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Effects of salinity and temperature on the metabolic and immune parameters of the banana shrimp *Fenneropenaeus merguiensis* (De Man, 1988)

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Abstract

This study investigated the activities of metabolic and immune enzymes in the hepatopancreas and muscle of the banana shrimp *Fenneropenaeus merguiensis* at different salinities (10, 15, 20, 25, and 30 ‰) and temperatures (21, 24, 27, 30, and 33°C). The shrimp (mean initial weight, 1.72±0.25 g) were cultured at different salinities or different temperatures for 15 d. All treatments were conducted in triplicate. Results showed that glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) activities in the hepatopancreas were the highest at a salinity of 20‰ (p<0.05). The GOT and succinate dehydrogenase (SDH) activities in the muscle were the highest at a salinity of 25 ‰ (p<0.05). The GOT and GPT activities in the hepatopancreas at a temperature of 24ºC were significantly higher than those at the other temperatures (p<0.05). The highest SDH activity in the muscle was observed at a temperature of 27 ºC (p<0.05). Different immune enzymes showed different responses to salinity and temperature. The highest superoxide dismutase (SOD) activity in the hepatopancreas, and the highest acid phosphatase (ACP) activity in the muscles was observed at a temperature of 24°C (p<0.05). By contrast, the lowest ACP activities in the hepatopancreas and muscles were observed at salinities of 25 and 20 ‰, respectively (p<0.05). These results indicated that suitable salinity and temperature can increase the metabolic enzyme activities, but the relationship of immune enzymes activities and ambient conditions is indeterminate.

Keywords: *Fenneropenaeus merguiensis*, Salinity, Temperature, Metabolic enzyme, Immune enzyme

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Introduction

Shrimp is one of main economically important aquaculture species worldwide. Shrimp production has increased rapidly with the development of aquaculture technology. With intensive culture development and environmental deterioration, the shrimp culture industry has suffered considerable economic losses worldwide as a result of diseases caused mainly by viruses and bacteria (Lightner, 2011; Thitamadee *et al*., 2016). Such losses are also attributed to increased susceptibility to diseases and other stress conditions for many reasons, such as inbreeding increases susceptibility to disease and other stresses (Doyle, 2016). Prior to 2000, *Penaeus monodon* was the dominant cultivated shrimp species in Asia. Then *Litopenaeus vannamei* quickly became the dominant cultivated species globally, primarily due to its success in avoiding problems with white spot disease outbreaks (Thitamadee *et al*., 2016). This emphasizes the need for diversification of shrimp culture. So other aquaculture shrimp species, e.g. banana shrimp *Fenneropenaeus merguiensis*, is paid more attention by many researchers due to the large size and export value (Hoang *et al*., 2002; Knibb *et al*., 2014; Qian *et al*., 2015; Iehata *et al*., 2017; Zhuo *et al*., 2017). Before *L. vannamei* was introduced to the several Asian countries, *F. merguiensis* is one of the most common species in China, which occurs throughout the Asian and Australian tropical and subtropical waters (Escobedo-Bonilla, 2016).

During the farming process of shrimp, environmental fluctuations associated with seasonal climatic changes were of major importance in triggering adjustments in the physiology and behaviour of aquatic organisms. Both salinity and temperature are important physical factors that are known directly effect on physiological responses of most invertebrate species. Ambient salinity and temperature can directly influence on growth, development, metabolism and immunity of aquaculture animals (Zacharia and Kakati, 2004; Cheng *et al*., 2005; Allan *et al*., 2006; Lin *et al*., 2012; Vaseeharan *et al*., 2013; Mizanur and Bai, 2014; Gao *et al*., 2016; Wu *et al*., 2017). Environmental temperature also influences on the metabolic enzyme activities of *Sebastes schlegeli*, led to comparatively lower activity at 15°C than 19 °C in glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) (Mizanur and Bai, 2014). The metabolic enzyme activities of *L. vannamei*, succinate dehydrogenase (SDH) activity, decreased after low-temperature treatment (Wu *et al*., 2017). The shrimp showed different immune enzymes activities, acid phosphatase (ACP) activities, after WSSV infection at different salinities. Increaseing temperature led to an increased superoxide dismutase (SOD), ACP activity in the thick shell mussel *Mytilus coruscus* (Hu *et al*., 2015). Higher temperatures (32 and 34°C) reduced immune capability of *L. vannamei* (Cheng *et al*., 2005). These parameters may be used as effective

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shrimp health indicators (Vaseeharan *et al*., 2013).

