The effect of acidified seawater on shell characteristics of blood cockle, *Tegillarca granosa*

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Received: May 2020 Accepted: November 2020

Abstract

Our ocean currently has been recorded to absorb about 25% of anthropogenic CO_2 on an annual basis. This has estimated the global average sea surface pH to decrease from 8.2 to 8.1 units since the pre-industrial revolution and to further drop between 0.1 to 0.3 units by the end of the 21st century. This possesses a potential impact on wide range of marine organisms' especially marine calcifiers where the CO_3^{2-} is a fundamental mineral for shell and skeleton formation. In a 7-day experiment, this study investigated the effect of different pH treatments, which were pH 7.10, pH 7.50 and control pH (pH 7.81) on shell properties of the blood cockle, *Tegillarca granosa*. The shell weight and shell density of *T. granosa* was significantly reduced at pH 7.10. The smaller mean ratio for weight and density at pH 7.10 indicated there was a large difference between the initial and final value for weight and density. Furthermore, the scanning electron micrograph revealed the rough outer shell surface (periostracum) of *T. granosa* under decreased pH treatment (pH 7.10). However, the ocean acidification level of pH 7.50 which predicted to occur by the year 2300 showed no significant decrease in shell weight and shell density of *T. granosa* compared to the control pH treatment (pH 7.81).

Keywords: Blood cockle, Climate change, Straits of Malacca, Shell structure

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[DOI: 10.18331/SFS2021.7.2.11]

Introduction

Our ocean currently has been recorded to absorb about 25% of anthropogenic CO_2 on an annual basis (Le Quéré *et al.*, 2018). Uptake of CO_2 by the ocean has altered the carbonate system and further upsets several parameters associated to carbonate system. Particularly by increasing the concentration of dissolved inorganic carbon (DIC), reducing the concentration of carbonate ions (CO_3^{2-}) and lower the pH (quantification of the activity of hydrogen ions, H^+). This ocean acidity is referred to as ocean acidification. The global average sea surface pH has been estimated to decrease from 8.2 to 8.1 units since the pre-industrial revolution and to further drop between 0.1 to 0.3 units by the end of the 21st century (Feely *et al.*, 2009; Stocker *et al.*, 2013; Fietzke *et al.*, 2015).

This phenomenon has threatened many marine organisms especially heavily calcified organisms where their ability to grow protective shells or skeletons was restricted because of the hydrogen ion (H^+) in the water column react with carbonate ion (CO_3^{2-}), causing reduction of concentration of CO_3^{2-} which act as important building block for production of their calcium carbonate shells (Feely *et al.*, 2004).

Marine bivalves have received much attention in ocean acidification research due to its highly susceptible to reduced calcification rate and dissolution of calcareous structures with lower pH (Fabry *et al.*, 2008; Xu *et al.*, 2016; Zhao *et al.*, 2017). This is because bivalves play a critical role in coastal ecosystem and also are ecologically and economically important economic resources for the fisheries and aquaculture industry (Shi *et al.*, 2017). Many studies has showed that decreased calcification rate and dissolution of calcareous structures caused reduction in shell mass, resulting in loss of structural integrity of the shells and reduced shell strength (McClintock *et al.*, 2009). As the consequence of acidification, they are vulnerable to dissolution of their $CaCO_3$ structure and this process is faster than they build their shells.

Hence, this study is aimed to investigate the effect of lowered pH (comparison before and after the acid treatment) on the shell characteristics of *T. granosa*.

Materials and methods

Experimental setup and seawater chemistry

This experiment was conducted at Centre for Marine and Coastal Studies (CEMACS), Universiti Sains Malaysia. The blood cockles (shell length 45+1.01 mm) were collected in October 2019 from intertidal site of Kuala Juru, Penang. Three replicate experimental aquariums were used to investigate pH levels. Each replicate experimental aquarium contained 20 blood cockles and filled with about 18 L filtered and natural seawater at desired pH values. By continuously bubbling with CO_2 gas mixture, the pH levels of seawater were set at the ambient pH value (7.8) and three lowered pH values (7.5 and 7.1),

[DOI: 10.18331/SFS2021.7.2.11]

which were projected for the years 2100 and beyond. The experiment lasted for seven days.

