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Effect of temporal increment in salinity of inland saline groundwater on growth performance, survival, metabolic and osmoregulatory responses of juveniles of *Labeo rohita* (Hamilton, 1822)

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ABSTRACT

Conjunctive use of saline groundwater and surface water has been employed in agriculture sector as an operational strategy to preserve water. In the present study, the effect of temporal increment in salinity of inland saline groundwater (ISGW) on growth, survival, physiological, and haemato-biochemical responses of rohu (*Labeo rohita*) juveniles was examined. The five treatments were assigned with a temporal IGSW salinity increment for 160 days *viz.,* T1 (0.1‰ on daily basis), T2 (0.1‰ for every two days), T3 (0.1‰ for every three days), T4 (0.1‰ for every four days), T5 (0.1‰ for every five days) with C1 (Control 1; 0.0‰) and C2 (Control 2: 4‰). No significant ($p > 0.05$) difference was found in growth performance between controls and T5, however, the growth rate was determined to be concealed significantly ($p < 0.05$) from T1 to T4. Both controls and T5 had the highest (100%) survival rates and the lowest was in T1 (5%). In all the treatments except T5, shortening the acclimatization time considerably altered growth and feed utilization in rohu. The hepatic aspartate aminotransferase, alanine aminotransferase, superoxide dismutase activity, and catalase activity in rohu significantly increased with decreasing acclimation period. The activity of Na^+/K^+-ATPase in gill significantly increased with decreasing acclimation period. Similar to this, with a shorter acclimatization period, serum lysozyme activity decreased significantly. The acclimation period had a substantial impact on serum biochemical and stress parameters (serum cortisol and serum glucose), and these values varied significantly between treatments. The result of the present study indicates that rohu can be successfully cultured in ISGW environments by a temporal increase in salinity level at 7.2‰ (T5) without statistically significant adverse effects on fish performance. In the present study, the temporal increment in salinity levels showed that 80 to 93% survival rates could be achieved in ISGW with salinities up to 12‰ suggesting that unutilised ISGW could be used safely for sustainable aquaculture.

1. Introduction

Researchers and policymakers are concerned about the growing population's food and nutritional security in the climate change scenario (Parry et al., 1999; Rosenzweig et al., 2004; Godfray et al., 2010), degradation of land and water (Oldeman, 1998; Pimentel, 2006), biodiversity loss (Foley et al., 2005; Lotze-Campen et al., 2008a, 2008b; Tscharntke et al., 2012), and freshwater scarcity (Rijsberman, 2006; Lotze-Campen et al., 2008a, 2008b). Salinization is a major issue worldwide and in India, 6.72 million ha of land is affected due to soil salinization (Arora et al., 2016; Arora and Sharma, 2017), and 0.2 million km^2 is affected by inland saline groundwater (ISGW) at salinity over 2‰ (Antony et al., 2021). Freshwater supply is limited in arid and semi-arid regions (Verma et al., 2013, 2014). There is a better scope to use ISGW for agriculture in order to fill the gap between freshwater demand and supply (Verma et al., 2010). In inland saline areas, the most culture species is shrimp, *Litopenaeus vannamei* (Prangnell and Fotedar, 2006; Roy et al., 2010), but now faced with several constraints; particularly in the areas of environment, legal, and disease (Sedhuraman et al., 2014; Salunke et al., 2020). Therefore, carp culture is the most eco-friendly practice in inland saline areas (Singh et al., 2020). The rohu, *Labeo rohita* (Hamilton, 1822), is the world's most important

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aquaculture species contributing about 5.1% of the total finfish (FAO, 2022) and very well-known major carp in India due to its ability to adapt to shallow waters, relatively high demand in the market, better growth rate, omnivorous feeding habits, and response to formulated feeds.

Salinity is one of the most essential abiotic factors that influences the growth, survivability and physiological condition of animals (Fiúza et al., 2015; Dawood et al., 2021; Mozanzadeh et al., 2021). Each fish species appears to have a species-specific optimal water salinity for growth, and additional factors including temperature, feed intake, sex, and developmental stage may also have an impact on growth performance (Boeuf and Payan, 2001). It has been demonstrated that variations in water consumption rates in various water salinities can impact the activity of digestive enzymes because these factors affect, the gut's salinity content, ion composition, and pH (Giffard-Mena et al., 2006; Psochiou et al., 2007). Teleost has highly efficient osmoregulatory mechanisms to maintain internal homeostasis. Fish gills, which are constantly exposed to external environments, are multifunctional organs important for various homeostatic activities such as gas exchange, ion regulation, and acid–base balance (Perry, 1997, 1998; Hirose et al., 2003; Evans et al., 2005). Na^+/K^+-ATPase (NKA) is crucial for maintaining intracellular homeostasis by providing a driving force for many other ion-transporting systems (Marshall and Bryson, 1998; Hirose et al., 2003; Hwang and Lee, 2007). Irrespective of salinity of the external medium, teleost fish typically maintain their plasma osmolarity within a specific range (290–340 mOsmol/L) (McCormick and Saunders, 1987), and failure to do so for an extended time period leads to mortality. Freshwater to saline water conversion necessitates a switch from net ion influx to net ion efflux, which is primarily controlled by the gills, but also involves the kidney, gut, and urine bladder (Evans, 1979; Foskett et al., 1983). Freshwater fish culture in low-saline areas could be a feasible option to aquaculture in the northern-western region of India where freshwater is scarce and salinity levels are high (Lakra et al., 2014). Previous studies have indicated that several growth-related parameters were lower in fish reared in high inland saline water (Kumar et al., 2017; Singh et al., 2020). Previously, we studied the effect of different salinity levels (transferred directly from freshwater to desired salinities) on various growth parameters and the health status of rohu and found that these parameters improved significantly up to 4‰ with 100% survival as compared to other treatments having salinity above 4‰. The growth and survival decreased in treatments above 4‰. Further, the complete mortality rohu was noticed at 14‰ after 80 days of rearing (Patel et al., 2022). Therefore, considering the Indian major carp (IMC) culture's prospects and needs in the ISGW region, the present study has been designed to investigate the salinity adaptation of rohu juveniles at five different acclimation intervals for a period of 160 days. The study introduces a novel approach for determining the salinity tolerance of fish exposed to temporal increment in salinity as culture progress from freshwater to lethal salinities. This method is an effective tool for determining the viability of any species for aquaculture in a variety of ISGW zones. The purpose of this study is to use ISGW of higher salinity for aquaculture and strategies to use more saline water to save freshwater for the future.

