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Effect of salinity changes on growth, survival and biochemical parameters of freshwater fish *Gibelion catla* (Hamilton, 1822)

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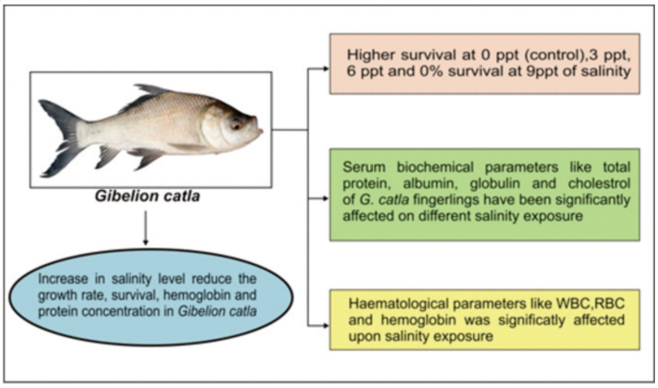
Abstract

Aim: The present investigation was conducted to access the effect of salinity stress on growth performance, survival and biochemical parameters of *Gibelion catla* under different salinity conditions with an idea to assess the potentiality of this species in inland saline affected areas.

Methodology: A 30-day-experiment was conducted in 1000 l (n=10) FRP tanks to study the effect of different salinity levels on the growth and biochemical parameters of catla species. A total of 120 fish (10.4 g) was randomly distributed into four treatments (0, 3, 6 and 9 ppt) with three replicates. The water quality parameters such as pH, temperature, nitrite, nitrate, ammonia, alkalinity and dissolved oxygen were recorded for each treatment on weekly basis, whereas fish sampling was carried out at 0^{th} and 30^{th} day of the experimental period to ascertain survival, weight gain, specific growth rate and feed conversion ratio of the fishes from each treatment.



Results: The highest growth rate was found in control subsequently in 3 and 6 ppt and the survival rate was 96.67, 83.33, 76.67 and 0% against 0, 3, 6 and 9 ppt, respectively. The red blood cells (3.65×10^6 µl) and haemoglobin concentration, (8.17 gm dl^{-1}) were also higher in control fish, followed by 3 and 6 ppt. However, white blood cells (24.40×10^6 µl) and glucose level (123.23 mg dl^{-1}) were higher at 6 ppt, compared to control. Plasma protein level of fish at control (0 ppt salinity) was significantly higher than those of fish exposed to 3 and 6 ppt salinities.

Interpretation: The present investigation revealed that an increase in salinity level had a significant impact on the growth and physiology of *Gibelion catla*. However, this species can be reared in low saline areas for some time which will not only help in the utilization of salt affected areas but will also help in the generation of employment and income.

