Examination of survival, physiological parameters and immune response in relation to the thermo-resistant heterosis of hybrid oysters derived from *Crassostrea gigas* and *C. angulata*

Gaowei Jiang [a], Jianmin Zhou [a], Geng Cheng [a], Lingxin Meng [a], Yong Chi [a], Chengxun Xu [a], Qi Li [a,b,*]

[a] *Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China*
[b] *Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China*

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ABSTRACT

High temperature is an important environmental stressor leading to summer mass mortality of oysters. Significant survival heterosis in summer were observed in hybrids between two phylogenetically closely-related oysters, *Crassostrea gigas* and *C. angulata*, but an explicit understanding of heterosis has been lacking. Here, we investigated the survival performance, oxygen consumption rate (OR) and enzyme activity (i.e., SOD, CAT and MDA) in *C. gigas* (GG), *C. angulata* (AA) and their hybrids *C. gigas* ♀ × *C. angulata* ♂ (GA) and *C. angulata* ♀ × *C. gigas* ♂ (AG) under acute heat stress containing five levels: 22, 26, 30, 34 and 38 °C. Compared with parental strains, the hybrid strains exhibited higher cumulative survival rate at all temperatures, indicating heterosis for thermal tolerance. Cox regression analysis showed that hybrid strains challenged with heat shock exhibited lower hazard ratio and longer expected lifetime. Moreover, OR in hybrid strains were significantly higher ($P < 0.05$) than those in parental strains under 34 and 38 °C. Arrhenius break-point temperature (ABT) and temperature coefficient ($Q10$) revealed that hybrids had broader temperature range of aerobic metabolism and lower sensitivity to rising temperature. In addition, the activities of SOD and CAT of hybrid strains were significantly higher than the parental strains at 34 and 38 °C-level treatment after 6 h and at 34 °C-level treatment after 3 h, whereas the MDA content were reduced, indicating their stronger antioxidant capacity. Accordingly, the enhanced aerobic capacity and antioxidant ability of hybrids under high temperature may contribute to the improvement of thermal tolerance. Our findings can facilitate our understanding of the physiological and immune mechanisms underlying thermo-resistant heterosis and facilitate the breeding of thermo-resistant oyster varieties.

1. Introduction

The Pacific oyster *Crassostrea gigas*, originated from Northern Asia, is a hardy and euryhaline species (Boudry et al., 1998; Ghaffari et al., 2019) and has become a key mariculture species globally because of its high growth and yield characteristics (Hedgecock and Davis, 2007). In recent decades, however, the farmed *C. gigas* has been heavily affected by recurrent summer mortality syndrome (SMS) (Lang et al., 2009; Fuhrmann et al., 2019). SMS is caused by multiple factors including elevated temperature, pathogens, and physiologic stress associated with reproduction (Dégremont et al., 2005; Solomieu et al., 2015). High temperature facilitates the growth of pathogens, inhibits oyster immune system, making them more susceptible to opportunistic pathogens

(Soletchnik et al., 2005; Yang et al., 2021) and is therefore considered to be an important incentive for SMS (Yang et al., 2017). At present, several breeding programs for thermal-tolerant oysters have been successfully implemented to address the problem associated with summer mortality (Hershberger et al., 1984; Ding et al., 2020; Juárez et al., 2021) and have been proved to be effective to improve the survival rate of the oyster during summer.

The majority of research on crossbreeding in plants and animals has been conducted to combine desirable traits (Falconer and Mackay, 1996). In aquaculture, hybridization has been frequently used to increase environmental tolerances when one parental species has a wide range of adaption or specific tolerance (Nelson and Hedgecock, 1980; Rahman et al., 2013). For instance, crosses between Mozambique and

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Nile tilapias resulted in a red tilapia with strong salinity tolerance (Lim et al., 1993); hybrid *Haliotis rufescens* × *H. discus hannai* exhibited improved thermostability than both purebred populations (Lafarga de la Cruz et al., 2012). *C. angulata*, a closely related species of *C. gigas*, naturally inhabits the low latitude regions of southern China, and is characterized by excellent warm-water adaptation (Huvet et al., 2002; Ghaffari et al., 2019; Jiang et al., 2021a). Strikingly, the reciprocal hybrids between *C. gigas* and *C. angulata* exhibited obvious heterosis in survival during the high-temperature months and thermotolerance (Jiang et al., 2021b). Yet, little is known about the physiological and immunological mechanisms of heterosis in thermotolerance of hybrid oysters.

Thermotolerance assessments of oysters are usually based on the survival curves, which ends with the death of the individuals (Dowd and Somero, 2013; Chen et al., 2016). The physiological processes (i.e., respiration and excretion) of marine ectotherms are influenced by elevated temperature (Magnuson et al., 1979) and is closely related to their thermal resistance (Clark et al., 2008). Elevated or high temperature increase the organism's oxygen consumption rate (OR), which is replenished by increased ventilation (Jansen et al., 2007). Q_{10} or Van't Hoff's temperature coefficient is frequently used to evaluate the temperature sensitivity of OR (Casas et al., 2018). However, extremely high temperature induces oxygen restriction, leading to a respiration peak, over which anaerobic respiration is stimulated, with a rapid decline in OR (Pörtner, 2002). The temperature at respiration peak, Arrhenius break-point temperature (ABT) (Parker et al., 2017), may represent the thermotolerance threshold of marine organisms (Jansen et al., 2007).

High temperature could induce organisms to produce excessive endogenous reactive oxygen species (ROS), resulting in oxidative damage (Lushchak, 2011), signal transduction disorder (Rahman and Rahman, 2021) and increased cellular apoptosis (Slimen et al., 2014). Malondialdehyde (MDA) is the part of final product of lipid oxidation by ROS, and its level is frequently used to measure the degree of oxidative stress (Dagoudo et al., 2021). To protect cells from detrimental effects, aquatic animals have evolved antioxidant defense systems to neutralize extra ROS and free radicals (Kang et al., 2005). The system consists of non-enzymatic component and antioxidant enzymes, the latter including the enzymes superoxide dismutase (SOD) and catalase (CAT) (Yu, 1994; Klein et al., 2017), which could degrade superoxide into oxygen and water (Tan et al., 2020). Thus, expression of antioxidant enzyme activities may reflect the immune resistance of aquatic organisms under heat stress (Rahman and Rahman, 2021).

