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Ocean acidification adversely influences metabolism, extracellular pH and calcification of an economically important marine bivalve, *Tegillarca granosa*

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ABSTRACT

Oceanic uptake of CO_2 from the atmosphere has significantly reduced surface seawater pH and altered the carbonate chemistry within, leading to global Ocean Acidification (OA). The blood clam, *Tegillarca granosa*, is an economically and ecologically significant marine bivalve that is widely distributed along the coastal and estuarine areas of Asia. To investigate the physiological responses to OA, blood clams were exposed to ambient and three reduced seawater pH levels (8.1, 7.8, 7.6 and 7.4) for 40 days, respectively. Results obtained suggest that OA suppresses the feeding activity and aerobic metabolism, but elevates proteins catabolism of blood clams. OA also causes extracellular acidosis and decreases haemolymph Ca^{2+} concentration. In addition, our data also suggest that OA impairs the calcification process and inner shell surface integrity. Overall, OA adversely influences metabolism, acid-base status and calcification of blood clams, subsequently leading to a decrease in the fitness of this marine bivalve species.

1. Introduction

Anthropogenic emission of carbon dioxide (CO_2) has elevated the atmospheric CO_2 concentration from a pre-industrial level of about 280 ppm to a present level of approximately 400 ppm (IPCC, 2014). About one third of the anthropogenic CO_2 is eventually absorbed by the ocean and subsequently leads to Ocean Acidification (OA) (Caldeira and Wickett, 2003). As a consequence of oceanic CO_2 uptake, the average surface seawater pH has already decreased by about 0.1 units, from a pre-industrial level of approximately 8.21 to a current level of 8.10 (Caldeira and Wickett, 2003). According to the prediction of Intergovernmental Panel on Climate Change (IPCC), the average seawater pH will drop by another 0.3–0.4 and 0.7–0.8 units by the end of 21st and 23rd century, respectively (IPCC, 2014). Besides declining seawater pH, OA also results in the alteration of seawater carbonate chemistry which subsequently decreases the saturation state of calcium carbonate ($CaCO_3$) (Feely et al., 2004). Over the past decade, biological processes and physiological functions of a wide variety of marine organisms have been found to be vulnerable to OA (Kroeker et al., 2010; Kurihara, 2008; Liu et al., 2016; Shi et al., 2016). However, there were species-specific variations in the sensitivity to OA (Kroeker et al., 2010; Ries et al., 2009). For example, calcification rates of a variety of calcifying organisms were reduced under the condition of OA even when $CaCO_3$ saturation state >1.0 (Chan et al., 2012; Gazeau et al., 2007; Li et al., 2016b; Melzner et al., 2011), whereas enhanced calcification rates were observed for other species (Irie et al., 2010; Ries et al., 2009; Wood et al., 2008). Similarly, though respiration rates were found to be decreased in many species under OA conditions (Wang et al., 2015; Hu et al., 2014; Pimentel et al., 2014), Thomsen and Melzner (2010) reported an increase in respiration rate in the blue mussel *Mytilus edulis* after 8-week exposure to OA. The species-specific variations were also detected in other biological processes, such as survival, growth, and reproduction (Kroeker et al., 2010). In addition, animals at different developmental stages may vary in their sensitivity to OA as well (Kroeker et al., 2010). For example, the calcium content was found to be increased whereas

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remained unchanged for the larvae and juvenile red king crabs, *Paralithodes camtschaticus*, respectively (Christopher Long et al., 2013a; Christopher Long et al., 2013b). Therefore, broader studies are necessary to envisage how marine organisms and ecosystem may be altered under future scenarios of OA.

