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Acute responses of spotted snakehead (*Channa punctata*) to salinity stress: A study of a freshwater fish to salinity challenges during intrusion of saline water

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Abstract

A climate driven changes in the coastal region of Bangladesh is saltwater intrusion in freshwater, which may directly affect the fishery production as well as economy and food security. Investigation on effect of different concentration of salinities (0, 5, 10, and 15ppt) on hemato-biochemical parameters, behavioral responses and gill morphology at certain time intervals (1, 6, 12, 24, 36, 48, and 72 h) were observed in a spotted snakehead, *Channa punctata*. In the present study the biochemical parameter and glucose exposed a significant (p<0.05) changes associated with different exposures at different time intervals. The hematological data of *C. punctata* revealed significant (p<0.05) decrease in red blood cell (RBC) count. The hemoglobin level in every treatment was lower till 24 h and then it increased up to 48 h but white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) showed significant (p<0.05) increase compared to the control. Increase and decrease of hematological indices expressed that fish exposure to different salinities were under stress. Unusual behavioral responses with various pathological signs in gills were also perceived. The results specified that the intrusion of saline water into the freshwater have deleterious effects on the hemato-biochemical parameters including gill morphology and behavior. So, the intrusion of saline water should be controlled to avert the losses.

Keywords: Freshwater fish, Hematological parameter, Physiological responses, Salinity

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Introduction

Bangladesh is a low-lying deltaic land located in South-Asia lies just less than 2 m above sea level (Schiermeier, 2014) with a 710 km coastline on the northern littoral of the largest bay of the world the Bay of Bengal. The coastal belt of Bangladesh is about 47201 km^2 or about 32% or almost one third of the total area of country, covering 19 districts dominantly under Barisal, Khulna and Chittagong Divisions, encompassing 147 sub districts, where 35.1 million people live accounting 28% of the total population of Bangladesh (WPO, 2004).

Bangladesh is one of the most disaster-prone countries in the world due to its unique geographical location and topography. Climatologically, the entire coastal zone of Bangladesh in worldwide recognized as an extremely vulnerable area than the other parts of the country because natural catastrophes ravage the country, particularly the coastal belt, almost every year. Bangladesh is perspiring with many environmental problems and its associated hazards, as she is particularly susceptible to the effects of climate change. Bangladesh is vulnerable to a combination of climatic variables, including floods, tropical cyclones, tornadoes, tidal bores, drought, flood, rainfall, salinity, and sea level rise. According to the Global Climate Risk Index, Bangladesh is ranked sixth among countries susceptible to climate change, while it was ranked first in 2012 (Harmeling and Eckstein, 2012). Water salinity and its distribution in the coastal area are increasing with the increase of sea level rise (World Bank, 2000). The main impacts of sea level rise on coastal water resources are fresh water availability reduction by salinity intrusion. Sea level rise is predicted to inundate 120,000 km^2 of Bangladesh (FPMU, 2013). Total 53% of the coastal areas are affected by different degrees salinity destroying normal characteristics of coastal soil and water. Salinity intrusion is spreading into the non-coastal areas as well.

In Bangladesh, salinity intrusion is a time varying event and occurs due to reduction of freshwater inflows from upstream; siltation of the rivers following the construction of the polder system, direct inundation by saline water through tidal flooding during wet season (June-October) and upward or lateral movement of saline ground water during dry season (November-May) fosters salinization process. Especially in the dry season, the problems turn into exacerbated when rainfall is inadequate and incapable of lowering the concentration of salinity on surface water and leaching out salt from soil. Saline water intrusion into the inland fresh water sources is causing severe threat to the existence of the species living around the world's greatest mangrove forest, the Sundarbans, Cyclone, Tsunami (26 December, 2004), Sidr (15 November, 2007) and Aila (27 May, 2009), Komen (30 July, 2015), Roanu (21 May, 2016),

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Mora (28 May, 2017) hit South and South Western part of Bangladesh along with about half of the tropical forest Sundarbansand destroyed the coastal embankment infrastructure and increased the salinity. The Sundarbans will be completely lost with 1-metre sea level rise (World Bank, 2000).

