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The behavioral and antioxidant response of the bivalve *Gomphina veneriformis* to sediment burial effect

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Abstract

A laboratory-based microcosm experiment was carried out to examine both the behavioral and antioxidant response of the clam *Gomphina veneriformis* under the conditions of 3 types of burial material (sand, silt, silt-sand mixture) with 3 burial depths (5 cm, 15 cm, 30 cm). The concentration of dissolved oxygen decreased significantly after 3 d of burial in all experimental groups. In silt and sand-silt mixture groups, the interstitial water quality became worsened with lower pH, and higher NH_4^+-N concentration, where clam mortality occurred simultaneously. However, clam samples in all sand groups and 5 cm, 15 cm sand-silt mixture groups survived well for 8 d. Obviously fewer individuals left in the bottom sand in the 15 cm, 30 cm silt groups and 30 cm sand-silt mixture groups than in the 5 cm groups. Therefore, it suggests that adding silt and increasing burial depth could stimulate the vertical movement of organisms and cause lethal effects. It was found that the burial depth was the key factor that influenced the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). The SOD and CAT activities in the gills and hepatopancreases of organisms both showed significant up-regulation in 30 cm burial depth after buried for 8 d. Higher enzyme activities were found in gills than in hepatopancreases, which indicated that the gills of the bivalve *G. veneriformis* were more susceptible to burial effects than hepatopancreases. Overall, this study shows that sediment burial could cause effects on the biological behavior and antioxidant enzyme activities.

Key words: *Gomphina veneriformis*, burial effect, biological response, physiological adaptation, antioxidant enzyme

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1 Introduction

Coastal zone is one of the hot spot regions which suffers the highest anthropogenic disturbance in the world, e.g., dredging, reclamation, industrial and sewage effluents, and oil pollution (Naser, 2011). The newly deposited sediment may clog and smother the habitats by infilling of interstices and make the habitat unsuitable for macroinvertebrates to live, which was called burial effects (Conroy et al., 2017). Burial effects from sand and mud are very common phenomena that can be induced by a wide variety of ways resulting from both natural and anthropogenic activities. During rainfall (storms or tidal events), mobilization and deposit of large volumes of sediment can occur in coastal areas. A range of human activities, e.g., reclamation projects, dredging, industrial disposal, and sewage sludge, can cause burial effects on material disposal areas (Conroy et al., 2017).

Macrobenthic assemblages are seriously affected by burial impacts because of their unique sedentary features, which cause them more sensitive to environmental changes. For these, they are widely used as ideal biological indicators to detect environmental impacts from several sources of stress and pollution (Naser, 2011). The burial impacts on macrobenthic assemblages are mostly associated with the burial sediment type, depth, the duration of burial, and the biological response of the organism. It might directly damage and kill some organisms or bury them within the sediment and then lead to biological responses by vertical migration or physiological adaptation (Yanez et al., 2008).

The clam *Gomphina veneriformis* used in our study belongs to Lamellibranchia, Veneridae of Bivalve. These clams are endemic species of the western coast of the Pacific and widely distributes along the coastline of Chinese sea area. They are commercially important due to its good taste and beautiful shells. Some previous studies have noted that these clams are digging

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and burrowing *par excellence* (You et al., 1992). They inhabit in sandy sediment and usually live under 1 cm to 2 m of the sediment (Park et al., 2012; You et al., 1992). The good excavator was selected to explore the tolerance to the burial effects.

Previous research have demonstrated vertical migration of organisms under various burial conditions. In the 1980s, Don Maurer reported that the vertical migration is an important factor in the recovery of benthic community's response to burial effects. Vertical migration and mortalities are closely related to synergistic effects of experimental parameters, e.g., sediment depths, burial time, particle size and temperature. Most species can burrow to recolonize to deal with the burial effects and mortalities generally increase with deeper sediment depth, longer burial time and sediment type (Maurer et al., 1981a, b, 1982, 1986). Chandrasekara and Frid (1998) found that the lethal depth and vertical movement ability were different between two gastropods, *Hydrobia ulvae* and *Lirrorina littorea* (Chandrasekara and Frid, 1998). Significant difference was found in survival numbers of organisms between control and experimental treatments with the highest percentage of survival happened in Polychaete *Perinereis nuntia* (Naser, 2011). Powilleit et al. (2009) explored the behavioral response of 6 brackish macroinvertebrates and found all the bivalves showed high escape potentials and could successfully escape from a 32–41 cm deposited sediment layer, while the mobility of Polychaete species varied significantly (Powilleit et al., 2009).

