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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 6 months of incubation, foraminiferal assemblages were kept and treated in natural sediment 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 of incubation, pore water alkalinity was much higher in comparison to the overlying seawater. Consequently, the saturation state of Ω_{calc} was much higher in the sediment than in the water column in nearly all pCO_2 treatments and remained close to saturation. As a result, the life cycle (population density, growth and reproduction) of living assemblages varied markedly during the experimental period, but was largely unaffected by the pCO_2 treatments applied. According to the size–frequency distribution, we conclude that foraminifera start reproduction at a diameter of 250 µm. Mortality of living *Ammonia aomoriensis* was unaffected, whereas size of large and dead tests decreased with elevated pCO_2 from 285 µm (pCO_2 from 430 to 1865 µatm) to 258 µm (pCO_2 3247 µatm). The total organic content of living *Ammonia aomoriensis* has been determined to be 4.3 % of $CaCO_3$ weight. Living individuals had a calcium carbonate production rate of 0.47 $g\,m^{-2}\,a^{-1}$, whereas dead empty tests accumulated a rate of 0.27 $g\,m^{-2}\,a^{-1}$. Although Ω_{calc} was close to 1, approximately 30 % of the empty tests of *Ammonia aomoriensis* showed dissolution features at high pCO_2 of 3247 µatm during the last 2 months of incubation. In contrast, tests of the subdominant species, *Elphidium incertum*, stayed intact. Our results emphasize that the sensitivity to ocean acidification of the endobenthic foraminifera *Ammonia aomoriensis* in their natural sediment habitat is much lower compared to the experimental response of specimens isolated from the sediment.

1 Introduction

Benthic foraminifera are the most diverse group of hard-shelled protists. They live at the sediment–water interface, especially 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 showed hampered calcification by 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; Uthicke et al., 2013). In contrast, some species showed no significant change of calcification under simulated future pCO_2, whereas other species benefit from elevated pCO_2

and showed an increase of growth (Vogel and Uthicke, 2012, McIntyre-Wressnig et al., 2013). Keul et al. (2013) revealed that not pCO_2, but rather CO_3^{2-} is the parameter that affects the test size and weights of *Ammonia* tests. All these studies cultured living benthic foraminifera as isolated specimens without any natural sediment.

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, and as a consequence of higher alkalinity, Ω_{calc} was slightly supersaturated. Under these conditions, the composition and population density of the benthic foraminiferal community were not affected by seasonally elevated bottom water pCO_2. Similar observations were described in the study of Kitidis et al. (2011), which showed that benthic ammonia oxidation rates in buffered sediments through A_T generation remained unaffected by ocean acidification in contrast to the overlying water column.

Benthic foraminifera are common in Kiel Fjord, western Baltic Sea, although seawater carbonate concentrations are permanently low and seasonally undersaturated with respect to Ω_{calc} (Thomsen et al., 2013). High biological activity and nutrient inputs characterize the area, and organic-rich mud prevails in Kiel Fjord (Nikulina et al., 2008). Degradation of organic matter between the sediment–water interface influences 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 to investigate the impact of natural high pore water pCO_2 on benthic foraminifera, which prevail with high population densities in the southwestern Baltic Sea (Haynert et al., 2012). The aim of this study was to investigate the response of the life cycle (comprising population density, their growth and reproduction) and species composition by using a pristine assemblage from Kiel Fjord to constantly elevated pCO_2 in their natural sedimentary environment during a long-term incubation over 6 months.

2 Materials and methods

2.1 Field sampling

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 consists of silty fine sand. The sampling site was close to a previous sampling station PF15–13 of Polovodova and Schönfeld (2008), where *Ammonia aomoriensis* (synonymous 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 plastic ring marked with a centimeter-scale was used to slice off the uppermost 1 cm of the surface sediment (Schönfeld et al., 2012). On board, the surface sediment was first passed through a 2000 µm screen in order to remove mollusk shells and pebbles. The samples were gently washed with seawater from Kiel Fjord. Surface sediment was transferred with a spoon into 300 mL Kautex™ wide-neck containers and covered with bottom water taken from the supernatant water of the coring tubes. Kautex™ wide-neck 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 for 4 weeks.

2.2 Preparation of culture vessels

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 4 weeks of acclimation, the sediment material in the different Kautex™ wide-neck containers was combined in one plastic container. The seawater of the stock cultures was removed except for 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 an even distribution pattern of benthic foraminifera within the sediment. After homogenization in the plastic container, new culture vessels (6.5 × 6.5 × 4.5 cm) were used. Each culture vessel was filled with 0.4 ±0.1 cm of sediment layer by using a spoon-shaped plastic spatula. The measured sediment height was 0.36 cm on average, which results in a given culture vessel area of 42.25 cm^2 in a mean sample volume of 15.2 cm^3.

After spreading of the sediment, the culture vessels were transferred into the experimental setup and filled up with a 4 cm overlying seawater column, cartridge-filtered (25 µm) 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. The acclimation of 20 days was deemed necessary to achieve a full recovery of the foraminiferal microhabitat pattern in a sub-centimeter scale (Ernst et al., 2002). During that time, 400 µL DT's Premium Blend were added weekly into each culture vessel.

After 20 days of acclimation, a nepheloid detritus layer of 0.1 cm separated the seawater from the underlying sediment pore water. The resulting pore water stagnation equates to 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.

2.3 Experimental setup

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) (Fig. 1a). Each culture vessel was flushed with 25 µm cartridge-filtered and UV-sterilized seawater from Kiel Fjord. The salinity values ranged from 15 to 16 during the experimental time. These relatively low salinities correspond to the natural conditions in the southwestern Baltic Sea. In comparison to the open oceans, this coastal region is characterized by low salinity (Wefer, 1976; Haynert et al., 2012). Observations from previous studies revealed that seawater salinity had no significant effect on change of diameter of *Ammonia* species, which inhabited hypo- to hypersaline waters (Bradshaw, 1957; Wefer, 1976; Haynert et al., 2012).

Four pCO_2 levels were adjusted by aeration of 5 L compact jerrycans with compressed and CO_2-enriched air (Fig. 1a). The target pCO_2 levels were 380, 1120, 2400 and 4000 µatm. The simulated pCO_2 levels equate to the environmental pCO_2 conditions in the southwestern Baltic Sea. During the course of the year, pCO_2 levels > 3000 µatm can be observed (Haynert et al., 2012), and even higher levels are likely to occur in the future (Melzner et al., 2013). Therefore, the treatment levels are adjusted to the present variability in order to test future scenarios. Altogether, we prepared 84 culture vessels, three replicates for four pCO_2 lines (430, 907, 1865 and 3247 µatm), and combined seven subsamples per pCO_2 treatment were terminated (Fig. 1b).

The preconditioned seawater from each tank flowed through the culture vessels, which were three times replicated for each pCO_2 level (Fig. 1b). The overflow seeped through the fissure between lid and vessel, and drained 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 that were inserted in each compressed air connection. Evaporation of 1 L per week was compensated by refilling the system with deionized water. Food (400 µL DT's Premium Blend) was added to each culture vessel every week. The experiment was terminated after 6 months (Fig. 1b).

