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BEHAVIORAL AND PHYSIOLOGICAL STRESS RESPONSES OF Java barb (*Barbonymus gonionotus*) TO ENVIRONMENTAL SALINITY CHALLENGE

Fahima Binte AMIN, Tania FARHANA, Golam Mohammod MOSTAKIM, Md. Mahiuddin ZAHANGIR, Mahbuba Monir MISHU, M. Sadiqul ISLAM

Department of Fisheries Biology & Genetics Bangladesh Agricultural University Mymensigh-2202, Bangladesh

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Corresponding author:

M. Sadiqul ISLAM, Department of Fisheries Biology & Genetics Bangladesh Agricultural University Mymensigh-2202, Bangladesh

E-mail: sadiqul1973@yahoo.com

Abstract:

Salinity impose stress on the physiology of the exposed freshwater fish population can modify their structure. In this study some indicators of behavioral and physiological stress responses were examined during exposure of Java barb (*Barbonymus gonionotus*) to different saline concentrations. Based on the result of median lethal salinity level (LC_{50}) (12.9ppt), one sub-lethal (12ppt) and two lethal salinity levels (14ppt and 16ppt) were selected to study the acute stress responses of fish for a period of 72h. A number of physiological responses, such as disturbance in body fluid, alterations of blood biochemical and hematological parameters and behavioral responses were detected with increasing ambient salinity. Fish exposed to different sub-lethal and lethal salinities exhibited clinical signs agitated behavior, respiratory distress, abnormal nervous behavior and death were recorded. From the present investigation, it was observed that higher salinity may affect the physiological and behavioral stress responses in freshwater fish.

Keywords: Behavior, Freshwater fish, Physiology, Salinity, Stress

Introduction

Environmental changes in organisms are always associated with a number of physiological responses. Theoretically these responses can be measured in fish and in other vertebrates in the form of changes in hormonal or substrate concentration in the plasma or alterations in the blood parameters (Donaldson, 1981). Salinity is an important factor in the environmental condition of fish by influencing osmotic pressure and metabolism (Mustafayev and Mekhtiev, 2008); causing changes physiological functions, habits, and survival of fish (Wang and Zhu, 2002).

Fish species which inhabits only the freshwater environments may be extinct in the coastal areas due to saltwater intrusion and increase of the salinity. As compared to terrestrial inhabitants, fish and other aquatic creatures are subject to a broader variety of stressors because their homeostatic mechanisms are highly dependent on prevailing conditions in their immediate surroundings. Examples of additional stressors for aquatic animals other than terrestrial animals include fluctuations in water salinity, pH, hardness, alkalinity, dissolved solids, water level or current, and exposure to waterborne pathogens or toxicants. Being an aquatic animal the fish have to face various stressors and have to cope with regular and unexpected fluctuation of different water parameters to survive, and adapt physiologically with alteration and modification of different organs and its behavior (Jasmin, 2013).

Java barb (*Barbonymus gonionotus*) a stenohaline freshwater fish species which was native to Asia but now spread worldwide and colonized in various environments was used in the experimental stages of the study. It can tolerate a range of different salinities from freshwater up to 13 ppt (BFRI, 1993), but the behavioral and physiological mechanisms that underlie the salinity tolerance of freshwater species are not well understood. The present study examines the possible effects that osmoregulatory processes can have on the behavioral and physiological responses in Java barb that are subjected to increasing levels of environmental salinity.

Materials and Methods

Healthy and moderate sized (8.02 ±1.44cm and 5.51 ±1.16g) Java barb (*B. gonionotus*) were collected from the local fish farm (Digarkanda, Mymensingh) and reared in the cemented rectangular tank and allowed to acclimatize to the laboratory conditions for 15 days to remove the suspected unhealthy subjects (handling and transportation stress) at 24-25°C. A total of 280 individuals were randomly assigned. Fishes were fed twice a day with zooplankton (enriched with *Daphnia*, *Keratella*, etc.) and commercial dry pellets (Krishibid Fish Feed Ltd.). The fishes were considered well adapted to laboratory conditions when mortality was recorded less than 1% during acclimatization period of 15 days. Fish of both sexes were used without discrimination.