To address this issue, we evaluated the survival, physiological parameters (OR) and antioxidant enzyme activity (SOD, CAT and MDA) in *C. gigas*, *C. angulata* and their reciprocal hybrids under acute thermal stress. The integration of phenotypic, physiological and immunological data is aimed to better understand the heterosis in thermotolerance of hybrids.

2. Materials and methods

2.1. Oysters

A diallel hybridization between *C. gigas* and *C. angulata* was carried out in June 2019. *C. gigas* were sourced from Rongcheng (Shandong Province, China; 37°11′N, 122°35′E), while *C. angulata* were gathered from Zhangzhou (Fujian Province, China; 24°28′N, 118°16′E). For each species, eggs from 40 females were pooled and divided equally into two 10-L beakers. Each beaker of eggs was fertilized with a mixture of sperm from 40 *C. gigas* or 40 *C. angulata*. Thus, four crosses were established: GG- *C. gigas* ♀ × *C. gigas* ♂, GA- *C. gigas* ♀ × *C. angulata* ♂, AG- *C. angulata* ♀ × *C. gigas* ♂, and AA- *C. angulata* ♀ × *C. angulata* ♂. After 12 months, yearling oysters of GG, AA and reciprocal hybrids GA and AG were obtained from Rongcheng and transferred to Yantai Haiyi hatchery, Shandong Province for heat shock treatment. For each strain, approximately 5000 individuals with similar size (Table 1) were cleaned to

Table 1
The summary for the growth characteristics and condition index of four strains.

Strains	Shell height (mm)	Whole weight (g)	Shell-free dry weight (g)	Condition index
GG	84.08 ± 5.70[ab]	78.71 ± 6.02[a]	0.89 ± 0.10[c]	3.70 ± 1.04[b]
bAA	81.65 ± 6.48[b]	73.63 ± 7.07[b]	0.84 ± 0.07[c]	3.55 ± 0.69[b]
GA	86.43 ± 4.87[a]	82.13 ± 9.40[a]	1.10 ± 0.10[a]	5.18 ± 1.12[a]
AG	85.05 ± 5.89[ab]	80.78 ± 6.95[a]	1.03 ± 0.07[b]	4.84 ± 1.03[a]

eliminate attaching organisms and acclimated in 24 m³ concrete pond with filtered, aerated seawater (salinity 31 psu, temperature 22 °C) for 10 days before the heat shock treatment. Oysters were fed daily with fresh *Phaeodactylum tricornutum* Bohlin and the animals were not fed one day before heat shock was applied.

2.2. Survival performance under acute heat stress

Ten 100-L polyethylene buckets were used for acute heat stress experiment, each divided into 4 equal sections separated by 3 polyethylene nets, forming a total of 40 trial areas. The experimental design was completely random with 20 treatments using a factor design (5 temperature × 4 strains) and 2 replicates per treatment. In the trial bucket, the temperature was increased from control temperature (22 °C) to designed temperatures (26, 30, 34 and 38 °C) at a rate of 1 °C h⁻¹ using a water bath unit with heaters or water chiller. During heat shock period, four strains (30 oysters per strain) were transferred directly to the same bucket with designed temperatures. Survival data was recorded every 6-h and stopped at 96 h. Then the cumulative survival rates of GG, AA, GA and AG were calculated. The oyster was regarded as dead when it did not respond to touch with an anatomic needle, and dead individuals are picked up. 30% of the seawater was exchanged daily, and the oysters were fed *P. tricornutum* three times a day.

2.3. Physiological parameters determination

Eight oysters from each strain (GG, AA, GA and AG) with similar size were randomly selected from the acclimation environment to determine oxygen consumption rate (OR) in a closed chamber (1 L). Oysters were fasted for 24 h before measurement. Each chamber contained one oyster, and every experiment consisted of eight replicates. All chambers were filled with air-saturated seawater at the control temperature (22 °C) and designed temperature (26, 30, 34, 38 °C) and blocked with liquid paraffin to avoid gas exchange with the external environment. One chamber containing empty shells was included to serve as a control to correct for autogenic trends. During heat shock, chambers were immersed in a thermostatic bath to maintain a constant temperature (± 0.2 °C). DO concentrations were determined (YSI, USA) at the beginning of heat shock and 1 h later. Subsequently, the soft body of oyster was separated from the shells and dried at 80 °C to measure the shell-free dry weight. OR (mg g⁻¹ h⁻¹) was calculated as the followings (Ghaffari et al., 2019):

$$OR = \frac{(DO_{0\,h} - DO_{1\,h})_{Treat} - (DO_{0\,h} - DO_{1\,h})_{Blank}}{DW \times t} \times V$$

where DO_{0h} and DO_{1h} are the DO concentrations at the beginning and 1 h, respectively; "Treat" represents chambers containing oysters; "Blank" represents chamber does not contain oysters; DW (g) is the shell-free dry weight; V (L) represents the volume of water; T (h) represents the measuring time.

The condition index (CI), an indicator the physiological or nutritional condition of oyster, was calculated as the following (Abbe and Albright, 2003):

$$CI = \frac{shell - free\ dry\ weight\ (g)}{whole\ wet\ weight\ (g) - shell\ wet\ weight\ (g)} \times 100$$

2.4. Antioxidant enzyme activity determination

The enzyme activity experiment consists of two parts: (1) after 6 h of heat shock treatment, nine oysters each strain were collected from the 22, 26 30, 34 and 38 °C buckets to examine the effects of different heat shock temperature on the enzyme activities of four strains; (2) nine oysters per strain from the 34 °C buckets were sampled at 0, 3, 6, 12, 24, 48, 72 and 96 h to evaluate the effects of different heat shock time on the enzyme activities of four strains. Oysters were gill-clipped and the gills were instantly frozen in liquid nitrogen and kept at −80 °C. All enzyme activity measurements were carried out with 3 replicates, and the gills from three individuals were pooled as one sample for each replicate. The gills were defrosted in −30 °C and 4 °C refrigerators, followed by blotting on filter paper and weighing. Samples were homogenized with ice-cold normal saline (1:10 dilution) in an ice bath and then centrifuged (3500 rpm for 10 min at 4 °C). The supernatant liquid was gathered and the activity of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and the concentrations of total proteins (TP) were measured (<12 h) using a test kit (Nanjing Jiancheng, China) according to the manufacturer's instructions (Meng et al., 2021). Briefly, SOD activity (U mgprot^{-1}) was measured by determining the ability of the xanthine/xanthine oxidase system to produce superoxide anions using hydroxylamine (Superoxide Dismutase assay kit – WST-1 method). CAT activity (U mgprot^{-1}) was measured by assessing the decomposition percentage of H_2O_2 at 412 nm (Catalase assay kit – Visible light). The content of MDA (nmol mgprot^{-1}) was determined by thiobarbituric acid (TBA) method and spectrophotometry at 532 nm (Malondialdehyde assay kit – TBA method). The activities of SOD, CAT and MDA were normalized by using the same protein concentration.