2.4 Determination of water chemistry parameters

Replicates (3n) of seawater pH_{NBS} (National Bureau of Standards pH scale), temperature and salinity were determined with a WTW 340i pH analyzer and a WTW Cond 315i conductivity meter every week for four pCO_2 treatments during 6 months of incubation time. 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, phosphate (PO_4^{3-}) and silicate (Si), were determined as duplicates (2n) monthly in one of the three replicates (1n) for each pCO_2 level. Sterile-filtered (0.2 µm pore size) nutrient samples were analyzed in 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, with reference to silicate concentrations, 2.5 to 6 % for silicate.

Total dissolved inorganic carbon (C_T) was determined every month and analyzed in sterile-filtered (0.2 µm pore size) water samples taken from one of the three replicates (1n) during the incubation time using an AIRICA autoanalyzer (Maranda GmbH, Kiel, Germany) with a precision of 2–4 µmol kg^{-1}. The accuracy of the 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).

After 6 months of incubation time, water chemistry parameters of pH_{NBS}, A_T, temperature and salinity were measured and analyzed from the supernatant seawater and sediment pore water in one of the three replicates (1n) per pCO_2 level. Seawater samples from 0–2 cm and 2–4 cm water layers were sterile-filtered (0.2 µm pore size) and transferred directly into 20 mL PVC bottles. For sediment pore water analyses, the entire sediment samples were transferred into 50 mL centrifuge tubes and centrifuged at 3000 rpm

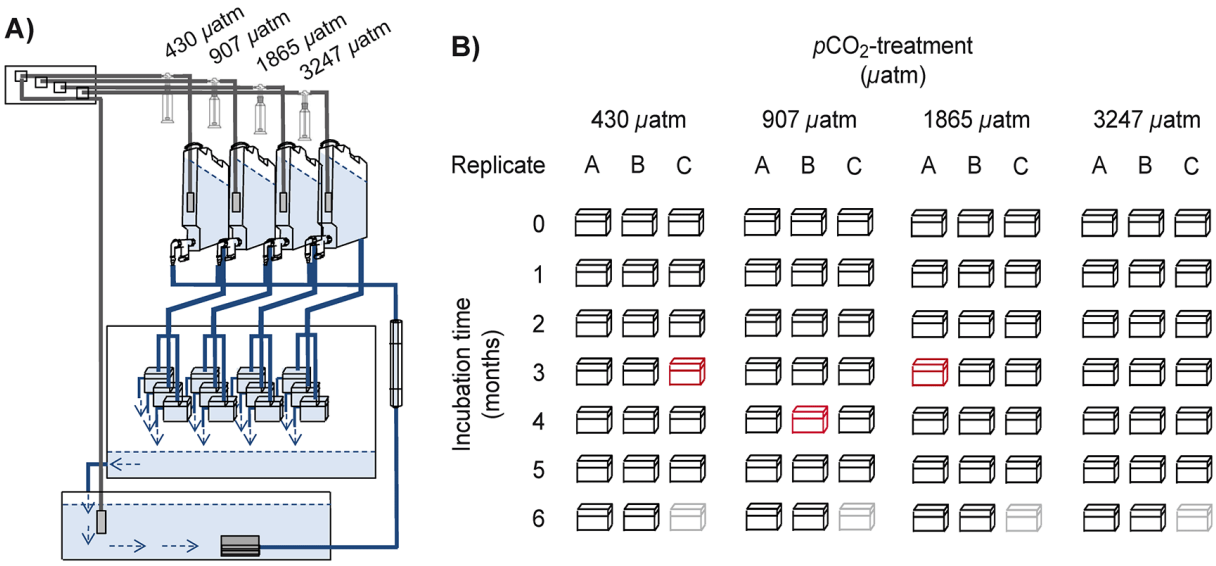

Fig. 1. (A) Scheme: experimental setup of *A. aomoriensis* in a closed flow-through system. Water equilibrated with four pCO_2 levels (430, 907, 1865 and 3247 µatm) supplied from the compact jerrycans into the three replicated culture vessels and drained into a catchment tank, from where is was pumped up again to the jerrycans. **(B)** Experimental design: altogether 84 culture vessels, replication for four pCO_2 treatments (430, 907, 1865 and 3247 µatm) during 6 months of incubation time. Red culture vessels: disappeared during incubation time. Culture vessels marked in light grey refer to treatments where carbonate chemistry parameters were measured in the supernatant seawater and sediment pore water in one of the three replicates after 6 months of incubation time.

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 0.02 M HCl and finished until a stable light pink color appeared. 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. Carbonate chemistry parameters were measured and calculated from seawater and sediment pore water for four pCO_2 levels after 6-month incubation.

2.5 Foraminiferal processing

In order to monitor changes of the foraminiferal community during the incubation time, the sediment of three culture vessel replicates for each pCO_2 line was analyzed monthly for foraminiferal assemblage composition. Due to the loss of some replicates during the incubation time (Fig. 1b), we added a replication (n) scheme for the measured parameters of population density or abundance, size class proportion, test diameter and $CaCO_3$ weight of *A. aomoriensis* in Table 1.

The complete benthic foraminiferal sediment samples were transferred from the culture vessels in 100 mL Kautex™ wide-neck containers, preserved and stained with rose Bengal ethanol (94 %) solution of 2 g L^{-1} for 3 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). Rose Bengal is an established and favorable method to discern between empty tests and living specimens. However, we would like to point out that staining with rose Bengal does not allow an accurate quantification of living and dead foraminifera (Bernhard et al., 2006). Stainable cytoplasm inside the tests is conserved for a long time; therefore it could be that the respective specimens had already terminated their metabolism. We have examined numerous examples from field and laboratory samples to be able to recognize the differences. A dead and altered protoplasm in *Ammonia* is orange instead of bright deep red and it shows no internal differentiation due to cell organelle. Nevertheless, we cannot rule out that population densities might be slightly overestimated in the current study (Bernhard et al., 2006).

Subsequently, the samples were gently washed with tap water through a 63 µm sieve. The 63–2000 µm and > 2000 µm fractions were dried at 60 °C for at least 24 h.

Table 1. Replication (n) scheme for the measured parameters of population density/abundance, size class proportion, test diameter and $CaCO_3$ weight of *Ammonia aomoriensis* for four pCO_2 treatments during 6 months of incubation time.

pCO_2-treatment	Incubation time (months)						
(µatm)	0	1	2	3	4	5	6
430	3n	3n	3n	2n	3n	3n	2n
907	3n	3n	3n	3n	2n	3n	2n
1865	3n	3n	3n	2n	3n	3n	2n
3247	3n	3n	3n	3n	3n	3n	2n

The size fraction 63–2000 µm was picked completely for living and dead foraminifera. Samples were not split. 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 *A. aomoriensis*, were mounted in Plummer cell slides with glue, counted and measured using an eyepiece reticle on the Wild M3C dissecting microscope. *A. aomoriensis* was kept in single cell slides for further analyses.

Test morphometry of *A. 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 *A. aomoriensis* tests, the size of tests is given as mean diameter, calculated from the minimum and maximum diameter.

The $CaCO_3$ weight of dead and living *A. aomoriensis* was measured using a micro-balance with an accuracy of 1 µg (Sartorius, M3P-000V001). Finally, tests of corroded specimens of *A. aomoriensis* and *Armorella sphaerica* were photographed with a MiniPixie (MPX2051UC) digital microscope.

2.6 Calculations

The census data were standardized to population densities 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 at 50 µm intervals and presented as the average of three replicates taken each month during the 6 months of incubation time.

