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The effect of hyperthermia on liver histology, oxidative stress and disease resistance of the Wuchang bream, *Megalobrama amblycephala*

Bo Liu [a], Pao Xu [a], Paul B. Brown [b], Jun Xie [a, *], Xianping Ge [a, **], Linghong Miao [a], Qunlan Zhou [a], Mingchun Ren [a], Liangkun Pan [a]

[a] Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, PR China
[b] Department of Forestry and Natural Resources, Purdue University, West Lafayette, 47907, Indiana, USA

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ABSTRACT

This study aimed to investigate the effects of hyperthermia on serum hormones, hepatic oxidization indices, hepatic heat shock protein (HSP60, 70, and 90) mRNA expression levels and liver cell ultrastructure in *Megalobrama amblycephala* before and after high temperature stress. Fish were exposed to the optimal temperature (25 ± 1 °C) or high temperature (32 ± 1 °C) and then challenged with *Aeromonas hydrophila*. The results showed that hyperthermic stress significantly increased serum adrenocorticotropic hormone (ACTH) at 0.5 and 2 d, serum cortisol (COR) at 0.5, 14, and 21 d and serum 3,5,3′-triiodothyronine (T3) at 1, 14, and 21 d after stress. Additionally, hyperthermia led to oxidative stress, as evidenced by a significant decrease in the hepatic anti-superoxide anion free radical concentration (ASAFER) at 1, 2, 7, and 21 d and in hepatic superoxide dismutase (SOD) activity at 1, 2, 14 and 21 d after stress; however, hepatic malondialdehyde content (MDA) increased at 1, 2, and 7 d after stress. Moreover, the expression of HSP60 at 1 d, HSP70 at 1 and 2 d, and HSP90 at 0.25, 0.5, 1 and 2 d after stress was higher in the stress group compared with the control group. The histological results clearly showed that hyperthermia resulted in fat and glycogen accumulation and structural alterations of the hepatocytes, mitochondria, and nuclei. The cumulative mortality increased in the high temperature stress group at 1 d after acute stress and at 2 and 7 d after chronic stress compared with the control group. Overall, 1 d or 2 d after hyperthermia stress damaged the hepatic ultrastructure and impaired mitochondrial bioenergetics. Dysfunction of the mitochondria subsequently mediated oxidative stress and improved HSP expression modulated the cellular anti-stress response, which in turn led to reduced efficacy of the immune system and increased mortality from *Aeromonas hydrophila* infection in *Megalobrama amblycephala*.

1. Introduction

Temperature is an important ecological factor. Temperatures above the optimum affect growth performance and physiological functions of fish, and they increase concentrations of harmful reactive oxygen species (ROS) [1–4]. Cellular ROS cause damage to DNA, a general disturbance of the cellular redox balance [5], and increase susceptibility to infection [6,7]. In addition, ROS, especially HO•, lead to a chain reaction and severe injury to plasma membranes, cause abnormal cellular function and loss of membrane integrity, and result in protein carbonylation and cellular aggregation or fragmentation [8].

To cope with these injuries, fish possess an efficient innate immune and antioxidant defense system to protect themselves against hyperthermia stress and maintain biochemical, molecular and physiological homeostasis. Antioxidant enzymes such as superoxide dismutase (SOD) and antisuperoxide anion free radical (ASAFR) directly detoxify harmful ROS and other compounds involved in ROS generation [9]. SOD is a well-known antioxidative enzyme that converts superoxide to hydrogen peroxide and oxygen, and catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen to remove the free radical of oxygen and reduce lipid peroxidation damage [10,11]. In addition to hyperthermia stress, the elevation of ROS is known to increase heat shock factor [12] and heat shock protein (HSP) expression levels

* Corresponding author. Present address: No.9 Shanshui East Road, FFRC CAFS, Wuxi 214081, PR China.
** Corresponding author.
E-mail addresses: liub@ffrc.cn (B. Liu), Xiej@ffrc.cn (J. Xie), gexp@ffrc.cn (X. Ge).

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[13], which prevent protein aggregation, assist in refolding any misfolded proteins, and maintain the integrity of the mitochondrial membrane [14–16]. Thus, the relationships between oxidative stress, antioxidants and HSPs play an important role in fish survival during exposure to elevated temperatures [17].

Previous studies have shown that histological examination of the liver could provide an index of the general condition of the fish [18,19]. Mitochondria are considered to be the major source of ROS production [20,21]. Moreover, ROS are also produced by the microsomal systems of the endoplasmic reticulum [22]. An understanding of the organelle ultrastructure could provide additional information regarding fish health and the metabolic condition. However, to our knowledge, the effects of hyperthermia-induced oxidative stress on the hepatic ultrastructure of *Megalobrama amblycephala* remain poorly studied.

