Marine Environmental Research 176 (2022) 105601

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

The survival and responses of blue mussel *Mytilus edulis* to 16-day sustained hypoxia stress

Qiao Li [a,b,*], Fang Zhang [a,b,c,d], Song Sun [a,b,c,d,e]

[a] CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China
[b] Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China
[c] University of Chinese Academy of Sciences, Beijing, China
[d] Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, China
[e] Jiaozhou Bay Marine Ecosystem Research Station, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

ARTICLE INFO

Keywords:
hypoxia
Mytilus edulis
Survival
Metabolic rate
Antioxidants
Immune response

ABSTRACT

The blue mussel *Mytilus edulis*, which is a worldwide commercial species distributed mainly from the intertidal zone to tens of meters deep, has been previously studied regarding its acute defense responses to air exposure and intermittent hypoxia. However, the effects of sustained hypoxia, such as caused by coastal eutrophication, remain to be explored. In the present study, the critical threshold of dissolved oxygen (DO) for experimental mussels exposed to 16 days of hypoxia was DO 0.7–0.8 mg L^{-1}, below which survival dropped drastically from nearly 80% to <38%. When hypoxia was combined with DO fluctuations or with poor water quality, the threshold rose to an average of DO 1.0 mg L^{-1}, which resulted in less than 80% survival. To find possible clues of physiological stress to account for mortalities, the metabolic rate and enzyme activities of Na^+/K^+ ATPase, superoxide dismutase, acid phosphatase, and alkaline phosphatase were further recorded.

1. Introduction

The blue mussel *Mytilus edulis*, which is a common species of the family Mytilidae, is widely distributed and cultured in coastal waters, where it plays an important role in the food web and carbon cycle and is a valuable fishery and mariculture commodity. The habitat of blue mussels spans from the shallows of the intertidal zone to tens of meters deep—a range with high variability in environmental conditions, including temperature, salinity, and dissolved oxygen (DO). Hypoxia is a common stressor for blue mussels owing to frequent air exposure in the intertidal zone and less often to water stratification during windless summer days.

Marine organisms under hypoxia face multiple types of stress, among which a reduced energy supply caused by the oxygen deficiency is the most crucial, because all life-sustaining systems need a minimum level of energy to function normally (Seibel, 2011). For blue mussels and other mollusks that are able to survive periodic exposure to air in the intertidal zone, depression of the metabolic rate combined with anaerobic respiration plays a key role. Gu et al. (2019) demonstrated that the respiration rate and feeding activity of blue mussels were significantly reduced at a DO concentration of 2.0 mg L^{-1}; if decreased to extremely low levels of DO < 1.0 mg L^{-1}, anaerobic respiration was adopted as an extra strategy in addition to metabolic depression. Amino acid adducts termed opines have been reported as an important source of energy to yield more adenosine triphosphate (ATP) under anaerobic metabolism (Stefano et al., 2015; Wang and Widdows, 1993).

To further relieve the energy shortage, an increasing amount of hypoxia-inducible factor 1 (HIF-1) is needed to induce the expression of genes related to oxygen transport and to energy production, such as through erythropoiesis and glycolysis (Wu, 2002). However, the stabilization and accumulation of HIF-1 rely on a certain amount of reactive oxygen species (ROS) to proceed, but which will put the organism in oxidative stress (Chandel et al., 2000). This kind of stress can be enormous in cases where environmental factors are complex and unstable, causing DO concentrations to fluctuate more frequently (Lawniczak et al., 2013). Because superoxide is generated by mitochondrial complex III under the stimulation of hypoxia, superoxide dismutase (SOD) plays an irreplaceable role to specifically catalyze the decomposition of excessive superoxide. In marine animals, studies have confirmed that SOD is essential to keep the organism from oxidative stress by

* Corresponding author. CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China.
E-mail address: liqiao@qdio.ac.cn (Q. Li).

https://doi.org/10.1016/j.marenvres.2022.105601
Received 18 November 2021; Received in revised form 9 March 2022; Accepted 11 March 2022
Available online 15 March 2022

significantly elevating its activity (Chen et al., 2007; González-Ruiz et al., 2021; Xiong et al., 2016). For blue mussels in the intertidal zone, the highest SOD activities were observed in individuals distributed highest above the 'mean lower low water', suggesting that this enzyme is closely linked with oxidative stress caused by air exposure (Lesser, 2016). In the Mediterranean mussel *Mytilus galloprovincialis*, which often coexists and hybridizes with blue mussel, the gene expression of SOD in gill tissue also significantly increased after 24-h hypoxia exposure (A Andreyeva et al., 2021).

Oxidative stress and energy shortage are not the only threats that blue mussels might face under hypoxic conditions. Because the decrease in DO leads to enormous physiological stress, cell lysis and hemocyte exudation are more likely to occur, which will reduce the total hemocyte count (THC), leaving the organism vulnerable to potential pathogens (Shen et al., 2019; Sui et al., 2016). Furthermore, the adverse effects of hypoxia on the endocrine and membrane proteins in bivalves, especially the disorder of noradrenaline and the regulation of integrins, may cause phagocytic activity to decrease, further depressing their immune function (Shen et al., 2019; Zhou et al., 2013). Owing to an absence of acquired immunity in blue mussels, innate immunity is crucial. Acid phosphatase (ACP) and alkaline phosphatase (AKP), which are the two lysosomal enzymes involved in innate immunity, are the foundation of the bacteria-killing matter secreted by phagocytes and they help to build the hydrolysis system to clear pathogens (Chen et al., 2007; Song et al., 2015); thus, the activation of ACP or AKP is an important signal for the immune response. In the hard-shelled mussel *Mytilus coruscus*, 72-h exposure to DO 2.0 mg L^{-1} strongly increased the ACP activity, and the increase was even larger when the hypoxia was combined with low pH (Sui et al., 2017). Similarly, in another bivalve, the scallop *Chlamys farreri*, which also inhabits the intertidal to subtidal zones, significantly higher AKP activity was observed just 12 h after the DO concentration dropped to 2.5 mg L^{-1} (Chen et al., 2007).

To date, most studies of blue mussels under hypoxic stress have been confined to a timescale of hours, and previous research has mainly looked at acute physiological or biochemical responses, leaving the impact on mussel survival as well as on their population densities unknown. As coastal eutrophication rapidly worsens in many regions, both the intensity and duration of hypoxic events increase tremendously, posing a great threat to blue mussels that far exceeds that caused by periodic exposure to air or natural DO fluctuations in summertime (Diaz and Rosenberg, 2008; Meryl Williams et al., 2011). This situation may worsen in high-density mariculture areas where the water exchange is often poor owing to high-density populations and consolidated aquaculture rafts. Thus, better knowledge is needed on the effects of hypoxia on the blue mussel since eutrophication-induced hypoxic events with much longer durations and growing severity are increasingly challenging its survival.

In the present study, we determined the critical threshold of DO concentration for the survival of blue mussel, in an experiment covering the range DO 0.5–2.0 mg L^{-1} during 16 h of exposure. We further assessed the impact of DO fluctuations and poor water quality as these are common stressors found in combination with hypoxic conditions. We also analyzed the changes of metabolic rate and the activities of several key enzymes to identify possible physiological stresses and defense responses of the blue mussel under sustained hypoxia. The findings will help to predict the fate of blue mussels under increased hypoxic events and provide scientific advice for mariculture management.

2. Material and methods

2.1. Experimental animals and the hypoxic environment

Blue mussels *Mytilus edulis* were purchased from the Lanshan mussel farm in Rizhao, Shandong Province, China. To avoid possible acclimatization to hypoxia, animals were kept at a low-density during transportation, as every 100 mussels were assigned to separate 300-L tanks, and each tank was equipped with two air stones connected to a mobile air pump. The *in situ* seawater in this farm was directly added to these transport tanks without warming or cooling. After being transported to the laboratory, the mussels were brushed softly to remove any attachments, and then transferred to holding tanks with the same population density as in transportation. The natural seawater used for holding and for the subsequent experiments was from Taipingjiao, Qingdao, Shandong Province. During the holding period, the temperature of seawater (salinity 30.0 ± 1.0) was set to 22 °C, and DO was maintained above 7.0 mg L^{-1} as air was continuously pumped in. *Chlorella vulgaris* (5.8×10^4 cells mL^{-1}) was supplied as food twice a day because this green microalgae is common in intertidal and subtidal zones, and it is often used in shellfish culture. To ensure acceptable water quality, the feces were carefully siphoned from the tanks each morning, and dead mussels were immediately removed once they were observed. One-third of the seawater was replaced daily, using 22 °C pre-warmed water to avoid possible stimulus from temperature fluctuation. Finally, only mussels with intact shells, a quick shell-closing response, and normal byssus threads were picked and transferred to experimental tanks for further study.

