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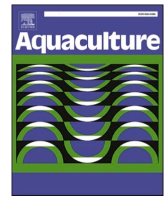

Growth performance, physio-metabolic, and haemato-biochemical status of *Labeo rohita* (Hamilton, 1822) juveniles reared at varying salinity levels using inland saline groundwater

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ABSTRACT

A 120-day experiment was conducted to investigate the effect of different inland saline groundwater salinities on growth performance, nutrient utilization, physiological-metabolic responses, and haemato-biochemical indices of juvenile *Labeo rohita*. The experiment setup comprised 24 circular tanks, each of 400 L capacity with 250 L water volume, stocked with juvenile L. *rohita* (2.04 ± 0.01 g; n = 25). The experimental tanks were categorised as seven treatments with different salinity levels viz., T1 (2‰), T2 (4‰), T3 (6‰), T4 (8‰), T5 (10‰), T6 (12‰), and T7 (14‰) and one control (0‰) with triplicates. No significant difference ($p > 0.05$) in growth performance was observed with increasing salinity from control to T2, but the growth rate was found to be suppressed significantly ($p < 0.05$) from T3 to T7. The highest and the lowest survival was recorded in control (100%) and T7 (0%), respectively. Increasing salinity above T2 (4‰) significantly retarded growth and feed utilization in L. *rohita*. Fish reared up to T2 (4‰) showed the highest amylase and protease activity, but those reared in T3 to T7 displayed the decreasing trend of enzyme activities with an overall significant difference ($p < 0.05$). With increasing salinity, the activity of Aspartate aminotransferase activity (AST), Alanine aminotransferase activity (ALT), Superoxide dismutase (SOD) and Catalase activity (CAT) in the liver of L. *rohita* were increased significantly ($p < 0.05$). Na^+/K^+-ATPase activity of gill significantly ($p < 0.05$) declined with increasing salinity. Similarly, total serum protein, albumin, globulin, Albumin:Globulin ratio, Ht%, haemoglobin and total erythrocyte count were reduced significantly ($p < 0.05$) with increasing salinity. In contrast, total leucocyte count was found to be significantly increased with increasing salinity. Serum biochemical response and stress indices (serum cortisol and serum glucose) were remarkably affected by salinity and differed significantly ($p < 0.05$) among treatments. The present study suggests that salinity level in the range of 0–4‰ is ideal for the culture of L. *rohita* in inland saline areas without affecting the overall fish performance. Further, it is also concluded from the study that survival of 72 to 88% could be obtained in inland saline groundwater having salinity up to 12‰, which suggests that degraded inland saline areas can be successfully utilized for sustainable aquaculture.

1. Introduction

The Indian fisheries sector contributes 7.28% of the agriculture Gross Domestic Production (Handbook, 2020). Government of India intends to increase fish production from 14.16 to 22 MMT by 2024–25 (PMMSY, 2020). This milestone can be achieved by utilizing untapped water resources. In arid and semi-arid regions, freshwater is limited (Verma et al., 2013, 2010) and after the introduction of irrigation canals in these areas, there are consequences in the rise in the water table and salinization (Verma et al., 2014, 2007). Salinization is a significant problem, and around 1125 million hectares of land are currently salt affected, with human induced salinization affecting approximately 76 million hectares spreading worldwide (Hossain, 2019). In India, 8.62 million hectares of land are severely impacted by soil salinity and 1.93 million square kilometres of land have been devastated by inland saline groundwater (ISGW) (Lakra et al., 2014). It is estimated that salt-affected lands in India are expected to increase to 20 million hectare by 2050 (Sharma et al., 2014). There is tremendous pressure to use

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inland saline groundwater (ISGW) for agriculture and aquaculture. Therefore, attempts to use ISGW for aquaculture as a critical technology to reduce freshwater use and offer economic opportunities for farmers in salt impacted areas are measures taken to solve this issue (Debroy, 2020; Singh et al., 2020).

In India, the ICAR-Central Institute of Fisheries Education, Rohtak centre, is dedicated to develop appropriate technology for commercial aquaculture in degraded inland saline environments. In this regard, several fish and crustacean species such as *Chanos chanos* (Raizada et al., 2005), *Penaeus monodon* (Antony et al., 2015), *Litopeaeus vannamei* (Jahan et al., 2018), *Pangasiandon hypothalamus* (Kumar et al., 2017), *Trachinotus blochii* (Pathak et al., 2019) have been evaluated for their suitability for culture using ISGW. Previous research works have shown that the salinity of culture water affected a variety of physiological responses in freshwater fish species such as *Colossoma macropomum* (Fiúza et al., 2015), *Pangasiandon hypothalamus* (Kumar et al., 2017) and *Cyprinus carpio haematopterus* (Singh et al., 2020).

L. rohita (Hamilton, 1822) is a major aquaculture species in the world, accounting for about 3.7% of total finfish production (FAO, 2020). It is commonly referred to as rohu, the most popular among Indian major carps, because of its capacity to flourish in shallow waters, high market demand, fast growth rate, omnivorous feeding behaviour, and adaptability to artificial diets. Due to its superior feeding niche, which extends from column to bottom, *L. rohita* is commonly stocked at higher levels than the other Indian major carps in most polyculture systems. The physiological responses of freshwater stenohaline species to the saline environment are gaining more attention as the use of inland saline water becomes more common for aquaculture practises, although they are little investigated. Out of many physicochemical properties which are considered as pivotal stress factors in aquaculture (Mazandarani et al., 2017), the salinity is a common one with significant effects on fish growth, survival, reproduction, digestive and stress enzymes, and osmotic regulation (Bøeuf and Payan, 2001; Kang'ombe and Brown, 2008; Fazio et al., 2013; Sui et al., 2016). Earlier studies have revealed that Indian major carps could tolerate salinities up to 10‰, and better growth occurred at salinity below 5‰ (Billard, 1999). Kumar et al. (2018a) conducted a short-term (10 days) study on salinity tolerance of L. *rohita* and found survival of L. *rohita* at a moderate salinity of about 6‰. In a similar work, (Sharma et al., 2020) observed substantial growth of L. *rohita* at 2.5‰ (90 days). However, the salt tolerance of L. *rohita* in inland saline groundwater for a prolonged period is not understood. The present study was conducted to evaluate the impact of changes in salinity level on growth responses, survival, digestive and metabolic enzyme activities, stress indices, haemato-biochemical parameters, and osmoregulation capacity in L. *rohita*.

2. Materials and methods

2.1. Experimental design, fish stocking and management

The present study was conducted for a period of 120 days in a wet laboratory of ICAR–Central Institute of Fisheries Education, Rohtak Centre, Haryana, India (28.86115° N, 76.47371° E) from July–October 2021. The experiment consisted of seven treatments with varying salinity levels (viz., 2, 4, 6, 8, 10, 12, and 14‰) and control in triplicates under a completely randomized design. The experimental system comprised 24 circular fibre reinforced plastic (FRP) tanks of capacity 400 L (1 × 0.5 m), each filled with 250 L of water. The tanks were first acid washed and then filled with potassium permanganate solution (4 mg L^{-1}), which were left overnight. The tanks were then thoroughly cleaned by hand, disinfected with bleaching powder, and rinsed with freshwater to remove residues of chlorine.

