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High temperature tolerance limits and changes of hemato-biochemical parameters of mrigal, *Cirrhinus mrigala*

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Abstract

The change in water temperature due to anthropogenic and climatic changes is expected to affect the physiology of fish. In this study, we determined high temperature tolerance (CT_{max}) and oxygen consumption rate of Indian major carp, Mrigal, *Cirrhinus mrigala* fingerlings followed by the acclimatization at three temperature regimes (31, 34, 37 °C). During the determination of CT_{max}, we analyzed the major hemato-biochemical indices - hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), and blood glucose levels in the sampled fish at the starting point and end point of the acclimated temperatures. Significantly decreased CT_{max} of the fish was found at 37 °C compared to 31 °C and 34 °C. Conversely, oxygen consumption rate of the fish significantly increased at 37 °C compared to 31 °C and 34 °C. The Hb levels and RBC content of the fish showed a significant decrease at the end point of thermal tolerance in each temperature group and even with the increase of acclimation temperature. On the other hand, WBC and blood glucose levels exhibited opposite scenario. Hematological and biochemical parameters have been acknowledged as valuable tools for monitoring fish health and monitoring any changes in the quality of water. The hematological studies on fishes have assumed greater significance due to the increasing emphasis on aquaculture. This research provides the knowledge of the characteristics of hematological parameters of freshwater fishes.

Keywords: mrigal fish, temperature, thermal tolerance

Introduction

Temperature has gained a paramount importance among multiple environmental variables for the development and growth of aquatic organisms. Cheung model depicts the projection that one degree Celsius temperature increase can reduce more than 3 million metric tons of prospective catches. Evolutionarily fishes are adapted to live within a specific range of environmental variation, and beyond that range poses a threat for their health (Barton *et al.* 2002) [4]. Almost all teleost species adopt their own special physiological and behavioral mechanisms that facilitate them to subsist against temperature associated stressful conditions (Prosser and Heath 1991) [22]. In the tropics, the normal range of water temperature to which fish adapted is 25–35 °C. Though temperature increase to an optimum level can be favorable for aquaculture pertaining to better growth and early maturity, exceeding that limit may negatively affect the growth by increasing metabolic rates and subsequent oxygen demand that assist pathogenic virulence. Moreover, water temperature affects fish survival, distribution, metabolism, immunity and natural reproduction (Shahjahan *et al.* 2017) [27], and high temperature can be stressful for fishes (Shahjahan *et al.* 2018) [28]. Therefore, to understand the impact of changes in temperature on aquatic animals including fish, thermal tolerance and acclimation studies have appeared a considerable tactics for scientists. Acclimation is the total ability of fishes to adapt themselves to short or long-term changes in their surrounding environment (Das *et al.* 2009) [12]. Critical thermal methodology (CTM) is extensively used for the quantification of upper and lower temperatures tolerance of aquatic organisms. Where fish are exposed to a continuous and constant increase or decrease in temperature until temperature

reaches the point of loss of equilibrium or lethal endpoint. The rate of oxygen consumption is another physiological response that can be integrated with the change in environmental parameters due to its direct correlation to metabolic activities and energy flow that affect homeostasis of organisms (Salvato *et al.* 2001) [25]. Hemato-biochemical parameters including blood glucose levels are increasingly used as indicators of physiological stress response to intrinsic or extrinsic changes in fish (Sharmin *et al.* 2015; Salam *et al.* 2015) [24, 29]. Variation in the number of white blood cells induced by different stressors has also been used as a biochemical immunosuppressive indicator for fish (Kopp *et al.* 2010) [19].

Its fry and fingerlings are easily available for culture and traits preferred by the consumers have made the species a suitable aquaculture candidate in Bangladesh. Furthermore, it is an ideal species for carp polyculture system and can be stocked with other carps like Catla (*Catla catla*) and Rui (*Labeo rohita*).

