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Effects of Water Temperature on Activities of Metabolic Enzymes of Juvenile *Siganus guttatus*

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ABSTRACT

Background: Temperature is one of the most important environmental factors affecting the survival, growth and metabolism of fish. The current study was aimed to study the effects of water temperature on the metabolic enzyme activities of juvenile *Siganus guttatus*.

Methods: The juveniles were domesticated at 28±1°C for two weeks and then the temperature was adjusted to the target temperature groups (31°C, 27°C, 23°C and 19°C) by the gradually increasing or decreasing temperature with the change rate of 2°C per day. The experiment lasted for 70 d. At the end of the experiment, the fish were anesthetized and all the livers were dissected on ice plate and stored in the refrigerator at -80°C for the determination of enzyme activity.

Result: The activities of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), hexokinase (HK) and pyruvate kinase (PK), lipoprotein lipase (LPL) and hepatic lipase (HL) tend to be increased with the reduction of temperature. The above enzymes activities in 19°C group were highest. The activity of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and citrate synthase (CS) was lowest in 19°C. These results suggests that 19°C had exceeded the suitable temperature range for juvenile *S. guttatus*. At low temperature, *S. guttatus* mainly use fat for energy, but less anaerobic metabolism for energy.

Key words: Metabolic enzymes, *Siganus guttatus*, Temperature.

INTRODUCTION

Temperature is one of the most important environmental factors affecting the survival, growth and metabolism of fish (Sun *et al*., 2006; Atkins and Benfey, 2008; Shi *et al*., 2019). In the process of fish adaptation to the environment, different physiological and biochemical strategies take place to cope with the temperature change (White *et al*., 2012; Mizanur *et al*., 2014). Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) are two of the most important transaminases in the organism, which can reflect the level of protein metabolism in the body. Glycolysis occurs in almost all cells and is one of the most important metabolic pathways in organisms. Hexokinase (HK) and pyruvate kinase (PK) are important allosteric modulators in the glycolytic process. Lactate dehydrogenase (LDH) is the last regulatory enzyme of anaerobic metabolism, its' activity reflects the strength of anaerobic metabolism (Zakhartsev, 2004). As the best energy substance, lipid plays an important role in the biological response to the unsuitable environment. Lipoprotein lipase (LPL) and hepatic lipase (HL), as important regulatory enzymes, directly affect the lipid metabolism of fish. Tricarboxylic acid cycle is the ultimate metabolic pathway for carbohydrates, lipids and proteins and most of the body's energy needs are largely provided by tricarboxylic acid cycle. The activities of succinate dehydrogenase (SDH) and citrate synthase (CS) reflect the levels of aerobic metabolism to a certain extent (Wang *et al*., 2002).

Siganus guttatus belongs to the family Siganidae within the order Perciformes, is mainly found in the tropical and subtropical Indian, Pacific and South China Sea, distributed from coral reefs to estuarine waters (Liu *et al*., 2009). *S. guttatus* is an omnivorous fish, which likes to eat a variety of algae, especially *Enteromorpha prolifera* (Westernhagen, 1973). *S. guttatus* can be cultured preferably due to its rapid growth and excellent disease resistance, which is applicable to a variety of ecological breeding modes (Gao *et al*., 2010). In recent years, *S. guttatus* as a new breeding species has aroused widespread concern and the scale of breeding has gradually expanded. In China, the artificial breeding and the trophic component of *S. guttatus* had already been studied (Song *et al*., 2018; Huang *et al*., 2018). To date, there are studies focusing on the reproduction, development and growth of the species (Wang *et al*., 2011; Liu *et al*., 2015), while the metabolism of *S. guttatus* at different temperature has not been reported. In order to explore the metabolic regulation ability of this species to adapt to the water temperature in different areas during the promotion from southern warm waters to northern cool waters. In this study, we discussed the changes of liver metabolic enzyme activity

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of this species under different temperature conditions, so as to provide basic data and theoretical guidance for the promotion and breeding of this species in different temperature areas.

MATERIALS AND METHODS

The artificially bred *S. guttatus* juveniles by Qionghai Research Center in Hainan Province were air-transported to East China Sea Fisheries Research Institute in Shanghai. The healthy juveniles, having uniform size with an average body weight of 0.83±0.03 g, were selected for the experiment.

The *S. guttatus* juveniles were domesticated at 28±1°C for two weeks and then the temperature was adjusted to the target temperature groups (31°C, 27°C, 23°C and 19°C) by gradually increasing or decreasing temperature with the change rate of 2°C per day (Liu *et al*., 2015). The juveniles were equally assigned into 3 replicates with 30 fish in each group in the tank of 670 L capacity. The experiment was carried out in East China Sea Fisheries Research Institute in 2015 and lasted for 70 d.