2. Material and methods

2.1. Experimental unit, design, and procedure

The current investigation was performed from 10 December 2021 to 19 May 2022 in the wet laboratory at the ICAR-CIFE, Rohtak (28.861150 N, 76.473710 E), Haryana, India. The experiment encompassed five treatments and two controls in triplicates (a total of 21 units) under a completely randomized design. The treatments were assigned with a temporal increment of 0.1‰ in salinity of IGSW on different days for a period of 160 days *viz.,* T1 (0.1‰ on daily basis; 20‰ after 160 days), T2 (0.1‰ for every two days; 12‰ after 160 days), T3 (0.1‰ for every three days; 9.3‰ after 160 days), T4 (0.1‰ for every four days; 8‰ after 160 days), T5 (0.1‰ for every five days; 7.2‰ after 160 days), C1 (0.0‰) and C2 (4‰). Twenty-one (21) circular fibre-reinforced plastic (FRP) tanks with a capacity of 300 L and 200 L of water made up the experimental unit. In each tank, a measuring scale was positioned to keep the water level constant. The tanks were filled with a potassium permanganate solution (4 mg/L) and left to sit overnight after being acid cleaned. After that, the tanks were hand-cleaned, bleached, and rinsed with freshwater to remove any excess chlorine. The inland saline water (16.2‰ salinity) was drawn from a borewell and filtered using a 100-filter bag to exclude unnecessary material before being transferred into two rectangular cemented tanks, each with a capacity of 9000 L and allowed to settle for a week. Water was then shifted to three circular tanks, each with a capacity of 1000 L and filled to 900 L. The stored natural ground saline water was used to get desired salinities. Initially, the treatment tanks (T1 to T5) and C2 were filled with 4‰ saline water while C1 tanks were filled with freshwater (zero salinity). After that, a salinity increment in T1 to T5 was initiated, while the salinity of water in C1 and C2 tanks remained steady at 0‰ and 4‰, respectively for the entire experimental period. Salinity increment was accomplished 2–3 h after the animal fed in the morning and was performed by draining an appropriate volume of water from each tank and gradually adding stored saline groundwater. The first control treatment utilized a similar procedure to interchange water, except that the water supply back to the tanks had the same salinity as the water taken out. Salinity was evaluated daily throughout the experimental period, and saline groundwater was provided as needed to maintain the desired salinities ($\pm$ 0.1‰). Healthy rohu juveniles (2.44 $\pm$ 0.01 g) were obtained from M/s. Lakra Fish Seed Farm in Rohtak, Haryana, India. Fish were treated with salt solution (20 g/L) for 2 min to reduce handling stress. Then treated fish were carefully transferred to two circular tanks (8000 L capacity) containing conditioned freshwater, and were covered with fibre mat to prevent fish's escape. Fish were acclimatized for one week with an appropriate aeration system. During the acclimatization period, fish were fed a commercial diet twice daily to satiation level. Acclimatized fish were distributed randomly to the control and treatment tanks with a stocking density of 20 fish per tank. Siphoning was performed on daily basis in all experiment tanks to clean faecal matter and extra feed. All the tanks were provided with proper aeration. Throughout the experiment, fish were fed commercial feed (30% crude protein) two times (8:00 and 17:00 h) a day up to satiation levels. The mortality rate (the number of fish that perished in the previous 24 h) was recorded. During the experimental period, different water quality parameters and growth performance of the animals in different treatments were examined at 20 days intervals. After the rearing period, selected tissues were collected from the animals for assaying various physio-metabolic and serum-biochemical parameters.

The experiment was carried out in conformity with guidelines established by the Ministry of Environment and Forests (Animal Welfare Division), Government of India, for using animals in scientific research.

