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Alterations in the Growth and Haematological Response of *Catla catla* (Hamilton, 1822) Exposed to Different Salinities

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Abstract

Investigation was carried out to study the effect of different salinities (3, 6, 9 ppt and control (0 ppt) on growth and haematological parameters of *Catla catla* juveniles having length and weight of 12.6±0.13cm and 18.26±0.07g respectively for about 30 days. The study revealed zero salinity (control) encouraged high growth (19.72±1.04 g), Specific Growth Rate (SGR) (0.52±0.13) and Protein Efficiency Ratio (PER) (3.08±0.26) at control than higher salinities. However, Food Conversion Ratio (FCR) was high at 9 ppt (7.30±2.04) and low at control (1.36±0.15). High leukocyte count was observed at 9 ppt (43.02±1.09) compared to control (36.87±0.50) whereas erythrocyte (2.29±0.19), haemoglobin (10.60±0.44) and haematocrit (31.53±1.12) were higher at control than at 9 ppt. Mean Corpuscular Volume (MCV) showed high at 3 ppt (141.14±09.98) while it was low at 9 ppt (131.79±13.46). Nevertheless, Mean Corpuscular Haemoglobin Concentration (MCHC) was high at zero salinity (33.64±1.17) and low at 9 ppt (28.34±1.050). Study inferred that alterations in the growth and haematological parameters occurred at higher salinities compared to lower salinity levels.

Keywords: *Catla catla*, growth, haematology, salinity

Introduction

Fisheries sector is the fast-growing sector in India in comparison with other allied sectors. Among all the countries, India ranked 2nd in the total aquaculture production (NFDB, 2017-18). Generally, cyprinids dominate the Global aquaculture production. Of which, *viz.,* catla (*Catla catla*), grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys* spp.), common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*), comprise more than half of the world's production. Further, nowadays carps are being cultured using bore well waters containing various salts, salty marshy soil ponds and areas which have tidal influence.

The alterations in the surrounding environmental parameters cause stress responses in the fish which can lead to secondary response like changes in the blood constituents. Salinity is considered as one of the most important parameters, which affects the metabolism of the fish that eventually influences the survival, growth and feed consumption (Raghuraman, 1973; De silva & Perera, 1976).

The ionic composition of the water affects the survival, growth and haematological parameters of the fish being cultured (Mansuri et al., 1979; Besra, 1997; Islam et al., 2014). Fry and fingerlings of *Catla catla* and *Labeo rohita* could with stand salinity levels up to 8 ppt without mortality even though the survival decreased with increase of salinity. (Ghosh et al. (1973) have reported that fry and fingerlings of *Catla catla* and *Labeo rohita* could with stand salinity levels up to 8 ppt without mortality even though the survival decreased with increase of salinity. It has also been observed that Indian major carps can survive upto the salinity of 10 ppt (Billard, 1999). Catla is considered as most preferred species for culture and important table fish in India since it has faster growth appreciable amount of protein

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and extensive demand. As a result, understanding the physiological changes and adaptation of the fish to varied environmental circumstances is critical for effective cultural operations.

Materials and Methods

The study was conducted in the wet laboratory of Department of Fisheries Resource Management, College of Fishery Science, Muthukur from December, 2019 to January, 2020 for about 30 days. Healthy juveniles having length and weight of 12.6±0.13 cm and 18.26±0.07g respectively were collected from the commercial nursery at Mollur of SPSR Nellore District, Andhra Pradesh, India. The fish were brought to the laboratory and acclimatized for a period of one week in 500 l collapsible tarpaulin tanks with aeration facilities. Experimental fish were fed with commercial pellet feed having protein content of 24% at 5% body weight twice a day. Faecal matter and unutilized feed materials were siphoned out every day and 25-30% of water exchange was done with fresh water on every alternative day.

