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Md. Shariful Islam
Senior Scientific Officer, Bangladesh Fisheries Research Institute, Freshwater Sub-Station, Chanchra, Jessore, Bangladesh

Mohammad Ashaf-Ud-Doulah
Senior Scientific Officer, Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh, Bangladesh

Md Salmon Hasan Biplob
Senior Upazilla Fisheries Officer
Department of Fisheries
Dharmapasha, Sunamganj
Bangladesh

Md. Saiful Islam
Scientific Officer
Bangladesh Fisheries Research Institute, Mymensingh
Bangladesh

Nafia Binte Ryhan
Lecturer
Department of Fisheries,
EXIM Bank Agricultural University, Chapainawabganj, Bangladesh

Corresponding Author:
Md. Shariful Islam
Senior Scientific Officer, Bangladesh Fisheries Research Institute, Freshwater Sub-Station, Chanchra, Jessore, Bangladesh
Email: sharif.bfri@gmail.com

Effects of temperature on parameters of *calbasu Labeo calbasu* fingerlings at laboratory condition

Md. Shariful Islam, Mohammad Ashaf-Ud-Doulah, Md. Salmon Hasan Biplob, Md. Saiful Islam and Nafia Binte Ryhan

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Abstract
Temperature is an important environmental factor, plays significant role in the physiology of fish and aquatic ecosystem. In this study, we observed growth, hemato-biochemical parameters and water quality of *calbasu*, *Labeo calbasu* exposed to three different temperature regimes, such as 31, 34 and 37 °C respectively for 30 days. Two hundred and twenty fish were used, kept in 9 Aquarium, and were exposed to the different temperature. Highest body weight gain and lowest feed conversion ratio (FCR) was recorded at 31 °C. The highest specific growth rate was recorded at 31 °C followed by 34 °C and 37 °C. Survival at different acclimation temperatures was between 90.00±0.00, 90.0±0.00 and 88.00±0.00%, from lower to higher acclimation temperatures. Dissolved oxygen decreased and free CO_2 increased significantly was found the study period. To determine the hemato-biochemical indices-hemoglobin (Hb) and blood glucose levels in the fish at each temperature. Hemoglobin (Hb) significantly decreased in response to temperature increases, while blood glucose levels displayed the opposite response. The present study revealed that high temperatures may be hazardous to *Labeo calbasu*.

Keywords: Temperature, growth, hematology

Introduction
Global warming is a threat for the aquaculture and fisheries. The increase in water temperature as a consequence of global warming has already started to affect physiological processes in fish, causing a decrease in fish abundance and even the extinction of certain species. Water temperature directly affects the growth and reproduction of fish. Rise of world temperature is thought to be ranged from 0.3 to 6.4 °C in 2090-2099 relative to 1980-1999 (IPCC, 2007) [12]. Global warming resulting from climate change is a threat to eco-systemic integrity (IPCC, 2014) [11]. Increasing surface water temperature and reduction in ice cover over the last decades are quantifiable effects of warming in aquatic ecosystems. The physiological processes in fish, such as growth, metabolism and reproduction, are severely impacted by changes of water temperature (Shahjahan *et al*., 2017) [15]. Elevated water temperature resulting from global warming has caused disrupt embryonic metabolism and physiology of rohu fish. Also displayed evidence of damaged embryos and Larvae of rohu showed developmental deformities (Ashaf-Ud-Doulah *et al*., 2021) [3].
Temperature also changes the physiological functions associated with the stress response of fish. Biochemical parameters, such as cortisol and glucose levels in plasma can be used as general stress indicators in fish. In addition, hematological parameters including hematocrit and hemoglobin are good indicators to assess fish health management in various conditions such as exposure to stress. Growth rate is regarded as the most important parameter that determines the economic aspects of commercial fish culture, which is impacted by various biotic and abiotic factors. Different physiological activities in fish are greatly influenced by water temperature. The effects of temperature changes on fish species may be predicted through physiological studies (Somero, 2010) [17]. Almost all biochemical and physiological activity is greatly affected by rising water temperature that causes stress and alteration of blood chemistry standards because of fish being aquatic poikilothermic animal. Chatterjee *et al*. (2004) [9] stated that high temperature increases the chemical reactions in fish body and greatly affect the physiological process when exceed the level of tolerance.