Key words: *Gibelion catla*, Plasma protein, Salinity, Specific growth rate

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Introduction

Salinization is considered a widespread threat to the structure and ecological functioning of inland and coastal ecosystem (Herbert *et al.,* 2015). The main cause of soil salinization in coastal and inland areas are water- logging, over irrigation and indiscriminate use of inorganic fertilizers (Beresford *et al.,* 2004). In addition, withdraw of freshwater for irrigation purposes and decreased river water flow during dry season also contribute to incremental salinity in freshwater and brackish water areas of country (Alam *et al.,* 2020). Environmental salinity is an important factor affecting the survivability of aquatic organisms (Saravanan *et al.,* 2018) and any fluctuation of water salinity level may affect different physiological processes of animals, which in turn may lead to poor growth and even mortality (Gholampoor *et al.,* 2011). Moreover, it is one of the most significant abiotic factors and its favourable range for survival and optimum growth of aquatic organisms is species-specific (Mubarik *et al.,* 2015). Murmu *et al.* (2019) studied the impact of salinity on Jayanti rohu fingerlings and reported that survival rate of rohu fingerlings were observed to be 100%, 95%, 80% and 75% corresponding 2, 4, 6 and 8 ppt salinities, respectively and 100% mortality occurred at 10 ppt. Increasing salinity up to a certain level is tolerated by *L. rohita* fingerling through osmoregulation, but extreme salinity stress can lead to alterations in the haematology as well as physiology and further inability to adapt, leads to metabolic down regulation, thereby affecting growth (Rani *et al.,* 2016). Gold fish, *Carassius auratus* and common carp have been reported to have moderate salinity tolerance level up to 6 ppt with 100 percent survival, while 94 and 100% mortality was recorded in 10 and 12 ppt salinity, respectively (Lawson and Alake, 2011; Mangat and Hundal, 2014). In recent years, haematology has been used as a tool to assess the physiological conditions in healthy or stressed fishes as varying salinity level have significant effects on erythrocyte, haemoglobin, leucocytes and on all biochemical parameters (Fazio *et al.,* 2013). Specific growth rate, body weight gain and feed conversion ratio were also significantly diminished with an increase in salinity level (Wang *et al.,* 1997). It has been reported that long term exposure to salinity can have significant impact on growth, survival, haematological and biochemical parameters of *Ctenopharyngodon idella* (Kilambi and Zdinak, 1980), *L. rohita* (Devika *et al.,* 2003; Akhtar *et al.,* 2013; Islam *et al.,* 2014; Sarma *et al.,* 2020), *Clarias batrachus* (Sahoo *et al.,* 2003; Verma *et al.,* 2011; Sarma *et al.,* 2013), *Notopterus notopterus* (Kavya *et al.,* 2015) and also in *Cyprinus carpio* (Salati *et al.,* 2011; Mangat and Hundal, 2014; Mubarik *et al.,* 2015; Singh *et al.,* 2018; Singh *et al.,* 2020; Iffat *et al.,* 2021).

But at present, limited information is available on the effects of salinity stress on growth, survival and biochemical parameters of *Gibelion catla*. Hence, information related to the growth, haematology and biochemical responses of this species after exposing to various salinities could be highly beneficial to understand the suitability of this specie for culturing in low saline waters. In this regards, present study was planned to determine the effect of salinity on survival, growth, haematology and biochemical parameters of *G. catla* under different salinities.

Materials and Methods

Total 300 healthy fingerlings of *Gibelion catla* (9.2±0.12 cm and 10.4±0.06 g), collected from fish seed rearing pond were brought to the carp culture unit of Aquaculture Production and Environment Division of ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar. The fishes were acclimatized in 1000 l FRP tanks for 7 days and subsequently, the rearing experiment was conducted for a period of 30 days. Continuous aeration was provided during the acclimation process as well as during the rearing experiment.

Preparation of experimental test solutions: A stock solution of 30 ppt saline water was prepared in the circular tank (500 L) by adding the commercial grade dried sea salt to freshwater (0.05 ppt). The 0 (freshwater used having salinity 50 mg l^{-1}), 3 (3,000 mg l^{-1}), 6 (6,000 mg l^{-1}) and 9 ppt (9000 mg l^{-1}) salinity were prepared by diluting the desired level of stock solution with freshwater. The salinity level was measured by a refractometer. To determine the effect of salinity on growth and survival of catla, individuals were exposed to 0, 3, 6 and 9 ppt salinity water with three replicates (10 fishes per tank) each for a period of 30 days. Sinking pelleted feed was (protein 28%) supplied to fishes during the acclimation as well as during the experimental period @ of 10% of total body weight in morning hours and 50% water replacement was done on weekly basis. The uneaten feed and excreted materials were removed from each tank on daily basis. Round the clock aeration was provided in all the tanks from a centralized air blower. Water quality parameters such as pH, temperature, nitrite, nitrate, ammonia, alkalinity and dissolved oxygen were recorded for each treatment on weekly basis following the standard methods of APHA (2017).