A hypoxic environment was created by pumping nitrogen into the water. For each system, an 800-L water supply tank equipped with a temperature regulator, a DO sensor, two air stones, and an ultraviolet (UV) lamp served to adjust the DO concentration and temperature. When the actual DO concentration was above the set value, compressed nitrogen was flowed in under computer control. Similarly, compressed air was flowed in when the actual value was below the set value. Above the supply tank, four 90-L experimental tanks were installed. A water purifier with activated carbon and filter cotton was connected to both the experimental tanks and water supply tank to remove metabolic wastes produced by the animals. While the system was running, water from the supply tank was pumped into the experimental tanks and then circulated back via the water purifier. The advantage of this design was to keep the experimental animals away from the disturbance of the gas injections or potential temperature fluctuation during the regulation of DO and water temperature.

2.2. Survival observations

To investigate the survival of blue mussels under different types of hypoxia events, three experiments were carried out.

In the first experiment, mussels were maintained under a series of constant DO concentrations to determine their tolerance to different degrees of hypoxia. According to pre-experiment results, most mussels could survive the hypoxia challenge when the DO concentration was above 1.0 mg L^{-1}. Thus, most DO concentrations were set below 1.0 mg L^{-1}, with DO 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5 and 2.0 mg L^{-1} being designated as treatment concentrations, and DO 7.0 mg L^{-1} as the control concentration. For each DO concentration, 160 mussels were assigned to four replicated tanks. Among them, three replicate tanks were for observation of their survival, while the fourth tank was for biochemical sampling (described below).

Before the mussels were transferred from the holding tanks to the experimental tanks, the temperature of seawater (salinity 30.0 ± 1.0) was pre-set to 22 °C, and the water flow was controlled at 200 L h^{-1}. Once the transfer was complete, all mussels were allowed to acclimatize to their experimental tank for 7 days. During the acclimation period, the DO of seawater (salinity 30.0 ± 1.0) was set to 7.0 mg L^{-1}. *Chlorella vulgaris* was supplied as food twice a day. One-third of the seawater was changed every 2 days until the end of the experiment. Dead mussels or any mussels that failed to attach to the tank by byssus threads were replaced with new ones. After 7 days of acclimation, the DO concentration was slowly and linearly reduced to the set value within 24 h, and thereafter maintained for 16 days.

During the 16-day period, the observation of survival was made at 9:00 daily. For each observation, all mussels were first inspected for

their appearance and behavior. Ones that featured largely opened shells, curled gills or a siphon were then touched three times with a glass rod. If the mussel showed no response, it was considered dead. Any dead mussels were immediately removed from the experimental tanks to keep the water quality from deteriorating, and were then measured for their shell height to determine the relationship between individual size and hypoxia tolerance.

When the 16-day period was over, the DO concentration in all treatment groups was slowly and evenly increased to a normoxic level of 7.0 mg L^{-1} within 24 h, and then maintained at this concentration for another 7 days. The observation of survival proceeded as before, to record the post-effects of hypoxia.

In the second experiment, mussels were placed in a hypoxic environment with fluctuating DO. In the 16-day experiment, DO cycle time was set to 24 h. In one cycle, the DO concentration was linearly increased from 0.5 mg L^{-1} to 1.5 mg L^{-1} over 12 h, and subsequently decreased back to 0.5 mg L^{-1} over 12 h. A total of 160 mussels were assigned to four replicate tanks. The temperature, flow rate, water-change operation together with the 7-day acclimation period were set in the same way as the first experiment. The observation of survival was made at 9:00 every day, following the same procedures as before.

In the third experiment, the factors of high population density and poor water quality were introduced to mimic the situation in a dense mariculture area. Specifically, for each group, a total of 320 mussels were assigned to four replicate tanks, and the water was never changed during the 16-day experiment. Furthermore, the UV lamp in the supply tank was turned off and the water purifier was removed from the system. The DO concentration was set at 0.5 mg L^{-1} for one group, and 1.0 mg L^{-1} for another, representing a severe hypoxic event and a milder one, respectively. The chemical oxygen demand and ammonia nitrogen of the seawater were monitored daily, and both fell below the minimum requirement of the Seawater Quality Standard GB3097-1997 after the 6th day. The temperature and water flow of the tanks were set as in the previous two experiments, and the observation of survival was conducted in the same manner. The results were compared with the two groups in the first experiment, which had experienced the same DO concentrations (0.5 and 1.0 mg L^{-1}) but a lower population density as well as water-quality control.

2.3. Measurement of oxygen consumption rate (OCR)

The oxygen consumption rate (OCR) of the mussels was being measured under the DO concentrations of 0.5, 1.0, 1.5 and 2.0 mg L^{-1} during the first experiment conducted to determine survival rates. To best record the change of OCR with DO, the measurements were also done during the 24-h DO-dropping period before the experiment commenced. In the DO-dropping period, the OCR was measured every 2 h, whereas during the following 24 days, the measurements were carried out once a day.

For each measurement, the OCR of mussels was measured tank by tank. The water in each tank was first reduced to 20–30 L, and then the water inlet and outlet valves were turned off. A foam board was placed on the surface of the water to prevent oxygen exchange, and a DO probe was inserted into the water through a hole in the board. The DO was measured and recorded every 60 s, by automatic computer control, and to avoid unevenness of DO, a magnetic stirrer in the tank was triggered for 20 s each time before data collection. Meanwhile, a 1-L beaker was filled with water from the same supply tank and then sealed to estimate the OCR of microorganisms. After 10 min of data collection, the valves of the experimental tank were reset to the previous positions, allowing the water to again recirculate at a rate of 200 L h^{-1}. The 10-min DO concentration data of the experimental tank was in turn extracted in 5-min groupings, to form data groups of 0–5 min, 1–6 min, 2–7 min, etc. All six data groups were then linear fitted (SPSS 16.0), and the group with the maximum determination coefficient was adopted for calculation of the OCR. The DO concentration dataset of the beaker was processed with the same method. Finally, the OCR of the specific tank was calculated as follows:

$$\mathrm{OCR} = \frac{(k - k_0) \times V}{N}$$

where k is the regression coefficient of the linear regression between DO and time in the experimental tank; k_0 is the regression coefficient of the linear regression between DO and time in the beaker; V is the water volume in the experimental tank during measurement; and N is the number of mussels in the tank.

2.4. Enzyme activity analysis

The activities of Na^+/K^+ ATPase, SOD, ACP and AKP were analyzed in the gill tissue of blue mussel during the three experiments conducted to observe survival. Specifically, in the first experiment, the enzyme activities of all groups were first determined under normoxic conditions before the DO began to drop. Then, on the 8th and 16th day of the experiment, the enzyme activities were further determined under DO concentrations of 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0 and 7.0 mg L^{-1}. Finally, after the DO concentrations were returned to normoxia, the enzyme activities were determined for the fourth time on the 20th day of the experiment. In the second and third experiments, the enzyme activities of all treatment groups were determined on the 16th day of the experiment to similarly assess the combined effects of DO fluctuations or high-density mariculture with hypoxia. For each time of the determinations in the first experiment, five mussels were randomly chosen from the biochemical sampling tank under each DO concentration, while in the second and third experiments, mussels were chosen directly from the tanks used for survival observations at the end of those experiments. To reduce physiological stress on the animals caused by sampling, the byssus threads of the chosen mussel were carefully cut from the surface it attached to. The mussel was then slowly transferred into a 500-mL beaker underwater. Finally, the water-filled beaker with mussel was taken to the laboratory for dissection. Gill tissue weighing approximately 0.1–0.2 g was taken from each mussel and immediately frozen in liquid nitrogen.

To prepare a sample solution, the tissue was homogenized in 4 °C for 10 min, with 0.9% normal saline serving as the extracting solution. The homogenate was then centrifugalized in the condition of 12 000 *g* and 4 °C for 10 min. The subsequent procedures strictly followed the instructions provided by the assay kit producer (i.e. total protein assay kit A045-4, adenosinetriphosphatase assay kit A016-2, total superoxide dismutase assay kit A001-1, acid phosphatase assay kit A060-2, and alkaline phosphatase assay kit A059-2; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The results are expressed as units per mg of protein.

The Appendix (Table A.1) outlines the entire experimental procedure and key details of each experiment.