$CaCO_3$ weight per individual/test of *A. aomoriensis* was calculated from the total $CaCO_3$ weight divided by the total number of individuals/tests. Carbonate production and accumulation of *A. aomoriensis* have been calculated from the difference of dry $CaCO_3$ weight of individuals or tests produced or accumulated during 6 months of incubation time. The production or accumulation rate in grams refers to an area of 1 m^2 per year.

From the control pCO_2 line of 430 µatm, 100 living *A. aomoriensis* from size fraction 200 to 300 µm were picked to calculate the total organic content, including cytoplasm and organic linings of the test, at the beginning of the experiment ($t = 0$) (Table S7 in the Supplement). The determination was performed by combustion off the organic material from the carbonate tests in a muffle furnace at 500 °C for 6 h. The amount of total organic content is calculated from the weight loss and given as a percentage of the total $CaCO_3$ weight of the individuals (Table S8 in the Supplement). This value was subtracted from total weight to obtain the $CaCO_3$ weight of shell carbonate in order to determine the absolute amount of $CaCO_3$ production and accumulation (Table S7 in the Supplement).

2.7 Statistics

Data were tested for normality using Shapiro–Wilks test. Homogeneity of variances was analyzed by applying Levene's test. Parametric tests were conducted when the assumptions of normality and homogeneity were matched. The difference between initial abundances at T0 and T1 was compared by using Student'S t tests for each pCO_2 level. The relationship of test diameter and weight was tested by ANCOVA with pCO_2 as a categorical factor. A number of specimens collected during the experimental period (4–24 weeks) was analyzed for differences between pCO_2 treatments using Kruskal–Wallis test, as data were not normally distributed. Similarly, differences between bulk water, bottom water and pore water were analyzed using Kruskal–Wallis test. Tests were carried out by using STATISTICA 8. ANOVA and regression analyses of test diameter and weights of living and dead *A. aomoriensis* were performed with StatView 4.02© Abacus Concepts Inc. All measured raw data of each treatment were used for statistical analyses.

3 Results

Water chemistry parameters, data set of population density or abundance, test diameter, test weight, and carbonate production or accumulation refers to means of the replicate measurements ± standard deviation (SD). The culture vessels, from which carbonate chemistry of the supernatant seawater and sediment pore water have been determined after 6 months of incubation time, were excluded.

3.1 Carbonate chemistry

Seawater temperature and salinity were stable during the experimental period. Average temperatures ranged from 16.4 ± 0.05 to 17.2 ± 0.27 °C and salinities from 15.1 ± 0.05 to 15.9 ± 0.30 (Table 2).

Phosphate and silicate concentrations changed markedly over the course of the experiment. Phosphate concentration increased 31-fold during the first 3 months from 0.19 ± 0.02

Table 2. 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 SD (1σ) refers to replicate measurements.

pCO_2 values of gas (µatm)	Incubation time (months)	Seawater measurements						Calculations from pH_{NBS} and C_T		
		T (°C)	S	pH_{NBS}	C_T (µmol kg^{-1})	PO_4^{3-} (µmol L^{-1})	Si (µmol L^{-1})	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

to 5.86 ± 0.05 µmol L^{-1} (Table 2). Mean silicate concentrations were in general high with 191.98 ± 84.18 µmol L^{-1} and exhibited a threefold increase from 138.94 ± 0.85 to 367.19 ± 0.69 µmol L^{-1} after 2 months (Table 2). Microscopic analysis showed that the detritus layer was composed of centric diatoms, which also dominated in the natural environment. By the end of the experiment, concentrations of phosphate and silicate decreased again and returned to the initial values that were observed at the beginning of the experiment.

As a consequence of four different pCO_2 treatments, mean pH values ranged between 8.21 ± 0.00 and 7.17 ± 0.01 (Table 2) and were stable throughout the incubation period. Total inorganic carbon increased with increasing pCO_2 from 2210.5 ± 74.61 to 2464.2 ± 99.94 µmol L^{-1} (Table 2). The calculated mean pCO_2 levels of the treatments were thereby 430 ± 73.76, 907 ± 74.74, 1865 ± 317.19 and 3247 ± 435.51 µatm (Table 2). 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 led to markedly lower pCO_2 levels than the target values. In the following, we refer our results to the pCO_2 levels that prevailed in the culture vessels (Haynert et al., 2011). Mean seawater alkalinity was 2379.3 ± 91.85 µmol kg^{-1} and did not differ between the treatments (Table 2). 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 ± 0.09 on average (Table 2).

During the experiment, a layer of organic detritus formed at the sediment–water interface. The carbonate chemistry measurements revealed strong differences of pCO_2 and Ω_{calc} between seawater and sediment pore water in the culture vessels (Fig. 2). The sediment pore water was characterized by higher pCO_2 and lower pH than the supernatant seawater in the culturing vessel (Fig. 2a and b). At the same time, pore water alkalinity (3127.8 ± 225.23 µmol kg^{-1}) was much higher than the bulk seawater alkalinity (0–2 cm water depths) of 2113.1 ± 6.86 µmol kg^{-1} and the water overlaying the sediment (2–4 cm water depths) with 2442.3 ± 9.98 µmol kg^{-1} (Kruskal–Wallis test, $H(2, 12) = 9.85$, $p < 0.01$) (Table S1 in the Supplement). The accumulation of A_T in the sediment caused a relative high saturation of Ω_{calc} and even in the highest pCO_2 treatment a slight supersaturation, $\Omega_{calc} > 1$ (Fig. 2c and d).

