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ORIGINAL ARTICLE

Physiological and gene expression responses of diploid and triploid Pacific oyster (*Crassostrea gigas*) to heat acclimation

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Abstract

High temperature is considered to be one of the important causes of mass mortality of diploid and triploid oysters in summer. In order to compare the tolerance adaptability of diploid and triploid oysters to heat stress, the activities of superoxide dismutase activity (SOD), catalase activity (CAT) and contents of malondialdehyde (MDA) and the expression of Hsp70 and metallothionein (MT) genes in diploid and triploid oysters under acute and chronic heat stress were studied. The results showed that the survival rate of diploid oysters was significantly higher than that of triploid oysters under acute and chronic heat stress ($p < 0.05$). Under acute heat stress, the SOD levels in gills of both diploid and triploid oysters increased significantly from hour 3 ($p < 0.05$), but there was no significant difference in SOD levels between diploid and triploid oysters at all time points. The SOD level in hepatopancreas of triploid oysters was significantly higher than that of diploid oysters at all time points except 48 h ($p < 0.05$). The CAT level of diploid and triploid oysters decreased sharply at 48 h and that of diploid oysters was significantly higher than that of triploid oysters ($p < 0.05$). Under acute heat stress, the increase in MDA content in triploid oysters was significantly higher than that in diploid oysters ($p < 0.05$). Under acute heat stress, the expression levels of Hsp70 in the gills and hepatopancreas were significantly increased in diploid and triploid oysters ($p < 0.05$), but increased varies between gills and hepatopancreas. The expression levels of MT in the gills and hepatopancreas were significantly decreased in diploid and triploid oysters ($p < 0.05$). The results obtained in this study provide physiological and immunological evidence to explain differences in high-temperature tolerance between diploid and triploid oysters and help us better understand the mass mortality of oysters that occurs during high temperatures in summer.

KEYWORDS
Crassostrea gigas, diploid, enzymatic activities, expression analysis, triploid

1 | INTRODUCTION

The Pacific oyster (*Crassostrea gigas*) is native to China, Japan and Korea and has been introduced worldwide as aquaculture species due to its rapid growth and wide tolerance to different environmental conditions (Ruesink et al., 2005). Due to its sterility, triploid oysters tend to have a higher growth rate, which increases the yield of oysters and the marketability of oysters throughout the year. Triploid oysters are welcomed by the majority of farmers and account for a rapidly increasing proportion in the oyster cultivation industry. Oyster is one of the largest farmed shellfish in the world. *C. gigas* is one of the important breeding species of oyster, playing an important role in world food security and high-quality animal protein supply. Oyster farming has faced some difficulties, among which the most prominent is the mass mortalities of oysters in summer (Ashton et al., 2020; Cotter et al., 2010; Huvet et al., 2004;

Malham et al., 2009; Patrick et al., 2006; Wendling & Wegner, 2013). This not only caused great economic losses to the oyster farmers but also posed severe challenges to the development of oyster industry. Mass mortality of oyster usually occurred in the summer, so the high-temperature characteristic of summer was thought to be one of the main causes of the problem (Ashton et al., 2020; Chavez-Villalba et al., 2010; de Kantzow et al., 2016; Wendling & Wegner, 2013). The change of temperature will cause physiological reaction of aquatic organisms, and the change of temperature over a certain range will lead to physiological disorder and even death of organisms (Dong et al., 2010; Tort et al., 2004). In order to explore the high-temperature tolerance of oysters, it is necessary to study the physiological changes of oysters under high-temperature stress.

Enzymes are important substances in biochemical reactions and important indicators reflecting physiological and immune status of organisms, which play an important role in aquatic organisms coping with temperature changes (Chen et al., 2007; Hao et al., 2014; Park et al., 2015; Rahman et al., 2019). The acute stimulation of temperature changes can lead to increased levels of reactive oxygen species (ROS) in Marine invertebrates, and excessive production of ROS and other pro-oxidants can lead to nutrient destruction and genetic damage (Abele et al., 2002; De & Victor, 2000; Xu et al., 1999). The lipid peroxidation product malondialdehyde (MDA) is often used as a marker of oxidative stress (Zanette et al., 2011). In order to cope with the damage caused by peroxides, organisms have evolved a variety of antioxidant defence mechanisms (antioxidant enzymes and non-enzymatic compounds), among which superoxide dismutase (SOD), catalase (CAT) and heat shock proteins (HSPs) play a major role. SOD and CAT are the most common antioxidant enzymes detoxifying reactive oxygen species (Park et al., 2015). In response to heat stress, enzyme activity reaction is not the only way, but a common response of various physiological processes, such as Heat shock proteins (HSPs).

Heat shock proteins are a kind of conserved protein, which plays an important role in enhancing the stress tolerance of cells and protecting cells from external damage. HSP70 is a representative protein of HSPs family, which is highly sensitive to thermal stress (Feder & Krebs, 1998; Hamdoun et al., 2003). Thermal stress has been found to stimulate HSP70 expression in some shellfish (Brun et al., 2008; Franzellitti & Fabbri, 2005; Hamdoun et al., 2003). In addition, metallothionein (MT) is also involved in the regulation of oxidative stress and plays a role when organisms are subjected to heat stress (Farcy et al., 2009). Under high-temperature stress, the physiological and immune responses of aquatic organisms are bound to occur, and the differences of physiological and immune responses reflect their tolerance adaptability to high temperature to a certain extent.