2.5. Data analysis.

In the experiments to observe survival, replicate survival data were combined, and survival rates under various DO concentrations or DO fluctuations were calculated every day throughout the experiments. Changes of survival rate with time and with DO concentration were plotted to reflect the tolerance of blue mussel to hypoxia. Furthermore, to explore differences in hypoxia tolerance among individuals of different size, the shell height of surviving mussels before and on the 16th day of the first experiment were compared (analysis of variance, ANOVA). Finally, the influences of DO fluctuations, high population density, and poor water quality on the hypoxia tolerance of the mussels were investigated with the Kruskal–Wallis H-test.

Similar to processing the survival data, replicate OCR data of each group were combined, and the changes in OCR with time were plotted to reflect the respiratory response of the mussels to hypoxia on a 24-day scale.

For the analysis of enzyme activities, means comparisons for the activities of Na^+/K^+ ATPase, SOD, ACP and AKP were made between multi-groups. For the preciseness of the conclusion, all data were tested for normality and homogeneity of variances before the means comparison. For the normality test, the method of Shapiro-Wilk was adopted, while Levene's test was used to check for homogeneity of variances. If the data passed all two tests, ANOVA was used to test for statistical significance, and the Tukey test was adopted for multiple comparisons. If the data failed to pass any of the two tests, the Kruskal–Wallis H-test was adopted to test for statistical significance, and the Mann–Whitney *U* test was used for multiple comparisons. No differences under hypoxia was given as the null hypothesis, and $p < 0.05$ was used as the significance threshold. The data analyses were conducted with SPSS 16.0, Origin 2018, and Excel.

3. Results

3.1. Survival under hypoxia

As shown in Fig. 1, as the DO concentration decreased, the survival rate of blue mussels decreased. However, the survival rate did not change linearly with DO. At DO concentrations of >0.8 mg L^{-1}, the treatment groups exhibited similar survival rates. Under moderate hypoxia of DO 2.0 mg L^{-1}, 97.5% of the mussels survived to the end of the 24-day experiment. However, under a far more challenging hypoxia situation of DO 0.9 mg L^{-1}, the survival rate was only ~10% less at 89.9%. Nevertheless, as the hypoxia intensified to < DO 0.9 mg L^{-1}, mussel mortalities rapidly increased. Under a DO concentration of 0.8 mg L^{-1}, the survival rate was 79.7%, but when the concentration reached 0.7 mg L^{-1} and 0.6 mg L^{-1}, survival dropped to 41.0% and 17.7%, respectively. This trend is depicted in Fig. 1B, revealing that a declining decrease in DO was required for the survival rate to be reduced by 10%. Moreover, even after the concentration was elevated back to DO 7.0 mg L^{-1} on the 16th day, the survival rate of some treatment groups still dropped over the following 7 days. Among these groups, the biggest drop was recorded under DO 0.7 mg L^{-1}. On the 16th day, 59.0% of the mussels exposed to that concentration survived the hypoxia challenge; however, during the normoxic period, another 18% died, leaving only 41% alive on the 24th (final) day of the experiment.

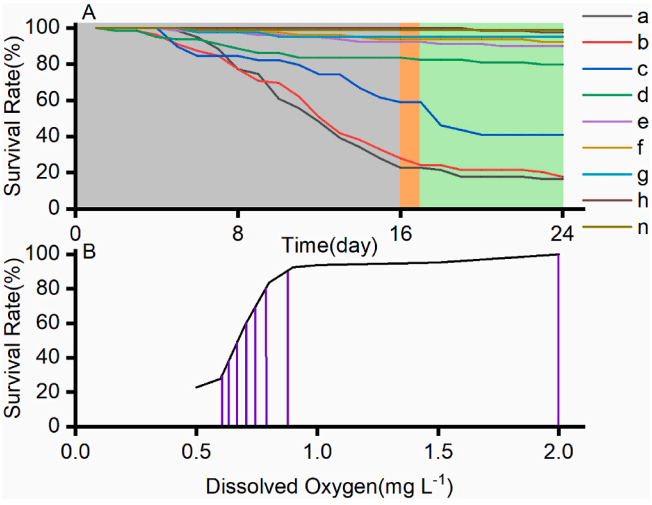

Fig. 1. Changes in the survival rate of blue mussels under hypoxia at different dissolved oxygen (DO) concentrations. (A) Changes in blue mussel survival rates with time. Lines labeled a, b, c, d, e, f, g, h and n represent the survival rates of mussels exposed to DO concentrations of 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0 and 7.0 mg L^{-1}, respectively. The gray area indicates the 16-day hypoxia period, and the orange area indicates the following 24-h period where the DO concentration of all treatment groups was elevated back to 7.0 mg L^{-1}. The green area marks the last 7-day period where the DO of all groups remained at 7.0 mg L^{-1}. (B) Changes in survival rate with DO after 16 days of sustained hypoxia stress. The intersection of the curved line and vertical lines correspond to survival rates of 100%, 90%, 80%, 70%, 60%, 50%, 40% and 30%, from right to left, respectively.

Fig. 2 shows shell height distributions before and after the first experiment. Mussels of different sizes did not exhibit different tolerance to hypoxia. Under DO 0.5 mg L^{-1}, the average shell height was 52.4 cm before the experiment and 52.2 cm afterwards (ANOVA, $p = 0.847$). Under DO 0.6 mg L^{-1}, the shell heights were 52.8 cm before and 53.2 cm after the experiment (ANOVA, $p = 0.694$).

In the second experiment conducted to observe survival, DO fluctuation was found to impact the survival rate. A hypoxic condition where DO fluctuated between 0.5 and 1.5 mg L^{-1} caused higher mortality than a constant concentration of DO 1.0 mg L^{-1} ($X^2 = 4.50$, $p = 0.034$) (Fig. 3). However, the survival rate under this DO fluctuation range was still higher than that under a constant concentration of DO 0.5 mg L^{-1} (which was the minimum concentration during the DO fluctuation).

In the third experiment conducted to observe survival, the hypoxic condition of DO 1.0 mg L^{-1} and the simulated situation of high-density mariculture significantly resulted in a lower survival rate than that under a single hypoxic stress of DO 1.0 mg L^{-1} ($X^2 = 4.50$, $p = 0.034$). However, when the DO concentration was further reduced to 0.5 mg L^{-1}, this combined effect was not obvious as the survival rates of the two groups were very close (Fig. 4).

3.2. Response of the OCR to hypoxic conditions

Fig. 5A–D shows that when the DO concentration was more than 5–6 mg L^{-1} in the first 12–14 h, the OCR remained relatively stable. After that, the OCR decreased continuously with decreasing DO. As the DO concentration decreased, the OCR decreased. When the DO concentrations for the four treatment groups finally reached the set valves of 0.5, 1.0, 1.5, and 2.0 mg L^{-1} at the 24th hour, the OCR values were 0.10, 0.19, 0.22, and 0.36 mg h^{-1} $ind.^{-1}$, respectively.

During the first 16 days of hypoxia stress, the OCR of the mussels was relatively stable, with only small fluctuations. After reoxygenation of the seawater on the 17th day, the OCR rose and reached the same level as before the experiment (Fig. 5).

3.3. Enzyme activities

The activity of Na^+/K^+ ATPase in gill tissue did not differ significantly among mussels kept at different DO concentrations, as measured on the 8th, 16th and 20th day. Nor did the activity change over time, except for mussels exposed to DO 1.5 mg L^{-1}; on the 8th day of the experiment, this treatment resulted in a significant increase in Na^+/K^+ ATPase activity, and significantly higher activity was also recorded on the 20th day ($p < 0.05$) (Fig. 6).

SOD enzyme activity displayed an upward trend from day 16 of the experiment (Fig. 6). Under a DO concentration of 0.6 mg L^{-1}, the SOD activity on the 16th day was 45% higher than that on the 8th day, and 34% higher on the 20th day than that on the 8th day, and these changes were significant ($p < 0.05$). Furthermore, the treatment DO 1.5 mg L^{-1} was found to have a strong promotion effect as the enzyme activity under this DO concentration reached the highest level among all groups ($p < 0.05$).