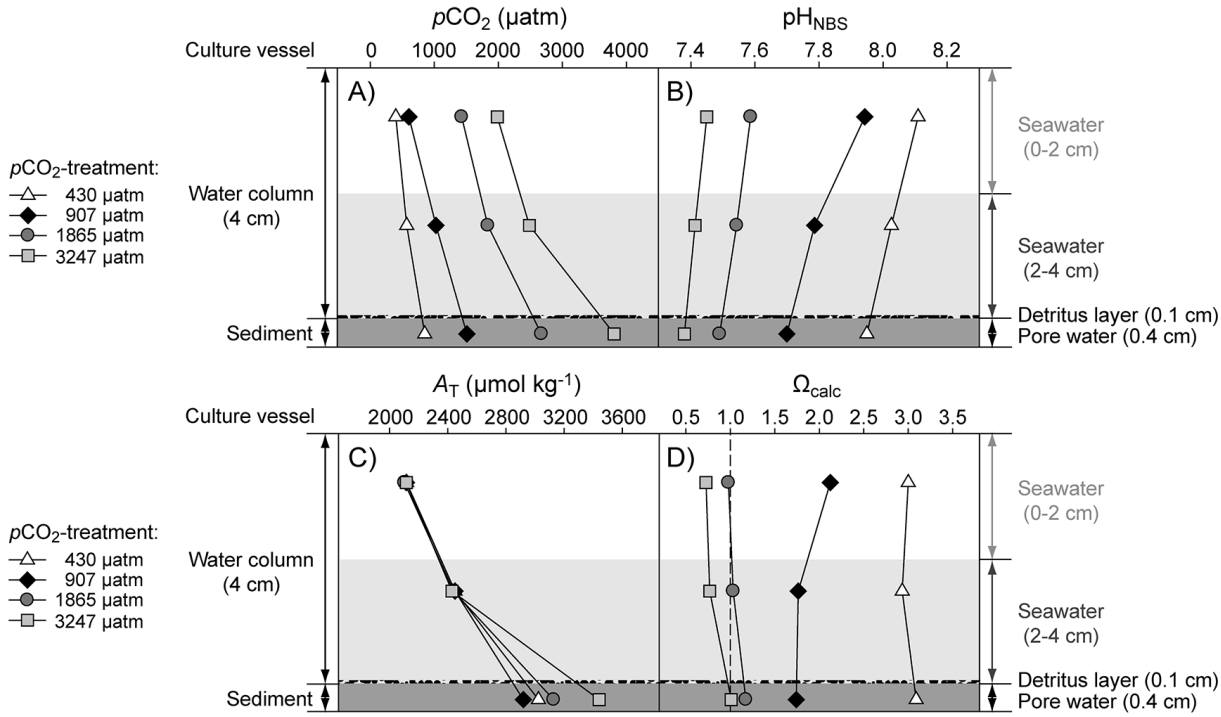

Fig. 2. Gradient of carbonate chemistry parameters of **(A)** partial pressure of CO_2 (pCO_2), **(B)** pH_{NBS}, **(C)** total alkalinity (A_T) and **(D)** saturation state of calcite (Ω_{calc}) in the seawater and sediment pore water for four pCO_2 treatments after 6 months of incubation time.

3.2 Species composition and foraminiferal assemblages

The assemblages comprised four calcareous species: *Ammonia aomoriensis*, the two subspecies *Elphidium excavatum excavatum* and *Elphidium excavatum clavatum*, as well as *Elphidium gerthi* and *Elphidium incertum*. Three arenaceous species were determined: *Ammotium cassis*, *Reophax dentaliniformis* and *Armorella sphaerica* (Tables 3 and 4).

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 (Tables 3 and 4). 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.

3.2.1 Population density and test diameter of living *Ammonia aomoriensis*

The population density and size–frequency distribution exhibited strong variations due to growth and mortality of foraminiferal faunas during the 6 months of incubation (Tables S2, S3 and S4 and in the Supplement). Size distributions revealed growth, which was characterized by a similar increase of test diameter and the loss of individuals by death during the same period.

Initial population density ($t = 0$) of living *A. aomoriensis* fauna varied from 189 to 771 ind. 10 cm^{-3} with a mean of 433 ± 166.16 ind. 10 cm^{-3} (Table S3 in the Supplement). The initial test diameter ($t = 0$) ranged from 169 ± 11.47 to 182 ± 3.52 µm, which equates to a mean diameter of 176 ± 5.41 µm (Table S4 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. 3a). *Ammonia aomoriensis* density declined strongly during the first month at pCO_2 of 1865 and 3247 µatm (Fig. 3a). The reduction, however, was only significant at 1865 µatm (*t* test, $p < 0.05$). An explosive increase of living *Ammonia aomoriensis* up to 610 ± 87.04, 1378 ± 22.78 and 518 ± 348.52 ind. 10 cm^{-3} was observed at pCO_2 of 907, 1865 and 3247 µatm after 3 months (Fig. 3a, Table S3 in the Supplement). A further increase up to 602 ± 308.96 ind. 10 cm^{-3} followed at 3247 µatm after 5 months (Fig. 3a, Table S3 in the Supplement). Nevertheless, overall population densities did not significantly differ between the pCO_2 levels, and densities of living foraminifera were not affected by treatment pCO_2 after 5 months of incubation (Kruskal–Wallis test, $H(3, 12) = 4.64$, $p > 0.05$). The strong increase of population densities correlated with the occurrence of high numbers of small individuals with a test diameter < 100 µm at pCO_2 of 1865 µatm after 3 months, and at pCO_2 of 3247 µatm after 3 and 4 months (Fig. 4). A less pronounced occurrence of individuals < 100 µm in the background was observed at a pCO_2 of 907 µatm after 4 and 5 months, though population densities were not affected (Fig. 4).

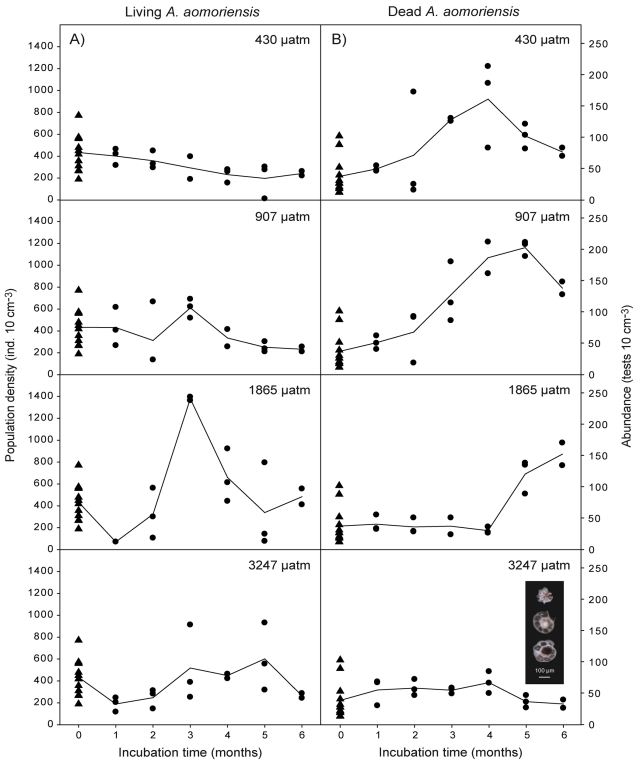

Fig. 3. (A) Population density of living and **(B)** abundance of dead *A. aomoriensis* at four pCO_2 levels (430, 907, 1865 and 3247 µatm) during 6 months of incubation time. The triangles represent the initial population densities/abundances ($T = 0$). Circles represent population densities/abundances of the measured replicates during the incubation time. Continuous line refers to mean of the replicate measurements.

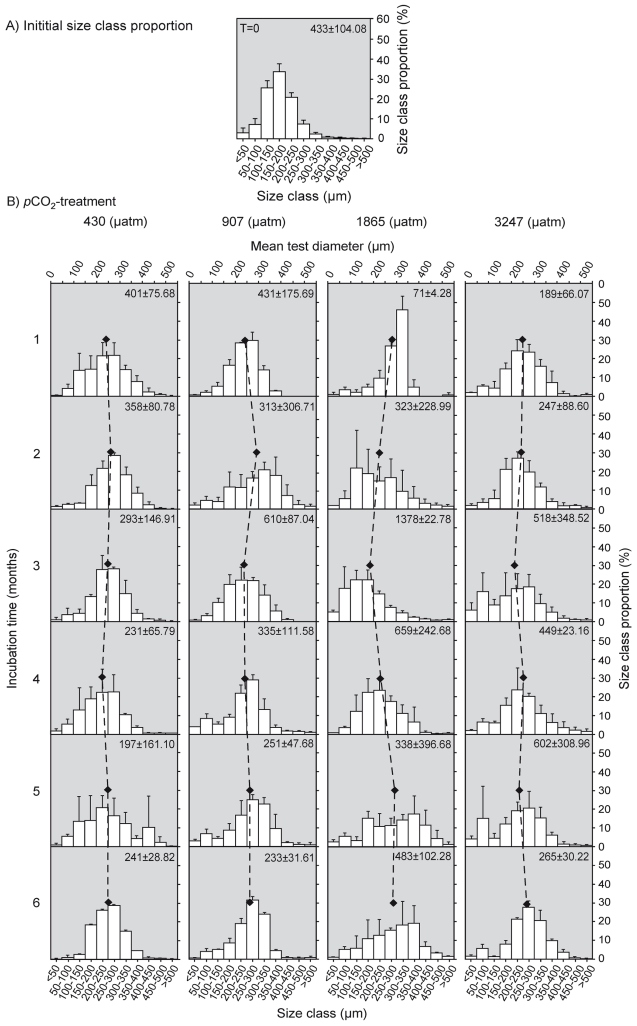

Fig. 4. Histogram of living *A. aomoriensis*, **(A)** initial size class proportions ($T = 0$) and **(B)** size class proportions for the tested four pCO_2 levels (430, 907, 1865 and 3247 µatm) during 6 months of incubation. Bars represent relative proportion of 11 size classes ranging from < 50 until > 500 µm in 50 µm intervals. Mean test diameter **(B)** is displayed by the black diamonds and dashed lines. The number in each figure represents the mean population density ±SD, which refers to replicate measurements (Table S3 in the Supplement).

