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RESEARCH PAPER

Effects of Increasing Salinity on Growth Performance, Hemato-biochemical Parameters, and Erythrocyte Structure of Freshwater Gourami (*Trichogaster fasciata*)

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Abstract

Climate change and sea-level rise cause seawater intrusion into freshwater ecosystems, risking increased salinity exposure for freshwater fish, endangering their long-term sustenance. To understand the effects of environmentally realistic increase in salinity exposure on freshwater fish, a total of 120 banded gourami, *Trichogaster fasciata* (8.54 g average initial weight) were exposed to four different salinities (0, 3, 6, and 9‰) for 90 days. The weight gain and specific growth rate values were significantly higher in the fish exposed to 3‰ compared to 0‰, and the lowest feed conversion ratio and survival rate were recorded in 3‰ and 9‰, respectively. The blood glucose and hemoglobin contents were found to be significantly higher and lower at 9‰ after 30 and 60 days, respectively, relative to 0‰. Moreover, significant low red blood cell counts were found at 6 and 9‰ after 30 and 90 days, respectively. Whereas, the white blood cell counts showed an opposite trend. Abnormalities of erythrocyte shape were significantly higher at 9‰, compared to 0‰. Results suggest that long-term exposure to high salinity (6, and 9‰) would cause osmotic stress and impair the physiological performance of freshwater gourami.

Introduction

Like other low-lying coastal countries, the freshwater ecosystems of Bangladesh are vulnerable to salinity intrusion. Sea levels rise due to climate change along with the increased frequency of some natural events namely storm surges and cyclones, which are believed to play an important role in coastal salinity intrusion (Rabbani et al., 2013; McCarthy et al., 2001). This salinity intrusion into the coastal areas of Bangladesh is already distressing the fertility of agricultural land and the sustainability of agricultural production, which will hamper food security and safety in the future (Islam et al., 2005). Moreover, the river water flow was reduced due to the unplanned construction of dams the upstream and withdrawal of freshwater for irrigation. These problems intensify during winter and dry seasons. This ultimately results in increased water salinity in freshwater and brackish water areas all over the world, especially in Bangladesh. Over the last few decades, agricultural activities have been replaced by shrimp aquaculture in vast coastal areas by allowing saline water entry into a previously freshwater environment (Ahmed, 2013). Increased water salinity alters the physical environment of coastal ecosystems, which may have negative impacts on

aquatic organisms. Over the last four decades, water salinity levels have increased by around 26% in the coastal regions of Bangladesh (Alam et al., 2017). It is assumed that in the near future, the freshwater zone of Bangladesh will reduce from 45% to 36%, the brackish water zone will reduce from 50% to 47%, and the saltwater zone will increase from 5% to 17% (Rahman et al., 2015).

For coastal areas, both natural and anthropogenic ally-driven salinity intrusion is a serious problem (Shameem et al., 2015). Bangladesh's coastal areas are diverse and competing in terms of land-use change; shrimp production is one of the noticeable land-use practices as saline intrusion increases (Nuruzzaman et al., 2014; Shameem et al., 2015). For Bangladesh coastal areas, increased salinity and saline water intrusion are emerging problems in coastal areas where land-use changes appear to be affecting aquaculture and liv elihoods (Hasan et al., 2020; Selim et al., 2021). Furthermore, as a result of saline water intrusion, a new environmental burden has emerged, with significant implications for aquatic biodiversity (Lai et al., 2015; Smajgl et al., 2015; Li et al., 2017; Saintilan et al., 2019).

Water salinity plays a significant role in affecting the survival and different physiological processes of aquatic organisms (Saravanan et al., 2018). Elevated salinity has been found to affect growth performance (Bœuf and Payan, 2001; Denson et al., 2003; Deane and Woo, 2009; Lowe et al., 2012; Nhan, 2016), osmoregulation, physiology, and stress responses (Gonzalez, 2012; Ytrestøyl et al., 2020), hemato-biochemical parameters (El-Leithy et al., 2019; Evans and Kültz, 2020; Dawood et al., 2022) and erythrocyte structure of a range of freshwater species important for both ecosystem and aquaculture (Jahan et al., 2019). For migratory fish, they were found to require more energy during migration from freshwater to saline water for adaptation which affects fish growth. To minimize energy expenditure for osmoregulation, and to ensure better growth performance, fish need optimum salinities (Bœuf and Payan, 2001; Ytrestøyl et al., 2020; Islam et al., 2020d, 2021b). Most freshwater fish is stenohaline and more susceptible to increased salinity which results in reduced growth and physiological performance (Bœuf and Payan, 2001).

Osmotic stress impacts in fish can be revealed by measuring a range of blood components and parameters (Maugars et al., 2018; Islam et al., 2021c). Hematological parameters are frequently considered an essential tool to assess the health condition as well as physiological stress caused by any internal or external factors that affect homeostasis in fish (Shahjahan et al., 2022). Fish blood is easy to collect and provides enough information about the physiological response of fish to environmental variations (Lohner et al., 2001; Cazenave et al., 2005; Elahee and Bhagwant, 2007; Sharmin et al., 2015; Salam et al., 2015). Recently, nuclear and cellular abnormalities of blood erythrocyte tests are being used to appraise the stress response to environmental changes (Shahjahan et al., 2013; Ashaf-Ud-Doulah et al., 2019; Islam et al., 2021a, 2021b, 2021c), which can be employed to unveil osmotic stress response in fish (Islam et al., 2021b).

Banded gourami, *Trichogaster fasciata* is a freshwater and estuarine tropical labyrinth fish of the Osphronemidae family (Sumon et al., 2019). It is an indigenous species and is widely distributed throughout the Indian sub-continent: Bangladesh (Sumon et al., 2016), Bhutan (Sehgal, 2015), India, Nepal, and Pakistan (Gupta, 2015). This fish is benthopelagic and carnivorous; generally prefers weedy littoral waters namely estuaries, lakes ponds, ditches, and rivers (Rahman et al., 2019). This species is considered an important target species for small-scale fishermen, who use a variety of traditional fishing gear (Kibria et al., 2005). This fish acts as an important protein source for people in Bangladesh and India (Rahman et al., 2019). Moreover, this fish species is used as a peaceful and beautiful aquarium fish, and traditionally, people like its good taste (Rahman et al., 2019). Moreover, banded gourami, *Trichogaster fasciata* is extensively utilized for various exposure studies (Sumon et al., 2016; 2019). Because of its short generation time, easy acclimation capacity, and availability of induced breeding techniques, this fish is considered suitable for aquatic research (Sumon et al., 2019). To date, there is no information on the growth and physiological fitness during exposure to climate change-induced salinity intrusion. Thus, a better understanding of potential compensatory measures and response mechanisms for banded gourami to saline water-induced osmotic stress is critical. In this study, experiments are conducted to demonstrate how environmentally realistic variable salinities over set periods affect the growth and physiological fitness of this fish species. It was hypothesized that the banded gourami would perform entirely within the range of optimal osmotic conditions, but that at stressful salinities, it may face growth and physiological constraints. Such changes may result in decreased growth performance, which may have an impact on banded gourami productivity. A series of hemato-biochemical parameters were measured to test the hypothesis. Individual fitness was determined by measuring growth performance, while stress responses were determined by examining hemato-biochemical parameters.

Materials and Methods

Experimental Fish

The fish were collected from a natural marshland (Borbeel, Kishorgonj, Bangladesh). The average initial weight and length were 8.54±0.58 g and 7.37±0.45 cm, respectively. *T. fasciata* was then transported alive in well-oxygenated polythene bags to the Wet Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, and was kept in a cement cistern with a

continuous water supply. The fish were acclimatized at 24±0.5°C under a controlled photoperiod regimen (12:12h, light: dark) for 21 days before the onset of the experiment. The fish were fed with a commercial diet (Mega Fish Feeds Ltd., Bangladesh; 32% crude protein, 13% moisture, 6% lipid, 13% ash, and 12% crude fiber) twice a day (9:00 and 16:00 hours) up to satiation.