The different levels of salinity concentrations used were achieved by serial dilution of brine water (150 ppt) with dechlorinated tap water, to get the desired concentrations. Pre-acclimatized fish were directly exposed to the different salinity levels (0, 2, 4, 6, 8, 10, 11, 12, 13, 14, 15 and 16ppt) for range finding test and determination of LC_{10-100} by static bioassay. The OECD Directive No. 203 (1992) 'Fish, acute toxicity test' was followed for the toxicity assay. Aeration was applied to the aquaria (45×30×30 cm^3) in order to obtain a homogeneous concentration of salt and to maintain standard DO concentration. Several inspections were made during the experimental period and dead fish if any, were removed immediately. Fish were considered as dead when respiratory movement of the opercula stopped and there was no response to touch. The salinity concentrations below the median lethal concentration (LC_{50}) value and above the LC_{50} values were considered as sub-lethal and lethal salinity concentrations, respectively.

To observe the behavioral stress responses, fish were exposed to tap water (dechlorinated) (0ppt) and considered as control, one sub-lethal concentration (12ppt) and two lethal concentrations (14ppt and 16ppt) of salinity for 72h. 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 (2, 4, 6, 12, 24, 48, 72h) and scored as 0 to 5 (very weak, weak, moderate, strong, and very strong) according to degree of responses.

To obtain blood samples, fish were caught gently in a small scoop net and then quickly taken out from the water and fish were anaesthetized by 5mg/l of clove oil to avoid any handling stress in order to minimize an error in normal blood values. Blood was obtained from the renal artery by cutting off the tail peduncle. Blood glucose was measured by using a digital blood glucose kit. A drop of blood sample was placed on the strips connected to the GLUCOLAB™ Auto-coding blood glucose test meter and results were recorded. The values were expressed in mmol/l. Hematological analyses were carried out by standard methods suggested by Blaxhall and Daisley (1973). To examine red blood cell (RBC) and white blood cell (WBC) counts, collected blood was gently pushed into sterilized eppendorf tubes containing anticoagulant (EDTA) to give a final concentration of 5mg EDTA per cm^3 blood. Blood samples were mixed gently and counts were made using a Neubauer hemocytometer.

Dehydration of fish body was measured to observe the effect of the sub-lethal and lethal concentrations of salinity. Weights were recorded from the beginning to end of the experiment at different time intervals (0, 1, 3, 6, 12, 24, 48, 72h).

Median lethal concentration (LC_{50}) values were calculated from the data obtained in acute toxicity bioassays, by Finney's (1971) method of "probit analysis". The data were subjected to an analysis of variance (ANOVA), followed by comparison of means using Tukey's HSD test to determine significance of each data treatment. In addition, the significance of differences of the values of RBC and WBC were determined by using "t test". Significant differences were indicated by P-values <0.05. All statistical analyses were performed using SPSS16.0.

Results and Discussion

Median concentration (LC_{50})

A study was carried out to find the range of salinity tolerance of Java barb from 0ppt to 16ppt. There was no mortality observed up to 10ppt but almost 100% mortality was observed at 16ppt in 72h exposure period. Based on range finding test the LC_{50} of salinity for Java barb was determined between 10ppt and 16ppt. At salinity concentration of 11ppt 20% mortality and at 13ppt 50% mortality was found, 100% mortality was occurred at 16ppt. Probit analysis showed that the LC_{50} of the fishes was about 12.90ppt.

