



Effect of salinity on survival, hematological and histological changes in genetically improved rohu (Jayanti), *Labeo rohita* (Hamilton, 1822)

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ABSTRACT

The recent climate change and anthropogenic activity affects the agricultural land/soil as well as aquaculture sector via salinization of the aquatic zone. The present study aimed to investigate the effect of salinity on genetically improved farmed rohu, *Labeo rohita* popularly known as Jayanti and evaluation of its physiological response towards combating salinity stress. Genetically improved rohu (Jayanti) fingerlings were reared in laboratory condition at different salinities 2, 4, 6, 8 and 10 ppt to assess the effect of salinity on survival for the first time in India. The study revealed that the Jayanti rohu fingerlings could tolerate salinity upto 8 ppt range. The survival rate of the fingerlings varied from 100%, 95%, 80% and 75% at 2, 4, 6 and 8 ppt salinities, respectively and 100% mortality occurred at 10 ppt. The survival rate was significantly higher in lower salinities than other acclimation salinities. The hematological parameters analysis depicted that blood parameters were affected by increase in salinity beyond 6 ppt. The histological analysis reported mild lesions on gills of fishes exposed to higher salinities at 8 ppt. The present study revealed that exposure to salinity moderately affects the survival and physiological response of genetically improved rohu and thus the potential of the improved rohu "Jayanti" to tolerate salinity levels upto 8 ppt. These results suggest that there is great prospective for culturing selectively bred rohu (Jayanti) species in low saline areas or salt affected areas with good survivability and open new avenues for further research and development.

Key words: Hematology, Histology, Jayanti, Rohu, Salinity, Survival rate.

INTRODUCTION

The aquaculture is the fastest growing sector in the food industry and thereby provides nutritional security worldwide (FAO 2009). Rapid coastal and inland aquaculture development is facilitated by growing demand for aquatic food products in conjunction with limited production from capture fisheries (Mourad *et al.*, 2012). The salinization of freshwater resources near coastline is reducing the area available for freshwater fish production. Since, 9.38 million hectares of salt-affected land comes under the hot semi-arid and arid eco-region of northern plains and central high lands, agricultural sectors have been affected and therefore it is essential to find alternatives for livelihood in these saline affected zones (IAB, 2000; Dagar, 2005).

The salinization occurred due to anthropogenic activities in agriculture sectors which in turn entered in to water bodies through leaching (Neumann, 1997; Rengasamy, 2006; Chaitanya *et al.*, 2014). In recent times a concentration of soluble salt in terrestrial and/or aquatic environments has been increasing and causes salinization of lands or fresh water area, which mainly occur due to naturally or anthropogenic activities (Larcher, 1995; Williams, 1999; Suzuki *et al.*, 1998; 2002; Roache *et al.*, 2006). This led to increase in the stress level on the aquatic fishes as well as on the environment due to the strengthening of aquaculture practices (Gabriel and Akinrotimi, 2011; Barman *et al.*, 2012; Mohapatra *et al.*, 2013). The stressor experienced includes

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biotic factors such as predation, space, reproductive activity and abiotic factors such as hypoxia, salinity, temperature and pollution (Walters and Plumb, 1980; Iwama *et al.*, 2006; Robertson, 1987; Sobhana, 2009; Barman *et al.*, 2012; Chakrapani *et al.*, 2017). They cause significant effects on fish physiology via the imbalance of hormones as well as enzymes and their survival (Portz *et al.*, 2006; Eddy *et al.*, 2006; Small and Bilodeau, 2005). Among that, salinity affecting the physiological response of aquatic fishes varies significantly (Fry *et al.*, 1971; Kinne *et al.*, 1971). Thus, this