Experimental Setup

After 21 days of acclimatization, the fish were exposed to four different salinities in triplicates: 0‰ (control), 3‰, 6‰, and 9‰ for 90 days based on previously estimated LC50 (11.59‰). A total of 120 fish were allocated in twelve 70 L plastic drums filled with water at designated salinities. Three plastic drums were used to prepare water for three salinities (3, 6, 9‰) separately. Thermostatic titanium water heaters (Hygger) were used to maintain the constant temperature (24±0.5°C). Each tank was connected to a continuous aeration system. Fish were fed twice a day up to satiation. Unfed feed and fecal wastes were siphoned daily. For each tank, water was exchanged once a week with previously prepared temperature and salinity-adjusted water. Fish were sampled on days 30, 60, and 90 of the experiment. Nine fish were sampled from each saline water treatment. The experiment was conducted following the guidelines of the Animal Care and Use Committee of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Growth and Survival

On each sampling day, fish length and weight were recorded to calculate growth performance and feed utilization efficiency as weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) as follows:

$$\text{Weight gain (WG)} = \text{Final weight (g)} - \text{initial weight (g)}$$

$$\text{Specific growth rate (SGR;\%)} = \frac{\text{In final weight (g)- In initial weight (g)}}{\text{Number of days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given (dry weight)}}{\text{Weight gain (wet weight)}}$$

$$\text{Survival rate (\%)} \frac{\text{Number of fish that survived at the end of the experiment}}{\text{Initial number of fish stocked}} \times 100$$

Measurement of Hemato-biochemical Parameters

On each sampling day (days 30, 60, and 90), the sampled fish (n=9) were anesthetized using MS-222 (5 mg/L) at pH ~7.0. From each fish, around 80 µL of blood was collected with the EDTA-rinsed plastic syringe through the caudal vein puncture and preserved in Eppendorf tubes containing anticoagulant 5 µL of EDTA (20 mM). To minimize handling stress the whole blood collection process was finished within 5 minutes (Ariful et al., 2019). The total count of RBCs and WBCs was done by the established Neubauer hemocytometer counting method (Ariful et al., 2019). Blood glucose and hemoglobin were measured by using a digital blood hemoglobin-glucose meter [EasyMate® GCHb (Taoyuan City, Taiwan)], a dual-function monitoring system using glucose and hemoglobin strips, respectively. Briefly, a drop of blood was put on one side of the test strip target area. In about 10 seconds, the measured value was displayed on the screen and recorded. To note, the methods and instruments used in this study were validated for fish in previous studies (Ashaf-Ud-Doulah et al., 2021; Islam et al., 2019; Jahan et al., 2019; Shahjahan et al., 2018).

Erythrocyte Nuclear Abnormalities (ENA) and Erythrocytic Cellular Abnormalities (ECA)

The blood was smeared on clean microscopic slides immediately after collection and air-dried for 10 minutes. The slides were cleaned with pure alcohol (100%) and dried for ten minutes. The slides were then stained with 5% Giemsa for 12-15 minutes before being rinsed with distilled water (4-5 minutes).The slides were air-dried overnight and mounted with DPX before being examined under an optical microscope (G-206, Italy) with a 100-objective lens. Six slides were prepared for each fish, and 2000 cells were scored from those slides. Only cells with intact cellular and nuclear membranes were considered for scoring. ECA and ENA were classified according to Carrasco et al. (1990).

Statistical Analysis

The collected data on growth and hematological parameters were analyzed statistically following the principles of CRD (Completely Randomized Design) using the MSTAT-C package program. Values are expressed as mean ± standard deviation (SD). The significance of the differences among the means across different treatments was calculated by LSD (Least Significant Difference) test. Furthermore, principal component analysis (PCA) was used for the tested parameters to understand the overall effects of salinity. For PCA analysis, data were log-transformed. The first two components' scores were plotted to create a PCA biplot. Origin 2020b was used for the PCA analysis (OriginLab, USA).

Results

Effect of Salinity on Growth and Survival

On day 90, the WG, SGR, FCR, and survival rate were significantly ($P<0.05$) higher in the fish reared at 3‰ followed by the fish in 0, 6, and 9‰ salinities (Table 1). The lowest FCR was recorded in 3‰, whereas

the highest FCR was observed for the fish in 9‰. The survival rate (%) was lowest (P<0.05) in 9‰, while no mortality was recorded for the fish in 0 and 3‰ salinities (Table 1).

Effects of Salinity on Blood Glucose Levels

On day 30, blood glucose levels (g/dL) increased significantly (P<0.05) in the fish reared at 9‰ compared to the fish at 0‰. Similarly, on day 60, the highest blood glucose level was recorded in the fish at 9‰, whereas the fish at 6‰ showed the highest blood glucose level on day 90. On day 60 and day 90, blood glucose levels were observed to be significantly lower (P<0.05) compared to those measured on day 30 in all the salinities (Figure 1).

Effects of Salinity on Blood Hemoglobin Levels

Throughout the entire period of the experiment, the blood Hb levels (g/dL) were suppressed significantly (P<0.05) in the fish reared in all three salinities (3, 6, and 9‰) compared to the control fish (0‰). On days 30 and 90, the highest and the lowest Hb levels were recorded in fish maintained at 0 and 6‰ salinities, respectively. However, the highest and lowest Hb levels were recorded in the fish reared at 0 and 9‰ salinities on day 60, respectively (Figure 2).

Effects of Salinity on Red Blood Cell (RBC)

On both days 30 and 60 RBCs counts (10^6/mm^3) were decreased significantly (P<0.05) in the fish acclimatized at 3, 6, and 9‰ salinities in comparison to the fish at 0‰. On day 60, the highest RBCs counts were recorded in the fish reared in 3‰ followed by 9‰, 0‰, and 6‰ (Figure 3).

Effects of Salinity on White Blood Cell (WBC)

On day 30, compared to the fish at 0‰, WBC counts (10^3/mm^3) were found to be significantly (P<0.05) increased in the fish reared at 9‰ followed by 6‰ and 3‰. On day 60, WBC counts in the fish at 3, and 6‰ were significantly (P<0.05) higher compared to the fish at 0‰ and 9‰. However, on day 90, no noticeable differences in WBC counts were observed among fish maintained at four tested salinities (Figure 4).

Table 1. Growth responses of banded gourami in four different salinity conditions on day 90.

Parameters	Salinity conditions			
	0 ‰	3 ‰	6 ‰	9 ‰
Initial BW (g)	8.37±0.87^a	8.43±0.67^a	8.47±0.78^a	8.39±0.92^a
Final BW (g)	12.92±0.98^b	14.65±0.1.25^a	12.38±0.56^b	10.77±0.83^c
Weight gain (g)	5.30 ± 1.32^b	5.76 ± 0.01^a	5.09 ± 0.09^b	3.05 ±1.01^c
SGR (%)	0.56±0.32^b	0.58±0.34^a	0.55±0.30^b	0.38±0.14^c
FCR	1.90 ± 0.14^b	1.60 ± 0.09^c	2.0 ± 0.05^b	2.25 ± 0.04^a
Survival (%)	100.00 ± 0.00^a	100.00 ± 0.00^a	95.00 ± 0.00^a	80.00 ± 0.00^b

Values with different alphabetical superscripts in a row differ significantly (P<0.05) among different salinity conditions. All values are expressed as mean ± SD.

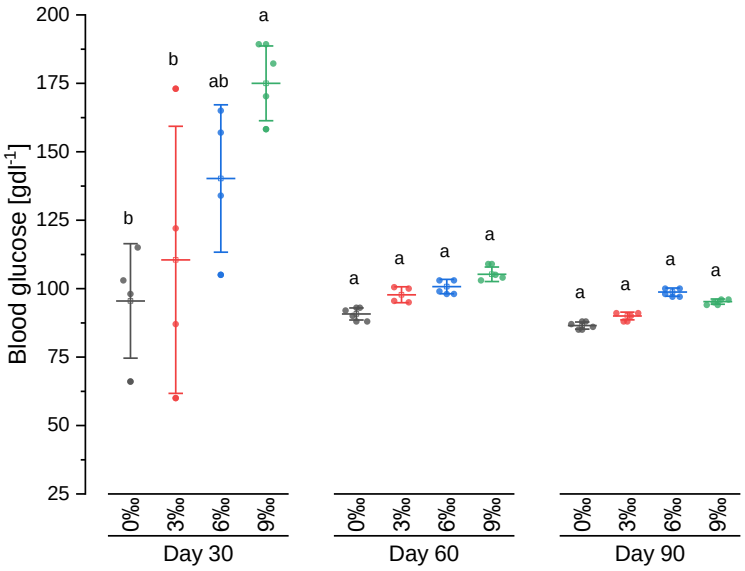

Figure 1. Changes in blood glucose levels (Mean ± SD) after exposure to different salinity concentrations. Values accompanied by different letters are statistically significantly different (P<0.05, n=9). Asterisks denote significant (P<0.05) differences among days of exposure.

Effects of Salinity on Erythrocyte Cellular Abnormalities (ECA) and Erythrocyte Nuclear Abnormalities (ENA)

Cellular abnormalities in the erythrocytes (ECAs), such as echinocytic (Figure 5b), elongated (Figure 5c), twins (Figure 5d), teardrop shape (Figure 5e), and fusion (Figure 5f), were recorded for fish from all tested salinities. ECA frequencies in fish blood smears are presented in Table 2. ECAs in fish at 0‰ were significantly (P<0.05) lower compared to fish at 3, 6, and 9‰. ECAs in treatments were found to increase significantly (P<0.05) with increasing salinities. On the other hand, the appearance of ECAs decreased in general over time (Table 2). ENAs such as binucleated (Figure 6a), micronucleated (Figure 6b), notched nuclei (Figure 6c), karyopyknotic nucleus (Figure 6d), nuclear bridge (Figure 6e), and nuclear bud (Figure 6f) were recorded in the blood of fish from both the control group (0‰) and treated groups (Table 3). Similar to ECAs, ENAs in the treatments were significantly (P<0.05) higher compared to the control group and the fish in 9‰ showed the highest value.