Hemato-biochemical parameters are measured frequently to assess the physiological status of fish exposed to different stressors related to environmental factors including temperatures (Sharmin *et al*., 2015) [14]. Fish erythrocytes possess hemoglobin that transports oxygen to all the tissues of fish body as a result it bears the probable cues of abnormal level and pathological indications therefore blood hemoglobin contents are frequently used to assess anemia and physiological well-being of fish (Hesser, 2011) [10]. The rise in environmental temperature reduces the dissolved oxygen content in the water which in turn increases the fish metabolic rate, and the fish adjust the adverse environmental condition by raising total hemoglobin level (Brix *et al*., 2004) [7]. Increased temperature affects physiological processes causing a decrease in fish abundance and even the extinction of certain species (Ashaf-Ud-Doulah *et al*., 2019) [1]. It has been reported that the survival, distribution, reproduction and normal metabolism of fish depend on aquatic environmental temperature (Shahjahan *et al*., 2017) [15].

Furthermore, it is an ideal species for carp polyculture system and can be stocked with other carps like catla (Catla catla), rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*). The purpose of the present experiment was to assess growth and hematological parameters in *Labeo calbasu* in different temperature regimes.

Materials and Methods

Experimental design

Healthy and active *Calbasu*, *Labeo calbasu* fry were collected from Bangladesh Fisheries Research Institute (BFRI) freshwater station, Mymensingh and placement in tanks (1000L) at ambient temperature in the Eco-physiology laboratory, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh for 15 days to recover from transportation stress. 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 25fry/100L water. Uniform rearing conditions were maintained in all the experimental groups except for the water temperatures at 31 °C, 34 °C and 37 °C.

Rearing for growth study

Fishes were reared in reinforced glass aquaria having 100 L of water for 15 days at 30 ± 0.5 °C in a controlled environment before starting the experiment. The fishes having average weight of 15.14 ± 0.70 g, respectively. The temperature were gradually increased by 1 °C/day to 30 °C, 33 °C and 36 °C for 60 days. The target temperature was achieved with the thermostat (REI-SEA, 300 watts, Japan). The aquarium was provided with filtration cum aeration device (Sobo-aquarium internal filter WP-850F) for self-cleaning and aeration throughout the study period. Water quality parameters pH, dissolved oxygen, ammonia and total alkalinity of each aquarium were maintained and recorded.

Feed and feeding

The fishes were supplied with commercial diet twice in a day and fed up to satiation.

Growth

Growth rate of fish was measured in terms of percentage weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) as given below:

Percentage weight gain = final weight-initial weight/initial weight×100

Specific growth rate = Ln final body weight-Ln initial body weight/duration of experiment (days) × 100

Feed conversion ratio (FCR) = Feed given (dry weight)/Weight gain (wet weight)

Survival = Number of fish harvested/Number of fish stocked × 100

Blood sampling

Fish were sacrificed at 7, 15 and 30 days after exposure to the three temperatures. On the sampling days, two fish were collected and sampled from each of the aquaria viz.; six fish sampled from each temperature regimes (n = 6). Fish were anesthetized by clove oil (5 mg/L) immediately after collection from aquaria. Blood from each fish was sampled from the caudalfin.

Measurement of hematological parameters

On each sampling day, haemoglobin (Hb) content (g/dl) and blood glucose level (mg/L) were assessed immediately after collection of blood from each fish (n = 6) with the help of haemoglobin and glucose strips respectively in a digital EasyMate®GHb (Model: ET 232, Hb/Glu double monitoring system, Bioptic technology Inc. Taiwan 35057).