2.2. Water quality parameters and different growth indices

The water temperature, dissolved oxygen, and pH of all the experimental tanks were measured twice daily (9 am and 6 pm), and salinity was measured twice before and after adding saline groundwater. In each experimental unit, a thermostat heater (RS Electrical Aquarium Heater-300 W) was used to maintain a consistent temperature, and a digital water thermometer was used to measure water temperature (Fisher Scientific, USA) and pH, dissolved oxygen (DO), and salinity measured by a probe (WQC-24, DKK-TOA Corporation, Japan). The total alkalinity and hardness of water were determined by the titrimetric method (APHA, 2005). The ammonia, nitrite, and nitrate were assessed by the spectrophotometric method, and the potassium (K^+) and sodium (Na^+) ions were measured by a flame photometer (SYSTRONICS, India). In contrast, calcium (Ca^{2+}) was assessed by the titration method (APHA, 2005). Overall growth performances *i.e.*, Weight gain (WG), Feed

Conversion Ratio (FCR), Specific Growth Rate (SGR), Feed Conversion Efficiency (FCE), and Survival in all the treatments were calculated according to the formula (Nuwansi et al., 2020, 2021) given below:

$$\text{WG (g)} = \text{Final body weight (g)} - \text{Initial body weight (g)} \quad (1)$$

$$\text{FCR} = \text{Feed consumption (g)} / \text{Weight gain (g)} \quad (2)$$

$$\text{SGR (\%/day)} = [(\text{Ln Final body weight} - \text{Ln Initial body weight}) / \text{days}] \times 100 \quad (3)$$

$$\text{FCE} = \text{Body weight gain (wet weight in g)} / \text{Feed given (dry weight in g)} \quad (4)$$

$$\text{Survival (\%)} = (\text{Number of fish harvested} / \text{number of fish stocked}) \quad (5)$$

2.3. Sampling for preparing tissue homogenate and serum

After 160 days of the experiment, three fish from each experimental tank (three experimental tanks in each treatment) were collected and anaesthetized with clove oil (50 μL/L) (Hajek et al., 2006). Blood was drawn from two fish from each treatment group and allowed to clot at room temperature before being centrifuged in sterilised vials to determine serum parameters. After centrifuging the clotted blood at 3000*g* for 10 min, the serum was carefully collected in a sterile Eppendorf tube and refrigerated at −20 °C for further investigation of serum biochemical characteristics (Haridas et al., 2017).

The euthanatized fish were dissected to acquire tissue samples (liver and gill) for enzyme assays. The tissue samples were homogenised in cold sucrose solution (0.25 M) using a Teflon-coated mechanical homogenizer (REMI Equipment tissue homogenizer) and centrifuged at 2800*g* for 10 min at 4 °C (Thermo Fisher Scientific) (Garg et al., 2022). The supernatant was immediately collected and stored at −20 °C for the enzyme assay. Lowry's method (Lowry et al., 1951) was used to calculate the protein content of various tissues, using bovine serum albumin (BSA) as a benchmark.

2.4. Metabolic enzyme assay

ERBA SGPT KIT (Code Number- 120207) and ERBA SGOT KIT (Code Number- 120204) colorimetric test kits were used to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in serum and liver, respectively.

2.5. Estimation of oxidative stress enzymes activity

The catalase (CAT) activities in the liver were measured using the H_2O_2 solution method (Takahara et al., 1960). The activity of superoxide dismutase (SOD) in the liver was calculated using the protocol of Misra and Fridovich (1972). The modified method of Post and Sen (1967) was followed to estimate the Na^+/K^+-ATPase enzyme activity in the gill.

2.6. Determination of cortisol and osmolality in serum

The serum cortisol level was measured using a commercial kit (carp-specific cortisol ELISA monoclonal kit; Cayman Chemical, USA) following the manufacturer's instructions. The ERBA kit was used to measure the serum glucose level according to Trinder's approach. A turbidometric assay utilizing lyophilized Micrococcus lysodeikticus was done to determine the lysozyme activity in serum as described by Ellis (1990). The osmolality was measured with a vapour pressure osmometer (VAPRO® MODEL 5600; ELITech, USA). To analyze the osmolality of different samples, 10 μL of sample and a 1000 mol/Kg osmolality standard were used. Osmoregulatory capacity (OC) is the difference between the fish serum's mean osmolality and their corresponding rearing media (Greenwell et al., 2003).

2.7. Statistical analysis

All data were statistically analyzed by using IBM SPSS program software (SPSS, 22). All experimental data (except water quality parameters) were subjected to one-way ANOVA with polynomial and quadratic contrast analysis, and post-hoc Duncan's multiple range tests were done to test overall, linear and quadratic trends at 5% probability level ($p < 0.05$). Levene's test was performed for the assumption of homogeneity of variance.

3. Results

3.1. Water quality parameters

The water quality parameters of different experimental tanks were calculated and represented in Table 1. There was no significant ($p > 0.05$) difference observed in water temperature (28.38 ± 0.10 to 28.49 ± 0.10 °C), pH (7.91 ± 0.09 to 7.96 ± 0.12), dissolved oxygen (6.18 ± 0.06 to 6.26 ± 0.06 mg/L), NH_3-N (0.01 ± 0.01 to 0.08 ± 0.01 mg/L), NO_2-N (0.002 ± 0.01 to 0.006 ± 0.01 mg/L), and NO_3-N (0.25 ± 0.01 to 0.44 ± 0.01 mg/L) throughout the experiment. However, total alkalinity (125.73 ± 1.64 to 240.11 ± 1.44 mg/L), total hardness (163.89 ± 1.85 to 3023.70 ± 1.26 mg/L), calcium (54.40 ± 1.17 to 332.34 ± 1.15 mg/L), potassium (2.41 ± 0.02 to 14.27 ± 0.02 mg/L), and sodium (16.84 ± 0.31 to 3761.11 ± 0.58 mg/L) showed a significant ($p < 0.05$) difference among all the experimental groups. The level of total alkalinity, total hardness, potassium, sodium, and calcium concentration were higher in T1. After 160 days of rearing period, the salinity levels among the different treatments were 16‰ (T1), 12‰ (T2), 9.3‰ (T3), 8‰ (T4), and 7.2‰ (T5).