Principal Component Analysis (PCA)

Based on the PCA-biplot, the first two dimensions account for 82.2% of the total variances (PC1, 66.9%, and PC2, 15.5%). Based on the evaluated parameters, the PCA-biplot revealed separated confidence ellipses among the sampling days. Almost all tested parameters in the PC1 vs. PC2 plane were clustered to the three

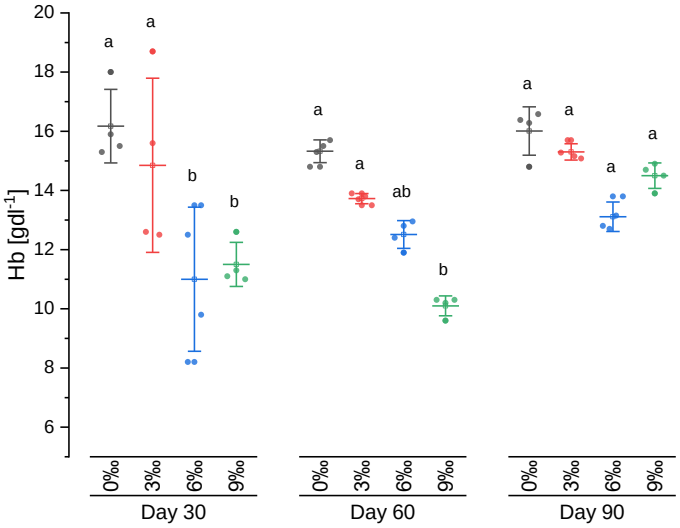

Figure 2. Changes in hemoglobin levels (Mean ± SD) after exposure to different salinity concentrations. Values accompanied by different letters are statistically significantly different (P<0.05, n=9). Asterisks denote significant (P<0.05) differences among days of exposure.

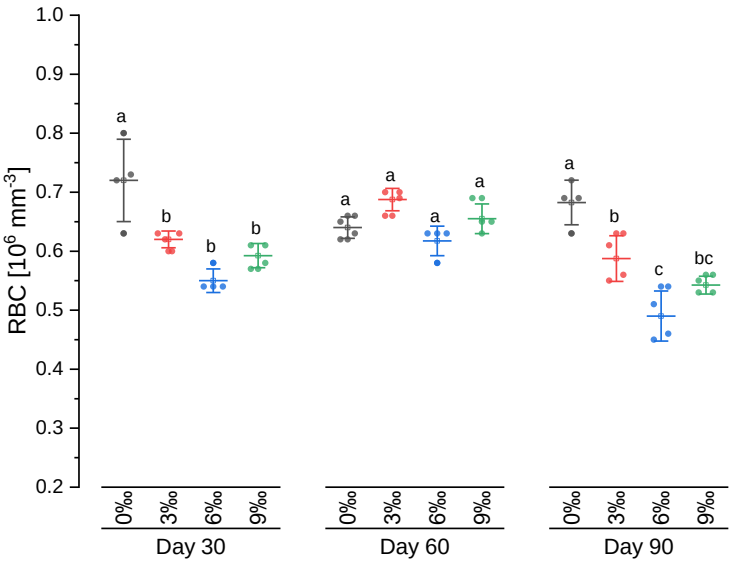

Figure 3. Changes in red blood cells (Mean ± SD) after exposure to different salinity concentrations. Values accompanied by different letters are statistically significantly different (P<0.05, n=9). Asterisks denote significant (P<0.05) differences among days of exposure.

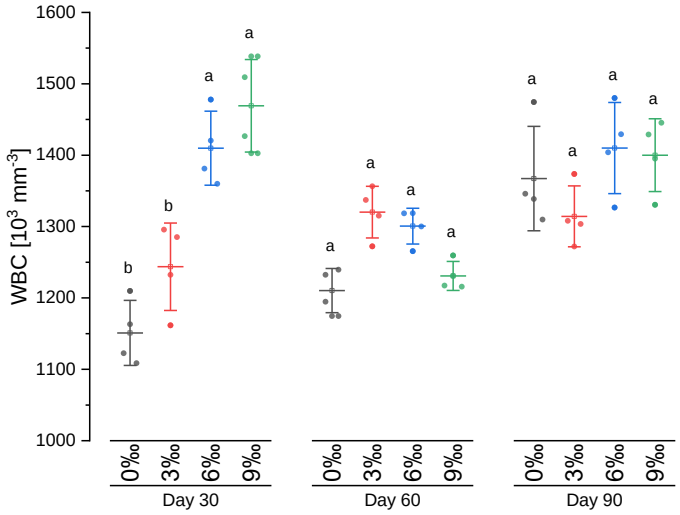

Figure 4. Changes in white blood cells (Mean ± SD) after exposure to different salinity concentrations. Values accompanied by different letters are statistically significantly different (P<0.05, n=9). Asterisks denote significant (P<0.05) differences among days of exposure.

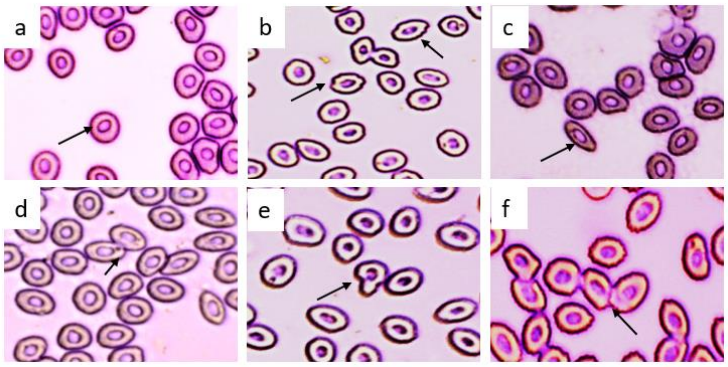

Figure 5. Various erythrocyte cellular abnormalities (ECA) in Giemsa stained blood smears of fish after exposure to different salinity concentrations; (a) regular cells, (b) echinocytic, (c) elongated shaped, (d) twin, (e) teardrop-shaped, and (f) fusion

Table 2. Frequencies of erythrocyte cellular abnormalities (ECA) of banded gourami after 30, 60 and 90 days of exposure to four different salinity conditions.

ECA	Treatments	Percentage of ECA		
		Days of exposure		
		Day-30	Day-60	Day-90
Tear-drop	0 ‰	0.80±0.04c	0.77±0.05bc	0.78±0.05ab
	3 ‰	1.54±0.05b	0.82±0.06ab	0.80±0.02a
	6 ‰	1.61±0.08b	0.73±0.05c	0.72±0.05b
	9 ‰	1.71±0.07a	0.84±0.02a	0.82±0.03a
Fusion	0 ‰	0.86±0.05c	0.73±0.04a	0.81±0.04a
	3 ‰	1.57±0.07b	0.80±0.10a	0.80±0.05ab
	6 ‰	1.67±0.11ab	0.77±0.05a	0.71±0.07b
	9 ‰	1.78±0.07a	0.86±0.05a	0.89±0.08a
Elongated	0 ‰	0.84±0.05c	0.80±0.05a	0.76±0.03a
	3 ‰	1.61±0.07b	0.86±0.06a	0.74±0.06a
	6 ‰	1.66± 0.06b	0.69± 0.07b	0.67±0.04a
	9 ‰	1.80±0.08a	0.78±0.06ab	0.75±0.05a
Echinocytic	0 ‰	0.77±0.06d	0.65±0.05b	0.61 ±0.03b
	3 ‰	1.47±0.09c	0.82±0.03a	0.77±0.05a
	6 ‰	1.59±0.08b	0.67±0.02b	0.72±0.05a
	9 ‰	1.78±0.05a	0.83±0.02a	0.78±0.04a
Twin	0 ‰	0.96±0.06c	0.84±0.04ab	0.73±0.03b
	3 ‰	1.52±0.08b	0.89±0.05a	0.81±0.07ab
	6 ‰	1.64±0.09b	0.79±0.07b	0.76±0.06b
	9 ‰	1.86±0.12a	0.90±0.06a	0.86±0.04a

Values of a single ECA in a column with different alphabetical superscripts are significantly (P<0.05) different. All values are 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.

sampling days. Glucose and most ECA and ENA types were characterized by higher PC1 scores. Whereas, RBC, Hb, WBC, and blebbed parameters were characterized by higher PC2 scores (Figure 7).