However, the physiological responses to different salinities and temperatures in *F. merguiensis* based on analysis of metabolic and immune enzymes are unclear. The studies on the effects of salinity and temperature on the metabolic (GPT, GOT and SDH) and immune (SOD and ACP) parameters of *F. merguiensis* will help to determine the optimal conditions for farming this species. Hence, the present study was conducted to examine variation in metabolic and immune parameters in response to different temperatures and salinities under laboratory conditions.

Materials and methods

Animals

A batch of healthy *F. merguiensis* (1.72±0.25 g) was collected from the marine biology research base of Guangdong Ocean University (Zhanjiang, Guangdong, PR China), where the experiments were carried out. The natural salinity and temperature of the seawater was 27 ‰ and 28 ºC, respectively.

Experiments design

Two different ambient parameters were tested respectively. The effect of salinity on the shrimp was investigated at five salinity levels (10, 15, 20, 25 and 30 ‰). Shrimp were randomly divided into five groups, and each group had three repetitions at a density of 50 shrimp per tank. Prior to the experiment, the salinity in designated tanks was gradually decreased or increased to the designated levels by adding fresh water or seawater crystal at a rate of 2-5 ‰ per day. When the salinity in the all groups reach to the designated levels, the experiment began. The experiment was conducted over 15 d. The salinity differences during the experiments were maintained within±0.5 ‰.

The effect of temperature on the shrimp was investigated at five different temperature levels (21, 24, 27, 30, and 33°C) and a constant salinity of 27 ‰. Shrimp were randomly divided into five groups, and each group had three repetitions at a density of 50 shrimp per tank. Prior to the experiment, the shrimp in designated tanks were acclimated to the designated temperature levels by adding ice packs or heater at a rate of 3ºC per day. The temperature was measured in each tank with thermometers. The temperature differences during the experiments were maintained within±0.5 ºC.

Shrimp were fed to satiation at a rate of 80 g kg^{-1} body weight per day. The daily amount was divided into three and fed to the shrimp at 8 am, 12 am and 6 pm. The water was aerated continuously and maintained at normal day-night illumination. Each tank was cleaned by syphoning off the accumulated faeces and feed remains every day. About 15-30 % of the total water volume was renewed daily to supplement the water syphoned off. The supplemental water was adjusted the temperature and salinity parameters to suit target tanks.

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Sample collection and Analysis

Samples for the analysis of metabolic and immune parameters were collected at 15 d after the start of the experiment. All shrimp were anesthetized before tissue excision using a previously described method (Luedeman and Lightner, 1992). The excised hepatopancreas and muscle tissues of *F. merguiensis* were homogenized in Tris-HCl buffer (pH 7.4) at 4°C. The homogenates were centrifuged at 4000 g for 10 min at 4°C, and the supernatant fluids were used directly for antioxidant parameter analysis (Yang *et al*., 2010). The supernatants were used for the analysis of GOT, GPT, SDH, ACP, and SOD. Only shrimp in the inter-moult period were used. All shrimp in the experiment received humane care in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The GOT, GPT, SDH, ACP, and SOD activities also were evaluated using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) according to the instructions of the manufacturer.

