Elevated CO₂ effects on morphology and survival rate in intertidal benthic foraminifera

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Abstract 28

Oceans are absorbing about one-third of the anthropogenic CO₂ from the atmosphere, and 29
the excess CO₂ leads to reduce the seawater pH, carbonate ion concentrations and saturation 30
states of biologically important calcium carbonate minerals. The ocean acidification affects many 31
calcifying organisms such as foraminifera. The present study assessed experimentally modified 32
ocean pH impacts on survival rate and shell morphology variation in three species of intertidal 33
benthic foraminifera. Foraminifera was collected from Parangipettai coastal waters, Tamilnadu, 34
India. Foraminiferal specimens were cultured for a period of five weeks at three different pH 35
treatments that replicated future scenarios of a high CO₂ and low pH. The experimental results 36
revealed that reduction of seawater pH significantly affected foraminiferal survival and 37
morphology. Scanning Electron Microscopic observations revealed significant changes in 38
foraminiferal morphology with clear evidence of dissolution and cracking processes on the test 39
surface, and reduction in teeth structure in treatments with decreasing pH. Hence, altering the 40
seawater chemistry might have extensive impacts on benthic foraminifera. 41

Keywords: Foraminifera, Scanning Electron Microscopy, pH, CO₂, ocean acidification 42

1. Introduction 48

The Ocean absorbs the anthropogenic CO₂ from the atmosphere and it changes the ocean's fundamental chemistry (Orr, 2011). The pH in ocean surface water has decreased by 0.1 units since the pre-Industrial Revolution and is now expected to drop further up to 0.4 units by the end of the 21st century (Calderia and Wicket, 2005). The atmospheric CO₂ concentration level of 280 ppm in the beginning of industrial revaluation increased to 393 ppm in 2012 (Maona Loa Observatory, 2012) and the level is currently increasing at the rate of 0.5 % per year (Forster et al., 2007). In this industrial era, oceans have absorbed approximately 30 % of CO₂ from the atmosphere due to the burning of fossil fuels and land use change (Sabine et al., 2004; Raven et al., 2005; IPCC, 2014). The observed CO₂ leads to reduce the seawater pH and carbonate ion concentration, and this process is known as ocean acidification (Calderia and Wicket, 2003).

Ocean acidification is likely to have a negative effect on calcifying marine organisms such as mollusks corals, some microalgae, foraminifera, and calcifying algae. Shells, skeletons, and other protective structures of the marine organisms are mostly made up of calcium carbonate materials (CaCO₃) (Zeebe and Sanyal, 2002; Riebesell and Tortall, 2011). Aragonite, calcite and magnesium calcite (Mg-calcite) are the three-major biogenic CaCO₃ occurring in the seawater (Gattuso and Hansson, 2011). The concentration of carbonate ions and the CaCO₃ saturation state are controlled the calcium carbonate precipitation where decrease processes with increasing the ocean acidification (Gattuso and Hansson, 2011).The response of marine calcifiers to decreasing calcium carbonate saturation state will be species-specific and depend on environmental parameters such as light, temperature, and available nutrients, the form of carbonate mineralogy and the mechanism of calcification (Feely et al., 2004) Although the chemistry of ocean acidification is well understood, and its impact on marine organisms and ecosystem remains in some cases poorly known (Gattuso et al., 2010). The changing seawater carbonate chemistry

directly affects calcareous skeletal structures of the marine organisms (Fabry et al., 2008), and the high $p\text{CO}_2$ treatment reduces calcification rate in benthic foraminifera (Kuroyanagi et al., 2009; Dissard et al., 2010; Fujita et al., 2011; Haynert et al., 2011; Uthicke et al., 2013).

Foraminifera is one of the most abundant groups of calcifying organisms, approximately precipitate Ca. 50% of biogenic calcium carbonate in the open oceans (Faber and Preisig, 1994; Schiebel, 2002). Most of the foraminifera test walls are organic, agglutinated or made of calcium carbonate in various chemical and structural modifications (Hemleben et al., 1986). Calcium carbonate forms are composed of either magnesium calcite, calcite or aragonite (Hemleben et al., 1986). Approximately 900 described genera deposit tests of calcium carbonate (Lipps, 1973). Foraminifera shells are composed of primary and secondary layers of calcite (Erez, 2003), primary layer consists of high- Mg calcite (Bentov and Erez, 2006) and the second layer consists of low-Mg calcite (Haynert et al., 2011). Calcite producing foraminifera are one of the most important groups of calcite producers. They precipitate annually 1.4 billion tons of calcite, which accounts for 25% of the total global calcite production (Langer, 2008).

