

# Biometry and dissolution features of the benthic foraminifer *Ammonia aomoriensis* at high pCO<sub>2</sub>

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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<sub>2</sub> concentrations. We exposed specimens to 5 seawater pCO<sub>2</sub> levels ranging from 618  $\mu$ atm (pH 7.9) to 3130  $\mu$ atm (pH 7.2) for 6 wk. Growth rates and mortality differed significantly among pCO<sub>2</sub> treatments. The highest increase of mean test diameter (19%) was observed at 618  $\mu$ atm. At partial pressures >1829  $\mu$ atm, the mean test diameter was observed to decrease, by up to 22% at 3130  $\mu$ atm. At pCO<sub>2</sub> levels of 618 and 751  $\mu$ atm, *A. aomoriensis* tests were found intact after the experiment. The outer chambers of specimens incubated at 929 and 1829  $\mu$ atm were severely damaged by corrosion. Visual inspection of specimens incubated at 3130  $\mu$ atm revealed wall dissolution of all outer chambers, only their inner organic lining stayed intact. Our results demonstrate that pCO<sub>2</sub> values of  $\geq$ 929  $\mu$ atm 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<sub>2</sub> levels in this range during summer and fall. Increasing atmospheric CO<sub>2</sub> 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.

**KEY WORDS:** Benthic foraminifera · Biometry · Test dissolution · Carbon dioxide · Baltic Sea

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## INTRODUCTION

The rise in atmospheric CO<sub>2</sub> concentrations has caused an increase in seawater pCO<sub>2</sub> over the past 250 yr (Takahashi 2004, Solomon et al. 2007). Surface ocean waters have taken up ~30% of anthropogenic CO<sub>2</sub> (Sabine et al. 2004, Khatiwala et al. 2009), causing a reduction in ocean pH and carbonate ion concentration (Orr et al. 2005, Cao & Caldeira, 2008). In response to this 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 & 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<sub>3</sub> per year on a global scale (Langer et al. 1997, Langer 2008), which amounts

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to about one-third of the production by planktonic foraminifers (Schiebel 2002).

In addition to CO<sub>2</sub>-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 & Gypens 2010, Cossellu & Nordberg 2010, Zhang et al. 2010). In comparison to the open ocean, the Baltic Sea exhibits lower salinities, lower [CO<sub>3</sub><sup>2-</sup>] and consequently lower calcium carbonate saturation states ( $\Omega$ ). In the western Baltic Sea, seasonal effects are superimposed (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<sub>2</sub> values over the year (Diaz & Rosenberg 2008, Conley et al. 2009, Thomsen et al. 2010). In response to low  $\Omega$  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 & Atkinson 2005, Orr et al. 2005, Moy et al. 2009, Thomsen & Melzner 2010). There is also evidence that planktonic foraminifers precipitate thinner test walls at reduced carbonate ion concentrations and higher atmospheric CO<sub>2</sub> 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, symbiont-bearing foraminifer *Marginopora kudakajimensis* during long-term incubation at 4 different pH<sub>NBS</sub> (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<sub>2</sub> concentrations of 120  $\mu$ atm (pH 8.4) and 2000  $\mu$ atm (pH 7.5; Dissard et al. 2009). Surprisingly, the specimens still calcified at concentrations <2000  $\mu$ atm. 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<sub>NBS</sub> of 7.5 (Le Cadre et al. 2003).

The consequences of future elevated atmospheric CO<sub>2</sub> concentrations for benthic foraminiferal calcification in shallow waters are thus not sufficiently studied. The purpose of this study was to investigate the calcifi-

cation response of the benthic foraminiferal *Ammonia aomoriensis* from the western Baltic Sea to different seawater pCO<sub>2</sub> levels.

## MATERIALS AND METHODS

**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 & Dunker 1994), deploying it from RV '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  $\mu$ m 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  $\mu$ l of a living algae mixture containing *Nannochloropsis oculata*, *Phaeodactylum tricorutum* 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.

**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 & 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.

**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  $\mu\text{m}$ ), larger and 'active' young specimens (size class 250 to 350  $\mu\text{m}$ ) and 'inactive' 'old' specimens of >200  $\mu\text{m}$  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  $\mu\text{m}$  and 250 to 350  $\mu\text{m}$ ) 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  $\mu\text{m}$  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<sup>-1</sup>) for 2 wk (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<sub>2</sub> levels and 3 replicates for each pCO<sub>2</sub> 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<sub>2</sub> gradient in

each pit.) A small amount (0.43 g) of carbonate-free quartz (>97 % SiO<sub>2</sub>) was dispersed in equal amounts among the pits with the intention of better mimicking the natural habitat of *Ammonia aomoriensis*. The microenvironment along the base plate of the vessel appeared to be less attractive to the specimens, since most of them stayed in their pit.

**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  $\mu\text{m}$ ) and UV-sterilized seawater from Kiel Fjord. In order to monitor the carbonate system, pH according to the National Bureau of Standards pH-scale (pH<sub>NBS</sub>), total alkalinity

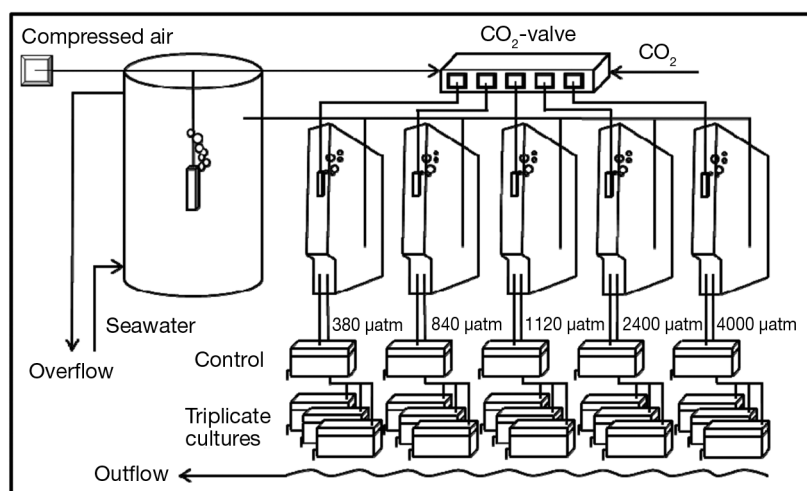


Fig. 1. *Ammonia aomoriensis*. Setup of culturing system. For explanation, see 'Experimental setup'

( $A_T$ ), 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  $\mu\text{atm}$ . The  $pCO_2$  range from 380 to 1120  $\mu\text{atm}$  corresponded to values recorded in Flensburg Fjord; the 2 higher levels were meant to simulate future scenarios of 2400 and 4000  $\mu\text{atm}$ . 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  $1.4 \text{ times h}^{-1}$ , the flow rate was adjusted to  $0.16 \text{ ml s}^{-1}$ . 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  $\mu\text{l}$  DT's Premium Blend algae mixture. The experiment lasted 6 wk.

**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  $>50 \text{ mm}$ , we could use only  $40\times$  magnification, which resulted in an error of  $\pm 12.5 \mu\text{m}$  (maximum distance to the next scale unit) in the size measurements. This is approx.  $\pm 4$  to  $\pm 7\%$  of the average diameter (from 180 to 280  $\mu\text{m}$ ) 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 wk 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).