Mass mortality in summer has occurred in both diploid and triploid oysters, but the high-temperature tolerance of diploid and triploid oysters is rarely studied. In order to compare the difference in high-temperature tolerance between diploid oyster and triploid oyster, this study evaluated the effects of acute and chronic heat stress on enzyme activities (SOD, CAT and MDA) and two heat resistance-related genes (HSP70 and MT) in diploid and triploid oysters, hoping to provide useful suggestions for oyster cultivation in summer.

2 | MATERIALS AND METHODS

2.1 | Experimental samples

The diploid and triploid *C. gigas* oysters were 18 months and collected from Sanggou Bay aquaculture farm in Rongcheng, Shandong province, China in October 2020. The triploid oysters were produced by crossing tetraploid and diploid. The coastal seawater temperature ranges from 14°C to 28°C between May and August in Rongcheng (COCMB, 2020). The haemolymph of the triploid oysters was sampled for ploidy, and the haemolymph of the diploid oysters was sampled as well as that of the triploid oysters. Oysters were maintained in filtered seawater (temperature 18 ± 0.2°C; salinity 31.5 ± 0.5 psu) for 10 days prior to the experiment. The oysters were fed *Spirulina* spp once a day. 1/2 water was replaced every day.

2.2 | Heat stress and samples collection

The diploid and triploid oysters were randomly divided into two treatment groups (acute and chronic heat challenge). In the acute heat stress challenge, 70 individuals each for diploid and triploid oysters were transferred from 18°C to 28°C and then treated for 216 h. In the chronic heat stress challenge, 70 individuals each for diploid and triploid oysters were transferred from 18°C to 23°C by increasing the temperature progressively (1–2°C/day) and then were kept at 23°C for 3 days. They then were transferred from 23°C to 28°C by increasing the temperature progressively (1–2°C/day), and then they were kept at 28°C for 3 days according to Dong et al. (2020). The salinity was maintained at 31.5 ± 0.5 psu throughout the experiments. Three replicates were set up for each treatment tank (800 L). At the process of the acute heat stress and chronic heat stress experiments, the gills and hepatopancreas of four oysters were randomly collected from diploid and triploid oysters, immediately frozen in liquid nitrogen, and stored at −80°C for subsequent RNA extraction to measure the expression level of heat shock protein 70 (Hsp70) and metallothionein (MT).

To assess the effects of acute heat stress and chronic heat stress on enzyme activity, the gills and hepatopancreas were collected from three randomly chosen oysters at 0, 3, 6, 12, 24, 48, 72, 96 h in acute heat stress group and three randomly chosen oysters after exposure to chronic heat stress at 18, 23 and 28°C respectively.

2.3 | Survival rate of oysters under heat stress

Thirty of seventy individuals in each group were used to estimate survival rate, and the remainder was used for tissue sampling. The

survival rate is the ratio of the number of surviving oysters to the 30 oysters after acute heat stress or chronic heat stress.

2.4 | RNA isolation and cDNA synthesis

Total RNA was extracted from hepatopancreas and gills using TRIzol Reagent (Invitrogen) following the manufacturer's protocol. The quality and quantity of RNA samples were assessed using 1% agarose gel electrophoresis and using a NanoDrop 2000c UV–Vis Spectrophotometer (Thermo Scientific). Each RNA sample from *C. gigas* was reverse transcribed with a complementary DNA (cDNA) synthesis kit (Takara, DRR047A). The cDNA product was diluted appropriately (10×) and stored at −20°C prior to use.

2.5 | Measurement of the expression of Hsp70 and MT

The forward and reverse Hsp70 and MT primers were designed using PRIMER 5.0. Table 1 lists the specific primers for Hsp70, MT and *elongation factor I* (*EF1*) (Renault et al., 2011) used in this study. The optimized two-step PCR program was 94°C for 5 min and then 40 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 15 s. The relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method (Schmittgen & Livak, 2008). The qRT-PCR was carried out using a Roche 480 Real-time PCR System (Roche).

2.6 | Enzyme activity assay

Prior to enzyme activity testing, the frozen gill and hepatopancreas were homogenized (10% w/v) on ice in 0.86% saline with a homogenizer (IKA) and immediately centrifuged at 2220 *g* for 15 min at 4°C. The SOD activity was determined with a commercial kit (Nanjing Jiancheng), using water-soluble tetrazole salt (WST-1) method. The CAT activity was detected according to Goth (1991) using a commercial kit (Nanjing Jiancheng). The content of MDA was determined according to Esterbauer and Cheeseman (1990) with a commercial kit (Nanjing Jiancheng).

