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Impact of Salinity on Growth and Physiological Responses of Striped Catfish *Pangasionodon hypophthalmus* Fingerling

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ABSTRACT

Background: A 90-day experiment was conducted to study the impact of salinity on growth and physiological response of *Pangasionodon hypophthalmus* fingerling at the Brackish water fish farm of Central Institute of Fisheries Education (CIFE), Kakinada Centre, Kakinada, East Godavari District, Andhra Pradesh, India.

Method: The study used different salinities condition such as 0 (control), 5 (T1), 10 (T2) and 15 (T3) ppt. Fingerlings (8.47±0.46 g) were gradually acclimatized to different salinities in 1000 l fibre reinforced plastic (FRP) tanks, in triplicates and fed with a commercial feed (crude protein 30%).

Result: Growth indicators such as specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FRR) and protein efficiency ratio (PER) revealed that growth and survival were negatively affected above 10 ppt salinity. Significantly higher amylase and protease activities were observed up to 10 ppt group. In contrast, significantly higher cortisol (97.71±1.94 ng/ml) and glucose (113.32±2.66 mg/dl) levels in the blood were recorded in 15 ppt group. Overall, the study proved that *P. hypophthalmus* can be cultured in low saline water (up to 10 ppt) without affecting its growth and physiological homeostasis.

Key words: Growth, Immunity, Metabolism, Pangasius, Physiology, Survival

INTRODUCTION

Aquaculture is supplying half of the animal protein-fish-for human consumption in the world and projected to supply 60% of the food fish in 2030 (FAO, 2020). Presently, the declined productivity of capture fisheries-from natural resources-is placing an additional pressure over the aquaculture sector. Fish growth and physiological activities are directly regulated by several ecological factors - salinity one among them (Boeuf and Payan, 2001).

Therefore, the development of salinity tolerant strains or rearing of saline tolerant freshwater species in coastal waters, need to be initiated soon to sustain the freshwater aquaculture production.

In general, salinity influences the growth by controlling standard metabolic rate, food intake, food conversion ratio and stimulates the hormone related to growth Sahoo *et al.* (2003). Further, a change in water salinity affects most of the physiological functions such as, immunity (Yanjiao *et al.,* 2011), digestibility Sahoo *et al.* (2003) and stress-moderating ability (Al-Khashali and Al-Shawi, 2013). In addition to that, in any aquaculture activity, stress is considered as a primary anti-economic factor which increases the chances of disease occurrence and mortality (Tsuzuki *et al.,* 2001). Therefore, the optimization of the rearing salinity is more important for improving the fish health and aquaculture production.

The striped catfish, *Pangasionodon hypophthalmus* (*Sauvage*, 1878) is considered as a well-established candidate species in freshwater aquaculture practices for its successful artificial propagation, acceptability to commercial feed, rapid growth, tolerance to low dissolved oxygen condition and good market demand (Hossain and Rahman, 2014). It also can survive in a salinity level of 10-15 ppt (Nguyen *et al.,* 2014; Kumar *et al.,* 2017) which can be potentially tapped for its culture expansion in low saline water and brackish water bodies. Moreover, this culture expansion can also address the emerging freshwater aquaculture issues such as increasing salinization and freshwater scarcity. Therefore, the present study designed to determine the effect of salinity stress on the growth and physiological responses of *P. hypophthalmus* fingerling.

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MATERIALS AND METHODS

A 90 days experiment was conducted at ICAR- Central Institute of Fisheries Education (CIFE), Kakinada Centre, Kakinada, East Godavari District, Andhra Pradesh, India. The experiment followed a completely randomized design (CRD) with one control (0 ppt) and three treatments (5, 10 and 15 ppt); each in triplicate. The treatment salinities such as 5, 10 and 15 ppt were prepared by mixing the fresh water with saline water (salinity 17 ppt) collected from the inlet canal of the brackish water fish farm, CIFE, Kakinada Centre. Twelve FRP tanks (1000 l) were used as experimental tanks and 40 fish (9.6±0.17 cm and 8.47±0.46 g) were stocked in each tank. Prior to stocking in treatments (5, 10 and 15 ppt), the fish were gradually acclimatized to saline waters by increasing 1 ppt salinity in every 24 h, until each group attained the salinities of 4, 9 and 14 ppt in which they lasted for two weeks. Finally, the fish were transferred to the treatment tanks of 5 (from 4 ppt), 10 (from 9 ppt) and 15 ppt (from 14 ppt) salinities and reared up to 90 days. The fish were fed twice (07.00 h and 17.00 h) in a day with a commercial feed (30% crude protein). Continuous aeration (from single point), daily cleaning and 20-30% daily water exchange were carried out to maintain water quality.