increase in salinity affects the aquatic ecosystems via ionic and osmotic stresses and oxidative stress to aquatic fishes is led by enhanced reactive oxygen species (ROS) level that induces damage to cellular constituents (Liu, 2007; Das *et al.*, 2004; Owens, 2001; Jampeetong and Brix, 2009; Lushchak, 2011). The cortisol and glucose are two most common stress indicators in fish (Martínez-Porchas *et al.*, 2009; Husen and Sharma, 2014) and elevation of cortisol level in plasma as well as Na⁺/K⁺/ATPase activity have been reported in salmonids species (Madsen and Bern 1993; Ayson *et al.*, 1995). The earlier evidence reported that salinity and temperature stress could affect the cortisol, enzymatic level of alanine-transmitters and apparatus transmits in goldfish, *Carassius auratus* (Al-Khashali and Al-Shawi, 2013). The effects of salinity on the growth, survival, hematological parameters and osmoregulation of tambaqui *Colossoma macropomum* juveniles were studied and demonstrated physiological changes significantly (Fiúza *et al.*, 2015). The preliminary evidence suggests that moderate salinities allow better performance of fishes with stable survival rates under stress in of *Odontesthes bonariensis* and *Odontesthes hatcheri* (Murayama *et al.*, 1977; Umezawa and Nomura, 1984; Strüssmann *et al.*, 1996).

To meet the increasing food demand, there is a need for a fish species bearing phenotypic characters of faster growth rate, stress resistance and disease resistance. Future demand and food security for aquaculture products can be attained by the selective breeding approach, if widely adopted (Gjedrem *et al.*, 2012; Janssen *et al.*, 2017). In the present study, we have performed experiment on genetically improved farmed rohu *Labeo rohita*, commonly known as Jayanti, which is an economically important freshwater fish in India as well as other Asian countries (Mahapatra *et al.*, 2016; Rasal *et al.*, 2017). The culture of Jayanti rohu is preferred over the normal rohu due to its higher growth performance and disease resistance as reported (Das Mahapatra *et al.*, 2016; Robinson *et al.*, 2014). An 18% percent average genetic gain per generation was achieved after eight generations of selection (Mahapatra *et al.*, 2016; Rasal *et al.*, 2017). There is no report of salinity tolerance of genetically improved rohu, Jayanti with respect to its survival and physiological changes.

Thus, this study attempts to study survival, hematological changes and histological evidences for understanding the physiological response under stress by exposing Jayanti rohu in different salinity level. The survival rate changes with respect to increase in salinity may provide a bio-energetic basis to evaluate performance of genetically improved rohu under culture conditions. This information will enable the aquaculture sector to take up this potential candidate species for culture in the salt affected areas.

MATERIALS AND METHODS

The experiment was carried out at the wet lab facility of Fish Genetics and Biotechnology Division of Indian Council of Agricultural Research-Central Institute of Freshwater Aquaculture (ICAR-CIFA) Bhubaneswar, Odisha.

Fish material and acclimatization

Total 200 numbers of advanced fingerlings produced in the selective breeding hatchery (2016 year class) were acclimatized for a period of one week prior to start of the experiment in the acclimatization tanks of capacity 1000 litres. The fishes at this time were fed with commercial floating feed (ABIS) containing 28% protein and 3% lipid. Continuous aeration was provided in these tanks and 50% replacement of water with fresh bore well water was done daily to avoid deposition of nitrogenous wastes and mortality thereafter. The water parameters were also found to be at par with the permissible range for the experiment to be carried out (Table 1).

Experimental design

Firstly, brine solution was prepared in the circular tanks (1000 L capacity) by adding the commercial grade of sodium chloride (NaCl) to freshwater until the desired levels of salinity were obtained. Sodium chloride (NaCl) was used in this study instead of seawater because these are most commonly employed to raise salinity during husbandry practices of these species (Tsuzuki *et al.*, 2000). The salinity level was measured by refractometer. The salinity (0, 2, 4, 6, 8 and 10 ppt) was prepared by mixing of desired levels of the brine solution with freshwater. Total 10 numbers of the acclimatized fingerlings were subjected to salinity regimes of 0, 2, 4, 6, 8 and 10 ppt in cemented tanks respectively (1000 L capacity) in triplicates. Water replacement was done by replacing 50% of the water with respective salinity at weekly interval.

