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Temperature and dissolved oxygen influence the immunity, digestion, and antioxidant level in sea cucumber *Apostichopus japonicus*

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Introduction

Aquatic species naturally live in water environment, therefore stress brought on by alterations in the environmental conditions directly affects them (Huo et al., 2021). The growth, survival and distribution of marine organisms are largely influenced by environmental factors like water temperature and dissolved oxygen (DO) (Coutant, 1985; Gobler et al., 2014). By the end of the century, it is expected that global temperatures will rise by at least 2°C, but ocean DO concentrations will drop by 4-7% (Matear and Hirst, 2003; Hoegh-Guldberg et al., 2007). Since oxygen becomes less soluble as temperature rises, heat stress and hypoxic stress frequently coexist (Huo et al., 2020).

The sea cucumber *Apostichopus japonicus* is an echinoderm with considerable commercial and ecological significance (Huo et al., 2020). As an aquatic poikilothermal animal, the physiological activities (*i.e.*, digestive function, immunity, and antioxidant defense) of sea cucumber are directly influenced by water temperature and dissolved oxygen. The suitable temperature range for *A. japonicus* growth is between 15°C and 18°C (Dong et al., 2006), and when the temperature exceeds 26°C and persists for more than 10 days, massive mortality would occur in farmed sea cucumbers. Moreover, hypoxia is typically seen as occurring when dissolved oxygen levels drop below 2 mg·L^{-1} (Wu, 2002). An earlier study illustrated that *A. japonicus* could survive at hypoxic condition (2 mg·L^{-1}) in a short time, but its physical status and movement would be affected (Huo et al., 2018). The *A. japonicus* mainly relies on non-specific immunity, and the humoral immune response is one of its main defense reactions (Shao et al., 2018). The coelomic fluid of *A. japonicus* is similar to lymphatic fluid, and the cells inside work together with various humoral immune factors to form an immune response. Therefore, it is necessary to investigate the variations of enzyme activity in the coelomic fluid of *A. japonicus* to reveal how that species reacts to environmental challenges.

The activity of digestive enzymes is one of the most commonly used indicators to evaluate the digestive capacity, nutritional biochemistry and physiological status of the organism (Zhang et al., 2014). *A. japonicus* would reduce feeding and the digestive tract would be degraded when water temperature increases and dissolved oxygen decreases (Xu et al., 2015; Huo et al., 2018), and the digestive functions were potentially negatively affected (Huo et al., 2018). Therefore, the digestive function of *A. japonicus* may be altered under environmental stress, and to investigate this change, we could check digestive enzyme activities.

Environmental stresses could lead to an increase in reactive oxygen species (ROS) in the organism (Das and Roychoudhury, 2014). To avoid the damage caused by ROS, organisms have evolved various types of antioxidant systems, including non-enzymatic antioxidants represented by vitamin C and vitamin E and enzymatic antioxidants represented by superoxide dismutase (SOD) and catalase (CAT) (Tan et al., 2020). The antioxidant enzyme family members are widely distributed in the organism and regulate ROS levels thus acting as antioxidants and play crucial roles in response to stress. It is necessary to identify the changes of antioxidant enzymes in *A. japonicus* under adverse environment. In this study, 16 enzymes related to immune defense, digestive function, and antioxidant level were measured to reveal the physiological response characteristics in *A. japonicus* exposed to environmental stress. Our findings would provide insight into the response and adaptation of sea cucumber under the context of global climate change.

Methods

Experimental *A. japonicus* were collected from the coast of Weihai, China, with a wet weight of 90-110 g. One-week acclimatation in a tank containing aeration sand-filtered seawater at a temperature of 16 ± 0.5°C before the formal experiment. The normal control (NC) group was maintained at a temperature of 16°C with sufficient aeration; the high temperature (HT) group (heat stress group) was maintained at a temperature of 26°C with sufficient aeration; the low dissolved oxygen (LO) group (hypoxic stress group) was maintained at a temperature of 16°C and dissolved oxygen concentration of 2 mg·L^{-1}; the high temperature and low dissolved oxygen (HL) group (heat combined with hypoxic stress group) maintains at a temperature of 26°C and a DO concentration of 2 mg·L^{-1}. The equipment used for temperature and DO change, and the changing rate were same with the previous study (Huo et al., 2020). Five replicates were set in each group and cultured in separate tanks during the experiment. After 48h exposure, the coelomic fluid of each *A. japonicus* was collected by sterile syringe and rapidly frozen in liquid nitrogen, and then transferred to a refrigerator at -80°C for storage.