TABLE 1 Primer sequence used in this study

Primer	Primer sequences	Purpose
HSP70-F	TCCAACCAAACAGACGCAGA	qRT-PCR
HSP70-R	GCTCGAACTTTCCGAGTAGGT	qRT-PCR
MT-F	CCGGATGTGGCTGCAAAGTC	qRT-PCR
MT-R	GTCCGCCTTCGTCCTTTGTT	qRT-PCR
EFI-F	CAAGAACGGAGATGCTGGTATGG	qRT-PCR
EFI-R	TTTCACTCTTTCCACCGGCTTT	qRT-PCR

2.7 | Statistical analysis

Significant differences were analysed using SPSS 23.0. Differences were considered statistically significant at $p < 0.05$. Independent sample *t* test was performed to evaluate differences in Hsp70 and MT expression levels and enzyme activities at different temperatures and time points in diploid and triploid oysters.

3 | RESULTS

3.1 | Survival rate

The survival rate of diploid and triploid oysters under heat stress is shown in Table 2. The survival rate of diploid oysters was significantly higher than that of triploid oysters under both acute and chronic heat stress ($p < 0.05$). Diploid oysters had the highest survival rate (74.27%), and triploid oysters had the lowest survival rate (16.30%) under chronic heat stress. For triploid oysters, the survival rate of acute heat stress was significantly higher than that of chronic heat stress ($p < 0.05$).

3.2 | Enzyme activity assays

3.2.1 | SOD, CAT and MDA in gills

The effect of heat stress on SOD level of diploid and triploid oysters is shown in Figure 1. Under acute heat stress, the SOD levels of both diploid and triploid oysters increased significantly from hour 3 ($p < 0.05$; Figure 1A), but there was no significant difference in SOD levels between diploid and triploid oysters at all time points. Under chronic heat stress, the SOD levels of both diploid and triploid oysters reached their maximum values at 23°C, with values of 130.46 and 126.73 U/mg prot respectively (Figure 1B). Similar to acute heat stress, there was no significant difference in SOD levels between diploid and triploid oysters.

Figure 2 shows the effects of heat stress on CAT levels in diploid and triploid oysters. There were no significant differences in CAT levels between diploid and triploid oysters under acute heat stress, except at hour 6 and hour 48 (Figure 2A). The CAT level of diploid oysters was significantly higher than that of triploid oysters at 6 and 48 h ($p < 0.05$), and at 48 h, the CAT level of diploid and triploid oysters decreased sharply and reached the minimum value, with values of 40.97 and 20.44 U/mg prot respectively ($p < 0.05$). Under chronic heat stress, there was no significant difference in CAT levels between diploid and triploid oysters (Figure 2B).

The MDA content of diploid and triploid oysters under heat stress is shown in the Figure 3. During 24 h of acute heat stress, The MDA content in triploid oysters behaved a general trend of increasing first and then decreasing, while the content of MDA in diploid oysters did not change significantly (Figure 3A). The MDA content of triploid oysters was significantly higher than that of diploid oysters at 0, 12 and

24 h ($p < 0.05$). The MDA content of diploid oysters reached the maximum (24.81 nmol/mg prot) at 3 h, while that of triploid oysters reached the maximum (43.75 nmol/mg prot) at 12 h. The trend of MDA content after 24 h was the same as that before 24 h, but there was no significant difference in MDA content between diploid and triploid oysters. Under chronic heat stress, the MDA content of diploid and triploid oysters tended to increase with temperature (Figure 3B).

TABLE 2 Survival rate of diploid and triploid *Crassostrea gigas* under heat temperature stress

	Survival rate/%	
Group	Acute heat stress	Chronic heat stress
Diploid	62.27 ± 8.81^{a}	74.27 ± 4.26^{a}
Triploid	42.95 ± 10.32^{bA}	16.30 ± 8.73^{bB}

Note: Different superscript lowercase letters in each column indicate significant difference ($p < 0.05$). Different superscript capital letters in each row indicate significant difference ($p < 0.05$).

3.2.2 | SOD, CAT and MDA in hepatopancreas

The effect of heat stress on SOD level of diploid and triploid oyster is shown in Figure 4. Under acute heat stress, the SOD levels of diploid and triploid oysters tended to decrease and sharply decreased at 48 and 96 h (Figure 4A). And the SOD level of triploid oysters was significantly higher than that of diploid oysters at all time points except 48 h ($p < 0.05$). Under chronic heat stress, the SOD levels of diploid and triploid oysters were significantly different only at 18°C ($p < 0.05$; Figure 4B).

