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Insight into deep-sea life – *Cibicidoides pachyderma* substrate and pH-dependent behaviour following disturbance

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ABSTRACT

Most palaeo-deep-water reconstructions are based on geochemical information stored in the calcareous shells of *Cibicidoides* species but hardly anything is known about their life cycle, population dynamics or ecology. The number of specimens of a single *Cibicidoides* species can locally be very limited and species may be lacking completely during certain intervals in the geological past. As a consequence, geochemical analyses are often carried out on lumped *Cibicidoides* spp. assuming that they share the same epizoic to epifaunal habitat and precipitated their shell in comparable offsets to surrounding bottom water mass properties. However, there is a growing body of evidence that particularly *Cibicidoides pachyderma* and its morphotypes *C. mundulus* and *C. kullenbergi*, may not be reliable bottom water recorders.

We have recently developed aquaria that allowed, for the first time, observations of *Cibicidoides pachyderma* var. *C. mundulus* under *in situ* pressure and temperature. Experiments were carried out with and without artificial sediments to simulate soft sediments and rocks, respectively. Seawater was set to pH 8 and pH 7.4 to simulate more or less particulate carbon export or more or less ventilation of bottom water. Our experiments demonstrate that *C. mundulus* may opt for an epifaunal or an infaunal habitat depending on elapsed time following physical disturbance, pH, current activity, the availability of sediments and growth. The specimen's initial response following transfer from atmospheric pressure into the high-pressure aquaria was to immerse into the sediment or to cover more or less parts of the test with aggregated sediments or algae. However, within 24 h a strong rheotaxis became apparent and most specimens moved to sites of increased current activity under normal pH conditions (pH 8). Only few specimens remained in algae cysts or in the sediment in the pH-8 experiment. On the contrary, all specimens under pH 7.4 agglutinated a firm sediment cyst around their test and remained infaunal throughout the experimental period of three months.

Independent of pH, growth was only observed in specimens that lived within an agglutinated cyst or infaunal. A solid thick cyst covered the specimens of the pH 7.4 experiment throughout the experiment and possibly restricted water exchange between the in-cyst water and the surrounding artificial bottom water mass. We suggest that a more fragile and possibly more porous sedimentary envelope may, at least temporally, have covered the infaunal specimens under pH 8 but no evidence for this was found upon termination of the experiment.

1. Introduction

Most *Cibicidoides* species are regarded as epibenthic or epizoic species (Jorissen et al., 1995). Therefore, they are assumed to precipitate their shell in accordance with the isotopic and chemical composition of the surrounding bottom water mass (e.g. Yu and Elderfield, 2008; Mackensen, 2008; Howe et al., 2016; Schmittner et al., 2017; Yu et al., 2010). *Cibicidoides* spp. are assumed to faithfully record bottom water properties such as $\delta^{13}C$ of the dissolved inorganic carbon (DIC) (Woodruff et al., 1980; Belanger et al., 1981; Duplessy et al., 1984). However, Gottschalk et al. (2016) showed species specific offsets for *C. wuellerstorfi* s.s., *C. wuellerstorfi* s.l., *C. pachyderma* (as *C. kullenbergi*), and *C. pachyderma* s.l. and that the offsets are not constant through time (e.g. larger during glacial conditions).

Although different possible scenarios to explain these offsets were presented by Gottschalk et al. (2016) it was and is impossible to deduce the reasons for such inter species-dependent larger or smaller variations in the $\delta^{13}C$-values with changing environmental conditions from fossil records or field studies alone.

For most *Cibicidoides* species an affinity to colonize exposed habitats

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can be deduced from field studies (e.g. Alexander and DeLaca, 1987; Lutze and Thiel, 1989; Linke and Lutze, 1993; Schönfeld, 2002). However, whether the animals are transported as swarmer stages or juveniles to pebbles, filtering metazoa or other elevated places directing in a water current or are able to actively mount to an elevated position could so far only been speculated (Schönfeld, 2002).

Rose Bengal-stained specimens are occasionally found at intermediate deep sediment levels (e.g. Hunt and Corliss, 1993; Wollenburg and Mackensen, 1998b). Without observational evidence it is difficult to judge if they lived there or if they were just buried by sediments during a recent disturbance and unable to emerge to the sediment surface.

Inspired by the pioneering work of Kitazato (1989); Turley et al. (1993); Hemleben and Kitazato (1995), and as a logical consequence of the work by Wollenburg et al. (2015), we have developed high-pressure aquaria for application under the stereomicroscope. The aquaria and associated equipment allow, for the first time, observations on deep-sea *Cibicidoides* spp. under *in situ* pressure. Here we describe the behaviour of *C. pachyderma* specimens following disturbance, their habitat selection, movement rates, taxis and the potential influence of pH on cyst-formation and growth.

2. Material and methods

2.1. Technical set-up

We have developed small high-pressure aquaria for observing deep-sea foraminifera with an inverted microscope and stereomicroscope, respectively. For the experimental set-up under the stereomicroscope used here, we designed a titanium aquarium with windows on just one side (Fig. 1). After the transfer of 5–15 specimens into the seawater filled aquarium it was covered with the window and closed with the window-screw (Fig. 1C-D). Four high-pressure aquaria were installed in a refrigerated worktable mounted on top of an electronic microscopic stage. This set-up allows for continuous observation and documentation of benthic foraminifera at *in situ* temperature and pressure (1–500 bar) (Fig. 2). Following the water depth/*in situ* pressure at which the specimens of the present study were collected we opted for an experimental pressure of 115–30 bar above the coring site at 860 m, for both experiments.

With a high-pressure pump (pump 1, ProStar218 Agilent Technologies) (Figs. 2–3), peak tubing, and multiple titanium valves a continuous isobaric and isocratic one-way seawater flow was directed through the serially arranged high-pressure aquaria (Fig. 3). Specimens were fed with 0.005 mg of a mixture of dried *Chlorella* and *Spirulina* algae every week (Table 1). The algae mixture was dispersed in seawater in an accessory high- pressure aquarium and placed on a magnetic stirrer. Using a second high-pressure-pump (pump 2) seawater with dispersed algae (0.005 mg dried algae/4 high-pressure aquaria) was fed into the high-pressure aquaria.

Fluorescent light (excitation wavelength of 470 nm, emission wavelength > 490 nm) was used to ensure that a sufficient quantity of alga had finally settled at the bottom of each aquarium. Temperature was continuously controlled by sensors connected to the outside of aquarium 1 and 3. With the aid of a combined O_2 and pH measuring device (WTW Multi 3620 IDS) and respective O_2 (WTW FDO®925) and pH (SenTix®980), sensors, pH and O_2 was measured in the seawater outflow three times per week.

2.1.1. Material

Surface sediments were collected with a multicorer (MUC) equipped with 8 tubes each with a diameter of 10 cm during RV Polarstern expedition PS101 on October 17. 2016. To collect a high quantity of *Cibicidoides*-type foraminifers we had chosen a coring site influenced by a strong activity of the Westspitsbergen Current on the Yermak Plateau (Arctic Ocean) (PS101/242-1, 79°27.09′N, 7°30.93′E, 856 m water depth). At this site salinity is 34,98 psu, temperature 2.5 °C and pH 7.6. Mean current activity in Fram Strait is 30 cm/s (Cokelet et al., 2008) and oxygen concentration at this water depth 296–300 mmol/L (Blindheim and Østerhus, 2005). After deployment, the MUC was operated at low speed (0.5 m/sec) to potentially allow for a pressure compensation inside the foraminiferal cells. As the air temperature was similar to the bottom water (3 °C), the sediments were extruded with a MUC extruder piston on deck. The surface centimetre of the sediments and pebbles were transferred in seawater-filled petri dishes (ø20 cm, height 10 cm) and covered with a lid. Stock-cultures were transported into a cold laboratory (3 °C) at the Alfred-Wegener-Institute and aerated with air pumps for common aquaria. Every other week half of the water in each petri dish was exchanged with fresh seawater. Once per month 0.005 mg dried *Chlorella/Spirulina* algae suspension, after sonicating for 1 min in a small vial with seawater, was dispersed over the sediment and pebbles in each petri dish using a small syringe. We started preliminary tests on November 1 and the experiments described here were started on November 28.

