

Increased water temperature altered hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish *Pangasianodon hypophthalmus*

Md. Shahjahan · Md. Helal Uddin · Victor Bain ·
Md. Mahfuzul Haque

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Abstract The increase of water temperature due to global warming is a great concern of aquaculturists and fishery biologists. In the present study, we examined the effects of high temperature on hematological parameters and blood glucose levels in striped catfish, *Pangasianodon hypophthalmus* exposed to three temperature conditions (28, 32, and 36 °C) for 7 days. Fish were sacrificed at days 1, 3, and 7. Erythroblasts (Ebs), erythrocytic cellular abnormalities (ECA), and erythrocytic nuclear abnormalities (ENA) were assayed using peripheral erythrocytes of the sampled fishes. Hemoglobin (Hb) and red blood cell (RBC) significantly ($P < 0.05$) decreased at 36 °C after 3 and 7 days of exposure, whereas white blood cell (WBC) showed opposite scenario. Blood glucose levels significantly ($P < 0.05$) increased at 36 °C on day 3. Frequencies of Ebs, ECA, and ENA were found to be elevated at increased temperature. Differential leucocytes count showed significant increases in neutrophil and decreases in lymphocytes in the highest temperature (36 °C). Dissolved oxygen decreased and free CO₂ increased significantly ($P < 0.05$) with increasing temperature, while the pH and total alkalinity of the water were almost unchanged throughout the study period. Therefore, the present study demonstrated that striped catfish feel better adaptation at 28 and 32 °C, while high temperature 36 °C is likely stressful to this fish species.

Keywords Striped catfish · Temperature · Hematological parameters · Blood glucose · Water quality parameters

Introduction

Water temperature is an important environmental factor that plays significant role in the physiology of fish. The increase of water temperature due to global warming is a great concern of aquaculturists and fishery biologists, and it has already started to affect physiological processes in fish, causing a decrease in fish abundance and even the extinction of certain species (Portner and Peck 2010; Cheng et al. 2013). Fish are very susceptible to physical and chemical changes of water which may be reflected in their blood components (Wilson and Taylor 1993). The survival, distribution, reproduction, and normal metabolism of fish depend on aquatic environmental temperature (Shahjahan et al. 2013, 2017), and inability of fish to adapt to temperature fluctuations may cause in death because of changes in metabolic pathways (Forghally et al. 1973). The normal range of water temperature in the tropics to which fish are adapted is 25–35 °C (Howerton 2001). The rate of biochemical processes roughly doubles for every 10 °C increase in temperature within the normal range (Boyd and Tucker 1998). Although it varies from species to species, temperature can increase to a point that may become harmful for growth and damage physiological processes (Pörtner et al. 2001).

M. Shahjahan (✉) · M. H. Uddin · V. Bain · M. M. Haque
Department of Fisheries Management, Bangladesh Agricultural
University, Mymensingh 2202, Bangladesh
e-mail: mdshahjahan@bau.edu.bd

Blood parameters are most important indicators of the physiological stress that express the endogenous or exogenous changes in fish (Santos and Pacheco 1996; Cataldi et al. 1998). Fish blood is easy to collect and blood parameters can provide sufficient information about physiological response of fish to environmental changes including any factors that affect homeostasis (Lohner et al. 2001; Cazenave et al. 2005; Elahee and Bhagwant 2007; Sharmin et al. 2015; Salam et al. 2015). The size and number of erythrocytes and the concentration of hemoglobin vary with ambient temperature (Andersen et al. 1985; McClanahan et al. 1986; Allen 1993; Hlavova 1993; Ytrestoyl et al. 2001). The increase in environmental temperature decrease dissolved oxygen concentration and increase metabolic activity, and fish adapt to this environmental condition by increasing their total hemoglobin content (Brix et al. 2004). Though a number of studies were conducted to make a better understanding of physiological responses of aquatic organisms after thermal stress (Fries 1986; Bevelhimer and Bennett 2000), the effects of high temperature on striped catfish (locally known as Thai pangas), *Pangasianodon hypophthalmus*, are not well studied, and there is no reported study in Bangladesh.

