Impact of elevated pCO_2 on benthic foraminifera from the southwestern Baltic Sea

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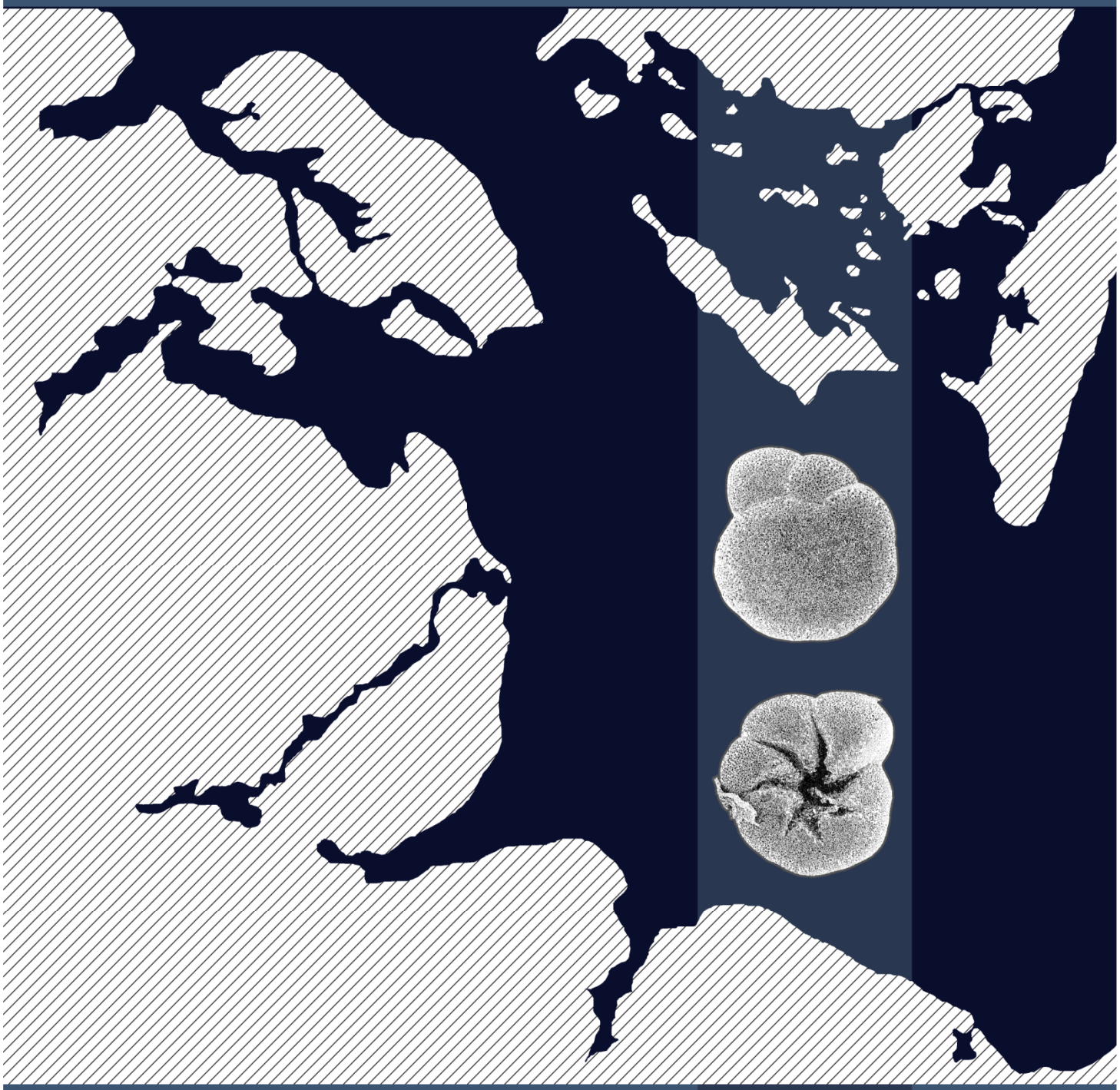

Dissertation

Impact of elevated pCO_2 on benthic foraminifera from the southwestern Baltic Sea

Auswirkungen erhöhter pCO_2-Werte auf benthische Foraminiferen aus der süd-westlichen Ostsee

Dissertation

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Kiel, den 30.04.2013

Kristin Haynert

SUMMARY

Increasing atmospheric CO_2 concentrations have a strong impact on the marine carbonate chemistry leading to a phenomenon called ocean acidification. Excess CO_2 dissolves in the surface water of the ocean, thereby the seawater pCO_2 increases, whereas the [CO_3^{2-}] and pH decrease. Reduced CO_3^{2-} concentrations may affect marine, especially calcifying, organisms such as benthic foraminifera, in that their ability to form calcareous tests might be affected. In comparison to open oceans, water pCO_2 levels are often not in equilibrium with the atmosphere in coastal regions, which are characterized by high CO_2 variability during the seasonal cycle. This has also been observed for the southwestern Baltic, an eutrophic marginal sea, where bacterial degradation of large amounts of organic matter cause O_2 depletion and CO_2 enrichment in the bottom water.

In the frame of this thesis, the impact of elevated pCO_2, temperature and salinity changes on the survival and calcification ability of the benthic foraminiferal species *Ammonia aomoriensis* was investigated in mid-term and long-term laboratory experiments. Under laboratory conditions, foraminifera were either isolated from the sediment or remained in their natural microhabitat. Further, the natural carbonate system variability and its impact on foraminiferal communities were monitored in a one-year field study.

Specimens of *Ammonia aomoriensis* were isolated from their natural sediment. They exhibited reduced survival and growth rates with increasing pCO_2 of up to 3130 µatm under laboratory conditions. At pCO_2 levels above 1800 µatm, dissolution caused a decrease of test diameter, and at the highest pCO_2, only the inner organic lining remained. Testing the combined effects of ocean acidification, temperature and salinity on living *Ammonia aomoriensis*, a significant reduction of test diameter was observed at a pCO_2 >1200 µatm (Ω_{calc}<1). Tests were mainly affected by undersaturation of calcite. This effect was partly compensated by a temperature rise, which increased Ω_{calc} and led to lower test degradation. In contrast, salinity did not have a significant effect on test growth. These results revealed that *Ammonia ammoriensis* exhibited a high sensitivity to elevated pCO_2 and accompanying calcium carbonate undersaturation when the specimens were kept without their protective sedimentary habitat.

During the field survey, large seasonal fluctuations of pCO_2 from 465 up to 3429 µatm were encountered in the bottom water of Flensburg Fjord in the southwestern Baltic Sea. The pCO_2 in the sediment pore water reached even higher values ranging from 1244 to 3324 µatm. However, and as a consequence of higher alkalinity (A_T), the calcium carbonate saturation state of the sediment pore water remained slightly supersaturated with respect to calcite for most of the year. Accordingly, during the monitoring period, no dynamic responses of foraminiferal population density and diversity to elevated sediment pore water pCO_2 were recognized. Benthic foraminifera may indeed cope with a high sediment pore water pCO_2 as long as the sediment pore water remains calcite supersaturated. This evidence from the field study was also supported by the results of a long-term laboratory experiment, in which a complete foraminiferal fauna in their natural sediment was exposed to elevated pCO_2 levels. Similar to field observations, the sediment pore water exhibited higher alkalinity and consequently higher saturation state of Ω_{calc} in comparison to the overlying seawater. Thereby the sediment chemistry created a microhabitat, which sustained

the growth and development of the foraminiferal community even at highly elevated pCO_2. The dominant species *Ammonia aomoriensis* exhibited growth and several reproduction events during the incubation time. Nevertheless, dissolution was observed on dead, empty tests of *Ammonia aomoriensis*, whereas tests of the second-ranked species *Elphidium incertum* stayed intact at high pCO_2 and $\Omega_{calc}<1$. This species-specific response could be due to differences in elemental composition and ultrastructure of the test walls.

Benthic foraminifera in their natural, sedimentary habitat tolerate elevated pCO_2 under laboratory conditions and the current high sedimentary pore water pCO_2, which prevails in the southwestern Baltic Sea. In this environment, organic-rich mud influences the carbonate chemistry, and thereby provides a suitable habitat for benthic foraminifera. Consequently, the calcifying *Ammonia aomoriensis* plays an important role in benthic carbonate production and accumulation in this area. These results emphasize the importance of understanding the carbonate chemistry in the natural environment of benthic foraminifera, which depends upon sediment composition and remineralization processes.

It is expected that enhanced future CO_2 uptake in the water column will cause a further rise of sedimentary pore water pCO_2 levels. As a consequence, undersaturation with respect to calcite will occur more frequently even in the sediment. This will most probably affect the dominant species *Ammonia aomoriensis*, which might induce changes in the benthic foraminiferal communities and their carbonate production in the southwestern Baltic Sea.

ZUSAMENFASSUNG

Der Anstieg der atmosphärischen CO_2-Konzentration hat einen starken Einfluss auf das Karbonatsystem der Ozeane und führt zur sogenannten Ozeanversauerung. Dabei führt die CO_2-Aufnahme des Oberflächenwassers zu steigenden pCO_2 Werten und gleichzeitig zu einer Abnahme der [CO_3^{2-}] und des pH-Wertes im Wasser. Die verringerte Konzentration an CO_3^{2-}-Ionen kann negative Auswirkungen auf die marine Fauna haben, wobei insbesondere kalzifizierende Organismen, wie benthische Foraminiferen bei der Schalenbildung beeinträchtigt werden könnten. Allerdings sind, im Gegensatz zum offenen Ozean, die CO_2-Konzentrationen in vielen küstennahen Meeresregionen, mitunter nicht im Gleichgewicht mit der Atmosphäre. Diese Gebiete sind durch saisonale CO_2-Schwankungen und teilweise sehr hohe pCO_2 Werte im Meerwasser charakterisiert. Dies trifft auch für das Untersuchungsgebiet der süd-westlichen Ostsee zu, wobei es sich hier um ein eutrophes Randmeer mit hohem bakteriellen Abbau von organischem Material handelt, welches zu einem starken O_2-Verbrauch und CO_2-Anreicherung im Bodenwasser führt.

Im Rahmen dieser Arbeit wurden die Effekte erhöhter pCO_2-Werte, Temperatur- und Salinitätsschwankungen auf das Überleben und die Kalzifizierung der benthischen Foraminifere *Ammonia aomoriensis* in Experimenten mit mittlerer und langer Inkubationszeit untersucht. In diesen wurden die Foraminiferen entweder aus dem Sediment isoliert oder in ihrem gewohnten Mikrohabitat gehältert. Weiterhin wurden die natürlichen Karbonatsystemvariabilitäten und deren Auswirkungen auf die Foraminiferen-Gemeinschaft in einer einjährigen Feldstudie untersucht.

Im Experiment zeigte *Ammonia aomoriensis*, wenn sie aus dem natürlichen Sediment isoliert wurde, eine deutliche Abnahme der Wachstum- und Überlebensraten mit steigendem pCO_2 bis 3130 µatm. Ab einem pCO_2 von mehr als 1800 µatm und gleichzeitiger Untersättigung von Kalzit führte die Auflösung der Schale zu einer deutlichen Abnahme des Gehäusedurchmessers. Unter sehr hohem pCO_2 blieb nur die innere organische Membran der Gehäuse erhalten. Gleiche Ergebnisse wurden in einer weiteren Studie erzielt, in welcher die kombinierten Effekte von Ozeanversauerung, Temperatur- und Salinitätsschwankungen auf die gleiche Art untersucht wurden. Diese zeigt wiederum eine deutliche Reduktion des Gehäusedurchmessers bei einem pCO_2 >1200 µatm (Ω_{Kalzit} <1). Gleichzeitig führte eine erhöhte Temperatur zu einer Zunahme des Ω_{Kalzit}, welches die Schalenauflösung entsprechend verringerte. Demgegenüber hatte die Salinität keinen nachweisbaren Einfluss auf das Wachstum. Diese Ergebnisse verdeutlichen, dass wenn die Individuen aus ihrem Sedimenthabitat isoliert wurden, *Ammonia ammoriensis* eine hohe Sensitivität gegenüber erhöhten pCO_2-Werten zeigt, insbesondere bei starker Untersättigung von Kalzit.

Während der Feldstudie wurden hohe saisonale pCO_2-Schwankungen von 465 bis 3429 µatm im Bodenwasser der süd-westlichen Ostsee in der Flensburger Förde beobachtet. Der pCO_2 im Sedimentporenwasser war im Mittel noch höher und variierte zwischen 1244 und 3324 µatm. Jedoch führten die hohen Alkalinitäten dazu, dass das Sedimentporenwasser für die meiste Zeit im Jahr leicht an Kalzit übersättigt war. Demzufolge waren die Auswirkungen des erhöhten pCO_2 im Sedimentporenwasser auf die Populationsdichten und Diversität der Foraminiferen-Gemeinschaft gering. Diese Erkenntnisse machen deutlich, dass benthische

Foraminiferen mit einem erhöhten pCO_2 im Sedimenporenwasser gut zurechtkommen, solange $CaCO_3$-Übersättigung besteht. Die Ergebnisse der Freilandstudie wurden durch ein weiterführendes Langzeit-Experiment bestätigt. In diesem wurde die Foraminiferen-Gemeinschaft in ihrem natürlichen Sediment erhöhten pCO_2-Bedingungen ausgesetzt. Wie bereits in der Feldstudie beobachtet, wies das Porenwasser des Sedimentes höhere Alkalinitäten und dementsprechend höhere Ω_{Kalzit}-Werte im Vergleich zum darüber liegenden Bodenwasser auf. Hierdurch schaffte die Porenwasserchemie ein Mikrohabitat, welches das Wachstum und die Entwicklung der benthischen Foraminiferen-Gemeinschaft, auch bei stark erhöhtem pCO_2 fördert. So zeigte die dominante Art *Ammonia aomoriensis* im Verlauf der Inkubationszeit ein ausgeprägtes Wachstum und mehrere Reproduktionsereignisse. Lediglich bei sehr hohem pCO_2, beziehungsweise Ω_{Kalzit} <1 wurde Schalenlösung an leeren Gehäusen beobachtet. Gehäuse von *Elphidium incertum* blieben jedoch unbeeinflusst. Diese art-spezifische Reaktion könnte in einem unterschiedlichen Elementaufbau und einer verschiedenen Ultrastruktur der Wandung begründet sein.

In ihrem natürlichen Habitat tolerierten benthische Foraminiferen erhöhte pCO_2-Werte, sowohl unter simulierten Laborbedingungen, als auch unter erhöhten Sedimentporenwasser pCO_2 in der süd-westlichen Ostsee. In dieser Umgebung beeinflusst das organikreiche Bodensediment die Karbonatchemie und schafft dabei ein geeignetes Habitat für benthische Foraminiferen. Unter diesen Bedingungen nimmt die kalzifizierende *Ammonia aomoriensis* eine bedeutende Rolle in der Karbonatproduktion ein. Die Ergebnisse unterstreichen die Notwendigkeit des Verständnisses der Karbonatchemie in der natürlichen Umgebung der Foraminiferen, und welche Sedimentzusammensetzung und Remineralisierungsprozesse ihr zugrunde liegen.

Bedingt durch die zukünftig steigende CO_2-Aufnahme der Ozeane werden die pCO_2-Werte im Sedimentporenwasser weiterhin ansteigen. Als Folge davon könnte die Kalzit-Untersättigung im Sedimentporenwasser zunehmen, welche zu einer drastischen Verschlechterung der Lebensbedingungen für die derzeit dominante Art *Ammonia aomoriensis* führen dürfte. Dies könnte wiederum zu tiefgreifenden Veränderungen innerhalb der benthischen Foraminiferen-Fauna in der südwestlichen Ostsee führen.

ABBREVATIONS

A_T	total alkalinity
Ca	calcium
$CaCO_3$	calcium carbonate
CI	confidence interval
CO_2	carbon dioxide
$CO_{2(aq)}$	aqueous carbon dioxide
CO_3^{2-}	carbonate
C_T	total dissolved inorganic carbon
CTD	conductivity temperature depth sensor package
d.f.	degrees of freedom
DIC	dissolved inorganic carbon
DIP	dissolved inorganic phosphate
EMP	electron micro-probe
FF	Flensburg Fjord
h	hour
H_2S	hydrogen sulfide
HCO_3^-	bicarbonate
Ind.	individual
KF	Kiel Fjord
Mg	magnesium
MS	mean squares
MUC	multiple corer
N or n	number of sample units (replicates)
N_2	nitrogen
O_2	oxygen
Ω_{calc}	saturation state of calcite
pCO_2	carbon dioxide partial pressure
pH_{NBS}	pH on the NBS (National Bureau of Standards) scale
PO_4^{3-}	phosphate
psu	practical salinity unit
rDNA	recombinant deoxyribonucleic acid
R^2	correlation coefficient
S	salinity
SD	standard deviation
SE	standard error
SEM	scanning electronic microscope
SiO_2	silicate
SS	sum of squares
T	temperature
UV	ultraviolet

I. Introduction

I.1 Motivation

Benthic foraminifera are the most diverse group of hard-shelled protozoa in marine environments. Owing to their short life cycle and their high abundances, they are able to respond quickly to environmental changes and are a valuable tool for climate change research. Their tests are readily preserved in the sediment and provide a record of environmental variability through time. For this reason, benthic foraminifera are of high importance in paleooceanographic reconstructions.

First observations of benthic foraminifera from the western Baltic Sea were documented by Rhumbler (1935). The impact of human activities (e.g. eutrophication and heavy metal emissions) and environmental variability (e.g. temperature, salinity, oxygen, and food supply) on benthic foraminifera were reported in previous studies from the Baltic Sea. These investigations focused on taxonomy and ecology (van Voorthuysen, 1960, Lutze, 1965, Haake, 1967), distribution pattern (Rottgardt 1952, Brodniewicz, 1965, Lutze, 1974, Hermelin, 1987), carbonate production and preservation (Wefer and Lutze, 1978), microhabitat preferences (Linke and Lutze, 1993), faunal dynamics (Lutze, 1968a, b, Wefer, 1976, Frenzel et al., 2009), test morphology (Grobe and Fütterer, 1981, Polovodova and Schönfeld, 2008), and faunal composition (Schönfeld and Numberger, 2007a, b, Nikulina et al., 2008, Polovodova et al., 2009).

Several attempts were made to culture living specimens in order to study the environmental tolerances, life cycle and reproduction of marginal marine benthic foraminifera (Bradshaw, 1957, 1961). These studies did not take the impact of seawater carbonate chemistry into account as possible important factor which regulates the foraminiferal diversity and abundance.

Today, one of the most important human impacts is the massive release of carbon dioxide from fossil fuel combustion which causes an acidification of the oceans (Zeebe and Wolf-Gladrow, 2001, Feely et al., 2004). In response to ocean acidification, the calcium carbonate saturation state for calcite will be lowered (Feely et al., 2004, Caldeira and Wickett, 2005). The reduced saturation state and carbonate ion concentrations will make it more difficult for calcifying benthic foraminifera to build their tests (Bradshaw, 1968, Erez, 2003). Nevertheless, calcareous benthic foraminifera are common in the southwestern Baltic Sea, where permanently low seawater carbonate concentrations and seasonal undersaturation prevail (Thomsen et al., 2010). Some tests of the dominant species *Ammonia beccarii* (*Ammonia aomoriensis* after Schweizer et al., 2010) exhibited dissolution features in Flensburg Fjord (Polovodova and Schönfeld, 2008).

Natural CO_2-rich habitats, such as the southwestern Baltic Sea, represent a suitable area to investigate the dynamic response of the foraminiferal fauna to elevated pCO_2. The results can serve as a valuable example for possible effects on calcifying benthic foraminifera and their faunal associations due to climate change.

I.2 Ocean acidification

The oceans cover approximately 70 % of the earth's surface and play an important role in global climate cycles. Ocean surfaces are in permanent exchange with the atmosphere and equilibrate with atmospheric carbon dioxide (CO_2), which has a strong impact on the carbonate chemistry.

The combustion of fossil fuels, deforestation and wetland drainage released about 300 Gt carbon and has led to an increase of atmospheric carbon dioxide (CO_2) from 285 ppm since the beginning of the Industrial Revolution to currently 397 ppm (Fig. I.3, A, Petit et al., 1999, Archer, 2005, Lüthi et al., 2008, http://co2now.org, data from Mauna Loa Observatory: NOAA-ESRL). Similarly the CO_2 partial pressure (pCO_2) increased in the oceans. About 50 % of the anthropogenic CO_2 release has already been taken up by the world oceans (Sabine et al., 2004). The hydration of the atmospheric CO_2 molecule with the water molecule H_2O forms carbonic acid (H_2CO_3) (Fig. I.1). Carbonic acid dissociates into bicarbonate (HCO_3^-) and releases a H^+ proton. This proton may react further with the seawater carbonate (CO_3^{2-}) and thereby forms bicarbonate (Fig. I.1, equation 1). The conversion can be described by two dissociation constants K_1 and K_2. The generation of protons during the dissociation steps increases seawater acidity and causes a decrease of the oceanic pH (Cao and Caldeira, 2008, Orr et al., 2009). The current global mean pH is 8.1, which is 0.1 pH units below the pre-industrial value of the open surface ocean (Sabine et al., 2004).

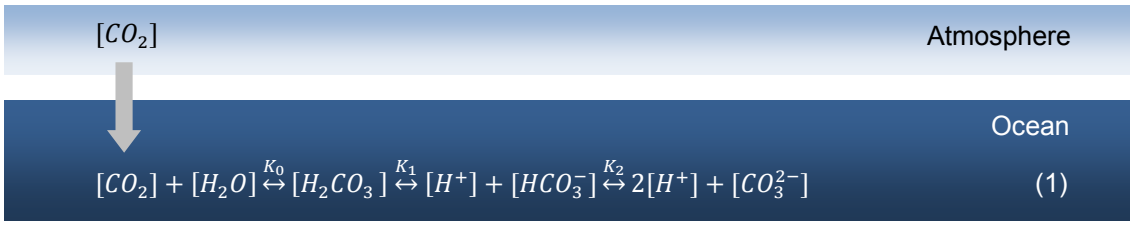

Fig. I.1: CO_2 dissociation in seawater.

The pH describes the concentration of protons and thus the acidity of seawater (equation 2). It can be measured on different scales: National Bureau of Standards (pH_{NBS}), seawater (pH_{SWS}), free (pH_F) and total (pH_T). Values on pH_{NBS} are about 0.15 units higher, while values on the SWS scale are about 0.01 units lower than values on pH_T.

$$pH = -log\ 10[H^+] \quad (2)$$

The decline in oceanic pH affects the distribution of all three carbonate system species: CO_2, HCO_3^- and CO_3^{2-}, which are in equilibrium with each other (Fig. I.2). All three components are the sum of dissolved inorganic carbon (C_T, equation 3). At current pCO_2 levels of approximately 380 µatm, $[CO_2]$ contributes about 1 %, $[HCO_3^-]$ 91 % and $[CO_3^{2-}]$ 8 % to C_T.

$$C_T = [CO_2] + [HCO_3^-] + [CO_3^{2-}] \quad (3)$$

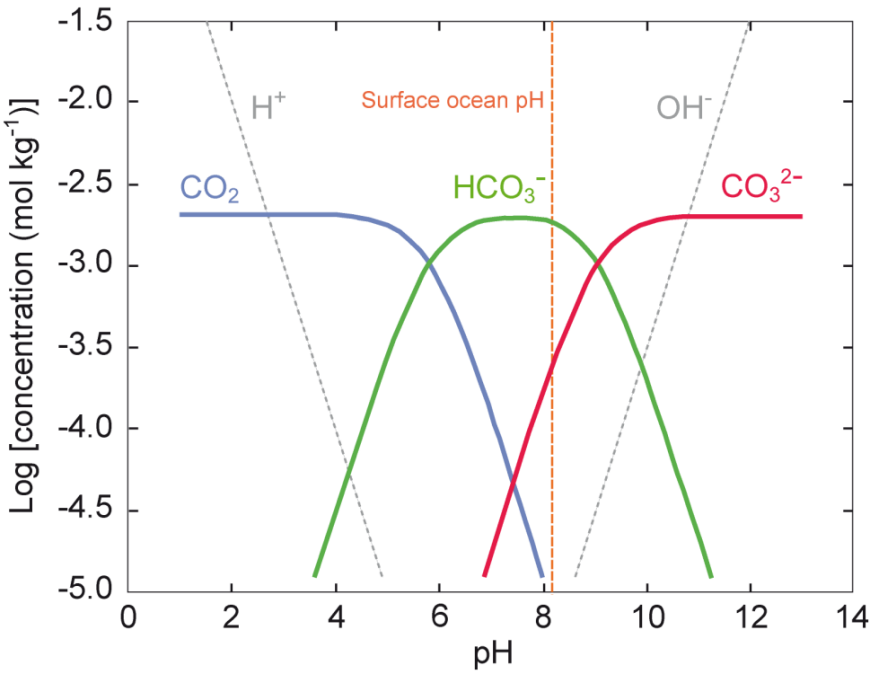

Fig. I.2: Bjerrum plot depicts the pH dependency of concentrations of the three carbonate system species CO_2 (blue line), HCO_3^- (green line) and CO_3^{2-} (red line). The orange dashed line corresponds to present day mean ocean surface pH (modified after Zeebe and Wolf-Gladrow, 2001).

The seawater pH is buffered by the total alkalinity (A_T), which is the sum of weak bases and balances the charge differences between anions and cations (= $[HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_3^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH_3] + [HS^-] + \ldots - [H^+] – [HSO_4^-] – [HF] – [H_3PO_4] \ldots$) (Dickson et al. 1981). The major component of this buffering system is the carbonate alkalinity (A_C), which is composed of $[HCO_3^-]$ and $[CO_3^{2-}]$ (equation 4).

$$A_C = [HCO_3^-] + 2[\,CO_3^{2-}] \quad (4)$$

Changes in the carbonate system species influence the calcium carbonate saturation state Ω, which is the product of $[Ca^{2+}]$ and $[CO_3^{2-}]$ divided by a stoichiometric solubility coefficient (K_{sp}), which is specific for each of the two major carbonate polymorphs, calcite or aragonite (equation 5) (Feely et al., 2004). At current CO_2 concentrations, oceans are supersaturated with respect to both polymorphs (Fig. I.3, D). The carbonate polymorph calcite is more stable and ocean saturation state is higher compared to aragonite (Cao and Caldeira, 2008).

$$\Omega = \frac{[Ca^{2+}] \times [CO_3^{2-}]}{K_{sp}} \quad (5)$$

The International Panel on Climate Change (IPCC) developed future scenarios for the amount of CO_2 emitted by human activities depending on social and technological development. Until the year 2100, the atmospheric CO_2 will increase to concentrations from 650 ppm up to 970 ppm (Fig. I.3, A, IPCC, 2007). This increase of CO_2 concentrations will in turn result in a pH decrease of 0.46 units by 2100 (Fig. I.3, A, Caldeira and Wickett 2005). In a future, more acidified ocean, the amount of $[CO_3^{2-}]$ will strongly decline, whereas $[CO_2]$ and $[HCO_3^-]$ will increase (Fig. I.3, B and C).

The shift in carbonate chemistry resulting in lowered [CO_3^{2-}] will decrease the calcium carbonate saturation state Ω (Fig. I. 3, D). The $CaCO_3$ saturation state also depends on temperature. Therefore, polar regions are characterized by lower saturation states and will be affected first by seawater undersaturation (Cao and Caldeira, 2008, Yamamoto-Kawai et al., 2009).

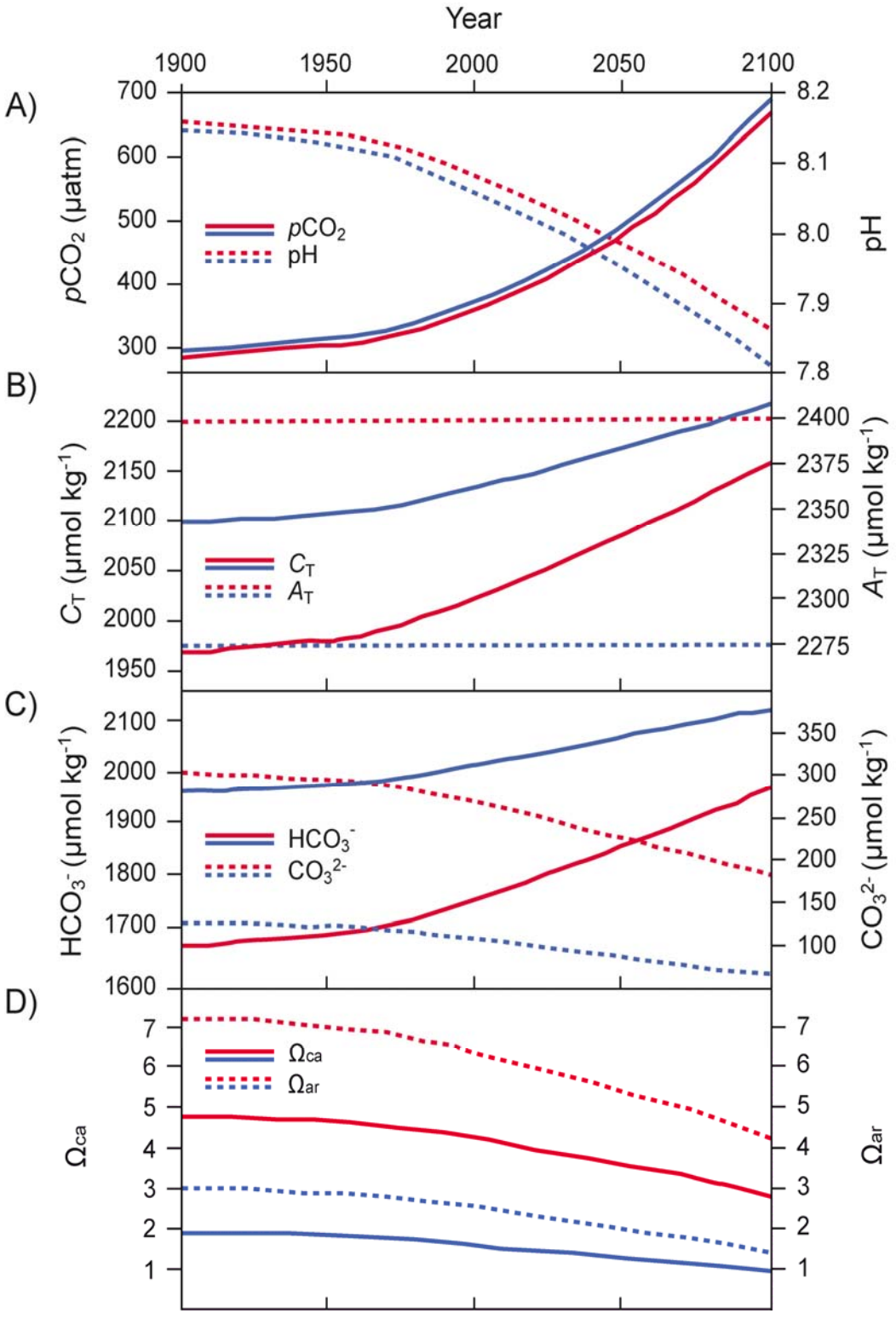

Fig. I.3: Development of the carbonate system parameters in warm (red lines) and cold (blue lines) waters of surface oceans from 1900 at the beginning of Industrial Revolution until future simulated scenarios in the year 2100 according to Riebesell et al. (2009), modified after Körtzinger (2010).

I.3 Carbonate chemistry in the southwestern Baltic Sea

The Baltic Sea is a semi-enclosed marginal sea with a surface area of 415.000 km^2 (HELCOM, 2003). The shallow water habitat has a mean depth of 60 m, with three exceptionally deep basins: Åland (290 m), Landsort (459 m) and Gotland Deep (239 m). Water exchange with the North Sea takes place through the Danish Straits (She et al., 2006). Through the high fresh water input from the surrounding hinterland and net precipitation, the surface water salinity declines from the west (Kattegat) to the north-east (Gulf of Finland and Gulf of Bothnia) from 20 to 2 (Rohde, 1998). The salinity difference promotes the formation of a halocline separating the low salinity surface water from high salinity bottom water. The salinity in the southwestern Baltic Sea, ranges between 10 and 20, with mean values of 15 in the surface and 18 in the bottom water.

The carbonate chemistry in coastal waters, such as Kiel and Flensburg Fjord, differs from the open ocean (Borges et al., 2006, Hofmann et al., 2011). In contrast to the relatively stable open ocean conditions, the near coastal pH is highly variable as a consequence of biogeochemical processes (Fig. I.4, Prytherch, 1929, Hinga, 2002, Feely et al., 2008, 2010, Provoost et al., 2010, Thomsen et al., 2010). Seasonal stratification and high productivity, enhanced by eutrophication, and the following degradation of organic matter effects a seasonal depletion of oxygen concentrations to 100 µmol kg^{-1} or even less in the bottom water (Babenerd, 1991, Hansen et al., 1999, HELCOM 2003, Conley et al., 2007). Due to the release of metabolic CO_2, which is coupled to the consumption of dissolved oxygen, hypoxic water masses are always characterized by elevated CO_2 concentrations. The study of Thomsen et al. (2010) showed strong seasonal fluctuations of surface pCO_2, and respectively pH, in Kiel Fjord (Fig. I.4). A pronounced stratification and occasional upwelling of CO_2-rich bottom waters cause pCO_2 values up to 2300 µatm. Thus, areas which are naturally acidified at a large scale could be a more suitable option to examine possible future community changes in an acidified ocean (Thomsen et al., 2010).

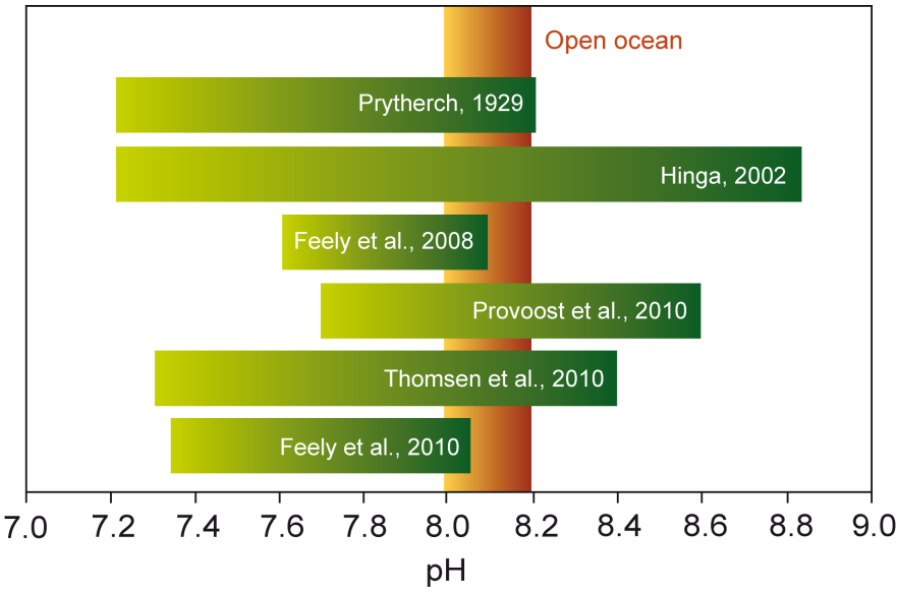

Fig. I.4: Previous field studies of natural pH fluctuations reported from coastal habitats worldwide (green horizontal areas). Vertical orange area represents open ocean pH fluctuations from Bermuda Atlantic and Hawaii Ocean Time Series (Bates and Peters, 2007, Dore et al., 2009), modified after Pansch (2012).

Furthermore, seawater alkalinity is related to salinity. Therefore, a reduced salinity in the southwestern Baltic Sea causes a relatively low alkalinity (1900-2100 µmol kg^{-1}). Consequently, the buffer capacity is reduced compared to the open oceans (2300-2400 µmol kg^{-1}, Beldowski et al., 2010). The input of reactive nitrogen and sulphur from fossil fuel combustion and agriculture and of acidic riverine water (Salisbury et al., 2008) may also reduce the coastal water alkalinity. Due to synergistic effects of acidification caused by coastal hypoxia and future atmospheric CO_2 increase, the surface water pCO_2 may seasonally reach pCO_2 values up to 4000 µatm (Melzner et al., 2012). These high values may prevail during even longer periods in the benthic environments. Therefore, the impact of ocean acidification may have stronger effects on benthic organisms of the Baltic Sea as compared to pelagic organisms of the open oceans.

I.4 Impacts of ocean acidification on marine organisms

It is expected that ocean acidification affects marine organisms and especially biogenic calcification (Gattuso and Hansson, 2011). Coccolithophorids (Riebesell et al., 2000) and corals (Gattuso et al., 1998, Langdon et al., 2000, Fine and Tschernov, 2007, Hoegh-Guldberg et al., 2007, Doney et al., 2009) exhibited reduced calcification rates under an increase of pCO_2. The same results were reported for other marine taxa such as echinoderms (Shirayama and Thornton, 2005), pteropods (Comeau et al., 2009, Lischka et al., 2011), bivalves (Ries et al., 2009, Thomsen and Melzner, 2010) and coralline red algae (Büdenbender et al., 2011).

Some studies demonstrated that the sensitivity differs between different life stages, whereby larval stages are more affected by elevated pCO_2 than juvenile and adult stages (Kurihara et al., 2008a). In most species, larval stages showed deformation and reduced survival, growth and calcification rates (Kurihara et al., 2007, 2008a,b, Gazeau et al., 2010, 2011, Miller et al., 2010, Talmage and Gobler et al., 2010, Hu et al., 2011, Stumpp et al., 2011). On the other hand, an increase of calcification has been observed for a sea star larva (Dupont et al., 2010). Even within the same oyster species, populations may exhibit different sensitivities (Parker et al., 2010, 2011).

However, most of these studies were performed on short time scales. Long-term acclimation of the cold-water coral *Lophelia pertusa* (Form and Riebesell, 2012) revealed a higher tolerance to acidification. Furthermore, the exposure of the coccolithophore *Emiliania huxley* over multiple generations caused a partial recovery of calcification rates and documented a high adaptation potential to ocean acidification (Lohbeck et al., 2012).

Most of the ocean acidification studies were performed under laboratory conditions and only for short time periods. Consequently the explanatory power of these experiments is limited. Naturally CO_2-rich habitats such as vents and coastal upwelling zones provide the possibility to overcome these limitations. Volcanic CO_2 vents cause a natural, local decline of pH (Tunnicliffe et al., 2009) and affect the benthic communities. They exhibited a decrease in abundance and species richness at low pH (Hall-Spencer et al., 2008, Cigliano et al., 2010, Dias et al., 2010, Fabricius et al., 2011). Similarly, coastal upwelling of CO_2 rich waters causes a

decrease of calcifying benthic communities under reduced pH, whereas non calcifying photosynthetic organisms such as seagrass and algae increased in abundance and growth (Feely et al., 2008, Hall-Spencer et al., 2008, Wootton et al., 2008, Fabricius et al., 2011). In contrast, Thomsen et al. (2010) reported a dominance of calcifying invertebrates such as the blue mussel *Mytilus edulis* in a seasonally pCO_2 enriched habitat with pCO_2 of approximately 1000 µatm. Consequently, naturally acidified habitats can serve as valuable examples to study future ocean acidification effects or non-effects on marine organisms. Previous results indicate that the response of elevated pCO_2 seem to be species- and life stage-specific. Acclimation and adaptation are also important issues to be considered for future predictions.

I.5 Benthic foraminifera

Foraminifera are amoeboid protists. To date, 2185 extant foraminiferal species were recorded (Murray, 2007). Only 45 of these species are planktonic, whereas the rest pursue a benthic lifestyle. They are covered by a test which consists either of calcite (rotaliids and milioliids), agglutinated sediment particles (textulariids) or organic material (allogromiids). On the basis of test morphology, 15 extant foraminiferal orders are recognized of which 7 are calcitic (Hansen, 1999, Sen Gupta, 2003). A dynamic and anastomosing net of fine strands of cytoplasm form the pseudopodia around the test. Reproduction in benthic foraminifera typically produces five to twenty, sometimes hundreds, of small offspring via either sexual or asexual reproduction (Lister, 1895, Myers, 1935a,b, 1936, Bradshaw, 1968, Goldstein, 1999).

Benthic foraminifera are highly abundant and diverse in all marine environments. They are able to quickly colonize new habitats and to respond to changing environmental conditions. Either they could live epifaunally on the sediment surface or elevated substrates, or infaunally inside the sediments (Lutze and Thiel, 1989, Corliss, 1985, Schönfeld, 2002). Some species are adapted to extreme environmental conditions, from salt marsh meadows (Hayward et al., 2011) up to deep sea trenches (Gooday et al. 2008, Alve et al., 2011).

Calcifying benthic and planktic foraminifera are one of the most important groups of calcite producers. They precipitate 1.4 billion tons of calcite per year, which accounts for 25 % of the total global calcite production (Langer, 2008). Calculations from data presented by Wefer and Lutze (1978) and Langer et al. (1997) revealed a benthic foraminiferal carbonate production of 0.04 Gt yr^{-1} in coral reefs and shallow water carbonate environments, and 0.03 Gt yr^{-1} on noncarbonated shelves. In total, we estimate a neritic foraminiferal carbonate production of 0.1 Gt yr^{-1}.

An increasing number of studies investigated the effects of elevated pCO_2 on benthic foraminifera (Table I.1). Most experiments were performed on single specimens under simulated elevated pCO_2 conditions. Determined parameters were growth or calcification, shell weight, size, thickness, fitness and survival of the individuals. The response was species-specific and varied from no pCO_2-effect (Hikami et al., 2011, Vogel and Uthicke, 2012, McIntyre-Wressnig, 2013) to a decrease (Le Cadre et al., 2003, Russell et al., 2004, Kuroyanagi et al., 2009, Allison et al., 2010, Dissard et al., 2010, Fujita et al., 2011, Hikami et al., 2011, Sinutok et al., 2011,

Uthicke and Fabricius, 2012) or even increased calcification under elevated pCO_2 (Fujita et al., 2011, Hikami et al., 2011, Vogel and Uthicke, 2012). Only two studies described community shifts of benthic foraminiferal assemblages in relation to different pH gradients (Cigliano et al., 2010, Dias et al., 2010).

Table I.1: Reference overview of ocean acidification studies and the response of elevated pCO_2/low pH on benthic foraminifera.

Reference	Species	Response parameter	Response to elevated pCO_2/low pH
Le Cadre et al., 2003	*Ammonia beccarii*	calcification	decrease
Russell et al., 2004	*Marginopora kudakajimensis*	shell weight	decrease
Kuroyanagi et al., 2009	*Marginopora kudakajimensis*	size, shell weight, growth	decrease
Allison et al., 2010	*Elphidium williamsoni*	shell thickness	decrease
Cigliano et al., 2010	foraminiferal assemblage	community shift	changes
Dias et al., 2010	foraminiferal assemblage	community shift	changes
Dissard et al., 2010	*Ammonia tepida*	weight	decrease
Fujita et al., 2011	*Baculogypsina sphaerulata*	shell weight	initial increase followed by decrease
	Calcarina gaudichaudii,	shell weight	initial increase followed by decrease
	Amphisorus hemprichii	shell weight	decrease
Hikami et al., 2011	*Calcarina gaudichaudii*	calcification	increase
	Amphisorus kudakajimensis	calcification	decrease
	Amphisorus hemprichii	calcification	no effect
Sinutok et al., 2011	*Marginopora vertebralis*	calcification	decrease
Uthicke and Fabricius, 2012	*Marginopora vertebralis*	calcification	decrease
Vogel and Uthicke, 2012	*Amphistegina radiata*	growth	no effect
	Heterostegina depressa	growth	no effect
	Marginopora vertebralis	growth	increase
McIntyre-Wressnig, 2013	*Amphistegina gibbosa*	growth, fitness, survival	no effect

I.6 Calcification and chamber formation in foraminifera

Foraminifera grow by adding new chambers. They precipitate the calcium carbonate for their test formation by vacuolization of seawater (Erez, 2003). Consequently, the calcification and chamber formation depends on the carbonate chemistry of the ambient seawater. During calcification of a newly formed chamber, a layer of rhomboedric crystallites develops as the first component of the chamber wall (Angell, 1979). During successive chamber formation, the organisms cover the pre-existing shell with a new layer of calcite. Therefore, young chambers have thinner walls and a fewer number of calcite lamella in comparison to old chambers (Erez, 2003).

According to different calcification mechanisms and their test structure, calcifying foraminifera are divided into two groups: miliolid and hyaline species (Fig. I.5 and I.6, de Nooijer et al., 2009). Miliolids (Fig. I.5) precipitate calcite as 2-3 µm long needles within cytoplasmic vesicles (Berthold, 1976, Hemleben et al., 1986). These needles are densely packed in random orientation and give the test an opaque appearance which provides the name of this taxon: porcelaneous. Before a new chamber is formed, the needles accumulate in the cell. After a continuous transport of needles to the exterior, the needles are incorporated within an organic

matrix and form the outer layer of the test. The organic template allows a parquetry-like orientation of secondary calcite crystals (Haake, 1971).

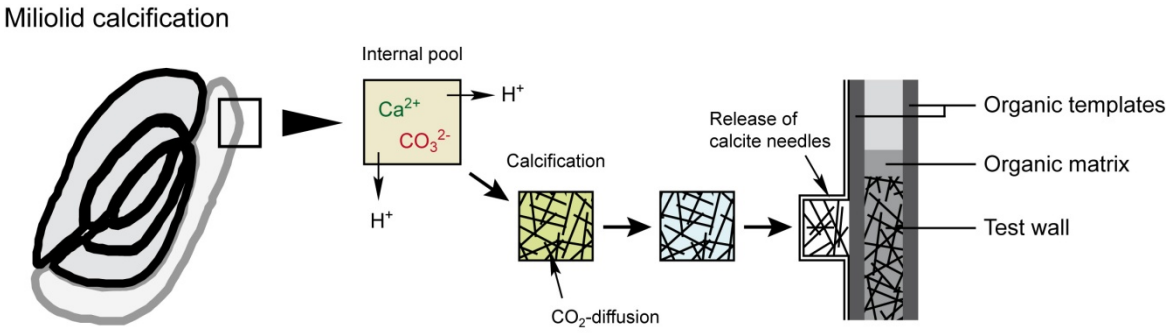

Fig. I.5: Model of miliolid calcification (modified after de Nooijer et al., 2009).

A similar calcification process is reported for some agglutinated foraminifera (Bender, 1992). These specimens form a protective cyst using detritus particles in which the chamber formation takes place. Particles are covered by an organic envelope and subsequently cemented. Finally, a biogenic calcite is deposited in voids between the grains which form a pore system of cylindrical cavities. After 24 hours, the chamber formation is completed (Bender and Hemleben, 1988, Bender, 1992).

Hyaline species include the Rotaliids, Buliminids and all planktonic species (Fig. I.6). They store the calcium carbonate used for calcification in separate internal pools (Anderson and Faber, 1884, ter Kuile and Erez, 1987, 1988, Erez, 2003, Toyofuku et al., 2008).

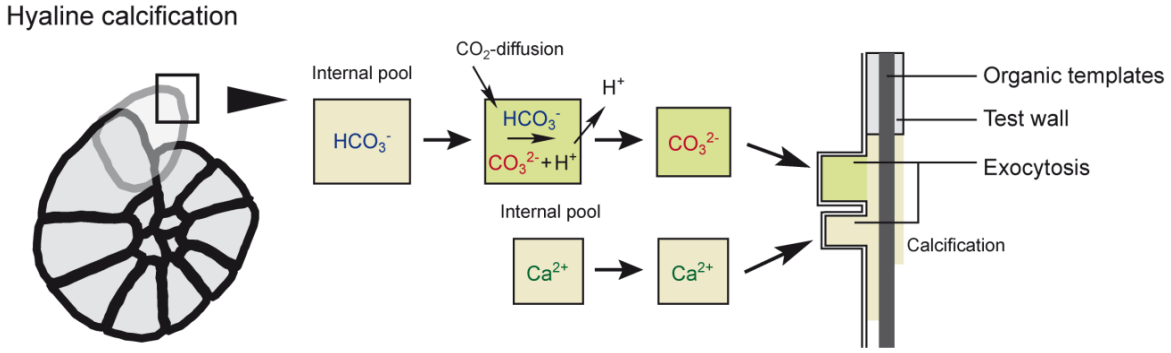

Fig. I.6: Model of hyaline calcification (modified after de Nooijer et al., 2009).

These pools are used for new chamber formation. A model proposed by Erez (2003) visualizes the vacuolization process in perforate foraminifera (Fig. I.7). Chamber formation starts with the production of a primary organic sheet (POS) in the shape of a new chamber (Hemleben et al., 1977, Angell, 1979, Erez, 2003). The primary organic sheet consists of cytoplasm, vacuole, pseudopod and optional symbionts. The sheet is exposed to the seawater (Fig. I.7, A).

Subsequently, seawater is taken up by the organism by formation of a vacuole (Fig. I.7, B). The self-vacuolization separates the test from the surrounding environment (Fig. I.7, C). The

formation of a new chamber takes place in the delimited space with a clear separation of intra- and extralocullar cytoplasm (Fig. I.7, D). Finally, the process of the secondary lamination involves a second vacuolization of seawater (Fig. I.7, E). Seawater CO_2 diffuses into the delimited space in the vacuols, across the membrane, and causes an internal storage of calcium carbonate (Bentov et al., 2009).

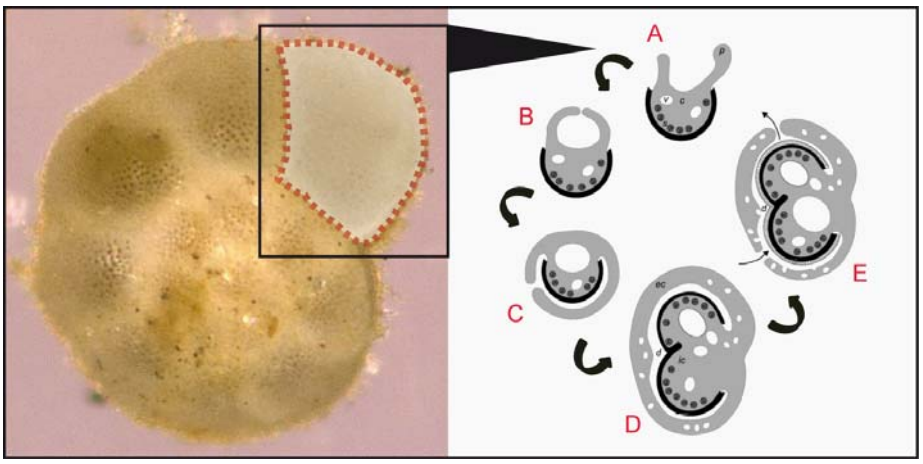

Fig. I.7: Schematic presentation of pseudopodia and vacuolization processes in perforate foraminifera (after Erez, 2003).

The seawater contains a Mg-content of approximately 50 mmol l^{-1}, i.e. a Mg/Ca ratio of 5.14 mmol mol^{-1} (Broecker and Peng, 1982). The shell carbonate of miliolid species contains relatively high Mg/Ca ratios from 100 to 150 mmol mol^{-1} (Toyofuku et al. 2000). But in general the foraminifera produce calcite with a low Mg content. Accordingly, most of the hyaline species have much lower Mg/Ca ratios between 1 and 20 mmol mol^{-1} (Raja et al., 2005, Toyofuku and Kitazato, 2005, Segev and Erez, 2006). Ratios below 5 mmol mol^{-1} indicate an active removal of Mg from the vacuolized seawater. This Mg-reduction takes place in the cytoplasm.

Foraminifera are able to elevate the pH at the site of calcification by approximately 1 unit above the current seawater pH of 8.1 (de Nooijer et al., 2009, Bentov et al., 2009). The elevation causes a shift in the carbonate chemistry from the predominant HCO_3^- (at a pH of 8.1) to [CO_3^{2-}], making up 90 % of the DIC at pH of 9.1 (Fig. I.2). The high pH increases the calcite saturation state inside the vesicles and thereby facilitates calcite precipitation (Lopez et al., 2009). Consequently, in an acidified ocean, the organisms may need more energy to produce the same amount of calcite, which in turn will reduce the gross calcite production by calcifying foraminifera (de Nooijer et al., 2009).

I.7 Research questions and outline

The aim of this study was to understand the sensitivity of benthic foraminifera to seawater acidification in their natural environment and its consequences. The conducted studies focused on the individual response of *Ammonia aomoriensis*, a dominant species in the Baltic Sea. Growth rates, mortality, test dissolution, survival, size class distribution, reproduction, test weight, test structure and elemental distribution, and carbonate production were investigated.

The following objectives and research questions (RQ) were addressed in publications and manuscripts, chapters I-IV.

RQ I: Is *Ammonia aomoriensis* able to maintain its calcification rates under ocean acidification?

RQ II: What carbonate chemistry conditions prevail in the natural habitat of benthic foraminifera in the southwestern Baltic Sea?

RQ III: Do seasonal variations in water chemistry influence the foraminiferal population dynamics and species composition?

RQ IV: Which carbonate chemistry parameter influences the calcification process in foraminifera?

RQ V: What are the long-term impacts of future ocean acidification on benthic foraminiferal assemblages in their natural environment?

RQ VI: How does *Ammonia aomoriensis* respond to combined effects of ocean acidification, temperature and salinity changes?

Outline of the publications and manuscripts:

Chapter I: Reports the individual response of growth, survival and test dissolution of the living benthic foraminifer *Ammonia aomoriensis* from Flensburg Fjord under different pCO_2 levels during a six weeks exposure period.

Chapter II: Documents the seasonal changes of carbonate chemistry and the ensuing response of foraminiferal population dynamics, variations of species composition, and diversity during a one year cycle with bi-monthly resolution in Flensburg Fjord. The main focus was on two calcifying species, *Ammonia aomoriensis* and *Elphidium incertum*.

Chapter III: We simulated the combined effects of different pCO_2, temperature and salinity levels on foraminiferal calcification in order to quantitatively assess the impact on test preservation, diameter and degradation of *Ammonia aomoriensis* during six weeks of incubation.

Chapter IV: This study represents the first long-term culturing experiment, which was performed with shallow-water foraminiferal assemblages in their natural sediment habitat from Kiel Fjord. The results revealed the impact of sediment pore water chemistry on the composition and population density of benthic foraminifera during a six month incubation. Size class distribution, reproduction, calcification rates, test structure and elemental distribution, and carbonate production were determined for the dominating species *Ammonia amoriensis*.

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II. Experimental design

Foraminiferal samples for the experiments and during the monitoring were taken with a Mini Corer, deployed from R/V *Littorina* or R/V *Polarfuchs* (Fig. II.1). The samples were collected in Flensburg Fjord (53°41´–55°00´N, 9°24´–10°10´E) and Kiel Fjord (54°19´–54°30´N, 10°06´–10°22´E), southwestern Baltic Sea (Fig. II.1). I sampled the 0-1 cm interval of surface sediments for both experimental and field studies.

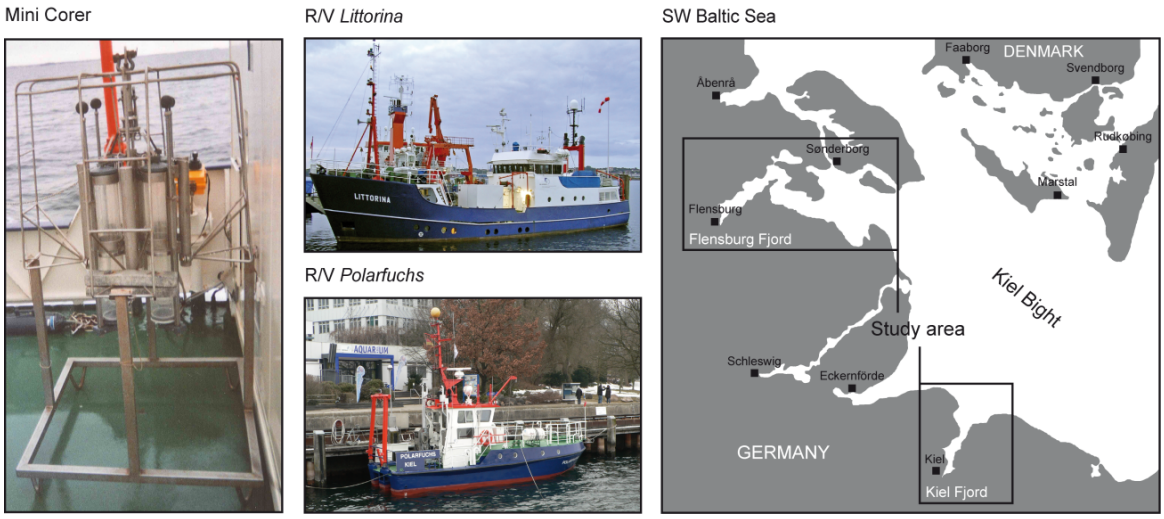

Fig. II.1: Locations of study areas (black framed): Flensburg Fjord and Kiel Fjord in the southwestern Baltic Sea. Mini Corer samples were taken with the R/V *Littorina* in Flensburg Fjord and R/V *Polarfuchs* in Kiel Fjord.

Four different approaches were chosen to study the effects of elevated pCO_2 on benthic foraminifera in the southwestern Baltic Sea. Three laboratory experiments (Fig. II.2) and one field study were performed:

(1) a 6-week **pilot culturing study** with *Ammonia aomoriensis* under 5 different pCO_2-levels.