2.1.2. Experimental set-up

Cibicidoides pachyderma (morphotype *C. mundulus)*[1] specimens, living attached to pebbles and revealing a strong cytoplasm staining were detached with a cactus-spine under a stereomicroscope. Using a small brush or pipette the specimens were then transferred into small (ø 3 cm) seawater-filled petri dishes in the cold laboratory. Of this stock, 12–15 and 5 specimens were transferred into each high-pressure aquarium for experiment (1) and (2), respectively (Fig. 1A). This *Cibicidoides* density is common at many Arctic sites (Wollenburg and Mackensen, 1998a; Wollenburg and Mackensen, 1998b; Wollenburg and Kuhnt, 2000).

Fine-grained siliceous oxide (1–5 µm) was used as artificial sediment that was provided in one aquarium of experiment (1) and all aquaria of experiment (2).

North Sea water is collected regularly off the coast of Helgoland. For each experiment 100 L of sterile-filtered (0.2 µm mesh) North Sea water was adjusted to a salinity of 34.98 psu by addition of 1 g Instant Ocean® sea salt per L and psu-offset. The natural pH of 8, after equilibration to the cold room atmosphere (at 2.5 °C and at atmospheric pressure), converts to pH 7.6 at 2.5 °C and a pressure of 115 bar (Culberson and Pytkowicz, 1968) for experiment (1). We stored this batch in ten 10 L Schott borosilicate bottles. The air filling the headspace while replacing the outflowing culture water was first ran through a wash bottle to minimize evaporation of the culture water. For experiments (2) 100 L sea water with a natural low pH of 7.4 after salting (at 2.5 °C and atmospheric pressure), converts to pH 6.9 at 2.5 °C and 115 bar (Culberson and Pytkowicz, 1968) was used. This sea water was stored in 5 L gas-tight bags (25 µm PVF Fluor polymer, Sain-Gobain) and pumped from these bags trough the aquaria.

With aid of a combined O_2 and pH measuring device (WTW Multi 3620 IDS) and respective O_2 (WTW FDO®925) and pH (SenTix®980) sensors, pH and O_2 was measured in the seawater outflow three times per week. For this purpose the sensors were installed in two small containers collecting ~ 20 ml and for minimum 2 h connected to the outflow from the overflow valve. As the water volume in the aquaria

[1] *Cibicidoides mundulus* is regarded as junior synonym to *C. kullenbergi* (see Holbourn et al., 2013). *C. kullenbergi* is characterised by strongly curved sutures on the umbilical side. However, as lectotypes of *C. kullenbergi* also reveal straight sutures on the umbilical side (Holbourn et al., 2013 and further references therein) and both species show a large variability in the curvature of their sutures in literature as well as from our own observations, we follow the suggestion of Morkhoven et al. (1986) and Holbourn et al. (2013) and consider *C. kullenbergi* as junior synonym to *C. mundulus*. As genetically *C. pachyderma* and *C. kullenbergi* form a single clade (Schweizer et al., 2009) we assign the *C. mundulus* specimens to *C. pachyderma* despite the fact that they lack the morphological diagnostic features of the latter species.

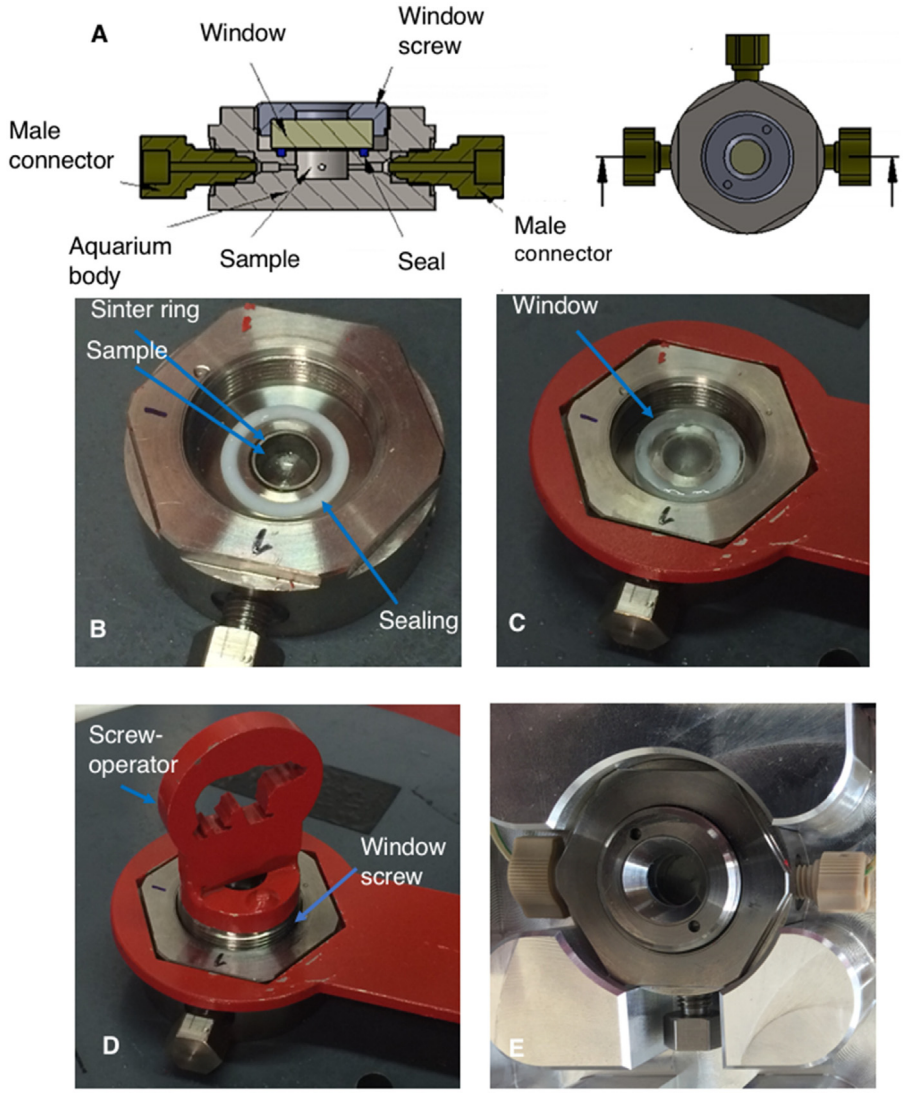

Fig. 1. Construction scheme of the Titanium high-pressure aquaria. **A)** Cross-section. Chamber height and width 4 mm and 9 mm, respectively. Gross volume is 0.318 ml but reduces to 0.251 ml by adding a 1 mm thick sinter ring (porosity 3 µm) to prevent loss of juveniles. **B**) High-pressure aquarium with seawater and foraminifers. **C-E)** Closing the high-pressure aquarium.

was theoretically[2] flushed twice per minute (at a flow rate of 0.6 ml/min), oxygen concentrations measured (340–396 mmol/L) were at all times above the ocean mean for this water depth/pressure and slightly above values recorded for this water depth and area (296–300 mmol/L Blindheim and Østerhus, 2005). The measured pH values dropped, on average, 0.1units when compared to the atmospheric pH values, presumably reflecting a slow adjustment of pH from high to atmospheric pressure (+ 0.47 and + 0.52 for experiment 1 and 2, respectively) (Culberson and Pytkowicz, 1968) and beyond that varied little between the measurements.

The aquaria were installed in the cooling table at 2.5 ± 0.2 °C and maintained at 115 ± 1 bar.[3] During feeding, when additional valves were operated, the pressure fluctuations increased to115 ± 3 bar.