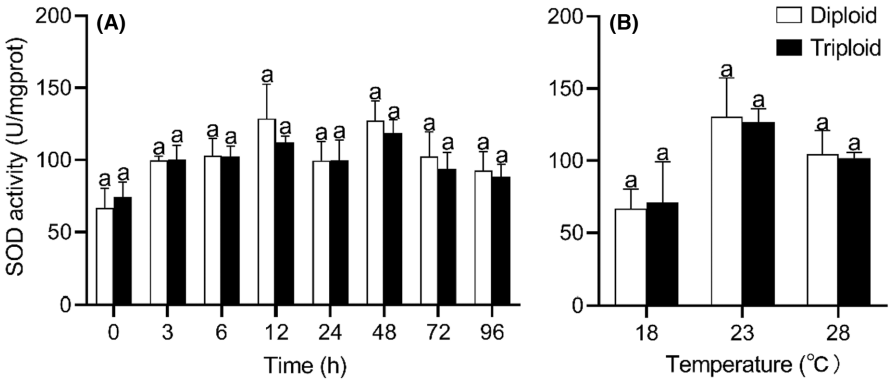

FIGURE 1 Effect of the acute heat temperature stress (a) and chronic heat temperature stress (b) on SOD activity in gills of diploid and triploid *Crassostrea gigas*. Different lowercase letters indicate significant differences ($p < 0.05$)

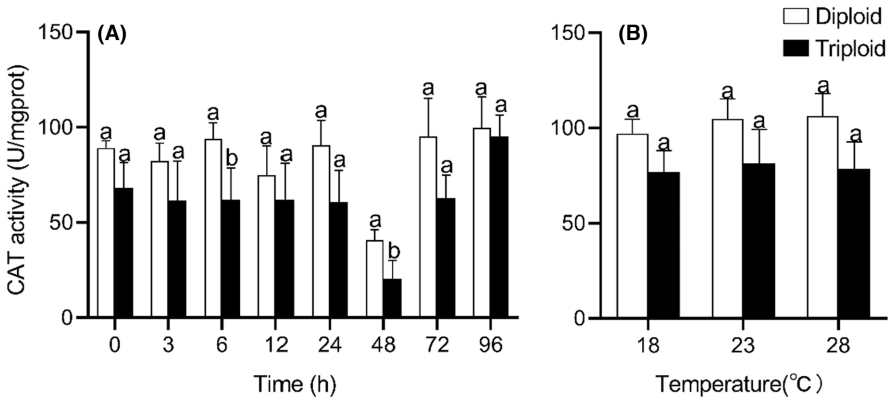

FIGURE 2 Effect of the acute heat temperature stress (a) and chronic heat temperature stress (b) on CAT activity in gills of diploid and triploid *Crassostrea gigas*. Different lowercase letters indicate significant differences ($p < 0.05$)

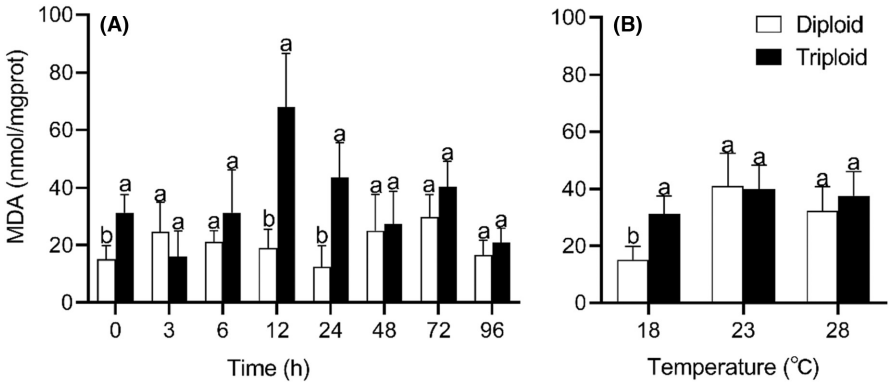

FIGURE 3 Effect of the acute heat temperature stress (a) and chronic heat temperature stress (b) on MDA in gills of diploid and triploid *Crassostrea gigas*. Different lowercase letters indicate significant differences ($p < 0.05$)

Figure 5 shows the effects of heat stress on CAT levels in diploid and triploid oysters. Within 24 h, the CAT levels behaved a general trend of decreasing first and then increasing under acute heat stress (Figure 5A). The CAT levels of diploid and triploid increased sharply at 24 and 72 h and that of triploid oysters was significantly higher than that of diploid oysters ($p < 0.05$), while the CAT levels of diploid and triploid oysters decreased sharply at 48 and 96 h. Under chronic heat stress, there was no significant difference in CAT level between diploid and triploid oysters, and the maximum CAT level of both diploid and triploid oysters appeared at 18°C (Figure 5B).

The MDA content of diploid and triploid oysters under heat stress is shown in the Figure 6. Under heat stress, the MDA content of diploid and triploid oysters was significantly different only at 12 h and the MDA level of triploid oysters was significantly higher than that of diploid oysters ($p < 0.05$; Figure 6A). The effect of heat stress on MDA levels of diploid and triploid oysters showed a similar pattern. Under acute heat stress, MDA levels of diploid and triploid increased first and then decreased within 48 h and reached the minimum (5.07 and 3.85 nmol/mg prot respectively) at 48 h. Then, the MDA level of diploid and triploid increased sharply at 72 h and reached the maximum (16.77 and 18.78 nmol/mg prot respectively) at the same time and then decreased sharply at 96 h. Chronic heat stress had no significant effect on MDA levels between diploid and triploid oysters, but MDA content in both diploid and triploid oysters increased with temperature (Figure 6B). The maximum MDA content of diploid and triploid oysters was 15.43 and 18.61 nmol/mg prot at 28°C respectively.