3.2. Growth parameters

Overall growth performances and survival of the rohu were significantly impacted by various salinity increment times in the present study, as shown in Table 2, Fig. 1 and Fig. 2. Fig. 3. depicts the salinity increment in various treatments during the course of the experimental period. After completion of the experiment, a significant ($p < 0.05$) difference was observed among the treatments for the mean of WG, FCE, and FCR of fish. However, no significant ($p > 0.05$) difference was found between fish cultured in the controls and all the treatments, all growth parameters were found to be better in T5. The WG was found to be significantly ($p < 0.05$) higher in C1 than the fish maintained at different salinity increment periods. The lowest WG (8.27 ± 0.02 g) was found in T1 and the trend of WG in other groups was T2 (11.94 ± 0.01 g) < T3 (13.80 ± 0.02 g) < T4 (14.49 ± 0.01 g) < T5 (15.34 ± 0.02 g) < C2 (17.56 ± 0.04 g) < C1 (17.58 ± 0.02 g). Similar patterns were also observed in the SGR, and FCE of the fish among the treatments. In contrast, the values for FCR were found to be increased in treatment groups as the acclimation period decreased, and the lowest value was observed higher in C1 (2.54 ± 0.01) followed by C2 (2.55 ± 0.02) < T5 (2.91 ± 0.04) < T4 (3.09 ± 0.04) < T3 (3.23 ± 0.06) < T2 (3.45 ± 0.05) < T1 (3.73 ± 0.01). The maximum survival (100%) was observed in the controls and T5 groups, and the survival of the T1 group was significantly ($p < 0.05$) lower than that of the other groups. Thereafter, it gradually declined as follows: T4 (93.33%) > T3 (91.66%) > T2 (80%) > T1 (5%), respectively (Table 3).

3.3. Metabolic enzymes, oxidative response and Na^+/K^+ ATPase activity

The level of the liver metabolic and oxidative enzymes, including ALT, AST, SOD, CAT, and gill Na+/K + -ATPase activity were measured and represented in Table 4. In the present study, ALT (5.12 ± 0.09), AST (3.98 ± 0.06), SOD (2.72 ± 0.07), CAT (2.50 ± 0.02), and Na^+/K^+-

Table 1
Physico-chemical parameters in tank water of rohu juveniles reared in different experimental units at the end of the 160 days of the experiment.

Parameters	Treatment							*P* value
	C1 (0.0‰)	C2 (4‰)	T1 (0.1‰ on daily basis)	T2 (0.1‰ for every 2 days)	T3 (0.1‰ for every 3 days)	T4 (0.1‰ for every 4 days)	T5 (0.1‰ for every 5 days)	
Temperature (°C)	28.49 ± 0.10	28.45 ± 0.05	28.38 ± 0.10	28.43 ± 0.05	28.42 ± 0.13	28.44 ± 0.10	28.46 ± 0.14	0.112
pH	7.91 ± 0.09	7.91 ± 0.11	7.92 ± 0.10	7.94 ± 0.11	7.96 ± 0.12	7.95 ± 0.19	7.93 ± 0.21	0.127
Dissolved oxygen (mg/L)	6.22 ± 0.06	6.21 ± 0.04	6.22 ± 0.05	6.25 ± 0.04	6.18 ± 0.06	6.20 ± 0.09	6.26 ± 0.06	0.212
Alkalinity (mg/L)	125.73^{a} ± 1.64	147.62^{b} ± 0.85	240.11^{g} ± 1.44	215.23^{f} ± 1.15	194.65^{e} ± 0.72	181.26^{d} ± 1.18	172.59^{c} ± 0.92	0.007
Hardness (mg/L)	163.89^{a} ± 1.85	1255.33^{b} ± 3.68	3023.70^{g} ± 1.26	2449.29^{f} ± 3.15	1854.82^{e} ± 5.36	1646.02^{d} ± 2.70	1528.96^{c} ± 2.80	0.002
Calcium (mg/L)	54.40^{a} ± 1.17	143.07^{b} ± 0.58	332.34^{g} ± 1.15	281.14^{f} ± 0.63	232.48^{e} ± 1.27	207.67^{d} ± 0.73	191.62^{c} ± 1.23	0.004
Potassium (mg/L)	2.41^{a} ± 0.02	7.37^{b} ± 0.02	14.27^{g} ± 0.02	12.24^{f} ± 0.02	11.84^{e} ± 0.02	10.37^{d} ± 0.03	08.26^{c} ± 0.03	0.002
Sodium (mg/L)	16.84^{a} ± 0.31	1183.60^{b} ± 1.78	3761.11^{g} ± 0.58	3444.29^{f} ± 1.15	2456.29^{e} ± 0.59	2226.15^{d} ± 1.53	1705.59^{c} ± 1.81	0.005
Nitrite (NO_2 − N) (mg/L)	0.003 ± 0.01	0.003 ± 0.01	0.002 ± 0.01	0.004 ± 0.01	0.005 ± 0.01	0.005 ± 0.01	0.006 ± 0.01	0.107
Nitrate (NO_3 − N) (mg/L)	0.25 ± 0.01	0.30 ± 0.01	0.28 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.38 ± 0.01	0.44 ± 0.01	0.132
Ammonia (NH_3−N) (mg/L)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.135

Note: All values are expressed as Mean ± SE (n = 3). Mean values in the same row with different superscripts differ significantly ($p < 0.05$) for each parameter.