Striped catfish is an important commercial cultured fish species that plays a significant role in nutrition, employment, and income generation not only in Bangladesh but also in some other countries of the world. It is a freshwater fish that can usually be found in the rivers of South East Asia, especially in Thailand, Vietnam, and Laos (Roberts and Vidthayanon 1991; Roberts and Baird 1995). It was introduced in Bangladesh from Thailand in 1989 (Ahmed 2007) and has subsequently become an important food fish for the country contributing 11.404% (0.42 million MT) of total fish production (FRSS 2016). This species is particularly important for their fast growth, lucrative size, and high market demand. Moreover, it can be stocked at a much higher density in ponds compared with other cultivable species. This fish species is considered as a suitable candidate for aquaculture due to higher growth rate, tolerance of wide environmental condition, good FCR value etc. The objectives of the present study were to know how increased water temperature alters the hematological parameters, blood glucose levels, and structure of erythrocytes in striped catfish.

Materials and methods

Experimental fishes

Healthy and active specimens of striped catfish (*Pangasianodon hypophthalmus*) fingerlings were collected from a local (Shoukhin) fish farm, Mymensingh, Bangladesh. The mean length and weight of the fishes were 9.87 ± 0.60 cm and 12.34 ± 0.78 g, respectively. The fishes were maintained in aquaria at 25 ± 0.5 °C under a controlled natural photo-regimen (12/12 h, light/dark) for a period of 21 days before the experiment. The fish were fed *pangas* starter feed (manufactured by Popular Poultry & Fish Feeds Ltd., Bangladesh) twice a day up to satiation.

Experimental design

Ten striped catfish fingerlings were stocked in each of the nine cleaned glass aquaria (75 cm × 45 cm × 45 cm) filled each with 100 L of tap water in the wet laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. Adequate aeration was maintained throughout the experimental period and the fishes were feed up to satiation twice a day. The fishes were exposed to three temperature conditions viz.; 28, 32, and 36 °C, each with three replications for 7 days. To acclimatize the fish to high temperature, temperature was gradually increased ($\Delta 1$ °C per 12 h) from normal temperature (25 °C) to the target temperature conditions (28, 32, and 36 °C). The required temperature was maintained by using thermostat (REI-SEA, Japan, 300 WATTS). Two fishes were sampled from each aquaria (i.e., 6 fishes from each temperature group; $n = 6$) on each sampling day, i.e., on days 1, 3, and 7 of the rearing period which served as day 1, day 3, and day 7 groups, respectively. The experiment was conducted following the guidance for Animal Experiments in the Faculty of Fisheries, Bangladesh Agricultural University.

Blood sampling

On each sampling day, the sampled fishes ($n = 6$) were anesthetized immediately after collection with clove oil (5 mg/L) and blood samples were collected from the caudal vein using heparinized plastic syringe. Whole blood withdrawal process took less than 1 min per fish which was considered important to avoid stress effects

in order to minimize any error in normal blood values. Collected blood was gently pushed into a sterilized microfuge tube containing anticoagulant (20 mM EDTA). Blood samples were mixed gently and discarded when encountered difficulty in taking them or clots seen in the vial during examination at the laboratory.

Measurement of hematological parameters

Immediately after collection of blood samples, hemoglobin (Hb) levels (g/dL) were measured using hemoglobin strips in a digital EasyMate® GHb, blood glucose/hemoglobin dual-function monitoring system (Model: ET-232, Bioptik Technology Inc. Taiwan 35057). The red blood cells (RBCs) and white blood cells (WBCs) were counted under a light microscope using an improved Neubauer Hemocytometer (Blaxhall and Daisley 1973).

Measurement of blood glucose

Blood glucose levels (mg/dL) were measured using glucose strips in a digital EasyMate® GHb, blood glucose/hemoglobin dual-function monitoring system.

Frequencies of *erythroblasts* (Ebs), erythrocytic nuclear abnormalities (ENA), erythrocytic cellular abnormalities (ECA), and differential leucocytes

Immediately after collection, blood was smeared on clean microscopic slides and air-dried for 10 min. The smear was fixed with methanol for 10 min and stained with 5% Giemsa and rinsed with distilled water. The slides were air-dried overnight and mounted with DPX, and then, the Ebs, ENA, and ECA were observed under an Optica optical microscope (G-206, Italy) using 100× objective lens. Three slides were prepared from blood of each fish and 2000 cells were scored from each slide. Only cells with intact cellular and nuclear membrane were scored. To minimize the technical variation, the blind scoring of Ebs, ENA, and ECA was performed on randomized coded slides.