Fortnightly, 10 fish were collected from each tank (n=30/ treatment) to record the length and weight of fish. The recorded data were used to evaluate the growth parameters. Monthly blood samples (0.1 ml) were collected from each treatment (n=9; each tank=3 fish) using a sterile 2 ml hypodermal syringe with pre-coated with EDTA (2.7%). Before drawing blood, fish were anesthetized using clove oil (5 µl/l). The blood was then transferred to 1 ml EDTA coated vials and used for NBT (Nitro blue tetrazolium) assay. For serum preparation (n=9), the blood was collected in a syringe without anticoagulant and transferred to serological tubes. The collected serum was used for further analysis. For immune and digestive enzymes, 20% liver tissue homogenate was prepared from the aseptically removed liver tissue (n=9) and the prepared supernatent was used for further analysis.

The serum glucose (Glucose kit, Trans Asia Bio-Medicals, India) and cortisol levels (Cortisol EIA Kit, Cayman Chemicals, USA) were measured using the kit as per the manufacturer's guidelines. Alanine transaminase (ALT kit, Cayman Chemicals, USA) and Aspartate aminotransferase assay (AST activity assay, Sigma-Aldrich, USA) were measured following the manufacturer's instructions.

Respiratory burst activity of the phagocytes was measured by NBT assay following the modified method (Anderson and Siwicki, 1995). The absorbance of the final reaction was measured on a spectrophotometer at 540 nm (GENESYS™ 10S UV-Vis Spectrophotometer). A turbidometric assay utilizing lyophilized *Micrococcus lysodeikticus* was done to determine the lysozyme activity in serum (Studnicka et al. 1986). The final OD was measured after incubating 1 h at 24°C. Lysozyme activity was expressed as units/ml where one unit is defined as the decrease in absorbance of 0.001/min. The total myeloperoxidase activity of serum was determined following the method by (Quade and Roth, 1997). The final OD value was measured at 450 nm in a microplate reader (Biotek, Power wave 360, USA).

The digestive enzymes of amylase and protease were determined using the DNS method (Bernfeld, 1955) and casein digestion method (Drapeau, 1976), respectively. One unit of amylase activity is defined as the number of moles of maltose released from starch per minute per milligram of protein at 37°C. Protease activity was determined using the tyrosine standard curve and expressed as micromole of tyrosine released per min per mg protein at 37°C.

RESULTS AND DISCUSSION

Growth performance parameters

Higher final average weight and specific growth rate were observed in fish reared in 0 ppt which was not significantly differed from the 5 and 10 ppt reared fish (Table 1). The study found a significant reduction in growth performance of the fish at higher salinity, 15 ppt group. In freshwater fish, it is proven that higher saline conditions negatively affects its growth and similar results were reported in *Clarias batrachus* (Sahoo *et al.,* 2003) *Heterobranchus longifilis* (Fashina-Bombata and Busari, 2003) and Nile tilapia (Iqbal *et al.,* 2012). Freshwater fish need to spend more energy at higher salinity to maintain an osmotic balance between the body fluid and external environment leading to reduced growth (Boeuf and Payan, 2001). However, the study did not find any significant difference in growth performance of

Table 1: Growth, feed utilization and survival (Mean±SE) of *P. hypophthalmus* fingerling reared in different salinities for an experimental duration of 90 days.