For each experiment 80 L batches of culture water were labelled with Calcein (10 mg/L) allowing for the documentation of experimentally precipitated calcite (Bernhard et al., 2004) and for a better visibility of foraminiferal protoplasm. To observe cell processes under fluorescent light, the aquaria were rinsed with unlabelled seawater from the remaining sterile-filtered batch of 20 L. This was done every 2–3 weeks for two days. As the samples were not exposed to any currents during the time of storage, seawater was pumped at a rate of 0.3 ml/min during the first month and at 0.6 ml/min for the last two months through the aquaria (Table 1). This equals a current speed of 0.1–2.5 cm/min and 0.2–5 cm/min for the aquaria as a whole and at the hole inside the sinter ring and at pumping rates of 0.3 ml/min and 0.6 ml/min, respectively. Current velocities at pumping rates of 0.6 ml/min match current activity values at the coring site in Fram Strait (Cokelet et al., 2008). To test a potential influence of current speed on movement and general behaviour of the *C. pachyderma* specimens water exchange was stopped twice for 24 h in week 6 of the pH 8 experiment. The experiments ran for three months.

[2] Pumping water into an existing water body creates a constant mixing not a constant complete exchange of old by fresh water.

[3] The ProStar218 HPLC pump used for our experiments is equipped with a pulsation damper to ensure a constant pumping of liquids. Minor pressure fluctuations noticed in pilot tests with just one high-pressure aquarium (see supplements) and no valves installed, are further reduced by the installation of 4 high-pressure aquaria in a row and many valves.

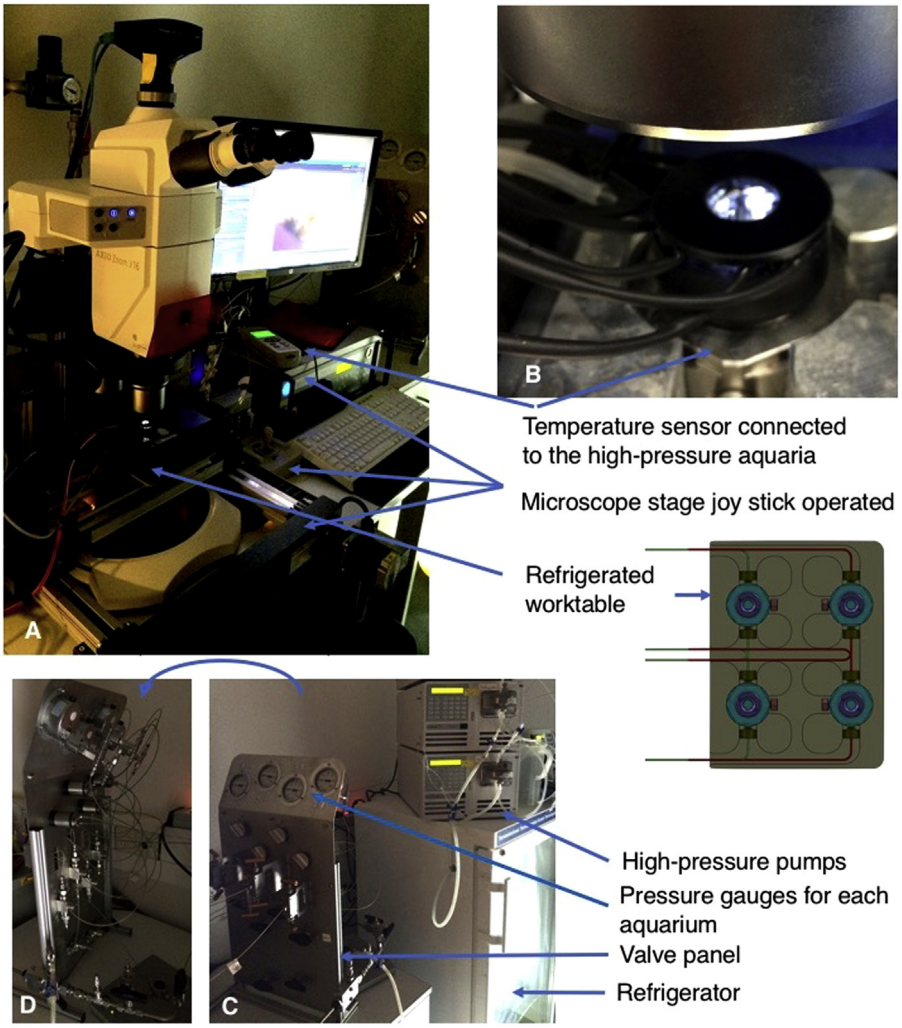

Fig. 2. Technical set-up of high-pressure culturing. **A)** Cooling table with 4 high-pressure aquaria mounted on a microscope stage under a Zeiss Axio Zoom V16. **B**) High-pressure aquarium with illumination and ventilation system. **C)** Seawater stored in a refrigerator is directed by 1 or, during feeding 2, high-pressure pumps through the high-pressure aquaria. The combination of multiple three- and four-way valves allows to direct seawater (and e.g. food) through all high-pressure aquaria in line or to separate one or more individual aquaria from seawater exchange. **D)** Back of valve board showing the connecting tubing.

3. Results

3.1. Experiment 1 at pH 8.0 - behaviour following detachment

Cibicidoides pachyderma was the most mobile *Cibicidoides* species in our experiments so far. Within less than 24 h after transfer all *C. pachyderma* specimens disappeared in the artificial sediment if provided (Fig. 4). In those aquaria without artificial sediment but dispersed and settled alga *C. pachyderma* immediately started to collect algae, forming mounds or cysts covering more or less parts of their test (Figs. 5 and 6). Even smaller *C. pachyderma* specimens were aggregated by larger ones during the first day of the experiment. That way, groups of up to eight specimens in one algae mound were formed (Fig. 5). Over the remaining days of the first week, especially small-sized specimens then left the *Cibicidoides*-groups, whereas, equal-sized *C. pachyderma*-couples often persisted to the end of the experiment (Fig. 5). In aquaria without artificial sediment, after two days most specimens that were not stuck in algal aggregates moved towards the porous sinter ring (Fig. 6). One week into the experiment, the majority of *C. pachyderma* were located in the vicinity of the sinter ring (out- and inside, top and bottom) in all aquaria (with and without artificial sediments) (Figs. 7 and 8). The specimen density was highest in the area of inflowing water, with a minor secondary maximum at the seawater outlet (Fig. 6). The fastest specimens made it to the hole inside the sinter ring were the inflowing seawater current is highest and once per month algae were entering. At all sinter ring holes a rhizopodial network of a nearby specimen collected algae from the inflowing current (Wollenburg et al., in prep.). Small *C. pachyderma* even moved into the holes (Fig. 7). Movement rates progressively slowed down over the first week and thereafter the overview pictures of the aquaria changed little for the remaining time (Fig. 5).

3.2. Experiment 1 at pH 8.0 - locomotion and velocity

In order to move *C. pachyderma* specimens position their aperture towards the bottom of the aquarium and orient the coiling axis of the test in a horizontal position (Fig. 9). Different to the resting or attached living mode when the test with a vertically oriented coiling axis offered a minimal load versus currents, during movement the test thus, became a large target for currents. For 3 months and five times per week overview photographs of all 4 aquaria and additional photographs of all specimens were taken. They allowed for an assessment of a 'minimum speed' more precisely the calculation of the minimum distance between two observations and the time elapsed for all visible specimens and times. Following the very active first two days, this 'minimum speed' during movement amounts to roughly 0.01–14 mm/d per moving specimen. When we were able to actually