Different erythroblasts, such as proerythroblast, basophilic erythroblast, polychromatophilic erythroblast, and orthochromatic erythroblast, were observed in the blood of fishes reared in different temperature conditions. *Frequencies of erythroblasts* of each stage in blood smears were counted.

ENA were classified according to Carrasco et al. (1990). Briefly, cells with two nuclei were considered as binucleated (BN). Blebbed (BL) had a relatively small evagination of the nuclear membrane and contained euchromatin. Nuclei with vacuoles and appreciable depth into a nucleus that did not contain nuclear material were recorded as notched (NT). Here, we have characterized nuclei with evaginations like a bud as nuclear bud (NBd) and nuclear bridge (NB) which was thin strands connecting individual nuclei.

The criteria for the identification of ECA must be dissimilar from the regular erythrocyte cell which is an oval-shaped structure with a condensed nucleus. ECA were classified as echinocytic, having serrated edges over the entire surface of the cell and much more uniform in shape; elongated, having notably more length than width, being long and slender; fusion, the joining of more than two cells to form heavier; twin, two cells joined by the cell surface; and tear-drop shape, a deformed erythrocyte which is tugged to a nipple at one end.

Differential leucocytes, such as neutrophil (Np), lymphocytes (Lc), monocytes (Mc), eosinophil (Ep), and basophil (Bp), were observed in the blood of fishes reared in different temperature conditions. *Frequencies of differential leucocytes* were counted in blood smears.

Water quality parameters

Some 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 by a DO meter (Model DO5509, Lutron, made in Taiwan) and pH by a portable pH meter (Model RI 02895, HANNA Instruments Co.). Free CO₂ (mg/L) was measured using phenolphthalein indicator and 0.0227 N NaOH titrant, and the total alkalinity (mg/L) was measured through titrimetric method using methyl orange indicator and 0.02 N H₂SO₄ titrant.

Statistical analysis

Values were presented as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among the different temperature and different sampling days. Statistical significance was set at $P < 0.05$. Statistical

analyses were performed using SPSS Version 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

Effects of high temperature on water quality parameters

Dissolved oxygen (mg/L), free CO₂ (mg/L), pH, and total alkalinity (mg/L) measured during the experimental period are presented in Table 1. Dissolved oxygen (mg/L) values were found to be decreased significantly ($P < 0.05$) with increasing water temperature, and free CO₂ (mg/L) values were found to be increased with increasing temperature. On the other hand, the values of pH and total alkalinity were remained almost unchanged throughout the study period irrespective of the temperature conditions (Table 1).

Effects of high temperature on hemato-biochemical parameters

Hb levels, RBCs, and WBCs in the blood of fishes were measured after 1, 3, and 7 days of exposure to three different temperature conditions. In the day 1 group, the values of Hb (g/dL) showed no distinct change in any temperature condition, while in the day 3 and 7 groups, Hb levels were significantly ($P < 0.05$) lowered at 36 °C

compared to 28 and 32 °C (Table 2). Similar to Hb, RBC ($\times 10^6/\text{mm}^3$) values were found to be decreased significantly ($P < 0.05$) at 36 °C compared to 28 °C in the day 3 and 7 groups, whereas no separable changes were found in the day 1 group in all temperature regimes (Table 2). On the other hand, the values of WBC ($\times 10^3/\text{mm}^3$) showed opposite scenario with Hb and RBCs values. In the day 1 group, the values of WBC ($\times 10^3/\text{mm}^3$) showed no distinct change in any temperature condition, while in the day 3 and day 7 groups, WBC values increased significantly ($P < 0.05$) at 36 °C compared to 28 °C (Table 2).

The blood glucose levels of fishes were also measured after 1, 3, and 7 days of exposure to three different temperature conditions. In fishes of day 3 group, blood glucose level (mg/dL) was significantly ($P < 0.05$) higher at 36 °C in comparison to 28 °C, but there was no significant difference between 32 and 36 °C (Table 2). On the other hand, in the fishes of day 1 and day 7 groups, blood glucose did not show any significant difference among the three temperature treated fishes (Table 2).

