for a brief period. Two parameters can decrease Ω_{calc} values: an increase of $[CO_2]$, which would also decrease pH and $[CO_3^{2-}]$, and/or a decrease of $[Ca^{2+}]$. In the first case, it has been demonstrated that foraminifera including *Ammonia* sp. have strong active control of their internal and external pH during the calcification process, and that calcification is not directly linked to the $[CO_3^{2-}]$ (De Nooijer et al. 2009; Glas et al. 2012; Toyofuku et al. 2017). Even if the pH and the $[CO_3^{2-}]$ were low in our experiments (**Tables 1 and 2**), both species were probably still able to compensate for this and maintain their test for a certain period of time. In the second case, a decrease in salinity will lower $[Ca^{2+}]$, as was the case in our experiments (**Tables 1 and 2**). It has been shown that *A. beccarii* is able to store Ca^{2+} ions internally, even if they still need some external Ca^{2+} ions to be able to calcify (Toyofuku et al. 2008). This storage capacity could be sufficient for the foraminifera to maintain a test for a short while. However, test dissolution will occur if the salinity is drastically decreased for a longer period, through exhaustion of Ca^{2+} ions. The complete process of biomineralization in benthic foraminifera is still under debate (review in De Nooijer et al. 2014; Toyofuku et al. 2017). However, our results suggest that even if the pH regulation during the calcification process is comparably easy to achieve for the foraminifera, it will be more difficult for the organisms to maintain tests and calcify under conditions of low salinity.

5. Conclusion

In our culture study, the two coastal species *Ammonia* sp. and *Elphidium crispum* could tolerate lowered salinity, pH, or a combination of the two, which reflects the environmental variation in their habitats. However, when cultured at a lower pH, both species seem to better resist test dissolution at open ocean salinities than in brackish conditions. This suggests that ocean acidification will probably have a larger impact on coastal ecosystems than on open ocean ecosystems, and that coastal ocean acidification and desalination have synergic effects on benthic foraminiferal calcification process and survival. As expected from previous studies, the foraminiferal response of lower pH and salinity was species-specific. In our case, *Ammonia* sp. will probably survive longer than *E. crispum* if an increase in the environment variability happens. Living dissolved specimens of juvenile *Ammonia* sp. occurred under the combined effect of low pH and very low salinity. However, recalcification did not occur when the specimens were returned to higher salinity and pH; this lack of response seems to depend on multiple environmental parameters. Finally, some specimens were able to maintain tests at Ω_{calc} <1, probably due to their biological active control of pH and to their capacity to store Ca^{2+} ions. This suggests that benthic foraminifera will not immediately be affected by coastal ocean acidification *per se*, but rather by a combination of decreasing salinity and lowered pH.

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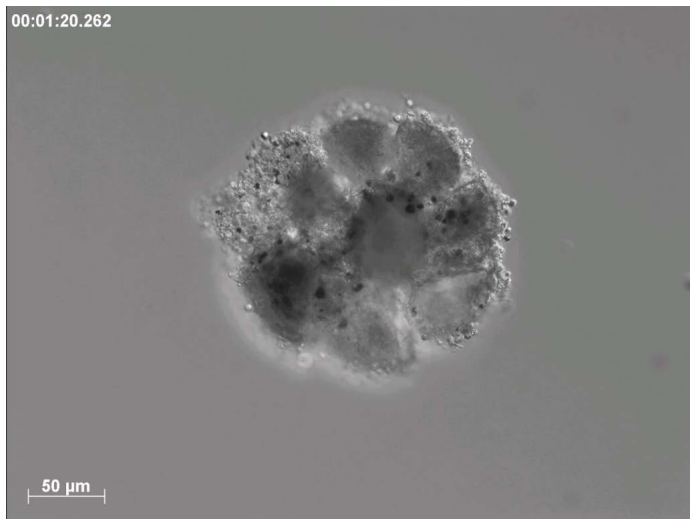
Supporting information



S1 movie. Living, dissolved specimen of juvenile *Ammonia* sp. The activity inside the cytoplasm proves the living state of the specimen.

Paper III

A bicentennial record of modern environmental changes in the Baltic Sea entrance

Laurie M. Charrieau, Helena L. Filipsson, Karl Ljung and Emma Kritzberg.

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A bicentennial record of modern environmental changes in the Baltic Sea entrance

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Abstract The Öresund strait is linking the North Sea and the Baltic Sea, which creates a transition zone for ecosystems in terms of salinity, temperature, carbonate chemistry, and oxygen concentration. In addition to the already extreme conditions, the area is responding to changes in nutrient loading, temperature, and pH. The aim of this study was to reconstruct environmental changes in the Öresund during the last c. 200 years. Sediment cores were collected from the north of the Island of Ven in the Öresund. The cores were radiometrically dated, and variations in benthic foraminiferal fauna, organic matter content and grain size distribution were the basis for reconstructing environmental changes. Four zones with characteristic foraminiferal assemblage reflecting the environmental conditions for each period could be distinguished. The largest changes in our data sets occurred ~1960, when the foraminiferal assemblage shifted from low diversity, dominance of the species *Stainforthia fusiformis* and muddy sediment to higher diversity, dominance of the *Elphidium* group and sandy sediment. This indicates an increased bottom water oxygenation and changes in the water circulation pattern, towards stronger currents in the area since the 1960s.

Key-words Benthic foraminiferal; Öresund; Environmental Reconstruction; Anthropocene

Introduction

The Öresund is a strait between Sweden and Denmark that links the open-ocean waters of the North Sea and the brackish waters of the Baltic Sea. The confluence of the water masses creates a North-South gradient as well as a strong vertical stratification of the water in terms of salinity, carbonate chemistry and dissolved oxygen concentration [O_2] (Leppäranta and Myrberg 2009). The depth of the halocline mainly depends of the inflows from the Baltic Sea, and a strong thermocline develops during spring and summer. Thus, the ecosystems in the Öresund are exposed and adapted to a unique transitional environment. The region is also characterized by intense human activities, with 4 million people living in the vicinity of the Öresund. Discharge from agricultural areas and industrial zones on both the Swedish and Danish sides, and the heavy impact of marine traffic – the strait is one of the busiest waterways in the world - generate significant pollution of the water. Consequently, the Öresund is assessed to be eutrophied, like most of the Baltic Sea,

and hypoxic events are frequent (Rosenberg et al. 1996; Conley et al. 2011; Wesslander et al. 2016). Moreover, increasing temperatures and declining pH, linked to global climate change and ocean acidification, have been reported for surface and bottom waters in the area (Andersson et al. 2008; Göransson 2017). Thus, ecosystems in the Öresund are currently under the combined impact of natural and anthropogenic stressors (Göransson et al. 2002), and most of the organisms living in the region are resistant to low oxygen concentration and high pollution levels (Henriksson 1969). The multiple stressors affecting the environment make this region particularly interesting to study. To our knowledge, no previous studies assess the environmental changes in the area during the last two centuries.