Water quality parameters

Water quality parameters such as dissolved oxygen (mg/L), free CO_2 (mg/L), pH, Ammonia (ppm) and total alkalinity (mg/L) were measured at every sampling day over the experimental period. Temperature, DO and pH were measured using a mercury thermometer, DO meter (Model DO5509, Lutron, made in Taiwan) and portable pH meter (Model number-RI 02895, HANNA Instruments Co.), respectively. The free CO_2 of water was determined by titrimetric method using phenolphthalein indicator and 0.0227N NaOH titrant. Total alkalinity of water was determined by titrimetric method using methyl orange indicator and 0.02N H_2SO_4 titrant. Ammonia was determined by ammonia test kit solution (HANNA Instruments Co.).

Statistical analysis

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. We set statistical level of significance at $p<0.05$. Statistical analyses ware carried out using Version 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

Growth performance

At the end of the experiment, weight gain (g), % weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival of the *Labeo calbasu* were measured (Table 1). Highest weight gain, % weight gain and SGR were observed at temperature of 31 °C, followed by 34 °C with the least values were observed at 37 °C. Lowest FCR value was found at 31 °C (2.10 ± 0.27), whereas significantly ($p < .05$) highest FCR was determined at 37 °C (2.70 ± 0.13) with moderate value at 34 °C (2.30 ± 0.11). The survival of the fish varied

from 90.00% ± 0.00% to 88.00% ± 0.00%. The highest survival (90.00 ± 0.00) was recorded in 31 °C and 34 °C followed by 37 °C (88.00% ± 0.00%). Fry survival at 31 ºC and 34 ºC was similar but was significantly lowered 37 ºC temperatures and was not lethal to the *Labeo calbasu* fry at the experimental acclimation temperatures.

Table 1: Growth responses of *Labeo calbasu* in different temperature treatments for one months

Growth Parameters	Temperature (ºC)		
	31	34	37
Initial BW (g)	10.60 ± 0.20^{a}	10.55 ± 0.43^{a}	10.52 ± 0.56^{a}
Final BW (g)	22.20 ± 1.90^{b}	18.05 ± 1.50^{ab}	15.55 ± 2.77^{a}
Weight gain (g)	11.60± 1.65^{b}	7.50 ± 2.12^{ab}	5.03 ± 3.36^{a}
% weight gain	109.43 ± 10.34^{b}	71.09 ± 11.78^{ab}	47.81 ± 9.20^{a}
SGR (%/day)	0.38 ± 0.04^{b}	0.25 ± 0.07^{ab}	0.16 ± 0.03^{a}
FCR	2.1 ± 0.27^{b}	2.3 ± 0.11^{ab}	2.7 ± 0.13^{a}
Survival (%)	90.00 ± 0.00^{a}	90.00 ± 0.00^{a}	88.00 ± 0.00^{a}

Hematological parameters

Hemato-biochemical parameters including Hb (g/dl) and blood glucose (mg/dl) were measured in samples collected at each of the temperature regimes. At 7, 15 and 30 days, the Hb concentration decreased significantly (p< .05) in fish blood at 34 °C and 37°C compared to that in the reared fish at 31 °C (Table 2), whereas no significant variation was found in Hb content of fish blood at days 30 within the treatment groups (Table 2). Blood glucose (mg/dl) level was significantly (p< .05) increased at days 7 and 15 at 37 °C compared to 31 °C but no significant changes were observed between 31 °C and 34 °C (Table 2). At days 30, blood glucose level was significantly (p< .05) lower at the highest temperature (37 °C). When blood glucose level was compared among different days of exposure, significant lower level of blood glucose was found at days 30 than those at days 7 and 15 in the highest temperature.