**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  $\pm 0.01$  for pH and  $\pm 0.1^\circ\text{C}$  for temperature. For salinity measurements, a WTW Cond 315i salinometer with a precision of  $\pm 0.1 \text{ psu}$  was used.

In order to calculate carbonate system parameters with  $CO_2SYS$  software, the phosphate concentration was measured weekly (Lewis & Wallace 1998). A 10 ml water sample was passed through a  $0.2 \mu\text{m}$  filter and was measured colorimetrically in a spectrophotometer (U 2000, Hitachi-Europe) at a wavelength of 882 nm according to Koroleff (1983). The precision of the phosphate measurements was  $\pm 0.2 \mu\text{mol l}^{-1}$ .

Samples for analysis of total alkalinity ( $A_T$ ) were sterile-filtered ( $0.2 \mu\text{m}$  pore size) and determined through potentiometric titration (Dickson, 1981) in a Metrohm Tiamo automatic titration device. The precision of the alkalinity measurements was  $2 \mu\text{mol kg}^{-1}$ . The carbonate system parameters  $pCO_2$  and  $\Omega_{\text{cal}}$  calcite values were calculated from measured  $A_T$ , pH, phosphate, temperature and salinity using the  $CO_2SYS$  software (Lewis & Wallace 1998). The equilibrium constants of Mehrbach et al. (1973), as refitted by Dickson & Millero (1987), were chosen.

Table 1. *Ammonia aomoriensis*. Carbonate chemistry of bottom water from sampling station FF3 from Flensburg Fjord ( $54^\circ 48.082' \text{ N}$ ,  $9^\circ 53.069' \text{ E}$ ), 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  $100 \mu\text{mol kg}^{-1}$ . Carbon dioxide partial pressure ( $pCO_2$ ) and Omega of calcite saturation state ( $\Omega_{\text{ca}}$ ) were calculated (cal) with the  $CO_2Sys$  program (Lewis & Wallace 1998) from measured  $A_T$ ,  $pH_{NBS}$ , temperature and salinity

Date (d/mo/yr)	Salinity	Temp. ( $^\circ\text{C}$ )	$A_T$ ( $\mu\text{mol kg}^{-1}$ )	$pH_{NBS}$	$pCO_2$ cal ( $\mu\text{atm}$ )	$\Omega_{\text{ca}}$ cal
03/06/09	20.2	10.3	$2125.5 \pm 87.6$	7.83	727.1	1.35
18/08/09	19.8	15.3	$2239.1 \pm 28.0$	7.47	1863.3	0.85
20/10/09	21.6	11.9	$2465.7 \pm 31.4$	7.31	2873.8	0.60
07/12/09	20.9	8.8	$2174.0 \pm 64.1$	7.81	769.1	1.42
15/02/10	16.9	-0.4	$1804.6 \pm 200.7$	7.94	465.1	1.05
19/04/10	18.8	5.6	$2374.9 \pm 90.8$	7.94	493.0	2.01

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<sub>NBS</sub> and alkalinity. Monitoring results will be reported elsewhere, but we refer to the pCO<sub>2</sub> values and their seasonal range in the present study (Table 1).

**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).

**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<sup>2</sup> for the fitted line. The error in the regression equations is  $\pm 1$  SE of the mean.

## RESULTS

### Water chemistry

Salinity ranged from 17 to 19.5 psu (mean  $\pm$  SE =  $18.4 \pm 0.8$ ) during the experimental period (Fig. 2, Table 2). Mean seawater temperature in the culture

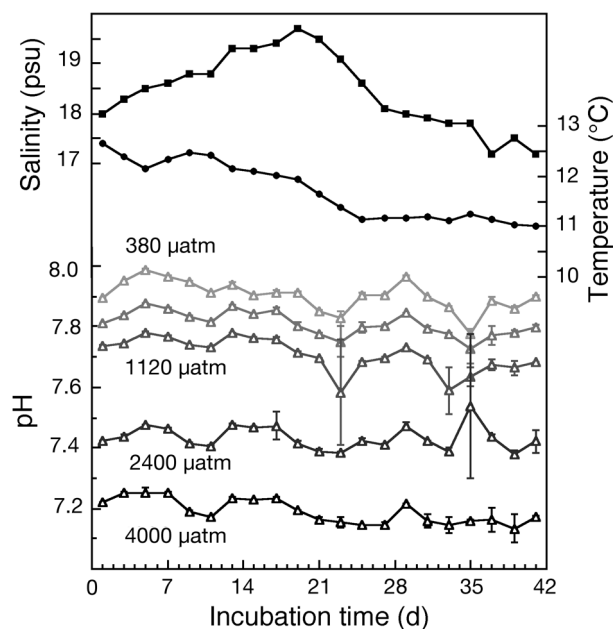


Fig. 2. *Ammonia aomoriensis*. Water chemistry during the experiment. Temperature and salinity are mean values of all measurements taken in each culture vessel flushed with different seawater pCO<sub>2</sub> levels as in Table 1. pH: means  $\pm$  SD, n = 3

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  $\pm 1$  SE) varied according to seawater pCO<sub>2</sub> from  $7.9 \pm 0.05$  to  $7.2 \pm 0.04$ . They were

Table 2. *Ammonia aomoriensis*. Carbonate chemistry of culture media, means  $\pm$ SD (n = 3) of several variables for 5 pCO<sub>2</sub> levels. Controls are mean values of all measurements made during the 6 wk incubation. pCO<sub>2</sub>, total carbon (C<sub>T</sub>), and Omega of calcite saturation state ( $\Omega_{ca}$ ) were calculated (cal) with the CO<sub>2</sub>Sys program (Lewis & Wallace 1998) from measured A<sub>T</sub>, pH<sub>NBS</sub>, PO<sub>4</sub><sup>3-</sup>, temperature and salinity