Growth performance: Sampling was accomplished at 0^{th} and 30^{th} day of the experimental period (8 fish per tank) to ascertain survival, weight gain, and specific growth rate and feed conversion ratio of fish by the following formulae:

$$\text{Survival (\%)} = \frac{\text{Number of fish survived after 30 days}}{\text{number of fish stocked}} \text{X100}$$

$$\text{Specific growth rate (\%)} = \frac{\text{Ln of Final weight-Ln of Initial weight}}{\text{Experimental day}} \text{X100}$$

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed by the fish}}{\text{Final weight-Initial weight}} \text{X100}$$

Collection of blood and serum: At the end of 30 days of the experimental rearing, 15 fish per treatment (5 per tank) were anaesthetized with clove oil (50 µl l^{-1}; Merck, Germany) and used for sampling purpose. From each fish, approximately 200–250 µl blood was drawn from the caudal vein by using a 1 ml hypodermal syringe and 24 gauge needles. Serum was collected without using anticoagulant and separated from blood by keeping the tubes in slanting position for around 2 hr and then centrifuged at 3,500 rpm for 10 min at 4°C followed by the collection of straw-coloured serum with a micropipette and stored at -20°C for further use. Total erythrocyte, leucocytes cells were counted in Neubauer improved, Haemocytometer (Marirnfeld, Germany) and Haemoglobin (Hb) concentration was determined by cyanmethemoglobin method using Drabkin's reagents (Drabkin and Austin, 1935).

Biochemical indices: Serum protein, albumin and cholesterol content were recorded according to biuret method, bromocresol green binding method and CHOD-POD method (Gornall *et al.,* 1949; Doumas *et al.,* 1971; Richmond, 1973). Globulin content was calculated by subtracting albumin values from total serum protein. Blood sugar was determined in serum by the GOD-PAP method (Trinder, 1969).

Data analyses: The experimental results were subjected to One-way ANOVA using Statistical Package, SPSS version 16. Duncan's Multiple Range Test was used to determine the differences among the treatment mean, which were significant at $P<0.05$. The study was carried out in triplicates and values were expressed as mean±SE.

Results and Discussion

India is having large area (1.71 million ha) under saline affected soil of varying soil salinities on which the growth of most crop plants is limited due to excess of soluble and insoluble salts (Mandal *et al.*, 2018). Most of the groundwater samples from semi-arid region of North India fall under hard water category and 40% samples were unacceptable for drinking purposes without any prior treatment, due to the high concentration of total dissolved salts (Khaiwal *et al*., 2019). Similarly, 15 ppt inland ground saline water has also been reported from Rohtak, Haryana, where Amur carp culture was attempted (Singh *et al*., 2020). Utilization of this saline water through fish culture can be the best alternation activity. However, suitability of species is the major concerned for utilization of saline waters. The *G. catla* is one of the most widely cultured species in India and hence, a long tern salinity exposure study (30 days) was undertaken and performance of *G. catla* under four saline conditions viz., 0 (control), 3, 6 and 9 ppt was evaluated.

Water quality parameters such as pH, temperature, nitrite, nitrate, ammonia, alkalinity and dissolved oxygen for different treatments are illustrated in Table 1. There was no significant difference in the water qualities among the treatments and were within the acceptable limit of the fish. This indicates that there were no other influence on the physiology of test fishes other than salinity. The survival rates of catla fingerlings were 96.67%, 83.33% and 76.67% when exposed to 0, 3 and 6 ppt salinities, respectively (Table 2). At 9 ppt salinity, the survival rate was 100% up to 12 days, and then dropped to 50% at 18 days and 100% mortality was recorded on 24^{th} day. In a similar type of study on *G. catla*, 100% survival rate up to 5 ppt salinity and 100% mortality beyond 8 ppt salinity at 45 days of rearing have been reported (Hoque *et al.,* 2020). However, Das *et al.* (2019) demonstrated that *G. catla* fingerlings can tolerate up to 9 ppt salinity without mortality; but survivability gradually decreased with increase in salinity levels.