2.5. Statistical analysis

A Cox proportional hazards model (Cox, 1972) was used to analyze the survival data with the "survival" package in R (R Core Team, 2016). The estimated survival time of each set was compared by log-rank test and survival curves were generated based on the package "ggsurvplot". Comparison of the lifetime estimates obtained from survival curves for each strain was performed with the log-rank test (Fernandes et al., 2018).

The follow formula was used to calculate the heterosis (*H*) in cumulative survival rate at 96 h (Cruz and Ibarra, 1997):

$$H = \frac{(X_{GA} + X_{AG})/2 - (X_{GG} + X_{AA})/2}{(X_{GG} + X_{AA})/2} \times 100(\%)$$

where X_{GA} and X_{AG} is the cumulative survival rate of the hybrid F1; X_{GG} and X_{AA} are the survival rate of the GG and AA lines.

To assess the effect of a 10 °C increase temperature on oxygen consumption, Q_{10} were calculated following the equation (Bayne and Newell, 1983):

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

where R_2 and R_1 are the corresponding OR at the higher and the lower temperatures, T_2 and T_1, respectively.

The ABT was determined using regression analyses of the natural logarithm of the OR against the absolute temperature (1000 / K) to generate the best-fit line on both sides of a putative break point (Stillman and Somero, 1996). The OR and temperature data were fitted using the SPSS 26.0 software. Before analysis of variance, data were log-transformed and checked for normality and homogeneity of variance. One-way analysis of variance (ANOVA) and Tukey's test were used to analyze the difference of OR and enzyme activity among different

strains. Two-way ANOVA was performed to test the effect of strain, temperature and their interaction on OR and enzyme activity parameter. *P* < 0.05 was considered statistically significant differences.

3. Results

3.1. Survival performance of oysters under thermal stress

Persistent mortality and considerable strains-specific differences were observed during 96 h heat shock (Fig. 1). Cox proportional hazards analysis showed that temperature, strain and their interaction had significant effects (*P* < 0.001) on survival. At 26 °C, two and one oysters died in GG and AA, respectively, but no death occurred in hybrids. At 34 °C, the mortality rates at 96 h in GA, AG, AA and GG were 36.67%, 41.67%, 68.33% and 83.33%, respectively. A similar difference observed at 38 °C, for which the 100% mortality occurred at 12 h in GG, at 24 h in AA, at 78 h in GA and at 66 h in AG respectively. The estimation of expected lifetime of hybrid strains were significantly longer (*P* < 0.001) than that of parental strains at 38 °C (Table 2). Moreover, hazard ratio was ranked in the following order: GA < AG < AA < GG (Table 2), indicating that the hybrid had higher level of tolerance to high temperatures. The heterosis (*H*) in cumulative survival rate increased with an increase in temperature, reached a maximum (151.72%) at 34 °C, and then decreased.

3.2. Oxygen consumption rate under acute heat shock

No mortality was seen during oxygen consumption rate measurement. Mixed model ANOVA results showed that there was a significant effect (*P* < 0.001) of strain, temperature and the interaction between strain and temperature on OR (Table 3). The OR of the four strains followed an upside-down U-shape over increased temperature, at first rising and then falling (Fig. 2). At 22 °C, GG had the lowest OR (1.37 ± 0.05 mg g^{-1} h^{-1}) among four strains (*P* < 0.05). The maximum of OR was observed at 26 °C in GG (2.30 ± 0.08 mg g^{-1} h^{-1}), whereas at 30 °C in other strains. Compared with parental strains, the two hybrid strains had a significantly higher (*P* < 0.05) level of OR under extreme high temperature (34–38 °C). Furthermore, both hybrids responded similarly to thermal shock, whereas GA (1.97 ± 0.05 mg g^{-1} h^{-1}) had a significantly higher OR than AG (1.78 ± 0.03 mg g^{-1} h^{-1}) at 38 °C.

The ABT of OR differed among four strains (Fig. 3). GA had the highest average ABT (30.48 ± 0.18), followed by AG (30.24 ± 0.05), AA were intermediate (29.64 ± 0.15) and GG (26.51 ± 0.13) was markedly inferior (*P* < 0.05). Linear regression equations of the Arrhenius plot for OR displayed a remarkable variation in slopes, in which hybrid strains were significantly lower than parental strains (*P* < 0.05). Overall Q_{10} values among four strains decreased with the increase of temperature (Fig. 4). Specifically, the Q_{10} value of GG was lower than 1.00 between 26 and 30 °C. In contrast, slightly increased or remained unchanged Q_{10} values were observed in GA and AG in the range of 26–30 °C, indicating the stable metabolic capacity of hybrids under heat stress. Maximum Q_{10} values showed a different trend among four strains in that GA had the Q_{10} maxima (1.99 ± 0.17) between 26 and 30 °C while GG (3.68 ± 0.64) and AA (2.91 ± 0.56) had the Q_{10} maxima between 22 and 26 °C. In addition, the Q_{10} value in GG (0.36–3.68) varied obviously throughout the experiment, whereas Q_{10} value in GA (0.68–1.99) and AG (0.56–1.66) fluctuated within a relatively narrow range.