Discussion

The present study investigated the hemato-biochemical response of banded gourami to three different elevated salinities to understand the impacts of climate change-induced salinity intrusion on fish physiology. Fish exposed to hypo-or hyper-salinities would require additional energy for osmotic adaptation, which could limit growth in comparison with the fish kept in an isosmotic condition (Boeuf and Payan, 2001; Tsuzuki et al., 2007; Herrera et al., 2009). Energy expenditure associated with osmoregulation may be different at various developmental stages. Moreover, fish's response to salinity is species-specific and this variation is dependent upon the relationships between osmoregulatory mechanisms and other physiological processes (Imsland et al., 2002; ONeill et al., 2011; Reid et al., 1998; Zou et al., 2019). The present study revealed that banded gourami could endure up to 6‰ without considerable mortality, whereas 9‰ salinity caused 20% mortality, which agrees with the conclusion drawn by Faizul and Christianus (2013). Ahmmed et al. (2017) reported that fingerlings of stinging catfish,

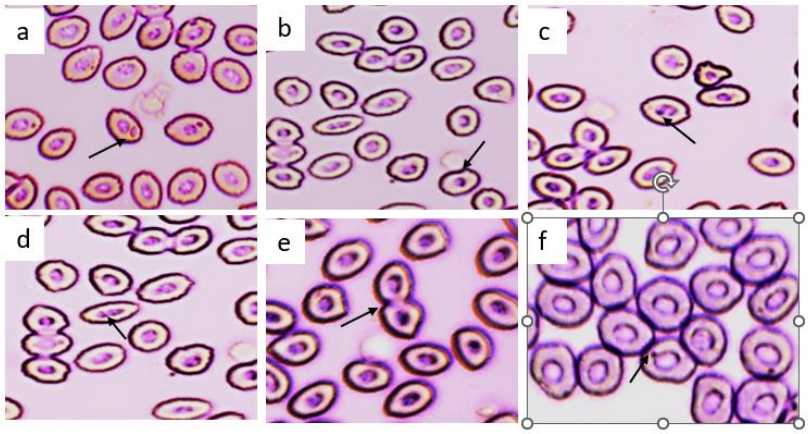

Figure 6. Various erythrocyte nuclear abnormalities (ENA) in Giemsa-stained blood smears of fish after exposure to different salinity concentrations; (a) binucleated, (b) micronucleus, (c) notched nuclei, (d) karyopyknosis nucleus, (e) nuclear bridge, and (f) nuclear bud.

Table 3. Frequencies of erythrocyte nuclear abnormalities (ENA) of banded gourami after 30, 60 and 90 days of exposure to four different salinity conditions.

ENA	Treatments	Percentage of ENA		
		Days of exposure		
		Day-30	Day-60	Day-90
Binuclei (BN)	0 ‰	0.17±0.06d	0.15±0.08c	0.12±0.04d
	3 ‰	0.79±0.03c	0.66±0.03b	0.51±0.02c
	6 ‰	0.98±0.02b	0.76±0.03a	0.58±0.01b
	9 ‰	1.70±0.07a	0.81±0.02a	0.64±0.01a
Micronucleus (MN)	0 ‰	0.15±0.02c	0.12±0.04c	0.10±0.03d
	3 ‰	1.51 ±0.02b	0.57±0.02b	0.52±0.02c
	6 ‰	1.57±0.12b	0.63±0.05b	0.62±0.05b
	9 ‰	1.72±0.05a	0.79±0.06a	0.73±0.05a
Notched nuclei (NT)	0 ‰	0.16±0.03d	0.13±0.02d	0.10±0.04d
	3 ‰	0.76±0.04c	0.51±0.06c	0.44±0.05c
	6 ‰	1.16±0.05b	0.67±0.04b	0.57±0.05b
	9 ‰	1.59±0.08a	0.79±0.08a	0.65±0.05a
Karyopyknotic nucleus (KN)	0 ‰	0.95±0.11c	0.82±0.04a	0.73±0.05bc
	3 ‰	1.49±0.12ab	0.89±0.07a	0.84±0.04a
	6 ‰	1.42±0.06b	0.67±0.07b	0.68±0.06c
	9 ‰	1.63±0.08a	0.86±0.09a	0.81 ± 0.07ab
Nuclear bridge (NB)	0 ‰	0.27±0.03c	0.23±0.04c	0.26±0.03c
	3 ‰	1.63±0.09a	0.92±0.04a	0.81±0.05a
	6 ‰	1.49±0.09b	0.81±0.04b	0.72±0.04b
	9 ‰	1.72±0.08a	0.86±0.08ab	0.77±0.04ab
Nuclear bud (NBd)	0 ‰	0.67±0.05b	0.58± 0.06c	0.54±0.05b
	3 ‰	1.72±0.10a	0.86±0.04a	0.74±0.05a
	6 ‰	1.79±0.08a	0.76±0.05b	0.73±0.06a
	9 ‰	1.81±0.08a	0.84±0.07ab	0.78±0.05a

Values of a single ENA in a column with different alphabetical superscripts are significantly ($P<0.05$) different. All values are 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

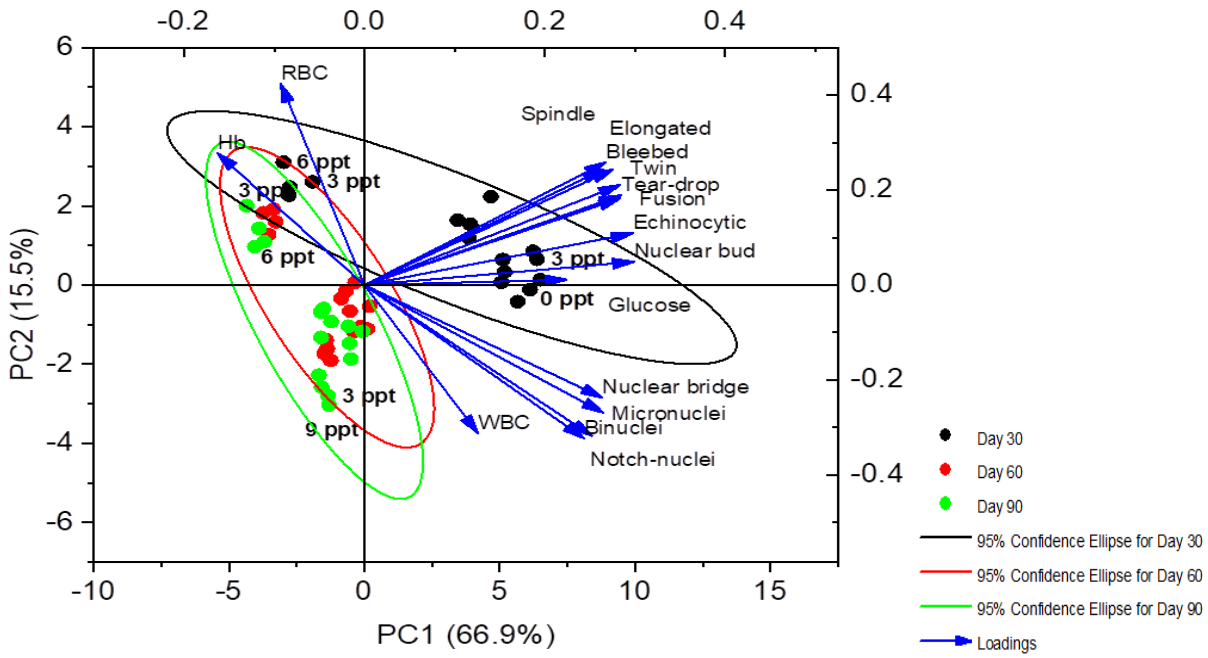

Figure 7. Principal Component Analysis (PCA) biplots. PCA representing the contribution of biochemical parameters and erythrocytic abnormalities at days 30, 60 and 90 for fish reared at 0, 3, 6 and 9‰. The variable coordination is presented by the complementary cases analysis showing distribution of days 30, 60 and 90. Legend: Day 30; Day 60; Day 90; black, red and green lines ellipses are for days 30, 60 and 90 respectively at 95% confidence level; blue arrows indicate PCA loadings along both component axes for haemato-physiological parameters determined; WBC, White Blood Cell; RBC, Red Blood Cell; Hb, Hemohlobin.

Heteropneustes fosslis exposed to ≥6‰ salinity resulted in a higher mortality rate and stunted growth. In addition, salinity levels ≥9‰ negatively affected the growth of goldfish, *Carassius auratus* (Altinok and Grizzle, 2001). The upper salinity tolerance limit of the stymphalia minnow, *Pseudophoxinus stymphalicus* was found to be around 11‰ (Biancoand and Nordlie, 2008). Kasim (1983) showed that the upper initial lethal salinities of white carp, *Cirrhinus mrigala*; fringed-lipped peninsula carp, *Labeo fimbriatus*; and common carp, *Cyprinus carpio* were 3.54‰, 7.07‰, and 8.13‰, respectively. For rosy barb, *Puntius conchonius*, maximum salinity tolerance was observed at 5-10‰ (Ramee et al., 2020). In the present research, the highest growth performance of banded gourami was recorded at 3‰. Several pieces of research revealed that oligohaline water (<5‰) can boost the growth of freshwater fish (Altinok and Grizzle, 2001; Fashina-Bombata and Busari, 2003; Overton et al., 2008). Fish do not need to spend more energy to adjust osmotic balance when exposed to the low saline condition because salt levels in the fish blood and the ambient water are in equilibrium. As a result, improved growth performance of banded gourami at 3‰ may be attributed to energy conservation and enhancement of basal and induced immune responses following exposure to low saline waters (Aihua and Buchmann, 2001; Schmitz et al., 2017a, 2017b).