Previous research on burial effects were mostly focused on the individual's ability and velocity of excavation. Nonetheless, few studies have followed the molecular mechanism of organisms under burial effects. The antioxidant defense is one kind of important defense mechanism of mollusks to minimize adverse stress caused by environmental disturbance (Cong et al., 2012). Various environmental stressors lead to the over-production of reactive oxygen species (ROS) in lipids, carbohydrates, and DNA of organisms, which ultimately result in oxidative stress. Meanwhile, the formation of ROS is prevented by an antioxidant system which consists of a series of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx) and so on (Park et al., 2012). As we know, most analyses of antioxidant response of organism have been focused on the exposure of trace metal and chemical toxicants, but only limited information is currently available regarding the burial effects on antioxidant defense and endocrine systems of bivalves at molecular level. In this study, the burial effects on the SOD and CAT activities in the two relevant tissues, gills and hepatopancreases of *G. veneriformis* were examined, besides the vertical migration of organisms. This study hypothesize the burial effects would cause the biological response of clams *G. veneriformis*, reflecting in both the vertical migration and physiological adaptation according to burial materials, depth, and burial duration.

2 Materials and methods

2.1 *Animal and sediment collection*

The clam individuals were collected from the intertidal zone of Yantai and transported to the laboratory. Before the experiment began, the clams were maintained in buckets filled with filtered seawater for 7 d. During the acclimatization period, aeration was provided continuously and the clams were fed with microalgae *Chlorella vulgaris* once a day. Seawater was completely changed daily and the water temperature was maintained at 20℃. After 7 d, the active individuals were selected for the experiment.

Sediment samples collected from the same coastal beach in Yantai were sieved and divided into three groups by sediment grain size: sand (S), silt (M), and 50% sand + 50% silt mixture (MS). All the sediment samples were pre-treated by the process of air-dried, sieved to <0.5 mm in size and exposed to sunlight outdoor prior to the experiment.

2.2 *Experimental setup*

The microcosm experiment was carried out in 10 plastic cubes (70 cm × 50 cm × 60 cm), which were filled with 10 cm layer of sieved sand sediment. Three cylindrical PVC tubes (inner diameter 19 cm, length 40 cm) were inserted into sediment 5 cm in each cube (Fig. 1). Ten individuals were firstly placed on the surface of the sediment in each tube to acclimate for 1 d. Then, they were buried by the treated sediment with different depth according to the experiment design (Fig. 1). Combing the two aspects, burial sediment type and burial depth, the experimental groups were named as C, S, MS and M, which represent the control group, 100% sand, 50% sand + 50% silt and 100% silt, respectively. For example, S5 means the treatment group with 5 cm burial depth and 100% sand burial material.

All the tubes were placed in a chamber with seawater changed every day. The experiment lasted for 8 d to analyze the subchronic sedimental burial effect on animals. There were 8 individuals per tube (24 individuals in total per treatment group) were collected and stored in –80°C for further biochemical analysis. The dissected tissues of the gills and hepatopancreases were flash frozen in liquid nitrogen and stored at –80°C prior to enzyme assay. Clam individuals were checked every day for vitality. They were considered as dead if their shell gaped and failed to shut again by an external stimulus, then picked out from the sediment.

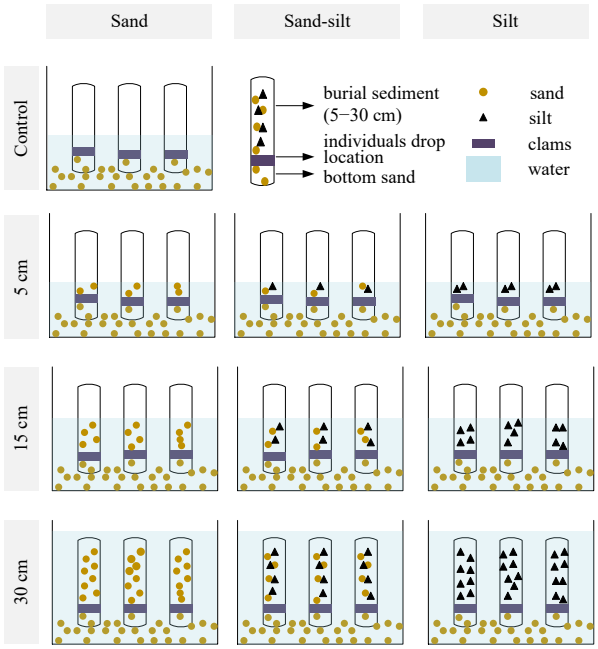

Fig. 1. The schematic diagram of experimental design.

