Disease and thermal tolerance ability biology essay

Science, Biology

Authors: Shuang Liang a, b, Xuan Luo a, b, Weiwei You a, b, Lianzhong Luo a, b, Caihuan Ke a, b,*a College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, Chinab State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China* Corresponding author. Department of Marine Technology and Ocean Engineering, College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China. Tel./fax: +86 592 2187420. E-mail address: chke@xmu. edu. cn (C. Ke). AbstractAbalone is a kind of high valued commercially molluscs which is cultured worldwide. Recently, mortality outbreaks related with severe temperature had happened frequently in abalone aquaculture, drawing our attention to the stress tolerance of abalone to extreme environments. It is widely known that hybridization is an effective way of genetic improvement in marine aquaculture, which could bring lots of benefits to the offspring, including growth rate, survival rate, thermal tolerance, disease resistance and so on. Interspecific hybrids between Haliotis discus hannai and Haliotis gigantean were produced previously. In this study, we compared the effects of low temperature (12℃) and high temperature (28℃) on the immune responses and thermal induced responses in the 4 groups (two interbreeding populations and two inbreeding populations), total haemocyte count (THC), respiratory burst, superoxide dismutase (SOD), acid phosphatase (ACP), alkaline phosphatase (AKP), myeloperoxidase (MPO) and HSP70 expression were determined on the day 1 and day 7 during the exposure to three different temperatures (12℃, 20℃, 28℃). THC was elevated during exposure to 28℃ in 4 groups on day 1 and day 7, while increased THC was also observed at 12℃ on day 7 in DD and

DG. Respiratory burst and SOD did not vary between groups, under 28℃, respiratory burst was significantly elevated during all days while SOD first rose then fell during 7-days exposure. AKP activity was elevated by 12℃ and 28℃ on day 1 and then obtained a recovery after 7 days, with a notable high level in DG. ACP activity was relatively steady under temperature stress, except for an increased high level in DG after 7 days exposure to 28℃. MPO activity was suppressed by both 12℃ and 28℃ exposure on day 1 and recovered on day 7. HSP70 expression in all the 4 groups could be up-regulated by 28℃ on day 1, while a significant decrease was observed in DD on day 7, the up-regulation of HSP70 by low temperature was also observed in this study. Overall, this study suggest that immune responses and HSP70 expression in abalone could be significantly influenced by temperature change and hybridization, these data indicate that interbreeding groups may perform better in disease and thermal resistance than inbreeding groups.

Keywords: Hybridization Abalone Immune response HSP701.

IntroductionAbalone, which belongs to genus Haliotis and family Haliotidae, is cultured worldwide because of its high commercial value [1, 2]. The rapid growth of abalone aquaculture industry has happened in recent years to cater for the decreasing of wild stocks and the increasing demands of abalone supply, nearly 45000 metric tons of abalones went into market in 2008, in which China has played an important role [3, 4]. The aquaculture of abalone in China started in the late 1980s and had been developed fastly since then [1, 5-7], till 2009, the annual output of China abalone aquaculture industry was approximately 23000 metric tons (From 2008 to 2009) [4]. Recently, however, mortality outbreaks caused by high temperature or

bacterial infection in abalone had occurred frequently, shown as seriously death of juvenile and adult abalones in both northern and southern provinces in China, which had brought heavy losses to China abalone market [1, 5-9]. Hybridization, intraspecies and interspecies, is an effective way of genetic improvement, which is widely used in aquaculture and fisheries in many kinds of fish and shellfish, including abalone, oyster, scallop, carp, catfish, salmonid, sparid, sunfish and so on [1, 2, 10-12]. Advances in growth and survival rate, thermal tolerance, disease resistance and other market-favored traits are usually obtained through hybridization, which is supported by heterosis theory (The offspring produced through hybridization either gain better phenotype traits than both parental groups, or perform on the average between their parents) [2, 10, 11]. Lots of researches have proved heterosis in stress (temperature and disease) tolerance in the hybrids. The " Pacific" scallop, offspring of the native weathervane scallop in Canada and introduced Japanese scallop, shows advantage in growth rate and disease resistance [12]. Two strains of oysters, MSX/Dermo-resistant strain and JOD-resistant are obtained after several generations of selective breeding, characterized by strong resistance to multinucleated sphere X (MSX) disease and Dermo disease for the former, strong resistance to juvenile oyster (JOD) disease and the ability of fast growing for the latter, separately, hybrids between the two strains are both Dermo-resistant and fast growing [13]. Hybridization between the white bass (Morone chrysops Rafinesque) ♀ and the striped bass (M. saxatilis Walbaum) ♂ created the sunshine bass, which has several advantages over both parents such as rapid growth rate, high survival rate, high temperature tolerance, disease resistant and so on .