Soil-water salinity and sea level rises will naturally affect the ecosystem and biodiversity of the coastal region of Bangladesh as well as hamper crop production but may bring some conveniences in the production of high valued fish products like the shellfishes especially shrimp and other brackish water fishes, but it is a threatening problem to the fishes and fisheries resources those are available only in the freshwater are going to be endangered and may be extinct due to salinity intrusion. Bangladesh has lost its abundance and many fish species became vulnerable or endangered for much natural and human interference. Increased salinity and change in water quality can instigate a change in species composition and distribution especially in coastal areas. There will be clear change in seasonal abundance of individual fish. Salinity intrusion will affect the readiness, maturity, and gonadal development of fishes in breeding season. Higher water salinity level may bring changes in physiology and sex ratios of fishes alter timing of spawning, migrations, and/or peak abundance, increased invasive species, diseases and algal blooms. These will lead to changes in timing and levels of productivity across marine and freshwater systems and reduced production of target species in marine and freshwater systems. Thus, strictly freshwater Cyprinidae, Anabantidae, Channidae, and many other fishes may likely suffer most (WFC, 2007). This would in turn encounter a change in fish culture practices in the affected areas.

Threats to the coastal areas because of salinity intrusion include the turn down of capital value of land, damage to infrastructure, salinization of water storage, loss of farm flora and fauna, the loss of nursery ground of many fish species, loss of shelter etc. Salinization is one of the foremost crucial issues for a low-lying country like Bangladesh. In fact, very little attention has been given on water salinity as a whole. However, none can ignore effects of salinity, which may occur in any country and any time. Therefore, it is important to pay attention on it locally and globally to bring sustainability.

The freshwater fishes, which have no or low ability to adapt in increasing saline water, some changes occur in their behavior, blood parameter, gill, liver, and kidney as they become stressed due to environmental change. But at advanced or tertiary phase responses involve systemic changes in which animals may become incapable of adapting to stressors, leading to adverse effects on the animal's overall health, including their performance, growth, reproduction, disease resistance, and behavior (Barton, 2002). Biochemical constituents and enzymes

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have been explored as potential biomarkers for variety of organisms as these parameters are highly sensitive and conserved between species and less variable (Agrahari and Gopal, 2009). Hence, there is a critical need to know the actual physiological consequences of salinity on an organism and to understand whether some of the freshwater species can be survived in the salinity-intruded areas. Therefore, the present study was undertaken to examine the acute effect of salinity on hematoma-biochemical parameters, behavioral and gill morphology of a freshwater spotted snakehead, *Channapunctata* kept under different salinity exposures.

Materials and methods

Adult and moderate sized (TL-24±1cm; W-70± 7g) spotted snakehead procured from the local fish market and nurtured in the rectangular cemented tank. Acclimatization period retained for five days. Live feed (*Tubifex*) and fish paste were provided, twice in a day for proper feeding. Fish of both sexes were used without discrimination. Brine water (120 ppt) was used to prepare three saline water solutions (5, 10, and 15ppt). Firstly, dechlorinated tap water was reserved in the pre-cleaned aquarium (45×30×30 cm^3) and then brine water poured into it to make the expected saline water for the study. The estimated level of salinity was tested by a refractometer (Optika, HRD, Italy). Pre-acclimatized 35 spotted snakehead were directly exposed to the different salinity levels (0, 5, 10, and 15ppt) on every tank. Sampling was done at 1, 6, 12, 24, 36, 48, and 72h after being exposed.