Table 2
Growth, survival and feed utilization parameters of rohu juveniles reared in different experimental units at the end of the 160 days of the experiment.

Treatment		Initial weight (g)	Final weight (g)	Weight gain (g)	Feed conversion ratio	Specific growth rate (%/day)	Feed conversion efficiency
C1 (0.0‰)		2.44 ± 0.01	20.02^{f} ± 0.02	17.58^{f} ± 0.02	2.54^{a} ± 0.01	1.31^{e} ± 0.02	0.39^{f} ± 0.01
C2 (4‰)		2.44 ± 0.01	20.00^{f} ± 0.04	17.56^{f} ± 0.04	2.55^{a} ± 0.02	1.31^{e} ± 0.02	0.39^{f} ± 0.01
T1 (0.1‰ on daily basis)		2.44 ± 0.02	10.71^{a} ± 0.02	8.27^{a} ± 0.02	3.73^{f} ± 0.01	0.92^{a} ± 0.02	0.27^{a} ± 0.01
T2 (0.1‰ for every 2 days)		2.44 ± 0.01	14.38^{b} ± 0.01	11.94^{b} ± 0.01	3.45^{e} ± 0.05	1.10^{b} ± 0.01	0.29^{b} ± 0.01
T3 (0.1‰ for every 3 days)		2.43 ± 0.01	16.24^{c} ± 0.03	13.80^{c} ± 0.02	3.23^{d} ± 0.06	1.18^{c} ± 0.02	0.31^{c} ± 0.01
T4 (0.1‰ for every 4 days)		2.44 ± 0.02	16.93^{d} ± 0.01	14.49^{d} ± 0.01	3.09^{c} ± 0.04	1.21^{c} ± 0.01	0.32^{d} ± 0.01
T5 (0.1‰ for every 5 days)		2.45 ± 0.01	17.79^{e} ± 0.01	15.34^{e} ± 0.02	2.91^{b} ± 0.04	1.24^{d} ± 0.02	0.34^{e} ± 0.02
Contrast analysis							
P value	*Overall*	0.987	0.001	0.001	0.001	0.001	0.001
	Linear	0.548	0.001	0.001	0.001	0.001	0.001
	Quadratic	0.862	0.001	0.001	0.001	0.001	0.001

Note: All values are expressed as Mean ± SE (n = 3). Mean values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter.

ATPase (7.79 ± 0.16) activities were found to be significantly higher in T1 group as compared the controls. However, there was no significant difference between C1 and C2.

3.4. Serum biochemical responses

The level of ALT, AST, glucose, and cortisol in the serum differed considerably among all treatments, and they were observed to increase as the water salinity increased (Table 5). There was no significant difference between C1 and C2. The values of ALT, AST, glucose, and cortisol were measured significantly ($p < 0.05$) higher in T1 (64.42 ± 0.12, 34.16 ± 0.23, 48.76 ± 0.28, and 45.80 ± 0.31 IU/L); as compared to controls. However, serum lysozyme activity was observed significantly ($p < 0.05$) lower in T1 (7.88 ± 0.39 μg/mL) as compared to controls.

3.5. Osmolality response

The osmolality of water and serum and osmoregulatory capacity were presented in Figs. 4, 5, and 6. A significant ($p < 0.05$) difference was found between water osmolality and osmoregulatory capacity in all experimental groups. The osmolality of water and serum was recorded significantly ($p < 0.05$) higher in T1 (411.86 ± 3.85 and 386.30 ± 2.57 mOsm/kg) and lower in C1 (13.63 ± 2.30 and 278.25 ± 3.12 mOsm/kg). However, significantly ($p < 0.05$) higher osmoregulatory capacity was observed in C1 (264.62 ± 2.09 mOsm/kg), and then it increased gradually while increasing the acclimation period in respective treatments.

4. Discussion

4.1. Water quality parameters

The quality of water is an essential factor that directly or indirectly impacts aquatic organisms' survival (Bhatnagar and Devi, 2013). Most water quality parameters were maintained at optimal values throughout the study and were suitable for fish farming in ISGW aquaculture. No significant differences in pH, temperature, nitrite nitrogen, dissolved oxygen, nitrate nitrogen, and ammonia across different treatments were noticed which is supported by various researchers in previous studies (Kumar et al., 2017; Patel et al., 2022; Pathak et al., 2019). Other water quality parameters such as salinity, total hardness, total alkalinity, potassium, calcium, and sodium also altered significantly ($p < 0.05$) when salinity levels were gradually increased in all treatments, which is consistent with earlier research (Patel et al., 2022; Singh et al., 2020).