ACP was the most activated enzyme under hypoxic stress (Fig. 6). The enzyme activity started to increase in most treatment groups as of day 8, and was significant for mussels exposed to DO 1.5 mg L^{-1} ($p < 0.01$). By day 16, as the enzyme was further activated, the increase in activity was significant for additional groups, namely mussels exposed to DO 0.8, 0.9, 1.0, and 1.5 mg L^{-1} ($p < 0.05$). Even 2 days after the elevated DO concentrations were returned to normoxia as of the 20th day, the treatment groups exposed to DO 0.9, 1.0, and 2.0 mg L^{-1} still maintained significantly higher ACP enzyme activity ($p < 0.05$). In contrast, a comparison of the enzyme activity between different DO

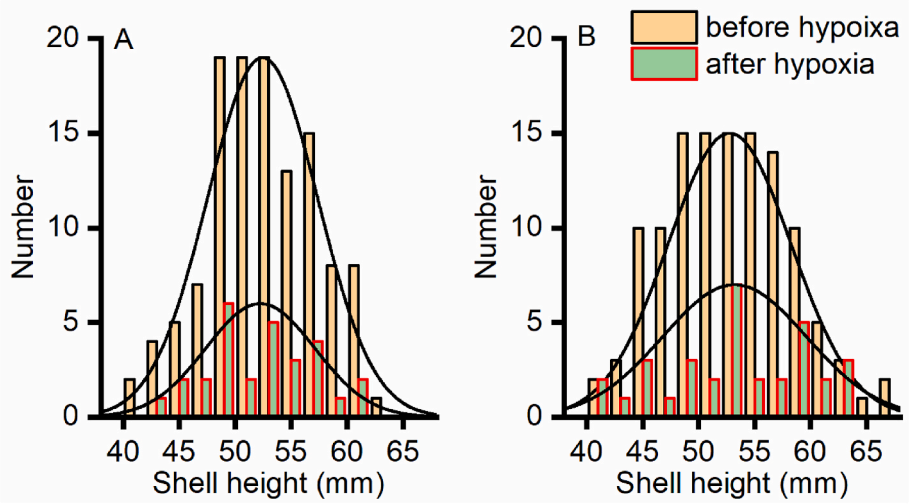

Fig. 2. Distribution of blue mussel shell heights before and after exposure to hypoxic conditions. Panels (A) and (B) correspond to the sizes blue mussels exposed to concentrations of DO 0.5 and 0.6 mg L^{-1}, respectively.

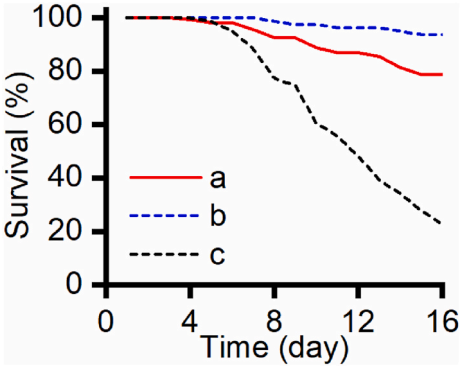

Fig. 3. Survival rate of blue mussels under hypoxia with DO fluctuations. The red line marked 'a' represents the survival rate under DO fluctuation within a cycle of 24 h. In the first 12 h, the DO was linearly increased from 0.5 mg L^{-1} to 1.5 mg L^{-1}, and over the following 12 h it was reduced back to 0.5 mg L^{-1}. The blue line and black line (labeled b and c) are the survival rates under a constant DO concentration of 1.0 mg L^{-1} or 0.5 mg L^{-1}, respectively, provided here as references.

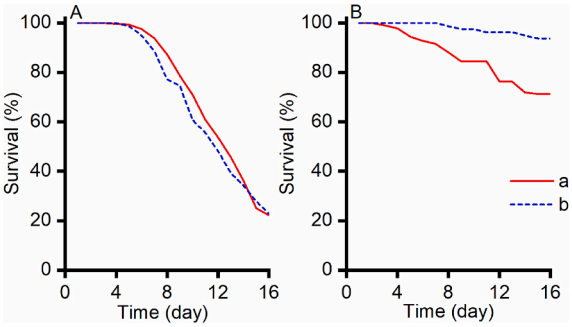

Fig. 4. Survival rate of blue mussels under the combined threats of hypoxia and high-density mariculture. Red lines (labeled a) represent the survival rates of blue mussels under the combined threat of hypoxia and high-density mariculture; blue lines (labeled b) represent survival rates under the single threat of hypoxia, provided here as a reference. The situation of high-density mariculture was simulated in the laboratory by doubling the population density and doing no water changes. Panels (A) and (B), respectively, correspond to mussels under DO concentrations of 0.5 and 1.0 mg L^{-1}.

concentrations at the same points in time showed that mussels exposed to DO in the range 0.8–2.0 mg L^{-1} were more likely to exhibit increased ACP activity. On the 16th day, mussels exposed to DO concentrations of 0.9, 1.0, and 2.0 mg L^{-1} showed significantly higher ACP activity, while on the 20th day the activity was significantly activated in mussels under DO concentrations of 0.8, 0.9, and 1.5 mg L^{-1} ($p < 0.05$).

AKP was the only enzyme examined that appeared to be inhibited during the experiment (Fig. 6). On the 8th day, AKP activity was significantly depressed in mussels exposed to DO concentrations of 0.6, 0.8, and 0.9 mg L^{-1} as compared with the level of enzyme activity before the experiment commenced (day 0). On the 16th day, the inhibitory effect on AKP occurred in mussels exposed to higher DO concentrations, namely 0.8, 0.9, and 1.0 mg L^{-1} ($p < 0.05$), and the activity was lowest under DO 1.0 mg L^{-1}. On the 20th day, the inhibitory effect persisted in mussels under DO concentrations of 0.7, 0.8, and 1.5 mg L^{-1}, revealing an aftereffect of hypoxia.

When the threat of hypoxia was combined with DO fluctuations, the AKP activity was significantly higher than that under a constant DO concentration (Fig. 7). Furthermore, when the more challenging situation of a high-density population and no water changes was combined with hypoxia stress at DO 1.0 mg L^{-1}, AKP was the only enzyme that exhibited significant differences in activity (Fig. 8).

4. Discussion

4.1. Survival under the threat of hypoxia

The blue mussel is considered hypoxia-tolerant because of its capacity to survive when exposed to the air during ebb tide (González et al., 2019). However, the hypoxia challenge caused by periodical air exposure is different from that induced by eutrophication in the coastal regions. In eutrophication-related hypoxia, the bottom oxygen-deficient water often remains under the control of the thermocline and halocline, for weeks to months, imposing sustained stress on the benthos.

Some of the results provided here reveal notable characteristics of the survival of blue mussels during and after a 16-day hypoxia event. First, the mussels could tolerate hypoxia for 4–5 days, with very few mortalities even at the extremely low DO concentration of 0.5 mg L^{-1}. In contrast, other studies have shown that acute hypoxia of less than 48 h duration could cause mass mortalities in various species of fish, crustaceans, and mollusks (Miller et al., 2002; Nagasoe et al., 2020; Shen et al., 2020). Therefore, the impact on blue mussels resembles the ability of some infaunal clams to well tolerate short-term hypoxia (Sun et al.,