Benthic foraminifera are widely used for environmental reconstructions, based on their rapid response to environmental changes, numerous occurrences, and often well-preserved tests in the sediment. For instance in the area, benthic foraminifera have been used for historical reconstructions of the Gullmar Fjord (Nordberg et al. 2000; Filipsson and Nordberg 2004a; Polovodova Asteman and Nordberg 2013) and the Koljö Fjord (Filipsson and Nordberg 2004b). In the Öresund, living foraminiferal assemblages have been studied by Hansen (1965) and Charrieau et al. (in revision), but no records of past assemblages have been performed. In this study, we use foraminiferal fauna analysis in combination with bulk geochemistry and grain size to reconstruct the benthic living conditions of the last two centuries in the Öresund.

Study site

The Öresund is a 118 km long narrow strait (Fig. 1). The water depth in the northern part is on average 24 m but it reaches 53 m south of the Island of Ven. The Öresund is an important link between the North Sea, Skagerrak, Kattegat and the Baltic Sea, and up to 30% of the water exchange in the region go through the Öresund (Leppäranta and Myrberg 2009); the remaining part goes through the Great and Little Belt. The width of the Öresund varies between 4 and 28 km, and the water has in general high current velocities. The Skagerrak and Kattegat are located

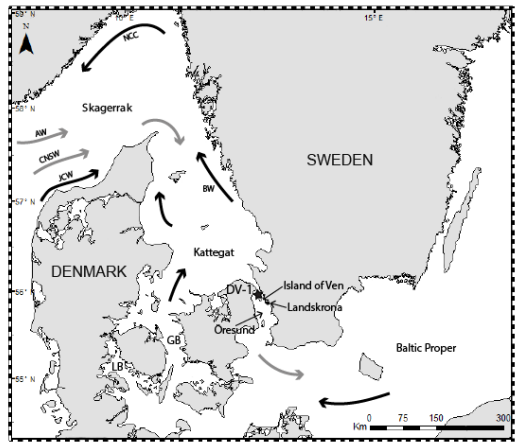

Fig. 1 Map of the studied area. The star shows the focused station of this study. General water circulation: main surface currents (black arrows) and main deep currents (grey arrows). GB: Great Belt; LB: Little Belt; AW: Atlantic Water; CNSW: Central North Sea Water; JCW; Jutland Coastal Water; NCC: Norwegian Coastal Current; BW: Baltic Water.

north of the Öresund (Fig. 1). The fully marine Skagerrak consists of water masses from the North Sea and the North Atlantic and in general a thin layer with water originating from the Baltic Sea and rivers draining into the sea; the water circulation forms a cyclonic gyre (cf. Erbs-Hansen et al. 2012). Part of the Skagerrak waters reach the Kattegat and the Baltic Sea, where they are successively diluted with the large amounts of freshwater (about 15,000 m^3/s, Bergström and Carlsson 1994) draining into the Baltic Sea from numerous large rivers. The low-saline Baltic Sea surface water exits the Kattegat through the Baltic Current that later joins the Norwegian Coastal Current in the Skagerrak (Fig. 1). The large fresh water inputs and the subsequent large salinity difference between the Kattegat and Baltic Sea result in a two-layer structure in the Öresund (Leppäranta and Myrberg 2009) (Fig. 2). The water stratification is influenced by the surface water from Arkona Basin (salinity 7.5-8.5), the surface water from the Kattegat upper layer (salinity 18-26) and the lower layer of the Kattegat (salinity 32-34). Salinity, temperature, pH and dissolved oxygen concentration both in the surface and bottom waters vary strongly by the season (Fig. 2).

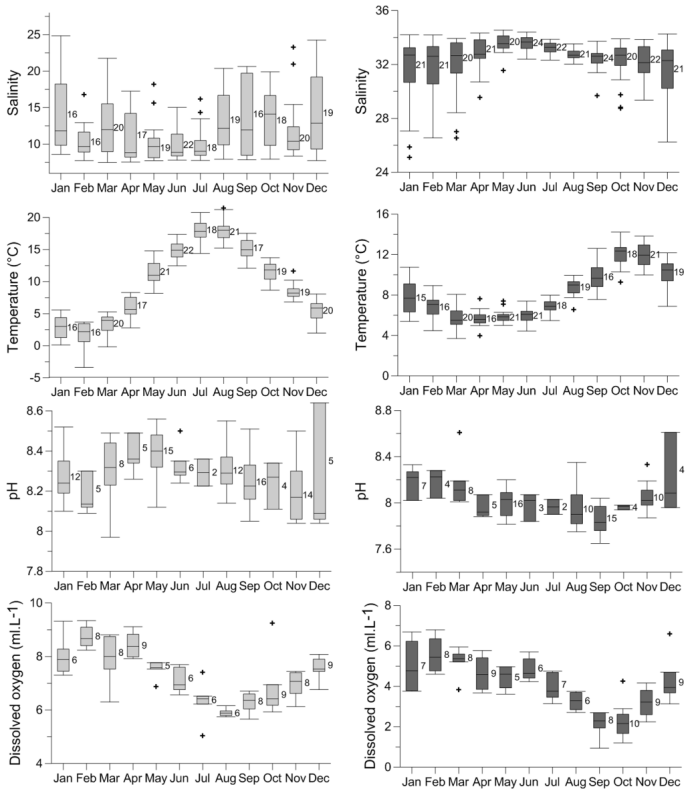

Fig. 2 Seasonal variability of salinity, temperature, pH, and dissolved oxygen concentration at the surface water (light grey) and at the bottom water (40-50m) (dark grey) of the Öresund. The data were measured between 1965 and 2016 by the SMHI (Swedish Meteorological and Hydrological Institute) at the station W LANDSKRONA. The numbers of measurements is indicated for each month.

Materials and Methods

Sampling

Sediment cores were collected in November 2013 during a cruise with R/V *Skagerak*. Here we present the data from down core records sampled at the Öresund station DV-1 (55°55.59'N, 12°42.66'E) (Fig. 1), north of the Island of Ven. The water depth was 45 m. In general, it is challenging to obtain suitable sediment cores in the Öresund, due the high current velocities and few areas of sediment deposition, but this site represents an accumulation area. Two cores (9-cm-inner-diameter) were collected using a GEMAX twin barrel corer. The corer allowed sampling of 30 and 36 cm long sediment cores (referred in this study as DV1-G and DV1-I, respectively) which were sliced into one centimeter sections. In the laboratory, water content was estimated by weighing the samples before and after freeze-drying. The samples from the DV1-G core were analyzed for carbon and nitrogen content, grain size distribution, and dated by the ^{210}Pb technique. The samples from the DV1-I core were analyzed which respect to foraminiferal fauna and carbon and nitrogen content.

Chronology

The age-depth model was established using the ^{210}Pb method on samples from the DV1-G core. The samples were measured with an ORTEC HPGe (High-Purity Germanium) Gamma Detector at the Department of Geology at Lund University, Sweden.

Corrections for self-absorption were made for ^{210}Pb following Cutshall et al. (1983). The instruments were calibrated against in-house standards and the maximum error was 0.5 year. Excess (unsupported) ^{210}Pb was measured down to 23 cm and the age model was calculated based on the Constant Rate of Supply (CRS) model (Appleby 2001).