(2) a 1-year **field study** with bi-monthly resolution to monitor the carbonate system variability and the response of benthic foraminiferal communities in their natural habitat at Flensburg Fjord.

(3) a 6-week **multiple stressors experiment** with *Ammonia aomoriensis*, which simulated the combined effects of 4 different pCO_2-levels and 3 different temperatures and salinities.

(4) a 6 month **long-term culturing experiment** using a foraminiferal assemblage in their natural sediment under 4 different pCO_2-levels.

The cultivation of the benthic foraminifera was performed in a flow-through system with filtered and UV-sterilized seawater (Fig. II.2). The assembly was slightly modified in the different experiments. In experiment **(1)** and **(3)**, a small amount of carbonate-free quartz sand was

dispersed in pits of the culture vessels. The sand provides shelter for the cultured species *Ammonia aomoriensis* similar to their natural habitat, without affecting the carbonate chemistry. In experiment **(4)**, benthic foraminifera were cultivated in their natural organic-rich sediment from Kiel Fjord. A detailed description of the experimental set up, material and methods for the individual experiments are described in the respective chapters.

	Pilot culturing study (1):	Multiple stressors experiment (3):	Long-term experiment (4):
Species:	*Ammonia aomoriensis*	*Ammonia aomoriensis*	foraminiferal assemblage study
Sampling location:	Flensburg Fjord	Kiel Fjord	Kiel Fjord
Cultivation sediment:	carbonate-free quartz sand	carbonate-free quartz sand	natural organic-rich mud
Duration:	6 weeks	6 weeks	6 month
pCO_2-level (µatm):	618, 751, 929, 1829, 3130	566, 1195, 2180, 3843	430, 907, 1865, 3247
Temperature (°C):	12	8, 13 18	17
Salinity:	18	15, 20, 25	16

Flow-through system:

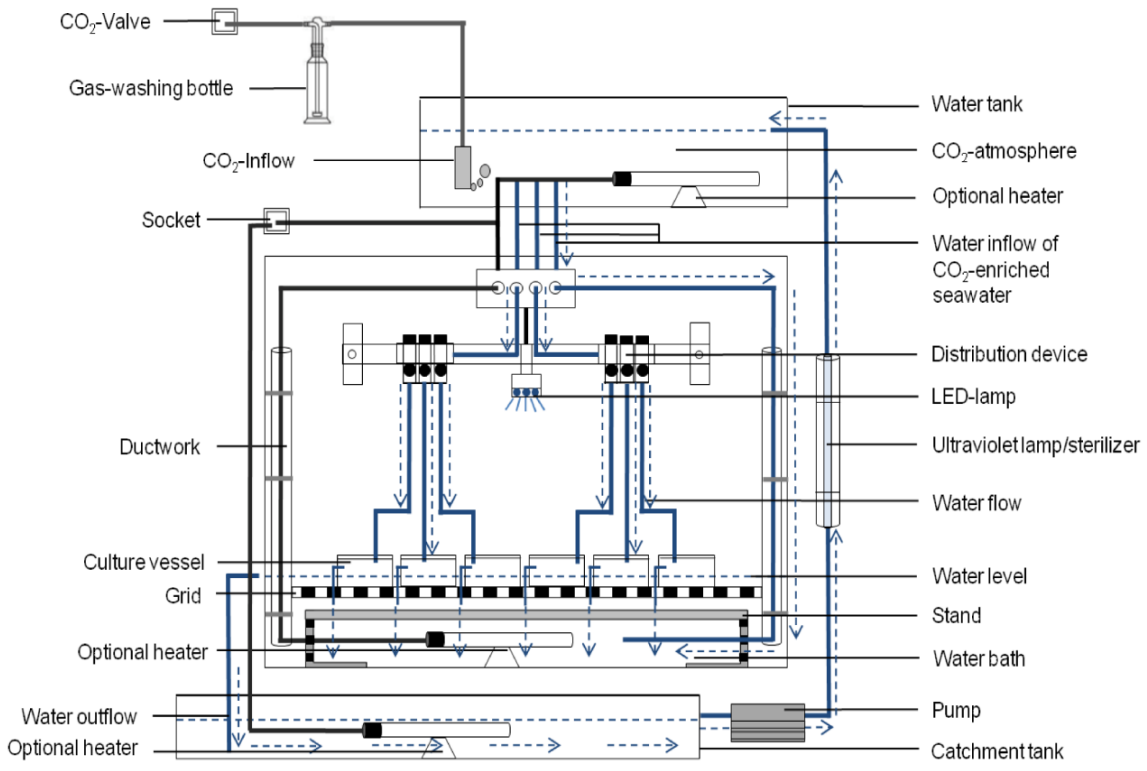

Fig. II.2: Principle design of the experimental flow-through system.

The experimental set up was implemented in temperature-constant climate rooms at GEOMAR (Fig. II.2). Water tanks, culture vessels, catchment tank and supplementary devices were placed on separate shelves of an iron storage rack (Fig. II.3, 1).

Gas-washing bottles were used to humidify the compressed air in order to avoid evaporation by seawater aeration (Fig. II.3, 2). Gas-flow of compressed control and CO_2 enriched air were regulated by a CO_2-valve directly installed in the climate room (Fig. II.3, 3). The water

flowed through tubes directly (Fig. II.3, 4) from the jerrycans into the culture vessels. The flow-through was regulated by a tap on each tube (Fig. II.3, 5).

In experiment **(1)** and **(3)**, living foraminifera were picked with a fine brush under a dissecting microscope (Fig. II.3, 6) and kept individually in recruitments pits of the culture vessels (Fig. II.3, 7). Different culture vessels were used for the experiment **(4)** with foraminifera in their natural sedimentary habitat (Fig. II.3, 5).

Fig. II.3: Experimental design: 1) experimental set up, 2) gas-washing bottle, 3) valves of the CO_2 gas-flow regulation unit, 4) water tubes, 5) taps regulated water flow, 6) picking of foraminifera and 7) culture vessels with recruitment pits .

III. Publications and manuscripts

Declaration of my contribution to the following chapters:

Chapter I:

Publication: Haynert, K., Schönfeld, J., Riebesell, U., Polovodova, I.: Biometry and dissolution features of the benthic foraminifer *Ammonia aomoriensis* at high pCO_2. Marine Ecology Progress Series 432: 53–67, doi: 10.3354/meps09138, 2011.

Statement:
The experiment was planned by myself and by Irina Polovodova. Sampling and maintenance of the experiment, and all analytical measurements were done by myself. I analyzed the experimental data and wrote the manuscript. Joachim Schönfeld determined the foraminifera and discussed the taxonomy. All co-authors helped improving and revising the manuscript.

Chapter II:

Publication: Haynert, K., Schönfeld J., Polovodova -Asteman, I., and Thomsen, J.: The benthic foraminiferal community in a naturally CO_2-rich coastal habitat in the southwestern Baltic Sea. Biogeosciences 9: 4421–4440, doi:10.5194/bg-9-4421-2012, 2012.

Statement:
The study was planned by myself, Jörn Thomsen and Irina Polovodova. I performed the study together with Jörn Thomsen. I collected the samples, measured, picked foraminiferal specimens and analyzed the data. Joachim Schönfeld scrutinized the taxonomy and provided calculations of carbonate production. The manuscript was written by myself together with Jörn Thomsen. All co-authors helped to improve and revise the manuscript.

Chapter III:

Manuscript: Haynert, K. and Schönfeld J.: Impact of changing carbonate chemistry, temperature and salinity on growth and test degradation of the benthic foraminifera *Ammonia aomoriensis*. Journal of Foraminiferal Research (resubmitted).

Statement:
I designed the study together with Joachim Schönfeld. Sampling and maintenance of the experiment and analytical measurements were done by myself. I analyzed the data and wrote the manuscript. Joachim Schönfeld helped by discussing the data and their interpretation.

Chapter IV:

Manuscript: Haynert, K., Schönfeld, J., Schiebel, R., Wilson, B., and Thomsen, J.; Response of benthic foraminifera to ocean acidification in their natural sediment environment: a long-term culturing experiment. First draft to be submitted to Biogeosciences.

Statement:
The experiment was planned and designed by myself and by Joachim Schönfeld. The collection of the samples, the experiment maintenance and the analytical measurements were done by myself. I analyzed the data set and wrote the first draft of the manuscript. All co-authors, Joachim Schönfeld, Ralf Schiebel, Brent Wilson and Jörn Thomsen helped substantially by discussing the data and their interpretation.

Chapter I

Biometry and dissolution features of the benthic foraminifer *Ammonia aomoriensis* at high pCO_2

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Abstract

Culturing experiments were performed with the benthic foraminifer *Ammonia aomoriensis* from Flensburg Fjord, western Baltic Sea. The experiments simulated a projected rise in atmospheric CO_2 concentrations. We exposed specimens to 5 seawater pCO_2 levels ranging from 618 µatm (pH 7.9) to 3130 µatm (pH 7.2) for 6 weeks. Growth rates and mortality differed significantly among pCO_2 treatments. The highest increase of mean test diameter (19 %) was observed at 618 µatm. At partial pressures >1829 µatm, the mean test diameter was observed to decrease, by up to 22 % at 3130 µatm. At pCO_2 levels of 618 and 751 µatm, *A. aomoriensis* tests were found intact after the experiment. The outer chambers of specimens incubated at 929 and 1829 µatm were severely damaged by corrosion. Visual inspection of specimens incubated at 3130 µatm revealed wall dissolution of all outer chambers, only their inner organic lining stayed intact. Our results demonstrate that pCO_2 values of ≥929 µatm in Baltic Sea waters cause reduced growth of *A. aomoriensis* and lead to shell dissolution. The bottom waters in Flensburg Fjord and adjacent areas regularly experience pCO_2 levels in this range during summer and fall. Increasing atmospheric CO_2 concentrations are likely to extend and intensify these periods of undersaturation. This may eventually slow down calcification in *A. aomoriensis* to the extent that net carbonate precipitation terminates. The possible disappearance of this species from the Baltic Sea and other areas prone to seasonal undersaturation would likely cause significant shifts in shallow-water benthic ecosystems in the near future.

I.1 Introduction

The rise in atmospheric CO_2 concentrations has caused an increase in seawater pCO_2 over the past 250 yr (Takahashi 2004, Solomon et al. 2007). Surface ocean waters have taken up ~30 % of anthropogenic CO_2 (Sabine et al., 2004, Khatiwala et al., 2009), causing a reduction in ocean pH and carbonate ion concentration (Orr et al., 2005, Cao and Caldeira, 2008). In response to this

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acidification, the calcium carbonate saturation state for calcite and aragonite will be lowered to half their present-day values by 2300 (Feely et al., 2004, Caldeira and Wickett, 2005). This reduced saturation state and reduction in carbonate ion concentration is expected to negatively affect shell and skeleton construction by calcifying organisms (e.g. Erez, 2003).

Benthic foraminifera are the most diverse group of hard-shelled protists. They live at the sediment-water interface, or within the sediments down to >12 cm depth (Corliss, 1985). Model calculations have inferred that benthic foraminifera account for from 5 to 30 % of carbonate production in shallow waters (Wefer, 1976, Langer, 2008). The benthic foraminiferal fauna is estimated to precipitate 0.2 Gt $CaCO_3$ per year on a global scale (Langer et al., 1997, Langer, 2008), which amounts to about one-third of the production by planktonic foraminifers (Schiebel, 2002).

In addition to CO_2-induced ocean acidification, anthropogenic eutrophication by river and groundwater discharge and by atmospheric deposition can lead to changes in carbonate chemistry, especially in coastal marine environments such as the Baltic Sea (Rosenberg, 1985, Conley et al., 2007, Levin et al., 2009, Borges and Gypens, 2010, Cossellu and Nordberg, 2010, Zhang et al., 2010). In comparison to the open ocean, the Baltic Sea exhibits lower salinities, lower CO_3^{2-} and consequently lower calcium carbonate saturation states (Ω). In the western Baltic Sea, seasonal effects are super imposed (Hansen et al., 1999). Vertical stratification, enhanced microbial activity and the ensuing consumption of dissolved oxygen by the decay of particulate organic matter causes hypoxic conditions in the bottom water and therefore strong seasonally varying pCO_2 values over the year (Diaz and Rosenberg, 2008, Conley et al., 2009, Thomsen et al., 2010). In response to low Ω and seasonal acidification, a reduced calcification of foraminifera is expected in Flensburg Fjord (Polovodova et al., 2009).

An increasing number of field and laboratory studies have shown that many calcareous organisms have lower calcification rates under simulated ocean acidification (e.g. Riebesell et al., 2000, Langdon and Atkinson, 2005, Orr et al. 2005, Moy et al. 2009, Thomsen and Melzner, 2010). There is also evidence that planktonic foraminifers precipitate thinner test walls at reduced carbonate ion concentrations and higher atmospheric CO_2 levels (Spero et al., 1997, Bijma et al., 1999, Moy et al., 2009). A recent study by Kuroyanagi et al. (2009) investigated growth rates of the tropical, symbiontbearing foraminifer *Marginopora kudakajimensis* during long-term incubation at 4 different pH_{NBS} (National Bureau of Standards pH) levels between 8.3 and 7.7. Their results indicated that growth rate, shell weight, and the number of newly added chambers decreased with a lowering of the pH. A further culturing experiment with the benthic foraminifer *Elphidium williamsoni* indicated the formation of significantly thinner chamber walls at a pH of 7.6 (Allison et al., 2010). Specimens of the boreal shallow-water species *Ammonia tepida* were cultured under atmospheric CO_2 concentrations of 120 µatm (pH 8.4) and 2000 µatm (pH 7.5; Dissard et al., 2009). Surprisingly, the specimens still calcified at concentrations <2000 µatm. This was in contrast to earlier experiments with living *A. beccarii* from Isle de Yeu, France. In the Ile de Yeu study, growth ceased and dissolution of the tests started at the same pH_{NBS} of 7.5 (Le Cadre et al., 2003).

The consequences of future elevated atmospheric CO_2 concentrations for benthic foraminiferal calcification in shallow waters are thus not sufficiently studied. The purpose of this study

was to investigate the calcification response of the benthic foraminiferal *Ammonia aomoriensis* from the western Baltic Sea to different seawater pCO_2 levels.

I.2 Materials and methods

I.2.1 Sampling and cultivation of foraminifera

Living *Ammonia aomoriensis* specimens were collected from Flensburg Fjord, western Baltic Sea (54°48´082´´N, 9°53´069´´E, 13 m water depth), in June 2009. This location is situated 0.316 n miles to the northwest of Station PF16–26, where, in June 2006, *A. aomoriensis* was reported (as *A. beccarii)* as dominating the living assemblages (Polovodova et al., 2009). The bottom sediment is a silty fine sand. We used a Mini Corer (inner diameter 100 mm; Kuhn and Dunker, 1994), deploying it from R/V *Littorina*. Altogether, 4 cores were taken. The first 2 cores were used for determination of carbonate system parameters and also to serve as backup material. Once aboard, the uppermost 1 cm of the third core was gently washed with seawater of 20 psu through a 63 µm mesh. The residue was kept in 300 ml Kautex wide-neck containers with seawater. The bottles were covered with Parafilm to avoid excess evaporation, and were then aerated and stored at 20 °C as stock cultures. The cultures were exposed to a 12 h light:12 h dark (12:12 L/D) cycle. Foraminifera were fed with 200 µl of a living algae mixture containing *Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella* (DT's Premium Blend) once a week. During feeding, the air pumps were switched off for 1.5 to 2 h to allow the algae to settle, and to facilitate successful feeding of the foraminifera.

I.2.2 Occurrence and identification of *Ammonia* species from Flensburg Fjord

In the western Baltic Sea, *Ammonia* spp. is common at 4 to 14 m water depth. It lives in muddy sands under brackish conditions with salinities ranging from 15 to 23 psu (Rottgardt, 1952, Lutze, 1965, Nikulina et al., 2008), and is found up to 6 cm deep in the sediment (Lutze ,1987). Initially, *Ammonia* spp. from Kiel Bight and adjacent fjords were identified as *Ammonia beccarii* (Linné, 1758), applying a broad understanding of this taxon (Schnitker, 1974). *Ammonia* spp. from European marginal seas have in fact mostly been identified as *Ammonia beccarii* or *Ammonia tepida* (Haake, 1962, Lutze, 1965, Murray, 1991, Debenay et al., 1998, Bouchet et al., 2007, Pascal et al., 2008). However, the diameter of our specimens from Flensburg Fjord was about 1.5 times larger than that of *Ammonia tepida* lectotypes from Puerto Rico (Cushman, 1926, Hayward et al., 2003). The Flensburg Fjord specimens commonly had 9 chambers in the last whorl, while *Ammonia tepida* lectotypes showed only 7 chambers, and the outline of Flensburg Fjord tests was less lobular than those from Puerto Rico. Topotypes of *Ammonia beccarii* from Rimini Beach, Italy, were much flatter, had 14 to 15 chambers in the last whorl, and showed a distinct ornamentation on both spiral and umbilical sides (Hayward et al., 2004). Such ornamentation is lacking in our specimens from Flensburg Fjord. *Ammonia* specimens from Flensburg and Kiel Fjord

were almost identical in shape and morphology (Polovodova and Schönfeld, 2008). A molecular identification of *Ammonia* specimens collected in the Kiel Fjord with rDNA sequences revealed that they belong to the phylotype T6, which, based on morphological characters, was referred to the Pliocene species *Ammonia aomoriensis* (Asano, 1951), which is likely to be extant (Hayward et al., 2004, Schweizer et al., 2010). The adjacent occurrence in the same marginal sea and the strong morphological similarity with specimens from Kiel Fjord suggests that *Ammonia* from Flensburg Fjord also represents the species *A. aomoriensis*. Ongoing molecular phylogenetic analysis by M. Schweizer, University of Edinburgh, is expected to provide more information about molecular identification of Flensburg Fjord *Ammonia* based on rDNA sequences.

I.2.3 Preparation of foraminifera

Living specimens were picked with a fine brush from the stock-cultures under a Wild M3C dissecting microscope. All individuals of *Ammonia aomoriensis* were divided into 3 groups of distinctively different behavior: small and 'active' young specimens (size class 150 to 250 µm), larger and 'active' young specimens (size class 250 to 350 µm) and 'inactive' 'old' specimens of >200 µm in diameter. The specimens were identified as being alive by their yellow cytoplasm content. Additionally, selected and presumably living specimens of 2 different size classes (150 to 250 µm and 250 to 350 µm) were aligned in a Petri dish and left for half an hour. This is an infallible method for distinguishing active from inactive individuals. Only those individuals that showed a lateral movement of at least 3 mm were considered active specimens. Larger, inactive specimens of >200 µm in diameter from both size classes showed a lateral movement of 0.5 to 1 mm only. These inactive specimens were adults, previously considered inappropriate for culturing (Barras et al., 2009).

All specimens, when we selected them, had the same stress condition, independent of activity level or size. We considered specimen movement – an individual and active response to disturbance of habitat – to be indicative of current physical condition, and on this basis we presumed physical condition to be the same for all specimens showing the same response behavior. After their classification, the selected specimens were exposed to calcein-stained seawater (4 mg l^{-1}) for 2 weeks (Bernhard et al., 2004, Barras et al., 2009) and then placed into 300 ml transparent polycarbonate culture vessels, 30 specimens to a vessel. Each vessel contained 10 small active, 10 larger active and 10 large inactive specimens (Fig. 1). This was done at 5 different pCO_2 levels and 3 replicates for each pCO_2 level and each size fraction.

Specimens were kept individually in 1 mm deep recruitment pits of 7 mm diameter, which were drilled into the base plate of the vessels. The recruitment pits were not enclosed, so that the specimens could move and seek shelter inside the pits. The basic idea was that the recruitment pits would help us locate the specimens for monitoring. We observed that most specimens moved around only within their pits, with only very few leaving the pits. (It might have been better to have had deeper or more enclosed pits, but this in turn might have caused a more unstable pCO_2 gradient in each pit.) A small amount (0.43 g) of carbonate-free quartz (>97% SiO_2) was dispersed in equal amounts among the pits with the intention of better mimicking the natural habitat of

Ammonia aomoriensis. The micro environment along the base plate of the vessel appeared to be less attractive to the specimens, since most of them stayed in their pit.

I.2.4 Experimental setup

Culturing of *Ammonia aomoriensis* was performed in a flow-through system following the concept of Hintz et al. (2004) (Fig. 1). The culture vessels were flushed with cartridge filtered (25 µm) and UV-sterilized seawater from Kiel Fjord. In order to monitor the carbonate system, pH according

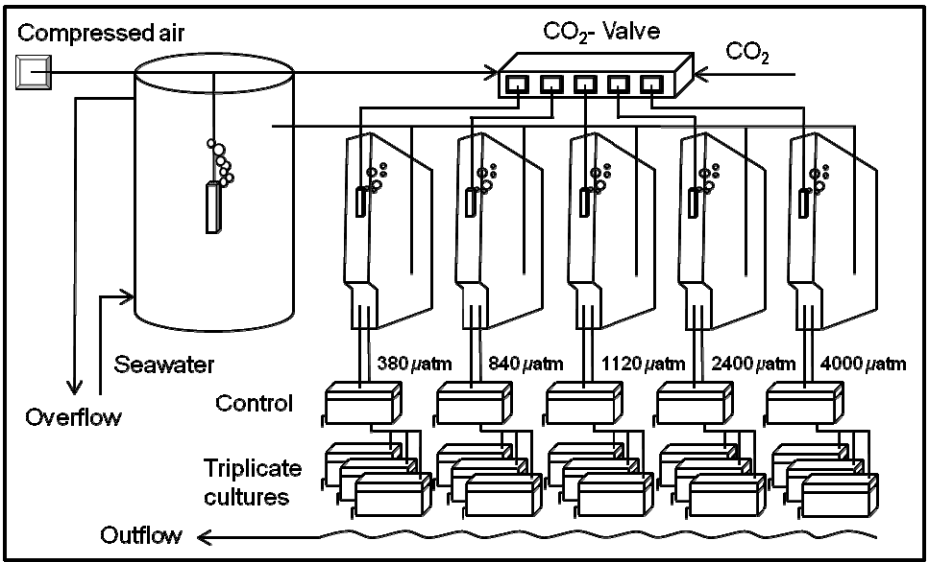

Fig. 1. *Ammonia aomoriensis.* Setup of culturing system. For explanation, see 'Experimental setup'.

to the National Bureau of Standards pH-scale (pH_{NBS}), total alkalinity (A_T), salinity, temperature and phosphate concentrations (PO_4^{3-}) were measured continuously in the flow-through system. The seawater was enriched with oxygen in a 30 l reservoir bin, and subsequently conditioned in five 5 l compact jerrycans with CO_2-enriched compressed air at partial pressures of 380, 840, 1120, 2400 and 4000 µatm. The pCO_2 range from 380 to 1120 µatm corresponded to values recorded in Flensburg Fjord; the 2 higher levels were meant to simulate future scenarios of 2400 and 4000 µatm. The preconditioned seawater from each jerrycan flowed through 4 culture vessels. Three vessels contained living foraminifera as triplicate experiments at the same pCO_2 exposure, and 1 vessel was left barren as a control for hydro-chemical monitoring. To replace the water in the aquaria 1.4 times h^{-1}, the flow rate was adjusted to 0.16 ml s^{-1}. The overflow seeped through the fissure between lid and vessel, draining off to a sink. Food was added every second day to the vessels containing foraminifera as 100 µl DT's Premium Blend algae mixture. The experiment lasted 6 weeks.

I.2.5 Population dynamics and biometry

The aquarium like culture vessels permitted monitoring of the individuals throughout the experiment. They were examined weekly under a dissecting microscope, and their presence, shape

and behavior noted. As the removal of culture vessels from the experimental setup for examination induced some disturbance, we refrained from surveillance at shorter intervals. Using an eyepiece reticle on the dissecting microscope, we measured the size of all specimens weekly in their recruitment pits. These measurements had to be made through the water column in the culture vessels. During these measurements, temperature was held stable by placing the culture vessel in a water bath with crushed ice, and monitoring the temperature.

Because of a working distance of >50 mm, we could use only 40x magnification, which resulted in an error of ± 12.5 µm (maximum distance to the next scale unit) in the size measurements. This is approx. ±4 to ±7 % of the average diameter (from 180 to 280 µm) of the examined specimens. The overall test diameter of specimens changed during the experiment. Size differences were calculated by measuring the test diameter at the beginning and at the end of the 6 weeks experiment. In addition to diameter measurements, we determined the number of new chambers formed during the incubation period. We did this at the end of the experiment by examining the specimens (according to Dissard et al., 2009) under an inverted fluorescence microscope (Zeiss Axiovert 100, wavelength: 530 nm). After the experiment, all individuals were stained with Rose Bengal to assess whether they still contained cytoplasm (indicating living specimens) or not (dead specimens).

I.2.6 Water chemistry

pH, as well as alkalinity, salinity, temperature and phosphate concentrations, were measured and compared in the culture vessels and controls. As an additional control we measured all parameters in the 30 l reservoir bin, in the five 5 l compact jerrycans and in the sink. pH_{NBS}, temperature and salinity were monitored every second day in the setup. We used a WTW 340i pH analyzer to measure pH and water temperature. The pH analyzer was calibrated with standard buffer solutions of pH 4.01, 7.00 and 10.00 (WTW standard, DIN/NIST buffers L7A). Precision was ±0.01 for pH and ±0.1 °C for temperature. For salinity measurements, a WTW Cond 315i salinometer with a precision of ±0.1 psu was used.

In order to calculate carbonate system parameters with CO2SYS software, the phosphate concentration was measured weekly (Lewis and Wallace, 1998). A 10 ml water sample was passed through a 0.2 µm filter and was measured colorimetrically in a spectrophoto meter (U 2000, Hitachi-Europe) at a wavelength of 882 nm according to Koroleff (1983). The precision of the phosphate measurements was ±0.2 μmol l^{-1}.

Samples for analysis of total alkalinity (A_T) were sterile-filtered (0.2 µm pore size) and determined through potentiometric titration (Dickson, 1981) in a Metrohm Tiamo automatic titration device. The precision of the alkalinity measurements was 2 μmol kg^{-1}. The carbonate system parameters pCO_2 and Ω calcite values were calculated from measured A_T, pH, phosphate, temperature and salinity using the CO2SYS software (Lewis and Wallace, 1998). The equilibrium constants of Mehrbach et al. (1973), as refitted by Dickson and Millero (1987), were chosen.

Near-bottom water samples were taken from Flensburg Fjord in order to determine salinity, temperature, pH and alkalinity in the natural habitat of *Ammonia aomoriensis*. The range of reproducibility of pH measurements from Flensburg Fjord bottom-water samples, collected

bi-monthly from June 2009 to April 2010, was from 0.04 to 0.1. Carbonate system parameters were calculated from measurements of pH_{NBS} and alkalinity. Monitoring results will be reported elsewhere, but we refer to the pCO_2 values and their seasonal range in the present study (Table 1).

Table 1. *Ammonia aomoriensis*. Carbonate chemistry of bottom water from sampling station FF3 from Flensburg Fjord (54° 48.082' N, 9° 53.069' E), showing duplicate average values of salinity, temperature, total alkalinity (A_T) and the pH according to the National Bureau of Standards pH-scale (pH_{NBS}). The precision of the alkalinity measurements was 100 µmol kg^{-1}. Carbon dioxide partial pressure (pCO_2) and Omega of calcite saturation state (Ω_{ca}) were calculated (cal) with the CO2Sys program (Lewis and Wallace, 1998) from measured A_T, pH_{NBS}, temperature and salinity.

Date (d/mo/yr)	Salinity	Temp. (°C)	A_T (μmol kg^{-1})	pH_{NBS}	pCO_2 cal (μatm)	Ω_{ca} cal
03/06/2009	20.2	10.3	2125.5 ± 87.6	7.83	727.1	1.35
18/08/2009	19.8	15.3	2239.1 ± 28.0	7.47	1863.3	0.85
20/10/2009	21.6	11.9	2465.7 ± 31.4	7.31	2873.8	0.60
07/12/2009	20.9	8.8	2174.0 ± 64.1	7.81	769.1	1.42
15/02/2010	16.9	-0.4	1804.6 ± 200.7	7.94	465.1	1.05
19/04/2010	18.8	5.6	2374.9 ± 90.8	7.94	493.0	2.01

I.2.7 Preparation for scanning electron microscopy

At the end of the experiment, the foraminifers were removed from the culture vessels using a fine brush and were transferred to Eppendorf-type micro centrifuge tubes. Fixation was accomplished in a solution of 2 g Rose Bengal in 1 l ethanol (98 %, technical quality) for 24 h. Finally, intact specimens were air-dried, prepared with an Emitech K550 (Au+Pd) sputter coater and photographed with a scanning electronic microscope (SEM; Cam Scan-CS-44).

I.2.8 Statistics

Changes in test diameter (see Fig. 3B) were analyzed by linear regression ($f = b + ax$) using SIGMA PLOT 10. Regression lines present Pearson correlation with confidence bands, which exhibit 95 % CI and correlation coefficient R^2 for the fitted line. The error in the regression equations is ±1 SE of the mean.

I.3 Results

I.3.1 Water chemistry

Salinity ranged from 17 to 19.5 psu (mean ± SE = 18.4 ± 0.8) during the experimental period (Fig. 2, Table 2). Mean seawater temperature in the culture vessels decreased steadily over the course of the experiment from 12.6 to 11 °C. The culture vessels were flushed with seawater, which was taken from Kiel Fjord with our aquarium system. Salinity and temperature of seawater changed seasonally (Thomsen et al., 2010). The fluctuations of these parameters during the experimental period therefore correspond to the natural variability in *Ammonia aomoriensis* habitat.

pH values (mean ± 1 SE) varied according to seawater pCO_2 from 7.9 ± 0.05 to 7.2 ± 0.04. They were reasonably stable during the whole incubation time and did not mirror variations in salinity and temperature (Fig. 2). Maxima and minima in mean values were caused by a few exceptionally high and low values in single replicates, which may represent measurement errors. Alkalinity and phosphate concentration also showed no significant change over the course of the experiment (Table 2). No systematic offsets or significant differences among experimental and control vessels were detected. While respiration and degradation processes are likely to be enhanced when food is added to the vessels, we saw no significant differences of measured or calculated parameters between controls and cultures. This suggests that the amount of food added was too small to change the abiotic conditions in the culture vessels at the given flow rates.

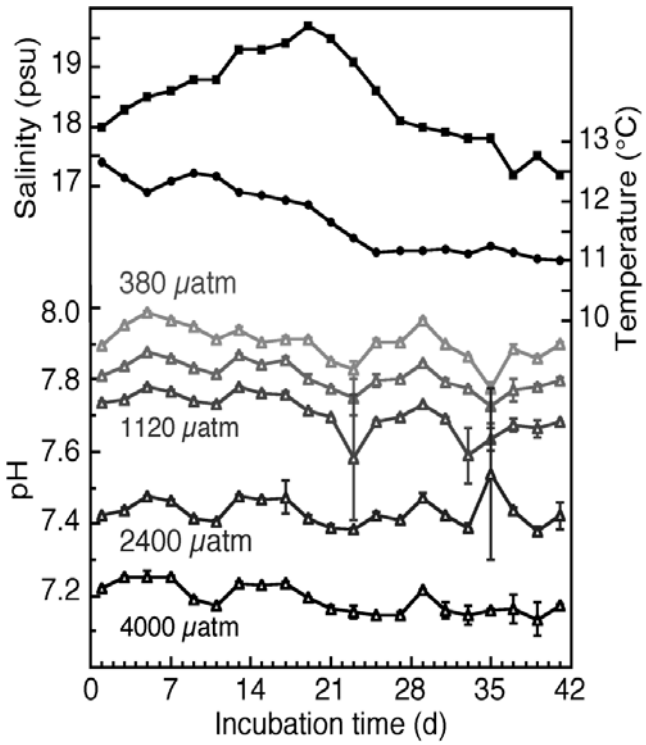

Fig. 2. *Ammonia aomoriensis*. Water chemistry during the experiment. Temperature and salinity are mean values of all measurements taken in each culture vessel flushed with different seawater pCO_2 levels as in Table 1. pH: means ± SD, n = 3.

The calculated pCO_2 in the culture vessels differed significantly from the pCO_2 baseline level in the compressed air (Table 2). For instance, in the jerrycan bubbled with compressed air without CO_2 addition, which should yield the ambient atmospheric partial pressure of 380 µatm, the measured value was 618 µatm. This was most likely due to a higher CO_2 concentration in subsurface waters of Kiel Fjord at the seawater system intake caused by seasonal phenomena, such as upwelling of hypoxic and hypercapnic waters (Thomsen et al., 2010). At higher pCO_2 levels, the measured values in the culture vessels were 11 to 24 % lower than the target values in the CO_2-charged compressed air (Table 2). The difference probably accounts for outgassing in the culture vessels due to the slow percolation rate. In the following, we refer to the pCO_2 values that were calculated from actually measured hydrochemical parameters in the culture vessels and not to the pre-adjusted values in the CO_2-enriched air (Table 2).

Table 2. *Ammonia aomoriensis*. Carbonate chemistry of culture media, means ±SD (n = 3) of several variables for 5 pCO_2 levels. Controls are mean values of all measurements made during the 6 weeks incubation. pCO_2, total carbon (C_T), and Omega of calcite saturation state (Ω_{ca}) were calculated (cal) with the CO2Sys program (Lewis and Wallace, 1998) from measured A_T, pH_{NBS}, PO_4^{3-}, temperature and salinity.

pCO_2 baseline level (μatm)	pCO_2 cal (µatm)	Salinity	Temp. (°C)	pH_{NBS}	A_T (μmol kg^{-1})	C_T cal (μmol kg^{-1})	Ω_{ca} cal	PO_4^{3-} (μmol l^{-1})
380	617.9 ± 8.5	18.4 ± 0.8	11.8 ± 0.6	7.90 ± 0.05	2040.1 ± 20.7	1980.2 ± 31.8	1.66 ± 0.25	0.99 ± 0.33
380_control	610.1 ± 94.0	18.4 ± 0.8	11.7 ± 0.6	7.91 ± 0.04	2043.1 ± 20.1	1983.1 ± 29.2	1.67 ± 0.22	0.99 ± 0.31
840	751.1 ± 22.8	18.4 ± 0.8	11.6 ± 0.6	7.81 ± 0.04	2039.0 ± 17.4	1999.5 ± 22.3	1.38 ± 0.16	1.02 ± 0.32
840_control	734.7 ± 67.8	18.4 ± 0.8	11.6 ± 0.7	7.81 ± 0.04	2036.0 ± 16.7	1996.4 ± 21.7	1.39 ± 0.14	1.04 ± 0.29
1120	929.1 ± 23.4	18.4 ± 0.8	11.7 ± 0.6	7.71 ± 0.06	2035.2 ± 19.8	2014.4 ± 23.2	1.14 ± 0.13	1.01 ± 0.33
1120_control	953.9 ± 147.4	18.4 ± 0.8	11.6 ± 0.5	7.71 ± 0.04	2034.0 ± 14.6	2018.0 ± 19.2	1.12 ± 0.15	1.06 ± 0.32
2400	1829.2 ± 33.5	18.4 ± 0.8	11.6 ± 0.6	7.43 ± 0.04	2036.2 ± 21.5	2075.2 ± 20.4	0.64 ± 0.08	1.01 ± 0.30
2400_control	1891.2 ± 227.0	18.4 ± 0.8	11.6 ± 0.6	7.42 ± 0.04	2042.0 ± 14.6	2095.7 ± 21.0	0.59 ± 0.06	1.00 ± 0.30
4000	3130.2 ± 33.6	18.4 ± 0.8	11.9 ± 0.5	7.19 ± 0.04	2039.7 ± 19.2	2156.2 ± 24.5	0.37 ± 0.04	1.00 ± 0.33
4000_control	3158.6 ± 235.1	18.4 ± 0.8	11.8 ± 0.5	7.18 ± 0.05	2036.9 ± 17.8	2159.8 ± 23.7	0.36 ± 0.03	0.96 ± 0.37

The Ω values (mean ± 1 SE) for calcite ranged from 1.66 ± 0.25 at a pCO_2 of 618 µatm to 0.37 ± 0.04 at a pCO_2 of 3130 µatm. The values <1.0 indicate carbonate dissolution at partial pressures above 929 µatm under the present settings of temperature and salinity.

The carbonate system parameter pCO_2 cal and Ω_{Ca} cal, as calculated from measured pH_{NBS} and A_T values, varied in the near-bottom water at the sampling site in Flensburg Fjord from 2874 µatm (pH 7.3) in October 2009 to 465 µatm (pH 7.9) in February 2010 (Table 1). In August and October 2009, Ω values for calcite were temporarily <1.0 (0.85 and 0.6, respectively). Therefore, our experiment covers the entire seasonal pCO_2 variability in the *A. aomoriensis* habitat in Flensburg Fjord, even though we did not capture the seawater pCO_2 level in February and April 2010 when the pCO_2 in Flensburg Fjord was ~130 µatm lower than at the lowest partial pressure in our experiment.

I.3.2 Test diameters

All specimens, whether inactive or active, were alive at the beginning of the experiment. They grew during the incubation, especially at low pCO_2 values. Active specimens from the 150 to 250 µm fraction displayed an increase of 19 % in diameter at a pCO_2 of 618 µatm, whereas mean diameter of active specimens from the 250 to 350 µm fraction increased by only 11 % (Fig. 3A). In comparison to active specimens, the mean diameter of the large inactive specimens (>200 µm) increased by only 2 % at a pCO_2 of 618 µatm during the course of the experiment (Fig. 3A).

The growth of specimens from the 150 to 250 µm fraction differed significantly, depending on pCO_2 treatment (Fig. 3B). The greatest increase in mean shell diameter of 35 µm was observed at the lowest pCO_2 level of 618 µatm. At pCO_2 levels of 751 µatm and 929 µatm, the mean diameter of *Ammonia aomoriensis* increased by only 29 and 13 µm, respectively. At a pCO_2 of 1829 and 3130 µatm, the test diameter was reduced by 5 and 41 µm due to test corrosion.

The shell diameter of active specimens from the 250 to 350 µm fraction differed also according to pCO_2 level (Fig. 3B). At a pCO_2 of 618 µatm, the mean shell diameter increased by 29 µm. The growth rate was highest at a pCO_2 of 751 µatm, where the increase was 39 µm.

Ammonia aomoriensis displayed a reduced growth under higher pCO_2 levels, viz. from 929 to 3139 µatm. While the mean shell diameter increased by 29 µm at a pCO_2 of 929 µatm, the specimens displayed barely any change in size at a pCO_2 of 1829 µatm during the 6 weeks incubation period. At a seawater pCO_2 of 3130 µatm the average shell diameter was reduced by 23 µm.

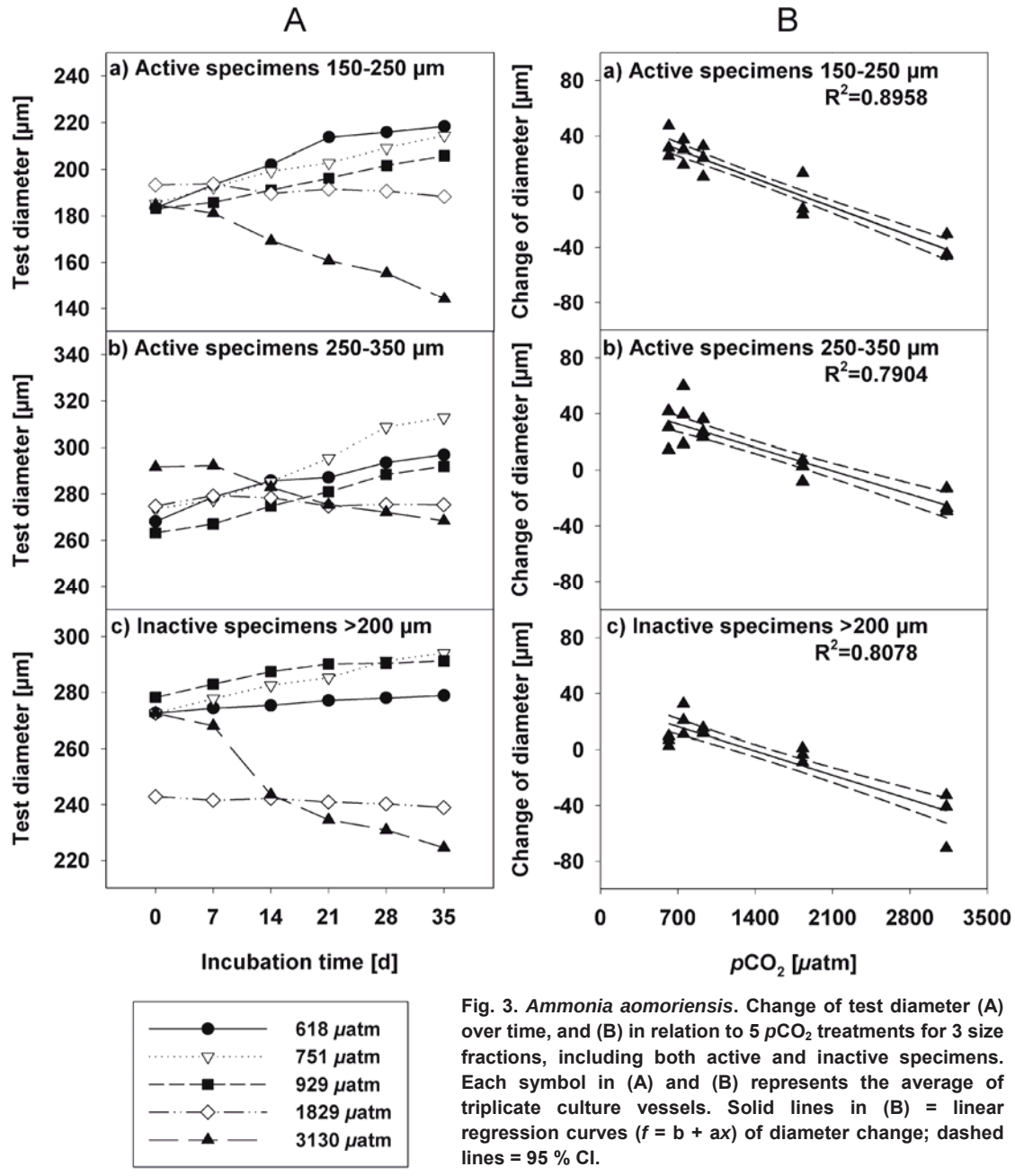

Fig. 3. *Ammonia aomoriensis*. Change of test diameter (A) over time, and (B) in relation to 5 pCO_2 treatments for 3 size fractions, including both active and inactive specimens. Each symbol in (A) and (B) represents the average of triplicate culture vessels. Solid lines in (B) = linear regression curves ($f = b + ax$) of diameter change; dashed lines = 95 % CI.

Inactive specimens from the >200 µm fraction showed only a slight change of test diameter at a pCO_2 of from 618 to 1829 µatm (Fig. 3B). The lowest increase (6 µm) was observed at a pCO_2 of 618 µatm, followed by an increase of 21 µm at a pCO_2 of 751 µatm and 13 µm at 929 µatm. Like active specimens, the shell diameter barely changed at a pCO_2 of 1829 µatm and, on average, decreased by 48 µm at a pCO_2 of 3130 µatm.

I.3.3 Loss and mortality rates

During the 6 weeks incubation period, some *Ammonia aomoriensis* specimens disappeared between weekly surveillance periods (Fig. 4). On these occasions the entire culture vessel was thoroughly screened twice for lost specimens. However, the missing individuals had neither moved out of their recruitment pits, nor had they crawled upwards on the sidewalls of the vessels (e.g. Lee and Anderson, 1993). Nor were encystation or clustering of juveniles around the mother individual observed (e.g. Lehmann, 2000, Heinz et al., 2005). The physical disturbance during removal of vessel, surveillance and sampling might have played a role in this occurrence. Allison et al. (2010), moreover, have described the possibility of flotation and escape of specimens attached to air bubbles with seawater outflow from the cultivation chambers. The inner organic lining, which is light and floats easily, may also be involved in escape by flotation. In our setup, however, air bubbles were trapped under the lid of the vessels, thus eliminating this option. It remains possible that some of the specimens were lost during sampling.

The loss resulted in no significant difference between the active specimens of size fractions 150 to 250 µm, 250 to 350 µm or inactive specimens >200 µm. In the pCO_2 range of 618 to 929 µatm, the losses of active and inactive specimens during the experimental period averaged from 7 to 11 of the 30 cultured specimens. Significantly higher losses were observed at a pCO_2 of 3130 µatm. From 30 specimens at the beginning of the experiment, the loss of active specimens of the 150 to 250 µm and 250 to 350 µm size fractions averaged 18 specimens. Among the inactive specimens >200 µm, an average of 22 out of 30 individuals were lost during the experiment (Fig. 4).

At the end of the experiment, the foraminifers were picked individually from the recruitment pits under water. Staining of these organisms with Rose Bengal revealed that most individuals incubated from 618 to 1829 µatm pCO_2 survived. Based on staining evidence, an average of 20 active and inactive specimens from each vessel had survived the experiment. However, of the 12 specimens remaining at experiment's end that had been subjected to a pCO_2 of 3130 µatm, an average of 10 contained no living cytoplasm at the end of the incubation and were presumed dead.

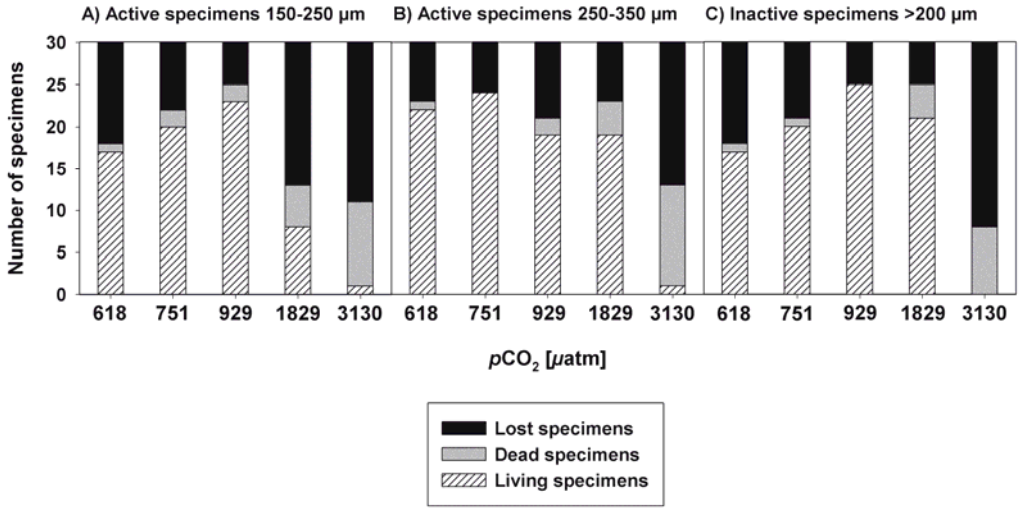

Fig. 4. *Ammonia aomoriensis.* Loss and mortality versus pCO_2 level for 3 size fractions (A, B, C), including both active and inactive specimens. Bars display means of lost, dead and live specimens.

I.3.4 SEM observations

The different stages of dissolution during the 6 weeks incubation time were revealed by SEM observations (Fig. 5). The tests of *Ammonia aomoriensis* exposed to a pCO_2 of 618 to 751 µatm were fully intact (Fig. 5A). The test walls exhibited a smooth surface, and the pore size and distribution on the shell wall remained unaffected. At pCO_2 levels of 929 and 1829 µatm the last 1 to 3 younger chambers were severely decalcified or destroyed (Fig. 5B,C). The surface of the walls showed fragmentary dissolution of the younger calcite layers, which were left only as scales (Fig. 6A,B). Furthermore, at a seawater pCO_2 of 1829 µatm, we observed the formation of cracks around the pores. (Fig. 6C). On some individuals, at a pCO_2 of 1829 µatm, the SEM examination revealed prograding incisions along the sutures. At a high pCO_2 of 3130 µatm, the tests had become heavily decalcified after 6 weeks (Fig. 5D). The tests showed an irregular shape caused by dissolution of the outer chamber walls (Fig. 6D). In all cases, the shell was interrupted and only the inner organic lining remained (Fig. 5D). The interlocular walls were isolated, and their internal calcite layers were separated (Fig. 6D). The remaining interlocular walls gave the tests a star-like appearance (Fig. 5D).

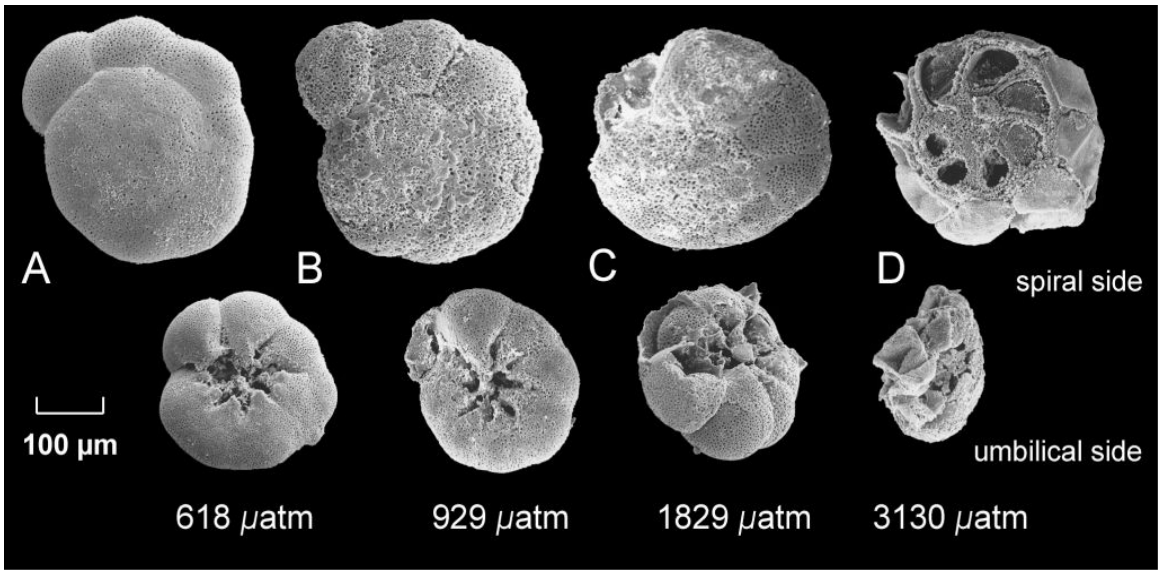

Fig. 5. ***Ammonia aomoriensis.*** **SEM images depicting different stages of dissolution on spiral and umbilical sides of the test at various pCO_2 levels (A to D).**

I.4 Discussion

I.4.1 Change of test diameter of *Ammonia aomoriensis* under elevated pCO_2

The increase in average test diameter indicated that the individuals had grown during the experiment. Growth was higher among the small young and active specimens than among the large active and inactive adults. At a control pCO_2 of 618 µatm, 78 % of the active specimens from the 150 to 250 µm size fraction grew during the experiment, as did 65 % of the specimens from the 250 to 350 µm size fraction. Only 44 % of inactive specimens of the >200 µm size fraction showed an

increase in test diameter, which implied growth by chamber addition during the 6 weeks incubation time (Table 3). Substantially more specimens grew in our experiment than during the experiments of Dissard et al. (2009), where ~60 % of the specimens kept at a low, pre-industrial CO_2 level added new chambers. With increasing pCO_2, the difference between initial and final test diameter of *Ammonia aomoriensis* decreased. A significant reduction in foraminiferal test diameter was observed at a high pCO_2 of 3130 µatm.

Foraminifera grow by adding new chambers. To assess chamber addition, specimens that had grown during the experiment were examined after the incubation time under a fluorescence microscope. All youngest chambers were stained with calcein (for comparison see Fig. 1A of Allison et al., 2010, p. 88). Since the intensity was hardly distinguishable from the elder part of the test, we could not assess with certainty the number of new chambers that had been formed during the experiment. The newly precipitated calcite of the final chambers contained calcein similar to the walls of the earlier chambers, even though it had not grown in calcein-stained water. We suppose that the calcein was incorporated and stored in vacuoles filled with seawater, from which the calcite for the new chamber wall of the foraminifer was formed. This might explain why the new chambers were fluorescent after the 6 weeks incubation time without calcein having been added to the percolating seawater. Another explanation might be that calcein was adsorbed to the organic lining and all calcein-stained chambers were either formed during pre-incubation time or the calcein was re-mobilised from the linings during formation of new chambers. As we observed that foraminifers were stained with calcein before placing them into culture vessels and they had definitely grown during the experiment, this explanation seems less likely. Furthermore, little is known about the internal dynamics and intermittent storage of calcein in living cells. Normally, calcein does not pass cell membranes. An adsorption to organic linings therefore does not appear likely.

Table 3. *Ammonia aomoriensis.* Newly added chambers from active specimens of 3 size fractions, including both active and inactive specimens, at a control pCO_2 of 618 µatm.

Number of specimens	Active specimens 150–250 µm	Active specimens 250–350 µm	Inactive specimens >200 µm
Survived the experiment	18	23	18
Showing no growth	4	8	10
Grown by 1 chamber	0	1	0
Grown by 2 chambers	6	6	6
Grown by 3 chambers	3	8	2
Grown by 4 chambers	5	0	0
Average chamber addition	**2.3**	**1.6**	**1.0**
Percentage showing growth	**78 %**	**65 %**	**44 %**

Due to the above-mentioned constraints, chamber formation of *Ammonia aomoriensis* could be assessed only by means of increase in test diameter. An examination of SEM images taken from 24 specimens of *A. aomoriensis* sampled in Flensburg Fjord, Kiel Fjord, and Eckernförde Bight (Schönfeld and Numberger, 2007a,b, Polovodova and Schönfeld, 2008, Nikulina et al., 2008, Polovodova et al., 2009, Schweizer et al., 2010) revealed an increase in diameter (average ± SD, n = 12) of 27 ± 16 µm per new chamber over the last 4 chambers. The height of the last 2 chambers, as seen from the spiral side, was 106 ± 23 µm. The increase in test diameter by adding new

chambers did not co-vary with the overall diameter. Therefore we conclude that *A. aomoriensis* growth by 13 to 39 µm at a pCO_2 of 618 µatm corresponded to the addition of 1 or 2 new chambers during the 6 weeks experimental period. In terms of chamber addition, active specimens of size fraction 150 to 250 µm added 2.3 chambers on average, and specimens of size fraction 250 to 350 µm added 1.6 new chambers. Inactive specimens added 1.0 new chamber on average (Table 3). An example of growth for one active individual of *A. aomoriensis* from size fraction 250 to 350 µm is presented in Fig. 7. During the incubation time, this individual grew by 33 µm. The growth took place in 2 increments of 16 µm, the first between days 7 and 14, and the second between days 14 and 21. On the basis of the aforementioned calculations, we concluded that this specimen had added 2 new chambers at a pCO_2 of 618 µatm.

Culture experiments assessing the growth of *Ammonia aomoriensis* from the western Baltic Sea have not been reported to date. The cultivation of *A. tepida* from San Antonio Bay, Texas, revealed a strong dependency of test growth on ambient temperature and salinity (Bradshaw, 1957). During the experimental period, we measured an average temperature of 11.7 °C and salinity of 18.4 psu. On the basis of Bradshaw's (1957) data applied to our experimental settings, we estimated a growth rate of 0.06 chambers per day, causing us to expect an addition of 2 to 3 new chambers over 6 weeks. In fact, our measurements indicated an addition of only 1 to 2 new chambers on average, which is slightly lower than could be estimated using Bradshaw's (1957) data, but it is nonetheless in general agreement with that estimate.

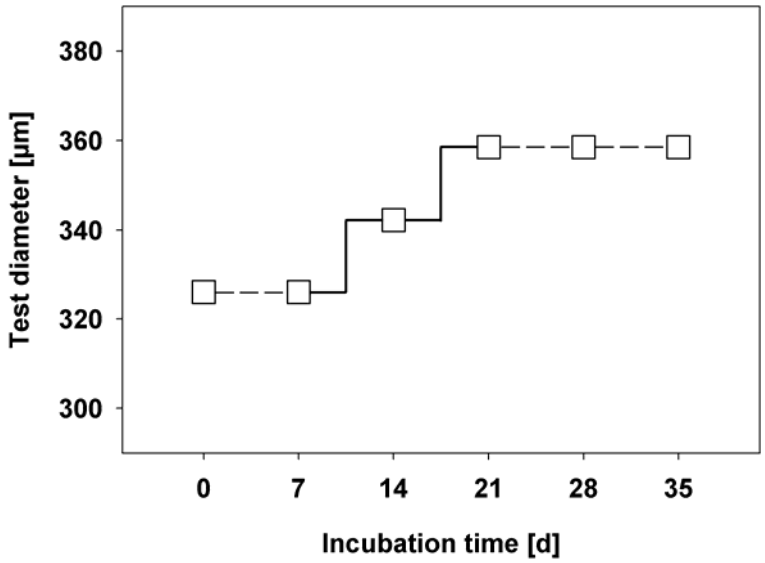

Fig. 7. *Ammonia aomoriensis.* Change in test diameter over time of a single active individual from size fraction 250–350 µm. Increment in diameter between Days 7 and 21 implies addition of 2 new chambers.