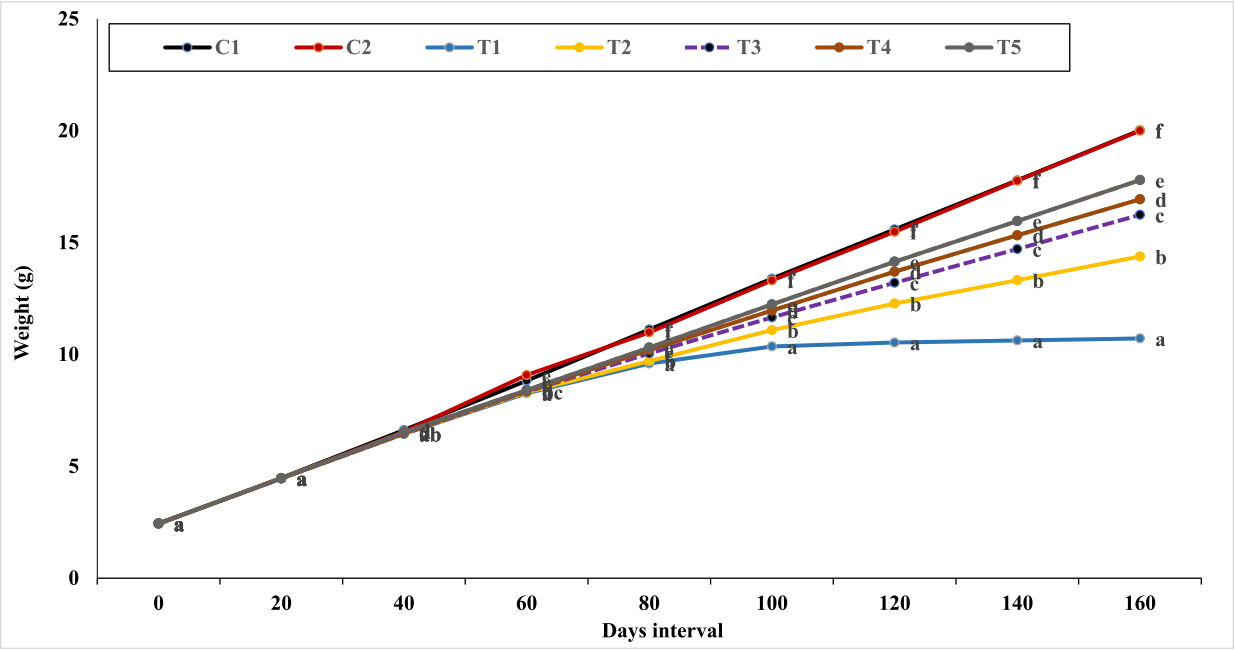

Fig. 1. Growth of rohu juveniles reared in different experimental units at 20 days of interval period. All values are expressed as Mean ± SE ($n = 3$) and the lines with different superscripts differ significantly ($p < 0.05$).

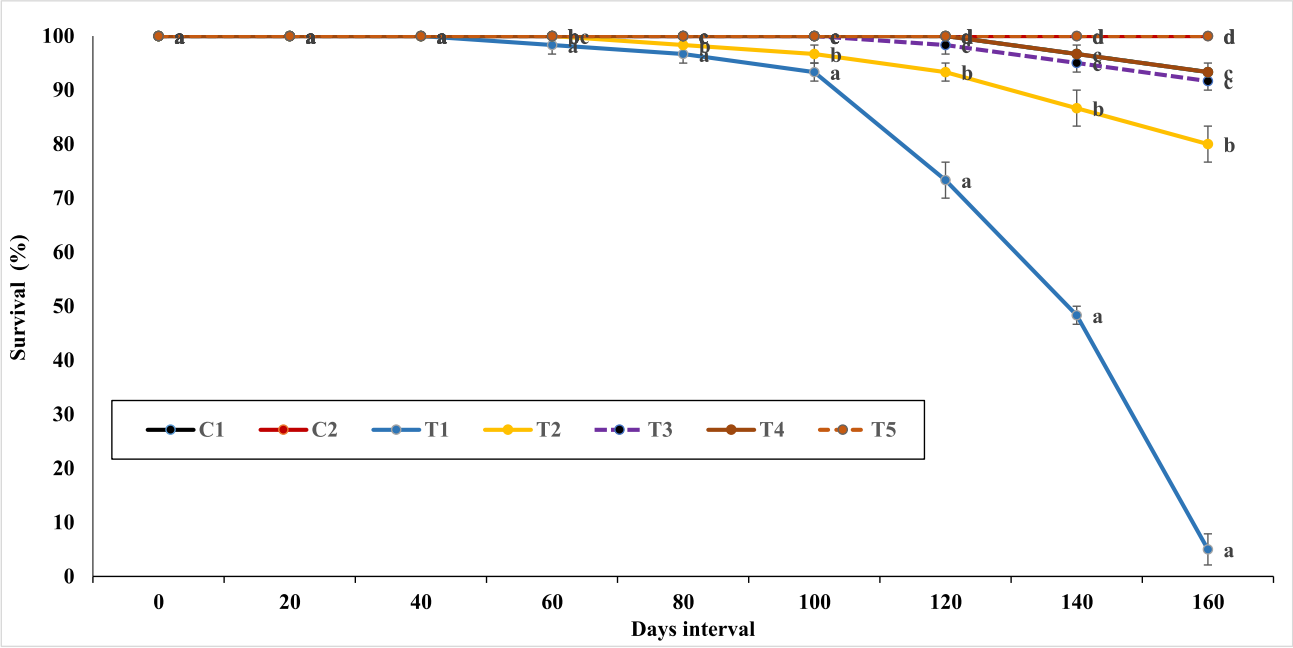

Fig. 2. Survival of rohu juveniles reared in different experimental units at 20 days of interval period. All values are expressed as Mean ± SE (n = 3) and the lines with different superscripts differ significantly ($p < 0.05$).