Statistical analysis

To determine the statistical difference, the results were analysed using one-way analysis of variance and Duncan's multiple comparison of the means (p<0.05). The statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The metabolism enzymes activities at different salinities

Metabolism enzymes activities in the hepatopancreas and muscle of the shrimp at different ambient salinities were measured (Fig. 1). The results showed that the GOT activities in the hepatopancreas at salinities of 20 and 25 ‰ were significantly higher than those in other salinity groups (10, 15 and 30 ‰) (p<0.05). The highest GOT and SDH activities in muscle were observed at a salinity of 25 ‰ (p<0.05). The GTP activity in the hepatopancreas at a salinity of 20 ‰ is higher compared to those in other groups (p<0.05), which is about 6 times as much as that at a salinity of 15 ‰. The GTP activity in the muscle at a salinity of 20 ‰ is higher than those at salinities of 15 and 25 ‰. However, the highest GTP activity appeared at a salinity of 10 ‰ (p<0.05).

The immune enzymes activities at different salinities

The immune activities of the shrimp at different ambient salinities were measured (Fig. 2). The SOD activity in the hepatopancreas at a salinity of 25 ‰ was higher than those at salinities of 15 and 20 ‰ (p<0.05). The SOD activity in the muscle at a salinity of 25 ‰ was higher than those at salinities of 10, 15, and 20 ‰, but which was lower than that at a salinity of 30 ‰ (p<0.05). However, the lowest ACP activity in the hepatopancreas and muscle were observed at salinities of 25 and 20 ‰, respectively (p<0.05).

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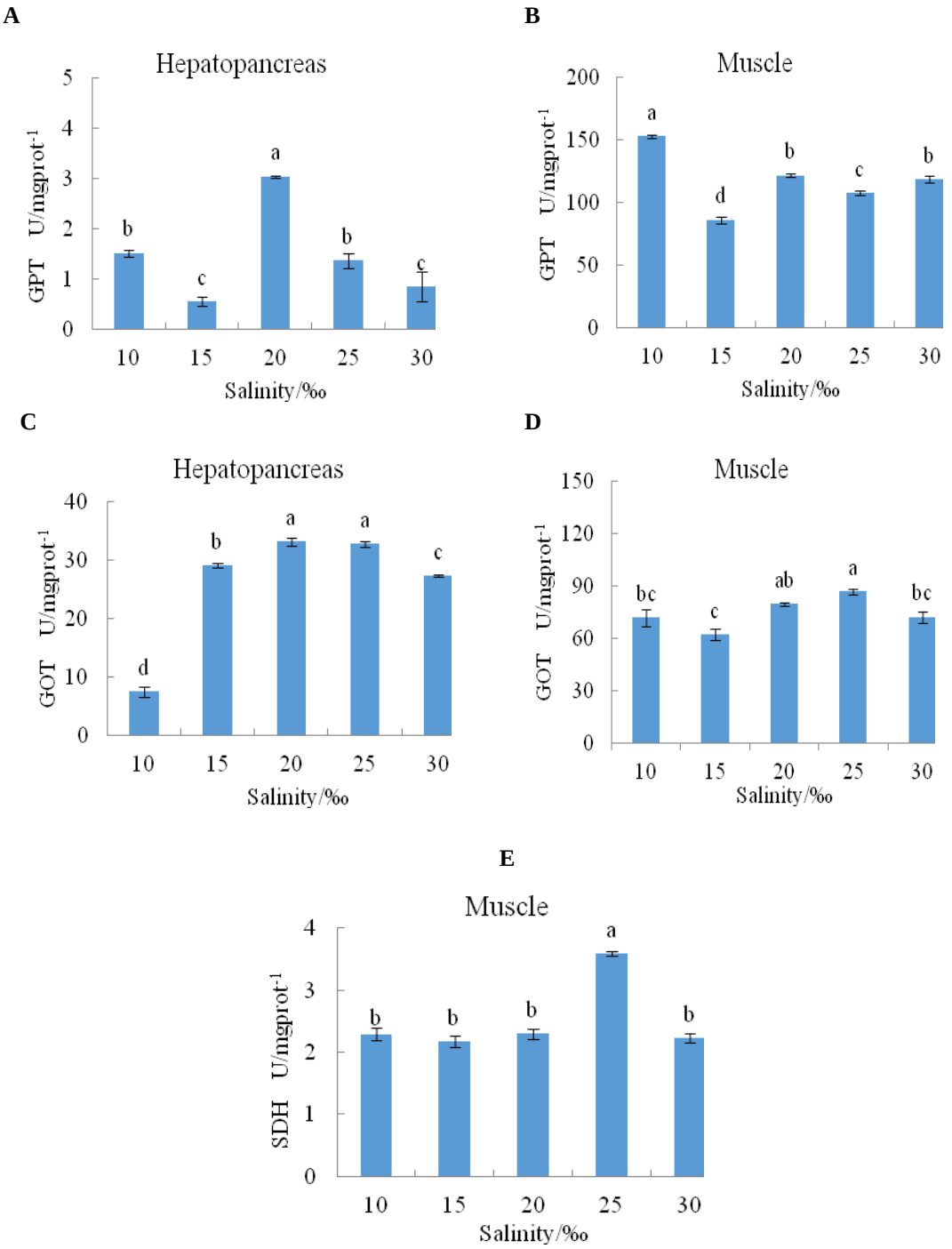