Lethal and sub-lethal effects of salinity on behavioral stress responses

The fish exhibited a normal behavioral activity without mortality when exposed to 0ppt. At sub-lethal concentration of salinity (12ppt) a low stress responses were noticed like aggression, stunned posture, erratic swimming etc. Stress responses like impatience or agitated activeness or erratic movement observed at lethal concentrations (14ppt and 16ppt) (Table 1, Figure 1). At the beginning of experiment (up to 3 hours) the fish showed frequent movement from surface to bottom, then aggression and sometime showed jumping activity at the lethal concentrations of salinity. They also expressed highly increased opercular movements accompanied by excessive secretion of mucus which indicates the respiratory distress. Fish were scattered here and there. Then the fish became very weak with a fade and gloomy body and eye color, settled at the bottom and finally died.

Effects of salinity on biochemical and hematological parameters

Variations on the biochemical parameter such as blood glucose levels at both treatments (6.5ppt, 50% of LC_{50} and 9.68ppt, 75% of LC_{50}) were found to be increased within an hour and highest about from 6-12h. Then glucose level gradually decreased and got back to its original states within 24h (Figure 2). A significant (P<0.05) decrease of RBC values were registered up to 24h in fish that were exposed to sub-lethal salinities (6.5ppt and 9.68ppt) compared to control (0ppt) group (Figure 3). On the other hand, the pattern was different for WBCs: values showed a significant increase up to 24h in a salinity of 9.68ppt (75% of LC_{50}), but subsequently returned to normal values within 72h as in the control group (Figure 4).

Table 1. Behavioral and clinical signs of Java barb after exposure 72h to different salinities

Behavioral signs	**Clinical signs**	**Salinity (ppt)**			
		0	**12**	**14**	**16**
Agitated behavior	Aggression	0	1	1	5
	Jumping	0	0	3	5
	SP	0	2	3	3
	FSBM	0	0	2	4
	ES	0	1	3	3
Respiratory distress	OM	0	0	2	4
	AG	0	0	4	5
	VPES	0	0	3	5
	EMS	0	0	3	5
Abnormal nervous behavior	SSM	0	1	2	4
	SM	0	2	4	4
	SD	0	0	3	5
	DP	0	0	4	3
	Death	0	0	3	5

Air gulping (AG), erratic swimming (ES), frequent surface to bottom movements (FSBM), vertical posture with exposed snouts (VPES), excessive mucus secretion (EMS), sluggish and swirling movements (SSM), state of motionless (SM), sudden darts (SD), different postures (DP), opercula movement (OM), stunned posture (SP); none (0), very weak (1), weak (2), moderate (3), strong (4), very strong (5)

Lethal and sub-lethal effects of salinity on osmotic challenge

In this study lethal and sub-lethal effect of salinity on the osmotic change was determined by observing variations of body weight of exposed fish to different salinity concentrations (Figure 5). At 12ppt, a gradual and slow decrease of weight was noticed up to 12h of exposure and within 24h body weight got back to its original state. On the other hand, at lethal concentrations of a sudden decrease of average weight (body fluid reduced up to 19%) of fish were recorded at the beginning hours of exposure. Then the weight decreased gradually and got back to its original state (Figure 5a, b).

The stress response of a freshwater Java barb to different salinity concentrations was evaluated using the levels of biochemical and hematological parameters of blood and behavior as indicators. Aquaculture urges for more accurate information on stress control, in order to be assured of good health status of fish, especially those transferred to a new environment. Environmental salinity fluctuation can act a stressor and leads to give information on behavioral and physiological responses.

Behavioral endpoints serve as valuable tools to distinguish and evaluate effects of exposure to environmental stressors, and fish behavioral alterations can provide important indices for ecosystem assessment (Kane *et al*., 2005). Throughout the study period fish exhibited low behavioral stress responses when exposed to sub-lethal concentration of salinity (12ppt). Impatience or agitated activeness or erratic movements were observed when exposed to lethal concentrations (14ppt and 16ppt). From the beginning up to 3 hours of the lethal salinity exposure 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. Lawson and Anetekhai (2011) and Aysel and Ayhan (2010) also noted similar behavioral stress responses after exposing fish in higher salinities.