3.3 | Effects of heat stress on the expression of Hsp70 and MT

3.3.1 | Expression of Hsp70 and MT in gills

Figure 7A shows the expression of Hsp70 of diploid and triploid oysters under acute heat stress. The expression of Hsp70 in diploid and triploid oysters performed the tendency that first rises and then decreases. The relative expression levels of Hsp70 of diploid and triploid oysters were significantly up-regulated from 3 h after heat stress began ($p < 0.05$). The relative expression level of Hsp70 in triploid oysters peaked at 6 h and was significantly higher than that in diploid oysters ($p < 0.05$), while the relative expression level of Hsp70 in diploid oysters peaked at 12 h and was significantly higher than that in triploid oysters ($p < 0.05$). Under chronic heat stress, the expression level of Hsp70 in diploid and triploid oysters at 28°C was significantly higher than that at 18 and 23°C ($p < 0.05$), but there was no significant difference in Hsp70 expression between diploid and triploid oysters at all temperature levels (Figure 7B).

The MT expression of diploid and triploid oysters showed a trend of first decline and then rise under acute heat stress (Figure 8A). The MT expression levels of diploid and triploid oysters were significantly down-regulated and reached the lowest point at 6 h ($p < 0.05$), but there was no significant difference in MT expression levels between diploid and triploid oysters at all time points. Under chronic heat stress, there was no significant difference in MT expression level between diploid and triploid oysters at 18 and 23°C, while MT

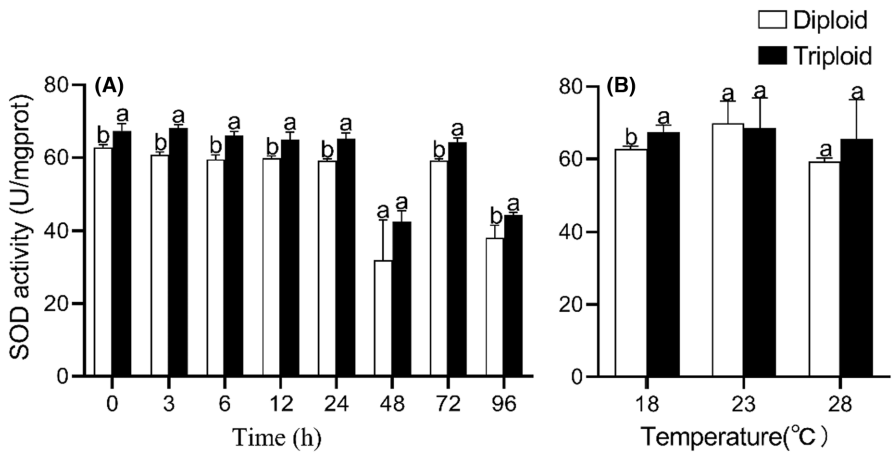

FIGURE 4 Effect of the acute heat temperature stress (a) and chronic heat temperature stress (b) on SOD activity in hepatopancreas of diploid and triploid *Crassostrea gigas*. Different lowercase letters indicate significant differences ($p < 0.05$)

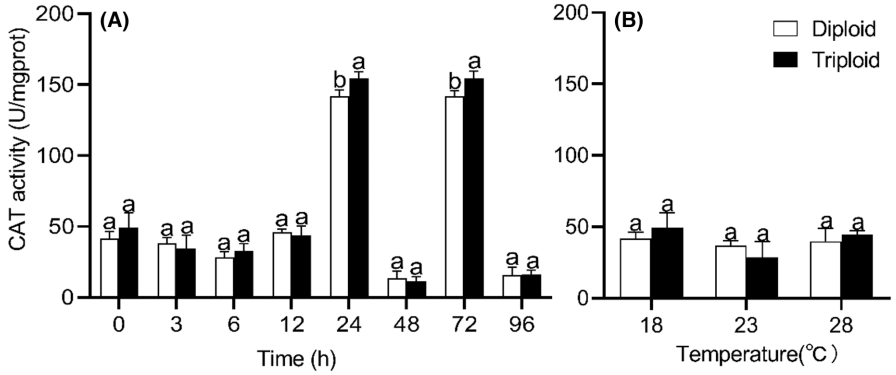

FIGURE 5 Effect of the acute heat temperature stress (a) and chronic heat temperature stress (b) on CAT activity in hepatopancreas of diploid and triploid *Crassostrea gigas*. Different lowercase letters indicate significant differences ($p < 0.05$)