3.3. Antioxidant enzyme activity

3.3.1. Effects of different temperature on enzyme activity

The SOD activity of four strains tended to increase with temperature escalation (Fig. 5A). Nevertheless, the maximum SOD activity (245.37 U mgprot^{-1}) in GG was observed at 34 °C, followed by a quick decline at 38 °C. Further, GG was significantly lower (*P* < 0.05) than the other strains at 38 °C. Using the two-way ANOVA model, SOD level was
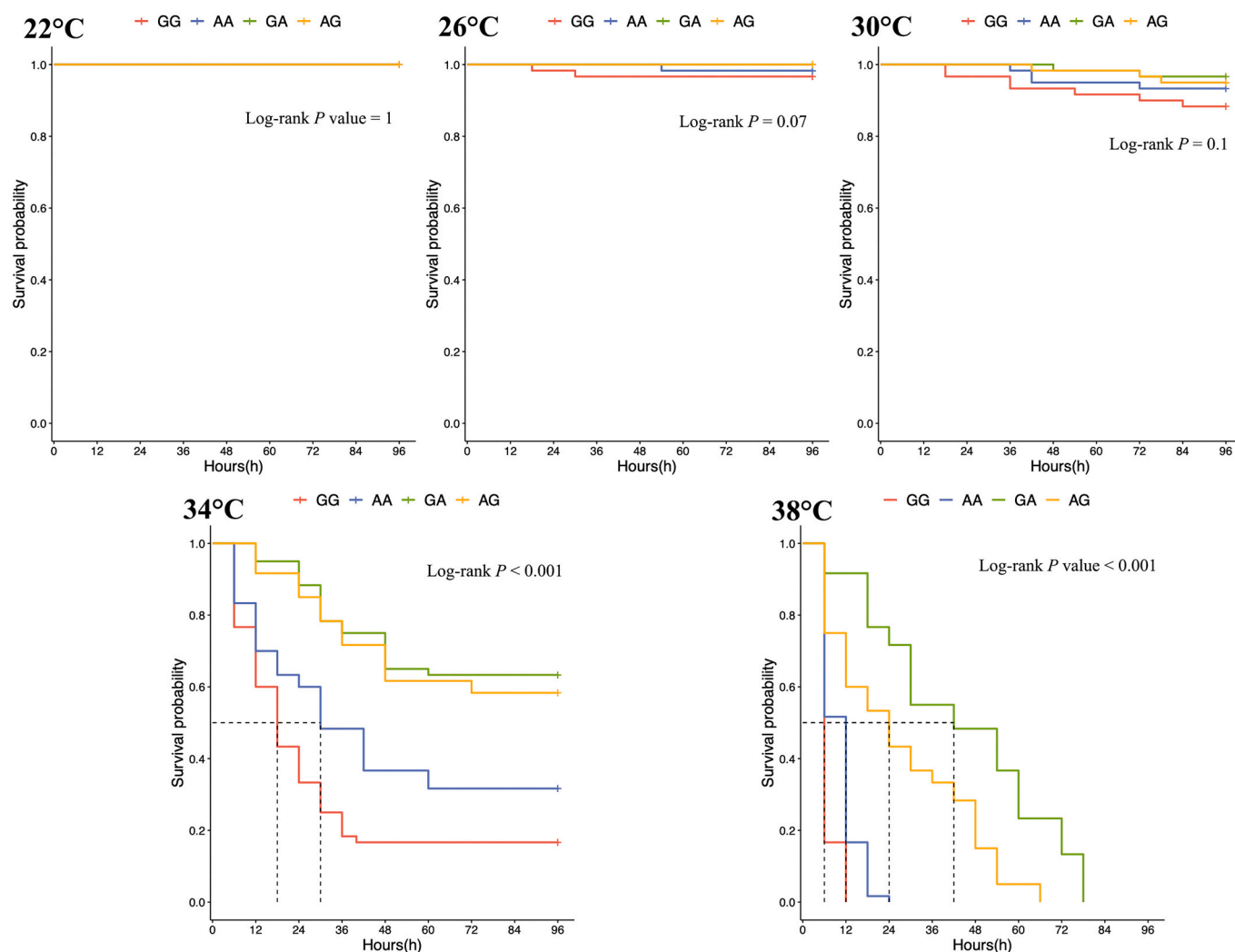
Fig. 1. Survival as a function of time of four strains GG, AA, GA and AG under different heat shock temperature levels (22, 26, 30, 34 and 38 °C).

Table 2
Cox proportional hazard model stratified by heat stress and estimation of expected lifetime (h) for four strains on 38 °C levels.

| Strains	Coefficient estimate	Hazard ratio (95% CI)	Estimation of expected lifetime
GG	0.563***	1.756 (1.606–1.921)	6 (6–6) a
AA	0.549***	1.732 (1.574–1.905)	12 (6–12) b
GA	0.482***	1.619 (1.471–1.783)	42 (30–54) c
AG	0.484***	1.622 (1.482–1.776)	24 (18–48) d

*** Strains were significantly correlated with survival time ($P < 0.001$); different lowercase letters in the same line indicating significantly different according to the log-rank test ($P < 0.001$).

strongly affected by strains, temperature and their interaction ($P < 0.001$; $P < 0.001$; $P < 0.01$, respectively; Table 3). Likewise, the CAT activity in GG and AA increased gradually as temperature elevated, reaching the maximum (143.52 U mgprot^{-1} and 154.62 U mgprot^{-1}, respectively) at 30 °C, and declined afterwards; in contrast, the CAT activities in GA and AG continued to rise with increasing temperature, and was significantly higher than the parental strains at 34 and 38 °C (Fig. 5B). Two-way ANOVA revealed clear effects of strain ($P < 0.001$), temperature ($P < 0.01$) and strain × temperature interaction ($P < 0.001$) on the CAT activity levels (Table 3). A dramatic increase in MDA content for GG (19.02–36.85 nmol mgprot^{-1}) and AA (18.34–33.89 nmol mgprot^{-1}) was found over temperature, while no obvious changes in MDA content between both GA and AG up to 34 °C (Fig. 5C). Strikingly, MDA levels in GA was significantly lower ($P < 0.05$) than the parental

Table 3
Two-way analyses of variance testing for temperature by strain interaction effects on oxygen consumption rate (OR) and antioxidant enzyme activity (SOD, CAT and MDA).