Foraminifera are an interesting group to examine in relation to ocean acidification experiments, they distributed worldwide, are environmentally sensitive, have short life-history and they are important ecosystem engineers (Jones et al., 1994). Some foraminifera species showed sensitivity to ecological conditions (Faber and Preisig, 1994). The most of the ocean acidification research on foraminifera has been published in the last 8 years with a variety of responses such as increased shell weight with increased carbonate ion concentration (Bijma et al., 1999, 2002), diversity reduction and species abundance shift from 24 to 4 species in natural pH levels (Dias et al., 2010), high $p\text{CO}_2$ site impact many foraminifera tests with corroded or pitted (Fabricius et al., 2011).

In recent years, the atmospheric $p\text{CO}_2$ levels have increased in the southwest Bay of Bengal (Shanthi et al., 2016). CO_2 flux indicates that the southeast Bay of Bengal acts as a significant sink of CO_2 from the atmosphere (Singh and Ramesh, 2015). In the present study area of coastal Parangipettai in southeast Bay of Bengal, the $p\text{CO}_2$ level ranged from 377 to 390 μatm and seawater $p\text{CO}_2$ levels ranged from 156 to 980 μatm . The present study investigated the survival and morphology of benthic foraminifera *Ammonia beccarii*, *A. tepida* and *A. dentata* in three different pH treatments that replicated future scenarios of a high atmospheric CO_2 and low pH.

2. Materials and methods

2.1 Sample Collection

The surface sediments scrapes (1-2 cm depth) containing benthic foraminifera were collected from the Parangipettai coastal waters, Bay of Bengal, Tamilnadu, India ($11^\circ 30' 45.06''$ N and $79^\circ 46' 29.18''$ E) during low tide in April 2016. The sediment sample was transferred to the laboratory, and then they were mixed and sieved over a set of 63 μm and 1000 μm screens.

2.2 Foraminiferal isolation

By simple observations of a small amount of sediment through a compound binocular microscope, live specimens of *A. beccarii*, *A. dentata* and *A. tepida* were identified. Those individuals showed colorful protoplasm extensively distributed across the entire foraminiferal tests, except in the last chamber. Despite the naturally intense protoplasm color is widely used as an indicator of the viable foraminiferal individuals (Bernhard 2000), pseudopodia activity was observed to confirm the viability. The procedure of sampling sieving and observation of live specimens was repeated for two days until a large number of target foraminifera was achieved

(10 -15 specimens/ cm³ of wet sediment). Approximately 100 cm³ of mixed sediment was placed in a series of 500 cm³ filtering flasks, and these glass containers were filled up with filtered natural seawater with ~ 33 ppt of salinity. Finally, 4500 living specimens were randomly selected and transferred to foraminiferal culture chambers. Experiment duration was a five-week period, and during the acclimation time the pH was reduced gradually until each treatment reached its required pH levels/*p*CO₂. Probes measuring pH and temperature continually monitored four reservoir tanks.

2.3 Experimental setup

The seawater pH was continually manipulated by bubbling air with a known equivalent atmospheric concentration of CO₂ (approx. 380, 1000 and >1200 µatm) into three reservoir tanks, separately. Therefore, the three selected pH levels of 8.1, 7.7 and 7.6 representing the range of pH predicted for future scenarios in a high CO₂ level. Seawater was continuously pumped from the 400 L tanks into culturing chambers.

Seawater samples were taken from each mesocosm tank to measure the total alkalinity (A_T). Collected seawater samples were stored in borosilicate glass bottles (250 ml) and it transferred to the laboratory. The total alkalinity concentration in seawater was analyzed by using an automatic alkalinity titration kit (Metrohm, Switzerland) at room temperature (29°C). The measured values of salinity, temperature, pH and total alkalinity (A_T) were used to calculate the carbon system parameters such as dissolved inorganic carbon (DIC) *p*CO₂, bicarbonate ions (HCO₃⁻) carbonate concentration (CO₃²⁻) and saturation states of calcite (Ω_{Calcite}) and aragonite (Ω_{Aragonite}) version of the program CO₂ Cal.

After completing the experimental period, all chambers were opened up and inserts were removed and placed to a petri dish. All the foraminiferal samples were fixed and stained immediately. Foraminifera was alive at the end of the experiment. Rose Bengal is a biological stain that absorbs onto proteins which are major cytoplasmic components and stains them rose color (Bernhard 2000). Ten live specimens with intact tests from each treatment were selected for SEM analysis after the morphological observation of stereomicroscopy. The specimens were mounted onto SEM tubs using double-sided adhesive tabs and subsequently, were imaged with a JSM 5610LV.