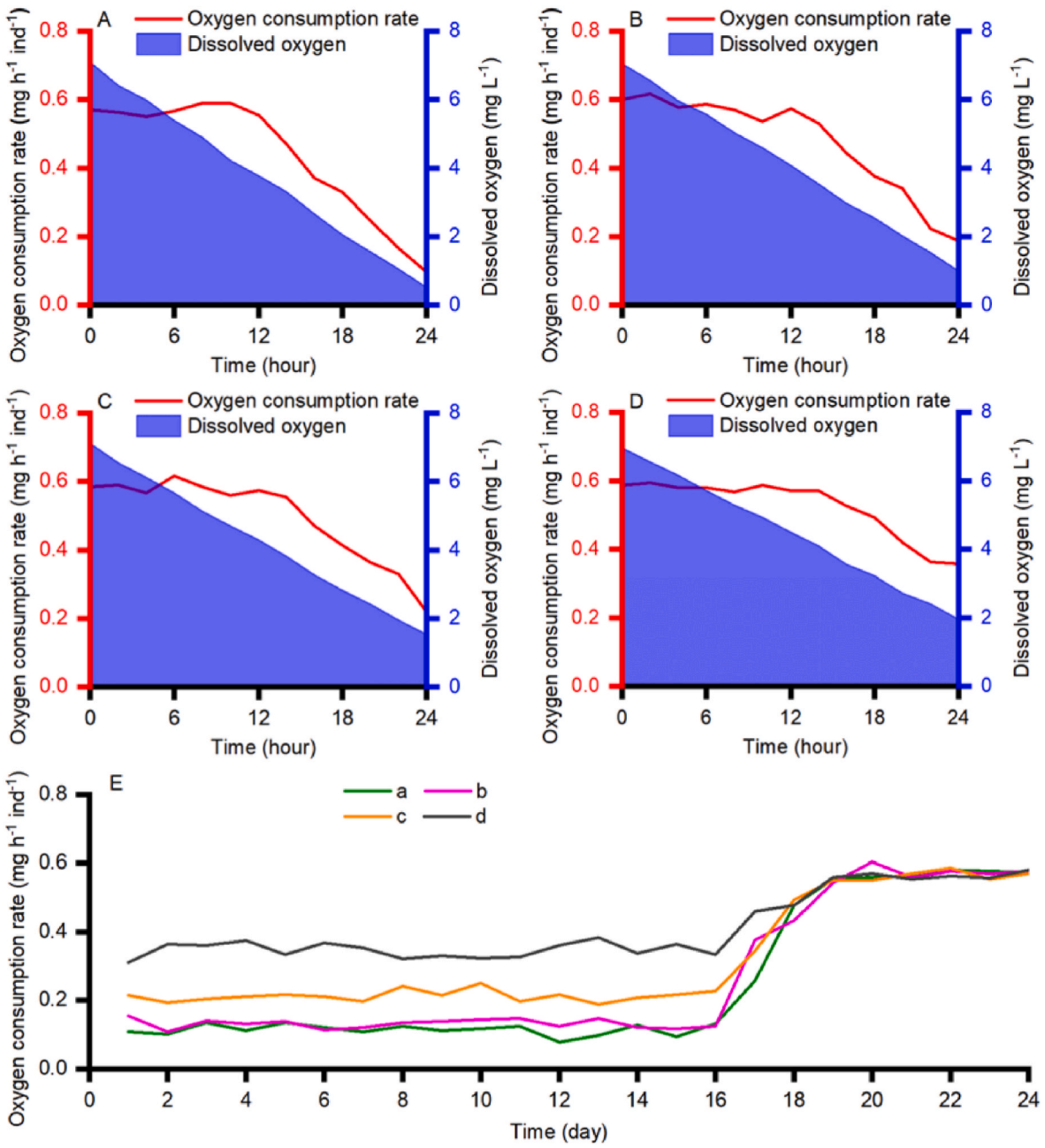

Fig. 5. Oxygen consumption rate of blue mussels under hypoxic conditions over time. Panels (A), (B), (C) and (D) show changes in the oxygen consumption rate of blue mussels during the DO-dropping period in the first experiment conducted to observe survival. Panel (E) shows the oxygen consumption rate of the mussels over the full 24-day experiment. Lines labeled a, b, c, and d correspond to mussels exposed to DO concentrations of 0.5, 1.0, 1.5, and 2.0 mg L^{-1}, respectively.

2021). Second, DO concentrations between 0.6 and 0.8 mg L^{-1} seem to constitute a critical range for the survival of blue mussel. Under the DO concentration of 0.8 mg L^{-1}, the mussels maintained a high survival rate of 83.5% at the end of the 16-day constant hypoxia pressure, whereas at DO 0.6 mg L^{-1} survival dropped dramatically to only 27.8%. This finding is clearly depicted in Fig. 1B as the slope of the survival curve reaches its maximum around that concentration range, which may represent the physiological threshold for blue mussel. Notably, researchers in other relevant studies were more likely to use critical pO_2, a DO value below which respiration changes from an oxygen-regulating to oxygen-conforming mode, to reflect the hypoxia-tolerance of marine organisms (Montgomery et al., 2019). However, in our study, the respiration rates under 0.5 mg L^{-1} and 1.0 mg L^{-1} were nearly the same, yet the survival rates varied greatly (Fig. 5). Thus, it may be more cautious to carry out observations of survival in addition to critical pO_2 tests to fully determine the hypoxia tolerance of a taxon. Third, the survival rates of some treatment groups continued to drop even after the DO was elevated back to normoxia. The ensuing mortalities might be attributable to the surge of oxidative pressure brought by the rising DO (Haider et al., 2020) or to irreversible physiological damage caused by the hypoxia.

Before conducting the experiments, we hypothesized that smaller individuals would exhibit higher tolerance than bigger ones under hypoxia because bigger mussels need more oxygen to maintain a standard metabolic rate. But as shown in Fig. 2, none of the shell height ranges to which the mussels belonged were linked to a significantly higher or lower survival rate. The finding of similar tolerance despite different sizes might be partly explained by the mussels' common

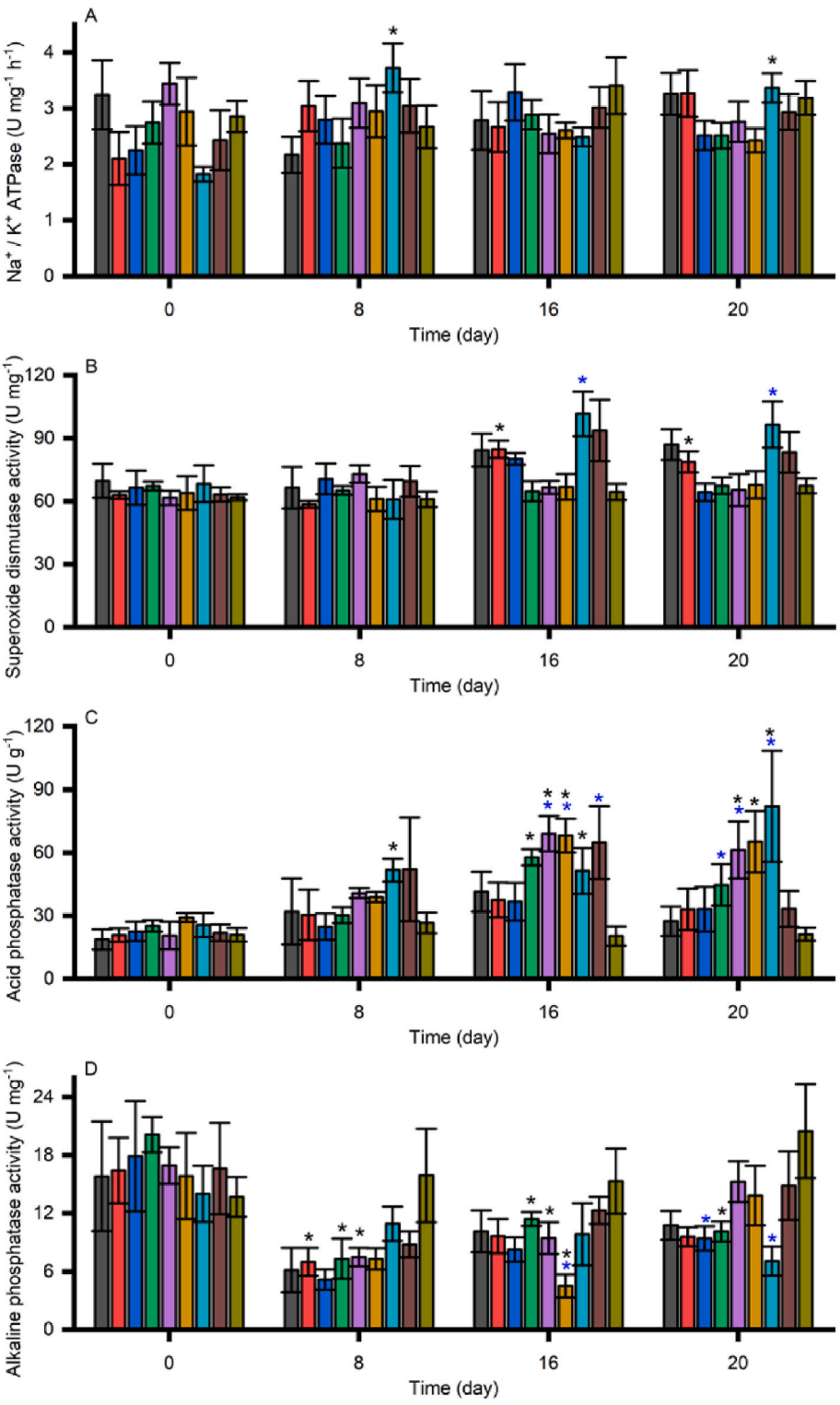

Fig. 6. Enzyme activities in gill tissue of blue mussels under hypoxia, over time. Panels (A), (B), (C) and (D) represent the responses of Na^+/K^+ ATPase, superoxide dismutase, acid phosphatase, and alkaline phosphatase, respectively. In each cluster of bars for each panel, the bars (from left to right) show the enzyme responses at DO concentrations of 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, and 7.0 mg L^{-1}, respectively. An black asterisk (*) indicates significance at $p < 0.05$ when comparing the results at the same DO concentration but at different times. A blue asterisk indicates significance at $p < 0.05$ when comparing the results at the same time but across different DO concentrations. Error bars indicate mean ± SE.

habitat and strong adaptability to ambient environments. Wang and Widdows (1991) previously found that even at its larval stage the blue mussel is able to regulate its feeding, growth, and metabolic rates, and can survive moderate to severe hypoxia events, testifying to the species' adaptability to environmental stressors. Moreover, Altieri (2006) further demonstrated the importance of habitat and the acclimation capability of the blue mussel by transplanting subtidal-dwelling mussels to the intertidal zone, which significantly improve their performance under hypoxic conditions. To conclude, oxygen deficiency is less likely to be a selection factor for mussels with different sizes.