The seawater temperature, salinity and pH were measured daily using a YSI Professional Plus (YSI Inc®). Seawater samples were also collected daily throughout the experiment period to determine total alkalinity (TA) value by using an open-cell acidimetric titration method following the Standard Operation Procedure (SOP) described by Dickson *et al*, 2007 with slight modification. The carbonate system parameters were calculated from the measured pH, salinity, temperature and total alkalinity using the open-source program CO2SYS (Pierrot *et al.*, 2006) with the established constants by Mehrbach *et al.* (1973) as refitted by Dickson and Millero (1987)

Shell characteristics

After a week of exposure, the shell weight and density were measured. Measurement of density of cockle shells was obtained using an analytical balance HR-250AZ with a density mode. After exposed to seven-day experiment, the shell from each pH treatment was observed under Hitachi Tabletop Microscope TM3000 to examine their outermost shell layer (periostracum).

Statistical analysis

In this study, the shell weight and shell density before and after the acid treatment was calculated for each cockle shell. Then, the data were presented in terms of ratio and analysed using one-way ANOVA with Tukey *post hoc* analysis to identify significant difference between pH treatments. Data were analysed by using Sigma Stat version 3.5, SPSS.

Results

The average minimum and maximum pH value over the seven days experiment was different between pH treatments. There was a difference in maximum pH value for pH 7.10 and pH 7.50 at p=0.017. The maximum pH values at pH 7.10 also differ from that of control pH treatment (pH 7.81) (p=0.000). The maximum pH value between pH 7.50 and control pH treatment (pH 7.81) was different with a significant level of p = 0.000.

The data obtained in this study was presented as ratio of final value/initial value. The ratio of final value/initial value that showed nearly 1 or equal to 1 meant the least or no difference between initial value (before acid treatment) and final value (after acid treatment). The ratio of final value/initial value that moving away from 1 means the large difference between initial value (before acid treatment) and final value (after acid treatment). At pH 7.10, the minimum value for ratio of final density/initial density was 0.99001 while the maximum value for ratio of final density/initial density was 0.99708. The ratio of 0.99001 indicated that the shell density reduced significantly after the acid treatment compared to the initial shell density before the acid treatment. The ratio of 0.99708 means that there is less difference between the initial shell

[DOI: 10.18331/SFS2021.7.2.11]

density and final shell density. At pH 7.50, the minimum ratio of final density/initial density (0.99752) and maximum ratio of final density/initial density (0.99996) indicated the final shell density did not show a great difference from initial shell density before the acid treatment. At control pH treatment (pH 7.81), the minimum ratio of final density/initial density (0.99674) which is less than of pH 7.50 (0.99752) indicated the shell reduced in density although no acid added into the treatment. The maximum value for ratio of final density/initial density (1.00089) revealed that the shell increased in density after the acid treatment. The range for the difference between final shell density and initial shell density at pH 7.10 was 0.00824-0.02809 g/cm^3. At pH 7.50, the range of density difference between final value and initial value was 0.00010-0.00701 g/cm^3 while at control pH treatment (pH 7.81). The density difference range was 0.00392-0.00922 g/cm^3.

As shown by the scanning electron microscope, the decreased pH altered the outer surface (periostracum) of *T. granosa*. The shell from pH 7.10 was characterized by the sign of external shell dissolution with the rough outer surface as shown in Figure 4.5 (a). This accompanied with the result showed in the previous section 4.3 where the shell density and shell weight of *T. granosa* were greatly reduced at pH 7.10 because the HCl corroded the shell surface and lead to loos of $CaCO_3$. The shells also showed a large density difference (0.00824-0.02809 g/cm^3) between initial value and final value where the shell highly dissolved its $CaCO_3$ under acidified condition. Although the range for shell density difference at pH 7.50 (0.00010~0.00701 g/cm^3) was not much different compared to the control pH treatment (pH 7.81) (0.00392~0.00922 g/cm^3), but from the scanning electron micrograph illustrated in Figure 4.5 (b), the shell exposed to pH 7.50 showed a little sign of corrosion on periostracum with a partially loss of prismatic arrangement. Based on Figure 4.5 (c), the shell exposed to control pH treatment (pH 7.81) had an intact periostracum with a compact prismatic structure and no shell dissolution observed on the outer shell surface (Figs. 1 to 4).