2.3 *Environmental parameters of sediment and pore water*

The Rhizon soil moisture sampler (Rhizosphere, Neatherlands) was inserted into the burial sediment to collect pore water samples in 3 depth layer: surface (5 cm to surface), middle (in the middle of the burial height), and bottom (5 cm to the bottom). During the experiment period, the pore water was sampled 4

times on Days 1, 3, 5 and 7, respectively. The temperature, dissolved oxygen (DO), and pH were measured by a portable water quality detector, YSI 600 QS-M-O (Yellow Springs Ohio 54387, USA). The NH_4^+-N, NO_3^--N and NO_2^--N concentrations were performed on a gas-segmented continuous flow system, Auto Analyzer 3 (SEAL Analytical, Germany). The grain size was measured by Mastersizer 2000 Laser Particle Sizer (Malvern Instruments, UK).

2.4 *Antioxidant enzyme activity analysis*

To assess antioxidant enzyme activity, 0.1 g of gills and hepatopancreases were individually homogenized in 0.9 volume of normal saline using an IKA homogenizer (Ultra Turrax IKA T10 basic, Germany). The homogenized samples were centrifuged at 10 000 r/min for 15 min at 4°C to remove tissue debris. The supernatant was collected for the analysis of enzyme activity and protein content. The antioxidant enzyme (SOD and CAT) activities were assayed by a multiscan spectrum microplate spectrophotometer (Infinite M200, TECAN) according to the manufacturer's protocols using enzyme kits (Jiancheng, China). The total protein concentration of the supernatant was calculated according to Bradford (1976) using bovine serum albumin to normalize enzyme activity data. All the enzyme activities were expressed as U/mg protein.

2.5 *Statistical analysis*

Three-way ANOVA analysis was used to test the difference of the environmental factors among groups, layers and experimental durations. Two-way ANOVA analysis was conducted to test whether the enzyme activities and the individual number distribution of species were affected by sediment grain size and burial depth. Mann-Whitney U paired test was used to test the difference of the enzyme activities between gills and hepatopancreases in the same groups. Data were tested for normality and homogeneity of variance using Shapiro-Wilk, and Levene's tests, respectively. If necessary, data were $\log_{10}$-transformed to meet these assumptions prior to analysis. Tukey's Honest Significant Difference test (Tukey's HSD) *post hoc* comparisons were conducted if the significant difference were found by the ANOVA analysis. The difference in size of clams among groups and layers was detected by the Kruskal test due to the data's non-normality, then dunntest was applied for the pairwise comparisons among groups.

3 Results

3.1 *Environmental parameters*

The grain size of all the sediment used in this experiment was less than 0.5 mm. The silt sediment was composed of 1.56% clay (<4 μm), 13.63% silt (4–63 μm) and 84.81% fine sand (>63 μm). The sand sediment was composed of 100% fine sand (>63 μm). The sand–silt mixture sediment was composed of 0.67% clay (<4 μm), 8.67% silt (4–63 μm) and 90.66% fine sand (>63 μm).

Although the seawater was refreshed every day and the temperature was controlled at 20°C in the experiment laboratory, the environmental parameters of interstitial water showed significant changes among groups, durations and layers due to burial effects (Fig. 2). All the environmental parameters changed significantly among layers (Three-way ANOVA, $p<0.05$). The interstitial water of bottom layer was with prominent lower temperature, pH, DO, and higher HH_4^+-N, NO_3^--N and NO_2^--N concentrations compared to the surface layer (Tukey's HSD, $p<0.05$). The pH (Three-way ANOVA, $F_{(9, 85)}=12.54$, $p<0.01$) and HH_4^+-N concentration (Three-way ANOVA, $F_{(9, 85)}=10.95$, $p<0.01$) also changed evidently among groups. Further analysis showed that the HH_4^+-N concentrations of 15 cm and 30 cm silt burial groups were especially higher than sand and control groups (Tukey's HSD, $p<0.05$); the pH of sand-silt and silt groups were especially lower than sand and control groups (Tukey's HSD, $p<0.05$). The DO (Three-way ANOVA, $F_{(3, 85)} = 24.52$, $p<0.01$), pH (Three-way ANOVA, $F_{(3, 85)} = 4.56$, $p<0.01$), NO_3^--N concentrations (Three-way ANOVA, $F_{(3, 85)}=6.95$, $p<0.01$) and NO_2^--N concentrations (Three-way ANOVA, $F_{(3, 85)} = 7.53$, $p<0.01$) changed significantly by time. Further pairwise comparison showed as follows: the pH was evidently lower on Day 5 and Day 7 compared to Day 1 (Tukey's HSD, $p<0.05$); the DO, NO_3^--N and NO_2^--N concentrations was high on Day 1 (Tukey's HSD, $p<0.05$) but decreased sharply on Day 3 and maintain the low concentration in Day 5 and Day 7. As shown in Fig. 2, the changes among groups and duration mainly happened on the middle and bottom layers but not surface layers.