A total of 16 enzyme activities involving immunity, digestion and antioxidant ability were measured in this study, including acid phosphatase (ACP), alkaline phosphatase (AKP) and lysozyme (LZM), lipase (LPS), α-amylase (AMS), pepsin (PEP), trypsin (TRY), SOD, glutathione peroxidase (GSH-PX), CAT, succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), total antioxidant capacity (T-AOC), malondialdehyde (MDA), peroxidase (POD), and phenol oxidase (PPO). All enzyme activities were determined within one month of sampling the coelomic fluid samples, and the commercial kits used in this study were purchased from Nanjing Jiancheng Biological Research Institute (Nan Jing, China) and tested according to the instructions. Specifically, the kit number for the enzymes assay were listed in Table S1. The obtained data were statistically analyzed by SPSS19 software (IBM Corp., Armonk, NY, USA). The significance of the differences between the treated and comparison groups for each enzyme was analyzed by *t*-test and the statistical significance threshold was set at $P < 0.05$. Bar graphs were plotted using Prism7 software (GraphPad Software Inc., USA).

Data description

As the immune response in *A. japonicus* is a typical non-specific immune response, enzymes like ACP, AKP, and LZM may be able to aid in the complete destruction of foreign compounds after they have passed through the organism's first line of defense (Wang et al., 2015). In this study, the activity of the three enzymes related to immune defense was measured (Figure 1). Compared with the normal environmental condition, the activity of ACP was significantly higher in *A. japonicus* under hypoxic stress ($P < 0.05$), and the changes of AKP activity were not significant under the three environmental stresses; the activity of LZM was significantly higher in *A. japonicus* under heat combined with hypoxic stress ($P < 0.05$). This could be that more adenosine triphosphate (ATP) was needed to maintain normal metabolic level when *A. japonicus* exposed to environment stress, and the inorganic phosphate required for ATP synthesis can be produced by the hydrolysis of phosphate by ACP and AKP (Zheng et al., 2014). The results suggested that supply of potential metabolic high energy demand was

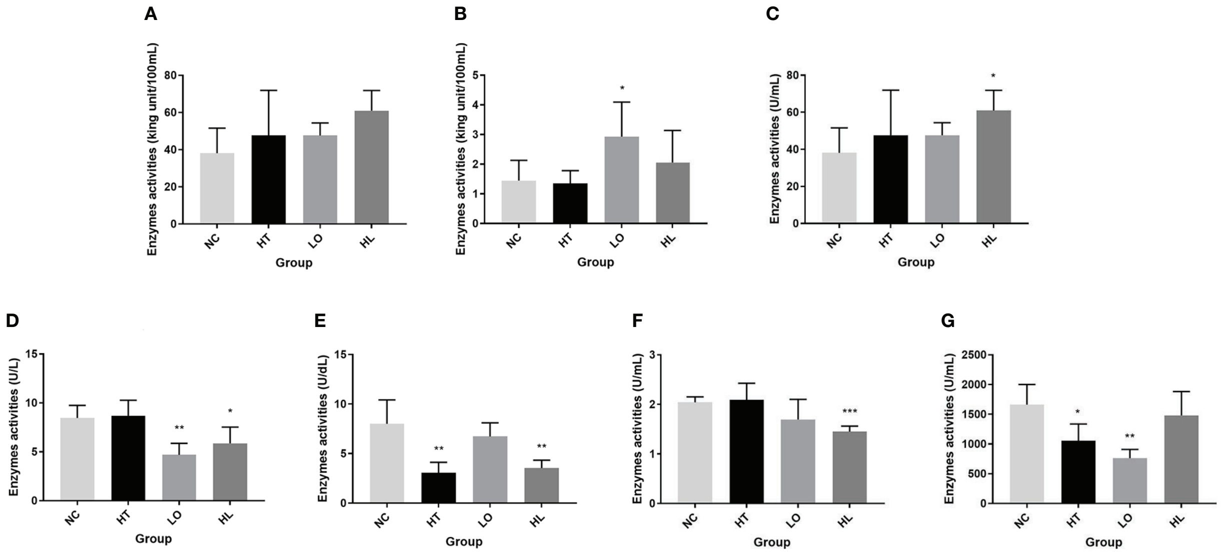

FIGURE 1
Activities of immune and digestive enzymes in *A. japonicus* under environmental stresses **(A)**. alkaline phosphatase (AKP); **(B)** acid phosphatase (ACP); **(C)** lysozyme (LZM); **(D)** lipase (LPS); **(E)** α-amylase (AMS); **(F)** pepsin (PEP); and **(G)** trypsin (TRY); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

enhanced, thus providing more energy to adapt to the adversity. Lysozyme is an important innate immune factor widely present in the endothelial cells and body fluids of echinoderms that kills germs and shields against bacterial infection (Canicatti and Roch, 1989). The increased LZM activity suggested that phagocytic activity of phagocytes may be elevated. A high temperature also caused an increase in serum lysozyme levels in Atlantic halibut *Hippoglossus hippoglossus* L. (Langston et al., 2002). According to the findings, the immune defense mechanisms were induced in *A. japonicus* in response to environmental stress, the organism's defense against foreign substances was enhanced.