pCO <sub>2</sub> baseline level (μatm)	pCO <sub>2</sub> cal (μatm)	Salinity	Temp. (°C)	pH <sub>NBS</sub>	A <sub>T</sub> (μmol kg <sup>-1</sup> )	C <sub>T</sub> cal (μmol kg <sup>-1</sup> )	$\Omega_{ca}$ cal	PO <sub>4</sub> <sup>3-</sup> (μmol l <sup>-1</sup> )
380	617.9 $\pm$ 8.5	18.4 $\pm$ 0.8	11.8 $\pm$ 0.6	7.90 $\pm$ 0.05	2040.1 $\pm$ 20.7	1980.2 $\pm$ 31.8	1.66 $\pm$ 0.25	0.99 $\pm$ 0.33
380_control	610.1 $\pm$ 94.0	18.4 $\pm$ 0.8	11.7 $\pm$ 0.6	7.91 $\pm$ 0.04	2043.1 $\pm$ 20.1	1983.1 $\pm$ 29.2	1.67 $\pm$ 0.22	0.99 $\pm$ 0.31
840	751.1 $\pm$ 22.8	18.4 $\pm$ 0.8	11.6 $\pm$ 0.6	7.81 $\pm$ 0.04	2039.0 $\pm$ 17.4	1999.5 $\pm$ 22.3	1.38 $\pm$ 0.16	1.02 $\pm$ 0.32
840_control	734.7 $\pm$ 67.8	18.4 $\pm$ 0.8	11.6 $\pm$ 0.7	7.81 $\pm$ 0.04	2036.0 $\pm$ 16.7	1996.4 $\pm$ 21.7	1.39 $\pm$ 0.14	1.04 $\pm$ 0.29
1120	929.1 $\pm$ 23.4	18.4 $\pm$ 0.8	11.7 $\pm$ 0.6	7.71 $\pm$ 0.06	2035.2 $\pm$ 19.8	2014.4 $\pm$ 23.2	1.14 $\pm$ 0.13	1.01 $\pm$ 0.33
1120_control	953.9 $\pm$ 147.4	18.4 $\pm$ 0.8	11.6 $\pm$ 0.5	7.71 $\pm$ 0.04	2034.0 $\pm$ 14.6	2018.0 $\pm$ 19.2	1.12 $\pm$ 0.15	1.06 $\pm$ 0.32
2400	1829.2 $\pm$ 33.5	18.4 $\pm$ 0.8	11.6 $\pm$ 0.6	7.43 $\pm$ 0.04	2036.2 $\pm$ 21.5	2075.2 $\pm$ 20.4	0.64 $\pm$ 0.08	1.01 $\pm$ 0.30
2400_control	1891.2 $\pm$ 227.0	18.4 $\pm$ 0.8	11.6 $\pm$ 0.6	7.42 $\pm$ 0.04	2042.0 $\pm$ 14.6	2095.7 $\pm$ 21.0	0.59 $\pm$ 0.06	1.00 $\pm$ 0.30
4000	3130.2 $\pm$ 33.6	18.4 $\pm$ 0.8	11.9 $\pm$ 0.5	7.19 $\pm$ 0.04	2039.7 $\pm$ 19.2	2156.2 $\pm$ 24.5	0.37 $\pm$ 0.04	1.00 $\pm$ 0.33
4000_control	3158.6 $\pm$ 235.1	18.4 $\pm$ 0.8	11.8 $\pm$ 0.5	7.18 $\pm$ 0.05	2036.9 $\pm$ 17.8	2159.8 $\pm$ 23.7	0.36 $\pm$ 0.03	0.96 $\pm$ 0.37

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.

The calculated  $p\text{CO}_2$  in the culture vessels differed significantly from the  $p\text{CO}_2$  baseline level in the compressed air (Table 2). For instance, in the jerrycan bubbled with compressed air without  $\text{CO}_2$  addition, which should yield the ambient atmospheric partial pressure of 380  $\mu\text{atm}$ , the measured value was 618  $\mu\text{atm}$ . This was most likely due to a higher  $\text{CO}_2$  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  $p\text{CO}_2$  levels, the measured values in the culture vessels were 11 to 24 % lower than the target values in the  $\text{CO}_2$ -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  $p\text{CO}_2$  values that were calculated from actually measured hydrochemical parameters in the culture vessels and not to the pre-adjusted values in the  $\text{CO}_2$ -enriched air (Table 2).

The  $\Omega$  values (mean  $\pm$  1SE) for calcite ranged from  $1.66 \pm 0.25$  at a  $p\text{CO}_2$  of 618  $\mu\text{atm}$  to  $0.37 \pm 0.04$  at a  $p\text{CO}_2$  of 3130  $\mu\text{atm}$ . The values  $<1.0$  indicate carbonate dissolution at partial pressures above 929  $\mu\text{atm}$  under the present settings of temperature and salinity.

The carbonate system parameter  $p\text{CO}_2$  cal and  $\Omega_{\text{Ca}}$  cal, as calculated from measured  $\text{pH}_{\text{NBS}}$  and  $A_{\text{T}}$  values, varied in the near-bottom water at the sampling site in Flensburg Fjord from 2874  $\mu\text{atm}$  (pH 7.3) in October 2009 to 465  $\mu\text{atm}$  (pH 7.9) in February 2010 (Table 1). In August and October 2009,  $\Omega$  values for calcite were temporarily  $<1.0$  (0.85 and 0.6, respectively). Therefore, our experiment covers the entire seasonal  $p\text{CO}_2$  variability in the *A. aomoriensis* habitat in Flensburg Fjord, even though we did not capture the seawater  $p\text{CO}_2$  level in February and April 2010 when the  $p\text{CO}_2$  in Flensburg Fjord was  $\sim 130$   $\mu\text{atm}$  lower than at the lowest partial pressure in our experiment.

## Test diameters

All specimens, whether inactive or active, were alive at the beginning of the experiment. They grew during the incubation, especially at low  $p\text{CO}_2$  values. Active specimens from the 150 to 250  $\mu\text{m}$  fraction displayed an increase of 19% in diameter at a  $p\text{CO}_2$  of 618  $\mu\text{atm}$ , whereas mean diameter of active specimens from the 250 to 350  $\mu\text{m}$  fraction increased by only 11% (Fig. 3A). In comparison to active specimens, the mean diameter of the large inactive specimens ( $>200$   $\mu\text{m}$ ) increased by only 2% at a  $p\text{CO}_2$  of 618  $\mu\text{atm}$  during the course of the experiment (Fig. 3A).