The saline water having salinity of 28ppt was brought to the laboratory from a coastal bore well of Krishnapatnam and stored in a plastic container after filtration. Salinity level required for the experiment was determined by conducting LC_{50} to find the lethal (LC_{50}) concentrations. Different salinities from 0 to 14 ppt (*viz.,* 0, 2, 4, 6, 8, 10, 12 and 14 ppt) chosen for lethal (LC_{50}) were prepared by adding the saline water gradually into dechlorinated fresh water to raise the salinity at the rate of 1ppt per day. The LC_{50} was conducted for 96 h in triplicates exposing 10 fish in each tank having capacity of 27 l with an observation for every 12 h interval. After 36 h of exposure 40% mortality was observed in 14 ppt tank. After 48 h of exposure 30% mortality was observed in 12 ppt tank. After 84 h, 100% and 60% mortality of fishes were observed in the tanks exposed to 14 ppt and 12 ppt salinity respectively. Hence, salinity levels ranging from 0 to 9 ppt were opted for the present experiment with an interval of 3 ppt and 0 ppt as control; thus, salinity treatment levels decided were 3, 6, 9 ppt. Total of 120 number of fish were distributed to 12 tanks having 270 l capacity each, filled with prepared saline solutions to a depth of 50 cm and aeration was provided. Water salinity was measured daily to maintain the required salinity level in all the treatments. Fishes were fed twice a day with commercial floating pellet feed having 24% protein at the rate of 5% of average body weight of the fish.

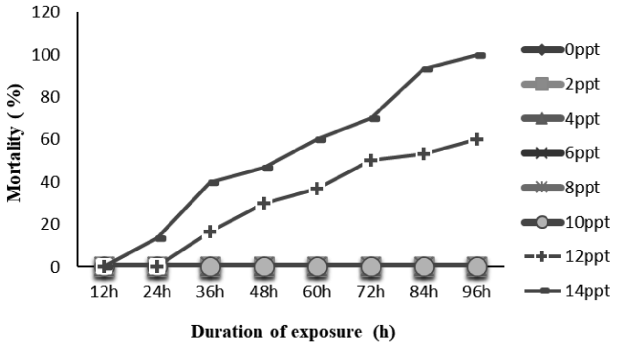

Fig. 1. Mortality percentage of Catla during the exposure to various salinity levels

The water quality parameters like Temperature (ordinary thermometer), pH (digital pH meter), Salinity (sea water refractometer), Ammonia (Phenate method), Dissolved Oxygen (Winkler's titrimetric method), Total Alkalinity (titration method) and Total hardness (EDTA titration method) were measured every day (APHA, 2012).

Every week fish were sampled randomly from all treatments to determine survival and growth parameters like Length-Weight, Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), and Food Conversion Ratio (FCR) based on standard formulae (Fagbenro & Arowosoge, 1991; Bandyopadhyay & Mohapatra, 2009). Similarly, blood samples were collected every week during morning hours to avoid diurnal variation in the blood components, from the caudal peduncle region of the experimental fish using 1 ml sterile syringe rinsed with anticoagulant (Heparin) fitted with 26 G needle and transferred into K2 EDTA coated tubes of 2 ml capacity. The haematological analysis was carried out and parameters like Total Erythrocyte Count (Wintrobe, 1967), Total Leukocyte count (Wintrobe, 1967), Haemoglobin Content (Wintrobe, 1975), Haematocrit Value (Wintrobe, 1975), Mean Corpuscular Volume (Jain, 1986), Mean Corpuscular Haemoglobin Concentration (Jain, 1986) were calculated.

After analysis of blood sample and growth parameters from each treatment, mean values of triplicate tanks of each treatment were calculated. Using the SPSS (16.0) statistical software, Two-way Analysis of Variance (Univariate) was applied to assess the effect of interaction between different salinity levels on growth and haematological parameters.