Dissard et al. (2009) reported that only half the specimens added new chambers during 6 weeks of laboratory cultivation. Their average rates of 0.9 to 1.7 new chambers per individual fit well with our results. The culturing of the benthic foraminifer *Elphidium williamsoni* showed that they formed from 1 to 3 new chambers at a pH range of 7.6 to 8.3 at 15 °C during an 8 weeks experimental period (Allison et al., 2010).

During our experimental period, we observed that tests dissolved more readily at a high

seawater pCO_2 than at a lower level. In our study the test diameter of *Ammonia aomoriensis* showed an increase with pCO_2 values up to 751 µatm. Above a critical pCO_2 level of 1829 µatm, however, dissolution features and a reduction of test diameter were observed. The inferred reduction of calcification might be a result of a presumably higher energetic cost to elevate the pH of intracellular vesicles where the first calcite crystals for the new chamber walls are precipitated (de Nooijer et al., 2008). This might explain the growth deceleration but not the size reduction observed at a pCO_2 at >1829 µatm. Alternatively, shell-wall thinning might be the result of dissolution under elevated pCO_2. SEM observations revealed the outer chamber walls to be 4 to 10 µm thick. Corrosion or even dissolution of the outer chamber walls would cause a reduction of the test diameter by 10 to 20 µm. The average chamber height is 106 µm. If the entire last whorl were to be dissolved, the shell loss would exceed by far the observed reduction of 23 to 49 µm in diameter. Therefore, it is reasonable to assume that the size reduction was due to a loss of outer shell wall and partial collapse of inner organic lining, which is in agreement with the SEM observations of dissolution features.

I.4.2 Dissolution features

Our results revealed a clear relationship between seawater pCO_2 and shell dissolution. The first dissolution features were recorded at a pCO_2 of 929 µatm and led to loss of the last-formed (thinner) chambers. At a pCO_2 of 3130 µatm all chambers were destroyed by complete calcium carbonate dissolution, only the inner organic lining stayed intact.

Under elevated pCO_2 conditions, we observed different stages of dissolution. Decalcification started with loss of the external walls of the last chambers at a pCO_2 level of 929 µatm. The younger chambers decalcify first because their walls consist of a lower number of lamellae and therefore are thinner (Le Cadre et al., 2003). The next stage of dissolution is total decalcification of the outer walls and the inner organic lining, with cytoplasm becoming visible at a pCO_2 of 3130 µatm. For instance, the individual presented in Fig. 5D had a test diameter of 342 µm at the beginning, and after 6 weeks incubation at pCO_2 of 3130 µatm the diameter was reduced to 32 µm.

The shell of foraminifers is composed of many calcitic layers – so called primary and secondary calcite – which cover the chambers (Erez, 2003). The thickness of each layer depends on the number of chambers per whorl (Reiss, 1957, Bentov and Erez, 2005). The needles of the primary calcite, which forms the inner lamella outlining the new chamber, usually consist of a high-Mg calcite. The secondary calcitic layer, which covers the inner lamella as well as the entire existing shell, consists of low-Mg calcite (Reiss, 1957, Erez, 2003). Dissolution of the secondary calcite, which is deposited among the primary calcite needles, leads to test transparency (Le Cadre et al., 2003). We even observed a scabbing of the external walls of all chambers at a pCO_2 of 929 µatm (pH 7.7), with the primary calcite dissolving first, and needles of the secondary calcite beginning to thin (Fig. 6A). At a higher pCO_2 level (1829 µatm), primary calcite dissolved first and caused formation of lacunae among the needles of the first layer of secondary calcite and the inner organic lining. At the same time, the needles of the secondary calcite thinned at their base (Fig. 6B). Furthermore, the pore diameter expanded on the external walls and cracks were formed on the surface. At the highest

pCO_2 treatment (3130 µatm), the primary calcite dissolved completely. In the first layer of secondary calcite from the inner side, the needles became generally thinner (Fig. 6D). The second layer of secondary calcite on the outer side corroded completely (Fig. 6D). In general, corroded tests became opaque. We conclude from our SEM observations that dissolution progressed both from the inner (cytoplasm) surface and the outer (seawater) surface. Acidified seawater probably diffuses through the pores from the external walls towards the inner organic lining. From the inner side the primary calcite corroded first. This is because this primary material consists of high-Mg calcite (Bentov and Erez, 2005), which is less resistant to dissolution than the needles of secondary, low-Mg calcite.

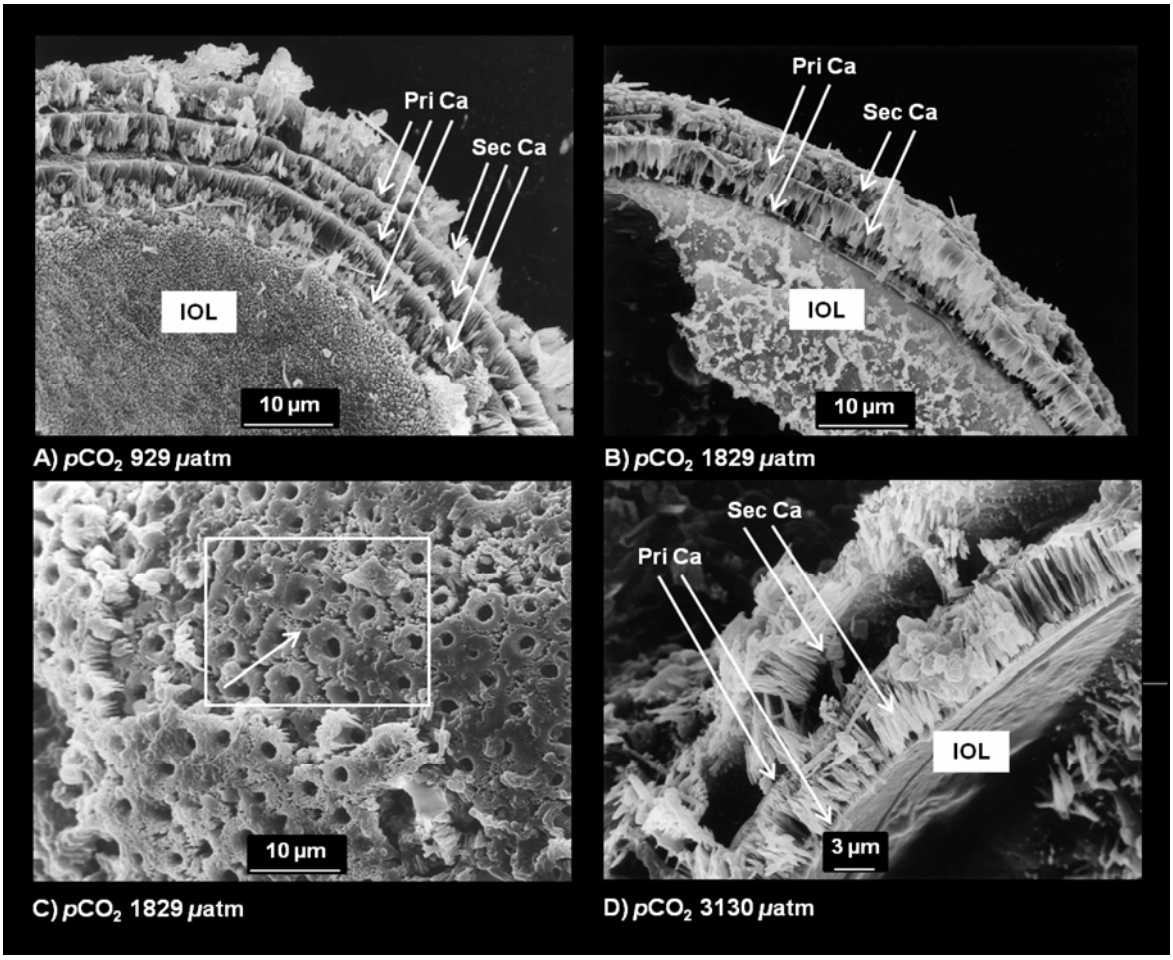

Fig. 6. ***Ammonia aomoriensis.*** **(A, B, D) SEM images showing layers of primary calcite (Pri Ca), secondary calcite (Sec Ca) and inner organic lining (IOL) after 6 weeks incubation under elevated pCO_2. (C) Note appearance of cracks at pCO_2 of 1829 µatm.**

Corroded walls have likewise been observed in living *Ammonia beccarii* from Isle de Yeu, France (Le Cadre et al., 2003). The specimens first retarded their pseudopodial network, then the test became opaque and the youngest chambers were destroyed. After 15 d, only the interlocular walls were preserved, and the inner organic layer covered the cytoplasm at the other parts of the test (Le Cadre et al., 2003).

The results of Le Cadre et al. (2003) demonstrate that *Ammonia beccarii* is able to rebuild its shells through recalcification when pH is increased following temporary exposure to low pH levels. In our laboratory experiment, however, *A. aomoriensis* was permanently exposed to low pH. According

to our observations, *A. aomoriensis* exhibited no evidence of counteracting dissolution through rebuilding its shells during the incubation time of 6 weeks.

Our laboratory experiment reproduced the dissolution phenomena observed in nature. Different stages of test dissolution of *Ammonia beccarii* were found in Gelting Bay, Flensburg Fjord. All observed *Ammonia* specimens were corroded and exhibited loss of the youngest chambers or the tests took on a star-like appearance with visible inner organic linings (Polovodova and Schönfeld, 2008, Plate 3, Figs. 2–6). Similar dissolution features were observed in the following: *A. batavus* from Sandebukta, Oslo Fjord (Alve and Nagy, 1986); *A. parkinsoniana, Elphidium excavatum* and *Palmerinalla palmerae* from Nueces Bay, Texas (Buzas-Stephens and Buzas, 2005); tropical, intertidal benthic foraminifera from Cleveland Bay, North Queensland (Berkeley et al., 2008); and estuarine foraminifera from South Alligator River, Northern Territory, Australia (Wang and Chappell, 2001).

The dissolution features observed in nature may have a variety of anthropogenic or natural causes (Le Cadre et al., 2003). Here we can only speculate that the lowering of pH in seawater of natural habitats is an important factor in test dissolution. Abrasion and predation, as well as early diagenesis, were previously considered as mechanisms that may act independently or amplify each other (Bradshaw, 1957, Martin et al., 1995, Alve and Murray, 1999, Moreno et al., 2007, Polovodova and Schönfeld, 2008). As these processes can be ruled out under laboratory conditions, the shell loss of cultured foraminifers can only be interpreted in light of carbonate chemistry impacts on the calcification and dissolution process (Stubbles et al., 1996a,b).

I.4.3 Loss rate and mortality

We observed no significant differences of loss between the active and inactive specimens. At a pCO_2 of 3130 µatm, however, where significantly higher losses were observed, the highest loss was seen in inactive specimens >200 µm, followed by small active specimens of the 150 to 250 µm size fraction. Subsequent treatment with Rose Bengal demonstrated that most of the specimens were devoid of cytoplasm at a pCO_2 of 3130 µatm. We observed that the test wall of *A. aomoriensis* was completely destroyed at a pCO_2 of 3130 µatm and that only the inner organic lining was left. Therefore it is possible that the inner organic lining, which is much lighter than a calcitic test, may float easily or disappear. This may explain why we did not recover some of the specimens after 6 weeks of incubation time. On the other hand, it is also possible that specimens were flushed away or escaped from the recruitment pits.

Loss and mortality rates revealed that inactive specimens or empty shells of foraminifera were affected first, followed by small active specimens of the 150 to 250 µm size fraction. This indicates that living cells may be able to counteract dissolution better than dead cells, at least up to a certain pCO_2 level. This emphasizes the potential for biological control, which is required to maintain inorganic tests and shells in an adverse abiotic environment.

Furthermore, we observed that the test walls of small specimens of the 150 to 250 µm size class sustained greater damage at high pCO_2 levels than did large specimens of the 250 to 350 µm size fraction. The surface:volume ratio of small tests is greater than that of large tests. Small

specimens have a relatively greater surface, which is affected by external corrosion and may therefore respond more sensitively to undersaturated conditions. Another possible reason for greater damage sustained by small specimens is the thickness of the test walls of such specimens. In comparison to adult and large specimens, young and small specimens have thinner walls and fewer calcite lamellae. Therefore, the test walls of small specimens could be more easily destroyed. Our results indicate that the test walls of *Ammonia aomoriensis* cracked or dissolved at the high pCO_2 of 3130 µatm, first in inactive or dead specimens, then in small and finally in large specimens.

I.4.4 Ecological effect

Ammonia species are the most successful colonizers in near-coastal environments, and well-known opportunists, able to tolerate environmental stress (e.g. Almogi-Labin et al., 1995, Debenay et al., 1998, 2009). In the western Baltic Sea, *A. beccarii* was considered an invasive species, which arrived from the North Sea and finally colonised the area in the 20th century (Polovodova et al., 2009, Schweizer et al., 2010).

Field studies of Polovodova et al. (2009) showed fine porosity on the tests of living *Ammonia* from Flensburg Fjord (Station PF16–25). In general, pores were seen to be joined in places, and tests showed signs of secondary calcification – e.g. regeneration scars. The observed porosity of *Ammonia* tests caused by dissolution may be explained by seasonal changes of the carbonate system under natural conditions (Table 1) and the ability of *Ammonia* to regenerate tests when conditions become less corrosive (Le Cadre et al., 2003). This is in contrast to the constantly elevated CO_2 levels simulated in our laboratory experiment.

While open ocean pCO_2 levels of 1829 (pH 7.4) and 3130 µatm (pH 7.2), as produced in this study, are not projected to develop due to ocean acidification in the near future, such conditions are already prevailing today in seasonally or permanently suboxic waters, including our sampling site in Flensburg Fjord. Because of the low buffering capacity of Baltic Sea water and the widespread seasonal undersaturation of portions of its bottom waters (Thomsen et al., 2010), the Baltic Sea is considered particularly vulnerable to acidification. Based on the results of this study, the resultant reduced calcification and shell dissolution of *A. aomoriensis* could lead to its disappearance from the Baltic Sea during the course of this century. This will also lead to changes in the community structure of benthic foraminifera (Watkins, 1961, Schafer, 1973, Ellison et al., 1986, Sharifi et al., 1991, Alve, 1991, Yanko et al., 1998, Thomas et al., 2000, Debenay et al., 2001) and may induce shifts in the benthic ecosystem of the SW Baltic Sea.

I.5 Conclusions

Ammonia aomoriensis exhibited reduced calcification and increased test dissolution at elevated pCO_2 levels and lowered pH. Decalcification started with loss of the outer, thinner chambers at a pCO_2 of 929 µatm. Total decalcification, when chambers were destroyed and the inner organic lining became visible, began at a pCO_2 of 3130 µatm. Our observations indicate that dissolution of

calcified structures progressed both from the inner (cytoplasm) surface and the outer (seawater) surface. Primary calcite is affected before secondary calcite. Observed loss and mortality rates suggest that living cells of *A. aomoriensis* are able to withstand and cope with dissolution up to a certain pCO_2 level. We have already achieved pCO_2 levels in the range from 1829 (pH 7.4) to 3130 µatm (pH 7.2) during the seasonal cycle in shallow areas of Flensburg Fjord. With progressing CO_2-induced acidification this may eventually lead to conditions inducing significant changes in the composition of benthic foraminiferal communities in our study area as well as in other regions experiencing naturally high bottom-water pCO_2 levels.

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Chapter II

The benthic foraminiferal community in a naturally CO_2-rich coastal habitat of the southwestern Baltic Sea

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Abstract

It is expected that the calcification of foraminifera will be negatively affected by the ongoing acidification of the oceans. Compared to the open oceans, these organisms are subjected to much more adverse carbonate system conditions in coastal and estuarine environments such as the southwestern Baltic Sea, where benthic foraminifera are abundant. This study documents the seasonal changes of carbonate chemistry and the ensuing response of the foraminiferal community with bi-monthly resolution in Flensburg Fjord. In comparison to the surface pCO_2, which is close to equilibrium with the atmosphere, we observed large seasonal fluctuations of pCO_2 in the bottom and sediment pore waters. The sediment pore water pCO_2 was constantly high during the entire year ranging from 1244 to 3324 µatm. Nevertheless, in contrast to the bottom water, sediment pore water was slightly supersaturated with respect to calcite as a consequence of higher alkalinity (A_T) for most of the year. Foraminiferal assemblages were dominated by two calcareous species, *Ammonia aomoriensis* and *Elphidium incertum*, and the agglutinated *Ammotium cassis*. The one-year cycle was characterized by seasonal community shifts. Our results revealed that there is no dynamic response of foraminiferal population density and diversity to elevated sediment pore water pCO_2. Surprisingly, the fluctuations of sediment pore water undersaturation (Ω_{calc}) co-vary with the population densities of living *Ammonia aomoriensis*. Further, we observed that most of the tests of living calcifying foraminifera were intact. Only *Ammonia aomorienis* showed dissolution and recalcification structures on the tests, especially at undersaturated conditions. Therefore, the benthic community is subjected to high pCO_2 and tolerates elevated levels as long as sediment pore water remains supersaturated. Model calculations inferred that increasing atmospheric CO_2 concentrations will finally lead to a perennial undersaturation in sediment pore waters. Whereas benthic foraminifera indeed may cope with a high sediment pore water pCO_2, the steady undersaturation of sediment pore waters would likely cause a significant higher mortality of the dominating *Ammonia aomoriensis*. This shift may eventually lead to changes in the benthic foraminiferal communities in Flensburg Fjord, as well as in other regions experiencing naturally undersaturated Ω_{calc} levels.

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II.1 Introduction

The combustion of fossil fuels and deforestation has already released about 300 Gt carbon (Archer, 2005). The release of carbon leads to rising atmospheric carbon dioxide concentrations, which causes an acidification of the oceans (Zeebe and Wolf-Gladrow, 2001). By 2100, the concentration of the ocean pCO_2 is expected to be approximately 750 µatm (Feely et al., 2004, Raven et al., 2005) and seawater pH is going to decrease by 0.4 units (Caldeira and Wickett, 2005). The reduced saturation state and carbonate ion concentration will cause a reduction in biogenic calcification of predominant organisms like corals, coccolithophorids and foraminifera (Gattuso et al., 1998, Kleypas et al., 1999, Bijma et al., 1999, Riebesell et al., 2000). Consequently, corrosive conditions are expected to affect the formation of carbonate skeletons of calcifying organisms (Erez, 2003, Raven et al., 2005).

Already today, calcifying organisms such as foraminifera are subjected to much more adverse carbonate system conditions in coastal marine environments as compared to the open ocean (Borges and Gypens, 2010). Especially environments such as the western Baltic Sea, which are subjected to a low salinity and alkalinity, are characterised by low [CO_3^{2-}] and consequently lower calcium carbonate saturation states (Ω_{calc}) (Thomsen et al., 2010). Furthermore, in this area seasonal stratification of water masses, respiration in deeper layers and eutrophication induce summer hypoxia in the bottom water layers. This causes high and variable pCO_2 and consequently low pH during the course of the year (Diaz and Rosenberg, 2008, Conley et al., 2009, Nikulina and Dullo, 2009, Thomsen et al., 2010). In such habitats, ongoing oceanic CO_2 uptake will cause a drastic increase of the prevailing pCO_2 levels with peaks up to 4000 µatm by the year 2100 (Melzner et al., 2012).

Many laboratory studies have shown that calcareous foraminifera exhibited lower calcification rates under simulated future scenarios of high seawater pCO_2 (Le Cadre et al., 2003, Kuroyanagi et al., 2009, Allison et al., 2010, Haynert et al., 2011, Fujita et al., 2011). To date, a low number of field studies reported that calcifying organisms are negatively affected by a high pCO_2 in natural habitats (Fabricius et al., 2011). In proximity to hydrothermal vents, where volcanic CO_2 causes a natural decline of pH, a significant decrease in abundance and species richness of calcareous foraminifera was observed between ambient pH levels of 8.09 to 8.15 and low pH-levels of 7.08 and 7.79 close to the vents (Cigliano et al., 2010).

Calcareous benthic foraminifera are common in the SW Baltic Sea, although seawater carbonate concentrations are permanently low and even seasonally undersaturated (Lutze, 1974, Wefer, 1976, Grobe and Fütterer, 1981, Polovodova et al., 2009, Thomsen et al., 2010, Haynert et al., 2011). Salinity, temperature, oxygen, and food availability were considered as important factors, which regulate the foraminiferal diversity and abundance (e.g. Rottgardt, 1952, Bradshaw, 1957, Lutze, 1965, Wefer, 1976, Alve and Murray, 1999, Frenzel et al., 2005). These studies, however, did not take into account the impact of seawater carbonate chemistry.

Living benthic foraminiferal assemblages in Flensburg Fjord were first described by Exon (1972). Some specimen of *Ammonia aomoriensis* from this area were reported as having thin

or opaque shell walls and extremely corroded tests (Polovodova et al., 2009). In some cases, the tests were completely destroyed and only the inner organic lining was left. Abrasion and predation were considered as possible mechanisms for test destruction, but test dissolution due to fluctuated pH has been suggested as the most likely cause for the corroded *Ammonia* tests in that area (Polovodova and Schönfeld, 2008). Indeed, similar signs of test dissolution were observed, when living specimen of *Ammonia aomoriensis* from Flensburg Fjord were exposed to elevated pCO_2 levels from 929 to 3130 µatm in a laboratory experiment (Haynert et al., 2011).

Natural CO_2-rich habitats can serve as valuable examples for possible effects on calcifying benthic community structures due to climate change (Hall-Spencer et al., 2008, Thomsen et al., 2010). Our study site in Flensburg Fjord, SW Baltic Sea represents an adequate study area for dynamic response of the foraminiferal fauna to elevated pCO_2. The consequences of naturally CO_2-enriched environments on benthic foraminifera are not sufficiently studied to date.

The aim of this study was to investigate the response of the foraminiferal population dynamics, as well as the variations of species composition and diversity to a high pCO_2 and low Ω_{calc} conditions over a one-year cycle. The main focus was on two calcifying species, *Ammonia aomoriensis* and *Elphidium incertum*. An effect of low sediment pore water carbonate saturation on population density and test dissolution of this species was assessed.

II.2 Study Site and sampling

Flensburg Fjord, located in the southwest of the Baltic Sea (53°41´–55°00´N, 9°24´–10°10´E), is a narrow and 50 km long inlet. The Fjord is subdivided into a 10–20 m deep inner fjord which extends from the city Flensburg to Holnis Peninsula. The area from Holnis Peninsula to Neukirchen/Kragesand is a 18–20 m deep middle fjord. The 10–32 m deep outer fjord comprises Soenderborg Bay, Gelting Bay and open waters to the east of Gelting Peninsula.

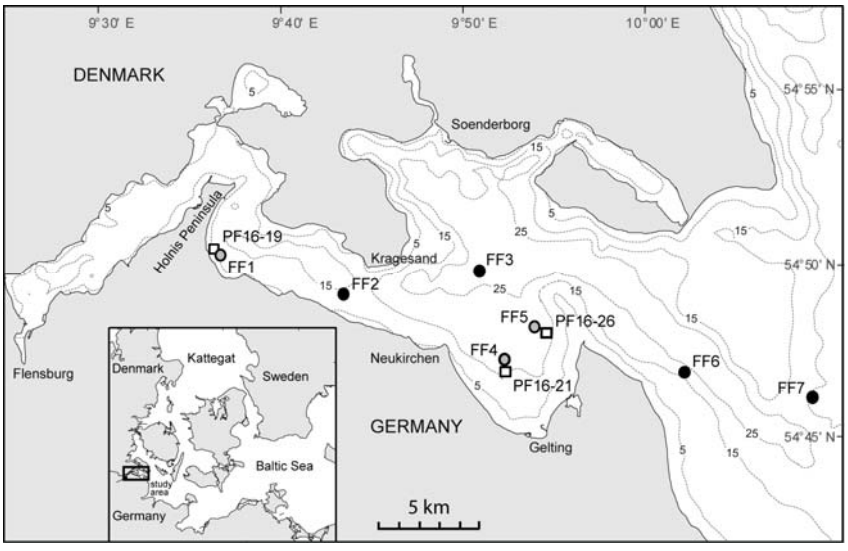

Fig. 1. Map of study area of Flensburg Fjord (design by courtesy of Anna Nikulina, GEOMAR). Insert indicates the location of study area within the SW Baltic Sea. Circles display sediment corer (FF1, FF4 and FF5) and water chemistry stations (FF1–FF7). White squares indicate sampling stations PF16–19, PF16–21 and PF16–26 of Polovodova et al. (2009) in June 2006.

Sediment and water samples were taken from seven stations (FF1 to FF7) on six bi-monthly cruises with R/V *Littorina* from June 2009 to April 2010 (Fig. 1). All seven stations (FF1 to FF7) were monitored for water carbonate chemistry. Sediment cores for foraminiferal studies were taken from stations FF1, FF4 and FF5. Station FF1 is located in a shallow near-coastal area, where sandy bottoms prevail (Table 1). At stations FF4 and FF5 muddy sands were encountered (Table 1). The cliff and submarine erosion are predominant sources for sediments, which are transported from the east by long shore drift toward the outer Flensburg Fjord (Exon, 1971).

Table 1. Sampling stations in Flensburg Fjord: specification, sampling device, latitude and longitude, water depth in metre, and sediment type at the corer stations (FF1, FF4 and FF5).

Station	Specification	Sampling device	Latitude [N]	Longitude [E]	Depth [m]	Sediment type
FF1	Corer station	MUC	54°50.50'	9°37.00'	13	sandy mud
PF16-19 (Polovodova and Schönfeld, 2008)	Corer station	Rumohr corer	54°50.20'	9°36.84'	10	sandy mud
FF2	Water chemistry station	CTD	54°49.00'	9°43.00'	18	-
FF3	Water chemistry station	CTD	54°50.00'	9°50.00'	27	-
FF4	Corer station	MUC	54°47.02'	9°51.37'	13	muddy sand
PF16-21 (Polovodova and Schönfeld, 2008)	Corer station	Rumohr corer	54°46.92'	9°51.26'	9	muddy sand
FF5	Corer station	MUC	54°48.02'	9°53.05'	13	muddy sand
PF16-26 (Polovodova and Schönfeld, 2008)	Corer station	Rumohr corer	54°48.28'	9°53.49'	8	muddy sand
FF6	Water chemistry station	CTD	54°47.00'	10°00.00'	22	-
FF7	Water chemistry station	CTD	54°46.00'	10°10.00'	23	-

II.3 Materials and methods

II.3.1 Foraminiferal processing

The foraminiferal communities were studied from surface sediments from stations FF1, FF4 and FF5. Benthic foraminiferal samples were taken with a Mini Muc K/MT 410 corer equipped with tubes of 60 cm length and 10 cm inner diameter. A plastic ring marked with 0.5 cm scale was used to slice the uppermost one centimetre of the sediment core. A thin grey spatula was gently moved between tube top and the plastic ring. The surface layer (0–1 cm) of sediment was safely removed from the core and transferred with a spoon into 300 ml Kautex^{TM} wide-neck containers. The sediment was preserved and stained with a Rose Bengal ethanol solution of 2 g l^{-1} according to Lutze and Altenbach (1991). Ethanol concentration was 94 %. Staining time was three weeks at minimum, to ensure that the protoplasm was completely impregnated with Rose Bengal in all tests of foraminifera that were living at the time of sampling.

In the laboratory, samples were first passed through a 2000 µm screen in order to remove molluscs shells and pebbles. Subsequently the samples were gently washed with tap water through a 63 µm sieve. The fractions 63–2000 µm and >2000 µm fractions were dried at 60 °C for at least 24 h. The fraction 63–2000 µm was split by using an Otto (1933) microsplitter to obtain aliquots of a manageable size. Subsequently, all size fractions were weighted and the fraction 63–2000 µm were quantitatively analysed for living and dead foraminifera. All Rose Bengal stained foraminifera were considered as living at the time of sampling, whereas unstained tests were considered as dead.

Living and dead specimens were picked from the respective aliquots, sorted by species, mounted in Plummer cell slides with glue, counted and measured. The dominant species were photographed by using a scanning electronic microscope (Cam Scan-CS-44) at the Institute of Geosciences, Kiel University.

In order to document the differences in test dissolution, foraminiferal tests were photographed using a scanning electronic microscope (Cam Scan-CS-44) and an electron probe microanalyser (Jeol JXA-8200 EPMA). Light micrographs were taken with a MiniPixie (MPX2051UC) digital microscope. The tests of living *A. aomoriensis* were subdivided into three dissolution stages: intact tests, dissolution of the last chamber, and dissolution of more than two chambers.

II.3.2 Carbonate chemistry

Temperature and salinity parameters of the surface and near-bottom water were recorded using a CTD48M probe (Sea&Sun Technology) at all stations (Tables 2 and 3). At water chemistry stations, samples for analyses of carbonate chemistry parameters were taken from the surface water at 1 m depth on stations FF1 to FF7 (Table 2), near-bottom water from 1 m above sea floor was taken at stations FF2, FF3, FF6 and FF7. Bottom water approximately 1 cm above the sediment surface and sediment pore water from 0 to 5 cm sediment depth was only collected at foraminifera sampling stations FF1, FF4 and FF5 (Table 3).

Surface and near-bottom water samples were taken using Niskin bottles and filled bubble free into 250 or 500 ml Duran^{TM} glass bottles. Samples were poisoned with 50 or 100 µl saturated mercury chloride solution and stored at room temperature until analysis. Total alkalinity (A_T) and total inorganic carbon (C_T) of the samples were measured by potentiometric titration using VINDTA autoanalyser and coulometric titration after CO_2 extraction using the SOMMA system, respectively (Mintrop et al., 2000, Dickson et al., 2007). Offset of total alkalinity (A_T) and total carbon (C_T) determinations (Tables 2 and 3) were assessed and corrected by measurements of certified reference material (Dickson et al., 2003). Seawater pH_{NBS}, pCO_2 and omega for calcite (Ω_{calc}) were calculated by using the CO2Sys-program developed by Lewis and Wallace (1998) (Tables 2 and 3). Dissociation constants K_1 and K_2 were chosen according to Mehrbach et al. (1973) as refitted by Dickson and Millero (1987) and the $KHSO_4$ dissociation constant after Dickson (1990).

Bottom water samples for carbonate system parameters were taken from the supernatant water of Minicorer-tubes and filled directly into 20 ml PVC bottles. For sediment pore water analyses, the sediment cores were sliced in 0.5 cm intervals up to 2 cm depth, below 2 cm the intervals were 1.0 cm up to 5 cm. Sediment samples from each interval were transferred to 50 ml centrifuge tubes and centrifuged at 3000 rpm for 30 to 40 min in order to separate the sediment pore water from the sediment. The extracted sediment pore water and the bottom water were transferred through 0.2 µm steril filters into 20 ml PVC bottles. Bottom and sediment pore water pH_{NBS} were measured using a WTW 340i with a precision of ±0.01. The pH electrode was calibrated using standard buffer solutions of pH 4.01, 7.00 and 10.00 (WTW standard, DIN/NIST buffers L7A). Subsequently, bottom and sediment pore water alkalinity was determined with a Metrohm titration instrument according to Ivanenkov and Lyakhin (1978). A greenish-brown Methyl-Red and Methylene-Blue indicator was

added, and titration was performed with 0.02 M HCl and finished until a stable light pink colour occurred. During titration, the sample was degassed by continuously bubbling nitrogen through the solution in order to remove the generated CO_2 or H_2S. The measured values were standardized using an IAPSO seawater solution. The precision of the alkalinity measurements was 0.37 %. The carbonate system parameters of bottom and sediment pore water, total carbon (C_T), pCO_2 and omega for calcite (Ω_{calc}) were calculated from measured pH_{NBS} and total alkalinity (A_T) according to dissociation constants as specified above.

II.4 Results

II.4 .1 Temperature and salinity

Surface and near-bottom water temperature and salinity from stations FF1 to FF7 in Flensburg Fjord were characterised by pronounced seasonal fluctuations, prevailing in the area of the Baltic Sea. Temperature ranged from -0.9 to 20 °C at the surface and from -0.8 to 15.3 °C at the bottom during the investigation period (Tables 2 and 3).

A stable thermocline from 7 to 8 m water depth stratified the water column between June and August 2009. From December 2009 to April 2010, the water column was well mixed with a temperature of 5 °C on average in both, surface and near-bottom water. The surface of Flensburg Fjord was covered by floating ice in February, during that time the lowest temperatures were observed, ranging from −0.9 to 1.1 in the surface and near-bottom water (Tables 2 and 3).

Mean salinity ranged from 13.3 to 21.1 at the surface, and 16.8 to 26.3 in the bottom water (Tables 2 and 3). The salinity increased from the surface (15.7) to the near-bottom water (21.4), caused a persistent pycnocline from spring to summer. Mixing in October caused a homogenous salinity in the water column of approximately 22. A slight halocline in December caused again a lower mean salinity of 18.1 in the surface and a higher value of 22.8 in the bottom water (Tables 2 and 3). In February, the boundary layer between the surface and near-bottom water was dissipated and a uniform salinity of 17 was observed.

II.4 .2 Carbonate chemistry

Carbonate chemistry measurements revealed a relatively stable surface pCO_2 during the whole year (Fig. 2a). In contrast, pH and pCO_2 in the bottom and sediment pore water showed a high variability during the seasonal cycle in Flensburg Fjord (Fig. 2b and c).

Surface pCO_2 (478 ± 197 µatm) was close to atmospheric levels with slightly lower values during the spring bloom, similarly pH (8.13 ± 0.15) was relatively high and stable (Table 2, Fig. 2a). In general, the western Baltic Sea is characterized by a low salinity, ranging from 13 to 21, and consequently a low alkalinity (A_T) of 1821 to 2057 µmol kg^{-1} prevailed in the surface water (Table 2). Consequently, the calcium carbonate saturation state for calcite (Ω_{calc}) was low in this area. During the monitoring, we recorded a mean surface Ω_{calc} of 1.84 ± 0.70 in 2009 and 2010.

Undersaturation of the surface water was observed in February, with Ω_{calc} values ranging from 0.40 to 0.94 (Table 2).

Table 2. Flensburg Fjord surface seawater chemistry speciation 2009 to 2010 at the sampling stations (FF1–FF7). Temperature and salinity were recorded using a CTD48M probe. Analyses for total alkalinity (A_T) and dissolved inorganic carbon (C_T) were measured by coulometric and potentiometric titration using SOMMA and VINDTA systems. pH_{NBS}, carbon dioxide partial pressure (pCO_2) and omega of calcite (Ω_{calc}) were calculated using the CO2Sys-software.

Surface water							
Station	Temperature [°C]	Salinity	pH_{NBS}	A_T [µmol kg^{-1}]	C_T [µmol kg^{-1}]	pCO_2 [µatm]	Ω_{calc}
FF1 (1 m)							
02.06.2009	16.0	15.8	8.24	1933.3	1820.3	368	2.62
18.08.2009	17.7	18.6	8.05	1973.8	1891.6	585	2.05
20.10.2009	10.3	21.1	8.10	2056.8	1975.0	492	1.93
07.12.2009	6.7	17.3	8.09	1972.9	1923.8	499	1.45
15.02.2010							
19.04.2010	7.6	15.7	8.06	1900.4	1862.4	532	1.30
FF2 (1 m)							
02.06.2009	15.9	15.6	8.23	1918.5	1810.0	375	2.54
18.08.2009	17.8	18.6	8.19	1988.2	1862.7	410	2.78
20.10.2009	10.9	20.8	8.03	2038.0	1972.4	575	1.70
07.12.2009	6.8	17.4	8.08	1980.3	1932.0	509	1.44
15.02.2010	-0.8	17.2	7.66	1904.8	1972.0	1249	0.40
19.04.2010	7.8	15.2	8.20	1889.1	1822.2	380	1.72
FF3 (1 m)							
02.06.2009	16.3	15.4	8.21	1907.0	1805.4	398	2.42
18.08.2009	18.2	18.1	8.27	1976.8	1826.6	336	3.24
20.10.2009	10.7	20.7	8.10	2030.8	1948.1	484	1.93
07.12.2009	7.9	18.4	8.03	1982.4	1939.2	577	1.37
15.02.2010	-0.9	17.1	8.23	1952.5	1897.5	325	1.46
19.04.2010	7.4	15.0	8.30	1890.2	1800.5	294	2.10
FF4 (1 m)							
02.06.2009							
18.08.2009							
20.10.2009							
07.12.2009	7.8	18.6	8.03	1980.3	1936.5	576	1.37
15.02.2010	-0.8	17.1	7.78	1912.0	1950.6	941	0.53
19.04.2010	8.0	14.3	8.34	1882.2	1784.0	271	2.27
FF5 (1 m)							
02.06.2009							
18.08.2009							
20.10.2009	10.7	20.7	8.09	2032.0	1954.2	502	1.88
07.12.2009	8.0	18.6	7.99	1980.0	1943.0	622	1.29
15.02.2010	-0.8	17.1	7.85	1949.6	1974.7	820	0.62
19.04.2010	7.3	14.9	8.22	1874.8	1805.0	355	1.76
FF6 (1 m)							
02.06.2009	15.6	15.0	8.21	1881.6	1785.2	392	2.32
18.08.2009	18.6	17.0	8.29	1934.9	1787.9	325	3.23
20.10.2009							
07.12.2009	7.9	19.0	8.08	1993.5	1935.5	507	1.57
15.02.2010	-0.8	16.8	8.25	1936.4	1877.9	307	1.50
19.04.2010	6.7	13.3	8.32	1830.2	1749.3	281	1.96
FF7 (1 m)							
02.06.2009	14.5	15.1	8.22	1890.9	1796.0	385	2.28
18.08.2009	20.0	15.9	8.25	1893.9	1762.5	361	3.00
20.10.2009	10.8	19.9	8.23	1957.8	1845.9	346	2.37
07.12.2009	7.0	17.2	8.16	1926.1	1860.7	408	1.66
15.02.2010	-0.9	16.2	8.05	1903.7	1889.0	495	0.94
19.04.2010	6.7	13.9	8.12	1821.0	1781.7	455	1.29

Table 3. Water chemistry parameters of the near-bottom water (1m above the sea floor) at stations FF2, FF3, FF6 and FF7 and of the bottom water (1 cm above the sediment surface) at stations FF1, FF4 and FF5 from June 2009 to April 2010. Temperature and salinity were measured by CTD48M probe at all stations from FF1 to FF7. At stations FF1, FF4 and FF5, the bottom water pH_{NBS} were measured using a WTW 340i. Analysis of total alkalinity (A_T) was determined with a Metrohm titration instrument. Dissolved inorganic carbon (C_T), carbon dioxide partial pressure (pCO_2), and omega calcite (Ω_{calc}) were calculated using the CO2Sys-program. At stations FF2, FF3, FF6 and FF7, analyses for total alkalinity (A_T) and dissolved inorganic carbon (C_T) were measured by coulometric and potentiometric titration using SOMMA and VINDTA systems. pH_{NBS}, carbon dioxide partial pressure (pCO_2) and omega of calcite (Ω_{calc}) were calculated using the CO2Sys-software.

Near-bottom and bottom water							
Station	Temperature [°C]	Salinity	pH_{NBS}	A_T [µmol kg^{-1}]	C_T [µmol kg^{-1}]	pCO_2 [µatm]	Ω_{calc}
Bottom water FF1 (13 m)							
02.06.2009	13.2	19.9	7.63	2388.4	2385.6	1337	1.19
18.08.2009	14.6	20.0	7.54	2199.5	2213.7	1536	0.95
20.10.2009	10.5	21.0	7.29	2465.7	2581.7	3074	0.53
07.12.2009	9.1	21.5	7.84	2174.0	2126.6	700	1.51
15.02.2010							
19.04.2010	5.7	18.7	7.86	2353.5	2322.2	739	1.41
Near-bottom water FF2 (18 m)							
02.06.2009	7.2	21.2	7.86	2060.1	2046.6	857	1.04
18.08.2009	11.6	21.6	7.40	2079.7	2173.1	2661	0.45
20.10.2009	11.0	21.4	7.93	2073.6	2030.7	754	1.40
07.12.2009	9.2	23.0	7.98	2053.7	1998.2	631	1.52
15.02.2010	-0.8	17.2	8.03	1951.5	1938.3	529	0.94
19.04.2010	4.3	19.4	8.03	2023.9	1987.5	557	1.26
Near-bottom water FF3 (27 m)							
02.06.2009	7.4	23.5	7.92	2123.3	2085.1	735	1.32
18.08.2009	11.1	23.4	7.45	2117.9	2193.1	2348	0.53
20.10.2009	13.2	23.4	7.49	2093.6	2149.8	2168	0.61
07.12.2009	9.0	24.0	8.02	2076.3	2006.2	568	1.70
15.02.2010	-0.2	17.5	8.08	1985.8	1958.9	478	1.11
19.04.2010	2.7	22.4	7.55	2022.4	2095.3	1631	0.45
Bottom water FF4 (13 m)							
02.06.2009	15.1	20.4	7.52	2367.3	2350.3	1267	1.34
18.08.2009	12.8	21.1	7.43	2236.1	2283.7	1982	0.72
20.10.2009	11.2	22.4	7.21	2360.4	2491.4	3429	0.45
07.12.2009	8.6	24.1	7.85	2187.8	2131.2	673	1.60
15.02.2010	-0.4	16.8	7.81	1816.8	1823.3	634	0.70
19.04.2010	4.8	19.0	7.88	2234.5	2201.5	655	1.35
Bottom water FF5 (13 m)							
02.06.2009	10.3	20.2	7.83	2125.5	2082.8	727	1.45
18.08.2009	15.3	19.8	7.47	2239.1	2270.8	1861	0.85
20.10.2009	11.9	21.6	7.29	2465.7	2573.0	3083	0.57
07.12.2009	8.8	20.9	7.81	2174.0	2138.9	769	1.36
15.02.2010	-0.4	16.9	7.94	1804.6	1784.3	465	0.98
19.04.2010	5.6	18.8	7.94	2374.9	2321.6	604	1.70
Near-bottom water FF6 (22 m)							
02.06.2009	7.6	22.0	7.87	2070.3	2051.0	843	1.10
18.08.2009	11.2	23.8	7.50	2115.9	2175.3	2091	0.59
20.10.2009							
07.12.2009	9.0	23.5	8.01	2056.1	1992.4	585	1.62
15.02.2010	-0.8	17.2	8.19	1960.1	1913.6	362	1.34
19.04.2010	2.9	21.4	7.68	2038.9	2081.0	1248	0.59
Near-bottom water FF7 (23 m)							
02.06.2009	8.7	26.3	7.87	2082.3	2043.1	796	1.30
18.08.2009	11.9	22.9	7.46	2089.3	2161.3	2326	0.53
20.10.2009	13.4	24.7	8.00	1985.0	1904.1	588	1.84
07.12.2009	9.0	22.9	8.02	2050.6	1986.8	577	1.62
15.02.2010	1.1	18.6	7.96	1974.5	1964.2	624	0.93
19.04.2010	2.9	22.8	7.63	2072.0	2122.5	1379	0.56

Stratification of the water column causes a strong CO_2-accumulation in the bottom water during summer and autumn. Therefore, large seasonal fluctuations of pCO_2, pH and Ω_{calc} were observed in the near-bottom and bottom water. One metre above the sediment, the mean near-bottom water pCO_2 was 1120 ± 82.86 µatm. In comparison, the bottom water pCO_2 (1 cm above sediment) increased to 1390 ± 71.63 µatm (Table 3). Highest pCO_2 levels reached up to 2000 µatm during August in the near-bottom water and up to 3000 µatm during October in the bottom water a few cm above the benthic boundary (Table 3, Fig. 2b). This caused lowest pH values of 7.40 and 7.21 in the near-bottom and bottom waters during August and October (Table 3). After mixing of the water column, pCO_2 decreased in winter to mean values of 550 ± 65.44 and 657 ± 132.07 µatm (Table 3). Similarly, mean pH showed the highest value of 7.91 in the near-bottom water and 7.85 in the bottom water (Table 3). The calculated mean Ω_{calc} values in the near-bottom water (1.08 ± 0.07) and bottom water (1.10 ± 0.05) were low compared to surface Ω_{calc} and varied between the studied stations (Table 3). The near-bottom and bottom waters of Flensburg Fjord were frequently undersaturated for Ω_{calc} with a lowest value of 0.45 in August and October (Table 3).

Table 4. Seawater carbonate chemistry of bottom water (1 cm above the sediment surface) and sediment sediment pore water (0–1 cm) at stations FF1, FF4 and FF5 during the one year cycle. Bottom and sediment pore water pH_{NBS} were measured using a WTW 340i. Total alkalinity (A_T) was determined with a Metrohm titration instrument. Dissolved inorganic carbon (C_T), carbon dioxide partial pressure (pCO_2), and omega calcite (Ω_{calc}) were calculated using the CO2Sys-software.

	bottom water	sediment pore water 0-1 cm	bottom water	sediment pore water 0-1 cm	bottom water	sediment pore water 0-1 cm	bottom water	sediment pore water 0-1 cm	bottom water	sediment pore water 0-1 cm
Station	pH_{NBS}	pH_{NBS}	A_T [µmol kg^{-1}]	A_T [µmol kg^{-1}]	C_T [µmol kg^{-1}]	C_T [µmol kg^{-1}]	pCO_2 [µatm]	pCO_2 [µatm]	Ω_{calc}	Ω_{calc}
					FF1					
02.06.2009	7.63	7.52	2388.4	2684.3	2385.6	2716.3	1337	1968	1.19	1.07
18.08.2009	7.54	7.53	2199.5	2833.6	2213.7	2858.8	1536	2058	0.95	1.22
20.10.2009	7.29	7.54	2465.7	3576.4	2581.7	3623.9	3074	2433	0.53	1.38
07.12.2009	7.84	7.61	2174.0	2694.9	2126.6	2709.8	700	1512	1.51	1.15
15.02.2010										
19.04.2010	7.86	7.54	2353.5	2610.3	2322.2	2668.7	739	1746	1.41	0.77
					FF4					
02.06.2009	7.52	7.36	2367.3	2726.6	2350.3	2806.8	1267	2965	1.34	0.80
18.08.2009	7.43	7.44	2236.1	2995.1	2283.7	3063.5	1982	2699	0.72	0.98
20.10.2009	7.21	7.69	2360.4	3062.2	2491.4	3050.2	3429	1709	0.45	1.76
07.12.2009	7.85	7.55	2187.8	2577.7	2131.2	2604.3	673	1631	1.60	0.99
15.02.2010	7.81	7.56	1816.8	2067.4	1823.3		634	1292	0.70	0.46
19.04.2010	7.88	7.43	2234.5	2861.0	2201.5	2972.4	655	2494	1.35	0.64
					FF5					
02.06.2009	7.83	7.69	2125.5	3281.1	2082.8	3274.1	727	1573	1.45	1.69
18.08.2009	7.47	7.60	2239.1	2496.7	2270.8	2494.8	1861	1545	0.85	1.29
20.10.2009	7.29	7.54	2465.7	3576.4	2573.0	3613.7	3083	2437	0.57	1.49
07.12.2009	7.81	7.70	2174.0	2740.2	2138.9	2731.1	769	1244	1.36	1.39
15.02.2010	7.94	7.61	1804.6	2494.0	1784.3		465	1593	0.98	0.63
19.04.2010	7.94	7.42	2374.9	3273.5	2321.6	3421.6	604	3324	1.70	0.78

The carbonate chemistry of the sediment pore waters strongly deviated from the conditions in the water column. Sediment pore water pCO_2 from the depth-interval 0 to 1 cm, did not fluctuate as strong as the bottom water. It was noticeable that the pCO_2 was high during the whole year and ranged from 1244 to 3324 µatm (Table 4, Fig. 2c). Mean sediment pore water pCO_2 of the 0–1 cm depth-interval was 2013 ± 610 µatm, pH (7.55 ± 0.10) was lower, but more stable in comparison to the water column (Table 4, Fig. 2c). In contrast, the pH-profile of the sediment pore water revealed considerable fluctuations within the 1 and 5 cm depth interval, ranging from 6.82 to 8.11 (Fig. 3).

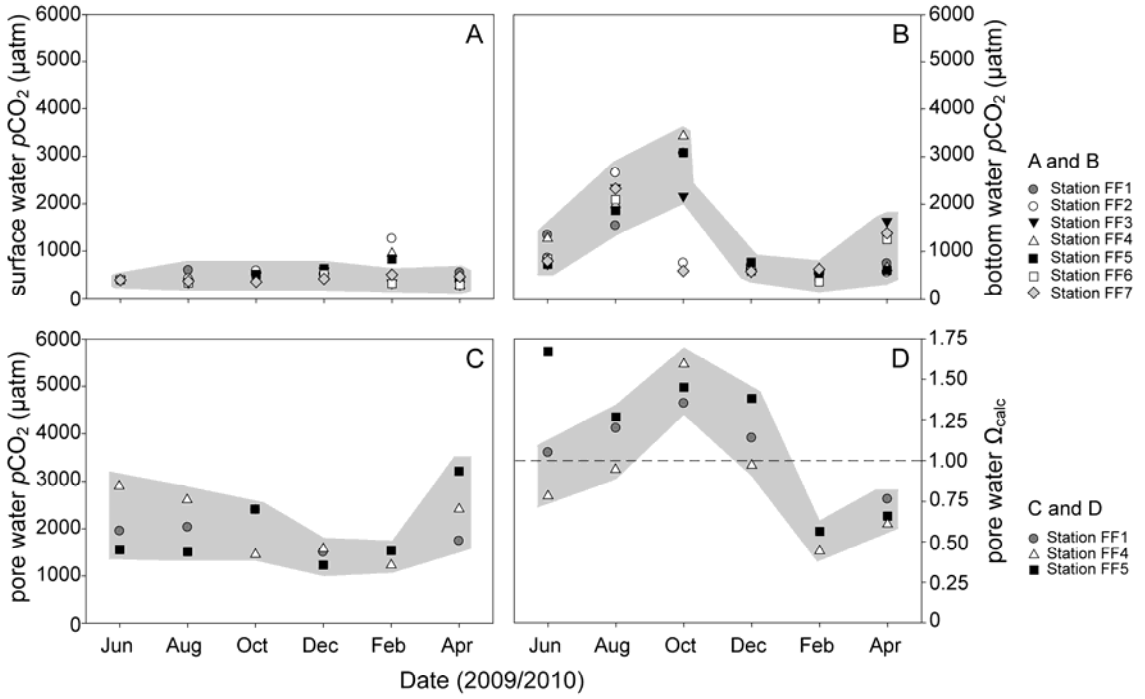

Fig. 2. (A and B) surface water, near-bottom water, and bottom water pCO_2 at sampling stations from FF1 to FF7; (C and D) sediment pore water pCO_2 and Ω_{calc} at stations FF1, FF4 and FF5 from June 2009 to April 2010.

Furthermore, the pH-fluctuations varied also between the sampling stations and during the seasonal cycle. No trend was observed in the 5 cm depth-interval. Compared to the bottom water A_T (2233 ± 190 µmol kg^{-1}), the sediment pore water alkalinity was much higher (2856 ± 400 µmol kg^{-1}) which causes a relative high, slightly supersaturated Ω_{calc} of 1.09 ± 0.38 (Table 4, Fig. 2d). Only sediments at station FF4 were consistently undersaturated for Ω_{calc} with the lowest value of 0.46 in February (Table 4, Fig. 2d).

II.4 .3 Foraminiferal population density and species composition

Population density of the living foraminiferal fauna in Flensburg Fjord ranged from 15 to 223 ind. 10 cm^{-3}, on average 68 ind. 10 cm^{-3}. The abundance of dead specimens ranged from 16 to 454 tests 10 cm^{-3}, on average 127 tests 10 cm^{-3}. The assemblages consisted of six calcareous species: *Ammonia aomoriensis*, *Elphidium albiumbilicatum, Elphidium excavatum clavatum, Elphidium excavatum excavatum, Elphidium gerthi* and *Elphidium incertum*, and two arenaceous species *Ammotium cassis* and *Reophax dentaliniformis* (Fig. 6). Foraminiferal faunas were dominated by *A. aomoriensis*, *E. incertum* and *A. cassis* (Fig. 4). The specimens of common to rare species, which were occasionally present in the foraminiferal assemblages, were combined to one group, called “Other” (Tables 5 and 6).

Calculations of two diversity indices, Shannon-Wiener-Index and Fishers alpha, exhibited low values at all stations which indicates a low diversity of living and dead assemblages. There is a maximum of 8 species constituting the community. Hence, any changes in assemblages composition will induce only insignificant differences of diversity.

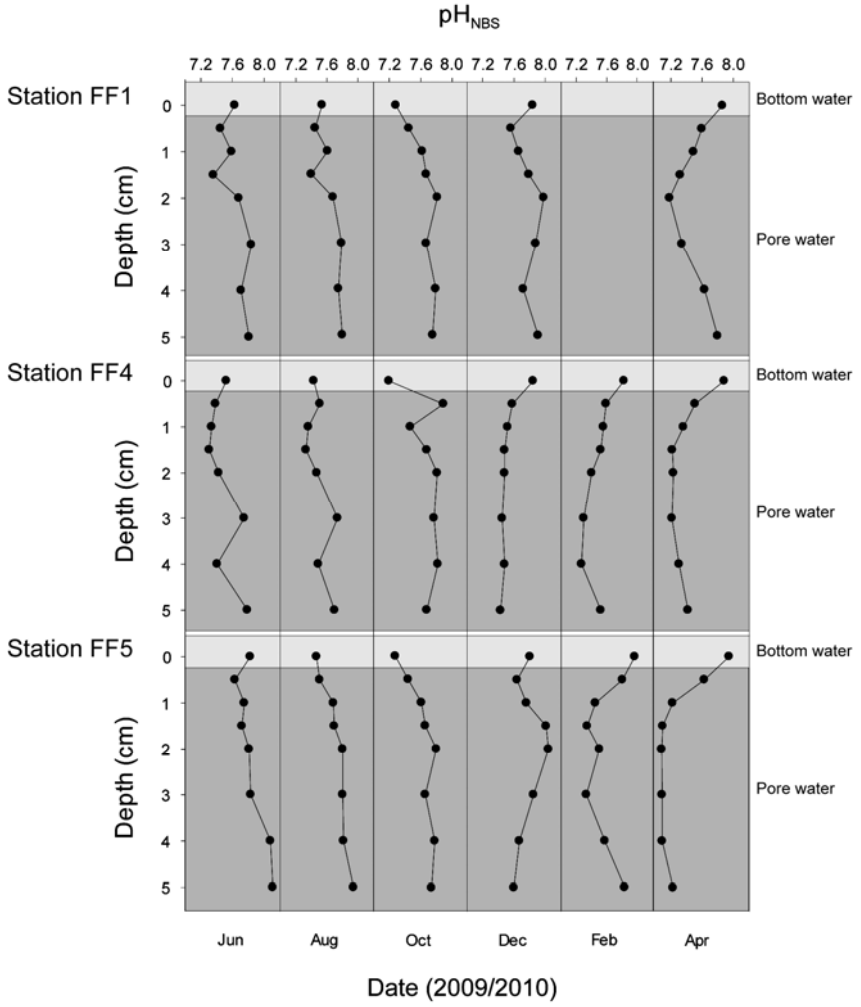

Fig. 3. Bottom and sediment pore water profiles of pH$_{NBS}$ plotted vs. sediment depth (cm) at stations FF1, FF4 and FF5 during the seasonal cycle (2009/2010).

II.4 .3.1 Living assemblages

Stations FF1 and FF5 showed a similar trend of population density and composition of living species during the seasonal cycle (Fig. 4). FF1 is located in the middle part of the fjord, where the sediment consists of sandy mud, whereas station FF5 is located in the outer fjord of Flensburg where muddy sand prevailed (Fig. 1). Maximum numbers of 101 and 129 ind. 10 cm^{-3} were observed in October at stations FF1 and FF5, when *A. aomoriensis* was frequent with 49 and 72 % (Fig. 4, Table S1 in the supplement). At station FF1, *A. aomoriensis* was also frequent in April with 61 %, and it was common with 17 % in August. *Elphidium incertum* dominated with 52 and 48 % during summer, and *A. cassis* was rather rare with 1 % (Fig. 4, Table S1 in the supplement). In contrast, *E. incertum* was the dominant species with 34 % on average during the whole year at station FF5 (Table 5). The arenaceous species *A. cassis* was very frequent in August and in February with 63 and 37 % (Table 5). At station FF4, which was also located in the outer Fjord, *E. incertum* was the dominant species during the whole year and showed a maximum of 94 % in April. In comparison, *A. aomoriensis* was rare, ranging from 0 to 9 % (Fig. 4). *A. cassis* achieved maximum proportions of 36 % in December (Table 5).

Table 5. List of living foraminiferal assemblages collected at the studied stations (FF1, FF4 and FF5) of Flensburg Fjord between June 2009 and April 2010, size fraction 63–2000 µm.