4.2. Growth parameters

Fish growth and health are major concerns in aquaculture and they are closely associated with the salinity of the rearing water (Tian et al., 2020). In the present investigation, the effect of temporal increment in salinity on the health status of juveniles of L. *rohita* was studied. Various analyses of different growth parameters such as weight gain, FCR, survival, SGR, FCE, *etc.* were performed. A similar study conducted by Nahar et al. (2016) in *Anabas testudineus* revealed complete mortality of animals in 21‰. In the present study, higher weight gain was observed in both the controls, but T5 displayed higher WG among the treatments. A similar pattern was noticed in SGR, FCE and survival. The earlier studies indicated a similar response when freshwater fish were reared at higher salinities (Fiúza et al., 2015; Gan et al., 2016; Islam et al., 2014). In a recent study conducted by Mozanzadeh et al. (2021) in Yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*), the growth performance was found to improve in the salinity range of 6–12‰; however, the growth and feed utilization declined after 24‰. Overton et al. (2008) reported reduced growth in a freshwater perch reared in 8‰ salinity and described the reason behind the lower growth as lower food consumption with a reduction in food conversion efficiency. The fish in our experiment showed a similar response with

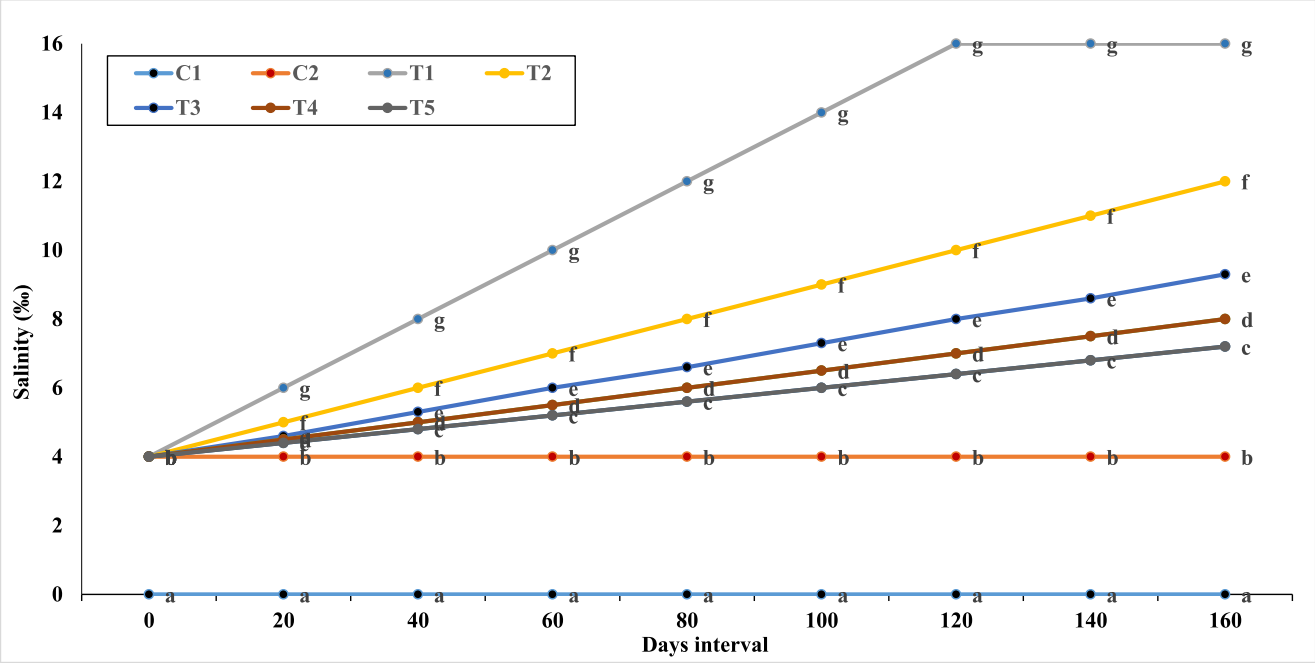

Fig. 3. Salinity increments in different experimental units at 20 days of interval period. All values are expressed as Mean ± SE (n = 3) and the lines with different superscripts differ significantly ($p < 0.05$).

reduced growth at a lower acclimation period that likely was associated with reduced food consumption at higher salinity, which might be due to depressed appetite and increased osmatic stress at higher salinity (Sahoo et al., 2003). In our previous studies, mortality of the L. *rohita* started at 6‰ (Patel et al., 2022), but the present study showed no mortality in T5 at 7.2‰, which might be because the fish adapted very well to this salinity due to the temporal increase in the salinity levels or prolonged acclimatization period (Kefford et al., 2004; Mubarik et al., 2019). Lower FCR indicates the appropriate condition of the rearing medium leading to the high performance of animals (Nahar et al., 2016). In the present study, low FCR was noticed in both the controls; and among the treatments, T5 showed the same. Higher FCR was seen in T1 treatment that may be resulted from alterations of the gut evacuation rate due to extra drinking water rate, low retention of nutrients and high excretion of metabolites at higher salinity with less adaptation period.