Many marine bivalves (e.g. blue mussels and oysters) play a critical role on the community structure of coastal ecosystems and are also important economic resources for the fishery and aquaculture industries. In recent years, an increasing attention has been paid to the potential of ecological and economic impacts of OA on marine bivalves (Scanes et al., 2014; Shi et al., 2016; Xu et al., 2016; Zhao et al., 2016). The blood clam, *Tegillarca granosa*, is an ecologically and economically significant marine bivalve that is widely distributed along the coastal and estuarine areas of Asia (Liu et al., 2014; Shao et al., 2016). According to the report of Food and Agriculture Organization of the United Nations (FAO), the aquaculture industry of blood clam was worth about $58 million in the year 2014 (FAO, 2016). The immune response of blood clams was found to be significantly weakened under future scenarios of OA (at pH 7.8 and 7.4), which may subsequently render individuals more susceptible to pathogens attacks such as those from virus and bacteria (Liu et al., 2016). The future scenarios of OA were reported to greatly increase the toxic metal (cadmium, Cd) accumulation in blood clams as well, suggesting a potential threat to seafood safety (Shi et al., 2016). Apart from weakening the immunity and enhancing toxic metal accumulation, OA also impairs sperm motility and fertilization success, which could decrease population recruitment of the species (Shi et al., 2016). However, to date, it remains unknown whether OA exert other additional effects on this marine bivalve species.

Therefore, the aim of this study is to investigate the effects of OA on feeding activity, metabolism, calcification, extracellular pH, and Ca^{2+} status of the blood clams, *T. granosa*. Animals were exposed to ambient (8.1) and three lowered seawater pH levels (7.8, 7.6, and 7.4) for 40 days, respectively. After the 40-day exposure, the clearance rates were measured to show the alteration in feeding activity. The respiration and ammonium excretion rates, and oxygen to nitrogen ratio (O:N ratio) were determined to investigate the effects of OA, if any, on the aerobic metabolism and protein catabolism. The haemolymph pH values and calcium (Ca^{2+}) concentration were investigated to estimate how OA affects extracellular acid-base status and Ca^{2+} hemostasis. In addition, the image analysis of inner shell surfaces and the determination of net calcification rate were performed to evaluate the effects of OA on shell integrity and calcification process. The data obtained will contribute to the management of the cultivation of economically significant marine bivalves, and to the better understanding of potential changes of coastal community in future.

2. Materials and methods

2.1. Animal collection and acclimation

Blood clams *T. granosa* (shell length of 20.03 ± 1.27 mm) were collected in August 2014 from an intertidal site of Yueqing Bay, Zhejiang, China, approximately 2 months after the spawning season (Liu et al., 2014). Animals were transported to the Qingjiang Station of Zhejiang Mariculture Research Institute. After cleaned off epibionts gently, animals were acclimated for at least a week in a 1000 L aquarium in filtered and UV-radiated natural seawater (pH: 8.10 ± 0.05, temperature: 28.0 ± 0.5 °C, salinity: 21.0 ± 0.3‰) with continuous aeration prior to the respective treatments. Animals were fed twice daily with microalgae *Platymonas subcordiformis* at a rate of ~5% dry tissue weight. Excess food and feces were removed daily through water change.

2.2. Experimental set-up and seawater parameters

Eight replicate experimental aquariums (volume = 22.5 L) were nested at each of the four investigated pH levels. Each replicate experimental aquarium contained 20 blood clams and filled with about 18 L filtered and UV-radiated natural seawater at desired pH values. The incubation of blood clams at the four pH levels were conducted one by one to leave enough time performing subsequent physiological measurements. By continuously bubbling with CO_2 gas mixture, the pH levels of seawater were set at the ambient pH value (8.1) and three lowered pH values (7.8, 7.6 and 7.4), which were projected for the years 2100 and beyond (Caldeira and Wickett, 2005; IPCC, 2014). The CO_2 gas mixture was obtained by mixing CO_2-free air and pure CO_2 gas at controlled flow rates using flow controllers (Zhao et al., 2016). Blood clams were fed with microalgae *P. subcordiformis* as described above. Two thirds of the seawater in each aquarium was removed by siphoning from the tank bottom, and the aquarium was then refilled with seawater pre-equilibrated to the desired pH values daily. The experiment lasted for 40 days. During the entire 40-day experiment period, no blood clam death was detected.