Living foraminiferal species 0-1 cm > 63 µm	June 02.06.2009	%	August 18.08.2009	%	October 20.10.2009	%	December 07.12.2009	%	February 15.02.2010	%	April 19.04.2010	%
Station FF1												
Species	25	25.3	37	16.7	132	48.7	23	43.4			69	61.1
Ammonia aomoriensis	1	1.0	2	0.9	42	15.5	2	3.8			1	0.9
Elphidium albiumbilicatum	5	5.1	28	12.7	53	19.6	4	7.5			7	6.2
Elphidium excavatum clavatum	16	16.2	33	14.9	24	8.9	16	30.2			18	15.9
Elphidium excavatum excavatum	1	1.0	16	7.2								
Elphidium gerthi	51	51.5	105	47.5	12	4.4	8	15.1			16	14.2
Elphidium incertum	99		221		263		53				111	
Total number of calcareous individuals											1	0.9
Ammotium cassis					8	3.0					1	0.9
Reophax dentaliniformis	0		0		8		0				2	
Total number of agglutinated individuals	99		221		271		53				113	
Total number of living specimens	6		6		6		5				7	
Species number	47		76		56		60				94	
Sample volume (cm^3)	0.5252		0.4585		0.4802		0.4791				0.5210	
Split (n)	40.1		63.4		100.8		18.4				23.1	
Population density (ind. 10 cm^{-3})												
Station FF4												
Species	3	8.3	9	9.3	2	2.6	4	4.5	2	1.1		
Ammonia aomoriensis			4	4.1								
Elphidium albiumbilicatum			11	11.3	1	1.3						
Elphidium excavatum clavatum			19	19.6	1	1.3						
Elphidium excavatum excavatum												
Elphidium gerthi	28	77.8	51	52.6	57	74.0	52	59.1	160	92.0	77	93.9
Elphidium incertum	31		94		61		56		162		77	
Total number of calcareous individuals	2	5.6	1	1.0	15	19.5	32	36.4	12	6.9	5	6.1
Ammotium cassis	3	8.3	2	2.1	1	1.3						
Reophax dentaliniformis	5		3		16		32		12		5	
Total number of agglutinated individuals	36		97		77		88	100	174		82	
Total number of living specimens	4		7		6		3		3		2	
Species number	59		95		100		93		73		59	
Sample volume (cm^3)	0.0663		0.2351		0.5080		0.4769		0.4913		0.0623	
Split (n)	92.0		43.4		15.2		19.8		48.5		223.0	
Population density (ind. 10 cm^{-3})												
Station FF5												
Species	13	14.9	12	7.0	63	72.4	64	28.6	6	6.6	5	5.7
Ammonia aomoriensis	8	9.2	2	1.2	2	2.3	19	8.5	1	1.1		
Elphidium albiumbilicatum	12	13.8			3	3.4	24	10.7			1	1.1
Elphidium excavatum clavatum	29	33.3	4	2.3	12	13.8	63	28.1	2	2.2	1	1.1
Elphidium excavatum excavatum	1	1.1			3	3.4	1	0.4				
Elphidium gerthi	18	20.7	44	25.7	4	4.6	50	22.3	47	51.6	69	79.3
Elphidium incertum	81		62		87		221		56		76	
Total number of calcareous individuals	1	1.1	108	63.2			1	0.4	34	37.4	11	12.6
Ammotium cassis	5	5.7	1	0.6			2	0.9	1	1.1		
Reophax dentaliniformis	6		109		0		3		35		11	
Total number of agglutinated individuals	87		171		87		224	100	91		87	
Total number of living specimens	8		6		6		8		6		5	
Species number	47		67		51		64		76		48	
Sample volume (cm^3)	0.1239		0.5340		0.1324		0.5071		0.5456		0.2996	
Split (n)	149.3		47.8		128.9		69.0		21.9		60.5	
Population density (ind. 10 cm^{-3})												

Table 6. Foraminiferal census data of dead species collected at the studied stations (FF1, FF4 and FF5) of Flensburg Fjord between June 2009 and April 2010, size fraction 63–2000 µm.

	Dead foraminiferal species 0-1 cm > 63 µm	June 02.06.2009	%	August 18.08.2009	%	October 20.10.2009	%	December 07.12.2009	%	February 15.02.2010	%	April 19.04.2010	%
Station FF1	Species												
	Ammonia aomoriensis	343	72.2	941	59.5	213	54.9	377	59.1			369	64.1
	Elphidium albiumbilicatum	1	0.2	3	0.2			2	0.3			2	0.3
	Elphidium excavatum clavatum	29	6.1	115	7.3	61	15.7	56	8.8			42	7.3
	Elphidium excavatum excavatum	8	1.7	183	11.6	68	17.5	114	17.9			92	16.0
	Elphidium gerthi	2	0.4			1	0.3	2	0.3				
	Elphidium incertum	87	18.3	296	18.7	38	9.8	63	9.9			64	11.1
	Total number of calcareous individuals	470		1538		381		614				569	
	Ammotium cassis	4	0.8	10	0.6			3	0.5			1	0.2
	Reophax dentaliniformis	1	0.2	33	2.1	7	1.8	21	3.3			6	1.0
	Total number of agglutinated individuals	5		43		7		24				7	
	Total number of specimens	475		1581		388		638				576	
	Species number	8		7		6		8				7	
	Sample volume (cm^3)	47		76		56		60				94	
	Split (n)	0.5252		0.4585		0.4802		0.4791				0.5210	
	Abundance (tests 10 cm^{-3})	192.4		453.7		144.3		221.9				117.6	
Station FF4	Species												
	Ammonia aomoriensis	1	8.3	19	52.8	142	49.1	353	85.9	38	40.4	7	38.9
	Elphidium albiumbilicatum												
	Elphidium excavatum clavatum			3	8.3	8	2.8			2	2.1		
	Elphidium excavatum excavatum	3	25.0	4	11.1	1	0.3						
	Elphidium gerthi			1	2.8								
	Elphidium incertum	1	8.3	1	2.8	55	19.0	26	6.3	38	40.4	8	44.4
	Total number of calcareous individuals	5		28		206		379		78		15	
	Ammotium cassis	6	50.0	6	16.7	63	21.8	11	2.7	9	9.6	2	11.1
	Reophax dentaliniformis	1	8.3	2	5.6	20	6.9	21	5.1	7	7.4	1	5.6
	Total number of agglutinated individuals	7		8		83		32		16		3	
	Total number of specimens	12		36		289		411		94	100	18	
	Species number	5		7		6		4		5		4	
	Sample volume (cm^3)	59		95		100		93		73		59	
	Split (n)	0.0663		0.2351		0.5080		0.4769		0.4913		0.0623	
	Abundance (tests 10 cm^{-3})	30.7		16.1		56.9		92.7		26.2		49	
Station FF5	Species												
	Ammonia aomoriensis	3	12.5	279	43.5	23	62.2	94	39.8	225	31.4	93	45.8
	Elphidium albiumbilicatum	1	4.2	11	1.7			2	0.8	2	0.3	1	0.5
	Elphidium excavatum clavatum	2	8.3	196	30.6	8	21.6	100	42.4	292	40.8	49	24.1
	Elphidium excavatum excavatum	10	41.7	4	0.6	2	5.4	1	0.4			3	1.5
	Elphidium gerthi		0.0					1	0.4				
	Elphidium incertum	7	29.2	137	21.4	3	8.1	33	14.0	165	23.0	53	26.1
	Total number of calcareous individuals	23		627		36		231		684		199	
	Ammotium cassis	1	4.2	6	0.9			2	0.8	22	3.1	3	1.5
	Reophax dentaliniformis			8	1.2	1	2.7	3	1.3	10	1.4	1	0.5
	Total number of agglutinated individuals	1		14		1		5		32		4	
	Total number of specimens	24		641		37		236		716		203	
	Species number	6		7		5		8		6		7	
	Sample volume (cm^3)	47		67		51		64		76		48	
	Split (n)	0.1239		0.5340		0.1324		0.5071		0.5456		0.2996	
	Abundance (tests 10 cm^{-3})	41.2		179.2		54.8		72.7		172.7		141.2	

II.4 .3.2 Dead assemblages

During the whole investigation period (except of June at FF4 and FF5), *A. aomoriensis* dominates the dead assemblages at stations FF1, FF4 and FF5 with 62, 46 and 39 % on average (Table S1 in the supplement, Fig. 4). At station FF1, abundance of dead foraminifera was consistently higher ranging from 118 to 454 tests 10 cm^{-3} in comparison to the other stations. At station FF1, *E. incertum* was common with 14 %, and *A. cassis* was very rare with 0.4 % on average throughout the year (Fig. 4). In contrast, *E. incertum* was common at stations FF4 and FF5, and depicted maximum values in February and April with 42 % on average at station FF4 (Table 6). The arenaceous species *A. cassis* was frequent with 50 % in June at station FF4, otherwise it was rare with 2 % on average at station FF5 (Table 6).

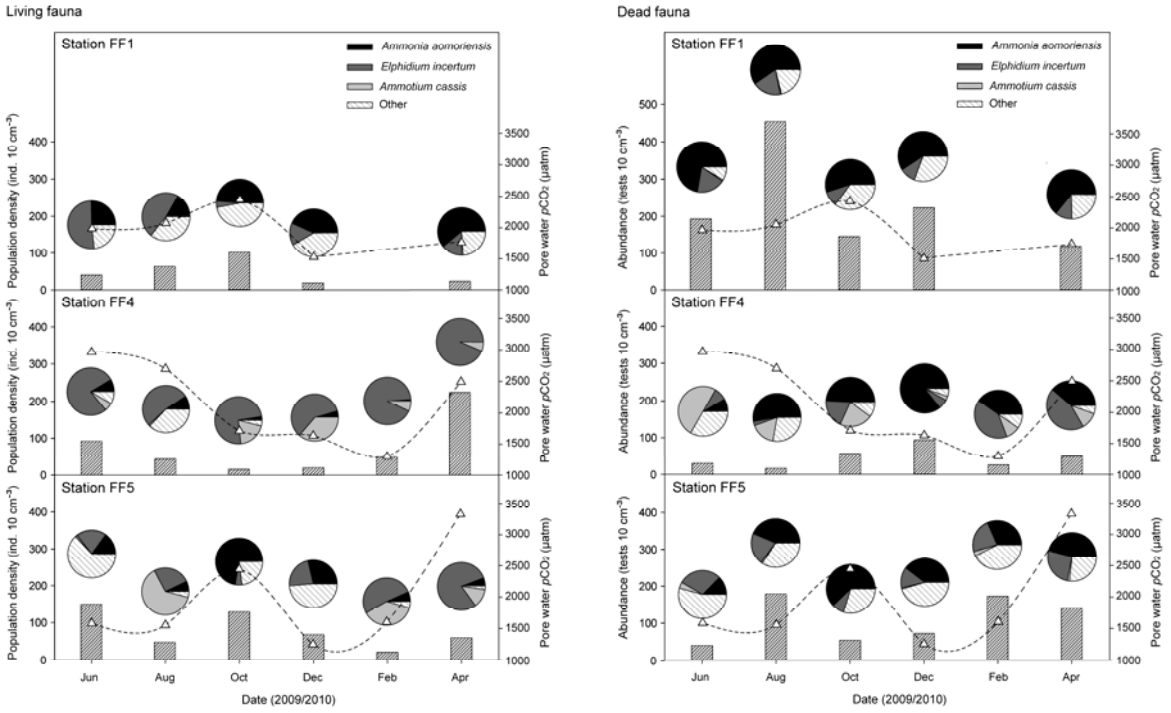

Fig. 4. Proportions and abundance of living and dead benthic foraminiferal species at stations FF1, FF4 and FF5 from June 2009 to April 2010. The bars present the population density and abundance of the living and dead fauna. Pie charts indicate the percentages of dominant species (Tables 5 and 6). Sediment pore water pCO_2 in Flensburg Fjord is displayed by white triangles.

II.4 .4 Co-variance of population density with respect to carbonate chemistry

The living and dead foraminiferal assemblages fluctuated seasonally. In particular the population density of the living fauna suggests a certain co-variance with pore water pCO_2. However, scale and magnitude of the fluctuation in both parameters revealed that the population density was not directly affected by changing pore water pCO_2, respectively Ω_{calc} (Fig. S1 in the supplement).

We tested the co-variance of the arenaceous species *A. cassis* and saturation state, but no correlation was recognised. The data provided no evidence that *A. cassis* could be affected by

changing carbonate chemistry. Therefore, we will focus on the calcareous species, which directly respond to changes of the carbonate chemistry.

Living calcareous *A. aomoriensis* and *E. incertum* revealed mean population densities of 16 ind. 10 cm^{-3} and 33 ind. 10 cm^{-3}. No correlation with the sediment pore water pCO_2 was recognised, neither in the living assemblages (Fig. 5), nor in the dead assemblages (Fig. S2 in the supplement). Furthermore, mean test diameter of living and dead *A. aomoriensis* also exhibited no relationship between test size and pCO_2 (Table S2 in the supplement).

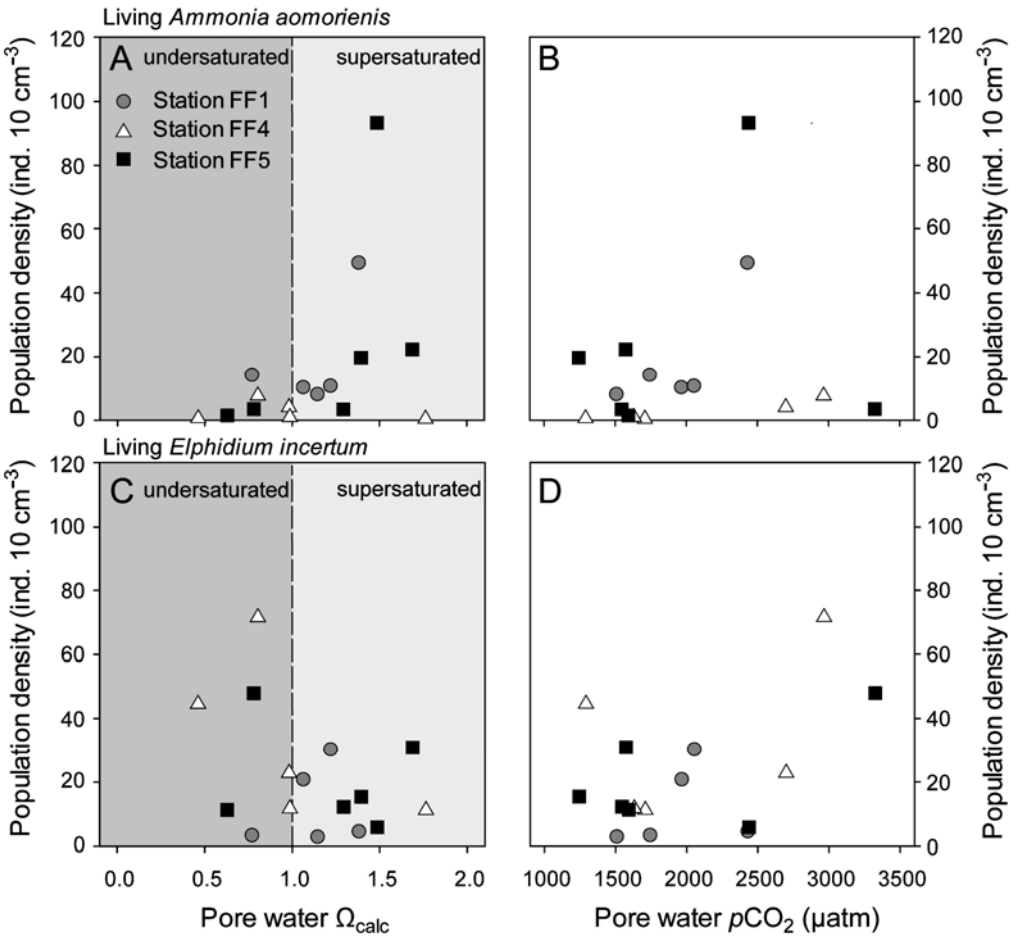

Fig. 5. Population density of living *A. aomoriensis* (A and B) and *E. incertum* (C and D) vs. sediment pore water Ω_{calc} (A and C) and pCO_2 (B and D). The different symbols present stations FF1, FF4 and FF5 during the one year cycle.

In contrast to pCO_2, population densities of living *A. aomoriensis* showed a co-variance with saturation state Ω_{calc}. Mean population density was comparatively low (5 ind. 10 cm^{-3}) when undersaturated conditions from 0.46 to 0.99 prevailed (Fig. 5). It was noticeable that station FF4 exhibited undersaturated conditions in sediment pore waters with an Ω_{calc} between 0.46 and 0.99 during the whole year, with the exception of October with Ω_{calc} of 1.76 (Table 4). During that time however, *A. aomoriensis* showed the lowest populations density of 3 ind. 10 cm^{-3} (Fig. 5; A). By comparison, stations FF1 and FF5 were most of the time supersaturated for Ω_{calc} from 1.07 to 1.69 (Table 4), and revealed mean population densities of 19 ind. 10 cm^{-3} and 35 ind. 10 cm^{-3}, respectively (Fig. 5; A).

In contrast, the population density of *E. incertum* showed no co-variance with sediment pore water Ω_{calc}. Under supersaturated Ω_{calc} conditions, the mean population density was lower with 15 ind. 10 cm^{-3}, in comparison to undersaturated values of Ω_{calc} with a population density of 53 ind. 10 cm^{-3}, on average (Fig. 5; C).

II.4 .5 Tests of living calcareous foraminifera

The test walls of the dominant calcareous species *A. aomoriensis* and *E. incertum* were examined. Different stages of tests of *A. aomoriensis* were classified as (1) intact tests (Fig. 6: 2), (2) dissolution of the last chamber (Fig. 7a: 4) and (3) dissolution of more than two chambers (Fig. 7a: 5).

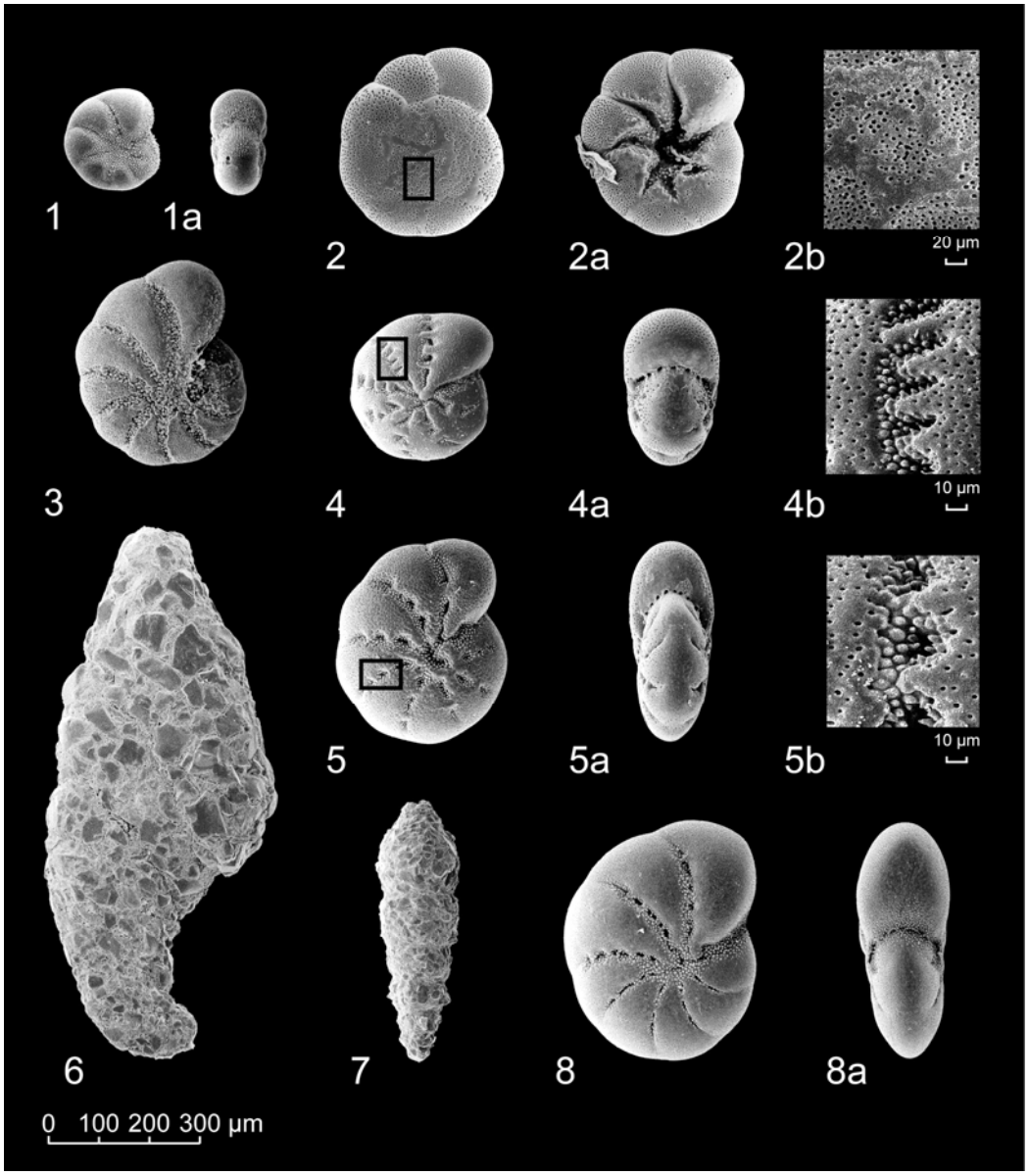

Fig. 6. Benthic foraminifera from Flensburg Fjord. 1 *Elphidium albiumbilicatum*: spiral (1) and apertural (1a) views; 2 *Ammonia aomoriensis*: spiral (2), umbilical (2a) and detailed view of the test wall (2b); 3 *Elphidium gerthi*: spiral view; 4 *Elphidium excavatum clavatum*: spiral (4), apertural (4a) and detailed view of the suture of two chambers (4b); 5 *Elphidium excavatum excavatum*: spiral (5), apertural (5a) and detailed view of the suture of two chambers (5b); 6 *Ammotium cassis*: top view; 7 *Reophax dentaliniformis*: top view; 8 *Elphidium incertum*: spiral (8) and apertural (8a) views.

Sixty four percent of the tests of living *A. aomoriensis* were intact and had a smooth and shiny surface, which was recognized in all samples during the one-year cycle (Fig. 6: 2). However, the remaining *A. aomoriensis* specimens exhibit different stages of test dissolution. At stations FF1 and FF5, 33 and 29 % of *A. aomoriensis* specimens exhibited dissolution of the last chamber. Dissolution of more than two chambers was observed in 4 and 13 % of the living specimens. All chambers were decalcified and in few individuals, only the inner organic lining was left.

In contrast, living *E. incertum* displayed no signs of dissolution. Occasionally, the last chambers of *E. incertum* were broken, which indicates impacts of mechanical forces, probably during sampling or processing (Fig. 7b: 6).

Furthermore, some test walls of living *A. aomoriensis* exhibited recalcified structures (Fig. 7b: 1–3). This recalcification was usually characterised by test deformities such as an irregular test shape (Fig. 7b: 2–3). The walls of the chambers were not completely covered by a newly formed calcite lamella, which indicated a fragmentary precipitation of calcite from the external to the internal test walls (Fig. 7b: 2). Old or compact and young or thinner chambers showed the same porosity (Fig. 7b: 2).

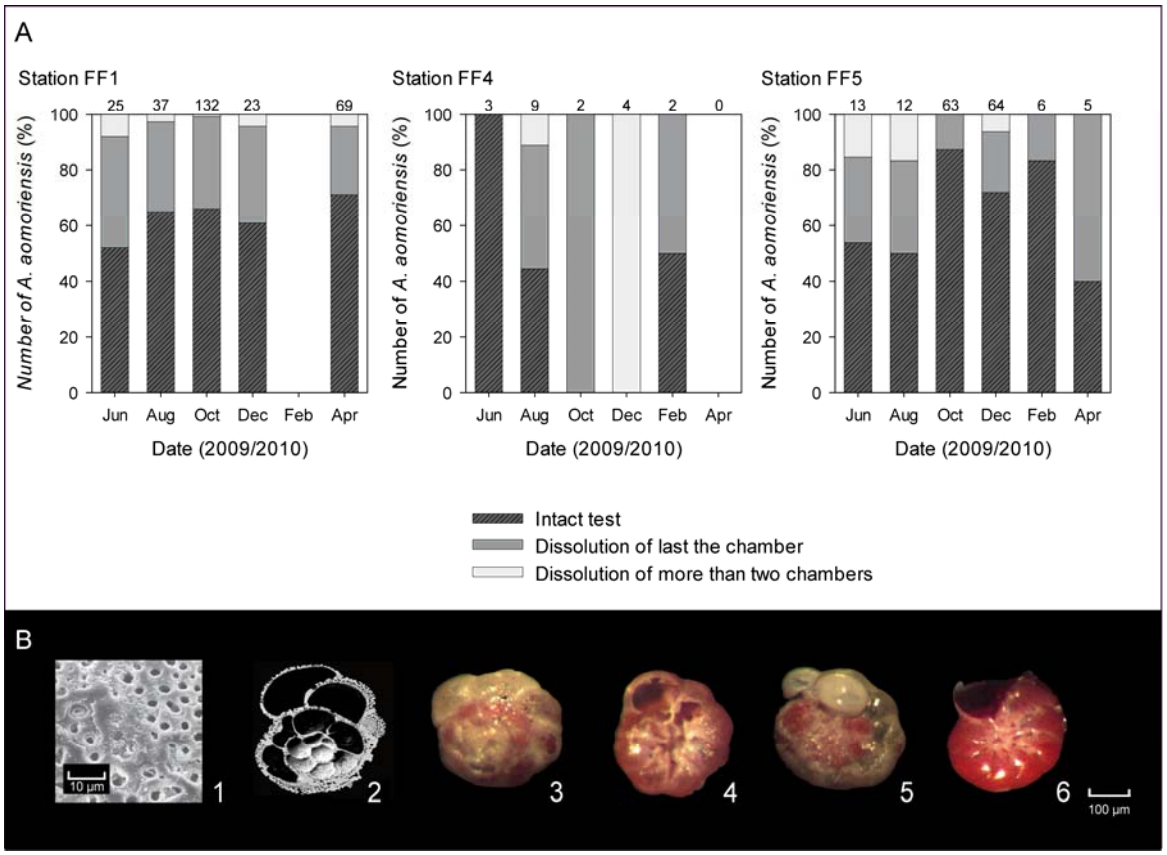

Fig. 7. (A): Three stages of preservation of living *A. aomoriensis*: intact tests, dissolution of the last chamber and dissolution of more than two chambers. Bars indicate the percentage of total species number of *A. aomoriensis* (Table 5) from June 2009 to April 2010. The number of counted *A. aomoriensis* specimens is present above each bar. (B): Subjacent SEM (1), EPMA (2) and light micrographs (3–6) of *A. aomoriensis* and *E. incertum* tests from Flensburg Fjord from Station FF5 in June 2009. 1–5 *A. aomoriensis*: detailed view of irregular test shape (1), spiral (2, 3 and 5) and umbilical (4) views of recalcifying (2 and 3) and dissolved tests (4 and 5). 6 spiral view of *E. incertum* with intact test, last chamber was broken.

II.5 Discussion

II.5 .1 Carbonate chemistry in Flensburg Fjord

Whereas, the surface pCO_2 of Flensburg Fjord is close to the atmospheric CO_2 concentrations, bottom water conditions were much more variable during the seasonal cycle. This seasonal variability of the carbonate chemistry is also found elsewhere in near coastal marine systems (Borges and Frankignoulle, 1999, Borges et al., 2006, Provoost et al., 2010, Thomsen et al., 2010, Hofmann et al., 2011).

These natural fluctuations are common in eutrophicated coastal habitats and estuaries (Diaz and Rosenberg, 2008, Conley et al., 2009, Nikulina and Dullo, 2009, Thomsen et al., 2010, Melzner et al., 2012). Furthermore, carbonate chemistry of the sediment pore water, especially in the living benthic foraminiferal habitat from 0–1 cm, strongly deviated from the conditions in the bottom water. Sediment pore water exhibited perennial high pCO_2 values ranging from 1244 to 3324 µatm. This is a consequence of C_T accumulation in the hypoxic water column and the surface sediments by aerobic processes. In contrast, in the deeper, anoxic sediments anaerobic bacterial decay of organic matter leads to production of metabolic bicarbonate (HCO_3^-) by nitrate and sulfate reduction and an increase of A_T (Yao and Millero, 1995). Whereas the HCO_3^- remains in the sediments, the gaseous end products H_2S or N_2 are either degassing or are bound as iron sulphides (Kristensen et al., 1998, Thomas et al., 2009).

The A_T in the surface waters ranges from 1800 to 2100 µmol kg^{-1} and thus is slightly lower than the buffer capacity of bottom water A_T (1800–2500 µmol kg^{-1}). However, the sediment pore water habitat of the benthic foraminifera exhibited a much higher alkalinity ranging from 2000 to 3500 µmol kg^{-1}. Remineralisation products cause C_T and A_T enriched sediment pore waters and an enhanced CO_2 buffer capacity (Thomas et al., 2009). Consequently, Ω_{calc} of the sediment pore waters was much higher than in the water column for most of the year. In contrast to stations FF1 and FF5, Ω_{calc} of station FF4 was undersaturated during most of the year. Both stations, FF4 and FF5, are located in Gelting Bay and have the same sediment, which is muddy sand. However, even slight differences in the sediment composition might cause different remineralisation processes (Kristensen et al., 1998, Asmus et al., 1998a,b), which could explain the Ω_{calc} undersaturation at station FF4.

II.5 .2 Foraminiferal community

The population density of the living assemblages showed fluctuations which can be attributed to the seasonality of food supply and degradation of organic matter (Schönfeld and Numberger, 2007a). In particular, high values of food supply during April and October could mirror spring and autumn blooms. The subsequent flux of algal debris to the sea floor is the dominating parameter structuring the population density and species composition of benthic foraminiferal faunas (Altenbach et al., 1999, Morigi et al., 2001, Gooday, 2003). As such, it is conceivable that

enhanced influx of organic matter provided sufficient food for a rich benthic community in Flensburg Fjord.

The composition of living and dead assemblages showed no correlation with pCO_2, respectively Ω_{calc}. This infers that either no extensive mortality occurred or dissolution of shells is prevented by the relative high carbonate saturation. Further, any shell loss of dead assemblages due to dissolution in seasonal undersaturated sediment pore waters was instantly compensated for by the delivery of empty tests from the living population through manifold reproduction.

In this study, we observed that living *A. aomoriensis* was frequent in muddy sediments at the middle station FF1 of Flensburg Fjord during the entire period of investigation. Only in October 2009, *A. aomoriensis* was dominant in muddy sands at the outer Fjord station FF5. This occurrence peak was possibly related to a favourable calcite saturation state at this location in October. Furthermore, oxygen and nutrient input could also favour an increase in population densities of *A. aomoriensis* (Polovodova et al., 2009). Also at the middle Fjord station FF1, the population density of *A. aomoriensis* varied apparently during the seasonal cycle. On the other hand, station FF4 in southern Gelting Bay showed a noticeable low population density of *A. aomoriensis*. This part of Flensburg Fjord was reported as a quiet area with low mixing events in the water column (Exon, 1971). Therefore, seasonal stratification and respiration in the deeper water causes hypoxic zones and carbonate undersaturation, which could influence the survival and calcification of *A. aomoriensis*. The oxygen depletion could also promote the low *Ammonia* population densities at station FF4 (Alve and Nagy, 1986, Buzas-Stephens and Buzas, 2005, Polovodova and Schönfeld, 2008), even though sufficient food is available.

The low-oxic conditions would also explain the dominance of *E. incertum* living in the uppermost sediment layer during the whole year at station FF4. *Elphidium incertum* has been described as an intermediate-infaunal species, which dwells in the sediment down to 3–6 cm depth (Linke and Lutze, 1993). Under unfavorable oxygen conditions, this species moves into the uppermost sediment layers (Wefer, 1976). In the current study, living *E. incertum* showed irregular spatial and temporal fluctuations in Flensburg Fjord. Higher population densities of *E. incertum* were observed in the middle Fjord station FF1 in June and in the outer Fjord station FF5 in April. The southern station FF4 in Gelting Bay, however, showed highest population densities of *E. incertum* in June and in April. Previous studies described that the reproduction of *E. incertum* preferentially takes place after phytoplankton blooms, which deliver high amounts of suspended organic particles to the sediment surface (Altenbach, 1985, Gustafsson and Nordberg, 1999). Indeed, we observed a dense layer of filamentous algae covering the sediment surface at all stations in June 2009. This algal mat probably induced and sustained the dense population of *E. incertum* in June, whereas the high and even rising population density in April was caused by the late spring diatom bloom in 2010 (Smetacek, 1985, Schönfeld and Numberger, 2007b).

The arenaceous species *A. cassis* was only common in the central and open parts of the outer Flensburg Fjord, where muddy sand prevailed. A higher number of living *A. cassis* was observed in October and December at station FF4. This transient peak correlated with the highest salinity values of 22.3 and 24.1 recorded at this station. It has been detected that the ability of *A. cassis* to live in the SW Baltic Sea is controlled by salinity (Lutze, 1965). A rising salinity due to

decadal, massive saltwater inflows from the Kattegat had led to increasing abundances of *A. cassis* in Kiel Bight during the following years (Schönfeld and Numberger, 2007a, Nikulina et al., 2008). A further important process, which influenced the reproduction of *A. cassis* was the availability of food particles, in particular their enrichment at hydrographic boundary layers and at the sediment surface bathed by these internal nepheloid layers (Wefer, 1976, Olsson, 1976). Given these favorable conditions, *A. cassis* bloomed and dominated the foraminiferal assemblages in August at station FF5.

II.5 .3 Comparison with earlier findings

Polovodova et al. (2009) took sediment samples in June 2006 and described the recent living foraminiferal distribution in Flensburg Fjord. Three of the sampling stations were adjacent to our stations in June 2009. The comparison of both data sets revealed changes in living faunal composition within three years (Fig. 8).

Station PF16–19 of Polovodova et al. (2009) was closely located to our station FF1. Both stations showed a similar species composition, 18 % *A. aomoriensis* and 57 % *E. incertum* in June 2006 and 15 and 52 % in June 2009, respectively (Fig. 8). This similar species proportions revealed that the environmental setting did not change substantially between 2006 and 2009 at station FF1 in the middle Fjord.

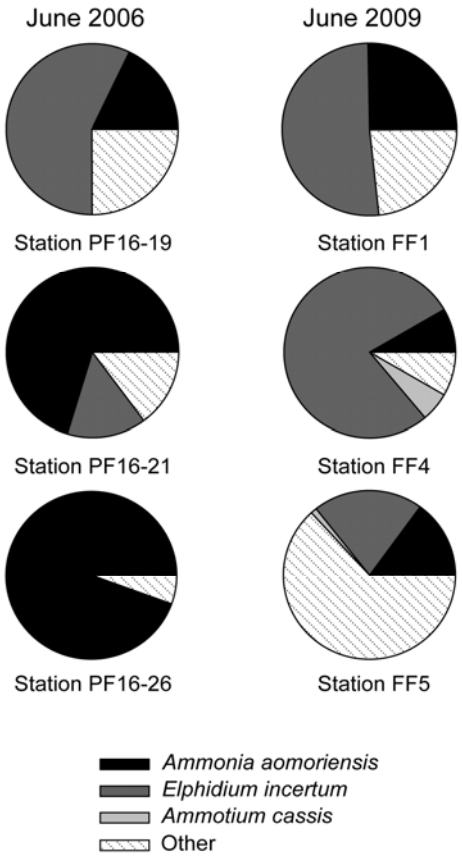

Fig. 8. Comparison of living benthic assemblages between the years 2006 and 2009.

Polovodova et al. (2009) stations PF16–21 and PF16–26 are close to our outer Fjord stations FF4 and FF5. From June 2006 to 2009, stations PF16–21 and FF4, and PF16–26 and FF5 showed a distinct faunal change. Living *A. aomoriensis* was dominant with 70 % (station PF16–21) and 94 % (station PF16–26), whereas in 2009 living *E. incertum* dominated with 78 % at station FF4 and was common at FF5 with 21 % (Fig. 8). Furthermore, a small population of *A. cassis* was observed before June 2009. This species comprised 6 % at station FF4 and 1 % at FF5.

On the one hand, this faunal change could reflect the year-to-year variability in parameters like salinity, food supply and oxygen content. The relationship with these parameters was documented for *A. beccarii*, *E. incertum* and *A. cassis* in previous studies (Wefer, 1976, Polovodova et al., 2009).

On the other hand, it is known that benthic foraminifera reveal irregular distribution pattern on the sea floor (Ellison et al., 1986, Schafer, 1973). The degree of patchiness varies, for instance a clumped distribution of many species reflects reproduction events (Buzas, 1968). Patchy colonization is a combination of many factors such as sediment composition (Bernstein et al., 1978, Bernstein and Meador, 1979) or microhabitat specialisation (Jumars, 1975). Patchiness of foraminiferal assemblages might play a certain role in the observed differences between the years and stations.

II.5 .4 Response of living calcareous foraminifera to undersaturated Ω_{calc}

It is expected that foraminifera will respond negatively to ocean acidification (Cigliano et al., 2010, Haynert et al., 2011). Some laboratory studies revealed hampered calcification and decreased survival at elevated pCO_2 (Le Cadre et al., 2003, Kuroyanagi et al., 2009, Allison et al., 2010, Haynert et al., 2011, Fujita el al., 2011), whereas other studies showed no significant change of calcification under simulated future pCO_2 scenarios (Dissard et al., 2010, McIntyre-Wressnig et al., 2011).

Population density of living *A. aomoriensis*, one of the dominating calcifying species, co-varies with sediment pore water undersaturation of Ω_{calc}. This finding is in agreement with observations from the laboratory, where mean test diameter of *A. aomoriensis* decreases in treatments with $\Omega_{calc}<1$, by up to 22 % (Haynert et al., 2011).

In contrast, the fitness and survival of the symbiont-bearing benthic foraminifera *Amphistegina gibbosa* and *Archaias angulatus* were not directly affected by elevated pCO_2 up to 2000 µatm (McIntyre-Wressnig et al., 2011). But it is important to note that during the whole six week incubation time, Ω_{calc} was supersaturated ranging from 5.4 to 1.5. These results confirm our in conclusion that living foraminifera are adapted to high pCO_2 levels, but respond most sensitive to an undersaturation of Ω_{calc}.

Furthermore, a previous study has been shown that *Ammonia tepida* revealed the highest calcification and survival rates at undersaturated conditions ($\Omega<1$) (Dissard et al., 2010). These results emphasise the need to understand the biological control of the calcification process in different foraminiferal species.

To date, only a low number of field studies investigated the response of calcifying organisms in natural CO_2-rich habitats. At CO_2 vents off Ischia (Italy), settlement and overall abundance and species richness of benthic foraminifera was significantly decreased at the low pH (7.08) site, which was undersaturated with respect to calcite (Ω_{calc} = 0.75) (Cigliano et al., 2010). In contrast, the current study exhibited that calcareous benthic foraminifera from Flensburg are able to survive and continue calcification under high pCO_2 and low pH values throughout the year. This infers no relationship between high pCO_2-levels and the calcification process itself.

Differences between Flensburg Fjord and Ischia might be explained by higher, slightly supersaturated Ω_{calc} values in the sediment of Flensburg Fjord. In contrast, high pCO_2 cause undersaturated conditions in the open seawater at Ischia. Nevertheless, the saturation state itself, neither pCO_2 nor pH, seems to be the parameter which has an intense effect on calcification and test integrity of benthic foraminifera. Therefore, it needs to be considered that foraminifera may not be subjected to undersaturation within sediments, which might cause a much lower vulnerability to increased atmospheric pCO_2 as observed in the Ischia study (Cigliano et al., 2010).

II.5 .5 Test dissolution

Biogenic calcification is expected to be highly affected by ocean acidification. Our study in Flensburg Fjord revealed no general impairment of calcification of living benthic foraminifera in a naturally CO_2-rich coastal environment. Only in undersaturated water dissolution features were observed, but the response was clearly species specific. For instance, *E. incertum* did not exhibit any signs of dissolution, whereas *A. aomoriensis* showed several stages of test corrosion.

Similar dissolution features were observed in marginal marine foraminifera from several settings: Sandebukta, Nueces Bay, Flensburg Fjord and Cleveland Bay (Alve and Nagy, 1986, Buzas-Stephens and Buzas, 2005, Polovodova and Schönfeld, 2008), and on estuarine foraminifera from South Alligator River (Wang and Chappell, 2001). All these dissolution phenomena may have different background reasons inferred by anthropogenic or natural conditions (Le Cadre et al., 2003). Abrasion and predation were suggested by different authors as forces, which may act independently or amplify the foraminiferal shell loss (Bradshaw, 1957, Martin et al., 1995, Alve and Murray, 1999, Polovodova and Schönfeld, 2008). However, we observed similar stages of dissolution in a previous laboratory study with manipulated carbonate system. The experiment results supported our hypothesis of calcite undersaturation as the major reason for dissolution of *A. aomoriensis* tests, also in Flensburg Fjord (Haynert et al., 2011).

In Flensburg Fjord, we observed recalcification structures on tests of *A. aomoriensis*, which are explained by seasonal fluctuations of Ω_{calc} in the sediment pore water. After periods of $\Omega_{calc}<1$, *A. aomoriensis* are seemingly able to rebuild their shell when Ω_{calc} returns to a supersaturated state >1. The same has been observed on tests of *Ammonia beccarii*, which begin to recalcify when pH was increased after a period of low pH levels (Le Cadre et al., 2003). The recalcification begins between the septal walls or around protruding cytoplasmic masses. Such a “repair” commonly leads to development of morphological abnormalities (Stouff et al., 1999, Le Cadre et al., 2000). Abnormal

tests of foraminifera were also observed in Rio Una (Brazil), resulting from natural periodical acidification (Geslin et al., 2002).

In order to investigate, whether dissolution and recalcification had an influence on the growth of the specimens during their entire lifespan, we measured the size distribution in a specimen of *A. aomoriensis*. The diameter of living and dead *A. aomoriensis* ranged in average from 306 µm in minimum up to a maximum of 461 µm. Mean diameter of the dead assemblage ranged from 269 µm in minimum up to a maximum of 433 µm. The sizes of *A. aomoriensis* are in good general agreement with populations from North Sea tidal flats (Hazeleger, 2010) in Quarternary sediments from the Dead Sea Rift, Israel (Almogi-Labin et al., 1995). Size distribution histograms differ between the successive sampling dates. Large proportions of small-sized tests or single modes usually indicate reproduction events (Swallow, 2000). In Flensburg Fjord, increase in size from one sampling event to another was not recognised. This can be regarded as corroborating evidence for generation times shorter than 88 days as reported by Bradshaw (1957, 1961). This infers that every *A. aomoriensis* population has to be regarded individually in the context of the environmental factors prevailing at the particular station about a couple of weeks before sampling. Therefore, certain foraminiferal species seem to cope much better with undersaturated conditions than others, which may eventually lead to future shifts in community structure.

Test dissolution in foraminifera is also known from the geological record (Alve, 1995, 1999). *Elphidium incertum* showed a higher resistance to undersaturation of Ω_{calc} in comparison to *A. aomoriensis*. Therefore, *A. aomoriensis* would be the better proxy for ocean acidification in the past. According to our results, calcification and recalcification of *A. aomoriensis* is a response to the environmental stress induced by changes in Ω_{calc}. High proportions of corroded tests of *A. aomoriensis* in sediment cores could indicate variations in ecological parameters, in particular elevated environmental stress. Therefore both, morphological abnormalities as well as dissolution features, might be useful proxies in paleoenvironmental reconstructions (Geslin et al., 2002).

II.5 .6 Impact of rising atmospheric CO_2 on the carbonate chemistry of a coastal habitat

Future ocean acidification will amplify pCO_2 levels, especially in hypoxic water masses (Brewer and Pelzer, 2009, Melzner et al., 2012). Already today, low [CO_3^{2-}] are encountered in the habitat of Flensburg Fjord. Additional CO_2 will cause further increases of seawater pCO_2 and lowering of CO_3^{2-} (Melzner et al., 2012). According to our calculations, increasing CO_2 levels will also cause a strong increase of sediment pore water (0–1 cm) pCO_2 by about 1500 µatm to mean values of 3550 ± 780 µatm (Fig. 9). At the same time, pH and Ω_{calc} will decrease to mean values of 7.42 ± 0.08 and 0.59 ± 0.20 (Fig. 9). This would lead to a constant undersaturation of sediment pore water Ω_{calc} during the whole year cycle (Fig. 9).

In consequence of increasing atmospheric CO_2 concentrations, a much higher pCO_2 increase is expected for seasonal hypoxic habitats such as Flensburg Fjord, in comparison to open ocean environments. Elevated pCO_2 or low pH may have not led to any drastic change of the benthic foraminiferal community structure yet. However, it is no doubt that certain species, in particular *A. aomoriensis*, have already exhibited high sensitivity to undersaturated states of present-day

environment. In the future, more adverse conditions may lead to a strong decline in *A. aomoriensis* population density.

More tolerant calcareous species, such as *E. incertum*, may potentially dominate the benthic foraminiferal communities under future elevated pCO_2 conditions. This shift will eventually lead to changes in the benthic foraminiferal communities of Flensburg Fjord. The same will probably apply to other regions too, which are going to experience naturally undersaturated Ω_{calc} levels.

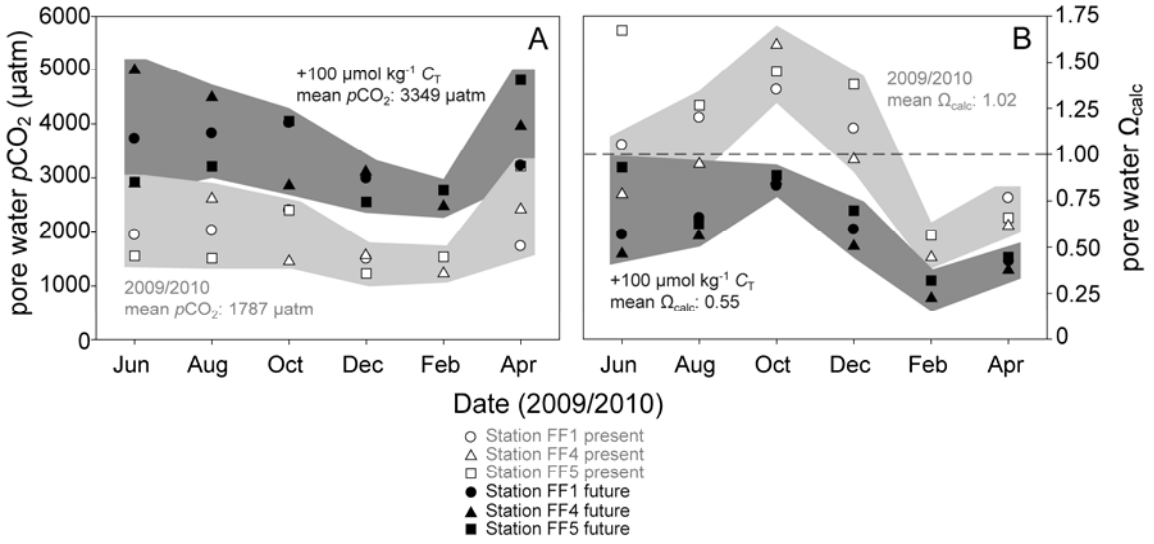

Fig. 9. Present (white symbols) and future (black symbols) sediment pore water (0–1 cm) pCO_2 (A) and Ω_{calc} (B) at stations FF1, FF4, and FF5. Future sediment pore water pCO_2 and Ω_{calc} were replotted from Table 4 and calculated after addition of 100 µmol kg^{-1} C_T to C_T from Table 4.

Furthermore, planktonic foraminifers also precipitate thinner test walls at reduced carbonate ion concentrations and higher atmospheric CO_2 levels (Spero et al. 1997, Bijma et al., 1999, Moy et al., 2009, Manno et al., 2012). Therefore, calcareous planktonic foraminifera in the water column may be more affected by the future pCO_2 increase in comparison to benthic foraminifera living in the surface sediments. The reduction of calcification of planktonic foraminifera may have a considerable impact on global carbonate production. At present, planktonic foraminifera export 2.9 Gt $CaCO_3$ per year from the photic zone on a global scale (Schiebel, 2002), whereas calcareous benthic foraminifera in neritic environments produce 0.1 Gt yr^{-1} (Table S3 in the supplement). This rate is about a magnitude lower than pelagic carbonate production. However, facing a future reduction in the export production of planktonic foraminifera, we may expect a relative increase of shallow-water benthic foraminiferal carbonate precipitation and thus a shift from pelagic to neritic carbonate production.

II.6 Conclusions

The present study is based on monitoring of the benthic foraminiferal assemblages in a naturally CO_2-rich coastal habitat of Flensburg Fjord. Bottom and sediment pore water pCO_2 showed

large seasonal fluctuations and sediment pore water pCO_2 was constantly high during the entire year. Nevertheless, as a consequence of higher alkalinity (A_T), the sediment pore water was often supersaturated with respect to calcite. These observations indicate that the benthic community was subjected to high pCO_2.

The living and dead foraminiferal assemblages fluctuated seasonally but showed no direct relationship with sediment pore water pCO_2, respectively Ω_{calc}. Instead, the population density of the living fauna showed fluctuations which can be attributed to the seasonality of food supply and organic matter degradation.

The population density of *A. aomoriensis*, one of the dominant calcifying species, co-varies with sediment pore water undersaturation of Ω_{calc}. In contrast, the co-occurring calcareous species *E. incertum* shows no relationship to $\Omega<1$. Also the dissolution response of the foraminiferal tests differs between the two species. Whereas *E. incertum* displays no signs of test dissolution, *A. aomoriensis* shows different stages of test dissolution. Test dissolution of *A. aomoriensis* could indicate environmental stress, such as undersaturation of Ω_{calc}. Therefore, dissolution features offer useful poxies for paleoenvironmental reconstructions.

The calculated future sediment pore water acidification in Flensburg Fjord is much higher than expected for the global ocean. We conclude that benthic foraminifera are relatively tolerant to current high pCO_2 conditions in Flensburg Fjord, which suggest that elevated pCO_2-levels do not lead to a drastic change in the foraminiferal communities. The modeled, future change of sediment pore water chemistry towards low, undersaturated Ω_{calc}, however, might increase the mortality of the dominating species *A. aomoriensis*, which will ultimately lead to changes in benthic foraminiferal communities for Flensburg Fjord.

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Supplements

TableS1. Population density and abundance of the living and dead dominant species: *A. aomoriensis*, *E. incertum* and *A. cassis* at station FF1, FF4 and FF5.

Living							
Station	Species (ind. 10 cm^{-3})	June 02.06.2009	August 18.08.2009	October 20.10.2009	December 07.12.2009	February 15.02.2010	April 19.04.2010
FF1	*Ammonia aomoriensis*	10.1	10.6	49.1	8.0		14.1
	Elphidium incertum	20.7	30.1	4.5	2.8		3.3
	Ammotium cassis	0.0	0.0	0.0	0.0		0.2
FF4	*Ammonia aomoriensis*	7.7	4.0	0.4	0.9	0.6	
	Elphidium incertum	71.5	22.8	11.2	11.7	44.6	209.4
	Ammotium cassis	5.1	0.4	3.0	7.2	3.3	13.6
FF5	*Ammonia aomoriensis*	22.3	3.4	93.3	19.7	1.4	3.5
	Elphidium incertum	30.9	12.3	5.9	15.4	11.3	48.0
	Ammotium cassis	1.7	30.2	0.0	0.3	8.2	7.6

Dead							
Station	Species (tests 10 cm^{-3})	June 02.06.2009	August 18.08.2009	October 20.10.2009	December 07.12.2009	February 15.02.2010	April 19.04.2010
FF1	*Ammonia aomoriensis*	138.9	270.1	79.2	131.2		75.4
	Elphidium incertum	35.2	85.0	14.1	21.9		13.1
	Ammotium cassis	1.6	2.9	0.0	1.0		0.2
FF4	*Ammonia aomoriensis*	2.6	8.5	28.0	79.6	10.6	19.0
	Elphidium incertum	2.6	0.4	10.8	5.9	10.6	21.8
	Ammotium cassis	15.3	2.7	12.4	2.5	2.5	5.4
FF5	*Ammonia aomoriensis*	5.1	78.0	34.1	29.0	54.3	64.7
	Elphidium incertum	12.0	38.3	4.4	10.2	39.8	36.9
	Ammotium cassis	1.7	1.7	0.0	0.6	5.3	2.1

Table S2. Mean test diameter of living and dead *A. aomoriensis* from June 2009 to April 2010.

A. aomorienis	Station	June [µm]	August [µm]	October [µm]	December [µm]	February [µm]	April [µm]
Living	FF1	415	393	385	378		392
	FF4	308	328	388	306	325	
	FF5	372	385	461	419	404	363
Dead	FF1	430	373	376	419		392
	FF4	400	269	375	308	369	357
	FF5	433	412	420	396	426	384

Table S3. Foraminiferal and total carbonate production, loss and accumulation on a global scale. Data sources are given in brackets.

	Planktonic foraminifera	Benthic foraminifera	Total Carbonate
Production	1.3 – 3.2, on average 2.9 Gt yr^{-1} **(1)** 1.2 Gt yr^{-1} **(2, 3)**	Coral reef environments: 0.04 Gt yr^{-1} **(4)** Non-carbonate shelves: 0.03 Gt yr^{-1} **(5)** Other shelf environments: 0.03 Gt yr^{-1} **(6)** Total neritic: 0.1 Gt yr^{-1} **(13)** Slopes and deep sea: 0.33 Gt yr^{-1} **(7)**	5.8 Gt yr^{-1} **(8)** 5.7 Gt yr^{-1} **(9)**
Loss	75 % **(1)**	Reef environments: 13 % **(4)** Neritic: >95 % **(10)**	Neritic: 75 % **(11)** Slopes: 40 % **(11)** Deep Sea: 55 % **(11)** Total: 40 % **(11)**
Accumulation	0.4 – 0.9 Gt yr^{-1} **(1)** 0.83 Gt yr^{-1} **(12)**	Coral reef environments: 0.035 Gt yr^{-1} **(4)** Non-carbonate shelves: 0.002 Gt yr^{-1} **(13)** Other shelf environments: 0.0075 Gt yr^{-1} **(13)** Total neritic: 0.045 Gt yr^{-1} **(13)** Slopes and deep sea: 0.15 Gt yr^{-1} **(13)** Total benthic foraminifera: 0.2 Gt yr^{-1} **(2)**	3.2 Gt yr^{-1} **(11)**

Sources: **(1)** Schiebel (2002), **(2)** Langer (2008), **(3)** probably export from the near surface ocean, **(4)** Langer et al. (1997), **(5)** 0.1 – 3, for deeper parts on average 2 g $CaCO_3$ m^{-2} yr^{-1} Wefer and Lutze (1978) at 15.3 x 10^6 km^2 Milliman (1993, his Table 1), **(6)** assigned to "Banks/Bays" by Milliman (1993) with the same carbonte production as non-carbonate shelves, **(7)** total accumulation of 0.2 Gt yr^{-1} minus **(2)** neritic accumulation plus loss due to pelagic export or dissolution, **(8)** Milliman et al. (1999), **(9)** Milliman and Droxler (1996), **(10)** Wefer and Lutze (1978), **(11)** Milliman (1993), **(12)** Catubig et al. (1998), **(13)** own calculations from the above figures.

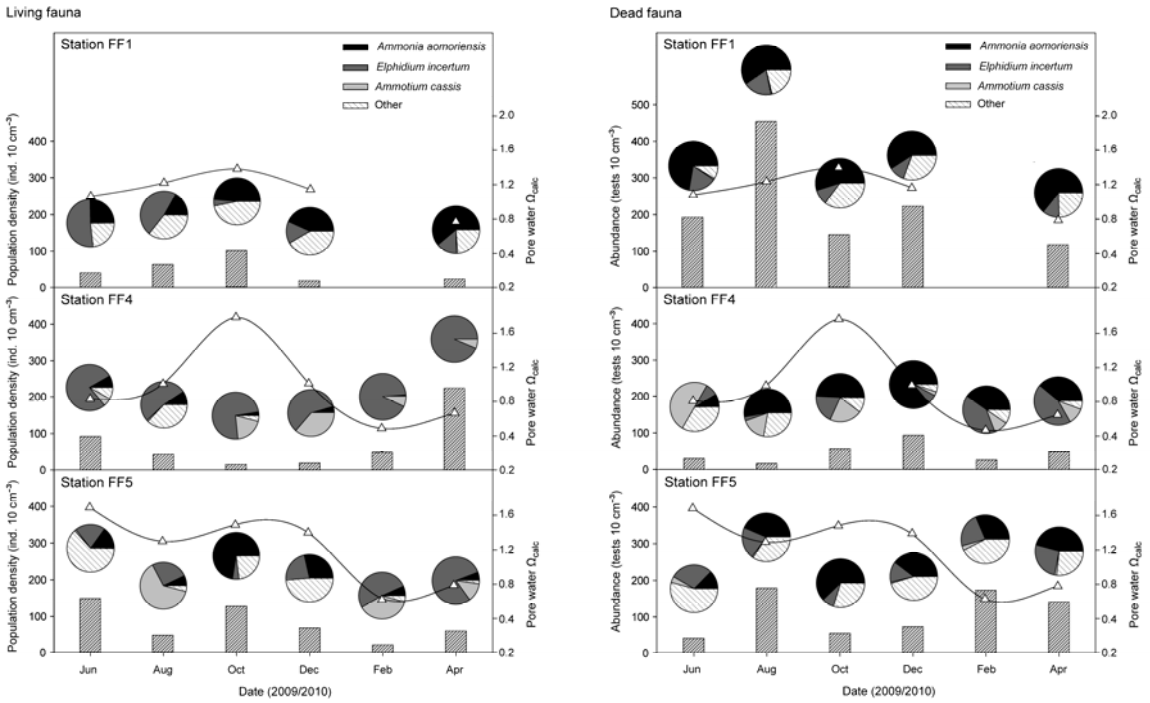

Fig. S1. Proportions and abundance of living and dead benthic foraminiferal species at stations FF1, FF4 and FF5 from June 2009 to April 2010. The bars present the population density and abundance of the living and dead fauna. Pie charts indicate the percentages of dominant species (Table 5 and 6). Pore water Ω_{calc} in Flensburg Fjord is displayed by white triangles.