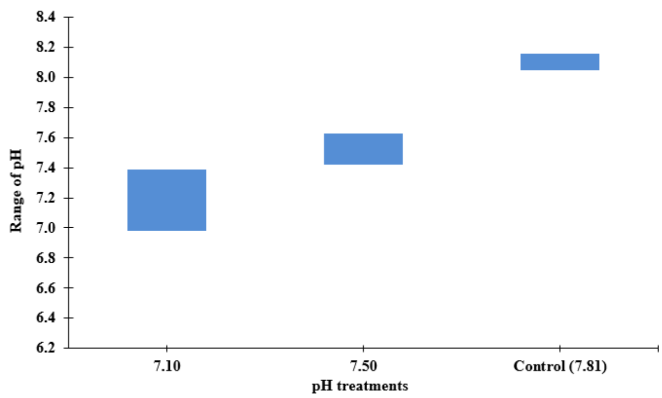

Figure 1: The pH range of seven days experiment in three pH treatments.

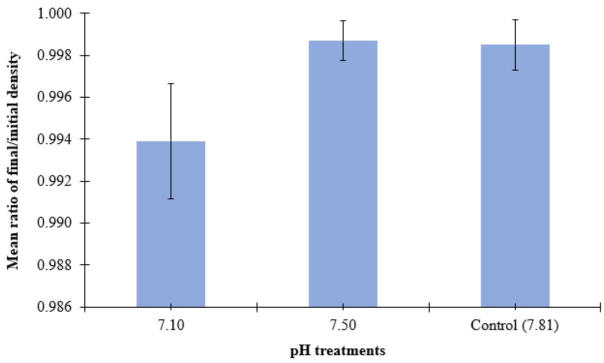

Figure 2: Mean ratio of final/initial density in three pH treatments.

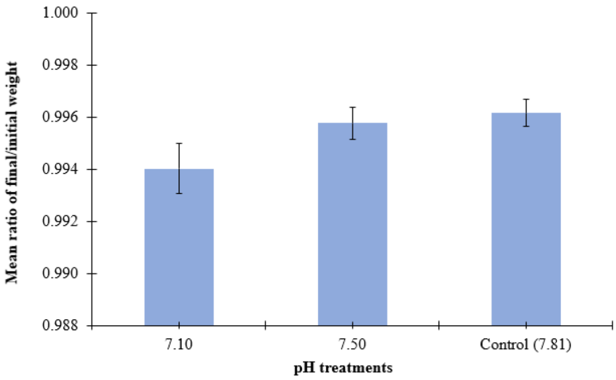

Figure 3: Mean ratio of final/initial weight in three pH treatments.