Similar phenomenon also exists in abalone. The hybrid, H. discus hannai ♀ × H. discus discus ♂, is superior in weight increase and shell growth at both 20℃ and 25-28℃, than its parents, showing higher adaptability to high temperature. Stronger tolerance of low temperature is found in the hybrid, H. discus hannai ♀ × H. kamtschatkana ♂, which gains higher growth rate in both 8℃ and 18℃ than parents [14]. HSP70 expression among four stocks of H. discus hannai, two inbred groups and two intraspecies groups, indicates that the hybrids have higher thermal temperature limit than the inbreds [15]. Overall, hybridization is an useful tool in aquaculture and fisheries, it's meaningful to study the interaction between hybridization and stress resistance. Water temperature is an important environment factor which is always related to disease outbreaks in aquaculture and fisheries [16-21]. The happening of diseases in halobios usually depends on two factors: The efficiency of host's immune response system and the ability of the pathogenic bacteria's invasion into the host's immune systems [17], which could both be influenced by water temperature [16]. Lots of researches have been done to determine the effects of temperature on immune parameters of halobios. For instance, green-lipped mussel, Prena viridis shows lower level in esterase, reactive oxygen species, lysosome content and phagocytosis under high temperatures [22]. In hard calm, Mercenaria mercenaria, cellular and humoral immune parameters such as total heamocyte count (THC), reactive oxygen species (ROS), phagocytosis, lysozyme exhibit significant variations according to different temperature [17]. Monthly change in immune responses of the European abalone, Haliotis tuberculata, was measured from early to late summer (June to September),

reduction of phagocytosis, phenoloxidase and increase of basal reactive oxygen species production, agglutination titres were determined [19]. Similar situation also happens in Haliotis rubra and Haliotis diversicolor, in which the increase of temperature could elevate the level of THC, Superoxide anion level (SO), susceptibility to infection by Vibrio parahaemolyticus or Vibrio harveyi, or antiviral and antibacterial ability [16, 23]. All these studies have greatly improved our knowledge of the relationships between temperature stresses and the living organisms' immune states, providing us with possible strategies to deal with frequently-happened summer diseases in aquaculture and marine fisheries. However, to the best of our known, few studies have been done focusing on the combined effects of hybridization and temperature on stress (disease and thermal) defenses in halobios, which requires further study. Haliotis discus hannai (DD) is an important commercial species in China since late 1980s [24], whose optimal temperature is 15~22℃ [25]. Haliotis gigantea (GG), also called Haliotis sieboldii, which was introduced from Japan into China in 2003, is a kind of warm-water species [25], with its crisp and tender meat, and its excellent disease resistance, this species have become an commercial species in China [25, 26], their offspring through interspecies hybridation: H. discus hannai ♀ × H. gigantean ♂ (DG) and H. gigantean ♀ × H. discus hannai ♂ (GD), were obtained in 2006 [2, 24]. Based on former jobs conducted by other researchers about heterosis in halobios□we hypothesized that the hybrids' resistances to temperature and immune system efficiencies are elevated through hybridization. Therefore, two reciprocal crosses and two parental groups described below were used as