3.2.2 Abundance and test diameter of dead *Ammonia aomoriensis*

At the beginning of the experiment ($t = 0$), the mean abundance of the dead fauna was 38 ± 29.37 tests $10\,cm^{-3}$, ranging from 13 to 103 tests $10\,cm^{-3}$ (Table S5 in the Supplement). At pCO_2 levels of 430 and 907 µatm, their abundance increased steadily until the fourth and fifth month, and subsequently decreased until the end of experiment (Fig. 3b). In contrast, at pCO_2 of 1865 and 3247 µatm, the number of *A. aomoriensis* tests did not change until the fourth month (Fig. 3b). Afterwards, the number of empty tests increased by 80 % at 1865 µatm until the end of the experiment (Fig. 3b). These results correlated with a strong decline of population densities from 1378 ± 22.78 to 338 ± 396.68 ind. $10\,cm^{-3}$ from the third to the fifth month (Fig. 3a, Table S4 in the Supplement). At 3247 µatm, test abundance decreased to 32 ± 9.30 tests $10\,cm^{-3}$ at the end of the experiment ($H(3, 12) = 9.67$, $p = 0.02$) (Table S5 in the Supplement).

The mean test diameter ranged between 290 ± 28.16 and 283 ± 32.50 µm at pCO_2 from 430 to 1865 µatm (Table S6 in the Supplement). At higher pCO_2 of 3247 µatm, test diameter was clearly lower with 258 ± 51.66 µm, which indicated a frequent mortality of large and adult foraminifera up to 1865 µatm, whereas at higher pCO_2 an increasing mortality of smaller and younger individuals was recognized (Table S6 in the Supplement). At high pCO_2 of 3247 µatm, light micrograph images of dead *A. aomoriensis* showed that approximately 30 % of the tests were completely destroyed during the last 2 months of the experiment. The strongest effect of test degradation was the characteristic star-like appearance, which left only the umbilicus area and the suture of the chambers intact (Fig. 3b).

Table 3. List of living foraminiferal species from size fraction 63–2000 µm of the four tested pCO_2 treatments during 6 months of incubation time. Number of species ± SD and mean percentage (%) refer to replicate measurements.

pCO_2 treatment	Living foraminiferal species > 63 µm	Incubation time (months) 0		1		2		3		4		5		6	
		Number of species ± SD	Mean %	Number of species ± SD	Mean %	Number of species ± SD	Mean %	Number of species ± SD	Mean %	Number of species ± SD	Mean %	Number of species ± SD	Mean %	Number of species ± SD	Mean %
430 µatm	Species														
	Ammonia aomoriensis	519.7 ± 145.5	100.0	609.3 ± 115.1	94.6	544.3 ± 122.9	97.3	445.0 ± 223.4	100.0	351.3 ± 100.1	98.9	299.0 ± 245.0	99.6	367.0 ± 43.8	99.0
	Elphidium excavatum excavatum			1.7 ± 0.6	0.3										
	Elphidium excavatum clavatum			0.3 ± 0.6	0.0										
	Elphidium incertum			31.7 ± 1.5	5.1	20.5 ± 10.6	2.7			4.7 ± 8.1	1.1	5.0	0.4	7.0	1.0
	Total number of calcareous individuals	519.7 ± 145.5		643.0 ± 114.1		558.0 ± 113.3		445.0 ± 223.4		356.0 ± 103.3		300.7 ± 246.3		370.5 ± 38.9	
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
	Total number of living specimens	519.7 ± 145.5		643.0 ± 114.1		558.0 ± 113.3		445.0 ± 223.4		356.0 ± 103.3		300.7 ± 246.3		370.5 ± 38.9	
	Species number	1		3 ± 0.6		2 ± 0.6		1		1		1		2 ± 0.7	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Population density (ind. 10 cm^{-3})	341.7 ± 95.6		422.7 ± 75.0		366.9 ± 74.5		292.6 ± 146.9		234.1 ± 67.9		197.7 ± 161.9		243.6 ± 25.6	
907 µatm	Species														
	Ammonia aomoriensis	602.7 ± 228.4	100.0	655.0 ± 267.2	98.6	475.3 ± 466.5	100.0	928.3 ± 132.4	100.0	510.0 ± 169.7	100.0	381.7 ± 72.5	100.0	354.0 ± 48.1	100.0
	Elphidium excavatum excavatum			0.3 ± 0.6	0.1										
	Elphidium excavatum clavatum														
	Elphidium incertum			8.7 ± 4.0	1.3										
	Total number of calcareous individuals	602.7 ± 228.4		664.0 ± 270.8		475.3 ± 466.5		928.3 ± 132.4		510.0 ± 169.7		381.7 ± 72.5		354.0 ± 48.1	
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
	Total number of living specimens	602.7 ± 228.4		664.0 ± 270.8		475.3 ± 466.5		928.3 ± 132.4		510.0 ± 169.7		381.7 ± 72.5		354.0 ± 48.1	
	Species number	1		2 ± 0.6		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})	396.2 ± 150.2		436.6 ± 178.0		312.5 ± 306.7		610.3 ± 87.0		335.3 ± 111.6		250.9 ± 47.7		232.7 ± 31.6	
1865 µatm	Species														
	Ammonia aomoriensis	628.7 ± 303.4	100.0	107.7 ± 6.5	100.0	491.7 ± 348.3	100.0	2096.5 ± 34.6	100.0	1002.7 ± 369.1	100.0	514.7 ± 603.3	100.0	735.0 ± 155.6	100.0
	Elphidium excavatum excavatum														
	Elphidium excavatum clavatum														
	Elphidium incertum														
	Total number of calcareous individuals	628.7 ± 303.4		107.7 ± 6.5		491.7 ± 348.3		2096.5 ± 34.6		1002.7 ± 369.1		514.7 ± 603.3		735.0 ± 155.6	
	Reophax dentaliniformis														
	Total number of agglutinated individuals	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
	Total number of living specimens	628.7 ± 303.4		107.7 ± 6.5		491.7 ± 348.3		2096.5 ± 34.6		1002.7 ± 369.1		514.7 ± 603.3		735.0 ± 155.6	
	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})	413.3 ± 199.5		70.8 ± 4.3		323.3 ± 229.0		1378.4 ± 22.8		659.2 ± 242.7		338.4 ± 396.7		483.2 ± 102.3	
3247 µatm	Species														
	Ammonia aomoriensis	886.3 ± 270.3	100.0	288.0 ± 100.5	90.1	375.3 ± 134.8	100.0	787.7 ± 530.1	100.0	682.7 ± 35.2	100.0	915.0 ± 469.9	100.0	402.5 ± 46.0	100.0
	Elphidium excavatum excavatum			2.0 ± 1.0	0.6										
	Elphidium excavatum clavatum			1.5 ± 0.7	0.6										
	Elphidium incertum			23.7 ± 9.8	8.7										
	Total number of calcareous individuals	886.3 ± 270.3		314.7 ± 91.2		375.3 ± 134.8		787.7 ± 530.1		682.7 ± 35.2		915.0 ± 469.9		402.5 ± 46.0	
	Reophax dentaliniformis			0.3 ± 0.6	0.5										
	Total number of agglutinated individuals	0.0		0.3		0.0		0.0		0.0		0.0		0.0	
	Total number of living specimens	886.3 ± 270.3		315.0 ± 90.7		375.3 ± 134.8		787.7 ± 530.1		682.7 ± 35.2		915.0 ± 469.9		402.5 ± 46.0	
	Species number	1		4 ± 1.0		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})	582.7 ± 177.7		207.1 ± 59.6		246.8 ± 88.6		517.9 ± 348.5		448.8 ± 23.2		601.6 ± 309.0		264.6 ± 30.2	