In this study, four enzymes related to digestive function were selected for activity measurement, including LPS, AMS, PEP and TRY. The results showed that the LPS activity was significantly reduced under hypoxia ($P < 0.01$) and heat combined with hypoxic stress ($P < 0.05$); AMS activity was highly significantly reduced under heat and heat combined with hypoxic stress ($P < 0.01$); PEP activity was extremely significantly reduced under heat combined with hypoxic stress ($P < 0.001$); and TRY activity was significantly reduced under heat stress ($P < 0.05$) and hypoxic stress ($P < 0.01$) (Figure 1). In the previous of yellowtail kingfish *Seriola lalandi*, TRY, LPS and AMS enzyme activities were altered by temperature but did not seem to be impacted by dissolved oxygen concentration (Bowyer et al., 2014); PEP and AMS activities also significantly changed by temperature in the leopard coral grouper *Plectropomus leopardus* (Sun et al., 2015). Under environmental stress, *A. japonicus* undergoes degeneration of the intestine and respiratory tree (Xu et al., 2015; Huo et al., 2018), and may even occur evisceration. Substantial changes in these digestive organs are also responsible for the decrease in digestive enzyme secretion and activity. The decrease in digestion-related enzyme activities in *A. japonicus* under environmental stress indicated that there was a negative impact of environmental stress on the digestive function of *A. japonicus*.

Environmental stress may lead to an increase in ROS, which could oxidize cellular components and damage cell membrane. The imbalance between the production and clearance of ROS will cause oxidative stress (Halliwell and Gutteridge, 2001; Kong et al., 2012). To assess the oxidative stress response of *A. japonicus* under environmental stress, nine enzyme activities were measured in this study (Figure 2), including SOD, GSH-PX, CAT, SDH, LDH, MDA, POD, PPO, and T-AOC. The T-AOC was significantly reduced under heat and heat combined with hypoxic stress ($P < 0.05$); SOD was significantly reduced, and MDA were extremely significantly increased under hypoxia and heat combined with hypoxic stress ($P < 0.001$). These suggested that oxidative stress brought on by environmental stress results in lipid peroxidation and caused oxidative damage to organisms. SDH activity was significantly reduced heat combined with hypoxic stress ($P < 0.05$), suggesting that aerobic oxidation capacity was suppressed, and the tricarboxylic acid cycle was impacted. POD and PPO activity were significantly increased under heat stress; the activity of CAT was significantly reduced under all three types of environmental stresses. PPO could oxidize phenolic substrates to unstable quinones (Cerenius et al., 2008); POD has the property of catalyzing the oxidation