research objects, which were exposed under different temperatures for 7 days, during which two cellular immune parameters: total haemocyte count (THC) and respiratory burst, together with four immune enzymes activities: superoxide dismutase (SOD), acid and alkaline phosphatase (ACP and AKP), myeloperoxidase (MPO) were examined to determine the immune system efficiencies in abalones, and heat shock proteins 70 (HSP70) expression was examined to evaluate the ability of thermal resistance in abalones. Through these experiments, we expected to get knowledge of the differences in the disease and thermal tolerance abilities between the parental populations and their hybridization offspring, thus to provide guidance for the practical application of heterosis in aquaculture and fisheries, and gives insight into the performance of abalones under changing temperature caused by realistic seasonal variations or other practical factors, such as daily water change in abalone factories or transportation of abalones for commercial trade. 2. Materials and methods2. 1. Abalone acclimation. Two interbreeding groups: H. discus hannai ♀ × H. gigantean ♂ (DG) and H. gigantean ♀ × H. discus hannai ♂ (GD) (crossings between 2 parental populations), and two inbreeding groups D ♀ × D ♂ (DD) and G ♀ × G ♂ (GG) (crossings within each parental population) were reared in equal standard abalone cages (12 abalones per cage) placed in the culture ponds in Zhangpu Hongyun Abalone Company. For acclimation, 24 cages of live adult abalones (60±10mm in shell length, 6 cages for each population) in good health were equally transferred into three 500L PVC tanks provided with aeration seawater, every tank contained 24 abalones for each population. The culture conditions were set at a temperature of 20 ± 1℃, salinity of 33 ‰ and pH of 7. 8 for 14

days before experiment. Abalones were fed with asparagus and the seawater was changed every 24h. Parameters of the seawater and the survival rate of the abalones were examined half a day. 2. 2. Temperature treatmentAfter a 2-weeks acclimation, one tank was kept at 20℃ (control group), the other two tanks were separately elevated to 28℃ using 1000 W titanium heater (Weinuo, China) or cooled down to 12℃ using cooling-water machine (Haili, China) at the rate of 2℃ per day (temperature-challenged groups), and then kept for 7 days at the three temperatures using electronic thermostat (Jingchuang, China). 2. 3. Haemolymph and muscle collectionFor each experimental condition, 6 individuals of each population (3 from each cage) were sampled and immediately anatomized. 3ml haemolymph of each abalone was collected from the epipodium using scalpel and eppendorf pipettor, and then divided into two equal parts, one part was flash-freezed by liquid nitrogen for immune enzyme activities determination, and the other was transferred into pre-cooled clean tube on ice for total haemocyte count (THC) and respiratory burst activity determination. About 1g foot muscle was collected from the eqipodium using scissors and then stored in -80℃ after liquid nitrogen flash-freezing for gene expression analysis. 2. 4. Total haemocyte count (THC)A sample of 50ul heamolymph of each individual was fully mixed with an equal volume of anticoagulant MASⅡ (glucose 20. 8 g/L, sodium citrate 8 g/L, EDTA 3. 36 g/L, NaCl, 45 g/L. Stored in 4℃) to avoid heamocytes from agglutination, according to previous study with some modifications [27]. For heamocyte count, a sample of 10ul mixture was added to a Neubauer hemacytometer (Jingchuang, China) and counted under a microscope under 40 × magnifications (Olympus, Japan). Three replicates

were counted for each sample and the results were converted as number of cells ml-1 haemolymph. 2. 5. Respiratory burstRespiratory burst activity of heamocytes was measured using the reduction of nitroblue tetrazolium (NBT) to formazan as previously described with some modifications [28]. Briefly, 100ul haemolymph in MASII was deposited in triplicate in 96-well micro plate, and then fully mixed with 10ul sodium alginate (0. 2 mg ml 1 in MASII) as stimulator, then mixed with 100ul NBT (0. 3%), 100ul MASII without haemolymph was used as control, after 120min reaction at 30℃, the supernatant was carefully removed and the pellet was resuspended in 100ul 100% methanol, then washed 3 times in 100ul 70% methanol and air-dried. 120ul of 2M KOH and 140ul of DMSO were added to dissolve the fromazan, the optical density was read at 630nm with a Bio-rad 680XR micro plate reader. The results were expressed as OD 630nm/hr. 2. 6. Immune enzymes activitiesThe assay of superoxide dismutase (SOD) activity was performed using a commercialized kit (Nanjing Jiancheng, China), according to the method previously described [29] with some modifications. Briefly, xanthine-xanthine oxidase assay was carried out in a 96-well micro plate in triplicate, and the activity of SOD (1 unit) was defined as the quantity of enzyme that inhibits the reduction of cytochrome by 50%. Acid phosphatase and alkaline phosphatase (ACP and AKP) activity were determined as previously described [30] using a kit (Nanjing Jiancheng, China) in triplicate. The optical density of phenol at 520nm after incubation was used to determine the ACP and AKP activity. Myeloperoxidase (MPO) activity was assayed according to previous study [31] using a kit (Nanjing Jiancheng, China). The assay was conducted in a 96-well micro plate in triplicate, the activity of MPO was