Fish exposed to stressful conditions alter various physiological parameters (Beyea et al., 2005). In the present study, the blood Hb levels were significantly suppressed in banded gourami at higher salinities (6, and 9‰) compared to fish at 0‰ and 3 %. This might have occurred due to the impairment of hematopoietic functions under stress imposed by high salinities (Elarabany, 2017; Masroor et al., 2018; Islam et al., 2020b). The changes in hematological parameters to the increased salinity are dissimilar and depend on the acclimation and adaptation capacity of individual species and the intensity of the change in salinity. Elarabany et al. (2017) reported that Hb content was reduced significantly in Nile tilapia, *Oreochromis niloticus* exposed to 8 and 12‰ salinities. In the present study, suppression of Hb might also be attributed to osmoregulatory dysfunction in fish while exposed to higher salinities (Fazio et al., 2013; Soltanian et al., 2016). Glucose plays a significant role in the bioenergetics of animals, through its conversion into energy (Elarabany et al., 2017; Dawood et al., 2020; Islam et al., 2021a; Shahjahan et al., 2022). In the present study, blood glucose levels were significantly increased in the fish at higher salinities (6, and 9‰) in comparison to the control group (0‰) and lower salinity (3‰). In stressful conditions, the chromaffin cells increase the production and release of catecholamine hormones, adrenaline, and noradrenaline, into the blood (Baker et al., 2013; Nadermann et al., 2019). This triggers an increase in cortisol levels in the blood circulation, resulting in increased glucose production through glucogenesis and glycogenolysis to meet the higher energy demand (Iwama et al., 1997). In this study, increased blood glucose levels in the fish reared at high salinities might have occurred due to the increased rate of glucose transportation from the liver

to the blood to meet the excess energy requirement for brisk and erratic movements (Islam et al., 2020a).

The fish in all salinities and RBC counts in the banded gourami were reduced significantly compared to the fish in the control group (0‰). Reduced RBC counts might have resulted from salinity-induced osmotic changes caused by ion leakage from the plasma (Alwan et al., 2009). The reduction in RBC counts following exposure to sub-lethal osmotic stress indicates reduced blood oxygen-carrying capacity. Das et al. (2006) reported that exposure to sub-lethal salinity concentrations resulted in the deformation and breakdown of certain RBC cells in Silver barb that impaired blood oxygen-carrying capacity, which can be ameliorated by rising oxygen affinity and hemoglobin capability as well as by boosting RBC production. An amplified WBC count recognizes leukocytosis, which is measured as an adaptive response for animals exposed to stressors. In fish, the severity of stress increases leukocytosis, which is stimulated by immunological defense upon exposure to a stressor(s). In the present study, increased WBC count in fish at sub-lethal salinities might be linked to increased antibody production to recover and survive while exposed to a stressful situation (Joshi et al., 2002). It also plays an important role in the quick exclusion of cellular fragments produced by the necrosis of tissue (John, 2007). As the body's defense mechanism during stressful conditions, the amount of WBC increases by triggering the leukopoietic process and enhancing the discharge of leukocytes into the blood flow (Begg and Pankhurst, 2004). After 30 days of exposure, significantly higher WBC counts in the higher salinity exposed banded gourami in this study might have occurred due to the disorder in the acid-base equilibrium, respiratory homeostasis, and ionic regulation. A good number of research findings revealed that the number of WBCs noticeably increased in fish due to stressful conditions, which is supportive of the present study (Akinrotimi et al., 2012; Far et al., 2012; Geetha, 2014).

In the present study, the occurrence of erythrocytic nuclear and cellular abnormalities in fish exposed to tested salinities could be from stress imposed by osmotic imbalance, which is corroborated by similar studies with temperature and salinity stress in fish (Shahjahan et al., 2018; Jahan et al., 2019; Islam et al., 2020a, 2020b). These ECA and ENA abnormalities in blood cells might have been induced by protein-lipid phase distribution interaction, changes in lipid layer viscosity, and increased lipid peroxidation of erythrocytes (Ariful et al., 2019; Shahjahan et al., 2019; Islam et al., 2020b). Moreover, fish exposed to genotoxic materials result in different ENA in the blood, which was reported by several previous studies (Ergene et al., 2007; Costa et al., 2008). The most important role in salinity adaptation and acclimatization is a homeoviscous adaptation, i.e., adaptation to environmental salinity by regulation of the viscosity of the cell membranes' lipid phase through changes in their fatty acid composition (Cossins, 1977; Cossins and Prosser, 1978). In the case of cold-blooded organisms, the lipid bilayer micro-viscosity, lipid phase allocation, protein micro-surrounding, interaction between proteins-lipids, as well as other characteristics of the structural membrane of erythrocytes is influenced by environmental salinity (Kreps, 1981). However, it can be summarized that such types of ECA and ENA may occur through morphological changes in the plasma membrane and distressing surface deformability, thereby making the erythrocytes more vulnerable to rupture during crossing through small capillaries. Previous studies revealed that during salinity adaptation, to maintain the structural stability of the erythrocyte membrane, fish regulate lipid composition by modifying the intra- and intermolecular mobility and interactions of cytoskeleton proteins (Fiol and Kültz, 2007; Evans, 2010).

Conclusion

The study showed that banded gourami stress responses to climate-induced environmentally realistic salinities vary among the fish in four tested salinities. Salinity stress can affect growth, hemato-biochemical parameters, and erythrocyte structure. A concurrent relationship has been found among growth, survival rate, and stress-linked immune-physiological responses. Moreover, a higher degree of ECA and ENA were recorded in the fish exposed to higher salinities (6, and 9‰). Most of the measured parameters showed negative trends with slight adaptive responses during the experiment. Based on our findings, further studies are recommended for a better understanding of the growth performance and physiology of this fish in saline waters.

Ethical Statement

The experimental protocol was approved by the Ethics Committee of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh (2018/727/BAU-FoF).

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Author Contribution

Md Helal Uddin & Most. Fatema Khatun: Conceptualization, Methodology, Writing – Original Draft Preparation; Md Jakiul Islam: Resources and Analysis; Md. Mahfuzul Haque: Investigation and Visualization; Som Niyogi: Review and Editing; Harunur Rashid: Supervision, Review and Editing.

Conflict of Interest

The authors have no conflict of interest.

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References

Ahmed, N. (2013). Linking prawn and shrimp farming towards a green economy in Bangladesh: Confronting climate change. *Ocean & Coastal Management*, 75, 33-42. https://doi.org/10.1016/j.ocecoaman.2013.01.002

Ahmmed, M. K., Ahmmed, F., Kabir, K. A., Faisal, M., Ahmed, S. I., & Ahsan, M. N. (2017). Biochemical impacts of salinity on the catfish, *Heteropneustes fossilis* (Bloch, 1794), and possibility of their farming at low saline water. *Aquaculture research*, 48 (8), 4251-4261. https://doi.org/10.1111/are.13246

Aihua, L., & Buchmann, K. (2001). Temperature-and salinity-dependent development of a Nordic strain of *Ichthyophthirius multifiliis* from rainbow trout. *Journal of Applied Ichthyology*, 17 (6), 273-276. https://doi.org/10.1046/j.1439-0426.2001.00279.x

Akinrotimi, O. A., Agokei, E. O., & Aranyo, A. A. (2012). Changes in blood parameters of Tilapia guineensis exposed to different salinity levels. *Journal of Environmental Engineering and Technology*, 1 (2), 4-12.

Alam, M. Z., Carpenter-Boggs, L., Mitra, S., Haque, M., Halsey, J., Rokonuzzaman, M., Saha, B., & Moniruzzaman, M. (2017). Effect of salinity intrusion on food crops, livestock, and fish species at Kalapara Coastal Belt in Bangladesh. *Journal of Food Quality*, 2017. https://doi.org/10.1155/2017/2045157

Altinokand, I., & Grizzle, J. M. (2001). Effects of brackish water on growth, feed conversion and energy absorption efficiency by juvenile euryhaline and freshwater stenohaline fishes. *Journal of fish Biology*, 59 (5), 1142-1152. https://doi.org/10.1111/j.1095-8649.2001.tb00181.x

Alwan, S. F., Hadi, A. A., & Shokr, A. E. (2009). Alterations in hematological parameters of fresh water fish, *Tilapia zillii*, exposed to aluminum. *Journal of Science and its Applications*, 3 (1), 12-19.