Table 4. List of dead foraminiferal species from size fraction 63–2000 µm of the four tested pCO_2 treatments during 6 months of incubation time. Number of tests ± SD and mean percentage (%) refers to replicate measurements.

pCO_2-treatment	Dead foraminiferal species > 63 µm	Incubation time (months) 0		1		2		3		4		5		6	
		Number of tests ± SD	Mean %	Number of tests ± SD	Mean %	Number of tests ± SD	Mean %	Number of tests ± SD	Mean %	Number of tests ± SD	Mean %	Number of tests ± SD	Mean %	Number of tests ± SD	Mean %
430 µatm	Species														
	Ammonia aomoriensis	37.0 ± 21.4	82.5	76.0 ± 7.0	72.4	109.0 ± 133.6	70.8	195.5 ± 4.9	91.1	245.3 ± 104.5	90.7	156.0 ± 30.0	81.2	117.0 ± 14.1	86.4
	Elphidium excavatum excavatum			2.3 ± 2.1	2.3	1.3 ± 1.5	0.9	1.0 ± 0.0	0.5	1.0 ± 1.7	0.3	12.0 ± 3.6	6.3	2.5 ± 2.1	1.7
	Elphidium excavatum clavatum	2.0 ± 1.0	6.2			0.3 ± 0.6	0.7					1.0 ± 1.7	0.6	0.5 ± 0.7	0.4
	Elphidium incertum	3.7 ± 2.1	8.2	21.0 ± 4.0	20.0	15.3 ± 2.5	21.9	13.0 ± 0.0	6.1	17.0 ± 2.6	7.0	16.7 ± 5.1	8.8	13.0 ± 5.7	9.3
	Total number of calcareous individuals	42.7 ± 22.5		99.3 ± 8.1		126.0 ± 134.3		209.5 ± 4.9		263.3 ± 107.7		185.7 ± 31.0		133.0 ± 21.2	
	Reophax dentaliniformis	1.3 ± 0.6	3.2	5.7 ± 2.1	5.4	4.3 ± 0.6	5.7	5.0 ± 1.4	2.3	5.7 ± 4.6	2.1	5.7 ± 2.1	3.1	3.0 ± 1.4	2.1
	Total number of agglutinated individuals	1.3 ± 0.6		5.7 ± 2.1		4.3 ± 0.6		5.0 ± 1.4		5.7 ± 4.6		5.7 ± 2.1		3.0 ± 1.4	
	Total number of individuals	44.0 ± 23.1		105.0 ± 8.5		130.3 ± 134.9		214.5 ± 3.5		269.0 ± 110.8		191.3 ± 29.6		136.0 ± 22.6	
	Species number	4 ± 0.0		4 ± 0.6		4 ± 0.0		4 ± 0.0		3 ± 0.6		4 ± 0.6		4.5 ± 0.7	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	28.9 ± 15.2		69.0 ± 5.6		85.7 ± 88.7		141.0 ± 2.3		176.9 ± 72.8		125.8 ± 19.4		89.4 ± 14.9	
907 µatm	Species														
	Ammonia aomoriensis	60.7 ± 64.5	79.5	79.0 ± 16.5	87.9	104.7 ± 64.7	87.5	195.0 ± 73.4	93.7	285.5 ± 54.4	94.9	310.0 ± 18.4	91.5	211.5 ± 21.9	90.0
	Elphidium excavatum excavatum	0.7 ± 0.6	1.5	1.7 ± 0.6	1.9	2.7 ± 0.6	3.6	4.7 ± 3.5	2.0	3.0 ± 1.4	1.0	9.3 ± 7.6	2.7	2.0 ± 0.0	0.9
	Elphidium excavatum clavatum	3.0 ± 1.0	6.7	1.0 ± 1.0	1.2			1 ± 0.0	0.5	1.0 ± 0.0	0.3	2.5 ± 0.7	0.5	0.5 ± 0.7	0.2
	Elphidium incertum	3.7 ± 2.1	9.4	5.3 ± 3.5	5.5	6.7 ± 0.6	8.7	5.7 ± 3.1	3.2	4.0 ± 0.0	1.4	10.3 ± 0.6	3.1	4.5 ± 2.1	1.9
	Total number of calcareous individuals	68.0 ± 63.6		87.0 ± 19.7		114.0 ± 64.1		206.3 ± 75.3		293.5 ± 55.9		331.3 ± 26.3		218.5 ± 23.3	
	Armorella sphaerica													0.7 ± 0.9	1.0
	Reophax dentaliniformis	1.3 ± 1.2	2.9	3.3 ± 2.3	3.5	0.3 ± 0.6	0.2	1.7 ± 2.9	0.6	7.5 ± 3.5	2.4	8.0 ± 4.0	2.3	15.0 ± 9.9	6.1
	Total number of agglutinated individuals	1.3 ± 1.2		3.3 ± 2.3		0.3 ± 0.6		1.7 ± 2.9		7.5 ± 3.5		8.0 ± 4.0		17.0 ± 7.1	
	Total number of individuals	69.3 ± 64.0		90.3 ± 21.8		114.3 ± 64.4		208.0 ± 78.1		301.0 ± 59.4		339.3 ± 29.0		235.5 ± 30.4	
	Species number	4.3 ± 1.2		5 ± 0.6		3 ± 0.6		4 ± 0.6		5 ± 0.0		5 ± 0.6		5 ± 1.4	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	45.6 ± 42.1		59.4 ± 14.3		75.2 ± 42.3		136.8 ± 51.3		197.9 ± 39.1		223.1 ± 19.1		154.8 ± 20.0	
1865 µatm	Species														
	Ammonia aomoriensis	41.0 ± 7.5	69.6	62.3 ± 19.7	87.6	55.7 ± 19.3	85.7	57.5 ± 29.0	88.4	46.7 ± 8.1	92.7	184.3 ± 41.9	86.4	232.5 ± 38.9	93.7
	Elphidium excavatum excavatum	2.7 ± 1.5	4.9	0.3 ± 0.6	0.6	6.0 ± 3.6	8.7	5.0 ± 0.0	8.7	1.7 ± 1.2	3.4	7.3 ± 2.1	3.6	0.5 ± 0.7	0.2
	Elphidium excavatum clavatum	5.0 ± 2.0	8.7	0.3 ± 0.6	0.3	0.3 ± 0.6	0.4	0.5 ± 0.7	0.6	1.0 ± 1.0	2.1	0.7 ± 1.2	0.3		
	Elphidium gerthi	0.3 ± 0.6	0.7												
	Elphidium incertum	8.3 ± 5.1	13.6	2.3 ± 1.5	3.1	2.0 ± 2.6	2.4	0.5 ± 0.7	0.6			15.3 ± 11.7	7.2	2.5 ± 0.7	1.0
	Total number of calcareous individuals	57.3 ± 9.6		65.3 ± 21.5		64.0 ± 26.0		63.5 ± 30.4		49.3 ± 7.6		207.7 ± 45.5		235.5 ± 38.9	
	Ammotium cassis					0.3 ± 0.6	0.4							0.5 ± 0.7	0.2
	Reophax dentaliniformis	1.3 ± 1.2	2.5	6.0 ± 3.5	8.4	1.3 ± 2.3	2.5	1.0 ± 0.0	1.7	1.0 ± 1.0	1.8	5.0 ± 3.6	2.5	12.0 ± 1.4	4.9
	Total number of agglutinated individuals	1.3 ± 1.2		6.0 ± 3.5		1.3 ± 2.3		1.0 ± 0.0		1.0 ± 1.0		5.0 ± 3.6		12.0 ± 1.4	
	Total number of individuals	58.7 ± 8.7		71.3 ± 23.1		65.7 ± 25.5		64.5 ± 30.4		50.3 ± 8.5		212.7 ± 43.8		248.0 ± 39.6	
	Species number	5 ± 1.0		4 ± 0.6		4 ± 1.2		4 ± 1.4		3.3 ± 0.6		4 ± 0.6		4 ± 1.4	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	38.6 ± 5.7		46.9 ± 15.2		43.2 ± 16.8		42.4 ± 20.0		33.1 ± 5.6		139.8 ± 28.8		163.1 ± 26.0	
3247 µatm	Species														
	Ammonia aomoriensis	92.3 ± 58.5	84.6	83.0 ± 32.9	77.3	87.7 ± 19.8	86.2	82.3 ± 7.4	84.3	101.0 ± 26.5	89.3	54.7 ± 15.0	87.3	49.0 ± 14.1	85.8
	Elphidium excavatum excavatum	2.7 ± 0.6	3.2	4.3 ± 5.8	3.7	2.7 ± 1.5	2.6	2.0 ± 1.0	2.1	0.7 ± 0.6	0.7	1.3 ± 0.6	2.1		
	Elphidium excavatum clavatum	4.3 ± 1.5	4.8	0.7 ± 1.2	0.5	1.0 ± 1.0	0.9			0.7 ± 0.6	0.7				
	Elphidium incertum	5.0 ± 1.0	6.3	13.3 ± 4.7	14.7	9.0 ± 3.0	8.7	7.7 ± 1.5	7.8	8.0 ± 5.3	7.0	4.3 ± 0.6	7.1	2.5 ± 2.1	4.0
	Total number of calcareous individuals	104.3 ± 57.8		101.3 ± 33.2		100.3 ± 24.6		92.0 ± 7.9		110.3 ± 27.0		60.3 ± 16.0		51.5 ± 16.3	
	Reophax dentaliniformis	1.0 ± 0.0	1.2	3.7 ± 2.1	3.7	1.3 ± 2.3	1.6	5.7 ± 2.5	5.8	2.7 ± 1.2	2.3	2.0 ± 1.0	3.5	5.5 ± 0.7	10.2
	Total number of agglutinated individuals	1.0 ± 0.0		3.7 ± 2.1		1.3 ± 2.3		5.7 ± 2.5		2.7 ± 1.2		2.0 ± 1.0		5.5 ± 0.7	
	Total number of individuals	105.3 ± 57.8		105.0 ± 33.8		101.7 ± 22.7		97.7 ± 8.5		113.0 ± 27.8		62.3 ± 15.6		57.0 ± 15.6	
	Species number	5 ± 0.0		4 ± 0.6		4 ± 0.0		4 ± 0.0		4 ± 1.2		4 ± 0.0		3 ± 0.0	
	Sediment volume (cm^3)	15.2		15.2		15.2		15.2		15.2		15.2		15.2	
	Abundance (tests 10 cm^{-3})	69.3 ± 38.0		69.0 ± 22.2		66.8 ± 14.9		64.2 ± 5.6		74.3 ± 18.3		41.0 ± 10.3		37.5 ± 10.2	

