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Effect of Salinity on Growth and Haematological Indices of *Pangasianodon hypophthalmus* (Sauvage, 1878)

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Abstract

Pangasianodon hypophthalmus juveniles having total length of 13.35±0.14cm and body weight of 17.52±0.16g were subjected to salinity levels of 4, 9 and 14 ppt and 0ppt as control, for determining their growth and hematological changes. The highest growth in length (14.57±0.92cm) and weight (20.89±2.51g) were observed in control while the lowest length (13.90±0.46cm) and weight (19.10±1.30g) in 14ppt. The highest SGR (1.19±0.15) and PER (3.63±0.37) were observed in control while lowest SGR (0.65±0.14) and PER (1.93±0.24) in 14ppt. The highest FCR observed was (2.18±0.27) in 14ppt and the lowest (1.16±0.16) in control. High count of TLC was recorded in 14ppt (28.37±0.65 (10^3/µl)) and the least in control (24.52±0.81 (10^3/µl)). The TEC was high (2.42±0.17 (10^6/µl)) in control and the least (1.99±0.20 (10^6/µl)) in 14ppt. The Hb was high in control (9.46±0.21g/dL) while least in 14ppt (6.93±0.43 g/dL). High Ht was observed in control (29.20±0.61%) whilst the least in 14ppt (24.07±1.83%). The highest MCV was observed in the salinity of 4ppt (125.07±9.12fL) and the lowest in 9ppt (121.01±6.25fL). On contrary, the high MCHC (33.73±0.92g/dL) was observed in 9ppt and the low (28.86±1.39g/dL) in 14ppt. The present research findings concluded that though *P. hypophthalmus* survived upto higher salinities of 14ppt, they exhibited poor growth performance when compared to lower salinities.

Keywords: Growth, haematology, *Pangasianodon hypophthalmus*, salinity

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Introduction

Aquaculture is a rapid growing animal based food-producing sector, predominantly in developing countries like India. Further, it is an important economic activity and a flourishing sub-sector with varied resources and potentials. India ranks second in global aquaculture production contributing 6.3% of the global fish production and 5% of the global fish trade (NFDB, 2018). One of the fastest growing types of aquaculture of the world is farming of catfish, especially *Pangasianodon hypophthalmus*. Among the world aquaculture productions, *P. hypophthalmus* alone contributed to about 5.1% with 2520.4 thousand tonnes of production in 2020 (FAO, 2022). It has been estimated that over 0.855 million tonnes of Pangasius catûsh are being produced annually in India (Singh, 2020).

Exploratory investigations envisage that changes in the environmental conditions of culture medium generally affect fish physiology and metabolic rates in various forms. Salinity is one of the important water quality parameters, which affects the physiological responses of the fish especially in metabolism, survival, growth and feed consumption of fish (Brocksen & Cole, 1972).

Various studies have been carried out to determine the salinity tolerance limits of various fish species to increase the fish production. *Oreochromis niloticus* grows faster up to 20ppt, but does not tolerate salinities beyond it (Baroiller et al., 2000). Asian catfish (*Pangasius hypophthalmus*) could be cultured in sea water up to 13ppt salinity and survive excursions up to 18ppt for at least 22 days (Castaneda et al., 2010). Kang'ombe & Brown (2008) exposed *Tilapia rendalli* to different salinity level (5, 10 and 15ppt) and better growth rates were reported in 10ppt followed by 5ppt salinity. Juvenile Spotted Scat, *Scatophagus argus* did not show significant difference in terms of survival but significantly differed in growth, body composition, oxygen consumption, and ammonia excretion when exposed to salinity range of 5-35ppt (Xu et al., 2020).

The fish exposed to various salinity levels alter the haematoloigcal parameters of the fish and their indices aid in understanding the correlation of blood characteristics to the phylogeny, activity, habitat and adaptability of fishes to the environment (Larsson et al., 1976). It could also provide reliable information on metabolic disorders, deficiencies and chronic stress status (Bahmani et al., 2001). The studies on the alterations in the hematological parameters of juvenile *O. niloticus* subjected to different salinities demonstrate denotable differences in blood parameters like blood glucose and hemoglobin (Bosisio et al., 2017). Many of the fish farms in India especially in Andhra Pradesh receive either hard water or salt water from natural source or borewell. Further, *P. hypophthalmus* is one of the predominant species being cultured in the state of Andhra Pradesh next to Indian major carps. In view of these, it is perceived that changes in the physiological parameters especially growth and haemtology of *P. hypophthalmus* in water containing different salinity levels have to be studied thoroughly for augmenting the culture of this species.