Treatments	Initial average body weight (g)	Final average body weight (g)	SGR (% day^{-1})	FCR	FER	PER	Survival (%)
0 ppt	8.43^{a}±0.29	39.60^{a} ±1.69	1.70^{a}±0.09	1.59^{b} ±0.02	0.63^{a} ±0.01	1.03^{a} ±0.01	95.67^{a} ±0.27
5 ppt	8.51^{a}±0.17	37.30^{a} ±1.41	1.63^{a}±0.08	1.62^{b} ±0.05	0.62^{a} ±0.02	0.95^{a} ±0.03	94.17^{a} ±0.27
10 ppt	8.47^{a}±0.28	36.50^{a} ±1.02	1.55^{a}±0.14	1.73^{b} ±0.04	0.58^{a} ±0.01	0.93^{a} ±0.02	92.33^{a} ±0.27
15 ppt	8.55^{a}±0.19	23.43^{b} ±2.06	0.98^{c}±0.08	2.29^{a} ±0.05	0.44^{b} ±0.01	0.50^{b} ±0.03	63.33^{b} ±1.96

SGR- Specific growth rate; FCR- Feed conversion ratio; FER- Feed efficiency ratio; PER- Protein efficiency ratio.

Values (mean±SE) in the same column with different superscripts differ significantly (P<0.05) for each parameter. One way ANOVA used following Duncan multiple range test.

0, 5 and 10 ppt, but a maximum growth was observed in 0 ppt. Since it is a freshwater fish, its osmoregulation energy demand could be minimal in freshwater condition and the conserved energy might have increased the growth of 0 ppt reared fish than the low saline water reared fish. Interestingly, almost similar SGR was observed in fish reared in 0, 5 and 10 ppt groups, indicating the suitability of using low saline water (up to 10 ppt salinity) for rearing *P. hypophthalmus* without affecting the growth.

The present study revealed that an increase in salinity increased the FCR and reduced FER. Similarly, short nose sturgeon, a freshwater fish, exposed to 5, 10 and 20 ppt salinities expressed a higher FCR than 0 ppt conditions (Jarvis *et al.,* 2001). Survival of fish in higher salinity depends on the ability of the fish to maintain osmoregulation (Sahoo *et al.* 2003). The reason for mortality in 15 ppt water may be due to the failure in osmotic regulation between the body fluid and the environment. It has been reported that *Pangasius* could grow well and survive up to 10-13 ppt (Castaneda *et al.,* 2010; Nguyen *et al.,* 2014; Kumar *et al.,* 2017). Similarly, in the present study, *P. hypophthalmus* exhibited a better survival up to 10 ppt. However, further increment in salinity (15 ppt) resulted in slower growth and reduced survival since it exceeds the reported physiological salinity tolerance limit (13 ppt) of *Pangasius* (Castaneda *et al.,* 2010).

Stress and immune parameters

The fish reared in 15 ppt showed significantly higher serum glucose (113.32±2.66 mg/dl), cortisol (97.71±1.94 pg/ml), liver AST (40.69±1.33 U/mg protein/min) and ALT (12.62±0.97 U/mg protein/min) activity throughout the culture trial (Table 2). Similarly, the striped catfish exposed to higher saline conditions (14 and 18 ppt) expressed elevated cortisol and glucose level than the low saline exposed group (up to 10 ppt) (Nguyen *et al.,* 2014). The increase in ALT and AST levels can be used as a salinity stress indicator in fish (Al-Khashali and Al-Shawi, 2013) because it initiates the amino acid metabolism in the liver to compensate the increased energy demand under changed physiological conditions (Ebeid *et al.,* 2005). Fish reared in 0, 5 and 10 ppt exhibited a lower stress response may be due to the osmoregulation ability of *P. hypophthalmus* in low saline waters. Previous reports on salinity tolerance of *Pangasius* proved that it could osmoregulate and survive in salinity level-up to 13-15 ppt (Kumar *et al.,* 2017).

In the present study, enhanced respiratory burst activity, lysozyme and myeloperoxidase were observed in low saline water reared fish (0, 5 and 10 ppt) (Table 3). Similarly, rainbow trout and Atlantic salmon reared in freshwater displayed an enhanced immune response (Fast *et al.,* 2002). In contrast to this, significantly lowered respiratory burst, lysozyme and myeloperoxidase activities were observed in 15 ppt. Similarly, rainbow trout transferred from freshwater to saline water displayed significantly reduced immune response (Marc *et al.,* 1995). In stressed condition, fish exhibits raised neuroendocrine factors such as cortisol (to attain homeostasis) which can suppress the respiratory burst

Table 2: Stress parameters (Mean±S.E) of *P. hypophthalmus* fingerling reared in different salinities for an experimental duration of 90 days.