In the field environment, oxygen deficiency is sometimes combined with other stressors. Here, we focused on situations with DO fluctuation and high population density. In shallow waters where these mussels are often distributed in large numbers, the risk of DO fluctuation is particularly great because hypoxic water caused by the decomposition of dead algae is mixed with oxygen-enriched water. A study that recorded a complete natural hypoxic event in Mikawa Bay, Japan, found that DO fluctuation occurred as frequently as four to seven times per week, and

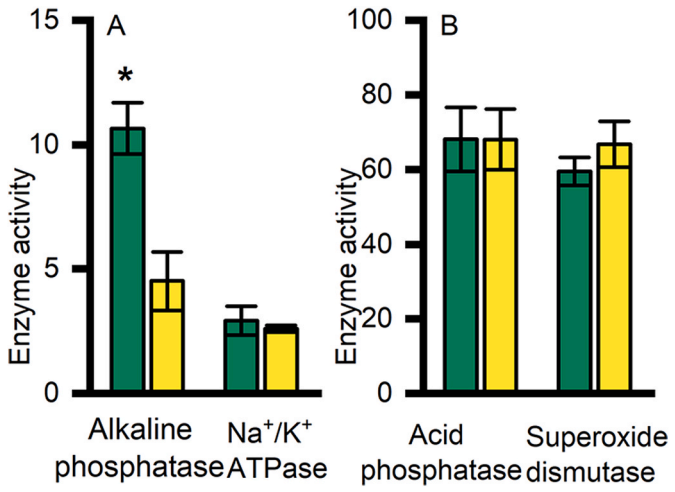

Fig. 7. Combined effects of dissolved oxygen fluctuation and hypoxia on enzyme activities in the gill tissue of blue mussels. (A) The activities of alkaline phosphatase and Na^+/K^+ ATPase, and (B) the activities of acid phosphatase and superoxide dismutase. Green bars represent the enzyme activities in gill tissue of mussels exposed to hypoxia with DO fluctuation. That is, in one 24-h cycle, the DO was linearly increased from 0.5 mg L^{-1} to 1.5 mg L^{-1} over 12 h, and then decreased back to 0.5 mg L^{-1} over the next 12 h. Yellow bars represent the enzyme activities of mussels exposed to a constant concentration of DO 1.0 mg L^{-1}.

mortalities of Manila clam *Ruditapes philippinarum* were reported even at DO saturation exceeding 30% (Uzaki et al., 2003). In the present study, we found that DO fluctuation around the central value of 1.0 mg L^{-1} in the range DO 0.5–1.5 mg L^{-1} in a 24-h cycle caused 15% more mortality than a constant concentration of DO 1.0 mg L^{-1}, suggesting potential oxidative stress was brought about by the changes in DO. In another common scenario, dense mariculture greatly increases the risks of disease, pollution, and hypoxia, which combined may more adversely affect marine organisms. In several mass-mortality events of cultured scallops in Shandong Peninsula, eastern China, hypoxia and the spread of pathogens were verified as the main causes (Guo and Luo, 2016; Wu et al., 2001). For blue mussel, a great disadvantage under such a scenario is that it is quite sensitive to infections by potential pathogens and mass mortalities often ensue (Benabdelmouna et al., 2018). In this study, we found that the survival rate of mussels under the combined threats of hypoxia and simulated high-density mariculture did not differ from mussels under the single threat of hypoxia when the DO concentration was 0.5 mg L^{-1}, implying that the energy restriction was more likely to be the main stressor here. In contrast, survival was significantly lower when the DO was at a more moderate level of 1.0 mg L^{-1} but the experimental conditions included a doubled population density, no water change, no water purifier, and no UV lamp illumination. Thus, we recommend that both the DO concentration and the spread of pathogens should be closely monitored in blue mussel mariculture, and that the stocking density should be relatively low under a risk of hypoxic conditions.

4.2. Physiological and biochemical responses

As mentioned, oxygen deficiency not only causes energy shortage but also challenges various basic biochemical activities. It is especially important to pay attention to antioxidants and the immune response because low DO often results in large amounts of ROS, reduced immune cell numbers, and diminished phagocytic activity (Chandel et al., 2000; Johnson, 2017; Nogueira et al., 2017). In the current study, we measured the OCR of blue mussels and the activities of four enzymes involved in these processes to reveal their strategies under hypoxia.

Previous studies have demonstrated that the blue mussel will depress its feeding and growth under hypoxic conditions, and the metabolic rate often decreases with the drop in DO (Artigaud et al., 2014; Sanders et al., 2014). Even if the DO concentration reaches an extremely low level, the animal is able to synthesize and use opines as an energy material to perform anaerobic respiration (Stefano et al., 2015). According to the OCR measurements here, DO concentrations of 5–6 mg L^{-1} seem to be the threshold below which the blue mussel shifts its respiratory mode from oxygen-regulating to oxygen-conforming. Considering the strong tolerance of the blue mussel to hypoxia, the present results may contradict some previous findings of a strong hypoxia tolerance being correlated with strong oxygen-regulating ability (Alexander and McMahon, 2004; Montgomery et al., 2019). For marine organisms that are able to well regulate their oxygen uptake under hypoxia, multiple physiological strategies are used to stabilize their OCR as well as to maintain aerobic respiration, given that aerobic respiration provides far more energy than anaerobic respiration (Grieshaber et al., 2005). Thus, it is reasonable to conclude that the energy requirements of blue mussel far exceed its need to maintain basic life activities. The energy allocated to feeding, digesting, growth, and reproduction should amount to a large portion under normoxia, and these energy requirements would be suppressed with the inhibition of OCR. Furthermore, anerobic respiration that uses the raw material of opines might play a key role when DO drops to an extremely low level. Nevertheless, the respiration rates of blue mussels were maintained at stable levels under the 16-day hypoxic stress despite some fluctuations, and values of OCR rapidly increased with the reoxygenation process, suggesting that a 16-day hypoxia event is less likely to cause respiratory dysfunction in surviving mussels. In addition to OCR measurements, the activity of Na^+/K^+ ATPase was analyzed during the 24-day experiment because it is essential to maintain membrane potential and to transport vital solutes. This important cellular function costs more than 30% of the total ATP, making it probably the most energy-consuming process in many cells. Thus, hypoxia poses a great challenge to this enzyme because the energy restriction requires the Na^+/K^+ ATPase to be depressed while the need for normal cell function requires it to remain activated. As shown in Fig. 6, the Na^+/K^+ ATPase enzyme activity did not show obvious upward or downward

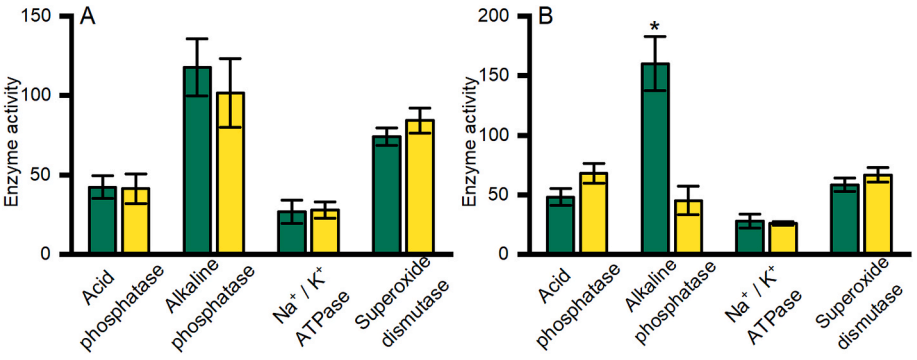

Fig. 8. Combined effect of high-density mariculture and hypoxia on enzyme activities in gill tissue of blue mussels. Green bars represent the enzyme activities of mussels under simulated high-density mariculture combined with hypoxia. Yellow bars represent the enzyme activities of mussels under the single threat of hypoxia. Panels A and B correspond to mussels exposed to DO concentrations of 0.5 and 1.0 mg L^{-1}, respectively. An asterisk (*) indicates significance at $p < 0.05$. Error bars indicate mean ± SE.

changes. A significant depression of this enzyme was observed a study of the less-tolerant sciaenid fish *Leiostomus xanthurus*, a finding that provides a clue that Na^+/K^+ ATPase is needed to maintain normal function of the sodium–potassium pump system to improve the chance of survival under hypoxia (Brinson, 2011). In general, it was not possible to portray the relationship between changes in survival rate and OCR or Na^+/K^+ ATPase responses because both parameters did not exhibit significant changes during the 16-day hypoxia stress.