Results and Discussion

Fish generally makes alteration in its morphology, hematology, biochemical composition and endocrinology when the environmental parameters get changed. Salinity is considered as one of the critical environmental limiting factors for the survival, growth and haematological parameters of freshwater cyprinid fishes especially like catla, rohu etc. In the present study, in all treatments, water quality parameters like pH, Temperature, total hardness, total alkalinity, Dissolved Oxygen were maintained uniformly except the salinity. Growth of experimental fish catla in terms of both length and weight decreased with increase of salinity from 3 ppt to 9 ppt. The highest length and weight observed were 13.45 ± 0.52 cm and 19.72 ± 1.04 g respectively in control while the respective lowest length and weight observed were 12.92 cm and 18.46 g in 9 ppt (Fig. 2). The growth in terms of length recorded between the salinities of 3 ppt and 6 ppt did not show any significance ($p>0.05$) despite overall significant difference could be observed between the treatments and control. Further, significant difference in length among weeks could be observed. Similarly, significant difference ($p<0.05$) could be observed in weight of fish grown at different salinities as well as weeks. Lower growth obtained in the experimental fish while exposing them to hyperosmotic water could be due to stress and thus production of huge quantity of stress harmone cortisol which always negatively affects the growth of the fish (Eckert et al., 2001). Further, failure of osmoregulatory system due to deterioration of osmotic and ionic regulatory mechanisms might have reduced the growth process (Deacon & Hecht, 1999).

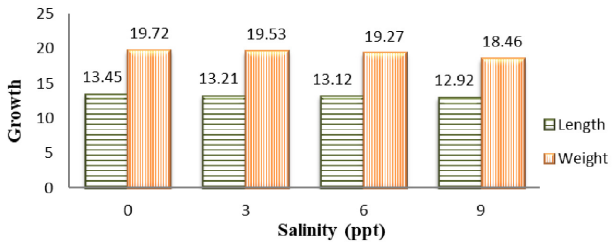

Fig. 2. Mean of Growth (Length and Weight) of *Catla catla* juveniles at different salinities

As mean growth of *Catla catla* decreased with increase of salinity, the mean values of Specific Growth Rate and Protein Efficiency Ratio of catla juveniles showed decreasing trend from the salinity of 3 ppt to 9 ppt (Fig. 3). The study did not reveal any significant difference ($p>0.05$) in SGR between control (0 ppt) and 3 ppt and between weeks though significant difference could be observed between control and other two salinities 6 and 9 ppt. However, significant difference in PER between treatments and weeks could be observed. The reduction in the SGR and PER rates could be explained as reported by Boeuf & Payan (2001) that if the fish were exposed beyond the optimum salinity, approximately 10-50% of their available energy would be directed to regulate their homeostatic balance. Further, high energy is required for the freshwater fishes for Na–K-ATPase to extract excess ions (Varsamos et al., 2005).

In contrary, mean values of Feed Conversion Ratio showed increasing trend from the salinity of 3 ppt to 9 ppt (Fig. 2). FCR was significantly differed between higher salinities of 6 and 9 ppt with control though no difference was found in 3 ppt. Similarly, significant difference in FCRs between weeks could be observed. In the present study, the high FCR obtained in 9 ppt could be due to changes in gut evacuation rate which might have caused by taking more salt water by the fish as an effort to overcome the loss of water to the hyperosmotic environment (Lambert et al., 1994). However, Wendelaar (2011) opined that reduction in food intake and conversion efficiency due to stress in hyperosmotic condition might have lowered the weight gain significantly ($p<0.05$) and lead to increase of FCR.