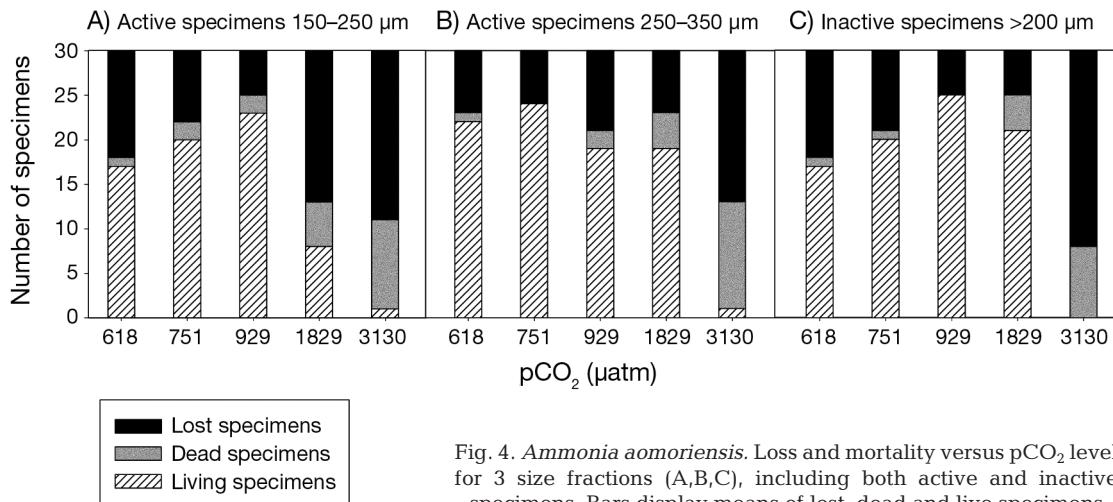
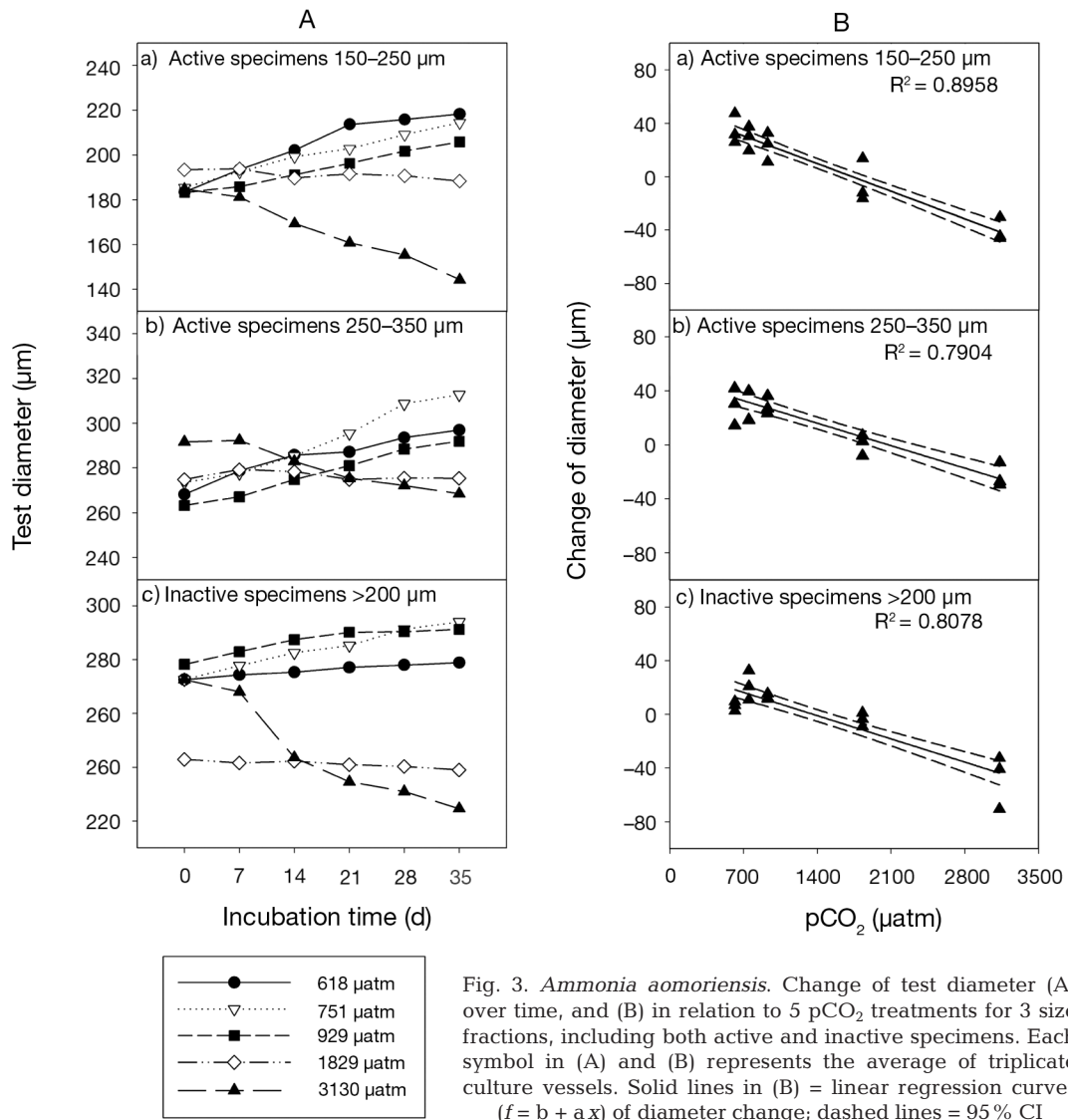
The growth of specimens from the 150 to 250  $\mu\text{m}$  fraction differed significantly, depending on  $p\text{CO}_2$  treatment (Fig. 3B). The greatest increase in mean shell diameter of 35  $\mu\text{m}$  was observed at the lowest  $p\text{CO}_2$  level of 618  $\mu\text{atm}$ . At  $p\text{CO}_2$  levels of 751  $\mu\text{atm}$  and 929  $\mu\text{atm}$ , the mean diameter of *Ammonia aomoriensis* increased by only 29 and 13  $\mu\text{m}$ , respectively. At a  $p\text{CO}_2$  of 1829 and 3130  $\mu\text{atm}$ , the test diameter was reduced by 5 and 41  $\mu\text{m}$  due to test corrosion.

The shell diameter of active specimens from the 250 to 350  $\mu\text{m}$  fraction differed also according to  $p\text{CO}_2$  level (Fig. 3B). At a  $p\text{CO}_2$  of 618  $\mu\text{atm}$ , the mean shell diameter increased by 29  $\mu\text{m}$ . The growth rate was highest at a  $p\text{CO}_2$  of 751  $\mu\text{atm}$ , where the increase was 39  $\mu\text{m}$ . *Ammonia aomoriensis* displayed a reduced growth under higher  $p\text{CO}_2$  levels, viz. from 929 to 3139  $\mu\text{atm}$ . While the mean shell diameter increased by 29  $\mu\text{m}$  at a  $p\text{CO}_2$  of 929  $\mu\text{atm}$ , the specimens displayed barely any change in size at a  $p\text{CO}_2$  of 1829  $\mu\text{atm}$  during the 6 wk incubation period. At a seawater  $p\text{CO}_2$  of 3130  $\mu\text{atm}$  the average shell diameter was reduced by 23  $\mu\text{m}$ .

Inactive specimens from the  $>200$   $\mu\text{m}$  fraction showed only a slight change of test diameter at a  $p\text{CO}_2$  of from 618 to 1829  $\mu\text{atm}$  (Fig. 3B). The lowest increase (6  $\mu\text{m}$ ) was observed at a  $p\text{CO}_2$  of 618  $\mu\text{atm}$ , followed by an increase of 21  $\mu\text{m}$  at a  $p\text{CO}_2$  of 751  $\mu\text{atm}$  and 13  $\mu\text{m}$  at 929  $\mu\text{atm}$ . Like active specimens, the shell diameter barely changed at a  $p\text{CO}_2$  of 1829  $\mu\text{atm}$  and, on average, decreased by 48  $\mu\text{m}$  at a  $p\text{CO}_2$  of 3130  $\mu\text{atm}$ .

## Loss and mortality rates

During the 6 wk 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 & 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  $\mu\text{m}$ , 250 to 350  $\mu\text{m}$  or inactive specimens >200  $\mu\text{m}$ . In the  $\text{pCO}_2$  range of 618 to 929  $\mu\text{atm}$ , 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  $\text{pCO}_2$  of 3130  $\mu\text{atm}$ . From 30 specimens at the beginning of the experiment, the loss of active specimens of the 150 to 250  $\mu\text{m}$  and 250 to 350  $\mu\text{m}$  size fractions averaged 18 specimens. Among the inactive specimens >200  $\mu\text{m}$ , 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  $\mu\text{atm}$   $\text{pCO}_2$  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  $\text{pCO}_2$  of 3130  $\mu\text{atm}$ , an average of 10 contained no living cytoplasm at the end of the incubation and were presumed dead.