3.2 *Size of individuals in experiment*

In order to alleviate the impact of body size on the experimental results, this study tried to choose individuals with similar size for experiment. The range of shell lengths was 2.3–3.9 cm and shell width 1.7–2.9 cm. However, there are still some differences on the average length and width of the clams among groups (shell length, chi-squared=19.28, $p<0.05$, Kruskal test; shell width, chi-squared=18.47, $p<0.05$, Kruskal test). The body size in M5 group was significantly larger than that in M30 group (Dunn test, $p<0.05$). However, there was no significant difference on the body size in any other two groups (Dunn test, $p>0.05$). Also, there was no significant difference in the body size among the layers in all 30 burial groups (Kruskal test, $p>0.05$) (Table 1)

3.3 *Vertical migration of individuals inside the sediment*

Ten individuals which were placed on the surface of the bottom sand for 1 d acclimation burrowed into the sand sediment at the beginning of the experiment. Most of the clam individuals showed upward vertical migration among all treatments after burial. The number of individuals in the bottom sand showed a significant difference among groups after buried for 8 d (One-way ANOVA, $F_{(9, 20)}=17.46$, $p<0.01$). Indeed, this difference was caused by the burial depth rather than the sediment type. The individual numbers in the M15 and M30 groups were fewer than that in the M5 group (Tukey's HSD test, $p<0.01$). The individual numbers in the MS30 group were fewer than that in the MS5 group (Tukey's HSD test, $p<0.01$). However, the difference among sediment types in the same burial depth was not significant (Table 2).

Except for the above differences in the individual distribution of species, the clam also showed different behavior in siphon tips. In Groups S5, MS5, M5, S15, and MS15, their siphon tips reached the sediment surface in 48 h after they were buried; conversely, the siphon tips reached the sediment surface 5 d later in Groups M15, S30, MS30, and M30.

3.4 *Survival rate of individuals buried inside the sediment*

All individuals kept alive in control groups (C), all sand groups (S) and 5–15 cm sand-silt mixture groups (MS). All the dead individuals were found in the burial sediment but not in the bottom sand in the groups with dead organisms. There are two dead individuals in Group M5. Four dead individuals distributed in 0–15 cm (to the bottom layer) in Group M5. Two dead individuals were found in the 5 cm (to the bottom) layer in Group