Hematological analyses were carried out by standard methods suggested by Blaxhall and Daisley (1973). At least five fishes from each treatment were sacrificed at fixed time intervals (1, 6, 12, 24, 36, 48, and 72h) for the hematological assessment. For obtaining blood samples, fish were quickly taken out from water in a gentle manner by a small scoop net and held on dissection tray with a cloth covering the head and blood samples from each fishes were withdrawn from the caudal vein using a heparinized plastic syringe. Whole blood withdrawal process took less than one min per fish to avoid stress effects to minimize an error in normal blood values. Collected blood was gently pushed into a sterilized eppendorf tubes containing anticoagulant (ethylene diamine tetra-acetic acid, EDTA) to give a final concentration of 5mg EDTA per cm^3 blood. Blood samples were mixed gently and discarded if any difficulty was encountered in taking them or if clots were seen in the vial during inspection in the laboratory. Glucose was measured by placing a drop of blood in the strip of a digital glucose meter, GLUCOLAB^{TM} through micropipette. The values were estimated as mmol/l. Hemoglobin estimation was done by applying a drop of blood on the test strips of digital EasyLife® Hb meter. The values

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wereexpressed in g/dl. Packed cell volume (PCV)/haematocrit (Hct) was determined by Wintrobe's haematocrit tube expressed in ml% using the following formula:

Hct percentage **=** (100/total sample length) x RBC length

Blood samples were collected from each treatment at different time intervals (1, 6, 12, 24, 36 48, and 72h) and were placed at Neubauer hemocytometer immediately after collection for counting of blood cells.
The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae as described by Jain (1986):

MCV = (PCV ÷ RBC) x 10
MCH (pg) = (Hb x 10) ÷ RBC
MCHC = (Hb ÷ PCV) x 100

Study on behavioral responses
To observe the behavioral stress responses, fish were exposed to dechlorinated tap water (0 ppt) and considered as control where as experimental fish were exposed to the different salinity levels (5, 10 and 15ppt). The abnormal stress behaviors were observed by visual assessment as suggested by Aysel and Ayhan (2010). Behavioral responses of fish such as convulsions, equilibrium status or imbalance, fin movement, hyperactivity and swimming rate were observed. Some other behavioral criteria were also observed as stress responses such as shoaling, body and eye color etc. Behavioral stress responses were observed at different time intervals (1, 6, 12, 24, 36, 48, and 72 h).

Study on morphological changes of gill
The effects of salinity on gill morphology of the spotted snakehead were observed under an Optica optical microscope (G-206, Italy) using 10x objective lens after exposure to different salinity concentrations at different time intervals (1, 6, 12, 24, 36, 48 and 72h). Fish gills were collected immediately and blood, mucus, dust like materials etc. were cleaned smoothly with a soft brush. Then the gills were placed on glass slide and observed under a microscope.

Statistical analysis
Statistical analysis was performed using the ANOVA to determine differences between treatments at 5% level of significance (p<0.05). The standard error of treatment was also estimated. All statistical analysis was carried out using SPSS 20.0 software.

Results
Effect of salinity on glucose level
In the present study, the glucose levels in control fish ranges 2.2 to 4.4 mmol/L (Table 1). Dramatic increase in the glucose level was seen at 5ppt with a slight fluctuation during 24h to 48h exposure compared to control and then it started to decrease. The highest level

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of glucose obtained at 5ppt was 10.9 mmol/l during 48h exposure. At 10ppt salinity the glucose level decreased gradually up to 12h exposure and then increased with an infrequent changes scoring highest value at 72h while at 15ppt there was a moderate changes in glucose level compared to control group in the whole study period (Table 1).

Effect of salinity on hemoglobin

The hemoglobin levels at 5, 10, and 15ppt were lower compared to control groups until 24h exposure and then it sharply increased up to 48h with a swift decrease at 72h exposure. The highest hemoglobin was found at 15ppt salinity just over 16 g/dL at 48h exposure (Table 1).

Effect of salinity on haematocrit (Hct)

The results of Hct (%) values are presented in Table 1. Hct (%) at 5ppt and 10ppt salinity decreased with a moderate fluctuation compared to control condition. After first hour of exposure to 15ppt the Hct (%) speedily decreased, then rose up and remained steady till 24h. After 24h of exposure, the value decreased hurriedly.