3.3 $CaCO_3$ weight of *Ammonia aomoriensis*

The total organic content of living individuals was found to be 4.3 %, which is an average value measured from 100 individuals of a specific size ranging from 200 to 300 µm (Table S7 in the Supplement). The total organic content of 4.3 % was subtracted from total $CaCO_3$ weight in order to determine the absolute amount of $CaCO_3$ (cf. Movellan et al., 2012). The initial $CaCO_3$ weight ($t = 0$) per individual of living *A. aomoriensis* was 1.56 ± 0.40 µg on average (Table S8 in the Supplement).

Test weight of living foraminifera was not affected by pCO_2 treatment, but with increasing test diameter, the dry weight per living individual increased constantly (ANCOVA, factor test diameter: F117.3, $p < 0.01$, factor pCO_2 F1.0, $p > 0.05$) (Fig. 5a). Similar results were obtained for the relationship of dead tests (ANCOVA, factor test diameter: F17.6, $p < 0.01$, factor pCO_2 F 0.34, $p > 0.05$). Earlier studies revealed an exponential relationship of foraminiferal size and weight due to their allometric growth (e.g., Altenbach, 1985; Schiebel and Movellan, 2012). Our data distribution showed only a slight curvature suggesting a quasi-linear relationship in the measured size range. Indeed, the correlation coefficient of the exponential regression was with 0.94 only slightly higher as 0.91 of the linear regression applied to living *A. aomoriensis* data (Fig. 5a). However, p value of the ANOVA table was < 0.0001 in both cases suggesting the relationship between test diameter and weight can be described by a simple linear model with sufficient accuracy. The correlation coefficient of linear regression of mean test diameter and weights of living and dead specimens at the beginning, and after every month retrieved from the 4 pCO_2 treatments was $r = 0.91$ and $r = 0.59$ ($n = 28$) (Fig. 5a and b). The p value from the ANOVA table was < 0.0001 and 0.0009 suggesting statistically significant relationships at the > 99.9 % confidence level in both cases. With the exception of living specimens from the 430 µatm treatment, regression line slopes and intercepts of individual CO_2 levels were in the range of lower and upper 95 % confidence intervals of the combined data set comprising all CO_2 treatments (Fig. 5a). It has to be noted, however, that both correlation coefficients and p values of the ANOVA tables were consistently lower in the data from empty tests. This pattern provides corroborating evidence that the relationship between mean test diameter and weights reliably displays the growth pattern of *A. aomoriensis*.