determined by measuring the H2O2-dependent oxidation of 3, 3', 5, 5'-tetramethylbenzidine at 650 nm. 2. 7. RNA exaction and cDNA synthesisTotal RNA was exacted from about 50mg foot muscle (Stored in -80℃) using TRIpure reagent (Invitrogen, USA) in a RNase-Free environment to prevent RNA from degrading, and then cDNA was synthesized following the instruction of PrimeScript RT reagent Kit With gDNA Eraser (Takara, Japan). The product (20ul) was stored in -80℃ for use. 2. 8. Real-time PCRPaired degenerate primers for HSP70 (table 1), according to previous studies [32] were used to amplify the HSP70 gene fragments in four abalone groups. Based on the fragments obtained (gene fragment sequence is almost the same in four groups), paired real-time PCR primers, rtHSP70F , rtHSP70R (table 1) were designed using Beacon Designer 7, and β-actin was used as an internal control as described previously [33]. Experiment was carried out in a 7500 fast qPCR system (ABI, USA). The reaction system contained 1ul of cDNA (10-times diluted), 1ul of each primer (10 pmol/L) and 10ul of 2×DyNAmo ColorFlash Master Mix (Thermo, USA), and the cycling parameters used were as follows: 95℃ for 7 min, 35 cycles at 95℃ for 20s and 60℃ for 1min, the fluorescent signal intensities were recorded at the end of each cycle. Melting curve analysis was performed from 60 to 95℃ with continuous fluorescence reading every 0. 5℃ increment to ensure the oneness of the amplification. The relative mRNA level of HSP70 was calculated based on the Ct values of this gene and β-actin normalized to that of the cDNA standard. 2. 9. Statistical analysis. Results were expressed as means ± SD. Analysis of all data was performed using SPSS. The effects of temperature, groups, exposure length and their interactions on immune

parameters or HSP70 were analyzed using three-way analysis of variance (ANOVA); At each sampling time point separately (day 1 or day 7), two-way ANOVA followed by LSD and S-N-K post hoc multiple comparisons were performed to analyze the differences between the effects of temperature or groups on immune parameters or HSP70, while pair-wise tests were performed to determine the differences between sampling time points (day 1 and day 7) in the same group under the same temperature. 3. ResultsNo mortality was observed during the 7 days exposure. For cellular immune factors, three-way ANOVA analysis revealed significant effects of temperature, groups and exposure length on THC (Table 2), while no significant effects of both temperature × groups (T × G) and temperature × length (T × L) interaction were determined (Table 2). The LSD post-hoc test showed significant increase of THC in abalones under 28℃ compared with those under 12℃ and 20℃ both on day 1 (28 vs 12 ℃, $p < 0.001$; 28 vs 20 ℃, $p < 0.05$) and day 7 (28 vs 12 ℃, $p < 0.001$; 28 vs 20 ℃, $p < 0.01$), while S-N-K homogeneous analysis showed that THC in DD and GG were significant lower than DG and GD on day 1 (DD and GG belong to homogeneous subset 1, DG and GD belong to subset 2 and 3 separately) and day 7 (DD and GG belong to subset 1, DG and GD belong to subset 2) (fig. 1A). Pair-wise tests revealed that THC in DD and DG increased significantly during 7 days under 12℃ and 28℃ ($p < 0.01$) (fig. 1A). As to respiratory burst, three-way ANOVA analysis demonstrated significant effects of temperature, exposure length and their interaction (T × L) on respiratory burst, which was not significantly affected by groups and T × G interaction (Table 2). On day 1, LSD Post-hoc tests showed that the level of respiratory