3. Results

3.1 Seawater carbonate chemistry

Seawater temperature (29 °C) and salinity (33 ppt) were stable during the incubation period. As a result of three different $p\text{CO}_2$ treatments, the pH values ranged from 8.10 to 7.6 (Table 2). The seawater total alkalinity ranged from 2240 to 2290 $\text{A}_\text{T} \mu\text{mol kg}^{-1}$. Bicarbonate levels increased with increasing $p\text{CO}_2$ levels. The higher $p\text{CO}_2$ treatment reduced the availability of carbonate ion in experimental seawater. The important calcite Ω_{calc} and aragonite Ω_{ar} minerals reduced with increasing levels of the $p\text{CO}_2$ (Table 2).

3.2 Survival rate

From a total of 4502 specimens transferred into the culturing chambers, only 3973 specimens were retrieved at the end of the experiment in all three species. This indicated a loss of 529 individuals throughout the experimental period (Table 1 and Fig. 1). This is attributed to the loss of specimens while transferring as well as the migration of specimens out the culturing

chambers. Survival rate was calculated as a percentage of the total number of surviving individuals compared with the total number of retrieved individuals at the end of the experiment.

Foraminifera culture chamber was opened and specimens were categorized as live or dead based on the rose Bengal staining. Any specimens that were inactive or contained no cytoplasm were considered as dead. The low survival rate was observed at pH 7.6 (Fig.1), and it was least in *A. dentata* among the three species. The highest survival rate was observed at ambient pH and pH 7.7 in all three species. The moderate survival rate was observed in pH of 7.6.

3.3 Scanning Electron micrographs (SEM) of foraminiferal tests

SEM images of all three *A. beccarii*, *A. tepida*, and *A. dentata* showed morphological differences among specimens cultured at different pH conditions (Fig. 2). These observations indicated a progressive alteration of the foraminiferal morphology. The test surface was smooth, and the pore size and chamber arrangements remained unaffected in all the three species of foraminifera at ambient pH 8.10. The most significant features observed on the test surface appearance were cracking and dissolution on the individuals exposed to the lowest pH levels. The foraminiferal specimens that were cultured at pH 7.7 and 7.6 showed the same cracking and dissolution of the surface ornamentation. The reduced pH treatments affected the foraminiferal folium part of the umbilical side and dissolved the chamber suture. More importantly, *A. dentata* was observed to be moderately affected in two pH treatments on whorl suture and dissolved test surface, and the cracked chamber was also found on the dorsal side of the species (Fig.2).

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4. Discussion 180

The seawater carbonate ions and calcite are important carbon species to make the test on 181
skeleton of calcifying organisms (Keul et al., 2013). The experimental seawater bicarbonate 182
concentration increases with increasing $p\text{CO}_2$, in contrast, the carbonate iron and mineral calcite 183
reduces with increasing $p\text{CO}_2$. 184

4.1 Survival rate 185

The present experiment was conducted to determine the short-term responses of three 186
benthic foraminiferal species (*Ammonia beccarii*, *A. tepida* and *A. dentata*) to three pH 187
treatments. All the pH treatments caused high mortality in the species due to negative effect of 188
reduced pH and also to the sudden exposure to experimental conditions from natural 189
environment. The mortality was well pronounced in *A. dentata* at pH 7.6, as compared to other 190
two species, and this reveals species-specific vulnerability to change in pH, in support of earlier 191
works (Fabry et al., 2008; Keul et al., 2013). The lower pH did not significantly affect the 192
survival rate in agglutinated and tectinous benthic foraminifera but negatively impacted the 193
survival rate in calcareous foraminifera (Bernhard et al., 2009a). The present study recorded 194
noticeable mortality rates in all the three species with decreasing pH, similarly to the previous 195
work of, McIntyre Wressing et al. (2011) who have reported that higher CO_2 concentration 196
affects survival rate in *Amphistegina gibbosa*. 197

4.2 Scanning electron micrograph of foraminifera 198

SEM analysis demonstrated that the morphology of all three species were sensitive to 199
decreasing pH conditions. In the ambient pH condition, the morphology of all three species were 200
very smooth with intact ornamentation such as tubercles and teeth lining the aperture. The 201

present study observed morphological variation such as, dissolution in ventral side of notch and 202
cracking in umbilical part of *A. tepida* and *A. dentata* pH 7.7 treatment, but there was no such 203
changes found in *A. beccarii* at 7.7 treatment. Khana et al. (2013) have recorded that shell 204
appearance of *Haynesina germanica* is not damaged at 586 ppm of CO₂ treatment as well as 205
teeth shape and size are intact, but only teeth numbers are reduced. However, they have found 206
that the average number of teeth, length and width of *H. germanica* teeth structure are reduced at 207
750 ppm of CO₂ treatment (Khana et al., 2013). Haynert et al. (2011) have reported that the test 208
surface of *Ammonia aomoriensis* is not affected when treated between 618 to 751 μ atm. The 209
present study recorded broken teeth with dissolved test surface to confirm the adverse effect of 210
lower pH on morphology of foraminiferans. A similar observation has been made in gastropods 211
with reduced toughness of inner shell at low pH of 7.7 (Duquette et al., 2017) and also with 212
reduced shell weight due to shell dissolution with reduced pH and increased CO₂ (Nienhuis et 213
al., 2016). The present study also recorded higher test weight loss in *A. dentata* in pH 7.7 and pH 214
7.6 treatments. 215