Table 2: Changes in hemato-biochemical parameters after exposure to different temperature during the experimental period

Parameters	Temperature (ºC)	Sampling days		
		7	15	30
Hb (g/dL)	31	9.20±0.31^{a}	9.70±0.61^{a}	9.50±0.20^{a}
	34	8.40±0.27^{b}	8.77±0.34^{b}	9.65±0.37^{a}
	37	7.23±0.26^{b}	8.40±0.15^{b}	8.60±0.53^{a}
Blood glucose (mg/dL)	31	95.77±8.11^{a}	92.00±7.48^{a}	88.33±9.54^{a}
	34	112.61±8.24^{ab,1}	102.75±9.22^{a,2}	91.00±8.60^{a,2}
	37	130.00±6.23^{b,1}	115.25±4.45^{a,2}	82.11±6.65^{a,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 row differ significantly (p<0.05) among days of exposure. All values expressed as mean ± SD (n = 6).

Water quality parameters

Water quality parameters (Dissolved oxygen, Free CO_2, pH, total alkalinity and ammonia) were measured during the study period at different temperatures. Dissolved oxygen varied from 5.2 ± 0.44 to 7.5 ± 0.52 mg/L. The highest dissolved oxygen value was 7.5 ± 0.52 mg/L in day 7 at 31 °C and the lowest value was 5.2 ± 0.44 mg/L in day 30 at 37 °C. Free carbon dioxide (CO_2) varied from 5.8 ± 0.23 to 10.0 ± 0.26 mg/L. The highest free CO_2 value was 10.0 ± 0.26 mg/L in day 30 at 37 °C and the lowest value was 5.8 ± 0.23 mg/L in day 30 at 31 °C. The values of free carbon dioxide (CO_2) and dissolved oxygen significantly increased at different treatments and days of exposure pH ranged from 7.55 ± 0.07 to 8.65 ± 0.21 during the study period. The highest pH value was 8.65 ± 0.21 in days 30 in 34 °C while the lowest pH value was 7.55 ± 0.07 in day 30 in 37 °C. There was no change in value of pH at different treatments and days of exposure. Total alkalinity varied from 125.0 ± 7.3 to 102.0 ± 6.2 mg/L. The highest value was 125.0 ± 7.3 in day 7 in 37 °C while the lowest value was 102.0 ± 6.2 in day 30 in 34 °C. At day 30, total alkalinity significantly decreased in 34 °C while at day 7, increased in 37 °C. Ammonia varied from 0.20 ± 0.05 to 0.50 ± 0.06 mg/L. The highest ammonia value was 0.50 ± 0.06 in day 7 in 37 °C while the lowest ammonia value was 0.20 ± 0.05 in several days and temperature. At day 7, ammonia levels (mg/L) significantly increased in 37 °C.

Table 3: Water quality parameters (Mean ± SD) during the study periods

Parameter	Temperature (ºC)	Days of exposure		
		7	15	30
Dissolved oxygen (mg/L)	31	7.5 ± 0.52	7.1± 0.18	7.0 ± 0.31
	34	6.7 ± 0.27	6.2± 0. 19	5.9 ± 0.33
	37	6.0 ± 0.11	5.6 ± 0.62	5.2 ± 0.44
Free CO_2 (mg/L)	31	6.5 ± 0.17	7.0± 0. 04	5.8 ± 0.23
	34	7.0 ± 0.34	7.0 ± 0.12	8.0 ± 0.06
	37	7.9 ± 0.19	8.5 ± 0.054	10.0 ± 0.26
pH	31	7.70 ± 0.20	7.73 ± 0.10	7.85 ± 0.35
	34	8.00 ± 0.0	8.33 ± 0.11	8.65 ± 0.21
	37	8.30 ± 0.30	8.60 ± 0.16	7.55 ± 0.07
Total alkalinity (mg/L)	31	120.0 ± 8.1	138.0 ± 7.1	118.0 ± 9.1
	34	110.0 ± 6.2	115.0 ± 8.7	102.0 ± 6.2
	37	125.0 ± 7.3	105.0 ± 6.1	112.0 ± 7.2
Ammonia	31	0.20 ± 0.05	0.25 ± 0. 05	0.30 ± 0.06
	34	0.30 ± 0.04	0.25 ± 0.06	0.40 ± 0.07
	37	0.50 ± 0.06	0.35 ± 0.10	0.40 ± 0.24