Effect of salinity on red blood cell (RBC)

The results of RBC (cells x 10^6/mm^3) values are presented in Table 1. The number of RBC exponentially decreased during the study period at 5, 10, and 15ppt with a mild fluctuation in comparison to control groups.

Effect of salinity on white blood cell (WBC)

The results of WBC (cells x 10^3/mm^3) values are presented in Table 1. The WBC significantly increased at 36, 48, and 72h exposure at both 5 and 10ppt treatments comparing to the control condition. On the other hand, the WBC significantly (p<0.05) increased at 12, 36 and 48h at 15ppt condition compared to control.

Effect of salinity on MCV, MCH, and MCHC

The mean corpuscular volume (MCV) is a measure of the average volume of red blood cell. In the present study, MCV for every treatment was higher than the control (Table 1). At 5ppt, MCV increased with slight fluctuation with the duration of exposure but it significantly (p<0.05) increased at 72h. There was a gradual increasing of MCV at 10ppt until 48h and after that, it decreased at 72h of exposure. In case of MCHC, it decreased after one hour of exposure in every treatment compared to control. MCHC significantly (p<0.05) increased at 36, 48 and 72h exposure at 5 and 10ppt salinity. On the contrary, at 15ppt, MCHC decreased for the first 24h of exposure with an increase at 12h compared to control and finally it increased dramatically until 72h (Table 1). In case of MCH, which is the average mass of hemoglobin per red blood cell in a sample of blood showed a significant (p<0.05) increase at every hour after being exposed to 5, 10 and 15ppt.

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Table 1: Mean hematological parameters of spotted snakehead exposed to different salinity concentrations (0, 5, 10 and 15ppt) Values are mean ± standard deviation, n=5, * Significant at $p<0.05$ level, RBC=Red blood cell, WBC=White blood cell, Hb=Hemoglobin, Hct=Haematocrit, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration.