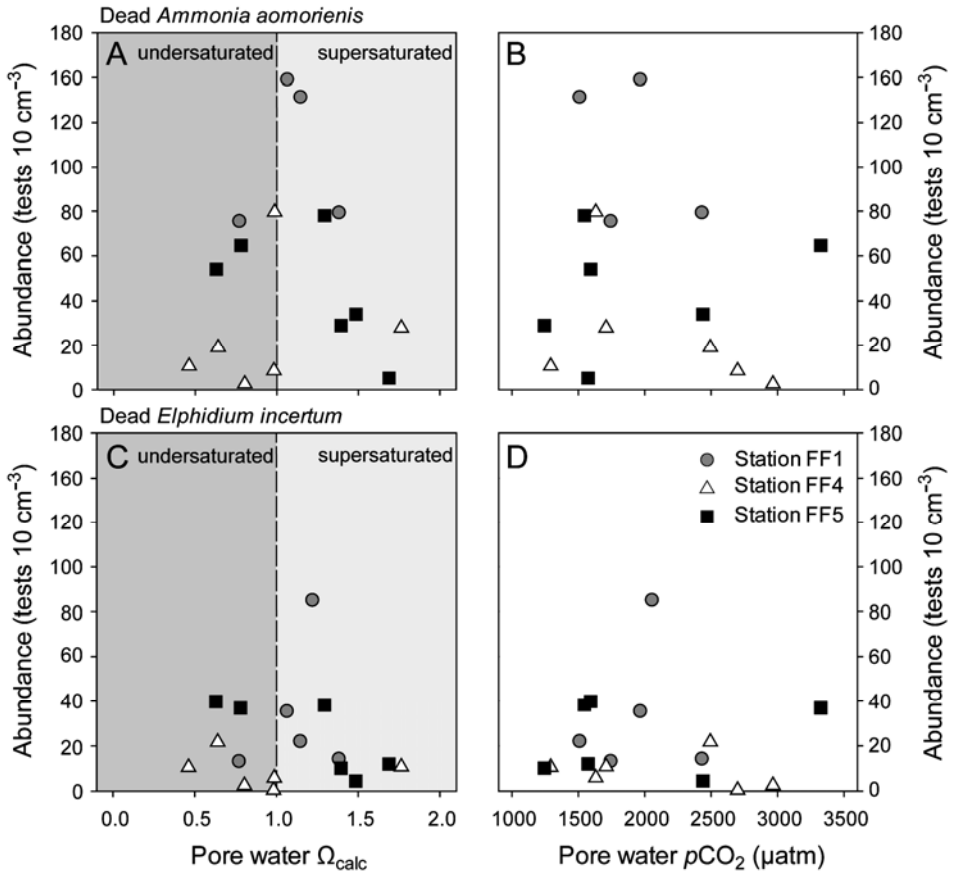

Fig. S2. Abundance of dead *A. aomoriensis* (A and B) and *E. incertum* (C and D) vs. sediment pore water Ω_{calc} (A and C) and pCO_2 (B and D). The different symbols present stations FF1, FF4, and FF5 during the one year cycle.

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Chapter III

Impact of changing carbonate chemistry, temperature and salinity on growth and test degradation of the benthic foraminifera *Ammonia aomoriensis*

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Abstract

The present study investigated the combined effects of ocean acidification, temperature and salinity on growth and test degradation of *Ammonia aomoriensis*. This species is one of the dominating benthic foraminifera in near-coastal habitats of the southwestern Baltic Sea, which could be particularly sensitive to changes in seawater carbonate chemistry. To assess potential responses to ocean acidification and climate change, we performed a fully crossed experiment with three temperatures (8, 13 and 18 °C), three salinities (15, 20 and 25) and four pCO_2 levels (566, 1195, 2108 and 3843 µatm) for six weeks. Our results highlight a sensitive response of *Ammonia aomoriensis* to an undersaturation of the seawater with respect to calcite. The specimens continue growth and increased their test diameter at pCO_2 <1200 µatm and Ω_{calc}>1. With increasing pCO_2, >1200 µatm (Ω_{calc}<1), a significant reduction of test diameter was observed. With respect to elevated temperature, Ω_{calc} increased which results in larger test diameter and lower test degradation. Maximal growth was observed at 18 °C. No significant relationship was observed between salinity and test growth. In the future, lowered and undersaturated Ω_{calc}, which results from increasing pCO_2 in bottom waters, may cause a significant decline of the population density of *Ammonia aomoriensis* in their natural environment. At the same, this effect might be partially compensated by temperature rise due to global warming.

III.1 Introduction

Rising atmospheric CO_2 concentrations up to 750 µatm will change the seawater carbonate chemistry and lead to rising seawater pCO_2 which will result in a seawater pH decrease by 0.4 units until 2100 (Feely et al., 2004, Caldeira and Wicket, 2005, Raven et al., 2005). Such pH decrease concomitantly will reduce the carbonate ion concentrations in the global oceans. Therefore, the mean calcium carbonate saturation state of the global ocean will be lowered to about half of their present-day values by the year 2300 (e.g. Feely et al., 2004, Caldeira and Wicket, 2005). Simultaneously, global temperatures have increased since beginning of the industrial revolution by 0.76 °C (IPCC, 2007). Model experiments indicated that global average

temperature will further increase by about 0.5 °C until the year 2200 (IPCC, 2007). This temperature rise is consistent with the calculated greenhouse effect due to measured, historical and recent rise in atmospheric carbon dioxide concentrations (Hansen et al., 1981).

In comparison to the open oceans, the southwestern Baltic Sea is characterized by low salinity (Wefer, 1976). Consequently, low alkalinity prevailed in this area. High pCO_2 levels are seasonally encountered in the bottom water, which leads in combination to exceptionally low carbonate ion concentrations (Thomsen et al., 2010, Haynert et al., 2012, Thomsen et al., 2013). Nevertheless, in the sediment pore water, Ω_{calc} was slightly supersaturated as consequence of higher alkalinity (A_T) for most time of the year. At the same time, sediment pore water pCO_2 is constantly high, ranging from 1244 to 3324 µatm (Haynert et al., 2012). Seasonally high pCO_2 levels are encountered and ongoing CO_2 uptake will cause an increase up to 4000 µatm in such neritic environments by the year 2100 (Melzner et al., 2012).

A reduced calcite saturation state will likely have consequences for calcifying foraminifera (Erez, 2003). Some laboratory studies have shown that benthic foraminifera exhibited different responses under simulated ocean acidification conditions. Most studies revealed a sensitivity to elevated pCO_2 (Le Cadre et al., 2003, Kuroyanagi et al., 2009, Allison et al., 2010, Dissard et al., 2010, Fujita et al., 2011, Haynert et al., 2011), whereas some species were not affected at rising pCO_2 (McIntyre-Wressnig et al., 2013, Vogel and Uthicke, 2012). A recent study of Keul et al. (2013) reported that not pCO_2, but rather CO_3^{2-} is the parameter which affects the test size and weights of *Ammonia* species. Most of these studies manipulated the carbonate system parameters without considering changes of other abiotic parameters, as for instance temperature and salinity. In contrast, the early, pioneering studies of Bradshaw (1957 and 1961) considered only temperature and salinity variations and did not change the carbonate chemistry of the seawater.

The impacts of the combined parameters of ocean acidification, salinity and temperature on their calcification were described in two further laboratory studies, the first with the benthic foraminifera *Ammonia tepida* and the second with the planktonic foraminifer *Neogloboquadrina pachyderma* (Dissard et al., 2010, Manno et al., 2012). The results of Dissard et al. (2010) exhibited that shell weights of *Ammonia tepida* decrease with decreasing $[CO_3^{2-}]$, and decrease with increasing temperature. However, specimens were still able to add new chambers to their tests at calcium carbonate undersaturation. The planktonic species *Neogloboquadrina pachyderma* revealed decreased calcification rates under low pH, too. Decrease in net calcification was attenuated when both, pH decreased and temperature increased simultaneously (Manno et al., 2012). The impact of ocean acidification and salinity on foraminifera calcification is poorly understood (Dissard et al., 2010). Previous studies have shown that salinity alters the incorporation of Mg which may indicate an impact on the calcification process (Groeneveld et al., 2008, Kisakürek et al., 2008).

It was referred to the Pliocene species *Ammonia aomoriensis* (Asano, 1951), which is likely to be extant (Hayward et al., 2004, Schweizer et al., 2010, Haynert et al., 2011). *Ammonia aomoriensis* (*Ammonia beccarii*) is one of the dominating species in the near-coastal

marine environment of the southwestern Baltic Sea and has a hyaline, and perforate calcareous test (Polovodova and Schönfeld, 2008, Nikulina et al., 2008, Polovodova et al., 2009, Haynert et al., 2012).

An earlier experiment exerted a strong influence of elevated pCO_2 on the calcification of *Ammonia aomoriensis* under constant salinity and temperature conditions (Haynert et al., 2011). In their natural environments of the southwestern Baltic Sea, seasonal changes of temperature and salinity influence the carbonate chemistry, especially the saturation state (Haynert et al., 2012). In order to further constrain the response scheme of this species to changes in saturation state under different physical environmental conditions, we designed a new experiment, where temperature, salinity and omega calcite could be tuned independently. The present study reports for the first time the results from a fully crossed experiment with pre-setting of three different temperatures and salinities, and four pCO_2 levels to quantitatively assess the impact on the test diameter of *Ammonia aomoriensis*.

III.2 Materials and methods

III.2.1 Field sampling

In April 2011, living specimens of *Ammonia aomoriensis* were collected from two stations in Kiel Fjord, southwestern Baltic Sea. At those stations, KF1 (54°20´713´´N, 10°10´160´´E, 13 m water depth) and KF2 (54°21´072´´N, 10°10´501´´E, 12 m water depth), silty fine sand prevailed. *Ammonia aomoriensis* is one of the dominating species of the living fauna in this area.

The benthic foraminiferal samples were taken with a Mini Muc K/MT 410 corer equipped with four tubes (each with 60 cm length and 10 cm inner diameter), deployed from R/V *Polarfuchs*. Altogether, 16 cores were taken. A plastic ring marked with a 1 cm-scale was used to slice off the uppermost one centimeter of the surface sediment (Schönfeld et al., 2012). The surface samples of the sediment cores were washed through a 63-µm sieve with the ambient seawater. The residue was immediately transferred into 300 ml Kautex™ wide-neck bottles and filled up with ambient bottom water. The Kautex™ bottles were covered with Parafilm to avoid evaporation and contamination. They were immediately brought to the laboratory at GEOMAR, where they were aerated and incubated at 15 °C for two days.

III.2.2 Isolation of *Ammonia aomoriensis*

Living *A. aomoriensis* from the size fraction 150 to 350 µm were picked with a fine brush from the stock-cultures under a Wild M3C dissecting microscope. The specimens were identified as being alive by their yellow cytoplasm content and their ability to move in Petri dishes, which was observed during a time period of one hour. After the assessment of living specimens, their maximum diameter was measured, and they were placed into 65 x 65 x 45 mm transparent

polycarbonate culture vessels at 10 specimens to a vessel. In total, 1440 specimens were picked and incubated individually.

The specimens were kept individually in 6 mm deep and open recruitment pits of 10 mm diameter, which were drilled into the base plate (57 x 57 x 10 mm) of the vessels. The specimens could move inside the pits and most of them stayed there. A small amount of carbonate-free medium quartz sand (0.2 g, 200–500 µm, >97 % SiO_2) was dispersed in the pits, so that the foraminiferal specimens could seek shelter similar to their natural habitat. The individuals were kept for three days at the corresponding temperature and salinity in the vessels for acclimatization until the start of the experiments. During the incubation period, no accumulation of waste material or growth of fungi on the foraminiferal specimens or in the vessels was observed.

III.2.3 Experimental setup

The culturing of *A. aomoriensis* was performed in a flow-through system as a fully crossed experiment. The setup was modified after Hintz et al. (2004) and Haynert et al. (2011). Three separate experiments with three different temperatures (8, 13 and 18 °C) were conducted sequently (Fig. 1). Each of the three experiments consisted of three independently recirculating systems with three different salinities (15, 20 and 25). Every of these systems included four replicates at the same pCO_2 for four different levels: 380, 1120, 2400 and 4000 µatm (Fig. 1). The selected temperatures and salinities cover the annual variability in *A. aomorienis* habitat in Kiel Fjord (Nikulina et al., 2008, Polovodova and Schönfeld, 2008). Observations in the natural habitat of the species exhibited high pore water pCO_2 (0–1 cm), which correspond with the selected pCO_2 range from 1120 to 4000 µatm (Haynert et al., 2012).

Each culture vessel was flushed with cartridge-filtered (25 µm) and UV-sterilized seawater. The seawater was obtained from different locations in the Baltic and North Sea: Kiel Fjord (salinity of 15), Husum (salinity of 20), and Helgoland (salinity of 25).

Due to the direct correlation between salinity and alkalinity, seawater alkalinity and thereby Ω_{calc} and similarly CO_3^{2-} differed significantly between the three salinity treatments. Due to the temperature dependency of the carbonate system dissociation constants, Ω_{calc} increased with increasing temperature. Therefore, we were not aiming to differentiate between Ω_{calc} and CO_3^{2-} as these necessarily co-vary in nature, but we were able to differentiate between the effects of Ω_{calc}/CO_3^{2-} and pCO_2/pH (Table 1).

Temperatures were adjusted by either ambient air in temperature controlled chambers (8 and 13 °C), or by conventional aquarium heaters (18 °C). Different pCO_2 levels were adjusted by aeration with compressed air enriched with CO_2 to target pCO_2 levels of 380, 1120, 2400 and 4000 µatm in four 5 l compact jerrycans (Fig. 1). The preconditioned seawater from each jerrycan flowed through four culture vessels. The flow rate was adjusted to 0.16 ml s^{-1} which is sufficient to replace the entire water volume of the vessels every 40 minutes. The overflow drained off to a 60 l catchment tank. In the catchment tank, the water was sparged with compressed air at a high rate in order to remove the excess CO_2 before the water is pumped back to the compact jerrycans.

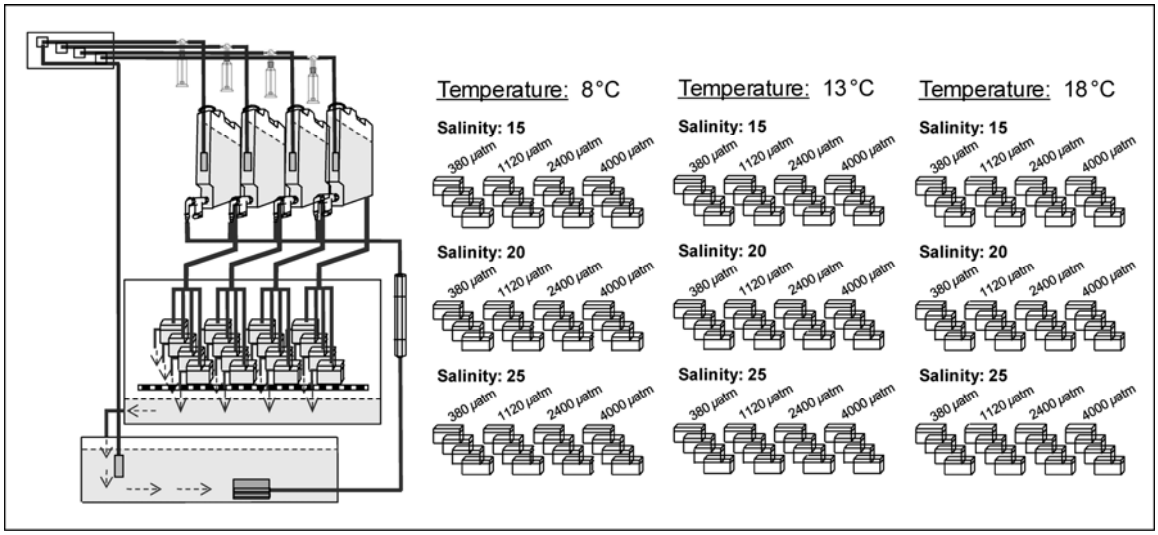

Fig. 1. Scheme: Culturing of *A. aomoriensis* in a closed flow-through system. Water equilibrated with a specific pCO_2 supplied four replicated aquaria and drained into a large tank, from where it was pumped up again. Additionally, 18 °C temperature was controlled by heaters in a water bath beneath the culture vessels and in the catchment tank. Experimental design: 3 experiments with 3 temperatures (8, 13 and 18 °C), each experiment included 3 independent setups with 3 salinities (15, 20 and 25). These separate setups included 4 replicates at the same pCO_2 for 4 different levels (380, 1120, 2400 and 4000 µatm).

In order to avoid evaporation by aeration of the seawater with compressed and thus dry air, the gas was humidified in gas-washing bottles which were inserted in each compressed air connection. However, 1 liter evaporated in the catchment tank per week, which was compensated by refilling the system with deionised water up to a marked level. Food was added every fourth day to each culture vessel. We used 200 µl DT's Premium Blend containing living *Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella*.

Temperature, salinity and pH_{NBS} (National Bureau of Standards pH-scale) were measured weekly in each culture vessel in order to monitor the water chemistry. Total alkalinity (A_T), phosphate (PO_4^{3-}) and silicate (Si) concentrations were determined bi-weekly in one of the 4 replicates for each pCO_2-, temperature- and salinity-level. After six weeks, the experiment was terminated. The specimens were inspected for growth and test degradation in the recruitment pits of the culture vessels under a dissecting microscope before they were taken out and dried in single cell slides. A previous experiment of Haynert et al. (2011) was used to compare their data of *A. aomriensis* growth and test degradation with the present study and to test the external reproducibility of our data.

III.2.4 Analysis of the water chemistry

pH_{NBS} and temperature were determined with a WTW 340i pH analyzer. The electrode was calibrated with standard buffer solutions of pH 4.01, 7.00 and 10.00 (WTW standard, DIN/NIST buffers L7A). Precision was ±0.01 for pH and ±0.1 °C for temperature. For salinity measurements, a WTW Cond 315i conductivity meter with a precision of ±0.1 salinity units was used. Nutrient concentrations of PO_4^{3-} and Si were analyzed colorimetrically in a spectrophotometer (U 2000,

Hitachi-Europe) at a wavelength of 882 nm and 810 nm according to Koroleff and Grasshof (1983). The measurement precision was ±0.2 µmol l^{-1} for phosphate and, in dependence on the concentrations, 2.5 to 6 % for silicate.

Total alkalinity (A_T) was analyzed immediately after sampling from sterile-filtered (0.2 µm pore size) water samples, taken from the culture vessels during the incubation time. Total alkalinity (A_T) was measured using a potentiometric titration device (Titrando 808, Metrohm, Bradshaw et al., 1981) and was calculated from the Gran function as described by Dickson et al. (2003). The precision was 2 µmol kg^{-1}.

Seawater carbonate system parameters pCO_2, C_T and Ω_{calc} were calculated from pH_{NBS}, A_T, temperature, salinity, phosphate and silicate with the software CO2SYS (Pierrot and Wallace, 2006) using the equilibrium constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

III.2.5 Growth and test degradation

Growth, the size of the tests i.e., maximum diameter on the dorsal side from the top of the youngest chamber over the umbilicus to the opposite chamber, was determined at the beginning and at the end of the 6 weeks experiment on all tests. The tests were counted and the diameter was measured by using an eyepiece reticle on the Wild M3C dissecting microscope. We applied only 40x magnification with the least possible error of ±12.5 µm. This resembles ±4 to ±7 % of the average diameter of the examined specimens. At the end of the experiment, all tests were removed from their recruitment pits with a fine brush and air-dried at room temperature. Finally, selected specimens were prepared for scanning electron microscopy (SEM) with an Emitech K550 (Au+Pd) sputter coater. They were examined with a Cam Scan-CS-44 (Institute of Geosciences, Kiel University) to investigate degradation and corrosion features of the tests.

III.2.6 Statistic

Data were analyzed by quadratic or linear regression with SIGMA PLOT 12.0. Regression lines presenting Pearson correlation with confidence bands, which exhibit 95 % confidence interval and the correlation coefficient R^2 for the fitted line. The error in the regression equations is ±1 sigma of the mean. All graphically presented values are means from 4 replicated culture vessels of each treatment. The numerical data of test diameter are given in the supplement (Table S1).

Multi-factorial analysis of ANOVA (STATISTICA 8) was performed to test the significance of correlations between temperature, salinity, pCO_2 and test diameter after the six weeks incubation time (Table 2). All measured raw data of each treatment were used for statistical analyses.

III.3 Results

III.3.1 Water chemistry

All treatment levels were constant and varied by less than 0.1 in temperature, salinity and pH units throughout the experimental period (Table 1, Fig. 2). The small temperature increase of 0.1 °C after 21 days results from changes of the climate room aeration (Fig. 2).

The calculated, ambient pCO_2 ranged between 481 and 685 µatm in the culture vessels (Fig. 3, Table 1). The elevated CO_2-levels of the 1120, 2400 and 4000 µatm treatments agreed more or less with the mean seawater pCO_2 in the culture vessels and ranged between 1195 ± 111.1, 2108 ± 352.7 and 3843 ± 234.7 µatm, respectively (Table 1).

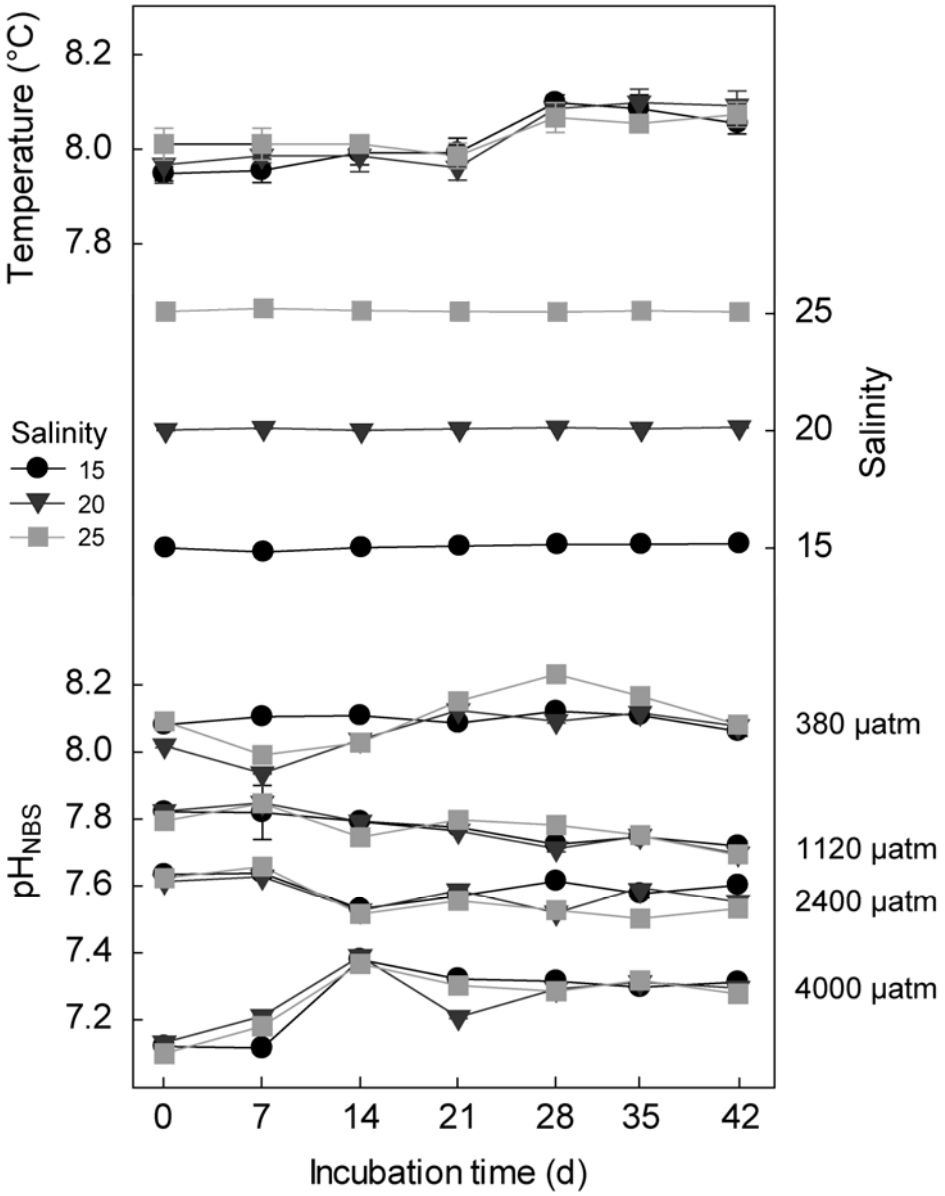

Fig. 2. Mean temperature, salinity and pH_{NBS} values (N = 4) taken for the 8 °C treatment during six weeks incubation time. The symbols represent three different salinities of the treatments: black circle = 15, dark grey triangle = 20, and light grey square = 25.

Table 1. Mean carbonate system parameters, measured and calculated from samples taken from seawater in the culture vessels of several variables for 3 temperatures (8, 13 and 18 °C), 3 salinities (15, 20 and 25) and 4 pCO_2 levels (380, 1120, 2400 and 4000 µatm). During the 6 weeks incubation time, mean concentrations of total carbon (C_T), partial pressure of CO_2 (pCO_2) and the saturation state of calcite (Ω_{calc}) were calculated from the measured mean values of temperature, salinity, pH_{NBS} (N = 4), total alkalinity (A_T), phosphate (PO_4^{3-}) and silicate (Si) (N = 1).

Treatment: T (°C)	S	pCO_2 (µatm)	Seawater measurements T (°C)		S		pH_{NBS}		A_T (µmol kg^{-1})		PO_4^{3-} (µmol l^{-1})		Si (µmol l^{-1})		Calculations from pH_{NBS} and A_T C_T (µmol kg^{-1})		pCO_2 (µatm)		Ω_{calc}	
8	15	380	8.0	(0.03)	15.0	(0.03)	8.10	(0.00)	1833.6	(166.8)	0.06	(0.01)	16.93	(3.99)	1790.4	(165.4)	481	(55.2)	1.34	(0.13)
		1120	8.0	(0.03)	15.0	(0.03)	7.77	(0.03)	1824.9	(172.9)	0.04	(0.01)	16.71	(4.71)	1846.5	(166.3)	1052	(49.4)	0.66	(0.13)
		2400	8.0	(0.03)	15.0	(0.03)	7.60	(0.00)	1830.6	(208.2)	0.06	(0.01)	16.34	(4.44)	1885.1	(211.7)	1498	(115.0)	0.47	(0.07)
		4000	8.0	(0.03)	15.0	(0.04)	7.27	(0.00)	1832.5	(171.8)	0.05	(0.02)	16.22	(4.08)	2001.7	(238.3)	3442	(1324.8)	0.22	(0.04)
8	20	380	8.0	(0.02)	20.0	(0.02)	8.06	(0.00)	2077.7	(37.1)	0.25	(0.27)	26.46	(1.87)	2018.6	(29.0)	549	(38.6)	1.61	(0.15)
		1120	8.0	(0.03)	20.0	(0.03)	7.77	(0.00)	2083.9	(41.5)	0.24	(0.26)	27.05	(2.00)	2096.8	(54.8)	1149	(186.6)	0.84	(0.10)
		2400	8.0	(0.03)	20.0	(0.00)	7.57	(0.00)	2093.8	(51.0)	0.24	(0.29)	28.00	(1.65)	2159.4	(59.2)	1861	(204.8)	0.54	(0.05)
		4000	8.0	(0.03)	20.0	(0.03)	7.26	(0.00)	2092.8	(44.4)	0.26	(0.28)	26.52	(2.39)	2261.0	(67.2)	3618	(928.7)	0.29	(0.07)
8	25	380	8.0	(0.02)	25.1	(0.02)	8.11	(0.00)	2387.8	(39.7)	0.13	(0.13)	28.75	(1.44)	2282.0	(23.1)	521	(98.8)	2.37	(0.49)
		1120	8.0	(0.02)	25.1	(0.03)	7.77	(0.00)	2394.3	(47.8)	0.16	(0.12)	28.28	(2.14)	2394.2	(53.2)	1234	(147.0)	1.10	(0.10)
		2400	8.0	(0.03)	25.1	(0.03)	7.56	(0.00)	2393.9	(38.0)	0.14	(0.15)	28.07	(2.15)	2454.3	(43.1)	2011	(227.0)	0.70	(0.08)
		4000	8.0	(0.03)	25.1	(0.02)	7.26	(0.01)	2370.9	(36.1)	0.14	(0.10)	29.31	(2.40)	2551.1	(87.9)	4055	(1172.2)	0.36	(0.08)
13	15	380	13.0	(0.03)	15.0	(0.03)	8.09	(0.00)	1829.2	(177.2)	0.04	(0.01)	17.16	(4.67)	1775.9	(183.3)	530	(110.5)	1.54	(0.07)
		1120	13.0	(0.01)	15.0	(0.03)	7.80	(0.00)	1803.3	(160.1)	0.04	(0.01)	16.67	(3.22)	1807.0	(153.0)	1021	(38.4)	0.83	(0.15)
		2400	13.0	(0.02)	15.0	(0.03)	7.54	(0.00)	1822.4	(177.5)	0.05	(0.03)	15.69	(3.27)	1882.9	(173.1)	1896	(104.5)	0.48	(0.10)
		4000	13.0	(0.15)	15.0	(0.03)	7.26	(0.00)	1827.3	(175.3)	0.05	(0.01)	14.87	(4.09)	1977.0	(234.2)	3662	(1324.4)	0.26	(0.04)
13	20	380	12.9	(0.00)	20.1	(0.03)	8.05	(0.19)	2064.0	(49.3)	0.23	(0.28)	24.46	(1.94)	1998.7	(10.4)	685	(316.1)	1.80	(0.58)
		1120	13.0	(0.03)	20.1	(0.03)	7.86	(0.19)	2090.3	(54.5)	0.21	(0.27)	24.38	(2.38)	2098.5	(57.9)	1324	(74.7)	0.93	(0.00)
		2400	13.0	(0.02)	20.1	(0.00)	7.53	(0.02)	2120.5	(69.9)	0.26	(0.27)	27.48	(2.97)	2180.5	(85.6)	2140	(326.8)	0.61	(0.06)
		4000	13.0	(0.00)	20.1	(0.02)	7.25	(0.00)	2075.9	(55.1)	0.22	(0.25)	24.41	(3.00)	2230.1	(62.5)	3972	(906.8)	0.33	(0.07)
13	25	380	13.0	(0.02)	25.0	(0.00)	8.07	(0.01)	2362.0	(39.8)	0.13	(0.10)	26.15	(3.15)	2250.2	(51.5)	596	(75.6)	2.50	(0.21)
		1120	13.0	(0.00)	25.1	(0.00)	7.74	(0.00)	2408.1	(31.5)	0.11	(0.12)	27.01	(3.58)	2395.5	(26.1)	1355	(155.7)	1.27	(0.16)
		2400	13.0	(0.03)	25.0	(0.00)	7.55	(0.00)	2390.6	(31.8)	0.09	(0.10)	25.86	(2.85)	2436.3	(38.6)	2154	(264.7)	0.82	(0.09)
		4000	13.0	(0.02)	25.0	(0.03)	7.27	(0.00)	2381.9	(53.6)	0.10	(0.10)	25.73	(3.93)	2521.5	(75.9)	3924	(734.5)	0.46	(0.07)
18	15	380	18.1	(0.03)	15.2	(0.03)	8.01	(0.01)	1937.3	(6.3)	0.65	(0.87)	1.14	(1.54)	1875.5	(11.5)	656	(69.5)	1.80	(0.16)
		1120	18.1	(0.04)	15.1	(0.03)	7.74	(0.02)	1916.3	(28.7)	0.60	(0.80)	0.75	(1.01)	1911.9	(25.0)	1202	(55.0)	1.03	(0.07)
		2400	18.1	(0.06)	15.1	(0.01)	7.51	(0.01)	1890.3	(52.0)	0.60	(0.79)	1.65	(2.32)	1946.8	(57.5)	2227	(182.7)	0.56	(0.01)
		4000	18.1	(0.03)	15.1	(0.02)	7.25	(0.01)	1895.0	(53.6)	0.61	(0.82)	1.69	(2.37)	2027.4	(54.4)	3936	(564.0)	0.33	(0.05)
18	20	380	18.1	(0.03)	20.1	(0.03)	8.05	(0.01)	1779.6	(0.3)	0.68	(0.92)	2.07	(2.49)	1696.0	(8.5)	526	(46.7)	1.91	(0.15)
		1120	18.1	(0.06)	20.1	(0.03)	7.73	(0.01)	1778.0	(11.0)	0.71	(0.95)	2.16	(2.70)	1770.4	(11.4)	1179	(53.3)	0.94	(0.04)
		2400	18.1	(0.03)	20.1	(0.03)	7.41	(0.01)	1788.9	(7.4)	0.70	(0.93)	2.02	(2.47)	1855.2	(11.9)	2493	(169.3)	0.47	(0.03)
		4000	18.1	(0.00)	20.1	(0.05)	7.20	(0.01)	1773.1	(4.9)	0.71	(0.95)	2.01	(2.71)	1896.4	(19.1)	3795	(419.6)	0.31	(0.03)
18	25	380	18.1	(0.02)	25.1	(0.02)	8.10	(0.00)	2232.3	(53.2)	0.58	(0.78)	1.91	(2.25)	2093.5	(55.3)	551	(41.4)	2.90	(0.12)
		1120	18.1	(0.03)	25.1	(0.02)	7.77	(0.01)	2245.2	(18.4)	0.57	(0.76)	1.60	(2.04)	2208.4	(20.1)	1240	(22.6)	1.48	(0.01)
		2400	18.0	(0.02)	25.1	(0.00)	7.44	(0.01)	2266.2	(1.6)	0.57	(0.76)	1.56	(2.19)	2322.2	(12.7)	2692	(242.9)	0.75	(0.06)
		4000	18.0	(0.03)	25.1	(0.02)	7.25	(0.01)	2258.0	(0.6)	0.56	(0.75)	1.33	(1.87)	2382.1	(10.9)	4185	(228.3)	0.49	(0.02)

Low salinity treatments of 15 and 20 simulated the coastal environment in the southwestern Baltic Sea, with a relative low mean alkalinity (A_T) of 1854 ± 7.3 and 1985 ± 2.2 µmol kg^{-1} (Table 1). As a consequence, at a seawater pCO_2 of above 1000 µatm, Ω_{calc} was <1.0. The carbonate chemistry conditions of the present study were similar to a previous data set (Haynert et al., 2011), at a temperature of 12 °C and a salinity of 18 (Fig. 3). In order to document the agreement of the growth response of *A. aomoriensis*, these data were also discussed in the current study.

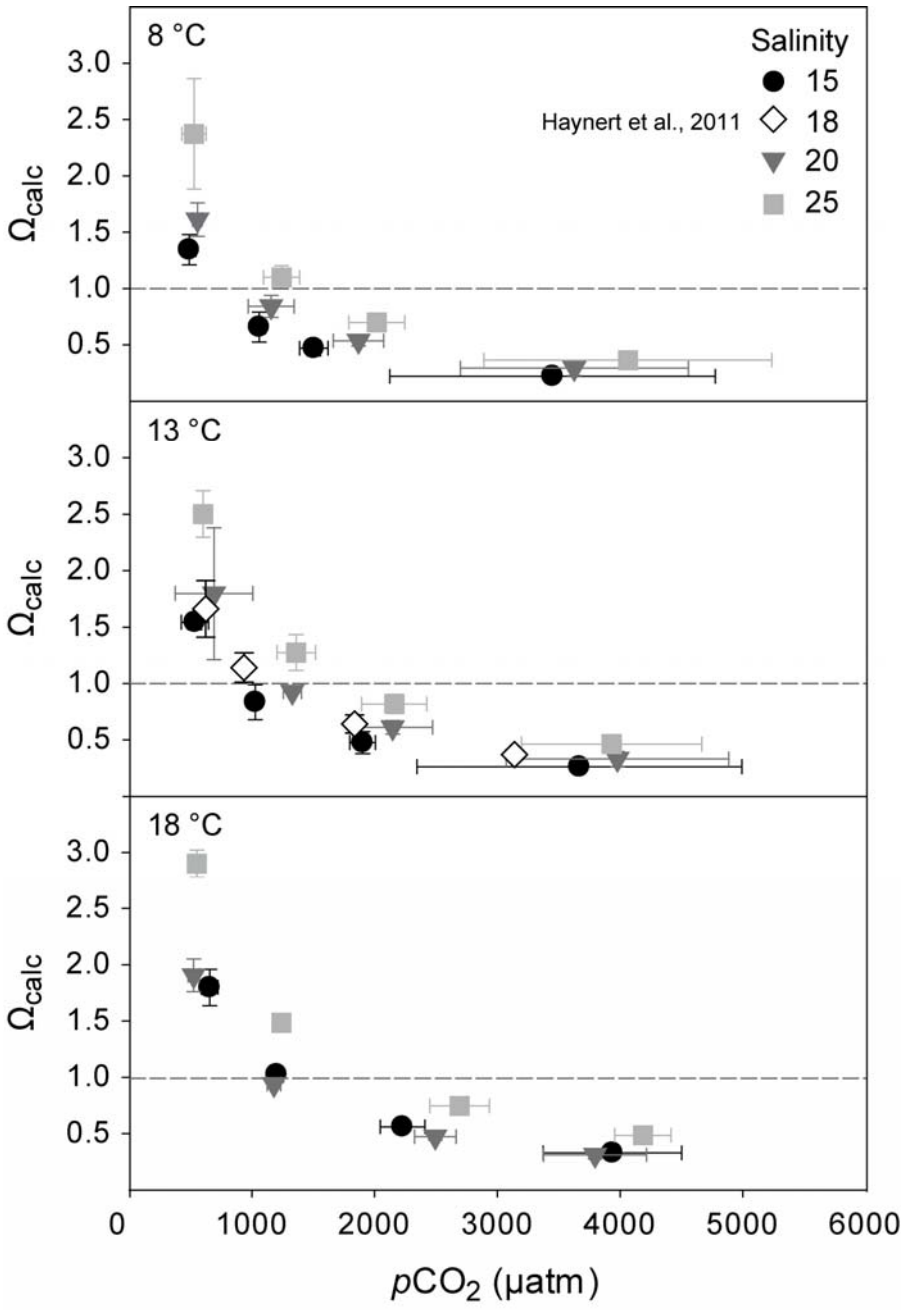

Fig. 3. Mean calculated Ω_{calc} in relation to 4 CO_2 partial pressure (µatm) treatments for 3 temperatures (8, 13 and 18 °C) and 3 salinities (15, 20 and 25) during the six weeks incubation time. Ω_{calc} and pCO_2 averages from an earlier culturing experiment replotted at a temperature of 13 °C and a salinity of 18 during six weeks incubation (Haynert et al., 2011). The grey dashed line symbolized the boundary between under- and supersaturated Ω_{calc} conditions. Vertical bars denote the standard deviation of Ω_{calc} while horizontal bars denote standard deviation for pCO_2.

The seawater from the salinity treatment of 25 was taken from the coastal environment of the North Sea, which exhibited higher mean seawater alkalinity (A_T) of 2341 ± 10.0 µmol kg^{-1}. Consequently, the seawater calcium carbonate saturation state for calcite was in general higher (Fig. 3, Table 1). Also temperature had a positive effect on carbonate saturation state, as higher temperature caused an increase of Ω_{calc} (Fig. 3, Table 1).

III.3.2 Change in test diameter

The highest increase of test diameter of *A. aomoriensis* was observed in the lowest pCO_2 treatment of 566 µatm (Fig. 4, C). An increase of mean test diameter by 36 ± 12.6 µm was observed at a pCO_2 of 566 up to 1195 µatm, respectively Ω_{calc} values >1.0 (Fig. 4, C and D). The intensity of test degradation was amplified by increasing pCO_2 and accordingly decreasing Ω_{calc} (Fig. 4, C and D). At pCO_2 levels above 1200 µatm, the mean diameter in most of the tests decreased by 23 ± 3.5 µm at all temperature and salinity conditions (Fig. 5). Earlier results of a laboratory study with *A. aomoriensis* exhibited the same dependency of test diameter and decreasing Ω_{calc} (Haynert et al., 2011). ANOVA test of significance emphasize the significant correlation of test diameter with pCO_2 (F= 2104.01, p <0.01) and temperature (F= 714.90, p <0.01) (Table 2).

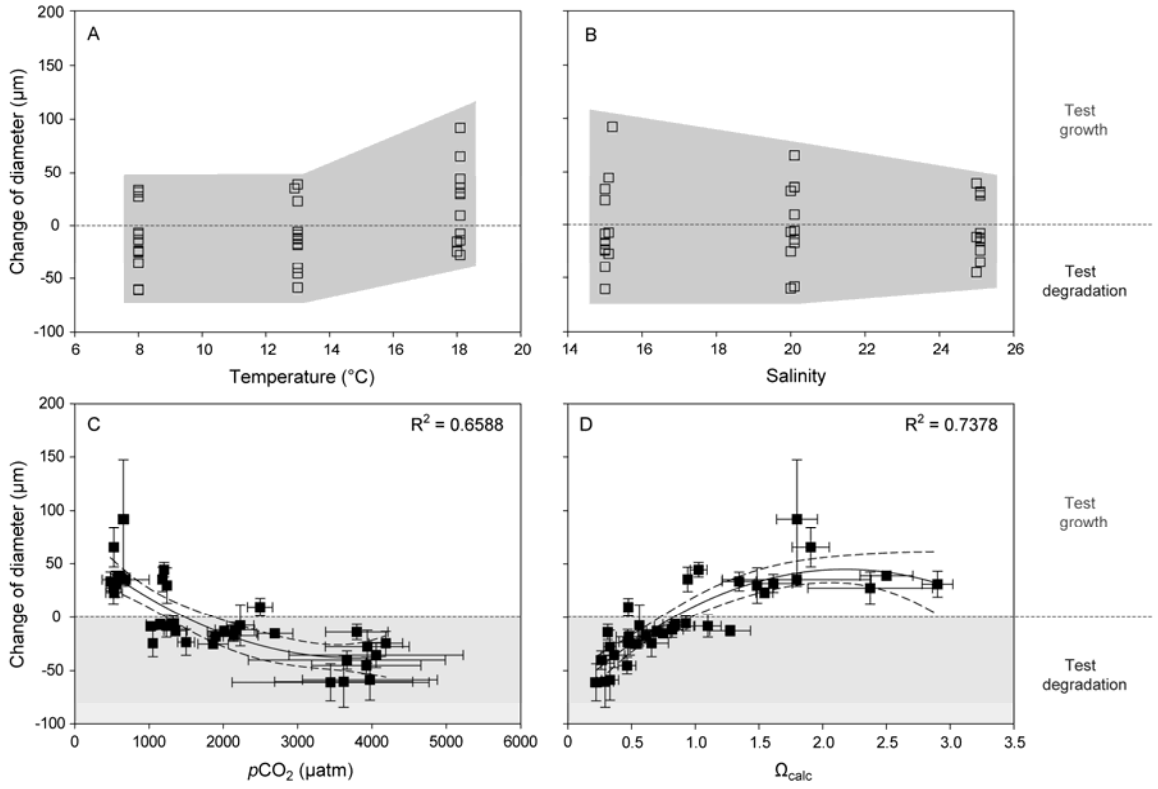

Fig. 4. Change of diameter vs. temperature (A), salinity (B), pCO_2 (C) and Ω_{calc} (D) for all treatments after six weeks. Vertical bars (C and D) denote the standard deviation of change of diameter while horizontal bars denote standard deviation for pCO_2 (C) and Ω_{calc} (D). Black solid line = quadratic regression curves of diameter change, dashed black lines = 95 % confidence interval.

Also the interaction between pCO_2 and temperature was significant (F= 37.79, p <0.01). The temperature of 18 °C had a positive effect on Ω_{calc}, accordingly a higher test growth of *A. aomoriensis* was recognized, namely by 44 ± 27.1 µm (Figs. 4, A and 5). In comparison, at temperatures of 8 and 13 °C, mean test diameter increased by 31 ± 3.1 and 32 ± 8.3 µm.

In comparison to pCO_2 and temperature, salinity apparently had no significant effect on change of test diameter (Table 2). Growth of *A. aomoriensis* decreased insignificantly from 48 ± 24.38 to 32 ± 4.98 µm up to pCO_2 of <1200 µatm (Ω_{calc}>1.0). At a pCO_2 of >1200 µatm respectively Ω_{calc}<1.0, degradation of the tests ranged similarly from -25 ± 9.69 to -21 ± 2.26 µm with increasing salinity from 15 to 25 (Figs. 4, B and 5).

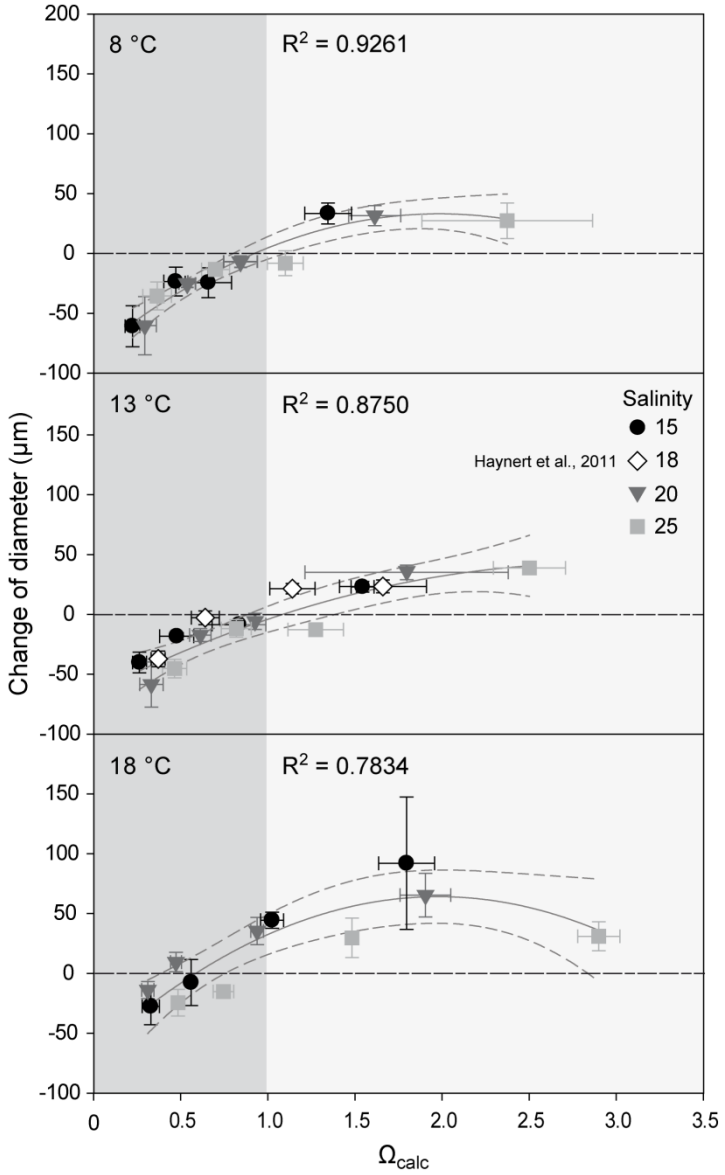

Fig. 5. Change of test diameter in relation to Ω_{calc} for 3 temperature (8, 13 and 18 °C), 3 salinity (15, 20 and 25) and 4 pCO_2 treatments ranging from 566 to 3843 µatm. Change of diameter and Ω_{calc} averages from an earlier culturing experiment replotted at a temperature of 13 °C and a salinity of 18 during six weeks incubation (Haynert et al., 2011). The different grey areas separate under- and supersaturated Ω_{calc} conditions. Black dashed line symbolized the boundary between growth and degradation of the test of *A. aomoriensis*. Vertical bars denote the standard deviation of diameter change while horizontal bars denote standard deviation for Ω_{calc} after six weeks (N = 4). Grey solid line = quadratic regression curves of diameter change, dashed grey lines = 95 % confidence interval.

Table 2. Multi-factorial ANOVA tests of significance indicating the effect of temperature, salinity and pCO_2 on test diameter of *A. aomoriensis*. Significant results are represented in bold. SS = Sum of Squares, d.f. = degrees of freedom, MS = Mean Squares.

Test diameter

	SS	d.f.	MS	F	*p*
Intercept	527.3	1.0	527.3	25.58	0.1127
pCO_2	**130100.1**	**3.0**	**43366.7**	**2104.01**	**0.0000**
Salinität	150.2	2.0	75.1	0.36	0.6955
Temperatur	**29470.3**	**2.0**	**14735.2**	**714.90**	**0.0000**
pCO_2 x Sal	2597.7	6.0	432.9	21.01	0.0590
pCO_2 x Temp	**4673.3**	**6.0**	**778.9**	**37.79**	**0.0019**
Sal x Temp	**5115.5**	**4.0**	**1278.9**	**62.05**	**0.0002**
pCO_2 x Sal x Temp	6061.1	12.0	505.1	24.51	0.0073
Error	22260.4	108.0	206.1		

III.3.3 SEM-Observations

SEM-observations were in agreement with the measured change of diameter after the six weeks incubation time (Pl. 1). At a pCO_2 of 566 µatm, the examined tests of *A. aomoriensis* were fully intact (Pl. 1, A). Test degradation started at the last chambers with thinner test walls at pCO_2 of 1195 µatm (Pl. 1, B). The intensity of test degradation increased with increasing pCO_2 and accordingly undersaturation for calcite (Pl. 1, B, C and D). The strongest effect of test degradation was observed at a high pCO_2 of 3843 µatm (Pl. 1, D). Some of the specimens depicted the characteristic star-like appearance, only the umbilicus area of the tests and the suture of the outer, thinner chambers remained (Pl. 1, E). The detailed view exhibited the inner organic lining with some pore fragments, the rest of the test was completely degraded (Pl. 1, E1 and E2).

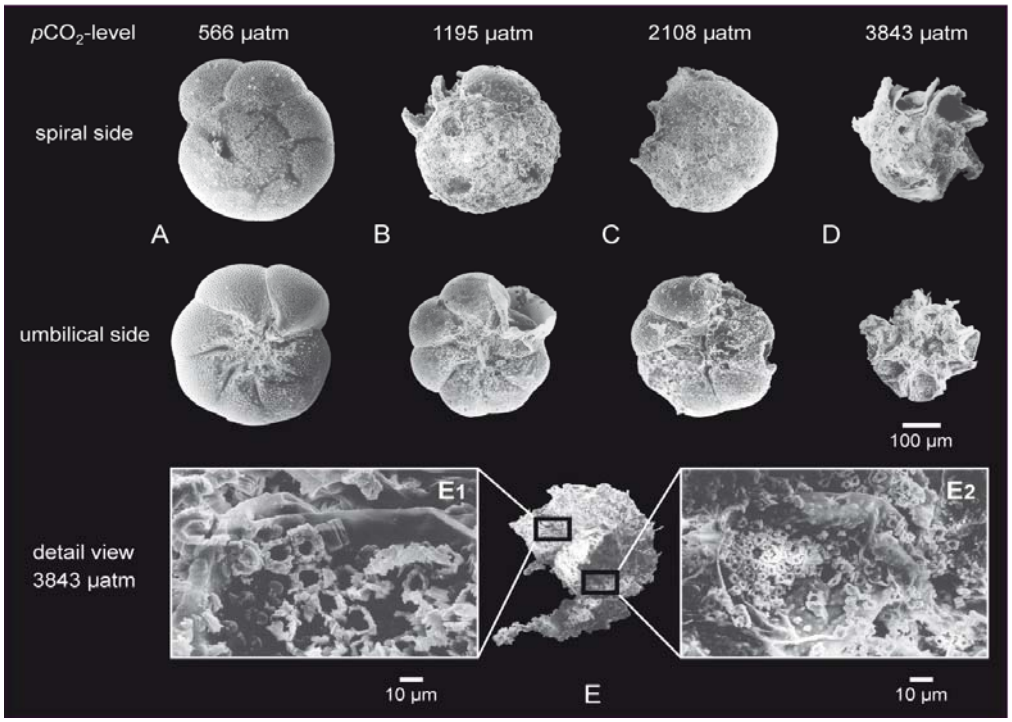

Plate 1. SEM images of *Ammonia aomoriensis* (Asano, 1951) depicting different stages of test degradation on spiral and umbilical side at 4 pCO_2 levels, ranging from 566 to 3843 µatm (A-D). Detailed view (E_1 and E_2) exhibits the inner organic lining (E) at a pCO_2 of 3843 µatm.

Single *A. aomoriensis* tests exhibited the formation of recalcification structures on the spiral side (Pl. 2). These scale-alike, platy structures resemble new layers of secondary calcite. In relation to temperature and salinity however, no visible effect of recalcification ability on tests of *A. aomoriensis* were recognized.

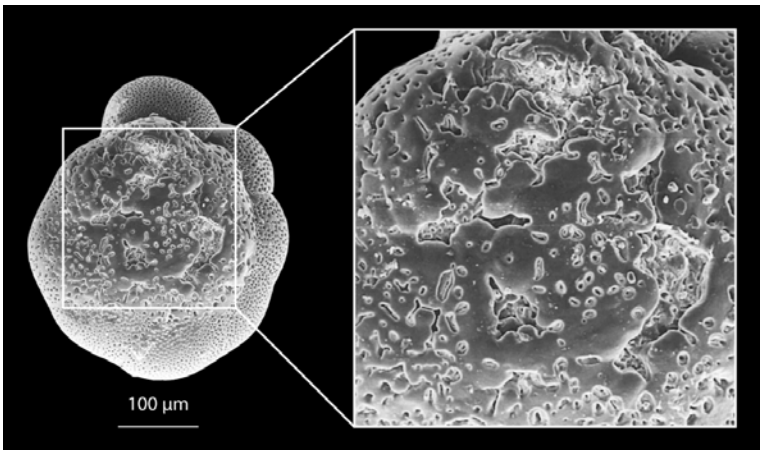

Plate 2. SEM images of recalcification structures on spiral side of *A. aomoriensis* at a pCO_2 of 1195 µatm.

III.4 Discussion

III.4.1 Vitality assessment

The number of remaining tests was counted at the end of the experiment. After the incubation time of six weeks, the tests were fragile, in particular at the highest seawater pCO_2 only the inner organic lining was left. The handling of the extremely corroded tests was very complicated and a lot of specimens were damaged during picking from the culture vessels and transfer to the cell slides. We therefore abstained from a treatment with chemicals or Rose Bengal. Observational assessment of survival as movements or cytoplasm flow (Bradshaw, 1961) would require a remediation phase, which the specimens would use to repair their tests. As this would bias the results, we simply dried them at the end of the experiment. Therefore, we could not asses the vitality of the specimens, and consequently no differentiation between active or inactive was determinable. However, many specimens contained yellow cytoplasm, which indicates that they were alive during the entire experimental period.

III.4.2 Test diameter of *Ammonia aomoriensis*

III.4.2.1 Response to manipulated carbonate chemistry

Seawater pCO_2 levels had a significant effect on the test growth and degradation of *A. aomoriensis*. These results agree with a previous culturing study of *A. aomoriensis* which reported a mean growth of 22 µm up to pCO_2 of 929 µatm and $\Omega_{calc}>1$ (Haynert et al., 2011).

Similarly, a significant reduction of test diameter was observed at high pCO_2 of 3130 µatm. Both laboratory studies emphasize the negative response under elevated pCO_2 and Ω_{calc}<1. Also in natural environments, as for instance Flensburg Fjord, located in the southwestern Baltic Sea, population density of living *A. aomoriensis* co-varies with pore water Ω_{calc} becoming lower at undersaturated conditions (Haynert et al., 2012). According to these results, we conclude that *A. aomoriensis* has developed a certain sensitivity to Ω_{calc}<1 or low carbonate ion concentration. These observations are in agreement with Keul et al. (2013), which exhibited a negative relationship on test weights and growth rates of *Ammonia* species with decreasing [CO_3^{2-}], respectively Ω_{calc}<1.

Similar observations were recorded by Gazeau et al., (2011). This study described that calcification rates of the larvae of the Pacific Oyster *Crassostrea gigas* were not directly affected by pH or pCO_2, but highly depended on the availability of carbonate ions. The oyster calcification decreased strongly below saturation levels. Other studies reported a positive relationship between saturation state and calcification rates: for instance, shell weights of planktonic foraminifera increased at higher saturation states (Spero et al., 1997, Bijma et al., 2002, Lombard et al., 2010). These results revealed that the response of organisms to change in seawater chemistry could be different between species. In particular, the strategies for carbon supply of calcification may vary between foraminiferal species.