Foraminifera analyses

The foraminiferal samples were prepared following standard micropalaeontological techniques (Murray 2006). Approximately 10 g of sediment per sample were wet sieved thought a 63 µm mesh screen and left to dry on filter paper at room temperature. Subsequently, the samples were dry sieved through 100 and 500 µm mesh screens and separated into the fractions 100-500 µm and >500 µm. The foraminifera from every second centimeter of the core were picked and sorted under a Nikon microscope. A minimum of 300 specimens per sample were picked and identified, if necessary the samples were split with an Otto splitter (Otto 1933). For taxonomy at the genus level, we mainly followed Loeblich and Tappan (1964) with some updates from more recent literature, e.g. Tappan and Loeblich (1988). For taxonomy at the species level, we mainly used Feyling-Hanssen (1964), Feyling-Hanssen et al. (1971) and Murray and Alve (2011). For original descriptions of the species, see Ellis and Messina (1940 and supplements up to 2013).

Recently, the eastern Pacific morphospecies *Nonionella stella* has been presented as an invasive species in the Skagerrak-Kattegat region (Polovodova Asteman and Schönfeld 2015). However, a comparison of *N. stella* DNA sequences from the Santa Barbara Basin (USA) (Bernhard et al. 1997) with the Swedish west coast specimens (Schweizer et al. unpublished results) demonstrates that they represent two closely related species but are not conspecific. Until this question is resolved we refer to the species found here as *N.* aff. *stella*, following Charrieau et al. (in revision). The species *Verneuilina media* (here referred to the genus *Eggerelloides*), which has often been reported in previous studies from the Skagerrak-Kattegat area, is morphologically close to *Eggerelloides scabrus* in the present material, and these two species have therefore been grouped as *E. medius/scabrus*. The taxon *Elphidium excavatum* forma *clavata* (cf. Feyling-Hanssen 1972), was referred to as *Elphidium clavatum* following Darling et al. (2016). *Elphidium clavatum* and *Elphidium selseyense* (Heron-Allen and Earland) are morphologically difficult to separate in this region, as transitional forms occur. The dominant species was *E. clavatum*, but we acknowledge that a few individuals of *E. selseyense* could have been included in the counts. The taxon *Ammonia beccarii* was referred to as *Ammonia batava*, following recent molecular work done on the taxon *Ammonia* in the Kattegat region (Groeneveld et al. in prep.).

Foraminiferal density was calculated and normalized to the number of specimens per cm^3. Densities of living + dead foraminifera for the first two centimeters of the core were taken from Charrieau et al. (in revision). Inner organic linings were reported separately and not included in the total foraminiferal counts. Benthic foraminiferal accumulation rates were calculated as follows:

$$\text{BFAR (number of specimens.cm}^{-2}\text{.yr}^{-1}\text{)} = \text{BF x SAR},$$

where BF is the number of benthic foraminifera per cm^3 and SAR is the sediment accumulation rate (cm.yr^{-1}). Foraminiferal species that accounted for >5% of the total fauna in at least one of the samples were considered as major species, and their density was used in statistical analysis. To determine foraminiferal zones, stratigraphically constrained cluster analysis was performed, using the size-independent Morisita's index to account for the large differences in the densities between samples. A dendrogram was then constructed based on arithmetic averages with the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). Correspondence analysis was also performed to determine significant foraminiferal species in each zone. Both statistical analyses were performed using the PAST software (Hammer et al. 2001).

Organic matter analyses

Total Organic Carbon (TOC) and Total Nitrogen (TN) content were measured for both DV1-G and DV1-I. Approximately 8 mg of freeze-dried sediment

was homogenized for each centimeter and placed in silver capsules. Removal of inorganic carbon was carried out by in-situ acidification (2M HCl) method based on Brodie et al. (2011). TOC and TN content were analyzed on a Costech ECS 4010 Elemental Analyzer at the Department of Geology, Lund University. The instrument was calibrated against in-house standards. The analytical precisions showed a reproducibility of 0.2% and 0.03% for TOC and TN contents, respectively. The molar C/N ratio was calculated.

Grain-size analyses

Grain-size analyses were performed on core DV1-G using 3.5 to 5 grams of freeze-dried sediment for each centimeter. Organic matter was removed by adding 15 mL of 30% H_2O_2 and heating during 3 to 4 minutes until the reaction ceased. After the samples had cooled down, 10 mL of 10% HCl was added to remove carbonates; thereafter the sediment was washed with milli-Q until its pH was neutral. In the last step, biogenic silica was removed by boiling the sediment in 100 mL 8% NaOH, and then washed until neutral pH was reached. The sand fraction (>63 µm) was separated by sieving and the mass fraction of sand of each sample was calculated. Grain sizes <63 µm were analyzed by laser diffraction using a Sedigraph III Particle Size Analyzer at the Department of Geology, Lund University. Three size groups, <4 µm (clay), 4–63 µm (silt) and 63-2000 µm (sand) were categorized.

Results

Age model

The unsupported ^{210}Pb showed a decreasing trend with depth in the DV1-G core (Fig. 3B). The peak observed in the ^{137}Cs around 9 cm corresponds to the Chernobyl accident in 1986 (Fig. 3C). The unsupported ^{210}Pb allowed direct dating of the core between 2013 and 1913. The ages of the lower part of the sediment record were deduced by linear

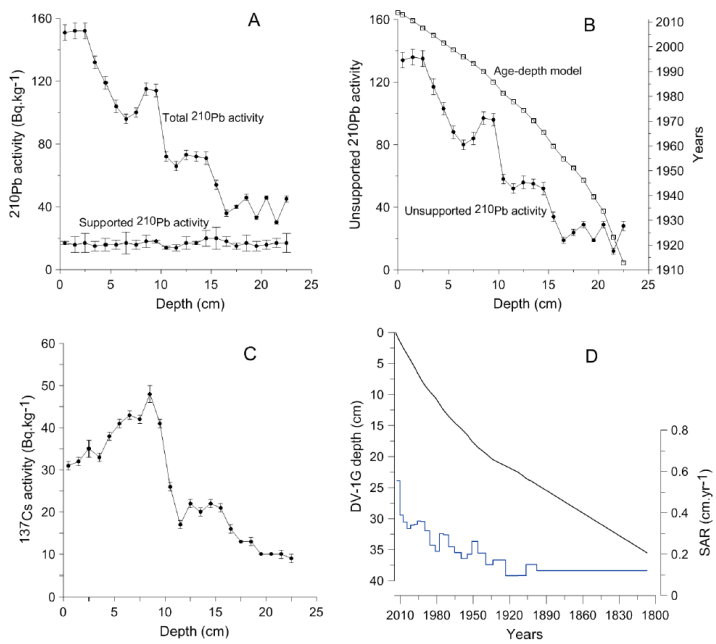

Fig. 3 Age-depth calibration for the sediment sequence from the Öresund (DV-1). A) Total and supported ^{210}Pb activity. B) Unsupported ^{210}Pb activity and the associated age-model. C) ^{137}Cs activity. The peak corresponds to the Chernobyl reactor accident in 1986. D) Age-depth model for the whole sediment sequence based on ^{210}Pb dates and calculated sediment accumulation rates (SAR).

extrapolation based on a sedimentation rate of 1.4 mm/year, corresponding to the linear mean sedimentation rate between the years 1913 and 1946 (Fig. 3D). The sedimentation rate was decreasing with depth and ranged between 1 and 5.6 mm/year, with an average of 2.2 mm/year.

Since TOC values were analyzed on both cores, the distinct TOC profiles were used to correlate the ^{210}Pb dated DV1-G core to the DV1-I core used for foraminiferal analyses (Fig. 4).