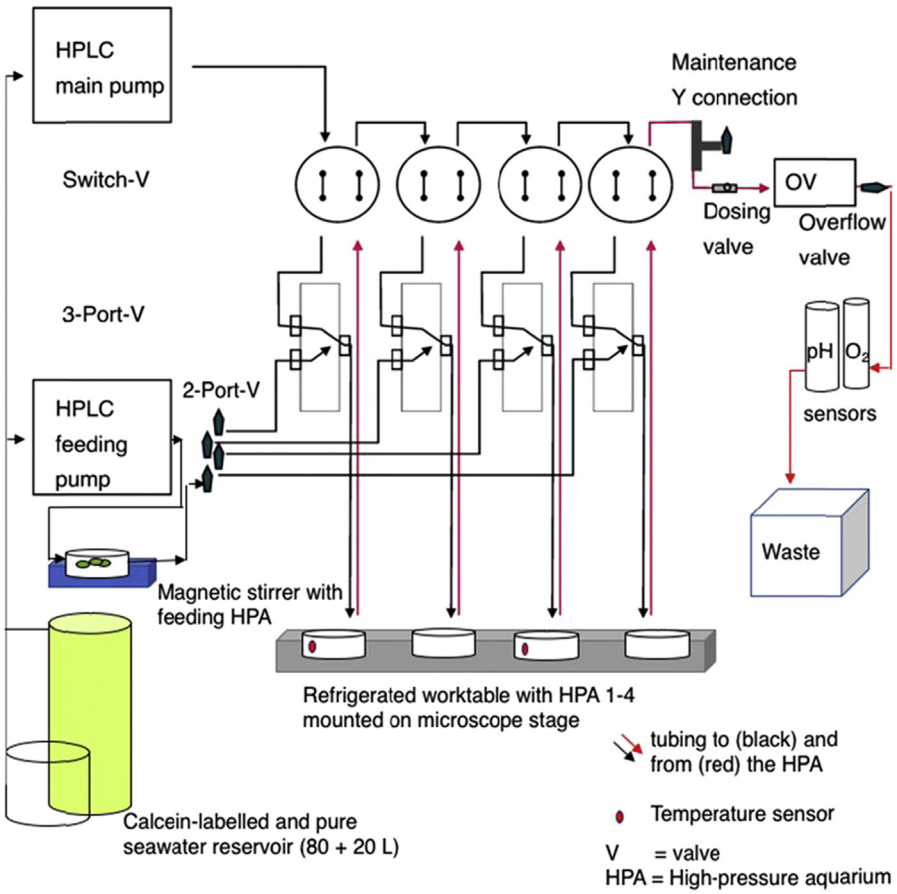

Fig. 3. Sketch of the seawater flow through the high-pressure aquaria (HPA) and the arrangement of pumps and valve systems. Black arrows indicate tubing to/seawater inflow, red tubing from/the outflow from the HPA. Switch-valves allow to separate one or more individual HPA from the serially arranged seawater flow from the main pump via aquarium 1 through 4 over a dosing and overflow valve out into the seawater waste container. Three-port-valves allow to direct water from the main and/or the "feeding" pump into each HPA separately. Two-port-valves finally terminate each seawater passage and can be closed to keep the pressure at the set level during maintenance or at times when this passage is not used. Illumination system and microscope is not shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 1
Basic parameters of the two experimental set-ups.

Experimental running time 3 months	Experiment 1		Experiment 2	
	First month	Last two months	First month	Last two months
Pressure (bar)	115	115	115	115
Temperature (°C)	2.5	2.5	2.5	2.5
pH at atmospheric pressure	8.0	8.0	7.4	7.4
Feeding (*Spirulina*/*Chlorella* 1:1)	Once a week	Once a week	Once a week	Once a week
maximum current velocity	0.3	0.6	0.3	0.6
no. specimens per aquarium	12, 15, 15, 15	12, 15, 15, 15	5, 5, 5, 5	5, 5, 5, 5

observe moving specimens (5×) during our work at the microscope they moved with a speed of roughly 10 µm/min (Fig. 9).

3.3. Experiment 2 at pH 7.4 - behaviour following detachment

In the treatment pH 7.4 all aquaria had artificial quartz sediment. Within 24 h after transfer, all *C. pachyderma* specimens immersed in the sediment and most specimens completely disappeared from sight for the next 3 months.

Three specimens were close enough to the sediment surface to identify their position under fluorescent light by either the emission of greenish fluorescence of their cytoplasm or a reddish fluorescence induced by ingested alga (Fig. 10). *C. pachyderma* specimens in the low pH treatment not only covered themselves with sediment but formed an agglutinated cyst (Fig. 10B-E).

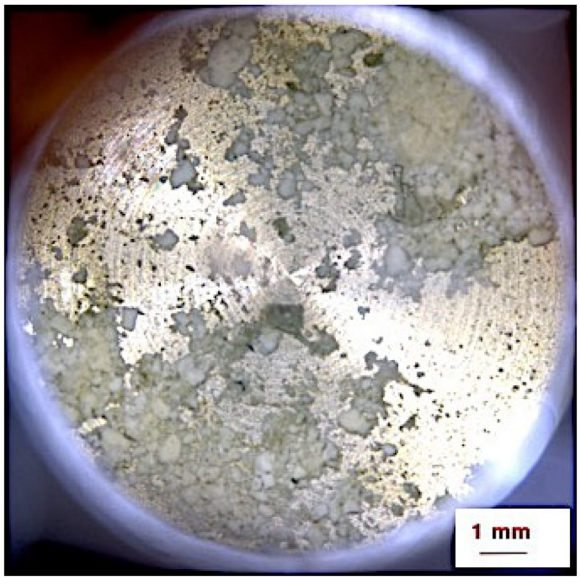

Fig. 4. Overview of aquarium 4 (experiment 1) with artificial fine-grained quartz sediment 24 h after the start of the experiments. *C. mundulus* specimens are covered with sediment and not visible.

Not a single specimen resurfaced from the sediment or even climbed the sinter ring to an elevated position for the entire 3 months of the experiment. Upon termination of the experiment we found all living[4] *C.*

[4] Assessment as 'living' required a fluorescent cytoplasm and pristine test (Figs. 10b, 11a-b).

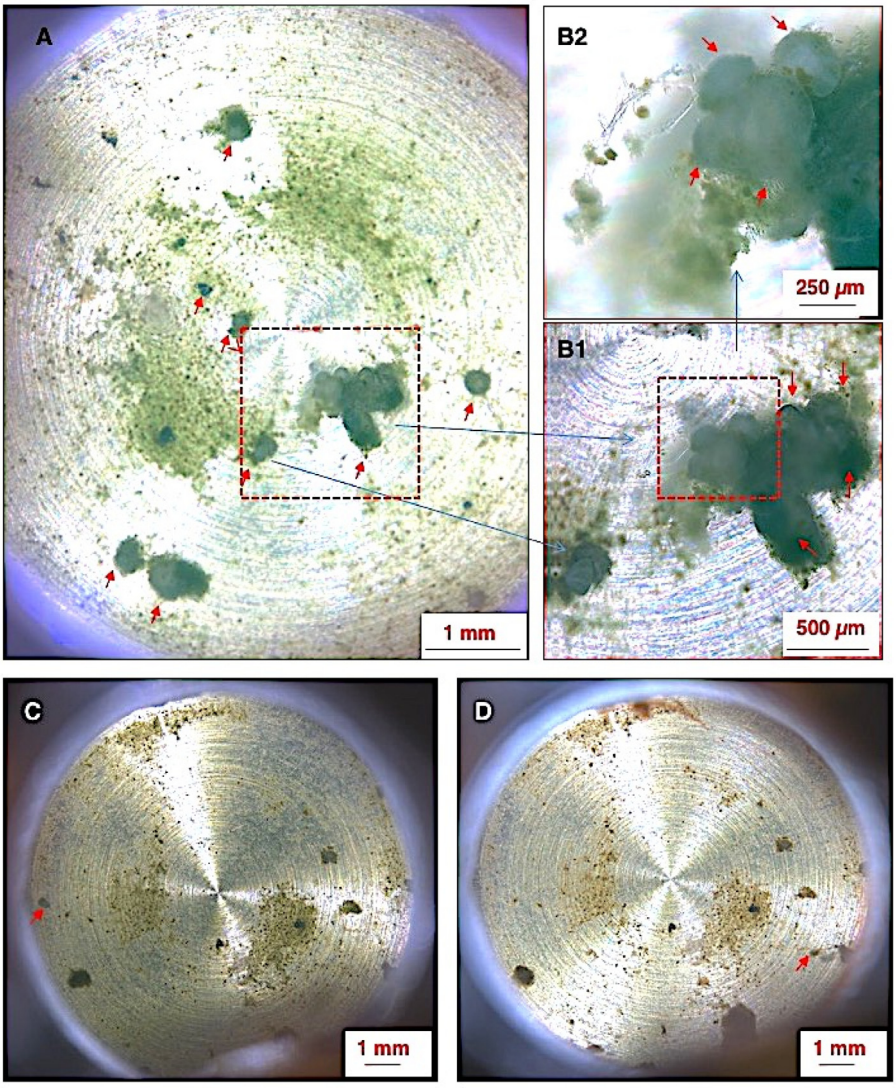

Fig. 5. A) Overview of aquarium 1 24 h after the start of experiment (1) (March 24.2017). *Cibicidoides pachyderma* specimens originally positioned in the aquarium's centre efficiently gathered the evenly distributed algae and eventually other *C. pachyderma* specimens to form mounds or loose cysts. **B1-B2)** Details of a mound containing 8*C. pachyderma* specimens. **C)** Overview on March 28.2017. **D)** Overview on June 23.2017. Red arrows indicate position of *C. pachyderma* specimens or *C. pachyderma* groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

pachyderma specimens (8 out of 20, see below) covered by a firmly agglutinated sedimentary cyst devoid of algal remains (Figs. 10 and 11).