### SEM observations

The different stages of dissolution during the 6 wk incubation time were revealed by SEM observations (Fig. 5). The tests of *Ammonia aomoriensis* exposed to a  $\text{pCO}_2$  of 618 to 751  $\mu\text{atm}$  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  $\text{pCO}_2$  levels of 929 and 1829  $\mu\text{atm}$  the last 1 to 3 younger chambers were severely decalcified or destroyed (Fig. 5B,C). The surface of the walls showed

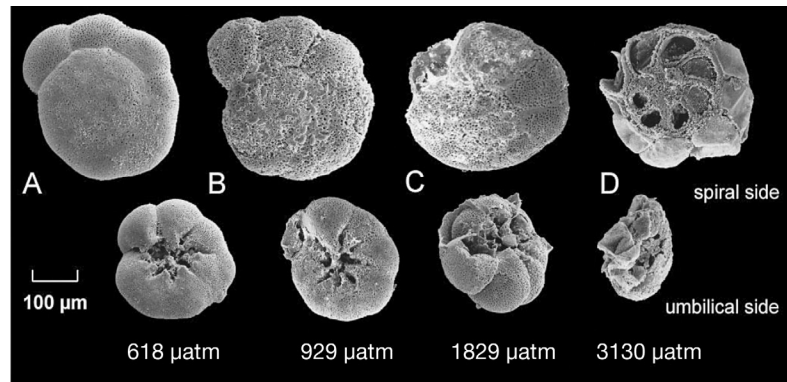


Fig. 5. *Ammonia aomoriensis*. SEM images depicting different stages of dissolution on spiral and umbilical sides of the test at various  $\text{pCO}_2$  levels (A to D)

fragmentary dissolution of the younger calcite layers, which were left only as scales (Fig. 6A,B). Furthermore, at a seawater  $\text{pCO}_2$  of 1829  $\mu\text{atm}$ , we observed the formation of cracks around the pores. (Fig. 6C). On some individuals, at a  $\text{pCO}_2$  of 1829  $\mu\text{atm}$ , the SEM examination revealed prograding incisions along the sutures. At a high  $\text{pCO}_2$  of 3130  $\mu\text{atm}$ , the tests had become heavily decalcified after 6 wk (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).

## DISCUSSION

### Change of test diameter of *Ammonia aomoriensis* under elevated $\text{pCO}_2$

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  $\text{pCO}_2$  of 618  $\mu\text{atm}$ , 78% of the active specimens from the 150 to 250  $\mu\text{m}$  size fraction grew during the experiment, as did 65% of the specimens from the 250 to 350  $\mu\text{m}$  size fraction. Only 44% of inactive specimens of the >200  $\mu\text{m}$  size fraction showed an increase in test diameter, which implied growth by chamber addition during the 6 wk 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  $\text{CO}_2$  level added new chambers. With increasing  $\text{pCO}_2$ , the difference between initial and final test diameter of *Ammonia aomoriensis*



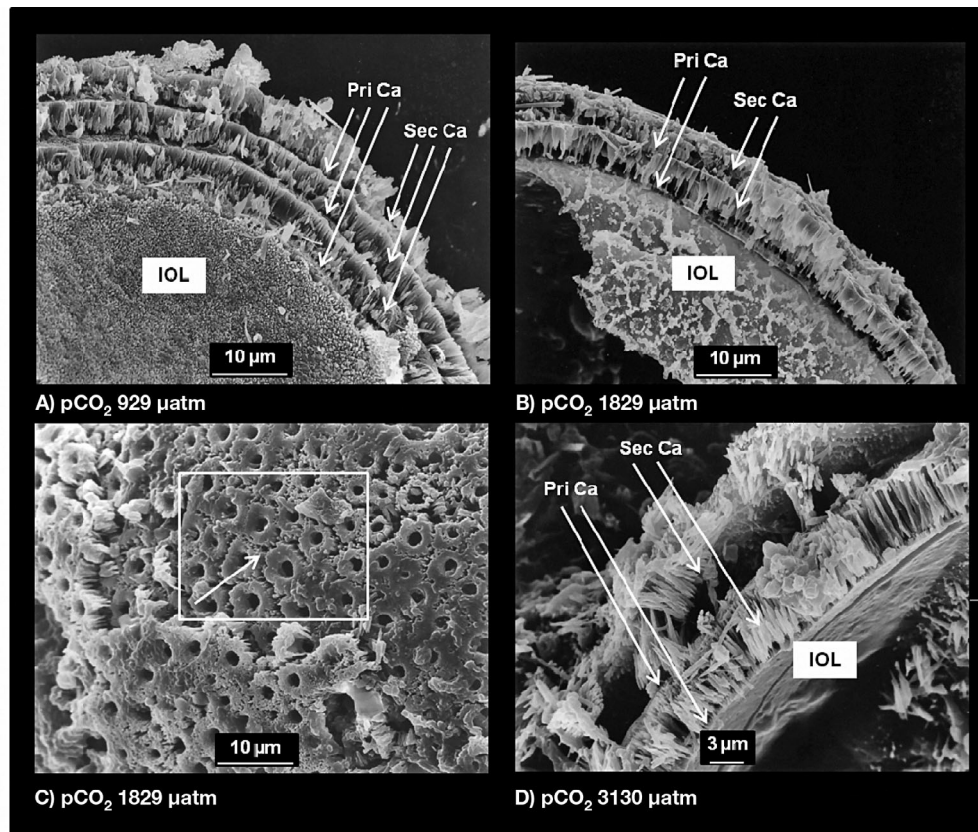


Fig. 6. *Ammonia aomoriensis*. (A,B,D) SEM images showing layers of primary calcite (Pri Ca), secondary calcite (Sec Ca) and inner organic lining (IOL) after 6 wk incubation under elevated pCO<sub>2</sub>. (C) Note appearance of cracks at pCO<sub>2</sub> of 1829 µatm

Table 3. *Ammonia aomoriensis*. Newly added chambers from active specimens of 3 size fractions, including both active and inactive specimens, at a control pCO<sub>2</sub> of 618 µatm

Number of specimens	— Active specimens		— Inactive specimens >200 µm
	150–250 µm	250–350 µm	
Surviving 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	<b>2.3</b>	<b>1.6</b>	<b>1.0</b>
Percentage showing growth	<b>78%</b>	<b>65%</b>	<b>44%</b>

decreased. A significant reduction in foraminiferal test diameter was observed at a high pCO<sub>2</sub> 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 wk 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 foraminifera 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.

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 & Numberger 2007a,b, Polovodova & Schönfeld 2008, Nikulina et al. 2008, Polovodova et al. 2009, Schweizer et al. 2010) revealed an increase in diameter (average  $\pm$  SD,  $n = 12$ ) of  $27 \pm 16 \mu\text{m}$  per new chamber over the last 4 chambers. The height of the last 2 chambers, as seen from the spiral side, was  $106 \pm 23 \mu\text{m}$ . The increase in test diameter by adding new chambers did not covary with the overall diameter. Therefore we conclude that *A. aomoriensis* growth by 13 to 39  $\mu\text{m}$  at a  $p\text{CO}_2$  of 618  $\mu\text{atm}$  corresponded to the addition of 1 or 2 new chambers during the 6 wk experimental period. In terms of chamber addition, active specimens of size fraction 150 to 250  $\mu\text{m}$  added 2.3 chambers on average, and specimens of size fraction 250 to 350  $\mu\text{m}$  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  $\mu\text{m}$  is presented in Fig. 7. During the incubation time, this individual grew by 33  $\mu\text{m}$ . The growth took place in 2 increments of 16  $\mu\text{m}$ , 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  $p\text{CO}_2$  of 618  $\mu\text{atm}$ .

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 wk. 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.