Sediment features

The TOC values in the two cores ranged from 1.95 to 3.81%, with a mean value of 3.17% (Fig. 4). A period of lower values was observed between 16 and 11 cm, with an average of 2.15%. The C/N ratio ranged between 9.50 and 10.86 (Fig. 4).

The clay size fraction dominated the sediment throughout the entire core and ranged from 45 to 66% (Fig. 4). The sand content was around 10% except and showed a pronounced increase between 16 and 11 cm, with an average of 28%.

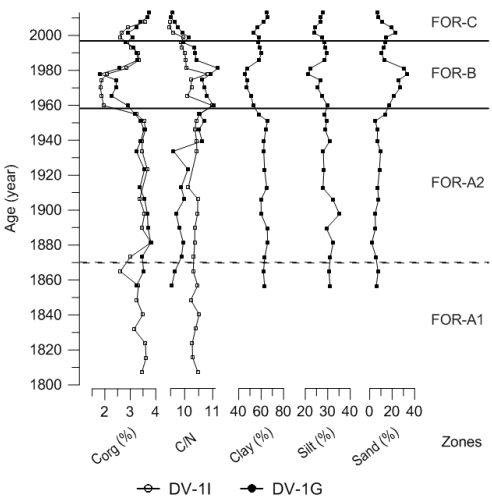

Fig. 4 Sediment parameters of the cores DV-1I and DV-1G (^{210}Pb dated): total organic carbon content (%), C/N ratio (%) and grain size (%). Foraminiferal zones reported.

Table 1 Significant foraminiferal species and scores according to the correspondence analysis.

Factor	Total variance (%)	Significant species	Score
1	51.06	*Nonionella* aff. *stella*	4.69
		Nonionoides turgida	4.01
2	28.62	*Ammonia batava*	1.43
		Stainforthia fusiformis	-1.35
3	13.58	*Elphidium albiumbilicatum*	-1.87
		Elphidium clavatum	-1.59
		Elphidium magellanicum	-1.40

Foraminiferal assemblages

The foraminiferal assemblages were composed of 74 species from the porcelaneous, hyalines and agglutinated forms. Eleven foraminiferal species had relative abundance higher than 5% in at least one sample and were considered as major species (Plate 1, Fig. 5).

The cluster analysis reveals three main foraminiferal zones (FOR-A, FOR-B, and FOR-C), separated into four subzones to which we assigned dates according to the age model: FOR-A1 (1807-1870), FOR-A2 (1870-1958), FOR-B (1958-1998) and FOR-C (1988-2013) (Fig. 5, 6).

The foraminiferal accumulation rate (BFAR) was on average 7 ±5 specimens.cm^{-2}.y^{-1} during zone FOR-A, 28 ±30 specimens.cm^{-2}.y^{-1} during zone FOR-B, and 17±22 specimens.cm^{-2}.y^{-1} during zone FOR-C. BFAR peaked at 92 specimens.cm^{-2}.y^{-1} around 1970 (Fig. 5). During FOR-A, the diversity was stable and low (Shannon index average 1.73 ±0.19), while during FOR-B and FOR-C it progressively increased towards the top of the core (Shannon index average 2.61 ±0.34). The correspondence analysis resulted in three factors explaining 93% of the variance, and in assemblages consisting in seven significant species (Table 1, Fig. 5).

Zone FOR-A1

The agglutinated species *Eggerelloides medius/scabrus* and the hyaline species *Stainforthia fusiformis* made major contributions to the assemblages (relative abundances up to 53% and 34%, respectively; Fig. 5). *Ammonia batava*, the three *Elphidium* species (*E. albiumbilicatum*, *E. clavatum*, and *E. magellanicum*), *Nonionellina labradorica* and the agglutinated species

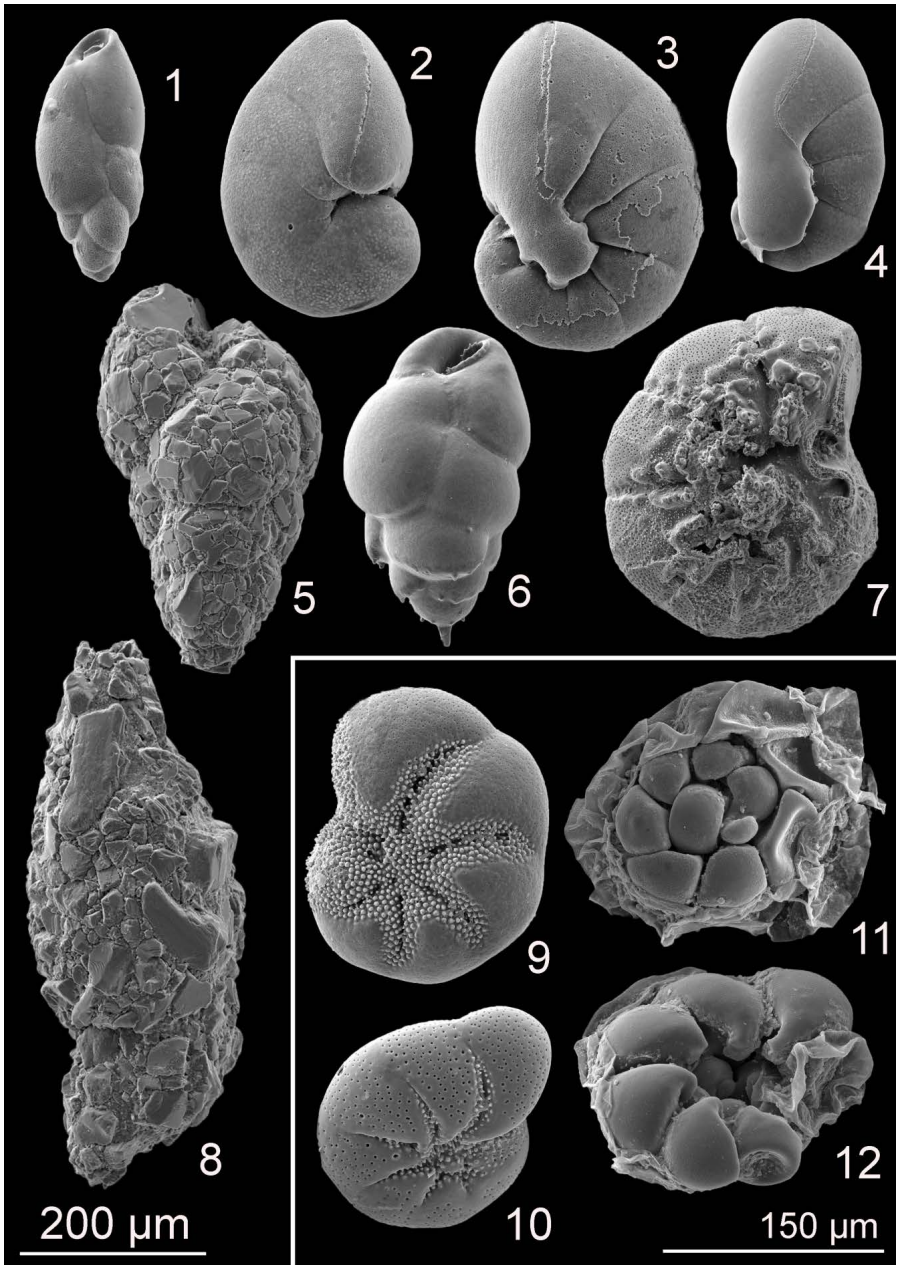

Plate 1 SEM pictures of the major foraminiferal species (>5%). 1. *S. fusiformis*; 2. *N. labradorica*; 3. *N*. aff. *stella*; 4. *N. turgida*; 5. *E. medius/scabrus*; 6. *B. marginata*; 7. *A. batava*; 8. *R. subfusiformis*; 9. *E. magellanicum*; 10. *E. clavatum*; 11-12. *Ammonia* sp.