Apart from the adverse situation of limited energy, the extra oxidative stress is another threat to survival. ROS are an important factor to induce the expression of genes related to erythropoietin and vascular endothelial growth, and their production often increases under hypoxia (Chandel et al., 2000). Increasing or decreasing concentrations of DO are also reported to induce excessive ROS generation, causing more oxidative stress during hypoxia events (Johnson, 2017; Zhou et al., 2013). According to existing knowledge, SOD plays a key role in antioxidative function in marine organisms, and its activity is often thousands of times that of other antioxidative enzymes (Johnson, 2017; Silva et al., 2021). Under hypoxia, SOD has an immediate response to decreasing DO because its activity is usually enhanced within hours after DO begins to drop. However, most studies have claimed that SOD activity will be further inhibited following the short time of activation (AY Andreyeva et al., 2021; Chen et al., 2007; Nogueira et al., 2017). Therefore, we wondered whether hypoxic stress could cause permanent dysfunction of the superoxide-eliminating system. For blue mussels here, an upward trend in the activity of this enzyme was recorded in some treatment groups on the 16th and 20th day, and none of these groups showed inhibited activity, which implies that blue mussels still have the antioxidative capacity to effectively decompose superoxide after a sustained hypoxic event. However, the values of SOD activity on the 16th and 20th days were almost the same, indicating that the reoxygenation process did not result in significant production of superoxide. This speculation was further supported in the second experiment because even the DO fluctuation did not result in a significant increase of SOD activity (Fig. 7). Thus, the oxidative stress induced by superoxide was less likely to be the main cause of greater mortalities in the second experiment; therefore, we needed to focus on other potential clues in further investigations.

Bivalves lack acquired immunity, and therefore nonspecific immunity is vital to protect them from pathogens. As important immune-response enzymes, ACP is involved in various lytic processes to kill and digest invaders, while AKP is an immunologic factor that participates in phosphate metabolism and transport across membranes (Mazorra et al., 2002; Seitkalieva et al., 2015). A few studies have reported that disease, cell invasion, and environmental stresses could significantly change the activities of both enzymes (Chi et al., 2019; Mazorra et al., 2002). Although some research on bivalves has found that ACP and AKP in the digestive gland had the highest activity, we choose gill tissue to measure blue mussel sensitivity to oxygen deficiency (Mazorra et al., 2002; Seitkalieva et al., 2015). As shown in Fig. 6, during the 16-day hypoxia, ACP activity gradually increased over time, indicating that lysosome plays a key role in response to hypoxic stress. In addition, the figure reveals that the standard errors under hypoxic conditions were bigger than those under normoxia, which could be a reflection of individual differences towards hypoxia. For AKP, the inhibited activity was detrimental to the mussels as inhibition of this enzyme signifies a loss of the barrier function of the plasma membrane, and it may contribute to eventual death of the animal during a hypoxic event. However, in the third experiment, it is unexpected that AKP activity was significantly higher under the combined threats of moderate hypoxia and simulated dense mariculture than that under the sole challenge of hypoxia, given that bacteria could propagate and spread more easily in the former situation. The further activation of AKP under DO fluctuations also lacks reasonable explanations, which might suggest a key role for AKP under these multiple stressors; therefore, this topic should be included in future research.

5. Conclusions

To investigate the survival of blue mussel under sustained hypoxia stress and its responses in terms of energy metabolism, antioxidant, and immunity, we conducted several sets of experiments along with physiological and biochemical measurements. The results allow several conclusions. (1) The blue mussel has a strong tolerance against hypoxia. The DO concentration range of 0.7–0.8 mg L^{-1} is a fateful threshold for this species as the survival rate dropped sharply within this range. (2) DO fluctuation and the adverse situation of a high population density and poor water quality would significantly reduce its survival rate under a moderate hypoxia event. (3) The blue mussel is an oxygen-conformer that depresses its respiration rate as well as the individual's total energy requirement when the DO concentration falls below 5–6 mg L^{-1} (4) Hypoxia results in increasing stress on immunity, suggesting that a compromised immune response is another important factor challenging the survival of blue mussel.

Author statement

Qiao Li: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft **Fang Zhang**: Validation, Resources, Writing - Review & Editing **Song Sun**: Conceptualization, Funding acquisition, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Li Zhou for providing the instruments needed to analyze the enzyme activities. We are grateful to Mengna Li for help with the enzyme analysis. This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences [grant number XDA19060201], the National Natural Science Foundation of China [No. 42130411], Key Deployment Projects of the Center for Ocean Mega-Science, Chinese Academy of Sciences [COMS2019Z01], and Mount Tai Scholar Climbing Plan to SS.

Appendix

Table A.1
Overview of the experimental design to observe survival, estimate the oxygen consumption rate (OCR), and measure enzyme activities in gill tissue of blue mussels under sustained hypoxic stress

	Experiment 1	Experiment 2	Experiment 3
Experimental stressors	Hypoxia	Hypoxia and DO fluctuation	Hypoxia, high-density population, and poor water quality

(*continued on next page*)

Table A.1 (*continued*)

		Experiment 1		Experiment 2	Experiment 3
Experimental period		24 days		16 days	16 days
Dissolved oxygen (DO) concentration		Days 1–16	DO 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, and 2.0 mg L^{-1} for treatment groups, and DO 7.0 mg L^{-1} for control group	Fluctuation between DO 0.5 and 1.5 mg L^{-1} for the treatment group, and a constant DO of 1.0 mg L^{-1} for the control group	Severe hypoxia at DO 0.5 mg L^{-1}, and moderate hypoxia at DO 1.0 mg L^{-1}
		Day 17	Reoxygenation of treatment groups		
		Days 18–24	DO 7.0 mg L^{-1} for all groups		
Population density		40 mussels in each 90-L tank		40 mussels in each 90-L tank	80 mussels in each 90-L tank
Water-quality control	**Purifier**	Equipped		Equipped	Not equipped
	UV lamp	On		On	Off
	Water change	One-third of the tank water was changed every 2 days.		One-third of the water was changed every 2 days.	Water was never changed.
OCR measurement		DO concentrations	DO 0.5, 1.0, 1.5, and 2.0 mg L^{-1}	None	None
		Time interval	Every 2 h during the drop in DO, and once daily during the following 24 days		
Enzyme analysis		DO concentrations	DO 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0 and 7.0 mg L^{-1}	On the 16th day	On the 16th day
		Sampling time	On day 0 and on the 8th, 16th, and 20th days		

References

Alexander Jr., J.E., McMahon, R.F., 2004. Respiratory response to temperature and hypoxia in the zebra mussel *Dreissena polymorpha*. Comp. Biochem. Physiol. Mol. Integr. Physiol. 137 (2), 425–434.

Altieri, A.H., 2006. Inducible variation in hypoxia tolerance across the intertidal–subtidal distribution of the blue mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. 325, 295–300.

Andreyeva, A., Gostyukhina, O., Kladchenko, E., Vodiasova, E., Chelebieva, E., 2021. Acute hypoxic exposure: effect on hemocyte functional parameters and antioxidant potential in gills of the pacific oyster, *Crassostrea gigas*. Mar. Environ. Res. 169, 105389.

Andreyeva, A.Y., Gostyukhina, O., Kladchenko, E., Afonnikov, D., Rasskazov, D., Lantushenko, A., et al., 2021. Hypoxia exerts oxidative stress and changes in expression of antioxidant enzyme genes in gills of *Mytilus galloprovincialis* (Lamarck, 1819). Mar. Biol. Res. 17 (4), 369–379.

Artigaud, S., Lacroix, C., Pichereau, V., Flye-Sainte-Marie, J., 2014. Respiratory response to combined heat and hypoxia in the marine bivalves *Pecten maximus* and *Mytilus spp*. Comp. Biochem. Physiol. Mol. Integr. Physiol. 175, 135–140.

Benabdelmouna, A., Garcia, C., Ledu, C., Lamy, P., Maurouard, E., Dégremont, L., 2018. Mortality investigation of *Mytilus edulis* and *Mytilus galloprovincialis* in France: an experimental survey under laboratory conditions. Aquaculture 495, 831–841.

Brinson, B.E., 2011. Lactate dehydrogenase and Na^+/K^+ ATPase activity in *Leiostomus xanthurus* (spot) in response to hypoxia. Explorations 4, 22–29.

Chandel, N.S., McClintock, D.S., Feliciano, C.E., Wood, T.M., Melendez, J.A., Rodriguez, A.M., et al., 2000. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1α during hypoxia: a mechanism of O_2 sensing. J. Biol. Chem. 275 (33), 25130–25138.