Erythroblasts (Ebs) induced by high temperature

Among different stages of erythroblasts, orthochromatic erythroblast, basophilic erythroblast, and proerythroblast were observed in the blood of fishes reared in different temperature. *Frequencies of*

Table 1 Water quality parameters (mean \pm SD) during the experimental period

Parameters	Temperature	Sampling days		
		1	3	7
Dissolved oxygen (mg/L)	28 °C	6.6 \pm 0.12 ^a	6.9 \pm 0.11 ^a	6.7 \pm 0.11 ^a
	32 °C	5.7 \pm 0.13 ^{ab}	5.7 \pm 0.10 ^{ab}	5.5 \pm 0.12 ^{ab}
	36 °C	4.0 \pm 0.10 ^b	4.3 \pm 0.12 ^b	4.8 \pm 0.10 ^b
Free CO ₂ (mg/L)	28 °C	4.5 \pm 0.10 ^a	5.3 \pm 0.11 ^a	5.2 \pm 0.10 ^a
	32 °C	6.2 \pm 0.12 ^{ab}	6.4 \pm 0.12 ^{ab}	6.5 \pm 0.11 ^{ab}
	36 °C	8.0 \pm 0.11 ^b	8.2 \pm 0.10 ^b	7.9 \pm 0.12 ^b
pH	28 °C	8.0 \pm 0.10	7.9 \pm 0.11	7.8 \pm 0.11
	32 °C	8.3 \pm 0.11	8.2 \pm 0.12	7.7 \pm 0.12
	36 °C	8.4 \pm 0.12	8.4 \pm 0.10	7.9 \pm 0.10
Total alkalinity (mg/L)	28 °C	102.0 \pm 0.11	112.0 \pm 0.10	96.0 \pm 0.11
	32 °C	106.0 \pm 0.10	96.0 \pm 0.11	102.0 \pm 0.10
	36 °C	100.0 \pm 0.12	96.0 \pm 0.12	94.0 \pm 0.12

Values of a single water quality parameter in a column with different alphabetical superscripts are significantly ($P < 0.05$) different

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

Parameters	Temperature	Sampling days		
		1	3	7
Hb (g/dL)	28 °C	8.20 ± 0.26 ^a	8.37 ± 0.21 ^a	8.07 ± 0.12 ^a
	32 °C	8.27 ± 0.25 ^a	8.07 ± 0.23 ^a	8.17 ± 0.21 ^a
	36 °C	7.57 ± 0.31 ^a	6.87 ± 0.32 ^b	6.23 ± 0.25 ^b
RBC (× 10 ⁶ /mm ³)	28 °C	0.84 ± 0.04 ^a	0.92 ± 0.02 ^a	0.88 ± 0.09 ^a
	32 °C	0.74 ± 0.10 ^a	0.85 ± 0.08 ^{ab}	0.94 ± 0.01 ^a
	36 °C	0.70 ± 0.10 ^a	0.63 ± 0.04 ^b	0.53 ± 0.07 ^b
WBC (× 10 ³ /mm ³)	28 °C	1.95 ± 0.03 ^a	2.21 ± 0.11 ^a	2.57 ± 0.12 ^a
	32 °C	2.11 ± 0.13 ^a	2.71 ± 0.13 ^{ab}	2.74 ± 0.15 ^{ab}
	36 °C	2.02 ± 0.12 ^a	3.23 ± 0.12 ^b	2.92 ± 0.16 ^b
Blood glucose (mg/dL)	28 °C	146.67 ± 30.73 ^a	129.67 ± 13.54 ^a	138.33 ± 28.42 ^a
	32 °C	139.33 ± 17.67 ^a	169.00 ± 8.53 ^{ab}	132.00 ± 10.32 ^a
	36 °C	151.67 ± 15.13 ^a	187.00 ± 31.61 ^b	129.33 ± 15.04 ^a

Values of a single hemato-biochemical parameter in a column with different alphabetical superscripts are significantly ($P < 0.05$) different. All values expressed as mean ± SD ($n = 6$)

erythroblasts in blood smears of fish treated with different temperature condition are shown in Table 3. Statistically significant ($P < 0.01$) increase in erythroblasts was detected in blood of fishes exposed to the highest temperature (36 °C).