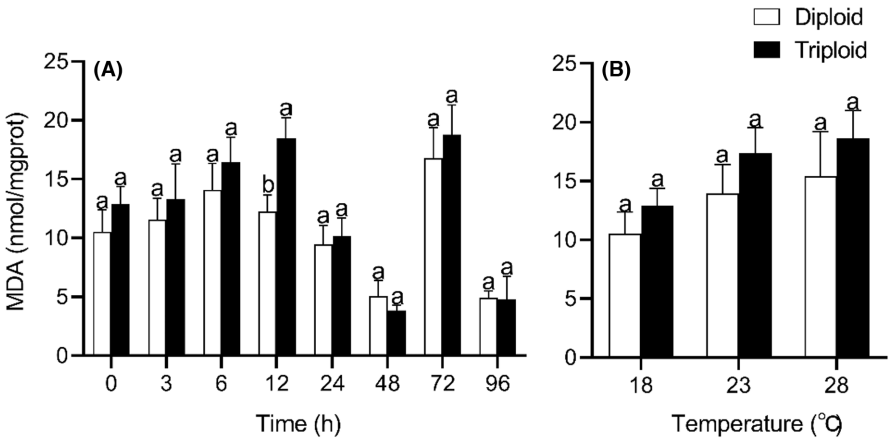

FIGURE 6 Effect of the acute heat temperature stress (a) and chronic heat temperature stress (b) on MDA in hepatopancreas of diploid and triploid *Crassostrea gigas*. Different lowercase letters indicate significant differences ($p < 0.05$)

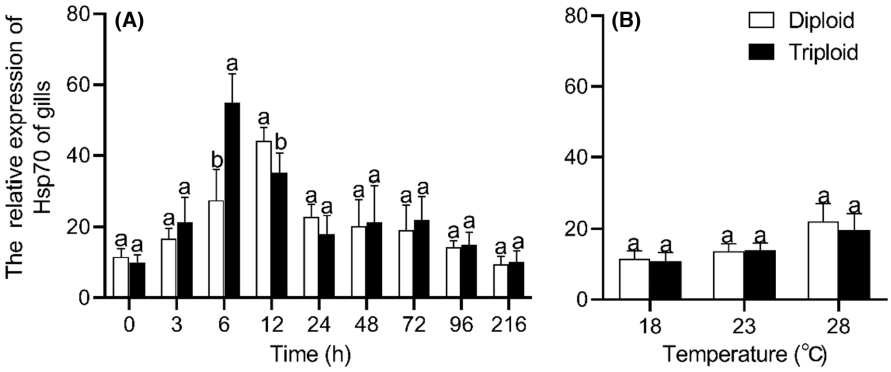

FIGURE 7 Expression analysis of Hsp70 in gills of diploid and triploid *Crassostrea gigas* after heat temperature stress (a) and chronic heat temperature stress (b). Different lowercase letters indicate significant differences ($p < 0.05$)

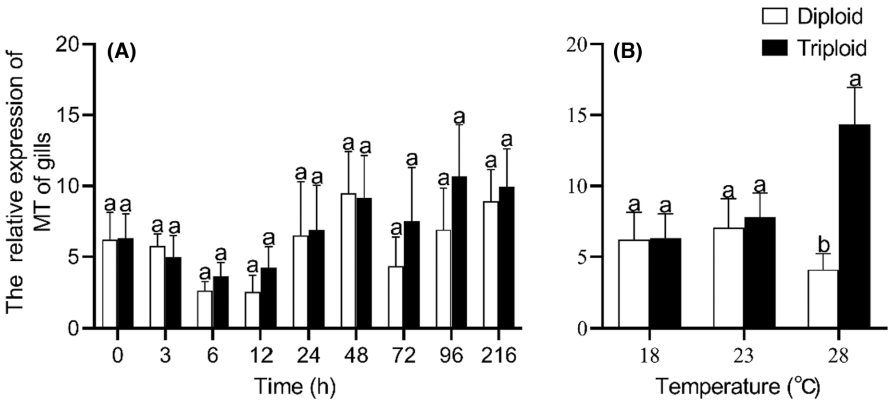

FIGURE 8 Expression analysis of MT in gills of diploid and triploid *Crassostrea gigas* after heat temperature stress (a) and chronic heat temperature stress (b). Different lowercase letters indicate significant differences ($p < 0.05$)

expression level of triploid oysters was significantly higher than that of diploid oysters at 28°C ($p < 0.05$) (Figure 8B).

3.3.2 | Expression of Hsp70 and MT in hepatopancreas

The Hsp70 expression of diploid and triploid oysters showed a trend of first decline and then rise under acute heat stress (Figure 9A). Under acute heat stress, Hsp70 expression levels were significantly different between diploid and triploid oysters at 3, 6 and 12 h ($p < 0.05$). At 6 h the relative expression level of Hsp70 in diploid oysters was significantly up-regulated and the relative expression level of Hsp70 in diploid oysters was significantly higher than that in triploid oysters. At 12 h, the relative expression level of Hsp70 in triploid oysters was significantly up-regulated, and the relative expression level of Hsp70 in triploid oysters was significantly higher than that in diploid oysters. Under chronic heat stress, the expression level of Hsp70 in diploid oysters was significantly higher than that in diploid oysters at 28°C ($p < 0.05$), while there was no significant difference between diploid and triploid oysters at 18 and 23°C (Figure 9B).