Ashaf-Ud-Doulah, M., Shahjahan, M., Islam, S. M., Al-Emran, M., Rahman, M. S. & Hossain, M. A. R. (2019). Thermal stress causes nuclear and cellular abnormalities of peripheral erythrocytes in Indian major carp, rohu *Labeo rohita*. *Journal of Thermal Biology*, 86, p.102450. https://doi.org/10.1016/j.jtherbio.2019.102450

Baker, M. R., Gobush, K. S., & Vynne, C. H. (2013). Review of factors influencing stress hormones in fish and wildlife. *Journal for Nature Conservation*, 21 (5), 309-318. https://doi.org/10.1016/j.jnc.2013.03.003

Begg, K., & Pankhurst, N. W. (2004). Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish *Acanthochromis polyacanthus*. *Journal of Fish Biology*, 64 (1), 133-145. https://doi.org/10.1111/j.1095-8649.2004.00290.x

Beyea, M. M., Benfey, T. J., & Kieffer, J. D. (2005). Hematology and stress physiology of juvenile diploid and triploid shortnose sturgeon (*Acipenser brevirostrum*). *Fish Physiology and Biochemistry*, 31, 303-313. http://dx.doi.org/10.1007/s10695-005-1552-y

Bianco, P. G., & Nordlie, F. (2008). The salinity tolerance of *Pseudophoxinus stymphalicus* (Cyprinidae) and *Valencia letourneuxi* (Valenciidae) from western Greece suggests a revision of the ecological categories of freshwater fishes. *Italian Journal of Zoology*, 75 (3), 285-293. https://doi.org/10.1080/11250000801931753

Bœuf, G., & Payan, P. (2001). How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 130 (4), 411-423. https://doi.org/10.1016/S1532-0456(01)00268-X

Carrasco, K. R., Tilbury, K. L., & Myers, M.S. (1990). Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. *Canadian Journal of Fisheries and Aquatic Sciences*, 47 (11), 2123-2136. https://doi.org/10.1139/f90-237

Cazenave, J., Wunderlin, D. A., Hued, A. C., & Bistoni, M. D. L. A. (2005). Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. *Hydrobiologia*, 537, 25-33.

Cossins, A. R. (1977). Adaptation of biological membranes to temperature. The effect of temperature acclimation of goldfish upon the viscosity of synaptosomal membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 470 (3), 395-411. https://doi.org/10.1016/0005-2736(77)90131-6

Cossins, A. R., & Prosser, C. L. (1978). Evolutionary adaptation of membranes to temperature. *Proceedings of the National Academy of Sciences*, 75 (4), 2040-2043. https://doi.org/10.1073%2Fpnas.75.4.2040

Costa, P. M., Lobo, J., Caeiro, S., Martins, M., Ferreira, A. M., Caetano, M., Vale, C., DelValls, T. A., & Costa, M. H. (2008). Genotoxic damage in *Solea senegalensis* exposed to sediments from the Sado Estuary (Portugal): effects of metallic and organic contaminants. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 654 (1), 29-37. https://doi.org/10.1016/j.mrgentox.2008.04.007

Das, P. C., Ayyappan, S. & Jena, J. K. (2006). Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. *Aquaculture*, 256 (1-4), 80-87. https://doi.org/10.1016/j.aquaculture.2006.02.019

Dawood, M. A., Alkafafy, M., & Sewilam, H. (2022). The antioxidant responses of gills, intestines and livers and blood immunity of common carp (*Cyprinus carpio*) exposed to salinity and temperature stressors. *Fish Physiology and Biochemistry*, 48(2), 397-408. https://doi.org/10.1007/S10695-022-01052-W/FIGURES/5

Dawood, M. A., Eweedah, N. M., Elbialy, Z. I., & Abdelhamid, A. I. (2020). Dietary sodium butyrate ameliorated the blood stress biomarkers, heat shock proteins, and immune response of Nile tilapia (*Oreochromis niloticus*) exposed to heat stress. *Journal of Thermal Biology*, 88, p.102500. https://doi.org/10.1016/j.jtherbio.2019.102500

Deane, E. E., & Woo, N. Y. (2009). Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: a review. *Reviews in Fish Biology and Fisheries*, 19, 97-120. https://doi.org/10.1007/s11160-008-9091-0

Denson, M. R., Stuart, K. R., Smith, T. I., Weirlch, C. R., & Segars, A. (2003). Effects of salinity on growth, survival, and selected hematological parameters of juvenile cobia *Rachycentron canadum*. *Journal of the World Aquaculture Society*, 34(4), 496-504. https://doi.org/10.1111/J.1749-7345.2003.TB00088.X

Elahee, K. B. & Bhagwant, S. (2007). Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. *Ecotoxicology and Environmental Safety*, 68 (3), 361-371. https://doi.org/10.1016/j.ecoenv.2006.06.003

Elarabany, N., Bahnasawy, M., Edrees, G., & Alkazagli, R. (2017). Effects of salinity on some haematological and biochemical parameters in Nile tilapia, *Oreochromus niloticus*. *Agriculture, Forestry and Fisheries*, 6 (6), 200-205. https://doi.org/10.11648/J.AFF.20170606.13

El-Leithy, A. A., Hemeda, S. A., El Naby, W. S. A., El Nahas, A. F., Hassan, S. A., Awad, S. T., El-Deeb, S. I., & Helmy, Z. A. (2019). Optimum salinity for Nile tilapia (*Oreochromis niloticus*) growth and mRNA transcripts of ion-regulation, inflammatory, stress-and immune-related genes. *Fish physiology and biochemistry*, 45, 1217-1232. https://doi.org/10.1007/S10695-019-00640-7/FIGURES/6

Ergene, S., Çavaş, T., Celik, A., Köleli, N., Kaya, F., & Karahan, A. (2007). Monitoring of nuclear abnormalities in peripheral erythrocytes of three fish species from the Goksu Delta (Turkey): genotoxic damage in relation to water pollution. *Ecotoxicology*, 16, 385-391. https://doi.org/10.1007/s10646-007-0142-4

Evans, T.G. (2010). Co-ordination of osmotic stress responses through osmosensing and signal transduction events in fishes. *Journal of Fish Biology*, 76 (8), 1903-1925. https://doi.org/10.1111/J.1095-8649.2010.02590.X

Evans, T.G., & Kültz, D. (2020). The cellular stress response in fish exposed to salinity fluctuations. Journal of Experimental Zoology Part A: *Ecological and Integrative Physiology*, 333 (6), 421-435. https://doi.org/10.1002/JEZ.2350

Faizul, M. I. M., & Christianus, A. (2013). Salinity and Stocking Density Effect on Growth and Survival of *Barbodes gonionotus* (Bleeker, I860) Fry. *Journal of Fisheries and Aquaticscience*, 8 (2), 419. https://doi.org/10.3923/jfas.2013.419.424

Far, M. S., Roodsari, H. V., Zamini, A., Mirrasooli, E., & Kazemi, R. (2012). The effects of diazinon on behavior and some hematological parameters of fry rainbow trout (*Oncorhynchus mykiss*). *World Journal of Fish and Marine Sciences*, 4(4), 369-375. DOI: 10.5829/idosi.wjfms.2012.04.04.61167

Fashina-Bombata, H. A., & Busari, A. N. (2003). Influence of salinity on the developmental stages of African catfish *Heterobranchus longifilis* (Valenciennes, 1840). *Aquaculture*, 224 (1-4), 213-222. https://doi.org/10.1016/S0044-8486(03)00273-4

Fazio, F., Marafioti, S., Arfuso, F., Piccione, G., & Faggio, C. (2013). Influence of different salinity on haematological and biochemical parameters of the widely cultured mullet, *Mugil cephalus*. *Marine and Freshwater Behaviour and Physiology*, 46(4), 211-218. http://dx.doi.org/10.1080/10236244.2013.817728

Fiol, D. F., & Kültz, D. (2007). Osmotic stress sensing and signaling in fishes. *The FEBS journal*, 274 (22), 5790-5798. https://doi.org/10.1111/J.1742-4658.2007.06099.X

Froese, R., & Pauly, D. (Eds.) 2018. Fish base 2018, World Wide Web electronic publication. Available at: http://www.fishbase.org

Geetha, S. (2014). Studies on the effects of water pH changes on hematological parameters in *Gerres filamentosus* (Cuvier). *International Journal of Environmental Sciences*, 4 (5), 983-986.

Gonzalez, R. J. (2012). The physiology of hyper-salinity tolerance in teleost fish: a review. *Journal of Comparative Physiology B*, 182, 321-329. https://doi.org/10.1007/S00360-011-0624-9/FIGURES/2

Gupta, S. (2015). A note on feeding and reproductive biology of banded gourami,*Trichogaster fasciata* (Bloch & Schneider, 1801). *International Journal of Research in Fisheries and Aquaculture*. 5, 147–150.

Hasan, M. H., Hossain, M. J., Chowdhury, M. A., & Billah, M. (2020). Salinity intrusion in southwest coastal Bangladesh: an insight from land use change. *In Water, Flood Management and Water Security under a Changing Climate: Proceedings from the 7th International Conference on Water and Flood Management*, 125-140. Springer International Publishing. https://doi.org/10.1007/978-3-030-47786-8_8

Herrera, M., Vargas-Chacoff, L., Hachero, I., Ruíz-Jarabo, I., Rodiles, A., Navas, J. I. & Mancera, J. M. (2009). Osmoregulatory changes in wedge sole (*Dicologoglossa cuneata* Moreau, 1881) after acclimation to different environmental salinities. *Aquaculture Research*, 40(7), 762-771. http://dx.doi.org/10.1111/j.1365-2109.2008.02147.x

Hussain, M.G. (2010). Freshwater fishes of Bangladesh: Fisheries, biodiversity and habitat. *Aquatic Ecosystem Health & Management*, 13(1), 85-93. https://doi.org/10.1080/14634980903578233

Imsland, A. K., Foss, A., Bonga, S. W., Van Ham, E., & Stefansson, S. O. (2002). Comparison of growth and RNA: DNA ratios in three populations of juvenile turbot reared at two salinities. *Journal of Fish Biology*, 60(2), 288-300. https://doi.org/10.1111/j.1095-8649.2002.tb00280.x

McCarthy, James J., Osvaldo F. Canziani, Neil A. Leary, David J. Dokken, & Kasey S. White, eds. Climate change 2001: impacts, adaptation, and vulnerability: contribution of Working Group II to the third assessment report of the Intergovernmental Panel on Climate Change. Vol. 2. Cambridge University Press, 2001.