Salinity challenge and monitoring survival

To determine the effects of salinity, advanced fingerlings were gradually exposed into 0, 2, 4, 6, 8 and 10 ppt salinity for 45 days rearing period in the laboratory. At first, the fish fingerlings were released into the cement tank at 0 ppt salinity. After 48 hours of acclimatization 30 fingerlings were released at the 0 ppt salinity cement tank (10 fingerlings per tank), while the rest of the fingerlings were kept inside the cement tank at 2% salinity for the next 48 hours. After another 48 hours of acclimatization 20 fish fingerlings were released at the 2 ppt the rest of the fingerlings were again kept inside the cement tank at 4 ppt saline water for the next 48 hours. Similarly, 48 hrs of acclimatization in 4 ppt, fishes were released into 6 ppt saline water and same procedure was followed up 10 ppt saline water. Sampling was done at 45th day of the experiment, which included recording survival rate (%) of fishes.

Water quality parameters such as pH, dissolved oxygen (DO) and temperature were monitored weekly in accordance with standard methods (APHA, 1988). The fish were fed with floating feed (ABIS, commercial feed) at 5% of total body weight.

Hematological analysis

Blood samples were collected (from 4-5 fishes per tank) randomly from each treatment group at an interval of 12

Table 1: Average water quality parameters in the cemented tanks of Jayanti rohu exposed to different salinities for a period of 45 days.

Parameter	Control	2 ppt	Salinity Conc. 4 ppt	6 ppt	8 ppt
Temperature (°C)	28.2 ± 0.18	29.6 ± 0.76	28.9 ± 0.85	29.0 ± 0.65	28.7 ± 0.70
DO (mg/l)	5.6 ± 0.84	5.5 ± 0.61	5.5 ± 0.55	5.3 ± 0.80	5.4 ± 0.85
pH	7.8 ± 0.25	7.9 ± 0.20	7.85 ± 0.18	7.8 ± 0.15	7.9 ± 0.23
Turbidity (NTU)	30.25	30.15	29.55	29.8	29.0

hrs. The fish were anaesthetized using MS222, weighed and around 1 ml blood was collected from caudal peduncle by heparinized syringe. The hematological analysis was carried out within one hour of sampling using Blood cell automated counter.

Data analysis

The survival percentage was also recorded as; (No of fishes survived after 45 days/Initial No of fishes stocked) X 100. The data were analyzed by ANOVA method (Weissgerber *et al.*, 2018). Data were expressed as Mean ± Standard Error of the means. Statistical software SPSS version 16.0 was used to analyze data with the levels of significance at $P < 0.05$. The histological analyses were performed using standard protocol for gill tissues of rohu. The tissue were sampled and fixed in Phosphate buffered saline (PBS) solution and then preserved in 70% ethanol. Further, after dehydration, tissue samples were embedded in paraffin and cut into series of cross and sagittal sections (5µm thick). Then, sectioned tissues were stained using Hematoxylin and Eosin (HE) stain and observed under the microscope (*Magnification* x 100).

RESULTS AND DISCUSSION

Fish encounter morphological, biochemical and endocrinological alterations in the acclimatization time, when there are fluctuations in environmental salinity. This leads to modification in oxygen consumption and energy demands. Every fish species has different optimum salinity ranges for growth and determination of the optimum conditions which will increase the production and brackish water areas can be used more efficiently. Growth and survival to changes in salinity may provide a bio-energetic basis to evaluate performance of rohu under different culture conditions. This is also relevant for the culture of salinity-tolerant aquaculture candidates which could be reared at different salinities. Salinity is considered as a limiting factor for the survival and growth of freshwater fish species like rohu. Thus, in order to evaluate the effect of different levels of salinity on Jayanti rohu, study was conducted to determine its survival and physiological values. All other parameters such as temperature, dissolved oxygen (DO), turbidity, pH and salinity were within the acceptable limit (Table 1) as given by / described by (APHA, 1988).

In the present work, to identify salinity tolerance level of selectively breed rohu carp, Jayanti, were exposed gradually to different levels of salinities and observed 100% mortality at 10 ppt level. Further, in order to determine effect

of salinity on physiology and survival of Jayanti rohu, 45 days of experiment were conducted via exposing fishes with 2, 4, 6, 7 and 8 ppt salinity level. In this study, we have analyzed the survival rate, hematological parameters and histological evaluation of the Jayanti rohu.