Parameter	Exposure time (h)	Control (0 ppt)	Concentration of salinity (ppt)		
			5	10	15
RBCs ($x\ 10^6/mm^3$)	1	**12.91±0.57**	**6.71±0.59***	**4.75±0.24***	**3.04±0.81***
	6	12.43±0.64	3.14±0.54*	4.42±0.62*	3.02± 2.68*
	12	13.84±1.40	3.77±0.45*	5.63± 3.48*	4.866±2.69*
	24	12.30±0.72	3.19±0.72*	2.56±1.58*	2.81±1.96*
	36	12.68±0.50	3.97±0.39*	3.34±2.33*	3.826±1.03*
	48	13.02±1.07	1.57±0.80*	2.98± 2.70*	1.873±0.61*
	72	12.94±1.31	0.72±0.54*	3.43±1.23*	-
WBCs ($\times10^4/mm^3$)	1	**4.27± 0.35**	**4.16± 0.34**	**3.97± 0.59**	**4.38±0.47**
	6	4.13± 0.16	4.86±0.21	4.96± 0.75	5.08±0.76
	12	3.94±0.96	5.52±0.26	5.56± 0.78	6.63±0.97*
	24	4.28±0.53	5.60±0.81	6.29± 0.66	6.23±0.89
	36	4.41± 0.23	6.75±0.31*	6.48± 0.54*	7.18±1.23*
	48	4.64±0.19	7.43±0.52*	6.91± 1.26*	7.94±0.85*
	72	4.39±0.53	6.71±0.91*	8.02± 0.49*	-
Glucose (mmol/l)	1	**2.36±0.12**	**4.10±0.76***	**4.63±1.51**	**3.20±0.74***
	6	2.80±0.36	3.03±0.76*	2.63±0.23*	2.60±0.01*
	12	4.03±0.21	4.20±1.58*	1.93±0.42*	2.60±1.04*
	24	3.33±1.10	2.96±1.09	3.00±1.83*	2.86±1.79*
	36	3.20±0.36	6.06±1.06	2.33±1.15*	3.23±0.25*
	48	3.20±1.00	8.53±2.35*	3.63±1.25*	3.33±0.51*
	72	3.50±0.88	6.53±2.79*	4.96±2.47*	-
Hemoglobin (g/dL)	1	**8.86±0.38**	**3.93±0.77***	**4.63±1.51***	**3.23±0.73***
	6	8.76±0.42	7.73±0.15	8.26±0.25	8.60±0.20*
	12	9.36±0..42	8.30±0.36	9.13±1.07	9.80±0.25*
	24	8.30±0.78	7.96±0.77	8.23±0.91	8.16±0.90*
	36	8.96±0.50	13.26±1.91*	12.70±1.30*	15.90±2.58*
	48	8.6±0.25	14.93±2.13*	11.56±1.50	16.60±2.40*
	72	8.7±0.76	8.4±0.52	9.33±1.25	-
Hct (%)	1	**40.22±15.56**	**36.37±0.49**	**33.47±1.30**	**30.92±4.19**
	6	39.06±15.35	26.76±0.88*	30.196±5.78*	37.12±6.78
	12	37.96±5.02	27.78±5.56*	29.31±2.23*	36.55±0.43
	24	36.78±11.74	22.44±0.82*	21.76±3.57*	37.63±5.68
	36	36.04±10.59	24.11±2.06*	25.18±3.67	27.71±2.65*
	48	37.27±10.88	26.84±1.59	25.08±1.93*	27.37±2.63*
	72	37.23±10.56	13.23±0.55*	16.26±6.16*	-
MCV (μm^3)	1	**38.82±2.83**	**54.47±5.13**	**70.58± 6.13**	**108.08± 36.79**
	6	31.56±12.43	86.51±11.16	70.47± 24.09	298.66± 360.84*
	12	27.57± 4.11	73.13± 7.02	78.85± 62.50	89.61± 40.08
	24	29.71± 8.19	72.11± 12.50	128.07± 104.45	225.35± 205.87*
	36	28.34±8.08	61.38± 10.71	154.57± 171.40	76.72± 24.21
	48	28.78±9.20	213.77± 129.76	169.30 ± 169.53	154.52± 42.27
	72	28.86± 8.08	316.24± 297.50*	47.31± 4.33	-
MCH (pg)	1	**6.87±0.32**	**5.83± 0.82**	**9.74± 3.23**	**11.46±4.76**
	6	7.06± 0.52	25.12± 4.25	19.00± 3.21	64.83±71.52
	12	6.79±0.39	22.13± 2.02	21.49± 13.41	23.87± 10.06
	24	6.76±0.69	26.01± 7.26	49.53± 42.55	46.25± 40.53
	36	7.06±0.21	33.89 ± 8.01	79.74±89.78	41.05± 16.31
	48	6.67±0.37	129.97 ± 106.16*	72.81± 66.84	96.47± 36.64*
	72	6.85±1.17	201.69 ± 187.31*	28.63 ± 6.24	-
MCHC (%)	1	**17.76±1.57**	**10.81± 2.02**	**13.88±4.62**	**10.36±1.09**
	6	25.52±11.96	28.92± 1.34	27.91± 4.19	23.72±4.62
	12	24.91± 2.87	30.54± 3.87	31.45± 5.73	26.91±0.94
	24	24.26±8.38	35.61± 4.33	38.35±6.17	22.97±5.84
	36	26.69±9.28	54.91±3.15*	51.42±11.02*	57.13± 4.14*
	48	24.85±8.24	56.1±11.69*	46.12± 4.78*	61.54±14.29*
	72	24.56±5.97	63.57±4.89*	60.71±13.21*	-

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The average mass of hemoglobin per red blood cell, MCH, was highest at 72h of exposure at 10ppt of salinity (Table 1).