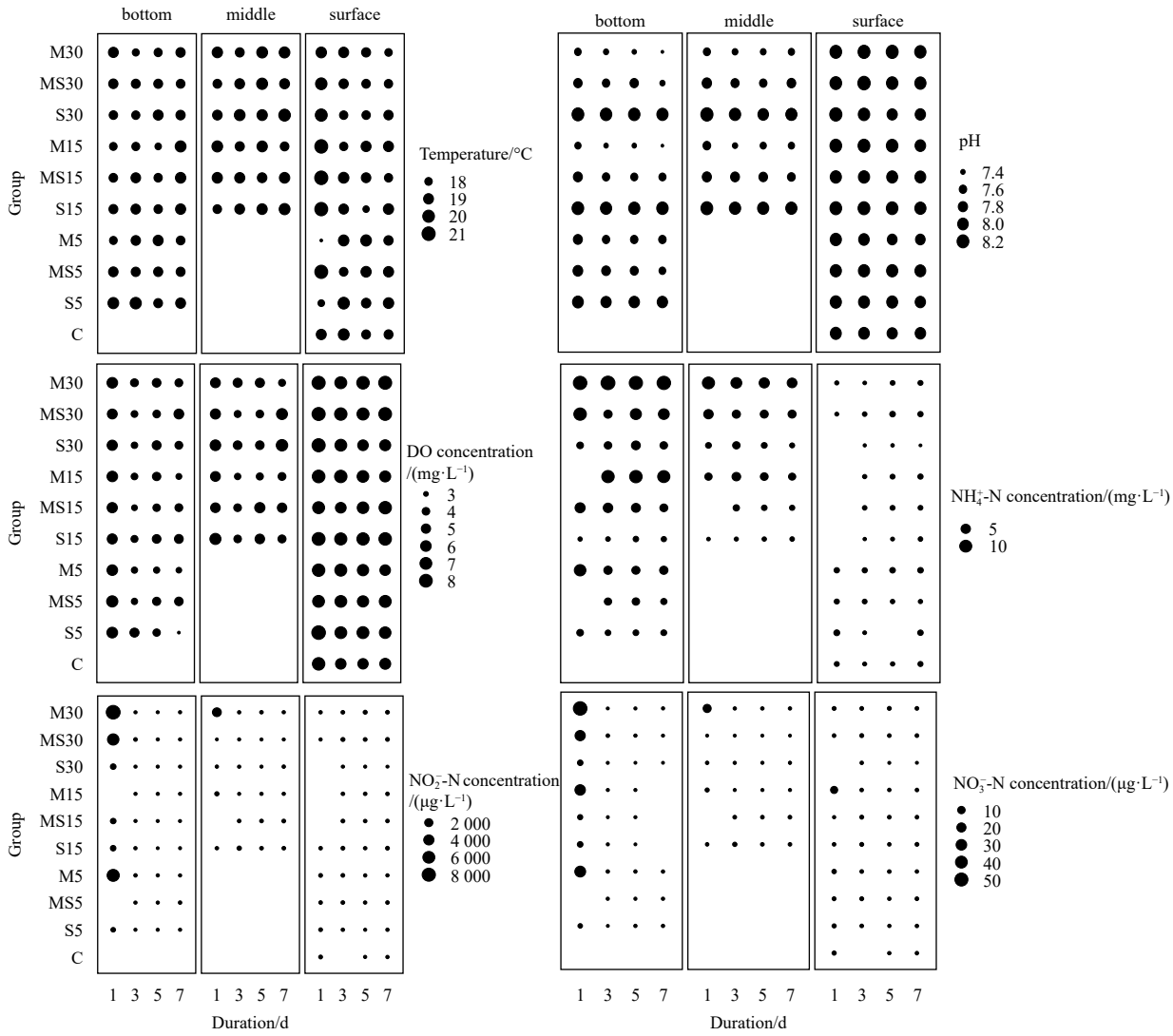

Fig. 2. Environmental parameters of pore water from the surface, middle and bottom sediment layers during the experiment.

Table 1. The average length and width of clam individuals distributed at different layers in 30 cm burial groups

Layer	S30		MS30		M30	
	Length/cm	Width/cm	Length/cm	Width/cm	Length/cm	Width/cm
25–30 cm	–	–	26.35	19.12	26.31	19.36
20–25 cm	29.33	20.04	–	–	25.82	18.80
15–20 cm	–	–	27.39	20.45	27.73	20.28
10–15 cm	24.44	17.56	27.02	19.87	25.74	18.66
5–10 cm	26.59	19.31	25.66	18.96	30.64	22.81
0–5 cm	28.16	20.95	28.42	20.89	25.64	18.78
–5 cm to 0 cm	26.95	19.89	24.81	18.15	25.10	18.51

Note: – means no data.

MS30. Three individuals distributed in 10 cm (to the bottom) layer in Group M30. The order of mortality rate was as follows: M15 (13.33%)>M30 (10%)>MS30 (6.67%)=M5 (6.67%).

3.5 *The activities of antioxidant enzymes: superoxide dismutase and catalase*

The SOD activities in gills were significantly impacted by different burial depths (Two-way ANOVA, $F_{(3, 57)}$=10.537, p<0.01) rather than by sediment grain size (Two-way ANOVA, $F_{(2, 57)}$= 2.602, p>0.05). Significant up-regulation of SOD activity was detected in the 30 cm burial depth groups compared to the control group (Tukey's HSD test, p<0.05), 5 cm burial depth groups (Tukey's HSD test, p<0.05) and 15 cm burial depth group (Tukey's HSD test, p<0.01) (Fig. 3).

The SOD activities in hepatopancreas were also closely related to the burial depths (Two-way ANOVA, $F_{(3, 57)}$ = 13.482, p< 0.01) but not sediment grain size (Two-way ANOVA, $F_{(2, 57)}$=0.578, p>0.05). The SOD activities were stimulated significantly in the 5 cm

Table 2. The average number (n=3) of individuals distributed at different layers when buried for 8 d

Layer	Experimental group									
	C	S5	MS5	M5	S15	MS15	M15	S30	MS30	M30
25–30 cm	–	–	–	–	–	–	–	–	2.00	1.33
20–25 cm	–	–	–	–	–	–	–	0.67	–	1.00
15–20 cm	–	–	–	–	–	–	–	–	1.67	1.00
10–15 cm	–	–	–	–	3.00	5.33	5.67	0.33	1.33	1.00
5–10 cm	–	–	–	–	0.67	1.33	1.67	2.00	0.67	1.67
0–5 cm	–	4.33	4.00	2.00	2.33	0.67	2.00	2.33	3.33	2.67
–5–0 cm	10.00	5.67	6.00	8.00	4.00	2.67	0.67	4.33	1.00	1.33

Note: – means no data.