Most previous culturing studies dealing with benthic foraminifera manipulated pH or pCO_2, without differentiating between effects of these manipulations. They did not consider the impact undersaturation of Ω_{calc} on calcification of foraminifera (Kuroyanagi et al., 2009, Allison et al., 2010, Fujita et al., 2011).

McIntyre-Wressnig et al. (2013) revealed that symbiont-bearing benthic foraminifera *Amphistegina gibbosa* and *Archaias angulatus* were not affected by elevated pCO_2 (1000 and 2000 µatm). Further symbiont-bearing foraminifera, the diatom-bearing *Amphistegina radiata* and *Heterostegina depressa*, and the dinoflagellate-bearing *Marginopora vertebralis* exhibited no negative calcification responses to pCO_2 up to 1925 µatm after six weeks (Vogel and Uthicke, 2012). In both studies, Ω_{calc} was supersaturated (Ω_{calc}>1.5) at the tested high pCO_2 levels. Any conclusions about their sensitivity to undersaturation are therefore not possible. It is evident, however, that endosymbionts pursuing photosynthesis may benefit from higher pCO_2. Accordingly, it is well conceivable that symbiont-bearing foraminifera are adapted to high pCO_2, but may still respond sensitive to undersaturation.

Furthermore, even though test degradation started at a pCO_2 level of about 1195 up to 2108 µatm (Ω_{calc}<1), some specimens of *A. aomoriensis* were able to rebuild their tests and formed recalcification structures under unfavorable conditions. This response was observed up to a pCO_2 level of 2108 µatm in the present study. The same recalcification feature was observed on *A. aomoriensis* tests in Flensburg Fjord after transient low Ω_{calc} periods (Haynert et al., 2012). It is evident that recalcification most likely took place when Ω_{calc} increased again during seasonal fluctuations as it has been observed in a natural environment (Haynert et al., 2012) or at a specifically driven pH-variability in laboratory experiments (Le Cadre et al., 2003). We assume that

recalcification of the specimens in the laboratory was possible due to adaptation to changing seawater chemistry, which they experienced during transfer from the natural environment to undersaturated laboratory conditions. It remains unclear, for how long the specimens were able to overcome test dissolution, which might be related to higher energetically effort and whether reproduction would still be possible under these conditions.

III.4.2.2 Response to temperature

Seawater temperature had a significant effect on the test diameter growth of *A. aomoriensis*. Maximum growth was observed at 18 °C, which is slightly above today's maximum temperatures (15.3 °C) observed in the bottom water in the southwestern Baltic Sea during summer (Haynert et al., 2012).

A similar positive correlation to temperature was observed by Bradshaw (1957 and 1961) for *A. beccarii tepida* (Cushman). While at 10 °C no growth was observed, growth increased with increasing temperatures of 20, 24–27 °C and reached an optimum at 30 °C. Only at much higher temperatures of >35 °C, a critical level was reached. No growth was observed and some specimens died. In contrast, another experiment using the benthic species *Rosalina leei*, exhibited that a successive increase of temperature from 25, 30 to 35 °C caused a retarded growth (Nigam et al., 2008). These results revealed that the growth of foraminifera was largely influenced by fluctuating environmental temperatures and that optimum condition may be different between the species.

III.4.2.3 Response to salinity

Our observations revealed that seawater salinity had no significant effect on change of diameter of *A. aomoriensis*. In the natural environment of Flensburg Fjord, salinity ranged from 16.8 to 26.3 in the bottom water during the seasonal cycle (Haynert et al., 2012). A similar salinity range from 15 to 25 was applied in the present study. The data provide no evidence for changes in growth that were directly induced by salinity variations. An earlier experiment revealed also no significant chances in test diameter to the closely related species *Ammonia tepida* at salinities ranging from 13 to 27 (Bradshaw, 1957). Studies of the recent distribution of *Ammonia beccarii* and related species, as for instance *A. aomoriensis*, designated them as euryhaline species, inhabiting hypo- to hyper saline waters (Wefer, 1976, Almogi-Labin et al., 1992, Alve and Murray, 1999, Frenzel and Oertel, 2002). Accordingly, the salinity levels of 15, 20 and 25, applied in our experiment, were within the tolerance range of this species, where no effects on growth or reproduction have been observed.

However, foraminifers precipitate the calcium carbonate for their test by vacuolization from external seawater [Ca^{2+}] and [CO_3^{2-}] Erez (2003). As seawater alkalinity is directly correlated to salinity, a higher salinity may in turn elevate the concentration and hence availability of carbonate ions. Therefore, increasing salinity caused a higher [CO_3^{2-}] availability, which may promote the

calcification process in foraminifera and, in turn, amplified their growth. Despite a higher availability of CO_3^{2-} at a salinity of 25, calcification of *A. aomoriensis* was not promoted. In particular, the thickness of the last new chambers and the solubility of tests exhibited also no differences between the salinities as applied in our treatments.

III.4.3 Dissolution of the tests

SEM-observations corroborated the relationship between seawater pCO_2, respectively Ω_{calc}, and dissolution of the tests. Elevated pCO_2 levels left their trace in substantial test degradation, especially at a calcite saturation state below 1, which was reached at a pCO_2 of about 1200 µatm. These results agree with the observations of a previous laboratory study (Haynert et al., 2011) and observations under natural conditions in Flensburg Fjord (Haynert et al., 2012), and emphasize the susceptibility of *A. aomoriensis* tests to low Ω_{calc}. The laboratory study of Le Cadre et al. (2003) reported corroded tests at low pH values from 7.5 to 7.0, which most probably induced undersaturation of the seawater with respect to Ω_{calc}.

Hyaline species like *A. aomoriensis* store calcium and carbonate in separate intracellular pools, which are used to precipitate new chambers extracellularly. For this calcification process, foraminifera elevated their intracellular pH (de Nooijer et al., 2009). Therefore, the amount of energy which is required to reach an optimal pH for calcification, depends on the initial saturation state in the ambient seawater (Bentov et al., 2009) as ambient seawater is the main source of $[Ca^{2+}]$ and $[CO_3^{2-}]$ (Erez, 2003). An adaptation to ambient undersaturation of calcite may also cause higher energetic cost, which in turn reduced the calcification potential in the specimens (Melzner et al., 2011). Foraminiferal calcification might be affected in a similar way, as also suggested by de Nooijer et al. (2009).

III.5 Conclusions

This study describes the combined effects of elevated pCO_2, and different temperature and salinity conditions on growth and test degradation of *A. aomorienis*. Our results highlight a large sensitivity of this boreal shallow water species to undersaturation of calcite. *A. aomoriensis* is able to grow at pCO_2 <1200 µatm respectively Ω_{calc}>1. A significant reduction in test diameter was observed at a pCO_2 >1200 µatm and simultaneously Ω_{calc}<1. Nevertheless, some specimens of *A. aomoriensis* are able to recalcify under undersaturation conditions.

Salinity had no effect on the diameter change of *A. aomorienis*, whereas increasing temperatures had a positive effect for calcifying foraminifera and facilitated their growth. Based on these results, the negative impact of ocean acidification could partially be mitigated by warming. However, the sensitivity to undersaturation of calcite and permanent low Ω_{calc}<1 could lead to significant decline of population density of *A. aomoriensis* in their natural environment in the southwestern Baltic Sea.

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Supplement

TableS1. Raw data of *A. aomoriensis* test diameter at the beginning and at the end of the experiment, and calculated change of diameter after six weeks incubation time.

T (°C)	S	pCO_2 (µatm)	Replicate	Specimen	Test diameter Beginning (µm)	Test diameter End (µm)	Change of diameter (µm)
8	15	380	A	1	375	450	75
				2	375	450	75
				3	300	325	25
				4	375	400	25
				5	400		
				6	400		
				7	375	425	50
				8	300	375	75
				9	475	475	0
				10	450		
			Mean		368	414	46
8	15	380	B	1	450		
				2	325	350	25
				3	400	450	50
				4	475	475	0
				5	425	475	50
				6	375		
				7	350	350	0
				8	375	450	75
				9	425	450	25
				10	500	500	0
			Mean		409	438	28
8	15	380	C	1	425	425	0
				2	400	425	25
				3	225	250	25
				4	500		
				5	400	450	50
				6	350	400	50
				7	325	375	50
				8	375	400	25
				9	375	375	0
				10	375		
			Mean		359	388	28
8	15	380	D	1	425	475	50
				2	400	400	0
				3	375	400	25
				4	400	450	50
				5	450	450	0
				6	425	475	50
				7	325	350	25
				8	450		
				9	275	325	50
				10	450		
			Mean		384	416	31
8	15	1120	A	1	450		
				2	350	325	-25
				3	250	250	0
				4	375	375	0
				5	375	375	0
				6	350	325	-25
				7	350		
				8	375	350	-25
				9	475	425	-50
				10	350		
			Mean		364	346	-18

8	15	1120	B	1	475	425	-50
				2	275		
				3	500	375	-125
				4	225	200	-25
				5	475	375	-100
				6	325	350	25
				7	425		
				8	375	350	-25
				9	325	325	0
				10	300		
			Mean		386	343	-43
8	15	1120	C	1	300		
				2	325	350	25
				3	450	400	-50
				4	300		
				5	325	300	-25
				6	275	275	0
				7	450		
				8	375	350	-25
				9	375	350	-25
				10	250	200	-50
			Mean		339	318	-21
8	15	1120	D	1	325	275	-50
				2	300	275	-25
				3	350	375	25
				4	425	400	-25
				5	375		
				6	400	400	0
				7	375	375	0
				8	425	400	-25
				9	375	350	-25
				10	375		
			Mean		372	356	-16
8	15	2400	A	1	250		
				2	400	400	0
				3	275	250	-25
				4	400	375	-25
				5	350	300	-50
				6	400		
				7	250	200	-50
				8	375	350	-25
				9	425		
				10	375		
			Mean		342	313	-29
8	15	2400	B	1	375	350	-25
				2	375	300	-75
				3	500		
				4	375	375	0
				5	350	325	-25
				6	400	350	-50
				7	475		
				8	425		
				9	400		
				10	350	300	-50
			Mean		371	333	-38
8	15	2400	C	1	375		
				2	475	425	-50
				3	375	375	0
				4	400		
				5	400	375	-25
				6	375		
				7	325	300	-25
				8	350	350	0
				9	400	400	0
				10	375		
			Mean		388	371	-17

8	15	2400	D	1	275	300	25
				2	400	350	-50
				3	225	250	25
				4	475		
				5	425	400	-25
				6	500	475	-25
				7	350	350	0
				8	425		
				9	350	325	-25
				10	375		
			Mean		361	350	-11
8	15	4000	A	1	400		
				2	250	175	-75
				3	325		
				4	325	300	-25
				5	350	325	-25
				6	400		
				7	350		
				8	450		
				9	400	350	-50
				10	375	325	-50
			Mean		340	295	-45
8	15	4000	B	1	425		
				2	375	325	-50
				3	400	350	-50
				4	400		
				5	375	325	-50
				6	250		
				7	400		
				8	500		
				9	325	250	-75
				10	325	300	-25
			Mean		360	310	-50
8	15	4000	C	1	450		
				2	325	275	-50
				3	325	225	-100
				4	400		
				5	475	475	0
				6	400		
				7	275	200	-75
				8	375	275	-100
				9	300	125	-175
				10	375		
			Mean		346	263	-83
8	15	4000	D	1	375	275	-100
				2	375	300	-75
				3	500		
				4	425		
				5	275	250	-25
				6	400		
				7	400		
				8	475	400	-75
				9	425	375	-50
				10	375		
			Mean		385	320	-65
8	20	380	A	1	375		
				2	325	375	50
				3	400	425	25
				4	425	450	25
				5	400		
				6	400	425	25
				7	425	475	50
				8	350		
				9	400	425	25
				10	425	425	0
			Mean		400	429	29

8	20	380	B	1	350	350	0
				2	400	450	50
				3	400		
				4	475		
				5	275	350	75
				6	450	450	0
				7	400		
				8	450	450	0
				9	225	325	100
				10	350	375	25
			Mean		357	393	36
8	20	380	C	1	375	400	25
				2	375	375	0
				3	275	300	25
				4	400		
				5	350	425	75
				6	500	500	0
				7	425		
				8	425	450	25
				9	425	425	0
				10	425		
			Mean		389	411	21
8	20	380	D	1	500	500	0
				2	400		
				3	450		
				4	225	300	75
				5	350	375	25
				6	300	350	50
				7	375	425	50
				8	400	450	50
				9	400	450	50
				10	350	375	25
			Mean		363	403	41
8	20	1120	A	1	450	450	0
				2	425		
				3	350	325	-25
				4	375	400	25
				5	425		
				6	475		
				7	475	475	0
				8	475	475	0
				9	300		
				10	375	375	0
			Mean		417	417	0
8	20	1120	B	1	375		
				2	275	300	25
				3	325	350	25
				4	425		
				5	400	375	-25
				6	400	375	-25
				7	425	400	-25
				8	300		
				9	350	350	0
				10	325	300	-25
			Mean		357	350	-7
8	20	1120	C	1	375	375	0
				2	375	350	-25
				3	325	300	-25
				4	350	325	-25
				5	300	300	0
				6	250	250	0
				7	275		
				8	350	350	0
				9	375	375	0
				10	375		
			Mean		338	328	-9

8	20	1120	D	1	375	375	0
				2	325		
				3	375	375	0
				4	500		
				5	350	350	0
				6	325		
				7	350	375	25
				8	450	425	-25
				9	275	225	-50
				10	300	275	-25
			Mean		354	343	-11
8	20	2400	A	1	325	300	-25
				2	375		
				3	200		
				4	375		
				5	350	325	-25
				6	225	225	0
				7	350	325	-25
				8	400		
				9	300	250	-50
				10	375	350	-25
			Mean		321	296	-25
8	20	2400	B	1	300	275	-25
				2	375	300	-75
				3	500	500	0
				4	400	375	-25
				5	450		
				6	475		
				7	400	375	-25
				8	425	400	-25
				9	400		
				10	400		
			Mean		400	371	-29
8	20	2400	C	1	300		
				2	325	300	-25
				3	350		
				4	275		
				5	450	425	-25
				6	450	400	-50
				7	350	350	0
				8	350	350	0
				9	300	275	-25
				10	325	300	-25
			Mean		364	343	-21
8	20	2400	D	1	375	350	-25
				2	375	375	0
				3	425		
				4	325	275	-50
				5	350	325	-25
				6	400		
				7	350	325	-25
				8	400		
				9	400		
				10	350		
			Mean		355	330	-25
8	20	4000	A	1	475		
				2	350		
				3	375	250	-125
				4	450		
				5	350		
				6	350	325	-25
				7	325	250	-75
				8	450	250	-200
				9	325		
				10	350	325	-25
			Mean		370	280	-90

8	20	4000	B	1	325	300	-25
				2	400		
				3	250		
				4	400	350	-50
				5	425		
				6	450	425	-25
				7	275	250	-25
				8	425		
				9	375		
				10	300		
			Mean		363	331	-31
8	20	4000	C	1	450		
				2	375	300	-75
				3	400	350	-50
				4	400		
				5	375	300	-75
				6	375		
				7	525		
				8	350	250	-100
				9	400		
				10	400	375	-25
			Mean		380	315	-65
8	20	4000	D	1	425		
				2	475		
				3	300		
				4	400		
				5	375	325	-50
				6	375	300	-75
				7	450	450	0
				8	300	250	-50
				9	500	400	-100
				10	350		
			Mean		400	345	-55
8	25	380	A	1	225	325	100
				2	275	300	25
				3	375	375	0
				4	450		
				5	300	375	75
				6	375		
				7	350	375	25
				8	375	400	25
				9	375		
				10	325	375	50
			Mean		318	361	43
8	25	380	B	1	350	375	25
				2	325	350	25
				3	350	375	25
				4	325	375	50
				5	425	325	-100
				6	275	300	25
				7	350		
				8	300	325	25
				9	450		
				10	575	575	0
			Mean		366	375	9
8	25	380	C	1	350	425	75
				2	500		
				3	275	325	50
				4	425		
				5	325	375	50
				6	425		
				7	350	375	25
				8	475	475	0
				9	325	375	50

				10	400	400	0
			Mean		357	393	36
8	25	380	D	1	400		
				2	500	500	0
				3	325	350	25
				4	500		
				5	375	400	25
				6	325	375	50
				7	475		
				8	375	400	25
				9	400	425	25
				10	425	425	0
			Mean		389	411	21
8	25	1120	A	1	275	250	-25
				2	475	475	0
				3	400	400	0
				4	450	425	-25
				5	300	300	0
				6	225	250	25
				7	500		
				8	425		
				9	500	500	0
				10	325		
			Mean		375	371	-4
8	25	1120	B	1	500	500	0
				2	375	400	25
				3	350		
				4	325		
				5	450	450	0
				6	350	325	-25
				7	350	375	25
				8	300		
				9	375	375	0
				10	400		
			Mean		400	404	4
8	25	1120	C	1	375	350	-25
				2	375	375	0
				3	400		
				4	425	400	-25
				5	300	275	-25
				6	300		
				7	425		
				8	350	325	-25
				9	375	350	-25
				10	375	375	0
			Mean		368	350	-18
8	25	1120	D	1	375	375	0
				2	325	300	-25
				3	400	400	0
				4	425	400	-25
				5	350	350	0
				6	450		
				7	375	350	-25
				8	425		
				9	400	375	-25
				10	325	300	-25
			Mean		372	356	-16
8	25	2400	A	1	400	375	-25
				2	375	375	0
				3	250		
				4	375	350	-25
				5	400	375	-25
				6	350		
				7	400		
				8	350	350	0
				9	325	325	0

				10	325		
			Mean		371	358	-13
8	25	2400	B	1	500	500	0
				2	425	400	-25
				3	300		
				4	275	250	-25
				5	425		
				6	450	425	-25
				7	400	375	-25
				8	275	300	25
				9	475		
				10	500		
			Mean		388	375	-13
8	25	2400	C	1	375		
				2	425	400	-25
				3	400		
				4	475		
				5	300	275	-25
				6	375		
				7	300	275	-25
				8	375	350	-25
				9	325		
				10	375	375	0
			Mean		355	335	-20
8	25	2400	D	1	400		
				2	375	350	-25
				3	250	225	-25
				4	400		
				5	300	300	0
				6	300		
				7	325	350	25
				8	400	375	-25
				9	375		
				10	475	475	0
			Mean		354	346	-8
8	25	4000	A	1	325	250	-75
				2	300	250	-50
				3	350	325	-25
				4	375	375	0
				5	250		
				6	375		
				7	350	275	-75
				8	425		
				9	400		
				10	425		
			Mean		340	295	-45
8	25	4000	B	1	400		
				2	475		
				3	450		
				4	400	375	-25
				5	250		
				6	400		
				7	275	250	-25
				8	300		
				9	375		
				10	325	275	-50
			Mean		333	300	-33
8	25	4000	C	1	425		
				2	525	500	-25
				3	450	425	-25
				4	350	325	-25
				5	325	325	0
				6	375		
				7	300		
				8	275	250	-25
				9	375		

				10	425		
			Mean		385	365	-20
8	25	4000	D	1	375	375	0
				2	425	375	-50
				3	400		
				4	375	350	-25
				5	475		
				6	400		
				7	225		
				8	400		
				9	375	275	-100
				10	375		
			Mean		388	344	-44
13	15	380	A	1	375	400	25
				2	375	375	0
				3	400		
				4	375	400	25
				5	350		
				6	375		
				7	525	525	0
				8	375	400	25
				9	375	425	50
				10	375	375	0
			Mean		396	414	18
13	15	380	B	1	400	425	25
				2	450		
				3	325	350	25
				4	325	350	25
				5	250	300	50
				6	400	400	0
				7	375		
				8	250	300	50
				9	325	375	50
				10	450	450	0
			Mean		341	369	28
13	15	380	C	1	400	425	25
				2	400	425	25
				3	350	375	25
				4	325		
				5	350	375	25
				6	375	375	0
				7	275	325	50
				8	300		
				9	450	450	0
				10	300	350	50
			Mean		363	388	25
13	15	380	D	1	375		
				2	300	325	25
				3	400	425	25
				4	350	375	25
				5	275		
				6	200	250	50
				7	250		
				8	400	400	0
				9	425	425	0
				10	375		
			Mean		346	367	21
13	15	1120	A	1	375		
				2	400	425	25
				3	375	375	0
				4	400		
				5	350	325	-25
				6	275	250	-25
				7	375		
				8	375	375	0
				9	325		

				10	375	350	-25
			Mean		358	350	-8
13	15	1120	B	1	450		
				2	325		
				3	375	375	0
				4	350	350	0
				5	375	375	0
				6	275	250	-25
				7	275	250	-25
				8	325		
				9	225	200	-25
				10	425	425	0
			Mean		329	318	-11
13	15	1120	C	1	325		
				2	400	375	-25
				3	400	400	0
				4	275	275	0
				5	375	350	-25
				6	400		
				7	350	350	0
				8	325		
				9	450	450	0
				10	375		
			Mean		375	367	-8
13	15	1120	D	1	275		
				2	425	400	-25
				3	425	425	0
				4	375		
				5	475	475	0
				6	325	300	-25
				7	300	300	0
				8	325	300	-25
				9	350		
				10	400	425	25
			Mean		382	375	-7
13	15	2400	A	1	425		
				2	300	250	-50
				3	300		
				4	450		
				5	375	350	-25
				6	425	425	0
				7	250		
				8	375	400	25
				9	425	400	-25
				10	325		
			Mean		380	365	-15
13	15	2400	B	1	375	350	-25
				2	425		
				3	375	375	0
				4	250	225	-25
				5	375		
				6	450		
				7	300	300	0
				8	325	300	-25
				9	375		
				10	350	325	-25
			Mean		329	313	-17
13	15	2400	C	1	325	300	-25
				2	275		
				3	250	225	-25
				4	400		
				5	325	300	-25
				6	400		
				7	400		
				8	250		
				9	450	450	0

				10	450	425	-25
			Mean		360	340	-20
13	15	2400	D	1	250	250	0
				2	350		
				3	250	225	-25
				4	375		
				5	375	375	0
				6	375	350	-25
				7	300	250	-50
				8	400	375	-25
				9	250	225	-25
				10	225		
			Mean		314	293	-21
13	15	4000	A	1	375	350	-25
				2	425		
				3	325	275	-50
				4	250	225	-25
				5	325	250	-75
				6	300		
				7	325		
				8	375	300	-75
				9	200		
				10	375		
			Mean		330	280	-50
13	15	4000	B	1	350		
				2	375		
				3	250	225	-25
				4	350		
				5	325	300	-25
				6	475	475	0
				7	375	350	-25
				8	300		
				9	350	300	-50
				10	375	325	-50
			Mean		358	329	-29
13	15	4000	C	1	375		
				2	375		
				3	450	425	-25
				4	375	375	0
				5	300	250	-50
				6	450		
				7	450	400	-50
				8	475	400	-75
				9	375		
				10	450		
			Mean		410	370	-40
13	15	4000	D	1	375	375	0
				2	450	350	-100
				3	300		
				4	325	300	-25
				5	250		
				6	375	325	-50
				7	375	350	-25
				8	325		
				9	450	400	-50
				10	400		
			Mean		392	350	-42
13	20	380	A	1	300	350	50
				2	375	375	0
				3	375	400	25
				4	350		
				5	325	375	50
				6	375	425	50
				7	350	400	50
				8	375		
				9	375	425	50

				10	425	425	0
			Mean		363	397	34
13	20	380	B	1	350		
				2	375		
				3	275	325	50
				4	375	400	25
				5	350	400	50
				6	325	350	25
				7	300		
				8	400	425	25
				9	400	425	25
				10	250	350	100
			Mean		339	382	43
13	20	380	C	1	225		
				2	375	375	0
				3	475	475	0
				4	375	425	50
				5	400	425	25
				6	400	425	25
				7	475	475	0
				8	300	350	50
				9	375		
				10	275	350	75
			Mean		384	413	28
13	20	380	D	1	225	275	50
				2	300	325	25
				3	375	425	50
				4	300	350	50
				5	400	450	50
				6	475	475	0
				7	375	400	25
				8	350	400	50
				9	450	450	0
				10	250	300	50
			Mean		350	385	35
13	20	1120	A	1	375	375	0
				2	275	250	-25
				3	375	375	0
				4	375		
				5	350	350	0
				6	450	425	-25
				7	475		
				8	400	375	-25
				9	325		
				10	325		
			Mean		371	358	-13
13	20	1120	B	1	400		
				2	500	500	0
				3	450	450	0
				4	375	400	25
				5	400	425	25
				6	425	425	0
				7	375		
				8	500	500	0
				9	375	350	-25
				10	250		
			Mean		432	436	4
13	20	1120	C	1	475	475	0
				2	250		
				3	275	300	25
				4	400		
				5	225	200	-25
				6	250	250	0
				7	400		
				8	325	300	-25
				9	450	425	-25

				10	300	300	0
			Mean		329	321	-7
13	20	1120	D	1	325	325	0
				2	325	300	-25
				3	350	350	0
				4	275	250	-25
				5	325		
				6	250	225	-25
				7	425	425	0
				8	250	275	25
				9	300		
				10	350		
			Mean		314	307	-7
13	20	2400	A	1	375	375	0
				2	325	300	-25
				3	250	225	-25
				4	375		
				5	400	350	-50
				6	250		
				7	425	425	0
				8	350		
				9	400	375	-25
				10	250		
			Mean		363	342	-21
13	20	2400	B	1	375	375	0
				2	350	375	25
				3	400	375	-25
				4	350		
				5	300	275	-25
				6	250	225	-25
				7	250	250	0
				8	325	300	-25
				9	400	400	0
				10	250		
			Mean		331	322	-9
13	20	2400	C	1	350	350	0
				2	400	375	-25
				3	450	450	0
				4	375	350	-25
				5	325	300	-25
				6	350		
				7	350	325	-25
				8	450	425	-25
				9	450		
				10	250		
			Mean		386	368	-18
13	20	2400	D	1	300	275	-25
				2	450	450	0
				3	375		
				4	350	325	-25
				5	325		
				6	300	275	-25
				7	300		
				8	350		
				9	250	225	-25
				10	325	300	-25
			Mean		329	308	-21
13	20	4000	A	1	450	375	-75
				2	300	250	-50
				3	325	250	-75
				4	325		
				5	250	225	-25
				6	375	300	-75
				7	350		
				8	375		
				9	325		

				10	425	375	-50
			Mean		354	296	-58
13	20	4000	B	1	325	300	-25
				2	300	250	-50
				3	200		
				4	375	300	-75
				5	325	300	-25
				6	375		
				7	300		
				8	525	525	0
				9	325	275	-50
				10	425		
			Mean		363	325	-38
13	20	4000	C	1	325	250	-75
				2	350	250	-100
				3	375	300	-75
				4	375	300	-75
				5	500		
				6	300	225	-75
				7	375		
				8	375		
				9	325	225	-100
				10	350		
			Mean		342	258	-83
13	20	4000	D	1	375		
				2	300	250	-50
				3	400		
				4	425		
				5	400		
				6	250	200	-50
				7	250	225	-25
				8	350		
				9	375	300	-75
				10	375	300	-75
			Mean		310	255	-55
13	25	380	A	1	325	375	50
				2	450	475	25
				3	400	425	25
				4	425	450	25
				5	300	375	75
				6	450	450	0
				7	275	350	75
				8	250		
				9	375	425	50
				10	275	300	25
			Mean		364	403	39
13	25	380	B	1	350	350	0
				2	400	450	50
				3	475	475	0
				4	425		
				5	425	450	25
				6	350	400	50
				7	375	425	50
				8	375		
				9	375	425	50
				10	300	375	75
			Mean		381	419	38
13	25	380	C	1	350	350	0
				2	300	375	75
				3	375	375	0
				4	375	425	50
				5	350	425	75
				6	350		
				7	325	400	75
				8	425	450	25
				9	250	300	50

				10	425	425	0
			Mean		353	392	39
13	25	380	D	1	325	375	50
				2	400	475	75
				3	300	375	75
				4	300	300	0
				5	375	425	50
				6	350	375	25
				7	250	300	50
				8	275	275	0
				9	225	250	25
				10	275	325	50
			Mean		308	348	40
13	25	1120	A	1	375	375	0
				2	300	325	25
				3	375	350	-25
				4	375	375	0
				5	325		
				6	300	300	0
				7	375		
				8	275	275	0
				9	350	325	-25
				10	375	350	-25
			Mean		341	334	-6
13	25	1120	B	1	275	250	-25
				2	250	250	0
				3	200		
				4	325	300	-25
				5	325	300	-25
				6	375		
				7	425	425	0
				8	400		
				9	275	250	-25
				10	350	325	-25
			Mean		318	300	-18
13	25	1120	C	1	325		
				2	400	375	-25
				3	450	450	0
				4	300	275	-25
				5	300	275	-25
				6	275	250	-25
				7	450	450	0
				8	375	375	0
				9	300		
				10	375		
			Mean		364	350	-14
13	25	1120	D	1	425	425	0
				2	275		
				3	325	300	-25
				4	300	300	0
				5	375	375	0
				6	400	375	-25
				7	325	300	-25
				8	400		
				9	325	300	-25
				10	300	300	0
			Mean		347	334	-13
13	25	2400	A	1	250	250	0
				2	400	400	0
				3	325	300	-25
				4	275	250	-25
				5	250		
				6	450	475	25
				7	300		
				8	400		
				9	375	350	-25

				10	425		
			Mean		346	338	-8
13	25	2400	B	1	325		
				2	300	300	0
				3	375		
				4	375	350	-25
				5	375	350	-25
				6	325	300	-25
				7	300	300	0
				8	325		
				9	350	325	-25
				10	375		
			Mean		338	321	-17
13	25	2400	C	1	425	375	-50
				2	275	250	-25
				3	400	375	-25
				4	350	350	0
				5	300	300	0
				6	350		
				7	375	350	-25
				8	400		
				9	350	325	-25
				10	475	475	0
			Mean		369	350	-19
13	25	2400	D	1	300	275	-25
				2	300	275	-25
				3	275		
				4	375	375	0
				5	275	300	25
				6	375		
				7	275	275	0
				8	350	350	0
				9	375	375	0
				10	400		
			Mean		321	318	-4
13	25	4000	A	1	350	300	-50
				2	350	300	-50
				3	400	350	-50
				4	375		
				5	300	250	-50
				6	400	350	-50
				7	500	400	-100
				8	400		
				9	250		
				10	325	300	-25
			Mean		375	321	-54
13	25	4000	B	1	275	200	-75
				2	325		
				3	375	350	-25
				4	425	375	-50
				5	400	375	-25
				6	375	300	-75
				7	325		
				8	425	375	-50
				9	400		
				10	300	250	-50
			Mean		368	318	-50
13	25	4000	C	1	300		
				2	325		
				3	250		
				4	475	425	-50
				5	375	350	-25
				6	300	250	-50
				7	300	275	-25
				8	325		
				9	300		

				10	425	375	-50
			Mean		375	335	-40
13	25	4000	D	1	275	250	-25
				2	350	300	-50
				3	300		
				4	350	300	-50
				5	325		
				6	400	375	-25
				7	450	375	-75
				8	375		
				9	425	425	0
				10	350		
			Mean		375	338	-38
18	15	380	A	1	475	500	25
				2	475		
				3	200		
				4	475		
				5	375		
				6	175		
				7	325	475	150
				8	400	450	50
				9	325	450	125
				10	275	325	50
			Mean		360	440	80
18	15	380	B	1	400	450	50
				2	250		
				3	200		
				4	313	550	238
				5	200		
				6	425	500	75
				7	200	600	400
				8	225		
				9	225	500	275
				10	250	250	0
			Mean		302	475	173
18	15	380	C	1	475	550	75
				2	325		
				3	300	375	75
				4	300		
				5	350	375	25
				6	225	300	75
				7	500		
				8	225		
				9	400	475	75
				10	300		
			Mean		350	415	65
18	15	380	D	1	450		
				2	225		
				3	200	250	50
				4	450	500	50
				5	200		
				6	400	450	50
				7	200		
				8	200	250	50
				9	425	500	75
				10	200	225	25
			Mean		313	363	50
18	15	1120	A	1	300	350	50
				2	300		
				3	350		
				4	225		
				5	225	250	25
				6	225	250	25
				7	425	500	75
				8	275		
				9	200	250	50

				10	325		
			Mean		275	320	45
18	15	1120	B	1	300	375	75
				2	200	250	50
				3	200	225	25
				4	325		
				5	400		
				6	200		
				7	350	400	50
				8	475	500	25
				9	275	300	25
				10	325		
			Mean		300	342	42
18	15	1120	C	1	425	450	25
				2	300	375	75
				3	300	300	0
				4	375		
				5	250	300	50
				6	475	500	25
				7	350	350	0
				8	375		
				9	200	400	200
				10	325		
			Mean		329	382	54
18	15	1120	D	1	300	300	0
				2	275	300	25
				3	400	425	25
				4	375	425	50
				5	500		
				6	525		
				7	300	400	100
				8	250		
				9	275		
				10	425	450	25
			Mean		346	383	38
18	15	2400	A	1	250	250	0
				2	250	275	25
				3	250	275	25
				4	350	350	0
				5	300	300	0
				6	425	425	0
				7	300		
				8	225		
				9	200	225	25
				10	275	250	-25
			Mean		288	294	6
18	15	2400	B	1	275	225	-50
				2	200	200	0
				3	250		
				4	650	400	-250
				5	250	250	0
				6	450		
				7	550	550	0
				8	175		
				9	375	375	0
				10	375	425	50
			Mean		382	346	-36
18	15	2400	C	1	175		
				2	350	375	25
				3	200		
				4	275	250	-25
				5	250	250	0
				6	325		
				7	400	400	0
				8	425	400	-25
				9	250	250	0

				10	300	300	0
			Mean		321	318	-4
18	15	2400	D	1	275	300	25
				2	375	375	0
				3	275	275	0
				4	325	325	0
				5	500	500	0
				6	200	200	0
				7	525	500	-25
				8	225	225	0
				9	300	300	0
				10	275	300	25
			Mean		328	330	3
18	15	4000	A	1	275		
				2	225		
				3	500	500	0
				4	400	375	-25
				5	375	350	-25
				6	250		
				7	450	450	0
				8	250		
				9	250	250	0
				10	225	175	-50
			Mean		367	350	-17
18	15	4000	B	1	500		
				2	275		
				3	350		
				4	525		
				5	550	550	0
				6	350		
				7	225		
				8	225		
				9	275	225	-50
				10	200		
			Mean		413	388	-25
18	15	4000	C	1	500		
				2	450	450	0
				3	350	300	-50
				4	375	325	-50
				5	250		
				6	475		
				7	525		
				8	325	350	25
				9	325		
				10	300		
			Mean		375	356	-19
18	15	4000	D	1	225		
				2	325	275	-50
				3	375	300	-75
				4	475		
				5	325		
				6	550		
				7	400	375	-25
				8	350		
				9	500		
				10	475		
			Mean		367	317	-50
18	20	380	A	1	425	450	25
				2	250		
				3	275	275	0
				4	200	250	50
				5	425	475	50
				6	525	525	0
				7	500	575	75
				8	450		
				9	325		

				10	425	500	75
			Mean		396	436	39
18	20	380	B	1	425	450	25
				2	225	350	125
				3	275	375	100
				4	225	300	75
				5	175	200	25
				6	375	450	75
				7	325		
				8	400	500	100
				9	250	375	125
				10	225	300	75
			Mean		286	367	81
18	20	380	C	1	250		
				2	475	525	50
				3	500	550	50
				4	525		
				5	200	300	100
				6	300	450	150
				7	275	375	100
				8	225	225	0
				9	300		
				10	250		
			Mean		329	404	75
18	20	380	D	1	400	450	50
				2	500		
				3	400		
				4	525	625	100
				5	300	350	50
				6	375	400	25
				7	225		
				8	275	375	100
				9	250	325	75
				10	200		
			Mean		354	421	67
18	20	1120	A	1	425	500	75
				2	400	450	50
				3	475	500	25
				4	325		
				5	250	300	50
				6	275		
				7	225		
				8	200	250	50
				9	225		
				10	250		
			Mean		350	400	50
18	20	1120	B	1	400	425	25
				2	275		
				3	300		
				4	275		
				5	525	575	50
				6	300		
				7	500	500	0
				8	200		
				9	400		
				10	250	275	25
			Mean		419	444	25
18	20	1120	C	1	425	450	25
				2	250	275	25
				3	175	200	25
				4	225	250	25
				5	250	300	50
				6	200	250	50
				7	350		
				8	625	625	0
				9	400	425	25

				10	300	325	25
			Mean		317	344	28
18	20	1120	D	1	225	225	0
				2	200	250	50
				3	325	375	50
				4	300	375	75
				5	175	225	50
				6	450		
				7	425	425	0
				8	275	300	25
				9	375	400	25
				10	525	600	75
			Mean		314	353	39
18	20	2400	A	1	275	300	25
				2	375	400	25
				3	275	275	0
				4	250		
				5	475	500	25
				6	450	475	25
				7	400	425	25
				8	250		
				9	275	300	25
				10	275		
			Mean		361	382	21
18	20	2400	B	1	200	225	25
				2	450	450	0
				3	350	325	-25
				4	250	275	25
				5	375	375	0
				6	325	325	0
				7	400	425	25
				8	225		
				9	300		
				10	325		
			Mean		336	343	7
18	20	2400	C	1	275	300	25
				2	225	250	25
				3	200	200	0
				4	400	375	-25
				5	200		
				6	250	250	0
				7	200		
				8	300		
				9	425	425	0
				10	325	325	0
			Mean		300	304	4
18	20	2400	D	1	375	375	0
				2	200	225	25
				3	325	325	0
				4	400	425	25
				5	500	500	0
				6	275	300	25
				7	225	250	25
				8	275	250	-25
				9	225	200	-25
				10	425		
			Mean		311	317	6
18	20	4000	A	1	400	375	-25
				2	350	350	0
				3	375	375	0
				4	575		
				5	200		
				6	400	400	0
				7	250	250	0

				8	375		
				9	300		
				10	250	250	0
			Mean		338	333	-4
18	20	4000	B	1	275	225	-50
				2	275		
				3	275		
				4	275	250	-25
				5	250	250	0
				6	475	475	0
				7	350	350	0
				8	250		
				9	250	250	0
				10	475	400	-75
			Mean		336	314	-21
18	20	4000	C	1	225		
				2	250	250	0
				3	250		
				4	300		
				5	300	275	-25
				6	275		
				7	350	325	-25
				8	325	325	0
				9	275		
				10	275	250	-25
			Mean		300	285	-15
18	20	4000	D	1	300		
				2	375	375	0
				3	350		
				4	250	225	-25
				5	225		
				6	250	250	0
				7	375	325	-50
				8	200	200	0
				9	225		
				10	200		
			Mean		290	275	-15
18	25	380	A	1	400		
				2	425	425	0
				3	300	350	50
				4	400	425	25
				5	300		
				6	225	250	25
				7	400	400	0
				8	225	250	25
				9	275		
				10	275		
			Mean		329	350	21
18	25	380	B	1	250	300	50
				2	375	400	25
				3	225	250	25
				4	275	300	25
				5	325		
				6	275	350	75
				7	275	300	25
				8	300		
				9	500	525	25
				10	175		
			Mean		311	346	36
18	25	380	C	1	200		
				2	450	475	25
				3	325	375	50
				4	250	300	50
				5	250	300	50
				6	325	375	50
				7	425		

				8	500	550	50
				9	250		
				10	200		
			Mean		350	396	46
18	25	380	D	1	275		
				2	275	275	0
				3	250	275	25
				4	225	250	25
				5	275	300	25
				6	450	475	25
				7	400		
				8	175		
				9	225	250	25
				10	275	300	25
			Mean		282	304	21
18	25	1120	A	1	450	450	0
				2	275		
				3	300		
				4	350	375	25
				5	500	525	25
				6	525	550	25
				7	475	500	25
				8	450		
				9	325	550	225
				10	550		
			Mean		438	492	54
18	25	1120	B	1	200		
				2	325	375	50
				3	200		
				4	175		
				5	225		
				6	225		
				7	275	300	25
				8	225	250	25
				9	350	350	0
				10	350	375	25
			Mean		305	330	25
18	25	1120	C	1	200	225	25
				2	300	325	25
				3	400	425	25
				4	250		
				5	250		
				6	350	350	0
				7	150	175	25
				8	475	500	25
				9	275	300	25
				10	400	400	0
			Mean		319	338	19
18	25	1120	D	1	275	300	25
				2	225		
				3	175		
				4	325	350	25
				5	225	250	25
				6	200		
				7	275	300	25
				8	250		
				9	275	300	25
				10	250	250	0
			Mean		271	292	21
18	25	2400	A	1	225	225	0
				2	450	450	0
				3	175		
				4	325	300	-25
				5	275	250	-25
				6	325		
				7	225		

				8	400	400	0
				9	425	400	-25
				10	175		
			Mean		350	338	-13
18	25	2400	B	1	225	200	-25
				2	375	350	-25
				3	325		
				4	425	425	0
				5	250	225	-25
				6	275	250	-25
				7	300		
				8	300	275	-25
				9	550	550	0
				10	275	250	-25
			Mean		334	316	-19
18	25	2400	C	1	300	275	-25
				2	175		
				3	325		
				4	400	400	0
				5	300	275	-25
				6	275	250	-25
				7	400		
				8	225	250	25
				9	375	350	-25
				10	350	350	0
			Mean		318	307	-11
18	25	2400	D	1	350		
				2	275		
				3	350	325	-25
				4	375	350	-25
				5	325	350	25
				6	250	250	0
				7	450	425	-25
				8	375	350	-25
				9	250	200	-50
				10	500	475	-25
			Mean		359	341	-19
18	25	4000	A	1	225	225	0
				2	350	250	-100
				3	250	250	0
				4	200		
				5	250		
				6	375	350	-25
				7	175		
				8	375	375	0
				9	200		
				10	375	325	-50
			Mean		325	296	-29
18	25	4000	B	1	275		
				2	300		
				3	375	375	0
				4	450	450	0
				5	275		
				6	275	250	-25
				7	275		
				8	175		
				9	275		
				10	300	250	-50
			Mean		350	331	-19
18	25	4000	C	1	275		
				2	175		
				3	350	350	0
				4	325		
				5	225		
				6	325	325	0
				7	350	300	-50

				8	300		
				9	225		
				10	375	375	0
			Mean		350	338	-13
18	25	4000	D	1	300		
				2	250	250	0
				3	500	425	-75
				4	300	250	-50
				5	375	325	-50
				6	200		
				7	200	175	-25
				8	225		
				9	250	225	-25
				10	225		
			Mean		313	275	-38

Chapter IV

Response of benthic foraminifera to ocean acidification in their natural sediment environment: a long-term culturing experiment

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Abstract

Calcifying foraminifera are expected to be endangered by ocean acidification, However, the response of a complete community kept in natural sediment and over multiple generations under controlled laboratory conditions has not been constrained to date. During six month incubation, foraminiferal assemblages were treated with pCO_2 enriched seawater of 430, 907, 1865 and 3247 µatm pCO_2. The fauna was dominated by *Ammonia aomoriensis* and *Elphidium* species, whereas agglutinated species were rare. After 6 months incubation, pore water alkalinity was much higher in comparison to the overlying seawater as a result of remineralization processes. Consequently, saturation state of Ω_{calc} was much higher in the sediment than in the water column in all pCO_2 treatments and remained close to saturation. As a result, the life cycle of living assemblages was largely unaffected by the tested pCO_2 treatments. Growth rates, reproduction and mortality, and therefore population densities and size-frequency distribution of *Ammonia aomoriensis* strongly varied during the experimental period. The growth rates ranged between 25 and 50 µm per month, which corresponds to an addition of 1 or 2 new chambers per month. According to the size-frequency distribution foraminifera start reproduction at a diameter of 250 µm. Mortality of large foraminifera was recognized, commencing at a test size of 285 µm at a pCO_2 ranging from 430 to 1865 µatm, and of 258 µm at 3247 µatm. The total organic content of living *Ammonia aomoriensis* has been determined to be 4.3 % of dry weight. Living individuals had a calcium carbonate production rate of 0.47 g m^{-2} a^{-1}, whereas dead empty tests accumulated at a rate of 0.27 g m^{-2} a^{-1}. Although Ω_{calc} was close to 1, some empty tests of *Ammonia aomoriensis*

showed dissolution features at the end of incubation. In contrast, tests of the second most dominant species *Elphidium incertum* stayed intact. This species specific response could be explained by differences in the elemental test composition, in particular the higher Mg-concentrations in *Ammonia aomoriensis* tests. Our results emphasize that the sensitivity to ocean acidification of endobenthic foraminifera in their natural sediment habitat is much lower compared to the experimental response of specimens isolated from the sediment.

IV.1 Introduction

Benthic foraminifera are the most diverse group of hard-shelled protists. They live at the sediment-water interface, preferential in the uppermost 0–1 cm, or within oxygenated sediments down to >12 cm depth (Corliss, 1985). It is expected that calcifying foraminifera will be adversely affected by the ongoing acidification of the oceans. Surprisingly, previous experimental studies did not report a consistent and uniform response, when living specimens were subjected to simulated ocean acidification. Most benthic foraminifera were negatively affected at high pCO_2 (Le Cadre et al., 2003, Kuroyanagi et al., 2009, Allison et al. 2010, Dissard et al., 2010, Fujita et al., 2011, Haynert et al., 2011). In contrast, some species showed an indistinct sensitivity under elevated pCO_2 (Vogel and Uthicke, 2012, McIntyre-Wressnig et al., 2013). A further new study of Keul et al. (2013) revealed that not pCO_2, but rather CO_3^{2-} is the parameter which affects the test size and dry weights of *Ammonia* species. All these studies cultured living benthic foraminifera as isolated specimens without their natural substrate.

To date, no ocean acidification studies were reported to pursue culturing experiments with benthic foraminifera in their natural sedimentary environment. The field study of Haynert et al. (2012) in Flensburg Fjord exhibited that the carbonate chemistry of sediment pore water differed strongly from the conditions in the overlying near-bottom water. Sediment pore water pCO_2 was constantly high, ranging from 1244 to 3324 µatm during the entire year. Nevertheless, as a consequence of higher alkalinity, Ω_{calc} was slightly supersaturated. Under these conditions the benthic foraminiferal community was not affected by seasonally elevated bottom water pCO_2.

Benthic foraminifera are common in Kiel Fjord, western Baltic Sea, although seawater carbonate concentrations are permanently low and are seasonally undersaturated with respect to Ω_{calc} (Thomsen et al., 2013). High biological activity and nutrient inputs characterized the area, and organic-rich mud prevailed in Kiel Fjord (Nikulina et al., 2008). Degradation of organic matter between the sediment-water interface influenced the underlying sediment chemistry (Graf et al., 1984), and therefore the habitat of benthic foraminifera. Benthic foraminifera in Kiel Fjord sediments were described in previous studies, initiated by Rhumbler (1935), followed by Rottgardt (1952), Lutze (1965), Wefer (1976), Nikulina et al. (2008) and Polovodova et al. (2008). These studies focused on taxonomy and distribution, and influences of temperature, salinity, oxygen, heavy metals and food supply, but did not consider possible impacts of seawater carbonate chemistry.

Kiel Fjord represents a suitable habitat, where benthic foraminifera were found with high population densities. The aim of this study was to investigate the response of foraminiferal population by using a pristine assemblage from Kiel Fjord to elevated pCO_2 in their natural sedimentary environment during a long-term incubation over six months.

IV.2 Materials and methods

IV.2.1 Field sampling and preparation

Foraminiferal samples were collected from station KF1 (54°20´713´´N, 10°10´160´´E, 13 m water depth) in Kiel Fjord, southwestern Baltic Sea, at the end of April 2011. At this location, the bottom sediment was silty fine sand. The sampling site was directly located to the station PF15–13 of Polovodova and Schönfeld (2008), where *Ammonia aomoriensis* (similar to *Ammonia beccarii* of Nikulina et al. (2008) and Polovodova et al., 2009) is one of the dominating species in the living assemblage.

Surface sediment samples were taken with a Mini Muc K/MT 410 corer equipped with four tubes of 60 cm length and 10 cm inner diameter (Kuhn and Dunker, 1994), deployed from R/V *Polarfuchs*. Altogether, 24 cores were taken. A graduated plastic ring was used to slice off the uppermost one centimeter of the surface sediment (Schönfeld et al., 2012). The surface layer was transferred with a spoon into 300 ml Kautex^{TM} wide-neck containers. The samples were covered with bottom water taken from the supernatant water of the coring tubes. On board, the containers were covered with Parafilm to avoid contamination by dust or evaporation. Afterwards, the samples were immediately brought to the laboratory at GEOMAR, where they were acclimated to culturing conditions at 17 °C room temperature for four weeks. During that acclimation period, stock cultures were aerated with compressed, humidified air and fed with 200 µl DT's Premium Blend containing living algae of *Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella* once a week.

After four weeks of acclimation, the seawater of the stock cultures was sucked off except a few millimeters above the sediment surface. The sediment was homogenized with a spoon for a few minutes. This technique was a simple and gentle way to achieve a considerable even distribution pattern of benthic foraminifera inside the sediment. After homogenization, each culture vessel was filled with 0.4 cm of sediment by using a spoon-shaped plastic spatula. At the end of the experiment, the measured sediment height was 0.36 cm on average, which results at a given culture vessel area of 42.25 cm^2 in a mean sample volume of 15.2 cm^3. We prepared 84 culture vessels, three replicate for four pCO_2 lines (380, 1120, 2400 and 4000 µatm), combined seven replicate sets per pCO_2 treatment were terminated. After spreading of the sediment, the culture vessels were transferred into the experimental set up and filled up with seawater from Kiel Fjord. For acclimatization and in order to restore the natural pore water chemistry in the sediments, the culture vessels were kept for 20 days at temperature of 17 °C and a salinity of 16 until the start of the experiment. This time was deemed necessary to achieve a full recovery of foraminiferal

microhabiat pattern in a sub-cm scale (Ernst et al., 2002). During that time, 400 µl DT's Premium Blend were added weekly into each culture vessel.

IV.2.2 Experimental design

The culturing of benthic foraminiferal assemblages from Kiel Fjord was performed in a closed flow-through system modified after Hintz et al. (2004) and Haynert et al. (2011). The culture vessels (6.5 x 6.5 x 4.5 cm) with an area of 42.25 cm^2 filled with a 0.4±0.1 cm sediment layer and an overlying seawater column of 4 cm. A nepheloid detritus layer of 0.1 cm separated the seawater from the underlying sediment pore water. The resulting pore water stagnation equates the conditions in their natural habitat, where foraminifera concentrate in the upper oxic surface layer of the sediment (Haynert et al., 2012). During field sampling we observed that the oxic sediment layer varied between 0.5 and 0.8 cm.

Each culture vessel was flushed with 25 µm cartridge-filtered and UV-sterilized seawater from Kiel Fjord. Four pCO_2 levels were adjusted by aeration of 5 l compact jerrycans with compressed and CO_2 enriched air. The target pCO_2 levels were 380, 1120, 2400 and 4000 µatm. Treatment levels were chosen in order to simulate present day and future pCO_2 peaks, which are transiently occurring today and are going to prevail for longer periods in future in Kiel Fjord (Thomsen et al. 2010, 2013, Melzner et al. 2012). The preconditioned seawater from each tank flowed through the culture vessels, which were three times replicated for each pCO_2 level. The overflow seeped through the fissure between lid and vessel, and draining off to a sink. The flow rate was adjusted to 0.16 ml s^{-1} which is sufficient to replace the water volume of the vessels 1.4 times per hour. The overflow drained off to a 60 l catchment tank. In the catchment tank, the water was sparged with compressed air at a high rate, in order to remove the excess CO_2 before the water was pumped back to the compact jerrycans. In order to avoid evaporation by aeration of the seawater with compressed and thus dry air, the gas was humidified in gas-washing bottles which were inserted in each compressed air connection. Evaporation of one liter per week was compensated by refilling the system with deionised water. Food (400 µl DT's Premium Blend) was added to each culture vessel every week.

Seawater temperature, salinity and pH_{NBS} (National Bureau of Standards pH-scale) were measured weekly in each culture vessel. Total dissolved inorganic carbon (C_T), phosphate (PO_4^{3-}) and silicate (Si) concentrations were determined monthly in one of the three replicates for each pCO_2-level. After six months, carbonate system parameters of total alkalinity (A_T) and pH_{NBS} of supernatant seawater and sediment pore water were measured in one of the three replicates per pCO_2-level.

In order to monitor changes of the foraminiferal community during the incubation time, the sediment of three culture vessel replicates were analyzed monthly for foraminiferal assemblage composition. The experiment ended after six month.

IV.2.3 Determination of water chemistry parameters

Seawater $pH_{NBS,}$ temperature and salinity were determined with a WTW 340i pH analyzer and a WTW Cond 315i conductivity meter. The pH_{NBS}-electrode was calibrated with standard buffer solutions of pH 4.01, 7.00 and 10.00 (WTW standard, DIN/NIST buffers L7A). Precision was ±0.01 for pH_{NBS} and ±0.1°C for temperature. The precision of the conductivity meter was ±0.1 salinity units. Nutrient concentrations were analyzed in sterile-filtered (0.2 µm pore size) water samples. Phosphate (PO_4^{3-}) and silicate (Si) were analyzed colorimetrically in a spectrophotometer (U 2000, Hitachi-Europe) at a wavelength of 882 nm and 810 nm according to Koroleff and Grasshof (1983). The measurement precision was ±0.2 µmol l^{-1} for phosphate and, in dependence of the concentrations, 2.5 to 6 % for silicate.

Total dissolved inorganic carbon (C_T) was analyzed in sterile-filtered (0.2 µm pore size) water samples taken from the culture vessels during the incubation time using an AIRICA autoanalyzer (Maranda GmbH, Kiel, Germany) with a precision of 2–4 µmol kg^{-1}. Accuracy of C_T measurements was ensured by using certified reference material provided by Andrew Dickson of the Scripps Institution of Oceanography (http://andrew.ucsd.edu/co2qc/). Seawater carbonate system parameters pCO_2, total alkalinity (A_T) and omega for calcite (Ω_{calc}) were calculated from pH_{NBS}, C_T, temperature, salinity, PO_4^{3-} and Si values using CO2SYS software by Lewis and Wallace (1998). Dissociation constants K_1 and K_2 were chosen according to Mehrbach et al. (1973) as refitted by Dickson and Millero (1987) and $KHSO_4$ dissociation constant after Dickson (1990).

Water chemistry parameters of pH_{NBS}, A_T, temperature and salinity were analyzed from the seawater and sediment pore water of selected culture vessels. Seawater samples from 0–2 cm and 2–4 cm water layers were sterile-filtered (0.2 µm pore size) and filled directly into 20 ml PVC bottles. For sediment pore water analyses, sediment samples were transferred into 50 ml centrifuge tubes and centrifuged at 3000 rpm for 15 min in order to separate the sediment pore water from the sediment. The extracted pore water was transferred through 0.2 µm sterile filters into 20 ml PVC bottles. pH_{NBS} and temperature were measured using the WTW 340i pH analyzer, salinity was determined with WTW Cond 315i conductivity meter. Total alkalinity (A_T) was determined with a Metrohm titration instrument according to Ivanenkov and Lyakhin (1978). A greenish-brown Methyl-Red and Methylene-Blue indicator was added, and titration was performed with 0.02M HCl and finished until a stable light pink colour occurred. During titration, the sample was degassed by continuously bubbling argon through the solution in order to remove the generated CO_2 or H_2S. The measured values were standardized using an IAPSO seawater solution. The precision of the alkalinity measurements was 0.37 %. Carbonate parameters pCO_2, C_T and Ω_{calc} of seawater and sediment pore water were calculated from measured pH_{NBS}, A_T, temperature, salinity, PO_4^{3-} and Si values according to dissociation constants as specified above. All chemical parameters are reported as mean values of replicate measurements (Table 1).

IV.2.4 Foraminiferal processing

Benthic foraminiferal sediment samples were transferred in 100 ml Kautex™ wide-neck containers, preserved and stained with Rose Bengal ethanol (94 %) solution of 2 g l^{-1} for three weeks following Lutze and Altenbach (1991). This period is sufficient to stain the protoplasm completely with Rose Bengal in all tests of foraminifera (Schönfeld et al., 2012).

Samples were first passed through a 2000 µm screen in order to remove mollusks shells and pebbles. Subsequently the samples were gently washed with tap water through a 63-µm sieve. The fractions 63–2000 µm and >2000 µm fractions were dried at 60 °C for at least 24 h. The size fraction 63–2000 µm was picked completely for living and dead foraminifera. Samples were not splitted. All Rose Bengal stained foraminifera were considered as living at the time of sampling, whereas unstained tests were considered as dead. Living and dead specimens were sorted by species. All species, except *Ammonia aomoriensis*, were mounted in Plummer cell slides with glue, counted and measured using an eyepiece reticle on the Wild M3C dissecting microscope. *Ammonia aomoriensis* was kept in single cell slides for further analyses.