Mean counts of total leukocyte in *Catla catla* juveniles exposed to different salinities (Fig. 4A) showed increasing trend from the salinity of 3 ppt to 9 ppt. TLCs' differed significantly ($p<0.05$) between treatments of 0 (C), 3, 6 and 9 ppt as well as weeks. Soegianto et al. (2017) observed that increase of TLCs count in *Oreochromis niloticus* was significant at higher salinities of 10 and 15 ppt than lower salinities Significant increase in leukocyte counts in the present study could be due to regulation of immunological function (Davis & Maerz, 2008; Bosisio et al.,2017). TLCs get increased with increase in lymphopoisis which in turn enhance the release of lymphocytes from lymphoid tissue (Johansson-Sjöbeck & Larsson, 1978). Stress due to hypo osmotic environment triggers the reduction of leukocytes counts especially lymphocytes and phagocytes which ultimately affect the immune system of the fish.

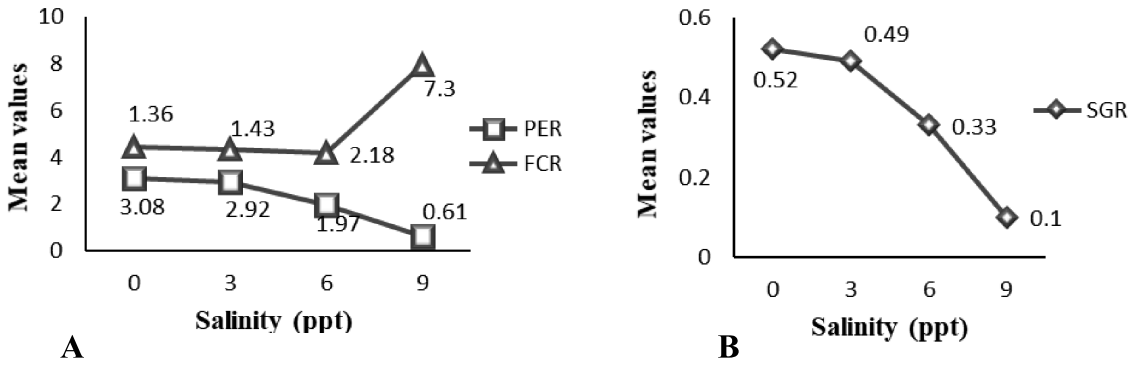

Fig. 3. Mean variation of Growth parameters of *Catla catla* juveniles at different salinities A) PER and FCR, B) SGR

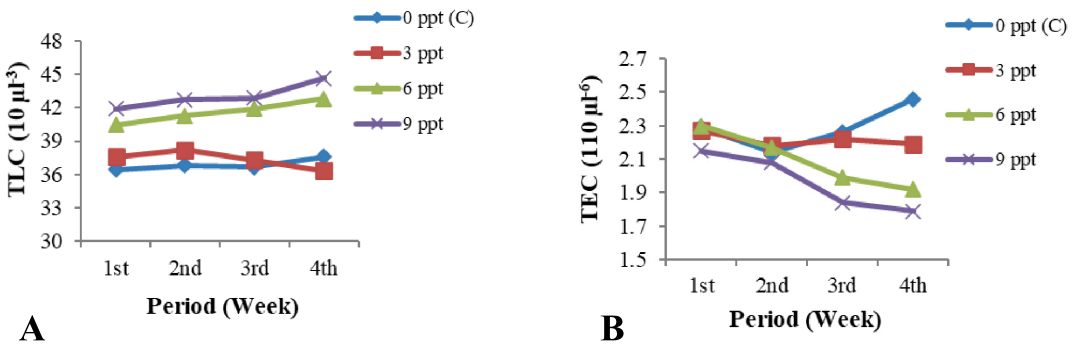

Fig. 4. Weekly analysis of blood composition of *C. catla* subjected to different salinities; A) Total Leukocyte Count (TLC), B) Total Erythrocyte Count (TEC)