Dissard et al. (2009) reported that only half the specimens added new chambers during 6 wk 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*

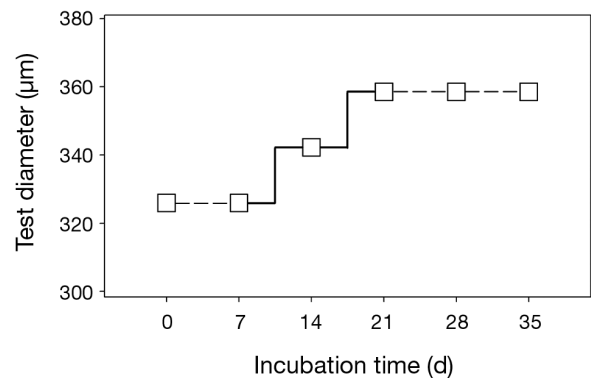


Fig. 7. *Ammonia aomoriensis*. Change in test diameter over time of a single active individual from size fraction 250–350  $\mu\text{m}$ . Increment in diameter between Days 7 and 21 implies addition of 2 new chambers

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 wk experimental period (Allison et al. 2010).

During our experimental period, we observed that tests dissolved more readily at a high seawater  $p\text{CO}_2$  than at a lower level. In our study the test diameter of *Ammonia aomoriensis* showed an increase with  $p\text{CO}_2$  values up to 751  $\mu\text{atm}$ . Above a critical  $p\text{CO}_2$  level of 1829  $\mu\text{atm}$ , 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  $p\text{CO}_2$  at >1829  $\mu\text{atm}$ . Alternatively, shell-wall thinning might be the result of dissolution under elevated  $p\text{CO}_2$ . SEM observations revealed the outer chamber walls to be 4 to 10  $\mu\text{m}$  thick. Corrosion or even dissolution of the outer chamber walls would cause a reduction of the test diameter by 10 to 20  $\mu\text{m}$ . The average chamber height is 106  $\mu\text{m}$ . If the entire last whorl were to be dissolved, the shell loss would exceed by far the observed reduction of 23 to 49  $\mu\text{m}$  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.

### Dissolution features

Our results revealed a clear relationship between seawater  $p\text{CO}_2$  and shell dissolution. The first dissolution features were recorded at a  $p\text{CO}_2$  of 929  $\mu\text{atm}$  and led to loss of the last-formed (thinner) chambers. At a

pCO<sub>2</sub> of 3130 µatm all chambers were destroyed by complete calcium carbonate dissolution, only the inner organic lining stayed intact.

Under elevated pCO<sub>2</sub> conditions, we observed different stages of dissolution. Decalcification started with loss of the external walls of the last chambers at a pCO<sub>2</sub> 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<sub>2</sub> of 3130 µatm. For instance, the individual presented in Fig. 5D had a test diameter of 342 µm at the beginning, and after 6 wk incubation at pCO<sub>2</sub> 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 & 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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 & Erez 2005), which is less resistant to dissolution than the needles of secondary, low-Mg calcite.

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 interocular 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 wk.

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 & Schönfeld 2008, Plate 3, Figs. 2–6). Similar dissolution features were observed in the following: *A. batavus* from Sandebukta, Oslo Fjord (Alve & Nagy 1986); *A. parkinsoniana*, *Elphidium excavatum* and *Palmerinalla palmerae* from Nueces Bay, Texas (Buzas-Stephens & 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 & 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 & Murray 1999, Moreno et al. 2007, Polovodova & 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).

### Loss rate and mortality

We observed no significant differences of loss between the active and inactive specimens. At a pCO<sub>2</sub> of 3130 µatm, however, where significantly higher

losses were observed, the highest loss was seen in inactive specimens  $>200\ \mu\text{m}$ , followed by small active specimens of the 150 to 250  $\mu\text{m}$  size fraction. Subsequent treatment with Rose Bengal demonstrated that most of the specimens were devoid of cytoplasm at a  $\text{pCO}_2$  of 3130  $\mu\text{atm}$ . We observed that the test wall of *Ammonia aomoriensis* was completely destroyed at a  $\text{pCO}_2$  of 3130  $\mu\text{atm}$  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 wk 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  $\mu\text{m}$  size fraction. This indicates that living cells may be able to counteract dissolution better than dead cells, at least up to a certain  $\text{pCO}_2$  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  $\mu\text{m}$  size class sustained greater damage at high  $\text{pCO}_2$  levels than did large specimens of the 250 to 350  $\mu\text{m}$  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  $\text{pCO}_2$  of 3130  $\mu\text{atm}$ , first in inactive or dead specimens, then in small and finally in large specimens.

### Ecological effect

*Ammonia* species are the most successful colonisers 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 (Stn 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  $\text{CO}_2$  levels simulated in our laboratory experiment.

While open ocean  $\text{pCO}_2$  levels of 1829 (pH 7.4) and 3130  $\mu\text{atm}$  (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.

### CONCLUSIONS

*Ammonia aomoriensis* exhibited reduced calcification and increased test dissolution at elevated  $\text{pCO}_2$  levels and lowered pH. Decalcification started with loss of the outer, thinner chambers at a  $\text{pCO}_2$  of 929  $\mu\text{atm}$ . Total decalcification, when chambers were destroyed and the inner organic lining became visible, began at a  $\text{pCO}_2$  of 3130  $\mu\text{atm}$ . 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  $\text{pCO}_2$  level. We have already achieved  $\text{pCO}_2$  levels in the range of from 1829 (pH 7.4) to 3130  $\mu\text{atm}$  (pH 7.2) during the seasonal cycle in shallow areas of Flensburg Fjord. With progressing  $\text{CO}_2$ -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<sub>2</sub> levels.