Test morphometry of *Ammonia aomoriensis* was analyzed with an automated image analysis system using a Leica Z16 APO microscope, and analySIS software (version 5.0) at the University of Angers, France (cf. Bollmann et al., 2004, Clayton et al., 2009). Resolution of images is 2.69 μm^2. Due to the oval shape of *Ammonia aomoriensis* tests, the size of tests is given as mean diameter, calculated from the minimum and maximum diameter.

The census data were standardized to population density given as individuals/tests per 10 cm^{-3} sediment. Histograms were created depicting the proportion of living *A. aomoriensis* in 11 size classes from <50 to >500 µm in 50 µm intervals and presented as average of three replicates taken each month during the six months incubation time.

Dry weight of dead and living *A. aomoriensis* was measured using a micro-balance with an accuracy of 1 µg (Sartorius, M3P-000V001). Dry weight per individual/test was calculated from total dry weight divided by total number of individuals/tests.

Finally, tests of *Armorella sphaerica* and corroded specimens of *A. aomoriensis* were photographed with a MiniPixie (MPX2051UC) digital microscope.

IV.2.5 Electron microprobe analysis

The two ranked species, *A. aomoriensis* and *E. incertum*, were prepared for **E**lectron **M**icro**P**robe (EMP) x-ray microanalysis (JEOL JXA 8200). We focused on the visual elemental distribution of Ca and Mg on cross-sections of the tests, which were cultured at pCO_2 of 3247 µatm and retrieved after six months incubation. The advantage of this technique is high spatial resolution (±1 µm), so that single foraminiferal tests can be analyzed (Kellner et al., 1998, Glock et al., 2012). Tests of both species were embedded under vacuum into Araldite™ epoxy resin by using a CitoVac™ vacuum embedding system (Struehrs™). Small drops of resin were used to fill the inner

part of the chambers. The tests were set under pressure to collapse air inclusions inside the resin and hardened in a drying cabinet at 60°C.

Using the Tegra-Pol-21 system (Struehrs^{TM}), the surface of the resin grinded down by hand with alumu-silica grinding paper until the proloculus of the specimen became visible. The surface was polished with different grades of alumo-silica and diamond paste (until 1 µm grain size) on a self rotating polishing plate.

Prior to the measurement, each cross-section was carbon coated. The microprobe was operated in a wavelength dispersive mode by using different Ka X-ray lines for each element. Two different detector crystals were used for the elements Ca (crystal: PETJ) and Mg (crystal: TAPH). The acceleration voltage was 15 kV and the beam current was 20 nA. The tests of *A. aomoriensis* and *E. incertum* were mapped by 0.5 µm step size and a dwell time of 500 ms. The results are illustrated as maps of relative measured intensities for both elements.

IV.2.6 Calculations

Carbonate production and accumulation of the major calcifying species *A. aomoriensis* have been calculated from the difference of dry carbonate weight of individuals or tests produced or accumulated during six months incubation time. The production or accumulation rate in gram refers to an area of 1 m^2 per year.

For 100 living *A. aomoriensis* from size fraction 200 to 300 µm, the total organic contents, including cytoplasm and organic linings of the test, was determined by combustion off the organic material from the carbonate tests in a muffle furnace at 500 °C for 6 hours. The amount of total organic contents is calculated from the weight loss and given as a percentage of the total dry weight of the individuals (Table S6 in the supplement). This value was subtracted from total weight to obtain the dry weight of shell carbonate (Table S5 in the supplement).

IV.2.7 Statistics

Two-factorial ANOVA was performed with the two parameters pCO_2 and incubation time with STATISTICA 8 (Table 4). All measured raw data of each treatment were used for statistical analyses. The data are given as mean ± standard deviation.

IV.3 Results

IV.3.1 Carbonate chemistry

Seawater temperature and salinity were stable during the experimental period. Average temperatures ranged from 16.4 to 17.2 °C and salinities from 15.1 to 15.9 (Table 1).

Phosphate and silicate concentrations changed markedly over the course of the experiment. Phosphate concentration increased 31-fold during the first three months from 0.19 to

5.86 µmol l^{-1} (Table 1). Mean silicate concentrations were in general high with 191.98 µmol l^{-1} and exhibited a threefold increase from 138.94 to 367.19 µmol l^{-1} after two months (Table 1). By the end of the experiment, concentrations of phosphate and silicate decreased again and achieved the initial values that were observed at the beginning of the experiment.

In dependency to seawater pCO_2, mean pH values ranged between 8.21 and 7.17 (Table 1) and were stable throughout the incubation period. Total inorganic carbon increased with increasing pCO_2 from 2210.5 to 2464.2 µmol l^{-1} (Table 1). The calculated mean pCO_2 levels of the treatments were thereby 430, 907, 1865 and 3247 µatm (Table 1). It has to be noted that CO_2 concentrations in the unchanged air effected a higher pCO_2, while the partial pressures at higher concentrations lead to markedly lower pCO_2 levels than the target values. In the following, we refer our results to the pCO_2 levels that were prevailed in the culture vessels (Haynert et al., 2011). Mean seawater alkalinity was 2379.3 µmol kg^{-1} and did not differ significantly between the treatments (Table 1). At a pCO_2 of 1865 µatm, seawater Ω_{calc} values decreased partially below 1. High pCO_2 of 3247 µatm caused permanent undersaturation of Ω_{calc} with 0.57 on average (Table 1).

The carbonate chemistry measurements revealed strong differences of pCO_2 and Ω_{calc} between seawater and sediment pore water in the culture vessels (Fig. 1). The sediment pore water was characterized by higher pCO_2 and lower pH than the supernatant seawater in the culturing vessel (Fig. 1, A and B). At the same time, pore water alkalinity (3127.8 µmol kg^{-1}) was much higher than the bulk seawater alkalinity (0-2 cm water depths) of 2113.1 µmol kg^{-1} and the water overlaying the sediment (2-4 cm water depths) with 2442.3 µmol kg^{-1}. The accumulation of A_T in the sediment caused a higher saturation of Ω_{calc} and even in the highest pCO_2 treatment a slight supersaturation, $\Omega_{calc}>1$ (Fig. 1, C and D, Table S1 in the supplement).

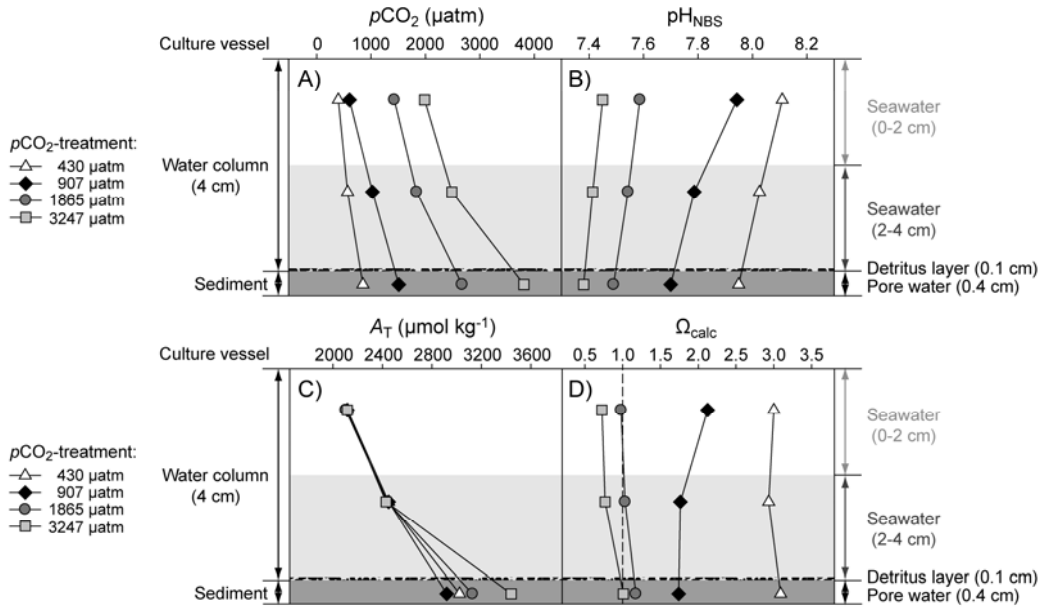

Fig. 1. Gradient of carbonate chemistry parameters of (A) partial pressure of CO_2 (pCO_2), (B) pH_{NBS}, (C) total alkalinity (A_T) and (D) saturation state of calcite (Ω_{calc}) in the seawater and sediment pore water for 4 pCO_2-treatments after six months incubation time.

Table 1. Carbonate chemistry parameters of each pCO_2-treatment in the culture vessels. Total alkalinity (A_T), partial pressure of CO_2 (pCO_2) and saturation state of calcite (Ω_{calc}) were calculated from measured temperature, salinity, pH_{NBS}, total carbon (C_T), phosphate (PO_4^{3-}) and silicate (Si). The standard deviation (1-sigma) refers to replicate measurements.

pCO_2-values of gas (µatm)	Incubation time (months)	Seawater measurements T (°C)	S	pH_{NBS}	C_T (µmol kg^{-1})	PO_4^{3-} (µmol l^{-1})	Si (µmol l^{-1})	Calculations from pH_{NBS} and C_T A_T (µmol kg^{-1})	pCO_2 (µatm)	Ω_{calc}
380	0	16.8 ± 0.05	15.7 ± 0.05	8.19 ± 0.01	2202.3	0.17 ± 0.02	139.24 ± 3.83	2377.5	359	3.98
	1	16.6 ± 0.05	15.8 ± 0.04	8.09 ± 0.01	2277.0	3.52 ± 0.26	262.17 ± 1.11	2422.5	472	3.30
	2	16.4 ± 0.05	15.9 ± 0.30	8.01 ± 0.01	2276.5	5.87 ± 0.10	366.76 ± 1.92	2397.0	568	2.75
	3	16.8 ± 0.16	15.5 ± 0.04	8.21 ± 0.00	2252.2	1.97 ± 0.04	144.35 ± 2.04	2439.8	352	4.23
	4	17.2 ± 0.24	15.3 ± 0.08	8.13 ± 0.01	2250.6	1.60 ± 0.01	149.94 ± 1.77	2406.0	425	3.62
	5	16.5 ± 0.00	15.1 ± 0.04	8.10 ± 0.01	2108.4	0.93 ± 0.26	138.26 ± 0.52	2238.4	430	3.06
	6	16.7 ± 0.06	15.2 ± 0.06	8.12 ± 0.00	2106.7	0.16	138.09	2245.6	406	3.25
1120	0	16.8 ± 0.04	15.6 ± 0.04	7.87 ± 0.02	2420.0	0.18 ± 0.01	138.36 ± 2.94	2487.6	851	2.15
	1	16.6 ± 0.05	15.8 ± 0.04	7.80 ± 0.01	2489.7	3.31 ± 0.09	261.87 ± 1.22	2545.5	1005	1.92
	2	16.4 ± 0.05	15.8 ± 0.04	7.78 ± 0.01	2389.0	5.82 ± 0.06	367.33 ± 0.67	2439.6	1022	1.73
	3	16.9 ± 0.10	15.5 ± 0.04	7.87 ± 0.01	2441.8	1.91 ± 0.08	148.48 ± 8.29	2514.6	842	2.22
	4	16.7 ± 0.14	15.6 ± 0.10	7.83 ± 0.02	2335.5	1.68 ± 0.17	150.47 ± 0.44	2391.7	897	1.89
	5	16.4 ± 0.05	15.2 ± 0.05	7.83 ± 0.01	2236.3	1.18 ± 0.42	139.61 ± 0.14	2286.9	867	1.77
	6	16.6 ± 0.06	15.2 ± 0.22	7.82 ± 0.00	2205.9	0.16	138.92	2254.8	866	1.74
2400	0	16.8 ± 0.04	15.6 ± 0.04	7.54 ± 0.02	2411.5	0.19 ± 0.03	140.00 ± 3.76	2389.7	1786	1.02
	1	16.6 ± 0.04	15.8 ± 0.00	7.47 ± 0.00	2539.7	3.32 ± 0.28	261.57 ± 1.75	2499.0	2210	0.90
	2	16.5 ± 0.05	15.8 ± 0.04	7.43 ± 0.01	2452.7	5.85 ± 0.12	368.11 ± 0.50	2404.9	2330	0.79
	3	16.8 ± 0.09	15.5 ± 0.04	7.54 ± 0.00	2407.0	2.00 ± 0.12	149.52 ± 8.27	2385.3	1810	1.00
	4	16.5 ± 0.15	15.3 ± 0.10	7.52 ± 0.01	2394.5	1.59 ± 0.02	150.12 ± 0.79	2365.7	1882	0.94
	5	16.5 ± 0.00	15.2 ± 0.05	7.62 ± 0.00	2291.0	0.87 ± 0.05	140.33 ± 0.38	2289.0	1427	1.13
	6	16.7 ± 0.06	15.2 ± 0.06	7.57 ± 0.00	2298.5	0.15	139.21	2283.5	1607	1.02
4000	0	16.8 ± 0.05	15.6 ± 0.04	7.32 ± 0.04	2496.5	0.21 ± 0.02	138.14 ± 2.83	2407.2	3031	0.63
	1	16.6 ± 0.05	15.8 ± 0.00	7.31 ± 0.01	2564.9	3.79 ± 0.32	262.93 ± 2.24	2472.8	3179	0.63
	2	16.4 ± 0.05	15.8 ± 0.03	7.17 ± 0.01	2492.9	5.90 ± 0.17	366.55 ± 0.88	2353.5	4178	0.43
	3	16.6 ± 0.05	15.5 ± 0.05	7.34 ± 0.00	2573.5	2.12 ± 0.33	154.18 ± 9.51	2489.1	2983	0.67
	4	17.1 ± 0.34	15.4 ± 0.03	7.30 ± 0.02	2461.2	1.59 ± 0.03	150.34 ± 0.74	2366.2	3178	0.59
	5	16.5 ± 0.05	15.1 ± 0.05	7.32 ± 0.01	2327.4	1.07 ± 0.04	133.99 ± 0.70	2240.8	2870	0.56
	6	16.6 ± 0.06	15.2 ± 0.06	7.25 ± 0.01	2332.8	0.17	136.62	2225.0	3310	0.49

IV.3.2 Species composition and foraminiferal assemblages

The assemblages comprised five calcareous species *Ammonia aomoriensis*, *Elphidium excavatum excavatum*, *Elphidium excavatum clavatum*, *Elphidium gerthi* and *Elphidium incertum*, and the arenaceous species *Ammotium cassis*, *Reophax dentaliniformis* and *Armorella sphaerica* (Table 2 and 3, Fig. 2).

The fauna was dominated by *A. aomoriensis* with 99 % of the living fauna and 85 % in the dead fauna, whereas *E. incertum* was rare with 1 % of living individuals and 7 % of dead tests (Table 2 and 3). All other species were very rare or were only occasionally found in the foraminiferal assemblages. Accordingly, we will focus on the dominant calcareous species *A. aomoriensis* in the following.

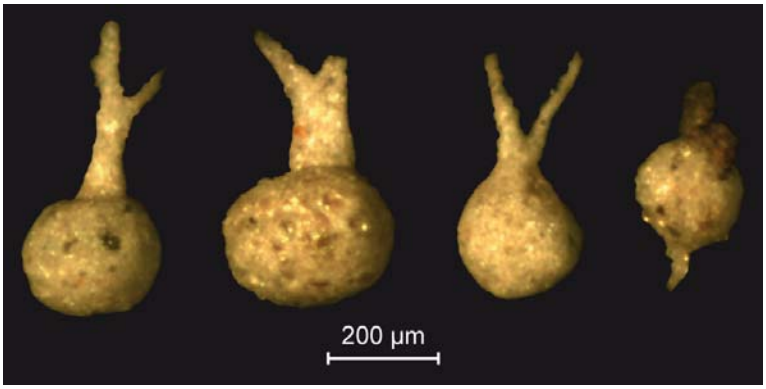

Fig. 2. Light micrograph images of the arenaceous species *Armorella sphaerica*.

IV.3.2.1 Population density, test diameter and reproduction of living *Ammonia aomoriensis*

The population density and size-frequency distribution exhibited strong variations due to growth, reproduction and mortality of foraminiferal faunas during the six months incubation. Incubation time had a significant effect on test diameter of living *A. aomoriensis* ($F(5,45)=18.14$, $p<0.01$) whereas pCO_2 had no effect as a single factor ($F(3,45)=0.61$, $p>0,05$,Table 4). However, the interaction of both, pCO_2 and incubation time was significant ($F(15,45)=2.14$, $p<0.03$). The size distribution revealed growth cohorts which were characterized by a fixed, simultaneously reproduction period. A further character of a cohort was the similar increase of test diameter and the loss of individuals by death during the same period.

Initial population density of living *A. aomoriensis* fauna varied from 342 to 583 ind. 10 cm^{-3} (Table S2 in the supplement) and mean test diameter ranged from 169 to 182 µm (Table S2 and 8 in the supplement). Lowest mean population densities were observed in the 430 µatm treatment with 295 ind. 10 cm^{-3}, intermediate with 367 and 407 ind. 10 cm^{-3} in 907 and 3247 µatm pCO_2-treatments, and highest mean densities of 524 ind. 10 cm^{-3} at a pCO_2 of 1865 µatm (Table S2 in the supplement). Whereas the population density at 430 µatm was relatively stable, the density fluctuated considerably in the 907, 1865 and 3247 µatm pCO_2-treatments (Fig. 3). *Ammonia aomoriensis* density declined strongly during the first month at pCO_2 of 1865 and

3247 µatm. An explosively increase of living *Ammonia aomoriensis* up to 610, 1378 and 518 ind. 10 cm^{-3} was observed at pCO_2 of 907, 1865 and 3247 µatm after three months. A further increase up to 602 ind. 10 cm^{-3} followed at 3247 µatm after five months (Fig. 3, A, Table S2 in the supplement). The strong increase of population densities correlated with dominant reproduction events at 1865 µatm after three months, and two sequent reproduction events at pCO_2 of 3247 µatm after three and four months as indicated by the occurrence of high numbers of small individuals with a test diameter <100 µm (Fig. 4, Table S3 in the supplement). A less pronounced reproduction event in the background was observed at a pCO_2 of 907 µatm after four and five months, though population densities were not affected (Fig. 4, Table S3 in the supplement). *Ammonia aomoriensis* was able to reproduce from size class of 250 µm up to 350 µm, at 350 µm 40 % of the cohorts had reproduced. After reproduction, the growth of individuals at 1865 µatm led to an increase of the mean test diameter from 185 to 287 µm until the end of the experiment (Table S4 in the supplement). The mean growth of *A. aomoriensis* varied between 25 and 38 µm per month during incubation, at the beginning specimens grew by up to 50 µm within one month.

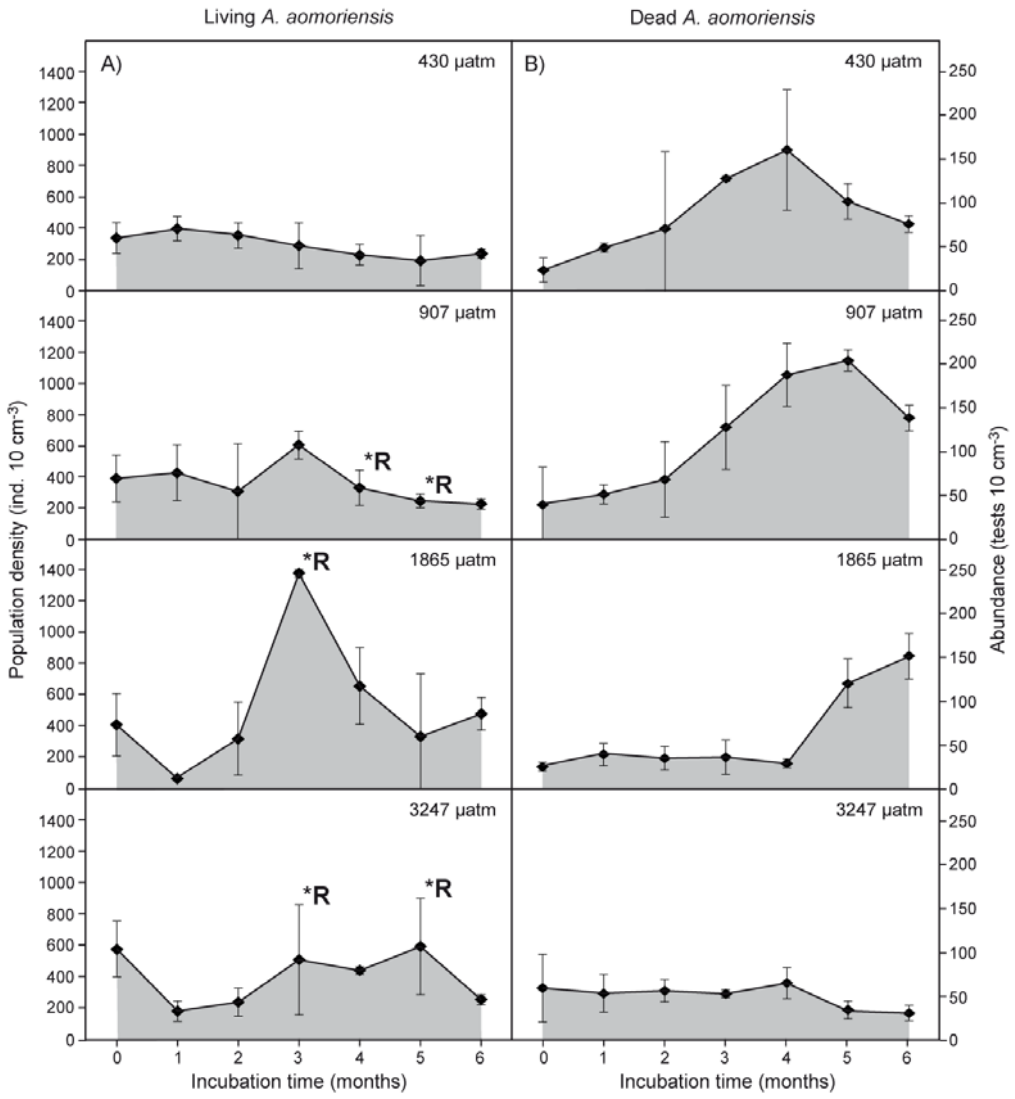

Fig. 3. (A) Population density and (B) abundance of living and dead *A. aomoriensis* at 4 pCO_2-levels during six months incubation time. The symbols represent the mean in three replicates (mean ± standard deviation). Reproduction events are indicated by starlet and bold R indicate.

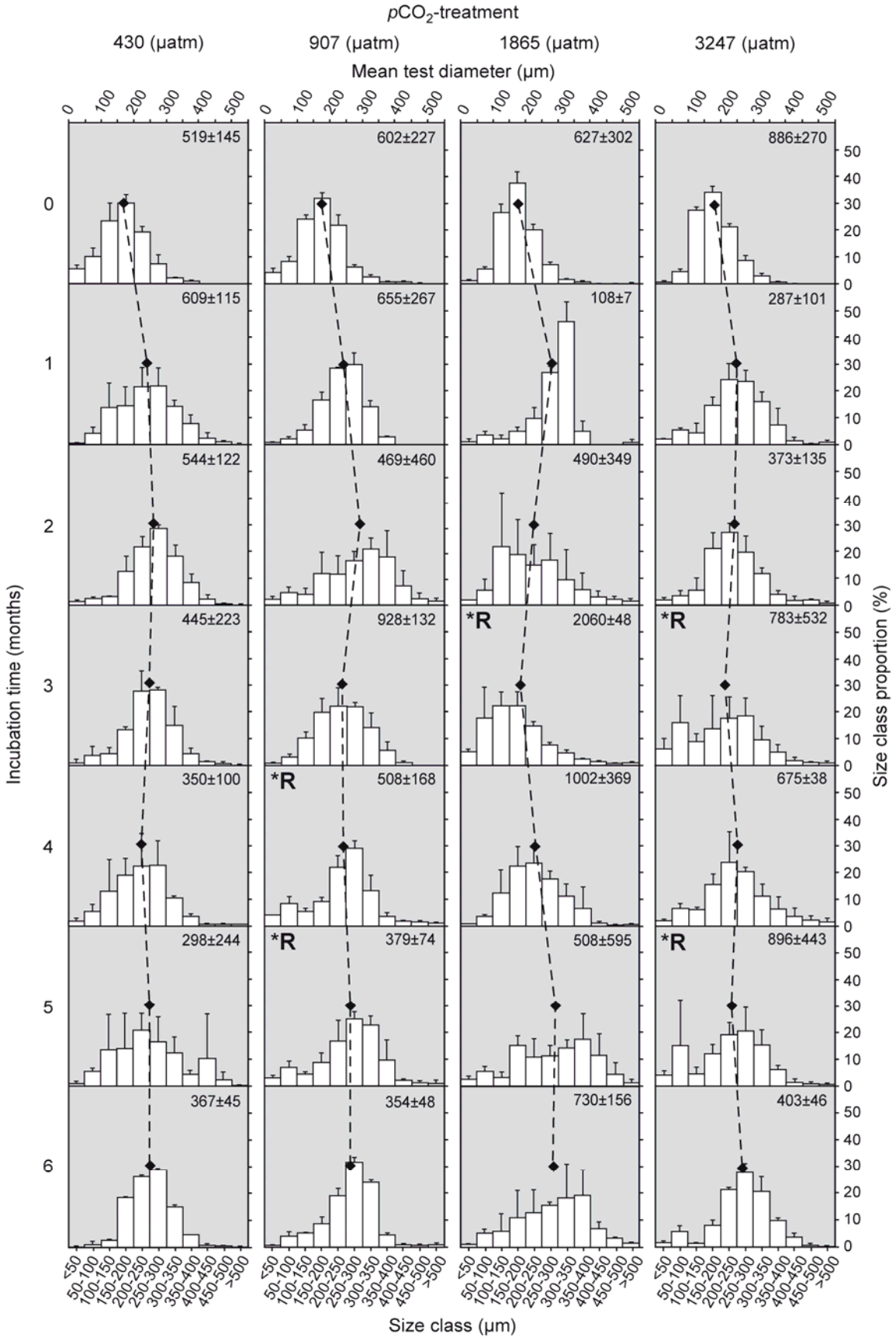

Fig. 4. Histogram of size class proprotions of living *A. aomoriensis* during six months incubation. Light grey bars represent relative proportion of 11 size classes ranging from <50 until >500 µm in 50 µm intervals. Mean test diameter is displayed by the black diamonds and dashed lines. The number in each figure represents the number of individuals (mean ± standard deviation of three replicates). Reproduction events are indicated by starlet and bold R indicate.

Table 2. List of living foraminifera collected from each culture vessel during six month incubation time, size fraction 63-2000 µm.

pCO_2-treatment	Living foraminiferal species >63 µm	Months June	%	July	%	August	%	September	%	October	%	November	%	December	%
430_A	Species														
	Ammonia aomoriensis	683	100.0	640	94.7	501	94.7	287	100.0	423	100.0	17	100.0	398	100.0
	Elphidium excavatum excavatum			2	0.3										
	Elphidium excavatum clavatum			1	0.1										
	Elphidium incertum			33	4.9	28	5.3								
	Total number of calcareous individuals	683		676		529		287		423		17		398	
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0		0	
	Total number of living specimens	683		676		529		287		423		17		398	
	Species number	1		4		2		1		1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	449.0		444.4		347.8		188.7		278.1		11.2		261.7	
430_B	Species														
	Ammonia aomoriensis	404	100.0	482	93.4	449	97.2	603	100.0	394	96.6	460	100.0	336	98.0
	Elphidium excavatum excavatum			2	0.4										
	Elphidium excavatum clavatum														
	Elphidium incertum			32	6.2	13	2.8			14	3.4			7	2.0
	Total number of calcareous individuals	404		516		462		603		408		460		343	
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0		0	
	Total number of living specimens	404		516		462		603		408		460		343	
	Species number	1		3		2		1		2		1		2	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	265.6		339.3		303.7		396.4		268.2		302.4		225.5	
430_C	Species														
	Ammonia aomoriensis	472	100.0	706	95.8	683	100.0			237	100.0	420	98.8		
	Elphidium excavatum excavatum			1	0.1										
	Elphidium excavatum clavatum														
	Elphidium incertum			30	4.1							5	1.2		
	Total number of calcareous individuals	472		737		683				237		425			
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0				0		0			
	Total number of living specimens	472		737		683				237		425			
	Species number	1		3		1				1		2			
	Sediment volume (cm^3)	15.2		15.2		15.2				15.2		15.2			
	Population density (ind. 10 cm^{-3})	310.3		484.5		449.0				155.8		279.4			
907_A	Species														
	Ammonia aomoriensis	543	100.0	938	98.6	204	100.0	788	100.0	630	100.0	362	100.0	388	100.0
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum			13	1.4										
	Total number of calcareous individuals	543		951		204		788		630		362		388	
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0		0	

	Total number of living specimens	543		951		204		788		630		362		388	
	Species number	1		2		1		1		1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	357.0		625.2		134.1		518.1		414.2		238.0		255.1	
907_B	Species														
	Ammonia aomoriensis	855	100.0	620	98.7	208	100.0	946	100.0			321	100.0	320	100.0
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum			8	1.3										
	Total number of calcareous individuals	855		628		208		946				321		320	
	Armorella sphaerica														
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0				0		0	
	Total number of living specimens	855		628		208		946				321		320	
	Species number	1		2		1		1				1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2				15.2		15.2	
	Population density (ind. 10 cm^{-3})	562.1		412.9		136.8		622.0				211.0		210.4	
907_C	Species														
	Ammonia aomoriensis	410	100.0	407	98.5	1014	100.0	1051	100.0	390	100.0	462	100.0		
	Elphidium excavatum excavatum			1	0.2										
	Elphidium excavatum clavatum														
	Elphidium incertum			5	1.2										
	Total number of calcareous individuals	410		413		1014		1051		390		462			
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0			
	Total number of living specimens	410		413		1014		1051		390		462			
	Species number	1		3		1		1		1		1			
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2			
	Population density (ind. 10 cm^{-3})	269.6		271.5		666.7		691.0		256.4		303.7			
1865_A	Species														
	Ammonia aomoriensis	728	100.0	101	100.0	162	100.0			674	100.0	1209	100.0	845	100.0
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum														
	Total number of calcareous individuals	728		101		162				674		1209		845	
	Ammotium cassis														
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0				0		0		0	
	Total number of living specimens	728		101		162				674		1209		845	
	Species number	1		1		1				1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2				15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	478.6		66.4		106.5				443.1		794.9		555.6	
1865_B	Species														
	Ammonia aomoriensis	288	100.0	114	100.0	856	100.0	2072	100.0	932	100.0	118	100.0		
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum														
	Total number of calcareous individuals	288		114		856		2072		932		118			

	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0			
	Total number of living specimens	288		114		856		2072		932		118			
	Species number	1		1		1		1		1		1			
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2			
	Population density (ind. 10 cm^{-3})	189.3		75.0		562.8		1362.3		612.8		77.6			
1865_C	Species														
	Ammonia aomoriensis	870	100.0	108	100.0	457	100.0	2121	100.0	1402	100.0	217	100.0	625	100.0
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium gerthi														
	Elphidium incertum														
	Total number of calcareous individuals	870		108		457		2121		1402		217		625	
	Ammotium cassis														
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0		0	
	Total number of living specimens	870		108		457		2121		1402		217		625	
	Species number	1		1		1		1		1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	572.0		71.0		300.5		1394.5		921.8		142.7		410.9	
3247_A	Species														
	Ammonia aomoriensis	850	100.0	375	94.7	475	100.0	591	100.0	704	100.0	845	100.0	435	100.0
	Elphidium excavatum excavatum			3	0.8										
	Elphidium excavatum clavatum														
	Elphidium incertum			18	4.5										
	Total number of calcareous individuals	850		396		475		591		704		845		435	
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0		0	
	Total number of living specimens	850		396		475		591		704		845		435	
	Species number	1		3		1		1		1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	558.8		260.4		312.3		388.6		462.9		555.6		286.0	
3247_B	Species														
	Ammonia aomoriensis	1173	100.0	178	82.0	222	100.0	384	100.0	702	100.0	1416	100.0	370	100.0
	Elphidium excavatum excavatum			1	0.5										
	Elphidium excavatum clavatum			2	0.9										
	Elphidium incertum			35	16.1										
	Total number of calcareous individuals	1173		216		222		384		702		1416		370	
	Reophax dentaliniformis			1	0.5										
	Total number of agglutinated individuals	0		1		0		0		0		0		0	
	Total number of living specimens	1173		217		222		384		702		1416		370	
	Species number	1		5		1		1		1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	771.2		142.7		146.0		252.5		461.5		931.0		243.3	
3247_C	Species														
	Ammonia aomoriensis	636	100.0	311	93.7	429	100.0	1388	100.0	642	100.0	484	100.0		
	Elphidium excavatum excavatum			2	0.6										
	Elphidium excavatum clavatum			1	0.3										

Elphidium incertum		18	5.4					
Total number of calcareous individuals	636	332		429	1388	642	484	
Reophax dentaliniformis								
Total number of agglutinated individuals	0	0		0	0	0	0	
Total number of living specimens	636	332		429	1388	642	484	
Species number	1	4		1	1	1	1	
Sediment volume (cm^3)	15.2	15.2		15.2	15.2	15.2	15.2	
Population density (ind. 10 cm^{-3})	418.1	218.3		282.1	912.6	422.1	318.2	

Table 3. List of dead foraminifera collected from each culture vessel during six month incubation time, size fraction 63-2000 µm.

pCO_2-treatment	Dead foraminiferal species >63 µm	Months June	%	July	%	August	%	September	%	October	%	November	%	December	%
430_A	Species														
	Ammonia aomoriensis	20	76.9	71	74.0	25	52.1	192	90.6	325	91.5	158	83.6	107	89.2
	Elphidium excavatum excavatum			4	4.2			1	0.5			16	8.5	1	0.8
	Elphidium excavatum clavatum	3	11.5			1	2.1							1	0.8
	Elphidium incertum	2	7.7	17	17.7	18	37.5	13	6.1	19	5.4	11	5.8	9	7.5
	Total number of calcareous individuals	25		92		44		206		344		185		118	
	Reophax dentaliniformis	1	3.8	4	4.2	4	8.3	6	2.8	11	3.1	4	2.1	2	1.7
	Total number of agglutinated individuals	1		4		4		6		11		4		2	
	Total number of individuals	26		96		48		212		355		189		120	
	Total number of species	4		4		4		4		3		4		5	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	17.1		63.1		31.6		139.4		233.4		124.3		78.9	
430_B	Species														
	Ammonia aomoriensis	61	87.1	84	74.3	39	68.4	199	91.7	127	88.2	125	76.7	127	83.6
	Elphidium excavatum excavatum			3	2.7	1	1.8	1	0.5			9	5.5	4	2.6
	Elphidium excavatum clavatum	1	1.4									3	1.8		
	Elphidium incertum	6	8.6	21	18.6	13	22.8	13	6.0	14	9.7	18	11.0	17	11.2
	Total number of calcareous individuals	68		108		53		213		141		155		148	
	Reophax dentaliniformis	2	2.9	5	4.4	4	7.0	4	1.8	3	2.1	8	4.9	4	2.6
	Total number of agglutinated individuals	2		5		4		4		3		8		4	
	Total number of individuals	70		113		57		217		144		163		152	
	Total number of species	4		4		4		4		3		5		4	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	46.0		74.3		37.5		142.7		94.7		107.2		99.9	
430_C	Species														
	Ammonia aomoriensis	30	83.3	73	68.9	263	92.0			284	92.2	185	83.3		
	Elphidium excavatum excavatum					3	1.0			3	1.0	11	5.0		
	Elphidium excavatum clavatum	2	5.6												
	Elphidium incertum	3	8.3	25	23.6	15	5.2			18	5.8	21	9.5		
	Total number of calcareous individuals	35		98		281				305		217			
	Reophax dentaliniformis	1	2.8	8	7.5	5	1.7			3	1.0	5	2.3		
	Total number of agglutinated individuals	1		8		5				3		5			
	Total number of individuals	36		106		286				308		222			
	Total number of species	4		3		4				4		4			
	Sediment volume (cm^3)	15.2		15.2		15.2				15.2		15.2			
	Abundance (tests 10 cm^{-3})	23.7		69.7		188.0				202.5		146.0			
907_A	Species														
	Ammonia aomoriensis	135	94.4	96	84.2	30	75.0	133	92.4	247	95.4	318	91.4	227	88.3
	Elphidium excavatum excavatum	1	0.7	2	1.8	3	7.5	1	0.7	2	0.8	6	1.7	2	0.8
	Elphidium excavatum clavatum	3	2.1	1	0.9			1	0.7	1	0.4	2	0.6		
	Elphidium incertum	2	1.4	9	7.9	7	17.5	9	6.3	4	1.5	10	2.9	6	2.3
	Total number of calcareous individuals	141		108		40		144		254		336		235	
	Reophax dentaliniformis	2	1.4	6	5.3					5	1.9	12	3.4	22	8.6
	Total number of agglutinated individuals	2		6		0		0		5		12		22	

	Total number of living specimens	543		951		204		788		630		362		388	
	Species number	1		2		1		1		1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	357.0		625.2		134.1		518.1		414.2		238.0		255.1	
907_B	Species														
	Ammonia aomoriensis	855	100.0	620	98.7	208	100.0	946	100.0			321	100.0	320	100.0
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum			8	1.3										
	Total number of calcareous individuals	855		628		208		946				321		320	
	Armorella sphaerica														
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0				0		0	
	Total number of living specimens	855		628		208		946				321		320	
	Species number	1		2		1		1				1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2				15.2		15.2	
	Population density (ind. 10 cm^{-3})	562.1		412.9		136.8		622.0				211.0		210.4	
907_C	Species														
	Ammonia aomoriensis	410	100.0	407	98.5	1014	100.0	1051	100.0	390	100.0	462	100.0		
	Elphidium excavatum excavatum			1	0.2										
	Elphidium excavatum clavatum														
	Elphidium incertum			5	1.2										
	Total number of calcareous individuals	410		413		1014		1051		390		462			
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0		0		0		0			
	Total number of living specimens	410		413		1014		1051		390		462			
	Species number	1		3		1		1		1		1			
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2			
	Population density (ind. 10 cm^{-3})	269.6		271.5		666.7		691.0		256.4		303.7			
1865_A	Species														
	Ammonia aomoriensis	728	100.0	101	100.0	162	100.0			674	100.0	1209	100.0	845	100.0
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum														
	Total number of calcareous individuals	728		101		162				674		1209		845	
	Ammotium cassis														
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0		0		0				0		0		0	
	Total number of living specimens	728		101		162				674		1209		845	
	Species number	1		1		1				1		1		1	
	Sediment volume (cm^3)	15.2		15.2		15.2				15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	478.6		66.4		106.5				443.1		794.9		555.6	
1865_B	Species														
	Ammonia aomoriensis	288	100.0	114	100.0	856	100.0	2072	100.0	932	100.0	118	100.0		
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum														
	Total number of calcareous individuals	288		114		856		2072		932		118			

	Reophax dentaliniformis			8	13.6	4	7.5	1	2.3	2	3.3	6	3.7		
	Total number of agglutinated individuals	0		8		4		1		2		6			
	Total number of individuals	66		59		53		43		60		163			
	Total number of species	4		3		3		3		4		4			
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2			
	Abundance (tests 10 cm^{-3})	43.4		38.8		34.8		28.3		39.4		107.2			
1865_C	Species														
	Ammonia aomoriensis	33	67.3	52	91.2	45	91.8	78	90.7	41	93.2	211	92.1	205	93.2
	Elphidium excavatum excavatum	4	8.2	1	1.8	3	6.1	5	5.8	1	2.3	5	2.2	1	0.5
	Elphidium excavatum clavatum	5	10.2					1	1.2	2	4.5				
	Elphidium gerthi	1	2.0												
	Elphidium incertum	4	8.2	2	3.5	1	2.0	1	1.2			5	2.2	2	0.9
	Total number of calcareous individuals	47		55		49		85		44		221		208	
	Ammotium cassis													1	0.5
	Reophax dentaliniformis	2	4.1	2	3.5			1	1.2			8	3.5	11	5.0
	Total number of agglutinated individuals	2		2		0		1		0		8		12	
	Total number of individuals	49		57		49		86		44		229		220	
	Total number of species	6		4		3		5		3		4		5	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	32.2		37.5		32.2		56.5		28.9		150.6		144.6	
3247_A	Species														
	Ammonia aomoriensis	41	75.9	45	68.2	109	86.5	85	86.7	75	90.4	70	88.6	39	84.8
	Elphidium excavatum excavatum	3	5.6	1	1.5	3	2.4	1	1.0	1	1.2	2	2.5		
	Elphidium excavatum clavatum	3	5.6			2	1.6			1	1.2				
	Elphidium incertum	6	11.1	17	25.8	12	9.5	9	9.2	4	4.8	5	6.3	1	2.2
	Total number of calcareous individuals	53		63		126		95		81		77		40	
	Reophax dentaliniformis	1	1.9	3	4.5			3	3.1	2	2.4	2	2.5	6	13.0
	Total number of agglutinated individuals	1		3		0		3		2		2		6	
	Total number of individuals	54		66		126		98		83		79		46	
	Total number of species	5		4		4		4		5		4		3	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	35.5		43.4		82.8		64.4		54.6		51.9		30.2	
3247_B	Species														
	Ammonia aomoriensis	80	85.1	101	80.8	70	86.4	88	83.0	100	84.7	40	83.3	59	86.8
	Elphidium excavatum excavatum	2	2.1	1	0.8	1	1.2	2	1.9	1	0.8	1	2.1		
	Elphidium excavatum clavatum	6	6.4	2	1.6					1	0.8				
	Elphidium incertum	5	5.3	15	12.0	6	7.4	8	7.5	14	11.9	4	8.3	4	5.9
	Total number of calcareous individuals	93		119		77		98		116		45		63	
	Reophax dentaliniformis	1	1.1	6	4.8	4	4.9	8	7.5	2	1.7	3	6.3	5	7.4
	Total number of agglutinated individuals	1		6		4		8		2		3		5	
	Total number of individuals	94		125		81		106		118		48		68	
	Total number of species	5		5		4		4		5		4		3	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	61.8		82.2		53.3		69.7		77.6		31.6		44.7	
3247_C	Species														
	Ammonia aomoriensis	156	92.9	103	83.1	84	85.7	74	83.1	128	92.8	54	90.0		
	Elphidium excavatum excavatum	3	1.8	11	8.9	4	4.1	3	3.4			1	1.7		
	Elphidium excavatum clavatum	4	2.4			1	1.0								

Elphidium incertum	4	2.4	8	6.5	9	9.2	6	6.7	6	4.3	4	6.7		
Total number of calcareous individuals	167		122		98		83		134		59			
Reophax dentaliniformis	1	0.6	2	1.6			6	6.7	4	2.9	1	1.7		
Total number of agglutinated individuals	1		2		0		6		4		1			
Total number of individuals	168		124		98		89		138		60			
Total number of species	5		4		4		4		3		4			
Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2			
Abundance (tests 10 cm^{-3})	110.5		81.5		64.4		58.5		90.7		39.4			

IV.3.2.2 Abundance and test diameter of dead *Ammonia aomoriensis*

The abundance of the dead fauna ranged from 24 to 61 tests 10 cm^{-3}. At pCO_2-levels of 430 and 907 µatm, their abundance increased steadily until the 4th and 5th month, and subsequently decreased until the end of experiment (Fig. 3, B, Table S2 in the supplement). In contrast, at pCO_2 of 1865 and 3247 µatm, the number of *A. aomoriensis* tests did not significantly change until the 4th month (Fig. 3, B). Afterwards, the number of empty tests increased by 80 % at 1865 µatm until the end of the experiment (Table S2 in the supplement). These results correlated with a strong decline of population densities from 1378 to 338 ind. 10 cm^{-3} from the third to the fifth month (Fig. 3, A). At 3247 µatm, test abundance decreased to 32 tests 10 cm^{-3} at the end of the experiment (Table S2 in the supplement).

The mean test diameter ranged between 290 and 283 µm at pCO_2 from 430 to 1865 µatm. At higher pCO_2 of 3247 µatm, test diameter was clearly lower with 258 µm (Table S4 in the supplement) which indicated a frequent mortality of large and adult foraminifera up to 1865 µatm, whereas at higher pCO_2 a frequent mortality of smaller and younger individuals was recognized. Test diameter of dead *A. aomoriensis* was significantly effected by incubation time ($F(5,46)=16.65$, $p<0.01$) but not by pCO_2 ($F(3,46, p>0,05)$ (Table 4).

Table 4. Two-way analysis of variance (ANOVA) revealed the effect of pCO_2 and incubation time on test diameter of *A. aomoriensis*. Significant results are represented in bold. SS = Sum of Squares, d.f. = degrees of freedom, MS = Mean Squares.

Test diameter of living *A. aomoriensis*

Factor	SS	d.f.	MS	F	*p*
pCO_2	1213.0	3.0	404.0	0.61	0.6100
Incubation time	59800.0	5.0	12000.0	**18.14**	**0.0000**
pCO_2 x time	21200.0	15.0	1412.0	**2.14**	**0.0250**

Test diameter of dead *A. aomoriensis*

Factor	SS	d.f.	MS	F	*p*
pCO_2	7351.0	3.0	2450.0	2.46	0.0750
Incubation time	83000.0	5.0	16600.0	**16.65**	**0.0000**
pCO_2 x time	21800.0	15.0	1450.0	1.45	0,1630

IV.3.3 Dry test weight

The initial dry weight per individual of living *A. aomoriensis* was 1.56 µg on average (Fig. 6, Table S5 in the supplement). With increasing test diameter, the dry weight per living individual increased constantly, but no significant differences of dry weight-diameter ratio were observed between the different pCO_2 treatments (Fig. 5, A). Dry weight per individual increased by 68, 57, 70 and 62 % at a pCO_2 of 430, 907, 1865 and 3247 µatm until the first month (Table S5 in the supplement). At 430 and 3247 µatm, dry weight per individual did not change significantly any more until the end of the experiment (Table S5 in the supplement). In contrast, the dry weight of *A. aomoriensis* increased up to 7.30 µg at a pCO_2 of 907 µatm after two months (Table S5 in the supplement), which correlated with an increase in test diameter by 116 µm on average (Fig. 4). At a pCO_2 of 1865 µatm, dry weight per individual decreased significantly to 1.30 µg after three months

(Table S5 in the supplement). These results correlated with the reproduction events and resulting dominance of small foraminifera of size classes <50 up to 200 µm (Fig. 4).

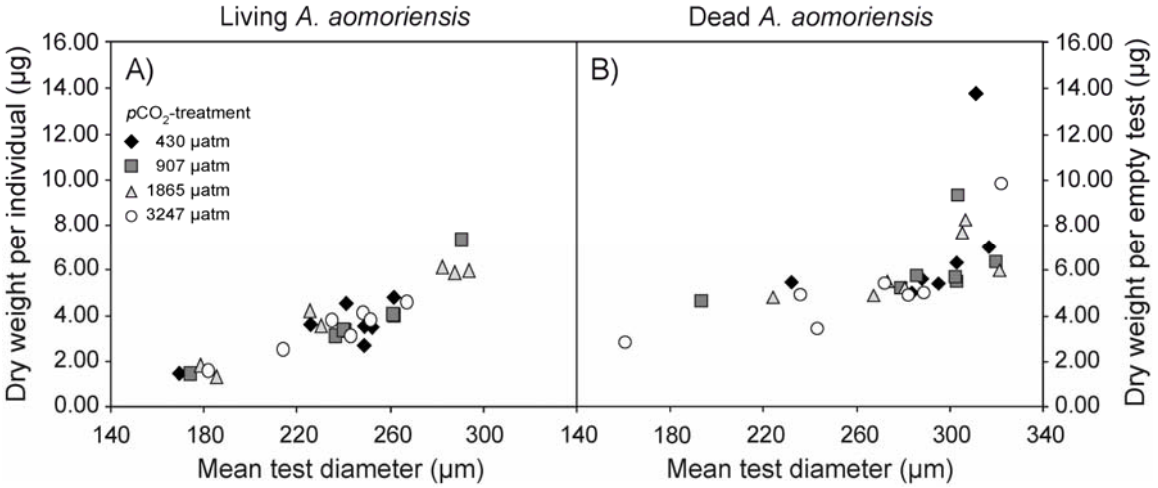

Fig. 5. Total dry weight of (A) living specimens including cytoplasm and (B) empty test of *A. aomoriensis* in relation to mean test diameter for the 4 tested pCO_2-treatments.

In comparison to living *A. aomoriensis*, mean dry weight per empty test was higher with 6.18 µg on average (Fig. 6, Table S5 in the supplement). This probably results from the more frequent reproduction or mortality of large specimens. Similar to the dry weight of specimens from the living fauna, no effect of any pCO_2 was observed with empty tests (Fig. 5, B). In accordance to changes in test diameter, the average dry weight per test changed during the experiment. At 430 and 907 µatm, dry weight per test increased by 60 and 50 % until the second month and remained stable with mean values of 5.63 and 5.84 µg until the end of the experiment (Table S5 in the supplement). In pCO_2-treatments 1865 and 3247 µatm, dry test weight increased by 41 and 71 % during the first month (Fig. 6, B), followed by a further slight increase up to 7.73 µg at 1865 µatm after five months (Table S5 in the supplement). These observations were in agreement to the decline of population densities of *A. aomoriensis* after the first and fifth month at 1865 µatm (Fig. 3, A).

IV.3.4 Carbonate production and accumulation

The total organic contents of living individuals was found to be 4.3 %, which was subtracted from total dry weight in order to determine the absolute amount of $CaCO_3$ (cf. Movellan et al., 2012) (Table S5 in the supplement). Production is considered as weight increase measured at subsequent sampling. Average production during the entire incubation period is given as mean value of monthly weight increases. Living *A. aomoriensis* exhibited the lowest mean $CaCO_3$ production of 595 µg at the lowest pCO_2 of 430 µatm, following by 886 µg at the highest pCO_2 of 3247 µatm after six months incubation. The three replicate treatments of 907 and 1865 µatm showed similarly higher mean $CaCO_3$ production of 1269 and 1256 µg with a

total culture vessel area of 42.25 cm^2 and an incubation time of six month. The calculated total carbonate production rate for living individuals was 0.47 g m^{-2} a^{-1} (Table S6 in the supplement).

The $CaCO_3$ accumulation of empty tests varied between the pCO_2 treatments (Table S6 in the supplement). The highest rate of 908 µg $CaCO_3$ was observed at 907 µatm, followed by 670 µg at 430 µatm. With increasing pCO_2 of 1865 and 3247 µtm, $CaCO_3$ rates decline to 435 and 239 µg after six months incubation with the above specified settings. The calculated total carbonate accumulation of empty tests was 0.27 g m^{-2} a^{-1} (Table S6 in the supplement).

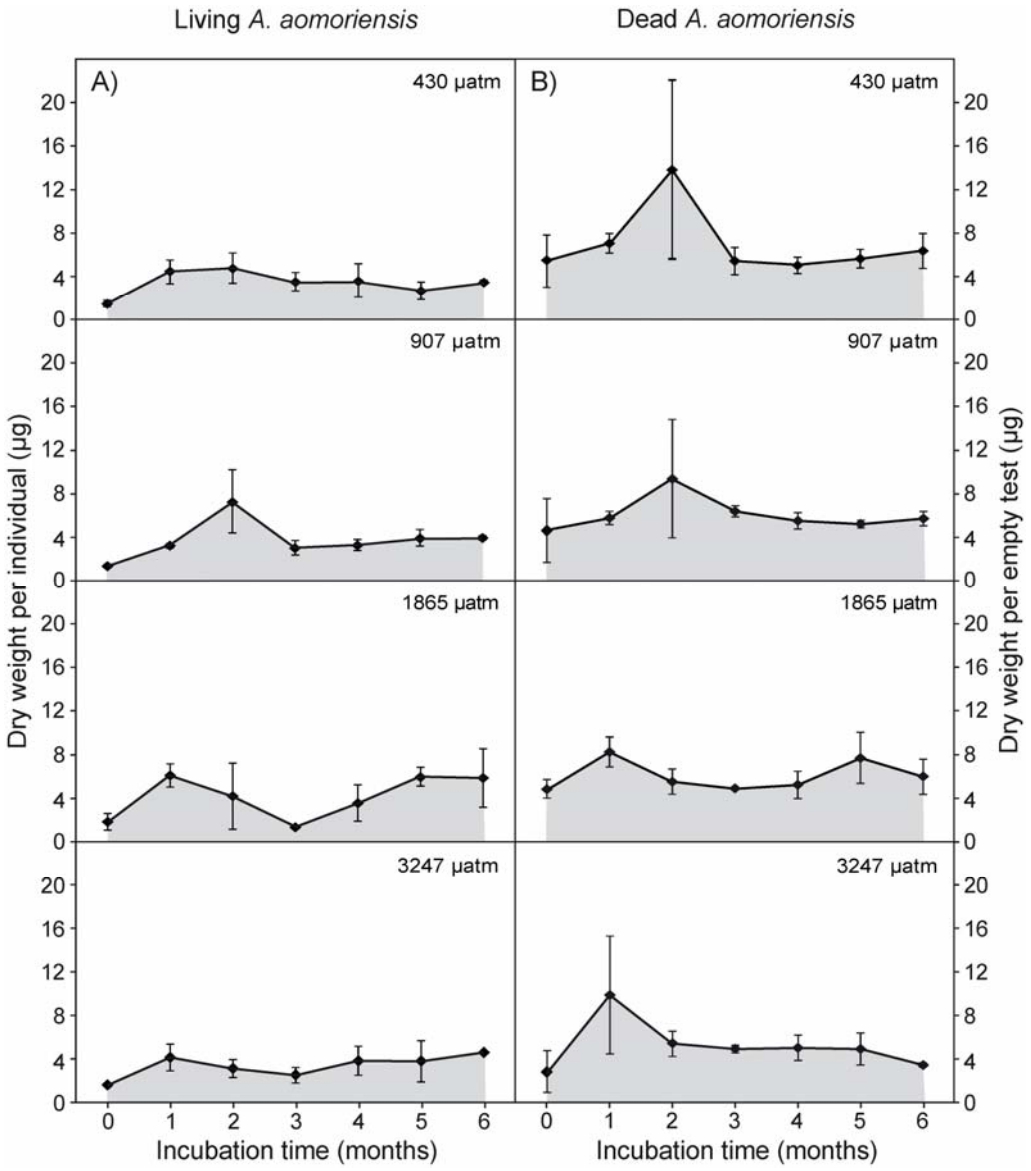

Fig. 6. Mean dry weight per individual including cytoplasm/empty test and standard deviation of three replicates of (A) living and (B) dead *A. aomoriensis* of the 4 tested pCO_2-levels during six months incubation.

IV.3.5 Observations of test structures and elemental distribution

At high pCO_2 of 3247 µatm, light micrograph images of dead *A. aomoriensis* showed that most of the tests were completely destroyed during the last two months of the experiment. The

strongest effect of test degradation was the charistic star-like appearance, which left only the umbilicus area and the suture of the chambers intact.

Cross-sections of test walls of *A. aomoriensis* (Fig. 7, A) and *E. incertum* (Fig. 7, B) revealed that the tests of both species were composed of a single calcium carbonate layer, which contained high Ca and low Mg calcite. In comparison to *E. incertum*, *A. aomoriensis* exhibited a higher Mg-content. Furthermore, the test wall of *A. aomoriensis* was highly porous, whereas *E. incertum* showed a smooth surface without any visible porosity.

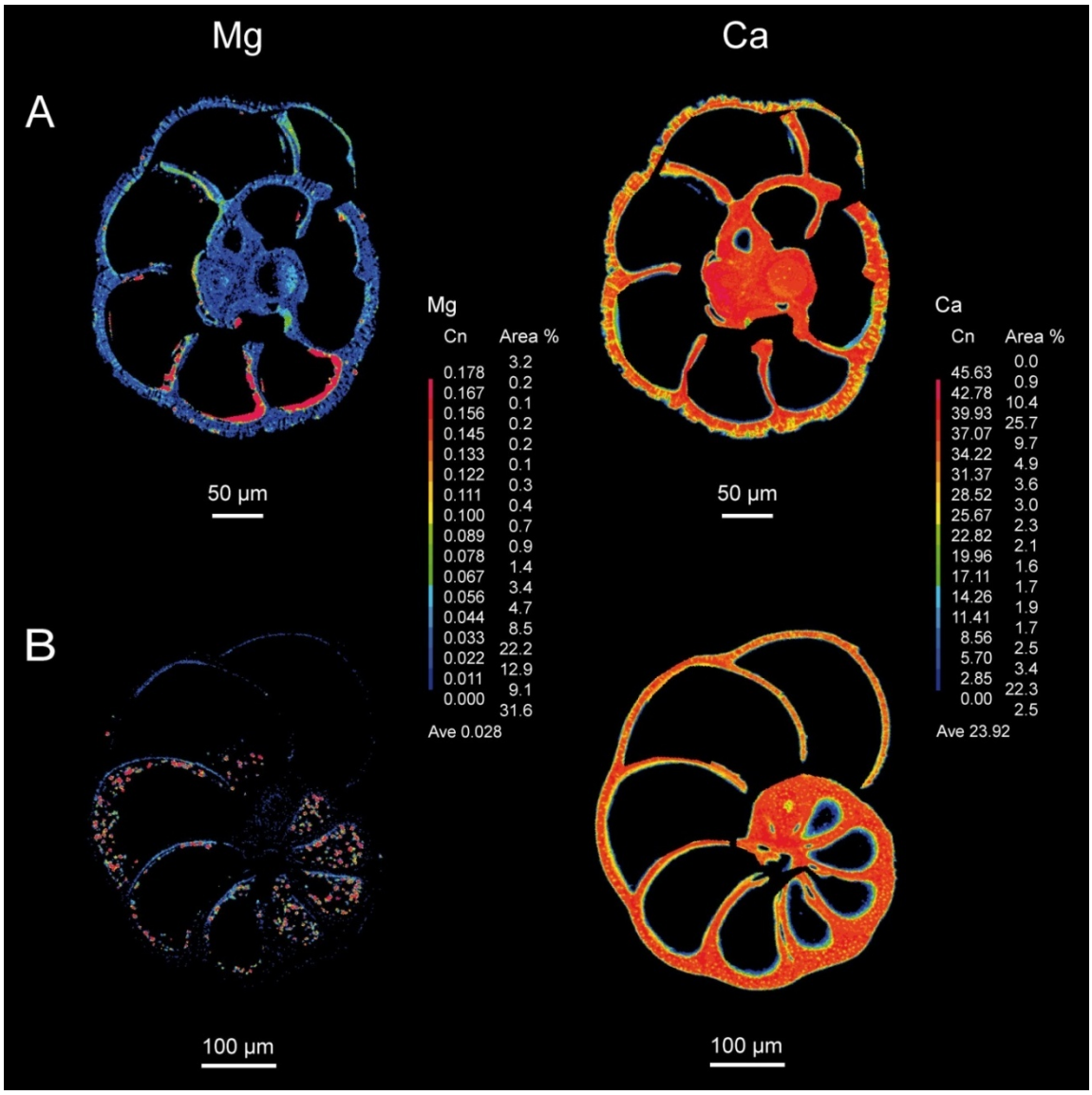

Fig. 7. EMP maps of Mg and Ca of test cross-sections from cultured (A) living *A. aomoriensis* and (B) living *E. incertum*. All intensity values are expressed in counts per second (cps) as shown in the color bars.