However, the decreasing trend of total erythrocyte count was observed (Fig. 4B) with increasing salinity from 3 ppt to 9 ppt. The count decreased initially at 0ppt during first week and later gradually increased which could be attributed that the fish attempted to mitigate the stress due to confined environment and dissolved oxygen fluctuation. TECs did not differ significantly between treatments of 0 (C) and 3 ppt though significant difference ($p<0.05$) could be observed in higher salinity of 6 and 9 ppt while compared with control. Nevertheless, the counts did not differ significantly between weeks in every treatment. The reduction of TECs might be due to inhibition of erythropoeis is as a result of exposure of fishes in hypo osmotic environment which lead to erythropenia in the

Table 1. Haematological Parameters of *Catla catla* juveniles exposed to different salinities

Salinity Parameters	0 ppt (C)	3 ppt	6 ppt	9 ppt
TLC (10 μl^{-3})	$36.87^{a} \pm 0.50$	$37.35^{b} \pm 0.75$	$41.62^{c} \pm 0.95$	$43.02^{d} \pm 1.09$
TEC (110 μl^{-6})	$2.29^{c} \pm 0.19$	$2.22^{c} \pm 0.16$	$2.09^{b} \pm 0.20$	$1.96^{a} \pm 0.23$
Haemoglobin (gdL^{-1})	$10.60^{d} \pm 0.44$	$10.37^{c} \pm 0.2$	$08.10^{b} \pm 0.38$	$07.26^{a} \pm 0.32$
Haematocrit (%)	$31.53^{d} \pm 1.12$	$31.12^{c} \pm 0.78$	$27.73^{b} \pm 0.72$	$25.64^{a} \pm 1.07$
MCV (fL)	$139.02^{a} \pm 14.67$	$141.14^{a} \pm 09.98$	$133.43^{a} \pm 11.00$	$131.79^{a} \pm 13.46$
MCHC (gdL^{-1})	$33.64^{b} \pm 1.17$	$33.34^{b} \pm 0.94$	$29.21^{a} \pm 1.09$	$28.34^{a} \pm 1.05$

experimental fishes (Nuzhat, 2016; Murmu et al., 2020)

The progressive reduction of Hb and Ht was observed from control to 9 ppt (Fig. 5). The respective high and low value of Hb obtained at control and 9 ppt was 10.60 gdL^{-1} and 7.26 gdL^{-1} whereas the respective Ht was 31.53 and 25.64%. Significant difference ($p<0.05$) in Hb and Haematocrit level was observed between treatments as well as weeks. The Hb results are in accordance with the finding of Das et al. (2004) in *Catla catla* fingerlings and Elarabany et al. (2017) in *Oreochromis niloticus.* Similarly, the reduction of Ht in the present study confirmed the findings of Morgan & Iwama (1991) and Altinok et al. (1998) in *Oncorhynchus tshawytscha* fry and in sturgeon *Acipenser oxyrinehus* respectively when animals were transferred from low to higher salinities. The reduction in Hb and Ht could be justified by reduction in the TECs due to ionic regulation in the blood plasma.

The MCV values followed increasing trend from control to 3 ppt and then decreased from 3 to 9 ppt (Fig. 6A). The drastic increase or decrease of MCV values might be due to fluctuation in blood parameters like TEC, Hb and Ht in the experimental fishes. No significant difference could be observed in MCV values between treatments as well as weeks. The MCHC showed decreasing trend from the salinity of 3 ppt to 9 ppt (Fig. 6B). MCHC results showed non-significant difference ($p>0.05$) between treatments of 0 ppt (C) and 3 ppt; and significant difference ($p<0.05$) between 6 ppt and 9 ppt when compared with control. However, no significant difference was observed between weeks. The findings of the present study agreed with the results of Elarabany et al. (2017) who found no significant differences in MCV and MCHC values in *O. niloticus*. Similarly, Akinrotimi et al. (2012) also could not find any significant changes in MCV and MCHC values while exposing *Tilapia guineensis* to different salinity levels. The reduction in MCV of blood may be attributed to occurrence of exosmosis.