Megalobrama amblycephala, also known as the blunt snout or Wuchang bream, is one of the principal species in Chinese freshwater culture systems. Production of this species in China reached approximately 0.73 million tons in 2013 [23]. The optimal temperature for the growth of *M. amblycephala* is 25–28 °C, but they commonly experience water temperatures of more than 32 °C; chronically elevated temperatures above 30 °C for 7–15 days or more in summer are common. Hyperthermia may increase *M. amblycephala* vulnerability to opportunistic bacterial pathogens and result in significant economic losses [24]. However, information regarding the physiological mechanisms, oxidative stress and pathogen invasion of *M. amblycephala* during increasing temperatures is not well understood. Therefore, we hypothesized that high temperature would affect the immune ability of the fish and increase the prevalence of bacterial infection. To test our hypothesis, the fish were exposed to the optimal temperature (25 ± 1 °C) or high temperature (32 ± 1 °C), and then challenged with bacteria. To compare the efficiency of their oxidative system and thermal resistance ability, we examined serum hormones, hepatic oxidization indices, hepatic HSP mRNA expression levels and liver cell ultrastructure during exposure. Using these experiments, we expected to gain an understanding of the relationship of high temperature-induced oxidative stress and disease resistance in fish challenged with bacteria and high-temperature stress. The results would provide guidance for understanding high-temperature stress and immune defense and the potential adverse effects of this condition on fish.

2. Materials and methods

2.1. Fish

We obtained 360 healthy *M. amblycephala* of a similar size (mean weight: 77.04 ± 2.18 g) from the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, China. The fish were placed in an indoor recirculating system containing 24 round fiberglass tanks (φ820 × 700 mm, N = 15 fish/tank) and acclimated for 20 d. The experimental facilities consisted of a thermo-regulated recirculating system and mechanical filtration units with recycled water at a rate of 3 L min^{-1}. All fish were starved for 24 h before the experiments. After acclimation, the fish in the 12 tanks were randomly divided into two groups (6 tanks per group) for acute or chronic high temperature stress experiments: control group (CT group, commercial diet with a water temperature of 25 ± 1 °C), high temperature stress group (HTS group, commercial diet with a water temperature of 32 ± 1 °C). The fish in the remaining 12 tanks were randomly divided into four groups (3 tanks per group) for the pathogenic infection experiment. One was the control group (commercial diet with a water temperature of 25 ± 1 °C) for *Aeromonas hydrophila* infection, and the other groups were exposed to three temperature stresses (commercial diet with a water temperature of 32 ± 1 °C) placed in water at 32 ± 1 °C on d 1, 2 and 7 and then challenged with *Aeromonas hydrophila.*

Daily removal of 5–6% water from the tanks was performed to remove leftover feed and excreta, and replenishment of the same volume and temperature of freshwater was provided in each group. During the experimental period, the water temperature in the each tank was monitored using data logger and adjusted using thermostatic water heaters (XiLong XL-888, range 20–34 °C).

An optimum extruded commercial diet with a proximate composition of 32.16% crude protein and 6.17% lipid (dry matter basis) was obtained from Wuxi Tongwei Feed Co. Ltd., China. During acclimation, the fish were hand-fed to apparent satiation three times daily (8:00–8:30, 12:00–12:30, and 16:00–16:30). During the experimental period, the water temperature for the control and high temperature stress groups was maintained at 25 ± 1 °C and 32 ± 1 °C, respectively. The water quality was monitored weekly to ensure that the conditions remained within ranges acceptable for fish growth; the dissolved oxygen concentration of the water was >6 mg/L, the ammonia-nitrogen concentration was <0.05 mg/L, the photoperiod was 12-h light and 12-h dark, and the pH remained between 7.60 and 7.80 throughout the study.

2.2. Challenge experiments

2.2.1. Acute high temperature stress

According to the procedure described by Fast et al. [25], there were two experimental groups: the control (CT) group and the stressed (HTS) group (6 replicate tanks in each group). The CT and HTS groups were maintained in the same water temperature of 25 ± 1 °C for 30 d of acclimation. The water temperature in the CT control group was subsequently maintained at 25 ± 1 °C. The fish in the HTS group were subjected to an acute heat shock and maintained at 32 ± 1 °C for 24 h. The water temperature of the 25 ± 1 °C system was quickly raised to 32 ± 1 °C within 3 h.

During the stress experiment, the water temperature was monitored using a data logger, and feed was withheld. Dissolved oxygen was not less than 6.0 mg/L, and ammonia nitrogen was lower than 0.05 mg/L. Minimal human interference prevented the fish from experiencing additional stress. Six samples (1 fish per replicate tank) were taken prior to the heat stress and at 0.5 d and 1 d after challenge.

2.2.2. Chronic high temperature stress

After the acute high temperature stress, the CT control and HTS group were maintained in the same water temperature of 25 ± 1 °C or 32 ± 1 °C for 21 d, respectively. During the chronic high temperature stress stage, the fish were hand-fed to apparent satiation once daily (8:00–8:30). The water temperature was monitored using a data logger; dissolved oxygen was not less than 6.0 mg/L, and ammonia nitrogen was lower than 0.05 mg/L. Six samples (1 fish per replicate tank) were collected on d 2, 7, 14 and 21 of the experiment.

2.2.3. Pathogen infection

Four groups (3 tanks per group) were assessed in the pathogen infection experiment: the control group with a water temperature of 25 °C, and three high temperature stress groups with a water temperature of 32 °C. Before application of the stress, and at 1 d (acute stress point), 2 d and 7 d (one of chronic stress points) after stress, the fish were challenged with the bacterial septicemia pathogen *A. hydrophila*. Thirty fish (3 tanks/group, N = 10 fish/tank) at each time point were used in the infection experiment. According to the method described by Liu et al. [26], the strain of *A. hydrophi1a* was activated using agar medium and then purified.