Behavioral responses of C. punctata to different salinity exposures

The behavioral responses of *C. punctata* to different salinity exposures and control groups were observed throughout the experimental period. In control group, the movements of the fish were normal and they moved in a school. No abnormal behavior due to stress was found. Gulping was absent. The 5ppt of salinity was found to be very little effective to the test fish. In this treatment the treated fish swam freely. Their movement was comparatively normal. For the first 20 minutes of exposure they were relaxed and then started gulping. Most of them remained agile and alive at the end of exposure period (72h). No significant abnormal behavior was observed. After 28h the fish started to show abnormality in their behavior. They become exhausted and started moving abnormally in the aquarium, they showed jerky movement and frequently run to the surface. Fast movement and gulping of fish were noticed immediately after exposure to 10ppt and after 20 minutes they become relaxed. After 1.5h of exposure all fish were being stable at one corner of the aquarium just continuously gasping, gill opening was less than normal. Some of them came to the upper zone of aquarium for gasping. The affected fish became very weak, gradually dropped on the bottom keeping their head upward. It is very likely that the treated fish were unable to carry out their normal physiological functions. The treated fish become totally relaxed after 15 minutes of exposure. The test fish frequently tried to jump out from the test media. They were seen to stay on the bottom of the aquaria and sometimes they come near the surface with jerking movement. They also showed fast and sudden erratic and circular movement and later stages they settled at the bottom of the aquarium with very slow opercular movement and occasionally attempting to swim but failed. The clinically observed signs on animals at this concentration were eye protrusion, darkening on the body surface and loss of scales on the body surface. Prior to death, their activity slowed down or generally inactive and no fish survived at 72h.

Morphological changes of gill

Morphological changes in the gill of fish due to salinity treatment were detected. As gill lamellae are the primary receptor of water as well as the ionic movement in water, it will be affected by any change in osmotic balance. In the present study, typical structure of gill was found in the control group. At 5ppt, no significant change of gill morphology was observed except an appearance of ruptured lamellae and lamellar fusion with slight slime secretion. However, at 10ppt and 15ppt, significant changes were observed

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compared to the fish in control such as ruptured and broken lamellae, curved and broken gill filament, gill necrosis etc. (Fig. 1).