Low survival of freshwater fish at elevated salinity may also be due to increasing osmotic maintenance requirements at higher salinities (Kilambi and Zdinak, 1980). Iffat *et al.* (2021) reported that common carp has the ability to maintain the mechanisms for osmotic and ionic regulation up to 15 ppt salinity but spend considerable energy in maintaining the higher plasma ion concentrations, which might have attributed to the reduction in survival rates. Moreover, higher salinity has been known to disrupt epithelium with diffuse oedema of both primary and secondary lamellae and this could be the reason for 100% mortality of fish fingerlings in 9 ppt salinity (Holliday and Jones, 1967). Low survival at higher salinities have also been reported in *Cyprinus carpio* (Salati *et al.,* 2011; Mangat and Hundal, 2014; Mubarik *et al.,* 2015; Singh *et al.,* 2018; Singh *et al.,* 2020; Iffat *et al.,* 2021), *Carassius auratus* (Lawson and Alake, 2011) and *L. rohita* (Islam *et al.,* 2014; Kumar *et al.,* 2018; Murmu *et al.,* 2019; Sarma *et al.,* 2020). In the present study, the specific growth rate (SGR %) and body weight gain (BWG) significantly decreased with increase in salinity levels. ($P<0.05$). Similar to the present study, it was found that the SGR % and percent weight gain progressively decreased in *L. rohita* on increasing the salinity levels from 0 ppt, 2.5 ppt, 3.5 ppt and 4.5 ppt, respectively (Sarma *et al.,* 2020). Negative impact of salinity on growth rate has also been reported in *Tilapia rendalli* (Kangombe and Brown, 2008), *Clarias batrachus* (Sarma *et al.,* 2013), *L. rohita* (Islam *et al.,* 2014) and common carp (Wang *et al.,* 1997; Singh *et al.,* 2020; Iffat *et al.,* 2021). Impaired growth of fish fingerlings observed at higher salinities may be due to loss of energy for osmoregulation (Jobling and Baardvik, 1994).

The feed conversion ratio was also estimated for all the treatments (Table 2). It was found that FCR was lowest in control and highest at 6 ppt salinity. The results are in close agreement with the previous study on *L. rohita*, where the lowest FCR was observed in 0 ppt and highest at 6 ppt saline treatment (Islam *et al.,* 2014). Decreasing trends in feed conversion ratio at higher salinities were also reported in *Oreochromis niloticus* and common carp (Chowdhury *et al.,* 2006; Singh *et al.,* 2020; Iffat *et al.,* 2021). The efficiency of feed conversion depends on many factors but the best response was probably related to optimizing the environment at which the fish is accustomed (Neill *et al.,* 2011). Moreover, increased FCR in catla reared at 6 ppt may also be due to low retention of nutrients and high excretion of metabolites by the fish maintained at high salinity (Barman *et al.,* 2005). Haematological parameters of fishes exposed to different

Table 1: Water quality parameters used for rearing *G. catla* fingerlings

	Salinity		
Parameters	**0 ppt**	**3 ppt**	**6 ppt**
pH	7.98±0.14	8.42±0.21	8.09±0.13
Temperature (°C)	28.8±0.06	28.63±0.09	28.17±0.32
Nitrite (mg l^{-1})	0.09±0.09	0.03±0.07	0.09±0.01
Nitrate (mg l^{-1})	0.04±0.004	0.03±0.002	0.03±0.008
Ammonia (mg l^{-1})	0.07±0.013	0.08±0.014	0.05±0.019
Alkalinity (mg l^{-1})	45.33±1.33	49.33±1.33	53.33±1.33
DO (mg l^{-1})	7.10±0.06	6.73±0.03	6.63±0.09

Table 2: Biological, haematological and biochemical parameters of *G. catla* fingerlings exposed to different salinities