Figure 10A shows the expression of MT of diploid and triploid oysters under acute heat stress. The expression of MT of diploid and triploid oysters was significantly down-regulated from 3 h after heat stress began ($p < 0.05$). The MT expression level of diploid oysters was significantly higher than that of triploid oysters at 3 and 6 h ($p < 0.05$) while the MT expression level of diploid oysters was significantly lower than that of triploid oysters at 72 and 216 h ($p < 0.05$).

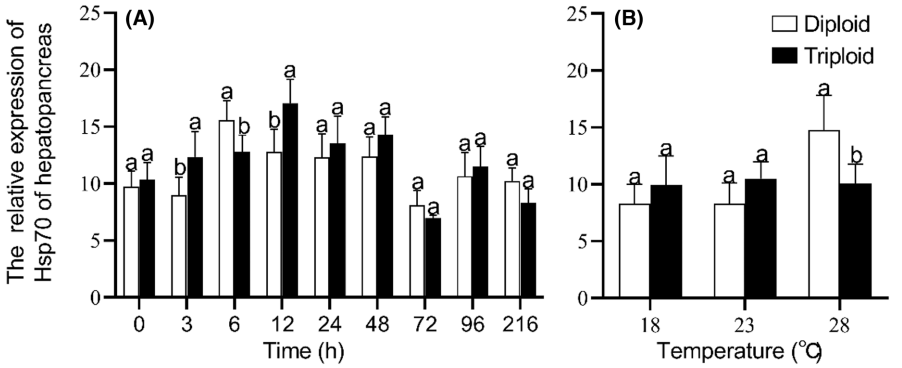

FIGURE 9 Expression analysis of Hsp70 in hepatopancreas of diploid and triploid *Crassostrea gigas* after acute heat stress (a) and chronic heat stress (b). Different lowercase letters indicate significant differences ($p < 0.05$)

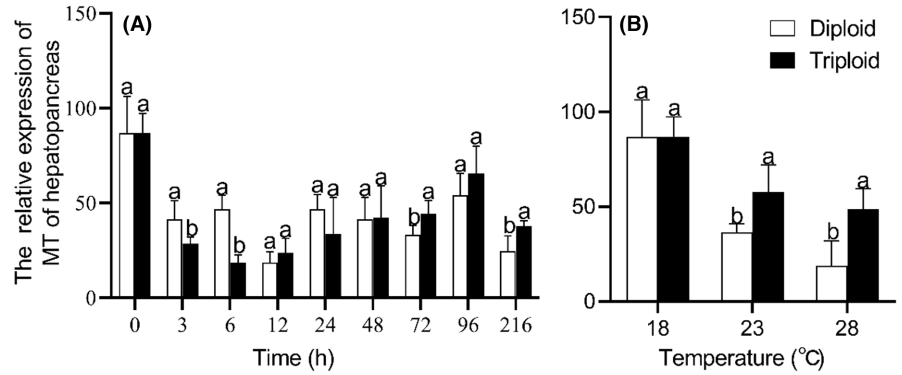

FIGURE 10 Expression analysis of MT in hepatopancreas of diploid and triploid *Crassostrea gigas* after acute heat stress (a) and chronic heat stress (b). Different lowercase letters indicate significant differences ($p < 0.05$)

Under chronic heat stress, the MT expression of diploid and triploid oysters decreased with temperature increasing Figure 10B. The MT expression level of triploid oysters was significantly higher than that of diploid oysters at 23 and 28°C ($p < 0.05$).

4 | DISCUSSION

Studies have reported mass mortality of oysters during the summer, involving diploid and triploid oysters (Cotter et al., 2010; Huvet et al., 2004), with high temperatures associated with mass mortality (Ashton et al., 2020; Chavez-Villalba et al., 2010; de Kantzow et al., 2016; Wendling & Wegner, 2013). In the past two decades, the triploid oysters have developed rapidly and played an important role in the development of oyster industry. However, there are few studies on the high-temperature tolerance of diploid and triploid oysters. Temperature has a significant effect on the physiological activities and relevant gene expression in molluscs (Farcy et al., 2007; Fearman & Moltschaniwskyj, 2010; Wang & Li, 2020). To some extent, the physiology and expression of related genes in oysters reflect their high-temperature tolerance. In this study, enzyme activity and related gene expression of diploid and triploid oysters were compared to reveal the tolerance of diploid and triploid oysters to high temperature.

4.1 | Survival rate

The difference in survival rate in this experiment reflects the higher temperature tolerance of diploid oysters than that of triploid oysters. Though the temperature set in the experiment was suitable for Pacific oyster, high-temperature stress, whether acute or chronic, results in high mortality of triploid and diploid oysters, this might indicate that the rate of temperature increase can significantly affect the survival rate of the oysters. During the actual cultivation process in the summer, oysters are usually subject to a combination of factors. Gagnaire et al. (2006) found that triploids had a higher survival rate than diploids and that the difference was greatest at gamete maturity. This suggested that the oyster viability might be more sensitive to gamete development stress than high temperature. Considering the difference in reproduction capacity between diploid and triploid oysters, we speculate that the decrease of triploid gonad development is beneficial to the survival rate in summer. Increasing the proportion of triploid oysters in aquaculture may be an effective way to decrease mass mortality of Pacific oyster in aquaculture areas that experience sustained high temperatures in summer.