The inland saline water of 15.5‰ salinity was collected from a borewell and filtered through a 100-filter bag to remove redundant debris before transferring into four rectangular cemented tanks of 9000 L (3 × 2 × 1.5 m) capacity each and allowed to settle for a week. Subsequently, water was transferred to seven circular tanks of capacity 1000 L (1.08 × 0.93 m) each and filled up to 900 L. The stored water was diluted with borewell water (freshwater) to get desirable salinities (viz., 2, 4, 6, 8, 10, 12, and 14‰). The experimental tanks were filled with water of different salinity levels. Healthy L. *rohita* juveniles (2.04 ± 0.01 g) procured from the Government fish seed farm, Haryana, India were acclimated in tanks and randomly distributed to treatment and control tanks at the rate of stocking density of 25 fish in each tank and provided with good aeration using a 2H.P. air blower (A1 AQUA A; Flow rate: 160 m^3hr^{-1}; 1200 RPM; A1 Blowers India Pvt. Ltd.). The experimental tanks were siphoned regularly to remove the faecal matter and uneaten feed. The animals were fed with commercial feed containing 32% crude protein (Abis feed company) twice a day (9 am and 6 pm) at satiation level for 120 days.

During the experimental period, different water quality parameters and growth performance of the animals in different treatments were assessed at 20 days intervals. After the rearing period, selected tissues were collected from the animals for assaying various physio-metabolic and haemato-biochemical parameters.

The experiment followed the guidelines of the CPCSEA (Committee for Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India, for undertaking scientific research on animals.

2.2. Physicochemical parameters of water

The water temperature, dissolved oxygen, pH, and salinity of all the experimental tanks were measured twice a day (9 am and 6 pm) daily by using a water thermometer (MERCK, Germany), a pH, dissolved oxygen (DO) and salinity probe (WQC-24, DKK-TOA corporation, Japan) respectively. The total alkalinity and hardness of water were determined by the titrimetric method (APHA, 2005). The ammonia, nitrite, and nitrate were evaluated by the spectrophotometric method, and the potassium (K^+) and sodium (Na^+) ions were measured using a flame photometer (SYSTRONICS, India) while calcium (Ca^{+2}) was measured by titration method (APHA, 2005).

2.3. Growth indices

During the experiment, fish were sampled at regular intervals of 20 days to determine the growth parameters such as body weight. For sampling, fish were starved for 24 h, and weight was measured using an electric balance with 0.01 g accuracy. Major growth parameters including weight gain, specific growth rate (SGR), weight gain rate (WGR), feed conversion efficiency (FCE), feed intake, feed conversion ratio (FCR), and survival rate were assessed as described in previous reports (Nuwansi et al., 2019).

2.4. Sampling, tissue homogenate and serum preparation

After 120 days of the experiment, five fish from each experimental tank were collected and anaesthetized with clove oil (50 μL L^{-1}). For the analyse of various haematological parameters, blood was collected from the fish by puncturing the caudal vein using a 1 mL medical syringe and immediately transferred to an EDTA (anti-coagulant) coated vial and kept at 4 °C for further analysis. Subsequently, the animals were euthanized and dissected to collect tissue (liver and intestine) samples for protein and various enzymes assays. The tissue samples were immediately homogenized in chilled sucrose solution (0.25 M) using a Teflon coated mechanical homogenizer (REMI Equipment tissue homogenizer) and centrifuged (Thermo Fisher Scientific) at 2800 ×*g* for 10 min at 4 °C. The supernatant was collected immediately and kept at −20 °C for enzyme assay. Estimation of the protein content of different tissue was carried out as per Lowry's method (Lowry et al., 1951) with bovine serum albumin (BSA) as standard.

For the analysis of serum parameters, blood was collected from three

fish of each treatment group and allowed to clot at room temperature and subsequently centrifuge in sterilized vials. The serum was separated following centrifugation of the clotted blood at 3000 ×*g* for 10 min (Nuwansi et al., 2019) and cautiously collected in a sterile Eppendorf tube and stored at −20 °C for further analysis of serum biochemical parameters.

2.5. Digestive enzyme assay

The intestinal protease activities were determined by the casein digesting method of Drapeau (1976). One unit of protease enzyme activity was defined as the amount of enzyme needed to release acid-soluble fragments equal Δ0.001 at 280 nm per min at 37 °C and pH 7.8 and described as micromole of tyrosine released/min/mg protein. Amylase activity intestinal tissue was appraised as the reducing sugars produced due to the action of glucoamylase and α-amylase on carbohydrate using dinitro-salicylic-acid (Rick and Stegbauer, 1974). Specific amylase activity was shown as maltose mole released from starch per min at 37 °C. Intestinal lipase activity was assessed following the procedure described by (Cherry and Crandall Jr, 1932), which involves titration of fatty acids formed by hydrolysis by a base using phenolphthalein as an indicator. The lipase activity was denoted as U per mg protein at 37 °C.

2.6. Estimation of protein metabolic and oxidative stress enzymes activity

AST and ALT activity of serum and liver was determined by using colourimetric assay kits, ERBA SGPT KIT (Code Number- 120207) and ERBA SGOT KIT (Code Number- 120204), respectively. The AST activity was expressed as nanomoles of oxaloacetate released/min/mg protein at 37 °C. The ALT activity was expressed as nanomoles of pyruvate released/min/mg protein at 37 °C. Catalase activities of liver tissue homogenates were determined by the method described by Takahara et al. (1960) using H_2O_2 solution.

Superoxide dismutase activity of liver tissue homogenates was estimated according to the method described by Misra and Fridovich (1972) based on the oxidation of epinephrine–adrenochrome transition by the enzyme. Na^+/K^+-ATPase activity in gill was estimated by the modified method of Post and Sen (1967), and expressed as nanomoles Pi released min^{-1} mg^{-1} protein at 37 °C.

2.7. Determination of serum and haematological parameters

Serum albumin, globulin, and total protein were assessed by using the commercial kits (ERBA® Diagnostics) following the manufacturer's instructions. The osmoregulatory capacity (O.C.) was determined by the method of Charmantier et al. (1989). The serum cortisol level was determined using a commercial kit (carp-specific cortisol ELISA monoclonal kit; Cayman Chemical, USA) following the manufacturer's instructions. Serum glucose level was assessed by Trinder's method using ERBA kit.

Haemoglobin concentration in blood was determined by the cyanmethemoglobin technique (Qualigens diagnostics) using Drabkin's solution. Briefly, Drabkin's working solution (5 mL) was mixed with a blood sample (20 μL) and the optical density (O.D.) was measured at a wavelength of 540 nm in a spectrophotometer (MERCK-Thermo Electron, WI, USA). A haemocytometer was used to count total erythrocytes, and leucocytes and counting was performed using a Neaubauer's counting chamber using the following formula.

$$\text{Number of cells}/\text{mm}^3 = (\text{Number of cells counted} \times \text{dilution})/(\text{Area counted} \times \text{depth of fluid}).$$

The packed cell volume (PCV) or haematocrit was measured by drawing the blood into microhaematocrit tubes via capillary action and sealing the tubes with a synthetic sealant. The PCV was calculated as a percentage using a microhaematocrit reader (Thomas Scientific).

2.8. Osmolality

The osmolality was assessed with a vapour pressure osmometer (VAPRO® MODEL 5600; ELITech, USA). Osmoregulatory capacity (OC) was determined as the difference between the mean osmolality of the fish serum and mean osmolality of their corresponding rearing media (Greenwell et al., 2003).

2.9. Statistical analysis

The data were analysed by using the SPSS version 22 statistical software. To determine the significant difference between the treatments, one way ANOVA and Duncan's multiple range tests were used at a significance level of $p < 0.05$.