Figure 1: The effects of salinity on the metabolism enzymes activities in the hepatopancreas (A, C) and muscle (B, D, E) of *Fenneropenaeus merguiensis.*

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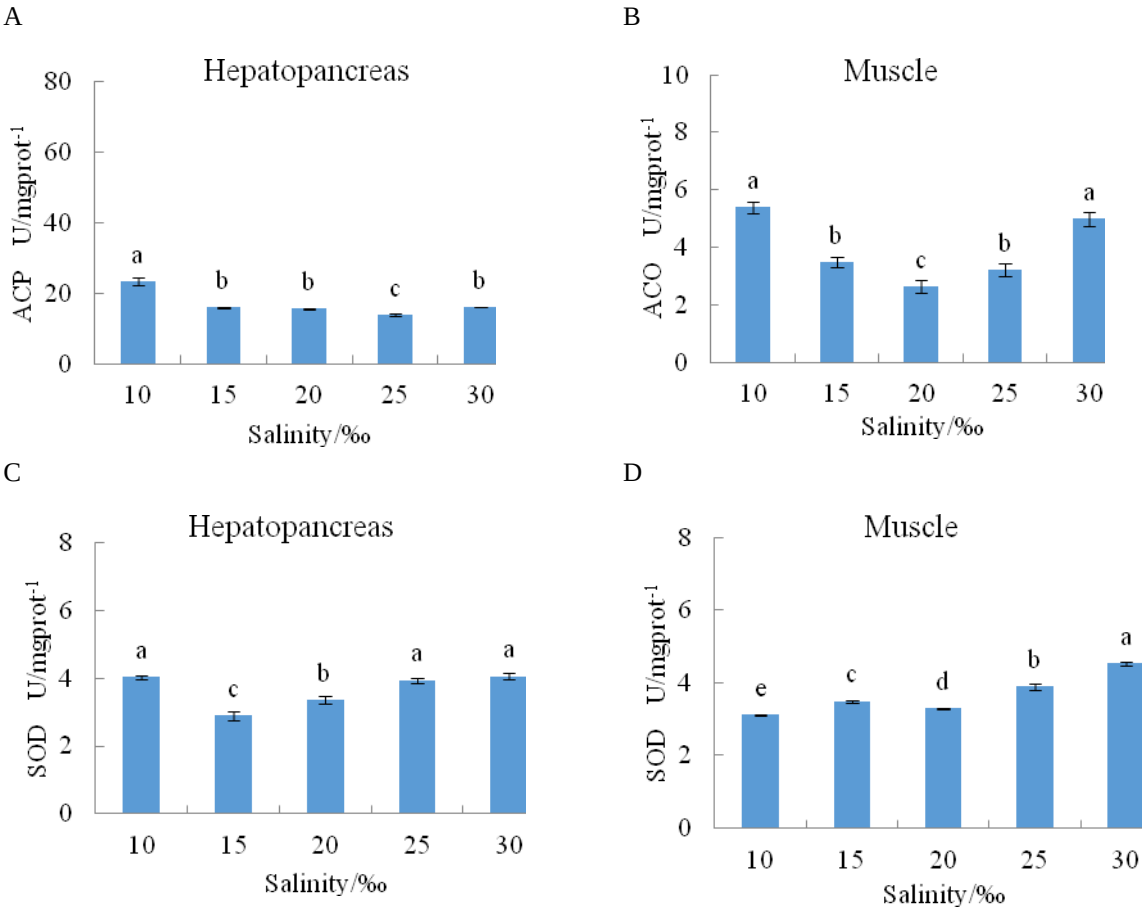