During the incubation, pH and salinity of each aquarium were measured twice daily. Total alkalinity of each aquarium was determined once a week. A Sartorius PB-10 pH meter (Sartorius, Germany) calibrated with standard US National Bureau of Standards (NBS) buffers was used to monitor the pH values. Salinity was measured using a conductivity meter (Multi 3410 WTW, Germany). Seawater temperature was real-time monitored and maintained at 28 °C using temperature regulators. Seawater samples were taken from experimental aquariums and immediately frozen at −80 °C for total alkalinity measurements, which was performed by potentiometric titration with an automatic titrator system (SM-Titrino 702, Metrohm). 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 (Dickson, 1990; Dickson and Millero, 1987; Mehrbach et al., 1973). The seawater parameters of the experimental trials are summarized in Table 1.

2.3. Physiological measurements

2.3.1. Clearance rate

After 40 days of exposure, blood clams were depurated for 12 h to empty their guts prior to the measurements of clearance rate. Five individual blood clams from each exposure aquarium (replicate) were randomly selected and transported into a 2 L chamber filled with filtered seawater pre-equilibrated to the corresponding pH values. An identical chamber without blood clams was used as the blank control. After acclimation for 30 min, the microalgae *P. subcordiformis* was added into the chamber to achieve an initial concentration of 2.7×10^5 cells mL^{-1}, the maximum concentration at which no pseudo-feces were produced in a preliminary experiment. The experiment lasted for 2 h. Microalgae cell concentrations at the beginning and end of the measurements were determined using a haemocytometer under a microscope (Nikon, E600). After measurements, soft t4a52a237(xp(undeod)-299.1(Sarms)-291.7(and12.9(re

Table 1
Seawater parameters during the 40-day incubation of blood clams, *T. granosa* (mean ± SD). T: temperature; Sal: salinity, TA: total alkalinity; pCO_2: CO_2 partial pressure; Ωara: aragonite saturation state; and Ωcal: calcite saturation state.

Target pH	pH_{NBS}	T (°C)	Sal (‰)	TA (μmol kg^{-1})	pCO_2 (μatm)	Ωara	Ωcal
8.1	8.13 ± 0.04	28.2 ± 0.2	21.58 ± 0.22	2085.67 ± 19.61	554.03 ± 5.37	2.25 ± 0.03	3.62 ± 0.04
7.8	7.80 ± 0.05	27.9 ± 0.3	21.63 ± 0.49	2097.47 ± 21.93	1202.05 ± 7.51	1.34 ± 0.04	1.95 ± 0.02
7.6	7.62 ± 0.03	28.2 ± 0.1	21.51 ± 0.43	2090.82 ± 20.33	1971.04 ± 17.89	0.85 ± 0.02	1.26 ± 0.02
7.4	7.39 ± 0.05	27.9 ± 0.4	21.49 ± 0.25	2072.43 ± 25.36	3120.11 ± 25.61	0.53 ± 0.03	0.83 ± 0.03

(cells mL^{-1}) at the beginning of the measurement, and C_2 is the microalgae concentration at time T (cells mL^{-1}), W is the dry weight of soft tissues (g) and T is the time elapsed (h).

2.3.2. Respiration rate, ammonium excretion rate, and oxygen to nitrogen ratio

As described above, blood clams were firstly depurated for 12 h to empty their guts prior to measurements. Five individual blood clams were then randomly selected and transported into a closed glass respirometers (2 L) full-filled with oxygen-saturated filtered seawater pre-equilibrated to the corresponding pH values. After acclimation for 30 min, the experiment started with the respirometers sealed off for 2 h. An identical respirometer without blood clams was used as the blank control. Dissolved oxygen concentration in each respirometer was maintained at above 70% saturation during the experiment. The initial and final dissolved oxygen concentrations in each respirometer were determined using an oxygen meter (Multi 3410 SET4, WTW, Germany). The ammonium (NH_4^+) excretion was measured with a spectrophotometer according to the phenol-hypochlorite method (Solórzano, 1969). Dry weight of soft tissues was obtained as described above. Respiration rate and ammonium excretion rate of blood clams were calculated using the following equation:

$$R(E) = V \times (C_1 - C_2) / (W \times T)$$

where R(E) is the respiration (or ammonium excretion) rate (mg g^{-1} h^{-1}), V is the volume of the seawater in the respirometer (L), C_1 and C_2 represent the dissolved oxygen (or ammonia) concentrations (mg L^{-1}) at the beginning and end of the measurement, W is the dry weight of soft tissues (g) and T is the time elapsed (h). The oxygen to nitrogen (O:N) ratio was obtained through dividing the measured respiration rate by ammonium excretion rate in atomic equivalents (Peng et al., 2016).

2.3.3. Net calcification rate

Together with the measurements of respiration and ammonium excretion rates, net calcification rate of the same group of blood clams was measured in parallel using the alkalinity anomaly technique (Smith and Key, 1975). The fresh weight of blood clams was measured with an electronic balance (Sartorius, Germany). The total alkalinity was determined by potentiometric titration as described above. The net calcification rate was calculated with the following formula modified from Gazeau et al. (2007):

$$G = \rho \times V \times (TA_1 - TA_2)/(2 \times FW \times T)$$

where G is the net calcification rate (μmol $CaCO_3$ g FW^{-1} h^{-1}), ρ is the seawater density (g L^{-1}), V is the volume of the seawater in the respirometer (L), TA_1 and TA_2 represent the total alkalinity before and after the measurement (μmol kg^{-1}), FW is the fresh weight of blood clams (g) and T is the time elapsed (h).

2.3.4. Haemolymph pH and Ca^{2+} concentration

Haemolymph samples of five individual blood clams from each exposed aquarium were extracted by pericardiac puncture using gas tight disposable syringes and transferred into 1.5 mL sterile tubes. Haemolymph samples were then centrifuged at 5000 g for 5 min. Supernatants were used for the determination of pH and Ca^{2+} concentration in cell free haemolymph. The pH values were obtained using a pH microelectrode (WTW, Germany). The Ca^{2+} concentrations were measured with a flame atomic spectrophotometer (WFX-130A, Beijing Rayleigh Analytical Instruments Co., Ltd, China) using the protocol described by Shi et al. (2016).

2.4. Shell analysis

Eight blood clams from each pH treatment (i.e. one blood clam per replicate aquarium) were haphazardly sampled after 40 days of experiment. Eight shells from each treatment (i.e. one shell per blood clam) were dissected, cleaned, and dried in an oven at 65 °C for 48 h. Stereomicroscopic images of inner shells were taken using a digital camera mounted on a stereomicroscope. Shells were further selected and fragmented along defined trajectories. Shell sections were mounted on pedestal stubs, coated with gold-palladium, and analyzed by a scanning electron microscopy (SEM, HITACHI SU8010). The outside shell surface was not analyzed in the present study because it is highly impracticable to clean off epibionts completely without damaging the shell.

2.5. Statistical analysis

One-way ANOVAs were conducted to show the effects of the various pH values on physiological parameters. Upon detecting significant differences at $p < 0.05$, a Tukey's post hoc test was performed to locate the differences. For all analyses, the assumptions of normality and homogeneity of variance were assessed using Shapiro-Wilk's test and Levene's test, respectively. In cases where the above assumptions were not satisfied by raw data, the data were log-transformed prior to analysis. All data were presented in mean ± standard deviation and all the statistical analyses were conducted using OriginPro 8.0.