The purpose of the present experiment was to assess how affects the high temperature tolerance (CT max) and oxygen consumption rate and major hemato-biochemical indices - hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), and blood glucose levels in the fish of Mrigal, *Cirrhinus mrigala*.

Materials and Methods

The materials used and methodologies followed are described under the following headings:

Experimental site and study period

The present study was conducted at the fish Ecophysiology laboratory, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh from March to April 2021.

Selection of test fish species

Apparently healthy and active fingerlings of Mrigal were collected from Bangladesh Fisheries Research Institute (BFRI) Freshwater Station, Mymensingh. The individual average weight and total length of fish were 18.26 ± 0.42 g and 15.36 ± 0.52 cm, respectively. The collected fingerlings were acclimatized in the laboratory for 15 days. The fingerlings were fed commercial diet containing 28% crude protein (Mega Fish Feeds Ltd., Bangladesh) twice daily at 10 am and 4 pm up to satiation. The experimental procedures followed the guidance approved by the Animal Care and Use Committee of Bangladesh Agricultural University, Mymensingh.

Experimental design

The experiment was conducted with three treatments each with three replications. The fish were exposed to three temperature conditions viz; 31 °C, 34 °C and 37 °C. Twenty fivemrigal fingerlings were stocked in each of the nine cleaned glass aquaria (75 cm × 45 cm × 45 cm) filled each with 100L of tap water. To acclimatize the fish to high temperature, temperature was gradually increased ($\Delta 1$ °C per 24 h) to the target temperature conditions (31 °C, 34 °C and 37 °C). The required temperature was maintained by using thermostat (REI-SEA, Japan, 300 WATTS). Adequate aeration was maintained throughout the experimental period.

Determination of high temperature tolerance limit of Rohu

The 225 uniform size fingerlings were equally distributed among three treatments (31 °C, 34 °C and 37 °C) each with three replications following a completely randomized design, with a stocking density of 25 fingerlings/100L water. The temperature was gradually increased by 1 °C/day to 31 °C, 34 °C and 37 °C for 20 days. To estimate thermal tolerance (critical thermal maxima; CT max) of Mrigal, randomly selected five fingerlings of each acclimation temperature was transferred to 50 L aquarium, the designated starting point. Then the fish was exposed to a constant increase of temperature (1 °C/30 min) till the loss of equilibrium (LOE), the designated end point.

Determination of whole-body oxygen consumption

Oxygen concentration in the water was measured every 15 min with a DO meter. Measurement was made at oxygen saturation >70% to avoid any possible influence of the dissolved oxygen concentration on fish oxygen consumption. Oxygen consumption rate (OCR) was calculated using the equation: $OCR = [(O_i - O_f) \times V / (T \times B)]$, where O_i and O_f correspond to the water oxygen content (mg O₂/L) at the beginning and the end of the measurement period, respectively; V is the tank volume (L); B is the fish biomass (g) and T is the measurement duration (h). Results were expressed in mg O₂/kg/h.

Measurement of hemato-biochemical parameters

During the determination of high temperature tolerance, several hemato-biochemical parameters, such, hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs) and blood glucose levels were measured in the sampled fish of starting point and end point of each acclimated temperature. Immediately after collection of blood samples, Hb levels (g/dL) were measured using hemoglobin strips in a digital EasyMate® GHb, blood glucose/hemoglobin dual-function monitoring system (Model: ET- 232, Biopik Technology Inc. Taiwan 35057). The RBCs and WBCs were counted using an improved Neubauer Hemocytometer under a light microscope. Similar to Hb, blood glucose levels (mg/dL) was measured using glucose strips in a digital EasyMate® GHb, blood glucose/hemoglobin dual-function monitoring system.