The osmotic gradient between the plasma and the environment is reduced in freshwater fishes when exposed to increase salinity. Kilambi and Zdinak (1980) stated decreasing survival rates with increasing salinity is characteristics of freshwater stenohaline fish, and it has been postulated that this is due to increasing osmotic pressures at higher salinities. In the present study a gradual and slow weight loss was noticed within 1-3 h of sub-lethal salinity exposure whereas at lethal concentrations a rapid decrease of weight was observed and then the weight increased gradually. Similar trend also reported by Augley *et al.* (2008) and Maceina and Shireman (1979). Norton and Davis (1977) suggested that the major limiting factor affecting the survival of freshwater fish in saltwater is the absence of efficient branchial and renal mechanisms that can readily adjust to hypertonic media. This inability to adjust to increase salinity results in increased electrolyte concentrations and osmotic stress (Furspan *et al.*, 1984) which causes an increase in osmotic pressure and deterioration of cell function.

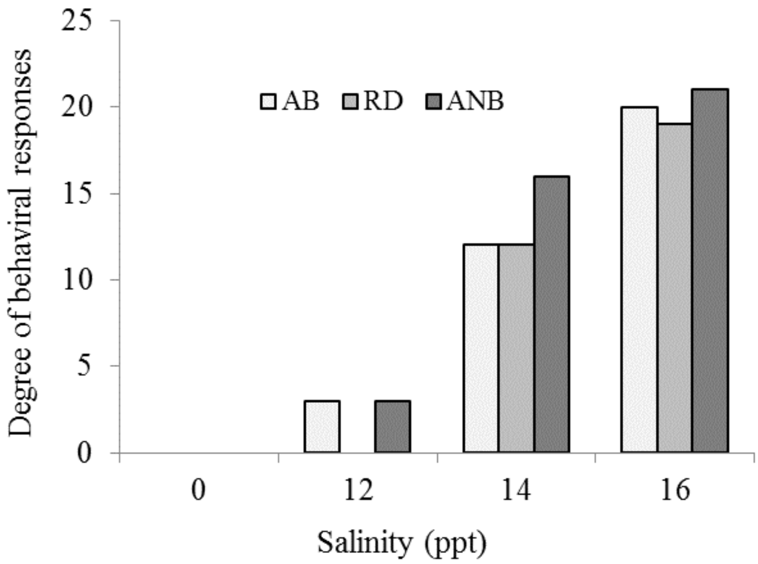


Figure 1. Degree of behavioral stress responses of Java barb at different salinity levels (AB= agitated behavior, RD= respiratory distress and ANB= abnormal nervous behavior

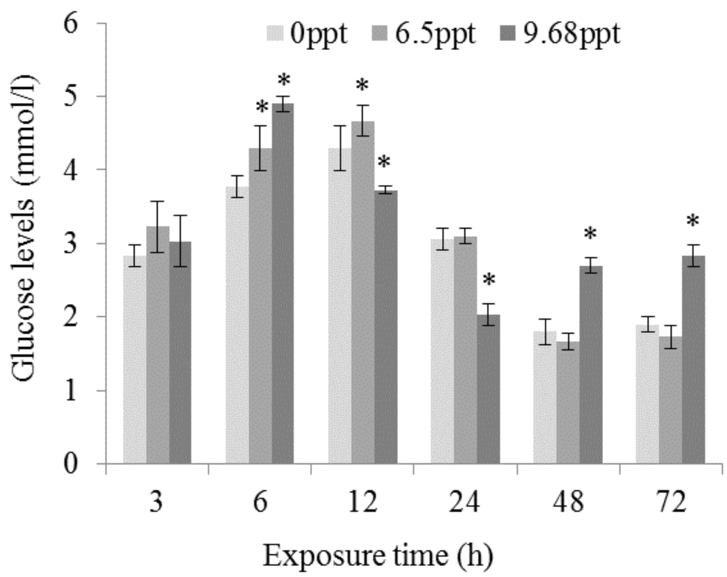


Figure 2. Sub-lethal effects of salinity on blood glucose levels at different time intervals in Java barb. Values are means ± SD. Asterisk (*****) indicates significant difference at P<0.05. *N* = 5 fish at each salinity