The results revealed that all the three foraminiferal species when exposed to the higher 216
CO₂ (pH 7.6) exhibited morphological deformations such as dissolution, rounding teeth, surface 217
cracking, dorsal and ventral side test dissolved, and these deformations might have affected the 218
survival of ferminiferans. This is accordance with White et al. (2013) who have reported that 219
high CO₂ exposure results in smaller shells and reduces the larval survival rate in *Argopecten* 220
irradiants 221

Similar to our observations, Haynert et al. (2011) have found that 1 to 3 younger 222
chambers of *A. aomoriensis* are severely decalcified at *p*CO₂ levels 929 to 1829 μ atm, in 223
addition to cracks found around the pores in the treatment. The authors also have recorded that 224

increasing high $p\text{CO}_2$ of 3130 μatm treatment reduces test diameter, the growth rate of *A. aomoriensis* and destroys all chambers by complete calcium carbonate dissolution (Haynert et al., 2011). Riebsell et al. (2000) have found that the increasing CO_2 concentration affects the coccoliths in term of malformed coccoliths, increases incomplete coccospheres, and reduces calcification rate by 36 to 83 %. This work has revealed that cultured coccolithophorids are highly sensitive to increased seawater $p\text{CO}_2$. Keul et al. (2013) have reported that *growth* rate in *Ammonia* species decreases with decreasing $[\text{CO}_3^{2-}]$ $\Omega_{\text{calc}} < 1$. The present observations are in support of previous laboratory studies, where *A. aomoriensis* and *A. beccarii* are affected by test degradation (Le Cade, 2003; Haynert et al., 2011). Therefore, CO_2 induced ocean acidification experimental studies reiterated the vulnerability of the benthic foraminifera to near future ocean acidification, by causing .morphological deformation and absence of important feeding structures in the organisms.

5. Conclusion

The experimental study provides a detailed understanding of the negative effects on survival rate, shell morphological changes in the benthic foraminifera in low pH experiment. The changes in the carbonate chemistry of natural coastal environment will have similar effects to this simulated in laboratory condition and hence, calcifying organisms will be more vulnerable to the near future ocean acidification, as reiterated by the present study.

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Figure 1. Survival rate of foraminifera specimens (*Ammonia beccarii*, *A. tepida* and *A. dentata*) in ambient and low pH treatment.

Figure 2. SEM micrographs of benthic foraminifera in three different pH ambient pH 8.10 pH 7.7 and pH 7.6 experiment. *A. beccarii* in Ambient pH 8.10 pH 7.7 and pH 7.6 no variation found (A, B, D, and G), surface dissolute (C), *A. tepida* pH 7.6, Ventral side dissolute and test cracked (E and F), pH 7.7 and pH 7.6 dorsal and ventral side test dissolved (H and I) pH 7.7 and pH 7.6.

Table 1. Number of individuals of *Ammonia beccarri* *A. tepida* and *A. tendata* cultured under different pH conditions ambient pH 8.1, pH 7.7 and pH 7.6. Individuals showing one or more new chambers added during the experimental period were considered as live individuals, survival rate (%) was calculated based on the number of living and dead over the experimental period.

Name of the species	Total number of individuals					Survival rate	Mortality rate
	Treatment	Start of the experiment	End of the experiment				
			Retrieved	Alive			
<i>Ammonia beccarri</i>	pH 8.1 (ambient)	495	452	341	74.62	25.38	
	pH 7.7	502	453	281	62.31	37.69	
	pH 7.6	498	445	145	32.44	67.56	
<i>A. tepida</i>	pH 8.1 (ambient)	505	411	301	72.36	27.64	
	pH 7.7	500	439	251	57.18	42.82	
	pH 7.6	496	457	125	27.11	72.89	
<i>A. dentata</i>	pH 8.1 (ambient)	500	432	301	69.68	30.32	
	pH 7.7	502	439	184	42.11	57.89	
	pH 7.6	504	445	105	23.81	76.19	

Table 2. The low pH experiment impacts on foraminifera individuals effects

Experiment finding on test morphology foraminifera species			
Treatment	<i>Ammonia beccarii</i>	<i>A. tepida</i>	<i>A. dentate</i>
pH 8.1 (Ambient)	no affects	no affects found	Effects Not found
pH 7.7	No affects	ventral side of notch slightly dissolved	Folium part of the umbilical side cracked
pH 7.6	surface ornamentation dissolute	surface cracking found	dorsal and ventral side test dissolved

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