	Salinity		
Parameters	**0 ppt**	**3 ppt**	**6 ppt**
Growth and survival			
Initial weight (g)	10.4±0.06	10.50±0.07	10.49±0.10
Final weight (g)	12.03±0.09^{B}	11.46±0.06^{A}	11.53±0.08^{A}
Feed conversion ratio	2.18±0.15^{A}	3.07±0.10^{B}	3.16±0.11^{B}
Specific growth rate	0.45±0.03^{B}	0.32±0.09^{A}	0.31±0.09^{A}
Body weight gain	1.53±0.09^{B}	1.06±0.02^{A}	1.05±0.02^{A}
Survival rate	96.67%	83.33%	76.67%
Haematological parameters			
RBC (×10^{6}µl)	3.65 ± 0.26^{C}	2.94 ± 0.03^{B}	1.64 ± 0.06^{A}
WBC (×10^{3}µl)	17.93 ± 0.88^{A}	22.44 ± 0.32^{B}	24.40 ± 0.25^{C}
Haemoglobin (gm dl^{-1})	8.17 ± 0.30^{C}	6.26 ± 0.05^{B}	3.39 ± 0.04^{A}
Serum biochemical parameter			
Total Protein (gm dl^{-1})	4.31±0.02^{C}	4.14±0.03^{B}	3.82±0.05^{A}
Albumin (gm dl^{-1})	3.13±0.03^{A}	3.26±0.01^{B}	3.34±0.02^{C}
Globulin (gm dl^{-1})	1.18±0.05^{C}	0.88±0.02^{B}	0.48±0.07^{A}
Cholesterol (mg dl^{-1})	159.22±5.12^{C}	156.56±1.35^{B}	148.44±5.24^{A}
Blood sugar (mg dl^{-1})	97.08±0.19^{A}	107.41±0.08^{B}	123.23±0.38^{C}

Values are expressed as mean ± SE. Mean with same letter in the same row is not significantly different ($P<0.05$)

salinities were illustrated in Table 2. From the study, it was found that the highest haemoglobin concentration was observed at control (8.17 gm/dl) and the level significantly decreased ($P<0.05$) at 3 ppt (23.4%) and 6 ppt (58.5%) salinities, respectively. Similar observations were also reported in *L. rohita* fingerlings, where the haemoglobin content was highest at 0 ppt and lowest at 6 ppt salinity level (Rani *et al.,* 2016).

The reduction in haemoglobin level could be correlated with behavioural observations made during the experiments which showed higher feeding activity at 0 and 3 ppt compared to 6 ppt salinity level. The effects of salinity on RBC in illustrated in Table 2. The highest concentration of RBC was recorded in control (3.65±0.26×10^{6}µL) and the level was decreased significantly at 3 ppt (19.5%) and 6 ppt(55.1%) salinities, respectively. A similar result was also observed in *L. rohita*, where the RBC decreased progressively from control to 6 ppt salinity (Rani *et al.,* 2016). A significant reduction of RBC counts at higher salinities might be due to haemolysis and shrinkage of blood cells. The lowest WBC counts were recorded in control fish and thereafter the level increased by 25.1% and 36.1% at 3 and 6 ppt, respectively. Similar to the current study, higher leucocytes were also found in *Oncorhynchus mykiss* and *L. rohita* fishes, exposed to higher salinity levels (Montero *et al.,* 1999; Rani *et al.,* 2016; Murmu *et al.,* 2019). Leukocytes, play an important role in enhancing non-specific immunity in fish (Akhtar *et al.*, 2013). Therefore, the occurrence of more leukocytes in the present study showed its involvement in the regulating immunological function and protective response of fish during the stress condition (Secombes and Fletcher, 1992).