Effect	OR			SOD			CAT			MDA		
	d.f.	MS	F-value	d.f.	MS	F-value	d.f.	MS	F-value	d.f.	MS	F-value
Strain	3	1.163	224.697***	3	2708.172	14.666***	3	6545.266	26.081***	3	169.290	14.798***
Temperature	4	4.863	939.547***	4	43,258.881	234.266***	4	1531.912	6.104**	4	240.351	21.010***
Strain × Temperature	12	0.526	101.594***	12	2980.099	16.139**	12	2027.384	8.079***	12	17.965	1.570^{NS}
Error	140	0.005	–	40	184.657	–	40	250.955		40	11.440	

The *P*-value associated with each *F*-value are indicated by asterisks (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; NS – not significant).
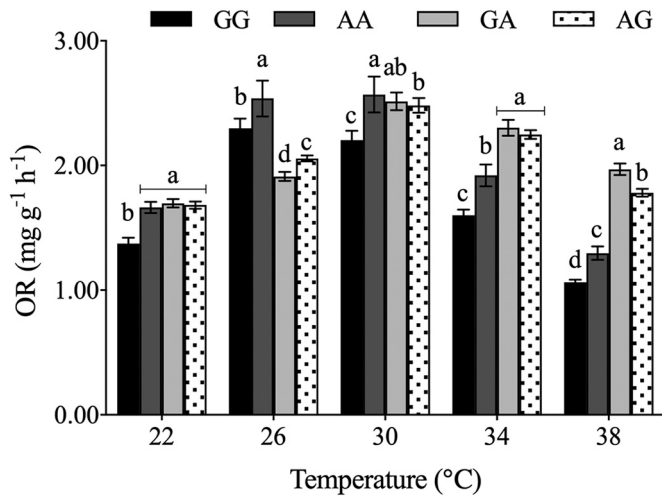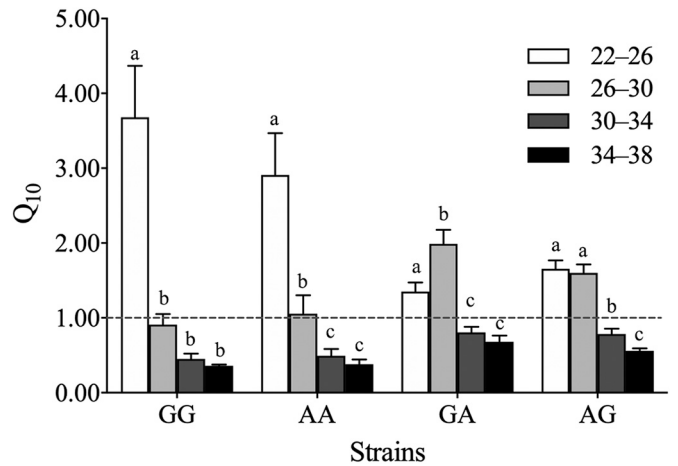
Fig. 2. Oxygen consumption rate (OR) of four strains under different heat shock temperature levels (22, 26, 30, 34 and 38 °C). Different lowercase letters denote significant difference between strains within given temperature (P < 0.05).

strains at 34 and 38 °C. Mixed model ANOVA results indicated significant effects (P < 0.001) of strains and temperature on MDA concentration (Table 3; Fig. 5C).

3.3.2. Effects of differing heat shock times on enzyme activity

SOD activity of the four strains were dramatically increased in the first 6 h followed by a slight declined at 12 h; subsequently, it showed a steady upward trend and remained high between 24 and 96 h (Fig. 6A). After exposure at 34 °C for 3 h, 72 h and 96 h, the levels of SOD were

significantly higher (P < 0.05) in GA and AG than in GG. In contrast, the activity of CAT in AA, GA and AG strains showed a trend of increasing first and then decreasing from 0 h to 6 h and 6 h to 96 h, respectively (Fig. 6B). Mean CAT activity during heat stress was significantly higher (P < 0.05) in GA or AG than in GG except at 9 h when mean activity among strains did not differ (P > 0.05). The activity of MDA among strains presented a trend of increasing during the early phase and then returned to the initial value. Overall, the MDA content of the hybrids did not change much regardless of time, with significantly lower levels at 6–12 h compared with GG. Two-way ANOVA demonstrated that both strain, temperature and their interactions significantly affected the