3.4 Carbonate production and accumulation

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 ± 818 µg at the lowest pCO_2 of 430 µatm, followed by 886 ± 728 µg at the highest pCO_2 of 3247 µatm after 6 months of incubation (Table S7 in the Supplement). The three replicate treatments of 907 and 1865 µatm showed similarly higher mean $CaCO_3$ production of 1269 ± 821 and 1256 ± 1153 µg with a total culture vessel area of 42.25 cm^2 and an incubation time of 6 months (Table S8 in the Supplement). The calculated total carbonate production rate for living individuals was 0.47 $g\,m^{-2}\,a^{-1}$ (Table S7 in the Supplement).

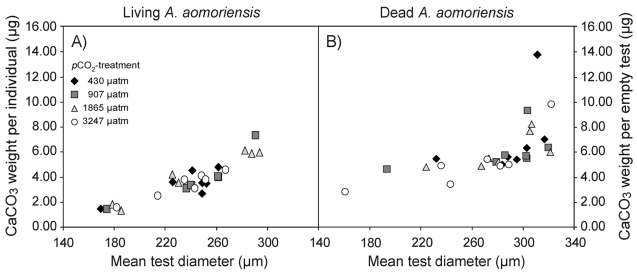

Fig. 5. Mean of total $CaCO_3$ weight of **(A)** living specimens including cytoplasm and **(B)** empty test of *A. aomoriensis* in relation to mean test diameter for the four tested pCO_2 treatments. Mean values refer to replicate measurements.

The $CaCO_3$ accumulation of empty tests varied between the pCO_2 treatments. 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 µatm, $CaCO_3$ rates decline to 435 and 239 µg after 6 months of incubation with the above specified settings. The calculated total carbonate accumulation of empty tests was 0.27 $g\,m^{-2}\,a^{-1}$ (Table S7 in the Supplement).

4 Discussion

4.1 Sediment chemistry

The sediment used in this experiment was from Kiel Fjord, western Baltic Sea, and is rich in organic matter (Nikulina et al., 2008). 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 2 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. This in turn would increase nutrient concentration increased in the overlying seawater.

The algal material of *Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella* 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 iron oxyhydroxides 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, resulting in mobilization of DIP from sediments (Krom and Berner, 1981). The decrease of both nutrients until the end of the experiment may have arisen from bacterial microbiological activity of bacteria, although this aspect has not yet been sufficiently studied.

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 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. The dark grey sediment color 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 were in agreement with observations from field studies (Thomas et al., 2009; Kitidis et al., 2011; Haynert et al., 2012).

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 ± 166.16 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). 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 foraminiferal growth in the southwestern Baltic Sea. This assumption is in agreement with a previous study, which reported that food supply plays an important role in relation to growth, chamber formation and reproduction of *Ammonia* species (Bradshaw, 1961). A 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 setup (Fig. S1 in the Supplement. 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 explained by the activation of dormant propagules (Alve and Goldstein, 2002) or an erratically occurrence of juveniles or adults.

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 2 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 increase 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 close to or even above 1. Accordingly, living *A. aomoriensis* specimens were relatively unaffected by high pCO_2 later on and continued to grow, even under highly elevated pCO_2 conditions (Haynert et al., 2012). The number empty shells declined under high pCO_2, most probably as a result of dissolution due to slight undersaturation in the sediment pore water. Obviously, living foraminifera possess the ability to counteract moderate corrosion but dissolve under more severe undersaturation. Thus, 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 could play an important role for the foraminiferal microhabitat. These observations are in agreement with the results of Kitidis et al. (2011), which showed that sediment ammonia oxidation rates, the key of microbial processes, were not affected by low CO_2-acidified seawater, in sharp contrast to water column NH_3 oxidation. They also suggested that buffering in sediments through A_T generation may explain the non-response to ocean acidification.

To date, no ocean acidification studies have been performed with benthic foraminifera in their natural sediment. 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; Fujita et al., 2011; Vogel and Uthicke, 2012; McIntyre-Wressnig et al., 2013; 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). However, the mechanistic study of Keul et al. (2013), performed without any natural sediment, reported that not pCO_2 but rather CO_3^{2-} is the parameter that affects the test size and weights of *Ammonia* species. The simulated high pCO_2 levels between 829 and 1301 µatm revealed simultaneously supersaturated Ω_{calc} ranging from 2.2 to 13.8. Under these conditions, no or a very little impact of pCO_2 was observed on the benthic foraminifera *Ammonia*.

Size distributions of tests may be explained by a combination of reproduction and mortality of individuals starting 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. The size distribution revealed growth cohorts that were characterized by small, juvenile individuals (< 100 µm). Strong cohorts with high numbers of individuals < 100 µm were observed at 1865 and 3247 µatm, with a strongest variability at 1865 µatm. According to the size–frequency distribution of a high number of small individuals, we assume that *A. aomoriensis* starts reproduction between a size of 250 µm and 350 µm. At 350 µm, 40 % of the growth cohorts either have produced juveniles 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, the different growth cohorts made it impossible to determine exactly either reproduction intervals or the number of juveniles per parent. However, the life cycle of *A. aomoriensis* has been insufficiently studied to date, and further field and laboratory experiments are necessary for a better understanding of the species-specific variability of *Ammonia* in general.

4.4 Carbonate production of *Ammonia aomoriensis*

The carbonate production of benthic foraminifera has been 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 test weight in benthic foraminifera. The estimated total organic content of living *A. aomoriensis* is 4.3 % of total $CaCO_3$ 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 A. Movellan (personal communication, 2013), 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. aomoriensis* amounts to $0.47\,g\,m^{-2}\,a^{-1}$, whereas the carbonate accumulation of dead tests equates to $0.27\,g\,m^{-2}\,a^{-1}$ during the incubation time. This 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 to 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*.

No less than 36 % of the produced tests are accumulated in the sediment, which is obviously higher than 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 ingestion 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 and potential predators; therefore a higher accumulation rate was recorded.

4.5 Dissolution features on foraminifera tests

Living *A. aomoriensis* and *E. incertum* exhibited no dissolution features during the whole incubation time. 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. The conditions in the natural sediment create a protective microhabitat for benthic foraminifera with a relatively high calcium carbonate saturation state.

However, some empty tests of *A. aomoriensis* were destroyed at a pCO_2 of 3247 µatm during the last 2 months of incubation. This may be because high pCO_2 and unfavorable $\Omega_{calc} < 1$ reduced the average diameter. In contrast, empty tests of *E. incertum* showed no signs of dissolution. This species-specific response agrees well with our field observation from Flensburg Fjord (Haynert et al., 2012).

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 that 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* in the southwestern Baltic Sea. Growth and mortality of living *A. aomoriensis* were unaffected by elevated pCO_2. Consequently, under the current microhabitat conditions, the dominant *A. aomoriensis* could maintain an important role in benthic carbonate production in the southwestern Baltic Sea. In contrast to the living fauna, approximately 30 % of empty tests of *A. aomoriensis* were subjected to dissolution at high pCO_2 of 3247 µatm and slight undersaturation of Ω_{calc}, whereas empty *E. incertum* tests remained intact. These results emphasize the importance in understanding 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 influences the pore water carbonate chemistry, and thereby provides a stable habitat for benthic foraminifera. Due to these characteristics, foraminiferal communities withstand present-day, seasonally high pCO_2 levels and might also tolerate moderate future pCO_2 increases.

Supplementary material related to this article is available online at http://www.biogeosciences.net/11/1581/2014/bg-11-1581-2014-supplement.pdf.

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