3. Results

3.1. Physio-chemical parameters of water

The physico-chemical parameters of water in each treatment group for an experimental period of 120 days are presented in Table 1. The values (mean ± SE) of temperature (28.18 ± 0.13–28.77 ± 0.06 °C), pH (7.94 ± 0.01–7.99 ± 0.01), dissolved oxygen (6.02 ± 0.01–6.05 ± 0.01 mg L^{-1}), NH_3-N (0.01 ± 0.01–0.09 ± 0.01 mg L^{-1}), NO_2 —N (0.002 ± 0.01–0.005 ± 0.01 mg L^{-1}) and NO_3 —N (0.14 ± 0.01–0.33 ± 0.01 mg L^{-1}) didn't exhibit any significant ($p > 0.05$) difference among all the treatments during entire the experimental period. However, significant ($p < 0.05$) difference in total alkalinity (115.73 ± 0.84–235.83 ± 1.29 mg L^{-1}), total hardness (157.07 ± 0.96–3226.17 ± 0.83 mg L^{-1}), calcium (53.93 ± 0.93–324.60 ± 1.27 mg L^{-1}), potassium (2.59 ± 0.03–15.88 ± 0.07 mg L^{-1}), and sodium (14.61 ± 0.31–3859.80 ± 1.86 mg L^{-1}) among the treatment groups was noticed. The highest level of total alkalinity, total hardness, potassium, sodium, and calcium was found in T7 (14‰).

3.2. Growth parameters

In the present study, different salinity levels significantly affected the growth, body indices and survival of the L. *rohita* and the same are depicted in Table 2. Considering the mean of WG, WGR, FCR, and FCE of fish, there was a significant difference among all the treatments at the end of 120 days. However, no significant difference was observed in fish reared in control, T1 and T2 treatments. The highest WG was observed in the control which was over 38 times significantly ($p < 0.05$) higher than fish maintained at highest salinity (i.e., T7 treatment). The lowest WG (0.16 ± 0.01) was observed in animals reared at T7 and increasing WG trends followed by T6 (0.54 ± 0.04) < T5 (2.98 ± 0.04) < T4 (3.23 ± 0.07) < T3 (3.81 ± 0.05) < T2 (5.85 ± 0.18) < T1 (5.98 ± 0.18) < control (6.19 ± 0.18). Similar trends were observed for the specific growth rate (SGR), WGR%, and FCE of the fish. In contrast, FCR was found to be the increased as the salinity increased, and the lowest value was recorded in control (3.01 ± 0.04) followed by T1 (3.02 ± 0.02) < T2 (3.05 ± 0.01) < T3 (3.79 ± 0.05) < T4 (4.06 ± 0.06) < T5 (4.38 ± 0.04) < T6 (4.66 ± 0.04) < T7 (6.64 ± 0.05); however, there was no

Table 1
Physico-chemical parameters in tank water of *Labeo rohita* juveniles reared in different experimental units at the end of the 120 days of the experiment.

Parameters	Treatment							
	Control	T1 (2‰)	T2 (4‰)	T3 (6‰)	T4 (8‰)	T5 (10‰)	T6 (12‰)	T7 (14‰)
Temperature °C	28.59 ± 0.10	28.69 ± 0.10	28.76 ± 0.12	28.77 ± 0.06	28.18 ± 0.13	28.48 ± 0.12	28.66 ± 0.30	28.60 ± 0.28
pH	7.94 ± 0.01	7.96 ± 0.01	7.96 ± 0.01	7.97 ± 0.01	7.97 ± 0.01	7.95 ± 0.01	7.96 ± 0.02	7.99 ± 0.01
Dissolved oxygen (mg L^{-1})	6.02 ± 0.01	6.02 ± 0.01	6.03 ± 0.01	6.04 ± 0.01	6.04 ± 0.01	6.05 ± 0.01	6.04 ± 0.01	6.03 ± 0.01
Alkalinity (mg L^{-1})	115.73^{a} ± 0.84	129.63^{b} ± 0.61	143.43^{c} ± 1.07	158.87^{d} ± 0.29	174.23^{e} ± 0.84	196.73^{f} ± 0.88	215.63^{g} ± 0.54	235.83^{h} ± 1.29
Hardness (mg L^{-1})	157.07^{a} ± 0.96	626.77^{b} ± 0.74	1163.67^{c} ± 1.81	1668.20^{d} ± 1.40	1833.40^{e} ± 3.49	2411.43^{f} ± 5.42	2877.53^{g} ± 1.51	3226.17^{h} ± 0.83
Calcium (mg L^{-1})	53.93^{a} ± 0.93	83.67^{b} ± 0.74	137.17^{c} ± 0.67	176.73^{d} ± 0.96	206.67^{e} ± 0 0.79	245.60^{f} ± 0 0.69	281.80^{g} ± 0.81	324.60^{h} ± 1.27
Potassium (mg L^{-1})	2.59^{a} ± 0.03	5.29^{b} ± 0.02	7.25^{c} ± 0.02	10.66^{d} ± 0.06	11.28^{e} ± 0.03	12.34^{f} ± 0.07	14.51^{g} ± 0.02	15.88^{h} ± 0.07
Sodium (mg L^{-1})	14.61^{a} ± 0.31	625.93^{b} ± 0.77	1163.93^{c} ± 1.84	1597.93^{d} ± 0.68	2458.47^{e} ± 1.11	2986.47^{f} ± 1.28	3362.57^{g} ± 1.13	3859.80^{h} ± 1.86
Nitrite (mg L^{-1})	0.002 ± 0.01	0.002 ± 0.01	0.002 ± 0.01	0.003 ± 0.01	0.003 ± 0.01	0.004 ± 0.01	0.005 ± 0.01	0.005 ± 0.01
Nitrate (mg L^{-1})	0.14 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.31 ± 0.01	0.33 ± 0.01
Ammonia (mg L^{-1})	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01

Note: All values in the same row with different superscripts differ significantly ($p < 0.05$) for each parameter. Data expressed as mean ± SE. One way ANOVA was used following Duncan multiple range tests in SPSS – 22.0.

Table 2
Growth, survival and feed utilization parameters of *Labeo rohita* juveniles reared in different experimental units at the end of the 120 days of the experiment.

Treatment	Initial weight (g)	Final weight (g)	Weight gain (g)	WGR (%)	SGR	FCR	Feed intake (g per fish)	FCE
Control	2.05 ± 0.05	8.24^{e} ± 0.20	6.19^{e} ± 0.18	301.89^{e} ± 8.80	1.16^{e} ± 0.02	3.01^{a} ± 0.04	18.72^{f} ± 0.27	0.34^{e} ± 0.01
T1 (2‰)	2.06 ± 0.03	8.04^{e} ± 0.19	5.98^{e} ± 0.18	297.21^{e} ± 8.58	1.15^{e} ± 0.02	3.02^{a} ± 0.02	18.04^{e} ± 0.44	0.33^{e} ± 0.01
T2 (4‰)	2.08 ± 0.02	7.93^{e} ± 0.17	5.85^{e} ± 0.18	285.34^{e} ± 6.57	1.12^{e} ± 0.02	3.05^{a} ± 0.01	17.84^{e} ± 0.47	0.33^{e} ± 0.01
T3 (6‰)	2.04 ± 0.06	5.85^{d} ± 0.05	3.81^{d} ± 0.05	189.33^{d} ± 2.76	0.88^{d} ± 0.01	3.79^{b} ± 0.05	14.46^{d} ± 0.05	0.27^{d} ± 0.01
T4 (8‰)	2.02 ± 0.10	5.25^{c} ± 0.07	3.23^{c} ± 0.07	157.38^{c} ± 3.26	0.79^{c} ± 0.02	4.06^{bc} ± 0.06	13.12^{c} ± 0.11	0.25^{d} ± 0.01
T5 (10‰)	2.07 ± 0.05	5.05^{c} ± 0.04	2.98^{c} ± 0.04	148.31^{c} ± 1.86	0.76^{c} ± 0.01	4.38^{bc} ± 0.04	13.0^{c} ± 0.08	0.23^{bc} ± 0.01
T6 (12‰)	2.04 ± 0.08	2.58^{b} ± 0.05	0.54^{b} ± 0.04	26.17^{b} ± 2.45	0.19^{b} ± 0.02	4.66^{e} ± 0.04	2.48^{b} ± 0.01	0.22^{b} ± 0.02
T7 (14‰)	2.02 ± 0.16	2.18^{a} ± 0.01	0.16^{a} ± 0.01	7.86^{a} ± 0.50	0.07^{a} ± 0.01	6.64^{f} ± 0.05	1.08^{a} ± 0.01	0.15^{a} ± 0.01
P value	0.001	0.012	0.001	0.002	0.001	0.001	0.002	0.001