Figure 2: The effects of salinity on the immune enzymes activities in the hepatopancreas (A, C) and muscle (B, D) of *Fenneropenaeus merguiensis.*

The metabolism enzymes activities at different temperatures

The temperature significantly affected on metabolism enzymes activities in the hepatopancreas and muscle of the shrimp (p<0.05) (Fig. 3). The results showed that the GOT and GPT activities in the hepatopancreas at a temperature of 24°C were significantly higher than those at other temperature conditions (p<0.05). Meantime the highest SDH activity in the muscle at a temperature of 27°C was observed (p<0.05). However, the GOT and GPT activities in the muscle performed different results, which were the highest level at a temperature of 21°C (p<0.05).

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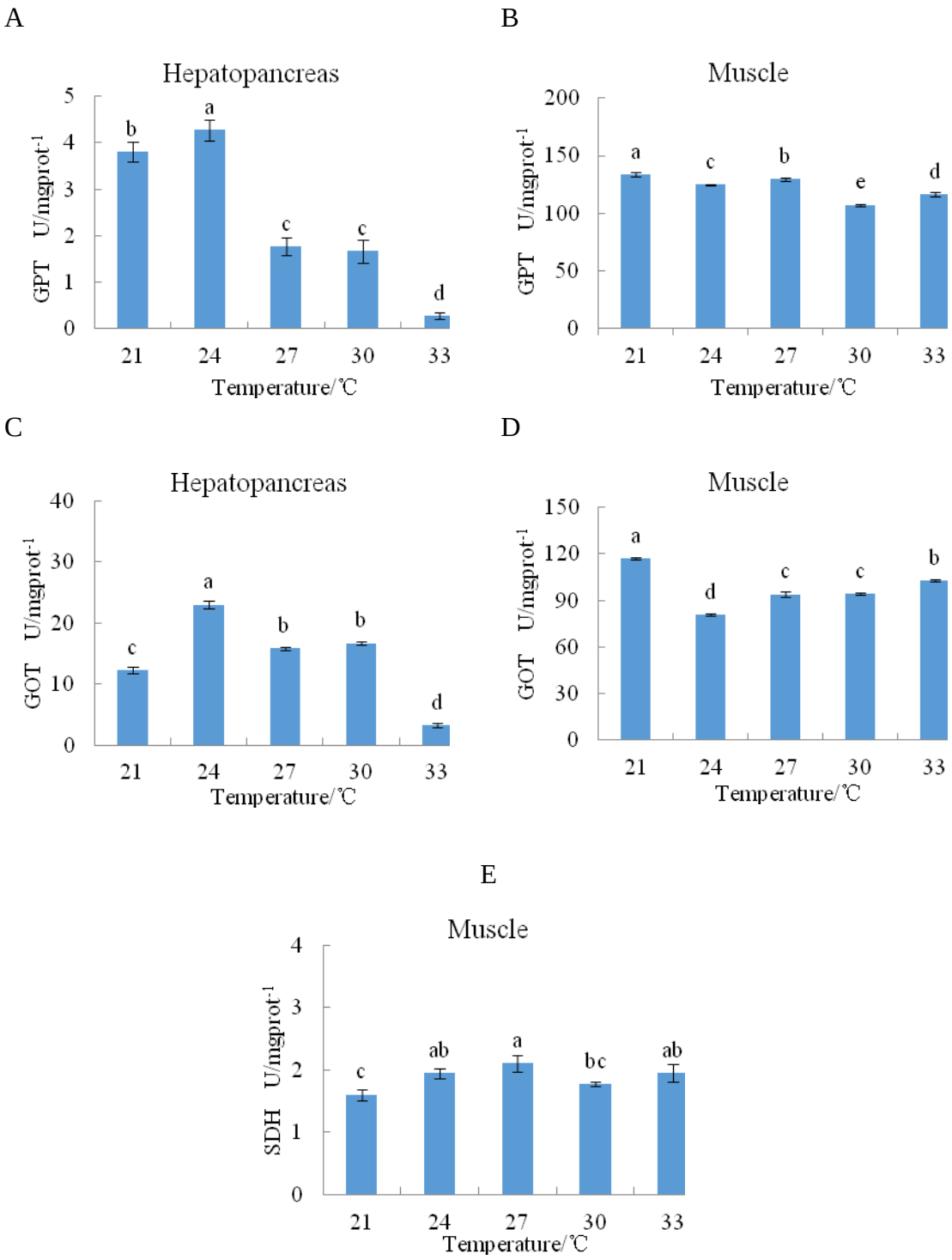