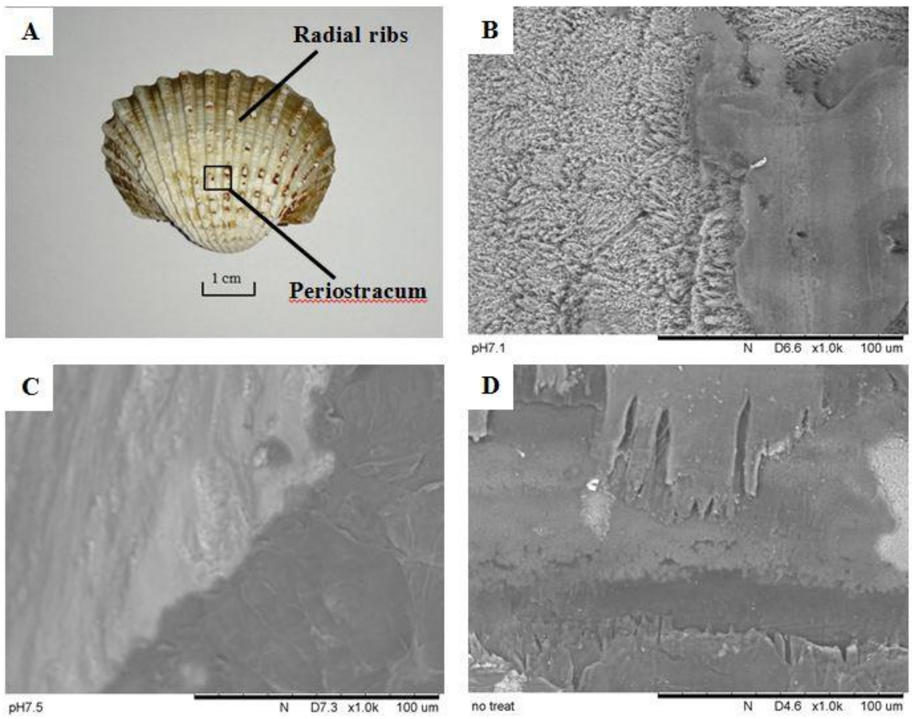

Figure 4: SEM micrographs of the periostracum of *Tegillarca granosa* shell (A) kept for a week in control, pH 7.81 (B); and acidified seawater, pH 7.54 (C) and pH 7.10 (D).

[DOI: 10.18331/SFS2021.7.2.11]

Discussion

Although ocean has an effective buffering effect to resist the pH change, the ocean pH would be expected to drop by 0.3-0.4 pH unit by the end of the century (Orr *et al*., 2005). This is because the rate that the ocean could buffer the acidic effect is slower than the rate of absorption of CO_2 into the ocean. The $CaCO_3$ compensation system, which has developed over historical time, is a slow process that can deal with natural variation of CO_2. However, human activities such as the increasing fossil fuels combustion and deforestation has caused a constant increase in production of CO_2 in recent year and CO_2 concentration has steadily increased since the industrial revolution. The current rate of change is about 10 times higher than the rate preceding the Paleocene-Eocene Thermal Maximum (PETM). This means that the $CaCO_3$ compensation system is unlikely to balance and the ocean would become acidic. The aragonite saturation state (Ω_{arg}) indicates the availability of CO_3^{2-} in seawater, which is widely used by marine calcifying organisms to construct their $CaCO_3$ structures. The surface seawater that exhibit Ω_{arg} more than 4 is optimal, 3.5 - 4.0 is adequate, 3.0 - 3.5 is marginal and less than 3.0 is critical (Guinotte *et al*., 2003). The saturation state of Penang seawater showed 2.06 indicated our water is undersaturated with respect to aragonite. This condition is critical by weakening the shellfish due to the seawater is corrosive to $CaCO_3$ and dissolution would happen instead of precipitation.

The ratio of final value/initial value was determined in this study to examine how much the difference in shell density and shell weight before and after the acid treatment. The smaller ratio value indicated there was a large difference between the initial value and final value whereas the larger the ratio value, the least the difference between the initial value and final value. For the case that the ratio value exceeds 1, this means there was an increase in the final value after the acid treatment. However, this situation should not happen because only empty shells of *T. granosa* used in this study and the shell itself did not carry out any biological responses. The reason to explain it might be due to the salt particles remained on the shell after the shell had dried. For the case that the shell showed decreased in final density after the control pH treatment (pH 7.81), this probably due to the seawater itself is corrosive to the shell and might be due to the more porous structures in the shell which caused damage when exposed to control pH treatment (pH7.81). The smaller mean ratio value at pH 7.10 revealed that the shell density and shell weight of *T. granosa* were greatly reduced after the acid treatment. This might be due to the more porous structure of *T. granosa* shell and dissolution occurred under acidified seawater. In acidic seawater, the shell showed loss of $CaCO_3$, which caused its mass and volume to decrease.