Erythrocytic nuclear abnormalities (ENA) induced by high temperature

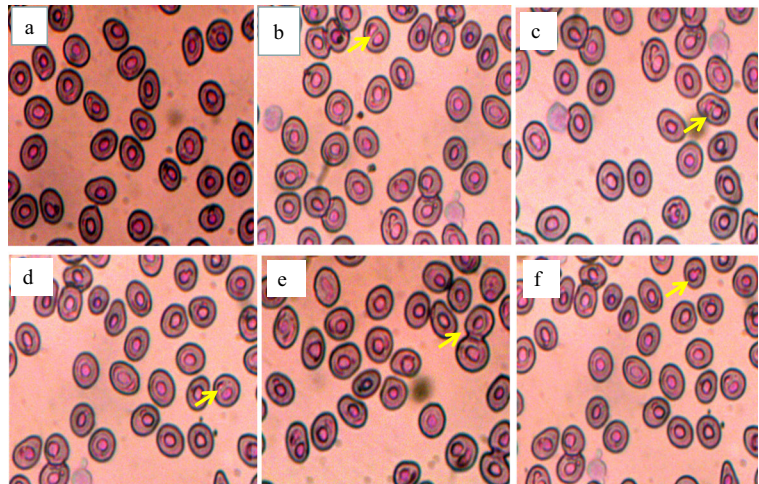
Several nuclear abnormalities in the erythrocytes, such as binuclei (BN) (Fig. 1b), blebbed (BL) (Fig. 1c),

Table 3 Differential erythrocyte count after 3 days of exposure to three different temperature conditions

Differential erythrocyte		Percentage of differential erythrocyte		
		28 °C	32 °C	36 °C
Ebs	Proerythroblast (PEb)	0.24 ± 0.01 ^a	0.85 ± 0.05 ^{ab}	1.21 ± 0.11 ^b
	Basophilic erythroblast (BEb)	0.80 ± 0.04 ^a	1.20 ± 0.06 ^{ab}	2.31 ± 0.12 ^{b*}
	Polychromatophilic erythroblast (PEb)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Orthochromatic erythroblast (OEB)	1.10 ± 0.00 ^a	1.85 ± 0.13 ^{ab}	2.72 ± 0.17 ^{b*}
ENA	Binuclei (BN)	0.89 ± 0.03 ^a	1.93 ± 0.09 ^{ab}	3.41 ± 0.18 ^{b*}
	Blebbed (BL)	0.68 ± 0.05 ^a	1.41 ± 0.06 ^{ab}	2.34 ± 0.12 ^b
	Notched nuclei (NT)	0.79 ± 0.07 ^a	1.93 ± 0.08 ^{ab}	2.34 ± 0.13 ^b
	Nuclear bridge (NB)	0.25 ± 0.01 ^a	0.95 ± 0.03 ^a	2.90 ± 0.15 ^{b*}
ECA	Nuclear bud (NBd)	0.65 ± 0.04 ^a	1.31 ± 0.05 ^{ab}	3.90 ± 0.18 ^{b*}
	Echinocytic	0.23 ± 0.01 ^a	0.94 ± 0.06 ^a	1.46 ± 0.13 ^b
	Elongated	0.00 ± 0.00	0.00 ± 0.00	0.98 ± 0.07
	Fusion	0.85 ± 0.05 ^a	1.39 ± 0.09 ^{ab}	3.85 ± 0.17 ^{b*}
	Tear-drop shaped	0.35 ± 0.03 ^a	0.98 ± 0.08 ^a	1.91 ± 0.16 ^b
	Twin	1.20 ± 0.08 ^a	2.18 ± 0.10 ^{ab}	5.23 ± 0.18 ^{b*}

Values with different alphabetical superscripts in a row differ significantly ($P < 0.05$, $*P < 0.01$) among three different temperature conditions. All values expressed as mean ± SD. Three slides were prepared from each fish and 2000 cells were scored from each slide and at least three fishes were analyzed from each group

Fig. 1 Various erythrocytic nuclear abnormalities (ENA) in giemsa stained blood smears of fish treated with three different temperature conditions: **a** regular cells, **b** binucleated, **c** blebbed, **d** notched nuclei, **e** nuclear bridge, and **f** nuclear bud



notched nuclei (NT) (Fig. 1d), nuclear bridge (NB) (Fig. 1e), and nuclear bud (NBd) (Fig. 1f), were observed in the blood of fishes reared in different temperature. Frequencies of erythrocytic nuclear abnormalities (ENA) in blood smears of fishes are shown in Table 3. Although a considerable number of ENA were observed at 32 °C, there was a significant ($P < 0.01$) increase in the frequencies of ENA at 36 °C treated groups compared to 28 °C treated group.