Note: All values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter. Data expressed as mean ± SE. One way ANOVA was used following Duncan multiple range tests in SPSS – 20.0.
SGR: specific growth rate; FCR: feed conversion ratio; FCE: feed conversion efficiency; WGR: Weight gain rate.

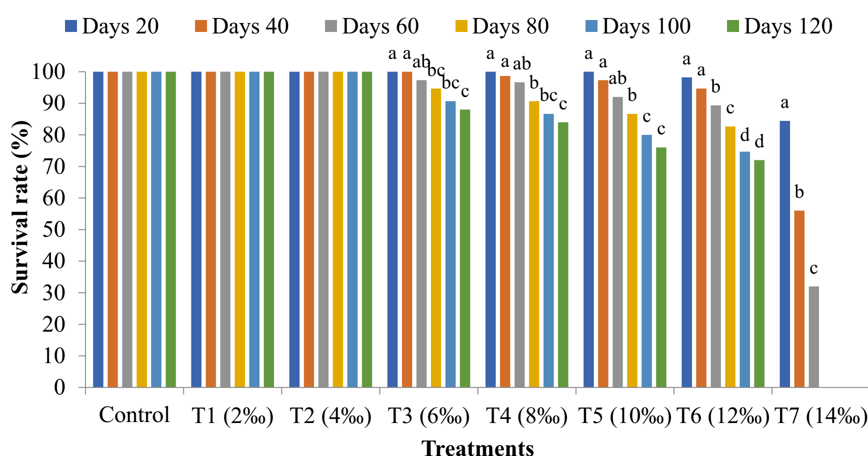

Fig. 1. Survival rate (%) of *Labeo rohita* juveniles reared in different treatments during 120 days of the experiment.

Table 3
Digestive enzyme activity of *Labeo rohita* juveniles reared in different experimental units at the end of the 120 days of the experiment.

Treatment	Protease (U mg protein^{-1})	Amylase (U mg protein^{-1})	Lipase (U mg protein^{-1})
Control	$10.69^d \pm 0.28$	$6.99^d \pm 0.20$	2.36 ± 0.83
T1 (2‰)	$10.60^d \pm 0.27$	$6.97^d \pm 0.19$	2.18 ± 0.79
T2 (4‰)	$10.47^d \pm 0.28$	$6.89^d \pm 0.21$	2.13 ± 0.96
T3 (6‰)	$8.77^c \pm 0.23$	$5.81^c \pm 0.16$	2.03 ± 0.88
T4 (8‰)	$8.62^c \pm 0.25$	$5.74^c \pm 0.18$	1.88 ± 0.99
T5 (10‰)	$5.93^b \pm 0.14$	$4.24^b \pm 0.07$	1.86 ± 0.62
T6 (12‰)	$3.99^a \pm 0.06$	$3.31^a \pm 0.18$	1.69 ± 0.79
T7 (14‰)	$3.92^a \pm 0.05$	$3.22^a \pm 0.17$	1.50 ± 0.88
P value	0.003	0.002	0.994

Note: All values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter. Data expressed as mean ± SE. One way ANOVA was used following Duncan multiple range tests in SPSS – 22.0.

Table 4
Metabolic and antioxidative enzyme activity in the liver and Na^+/K^+ ATPase activity in the gill of *Labeo rohita* juveniles reared in different experimental groups at the end of the 120 days of the experiment.

Treatments	ALT	AST	SOD	CAT	Na^+/K^+ ATPase
Control	$1.67^a \pm 0.25$	$1.13^a \pm 0.10$	$1.17^a \pm 0.02$	$0.58^a \pm 0.02$	$5.84^d \pm 0.16$
T1 (2‰)	$1.88^a \pm 0.24$	$1.26^a \pm 0.15$	$1.18^a \pm 0.03$	$0.60^a \pm 0.02$	$5.71^d \pm 0.21$
T2 (4‰)	$2.11^a \pm 0.28$	$1.47^a \pm 0.24$	$1.19^a \pm 0.02$	$0.62^a \pm 0.02$	$5.66^d \pm 0.19$
T3 (6‰)	$3.19^b \pm 0.22$	$2.12^b \pm 0.19$	$2.26^b \pm 0.05$	$1.18^b \pm 0.25$	$4.08^c \pm 0.10$
T4 (8‰)	$3.42^b \pm 0.24$	$2.44^b \pm 0.29$	$2.30^b \pm 0.04$	$1.20^b \pm 0.32$	$3.96^c \pm 0.11$
T5 (10‰)	$4.03^c \pm 0.05$	$3.22^c \pm 0.31$	$2.65^c \pm 0.01$	$1.95^c \pm 0.14$	$3.67^b \pm 0.05$
T6 (12‰)	$5.02^d \pm 0.12$	$3.90^d \pm 0.17$	$2.76^d \pm 0.03$	$2.60^d \pm 0.14$	$3.05^a \pm 0.10$
T7(14‰)	$5.31^d \pm 0.13$	$4.09^d \pm 0.17$	$2.78^d \pm 0.03$	$2.62^d \pm 0.24$	$2.92^a \pm 0.08$
P value	0.002	0.004	0.001	0.001	0.002

Note: All Values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter. Data expressed as mean ± SE, n = 3. One way ANOVA was used following Duncan multiple range tests in SPSS – 22.0.
Alanine aminotransferase activity (ALT) expressed as nanomoles of pyruvate formed per mg protein per min. at 37 °C.
Aspartate aminotransferase activity (AST) expressed as nanomoles of oxaloacetate formed per mg protein per min at 37 °C.
Superoxide dismutase (SOD) activity is expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min (equivalent to 1.67–05 katal/mg protein or, 103 U per mg protein).
Catalase activity (CAT) is expressed as nanomoles H_2O_2 decomposed/min/mg protein (equivalent to 1.67–11 katal/mg protein Or, 1.00–03 U per mg protein).
Na^+/K^+ ATPase as nanomoles Pi released min^{-1} mg^{-1} protein at 37° C.

significant ($p > 0.05$) difference within control, T1, and T2, T4 and T5 treatments. The survival of the T7 group was significantly ($p < 0.05$) lower as compared to other groups, and the highest value (100%) was displayed in the control, T1, and T2 group and then gradually decreased in T3 (88%) > T4 (84%) > T5 (76%) > T6 (72%) respectively (Fig. 1).