Fig. 4. Q_{10} coefficients of four strains calculated from different temperature ranges. The dotted line represents the value of Q_{10} as 1.
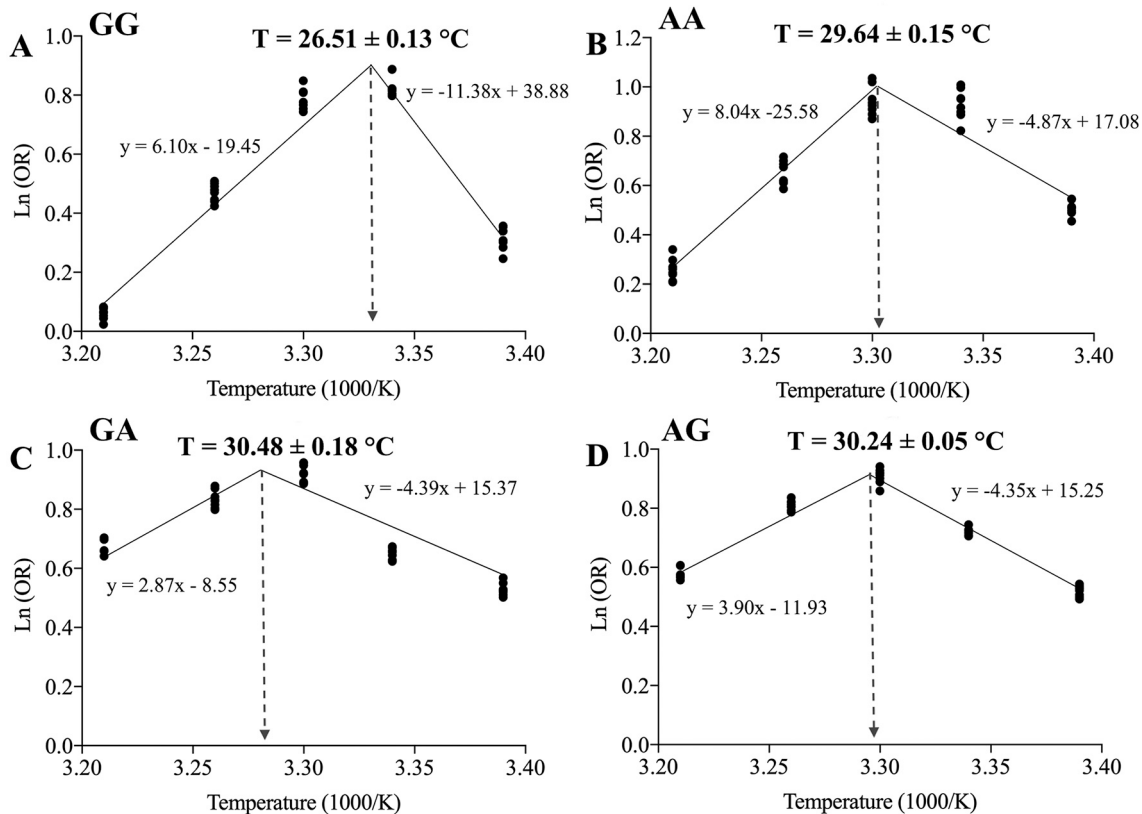
Fig. 3. Arrhenius break-point temperatures (ABT) (vertical arrows) of oxygen consumption rate (OR) for representative individuals of four strains (mean ± standard deviation). Trend lines represent linear regressions. Linear fitting equations for regression lines are plotted.
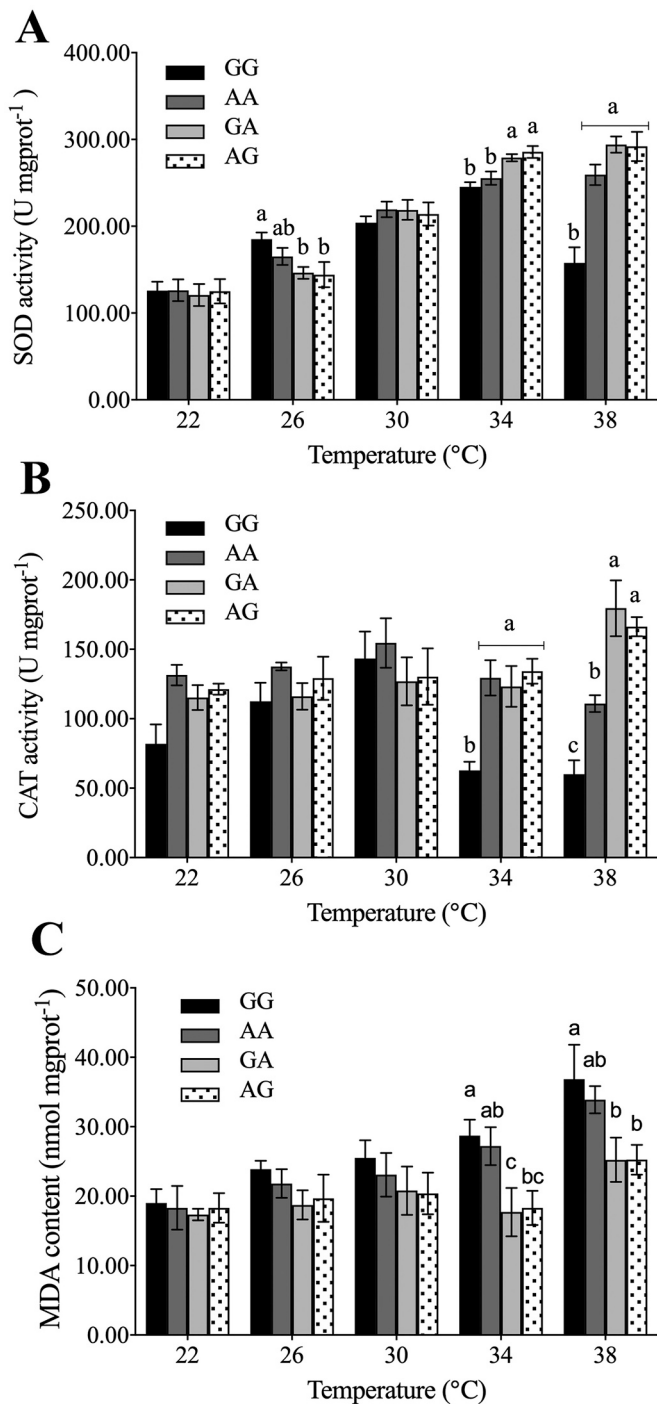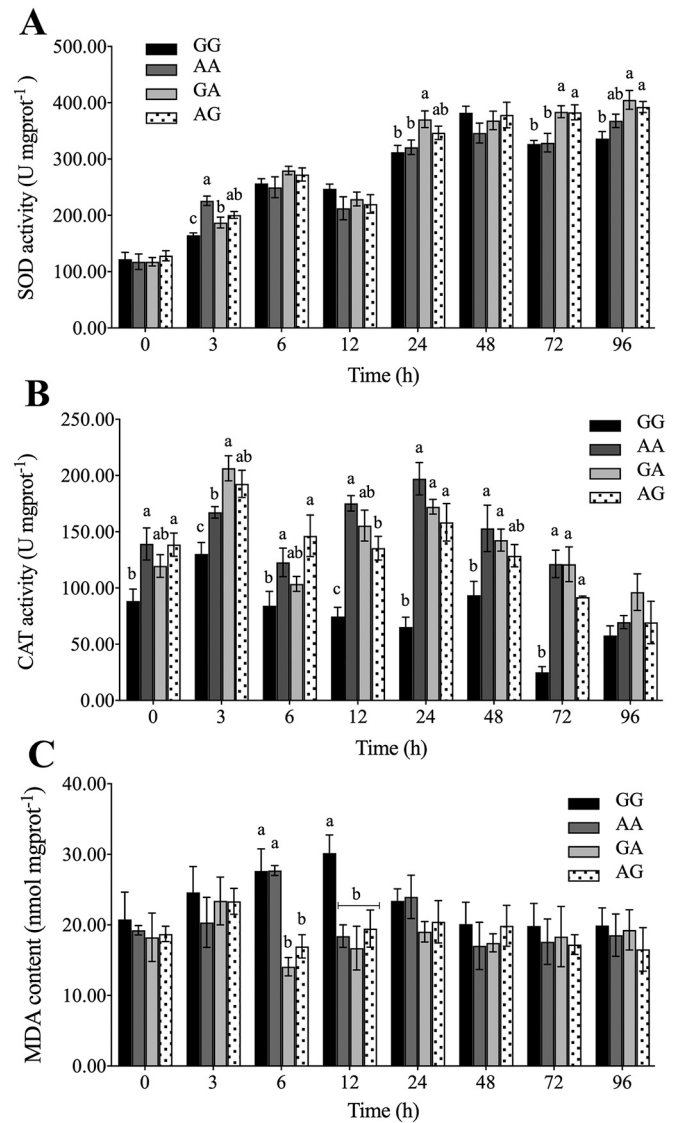
Fig. 5. Changes in enzyme activities of four strains exposed to different heat shock temperature levels (22, 26, 30, 34 and 38 °C) for 6 h. Different lowercase letters denote significant difference between strains within given temperature ($P < 0.05$).