burst in abalones significantly increased under 28 ℃ compared with those under 12 ℃ and 20 ℃ (28 vs 12 ℃, $p < 0.05$; 28 vs 20 ℃, $p < 0.001$), until day 7, the levels of respiratory burst under 12 ℃ and 20 ℃ significantly decreased, and the level of respiratory burst in DD under 28 ℃ decreased according to pair-wise tests ($p < 0.05$) (fig. 1B). For immune enzymes activities, three-way ANOVA analysis showed that all the five factors significantly affected the activities of the 4 kinds of enzymes (Table 2). SOD activity peaked on day 1 and then dropped on day 7, compared with 12 ℃ and 20 ℃ (12 ℃, 20 ℃ belong to subset 1 and 28 ℃ belong to subset 2 in S-N-K homogeneous analysis both on day 1 and 7) (fig. 2A). Temperature and groups did not significantly affect the ACP activities on day 1 and day 7 separately (two-way ANOVA) but an increase in DG on day 7 was noted compared to other groups (DG belongs to subset 1, and the others belong to subset 2) (fig. 2B). S-N-K homogeneous analysis showed that DG showed higher AKP activities under all temperature exposures on day 1 than other 3 groups (DG belong to subset 1, and the others belong to subset 2), no significant effects of temperature or groups on AKP activity were observed on day 7 (fig. 2C). Higher MPO activity was observed in abalones under 20 ℃ compared to 12 ℃ and 28 ℃ on day 1 (20 vs 12 ℃, $p < 0.001$; 20 vs 28 ℃, $p < 0.05$) (fig. 2D). For gene expression, three-way ANOVA demonstrated that both temperature and groups and their interaction (T × G) clearly affected the expression of HSP70 in abalone (Table 2). On day 1, HSP70 levels were higher in 4 groups under 28 ℃ compared to those under 20 ℃ (LSD post hoc tests, 28 ℃ vs 20 ℃, $p < 0.01$), with a higher level of HSP70 observed in all the 4 groups under 28℃ except for DG compared with those

in 20 ℃, and between groups, the level of HSP70 was higher in DD than other 3 groups under 28 ℃ on day 1 (DD belongs to subset 1, the others belong to subset 2) (fig. 3). No significant difference in HSP70 expression between 12 ℃ and 20 ℃ was observed on day 1 but a slight (not significant) increase of HSP70 expression was noted in DD under 12 ℃ compared with those under 20℃ (fig. 3). After 7 days, the levels of HSP70 were significantly elevated in SS ($p < 0.01$) and DS ($p < 0.05$), slightly elevated in SD, and significantly reduced in DD ($p < 0.05$) under 28 ℃ compared with levels measured on day 1, which caused lowest level of HSP70 in DD compared with other three groups (fig. 3). Under 12 ℃, there exhibited no significant difference across days, but a general rise in HSP70 expression was observed in DD, GG and DG on day 7 (fig. 3). 4. DiscussionIn mariculture, there exists a delicate balance between organisms, environments and pathogens, breaking of this balance often leads to disease outbreaks [17, 34], which may bring great losses to aquaculture, hence, it’s meaningful to study the duplicate interactions between these three elements. Change of environment parameters, for example, temperature, pathogens abundance, salinity, dissolved oxygen, chemical compound concentration and so on, often forms stresses which would influence the physiological status of marine invertebrates [22, 35-43], the adaptive capacity and recovery rate under pressure are considered as important indexes for evaluating aquaculture species. Hybridization is considered as an effective tool in fish and shellfish aquaculture for genetic improvement, which regularly results in many dominant phenotypic traits in the offspring [1, 2, 11, 12, 44], including thermal and disease resistance, two of the most important traits in