At the end of the experiment, the fish were anesthetized with MS-222 (200 mg·L^{-1}). All the livers were dissected on ice plate and stored in the refrigerator at -80°C. The liver tissues were taken out and weighed before determination of the enzyme activity. The precooled saline (0.65%) was added into the liver tissue according to the mass-volume ratio of 1:9. The homogenate was homogenized in ice bath by high-speed tissue homogenizer. The homogenate was centrifuged at 4°C for 10 min at 3500 rpm, the supernatant was placed in the refrigerator (4°C) for further use. The determination of enzyme activity was completed within 8 hours.

The determination of enzyme activity and definition of enzyme activity unit were according to Song (2015) methods, as follows:

GPT and GOT activity units

At 25°C, the pyruvate produced by the reaction of 1 min per mg protein in the tissue oxidizes NADH to NAD^+, resulting in the decrease of absorbance value by 0.001 as an activity unit.

PK activity unit

At 37°C and pH 7.6, 1 μmol of PEP per gram of tissue protein per minute is converted into pyruvate as an activity unit.

HK activity unit

At 37°C and pH 7.6, 1 mmol·L^{-1} of NADPH is generated per gram of tissue protein in the reaction system per minute as an activity unit.

LDH activity unit

At 37°C, 1 μmol pyruvate is produced by the interaction of tissue protein and matrix for 15 min as an activity unit.

LPL and HL activity unit

Each mg of histone produces 1μmol of free fatty acid per hour in the reaction system as an activity unit.

SDH activity unit

At 37°C, the absorbance of the reaction system is reduced by 0.01 to 1 per mg protein per minute as an activity unit.

CS activity unit

At 37°C, CS enzyme activity is calculated by measuring the decrease of DTNB in the reaction system within 15 minutes as an activity unit.

One-way ANOVA was used to determine the differences of enzyme activities among various groups. Data are presented as means ± SD at statistically significant level of $P<0.05$. All statistics were performed using SPSS (version 19.0).

RESULTS AND DISCUSSION

Effects of temperature on GPT and GOT activity

Water temperature is one of the most important ecological factors affecting the behavior and physiological process of aquatic organisms (Xia and Li, 2010). GPT and GOT are the two most important transaminases in organisms, which play an important role in the metabolism of amino acids and the transformation of nutrients.

The activity of GPT and GOT in the liver of juvenile *S. guttatus* increased gradually with the decrease of temperature (Fig 1). GPT and GOT activities increased with the decrease of temperature in the tissues of the organisms within the moderate temperature range (Hochachka and Somero, 1984). Zhang *et al*. (2010) also found that the GPT activity in the blood of *Scortum barcoo* decreases with the temperature increasing from 16°C to 28°C. GPT and GOT activities show decreasing trend with the increase of temperature, which is consistent with the results in this study. A significant increase in GPT activity indicates an interruption in liver metabolism, while a significant increase in GOT activity shows an interruption in heart or muscle metabolism. The warm-water fish *S. guttatus* needs to maintain a higher basic metabolism to adapt to the lower temperature and the protein metabolism is more active, the transaminase activity is also increased.

Effects of temperature on HK, PK and LDH activity

HK and PK are the most important regulatory enzymes in glycolysis and their activities play an important role in maintaining energy flow, which is helpful for fish to adapt to different temperature (Low and Somero, 1976). HK catalyzes the first step of glycolysis to produce glucose-6-phosphate. PK catalyzes the final step of glycolysis, transferring the phosphate group of phosphoenolpyruvic acid (PEP) to adenosine diphosphate (ADP), to form adenosine triphosph (ATP), which releases pyruvic acid. The stability of PK spatial conformation is related to the temperature and the fluctuation of temperature may lead to the change of PK activity (Lockwood and Somero, 2012). The response of HK and PK to temperature is very complex and the response is different among different species. Some studies showed that

the activities of HK and PK decrease with the reduction of temperature (Fudge *et al*., 1997; Valerie and Douglas, 1997), while some studies showed that the HK and PK activity increases gradually with the decrease of temperature (Valerie and Douglas, 1997; He *et al*., 2015). The activity of HK and PK in liver of juvenile *S. guttatus* at 19°C is significantly higher than that of other temperature groups (Fig 2), which was similar with the results in *Fundulus majalis* for HK (Valerie and Douglas, 1997) and in *Thunnus thynnus* for PK (Fudge *et al*., 1997). The above results indicated that HK and PK activity exhibited species specificity with the change of temperature. The metabolism of fish changes adaptively under different acclimation temperatures. The activity of HK and PK increases significantly with the decrease of temperature for the juvenile *S. guttatus*, which indicates that the activity of glycolytic process might be more active under low temperature.