Figure 3: The effects of temperature on the metabolism enzymes activities in the hepatopancreas (a, c) and muscle (b, d, e) of *Fenneropenaeus merguiensis.*

The immune enzymes activities at different temperatures

The immune enzymes activities in the hepatopancreas and muscle of the shrimp at different ambient temperatures were measured (Fig. 4). A different immune enzyme in different tissues performs different trends. The highest SOD activities in the hepatopancreas, and the highest ACP activity in the muscle were observed at a temperature of 24°C (p<0.05). The

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ACP activity in the hepatopancreas decreased with the increase of temperature. As for muscle, the significantly higher SOD activity was observed at a temperature of 33°C (p<0.05), while no significant difference was observed between temperatures of 21, 24, 27 and 30°C (p>0.05).

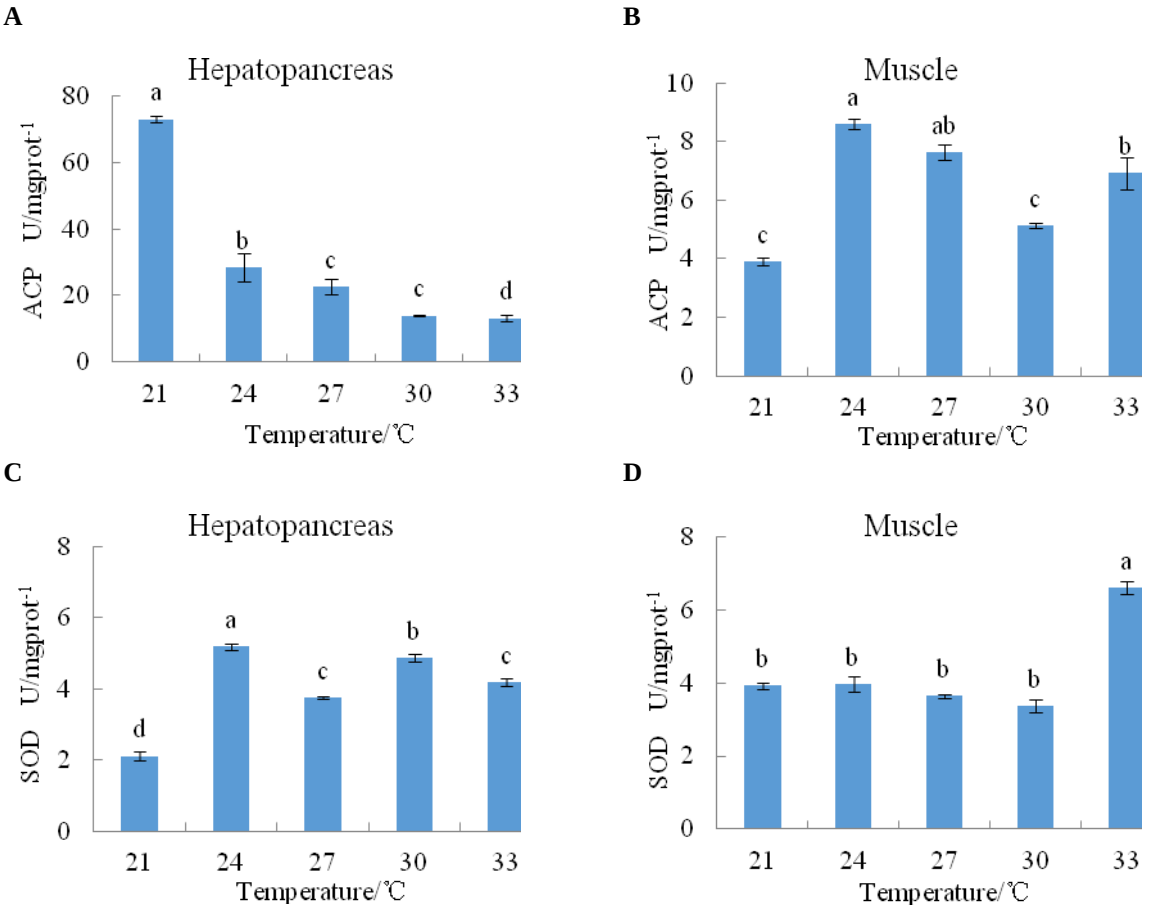

Figure 4: The effects of temperature on the immune enzymes activities in the hepatopancreas (a, c) and muscle (b, d) of *Fenneropenaeus merguiensis*.

Discussion

GOT and GPT are the most important amino-transferases which take part in amino acids linked to the citric acid cycle and the transfer of amino groups from one specific amino acids to another (Wu *et al*., 2008). GOT, also called aspartate aminotransferase (AST). GPT also called alanine aminotransferase (ALT). GOT and GPT activities are usually used as general indicators of the functioning of the vertebrate liver. They also were a direct indicator of shrimp health (Pan et al., 2003). SDH is an important enzyme in aerobic metabolism and involved in both the citric acid cycle and the respiratory electron transfer chain. Its activity can roughly reflect the level of aerobic metabolism (Rutter *et al*., 2010). There are many early reports about effects of dietary supplements or pollutants on GOT, GPT and SDH activities of aquaculture animals (Galindo-Reyes *et al*., 2000; Vijayavel and Balasubramanian, 2006; Wu *et al*., 2008). Hepatopancreas is not only a digestive organ possesses abilities of