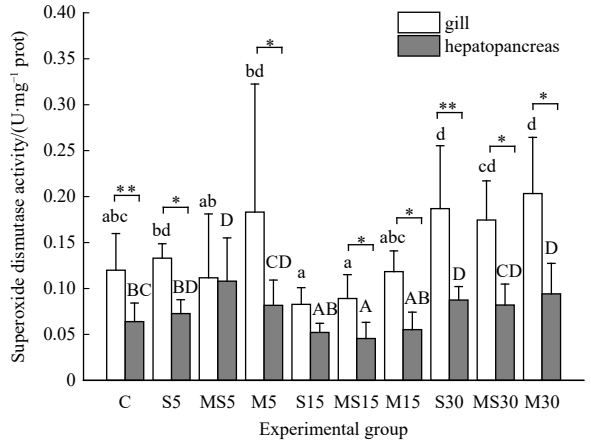

Fig. 3. The superoxide dismutase (SOD) activities in gills and hepatopancreases of *G. veneriformis* in each experimental group. Error bars indicate standard deviation (n>5). The bars marked with different letters indicates there are significant differences among experimental groups, same letters no difference. Lower-case letters indicate difference analysis of SOD activities among groups in gills and capital letters in hepatopancreases. Asterisks indicated different SOD activities between gills and hepatopancreas in the same group. *p<0.05; **p<0.01.

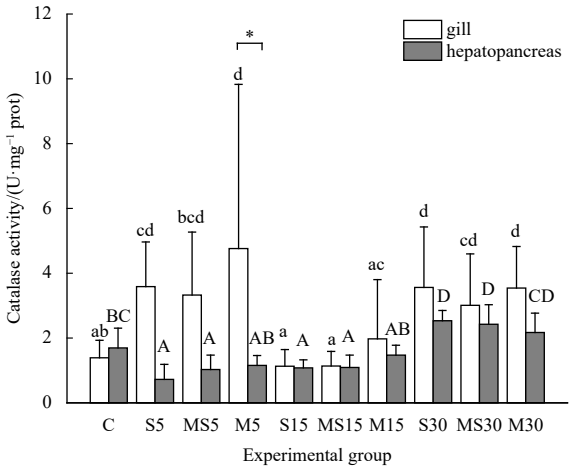

Fig. 4. The catalase (CAT) activities in gills and hepatopancreases of *G. veneriformis* in each experimental group. Error bars indicate the standard deviation (n>3). The bars marked with different letters indicates there are significant differences among experimental groups, same letters no difference. Lower-case letters indicate difference analysis of CAT activities among groups in gills and capital letters in hepatopancreases. Asterisks indicated different CAT activities between gills and hepatopancreas in the same group. *p<0.05; **p<0.01.

and 30 cm depth groups compared to the 15 cm group (Tukey's HSD, p<0.01) (Fig. 3). The value of SOD activities in gills was significantly higher than in hepatopancreases in most groups except Groups MS5 and S15 (Mann–Whitney U paired test, p>0.05) (Fig. 3).

The CAT activities in gills and hepatopancreas changed significantly among groups with different burial depths (Two-way ANOVA, $F_{(3, 50)}$=11.588, p<0.01; $F_{(3, 50)}$=28.797, p<0.01) but not significantly among groups with different sediment grain size (Two-way ANOVA, $F_{(2, 50)}$=1.985, p>0.05; $F_{(2, 50)}$=0.202, p>0.05). The CAT activities in gills was higher than that in hepatopancreases. Especially, the value of CAT activities in gills was significantly higher than that in hepatopancreases in Group M5 (Mann–Whitney U paired test, p<0.05) (Fig. 4).

The CAT activities in gills in the 5 cm and 30 cm groups were stimulated significantly. CAT activities in 5 cm and 30 cm groups were higher than that in the control group (Tukey's HSD, p=0.03<0.05); Tukey's HSD, p=0.02<0.05) and 15 cm groups (Tukey's HSD, p<0.01; Tukey's HSD, p<0.01). However, no significant difference was found between the control group and 15 cm groups (Tukey's HSD, p>0.05), or between 5 cm and 30 cm groups (Tukey's HSD, p>0.05) (Fig. 4).

The value of CAT activities in hepatopancreases in the 30 cm depth group was extremely higher than the control group (Tukey's HSD, p=0.02<0.05), the 5 cm group (Tukey's HSD, p<0.01) and 15 cm group (Tukey's HSD, p<0.01). The CAT activities in 5 cm groups were restrained significantly than the control group (Tukey's HSD, p<0.01) (Fig. 4).