The data for survival rate is depicted in the Table 2. During initial 48 hours of observation, the fishes were apparently normal and there was no mortality even at 10 ppt. The survival rate for rohu fingerlings were observed to be 100%, 95%, 80% and 75% at 2, 4, 6 and 8 ppt salinities, respectively. However, there was no significant difference ($P > 0.05$) in weight gain among the rohu fishes exposed to different salinities even though, the fishes were active and showed normal feeding and swimming behavior. Recent studies demonstrated that salinity change can induce stress in aquatic animals by interfering with physiological homeostasis, routine biological processes and accelerates oxidative damage (Kültz, 2015). In such an alarming scenario, culture of salinity-tolerant aquatic animals which could be reared at different salinities could be an effective solution (Luz *et al.*, 2008). The physiological responses of freshwater stenohaline species to saline environments are attracting increased interest, particularly with respect to using saline water for the optimization of aquaculture practices (Wang *et al.*, 1997; Overton *et al.*, 2008; Imanpoor *et al.*, 2012; Küçük *et al.*, 2013). The preliminary evidence suggests that moderate salinities allow better performance, in particular reference to the attainment of stable survival rates under stress conditions (Murayama *et al.*, 1977; Umezawa and Nomura, 1984; Strüssmann *et al.*, 1996). Tsuzuki *et al.*, 2000 studied the salinity tolerance of sub-adults of *O. bonariensis* and *O. hatcheri* and obtained preliminary information on their osmoregulatory and compensatory stress responses under different NaCl concentrations and found that *O. bonariensis* seems to tolerate high salinities better than *O. hatcheri*, whereas the reverse occurs in low salinities. Recently, it was studied that

Table 2: Survival rate (in percentage) of Jayanti rohu fingerlings exposed to different salinities.

Treatments	Survival % of fingerlings
Control (0 ppt)	100
2 ppt	100
4 ppt	95
6 ppt	80
8 ppt	75
10 ppt	0

Table 3: Hematological parameters of Jayanti rohu exposed with different concentrations of salinities.

Parameter	Control (n=12)	2 ppt (n=12)	Salinity Conc. 4 ppt (n=12)	6 ppt (n=12)	8 ppt (n=12)
RBCs ($10^6/\mu\text{l}$)	0.89 \pm 0.09 ^a	0.86 \pm 0.14 ^a	0.73 \pm 0.18 ^a	0.56 \pm 0.10 ^b	0.06 \pm 0.13 ^c
HCT (%)	21.23 \pm 0.34 ^a	19.34 \pm 0.41 ^a	19.20 \pm 0.29 ^a	18.34 \pm 0.18 ^b	18.30 \pm 0.25 ^b
Platelets ($10^5/\mu\text{l}$)	6.56 \pm 2.78 ^a	6.89 \pm 1.86 ^a	7.12 \pm 2.45 ^a	7.93 \pm 2.67 ^b	8.35 \pm 2.98 ^b
Hemoglobin (g/dl)	3.12 \pm 0.26 ^a	3.10 \pm 0.28 ^a	2.92 \pm 0.31 ^a	2.87 \pm 0.34 ^a	0.81 \pm 0.23 ^b
MCV(fl)	85.23 \pm 0.92 ^a	84.45 \pm 0.9 ^a	79.87 \pm 0.89 ^b	77.5 \pm 0.84 ^b	83.3 \pm 1.02 ^a
MCH (pg)	51.23 \pm 0.8 ^a	50.67 \pm 0.92 ^a	51.00 \pm 0.96 ^a	50.1 \pm 0.78 ^a	50 \pm 1.34 ^a
MCHC (g/dl)	56.12 \pm 1.25 ^a	57.87 \pm 0.89 ^a	56.98 \pm 0.97 ^a	58.7 \pm 1.20 ^a	60 \pm 1.13 ^a

Values are means \pm S.E.M. Values with different superscript letters within each row are significantly different (analysis of variance, $P < 0.05$).

salinity higher than 5 ppt has significant effects on the growth, survival, haematological parameters and osmoregulation of tambaqui (*Colossoma macropomu*) juveniles (Fiúza *et al.*, 2015). James *et al.*, (1969) studied that the maximum salinity tolerance was 11 ppt for channel cat fish, 12 ppt for blue cat fish and 13 ppt for white cat fish and their production should not be attempted if salinity exceeds these levels. Ghosh *et al.*, (1973) observed that common carp can be profitably cultured up to 5 ppt salinity and there was no adverse effect on the fish even when the salinity was raised to 7 ppt. Mohammadi *et al.*, (2011) revealed that broodfish of Rainbow Trout, *Oncorhynchus mykiss* that were reared for 6 months in underground brackish water (11 ppt) successfully matured and spawned and their gonad quality was better than the broodfish in freshwater. Our results also suggested the potential of Jayantirohu fingerlings to be cultured in saline environment.