IV.4 Discussion

This study describes the impact of elevated pCO_2 on a natural foraminiferal community from the southwestern Baltic Sea which was exposed to 4 different pCO_2 levels for six months. It is the first study which investigated the multi-generational response using natural sediment with living

foraminiferal fauna of natural composition. The foraminiferal community developed and reproduced normally in all treatments, as the specific sediment chemistry caused, compared to the bulk seawater, a relative high saturation state with respect to omega calcite, and thereby prevented the tests of living individuals from dissolution.

IV.4.1 Sediment chemistry

The sediment used in this experiment was sampled from Kiel Fjord, western Baltic Sea, which is rich in organic matter (Nikulina et al., 2008). During the experiment, a layer of organic detritus formed at the sediment-water interface. Organic substances influence the microbial activity, the rates and pathways of organic matter remineralization and nutrient recycling (Jonsson et al., 1990, Conley and Johnstone, 1995), and thereby influenced the underlying sediment chemistry (Graf et al., 1984). The remineralization of organic matter is mainly attributed to a wide array of hydrolytic and fermentative bacteria (Turley et al., 2000), which break complex multi-carbon compounds down to smaller, more soluble and digestible substances. Bacterial activity is often attributed as a limiting step in degradation of organic matter and extent of degradation (Tyson, 1995; Arnosti, 2004).

It is conceivable that silicate and phosphate concentrations were highly variable in the stagnated water body of the culture vessels throughout the experiment due to the high microbiological activity. Concentrations of both nutrients increased during the first two months and afterwards decreased until the end of the experiment.

One aspect could be the disturbance of the sediment after homogenization at the beginning of the experiment. This process could cause a remobilization of the bounded nutrients in the pore water, which diffused gradually through the filter of the nepheloid layer. Therefore, nutrient concentration increased in the overlying seawater.

However, silicate concentrations remain relatively high. Microscopic analysis showed that the detritus-layer was composed of centric diatoms, which also dominated in the natural environment. The algal material of *Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella* was added as food did not accumulate on the sediment surface. This in turn could imply that the added algae material was directly dissolved, which could explain the high silicate concentrations. Otherwise, increasing silicate concentrations may result from remineralization of diatoms (Conley and Johnstone, 1995). Sediment samples were taken in April, when high nutrient and productivity is usually encountered (Wulff et al., 1986, Elmgren, 1989, Wulff et al., 1990, Wollast, 1998, Thomas et al., 2003, Pätsch and Kühn, 2008). The spring bloom, ranging from the end of February to early April in the southwestern Baltic Sea, is dominated by diatoms, therefore maximal diatom biomass and biogenic silica flux to the sea floor (Wasmund et al., 2005, 2006). After deposition at the sediment surface, remineralization of the dead diatoms may have caused a substantial increase of silicate in the culture vessels during the experiment (Conley and Johnstone, 1995).

The high degradation of organic matter such as diatoms may have also caused an increase of phosphate concentrations (Balzer, 1986). In oxic sediments dissolved inorganic

phosphate (DIP), is usually bound to calcium, chemisorbed by ironoxyhydroxides in distinct iron compounds and causes high phosphate accumulations in the sediment (Nissenbaum, 1979, Filipek and Owen, 1980, Krom and Berner, 1981). However, high O_2 consumption by degradation of organic matter may have reduced the oxygen concentrations in the sediment which result in mobilization of DIP from sediments (Krom and Berner, 1981). The decrease of both nutrients until the end of the experiment may be reasoned by microbiological activity of bacteria, which is insufficient studied to date.

Degradation processes of organic matter at the sediment-water interface consumed O_2 and produced CO_2, and thus resulted in higher sediment pore water pCO_2 in comparison to the seawater. Similarly, the sediment was characterized by much higher alkalinity values than the seawater (3127 vs. 2379 µmol kg^{-1}). Both, aerobic or anaerobic degradation of organic matter release dissolved inorganic carbon (DIC). Whereas aerobic degradation has no significant effect, anaerobic degradation increases alkalinity (A_T) by denitrification and sulfate reduction (Yao and Millero, 1995, Thomas et al., 2009). Therefore, we assume that O_2 consumption at the sediment-water interface exceeded the delivery of O_2 via diffusion, which could induce anaerobic conditions in the sediment pore water. A dark grey sediment layer on the bottom of the culture vessels supported our assumption. This in turn enhances the CO_2 buffer capacity and consequently causes a relatively high Ω_{calc} (Thomas et al., 2009). These differences of the carbonate chemistry between water column and sediment was in agreement with observations from field studies (Thomas et al, 2009, Haynert et al., 2012).

IV.4.2 Foraminiferal communities

The species composition of living and dead assemblages which were used in the experiment were similar to the assemblages prevailing in Kiel and Flensburg Fjords (Nikulina et al., 2008, Polovodova et al., 2008, 2009, Haynert et al., 2012).

At the beginning of the experiment, the population density of the living fauna (433 ind. 10 cm^{-3}) was very similar to the mean population density of 448 ind. 10 cm^{-3}, found in the nearby sampling station PF15–13 in December 2005 (Nikulina et al., 2008, Polovodova et al., 2008). The living assemblages at both stations were dominated by *A. aomoriensis* (*A. beccarii* of Nikulina et al., 2008). But in December 2005, *E. excavatum clavatum* was common and *E. excavatum excavatum* and *E. incertum* were rare, whereas in April 2011, the living fauna consisted almost exclusively of *A. aomoriensis*. These differences were probably caused by the interannual variation in the community structure (Lutze, 1974, Wefer, 1976, Polovodova et al., 2009, Haynert at al., 2012).

The dominance of living *A. aomoriensis* is probably explained by the diatom dominated spring bloom, which enables high food supply and thereby facilitates reproduction. Similar relationship between diatom spring blooms and reproduction events was reported for *E. excavatum clavatum* at deeper waters off Boknis Eck, southwestern Baltic Sea (Schönfeld and Numberger, 2007).

In addition, the arenaceous species *Armorella sphaerica* occurred by incident in the set up. The species was recorded in Kiel Bay by Rhumbler (1935) and later by Brodniewicz (1965) in the southern Baltic Sea. Its sporadic occurrence and sudden appearance in our experiment could be possibly explained by the activation of dormant propagules (Alve and Goldstein, 2002). Alternatively, the species is common but rare in Kiel Fjord and has been eventually overlooked in previous studies. In our experiment, however, suitable conditions, such as food supply and oxygenation could have induced the activation of resting stages.

IV.4.3 Response of *Ammonia aomoriensis* life cycle

In the first month of the experiment, population densities of living *A. aomoriensis* declined strongly at a pCO_2 of 1865 and 3247 µatm. This decline might indicate conditions of $\Omega_{calc}<1$ in the sediment. The sediment was put in the vessels 20 days prior to CO_2 manipulation. Nevertheless, the remineralization processes in the sediment were still ongoing as indicated by the strong increases of seawater phosphate and silicate concentration during the first two months. Therefore, high pore water alkalinity and thereby supersaturation of calcium carbonate might have not been established in the early phase of the experiment. Undersaturation has been already shown to decrease growth of *A. aomoriensis* and thereby increased mortality and test dissolution (Haynert et al., 2011). Similarly, a decrease of *Ammonia* species growth was observed with decreasing $[CO_3^{2-}]$, respectively $\Omega_{calc}<1$ (Keul et al., 2013).

During the course of the experiment however, the progressing remineralization processes in the sediment may have increased saturation state above 1. Accordingly, *A. aomoriensis* was relatively unaffected by high pCO_2 later on and continued to grow and reproduce, even under highly elevated pCO_2 conditions (Haynert et al., 2012).

In comparison, previous ocean acidification studies cultured living benthic foraminifera without their natural sediment (Le Cadre et al., 2003, Kuroyanagi et al., 2009, Dissard et al., 2010, Allison et al., 2010, Haynert et al., 2011, McIntyre-Wressnig et al., 2013, Fujita et al., 2011, Vogel and Uthicke, 2012, Keul et al., 2013). Under these laboratory conditions, foraminifera were directly exposed to the seawater which might result in low and even undersaturation of omega calcite at high seawater pCO_2 (Haynert et al., 2011).

The current study underscores, that endobenthic foraminifera from marginal low saline habitats responded completely different under elevated pCO_2 in culturing experiments, depending whether they were kept with or without their natural sediment. Furthermore, high or low organic matter content of the substrate, grain size composition (Conley and Schelske, 1989), bacterial remineralization (Turley et al., 2000), and oxic or anoxic conditions (Jonsson et al., 1990) play a certain role in sediment and pore water chemistry, and thereby may influence the living conditions of benthic communities. In fine-grained sediments with a high content of organic substances these processes play an important role for the foraminiferal microhabitat. It is reasonable to assume, that the effects on carbonate chemistry are less pronounced in coarse sediments. These results emphasize the importance to understand the sediment carbonate chemistry in the context of their natural settings.

During the six months incubation, the populations of all treatments revealed strong variations of their density and size-frequency distribution due to growth, mortality and reproduction events. The shift of mean size distribution between the monthly sampling intervals enabled the calculation of growth rates which varied between 25 and 38 µm per month and agreed with earlier growth rates estimates reporting 13 to 39 µm (Haynert et al., 2011). This growth corresponds to the addition of one or two chambers per month, as one added chamber equates 27±16 µm in test diameter (Haynert et al., 2011). An earlier study described, growth rate as being uniform in all size classes. Small individuals grew more rapidly, whereas adult individuals slow down the growth before reproduction (Bradshaw, 1957). Furthermore, changing habitat conditions, in particular food supply, play an important role at chamber formation (Bradshaw, 1961). It appears plausible that larger and a greater number of chambers will be formed under favorable conditions.

Size distributions of tests revealed that most of the individuals reproduced or died at a mean size of 285 µm at a pCO_2 ranging from 430 to 1865 µatm. At the highest pCO_2 of 3247 µatm, mean test diameter was slightly lower with 258 µm. Apart from growth, reproduction events had a pronounced effect on the mean size of the population. Cohorts of small, juvenile individuals (<100 µm) could be identified. Whereas reproduction events, could not be detected at 430 µatm, strong cohorts with high numbers of individuals were observed at 1865 and 3247 µatm, with a strongest variability at 1865 µatm.

Ammonia aomoriensis reproduced at sizes between 250 µm and 350 µm. At 350 µm, 40 % of the cohorts either have reproduced or died. These results agreed with a previous study of Bradshaw (1957) where the mean test diameter of *Streblus beccarii* var. *tepida* ranged between 266 and 357 µm at reproduction. In dependency of environmental conditions, reproduction follows approximately 28-day intervals with about 28 young juveniles per parent under optimal conditions (Bradshaw, 1957). In the current study we observed reproduction events, but the different cohorts made it impossible to exactly determine the intervals of reproduction, respectively the number of juveniles per parent.

In dependency of asexual and sexual reproduction, the generations consisted of different forms as influenced by test dimorphism (Murray, 2012). The megalospheric form is the result of asexual reproduction, whereas the microspheric form is the product of sexual reproduction (Lister, 1903). However, the reproduction of *A. aomoriensis* is insufficiently studied to date, further field and laboratory experiments would be necessary to better understand the life cycle of *A. aomoriensis*, and the species specific variability of *Ammonia* in general. However, since reproduction was observed in all pCO_2 treatments, the conditions prevailing in the sediment were sufficient to allow successful reproduction even at 3247 µatm pCO_2. This supports findings, that natural foraminiferal communities in the southwestern Baltic Sea can withstand permanent high pCO_2 levels of above 2000 µatm when they are fully sheltered in fine-grained sediment (Haynert et al., 2012).

IV.4.4 Carbonate production of *Ammonia aomoriensis*

The carbonate production of benthic foraminifera was described in previous studies (e.g. Phleger and Soutar, 1973, Muller, 1974, Wefer and Lutze, 1978, Hallock, 1981, Bosence, 1989, Langer et al., 1997) which determined the foraminiferal carbonate content of sediment samples from different habitats. In contrast, in our study the main focus was on living benthic foraminifera containing cytoplasm. Little is known about the fractions of cytoplasm and tests weight in benthic foraminifera. The estimated total organic content in living *A. aomoriensis* is 4.3 % of total dry weight which is in agreement with the estimated protein content of 20 % and 5 % organic dry content in *Ammonia tepida* (Movellan et al. 2012). The study of Wefer and Lutze (1976) observed a ratio of 1:1 of protoplasm and test weight in benthic foraminifera which referred to wet weight (see Table 1 in Wefer and Lutze, 1976) The cytoplasm water content plays an important role in this determination as TEM micrograph images documented a high amount of seawater vacuoles in the foraminifera chambers (see Fig. A3.3.2 in Glock, 2011). Therefore and in agreement with Movellan (personal communication), we assume a water content of approximately 76 % in foraminiferal cytoplasm. However, the percentage of water and cytoplasm content varies in relation to test size and weight, and due to the natural variability of the microenvironment and the species-specific morphometry (Movellan et al., 2012).

The carbonate production of living *A. aomoriensi*s amounts 0.47 g m^{-2} a^{-1}, whereas the carbonate accumulation of dead tests equates 0.27 g m^{-2} a^{-1} during the incubation time. The estimate of $CaCO_3$ production is much higher than those reported for natural foraminiferal assemblages in the western Baltic Sea. Production rates of calcareous benthic foraminifera rates ranged from 0.01 to 0.03 g m^{-2} a^{-1} in the shallow water habitat from 5–15 m depth of Kiel Bay (Wefer and Lutze, 1978). However, this low carbonate production was probably caused by a strong seasonality of food supply and mechanical stress on shoals with lag sediments, where these samples were taken. Muddy sediments such as in Kiel Fjord, with high organic matter content as potential source of food showed a markedly higher foraminiferal carbonate production of up to 3.12 g m^{-2} a^{-1} (Wefer and Lutze, 1978, Nikulina et al., 2008). Therefore, both favorable food supply and sustaining high population densities during the incubation facilitated the observed high $CaCO_3$ productivity of *A. aomoriensis*.

Not less than 36 % of the produced tests are accumulated in the sediment, which is obviously higher as reported by Wefer and Lutze (1978), the accumulation rate ranged between 0 and 4 % in the natural habitat. In the transitional environments from fine sand to mud, the accumulation rate varied from 1 to 1.2 % (see Table 2 in Wefer and Lutze, 1978). Under natural conditions, mechanical forces play an important role and affected the tests, as well as dissolution and ingestions by macro- and meiofauna (Wefer and Lutze, 1978). In the present study, *A. aomoriensis* was cultivated in a protective habitat without any mechanical impacts of the empty tests, therefore a higher accumulation rate was recorded.

IV.4.5 Impact on foraminifera tests

Living *A. aomoriensis* and *E. incertum* exhibited no dissolution features during the whole incubation time. The conditions in the natural sediment create a protective microhabitat for benthic foraminifera with a relatively high calcium carbonate saturation state. These observations are in contrast to previous laboratory studies, where *A. aomoriensis* and *A. beccarii* exhibited a relationship between pCO_2, respectively pH, and test degradation (Le Cadre, 2003, Haynert et al., 2011). In comparison to the present study, living foraminifera were isolated from their natural sediment, therefore the tests of living specimens were directly affected by the carbonate chemistry conditions of the seawater.

However, some empty tests of *A. aomoriensis* were destroyed at a pCO_2 of 3247 µatm during the last two months of incubation. This could be reasonable by high pCO_2 and unfavorable saturation state of $\Omega_{calc}<1$, thereby reducing the average diameter. In contrast, empty tests of *E. incertum* showed no signs of dissolution. This species specific response agreed well with our field observation from Flensburg Fjord (Haynert et al., 2012). In order to explain these differences, cross sections of both species were analyzed using EMP. The element maps suggested that tests of both foraminiferal species are composed of a single layer of secondary calcite, which is characterized by a relative low Mg-content (Erez, 2003). Nevertheless and in comparison to *E. incertum*, the test of *A. aomoriensis* displayed a higher Mg-content and was highly porous. The crystals, rich in Mg are oriented in a structure of small radial needles (Hansen and Reiss, 1972, Bellemo, 1974), which are sensitive and dissolved first at undersaturated conditions. In combination with a greater test surface, *A. aomoriensis* was therefore less resistant to dissolution than *E. incertum*. EMP observations are thus considered to provide valuable constraints for the sensitivity of a species towards dissolution stress.

IV.5 Conclusions

In the present ocean acidification study, benthic foraminifera were cultured in their natural sediment over a period of several generations. Under those laboratory conditions, the alkalinity (A_T) and therefore the CO_2 buffer capacity in the sediment differed strongly from the conditions in the water column. Thereby the sediment chemistry created a microhabitat, which supported the growth and development of a benthic foraminiferal community even at highly elevated pCO_2. Fine-grained sediments with high organic matter content facilitated a high carbonate production of *A. aomoriensis*. Growth, reproduction and mortality of *A. aomoriensis* were unaffected by elevated pCO_2. Consequently, under the current microhabitat conditions, the dominant *Ammonia aomoriensis* could maintain an important role in benthic carbonate production and accumulation in the southwestern Baltic Sea. However, at high pCO_2 and slight undersaturation of Ω_{calc}, empty tests of *A. aomoriensis* were subjected to dissolution, whereas empty *E. incertum* tests were kept intact. The species specific response could be explained by differences in test composition and microstructure. These results emphasize the importance to understand the

sediment carbonate chemistry in the natural environment of benthic foraminifera, which depend on sediment type, grain size, organic matter, remineralization and chemical conditions in the pore water. In Kiel Fjord, organic-rich and fine-grained sediments prevail which influence the pore water carbonate chemistry, and thereby provide a stable habitat for benthic foraminifera. Due to these characteristics, foraminiferal communities withstand present day, seasonally high pCO_2 levels and might be also be able to tolerate moderate future pCO_2 increases.

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Supplements

Table S1. Carbonate chemistry parameters were measured and calculated from samples of seawater (SW) and sediment pore water (PW) for 4 pCO_2-levels after six months incubation. Total carbon (C_T), partial pressure of CO_2 (pCO_2) and saturation state of calcite (Ω_{calc}) were calculated from measured temperature, salinity, phosphate (PO_4^{3-}), silicate (Si), total alkalinity (A_T) and pH_{NBS}.

pCO_2-treatment (µatm)	Seawater measurements				A_T (µmol kg^{-1})			pH_{NBS}			Calculations from A_T and pH_{NBS}								
	T (°C)	S	PO_4^{3-} (µmol l^{-1})	Si (µmol l^{-1})	SW (0-2 cm)	SW (2-4 cm)	PW	SW (0-2 cm)	SW (2-4 cm)	PW	C_T (µmol kg^{-1}) SW (0-2 cm)	C_T (µmol kg^{-1}) SW (2-4 cm)	C_T (µmol kg^{-1}) PW	pCO_2 (µatm) SW (0-2 cm)	pCO_2 (µatm) SW (2-4 cm)	pCO_2 (µatm) PW	Ω_{calc} SW (0-2 cm)	Ω_{calc} SW (2-4 cm)	Ω_{calc} PW
430	16.7	15.2	0.16	138.09	2117.5	2450.1	3023.5	8.11	8.03	7.95	1987.9	2335.2	2918.3	393	563	845	3.00	2.93	3.08
907	16.6	15.2	0.16	138.92	2115.9	2450.1	2919.3	7.94	7.79	7.70	2036.7	2408.0	2900.1	599	1022	1504	2.12	1.76	1.74
1865	16.7	15.2	0.15	139.21	2102.8	2439.7	3127.8	7.59	7.54	7.49	2111.7	2463.9	3181.0	1421	1830	2666	0.97	1.03	1.17
3247	16.6	15.2	0.17	136.62	2116.1	2429.3	3440.6	7.45	7.41	7.38	2159.5	2491.6	3547.2	1980	2480	3804	0.72	0.76	1.01

Table S2. Mean population density/abundance and standard deviation (SD) of the 3 replicates for living and dead *A. aomoriensis* of the 4 testes pCO_2-levels during six months experimental time.

Living *A. aomoriensis* (ind. 10 cm^{-3})								
Incubation time (months)	pCO_2-treatment 430 µatm		907 µatm		1865 µatm		3247 µatm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	341.7	95.65	396.2	150.18	413.3	199.51	582.7	177.74
1	400.6	75.68	430.6	175.69	70.8	4.28	189.3	66.07
2	357.9	80.78	312.5	306.71	323.3	228.99	246.8	88.60
3	292.6	146.91	610.3	87.04	1378.4	22.78	517.9	348.52
4	231.0	65.79	335.3	111.58	659.2	242.68	448.8	23.16
5	196.6	161.10	250.9	47.68	338.4	396.68	601.6	308.96
6	241.3	28.82	232.7	31.61	483.2	102.28	264.6	30.22
Dead *A. aomoriensis* (tests 10 cm^{-3})								
Incubation time (months)	pCO_2-treatment 430 µatm		907 µatm		1865 µatm		3247 µatm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	24.3	14.05	39.9	42.43	27.0	4.96	60.7	38.45
1	50.0	4.60	51.9	10.86	41.0	12.92	54.6	21.65
2	71.7	87.81	68.8	42.52	36.6	12.72	57.6	12.99
3	128.5	3.25	128.2	48.24	37.8	19.06	54.1	4.85
4	161.3	68.71	187.7	35.80	30.7	5.35	66.4	17.43
5	102.6	19.76	203.8	12.07	121.2	27.57	35.9	9.87
6	76.9	9.30	139.1	14.41	152.9	25.57	32.2	9.30

Table S3. Mean percentage of size class proportions from <50 to >500 µm in 50 µm intervals of living *A. aomoriensis* and standard deviation (SD) of the three replicates during six months incubation.

pCO_2 treatment	Incubation time (months)	Size class proportions (%)																					
		<50 µm		50-100 µm		100-150 µm		150-200 µm		200-250 µm		250-300 µm		300-350 µm		350-400 µm		400-450 µm		450-500 µm		>500 µm	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
430 µatm	0	5.7	1.47	10.2	3.38	23.5	6.62	30.2	3.19	19.4	2.17	7.3	3.34	2.1	0.35	1.1	0.52	0.4	0.33	0.1	0.11	0.0	0.08
	1	0.4	0.22	4.0	2.14	13.6	9.16	14.3	7.01	21.3	7.17	21.7	6.71	14.0	2.14	7.7	3.15	2.1	2.13	0.8	0.41	0.2	0.21
	2	1.4	0.61	2.6	0.68	3.1	0.29	12.7	5.79	21.7	3.94	28.6	1.48	18.4	4.06	8.4	3.11	2.2	1.53	0.5	0.56	0.3	0.30
	3	0.9	1.29	3.7	3.24	4.4	2.19	13.3	1.11	27.7	7.61	28.2	0.95	14.9	7.00	4.5	2.07	1.5	0.41	0.8	0.87	0.2	0.23
	4	1.7	1.13	5.3	2.78	13.0	11.93	18.8	6.42	22.4	12.46	22.7	9.26	10.5	0.80	3.6	0.89	0.5	0.36	0.7	0.53	0.6	0.18
	5	0.8	0.88	5.5	1.30	13.4	13.36	14.0	13.36	20.8	6.36	16.4	9.34	12.3	5.94	4.3	1.53	10.3	16.58	2.3	3.13	0.1	0.14
	6	0.3	0.36	1.2	1.21	2.7	0.33	18.5	0.45	26.4	0.70	28.9	0.73	15.2	0.49	4.9	0.18	1.0	0.70	0.7	0.28	0.3	0.42
907 µatm	0	4.1	1.76	8.2	1.81	24.0	1.47	31.8	2.07	21.7	3.72	6.2	0.97	2.5	0.92	0.7	0.27	0.7	0.45	0.2	0.32	0.0	0.00
	1	0.9	0.35	2.2	0.89	5.3	2.06	16.6	3.01	28.4	0.30	29.8	4.28	13.9	2.37	2.7	0.36	0.1	0.11	0.1	0.14	0.0	0.00
	2	2.1	1.24	4.7	2.10	4.0	2.38	11.7	8.03	11.4	6.89	16.5	3.55	20.9	4.26	17.9	9.69	7.1	5.55	2.2	1.98	1.4	1.22
	3	0.8	0.30	3.2	1.18	10.2	2.37	19.9	5.30	22.3	6.78	22.0	1.60	14.1	5.51	5.8	3.16	1.2	0.52	0.2	0.16	0.2	0.08
	4	4.1	0.73	8.5	2.60	5.4	1.13	9.3	1.51	22.1	4.37	29.2	2.71	13.2	5.64	3.6	1.47	1.7	0.44	1.7	0.55	1.1	0.47
	5	2.9	0.90	6.9	2.64	4.2	0.76	8.7	3.78	16.6	7.93	24.9	2.93	22.5	3.30	9.6	7.63	1.9	0.59	1.0	0.84	0.8	1.38
	6	0.6	0.08	4.1	1.75	5.1	0.29	8.7	2.61	19.0	2.78	31.5	1.71	24.1	0.87	4.6	0.77	0.9	0.52	0.7	0.51	0.8	0.69
1865 µatm	0	1.0	0.44	5.4	1.11	26.4	3.05	37.5	4.49	20.0	2.01	6.9	1.10	1.6	0.31	0.5	0.18	0.2	0.18	0.2	0.18	0.3	0.28
	1	1.0	0.99	3.5	1.30	2.1	1.30	4.9	1.78	9.8	4.25	26.9	2.49	46.2	7.46	5.0	3.86	0.0	0.00	0.0	0.00	0.7	1.14
	2	1.8	1.71	5.5	4.03	21.7	19.99	18.7	13.03	14.8	7.46	16.6	9.73	9.2	11.30	5.6	6.35	3.0	2.23	2.0	1.15	1.2	1.10
	3	5.2	1.05	17.8	11.47	22.3	5.21	22.2	5.21	14.6	1.58	7.6	0.95	4.6	1.14	2.4	0.43	1.5	0.28	0.7	0.15	1.0	0.54
	4	0.9	0.14	3.5	0.56	12.3	8.53	22.4	7.36	23.5	5.72	17.5	2.93	11.2	4.62	6.6	8.05	1.0	0.73	0.5	0.33	0.6	0.46
	5	2.7	1.24	5.8	1.80	3.6	2.05	15.4	3.55	11.0	6.80	11.5	3.89	14.5	3.05	17.8	9.71	11.7	8.09	4.6	4.29	1.4	1.28
	6	1.0	0.21	5.1	1.39	5.9	6.69	10.9	9.97	12.6	8.40	15.6	1.24	18.3	12.66	19.2	9.37	6.8	2.53	3.2	0.47	1.4	0.38
3247 µatm	0	0.6	0.50	4.4	0.82	27.3	1.24	34.0	2.28	21.3	1.14	8.5	1.77	2.9	0.92	0.7	0.30	0.2	0.25	0.1	0.06	0.0	0.00
	1	1.9	0.22	5.5	0.66	4.2	3.72	14.7	3.27	24.3	6.03	23.6	4.14	15.9	3.56	7.3	6.00	1.4	1.42	0.2	0.17	0.9	0.85
	2	2.0	1.05	3.6	1.81	5.6	4.52	21.2	5.91	27.2	3.37	19.9	6.04	11.9	2.07	4.0	1.18	1.7	1.49	2.0	0.42	0.9	0.77
	3	6.2	3.71	16.0	10.17	9.0	3.07	13.9	12.40	17.7	8.01	18.5	6.70	9.7	4.83	5.0	2.65	2.0	1.21	1.1	0.39	1.0	0.89
	4	2.1	0.57	6.6	1.88	6.3	1.03	15.5	3.95	23.9	11.78	20.3	1.49	11.2	4.55	6.5	4.64	3.8	3.20	2.3	1.47	1.5	1.24
	5	4.1	1.78	15.1	17.36	4.5	2.61	12.1	3.62	19.2	4.43	20.7	9.00	15.4	5.82	6.1	1.71	1.3	1.28	0.8	0.65	0.7	0.53
	6	1.5	0.54	5.6	2.17	1.3	0.37	8.0	2.08	21.2	0.92	27.6	3.33	20.6	5.69	9.9	0.98	3.5	1.53	0.7	0.62	0.1	0.19

Table S4. Test diameter (mean and standard deviation (SD) of the 3 replicates) of living and dead *A. aomoriensis* during six month.

Living *A.aomoriensis*

Incubation time	pCO_2-treatment 430 µatm		907 µatm		1865 µatm		3247 µatm	
(months)	Mean (µm)	SD	Mean (µm)	SD	Mean (µm)	SD	Mean (µm)	SD
0	169	11.47	174	4.09	178	5.98	182	3.52
1	241	15.78	240	1.45	282	10.09	248	17.17
2	261	4.69	290	11.63	225	20.91	243	25.84
3	249	3.89	236	11.29	185	22.90	214	39.72
4	225	13.55	240	2.82	230	12.06	251	31.63
5	249	19.99	261	18.72	294	19.69	235	45.99
6	252	4.23	261	0.66	287	12.42	267	10.70

Dead *A.aomoriensis*

Incubation time	pCO_2-treatment 430 µatm		907 µatm		1865 µatm		3247 µatm	
(months)	Mean (µm)	SD	Mean (µm)	SD	Mean (µm)	SD	Mean (µm)	SD
0	232	23.93	193	10.55	224	23.19	160	21.25
1	317	5.92	286	16.03	307	3.52	322	6.04
2	311	24.58	303	12.75	273	8.92	272	7.71
3	295	16.94	320	10.10	267	26.02	236	47.59
4	283	16.47	303	16.60	280	17.32	289	15.70
5	288	2.83	279	7.96	305	25.22	282	45.56
6	303	10.06	302	12.18	321	31.54	243	8.57

Table S5. Dry weight per individual/test of living and dead *A. aomoriensis* calculated from total dry weight and number of individuals for each replicate during six months investigation period. For living individuals, 4.29 % organic percentage subtracted from total dry weight.

		Living *A. aomoriensis*					Dead *A. aomoriensis*		
pCO_2-treatment	Incubation time (months)	Total weight (µg)	4.29 % organic percentage (µg)	Total weight subtracted by 4.29 % organic percentage (µg)	Number of individuals	Weight per individual (µg)	Total weight (µg)	Number of tests	Weight per test (µg)
430_0A	0	1111	48	1063	683	1.56	142	20	7.10
430_0B	0	698	30	668	404	1.65	168	61	2.75
430_0C	0	551	24	527	472	1.12	201	30	6.70
907_0A	0	913	39	874	543	1.61	192	134	1.43
907_0B	0	1213	52	1161	855	1.36	124	18	6.89
907_0C	0	581	25	556	410	1.36	162	28	5.79
1865_0A	0	958	41	917	728	1.26	199	42	4.74
1865_0B	0	821	35	786	288	2.73	275	48	5.73
1865_0C	0	1296	56	1240	870	1.43	135	33	4.09
3247_0A	0	1339	57	1282	850	1.51	200	41	4.88
3247_0B	0	1898	81	1817	1173	1.55	218	80	2.73
3247_0C	0	1092	47	1045	636	1.64	163	156	1.04
430_1A	1	2262	97	2165	640	3.38	555	71	7.82
430_1B	1	2835	122	2713	482	5.63	511	84	6.08
430_1C	1	3301	142	3159	706	4.48	535	73	7.33
907_1A	1	3082	132	2950	938	3.14	491	96	5.11
907_1B	1	2324	100	2224	620	3.59	483	78	6.19
907_1C	1	1372	59	1313	407	3.23	388	63	6.16
1865_1A	1	775	33	742	101	7.34	821	85	9.66
1865_1B	1	630	27	603	114	5.29	346	50	6.92
1865_1C	1	635	27	608	108	5.63	431	52	8.29
3247_1A	1	1351	58	1293	375	3.45	723	45	16.07
3247_1B	1	1020	44	976	178	5.48	719	101	7.12
3247_1C	1	1087	47	1040	311	3.35	669	103	6.50
430_2A	2	3360	144	3216	501	6.42	562	25	22.48
430_2B	2	1931	83	1848	449	4.12	510	39	13.08
430_2C	2	2680	115	2565	683	3.76	1571	263	5.97
907_2A	2	1430	61	1369	204	6.71	469	30	15.63
907_2B	2	2274	98	2176	208	10.46	824	141	5.84
907_2C	2	5023	215	4808	1014	4.74	956	143	6.69
1865_2A	2	1282	55	1227	162	7.57	399	78	5.12
1865_2B	2	1531	66	1465	856	1.71	304	44	6.91
1865_2C	2	1545	66	1479	457	3.24	208	45	4.62
3247_2A	2	1089	47	1042	475	2.19	609	109	5.59
3247_2B	2	898	39	859	222	3.87	459	70	6.56
3247_2C	2	1431	61	1370	429	3.19	360	84	4.29
430_3A	3	1233	53	1180	287	4.11	1224	192	6.38
430_3B	3	1821	78	1743	603	2.89	903	199	4.54

430_3C	3							928	133		6.98
907_3A	3	2104	90	2014	788	2.56		1112	176		6.32
907_3B	3	3754	161	3593	946	3.80		1652	276		5.99
907_3C	3	3220	138	3082	1051	2.93					
1865_3A	3							183	37		4.95
1865_3B	3	2823	121	2702	2072	1.30		384	78		4.92
1865_3C	3	2870	123	2747	2121	1.30		434	85		5.11
3247_3A	3	1222	52	1170	591	1.98		462	88		5.25
3247_3B	3	1329	57	1272	384	3.31		338	74		4.57
3247_3C	3	3198	137	3061	1388	2.21		1368	325		4.21
430_4A	4	1694	73	1621	423	3.83		712	127		5.61
430_4B	4	784	34	750	394	1.90		1511	284		5.32
430_4C	4	1238	53	1185	237	5.00		1507	247		6.10
907_4A	4	1972	85	1887	630	3.00					
907_4B	4							1625	324		5.02
907_4C	4	1509	65	1444	390	3.70		180	43		4.19
1865_4A	4	3828	164	3664	674	5.44		372	56		6.64
1865_4B	4	2680	115	2565	932	2.75		204	41		4.98
1865_4C	4	3503	150	3353	1402	2.39		361	75		4.81
3247_4A	4	1669	72	1597	704	2.27		630	100		6.30
3247_4B	4	3301	142	3159	702	4.50		521	128		4.07
3247_4C	4	3077	132	2945	642	4.59		875	158		5.54
430_5A	5							635	125		5.08
430_5B	5	1007	43	964	460	2.10		1155	185		6.24
430_5C	5	1418	61	1357	420	3.23		1712	318		5.38
907_5A	5	1458	63	1395	362	3.85		1610	289		5.57
907_5B	5	1596	68	1528	321	4.76		1571	323		4.86
907_5C	5	1618	69	1549	462	3.35		2068	206		10.04
1865_5A	5	6637	285	6352	1209	5.25		1050	136		7.72
1865_5B	5	852	37	815	118	6.91		1144	211		5.42
1865_5C	5	1276	55	1221	217	5.63		242	70		3.46
3247_5A	5	3212	138	3074	845	3.64		254	40		6.35
3247_5B	5	2819	121	2698	1416	1.91		273	54		5.06
3247_5C	5	2915	125	2790	484	5.76		561	107		5.24
430_6A	6	1370	59	1311	398	3.29		955	127		7.52
430_6B	6	1273	55	1218	336	3.63		1194	227		5.26
907_6A	6	1692	73	1619	388	4.17		1225	196		6.25
907_6B	6	1297	56	1241	320	3.88		1868	260		7.18
1865_6A	6	6849	294	6555	845	7.76		1004	205		4.90
1865_6C	6	2570	110	2460	625	3.94		131	39		3.36
3247_6A	6	2042	88	1954	435	4.49		213	59		3.61
3247_6B	6	1789	77	1712	370	4.63					

Table S6. Proportion of total organic content, mean $CaCO_3$ production and accumulation of monthly differences in weight (mean and standard deviation (SD) of the 3 replicates) of living and dead *A. aomoriensis* during six months incubation time. Calculation of total carbonate production and accumulation rates refers to gram per area of 1 m^2 and year.

Total organic content

Number of specimens	Initial dry weight (mg)	Dry weight after combustion (mg)	Weight loss (mg)	Proportion of organic content (%)
100	0.723	0.692	0.031	**4.29**

Living fauna

Incubation time (months)	pCO_2-treatment 430 µatm Mean (µg)	SD	907 µatm Mean (µg)	SD	1865 µatm Mean (µg)	SD	3247 µatm Mean (µg)	SD	Number of replicates
0	Initial dry weight of 995 ± 361 µg was subtracted from the total weight–4.29 % (Table S5).								12
1	1685	498	1168	820	-344	79	109	167	3
2	1548	684	1790	1798	396	142	96	258	3
3	-20	889	1902	806	822	1573	839	1064	3
4	191	435	116	987	2199	566	1573	847	3
5	-221	698	496	83	1802	3086	1859	196	3
6	388	66	2144	267	2662	2896	839	171	3
Mean of monthly difference in weight	595	818	1269	821	1256	1153	886	728	

Total carbonate production

	(µg)	per year (µg)	(mg m^2 a^{-1})	(g m^2 a^{-1})
Total mean	1001	2003	474.08	**0.47**
SD	324			

Dead fauna

Incubation time (months)	pCO_2-treatment 430 µatm Mean (µg)	SD	907 µatm Mean (µg)	SD	1865 µatm Mean (µg)	SD	3247 µatm Mean (µg)	SD	Number of replicates
0	Initial dry weight 182 ± 42 µg was subtracted from the total weight–4.29 % (Table S5).								12
1	352	22	272	57	351	253	522	30	3
2	699	598	568	252	122	96	294	125	3
3	527	635	1049	376	7	192	230	65	3
4	1015	426	862	906	70	105	322	135	3
5	707	260	1449	73	1239	563	75	16	3
6	722	279	1247	22	819	611	-10	58	3
Mean of monthly difference in weight	670	222	908	436	435	493	239	189	

Total carbonate accumulation

	(µg)	per year (µg)	(mg m^2 a^{-1})	(g m^2 a^{-1})
Total mean	563	1126	266.57	**0.27**
SD	290			

IV. Conclusions and outlook

IV.1 Conclusions

I investigated the response of benthic foraminiferal assemblages from the shallow water habitat of the southwestern Baltic Sea to ocean acidification. The foraminiferal community in this area is dominated by calcareous species, which are opportunistic and tolerant to a large number of environmental conditions such as food supply, heavy metal concentrations, and salinity variations. However, previous experimental studies revealed that foraminifera are not well-adapted to changing temperatures and pCO_2/pH fluctuations and may therefore be sensitive to climate change. In particular, calcification and survival of benthic shallow-water foraminifera is expected to be affected by ocean acidification.

Within the framework of this thesis, mid-term and long-term laboratory experiments, spanning multiple generations and a monitoring field study were performed. The experiments simulated the effects of ocean acidification and the combined effects of multiple stressors on benthic foraminifera.

In the southwestern Baltic Sea, organic-rich mud prevails. Organic substances fuel the microbial activity and thereby influenced the underlying sediment chemistry. Degradation processes of organic matter at the sediment-water interface consume O_2 and produce CO_2, and thus result in high sediment pore water pCO_2 which fluctuated during the seasonal cycle from 1244 to 3324 µatm. Under low oxygen conditions, anaerobic bacterial decay of organic matter leads to production of metabolic bicarbonate (HCO_3^-) by nitrate and sulfate reduction. Thereby, the alkalinity (A_T) increases in the sediment pore water enhance the CO_2 buffer capacity. As a consequence, alkalinity values between 2000 to 3500 µmol kg^{-1} prevailed in the sediment pore water, which was supersaturated with respect to calcite for most time of the year, even when the overlying near-bottom water was undersaturated.

Accordingly, endobenthic foraminifera were able to cope with elevated pCO_2 levels, as long as sediment pore waters remain supersaturated. Laboratory and field observations revealed a clear relationship between calcification of *Ammonia aomoriensis* and Ω_{calc}. At saturation state $\Omega_{calc}>1$, *Ammonia aomoriensis* was able to maintain their calcification and built new chambers, whereas a slow-down of calcification and the beginning of test dissolution was detected under a saturation state of $\Omega_{calc}<1$ (Fig. IV.1.1). Complete decalcification began at $\Omega_{calc}<0.5$, when chambers were destroyed and the inner organic lining became visible. Consequently, my results highlighted that not pCO_2, but rather Ω_{calc} or CO_3^{2-} is the parameter which affects the calcification of *Ammonia aomoriensis* (Fig. IV.1.1).

In contrast, the co-occurring calcareous species *Ephidium incertum* shows no relationship to Ω_{calc}. This species specific response might be explained by differences in test composition and microstructure. Furthermore, in relation to the composition of the foraminiferal assemblages, no effect was observed during the seasonal cycle of carbonate chemistry variability in Flensburg Fjord. This infers that either no extensive mortality occurred or dissolution of living specimens is prevented by the relative high carbonate saturation. Nevertheless, some empty tests of

Ammonia aomoriensis exhibited dissolution features which could be due to seasonal undersaturation in pore water of the sediments.

Finally, under the current microhabitat conditions, the dominant *Ammonia aomoriensis* could maintain an important role in benthic carbonate production and accumulation in the southwestern Baltic Sea. However, in future, pore water might be undersaturated for longer periods of the year which might result in higher mortalities and test dissolution of this currently dominating species, which will ultimately lead to changes in benthic foraminiferal communities. Such endobenthic faunal change may also affect other regions experiencing naturally undersaturated Ω_{calc} levels, as well as preferentially will endanger epibenthic and planktonic species.

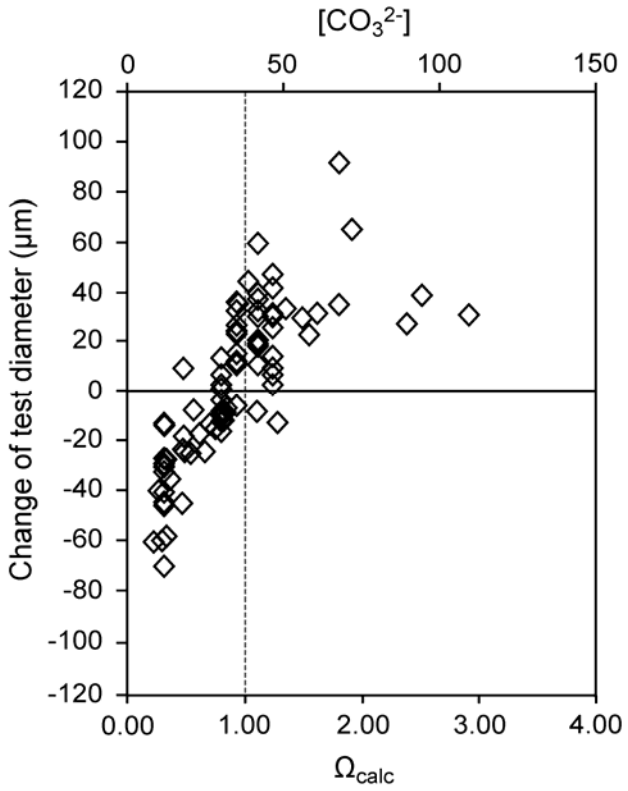

Fig. IV.1.1: Change of diameter of *Ammonia aomoriensis* in relation to $[CO_3^{2-}]$ and Ω_{calc} averages from both culturing experiments during six month incubation time.

The results of the study underscores that the response of the calcareous species *Ammonia aomoriensis* to elevated pCO_2 varied completely depended whether specimens were kept without or within their natural organic-rich mud (Fig. IV.1.2). When specimens were exposed to high pCO_2 levels without their protective sediment, *Ammonia aomoriensis* is directly affected by the ambient carbonate chemistry of the seawater. Strong undersaturation of the seawater with respect to Ω_{calc} led to high mortalities and test dissolution. In contrast, chemical processes in the sediment created a microhabitat, which supported growth and reproduction even at highly elevated pCO_2 without a drastic increase in mortality. These results emphasize the importance to understand the sediment carbonate chemistry in the near-coastal microhabitat of benthic foraminifera, which depend on sediment type, grain size, organic matter, remineralization, chemical and abiotic conditions in the sediment pore water (Fig. IV.1.2). Further, in order to predict the

response of benthic foraminifera to ocean acidification it is crucial to perform experiments under natural conditions as they provide a stable microhabitat even at high pCO_2.

Ocean acidification is only one aspect of global change and will be accompanied by warming, and desalination in the Baltic Sea. Consequently, the combination of these parameters may stress the tolerance of *Ammonia aomoriensis* to environmental variability in the southwestern Baltic Sea. My experimental results revealed that the tested salinity levels had no effect on the diameter change of *Ammonia aomorienis*, whereas increasing temperatures had a positive effect and favours their growth. It may be possible that the negative impact of ocean acidification could partially be mitigated by warming.

	Laboratory experiments		Natural environment
	Cultivation without sediment	**Cultivation within sediment**	**Present field conditions**
Sediment type:		organic-rich mud	organic-rich mud
Carbonate chemistry:	constant: high pCO_2/low pH low alkalinity (A_T) undersaturation of Ω_{calc}	constant: high pCO_2/low pH high alkalinity (A_T) supersaturation of Ω_{calc}	variable and elevated: pCO_2 alkalinity (A_T) saturation state of Ω_{calc}
Abiotic parameter:	constant: temperature salinity	constant: temperature salinity	variable: temperature salinity
Response of calcifying foraminifera:	**SENSITIVE**	**TOLERANT**	**TOLERANT**

Fig. IV.1.2: Response scheme: environmental parameters under laboratory and present field conditions which influence the ocean acidification sensitivity of calcifying foraminifera.

IV.2 Outlook

The present thesis investigated the foraminiferal calcification response to changing chemical environments as a result of ocean acidification. However, the experimental results could not reveal in which ontogenetic stage of *Ammonia aomoriensis* the calcification process is mainly affected by unfavorable Ω_{calc}/CO_3^{2-} conditions. Therefore, additional studies with different life stages of *Ammonia aomoriensis* may provide a possibility to investigate the different sensitivities between juveniles and adult stages under $\Omega_{calc}<1$.

Little is known about the general mechanisms of the species-specific calcification in benthic foraminifera. Therefore, more interdisciplinary work is needed in order to better understand biomineralization response scheme in relation to test composition and microstructure in order to predict, how foraminiferal communities might change in the future. In this context, recalcification structures were observed on *Ammonia aomoriensis* tests when exposed to undersaturated conditions, which might indicate a responsive adaptation to unfavourable conditions. Subsequent studies need to clarify, for how long the specimens are able to withstand test dissolution which might be related to higher energetic effort.

The results revealed the relationship of the sediment facies to the carbonate chemistry conditions in the microhabitat of benthic foraminifera. According to these results, a long-term field study should be performed which vary with respect to sedimentary composition, such as muddy, sandy and coralline sediments. The results could emphasize the high dependency of the foraminiferal assemblage composition in relation to saturation state in their natural environment.

Ammonia aomoriensis is an opportunistic species and often found in strongly eutrophicated environments with high nutrient concentrations and algae biomasses. Previous studies exhibited that food supply plays an important role on growth, chamber formation and reproduction of benthic foraminifera. Therefore, the importance of the physiological responses and consequently, the substantial effects of changing energy availability in combination with a manipulation of the carbonate chemistry on the life cycle of foraminifera need to be constrained.

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… und das Beste kommt zum Schluss: am allermeisten möchte ich *Jörn* danken. Du bist an meiner Seite und begleitest mich jeden Tag durchs Leben. Deine Unterstützung hat mir viel Kraft und Motivation gegeben, vor allem aber ein unersetzliches Gefühl von Liebe, Glück, Geborgenheit und Zufriedenheit.

Kristin Haynert

Date of Birth: 06. February 1983

Nationality: German

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EDUCATION

2009–present **PhD student**
Micropaleontology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, Advisor: J. Schönfeld, M. Frank
Thesis title: "Impact of elevated pCO_2 on benthic foraminifera from the southwestern Baltic Sea" (project: Excellence Cluster 'Future Ocean' of Kiel University, German Research Foundation)

2008 **Diplom Biologist (equivalent to MSc)**
IOW (Leibniz Institute for Baltic Sea Research), Marina Biology, Warnemünde, Germany, Advisor: M. Nausch, M. Voss, J. Dippner
Thesis title: "Effects of elevated pCO_2 of the abundance and diversity of phytoplankton in the Baltic Sea" (project: SOPRAN)

2002–2008 **Undergraduate studies in Biology**
University of Rostock, Germany
Major: botany, Minors: marine biology, ecology, land improvement and environmental protection

2002 **Highschool Degree (Abitur)**
Niedersorbisches Gymnasium (highschool), Cottbus, Germany

SCIENTIFIC EXPERTISE

- **Experience working with living benthic foraminifera**
  - Cultivation of boreal and tropical specimens
  - Simulation of changing pCO_2, temperature and salinity levels in laboratory experiments
  - Taxonomy and ecology, western Baltic Sea
  - Growth, survival and mortality
  - Calcification
- **Experience working with Phytoplankton**
  - Cultivation and simulation of changing pCO_2
  - Off-Shore-Mesocosm studies
  - Taxonomy and ecology, Baltic Sea
  - Growth, survival and mortality
- **Nutrient and carbonate chemistry**

RESEARCH EXPERIENCE

02/2013
07/2012
02 – 03/2011
Research stay
Department of Chemical Engineering, University of the West Indies at St. Augustine, Trinidad and Tobago, Advisor: B. Wilson
Dynamics of benthic foraminifera in mangroves, Caroni Swamp

06/2012 **Research stay**
Department of Geology, University of Angers, France, Advisor: R. Schiebel
Analysis of benthic foraminifera

08/2012 **Workshop - FOBIMO (FOraminiferal BIo-MOnitoring)**
06/2011 Fribourg, Swizerland
Workshop on the reliability of benthic foraminifera as a tool in bio-monitoring studies

03/2011 – 04/2012 **Scientific cruise**
Chief scientist on R/V *Polarfuchs* in Kiel Fjord, western Baltic Sea
Sampling for foraminiferal analyses

06/2009 – 04/2010 **Scientific cruise**
Participation on R/V *Littorina* cruises in Flensburg Fjord, western Baltic Sea, Chief scientist: J. Thomsen
Carbonate system monitoring, sampling for foraminiferal analyses

02 – 03/2009 **Scientific cruise**
Participation on R/V *Meteor* cruise M81/1 from Las Palmas to Trinidad and Tobago, Chief scientist: M. Frank
Trace metals and their isotopes in the Tropical Atlantic Ocean

01 – 06/2009 **Research Associate**
University of Rostock, Management of rural areas, Germany, Advisor: H. Behm
Area of research: Conversation of the EU-Water Framework Directive with impacts of landscape management.

09 – 12/2008 **Research Associate**
IOW (Leibniz Institute for Baltic Sea Research), Marine Biology, Warnemünde, Germany, Advisor: M. Nausch
Area of research: Monitoring of the Phytoplankton community in the Baltic Sea (project: SOPRAN)

07/2007 **Scientific cruise**
Participation on F.S. *Alkor* cruise in the Gotland Sea, Baltic Sea, Chief scientist: U. Riebesell, M. Voss
Off-Shore-Mesocosm studies for future ocean simulations (project: SOPRAN)

OTHER RELEVANT EXPERIENCE

TEACHING

11/2012 - 01/2013 **Course "Foraminiferen im Schleswig-holsteinischen Wattenmeer"**
11/2011 - 01/2012 Field excursion, practical course and seminar
GEOMAR, Kiel, Germany
Instructor: J. Schönfeld

MENTORING

04/2011 – 06/2012 **Mentoring of visiting scientists**
Sampling in the field and practical lab work
J. Erez, P. Munz, B. Wilson, A. Costelloe

10/2010 – 04/2012 **Mentoring of undergraduate students**
Cultivation, carbonate chemistry and benthic foraminifera
C. Nissen, T. Struve, E. Klünker

10/2011 – 11/2011 **Mentoring of apprentices**
Introduction in practical chemical lab work
N. Janssen

SCIENTIFIC PRESENTATIONS

PEER REVIEWED PUBLICATIONS (PUBLISHED)

Haynert, K., Schönfeld J., Polovodova -Astemann, I., and Thomsen, J.: *The benthic foraminiferal community in a naturally CO_2-rich coastal habitat in the southwestern Baltic Sea.* **Biogeosciences** 9: 4421–4440, doi:10.5194/bg-9-4421-2012, **2012**.

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., Korsun, S., Spezzaferri, S., Abramovich, S., Almogi-Labin, A., Armynot du Chatelet, E., Barras, C., Bergamin, L., Bicchi, E., Bouchet, V., Cearreta, A., Di Bella, L., Dijkstra, N., Trevisan Disaro, S., Ferraro, L., Frontalini, F., Gennari, G., Golikova, E., **Haynert, K.**, Hess, S., Husum, K., Martins, V., McGann, M., Oron, S., Romano, E., Mello Sousa, S., Tsujimoto A.: *The FOBIMO (FOraminiferal BIo-MOnitoring) initiative-Towards a standardized protocol for soft-bottom benthic foraminiferal monitoring studies.* **Marine Micropaleontology** 94–95: 1-13, **2012**.

Haynert, K., Schönfeld, J., Riebesell, U., Polovodova, I.: *Biometry and dissolution features of the benthic foraminifer* Ammonia aomoriensis *at high pCO_2*. **Marine Ecology Progress Series** 432: 53–67, doi: 10.3354/meps09138, **2011**.

PEER REVIEWED PUBLICATIONS (RESUBMITTED)

Haynert, K. and Schönfeld J.: *Impact of changing carbonate chemistry, temperature and salinity on growth and test degradation of the benthic foraminifer* Ammonia aomoriensis*.* Resubmitted in **Journal of Foraminiferal Research.**

IN PREPARATION

Haynert, K., Schönfeld, J., Schiebel, R., Wilson, B., and Thomsen, J.; Response of benthic foraminifera to ocean acidification in their natural sediment environment: a long-term culturing experiment. First draft to be submitted to **Biogeosciences**.

ORAL PRESENTATIONS

05/2011 **Seminar-Paleoceanography, GEOMAR, Kiel, Germany**
Haynert, K. *Response of benthic foraminiferal species* Ammonia aomoriensis *at high pCO_2.*

01/2011 **Seminar-Biomineralization, GEOMAR, Kiel, Germany**
Haynert, K., Glock N., *Biomineralization in foraminifera.*

09/2010 **International Symposium on Foraminifera, Bonn, Germany**
Haynert, K., Schönfeld, J., *Responses of the benthic foraminiferal species* Ammonia beccarii *to a high CO_2 ocean.*

POSTER PRESENTATIONS

02/2012 **Ocean Science Meeting, Salt Lake City, Utah, USA**
Haynert, K., Schönfeld, J., Thomsen J., and Polovodova-Asteman, *Response of benthic foraminifera to a naturally CO_2-rich coastal habitat in Flensburg Fjord (SW Baltic Sea).*

03/2010 **Clusterretreat, Schleswig, Germany**
Haynert, K., Schönfeld, J., *Benthic foraminifer species* Ammonia beccarii *in a high CO_2* world.

MEMBERSHIP

FOBIMO (FOraminiferal BIo-MOnitoring)
Group of 37 scientists from 24 research groups and 13 countries.
Aim: General acceptance of benthic foraminifera as a reliable tool in bio-monitoring studies.

REVIEWER FOR THE FOLLOWING JOURNAL

2012 Marine Ecology Progress Series

FURTHER PROFESSIONAL EDUCATION

LANGUAGE SKILLS

German, English, Sorbian

CERTIFICATIONS

2001 Driver's license
1998 PADI Advanced Open Water Diver
1997 PADI Open Water Diver