4 Discussion

4.1 *Burial effect on the vertical movement and survival of G. veneriformis*

The majority of organisms have an innate burrowing ability to regain their preferred positions when they are buried in the sediment (Maurer et al., 1981b, 1986; Trueman, 1983). Organisms in all groups showed upward movement when they were buried in sediment. However, the ability and velocity of vertical movement varied among groups depending on the burial materials and depths. For example, the delay of siphon appearance on the surface in both the 15 cm (M15) and 30 cm (M30) silt groups indicated that the increase of the silt component and burial depth would stress the difficulty for vertical movements of organisms. The number of individuals in the bottom sand of 30 cm

sand-silt and silt burial groups was fewer than that of 5 cm burial groups, which indicated the addition of silt component in burial sediment also could stimulate the upward movement of organisms for survival. In fact, the ability of organisms to escape from burials varies with multiple factors, especially their habits and morphology (Chandrasekara and Frid, 1998). Infauna taxa are able to escape from 10 cm or deeper burial depth (Bellchambers and Richardson, 1995; Jackson and James, 1979). Whereas epibenthic fauna may be unable to escape from 1 cm burial depth (Kranz, 1974). The species with a large cylindrical foot, such as some bivalves, are more likely to escape from burials than species with a reduced foot, such as some gastropod with an extremely flattened foot or plough-shaped foot (Trueman, 1983). Chandrasekara and Frid (1998) found two epibenthic gastropod species, *H. ulvae* and *Littorina littorea*, which were unable to regain the surface under 5 cm depth in natural sediment. Therefore, the ability of upward movement was species-specific. This study found the bivalve *G. veneriformis* could regain the surface successfully in 30 cm burial depth even buried in silt sediment.

Although most of the individuals were able to regain the surface and survive well for 8 d in the present work, death occurred in all the silt burial groups and 30 cm sand-silt mixture groups. It was supposed that the finer sediment fraction and the deeper burial depth could cause more damage to the organisms. The negative effects of silt and deeper burial depth could also be indicated clearly in the chemical parameters of interstitial water in the bottom layer. Groups M5, M15, MS30, and M30 were in worse water quality in the bottom layer with significantly lower pH and the higher concentrations of NH_4^+-N, NO_2^--N and NO_3^--N where the lethal rate increased simultaneously. As a result, the changes of chemical parameters are a good explanation for the death of individuals.

Bivalves are regarded as burrowing *par excellence* (Trueman, 1983). Lots of experiments have demonstrated their burrowing behavior and vertical movement after burial (Kranz, 1974; Maurer et al., 1981a; Stanley, 1970; Trueman, 1983). Kranz (1974) concluded that the burrowing activities of bivalves differed from bivalves' biological factors (e.g., size of individuals, foot morphology), life habits (e.g., living depths, fill types) and environmental parameters (e.g., native sediment, temperature, salinity, and oxygen concentration). The bivalve *G. veneriformis* belongs to the life form of shallow burrowing with siphonate suspension feeders (Kranz, 1974), which is able to inhabit from 10 cm to 50 cm of its native sediment. As mentioned above, the migration of species is associated with multiple variables, which make it not easy to compare with other studies. For example, Chandrasekara and Frid (1998) pointed out that the maximum overburden of the *Hydrobia ulvae* was 5 cm, while Bijkerk (1988) reported that the lethal burial depth of *H. ulvae* was 9 cm for sand and 20 cm for mud (cited by Bolam (2011)). In a word, the results of this study were consistent with the general conclusions that the changes in native sediment type (finer sediment fraction) and deeper burial depths caused significant effects to the survival and movement of species (Conroy et al., 2017; Kranz, 1974).

4.2 *Adaptation mechanism of G. veneriformis to the burial effects*

The silt adding have caused environmental changes by lower pH, higher NH_4^+-N, NO_2^--N and NO_3^--N concentrations in the interstitial water of the bottom layer as well as the increase of the lethal rate in groups with silt burials. However, the grain size did not cause significant difference among the three types of sediment due to the higher percent (over 80%) of fine sand in all the groups, which may not reach a threshold creating a significant difference among antioxidant enzymes of the species. Therefore, the differences of the SOD or CAT activities among sediment grain size were not significant. Conversely, the activities of SOD and CAT were significantly increased in organisms at the 30 cm burial depth, which might be associated with the oxidative stress induced by the burial effects.