mariculture. In the present study, we studied the changing patterns of some immune-related parameters and heat shock protein 70 in Haliotis discus hannai, Haliotis gigantance and their hybrids under short-term thermal stress. Results indicated that both temperature and heterosis were closely related to stress responses in abalone. 4. 1. THCLike other marine invertebrates, the immune system of abalone is mainly consisted of cell mediated response and humoral response, and haemocyte plays the most important role in this system, characterized by chemotaxis, recognition of antigen and then elimination of foreign substances or infected cells by phagocytosis, respiratory burst, synthetizing of antimicrobial compound and so on [16, 21, 45, 46]. An open circulatory system exists in marine invertebrates, leading to a wide spread of haemocytes both in tissues and heamolymph, hence, it is widely considered that the elevating of heamocytes is owing to the hyperplasia or translocation of cells between tissues and haemolymph [45-48]. In our study, exposure to high temperature could significantly elevates the THC in abalones, agreed with many studies on other marine invertebrates, such as Mactra veneriformis, Chlamys farreri, Haliotis diversicolor supertexta, Haliotis rubra [16, 23, 45, 47]. What deserve greater concern are the THC variations in DD and DG under 7 days exposure to low temperature and the differences in the changing patterns of THC between the 4 groups. Under 12℃, slight decrease in THC was observed in 4 groups (fig1A), however, after 7 days exposure, THC in DD and DG were significantly increased, differed from GG and GD, who had steady THC levels under low temperature. In consistent with our results, white shrimp Litopenaeus vannamei reared at 20℃ showed decreased THC compared to

those reared at 24℃ and 28℃ [49], the freshwater crayfish Pacifastacus leniusculus had a significant lower THC in 4℃ rather than 12℃ and 22℃ [50], seasonal analysis of the immune paraments of Manila clam Venerupis revealed lowest THC in the low-temperature month October and highest THC in the high-temperature month April [51]. On the contrary, " increased THC with decreased temperature" pattern could also be found in the small abalone Haliotis diverscolor and hard clam Mercenaria mercenaria [17, 23]. Hence, THC changes under low temperature may be different among different species and various exposure conditions. As THC is also responsible for some metabolism activity in abalone such as transportation and digestion of nutrients, excretion, shell growth and so on [46], we hypothesized that decrease of THC under short-term cold shock may be related to decrease of metabolism in organisms, and long-term cold exposure may formulate a kind of stress which would elevate the THC in organisms as a kind of stress responses. More information could be discovered from the results in this study: (1) Under optimal temperature 20℃, DG and GD had higher THC compared with DD and GG; (2) THC levels in DG and GD were less sensitive to temperature changes than DD and GG, all these information led us to hypothesize that hybrids may be more resistant to thermal stress and more efficient in their immune systems compared with their parents. 4. 2. Respiratory burst and SOD, MPO activityIn marine invertebrates, activation of immune responses often happens when pressure exsits, resulting in phagocytosis to eliminate the foreign substances, during phagocytosis, the host’s NADPH-oxidase is activited, producing reactive oxygen species (ROS), including hydroxide ions (OH), hydrogen peroxide (H2O2), superoxide anion

(O2-) and singlet oxygen (1O2), to play an important part in eliminating, this process is called respiratory burst [22, 34, 46, 49]. However, production of ROS could be harmful to both foreign particles and the host's self cells, for instance, antioxidant enzymes such as superoxide dismutases (SOD) and myeloperoxidase (MPO) are important in resisting oxidase stress brought by ROS in the host [20, 34, 45, 47, 52-54], the antioxidant enzymes and respiratory burst work together to keep homeostasis in organisms. In this study, we found no significant differences in respiratory burst levels and SOD activities between 4 groups, respiratory burst levels were higher at 28℃ both on day 1 and day 7 compared to 12℃ and 20℃, while activity of SOD under 28℃ went up on day 1 and come down on day 7. Previous studies focusing on the effects of temperature on ROS showed diversiform phenomena in marine invertebrates, for example, decreased ROS under high temperature could be found in green-lipped mussel Perna viridis, white shrimp Litopenaeus vannamei, intertidal mud clam Mya arenaria [22, 49, 55], contrasting results with ROS being reduced under high temperature were found in the Zhikong scallop Chlamys farreri, the taiwan abalone Haliotis diversicolor and the Antarctic bivalve Laternula elliptica [23, 47, 56], showing that the effect of temperature on ROS cannot be generalized between molluscan species, however, responses of SOD to thermal stress seemed to be relatively alike, with SOD activity fell, or first rose then fell under high temperature [20, 47, 53, 54, 57]. Combined with the phenomena observed in this study, we guessed that high temperature could active the ROS producing system and antioxidant systems in marine invertebrates, causing elevation in ROS and SOD activity, however, long-term exposure