3.3. Digestive enzyme response

Water salinity significantly affected the digestive enzyme activity of L. *rohita* grown in different treatment groups at the end of 120 days as shown in Table 3. The amylase activities in control group (6.99 ± 0.20) was found to be significantly ($p < 0.05$) different among the treatments T3 (5.81 ± 0.16), T4 (5.74 ± 0.18), T6 (3.31 ± 0.18), and T7 (3.22 ± 0.17); however no significant difference was noticed among T1 (6.97 ± 0.19) and T2 (6.89 ± 0.21). Similar trends were observed in protease enzyme activities; the highest and lowest values were noticed in control (10.69 ± 0.28) and T7 (3.92 ± 0.05), respectively. Interestingly, the lipase activity did not differ significantly ($p > 0.05$) among all the treatments; the highest and lowest lipase activity was observed in control (2.36 ± 0.83) and T7 (1.50 ± 0.88), respectively.

3.4. Metabolic and oxidative enzymes response in liver and Na^+/K^+ ATPase response in gill

The observed values of metabolic and oxidative enzymes in the liver, such as ALT, AST, CAT, SOD and Na^+/K^+-ATPase activity in gill, are depicted in Table 4. These parameters differed significantly ($p < 0.05$) among all the treatment groups. The ALT activity was highest in T7 (5.31 ± 0.13), and the lowest value was observed in control (1.67 ± 0.25). The highest and lowest AST activity was observed in T7 (4.09 ± 0.17) and control (1.13 ± 0.10), respectively. The antioxidative enzyme, such as SOD, was higher in T7 (2.78 ± 0.03) and lower in control (1.17 ± 0.02). Moreover, the highest and lowest CAT activity was noticed in T7 (2.62 ± 0.24) and control (0.58 ± 0.02), respectively. Overall, the metabolic and oxidative stress indicative enzymes were significantly ($p < 0.05$) lower in control and gradually increased with increasing salinity levels in T1 and T2, T3 and T4, T6 and T7 treatments, respectively. The significantly ($p < 0.05$) highest and lowest Na^+/K^+-ATPase activity of gill was recorded in control (5.84 ± 0.16) and T7 (2.92 ± 0.08).

3.5. Serum biochemical response

The serum biochemical parameters such as ALT, AST, ALP, glucose and cortisol differed significantly ($p < 0.05$) among all the treatments, and their levels increased as the salinity of water increased (Table 5). Significantly ($p < 0.05$) higher value of ALT, AST, ALP, glucose and cortisol were observed in T7 (35.53 ± 1.21, 76.13 ± 1.26, 56.89 ± 0.74, 63.33 ± 0.78 and 54.59 ± 0.49), whereas control displayed lower values (22.27 ± 1.24, 43.94 ± 1.32, 32.26 ± 0.95, 30.33 ± 0.48, 21.43 ± 0.55).

Table 5
Serum biochemical response of *Labeo rohita* juveniles reared at different experimental groups at the end of the 120 days of the experiment.

Treatment	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})	Glucose (mg dL^{-1})	Cortisol (ng mL^{-1})
Control	$22.27^a \pm 1.24$	$43.94^a \pm 1.32$	$32.26^a \pm 0.95$	$30.33^a \pm 0.48$	$21.43^a \pm 0.55$
T1 (2‰)	$22.88^a \pm 1.23$	$45.77^a \pm 0.64$	$33.50^a \pm 2.31$	$30.66^a \pm 0.55$	$22.76^a \pm 0.36$
T2 (4‰)	$23.54^a \pm 1.65$	$46.23^a \pm 0.27$	$34.85^a \pm 2.35$	$30.90^a \pm 0.61$	$23.54^a \pm 1.61$
T3 (6‰)	$28.23^b \pm 0.94$	$54.56^a \pm 1.10$	$42.96^b \pm 0.42$	$42.00^b \pm 0.67$	$32.56^a \pm 0.51$
T4 (8‰)	$29.49^{bc} \pm 0.70$	$56.32^a \pm 0.84$	$46.54^b \pm 0.97$	$42.76^b \pm 0.60$	$33.00^a \pm 0.73$
T5(10‰)	$32.57^c \pm 0.81$	$65.13^a \pm 0.93$	$51.97^c \pm 0.37$	$51.76^c \pm 0.35$	$46.75^a \pm 0.22$
T6 (12‰)	$33.86^d \pm 1.03$	$74.15^a \pm 0.87$	$54.50^{cd} \pm 0.31$	$61.88^d \pm 0.96$	$53.24^a \pm 0.50$
T7 (14‰)	$35.53^d \pm 1.21$	$76.13^a \pm 1.26$	$56.89^d \pm 0.74$	$63.33^d \pm 0.78$	$54.59^a \pm 0.49$
P value	0.003	0.004	0.002	0.001	0.002

Note: All values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter. Data expressed as mean ± SE, n = 3. One way ANOVA was used following Duncan multiple range tests in SPSS – 22.0. Alanine aminotransferase activity (ALT); Alanine aminotransferase activity (ALT); Alkaline phosphatase (ALP).

Table 6
Serum biochemical response and haematological parameters of *Labeo rohita* juveniles reared at different experimental groups at the end of the 120 days of the experiment.

Treatment	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Albumin: Globulin ratio	Haematocrit value (%)	Haemoglobin concentration (g dL^{-1})	Total erythrocyte count (10^6 cells mm^3)	Total leucocytes count (10^3 cells mm^3)
Control	$1.73^d \pm 0.04$	$0.40^c \pm 0.06$	$1.33^d \pm 0.01$	$0.30^c \pm 0.02$	$25.19^d \pm 0.03$	$8.26^d \pm 0.07$	$1.81^d \pm 0.02$	$6.10^a \pm 0.03$
T1 (2‰)	$1.72^d \pm 0.03$	$0.40^c \pm 0.07$	$1.32^d \pm 0.01$	$0.30^c \pm 0.03$	$25.16^d \pm 0.05$	$8.23^d \pm 0.06$	$1.78^d \pm 0.02$	$6.12^a \pm 0.03$
T2 (4‰)	$1.71^d \pm 0.03$	$0.39^c \pm 0.07$	$1.31^d \pm 0.01$	$0.30^c \pm 0.04$	$25.13^d \pm 0.04$	$8.21^d \pm 0.06$	$1.77^d \pm 0.02$	$6.14^a \pm 0.02$
T3 (6‰)	$1.48^c \pm 0.01$	$0.26^b \pm 0.02$	$1.22^c \pm 0.01$	$0.21^b \pm 0.01$	$21.29^c \pm 0.04$	$6.89^c \pm 0.02$	$1.60^c \pm 0.04$	$6.39^b \pm 0.04$
T4 (8‰)	$1.46^c \pm 0.01$	$0.25^b \pm 0.01$	$1.21^c \pm 0.01$	$0.21^b \pm 0.01$	$21.25^c \pm 0.05$	$6.86^c \pm 0.03$	$1.56^c \pm 0.03$	$6.41^b \pm 0.04$
T5 (10‰)	$1.25^b \pm 0.01$	$0.19^{ab} \pm 0.02$	$1.12^b \pm 0.01$	$0.18^b \pm 0.01$	$18.20^b \pm 0.02$	$4.16^b \pm 0.01$	$1.31^b \pm 0.01$	$7.14^c \pm 0.02$
T6 (12‰)	$1.17^a \pm 0.01$	$0.12^a \pm 0.03$	$1.05^a \pm 0.01$	$0.11^a \pm 0.01$	$15.40^a \pm 0.02$	$3.13^a \pm 0.03$	$0.99^a \pm 0.06$	$8.61^d \pm 0.03$
T7 (14‰)	$1.14^a \pm 0.01$	$0.11^a \pm 0.03$	$1.03^a \pm 0.01$	$0.10^a \pm 0.01$	$15.38^a \pm 0.02$	$3.11^a \pm 0.03$	$0.90^a \pm 0.05$	$8.66^d \pm 0.01$
P value	0.001	0.003	0.001	0.001	0.005	0.001	0.002	0.002

Note: All values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter. Data expressed as mean ± SE, n = 3. One way ANOVA was used following Duncan multiple range tests in SPSS – 22.0.