3.3.1. Mortality and growth

Fifty-four living specimens were placed in the pH-8 treatment. Forty-six specimens were positioned with the spiral side facing the aquarium floor, eight with the spiral side towards the water column. Four of these 'wrongly-positioned' specimens died the others flipped after 4–6 weeks. A total of 20*C. pachyderma* specimens were placed in the pH-7.4 treatment, all positioned with the spiral side facing the aquarium floor. Eight specimens survived the low pH-treatment, the others died during the experiment and their tests revealed a whitish colour, a sign of initial carbonate dissolution that contrasts with the translucent tests of the living ones (Fig. 11). Each of the 8 surviving specimens showed growth of one to two chambers (Fig. 11). In contrast, in the pH-8 treatment growth was only observed in specimens that settled in artificial sediment and not in any of the 47 living specimens that climbed the sinter ring or rested in algal aggregates.

4. Discussion

Based on Rose Bengal-stained specimens at spatial variable depths in the sediment column *C. pachyderma,* with its respective morphotypes, has been assigned to an epizoic (e.g. Lutze and Thiel, 1989) or facultative endobenthic (e.g. Licari, 2006) living mode. Food availability may be a living mode determining parameter. Hereby, it is assumed that at oligotrophic sites such as the oligotrophic flank of the Walvis Ridge (200–3700 m) *C. pachyderma* (*C. kullenbergi* morphotype) lives epifaunal or shallow infaunal (Schmiedl et al., 2000). In contrast, at eutrophic sites this species lives predominantly endobenthic. For instance, at intermediate to deep-water sites in the Benguela upwelling an average living depth (ADL) (Jorissen et al., 1995) of 1.5 cm and living specimens up to 5 cm below the sediment surface have been reported (Licari, 2006). Based on our observations we argue that the infaunal habitat of *C. pachyderma* in these areas is probably related to the low pH (< 7.4) caused by the decomposition of organic matter at the sediment water interface (Flohr et al., 2014) and not to nutrition. The infaunal habitat and a rigid sedimentary cyst obviously helps the foraminifera to escape low.

In the modern Arctic Ocean where we collected the samples for this

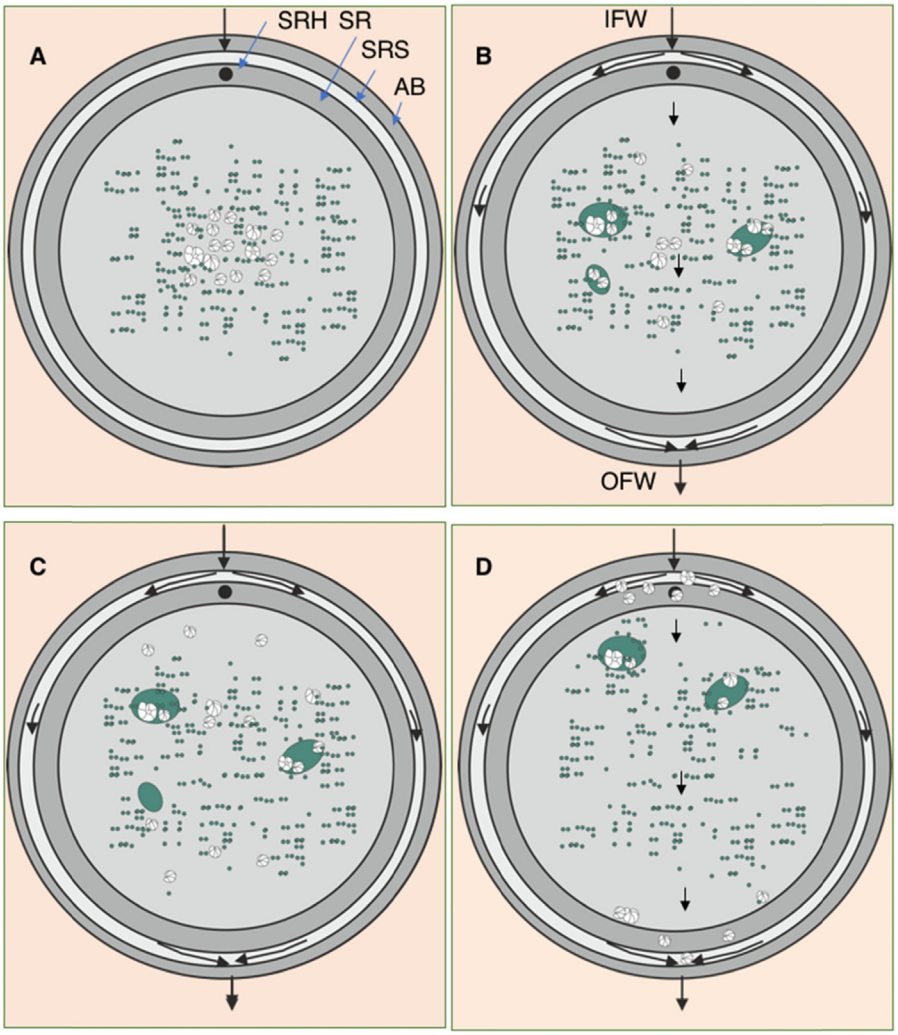

Fig. 6. Schematic sketches showing the behaviour and movement patterns of *C. pachyderma* specimens during the substrate-free aquaria of experiment (1). **A)** Start of the experiment: All *C. pachyderma* specimens were placed in the centre of the aquarium. **B)** 24 h after the start of the experiment. The specimens had accumulated algae forming mounds and loose cysts, and forming groups of up to 8 individuals. **C)** 48 h after the start of the experiment. Rheotaxis became apparent and the first individuals detached from the foraminiferal groups and moved towards areas of maximum current activity (positive and negative rheotaxis). **D)** One week after the start of the experiment most specimens had optimized their position with respect to highest current activity and competing specimens. High densities are observed on and behind the sinter ring with maxima in the area of inflowing seawater. Green dots indicate settled *Spirulina* and *Chlorella* algae. AB = aquarium sidewall, SR= sinter ring, SRS = space between AB and SR, SRH = hole inside the sinter ring, the inflow of seawater and algae, IFW = inflow of seawater, OFW = outflow of seawater. Arrows indicate direction of water flow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

study, *C. pachyderma* (*C. mundulus* morphotype only) is confined to areas of minimum annual sea ice cover and thus moderate carbon fluxes north and west of Spitsbergen (Lalande et al., 2014). Although the specimens for this study were collected from densely colonized pebbles, living specimens were also found in the upper centimetre of the sediment. Therefore, an exclusive epizoic habitat for the Arctic *C. mundulus* morphotype cannot be concluded from our field samples.

The samples for this study were collected on October 17 during the return trip, and over a time span of 8 days transported to the Alfred Wegener Institute in Bremerhaven. After two short pilot tests the experiments described here started with the pH 7.4 treatment on November 28, followed by the pH 8.0 treatment on February 27. It could be argued that after maintaining the samples for several weeks at atmospheric pressure may have affected the behaviour of the specimens at least at the beginning of experiments. However, the change from the initial infaunal to a epifaunal mode of living observed in the pH 8 experiments was not observed in the pH 7.4 treatment although this experiment was carried out 3 months earlier. Furthermore, the specimens used for this study were picked from the upper part of a large pebble that in the field faced into the water column, and all specimens that we had picked were covered by a loose sediment coverage/cyst on this pebble. Therefore, the behaviour of foraminifera in the pH 8 treatment reflected the *in situ* behaviour.