3. Results

3.1. Effects of OA on physiological energetics

A significant effect of OA on clearance rates was detected (one-way ANOVA, $p < 0.01$) (Fig. 1a). After 40 days of experiment, OA lead to a significant decrease in the clearance rates. As compared with those at pH 8.1, the clearance rates were significantly decreased to approximately 78%, 66%, and 53% at pH 7.8, 7.6, and 7.4, respectively. In addition, a significant influence on the respiration rate by OA was also detected (one-way ANOVA, $p < 0.01$) (Fig. 1b). Blood clams exhibited a significant reduction in respiration rates with declining seawater pH values. In comparison with those at pH 8.1, the respiration rates of blood clams incubated in seawater at lowered values of pH (7.8, 7.6, and 7.4) were significantly reduced to approximately 95%, 89%, and 84%, respectively. OA also showed a significant influence on the ammonium excretion rates (one-way

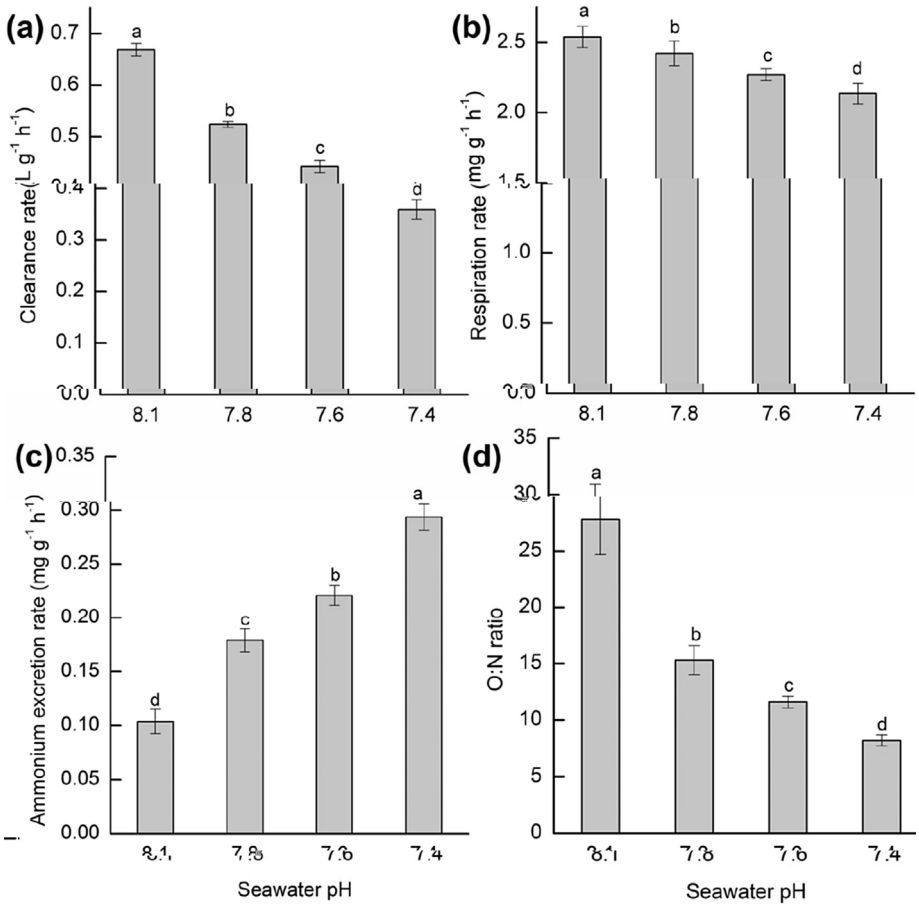

Fig. 1. Clearance rates (a), respiration rates (b), ammonium excretion rates (c), and the corresponding oxygen to nitrogen (O:N) ratio (d) of blood clams after 40 days experiment (N = 40 blood clams per pH treatment consisting of 8 replicates, mean ± SD). Means not sharing the same superscript are significantly different (Tukey's HSD, $p < 0.05$).