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absorption, digestion, storage, and secretion, but also a major site where biotransformation undergo in crustaceans (Wu *et al*., 2008). GOT and GPT levels in hepatopancreas and muscle also were determined as biochemical evidence to confirm metabolism level. The increase of GPT enzyme activity suggests an increased turnover of amino acids in the body (Li *et al*., 2008). Hepatic GPT in *Pseudobagrus ussuriensis* seems to be affected by feeding rates and the unfed fish showed the lowest activity of GPT (Bu *et al*., 2017). The activities of protein metabolism enzymes (GPT and GOT) in liver were reduced with increasing dietary canola meal level, which indicated the utilization of dietary protein decreased (Cheng *et al*., 2010). In the present study, the higher GPT and GOT activities in the hepatopancreas were observed at a salinity of 20 ‰. The highest SDH and GOT activities in the muscle were observed at a salinity of 25 ‰ (p<0.05). These results hinted that the suitable salinity for *F. merguiensis* is 20-25 ‰, and higher metabolism enzyme observed in this range. According to the growth and survival data, the optimum salinity for juvenile banana prawns *F. merguiensis* is 25 ‰ (Staples and Heales, 1991). The GOT and GPT activities in the hepatopancreas at a temperature of 24°C were significantly higher than those at the other temperature conditions (p<0.05). The SDH activity in the muscle at a temperature of 21°C was lower than those at temperatures of 24°C and 27°C (p<0.05). The activities of GPT in gill and digestive glands increased with elevated temperature (Hu *et al*., 2015). Hepatic GPT and GOT activities in fish *Labeo rohita* were significantly decreased in the groups exposed to a higher temperature (32°C) compared with the groups at a lower temperature (26°C) (Kumar *et al*., 2013). The SDH activities in the gill, muscle, and hepatopancreas of *L. vannamei* decreased with decreased in temperature (Wu *et al*., 2017). These results indicated that aerobic metabolism was probably weakened which were caused by respiratory disorder under low temperature conditions (Wu *et al*., 2017). These results hinted the suitable temperature for *F. merguiensis* is about 24-27°C. Similar results were obtained based on the growth and survival data (Qian *et al*., 2015).