In our experiments, the immediate response of dislocated and transferred *C. pachyderma* specimens was to immerse in the provided artificial sediment. This reaction may be stimulated by stress and inherited from their Neoproterozoic naked or softwalled ancestors (Pawlowski et al., 2003) that choose to live in rather than on the sediment. Support for this suggestion comes from the fact that *C. pachyderma* in the sediment-free aquaria immediately began to cover their tests by aggregating of whatever was in reach of their rhizopoda (algae and other foraminifers). The formation of such a cyst or 'sediment' envelope protects the shell and extracellular cell body and can be regarded as very basic behaviour with a long evolutionary history (Heinz et al., 2005 and further references therein). Although cyst formation has not been described for *C. kullenbergi, C. mundulus* and *C. pachyderma* the formation of cysts even covering an extended rhizopodial network have been described for various other *Cibicidoides* species and may thus be a common feature of the genus (e.g. Nyholm, 1961; Lutze and Thiel, 1989; Heinz et al., 2005; Hancock et al., 2015). Furthermore, the experimental specimens were freed from an agglutinated covering prior the experiments.

Under the 'normal marine' conditions (pH 8.0), cyst formation and algae aggregation stopped approx. 24 h after the onset of the experiment. At the same time the first specimens resurfaced from the sediment or left the large phytodetritus-foraminifera aggregates in aquaria with and without sediments, respectively. After resurfacing, specimens demonstrated a strong rheotactic response and moved rather straight towards the place of highest current velocity or close to it (Figs. 7 and 8). Zigzagging was not observed but 3 specimens on the bottom of the aquarium close to a sinter ring were observed to move repeatedly behind and in front of the sinter ring possibly in search for the best conditions (Fig. 5C-D). If movement was observed, the movement rates of *C. pachyderma* (morphotype *C. mundulus*) in our high-pressure

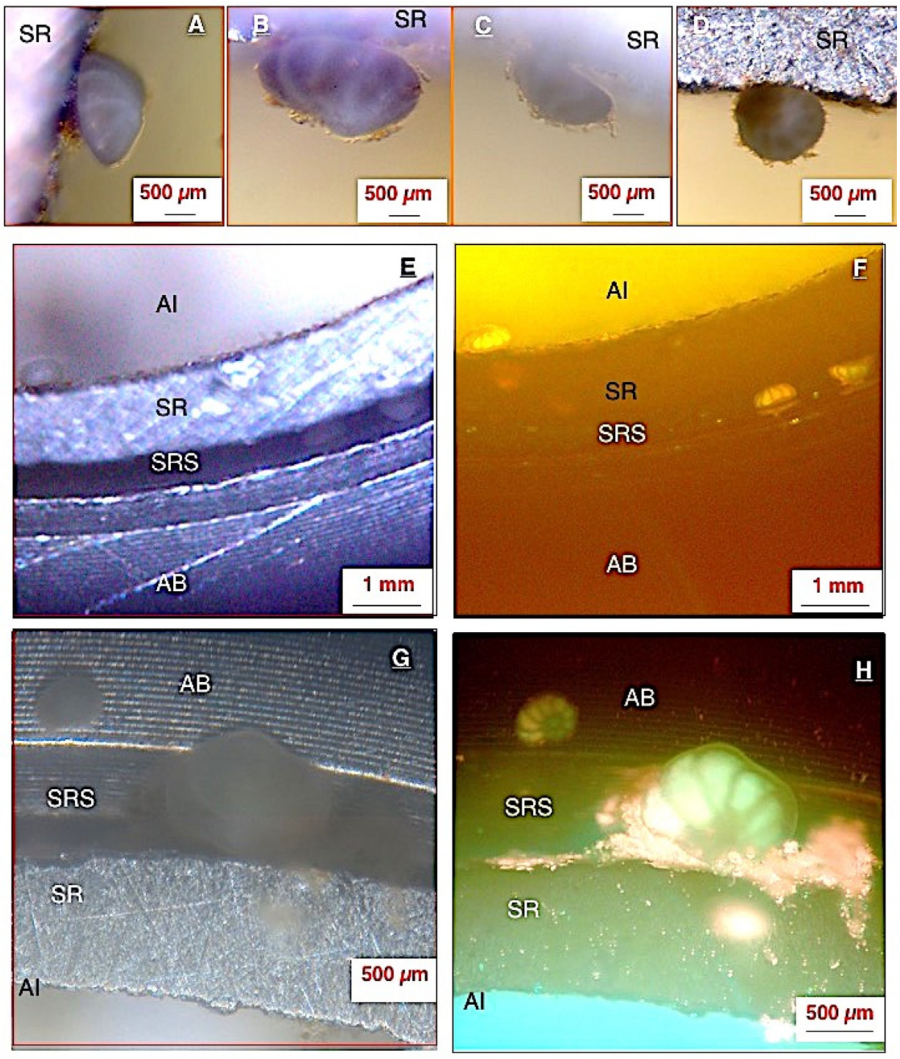

Fig. 7. *Cibicidoides pachyderma* specimens in a more or less fixed position on and behind the sinter ring from day 3–7 to the end of experiment (1). **A-D)** Specimens positioned on the upper part of the sinter ring, examples from aquarium 1, 2, 3, and 4, respectively. **E-F)** *Cibicidoides pachyderma* specimens positioned on the sinter ring facing to the interior of the aquarium and inside the lumen between sinter ring and aquarium sidewall (examples from aquarium 2). E) *Cibicidoides pachyderma* specimens under normal light. F) *Cibicidoides pachyderma* specimens under fluorescent light revealing a bright fluorescence of foraminiferal protoplasm. **G-H)** *Cibicidoides pachyderma* specimens positioned on the sinter ring facing the aquarium glass and above the lumen between sinter ring and aquarium sidewall (examples from aquarium 1). G) *Cibicidoides pachyderma* specimens under normal light. H) *Cibicidoides pachyderma* specimens under fluorescent light revealing a bright fluorescence of foraminiferal protoplasm. AB = aquarium sidewall, AI = aquarium interior, SR = sinter ring, SRS = space between AB and SR.

aquaria matches those described for *C. pachyderma* (morphotype *C. pachyderma*) from shallow water sites and cultured at atmospheric pressure (1–23 mm/d, Bornmalm et al., 1997). However, once positioned in what appears to be an optimal place with respect to the current, specimens remained stationary independent of current velocity (0 or 50 cm/s) or whether food was provided or not.

At most sites and in most down-core records the $\delta^{13}C$ values of *C. pachyderma* and *C. wuellerstorfi* are comparable with no or only minor offsets between both species (Gottschalk et al., 2016 and further references therein). However, at sites (e.g. Licari, 2006; Eberwein and Mackensen, 2006) or times (Martínez-Méndez et al., 2013; Gottschalk et al., 2016) with high carbon export fluxes significantly lower $\delta^{13}C$ values are noted for *C. pachyderma*. From the combined analyses of down-core distribution of Rose Bengal-stained specimens and corresponding differences in the $\delta^{13}C$ of *C. pachyderma* and *C. wuellerstorfi*, Licari (2006) concluded that *C. pachyderma* is a facultative epi-, yet, preferential endobenthic dweller with a preference for chamber formation in the sediment. The results from our treatment at pH 8 (normal conditions) demonstrated two, at first glance, contradicting behaviours. Cyst formation and rheotaxis are suggestive for an infaunal and epizoic habitat, respectively. Whereas, in the low pH treatment the specimens stayed infaunal throughout the experiment, the majority of specimens in the normal pH treatment became epifaunal within 24 h. The specimens that finally occupied locations of high current velocity in the vicinity of the sinter ring usually lacked a sediment envelope, whereas a firm sediment layer or cyst covering at least two thirds of the tests was characteristic for specimens collected from high current locations such as pebbles on the ocean floor. Only two specimens on top of the sinter ring had started to accumulate algae and sediments when we terminated the experiment (Figs. 7H; 8A-B). We assume that the lack of sediment at elevated positions in the aquaria in our experimental setup limited a more pronounced cyst-formation.