Islam, M. J., Kunzmann, A., & Slater, M.J. (2021a). Extreme winter cold-induced osmoregulatory, metabolic, and physiological responses in European seabass (*Dicentrarchus labrax*) acclimatized at different salinities. *Science of the Total Environment*, 771, p.145202. https://doi.org/10.1016/j.scitotenv.2021.145202

Islam, M. J., Kunzmann, A., Bögner, M., Meyer, A., Thiele, R., Slater, M. J. (2020d). Metabolic and molecular stress responses of European seabass, *Dicentrarchus labrax* at low and high temperature extremes. *Ecological Indicators*, 112, 106118. https://doi.org/10.1016/j.ecolind.2020.106118

Islam, M. J., Kunzmann, A., Slater, M. J. (2021c). Extreme winter cold-induced osmoregulatory, metabolic, and physiological responses in European seabass (*Dicentrarchus labrax*) acclimatized at different salinities. *Science of the Total Environment,* 771, 145202.

https://doi.org/10.1016/j.scitotenv.2021.145202

Islam, M. J., Kunzmann, A., Thiele, R., & Slater, M. J. (2020a). Effects of extreme ambient temperature in European seabass, *Dicentrarchus labrax* acclimated at different salinities: Growth performance, metabolic and molecular stress responses. *Science of the Total Environment*, 735, p.139371. https://doi.org/10.1016/j.scitotenv.2020.139371

Islam, M. J., Slater, M. J., Bögner, M., Zeytin, S., & Kunzmann, A. (2020b). Extreme ambient temperature effects in European seabass, *Dicentrarchus labrax*: Growth performance and hemato-biochemical parameters. *Aquaculture*, 522, p.735093. https://doi.org/10.1016/j.aquaculture.2020.735093

Islam, M. J., Slater, M. J., Kunzmann, A. (2020d). What metabolic, osmotic and molecular stress responses tell us about extreme ambient heatwave impacts in fish at low salinities: The case of European seabass, *Dicentrarchus labrax*. *Science of the Total Environment,* 749, 141458. https://doi.org/10.1016/j.scitotenv.2020.141458

Islam, M. J., Slater, M. J., Thiele, R., & Kunzmann, A. (2021b). Influence of extreme ambient cold stress on growth, hematological, antioxidants, and immune responses in European seabass, *Dicentrarchus labrax* acclimatized at different salinities. *Ecological Indicators*, 122, p.107280. https://doi.org/10.1016/j.ecolind.2020.107280

Islam, M. S., Milstein, A., Wahab, M. A., Kamal, A. H. M., & Dewan, S. (2005). Production and economic return of shrimp aquaculture in coastal ponds of different sizes and with different management regimes. *Aquaculture International*, 13, 489-500. https://doi.org/10.1007/s10499-005-9000-7

Iwama, G. K., Takemura, A., & Takano, K. (1997). Oxygen consumption rates of tilapia in fresh water, sea water, and hypersaline sea water. *Journal of Fish Biology*, 51(5), 886-894. https://doi.org/10.1111/j.1095-8649.1997.tb01528.x

Jahan, A., Nipa, T. T., Islam, S. M., Uddin, M. H., Islam, M. S., & Shahjahan, M. (2019). Striped catfish (*Pangasianodon hypophthalmus*) could be suitable for coastal aquaculture. *Journal of Applied Ichthyology*, 35(4), 994-1003. https://doi.org/10.1111/jai.13918

John, P.J. (2007). Alteration of certain blood parameters of freshwater teleost *Mystus vittatus* after chronic exposure to Metasystox and Sevin. *Fish physiology and Biochemistry*, 33, 15-20. https://doi.org/10.1007/s10695-006-9112-7

Joshi, P., Harish, D., & Bose, M. (2002). Effect of lindane and malathion exposure to certain blood parameters in a fresh water teleost fish *Clarias batrachus*. *Pollution Research*, 21(1), 55-57.

Kasim, M. H. (1983). Salinity tolerance of certain freshwater fishes. Indian Journal of Fisheries, 30, 46-54.

Kibria, G., Ahmed, M., Kibria, M. G., Ahmed, K. K. U. (2005). Diversity of selective and non-selective fishing gear and their impact on inland fisheries in Bangladesh. *WorldFish Center Newsletter,* 28.

Kreps, E. M. (1981). Lipidy Kletochnykh Membran (Lipids of Cell Membranes), Nauka, Leningrad, 339.

Lai, S., Loke, L. H. L., Hilton, M. J., Bouma, T. J., Todd, P. A. (2015). The effects of urbanization on coastal habitats and the potential for ecological engineering: A Singapore case study. *Ocean & Coastal Management* 103, 78–85. https://doi.org/10.1016/j.ocecoaman.2014.11.006

Li, D., Long, D., Zhao, J., Lu, H., Hong, Y. (2017). Observed changes in flow regimes in the Mekong River basin. *Journal of Hydrology*, 551, 217–232. https://doi.org/10.1016/j.jhydrol.2017.05.061

Lohner, T. W., Reash, R. J., Willet, V. E., & Rose, L.A. (2001). Assessment of tolerant Sunfish populations (*Lepomis* sp.) inhabiting selenium-laden coal ash effluents: 1. Hematological and population level assessment. *Ecotoxicology and Environmental Safety*, 50(3), 203-216. https://doi.org/10.1006/eesa.2001.2097

Lowe, M. R., Wu, W., Peterson, M. S., Brown-Peterson, N. J., Slack, W. T., Schofield, P. J. (2012). Survival, growth and reproduction of non-native Nile tilapia II: fundamental niche projections and invasion potential in the northern Gulf of Mexico. *PLoS One* 7, e41580. https://doi.org/10.1371/journal.pone.0041580

Masroor, W., Farcy, E., Gros, R., & Lorin-Nebel, C. (2018). Effect of combined stress (salinity and temperature) in European sea bass *Dicentrarchus labrax* osmoregulatory processes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 215, 45-54. https://doi.org/10.1016/j.cbpa.2017.10.019

Maugars, G., Manirafasha, M. C., Grousset, E., Boulo, V., Lignot, J. H. (2018). The effects of acute transfer to freshwater on ion transporters of the pharyngeal cavity in European seabass (*Dicentrarchus labrax*). *Fish Physiology and Biochemistry*, 44, 1393–1408. https://doi.org/10.1007/s10695-018-0529-6

Mitra, K., Suresh, V., Vinci, G., … N.M.-A.F., 2007, undefined, 2007. Biology and fishery of banded gourami, *Colisa fasciata* (Bloch and Schneider 1801) in a floodplain wetland of Ganga river basin. researchgate.net. https://doi.org/10.33997/j.afs.2007.20.4.007

Nadermann, N., Seward, R. K., & Volkoff, H. (2019). Effects of potential climate change-induced environmental modifications on food intake and the expression of appetite regulators in goldfish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 235, 138-147. https://doi.org/10.1016/j.cbpa.2019.06.001

Nhan, N.H. (2016). Tidal regime deformation by sea level rise along the coast of the Mekong Delta. *Estuarine, Coastal and Shelf Science*, 183, 382–391. https://doi.org/10.1016/j.ecss.2016.07.004

Nuruzzaman, A., Rabbi, F., Mahmuduzzaman, M., Ahmed, U., Nuruzzaman, A. K. M., Ahmed, S. (2014). Causes of salinity intrusion in coastal belt of Bangladesh. researchgate.net 4, 8–13. https://doi.org/10.5923/s.plant.201401.02

O'Neill, B., De Raedemaecker, F., McGrath, D., & Brophy, D. (2011). An experimental investigation of salinity effects on growth, development and condition in the European flounder (*Platichthys flesus*. L.). *Journal of Experimental Marine Biology and Ecology*, 410, 39-44. https://doi.org/10.1016/j.jembe.2011.10.007

Overton, J. L., Bayley, M., Paulsen, H., & Wang, T. (2008). Salinity tolerance of cultured Eurasian perch, *Perca fluviatilis* L.: effects on growth and on survival as a function of temperature. *Aquaculture*, 277(3-4), 282-286. https://doi.org/10.1016/j.aquaculture.2008.02.029

Rabbani, G., Rahman, A., Mainuddhin, K., Shoef, I. J. (2013). Loss and Damage from Salinity Intrusion in Satkhira District, Coastal Bangladesh. Bangladesh Centre for Advanced Studies (BCAS), Dhaka.