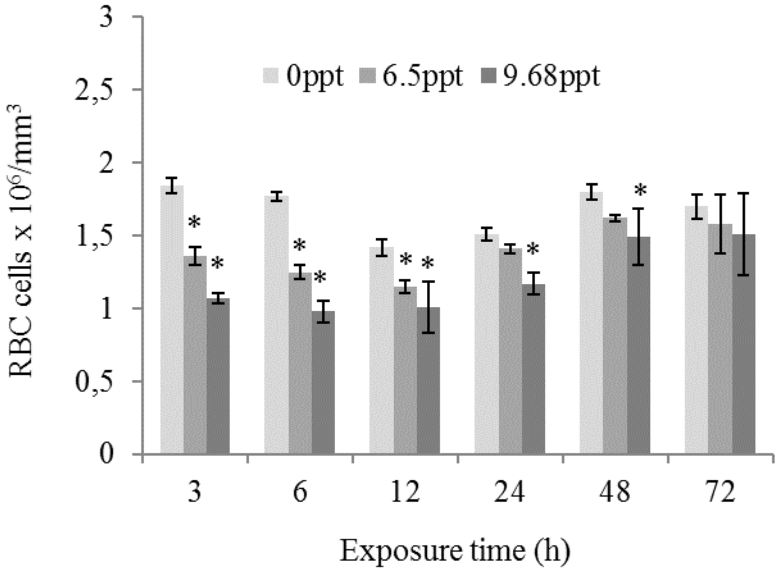


Figure 3. Effects of sub-lethal exposure of salinity on RBC levels at different time intervals in Java barb. Values are means ± SD. Asterisk (*) indicates significantly different (one-way analysis of variance, P<0.05, *N* =5 fish at each salinity)

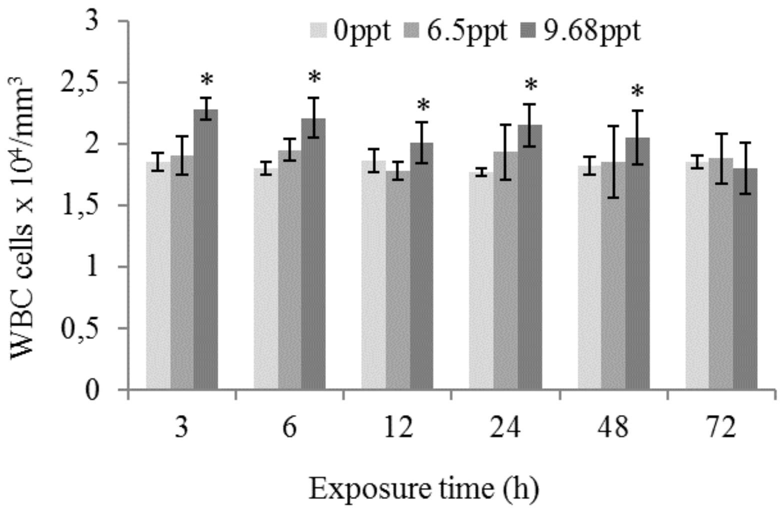
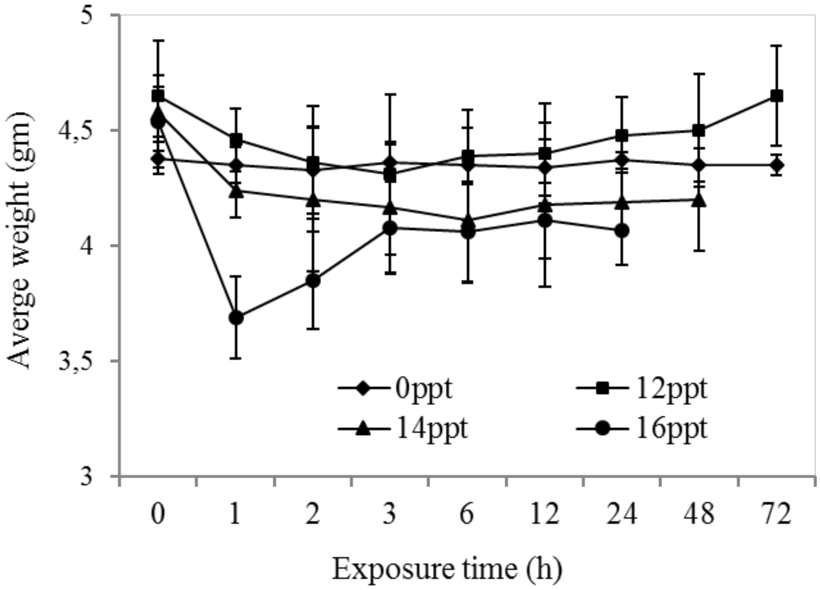