The specimens selected for both treatments were equally vital i.e. they were mobile and had ingested laboratory fed *Chlorella/Spirulina* algae as indicated by the bright greenish staining of the protoplasm contrasting the brownish colour resulting from a typical Arctic diatom food source. However, growth was only observed in aquaria containing artificial sediment, and in specimens that lived in the sediment. In the pH 7.4 treatment, two chambers were added on average when the specimens were covered by a complete and firm sedimentary envelope (Figs. 10 and 11). A less rigid cyst may have covered the 3 specimens during growth in the pH 8.0 treatment but were not present anymore when the experiment was terminated. This is in line with field observations and culture experiments for some shallow-water and deep-water taxa that demonstrate the importance of a sedimentary envelope, at least covering the apertural area, during growths (see Heinz et al., 2005 for a comprehensive overview).

A cyst, in association with the cytoplasmic envelope, provides protection and a barrier from the ambient environment that allows to create a distinct microhabitat for cell processes such as chamber formation. It has been shown that the cytoplasmic envelope serves to separate the site of calcification from the surrounding seawater and

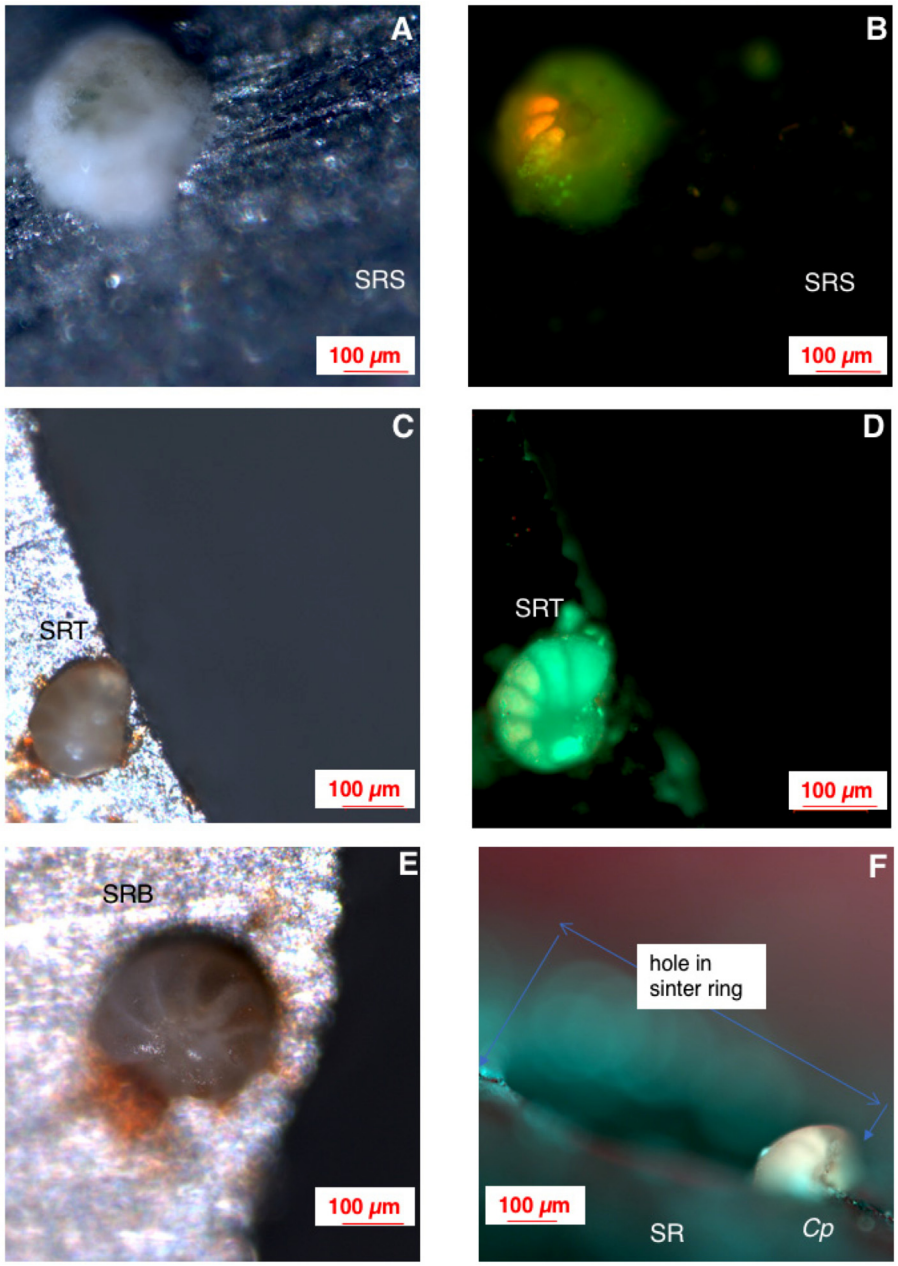

Fig. 8. Examples of *C. pachyderma* specimens firmly attached to various parts of the sinter rings immediately after the termination of experiment (1), photographed in air. Pictures were taken under normal light (left column) and fluorescent light (right column). **A-B)** Aquarium 4 with artificial sediments, *C. pachyderma* specimen attached to the outer part of the sinter ring facing the lumen between sinter ring and aquarium sidewall. The specimen shows a thin agglutinated cyst that covers the marginal part of its test. B) The red colour apparent under fluorescent light results from ingested algae. The bright greenish fluorescent dots in front of the last chamber are the outer tips of a rhizopodial network inside the cyst that was likely extended to define the shape of new chamber prior to precipitation. **C-D)** Aquarium 2 showing a *C. pachyderma* specimen originally positioned in the space between sinter ring and aquarium glass. C) The specimen had fixed itself on top of the sinter ring potentially with an organic glue indicated by the reddish-brown colour,[5] like other *Cibicides* taxa (e.g. Hancock et al., 2015; Dubicka et al., 2015). D) Analyses of the same specimen under fluorescent light revealed that food/alga was stored in the specimen except for the last 2 chambers. **E)** Aquarium 3 showing a *C. pachyderma* specimen that had fixed itself upside down to the bottom of the filter ring. Also note the reddish-brown colour of the presumed specimen's organic adhesive (see above). **F)** Aquarium 1 showing a *C. pachyderma* specimen under fluorescent light that had fixed itself inside the hole of the sinter ring. SR = sinter ring, SRS = space between aquarium sidewall and sinter ring, SRT = top of the sinter ring, SRB = bottom of the sinter ring. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

possibly controls the flux of CO_2, H^+ and Ca^{2+} (Toyofuko et al., 2017). As a consequence of the outward proton flux, the pH in the water around the specimen drops by 0.5–1.3 units to a minimum of 6.3 (Glas et al., 2012; Toyofuko et al., 2017). A firm sedimentary envelope around a specimen may cage this water exchange reservoir. Biotic and abiotic processes even beyond the calcification process may besides pH also alter the carbonate chemistry and $\delta^{13}C_{DIC}$ of the water exchange reservoir. By changes in pH alone a significant deviation between bottom water $\delta^{13}C_{DIC}$ and $\delta^{13}C_{foraminifera}$ can be expected (Hesse et al., 2014) leaving aside that also the in-cyst $\delta^{13}C_{DIC}$ might be altered. As stated earlier negative offsets between bottom water $\delta^{13}C_{DIC}$ and $\delta^{13}C_{C.\ pachyderma}$ as well as $\delta^{13}C_{C.\ wuellerstorfi}$ and $\delta^{13}C_{C.\ pachyderma}$ in areas or at times of high carbon flux have been described (see Gottschalk et al., 2016 and further references therein). Provided that *C. wuellerstorfi* lacks a comparable firm sedimentary envelope to *C. pachyderma,* these offsets at times of high carbon flux and thus likely low pH could at least in parts be caused by such a cyst-effect for *C. pachyderma*. However, more experimental data are needed to confirm such hypotheses from these very first insights.[5]

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[5] The reddish-brown colour may stem from incorporated Calcein that in dry state has the same colour.