ANOVA, $p < 0.01$) (Fig. 1c). Ammonium excretion rates were significantly increased by decreasing seawater pH values. Compared with those at pH 8.1, ammonium excretion rates were significantly elevated to approximately 172%, 213%, and 283% at pH 7.8, 7.6, and 7.4, respectively. Due to both reduced respiration rates and increased ammonium excretion rates, the O:N ratios were significantly reduced at lowered seawater pH values (one-way ANOVA, $p < 0.01$) (Fig. 1d). The O:N ratios were approximately 28, 15, 12, and 8 at pH 8.1, 7.8, 7.6, and 7.4, respectively.

3.2. Effects of OA on haemolymph pH and Ca^{2+} concentration

OA exerted a significant impact on the haemolymph pH, which was found to be decreased with the reduction of seawater pH (Fig. 2a) (one-way ANOVA, $p < 0.01$). In comparison with pH 8.1 treatment, haemolymph pH levels were significantly lower in pH 7.6 and 7.4 treatments. Similarly, haemolymph Ca^{2+} concentration was also found to be significantly affected by OA (Fig. 2b) (one-way ANOVA, $p < 0.01$). Though no significant difference of Ca^{2+} concentration was found between pH 8.1 and 7.8 treatments, the Ca^{2+} concentrations were significantly reduced in pH 7.6 and 7.4 treatments.

3.3. Effects of OA on calcification and shell integrity

Net calcification rates were also profoundly affected by OA (one-way ANOVA, $p < 0.01$) (Fig. 3). Compared to those at pH 8.1, net calcification rates were significantly declined to approximately 86%, 76%, and 57% at pH 7.8, 7.6, and 7.4, respectively. The stereomicroscopic and SEM analysis illustrated that the inner shell surfaces of most blood clams from the lowered pH (7.8, 7.6, and 7.4) treatments were partially corroded and dissolved, while inner shell surfaces were unaffected in blood clams from pH 8.1 treatment (Fig. 4). In addition, seawater pH has significantly influenced the magnitude of corrosion and dissolution of inner shell surfaces. The most serious corrosion and dissolution showed a high occurrence in the shells of blood clams grown under pH 7.4, than those cultivated under pH 7.8 and 7.6.

4. Discussion

The significant reduction of clearance rate in the present study suggests that the feeding activity of blood clam was affected under the simulated future OA scenarios. These findings are consistent with recent literatures reporting negative effects of OA on other marine species, such as the reduced feeding rate of the mussel *M. coruscus* after 14-day exposure to acidified seawater at temperature 25 °C (Wang et al., 2015), and the decreased food uptake of Manila clam *Ruditapes philippinarum* brought about by OA (Xu et al., 2016). The OA induced suppression of feeding activity may limit the capacity of energy acquisition and nitrogen uptake from food sources. The deficit of energy supply and nitrogen could subsequently impose an adverse effect on the abilities of maintaining ion and acid-base homoeostasis, and protein synthesis.

Previous observations found that OA generally reduced respiration rates of marine invertebrates, such as the hard shell mussel