Reophax subfusiformis were also major species with abundances up to 7%.

Zone FOR-A2

Stainforthia fusiformis dominated the assemblage with relative abundances up to 56% and *E. medius/scabrus* was still highly present, up to 48% (Fig. 5). *Ammonia batava*, the three *Elphidium* species and *N. labradorica* were present but with lower abundances (maximum 5%) than in the zone FOR-A1. *Bulimina marginata* started to be more abundant in this zone with an average abundance of 2%. *Reophax subfusiformis* was still a part of the assemblage and ranged between 1 and 8%.

Zone FOR-B

The zone was characterized by a drastic drop in the relative abundance of *S. fusiformis* from 31 to 2%, with a small increase in the second part of the zone to maximum 9% (Fig. 5). *Eggerrelloides medius/scabrus* was still dominant but gradually decreased in the zone from 49 to 24%. The highest abundance for *A batava* over the core was in this zone but it was slowly decreasing as well, from 9 to 4%. The *Elphidium* group was more abundant than in the FOR-A zones and they were increasing, especially for *E. clavatum* (increasing up to 23%). *Bulimina marginata*, *N. labradorica* and *R. subfusiformis* were also present between 2 and 6%.

Zone FOR-C

The dominant species in this zone were *E. clavatum* (up to 24%), *Nonionella* aff. *stella*, which had not occurred in the record until now, up to 14%, and *R. subfusiformis* (up to 13%; Fig. 5). *Eggerelloides medius/scabrus* had its lowest abundance over the core with maximum 13%. *Nonionoides turgida*, which was present in very low abundances along the core, increased to 9% at the end of the zone. *Bulimina marginata*, the other two *Elphidium* species, *N. labradorica* and *S. fusiformis* were still present (between 1 and 9%). *Ammonia batava* declined and was absent at the end of the zone.

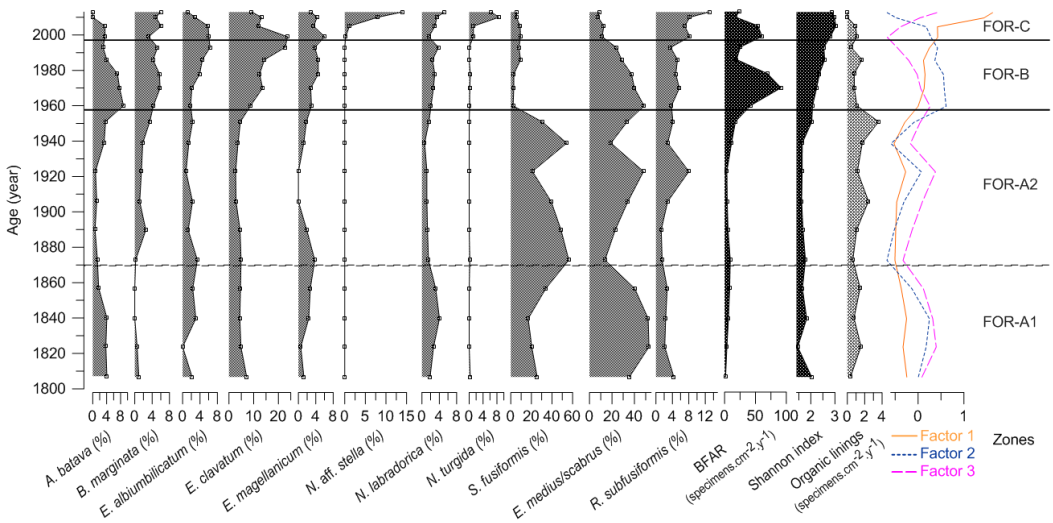

Fig. 5 Relative abundances (%) of the foraminiferal major species (>5 %), benthic foraminiferal accumulation rate (BFAR, specimens.cm^{-2}.yr^{-1}), Shannon index, organic linings (specimens.cm^{-2}.yr^{-1}) and factors from the correspondence analysis. Foraminiferal zones based on cluster analysis. Note the different scale on the x axes.

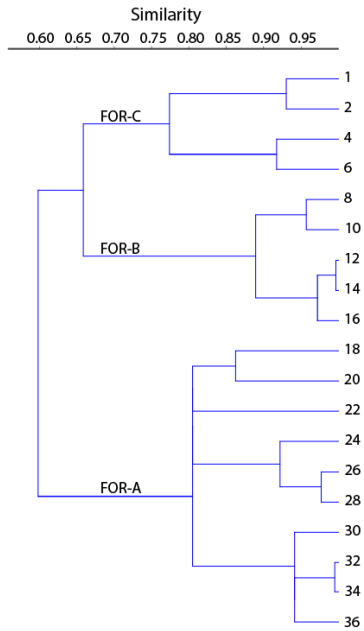

Fig. 6 Dendrogram produced by the cluster analysis based on the Morisita index and the UPGMA clustering method.

Organic Linings

Decalcified specimens were observed throughout the core and the morphology of the remaining inner organic linings allowed the identification of the taxon *Ammonia* (Plate 1). The accumulation of decalcified specimens varied between 0 and 9 specimens.cm^{-2}.y^{-1} with an average of 1 specimen.cm^{-2}.y^{-1} (Fig. 5).

Discussion

Our environmental interpretations of the foraminiferal assemblages were based on the ecological characteristics of each major species (Table 2). In our environmental reconstructions, we could infer environmental changes regarding oxygen concentration, salinity, organic matter content, and pollution levels.

1807 – 1870 CE

All the major species found in this period are tolerant to low oxygen conditions, especially the two main species *S. fusiformis* and *E. medius/scabrus* (Table 2). *Stainforthia fusiformis* is an opportunistic species used to hypoxic and potentially anoxic conditions (Alve 1994), and *E. medius/scabrus* specimens were found alive down to 10 cm in the sediment, where no oxygen was available (Cesbron et al. 2016). *Stainforthia fusiformis* and *N. labradorica* are also able to denitrify. The fact that species tolerant to low oxygen conditions dominated, and the presence of species that has the capacity to denitrify, suggest that low oxygen conditions were prevailing during this period. Most of the major species found during this period, such as the *Elphidium* group, *R. subfusiformis* and *A. batava* tolerate lower salinities, and are typical of brackish environments. The absence of *B. marginata*, a typical marine species, suggests a salinity lower than in the open ocean. However, the salinity was probably not below 30, which is the lower limit for *N. labradorica* and *S. fusiformis* which were present throughout the period. Furthermore, *S. fusiformis* prefers organic rich substrate and clayey sediment, which was measured in our core during this time period. In summary, this period appears to have been characterized by low oxygen concentration, high organic matter content, and salinity around 30.