Table 7
Osmoregulatory response of *Labeo rohita* juveniles reared at different experimental groups at the end of the 120 days of the experiment.

Treatment	Water osmolality (mOsm kg^{-1})	Serum osmolality (mOsm kg^{-1})	Osmoregulatory capacity (mOsm kg^{-1})
Control	$11.88^a \pm 0.87$	$299.96^a \pm 1.88$	$288.07^h \pm 1.02$
T1 (2‰)	$56.23^b \pm 0.56$	$302.16^a \pm 2.53$	$245.92^g \pm 2.96$
T2 (4‰)	$94.82^c \pm 0.21$	$304.83^a \pm 3.30$	$210.01^f \pm 3.17$
T3 (6‰)	$152.27^d \pm 1.18$	$326.27^b \pm 2.64$	$173.99^e \pm 2.49$
T4 (8‰)	$243.06^e \pm 1.47$	$326.27^b \pm 2.22$	$86.46^d \pm 1.81$
T5 (10‰)	$292.29^f \pm 1.22$	$338.07^c \pm 1.07$	$45.77^c \pm 0.84$
T6 (12‰)	$332.55^g \pm 1.23$	$346.05^d \pm 2.70$	$13.50^b \pm 1.60$
T7 (14‰)	$376.56^h \pm 0.87$	$350.25^d \pm 2.30$	$-26.31^a \pm 1.43$
P value	0.001	0.002	0.001

Note: All values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter. Data expressed as mean ± SE, n = 3. One way ANOVA was used following Duncan multiple range tests in SPSS – 22.0.

3.6. Haemato-biochemical assay

The haemato-biochemical parameters such as total protein, albumin, globulin and A/G ratio, haematocrit value, haemoglobin concentration (Hb), total erythrocyte count (TEC), and total leucocytes count (TLC) values are depicted in Table 6. Overall, haemato-biochemical parameters differed significantly ($p < 0.05$) among all the treatment groups with increasing salinity levels. Significantly ($p < 0.05$) higher value of total protein, albumin, globulin and albumin: globulin ratio was observed in control (1.73 ± 0.04, 0.40 ± 0.06, 1.33 ± 0.01 and 0.30 ± 0.02) and lower value was recorded in T7 (1.14 ± 0.01, 0.11 ± 0.03, 1.03 ± 0.01 and 0.10 ± 0.01). The highest value of haematocrit value, haemoglobin concentration, and total erythrocyte count were recorded in control (25.19 ± 0.03, 8.26 ± 0.07 and 1.81 ± 0.02); however, the lowest value was found in T7 treatment group (15.38 ± 0.02, 3.11 ± 0.03 and 0.90 ± 0.05). A significantly ($p < 0.05$) higher value of total leucocytes count was reported in T7 (8.66 ± 0.01) and a lower value was observed in control (6.10 ± 0.03).

3.7. Osmolality response

The Osmolality response in terms of water osmolality, serum osmolality and osmoregulatory capacity values are depicted in Table 7. Overall, the water osmolality and osmoregulatory capacity differed significantly ($p < 0.05$) among all the treatment groups ($p < 0.05$). The significantly ($p < 0.05$) higher value of water osmolality and serum osmolality was noticed in T7 (350.25 ± 2.30 and 376.56 ± 0.87) and lower value in control (11.88 ± 0.87 and 299.96 ± 1.88). However, osmoregulatory capacity was significantly ($p < 0.05$) higher in control (288.07 ± 1.02), and it gradually decreased with increasing salinity levels in respective treatments.

4. Discussion

4.1. Physio-chemical parameters of water

Water quality is a critical factor that affects the survivability of aquatic organisms directly or indirectly. During the experimental period, the mean value of various water quality parameters such as temperature, pH, dissolved oxygen, NH_3-N, NO_2-N, and NO_3-N was maintained relatively steady within the acceptable ranges for fish production in inland saline waters. Other physico-chemical parameters, such as total alkalinity, total hardness, calcium, potassium, and sodium varied significantly ($p < 0.05$) with increased salinity levels and have

Table 8
Salinity tolerance and optimal salinity for growth of widely cultivated freshwater species.

Fish	Study duration (days)	Optimal salinity (‰)	Tolerance salinity (‰)	References
Gift tilapia	45	7.5	20	Qiang et al. (2013)
Silver carp	60	3–4	10	von Oertzen (1985)
Grass carp	30	(0.05)	25	Kilambi (1980), Chervinski (1977)
Rohu	90	2.5	4.5	Sharma et al. (2020)
Rohu	30	(0.05–2)	12	Pillai et al. (2003)
Magur	23	(0.05-2)	8	Sahoo et al. (2003)
Pangasius	60	10	15	Kumar et al. (2017)
Singhi	90	(0-6)	9	Ahmmed et al. (2017)
Channa	60	5	13	Dubey et al. (2016)
Gold	21	2	10	Luz et al. (2008)
Common carp	60	(0.05–6)	12	Mangat and Hundal (2014)

shown variations in ionic composition also, which is in accordance with the earlier research works (Kumar et al., 2017; Sharma et al., 2020; Singh et al., 2020). It has been reported that ISGW ionic concentrations varied from place to place, even at the same salinity level. The composition of minerals/ions in ISGW differs from freshwater with hardness (Ca^{+2} and Mg^{+2}) being reasonably high in ISGW as compared to freshwater (Prangnell and Fotedar, 2006; Saoud et al., 2003).

4.2. Growth parameters

Earlier studies have discovered that fish grow better in brackishwater than in freshwater or seawater (Imsland et al., 2001; Küçük et al., 2013; Luz et al., 2008). Previous research works in several fish species have shown that increasing salinity in freshwater boosts the growth rate to a certain point, after which it drops, indicating a fish species' tolerance threshold (Table 8). Although fish may survive at higher salinities, their growth is adversely affected after a certain level of salinity. In the present study, the growth performance of animal was not influenced at salinity up to 4‰, however, significant decline in growth ($p < 0.05$) was observed at salinity range of 4–14‰. The growth data revealed a substantial difference when analysing the fish reared at more than 4‰ salinity to the control group. Active feeding was observed in the present investigation at lower salinities up to 8‰. After that, the feeding intensity gradually reduced as salinity increased, and finally, there was no feed intake at 14‰ after some days demonstrating that appetite was influenced by salinity. A decline in growth was observed by Luz et al. (2008) in goldfish, and Gan et al. (2016) in *Oreochromis niloticus* in response to higher salinities. Reduced feed intake in L. *rohita* due to more prolonged exposure to higher salinity significantly affected FCR and SGR, which was also reported in *C. macropomum* juveniles (Fiúza et al., 2015). In the present study, the survival rate of L. *rohita* was significantly ($p < 0.05$) influenced by more prolonged exposure to different salinities. Complete mortality occurred at 14‰ after 80 days, and no mortality was observed in fish reared up to 4‰ in 120 days. In an earlier study on salinity tolerance of L. *rohita*, 100% survival has been reported upto 6‰ salinity during the 90 days experimental period and using ordinary salt as water medium (Islam et al., 2014). However, Sharma et al. (2020) noticed 47.5% mortality in L. *rohita* at 4.5‰ during the 90 days exposure period. The variation in survival rate was attributed to differences in the size of fish utilized in the experiment, the quality of feed used and the experimental methodology. Similar to the present study, the low survival of animals at higher salinity has been reported in *C. macropomum* (Fiúza et al., 2015), *O. niloticus* (Gan et al., 2016), *Pangasianodon hypophthalmus* (Kumar et al., 2017), and L. *rohita* (Sharma et al., 2020).