Fig. 6. Changes in enzyme activities of four strains exposed to different heat shock time levels (0, 3, 6, 12, 24, 48, 72 and 96 h) at 34 °C. Different lowercase letters denote significant difference between strains within given time ($P < 0.05$).

activity or content of the three enzymes ($P < 0.001$ or $P < 0.01$; Table 4).

4. Discussion

This study was performed to assess the survival, physiological parameters and immune responses of hybrid oyster under acute heat shock, with an aim of explaining their survival advantage compared to the parental species. We found that the hybrid strains showed larger tolerance to thermal stress since they exhibited higher survival rate and

more stable aerobic metabolism when exposed to high temperatures conditions. Meanwhile, the hybrid strains showed enhanced antioxidant capacity and less oxidative damage at extremely temperature.

4.1. Comparison of mortalities under heat stress among cohorts

C. gigas from the coast of northern China is recurrently exposed to massive mortality events. It has been estimated that >60% of *C. gigas* farmed in Shandong were lost in the summer of 2019, when seawater temperatures exceeded 25 °C (Yang et al., 2021). Nonetheless, the survival rate of hybrid oysters was >80% during warmer months (Jiang et al., 2021b). This resistance to high water temperature was supported in the current research, since an increase in temperature from 22 °C to 34 °C leads to an obvious increase in survival heterosis. The Cox proportional hazards analysis showed that the hazard ratio of hybrids was lower compared to parental species under heat stress. This result was consistent with the estimation of expected lifetime, indicating that hybrids were more vigorous to rising temperature than their parental species. Previous studies also found improved environmental resistance

Table 4

Two-way analyses of variance testing for time by strain interaction effects on antioxidant enzyme activity (SOD, CAT and MDA).

Effect	d.f.	SOD		CAT		MDA	
		MS	F-value	MS	F-value	MS	F-value
Strain	3	3763.696	14.879***	22,880.284	107.173***	116.712	10.466***
Time	7	105,404.950	416.692***	12,162.746	56.971***	38.508	3.453**
Strain × Time	21	1174.850	4.644***	1276.798	5.981***	27.963	2.507**
Error	64	252.956		213.488		11.152	

The P-value associated with each F-value are indicated by asterisks (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; NS – not significant).

in aquatic hybrids, such as *Ictalurus punctatus × I. furcatus* with low-oxygen tolerance (Dunham et al., 2014), *Pomacea canaliculata × P. maculate* with low-temperature tolerance (Matsukura et al., 2016) and *Haliotis rubra × H. laevigata* with heat tolerance (Alter et al., 2017). Besides, the Cox proportional hazards analysis revealed that strain, temperature and their interaction had significant influence on cumulative survival rate, indicating the close dependence of strain and temperature on oyster survival. This genotype-environment interaction was associated with the biogeographical distribution of parental lines (Dunham et al., 2014), which may affect the heat tolerance of hybrids (Dahlhoff and Somero, 1993). As the main *C. gigas* producing area in China, Shandong Province has an average seawater temperature of 2–26 °C; in contrast, *C. angulata* is a local species in Fujian Province where temperatures are higher, ranging from 17 °C to 30 °C (Jiang et al., 2021b). Consequently, the high heat resistance of hybrid oysters may be inherited from one of its parents, the warm-adapted *C. angulata* (Alter et al., 2017). In a similar case in abalone, Liang et al. (2014) reported *H. discus hannai × H. gigantea* successfully inherited the high-temperature resistance of *H. gigantea*. However, in this study, GA was more tolerant of high temperature compared to AG, which may suggest that the maternal effect was small and the paternal effect was large. Similarly, Šimková et al. (2015) found that the hybrids with common carp in the paternal position exhibited greater viability than the hybrids with common carp in the maternal position.

4.2. Change of oxygen consumption in response to heat stress

For several marine animals such as largemouth bass (Díaz et al., 2007), small clam (Jansen et al., 2007) and Yesso scallop (Jiang et al., 2016), findings showed that metabolic rate was positively associated with seawater temperature. However, a few studies have reported a negative relationship between metabolic rate and environment temperature in some freshwater animals, including limpets (McMahon, 1973) and sculpins (Walsh et al., 1997). In this study, the OR of the four oyster strains first rose and then fell with rising temperature. Upregulated respiration rate was thought to be associated with increased digestion rate and protein synthesis rate (Jiang et al., 2016), but also indicated the activation energy needed for enzyme catalytic reaction was lower at this moment (Hochachka and Somero, 2002). Meanwhile, the decrease of OR may be a protection mechanism against excessive metabolism under high-temperature conditions (Jansen et al., 2007). Animals possessing the capacity to reduce total metabolism was thus thought to be likely to deal better with extreme temperature than animals without such capacity (Liao et al., 2021). In contrast, other studies concluded that species that could maintain their aerobic capacity at higher temperature have a better heat resistance than species that experience a decline in aerobic performance as temperature rises (Ding et al., 2020; Ghaffari et al., 2019). This may be due to the different reaction norms of animals under short-term and long-term stress, allowing them to survive better under different acclimation conditions (Glanville and Seebacher, 2006). The results in this study were consistent with the latter view, as the hybrids with higher survival rates exhibited higher OR at 30–38 °C. Further, GA had higher metabolic rate compared with AG at 38 °C, indicating stronger aerobic capacity in GA under extreme heat.