LDH activity is closely related to the clearance and tolerance of lactic acid during anaerobic exercise, which is one of the indexes of anaerobic metabolism (Zakhartsev *et al*., 2004). Temperature has a significant effect on the structure and function of LDH (Nikolai *et al*., 1994). The anaerobic metabolism changes adaptively under low temperature, but different species respond differently to temperature. The LDH activity in *Ctenopharyngodon idellus* increased with the decrease of temperature, while that in *Cyprinus carpio* decreased with the decrease of temperature (He *et al*., 2015). The complex changes of LDH activity with the decrease of temperature may be related to the less participation of anaerobic fermentation in the process of energy metabolism (Somero and Hochachka, 1968). At 19°C, LDH activity of juvenile *S. guttatus* is significantly lower than that of the other groups (Fig 2). It may be that 19°C has exceeded the suitable temperature range for this species, which leads its abnormal metabolism.

Effects of temperature on LPL and HL activity

LPL is a rate-limiting enzyme that catalyzes the hydrolysis of triglyceride (TG) and is also a key enzyme in regulating lipid deposition and lipid metabolism. It is involved in the metabolism of various lipoproteins and it degrades the lipid-rich lipoprotein in the plasma. It is also involved in the transformation of carrier proteins and phospholipids between very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) (Mead *et al*., 2002). The tissue division of LPL in different fishes shows some differences. LPL in adipose tissue of *Sparus aurata* is several times higher than in liver and skeletal muscle (Saera-Vila *et al*., 2005) and

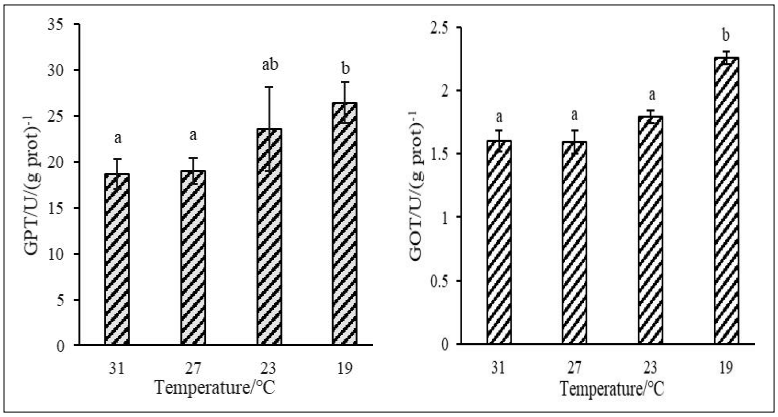

Fig 1: Activity of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) in livers of juvenile *Siganus guttatus* under different temperature levels. Different letters indicate significant differences in different temperature groups (P<0.05).

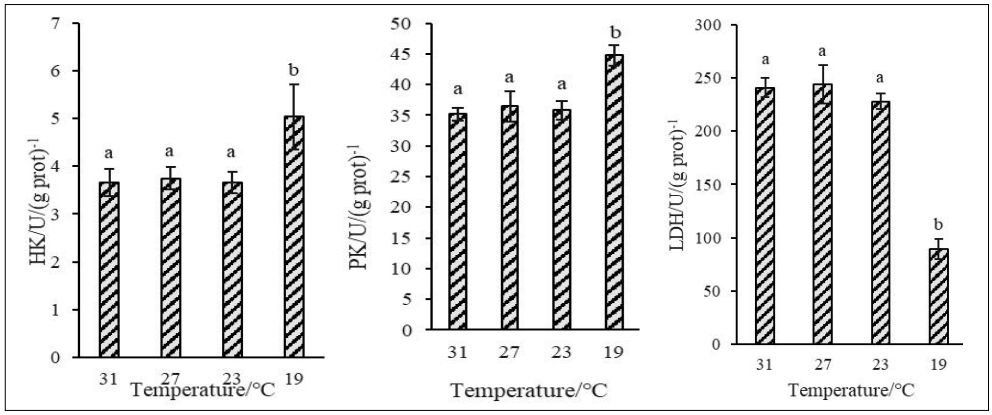

Fig 2: Activity of hexokinase (HK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) in livers of juvenile *Siganus guttatus* under different temperature levels. Different letters indicate significant differences in different temperature groups (P<0.05).

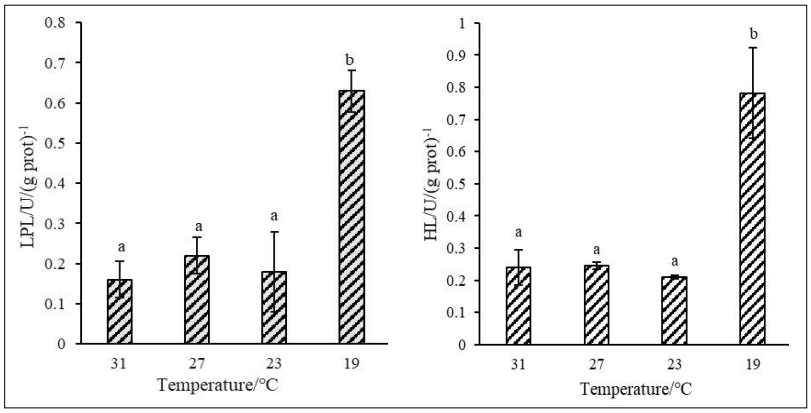

Fig 3: Activity of lipoprotein lipase (LPL) and hepatic lipase (HL) in livers of juvenile *Siganus guttatus* under different temperature levels. Different letters indicate significant differences in different temperature groups (P<0.05).