Monitoring water quality parameters

Water quality parameters such as dissolved oxygen (mg/L), free CO₂ (mg/L), pH and total alkalinity (mg/L) were measured during the experimental period. Dissolved oxygen (mg/L) was measured with a DO meter (Model DO5509, Lutron, made in Taiwan) and pH using a portable pH meter (Model RI 02895, HANNA Instruments Co.) by directly inserting the probe into the aquaria. The sample water was collected and brought to the nearby Laboratory of Water Quality for analyzing free CO₂ and total alkalinity. Free CO₂ (mg/L) was monitored using phenolphthalein indicator and 0.0227N NaOH titrant, and the total alkalinity (mg/L) was measured by titrimetric method using methyl orange indicator and 0.02N H₂SO₄ titrant.

Statistical analyses

All the values were represented as mean ± standard deviation. To test the statistically significant difference among the

different temperature conditions, one-way analysis of variance (ANOVA) was carried out followed by Tukey's post hoc test. Mann-Whitney U test with a Bonferroni correction was used to assess the significant difference among the days of exposure to different temperature treatments. Statistical level of significance was set at $p < 0.05$. Statistical analyses were carried out using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

Thermal tolerance and oxygen consumption

Primarily the upper thermal tolerance (CT max) of *Cirrhinus mrigala* fingerlings were assessed considering the point where

fish showed loss of equilibrium (LOE). Data related to thermal tolerance and oxygen consumption of *C. mrigala* fingerlings are shown in Table 1. Thermal tolerance level (CT max) showed variation among acclimation temperature (31, 34, 37 °C). Significantly ($p < 0.05$) decreased CT max (42.5 ± 0.27) was found at 37 °C compared to 31 °C (46.5 ± 0.79) and 34 °C (44.0 ± 0.59) acclimated temperatures. Conversely, significantly ($p < 0.05$) increased level of oxygen consumption rate (mg O₂/kg/h) of *C. mrigala* fingerlings was recorded with increasing acclimation temperature (Table 1). Mean oxygen consumption rate at 31, 34 and 37 °C were 55.20 ± 0.05 , 67.49 ± 0.31 , 79.72 ± 0.87 mg O₂/kg/h, respectively.

Table 1: High temperature tolerance (CT max) and oxygen consumption of Mrigal fingerlings acclimated at three different temperature conditions

Parameters	Acclimation temperature (°C)		
	31	34	37
CT max	46.5 ± 0.79^a	44.0 ± 0.59^a	42.5 ± 0.27^b
Oxygen consumption (mg O ₂ /kg/h)	55.20 ± 0.05^a	67.49 ± 0.31^{ab}	79.72 ± 0.87^c

Values with different alphabetical superscripts in a row differ significantly ($p < 0.05$) among acclimation temperature (°C). All values expressed as mean \pm SD.

Changes in hemato-biochemical parameters

Hemato-biochemical parameters during the determination of high temperature tolerance are presented in Table 2. Both Hb levels and RBC content showed a significant decrease at the end point of thermal tolerance at each acclimated temperature

groups and even with the increase of acclimation temperature. On the other hand, WBC content exhibited opposite scenario. Sharp rise of glucose levels were also found at the endpoint of temperature tolerance at each acclimated temperature groups.

Table 2: Changes in hemato-biochemical parameters during the determination of high temperature tolerance

Parameters	Sampling point	Acclimation temperature (°C)		
		31	34	37
Hb (g/dL)	Starting	$12.00 \pm 1.41^{a,1}$	$11.93 \pm 0.55^{a,1,2}$	$11.50 \pm 0.54^{a,2}$
	End	$10.70 \pm 0.73^{b,1}$	$10.45 \pm 1.37^{b,1,2}$	$8.90 \pm 0.71^{b,2}$
RBC ($\times 10^6/\text{mm}^3$)	Starting	$1.84 \pm 0.35^{a,1}$	$1.91 \pm 0.20^{a,1}$	$1.35 \pm 0.25^{a,2}$
	End	$1.39 \pm 0.41^{b,1}$	$1.32 \pm 0.09^{b,2}$	$1.03 \pm 0.07^{b,2}$
WBC ($\times 10^3/\text{mm}^3$)	Starting	$1.23 \pm 0.08^{a,1}$	1.19 ± 0.10^a	$1.31 \pm 0.14^{a,2}$
	End	$1.67 \pm 0.10^{b,1}$	1.85 ± 0.10^b	$1.93 \pm 0.09^{b,2}$
Blood glucose (mg/dL)	Starting	$75.71 \pm 6.34^{a,1}$	$76.00 \pm 8.42^{a,1}$	$81.30 \pm 7.82^{a,2}$
	End	$120.60 \pm 9.39^{b,1}$	$139.33 \pm 10.0^{b,2}$	$151.33 \pm 6.63^{b,2}$