Materials and Methods

Juveniles of *P. hypophthalmus*, having length and weight of 13.35±0.14cm and 17.52±0.16g respectively were collected from a commercial nursery, Narukur, SPSR Nellore District, Andhra Pradesh and brought to the wet laboratory of Department of Fisheries Resource Management, College of Fishery Science, Muthukur. Collected fish were stocked in 500L of collapsible tarpaulin tanks and were acclimatized to the laboratory conditions for a period of one week using fresh tap water. Juvenile fish were fed twice a day to satiation with floating pellet feed having 24% protein. Every day faecal and unutilized feed materials were siphoned out, while 25-30% of water exchange was carried out with fresh water on every alternative day.

The saline water having 28ppt was brought from a coastal bore well of Krishnapatnam. Prior to the experiment, the water was filtered and stored and then it was made up to the required salinities i.e., 4, 9 and 14ppt for conducting the experiment. The above salinities were prepared by gradual addition of saline water in the fresh water at the rate 2ppt per day to reach the required salinity levels. The salinity was measured using seawater refractometer (HANA, HI 96822).

The experiment was carried out in collapsible tarpaulin tanks having dimensions of 0.7m in diameter and 0.7m in height with water holding capacity of 270L. The tanks were filled up to a depth of 50cm with saline water of 4, 9, 14ppt and control (0 ppt) respectively in triplicates. Each experimental tank was stocked with 10 numbers of fish which were selected randomly from the acclimatized tank. Fish were fed twice a day with commercial pellet feed having protein content of 24% and fed. Water quality parameters like temperature, dissolved oxygen (DO), pH, ammonia, total alkalinity, total hardness and salinity were measured every day (APHA, 2012). Similarly, changes in growth, survival and haematology of the fish were studied every week.

Experimental fish were sampled randomly every week from each treatment to estimate the growth parameters like length, weight, specific growth rate (SGR), protein efficiency ratio (PER), and food conversion ratio (FCR) using the standard formula (Fagbenro & Arowosoge, 1991; Bandyopadhyay & Mohapatra, 2009). Blood samples were also collected every week from the experimental fish to estimate the changes in haematological parameters during the morning hours to evade diurnal variations in the blood components. Blood were collected by holding the fish gently with care to avoid additional stress during handling. The fish were held firmly on a sponge bed, and blood was collected using 1ml syringe rinsed with anticoagulant (Heparin) fitted with 26G needle from the caudal peduncle. Blood sample was immediately flushed into the K_2 EDTA coated blood collection vials of 2ml capacity. Collected blood samples were analysed for estimating haematological parameters like Total Erythrocyte Count (TEC) and Total Leukocyte count (TLC) as described by Wintrobe (1967), Haemoglobin Content (Hb) and Haematocrit Value (Ht) by using standard formulae given by Wintrobe (1975), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) by using standard formula given by Jain (1986).

The collected growth and haematological data were analyzed by a statistical tool of two-way analysis of variance using the SPSS (16.0) Statistical software to study the effect of interaction between different salinity levels and their respective time intervals on all the haematological and growth parameters.

Results and Discussion

Salinity is one of the important environmental factors, which is closely associated with metabolism of fish that affect survival, feed digestibility, protein efficiency, food conversion and growth of the fish. In present study, growth rate in terms of both length and weight showed a decreasing trend from salinity of 4ppt to 14 ppt (Fig. 1). The highest length and weight observed were 14.57±0.92cm and 20.89±2.51g respectively in control while the respective lowest length and weight observed were 13.90±0.46cm and 19.10±1.30g in 14ppt. In the case of growth study, length showed significant difference ($p<0.05$) among treatments and in terms of weight, no significant difference ($p>0.05$) could be observed between control (0ppt) and 4ppt, but significant difference ($p<0.05$) was observed between treatments of 9 and 14ppt. Likewise reduction in growth rate was observed by Jomori et al. (2012) in *Piaractus mesopotamicus,* when exposed to higher salinities. As osmoregulation is an energy-demanding process (Deacon & Hecht, 1999), minimum osmoregulatory stress and osmoregulatory costs are obtained by maintaining the iso-osmotic salinities and thereby increasing the availability of energy for growth and survival of fish (Moustakas et al., 2004). In addition, cortisol combine with growth hormone produces high level of stress hormones, which negatively affect the growth of the fish in hyperosmotic water (Eckert et al., 2001).