Mass ROS will be produced under the oxidative stress when the organisms are suffered to the burial effects, which will cause peroxide injury on protein, nucleic acid, lipid of organisms. The SOD is the first line of defense against ROS by catalyzing it to hydrogen peroxide (H_2O_2). CAT is the major antioxidant to catalyze the conversion of H_2O_2 to water and oxygen (Basha and Rani, 2003; Ross et al., 2001). The defense of SOD, CAT and other metabolic mechanisms in oxidative stress were reported when invertebrates were exposed to contamination such as trace metal and chemical toxicants, environmental factors such as temperature, hypoxia, water turbidity and so on (Greco et al., 2011; Nunes et al., 2017; Suzuki et al., 2018; Woo et al., 2013; Wu et al., 2012). In this study, significantly higher activities of SOD and CAT in *G. veneriformis* were observed in all 30 cm buried depth groups. Ji (2014) made a series of observations on the changes of SOD and CAT activities in gills and viscera of *Ruditapes philipinarum* every 6 h from 96 h and found that the reaction of SOD and CAT activities in gills and viscera varied by burial sediment granularity and buried depths. They realized that the antioxidant enzyme activities were firstly mainly restrained and then induced in low to middle burial depth (2–8 cm), but were mainly inhibited in high burial depths (10–12 cm) when the organisms were buried in 125–63 μm sediment. The antioxidant enzyme activities mainly showed an induced-restrained cycle when the organisms were buried in 125–63 μm of sediment (Ji, 2014). In our work, the SOD and CAT activities of gills and hepatopancreases showed an up-regulation mode when buried in 5 cm and 30 cm depths for 8 d. This might because of the "hormesis" induced by low-level stress (Stebbing, 1982). The antioxidant may also have experienced inhabitation firstly and then stimulation, but only the stimulation phase at the end of the experiment was observed. To avoid disturbance during the burial duration, the samples had not been collected until the end of the experiment.

The SOD and CAT activities of bivalve showed tissue-specific characters in this study. The gills of bivalve were more susceptible to burial effects than hepatopancreas and the SOD and CAT activities in gills were higher than that in hepatopancreas. This phenomenon was also found in bivalve when they were exposed to high suspended solids (Yang et al., 2017). During the dissection, this study found the gills of organisms were filled with fine solids. The bivalve requires a flow of water through their gills for respiration and feeding, so the gills are more susceptible to the damage of interstitial water with lower pH, higher NH_4^+-N concentration and finer sediment. However, It is also reported that the enzyme activities were higher in viscera than in gills because viscera holds digestive organs, liver and more types of enzymes while the gills were mainly for gaseous exchange and filter-feeding (Chen et al., 2002; Ji, 2014). The reaction of antioxidant enzyme systems varies with species and tissues and can be also influenced by experimental environmental parameters. Thus, it is unreasonable to compare the results of different research by neglecting the experimental species and environmental factors.

There are still some limitations about this study and some questions still need to be answered before getting some sound explanations. For example, the significant up-regulation of SOD

and CAT activities of organism was observed at 5 cm and 30 cm but not at 15 cm depths. Limited references make it more difficult to achieve some clear explanations for these results. Further mechanism analysis is urgently needed to make clear how and why the organisms respond and survive in the burial effect.

Body size is closely linked to several functional traits, such as growth, reproduction and mortality influencing ecosystem functioning (Séguin et al., 2014; Woodward et al., 2005). This study has tried to select the clam individuals with similar body size to alleviate its effect on the results. Although, there are still some difference on the body size during these experiments, it did not make much confusion to these experimental results. Body size was not the main factor influencing the vertical movement in all 30 cm burial groups. Additionally, the sediment type also most possibly influenced the animals' behavioral and antioxidant response during burying process. In the present study, the aim was to simulate the natural status and practical application, so the natural sand and silt mud with high percentage of fine sand (>63 μm) collected from Yantai coastal beach were tested. To better understanding the burial effects to marine macrobenthos, the different sediment types with different grain size should be considered in further study.

5 Conclusions

Clam *G. veneriformis* presented good vertical movement ability and high tolerance to the burial effects. Most individuals could burrow upward or reconnected to the surface layer at 30 cm burial depth in 8 d. However, the vertical movement ability varied with the burial materials and depths: the increased silt component and burial depth enhanced the difficulty for vertical movements of organisms and also stimulated the upward movement for survival.

The pore water at the bottom and middle layers of silt and silt-sand groups was in a poor quality condition with lower pH and higher NH_4^+-N concentrations, where higher mortality rate occurred simultaneously. The oxygen decreased over time at the bottom in all groups, and the SOD and CAT activities showed up-regulation at 30 cm burial depth, which indicated the animal would die over time under these conditions.

Quantifying the animal response to burial effects will not only aid in predicting the ecological impacts caused by dredging and reclamation activities, but also providing the basis information for the sustainable use of the biotic resource in coastal zone and the health of marine ecosystems. However, there are still large gaps in the knowledge about the mechanisms of benthic animal response to burial effects for further study.

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