would cause severe oxidate stresses in organisms which would cause damage to the host’s self cells, resulting in decrease in SOD activity. Low temperature did not significantly affect both ROS and SOD activity in all the 4 groups, while the changing pattern of ROS during 7 days under 12℃ and 20℃ may be attributed to an recovery from temperature change, cause the environment temperature (15℃) was 5℃ less than acclimation temperature (20℃), indicating that ROS reaction to temperature change is relatively slow. To the best of our known, studies on the effect of temperature on MPO are few. In this study, we observed significantly decrease or increase of MPO activity exposed to low temperature or high temperature on day 1, especially in DD and GG, what is noteworthy is that (1) MPO activities in DD and GG were higher than these in DG and GD under 20℃ and 28℃; (2) MPO activities in DG and GD did not vary much under different temperatures. As MPO also play a role in antioxidant, from these results, we hypothesized that hybrids may have less inner oxidate stress compared to their parents, both under optimal temperature and severe temperatures. 4. 3. ACP and AKP activity. ACP and AKP are both important lysosomal enzymes in marine invertebrates, ACP mainly acts in catalyzing the hydrolysis of various phosphate esters and phosphoproteins, and AKP mainly acts in the transfer of phosphate groups or metabolism, any of the 2 enzymes plays a role in non-specific immunology, characterized by digestion in foreign particles, aid in phagocytosis and so on [20, 44, 47, 58, 59]. Previous studies showed an increase of the 2 enzymes after challenged by a novel pathogen spiroplasma MR-1008 or injection with the pathogen Vibrio parahaemolyticus [52, 60], further confirmed the immune effects of ACP and AKP. In our study, on day 1,

we observed an increase of AKP activities in 4 all groups with elevated temperature, companied with a significantly higher AKP activity in DG under all temperatures, while on day 7, a significant increase of ACP activity in DG under high temperature on was noted. Fluctuation of ACP and AKP activities with temperature was studied in previous jobs mainly in scallops, while both of them were often elevated under exposure to high temperatures [20, 47, 57, 58]. From the present study, we could infer that AKP is more sensitive to temperature change compared to ACP, which may be caused by the optimal temperatures previously determined for AKP and ACP activities to be 35℃ and 55℃ [61], in this study, 28℃ obviously could not create the best environment for ACP activity. Moreover, higher ACP and AKP activities in DG are noteworthy in this study, probably indicating a higher immune ability of DG, which needs further confirmation, however. 4. 4. HSP70 expression. Heat shock proteins (HSPs) are highly conserved proteins distributed universally from bacteria to human, they are a subset of molecular chaperones, functioning in protecting normal proteins from degeneration, catalyzing the folding of normal proteins and the refolding of abnormal proteins, removing the irreversible damaged proteins and so on to keep cellular homeostasis in organisms [15, 32, 62, 63]. HSP70 is an important kind of HSPs named according to its molecular mass, which has been reported to play an important role in resisting foreign stresses, such as temperature and salinity change, tissues injury, radiation, heavy metal pollution, bacterial infection, hypoxia stress and so on [62, 63]. HSP70 responses to temperature stress have been extensive studied in marine invertebrates and it is widely known that HSP70 can be greatly up-regulated

by acute exposure to high temperature [32, 62, 63, 64-70]. In this study, we observed significantly increase of HSP70 expressions in 4 groups exposed to high temperature on day 1, which is consistent with most studies described below. Highest HSP70 level was observed in DD, which is considered to be that 28℃ is a danger temperature for DD, which needs this population to produce great amounts of HSP70 to keep homeostasis. However, after 7 days, a dramatic reduction in HSP70 at 28℃ happened in DD, along with relatively smooth rise in other 3 groups, however, consistent results were obtained previously in Haliotis discus hannai, in that study, abalones reared at 30℃ for 4 months still showed highest HSP70 expression among all the acclimation temperatures [33], we consider this difference to be caused by difference in materials, in that study, abalones used for experiment were from a selectively bred population named P-97, the offspring obtained through intraspecies hybridization between Chinese population and Japanese population, which had been identified to be advantageous in growth and stress tolerance. Hence, in this study, we considered the drop of HSP70 level in DD to be intolerance of the DD to long-term exposure under high temperature, which had caused the breakdown of homeostasis in DD, while other 3 groups still maintained relatively high levels of HSP70 after 7 days' exposure to 28℃, indicating that they had a wider thermal tolerance range compared with DD, which may be attributed to the fact that DD is a kind of species which prefers lower temperature while GG is a kind of warm-water species [25], and the hybrids successfully inherit the thermal tolerance trait of GG. Another interesting result is that, under 12℃, HSP70 level of DD and GG slightly increased, indicating that low temperature stress could also