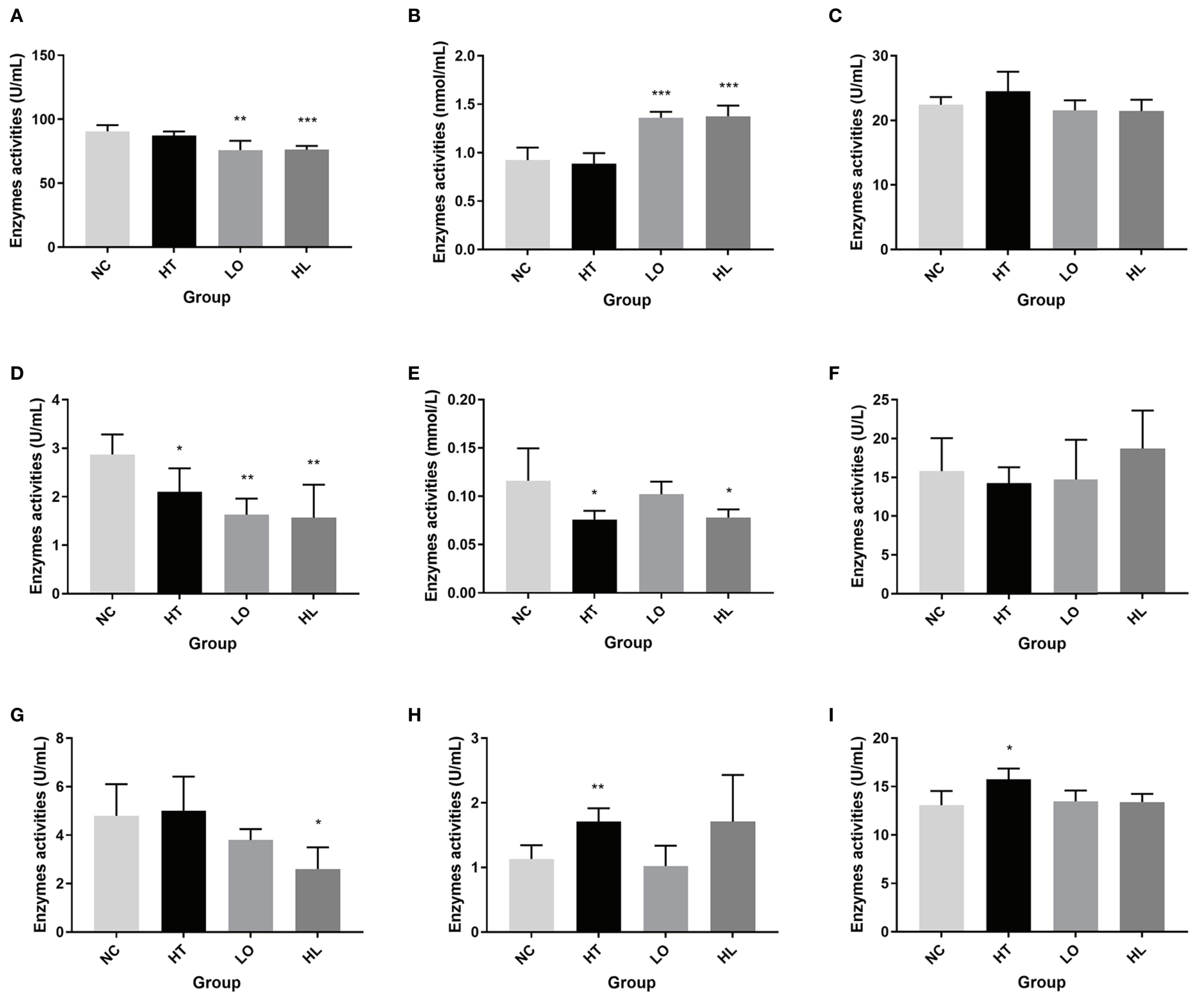

FIGURE 2
Activities of antioxidative enzymes in *A. japonicus* under environmental stresses **(A)**. superoxide dismutase (SOD); **(B)** malondialdehyde (MDA); **(C)** glutathione peroxidase (GSH-PX); **(D)** catalase (CAT); **(E)** total antioxidant capacity (T-AOC); **(F)** lactate dehydrogenase (LDH); **(G)** succinate dehydrogenase (SDH); **(H)** peroxidase (POD); and **(I)** phenol oxidase (PPO); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

reaction between hydrogen peroxide (H_2O_2) and hydrogen donors (Şişecioğlu et al., 2010), and CAT could catalyze the intracellular hydrogen peroxide decomposition (Wang et al., 2016). Therefore, by altering their activity to reduce the cell damage caused by excessive free radicals, *A. japonicus* could adapt to the adversity. The activity of GSH-PX and LDH did not significantly change; It is possible that these two enzymes did not play such crucial role in the oxidative stress defense in *A. japonicus*. It is also possible that these two indicators are not sensitive to environmental stress in *A. japonicus* coelomic fluid. The oxidation status influenced by environmental changes has been reported in aquatic animals, including the shrimp *Litopenaeus vannamei* (Liu et al., 2015), the crab *Paralomis granulosa* (Romero et al., 2011), the fish *Carassius auratus* (Sun et al., 2012), *Micropterus salmoides* (Sun et al., 2020) and the scallop *Chlamys farreri* (Chen et al., 2007). For example, the results of decreased SOD and CAT were in accordance of the crucian carp *Carassius auratus* in hypoxic condition (Sun et al., 2012). Increased T-AOC and GSH-Px activities were also found in heat-stressed largemouth bass *Micropterus salmoides* (Sun et al., 2020). It was suggested that various environmental stresses resulted in varing degrees of oxidative stress response, and these enzymes with altered activity may be vital in protecting *A. japonicus* from oxidative damage.

To conclude, the coelomic fluid is an important component in sea cucumbers to defense against undesirable environments. When exposed to the environmental stress, digestive function was suppressed, immune system was induced, and the antioxidant enzymes changed in varying degrees in *A. japonicus*. The integrated regulation of immunity, digestion, oxidative stress, and other associated enzymes is a series of adaptive mechanisms made by the *A. japonicus* to adapt to the extreme environment.

Our results provide a better understanding of how the *A. japonicus* survives in adversity in the context of global change.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Since culture commercial sea cucumber were used, ethical review and approval not required.

Author contributions

DH: Conceptualization, Investigation, Data curation, Methodology, Validation, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing. FS: Validation, Writing - review & editing. LZ: Supervision, Writing - review & editing. HY: Supervision, Funding acquisition, Writing - review & editing. LS: Formal analysis, Funding acquisition, Writing - review & editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1094814/full#supplementary-material

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