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#### LITERATURE CITED

- Allison N, Austin W, Paterson D, Austin H (2010) Culture studies of the benthic foraminifera *Elphidium williamsoni*: Evaluating pH,  $\Delta[\text{CO}_3^{2-}]$  and inter-individual effects on test Mg/Ca. *Chem Geol* 274:87–93
- Almogi-Labin A, Siman-Tov R, Rosenfeld A, Debar E (1995) Occurrence and distribution of the foraminifer *Ammonia beccarii tepida* (Cushman) in water bodies, recent and quaternary, of the Dead Sea rift, Israel. *Mar Micropaleontol* 26:153–159
- Alve E (1991) Benthic foraminifera in sediment cores reflecting heavy metal pollution in Sorfjord, Western Norway. *J Foraminiferal Res* 21:1–19
- Alve E, Murray JW (1999) Marginal marine environments of the Skagerrak and Kattegat: a baseline study of living (stained) benthic foraminiferal ecology. *Palaeogeogr Palaeoclimatol Palaeoecol* 146:171–193
- Alve E, Nagy J (1986) Estuarine foraminiferal distribution in Sandebukta, a branch of the Oslo Fjord. *J Foraminiferal Res* 16:261–284
- Asano K (1951) Rotaliidae. In: Stach LW (ed) *Illustrated catalogue of Japanese Tertiary smaller foraminifera*. Hosokawa Printing, Tokyo, p 1–21
- Barras C, Geslin E, Duplessey JC, Jorissen FJ (2009) Reproduction and growth of the deep-sea benthic foraminifer *Bulimina marginata* under different laboratory conditions. *J Foraminiferal Res* 39:155–165
- Bentov S, Erez J (2005) Novel observations on biomineralization processes in foraminifera and implications for Mg/Ca ratio in the shells. *Geology* 33:841–844
- Berkeley A, Perry CT, Smithers SG, Horton BP (2008) The spatial and vertical distribution of living (stained) benthic foraminifera from a tropical, intertidal environment, north Queensland, Australia. *Mar Micropaleontol* 69:240–261
- Bernhard JM, Blanks JK, Hintz CJ, Chandler GT (2004) Use of the fluorescent calcite marker calcein to label foraminiferal tests. *J Foraminiferal Res* 34:96–101
- Bijma J, Spero HJ, Lea DW (1999) Reassessing foraminiferal stable isotope geochemistry: impact of the oceanic carbonate system (experimental results). In: Fisher G, Wefer G (eds) *Use of proxies in paleoceanography: examples from the South Atlantic*. Springer, Heidelberg, p 489–512
- Borges AV, Gypens N (2010) Carbonate chemistry in the coastal zone responds more strongly to eutrophication than ocean acidification. *Limnol Oceanogr* 55:346–353
- Bouchet VMP, Debenay JP, Sauriau PG, Radford-Knoery J, Soletchnik P (2007) Effects of short-term environmental disturbances on living benthic foraminifera during the Pacific oyster summer mortality in the Marennes-Oléron Bay (France). *Mar Environ Res* 64:358–383
- Bradshaw J (1957) Laboratory studies on the rate of growth of the foraminifer *Streblus beccarii* (Linné) var. *tepida* (Cushman). *J Paleontol* 31:1138–1147
- Buzas-Stephens P, Buzas MA (2005) Population dynamics and dissolution of foraminifera in Nueces Bay, Texas. *J Foraminiferal Res* 35:248–258
- Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J Geophys Res* 110:C09S04, doi: 10.1029/2004JC002671
- Cao L, Caldeira K (2008) Atmospheric CO<sub>2</sub> stabilization and ocean acidification. *Geophys Res Lett* 35:L19609, doi: 10.1029/2008GL035070
- Conley DJ, Carstensen J, Ærtebjerg G, Christensen PB, Dalsgaard T, Hansen JLS, Josefson AB (2007) Long-term changes and impacts of hypoxia in Danish coastal waters. *Ecol Appl* 17:S165–S184
- Conley DJ, Björck S, Bonsdorff E, Carstensen J and others (2009) Hypoxia-related processes in the Baltic Sea. *Environ Sci Technol* 43:3412–3420
- Corliss BH (1985) Microhabitats of benthic foraminifera within deep-sea sediments. *Nature* 314:435–438
- Cossellu M, Nordberg K (2010) Recent environmental changes and filamentous algal mats in shallow bays on the Swedish west coast: A result of climate change? *J Sea Res* 63:202–212
- Cushman JA (1926) Recent foraminifera from Porto Rico. *Carnegie Inst Washington Publ* 342:73–84
- de Nooijer LJ, Toyofuku T, Oguri K, Nomaki H, Kitazato H (2008) Intracellular pH: distribution in foraminifera determined by the fluorescent probe HPTS. *Limnol Oceanogr Methods* 6:610–618
- Debenay JP, Beneteau E, Zhang J, Stouff V, Geslin E, Redois F, Fernandez-Gonzalez M (1998) *Ammonia beccarii* and *Ammonia tepida* (Foraminifera): morphofunctional arguments for their distinction. *Mar Micropaleontol* 34:235–244
- Debenay JP, Tsakiridis E, Soulard R, Grosse H (2001) Factors determining the distribution of foraminiferal assemblages in Port Joinville Harbour (Ile d'Yeu, France): the influence of pollution. *Mar Micropaleontol* 43:75–118
- Debenay JP, Della Patrona L, Goguenheim H (2009) Colonization of coastal environments by foraminifera: insight from shrimp ponds in New Caledonia (SW Pacific). *J Foraminiferal Res* 39:249–266
- Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321:926–929
- Dickson AG (1981) An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. *Deep-Sea Res A* 28:609–623
- Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Res A* 34:1733–1743
- Dissard D, Nehrke G, Reichert GJ, Bijma J (2009) Impact of seawater pCO<sub>2</sub> changes on calcification and on Mg/Ca and Sr/Ca in benthic foraminifera calcite (*Ammonia tepida*): results from culturing experiments. *Biogeosciences Discuss* 6:3771–3802
- Ellison R, Broome R, Olgilvie R (1986) Foraminiferal response to trace metal contamination in the Patapsco River and Baltimore Harbour, Maryland. *Mar Pollut Bull* 17:419–423
- Erez J (2003) The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. *Rev Mineral Geochem* 54:115–149
- Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO<sub>2</sub> on the