Discussion

Water temperature is among the environmental factors that very directly affect various physiological processes of fish. Each species of fish has an optimal range of temperatures in which they function comfortably, and fluctuation beyond this range is stress-inductive, disrupting normal activities (Beitinger, *et al*., 2000) [5]. In this experiment, we investigated the effects of temperature on growth, hemato-biochemical parameters of *Labeo calbasu*. Growth is affected by temperature. In addition, alterations were observed in hemato-biochemical parameters due to chronic exposure to temperatures, suggesting stress in the experimental fish. Our results are in consistent with the findings of Ashaf-ud-doulah *et al*., (2020) [2] who found highest growth within 30 °C-33 °C temperature range and lowest in 36 °C for *L. rohita* fry. According to Cech and Brauner (2011) [8], with the increase in temperature, declining oxygen solubility in water causes stress to fish. The best FCR was observed in the fish kept at 33 °C temperature. These results are consistent with the findings of Andrews and Stickney (1972) [4], who reported that channel catfish, Ictalurus punctatus, fingerlings reared at a temperature range of 18-34 °C registered improvement in FCR, with the best values obtained at 30 °C.

Hb levels are significantly reduced in response to high temperatures. Similar results were observed in the *L. rohita* fry Ashaf-ud-doulah *et al*., 2019 [1]. Thermal stress caused by elevated temperatures alters the Hb and RBC content in Rohu fish, (Ashaf-ud-doulah *et al*., 2020) [2], which is similar to our present findings. Elevated temperature increased the Blood glucose and WBC content in blood of the Rohu fish, *Labeo*

rohita (Ashaf-ud-doulah *et al*., 2020) [2]. Blood glucose and cortisol level are recognized indicators used to assess stress status in fish regularly (Syawal *et al*., 2012) [18]. We observed increases in blood glucose level in the fish exposed to high temperature, possibly resulting from glycogenolysis to facilitate increased energy demands imposed by stressful conditions. Blood glucose level significantly increased under stress in Rohu (Ashaf-ud-doulah *et al*., 2020) [2] supported our findings. These observations are in agreement with the study by Shahjahan *et al*. (2018) [16], in which blood glucose levels of P. hypophthalmus fingerlings were found to increase at high rearing temperature (36 °C) compared to low temperature (28 °C).

In the present study, Dissolved oxygen was significantly decreased while free CO_2 were increased after rising temperature. The oxygen demand of the fish increases as temperature increases (Ravichandra, 2012) [13]. CO_2 can build up to significantly high levels in systems with large numbers of fish and relatively slow water turnover. pH was positively correlated with electrical conductance and total alkalinity. The influence of alkalinity and acidity upon fish has also received some consideration, especially in reference to their reactions. But in the present study, there were no significant change at pH and total alkalinity in high temperature among treatment. There are some reasons to believe that ammonium ion can contribute significantly to ammonia toxicity under some conditions. According to Brahmane *et al*. (2014) [6], dissolved oxygen concentration decreased and pH increased significantly (p<0.05) with increasing temperature, while the pH and total alkalinity of the water were almost unchanged (Shahjahan *et al*., 2018) [15].

Conclusion

The present study demonstrated that water temperature had a significant affect the growth, Hematological and water quality parameters of *calbasu*. The results indicate that the best water temperature for the growth of *calbasu* is 31 °C and fish showed good growth performance. Taken altogether, this study settled that high temperature is stressful to *calbasu*. Acclimated to different temperature could improve prospects for sustainable aquaculture management, especially during this critical climate change situation.

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