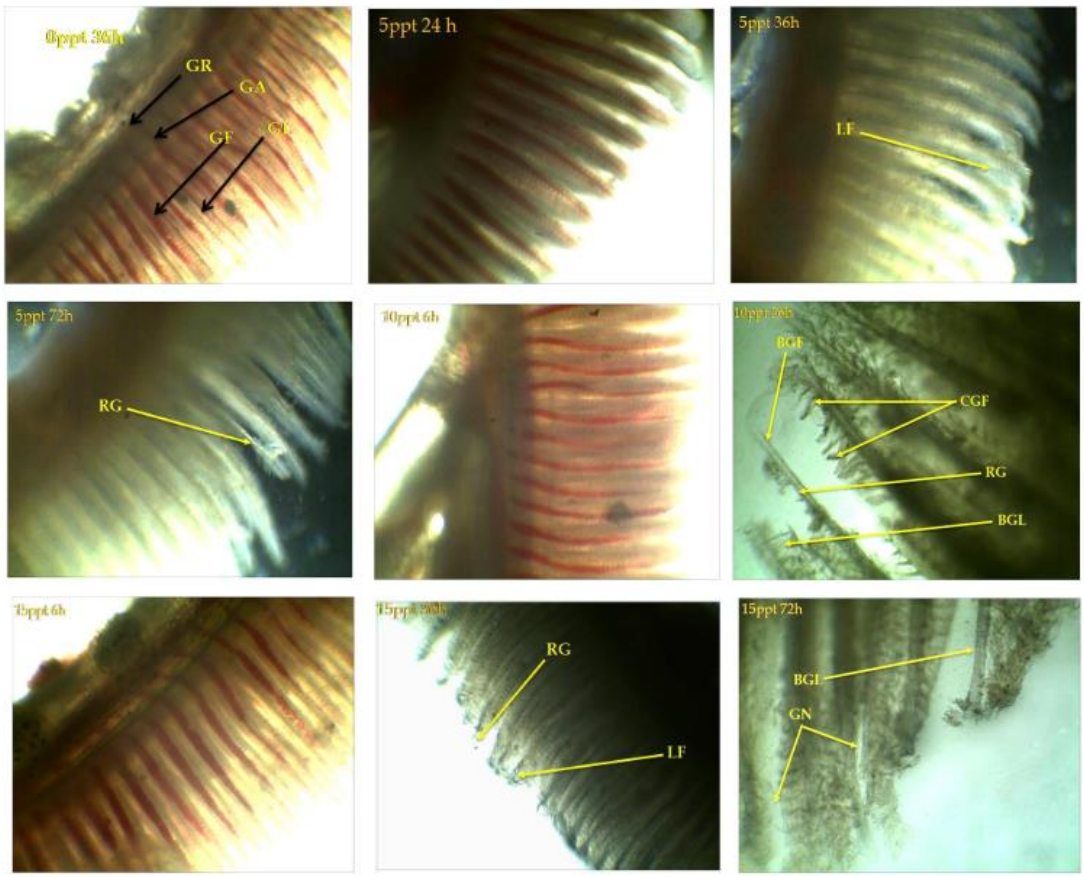


Figure 1: Microscopic view of gill morphology at different salinity levels atdifferent time intervals (6, 24, 36, and 72h). Arrows are indicating gill lamellae (GL), gill raker (GR), gill arch (GA), gill filament (GF) and, ruptured gill (RG), lamellar fusion (LF), (BGL), broken gill filament (BGF), curved gill filament (CGF), ruptured gill (RG), lamellar fusion (LF), gill necrosis (GN).

Discussion

Salinity represents a critical environmental factor for all aquatic organisms, including fishes. A change in the saltiness of habitat water causes salinity stress because, if not compensated for, it interferes with physiological homeostasis and routine biological processes. The present study revealed the acute responses of *C. punctata*to salinity stress.

Blood glucose is a highly reliable stress response parameter that is strongly influenced by handling and environmental stresses as well as seasonal variations, and nutritional status (Prasad and Charles, 2010). The elevation of blood glucose following stress functions to provide energy for the 'fight-or-flight' reaction (Wedemeyer *et al.*, 1990). In the

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present experiment, the highest glucose level at 5ppt was 10.9 mmol/l at 48 hour. At 10ppt, the glucose level decreased gradually at first 12 hours and then increased with an infrequent changes scoring highest value at 72 hours while at 15ppt there was a moderate changes in glucose level compared to control group in the study period. In stressful conditions (internal or external) the chromaffin cells elevated the secretion of catecholamine hormones, adrenaline and noradrenaline toward blood circulation (Reid *et al*., 1998) which in turn in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways (Iwama *et al*., 1997) to cope with the energy demand produced by the stressor for the "fight of flight" reaction. The stored glucose in different cells breaks down and passes through the blood ultimately increases the glucose level with duration of exposure to different concentration. The elevated blood glucose levels reflect an increase in the rate of transportation of glucose probably from the liver to muscle where high energy demand was met due to brisk and erratic movements (Ravichandran *et al*., 1995).

In the present study hemoglobin decreased until 24h in every treatment, which may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production, which resembles to the finding of Ahmad *et al*. (1995). The release of immature cells from haemopoietic tissue into the blood strength as well as disruption of iron metabolism may lead to a defective hemoglobin synthesis. The validity of the significant decrease in the concentration of Hb may also be due to an increase either in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis (Reddy and Bashamohideen, 1989) which supports the judgment of the current study.

In this study, Hct (%) value significantly decreased with increasing the concentration of saltwater and exposure duration. The present finding is harmonious with corresponding's of Gabriel *et al.*(2011) andAkinrotimi *et al*. (2012). The validity of the judgment of decreased Hct (%) is due to major reduction in RBC. The reduction in RBC count and Hb are often accompanied by a decrease in Hct and demonstrates the physiological dysfunction of the hemopoietic system. In order to overcome hypoxic conditions in stressful media, fish usually responded by increasing the MCV and MCH of erythrocytes.