1870 – 1958 CE

Stainforthia fusiformis was largely dominating the assemblage during this period, which may suggest even lower oxygen conditions. This would also go along with the low species diversity. The low species diversity, as indicated by the low Shannon index in this section of the core, is usually linked with low salinity (Sen Gupta 1999). However, the occurrence of the marine species *B. marginata* suggests that the salinity was at least 32. Low oxygen is frequently associated with high organic matter contents, since oxygen is consumed during remineralization of organic matter. However, no increase in TOC was observed in our core in this zone compared to the previous one (Fig. 4). At the time of the industrial revolution, the Öresund was used as a sewage

Table 2 Ecological significance of the benthic foraminiferal assemblages (major species).

Species	Ecological significance	Reference
Ammonia batava	Salinity 15-35, T 0-29^{0}C, high tolerance to varying substrate and TOC	Alve and Murray (1999); Murray (2006)
Bulimina marginata	Salinity 30-35, T 5-13^{0}C, muddy sand	Murray (2006)
Elphidium albiumbilicatum	Salinity 16-26, typical brackish species	Alve and Murray (1999)
Elphidium clavatum	Salinity 10-35, T 0-7^{0}C, high tolerance to varying substrate and TOC, subtidal	Alve and Murray (1999); Murray (2006)
Elphidium magellanicum	Coastal species	Sen Gupta (1999)
Nonionella aff. stella	Tolerates low oxygen conditions, kleptoplastidy, able of denitrification, invasive in the Skagerrak-Kattegat	Piña-Ochoa et al. (2010); (Bernhard et al. 2012); Charrieau et al. (in revision)
Nonionellina labradorica	Salinity > 30, T 4-14^{0}C, high latitudes, kleptoplastidy, able of denitrification	Cedhagen (1991)
Nonionoides turgida	Opportunistic species, tolerates low oxygen conditions and prefers high food availability	Van der Zwaan and Jorissen (1991)
Stainforthia fusiformis	Opportunistic species, tolerates very low oxygen conditions, salinity > 30, able of denitrification, prefers organic rich substrates, fast reproduction cycle	Alve (1994); Filipsson and Nordberg (2004); Piña-Ochoa et al. (2010)
Eggerelloides medius/scabrus	Salinity 24-35, T 8-14^{0}C, high tolerance to hypoxia, sandy-muddy sand, tolerance to various kind of pollution	Alve and Murray (1999); (Alve 1990); Murray (2006); (Cesbron et al. 2016)
Reophax subfusiformis	Tolerance to environmental variations	Sen Gupta (1999)

recipient for a mixture of domestic and industrial wastes, industrial cooling water and drainage water (Henriksson 1968), and the amount of marine traffic increased considerably during this time period. These diverse types of pollution could have modified the water properties, for example regarding the carbonate chemistry and pH. Indeed, this zone is characterized by the highest concentrations of organic linings in the core (see also section 5.5). Pollution and low oxygen concentration could explain the low species richness as well as the dissolution of tests. Other species that were present, i.e. the agglutinated species *E. medius/scabrus* and *R. subfusiformis*, are tolerant to pollution.

1958- 1998 CE

During this period, the relative abundance of *S. fusiformis* decreased, which may indicate more oxic conditions. In line with this are the coarser grain size and the lower TOC values, which suggest that the area was affected by stronger currents than previously, causing changes in sedimentation pattern. The foraminiferal accumulation (BFAR) reached the highest values in this period and diversity was higher than during earlier periods. Species that tolerate sandy environments and varying TOC dominated the assemblage, such as *A. batava*, the *Elphidium* group, *B. marginata*, and *E. medius/scabrus*. The decline in relative abundance of *S. fusiformis* and the high diversity suggests that the water was more oxygenated and with open ocean salinity.

1998 – 2013 CE

This period is characterized by the appearance of two new major species: *N. turgida* and *N.* aff. *stella. Nonionella* aff. *stella* is considered as an invasive species in the region, which arrived by ship ballast tanks around 1985, and rapidly expanded to the Kattegat and Öresund (Polovodova Asteman and Schönfeld 2015; Charrieau et al. in revision). According to our dated core, the species arrived in the Öresund ~2000 CE (Fig. 5). The ability of the species to denitrify and its tolerance to varying environment may explain its rapid increase during this period. *Nonionoides turgida* is an opportunistic species that prefers high levels of organic matter in the sediment, which are indeed increasing on this period (Fig. 4). This period is thus characterized by high oxygen concentration, high organic matter content, and open ocean salinity.

Dissolution

The inner organic linings of the taxon *Ammonia* were observed along the whole core, except in the top two

centimeters (Fig. 5). Inner organic linings of the taxa *Ammonia* and/or *Elphidium* were noticed in previous studies among dead fauna in the region (Jarke 1961; Hermelin 1987: Baltic Sea; Christiansen et al. 1996; Murray and Alve 1999: Kattegat). Dissolution of calcareous foraminiferal tests has been considered as a taphonomic process, affecting the test of the specimens after their death (Martin 1999; Berkeley et al. 2007). However, living decalcified foraminifera were observed in the Arcachon Bay (Cesbron et al. 2016) and in the south Baltic Sea (Charrieau et al. in revision), proving that test dissolution can also happen before the specimens die. In any case, low pH and low calcium carbonate saturation are suggested as involved in the observed dissolution (Jarke 1961; Christiansen et al. 1996; Murray and Alve 1999; Cesbron et al. 2016; Charrieau et al. in revision). Test dissolution may occur on all calcitic species, but only the organic linings of *Ammonia* were found, probably because these were more robust to physical stress such as abrasion.

Conclusion

In this study, we described an environmental record from the Öresund, based on benthic foraminifera - and geochemical data. The exceptional dating allows the reconstruction of the environment on the last 200 years. Four foraminiferal zones were differentiated and associated with environmental changes in terms of salinity, dissolved oxygen concentrations, and organic matter content. The main event is a major shift in the foraminiferal assemblage ~1960, when *S. fusiformis* stopped dominating the assemblage. This period also corresponds to an increase in grain-size, resulting in a higher sand content. The grain-size distribution suggests changes in the current velocities. Organic linings of *Ammonia* were observed throughout the core, probably linked to low pH and calcium carbonate saturation, affecting test preservation.

Supplementary data

Appendix A with total foraminiferal faunas normalized to 50 cm^3 along the DV core is available in the online version of the article.

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Paper IV

Improved wet splitter for micropalaeontological analysis, and assessment of uncertainty using data from splitters

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Improved wet splitter for micropalaeontological analysis, and assessment of uncertainty using data from splitters

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ABSTRACT - Analyses of foraminiferal assemblages have often been implemented on dry samples, which are easy to split. In some cases, the wet picking method is preferred as it allows preservation of more foraminiferal forms and facilitates the picking of live foraminifera. However, the increased execution time needed for wet-picking may cause micropalaeontologists to refrain from employing it in a routine way. Here we present an improved and cost-effective wet splitter (including a 3D printing file) for micropalaeontological samples aimed to reduce picking time while keeping information loss to a minimum. We demonstrate small sample losses as well as statistical consistency across splits. We show that the time saved picking a subset will always be larger than the relative increase in statistical uncertainty.