The loss of mass and volume of shell affected the density because the determination of density required both mass and volume. The larger mean ratio for both density and weight at pH 7.50 indicated the shell density and shell weight of *T. granosa* were not much reduced although there was a significant difference in the average minimum and maximum pH values when compared to the control pH treatment (pH 7.81). This might be due to the denser and compact microstructure arrangement of *T. granosa* shell when exposed to pH 7.50. The shells could minimize the loss of $CaCO_3$ and did not show great reduced in mass and volume. However, other study showed that the shell density has decreased with lower pH. By using the micro-computed tomography (Micro-CT), it showed that the shell mineral density of oyster reduced significantly in decreased pH (pH 7.80 and pH 7.50). Three-dimensional (3D) density maps also revealed the larger proportions of lower mineral density regions or pores in the oyster shells, which exposed to pH 7.80 and pH 7.50 (Yao, *et al*., 2018). This is different from the finding of this study that showed that there was no significant reduced in shell density of *T. granosa*. This might be due to the different life stages of organism used in the study where the larvae and juvenile stages of oyster may be able to withstand the extreme low pH condition. The shell density and shell weight of *T. granosa* did not show large changes in control pH treatment (pH 7.81) since any acid added into it.

The outer surface (periostracum) of the *T. granosa* experienced shell corrosion when exposed to pH 7.10. The acidified seawater in treatment of pH 7.10 contained high concentration of H^+ and this would make *T. granosa* to dissolve their shell by releasing CO_3^{2-} to neutralise the acidic condition of the seawater. Shell dissolution on the outside of the shell also observed in cockle, *Cerastoderma edule* at 6,600 μatm (pH 7.0) (Vanreusel *et al*., 2016). There was no visible sign of shell corrosion with a slightly loss of prismatic structures in *T. granosa* from treatment of pH 7.50. The result of this study was different from other findings which indicated the moderate degrees of acidification can lead to severe shell damage. For example, shell dissolution was visible and hole can be observed in small sized cockle, *Cerastoderma edule* at 2,900 μatm (pH7.40) (Vanreusel *et al*., 2016). This probably due to the difference in size and life history stage of the organisms when exposed to extreme environment. Similar impact was observed in clam, *Yoldia eightsi* and *Laternula elliptica* where their outer surface of shell had suffered significant dissolution at pH 7.40 seawater (McClintock *et al*., 2009). The extent of shell damage exhibited by bivalve molluscs is species-specific. This can relate the outer shell corrosion with the absence of thick and intact periostracum in bivalve molluscs. Due to the very thin periostracum (ca. 2 μm) of *Cerastoderma edule* (Harper, 1997) and its shell, which exclusively composed of aragonite, is most prone to dissolution (Glover and Kidwell, 1993). In contrast to mytilids, which are protected by a periostracum of more than 20 μm (Harper, 1997), they can survive in seawater that is strongly undersaturated for $CaCO_3$ (Ω_{arg}<0.2) as long as their periostracum is intact.

Conclusions

In conclusion, the study of acidified seawater on *T. granosa* revealed that the shell properties such as shell weight, shell density and shell microstructure were significantly affected by extreme condition (pH 7.10). The weight and density of *T. granosa* was greatly reduced at lower pH treatment (pH 7.10). The smaller mean ratio of final density/initial density showed that the final shell density was decreased significantly when compared to initial shell density before pH treatment. The smaller mean ratio of final weight/initial weight indicated that there was a large difference of shell weight before and after the pH treatment. The moderate level of acidified seawater (pH 7.50) did not show significant impact on shell weight and shell density of *T. granosa* and no significant difference in relative to control pH treatment (pH 7.81). *T. granosa* showed a visible rough outer shell surface (periostracum) at pH 7.10 based on scanning electron microscope analysis. The shell from pH 7.50 showed a little sign of shell corrosion on the outer surface when compared to the control pH treatment (pH 7.81).

Acknowledgments

This study was financially supported by RUI Grant 1001/ PPANTAI/8011088 (Universiti Sains Malaysia). We thank the staffs and the facilities provided by Centre of Marine and Coastal Studies (CEMACS), USM

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[DOI: 10.18331/SFS2021.7.2.11]

[DOI: 10.18331/SFS2021.7.2.11]

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[DOI: 10.18331/SFS2021.7.2.11]