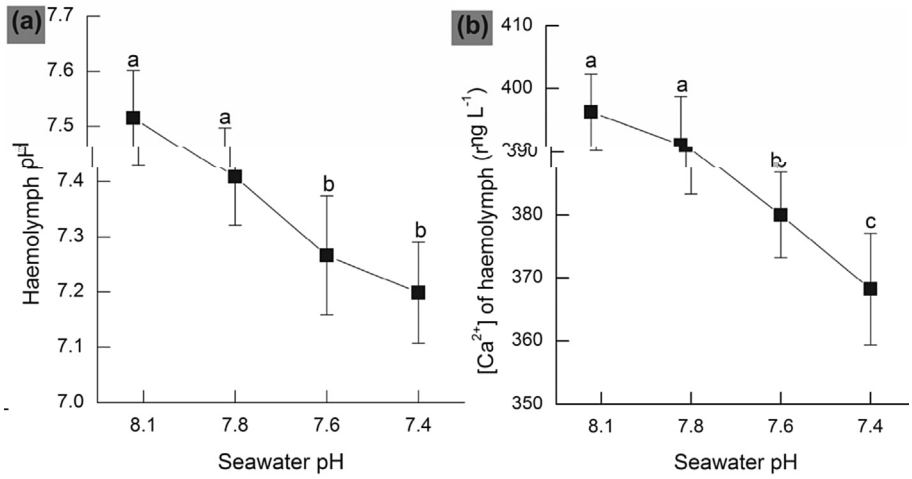

Fig. 2. Haemolymph pH (a) and calcium concentration ([Ca^{2+}]) (b) of blood clams after 40 days experiment (N = 40 blood clams per pH treatment consisting of 8 replicates, mean ± SD). Means not sharing the same superscript are significantly different (Tukey's HSD, $p < 0.05$).

M. coruscus (Wang et al., 2015) and the brittle star *Amphiura filiformis* (Hu et al., 2014). Similarly, the present study found that respiration rates of blood clams were markedly declined with the reduction of seawater pH values. Since respiration rate is commonly used as an indicator of metabolic energy expenditure (Xu et al., 2016), the results obtained suggest that aerobic metabolism and energy expenditure were suppressed. It has been shown that most marine invertebrates adopted metabolic depression as a major strategy to cope with abiotic environmental stress (Guppy, 2004; Guppy and Withers, 1999; Langenbuch and Pörtner, 2002). Since no individual death was found in the present study, down-regulation of respiration rates might be an adaptation response of blood clams to survive through OA stress.

Besides reduced clearance and respiration rates, the results of present study demonstrate that the ammonium excretion rate of blood clams increased under the simulated future OA scenarios. Due to the reduced respiration rate and increased ammonium excretion rate, the O:N ratio of blood clams was significantly down-regulated in response to acidified seawater. These findings are in accordance with the results reported on the blue mussel *M. edulis* (Thomsen and Melzner, 2010) and the brittle star *A. filiformis* (Hu et al., 2014) as well. Commonly, the O:N ratio is utilized as an indicator for the respective fractions of three metabolic substrates (carbohydrates, lipids and proteins) used in energy metabolism (Mayzaud and Conover, 1988). In general, higher O:N ratios indicate a higher fraction of catabolism of carbohydrates and lipids, whereas lower O:N ratios suggest an elevated turnover of proteins (Mayzaud and Conover, 1988). The O:N ratio of blood clams is approximately 28 at pH 8.1, a value indicating a preference of carbohydrates and lipids catabolism (Wang et al., 2015). The lower O:N ratios (15, 12, and 8 at pH 7.8, 7.6, and 7.4, respectively) suggest that amino acid catabolism covers the largest fraction, if not all, of the energy expenditure (Langenbuch and Pörtner, 2002). The enhanced amino acid catabolism despite low respiration rate as seen in the present study might indicate preferred catabolism of low O:N ratio amino acids such as asparagine (O:N ratio = 3.3), glutamine (O:N ratio = 1.5) and their dicarboxylic acids (aspartic and glutamic acid) (Langenbuch and Pörtner, 2002). The oxidative decarboxylation of these two dicarboxylic acids would lead to a high net formation of bicarbonate (HCO_3^-), supporting the regulation of acid-base balance (Langenbuch and Pörtner, 2002). Nevertheless, the increased ammonium excretion rate in combination with reduced O:N ratio indicate that blood clams shift towards amino acids catabolism under future OA scenarios.