4.2 | Enzyme activity assays

Superoxide dismutase was an important enzyme defence factor in oxidative stress reaction (Downs et al., 2001), and the SOD level and response speed could reflect the tolerance of organism to environmental stress. Under high temperatures, SOD levels rise in organisms to neutralize excess ROS (Rahman et al., 2019). In this study, acute heat stress resulted in a sharp increase in SOD levels in the gills of diploid and triploid oysters, suggesting that SOD played an important role in avoiding organism damage from high-temperature stress. The reaction patterns of SOD in the gills of

diploid and triploid oysters were similar, and the difference was not significant, demonstrating that there was no significant difference in the ability of triploid and diploid oysters to prevent oxidative damage in the gills. Moreover, in hepatopancreas, the SOD level of triploid oysters was generally higher than that of diploid oysters. In terms of SOD level, it was not the low antioxidant capacity of the triploid oysters that led to the high mortality of the triploid oysters.

In this study, CAT was different from SOD in that its reaction speed was slower and the reaction level was very different, indicating that SOD might be more sensitive to high-temperature stress than CAT. Under high-temperature stress, the CAT level in gills and hepatopancreas of diploid and triploid oysters showed the same trend and the CAT level remained stable or even slightly decreased in gills and hepatopancreas of diploid and triploid oysters. This might be related to the fact that high temperatures can disrupt antioxidant systems, leading to a decrease in antioxidant enzyme expression (Rahman & Rahman, 2021). The difference in CAT levels between diploid and triploid oysters at 6 and 48 h was beneficial to the survival of diploid oysters, which might be one reason for the high survival rate of diploid oysters under heat stress.

As a product of lipid peroxidation, MDA levels are often used as a marker of oxidative stress. Under acute heat stress, MDA content in gills and hepatopancreas of triploid oysters increased more than that of diploid oysters, suggesting that high temperatures put more pressure on triploid oysters than diploid oysters. Considering the changes of SOD and CAT levels, we speculated that the high mortality rate of triploid oysters might be because of the increased content of MDA.

4.3 | Effects of heat stress on the expression of Hsp70 and MT

In addition to antioxidant enzymes, shellfish could reduce heat damage by translating and synthesizing heat-resistant proteins. Hsp70 is an important molecular chaperone involved in the acute response to temperature stress (Lindquist & Craig, 1988). Compared with hepatopancreas, gills, which are directly in contact with the surrounding environment, are more vulnerable to heat stress. In this study, the difference of Hsp70 expression between gill and hepatopancreas of diploid and triploid oysters under acute heat stress reflects the different sensitivity, which may be related to the different sensitivity thresholds and tissue specificity of animals (Hofmann, 1999). In gills, Hsp70 of the triploid oyster was more responsive to high temperature and expressed at a higher level than that of the diploid oyster, suggesting that the triploid oyster gills were more sensitive to high-temperature stress. However, an oversensitive response to high temperatures might be detrimental to the body, leading to a persistent stress state of the organism as temperatures fluctuate. This might be one of the reasons why the survival rate of triploid oysters under acute heat stress is higher than that under chronic heat stress.

Metallothionein could scour oxygen free radicals and play a role in preventing oxidative stress (Sato & Bremner, 1993). In this study, the expression level of MT in hepatopancreas of diploid and triploid oysters is higher than that in gills, indicating that MT expression level was tissue-specific in oysters. This was consistent with the tissue-specific expression of MT in abalone (Lee & Nam, 2016). The expression of MT in diploid and triploid oyster hepatopancreas in this study was inhibited and decreased in early phase of high-temperature stress, indicating that the scavenging ability of MT on reactive oxygen species might be limited by temperature. This phenomenon has also been found in abalone during acute stress, which is considered to be rapid overload caused by acute stress, in which stress levels may exceed normal regulatory capacity (Lee & Nam, 2016).

5 | CONCLUSION

We analysed and compared the physiological and gene expression responses of diploid and triploid oysters to heat stress. The results showed that high-temperature stress had strong effects on physiology and enzyme activity, especially acute high-temperature stress. The survival rate of diploid oysters was significantly higher than that of triploid oysters under both acute and chronic heat stress. In terms of SOD, CAT and MDA levels, the main reason for the high mortality of triploid oysters may be the increased content of MDA, rather than the difference of SOD and CAT levels. The expression levels of Hsp70 and MT in diploid and triploid oysters were significantly affected by heat stress, and the expression levels were tissue-specific. This study provides physiological and immunological evidence to explain differences in high-temperature tolerance between diploid and triploid oysters and helps us better understand the mass mortality of oysters that occurs during high temperatures in summer.

AUTHOR CONTRIBUTIONS

Qi Li designed the study. Yongguo Li and Chengxun Xu performed the study. Yongguo Li analysed the data and wrote the paper. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the first author upon request.

ETHICAL APPROVAL

The present study was performed according to the standard operation procedures of the Guide for the Use of Experimental Animals of

the Ocean University of China. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Ocean University of China.

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