Group	Cortisol (ng/ml)			Serum Glucose (mg/dl)			ALT (U/mg protein/min)			AST (U/mg protein/min)		
	30th Day	60th Day	90th Day	30th Day	60th Day	90th Day	30th Day	60th Day	90th Day	30th Day	60th Day	90th Day
0 ppt	62.97^{a}±1.83	68.22^{a}±1.69	63.69^{a}±1.31	74.59^{b}±1.71	86.42^{a}±1.60	77.98^{b}±1.49	7.36^{b}±0.65	7.80^{b}±0.33	6.17^{b}±0.32	22.41^{b}±1.21	25.67^{c}±1.01	21.14^{b}±1.29
5 ppt	63.19^{a}±1.91	70.96^{b}±1.40	65.4^{b}±1.96	75.48^{b}±1.80	88.20^{b}±1.78	79.79^{a}±1.49	7.83^{b}±0.50	8.17^{b}±0.47	6.71^{b}±0.51	25.71^{b}±1.36	28.92^{b}±1.16	22.93^{b}±1.53
10 ppt	65.4^{b} ± 1.45	72.79^{b} ±2.61	66.67^{b}±1.64	77.28^{b}±1.53	90.94^{b}±2.68	81.30^{b}±1.69	8.54^{b}±0.85	8.26^{b}±0.36	7.40^{b}±0.91	25.96^{b}±1.13	30.99^{b}±2.23	25.67^{b}±2.84
15 ppt	87.65±2.38	97.71^{a}±1.94	82.56^{a}±1.97	98.71^{a}±1.76	113.32^{a}±2.66	98.13^{a}±1.60	11.32^{a}±0.62	12.62^{a}±0.97	9.65^{a}±0.96	34.79^{a}±1.31	40.69^{a}±1.33	32.59^{a}±0.90

Values in the same row with different superscripts differ significantly (P<0.05) for each parameter. One way ANOVA used following Duncan multiple range test in SAS-9.3.

Table 3: Immune parameters (Mean±S.E) of *P. hypophthalmus* fingerling reared in different salinities for an experimental duration of 90 days.

Group	Respiratory burst activity (OD at 540 nm)			Lysozyme activity (Units/ml)			Myeloperoxidase (OD at 450 nm)		
	30th Day	60th Day	90th Day	30th Day	60th Day	90th Day	30th Day	60th Day	90th Day
0 ppt	1.48^{a}±0.09	1.37^{a}±0.05	1.37^{a}±0.06	88.67^{a}±1.76	75.33^{a}±2.81	79.33^{a}±3.37	1.43^{a}±0.45	1.15^{a}±0.90	1.26^{a}±0.31
5 ppt	1.45^{a}±0.02	1.35^{a}±0.05	1.30^{a}±0.02	86.67^{a}±2.47	73.67^{a}±1.37	75.33^{a}±2.42	1.34^{a}±0.91	1.08^{a}±0.28	1.14^{a}±0.44
10 ppt	1.41^{a}±0.20	1.26^{a}±0.02	1.28^{a}±0.12	85.86^{a}±1.53	72.02^{a}±2.45	74.05^{a}±2.25	1.26^{a}±0.34	1.02^{a}±0.45	1.05^{a}±0.25
15 ppt	1.26^{b}±0.05	1.16^{b}±0.07	1.14^{b}±0.03	64.00^{b}±0.72	58.00^{b}±1.15	60.00^{b}±0.85	0.86^{b}±0.33	0.50^{b}±0.67	0.64^{b}±0.85

In each month interval, values in the same coloumn with different superscripts differ significantly (P<0.05) for each parameter. One way ANOVA used following Duncan multiple range test in SAS-9.3.