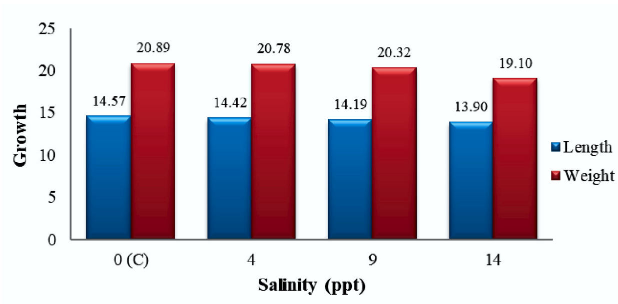

Fig. 1. Mean Growth of *P. hypophthalmus* juveniles at different salinities

SGR and PER showed a decreasing trend from salinity of 4ppt to 14ppt (Fig. 2). The maximum SGR (1.19±0.15) and PER (3.63±0.37) were observed in control, while the minimum SGR (0.65±0.14) and PER (1.93±0.24) were recorded in 14ppt. The finding revealed no significant difference in SGR and PER between 4ppt and 9ppt despite significant difference ($p<0.05$) between control and 14ppt was recorded. The observed diminution of SGR and PER in high salinity ranges might be due to utilization of approximately 10-50% of the available energy by the fish for regulating their homeostatic equilibrium (Boeuf & Payan, 2001) In addition, the freshwater fish requires more energy for Na^+–K^+-ATPase to extract excess ions (Eckert et al., 2001; Varsamos et al., 2005).

In the experiment, the FCR has steadily increased from the salinity of 4ppt to 14ppt (Fig. 2). It was high (2.18±0.27) in the salinity of 14ppt and the low (1.16±0.16) in control. The results showed no significant difference ($p>0.05$) among FCRs between the salinity of 4ppt and 9ppt, but significantly differed ($p<0.05$) when compared with control and 14ppt. However, significant difference ($p<0.05$) in FCR could be observed between 14ppt and control. The significant increase of FCR in 14ppt could have been due to changes in gut evacuation rate, caused by excess intake of saline water in an effort to overcome the loss of water to the hyperosmotic environment (Lambert et al., 1994). Further, reduction in food intake and food conversion efficiency might also be attributed to the stress caused in higher salinities (Wendelaar-Bonga, 2011). Fish exposed to 14ppt could have likely utilized their energy away from growth to other activities, especially in dealing the osmoregulation (Tort, 2011).

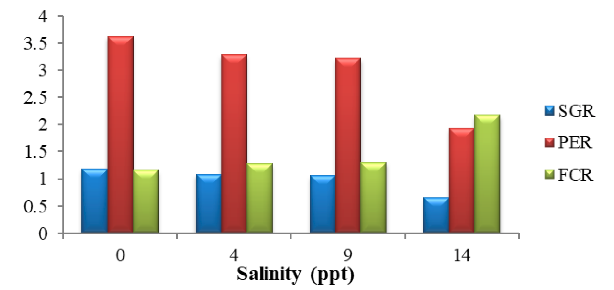

Fig. 2. Mean variation of SGR, PER and FCR of *P. hypophthalmus* juveniles at different salinities

The Total Leukocyte count increased from salinity of 4ppt to 14ppt (Table 1). The highest count of TLC observed was 28.37±0.65 (10^3/µl) in the salinity of 14ppt and the lowest was 24.52±0.81 (10^3/µl) in control. Further, the rising trend of TLC was observed from first week to fourth week in all treatments. Statistically significant difference ($p<0.05$) could be observed in TLCs between treatments of 0ppt (C), 4ppt, 9 ppt and 14ppt whereas, significant difference was observed between 14ppt and other treatments throughout all weeks (Fig. 3A). According to Javed & Usmani (2013) increase in TLC with increase in salinity might be as a response to stress as reported in many cases. Alterations in TLC with salinity could be correlated with the immunological defense mechanism of the fish (Saravanan & Harikrishnan, 1999). Thus, an increase in the count of TLC might also be due to release of cells accumulated in the spleen, to combat the stressors (Houston et al., 1996; Ajani, 2008). Increased lymphopoeisis and enhanced release of lymphocytes from lymphoid tissue (Johansson-Sjöbeck & Larsson, 1978) could also be possible cause for increased TLCs in the fish at higher salinities.