INTRODUCTION

Splitting samples into smaller subsamples is often necessary in micropalaeontological studies. Indeed, the general high abundance of microfossils - which makes them excellent tools to reconstruct past environments - also results in very time-consuming faunal analyses. For assemblage analyses of benthic foraminifera, Patterson & Fishbein (1989) recommend a count of at least 300 specimens in order to identify the species comprising 10% or more of an assemblage. Studies using fossil foraminifera are most often carried out on dry material; if the samples need splitting, the well-known "Otto" microsplitter (Otto 1933) is typically used. However, in many cases it is necessary to keep the samples in a liquid, for example to reduce the risk of destroying fragile (poorly cemented) or thin-shelled forms during the drying process. Additionally, the number of studies focusing on living foraminifera is increasing, which suggests an increase in the use of wet samples. Indeed, the methods employed to distinguish live from dead foraminifera such as the rose Bengal stain (Walton 1952) or the more recent CellTracker™ Green (Bernhard et al. 2006), are more efficient when the specimens are in a liquid, as the stain/labelling becomes easier to discern. Moreover, the non-fossilising soft-shelled forms will also be preserved. Wet-picking for assemblage studies is considerably more time-consuming than comparable work using dry samples (Murray 2006), adding to the necessity for a reliable wet splitter. A wet splitter device was first described by Elmgren in 1973 and subsequently improved by Scott & Hermelin (1993). However, the improved design by Scott & Hermelin (1993) is also sub-optimal for several reasons. First, sample losses may occur due to leakage, as well as to sticking to compartment wall edges and their crossing point (Scott & Hermelin 1993). Second, the relatively short cylinder could potentially prevent heavy particles such as foraminifera from being homogeneously distributed in the water column before settling. Third, the off-centre drainage system results in non-symmetrical water swirls, which may bias the spatial distribution of particles between the splits. No quantitative or

statistically consistent results regarding studies of these effects have been reported for the previous devices.

Here we present a modified device built at the Department of Geology, Lund University, Sweden. The main improvements are: a fully hermetic and symmetric splitter design with a central drainage system, and very thin, polished walls made possible by the advancement of 3D printing techniques. For the first time, a comparison both between all the splits and with the known input sample was made, enabling assessment of loss and inhomogeneity.

DESCRIPTION OF THE DEVICE

We designed a new splitter (Fig. 1) based on the one by Scott and Hermelin (1993). The device is composed of two parts: a one-meter PVC cylinder with an outside diameter of 100 mm, and a plastic base created in one piece using a 3D printer. The base is divided into eight sections with 1 cm diameter outlets hermetically sealed by rubber stoppers. To avoid particles sticking to the edges of the walls that separated the sections, the walls of the base were made as thin as possible (< 1 mm thick) with v-shaped upper edges, and the base was polished and varnished. The draining system is a siphon, composed of a PVC pipe with a diameter of 6 mm, linked to the centre of the base (Fig. 1).

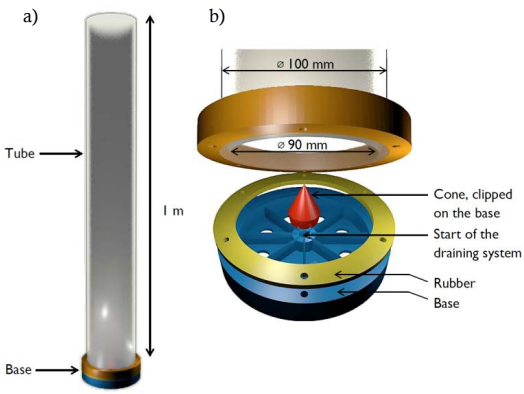

Fig. 1. Different views of the wet-splitter a) full view b) base details.

There are three main innovations. Besides the novel design of the section walls, we have added a rubber ring between the base of the device and the cylinder (Fig. 1b), which considerably reduces the potential problem of leakage, as reported from previous devices (Scott & Hermelin 1993). Six screws keep the cylinder and the base together. Furthermore, the draining pipe is connected to the mid-point of the base, symmetrising the effect of the small swirl formed during drainage. Finally, adding a cone at the mid-point of the eight sections results in 1) preventing particles from settling in the central drainage hole, where they would be lost when the water is drained; and 2) even distribution of the particles among the eight sections (Fig. 1b). The mid-point cone effectively reduces the area where particles could get stuck compared with previous designs.

ASSEMBLY AND USAGE

To operate the splitter, the user needs to assemble the cylinder, the rubber ring, and the base, and then firmly tighten the screws. After filling the cylinder with water, strong turbulence is created by stirring, ensuring an equal distribution of the particles in the water column. The user should rapidly pour the sample into the cylinder and let it settle for at least one hour. Once the water has been slowly siphoned through the drainage pipe, the two parts of the device can be carefully separated. The rubber stoppers of the eight sections can be removed, and each split is collected into individual vials using a squeeze bottle.

PERFORMANCE TESTS

The following section describes a series of tests carried out to quantify the accuracy that can be expected when using the device. The statistical uncertainties were first assessed thanks to a statistical model and to the Poisson distribution (see details in SI). The existence of the established statistical model enables a quantitative interpretation of the advantage of using a splitter. If picking only a fraction 1/n of the total sample, the time spent picking will decrease by a factor n. The Poisson distribution implies that the relative statistical uncertainty in the measurement, given by $1/\sqrt{\mu}$, will then increase by $\sqrt{(n)}$. This is shown with both the general mathematical expression and an explicit example in Table 1. This quantifies the

Table 1. Illustration of the increase in statistical uncertainty from estimating the total number of specimens (N) from a picked sub-sample (comparing the two last rows). Both the general expression and a made-up example are shown. The total number in the example is arbitrarily set to a number close to 400 for illustration purposes. The relative uncertainty is increased by $\sqrt{(n)}$, while the time saved increases by n.

	General expression (expected)	Example numbers
Fraction picked (relative time spent)	$1/n$	1/4
Number of specimens in split	N/n	100
Statistical uncertainty	$\sqrt{(N/n)}$	$\sqrt{100}=10$
Estimated total number in sample	$n(N/n\pm\sqrt{(N/n)}) = N\pm\sqrt{(n)}\sqrt{(N)}$	$4(100\pm10)=400\pm40$
Counted total number in sample	$N\pm\sqrt{(N)}$	$392\pm\sqrt{392}\approx392\pm20$

trade-off, where the time saved on picking a subset will always be larger than the relative increase in statistical uncertainty. The acceptable level of statistical uncertainty has to be decided for the individual sample and analysis goals.

Sample tests: method and results

Two sets of tests were executed to assess the efficiency of the splitter. We used marine sediment samples that were sieved through different mesh screens. The efficiency (denoted by ε in Fig. 2) was defined as the fraction of the input sample recovered when summing over all splits after the splitting procedure. In order to assess whether differences between splits were statistically significant, which would indicate leaks or other inhomogeneities in the construction, a statistical uncertainty was assigned to the measurements according to the previously verified Poisson distribution fits (e.g. a measured value of 9 has an uncertainty $\sqrt{9} = 3$). In the first set, a known number of sediment grains with sizes of 100-500 or > 500 µm were poured into the device and then each split was picked (Table 2). In all the cases, the total number of grains was always recovered, yielding 100% efficiency (Table 2). However, since time constraints limit counting tests to small numbers of grains, there are potentially large statistical fluctuations in such efficiency measurements, potentially hiding smaller systematic effects. Thus, in a second set, we used larger, well-sorted sediment samples with grain sizes of > 20, > 63, > 100 and > 250 µm, respectively, with a maximum grain size of 600 µm. Here, the weight of each split was measured and compared to the expected weight from the known input weight (Table 2). For all the tests, the weight measured in each split agreed with the average overall splits within statistical

Table 2. Grain size, number of particles, input weight and splitter efficiency for the first and second sets of tests. *tests presented in Fig. 2.