The metabolism enzymes in different tissues showed different change trends while the aquaculture exposed to the different ambient conditions. The liver is richer in GOT and GPT than serum. Serum GOT and GPT levels increased with hepatopancreatic damage, and are important diagnostic tools in human and animals, and are used to indicators of environmental risks (Allah and Hameid, 2009). Hemolymphatic GOT and GPT activities in *L. vannamei* were increased after exposure to heavy metals. The hepatopancreas is rich in GOT and GPT, and damage to it can result in the liberation of large quantities of these enzymes into the blood (Wu *et al*., 2008). So tissue type should be taken care of when

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comparing and dissecting the GOT and GPT activities.

ACP can be used as a reliable index in the assessment of the immune status of penaeid prawns (Sarlin and Philip, 2011). *L. vannamei* fed a diet containing polysaccharide extract had significantly increased the ACP activities in the gills and hepatopancreas when compared to controls (Liu *et al*., 2011). The ACP activities in *P. monodon* fed with guava leaves diet also were positively regulated (Yin *et al*., 2014). In the current study, the highest ACP activity in the muscle was observed at a temperature of 24°C (p<0.05) However, the lowest ACP activities in the hepatopancreas and muscle were observed at salinities of 25 and 20 ‰ (p<0.05). Higher ACP activity was observed for shrimp at a salinity of 35 ‰ (Selven and Philip, 2013). In general, ACP activity was higher at intermediate salinities (Wang *et al*., 2016). According to Wang *et al*. (2016), one reason could be that the animal enhanced liver metabolism and energy supply as subjecting to long-term stress of such inappropriate conditions. The other reason was the long-term stress lead to reduced immunity, and pathogen invasion caused a corresponding stress response (Wang *et al*., 2016).

Shrimp fed with guava leaf diets experienced an increased SOD activity in the hepatopancreas (Yin *et al*., 2014). In the present study, the SOD activities in the hepatopancreas and muscle at a salinity of 25 ‰ were higher than those at salinities of 15 and 20 ‰. The results indicated shrimp at a salinity of 25 ‰ should be healthier than other salinity. However, the SOD activity in the muscle at a salinity of 10 ‰ also was higher than that at salinities of 15 and 20 ‰. Higher SOD activities in shrimp at lower salinity might indicate that the stress of low salinity resulted in an accumulation of radicals to a higher level in shrimp (Li *et al*., 2008). The higher the SOD value is, the more superoxide radicals need to be reacted (Liñán-Cabello *et al*., 2003). SOD activities are related to the status of the organisms affected by different factors including dietary nutrition, environmental factors, etc. (Winston and Giulio, 1991). However, the physiological relationship between these parameters and shrimp health is not yet established (Niu *et al*., 2016). Therefore, more detailed investigation between shrimp health state and immune parameters should be needed in further study.

In conclusion, the present study indicates that salinity and temperature alter metabolic and immune enzyme activities in hepatopancreas and muscle of *F. merguiensis*. Unsuitable salinity and temperature reduce metabolic enzyme activities.

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