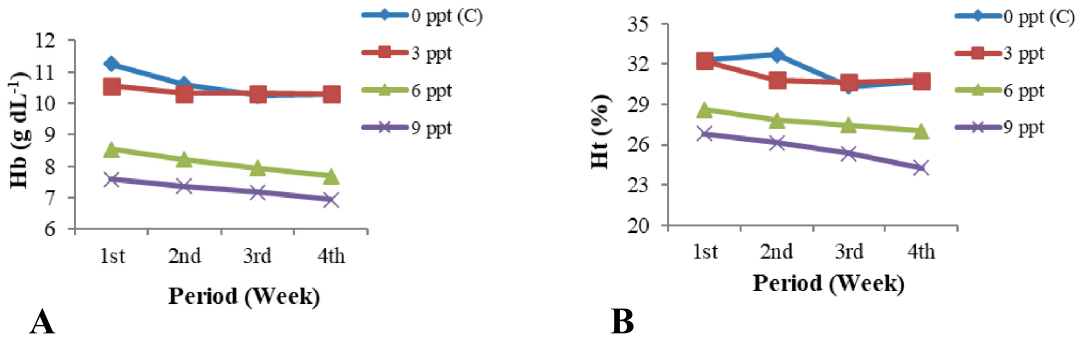

Fig. 5. Weekly analysis of blood composition of *C. catla* subjected to different salinities; A) Total Haemoglobin (Hb), B) Total Haematocrit (Ht)

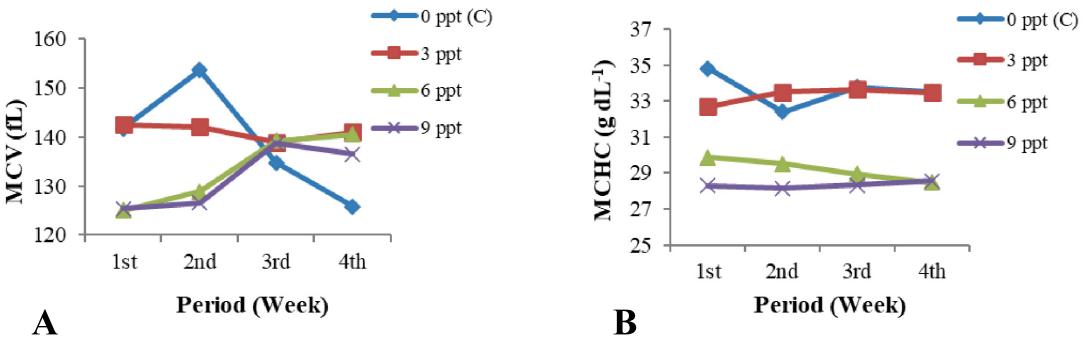

Fig. 6. Weekly analysis of blood composition of *C. catla* subjected to different salinities; A) Mean Corpuscular Volume (MCV), B) Mean Corpuscular Haemogolobin Concentration (MCHC)

The hyperosmotic environment reduced the growth rate, SGR and PER in catla since high level of energy was directed towards regulation of physiological function in the fish. The food conversion ratio was decreased at higher salinities due to stress and reduction in food intake. No change in the behaviour and health of the fish could be noticed at 3 ppt. But in higher salinities, the fish could not survive and showed adaptive alterations in their feeding and behavior through osmoregulation. Fish could not exhibit any alteration in heamatological parameters *viz.,* TEC, MCV and MCHC upto 3 ppt. However, higher salinities decreased the haematological value since the salinity induced osmoregulatory dysfunctions which lead to failure of erythrocyte fragility and erythropoeisis. Total Leukocyte count increased with increasing salinity as a function of immunological activity to overcome the salinity stress. Hb and Ht decreased with increasing salinity due to the reduction in the erythrocyte counts. The reduction in Hb, Ht values and erythrocyte counts influenced the alterations in the values of MCV and MCHC. The study revealed that the fish *Catla catla* could survive up to a salinity range of 9 ppt despite poor in growth performance.

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