The erythrocyte count in the blood samples of experimental fish decreased from salinity of 4ppt to 14ppt. A high count of TEC (2.42±0.17 (10^6/µl)) was observed in control, while the lowest count (1.99±0.20 (10^6/µl)) was at 14ppt. TECs did not vary significantly in treatments of 4ppt and 9ppt when compared to control despite significant difference ($p<0.05$) could be observed between 14ppt and control (Table 1). However, significant difference in TEC was observed in 14ppt during all weeks except for the first week (Fig. 3B). Similar results was found by Santhakumar et al. (1999) when experiment conducted on the effect of sublethal concentration of monocrotophos on erythropoietic activity in *Anabas testudineus* and observed significant decrease in the RBC. Nuzhat (2016) stated that the decrease in total erythrocyte count indicated the occurrence of anemia associated with erythropenia. The reduc-

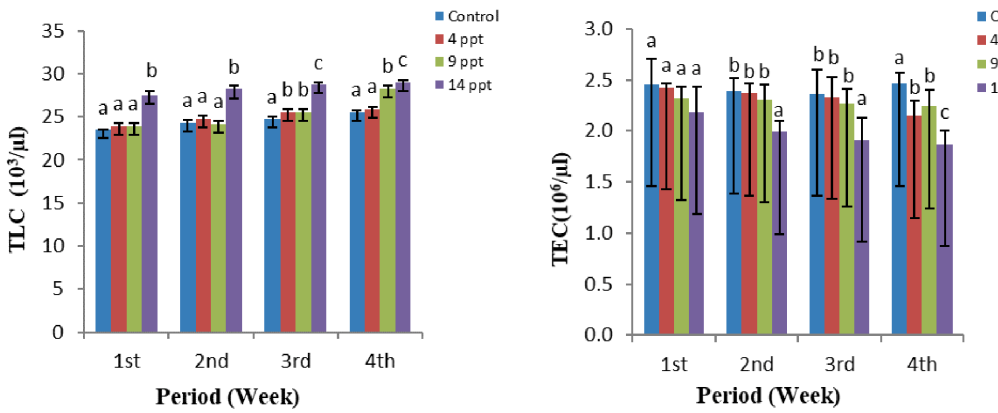

Fig. 3. Weekly analysis of blood composition of *P.hypophthalmus* subjected to different salinities; A) Total Leukocyte Count (TLC), B) Total Erythrocyte Count (TEC)

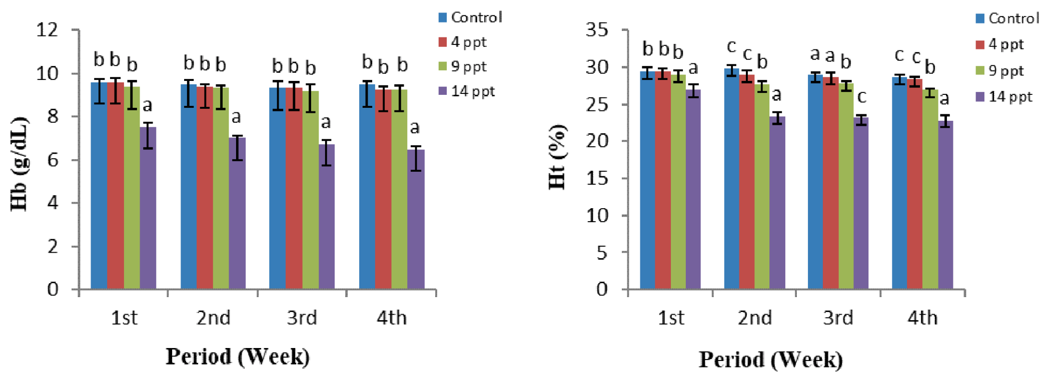

Fig. 4. Weekly analysis of blood composition of *P. hypophthalmus* subjected to different salinities; A) Total Haemoglobin (Hb), B) Total Haematocrit (Ht)

tions in TEC in the present study could be attributed to salinity-induced osmoregulatory dysfunction which in general leads to erythrocyte fragility (Montero et al., 1999; Girling et al., 2003).

The decreasing trend of Hb and Ht was observed from salinity of 4ppt to 14ppt. The highest value of haemoglobin (9.46±0.21g/dL) and haematocrit (29.20±0.61%) was observed in control (0 ppt), whereas, the lowest value of haemoglobin (6.93±0.43g/dL.) and haematocrit (24.07±1.83%) was observed at salinity of 14ppt. Hb did not show any significant difference between treatments (4ppt and 9ppt) with control (0ppt) while significant difference (p 0.05) was observed among 14ppt and control (Table 1). Similarly, significant difference ($p<0.05$) in haematocrit values were observed among the treatment groups of 9ppt and 14ppt salinity with control (0ppt), though no significant difference was found between control and 4ppt (Table 1). However, higher significant difference in values of Hb and Ht were observed in 14ppt among all weeks (Fig. 4A & 4B). The Hb and Ht results are in accordance with the findings of Mohamed et al. (2021) and Zarejabad et al. (2010) in *O. niloticus* and *Huso huso* respectively when animals were transferred from low to higher salinities. The reduction in the haemoglobin content could be due to the prevailing anoxic condition, as there will be suppression and exhaustion in the haemopoietic potential under such conditions (Sawhney & Johal, 2000). The reduction of haematocrit percentages in the present study might be attributed to reduced volume of RBCs which would have occurred due to osmotic changes caused by ion leakage from the plasma (Alwan et al., 2009).