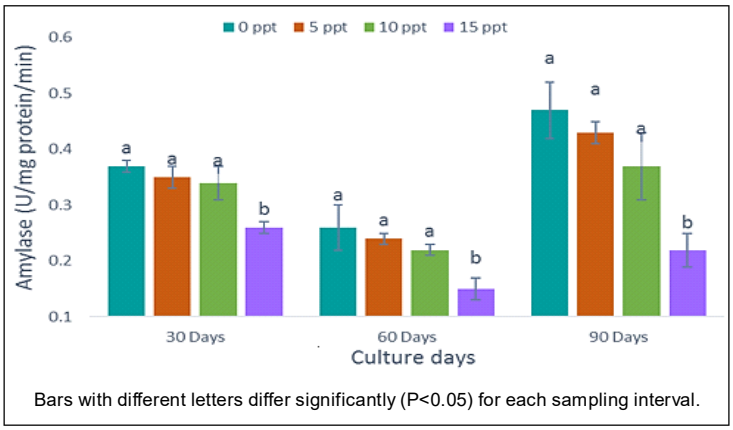

Fig 1: Amylase activity of (U/mg protein/min) *P. hypophthalmus* fingerling reared in different salinities.

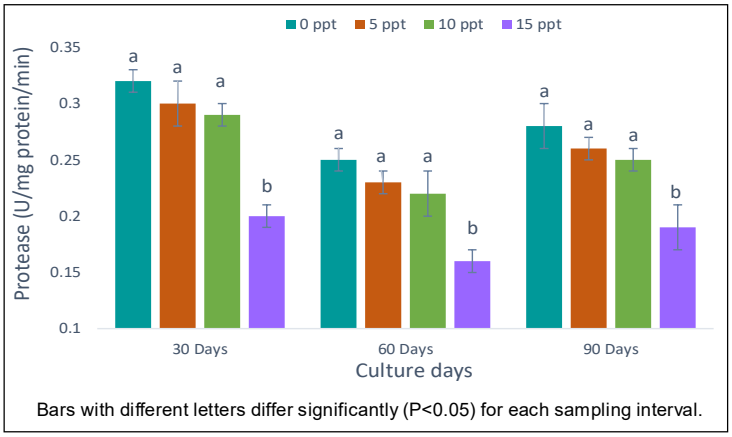

Fig 2: Protease activity of (U/mg protein/min) *P. hypophthalmus* fingerling reared in different salinities.

activity of blood leucocytes, spleen and head kidney (Tort, 2011). In chronic stress, fish distribute more energy to mitigate the stressor which may lead to the lower availability of energy for the growth and production of antibodies, proteins and different types of leukocytes and thus proceed to lower efficacy or suppression of the immune system. The present experiment considered as a chronic salinity stress study (because of the 90-day trial) which reveals that *P. hypophthalmus* fingerling can survive up to 15 ppt, but the higher energy expenditure for stress mitigation (chronic salinity stress) hinders the normal growth and other metabolic activities of the fish.

Digestive enzymes

The higher amylase activity was observed in 0 ppt (0.47± 0.47 U/mg protein/min) followed by 5 ppt (0.35±0.32 U/mg protein/min) (Fig 1). A similar trend was observed in protease activity (Fig 2) among the various salinity treatment groups. Most of the fish maintain acidic stomach and alkaline intestine because the initial digestion of ingested food, digestive enzyme activity and absorption of nutrients depends on the stomach and intestinal pH (Devi and Aravindan, 1997). Exposing the fish into saline condition creates an alkaline state and changes the intestinal pH,

which affects the digestive enzyme activity and thus leads to variations in the digestion and absorption of nutrients (Tsuzuki *et al.,* 2007). In the present study, fish raised in 15 ppt might have failed to maintain the intestinal pH and affected the digestive enzyme activity because of salinity stress which can be further correlated with their lowered FER and PER values. In addition to digestion, the fish intestine plays a central role in osmoregulation during salinity stress by mediating the fast movement of food in the gut (Barman *et al.,* 2005). The present study revealed the absence of salinity stress up to 10 ppt salinity, which might have increased the time available for digestive enzymes to completely digest the feed and absorb the nutrients due to normal movement of food in the gut.

CONCLUSION

Overall, the results of the present study proved that striped catfish *P. hypophthalm*us has optimal growth potential, stress mitigating ability and balanced physiological activities up to 10 ppt salinity. However, a further increase in salinity negatively affects the growth and survival of this species. Therefore, the study concludes that *P. hypophthalmus* can be cultured in coastal water areas, salinity level up to 10 ppt, without much adverse effects on growth and physiological homeostasis of the fish.

Conflict of interest

The authors declare that there is no conflict of interest.

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