Rahman, M. T., Ziku, A. L. M. E., Choudhury, T. R., Ahmed, J. U., Mottaleb, M. A. (2015). Heavy metal contaminations in vegetables, soils and river water: a comprehensive study of Chilmari, Kurigram, Bangladesh. *The International Journal of Environment, Ecology, Family and Urban Studies*, 5, 29–42.

Rahman, M., Islam, M., Hossain, M., Hasan, M., Khatun, D., Rahman, O., Parvin, M., Mawa, Z., & Chowdhury, A. A. (2019). Morphometric and Meristic Characteristics of the Banded Gourami, *Trichogaster fasciata* (Bloch & Schneider, 1801) in a Wetland Ecosystem from Northwestern Bangladesh. *Jordan Journal of Biological Sciences*, 12 (5).

Ramee, S. W., Lipscomb, T. N., DiMaggio, M. A. (2020). Evaluation of the effect of larval stocking density, salinity, and temperature on stress response and sex differentiation in the Dwarf Gourami and Rosy Barb. *Aquaculture Reports*, 16, 100287. https://doi.org/10.1016/J.AQREP.2020.100287

Reid, G., Gurney-Smith, H., Marcogliese, D., Knowler, D., Benfey, T., Garber, A., Forster, I., Chopin, T., Brewer-Dalton, K., Moccia, R., Flaherty, M., Smith, C., De Silva, S. (2019). Climate change and aquaculture: considering biological response and resources. *Aquaculture Environment Interactions*, 11, 569–602. https://doi.org/10.3354/aei00332

Reid, S. G., Bernier, N. J., & Perry, S. F. (1998). The adrenergic stress response in fish: control of catecholamine storage and release. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 120(1), 1-27. https://doi.org/10.1016/S0742-8413(98)00037-1

Saintilan, N., Rogers, K., Kelleway, J. J., Ens, E., Sloane, D. R. (2019). Climate Change Impacts on the Coastal Wetlands of Australia. *Wetlands* 39, 1145–1154. https://doi.org/10.1007/S13157-018-1016-7/FIGURES/3

Salam, M. A., Shahjahan, M., Sharmin, S., Haque, F., & Rahman, M. K. (2015). Effects of sub-lethal doses of an organophosphorus insecticide sumithion on some hematological parameters in common carp, *Cyprinus carpio*. *Pakistan Journal of Zoology*, 47(5).

Saravanan, M., Ramesh, M., Petkam, R., & Poopal, R. K. (2018). Influence of environmental salinity and cortisol pretreatment on gill Na+/K+− ATPase activity and survival and growth rates in *Cyprinus carpio*. *Aquaculture reports*, 11, 1-7. https://doi.org/10.1016/j.aqrep.2018.04.002

Schmitz, M., Baekelandt, S., Bequet, S., & Kestemont, P. (2017a). Chronic hyperosmotic stress inhibits renal Toll-Like Receptors expression in striped catfish (*Pangasianodon hypophthalmus*, Sauvage) exposed or not to bacterial infection. *Developmental & Comparative Immunology*, 73, 139-143. https://doi.org/10.1016/j.dci.2017.03.020

Schmitz, M., Ziv, T., Admon, A., Baekelandt, S., Mandiki, S. N., L'Hoir, M., & Kestemont, P. (2017b). Salinity stress, enhancing basal and induced immune responses in striped catfish *Pangasianodon hypophthalmus* (Sauvage). *Journal of proteomics*, 167, 12-24. https://doi.org/10.1016/j.jprot.2017.08.005

Sehgal, K. L. (2015). Coldwater Fish and Fisheries in the. FAO (Food and Agriculture Organization of the United Nations) Fisheries Technical Paper 1–18.

Selim, S.A., Glaser, M., Tacke, F.I., Rahman, M., Ahmed, N. (2021). Innovative Aquaculture for the Poor to Adjust to Environmental Change in Coastal Bangladesh? Barriers and Options for Progress. *Frontiers in Marine Science*, 8, 175. https://doi.org/10.3389/FMARS.2021.635281/BIBTEX

Shahjahan, M, Kitahashi, T., Ogawa, S., Parhar, I. S. (2013). Temperature differentially regulates the two kisspeptin systems in the brain of zebrafish. *General and Comparative Endocrinology,* 193, 79–85. https://doi.org/10.1016/j.ygcen.2013.07.015

Shahjahan, M., Islam, M. J., Hossain, M. T., Mishu, M. A., Hasan, J., Brown, C. (2022). Blood biomarkers as diagnostic tools: An overview of climate-driven stress responses in fish. *Science of the Total Environment* 156910. https://doi.org/10.1016/J.SCITOTENV.2022.156910

Shahjahan, M., Kitahashi, T., Ogawa, S., & Parhar, I. S. (20130. Temperature differentially regulates the two kisspeptin systems in the brain of zebrafish. *General and Comparative Endocrinology*, 193, 79-85. https://doi.org/10.1016/j.ygcen.2013.07.015

Shahjahan, M., Rahman, M. S., Islam, S. M. M., Uddin, M. H., Al-Emran, M. (2019). Increase in water temperature increases acute toxicity of sumithion causing nuclear and cellular abnormalities in peripheral erythrocytes of zebrafish *Danio rerio*. *Environmental Science and Pollution Research*, 26, 36903–36912. https://doi.org/10.1007/s11356-019-06886-1

Shahjahan, M., Uddin, M. H., Bain, V., & Haque, M. M. (2018). Increased water temperature altered hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish *Pangasianodon hypophthalmus*. *Fish Physiology and Biochemistry*, 44, 1309-1318. https://doi.org/10.1007/s10695-018-0522-0

Shameem, M. I. M., Momtaz, S., Kiem, A. S. (2015). Local perceptions of and adaptation to climate variability and change: the case of shrimp farming communities in the coastal region of Bangladesh. *Climate Change* 133, 253–266. https://doi.org/10.1007/s10584-015-1470-7

Sharmin, S., Shahjahan, M., Hossain, M. A., Haque, M. A., & Rashid, H. (2015). Histopathological changes in liver and kidney of common carp exposed to sub-lethal doses of malathion. *Pakistan Journal of Zoology*, 47(5).

Smajgl, A., Toan, T. Q., Nhan, D. K., Ward, J., Trung, N. H., Tri, L. Q., Tri, V. P. D., Vu, P. T. (2015). Responding to rising sea levels in the Mekong Delta. *Nature Climate Change*. 5, 167–174. https://doi.org/10.1038/nclimate2469

Soltanian, S., Vazirzadeh, A., & Fallahi, R. (2016). Effects of sudden salinity changes on short-term hematological and biochemical responses in Mudskipper *Periophthalmus waltoni* Koumans 1941 (Gobiidae: Perciformes). *Iranian Journal of Ichthyology*, 3(1), 31-42.

Sumon, K. A., Saha, S., van den Brink, P. J., Peeters, E. T., Bosma, R. H., & Rashid, H. (2016). Acute toxicity of chlorpyrifos to embryo and larvae of banded gourami *Trichogaster fasciata*. *Journal of Environmental Science and Health*, Part B, 52 (2), 92-98. https://doi.org/10.1080/03601234.2016.1239979

Sumon, K. A., Yesmin, M. F., Van den Brink, P. J., Bosma, R. H., Peeters, E. T. H. M., Rashid, H. (2019). Effects of long-term chlorpyrifos exposure on mortality and reproductive tissues of Banded Gourami (*Trichogaster fasciata*). *Journal of Environmental Science and Health, Part B* 54, 549–559.

https://doi.org/10.1080/03601234.2019.1614399/SUPPL_FILE/LESB_A_1614399_SM5606.DOCX

Talwar, P. K., Jhingran, A. G. (1991). Inland fishes of India and adjacent countries–Oxford and IBH publishing Co. Pvt. Ltd. New Delhi, Bombay, Calcutta.

Tsuzuki, M. Y., Sugai, J. K., Maciel, J. C., Francisco, C. J., & Cerqueira, V. R. (2007). Survival, growth and digestive enzyme activity of juveniles of the fat snook (*Centropomus parallelus*) reared at different salinities. *Aquaculture*, 271(1-4), 319-325. https://doi.org/10.1016/j.aquaculture.2007.05.002

Ytrestøyl, T., Takle, H., Kolarevic, J., Calabrese, S., Timmerhaus, G., Rosseland, B. O., Teien, H. C., Nilsen, T. O., Handeland, S. O., Stefansson, S. O., Ebbesson, L. O. E., Terjesen, B. F. (2020). Performance and welfare of Atlantic salmon, *Salmo salar* L. post-smolts in recirculating aquaculture systems: Importance of salinity and water velocity. *Journal of World Aquaculture Society*, 51, 373–392. https://doi.org/10.1111/jwas.12682

Zou, H., He, F., Lan, Z., Tang, L., Lu, W. (2019). The personality of Japanese flounder (*Paralichthys olivaceus*) and gene expression related with osmoregulatory capacity in the gills. *Aquaculture*, 500, 221–227. https://doi.org/10.1016/j.aquaculture.2018.10.013