The MCV observed was high (125.07±9.12fL) at salinity of 4ppt, while it was low (121.01± 6.25fL) in 9ppt and an irregular trend of MCV was observed among treatments (Table 1). MCV showed no significant difference among treatments with salinities 4, 9 and 14ppt and control. Similarly, no significant difference was observed between weeks (Fig. 5A). MCHC was in increasing trend from 4ppt to 9ppt reared fish, then started decreasing at 14ppt (Table 1). The highest value observed was 33.73±0.92g/dL in 9ppt and the lowest was 28.86±1.39 g/dL in 14ppt. MCHC did not show any significant difference between control (0ppt) and 4ppt but significant difference ($p<0.05$) was observed between treatments as well as 9 ppt and 14 ppt when compared with control. However, significant difference was observed in MCHC values of 14ppt during weekly analysis. The increase in MCV after exposure to high salinities, attributed to be coupled with low haemoglobin content which indicated that the red blood cells might have shrunken, either due to hypoxia or microcytic anaemia (Adeyemo, 2005). The significant decrease in the MCHC probably might be an indication of swelling of red blood cell and/or decrease in haemoglobin synthesis (Milligan & Wood, 1982; Adeyemo, 2005).

The reduction in the growth rate, SGR and PER under high salinities might be due to utilization of high energy to maintain the iso-osmotic condition and survival of fish in hyperosmotic environments which might further affect the growth of fish. There was no observed change in the behavior, feeding and health of the fishes which made them being active upto 9ppt. The experimental fish managed to

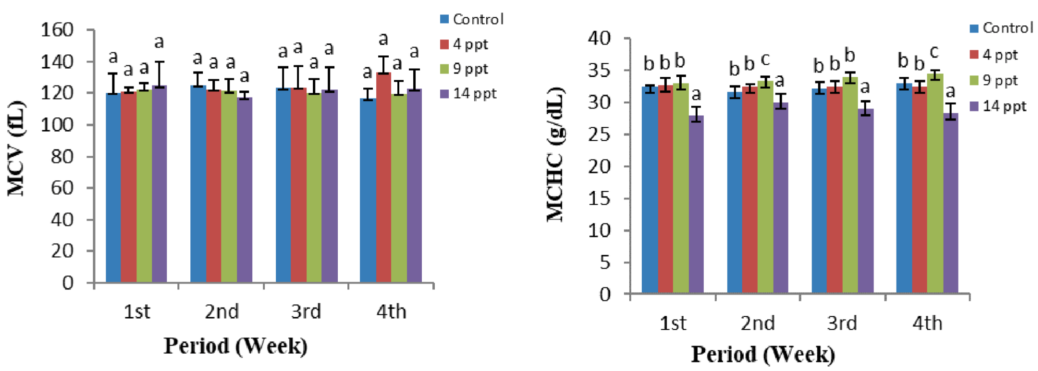

Fig. 5. Weekly analysis of blood composition of *Pangasianodon hypophthalmus* subjected to different salinities; A) Mean Corpuscular Volume (MCV), B) Mean Corpuscular Haemogolobin Concentration (MCHC)

survive at higher salinity and could manage the osmotic stress through osmoregulation by developing adaptive alterations in the behavior. In comparison with control (0ppt), the least alterations were found at 4ppt and 9ppt in terms of TEC, Hb and Ht except in 14ppt where high reductions were observed. The high reduction in blood components in higher salinities was due to induced osmoregulatory dysfunction which caused erythrocyte fragility and failure of the erythropoeisis. The value of TLC increased with increase in salinity in correlation with immunological response to combat the salinity stress which initiates the lymphopoisis and enhanced the release of lymphocytes from lymphoid tissue. Further, it could be observed that reductions in Hb and Ht lead to changes in the values of MCV and MCHC. The present study revealed that, in spite of poor growth performance in higher salinities, the *P. hypopthalmus* managed to survive upto the salinity range of 14ppt.

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