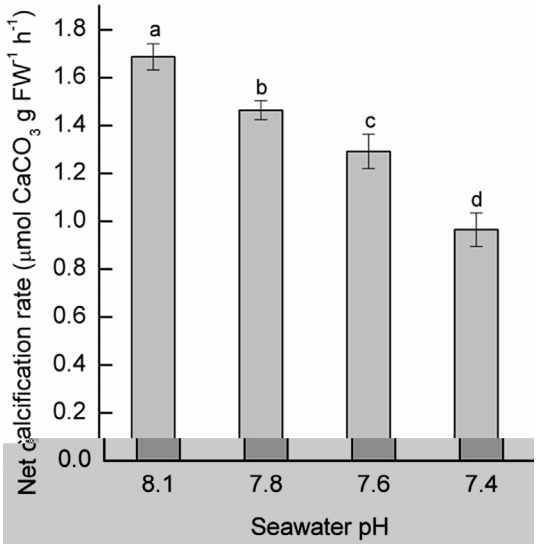

Fig. 3. Net calcification rates of blood clams after 40 days experiment (N = 40 blood clams per pH treatment consisting of 8 replicates, mean ± SD). Means not sharing the same superscript are significantly different (Tukey's HSD, $p < 0.05$).

The haemolymph pH values of blood clams declined from about 7.51 to 7.19 with seawater pH decreasing from 8.1 to 7.4. Similar effects were also found among other marine invertebrates, such as blue mussel *M. edulis* (Michaelidis et al., 2005; Thomsen et al., 2013) and sea urchin *S. droebachiensis* (Stumpp et al., 2012). These findings indicate that OA leads to extracellular acidosis. The ammonium excretion by amino acid catabolism has been hypothesized to serve as an acid-base regulation mechanism through proton (H^+) removal in many marine invertebrates (Shih et al., 2008; Wu et al., 2010), such as the blue mussel *M. edulis* (Thomsen and Melzner, 2010) and the sea urchin *Strongylocentrotus droebachiensis* (Stumpp et al., 2012). Therefore, in fact, the extracellular acidosis has already been partially attenuated by the simultaneously up-regulated ammonium excretion. Otherwise, a more serious extracellular acidosis would be expected. Due to the critical role of extracellular pH on metabolic enzymes, the detected extracellular acidosis could be one of the reasons causing metabolic depression. In addition, the extracellular acidosis would also

negatively affect $CaCO_3$ formation and the subsequent calcification process through reducing the saturation state of $CaCO_3$, which may therefore lead to the observed reduction in net calcification rates of blood clams under simulated future OA conditions.

Additionally, the inner shell surfaces of blood clams were found to be partly corroded and dissolved under future OA scenarios, which are in accordance with observations reported in other marine bivalves, such as the blue mussel *M. edulis* (Asplund et al., 2014; Li et al., 2015a; Melzner et al., 2011) and the striped venus clam *Chamelea gallina* (Bressan et al., 2014). It has been suggested that the dissolution of $CaCO_3$ shell, which releases HCO_3^-, could be a compensation mechanism to attenuate extracellular acidosis (Lindinger et al., 1984; Michaelidis et al., 2005). At the same time, the dissolution of $CaCO_3$ shell will also release free dissolved Ca^{2+} and may therefore increase the concentration of Ca^{2+} in haemolymph (

such as the blue mussel *M. edulis* (Fitzer et al., 2016, 2014a, 2014b). Overall, the observed OA-induced impairment of calcification process and inner shell surface integrity could be partly accounted by 1) insufficient energy supply and allocation, 2) the incompletely compensated extracellular acidosis, and 3) a nitrogen limitation.

In conclusion, the present study demonstrates that OA would pose significant adverse effects on the feeding activity, metabolism, calcification, and maintenance of haemolymph acid-base balance and Ca^{2+} homeostasis of the blood clam, *T. granosa*. In combination with weakened immunity, accumulation of toxic metal and impairment of fertilization as reported by our previous studies (Liu et al., 2016; Shi et al., 2016), the projected OA would dramatically affect the fitness of this marine bivalve species, posing threats to both the aquaculture industry and the stability of coastal community.

Competing interests

The authors declare no competing financial interests.

Authors' contributions

X.Z. and G.L. designed and performed the whole experiments. W.S., Y.H., S.L., and C.G. contributed to animal incubation, and data collection. Other than the first author (X.Z.) and corresponding author (G.L.), W.F. and X.C. also contributed to manuscript writing. All authors gave final approval for publication.

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