Figure 4. Effects of sub-lethal exposure of salinity on WBC levels at different time intervals in Java barb. Values are means ± SD. Asterisk (*) indicates significantly different (one-way analysis of variance, P<0.05, *N* =5 fish at each salinity)

(a)



(b)

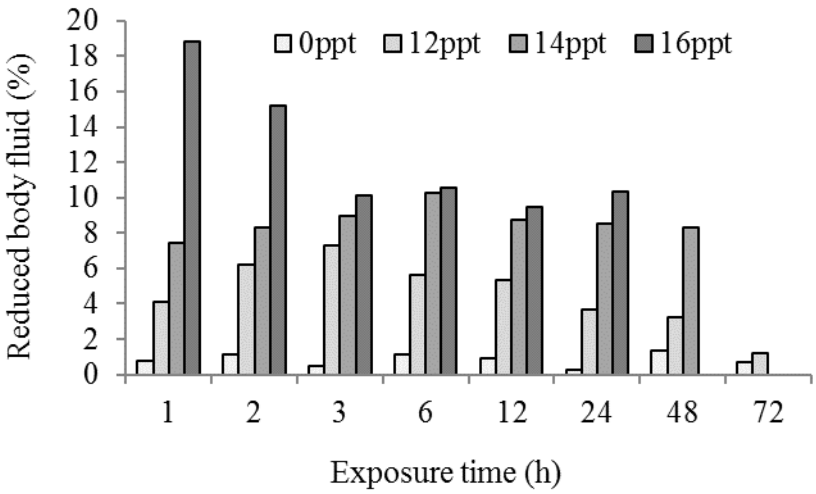


Figure 5. a) Hydration levels and b) percentage of reduced body fluid of fishes during exposure to sub-lethal and lethal concentrations of salinity for a period of 72h. Values are means ± SD. *N* = 5 fish at each salinity.

The significant decrease in RBC counts at sub-lethal concentration might be due to haemolysis and shrinkage of blood cells by the effects of salinity. The reduction in the RBC counts of Java barb following exposure to salinity indicated a reduced blood oxygen carrying capacity (Das *et al*., 2006). In the context of distortion and lysis of certain RBC cells as observed at sub-lethal treatments, the reduced blood oxygen carrying capacity can be compensated either through increasing oxygen affinity and capacity of hemoglobin and/or increase in RBC production (Das *et al.*, 2006).

An increased WBC counts established leukocytosis, which is considered to be an adaptive value for the tissue under environmental stress. 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 noradrenaline, 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 Java barb 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).

Conclusion

The present study which aimed to mimic of fluctuating salinities, has shown that short-term exposure of fish to salinities as high as 14ppt led to extreme physiological disturbances as revealed in changes observed in their behavioral and physiological characteristics. From the overall study the perceptible concern might be that the Java barb can tolerate a definite range of salinity level without being affected significantly, but extreme changes in salinity may cause some effects on physiology and behavior of the fish.

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