In the present study the RBC decreased significantly at 5, 10 and 15ppt compared to control due to stresses for different concentration of saline water. The reduction in the RBC count of silver barb following exposure to sub-lethal concentration of salinity indicated a reduced blood oxygen carrying capacity (Das *et al*., 2006) which also granted the findings. In the context of distortion and lysis of certain RBC cells as observed at sub-lethal treatment, the

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reduced blood oxygen carrying capacity can be compensated either through increasing oxygen affinity and capacity of hemoglobin and/or through increase in RBC production (Das *et al.*, 2006).

Escalation in total WBC count in the present study was a result of direct stimulation for its defense from diseases due to the exposure to different salinity. An increased WBC count established leukocytosis, which is considered to be of an adaptive value for the tissue under chemical stress. Leukocytosis is directly proportional to severity of stress condition in maturing fish and is a result of direct stimulation of immunological defense due to the exposure. The increase in WBC count at sub-lethal concentrations can be correlated with an increase in antibody production, which helps in survival and recovery of the fish exposed to toxicants (Joshi *et al*., 2002). This also helps in the removal of cellular debris of necrosed tissue at a faster rate (John, 2007). As a protective response of the body during stress, WBC increases through stimulation of leukopoietic process and enhanced release of leukocytes to the blood circulation. The released catecholamines, adrenaline and nor-adrenaline, increase the conversion of liver glycogen to blood glucose to satisfy the greater energy demands of the body to stress (Begg and Pankhurst, 2004). The variation of the WBC and increased blood glucose levels in the present study indicated elevated stress levels in the spotted snakehead, which were most likely due to the disturbance in the acid-base balance, respiratory homeostasis, and ionic regulation during exposure to sub-lethal salinity. Consistent supports to the above with several results, which showed a significant increase in the WBC (Akinrotimi *et al*., 2012; Far *et al*., 2012; Geetha, 2014).

A significant increase in hematological indices MCV, MCH, and MCHC levels were observed in the test fish after 1h exposure compared to the control. Khoshbavar-Rostami *etal*. (2006) also found stressors increase MCV and MCH level at certain period in *Acipenser stellatus* that supports the finding of the study.

Throughout the study period, fish exhibited low behavioral stress responses when exposed to salinity of 5ppt. Impatience or agitated activeness or erratic movements were observed when exposed to concentrations (10ppt and 15ppt). From the beginning fish showed frequent movement from surface to bottom, aggression and sometime showed jumping activity. In addition, fish expressed highly increased opercular movements accompanied by excessive secretion of mucus which indicating respiratory distress of fish. Aysel and Ayhan (2010) and Fahima *et al*. (2016) also noted similar behavioral stress responses after exposing fish in higher salinities.

At 5ppt, there was no significant change of gill morphology observed except ruptured lamellae with slight mucus secretion. However, at lethal

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concentration (15ppt) significant change was observed compared to the fish in control. The most common symptoms of the salinity effects on gill morphology of spotted snakehead included ruptured and broken lamellae, necrosis, curved and broken filament etc. Lin *et al*. (2004), Sardella *et al*. (2004), Evans *et al*. (2005), and Yahona and Pablo (2007) also reported similar interpretations.

Due to climate change, habitat degradation and anthropogenic activities, the severity and frequency of salinity stress are increasing in many parts of the world and may eventually exceed the coping ability of an unknown number of species. So it is concluded that the intrusion of salinity into freshwater has severe effects on the normal physiology of tested fishes causing stresses. Hence, the present investigation results confirm that stress due to salinity intrusion into freshwater does create hematological disturbances, erythrocyte destruction (hemolysis) and leukocytosis in fish, affecting the immune system and making the fish vulnerable to diseases. Therefore, the fish is provided as a bioindicator of deteriorating water quality and care should be taken to monitor the effects of sudden saltwater intrusion to freshwater territory. Sea level rise has become a serious and emergent problem in the past two decades. Preventive measure should be taken to minimize the losses of the fish in coastal area to avoid this unexpected salinity invasion.

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