Values of a single hemato-biochemical parameter in a column with different alphabetical superscripts are significantly ($p < 0.05$) different. Values with different numeric superscripts in a row differ significantly ($p < 0.05$) among acclimation temperature (°C). All values expressed as mean \pm SD (n = 6).

Water quality parameters

Values of water quality parameters (Mean \pm SD) for dissolved oxygen, free CO₂, pH and total alkalinity (mg/L) are shown in Table 3. Dissolved oxygen (mg/L) decreased significantly ($p < 0.05$) with increasing water temperature, while free CO₂

(mg/L) increased with increasing temperature. On the other hand, the values of pH and total alkalinity remained nearly unchanged throughout the study period irrespective of the temperature conditions (Table 3).

Table 3: Water quality parameters at three different temperature

Parameters	Sampling point	Acclimation temperature (°C)		
		31	34	37
DO	Starting	7.40	5.80	5.60
	End	2.93	2.62	1.83
Free CO ₂	Starting	9	13	13
	End	8	14	14.5
pH	Starting	7.47	7.22	7.57
	End	7.68	7.06	7.23
Alkalinity	Starting	186	183	189
	End	167	176	191

Discussion

Every fish has a comfort zone of temperature that it can tolerate, beyond which it copes with thermal stress. Such temperature fluctuation may produce a significant disturbance in the normal functions of survival of fish (Beitinger *et al.* 2000) [5]. In the present study, higher CT max values were observed in the *C. mrigala* fingerling exposed to comparatively lower acclimation temperatures (31 °C and 34 °C) followed by a significant decrease in higher acclimation temperature (37 °C). Previously, thermal tolerance studies were performed in *L. rohita* early fry (Chatterjee *et al.* 2004; Das *et al.* 2004; Das *et al.* 2005) [9, 10, 11] showing significant increased CTmax values with the increasing acclimated temperatures, when early fry was acclimated to 25 °C, 30 °C and 35 °C, which are bit inconsistent with our results. Several studies suggested that the value of CT max is affected by a range of variables, the difference in the rate of temperature changes during temperature tolerance studies, the animal size and condition factor (Baker and Heidinger 1996) [3], and the presence of lethal compounds (Beitinger *et al.* 2000) [5]. The decreased CTmax value in high acclimation temperature (36 °C) in contrast to earlier results indicate a temperature limit which may be beyond the optimum range as increased level of plasma cortisol which is used as stress indicator that was found in the higher acclimated temperature (36 °C) in early fry of *L. rohita* (Das *et al.* 2009) [12]. Therefore, our results suggest that high acclimation temperature may suppress the capability of the thermal tolerance of this species.

The metabolic rates of freshwater fish that are usually measured in terms of oxygen consumption show a strong dependency on acclimation temperature because of their immediate effect on the energy flow (Hochachka and Somero 1971; Kita and Setoguma 1996) [16, 18]. Thus, the results of the present study indicate that oxygen consumption of *C. mrigala* is dependent on acclimation temperature. Similar findings were observed on *L. rohita* early fry (Das *et al.* 2005; Brahmane *et al.* 2014) [6, 11], along with *Catla catla*, and *Cirrhinus mrigala* (Das *et al.* 2004) [10], early fingerlings of *L. rohita* and *Cyprinus carpio* (Chatterjee *et al.* 2004) [9], Crucian carp, *Carassius carassius* and *C. auratus* (Sollid *et al.* 2005) [30]. The straight relationship between oxygen consumption and acclimation temperature had also been reported for *Macrobrachium rosenbergii* (Manush *et al.* 2004) [20], *Pangasius pangasius* (Debnath *et al.* 2006) [13], juvenile *Micropterus salmoides* (Díaz *et al.* 2007) [14], *Anabas testudineus* (Sarma *et al.* 2010) [26], and *Tor putitora* (Akhtar *et al.* 2013) [1].