In our study, the ABTs were 26.51 °C and 29.64 °C for GG and AA

strain, respectively. One worry is that the ABT measured for GG in this research is close to seawater temperature (26 °C) during summer in Shandong Province, rendering *C. gigas* vulnerable to temperature rise caused by climate warming. It is remarkable that the ABTs of GA and AG were 3.98 °C and 3.73 °C higher than that of GG, respectively ($P < 0.05$). The higher value of ABTs in hybrids (and AA) suggested that they could maintained normal aerobic respiration at higher temperature and therefore had strong thermal adaptability compared to GG. Likewise, a comparative study of thermal tolerance in porcelain crabs have shown a better adaptability in *Petrolisthes cinctipes* than in *P. eriomerus* (Stillman and Somero, 1996). Contrary to our results, however, Ghaffari et al. (2019) reported relatively higher ABT of oxygen consumption rates in *C. gigas* (29.22 °C) and *C. angulata* (33.09 °C). This is not surprising as ABT is influenced by several factors, such as sizes, weights, ages and spawning performance of individuals, as well as measuring method (Xing et al., 2021). Also, as Stenseng et al. (2005) found that the ABT of congeneric marine snails increased after warm acclimation, it is reasonable to presume that low-temperature acclimation during winter in north China resulted in a decrease in ABT of the oysters used in this study.

Compared with GG (3.47), the hybrids had a lower Q_{10} value between 22 °C and 26 °C (GA: 1.25; AG: 1.66). A lower value of Q_{10} over a range of temperatures indicated that the organism's respiration was not responsive to temperature rise (Nie et al., 2017). The insensitivity of hybrids to environmental variables may contribute to less metabolic expense and enhanced environmental adaptability (Alter et al., 2017). Moreover, as observed previously in American oyster (Dame, 1972), the Q_{10} of parental strains decreased significantly with temperature, suggesting a reduced metabolic rate for energy balance (Jansen et al., 2007). In contrast, the Q_{10} of GA rose from 1.35 between 22 and 26 °C to 1.99 between 26 and 30 °C and then decline. The recorded values (∼ 2) of Q_{10} were similar to the results reported in Manila clam, and may be indicative of a superior adaptability of hybrid to higher temperatures (Nie et al., 2017). This is supported by the research of Kita et al. (1996), which indicated that the temperature at which Q_{10} for OR starts to decrease as temperature is the optimum range for growth. Furthermore, the temperature ranges of GG, GA and AG that Q_{10} value below 1 were 26–38 °C, 30–38 °C and 30–38 °C, respectively. Several studies revealed that the Q_{10} value below 1 happen when the ambient temperature exceeds the breakpoint temperature (Pörtner, 2001; Casas et al., 2018).

4.3. Non-specific immune response under shock stress

In this study, the activities of antioxidant enzyme were strongly influenced by strain, temperature and thermal stress time. Specifically, the activities of SOD and CAT in GG and AA increased at first, but then decreased with increasing temperature or time. The increased activity of antioxidase indicated the protective mechanism of organism for reducing oxidative damage under heat stress (Ding et al., 2020). Meanwhile, extreme high-temperature (Rahman and Rahman, 2021) or long-term heat exposure (Jiang et al., 2016) could severely damage antioxidant system and cause a decrease in oxidase activity. It seems that the change of SOD activity under heat shock is relatively similar for diverse species, showing a pattern of "increase first and then fall" (Liang et al., 2014). Nonetheless, the SOD activity of hybrid strains increased

steadily over temperature or time (12–96 h), which differs from this pattern. Moreover, the increasing activity of SOD with exposure to stress over time was also found in muscle tissue of goldfish (Lushchak and Bagnyukova, 2006), indicating that no switch from aerobic to anaerobic metabolism occurred in the organism (Ding et al., 2020). Apart from that, there were other prominent performances of hybrids may be related to their survival advantage under heat shock: (1) GA and AG gained significantly higher ($P < 0.05$) activities in SOD and CAT than GG at extremely high temperature (34 and 38 °C, Fig. 5). (2) SOD and CAT activities of GA and AG were significantly higher than those of GG at 3 h (34 °C, Fig. 6). Accordingly, these two characteristics of hybrids may mean earlier response time and higher response intensity of antioxidant system at extreme temperatures, implying an enhanced capacity to maintain cellular functions of the antioxidative system during thermal stress (Rahman and Rahman, 2021; Dagoudo et al., 2021).

Another striking finding in this research was that the hybrids had lower level (Fig. 5) and less variation (Fig. 6) in MDA content at 34 and 38 °C compared to the parental strains. MDA is a lipid peroxidation product of polyunsaturated fatty acids in biofilms and represents the degree of biological oxidative damage (Qiang et al., 2018). Increased MDA content have been reported in whiteleg shrimp (Duan et al., 2018), Yesso scallop (Jiang et al., 2016) and red swamp crayfish (Guo et al., 2020) in response to short-term heat stress. Notably, Tan et al. (2020) found there was a significant negative correlation ($r2 = -0.66$, $P < 0.05$) between MDA and SOD in brown scallops, a low-survival population compared to the golden scallops. Thus, the lower MDA content of hybrids indicated less oxidative damage, which corresponded with their high survival performance under thermal stress.

5. Conclusions

This study demonstrated that the hybrids showed higher cumulative survival rate under thermal stress compared with their parental species, *C. gigas* and *C. angulata*. The significant heterosis of survival rate suggested that crossbreeding improved the thermal tolerance of oysters remarkably. Meanwhile, the hybrids exhibited broader temperature range of aerobic metabolism and lower sensitivity to temperature rise, which contribute to the stable and great aerobic capacity of hybrids under high temperature environment. Further, compared with the parental strains, the antioxidant system (SOD and CAT) of hybrids exhibited a more rapid and intensive response. Also, the hybrids maintained lower levels of MDA content, indicating less oxidative damage. These findings can facilitate our understanding of the physiological and immune mechanisms underlying heterosis in heat resistance of hybrids and provide a new viewpoint for the future breeding of thermo-resistant oyster.

CRediT authorship contribution statement

Gaowei Jiang: Investigation, Conceptualization, Formal analysis, Writing – original draft. **Jianmin Zhou:** Investigation. **Geng Cheng:** Investigation. **Lingxin Meng:** Investigation. **Yong Chi:** Investigation. **Chengxun Xu:** Supervision. **Qi Li:** Supervision, Conceptualization, Resources, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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