4.3. Digestive enzyme response

It is assumed that water salinity can affect the activation of each enzyme's zymogen independently in the gut lumen as well as change in the gut's physicochemical state by modifying the pH, ion concentrations, or ions composition of the gut contents (Moutou et al., 2004; Usher et al., 1988). However, rearing of fish at varying water salinity levels may influence the drinking rates, which may affect the saltiness of the gut content, the rate of gut evacuation, and ultimately affect digestive enzyme activity (Usher et al., 1988). The findings of the present study revealed that the proteases and amylase activity significantly ($p < 0.05$) decreased with increasing water salinity in L. *rohita* juveniles. A similar response over increasing water salinity has been observed by Asha-Devi and Aravindan (1997) in *Oreochromis mossambicus,* Moutou et al. (2004) in *Sparus aurata*, Vargas-Chacoff et al. (2015) in *Eleginops maclovinu,* Singh (2019) in *C. carpio haematopterus,* Debroy (2020) in *Anabas testudines*, Mozanzadeh et al. (2021) in *Acanthopagrus latus* and *Lates calcarifer*. Lipase activity did not vary considerably in this investigation, which corresponds with the study of Singh (2019) in *C. carpio haematopterus,* Debroy (2020) in *Anabas testudines*. Findings of the present study indicated that the increased salinity has a negative impact on digestive enzyme activity resulting in reduced growth performance and increased FCR.

4.4. Metabolic and oxidative enzymes response in liver and Na^{+}/K^{+}-ATPase response in gill

Increased levels of ALT and AST enzymes in the blood indicate liver damage, necrosis, liver malfunction, and tissue degeneration; they also indicate the reflections of protein metabolism changes (Chowdhury et al., 2020; Dawood et al., 2021; Hossain et al., 2018). In the present experiment, the ALT, AST, and ALP enzymes activities in the liver of L. *rohita* juveniles varied significantly with respect to salinity in different treatments groups. In the present study, it has been observed that metabolic enzymes activity in the liver increased with increasing water salinity levels. The study also revealed the elevation of metabolic enzymes in liver tissue (such as ALT, AST, and ALP) of *L. rohita* with high salinity; this could be due to impaired liver function caused by the prolonged salinity exposure. Ma et al. (2021) reported that hepatocytes in the liver destabilized and broken-down during stress, resulting in an increased release of liver enzymes. The liver damage in rainbow trout with increasing water salinity has been reported by (Hoseini et al., 2019). It has also been reported that increasing water salinity led to stressful situations like osmotic disruption due to oxidative stress and the production of reactive oxygen in the aquatic organism (Ghelichpour et al., 2020). Fish exposed to higher concentrations of environmental stressors have shown increasing ALT, AST, and ALP levels (De Smet and Blust, 2001; Kumar et al., 2018b; Li et al., 2018; Dawood et al., 2021; Ma et al., 2021).

SOD and catalase are enzymes that are served to restore lipids from oxidizing. The superoxide anion is turned into hydrogen peroxide by SOD (Tao et al., 2013), which is then reduced down into water and oxygen by catalase, preventing lipid peroxidation (Tavares-Sánchez et al., 2004). Recent research has demonstrated salinity variations to promote oxidative stress, which reduces antioxidant defences. In general, higher SOD and CAT activity suggest that more radicals must be transformed (Chien et al., 2003). As a result, significantly increased SOD and CAT activities in fish at higher salinity indicate that stress at higher salinity caused radical build-up at higher levels in L. *rohita*. It can be further attributed that above the optimum salinity level, it induces stress to the organism due to oxidative stress in fish liver tissue. CAT activity increased when fish got stressed by salinity. The findings of the study revealed that differing salinity levels substantially impacted SOD and CAT enzymes activities. When salt concentrations were increased among the treatments (0.05–14‰), SOD and CAT activity in the liver significantly ($p < 0.05$) increased in T7 (14‰) compared to the control, T1, and T2. Gan et al. (2016) reported similar findings in tilapia, where SOD and CAT activities were relatively high at 16‰ and 24‰ when compared to 8 and 0‰. Sui et al. (2016) found that increasing water salinity augmented SOD levels in blood parrotfish (*Cichlasoma synspilum x Cichlasoma citrinellum*) during 6 h of salinity exposure. Martínez-Álvarez et al. (2002) reported that SOD and CAT activity significantly ($p < 0.05$) increased with rising salinity in *Acipenser naccarii.* SOD and CAT activity increased when euryhaline fish were cultured at low saline water, such as *Pampus argenteus* (Yin et al., 2011) and *Dicentrarchus labrax* (Islam et al., 2020). In our investigation, high SOD and CAT activities indicate incremental stress in fish as ambient salinity gradually increased.

The presence of a gradient of concentration between the environment and the organism is imposed by exposure to fresh, brackish and saline water, Na^{+}/K^{+}-ATPase activity plays a vital role in maintaining homeostasis (Laverty and Skadhauge, 2012). NKA activity was shown to decrease trends when salinity increased significantly. The highest activity of L. *rohita* was found in 0–4‰, and the salinity range up to 4‰ indicated the minimal differences between ambient water and body fluid. Furthermore, at this salinity*, L. rohita* was likely to save energy on

osmoregulation and utilize it for other functions. NKA activity decreasing with salinity stress exposed similarly trends observed in *Synechogobius ommaturus* (Shui et al., 2018), *D. labrax* (Sinha et al., 2015) and *Oncorhynchus tshawytscha* (Stewart et al., 2016) and *Cichlasoma managuense* (Ai et al., 2020).

4.5. Serum biochemical response

Even though ALP is abundant in the biliary system and erythrocytes, injury to these cells causes the enzyme to escape into circulation (Banaee et al., 2014; Taheri Mirghaed et al., 2019). A surge in water salinity can harm the biliary system of fish and erythrocytes. The current findings revealed that the fish reared at high salinity levels had considerably higher contents of ALT, AST, and ALP enzymes. In the present investigation, the fish suffered from hepatic impairment in saline water of more than 4‰. These compounds may cause hemolysis and increase ALP levels in the blood. The findings are in agreement with Liu et al. (2013), who noticed that 2–5 fold of serum ALT enzyme activity in juvenile chum salmon reared in high-salinity groups than in low-salinity groups. Luo et al. (2017) found a substantial rise in AST with increasing salinity in genetically enhanced tilapia (*O. niloticus*) grown in biofloc production systems at three salinity levels (0, 10, and 20‰). Sultan (2007) also found that increasing salinity increased plasma ALT and AST concentrations in young *A. latus* maintained in different (3, 23, 30‰) salt solutions. AlKatrani et al. (2018) reported increasing trends of AST and ALT against salinity in *Oreochromis aureus*. Wei et al. (2010) reported a substantial difference between the serum ALT and AST enzymes in *Oncorhynchus keta* in saline water and their presence in freshwater. Abdel-Rahim et al. (2019) measured higher serum AST, ALT and ALP values in *Argyrosomus regius.*

Elevation of blood glucose indicates stress and increased energy expenditure when freshwater fish are transferred to saltwater (Hoseini et al., 2019). According to Karsi and Yildiz (2005), the stress in fish causes a primary reaction involving neurohormonal activation and increased corticosteroid and catecholamine secretions. Plasma glucose is an organic component of the blood that is commonly employed as a stress indicator in fish that have been subjected to environmental changes (Kim et al., 2019). In the present study, a significant increase in serum glucose levels was observed with increasing salinity which is similar to the study of Fiess et al. (2007) in *O. mossambicus*, Ahmmed et al. (2017) in *Heteropneustes fossilis,* Souza-Bastos and Freire (2009) in *Rhamdia quelen*, Sarma et al. (2013) in *Clarias batrachas*, Li et al. (2007) in *O. mossambicus*.