Set1	Fraction (µm)	Number of particles	Efficiency (%)
	100-500	80	100
	>500	80	100
	>500	160	100
	>500	160	100
Set 2	Fraction (µm)	Input weight (mg)	Efficiency (%)
	>20	1600	95.1*
	>20	800	95.0
	>63	800	92.4*
	>63	1600	97.9
	>63	1600	97.6
	>63	1600	95.5
	>100	400	92.4
	>100	400	99.1*
	>250	328	92.1*

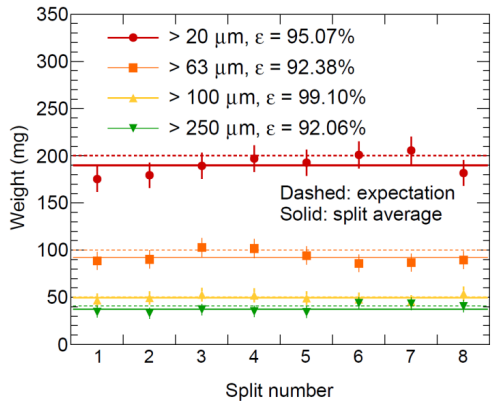

Fig. 2. The weight measured in each split, compared to the expectation without losses (dashed line) and the average across the splits in each test (solid line) for a few representative example samples. The vertical bars on the data points represent the statistical uncertainty. The grain size and the efficiency $\boldsymbol{\varepsilon}$ for each test are given in the legend.

uncertainties (Fig. 2, solid line), and no systematic differences between the splits were observed. The deviations from the average were verified to be normally distributed, as is expected from random fluctuations. There were small losses of sediment (Fig. 2), attributed to losses in the water and on the compartment walls. An average efficiency of 95% was seen, and the efficiency was independent of grain size (Table 2). The absolute losses were seen to have a positive but steadily decreasing dependence on the input weight, which is interpreted as a saturation of possible losses. Where high accuracy is needed, initially performing this type of test, probing the grain size and weight dependence of the efficiency of the device, allows for corrections of the subsequently measured foraminiferal data.

CONCLUSIONS

The improved wet splitter described in this paper splits samples with small sample losses and without introducing systematic differences between sample splits. Comparisons across all splits and with the known total input show that, for a range of particle sizes, picking a subset of the splits gives statistically compatible results to picking all of them. The verified statistical model used quantifies the associated larger relative statistical uncertainties from picking only a subsample. With both the time saved and increased statistical uncertainties thus known, the optimal balance can be decided on a use-case basis. Furthermore, efficiency losses are predictable and we present a method to measure them. We recommend the use of the wet splitter to analyse foraminiferal assemblages in a more time-efficient manner.

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SUPPLEMENTARY FILE

Statistical model and simulation method

To determine if differences between measurements are significant, it is necessary to know the size of the expected fluctuations. The statistical uncertainty must

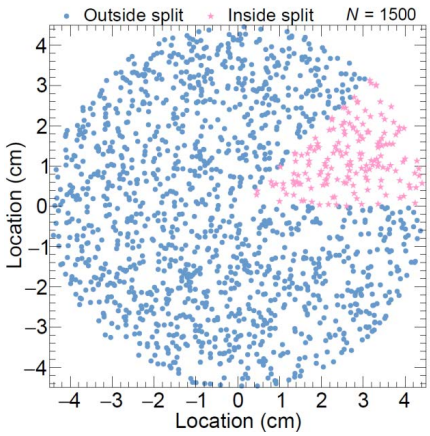

Fig. SI 1. Simulated random spatial distribution of $N = 1500$ particles on a modelled simplified splitter bottom cross section.

therefore be known, which in turn requires a statistical model in this case derived using computer simulation. The simulation was a simple toy model in Python, using a publicly available random number generator and statistics library. The cross section of the splitter bottom was modelled as a circle with eight identical circle sectors, and the locations of N particles (mimicking e.g. foraminifera) from a simulated fill in the splitter were drawn from a uniform random spatial distribution (Fig. SI 1). The number of particles ending up in one of the splits was recorded for 1000 simulated trials. The resulting particle distribution is shown in Fig. SI 2 for, as an example, $N = 4$ and $N = 40$. A Poisson distribution fit to each of the distributions is also shown; this distribution describes the probability of obtaining a given number of events in a time or space interval if they are independent of the time since - or location of - the last event and occur at some average rate. In a splitter where care has been taken to create a homogeneous particle distribution in the water, this precisely describes the expected situation of particles settling on the base: where one particle lands is not influenced by where the previous one landed. The Poisson distribution has only one parameter: the expectation value μ (which roughly translates to the average value) also immediately gives the variance (such that $\sqrt{\mu}$ describes the width of the distribution, indicating how large statistical fluctuations are expected). The Poisson distribution fits verify that the number of particles in one split is well described by a Poisson distribution with $\mu = N/8$. This was further verified for a range of choices of N.

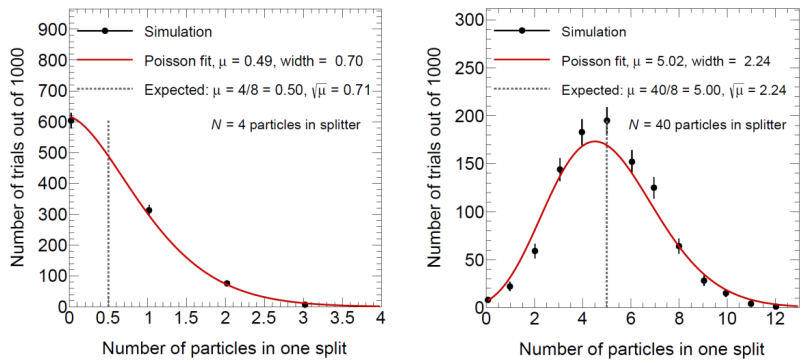

Fig. SI 2. Sampling distributions of number of particles in one eighth of the splitter (black dots) as obtained from simulation of splitting a sample consisting of a) $N = 4$ and b) $N = 40$ particles. The distributions are overlaid with Poisson distribution fit (red solid line). The expected number given by $N/8$ is indicated by the dashed grey vertical line.

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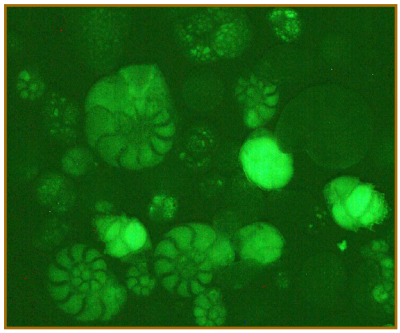

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