induce the expression of HSP70, agreed with the phenomenon previously described in Haliotis discus hannai [33]. Well, the HSP70 level in DG under 12℃ showed as " first fall then rise", we infer that the suppression in HSP70 expression at the beginning is accorded with the suppression on metabolism level in organisms, brought by low temperature, after 7 days acclimation under low temperature, the organisms adapted to the cold environment to some extent, causing resuscitation in HSP70 expression. Also, significantly change of HSP70 level in GD under low temperature was not observed, indicating that 12℃ could not formulate a stress to elevate the HSP70 level in GD, which could explained by heterosis theory, that GD inherits the cold resistant trait from DD, and performs better than DD. In conclusion, this is the first study that compared the combined effects of temperature and hybridization on the stress handling ability in abalone, including immune system efficiency and thermal tolerance ability. Results showed a significantly influence of hybridization on some immune parameters in abalone, including THC, AKP, ACP and MPO activity, with hybrids either gained higher levels in some parameters under ambient temperature or extreme temperature, or showed less fluctuation in some parameters when coped with temperature stress, these phenomena indicate that interbreeding groups (DG and GD) may be more efficient in their immune systems than their parents. HSP70 analysis also showed some advantages brought by hybridization, reflected in the offspring’s performance dealing with temperature stress, with the hybrids either gain higher levels of HSP70 under long-time exposure to extreme temperatures, or show less fluctuations dealing with temperature stress. In summary, this study may provide a

confirmation of heterosis theory in abalone, we inferred from the data in this experiment that the two interbreeding groups may perform better in actual mariculture and production. Further studies should focus the molecular mechanism which is responsible for heterosis theory, and the possible different types of gene regulation pathways/networks in inbreds and hybrids are needed to be clarified to depth our recognition. AcknowledgeThis work was supported by ? Table 1Primers used in the present studyPrimer Sequence (5'-3') Product size (bp)degHSP70F GGKTCCACDCGTATTCCAAAG 950degHSP70R ATCRACCTCCTCRATGGTTGGrtHSP70F AGGAGGAGATAGAGCGTAT 181rtHSP70R TCGGTGATGGTCTTCTTGβ-actinF GGTATCCTCACCCTCAAGT 158β-actinR GGGTCATCTTTTCACGGTTGTable 2Summary of three-way ANOVA results assessing the effect of temperature (12℃, 20℃, 28℃), groups (DD, GG, DG, GD) and sampling time point (day 1 and day 7) on immune parameters and HSP70 in abalone. Temperature (T)Groups (G)Length (L)T × GT × LCellular immunityTHC

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NSNSRespiratory burst

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NS

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Enzyme activitySOD

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ACP

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MPO

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Gene expressionHSP70

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NSNS represents non-significant differences, symbols represent significant differences at p < 0. 05 (*), p < 0. 01 (**) or p < 0. 001 (***). AC: UsersLeungDesktop 文章作图 THC. tifBC: UsersLeungDesktop 文章作图 RB. tifFig. . The effect of temperature and sampling time point on cellular immune responses in 4 groups. (A) total haemocyte count (THC, ×106 cells ml-1), (B) respiratory burst (OD 630nm)AC: UsersLeungDesktop 文章作图 SOD. tifBC: UsersLeungDesktop 文章作图 ACP. tifCC: UsersLeungDesktop 文章作图 AKP. tifDC: UsersLeungDesktop 文章作图 MPO. tifFig. 2. The effect of temperature and sampling time point on enzymes activities in 4 groups. (A) superoxide dismutase (SOD, U ml-1), (B) acid phosphatase (ACP, U ml-1), (C) alkaline phosphatase (AKP, U 100ml-1), (D) myeloperoxidase (MPO, U L-1). C: UsersLeungDesktop 文章作图 HSP70. tifFig. 3. The effect of temperature and sampling time point on HSP70 expression levels (Fold) in 4 groups. The

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