Individuals may have been chronically stressed, resulting in increased salinity or other stressors, and survivors downregulated cortisol release via a negative feedback loop in the HPI axis to avoid the harmful effects of consistently excessive cortisol levels (Rich and Romero, 2005; Romero, 2004). General stress indicators in fish usually involve cortisol and glucose (Pacheco and Santos, 2001; Santos and Pacheco, 1996). Kammerer et al. (2010) observed an increase in plasma cortisol as early as 3 h following the onset of saltwater stress. Higher saline conditions significantly altered fish physiological status as a crucial stressor as evidenced by the increased cortisol level of L. *rohita* observed in the present study. Similarly, increasing salinity ultimately elevate the cortisol levels, which was observed by Ron et al. (1995) in *O. mossambicus*, Luz et al. (2008) in *C. auratus*, Tsui et al. (2012) in *E. malabaricus,* Al-Khashali and Al-Shawi (2013) in *C. auratus*, Fiúza et al. (2015) in *C. macropomum,* Jumah and Traifalgar (2015) in *O. niloticus*, Ranjbar and Mohammad Nejad (2020) in *O. mykiss*. In the present study, plasma cortisol was higher in L. *rohita* where salinity was more than 4‰. Our present findings imply that fish reared at more than 4‰ salinity suffered from chronic stress.

4.6. Haemato-biochemical assay

A wide range of proteic components such as albumin, immunoglobulins, complements, proteins, cytokines, transferrin, and lectins, serum/plasma total to protein level is an important criterion for the good health status (Magnadóttir, 2006). In the present study, total serum protein declined in L. *rohita* among all the treatments, inferring those proteins were oxidized for gluconeogenesis and releasing energy for osmoregulation at higher salinity (Luz et al., 2008). In the present study, the serum total protein content decreased gradually as water salinity increased, as also observed by Kelly et al. (1999) in *Mylio macrocephalus*, Martínez-Álvarez et al. (2002) in *A. naccarii*, Luz et al. (2008) in *Carassius auratus* and Abdel-Rahim et al. (2019) in *A. regius.*. Similarly, the decline in protein following exposure to an environmental stressor has been reported in L. *rohita* (Christobher et al., 2016).

The response of salinity on fish haematological characteristics has been studied earlier (Soltanian et al., 2016). Increased salinity in the environment caused stress to fish and led to alterations in haematological parameters, indicating a breakdown in homeostasis or a compensatory response to salinity changes Salati et al. (2010). In the present investigation, it was observed that at higher saline conditions (4–14‰), haematological parameters such as RBC, HCT, and H.B. concentration drastically reduced in L. *rohita*. The impact of salinity on RBCs, HCT, and Hb was also observed in a number of stressed species such as hybrid tambacu (*Piaractus mesopotamicus Holmberg* x *C. macropomum Cuvier*) (Tavares-Dias et al., 2000), *Rachycentron canadum* (Denson et al., 2003), *Pangasius hypophthalmus* (Usha, 2011), *Periophthalmus waltoni* (Soltanian et al., 2016), Improved Jayanti rohu (Murmu et al., 2020) and *Paralichthys olivaceus* (Kim et al., 2021). The present study revealed that the reduction in RBC is detected by osmotic changes induced by ion leakage from the plasma, which might explain low haematocrit percentages in stressed L. *rohita*. In fish, the number of white blood cells is a reliable measure of physiological stress (Svobodová et al., 2001). In the current investigation, increasing trends in WBC with increased salinity levels were observed. Similar findings were also reported in rainbow trout (Hosseinzadeh Sahafi et al., 2013) and Improved Jayanti rohu (Murmu et al., 2020).

4.7. Osmolality response

The increasing salinity level in different treatments proportionately enhanced the water osmolarity. At different salinity levels ranging from 0 to 14‰, Luz et al. (2008), Oyoo-Okoth et al. (2011), Kumar et al. (2017), and Singh et al. (2020) reported a similar trend in water osmolarity. Blood plasma osmolality was significantly higher in L. *rohita* reared above 4‰ in ISGW. Similarly, blood plasma osmolality stayed at 300 mOsm kg^{-1}, which is typical of freshwater fish, despite a trend of rising plasma osmolality with increasing water salinity (Luz et al., 2008; Overton et al., 2008; Oyoo-Okoth et al., 2011; Fiúza et al., 2015; Kumar et al., 2017; Singh et al., 2020). Increasing salinity has a negative effect on plasma osmolality. As a result, it implies that L. *rohita* cannot regulate plasmatic osmolality and maintain homeostasis in these conditions without causing osmotic problems. It has been reported that the osmoregulatory ability of aquatic animals at various salinities provided a better reflection of changes caused by salinity stress (Lignot et al., 2000). In our study, the highest osmoregulatory capacity was found in the control group, followed by a decreasing trend in the subsequent groups with increasing salinities. The findings of the study revealed that salinity had a minor impact on the long-term survival of the animals up to 12‰. However, in 14‰, negative osmoregulatory capacity was observed. Similar findings have been reported in other freshwater fish showing a negative correlation with water salinity (Singh, 2019; Debroy, 2020). According to the present findings*, L. rohita* could manage osmoregulation only at 0–4‰ salinities; and above these salinities, osmoregulatory disruption occurred, resulting in poor growth performance and decline in aquaculture production.

5. Conclusion

From the present study's findings, it is concluded that the L. *rohita*, a freshwater fish, can survive in moderate saline water up to 10‰. The fish adapted well up to 4‰ inland saline water and maintained their growth, survival, physio-metabolic responses, and haemato-biochemical status in better condition. However, prolonged exposure to higher salinities severely impaired the growth and health status of the animal. In areas where the freshwater source is lacking, and saline water of 6–12‰ is available, above 72 to 88% survival of the animals can be achieved with minimal impairment in growth and other parameters, which will become very important to improve the production and productivity in degraded saline areas for sustainable aquaculture. This aquaculture practice would minimize freshwater use and could be a sustainable approach towards uplifting socio-economic status by tapping unutilised saline water resources for fish culture.

CRediT authorship contribution statement

Ravi Kumar Patel: Conceptualization, Formal analysis, Writing - original draft. **A.K. Verma:** Conceptualization, Supervision, Writing - review & editing. **K.K. Krishnani:** Conceptualization, Formal analysis. **K. Sreedharan:** Supervision, Formal analysis. **M.H.Chandrakant**: Methodology, Writing - review & editing.

Declaration of Competing Interest

None.

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