Significant reductions of Hb and RBC were caused due to the failing of hematopoietic system under stressed condition resulting from high temperature. Dealing with thermal stress in either lower temperature (24 °C) or raised temperature (36 °C) and resulting hematological parameters are a common response to hypoxia or anoxia (Carvalho and Fernandes 2006; Hedayati and Tarkhani 2014) [7, 15]. Temperature can cause stress because oxygen solubility drops in water with increasing temperature (Cech and Brauner 2011) [8]. Thermal stress and altering the Hb and RBC content in the Neotropical fish, *Prochilodus scrofa* due to the exposure to raised temperatures (Carvalho and Fernandes 2006) [7] is in agreement with the present study. A decline in the value of Hb and RBC were also observed followed by the exposure in high temperature in the striped catfish, *Pangasianodon hypophthalmus* (Shahjahan *et al.* 2018; Islam *et al.* 2019) [17, 28].

WBCs, the indicators of fish health status, play a significant role in improving nonspecific or innate immunity (Roberts 1978) [23]. Determination of blood glucose is another important indicator to assess the effects of various stressors (Pacheco and Santos 2001) [21]. These observations are in agreement with the study by Shahjahan *et al.* (2018) [28], where blood glucose levels of *P. hypophthalmus* fingerlings were found to increase at high rearing temperature (36 °C) compared to low temperature (28 °C). Similar findings were also observed in *L. rohita* (Alexander *et al.* 2011) [2] with increased levels of blood glucose in fish exposed to high temperature. Thus, our observations indicate that high acclimation temperature may provoke hematocytic stressful condition of *C. mrigala* fingerling and these resulting hematopoietic alterations are compensatory response (Zafalon-Silva *et al.* 2017) [31].

Summary and Conclusion

Among the environmental factors, temperature is an important factor directly affecting molecular, biochemical and physiological processes, particularly in ectothermic vertebrates. The ability to tolerate various temperature ranges differs with species. Water temperature significantly affects some physiological fish processes such as growth and metabolism.

Hematological indices are of different sensitivity to various environmental factors and chemicals. Hematology and clinical chemistry analysis, although not often used in fish medicine, can provide substantial diagnostic information. Studies have shown that when the water quality is affected by different temperature, any physiological changes will be reflected in the values of one or more of hematological parameters.

Temperature has greatly effect on fish body and it's physiological function. Blood cells are greatly affected morphologically due to the high temperature. The present research work was conducted to evaluate the changes of hemato-biochemical parameters in Mrigal during the determination of high temperature tolerance. The experiment was conducted with three treatments. Three temperature conditions such as 31 °C, 34 °C and 37 °C representing treatment one (T₁), treatment two (T₂) and treatment three (T₃) respectively were selected for the experiments.

In conclusion, the effect of three acclimated temperatures on high temperature tolerance, hemato-biochemical parameters were evaluated. Several water quality parameters such as dissolved oxygen, free CO₂, pH, and total alkalinity were recorded during the experiment periods. Though oxygen consumption rate was increased with the increase in acclimated temperatures, CT max was reduced in the high acclimated temperature. Hb and RBC levels decreased but WBC and blood glucose levels increased in fish exposed to high acclimation temperature. Finally, in order to maintain the sustainability of fish production in an apparent climate change situation with gradually increasing temperature, outcomes of the physiological reactions of fish to different acclimation temperatures would assist to better manage the aquaculture.

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