Erythrocytic cellular abnormalities (ECA) induced by high temperature

The erythrocytic cellular abnormalities (ECA) observed in the different temperature treated striped catfish were echinocytic (Fig. 2b), elongated (Fig. 2c), fusion (Fig. 2d), tear-drop shaped (Fig. 2e), and twin (Fig. 2f). Frequencies of erythrocytic cellular abnormalities (ECA) in fish treated with different temperature conditions are shown in Table 3. Similar to ENA, statistically significant increase ($P < 0.01$) in ECA was detected in the blood of fishes exposed to the highest temperature (36 °C).

Differential leucocytes count in three different temperature conditions

Among different leucocytes, neutrophil, lymphocytes, monocytes, and eosinophil were observed in the blood of fishes reared in different temperature. No basophils were observed in any temperature conditions. *Frequencies of differential leucocyte in blood smears of fish*

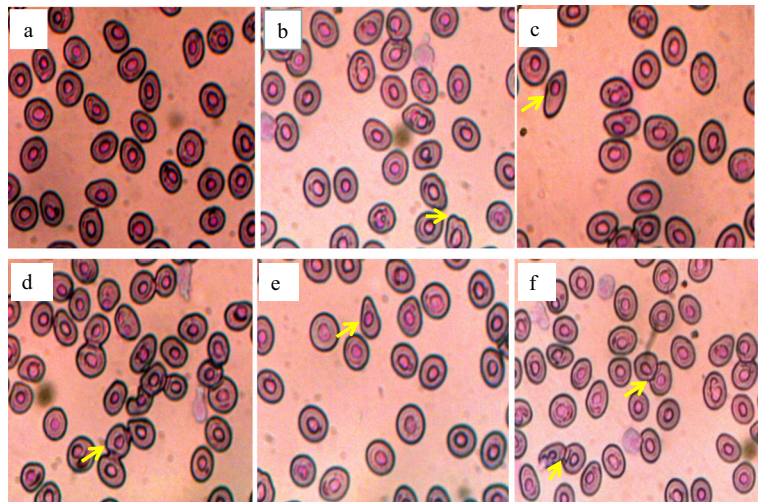
treated with different temperature condition are shown in Table 4. Neutrophil and lymphocytes showed opposite scenario. Statistically significant ($P < 0.01$) increases in neutrophil and decreases in lymphocytes were noticed in blood of fishes exposed to the highest temperature (36 °C).

Discussion

Among the different environmental factors, temperature significantly affects various physiological processes in fish. To understand the role of temperature on fish physiology, we examined the effects of high temperature on hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish. No fish mortality was observed during the experimental period. However, differential changes observed in hemato-biochemical parameters and erythrocytes structure at high temperature indicate the stressful impacts of high temperature on fish physiology.

Stress causes changes in different physiological parameters in fish (Beyea et al. 2005). In the present study, significant decrease in Hb and RBC in striped catfish might have occurred due to the failure of hematopoietic system under stressed condition exerted by high temperature. Surprisingly, increase of frequencies of erythroblasts in fishes exposed to high temperature condition supported the failure of hematopoietic system under stressed condition exerted by high temperature. The response of hematological parameters to the increased temperature varies from species to species of fish and

Fig. 2 Various erythrocytic cellular abnormalities (ECA) in giemsa stained blood smears of fish treated with three different temperature conditions: **a** regular cells, **b** echinocytic, **c** elongated shaped, **d** fusion, **e** tear-drop shaped, and **f** twin



depends on the ability of adaptation of individual species and on the duration of exposure and scale of the change in temperature. In the tilapia (*Oreochromis niloticus*), the mean value of Hb and RBC was found to be increased at increased temperature, while in the common carp and trout, these values remained constant (Smit et al. 1981). It has been reported that thermal stress either by lower temperature (24 °C) or by raised temperature (30–36 °C) causes changes in the hematological parameters in fishes leading to hypoxia or anoxia (Carvalho and Fernandes 2006; Hedayati and Tarkhani 2013). Temperature can cause stress to fish because increase in temperature decrease oxygen solubility in water and hence availability to fish (Cech and Brauner 2011). Hematological parameters are used to assess the functional status of the oxygen carrying capacity of the blood stream (Shah and Altindag 2004) and the physiological status of fish under the stressed condition

Table 4 Differential leucocyte count after 3 days of exposure to three different temperature conditions