Further, haematological and histological studies depicted the effect of salinities on rohu fingerlings. The RBCs (Red blood cells), Hb (Haemoglobin), Platelets count were determined and MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated. Results in Table 3 depict significant changes in RBC counts was also reduced in higher salinity exposed fishes as compared to control group. The RBC counts were not statistically significant in control groups, 2 and 4 ppt salinity level fishes, whereas it was significantly reduced beyond 6 ppt salinity. Similarly, haemoglobin content was also drastically reduced in the fishes exposed with high salinity as compared control. The haemoglobin content were also not statistically significant in control groups, 2 and 4 ppt salinity level fishes, while it was drastically reduced beyond 6 ppt salinity level. The HCT value were also not statistically significant in control groups, 2 and 4 ppt salinity level fishes and significant results obtained in 6 and 8 ppt salinity level fishes. In earlier studies, effects of salinities were observed in Nile tilapia, *O. niloticus* and hematological parameter showed significant changes in higher salinity exposed fishes as compared to control fishes (Elarabany, *et al.*, 2017). In other studies, several haematological parameters have been used as stress indicators to evaluate the effect of salinity on physiological homeostasis of fish (Verdegem *et al.*, 1997;

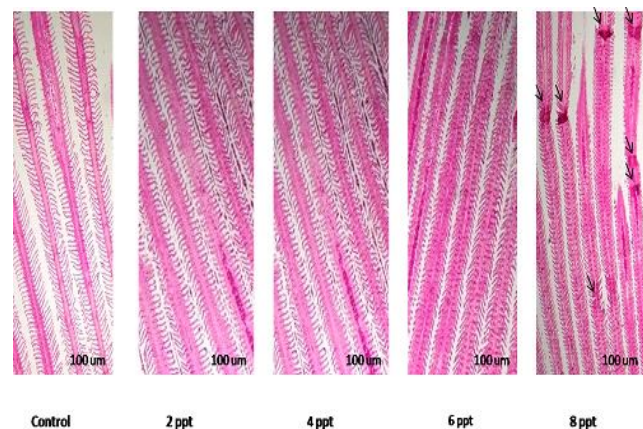


Fig 1: Histology of gill tissues of Jayanti rohu exposed to different salinities along with control (Arrow indicates mild lesion or mild damage to chloride cells of lamella).

Altinok *et al.*, 1998; Denson *et al.*, 2003; Luz *et al.*, 2008). Further, histological investigation was performed using gill tissues of different treatment group of fishes and results are in line with earlier report that increase in salinity level affects the ion exchange mechanism in the gills of fishes. In this work, we could not observe any major damage to gills of fishes upto 6 ppt. Only at 8 ppt, mild lesions were evident on gills (Fig 1). The earlier studies indicated that juveniles of rohu could grow normally upto 4 ppt salinity level, but less growth was observed as salinity increased above 4 ppt. (Baliarsingh *et al.*, 2018). Overall this study suggests that Jayanti rohu can be cultured in salt affected areas or saline affected zones upto 8 ppt and there is great potential to incorporate this species in low saline aquaculture.

CONCLUSION

Thus, present study revealed that genetically improved rohu (Jayanti) can tolerate salinity upto 8 ppt with good survival rate. This is first kind of report on selectively bred rohu, Jayanti using different level of salinities. It could be great species in low saline area where agriculture crops are difficult to cultivate and can be a potential candidate to culture in low saline zones. Thus, this comprehensive study using hematological and histological aspects would be the basis

to sustain the aquaculture production in the climate change scenarios with reference to saline zone. This could also be helpful in preparation of management plan for carp culture purpose in areas, where water salinity remains within the study level.

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