Chen, J., Mai, K., Ma, H., Wang, X., Deng, D., Liu, X., et al., 2007. Effects of dissolved oxygen on survival and immune responses of scallop (*Chlamys farreri* Jones et Preston). Fish Shellfish Immunol. 22 (3), 272–281.

Chi, C., Zhang, C., Liu, J., Zheng, X., 2019. Effects of Marine Toxin domoic acid on innate immune responses in Bay Scallop *Argopecten irradians*. J. Mar. Sci. Eng. 7 (11), 407.

Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science 321 (5891), 926–929.

González, P.M., Rocchetta, I., Abele, D., Rivera-Ingraham, G.A., 2019. Hypoxically induced nitric oxide: potential role as a vasodilator in *Mytilus edulis* gills. Front. Physiol. 9, 1709.

González-Ruiz, R., Peregrino-Uriarte, A.B., Valenzuela-Soto, E.M., Cinco-Moroyoqui, F. J., Martínez-Téllez, M.A., Yepiz-Plascencia, G., 2021. Mitochondrial manganese superoxide dismutase knock-down increases oxidative stress and caspase-3 activity in the white shrimp *Litopenaeus vannamei* exposed to high temperature, hypoxia, and reoxygenation. Comp. Biochem. Physiol. Mol. Integr. Physiol. 252, 110826.

Grieshaber, M., Hardewig, I., Kreutzer, U., Pörtner, H.-O., 2005. Physiological and metabolic responses to hypoxia in invertebrates. Rev. Physiol. Biochem. Pharmacol. 125, 43–147.

Gu, H., Shang, Y., Clements, J., Dupont, S., Wang, T., Wei, S., et al., 2019. Hypoxia aggravates the effects of ocean acidification on the physiological energetics of the blue mussel *Mytilus edulis*. Mar. Pollut. Bull. 149, 110538.

Guo, X., Luo, Y., 2016. Chapter-scallops and scallop aquaculture in China. Developments in aquaculture and fisheries. Science 40, 937–952.

Haider, F., Falfushynska, H.I., Timm, S., Sokolova, I.M., 2020. Effects of hypoxia and reoxygenation on intermediary metabolite homeostasis of marine bivalves *Mytilus edulis* and *Crassostrea gigas*. Comp. Biochem. Physiol. Mol. Integr. Physiol. 242, 110657.

Johnson, C.J., 2017. How Does the Green Eelgrass Isopod *Pentidotea Resecata* Protect its Tissue against Highly Fluctuating Oxygen Conditions? Walla Walla University.

Lawniczak, M., Romestaing, C., Roussel, D., Maazouzi, C., Renault, D., Hervant, F., 2013. Preventive antioxidant responses to extreme oxygen level fluctuation in a subterranean crustacean. Comp. Biochem. Physiol. Mol. Integr. Physiol. 165 (2), 299–303.

Lesser, M.P., 2016. Climate change stressors cause metabolic depression in the blue mussel, *Mytilus edulis*, from the Gulf of Maine. Limnol. Oceanogr. 61 (5), 1705–1717.

Mazorra, M., Rubio, J., Blasco, J., 2002. Acid and alkaline phosphatase activities in the clam *Scrobicularia plana*: kinetic characteristics and effects of heavy metals. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 131 (2), 241–249.

Meryl Williams, M., Harper, N., Chaitovitz, C., Dansie, A., Diaz, R., Heidemeier, J., et al., 2011. Hypoxia and Nutrient Reduction in the Coastal Zone: Advice for Prevention, Remediation and Research. Global Environment Facility.

Miller, D., Poucher, S., Coiro, L., 2002. Determination of lethal dissolved oxygen levels for selected marine and estuarine fishes, crustaceans, and a bivalve. Mar. Biol. 140 (2), 287–296.

Montgomery, D.W., Simpson, S.D., Engelhard, G.H., Birchenough, S.N., Wilson, R.W., 2019. Rising CO_2 enhances hypoxia tolerance in a marine fish. Sci. Rep. 9 (1), 1–10.

Nagasoe, S., Tokunaga, T., Yurimoto, T., Matsuyama, Y., 2020. Survival and behavior patterns associated with hypoxia at different life stages of the pen shell *Atrina cf. japonica*. Aquat. Toxicol. 227, 105610.

Nogueira, L., Mello, D.F., Trevisan, R., Garcia, D., da Silva Acosta, D., Dafre, A.L., et al., 2017. Hypoxia effects on oxidative stress and immunocompetence biomarkers in the mussel *Perna perna* (Mytilidae, Bivalvia). Mar. Environ. Res. 126, 109–115.

Sanders, T., Widdicombe, S., Calder-Potts, R., Spicer, J.I., 2014. Environmental hypoxia but not minor shell damage affects scope for growth and body condition in the blue mussel *Mytilus edulis* (L.). Mar. Environ. Res. 95, 74–80.

Seibel, B.A., 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. J. Exp. Biol. 214 (2), 326–336.

Seitkalieva, A., Menzorova, N., Rasskazov, V., 2015. Phosphatases of echinoderms and bivalve mollusks of the Japan and Okhotsk seas. Russ. J. Mar. Biol. 41 (1), 51–59.

Shen, Y., Huang, Z., Liu, G., Ke, C., You, W., 2019. Hemolymph and transcriptome analysis to understand innate immune responses to hypoxia in Pacific abalone. Comp. Biochem. Physiol. Genom. Proteonomics 30, 102–112.

Shen, Y., Huang, M., You, W., Luo, X., Ke, C., 2020. The survival and respiration response of two abalones under short-term hypoxia challenges. Aquaculture 529, 735658.

Silva, A.R., Silveira Guerreiro, A., Martins, S.E., Sandrini, J.Z., 2021. DCOIT unbalances the antioxidant defense system in juvenile and adults of the marine bivalve *Amarilladesma mactroides* (Mollusca: Bivalvia). Comp. Biochem. Physiol. C Toxicol. Pharmacol. 250, 109169.

Song, Q., Li, Y., Zhou, H., 2015. Spatial and temporal changes of immune-related enzymes activities in *Litopenaeus vannamei* under hypoxia-reoxygenation stress. J. Trop. Biol. 6 (4), 353–358.

Stefano, G., Mantione, K., Casares, F., Kream, R., 2015. Anaerobically functioning mitochondria: evolutionary perspective on modulation of energy metabolism in *Mytilus edulis*. Invertebr. Surviv. J. 12 (1), 22–28.

Sui, Y., Kong, H., Shang, Y., Huang, X., Wu, F., Hu, M., et al., 2016. Effects of short-term hypoxia and seawater acidification on hemocyte responses of the mussel *Mytilus coruscus*. Mar. Pollut. Bull. 108 (1–2), 46–52.

Sui, Y., Hu, M., Shang, Y., Wu, F., Huang, X., Dupont, S., et al., 2017. Antioxidant response of the hard shelled mussel *Mytilus coruscus* exposed to reduced pH and oxygen concentration. Ecotoxicol. Environ. Saf. 137, 94–102.

Sun, X., Tu, K., Li, L., Wu, B., Wu, L., Liu, Z., et al., 2021. Integrated transcriptome and metabolome analysis reveals molecular responses of the clams to acute hypoxia. Mar. Environ. Res. 168, 105317.

Uzaki, N., Kai, M., Aoyama, H., Suzuki, T., 2003. Changes in mortality rate and glycogen content of the Manila clam *Ruditapes philippinarum* during the development of oxygen-deficient waters. Fish. Sci. 69 (5), 936–943.

Wang, W., Widdows, J., 1991. Physiological responses of mussel larvae *Mytilus edulis* to environmental hypoxia and anoxia. Mar. Ecol. Prog. Ser. 70 (3), 223–236.

Wang, W., Widdows, J., 1993. Metabolic responses of the common mussel *Mytilus edulis* to hypoxia and anoxia. Mar. Ecol. Prog. Ser. 95, 205.
Wu, R.S., 2002. Hypoxia: from molecular responses to ecosystem responses. Mar. Pollut. Bull. 45 (1–12), 35–45.
Wu, Y., Zhou, C., Zhang, Y., Pu, X., Li, W., 2001. Evolution and causes of formation of *gymnodinium sanguineum* bloom in yantai sishili bay. Oceanol. Limnol. Sinica 32 (2), 159–167.
Xiong, X., Huang, G., Peng, Y., Liu, X., 2016. Effect of hypoxia on growth performance, energy metabolism and oxidative stress of *Mugil cephalus*. J. Fish. China 40 (1), 73–82.
Zhou, Z., Ding, Q., Liu, Q., Wang, Y., Hu, M., 2013. Stresses of hypoxia and its effects on immune system of bivalve. Asian J. Ecotoxicol. (3), 324–330.