Differential leucocyte	Percentage of differential leucocytes		
	28 °C	32 °C	36 °C
Neutrophil (Np)	15.0 ± 1.0 ^a	22.0 ± 3.0 ^{ab}	70.0 ± 5.0 ^{b*}
Lymphocytes (Lc)	18.0 ± 1.0 ^b	13.0 ± 4.0 ^{ab}	8.0 ± 1.0 ^a
Monocytes (Mc)	2.0 ± 1.0 ^a	3.0 ± 1.0 ^a	2.0 ± 1.0 ^a
Eosinophil (Ep)	1.0 ± 0.0 ^a	1.0 ± 0.0 ^a	2.0 ± 1.0 ^a

Values with different alphabetical superscripts in a row differ significantly ($P < 0.05$, $*P < 0.01$) among different temperature in differential leucocytes. All values expressed as mean ± SD. Three slides were prepared from each fish

(Fernandez and Mazon 2003). In the present study, the dissolved oxygen (mg/L) values were found to be decreased with increasing temperature which caused stressful condition to the fish.

Remarkably, there was a significant increase in WBC count in the blood of fishes exposed to increased temperature, which can be correlated with an increase in antibody production that helps in survival of the fish in adverse environmental condition. Interestingly, differential leucocyte count in the present study showed significant increases in neutrophil and decreases in lymphocytes in blood of fishes exposed to the highest temperature (36 °C). It has been reported that increases in numbers of neutrophils (neutrophilia) and decreases in lymphocyte numbers (lymphocytopenia) were observed due to stress in boars (Bilandzic et al. 2006). Since numbers of neutrophils and lymphocytes are affected by stress in opposite directions (Davis et al. 2008), the present study suggested that high temperature (36 °C) is possibly stressful to striped catfish.

Blood glucose levels are general stress indicators in fish (Pacheco and Santos 2001). In the present study, increased glucose levels recorded in the fish exposed to high temperature might be due to the mobilization of glycogen into glucose to meet the increased demand for energy under stressed condition at high temperature. Glucocorticoids and catecholamine hormones are known to produce hyperglycemia in animals and stress stimuli elicit rapid secretion of these hormones from adrenal tissues of the fish (Pickering 1981). Such increase may be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands (Winkaler et al. 2007). The high

temperature may change the functions of vital organs like liver and kidney, disrupting the homeostatic condition of the fish body.

In the present study, we have observed various morphological (cellular and nuclear) alterations in blood cells of fish exposed to high temperature. Blood smears of fish treated with high temperature results in erythrocytic cellular abnormalities (ECA) and erythrocytic nuclear abnormalities (ENA). In poikilo-thermal organisms, environmental temperature regulates the lipid bilayer micro-viscosity, the phase distribution of lipids, the micro-surrounding of proteins, the protein-lipid interactions, and other characteristics of the membrane structural organization of erythrocytes (Kreps 1981; Avrova 1999). Thus, the most important role in the temperature compensation is played by homeo-viscous adaptation, i.e., adaptation to environmental temperature by regulation of viscosity of the cell membrane lipid phase through changes of their fatty acid composition (Cossins 1977; Cossins and Prosser 1978). Similarly, ENA, such as elongated, fusion, tear-drop shaped, and twin, might be resulted due to concentration-dependent increase of lipid peroxidation products in erythrocytes of exposed fish to high temperature (Bai et al. 2014; Ghaffar et al. 2015). Echinocytic is another abnormality that resulted due to interruption of the lipid solubility of membranes of erythrocytes that ultimately leads to apoptosis (Walia et al. 2013). Moreover, it can be hypothesized that such types of ECA may undergo morphological alterations in the plasma membrane affecting surface deformability and make the erythrocytes more susceptible to burst when crossing small capillaries. Considering the previous findings, it can be concluded that the mechanism maintaining the structural stability of erythrocyte membrane during temperature acclimatization is based, apart from regulation of lipid composition, on modification of the intra- and intermolecular mobility and interactions of cytoskeleton proteins.

In conclusion, we examined the effects of high temperature on hemato-biochemical parameters and peripheral erythrocytes structures in striped catfish. Though striped catfish is known to tolerate wide variation of environmental parameters, in the present study, high temperature decreased the Hb and RBC levels and increased the WBC and blood glucose levels of the fish. Frequencies of Ebs, ECA, and ENA were found to be elevated at increased temperature. Taken altogether, this study demonstrated that high temperature is stressful to striped catfish.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests. The authors themselves are responsible for the content of the paper.

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