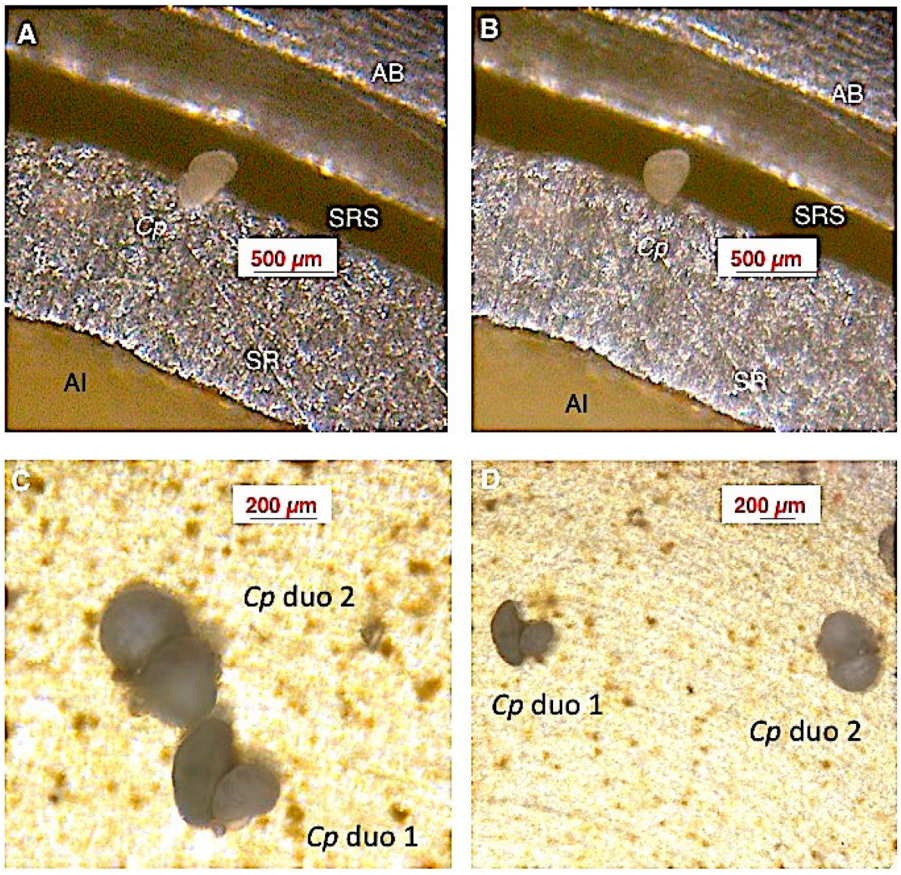

Fig. 9. Movement of *C. pachyderma* in experiment (1). **A-B)** *Cibicidoides pachyderma* on its way to a position in the lumen between sinter ring and aquarium sidewall (aquarium 1). Photos are taken 3 min apart. **C-D)** Movement of two *Cibicidoides pachyderma* coupless on day 5–6 in aquarium 2. Photos were taken 20 h apart. AB = aquarium sidewall, AI = aquarium interior, SR sinter ring, SRS = space between AB and SR.

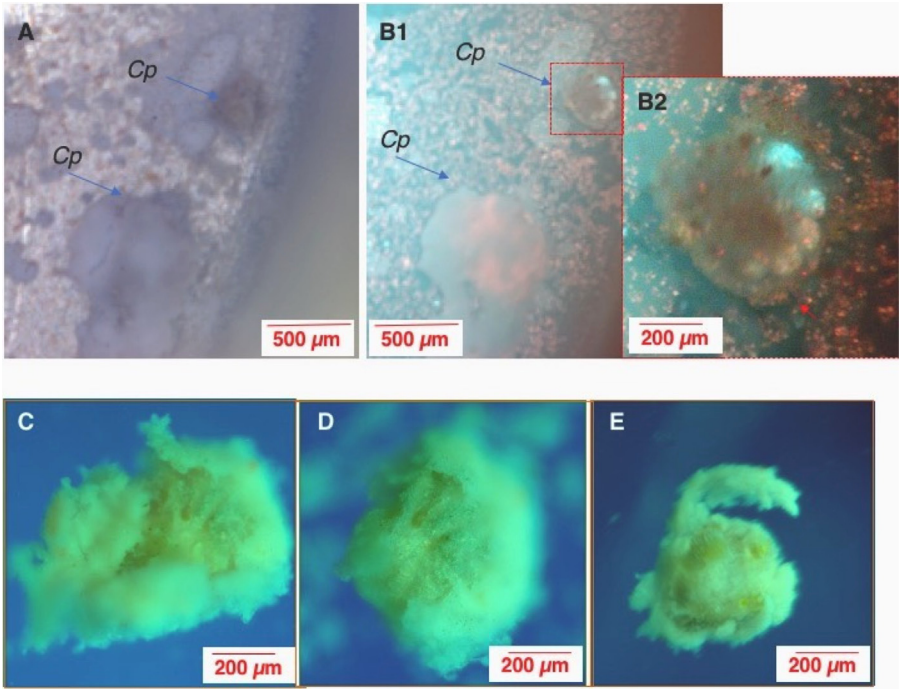

Fig. 10. pH 7.4 treatment (experiment 2). **A-B)** Two *C. pachyderma* specimens covered by a thin layer of sediment. A) Normal light. B) Fluorescent light. The blue arrows point to the firm sediment cyst surrounding the specimen. **C-E)** *Cibicidoides pachyderma* specimens partly freed from their sedimentary cyst with a brush after the termination of the experiment, photographed in air under normal light. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

[5] The reddish-brown colour may stem from incorporated Calcein that in dry state has the same colour.

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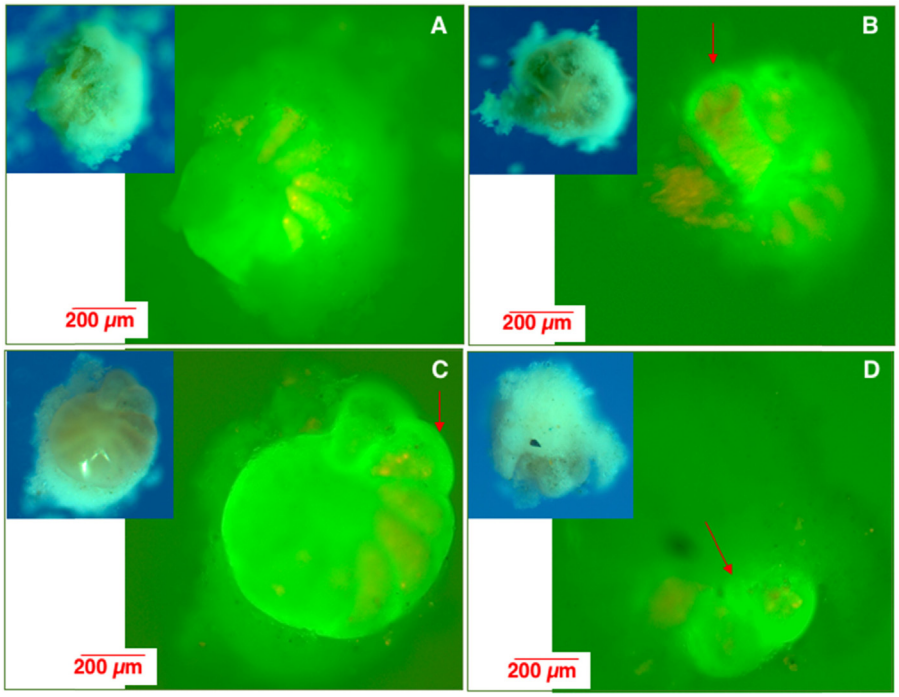

Fig. 11. Sedimentary cyst and growth of specimens from the pH 7.4 treatment (experiment 2), photographed in air. Each panel shows the same specimen once photographed under normal light (blue background) and once under fluorescent light (greenish image), respectively. Growth is indicated by a bright greenish fluorescence of calcite precipitated during the incubation. The red arrows indicate the penultimate chambers that were formed during the experiment (in specimen B, C, and D). For species A this is obscured due to the strong fluorescence of calcite precipitated on older test parts, and fluorescent cytoplasm. The size bar refers to the fluorescent image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr.2018.07.006.

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