- CaCO<sub>3</sub> system in the oceans. *Science* 305:362–366
- Haake FW (1962) Untersuchungen an der Foraminiferen-Fauna im Wattgebiet zwischen Langeoog und dem Festland. *Meyniana* 12:25–64
- Hansen HP, Giesenhagen HC, Behrends G (1999) Seasonal and long-term control of the bottom water oxygen deficiency in a stratified shallow water coastal system. *J Mar Sci* 56:65–71
- Hayward BW, Buzas MA, Buzas-Stephens P, Holzmann M (2003) The lost types of *Rotalia beccarii* var. *tepida* Cushman 1926. *J Foraminiferal Res* 33:352–354
- Hayward BW, Holzmann M, Grenfell HR, Pawlowski J (2004) Morphological distinction of molecular types in *Ammonia*: towards a taxonomic revision of the world's most common and misidentified foraminifera. *Mar Micropaleontol* 50: 237–271
- Heinz P, Geslin E, Hemleben C (2005) Laboratory observations of benthic foraminiferal cysts. *Mar Biol Res* 1:49–159
- Hintz CJ, Chandler GT, Bernhard JM, McCorkle DC, Havach SM, Blanks JK, Shaw TJ (2004) A physicochemically constrained seawater culturing system for production of benthic foraminifera. *Limnol Oceanogr Methods* 2:160–170
- Khaliwala S, Primeau F, Hall T (2009) Reconstruction of the history of anthropogenic CO<sub>2</sub> concentrations in the ocean. *Nature* 462:346–350
- Koroleff F (1983) Determination of nutrients. In: Grasshoff K, Ehrhardt M, Kremling K (eds) *Methods of seawater analysis*. Verlag Chemie, Weinheim, p 125–187
- Kuhn G, Dunker E (1994) Der Minicorer, ein Gerät zur Beprobung der Sediment/Bodenwasser-Grenze. *Greifswalder Geowissenschaftliche Beiträge* 2:99–100
- Kuroyanagi A, Kawahata H, Suzuki A, Fujita K, Irie T (2009) Impacts of ocean acidification on large benthic foraminifera: results from laboratory experiments. *Mar Micropaleontol* 73:190–195
- Langdon C, Atkinson MJ (2005) Effect of elevated pCO<sub>2</sub> on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. *J Geophys Res* 110:C09S07, doi: 10.1029/2004JC002576
- Langer MR (2008) Assessing the contribution of foraminiferan protists to global ocean carbonate production. *J Eukaryot Microbiol* 55:163–169
- Langer MR, Silk MT, Lipps JH (1997) Global ocean carbonate and carbon dioxide production: the role of reef foraminifera. *J Foraminiferal Res* 27:271–277
- Le Cadre V, Debenay JP, Lesourd M (2003) Low pH effects on *Ammonia beccarii* test deformation: implications for using test deformations as a pollution indicator. *J Foraminiferal Res* 33:1–9
- Lee JJ, Anderson OR (1993) *Biology of foraminifera*. Academic Press, London
- Lehmann G (2000) Vorkommen, Populationsentwicklung, Ursache fleckenhafter Besiedlung und Fortpflanzungsbiologie von Foraminiferen in Salzwiesen und Flachwasser der Nord- und Ostseeküste Schleswig-Holsteins. PhD thesis, Universität Kiel, available at [http://eldiss.uni-kiel.de/macau/receive/dissertation\\_diss\\_00000413](http://eldiss.uni-kiel.de/macau/receive/dissertation_diss_00000413)
- Levin LA, Ekau W, Gooday AJ, Jorissen F and others (2009) Effects of natural and human-induced hypoxia on coastal benthos. *Biogeosciences* 6:2063–2098
- Lewis E, Wallace DWR (1998) Program developed for CO<sub>2</sub> system calculations, ORNL/CGIAC-105. Oak Ridge National Laboratory, US Department of Energy
- Linné C (1758) *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Tomus I. Editio decima, reformata. Laurentii Salvii, Holmiae. 10th edn, p 824
- Lutze GF (1965) Zur Foraminiferen-Fauna der Ostsee. *Meyniana* 15:75–142
- Lutze GF (1987) Benthische Foraminiferen: Vertikale Verteilung in den obersten Sedimentlagen und Probleme bei der Entnahme von Standard-Proben. *Sonderforschungsbereich 313 der Universität Kiel* 6:79–87
- Martin RE, Harris MS, Liddell WD (1995) Taphonomy and time-averaging of foraminiferal assemblages in Holocene tidal flat sediments, Bahia la Choya, Sonora, Mexico (northern Gulf of California). *Mar Micropaleontol* 26: 187–206
- Mehrbach C, Culberso CH, Hawley JE, Pytkowicz RW (1973) Measurement of the apparent dissociation-constants of carbonic acid in seawater at atmospheric-pressure. *Limnol Oceanogr* 18:897–907
- Moreno J, Valente T, Moreno F, Fatela F, Guise L, Patinha C (2007) Occurrence of calcareous foraminifera and calcite-carbonate equilibrium conditions: a case study in Minho/Coura estuary (north Portugal). *Hydrobiologia* 587: 177–184
- Moy AD, Howard WR, Bray SG, Trull TW (2009) Reduced calcification in modern Southern Ocean planktonic foraminifera. *Nat Geosci* 2:276–280
- Murray JW (1991) *Ecology and paleoecology of benthic foraminifera*. Longman, Harlow
- Nikulina A, Polovodova I, Schönfeld J (2008) Foraminiferal response to environmental changes in Kiel Fjord, SW Baltic Sea. *Earth (Waukesha)* 3:37–49
- Orr JC, Fabry VJ, Aumont O, Bopp L and others (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437:681–686
- Pascal PY, Dupuy C, Richard P, Niquil N (2008) Bacterivory in the common foraminifer *Ammonia tepida*: isotope tracer experiment and the controlling factors. *J Exp Mar Biol Ecol* 359:55–61
- Polovodova I, Schönfeld J (2008) Foraminiferal test abnormalities in the western Baltic Sea. *J Foraminiferal Res* 38: 318–336
- Polovodova I, Nikulina A, Schönfeld J, Dullo WCh (2009) Recent benthic foraminifera in the Flensburg Fjord. *J Micropaleontol* 28:131–142
- Reiss Z (1957) The Bilamellidea, nov. superfam. and remarks on Cretaceous Globorotaliids. *J Foraminiferal Res* 8: 127–145
- Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO<sub>2</sub>. *Nature* 407: 364–367
- Rosenberg R (1985) Eutrophication: The future marine coastal nuisance? *Mar Pollut Bull* 16:227–231
- Rottgardt D (1952) Mikropaläontologische wichtige Bestandteile rezenter brackischer Sedimente an den Küsten Schleswig-Holsteins. *Meyniana* 1:169–228
- Sabine CL, Feely RA, Gruber N, Key RM and others (2004) The oceanic sink for anthropogenic CO<sub>2</sub>. *Science* 305: 367–371
- Schafer CT (1973) Distribution of foraminifera near pollution sources in Chaleur Bay. *Water Air Soil Pollut* 2:219–233
- Schiebel R (2002) Planktic foraminiferal sedimentation and the marine calcite budget. *Global Biogeochem Cycles* 16:1065, doi:10.1029/2001GB001459
- Schnitker D (1974) Ecotypic variation in *Ammonia beccarii* (Linné). *J Foraminiferal Res* 4:217–223
- Schönfeld J, Numberger L (2007a) Seasonal dynamics and decadal changes of benthic foraminiferal assemblages in

- the western Baltic Sea (NW Europe). *J Micropaleontol* 26:47–60
- Schönfeld J, Numberger L (2007b) The benthic foraminiferal response to the 2004 spring bloom in the western Baltic Sea. *Mar Micropaleontol* 65:78–95
- Schweizer M, Polovodova I, Nikulina A, Schönfeld J (2010) Molecular identification of *Ammonia* and *Elphidium* species (Foraminifera, Rotaliida) from the Kiel Fjord (SW Baltic Sea) with rDNA sequences. *Helgol Mar Res* 65:1–10
- Sharifi AR, Croudace LW, Austin RL (1991) Benthic foraminifera as pollution indicators in Southampton Water, southern England, UK. *J Micropaleontol* 10:109–113
- Solomon S, Qin D, Manning M, Chen Z, Marquis M (eds) (2007) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York
- Spero HJ, Bijma J, Lee DW, Bemis BE (1997) Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. *Nature* 390:497–500
- Stubbles SJ, Green JC, Hart MB, Williams CL (1996a) The ecological and palaeoecological implications of the presence and absence of data: evidence from benthic foraminifera. *Proc Ussher Soc* 9:54–62
- Stubbles SJ, Hart MB, Williams CL, Green JC (1996b) Responses of foraminifera to presence of heavy metal contamination and acidic mine drainage. Conference on minerals, metals and the environment II. Institution of Mining and Metallurgy, Prague, p 217–235
- Takahashi T (2004) The fate of industrial carbon dioxide. *Science* 305:352–353
- Thomas E, Gapotchenko T, Varekamp EC, Mecray EL, Buchholtz ten Brink MR (2000) Maps of benthic foraminiferal distribution and environmental changes in Long Island Sound between the 1940s and the 1990s. In: Paskevich VF, Poppe LJ (eds) US Geological Survey Open-File Report 00-304, chap. 9. USGS, Woods Hole, MA, available at <http://pubs.usgs.gov/of/2000/of00-304/htmldocs/chap09/index.htm>
- Thomsen J, Melzner F (2010) Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. *Mar Biol* 157:2667–2676
- Thomsen J, Gutowska MA, Saphörster J, Heinemann A and others (2010) Calcifying invertebrates succeed in a naturally CO<sub>2</sub>-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences* 7:3879–3891
- Wang P, Chappell J (2001) Foraminifera as Holocene environmental indicators in the South Alligator River, northern Australia. *Quat Int* 83-85:47–62
- Watkins JG (1961) Foraminiferal ecology around the Orange County, California, ocean sewer outfall. *Micropaleontology* 7:199–206
- Wefer G (1976) Umwelt, Produktion und Sedimentation benthischer Foraminiferen in der westlichen Ostsee. Reports Sonderforschungsbereich 95 Wechselwirkung Meer-Meeresboden 14:1–103
- Yanko V, Ahmad M, Kaminski M (1998) Morphological deformities of benthic foraminiferal tests in response to pollution by heavy metals: implications for pollution monitoring. *J Foraminiferal Res* 28:177–200
- Zhang J, Gilbert D, Gooday AJ, Levin L and others (2010) Natural and human-induced hypoxia and consequences for coastal areas: synthesis and future development. *Biogeosciences* 7:1443–1467

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