Blood glucose level, total protein, albumin, globulin and cholesterol level in fish exposed to different salinities are illustrated in Table 2. In the present study, blood sugar level was significantly (*P*<0.05) increased by 10.6% and 26.9% against 3 and 6 ppt indicated hyperglycaemic condition in catla fingerlings upon salinity stress. Hyperglycaemia in the fishes in response to salinity stress has been reported earlier (Sarma *et al.,* 2013; Shahkar *et al.,* 2015; Soltanian *et al.,* 2016; Yan, 2017; Ghelichpour *et al.,* 2018; Guo *et al.,* 2020). It has been established that stress in fish can initiate neuro-hormonal stimulation resulting in an increase in corticosteroid and catecholamine secretions (Karsi and Yildiz, 2005). These stress hormones in conjunction with cortisol can mobilise and elevate glucose production in fish through gluconeogenesis and glycogenolysis pathways to cope with increase energy demand of the fish due to the stressors (Iwama *et al*., 1999) or can also be attributed to the stress response and/or increased energy demand for maintenance of hydro-mineral balance at higher salinity (Jeanette *et al.*, 2007). Glucose homeostasis is also very important because glucose is a significant fuel for the functioning of specific tissues, including the erythrocytes, gills, gonads, and brain (Chen *et al*., 2017). Under sub-optimum and stressful conditions, the chromaffin cells release catecholamine in response to environmental stress, which enhances the conversion of liver glycogen into blood glucose (Nascimento *et al.,* 2012) as fish requires more energy to deal with environmental stress (Mirghaed and Ghelichpour, 2019).

Serum protein level also decreased significantly (P<0.05) in the present study as salinity increased. The highest serum protein was found in control fish and decreased later by 4% and 11.4% upon exposure to 3 and 6 ppt salinity levels, respectively. Several authors have reported that the protein level significantly decreases with increase in salinity level (Kelly and Woo, 1999; Jeanette *et al.,* 2007; Elarabany *et al.,* 2017). Probably due to reduced/ perturbation of protein synthesis, increase in proteolytic activity and possible utilization on their product for the metabolic purpose (Martinez *et al.,* 2002). Similarly, globulin level also decreased significantly (*P*<0.05) when fishes were treated with 3 and 6 ppt salinity levels. This result is in agreement with the previous finding on Tilapia zilli under different salinity levels (Farghaly *et al.,* 1973). Albumin and globulin are the two major proteins present in the blood serum and play a significant role in the immune response in fish. The decrease in the level of globulin in the present study may be due to the use of globulin by the fish for protective purpose. It has been reported that α-globulin, present in normal serum of rainbow trout, is capable of neutralizing the toxic effects of the extracellular products (ECP) and lesions would probably be produced only when the ECP exhausts the a-globulin either locally or systemically (Ellis *et al.*, 1981).

On the contrary, albumin level significantly increased, maximum recorded at 6 ppt and minimum at control. Akhtar *et al.* (2012) and Peyghan *et al.* (2014) observed that the albumin level decreased at higher salinity level in *L. rohita* and *Ctenopharyngodon idella*. In carps, generally structural reorganization of albumins was observed under adaptation of fish to increased salinity concentration (Andreeva, 2010). Albumin is entirely produced by liver, so increased albumin level in total protein could be attributed toward the protein synthesis for utilization to meet high energy demand (Javed and Usmani, 2015). In the present study, it was observed that significantly (*P*<0.05) highest level of cholesterol was found at control followed by 3 and 6 ppt saline treatment. Similar results were observed in *Solea senegalensis, Notopterus notopterus* and *Cyprinus carpio* at higher salinity levels and suggested that these biochemical energy source decrease as salinity level increases (Arjona *et al.,* 2009; Kavya *et al.,* Jahan *et al.,* 2020).

In conclusion, the present study reflects that *G. catla* fingerlings have the overall ability to tolerate up to 3 ppt salinity with a good survival rate and minimum physiological impact. Hence, information derived from the study will be helpful in utilization of saline water for carp culture, which otherwise will have limited agricultural use. However, further comprehensive study to access the impact of salinity on different life stages of this species and duration of exposure are necessary before utilizing low saline waters for farming purpose.

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Add-on Information

Authors' contribution: S.K. Ahirwal: Planning, conceptualization and execution; **P.C. Das:** Planning and conceptualization; **K. Sarma:** Conceptualization and data interpretation and writing; **T. Kumar:** Data analysis and writing**; J. Singh:** Data analysis and writing; **S.P. Kamble:** Data collection and assisting in carrying out the study.

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