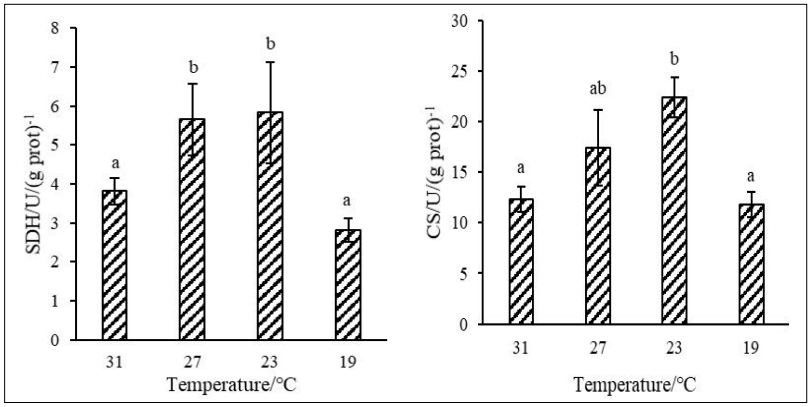

Fig 4: Activity of succinate dehydrogenase (SDH) and citrate synthase (CS) in livers of juvenile *Siganus guttatus* under different temperature levels. Different letters indicate significant differences in different temperature groups (P<0.05).

LPL is mainly expressed in liver of *Oncorhynchus mykiss* (Albalat *et al*., 2006). HL is synthesized in hepatocytes and acts as a ligand to promote the entry of low-density lipoprotein (LDL) and chylomicron (CM) into hepatocytes and is directly involved in the reverse transport of cholesterol in HDL and the breakdown of residual HDL particles (Zhu *et al*., 2010). Temperature has a significant effect on lipid metabolism. The low temperature conditions at 10°C can promote fatty acid oxidation for *Sparus aurata* (Kyprianou *et al*., 2010). Studies have shown that in response to environmental stress, the expression of fatty acid binding protein in fish livers and the use of fat by liver cells have increased. With the cooling of season and the decrease of temperature, the lipid oxidation and storage capacity in *Oncorhynchus mykiss* increased (Albalat *et al*., 2006). LPL and HL activities in the liver of juvenile *S. guttatus* are significantly increased at 19°C (Fig 3). It is suggested that the lipid metabolism of juvenile *S. guttatus* is enhanced at 19°C.

Effect of temperature on SDH and CS activity

As an important metabolic pathway, tricarboxylic acid cycle plays an important role not only in the oxidative degradation of glucose, lipid and protein, but also in the anabolism of many important biomolecules. The only enzyme embedded in tricarboxylic acid cycle of SDH can reflect the level of aerobic metabolism to some extent (Wang *et al*., 2002). Most studies show that SDH activity increases with the decrease of temperature (Hazel 1972a, b). CS, as an aerobic metabolic enzyme in mitochondria, catalyzes the synthesis of citric acid. Its activity may reflect the ability of the body to provide energy through aerobic metabolism. CS activities in most of fish increases with the decrease of acclimation temperature (Kawall *et al*., 2002; Lucassen *et al*., 2006), which is considered to be an adaptive response of fish to the adverse effects of low temperature on metabolic rate by increasing metabolic capacity (Hazel and Prosser, 1974). SDH and CS activities in liver of juvenile *S. guttatus* increase gradually with the decrease of temperature from 31°C to 23°C (Fig 4), which is consistent with the above results. However, SDH and CS activities in liver of juvenile *S. guttatus* at 19°C decreased (Fig 4), which indicated that the long-term exposure to the low temperature may have adverse effects on its growth, immunity and other aspects.

CONCLUSION

The water temperature from 23°C to 31°C may be within the suitable temperature range for the juvenile *S. guttatus*

and there is no significant difference in liver metabolic enzyme activity within this range. When the temperature is 19°C, the lipid-metabolizing enzymes activities of LPL and HL in liver increase obviously, indicating that the fish may use more energy from fat to resist low temperature. The obvious decrease of LDH activity at 19°C may be due to long-time energy-supplying by aerobic oxidation of fat, which showed that the fish may adaptively regulate the activity of anaerobic metabolic enzymes to save energy to cope with the low temperature.

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