4.3. Metabolic enzymes, oxidative responses and Na^+/K^+-ATPase responses

The ALT and AST enzymes are used to assess the impairment of tissues such as the liver, kidneys, and gills. Moreover, the high concentration of these enzymes signifies that sugar is being digested to generate aspartate and alginate, which allows fish to adapt to stressful situations (Gowda et al., 2009; Hegazi et al., 2014). Our study revealed elevated liver enzymes (ALT and AST) in the groups of fish kept at high salinity (T1) indicating that the salinity-induced impairment of liver function and the same had been reported by other researchers (Dawood et al., 2021; Ghelichpour et al., 2020; Hoseini et al., 2019; Ma et al., 2021). Teleost fish are recognized for their ability to convert amino acids into glucose. As a result, the increased activity of AST and ALT suggested that aspartate and alanine are being mobilised for gluconeogenesis to produce glucose to deal with stress (Chatterjee et al., 2006). Fish reared in T5 showed no significant difference compared to both controls, and our results indicated normal liver functioning and fish showed good adaptation against rising salinity.

Antioxidant enzymes like CAT and SOD are essential for combating oxidative damage brought on by metabolic and environmental variables (Wendelaar Bonga, 1997), and they represent the mainstay of physiological antioxidant protection in fish's immune defence system (Martínez-Álvarez et al., 2005; Zhao et al., 2020). Aquatic species have a clear link between oxidative stress and immune suppression (Lu et al., 2014; Zhang et al., 2017). In our study, CAT and SOD activity increased significantly when the rearing salinity was increased. The increased antioxidant activity could be associated with higher vigorous metabolic activity (Islam et al., 2020). The excess H_2O_2 generation by peroxisomal metabolising enzymes and the generation of H_2O_2 at mitochondrial levels by SOD may be the cause of the increased oxidative stress enzyme activity (Kumar et al., 2019). No significant difference was observed between T5 and both the controls, indicating that animals are well adapted against increasing salinity in this treatment. Adaptive response of SOD and CAT activity in response to increasing salinity was observed by Gan et al. (2016) in tilapia, Sui et al. (2016) in blood parrotfish, Handayani et al. (2017) in *Oreochromis niloticus,* Islam et al. (2020) in *Dicentrarchus labrax,* Mozanzadeh et al. (2021) in *Acanthopagrus latus* and Moniruzzaman et al. (2022) in *Notopterus chitala*. In the present investigation, increasing SOD and CAT activity levels may have resulted from higher ROS generation along with higher salinity in all treatments except T5.

Na^+/K^+-ATPase (NKA) is a crucial membrane protein in aquatic organisms that drives ion control and mediates whole-body osmoregulation. Therefore, salinity has a major effect on Na^+/K^+-ATPase activity (Oruc, 2012: Wang et al., 2016). The gill's Na^+/K^+-ATPase is an ion pump that facilitates ion transport across the membrane and aids fish in maintaining their body's osmoregulation (Clausen et al., 2017). This NKA enzyme, which is found in branchial chloride cells, can produce a chemical gradient to remove excess internal and extracellular Na^+ and Cl^- in a hyperosmotic environment while absorbing Cl^- in a hypo-osmotic environment (Hirose et al., 2003; Wood, 2011). Our study revealed that fish from the treatment groups with high salinity exhibited considerably higher Na^+/K^+-ATPase levels than T5 and both controls as observed by other researchers (Hiroi and McCormick, 2007; Ruiz-Jarabo et al., 2022; Sangiao-Alvarellos et al., 2003). Similarly, Sangiao-Alvarellos et al. (2003) found gill, Na^+/K^+-ATPase activity shows a "U-shaped" salinity dependency, with higher values in low (5‰) and high (55‰) salinities with respect to SW (38‰) and BW (15‰) and study also observed that gill energy metabolism changes in *S. aurata* acclimated to

Table 3
Growth and survival of rohu juveniles reared in different experimental units at 20 days of interval period.

Treatments	0 days			20 days			40 days			60 days			80 days		
	Salinity (‰)	Survival (%)	Weight (g)	Salinity (‰)	Survival (%)	Weight (g)	Salinity (‰)	Survival (%)	Weight (g)	Salinity (‰)	Survival (%)	Weight (g)	Salinity (‰)	Survival (%)	Weight (g)
C1	0.0	$100.00^{a} \pm 0.00$	$2.44^{a} \pm 0.01$	0.0	$100.00^{a} \pm 0.00$	$4.48^{a} \pm 0.01$	0.0	$100.00^{a} \pm 0.00$	$6.62^{d} \pm 0.01$	0.0	$100.00^{b} \pm 0.00$	$8.83^{e} \pm 0.01$	0.0	$100.00^{c} \pm 0.00$	$11.12^{f} \pm 0.01$
C2	4.0	$100.00^{a} \pm 0.00$	$2.44^{a} \pm 0.01$	4.0	$100.00^{a} \pm 0.00$	$4.47^{a} \pm 0.01$	4.0	$100.00^{a} \pm 0.00$	$6.59^{d} \pm 0.02$	4.0	$100.00^{b} \pm 0.00$	$8.80^{e} \pm 0.01$	4.0	$100.00^{c} \pm 0.00$	$11.09^{f} \pm 0.01$
T1	4.0	$100.00^{a} \pm 0.00$	$2.44^{a} \pm 0.02$	6.0	$100.00^{a} \pm 0.00$	$4.47^{a} \pm 0.01$	8.0	$100.00^{a} \pm 0.00$	$6.44^{a} \pm 0.01$	10.0	$98.33^{a} \pm 1.66$	$8.26^{a} \pm 0.01$	12	$96.66^{a} \pm 1.67$	$9.59^{a} \pm 0.00$
T2	4.0	$100.00^{a} \pm 0.00$	$2.44^{a} \pm 0.01$	5.0	$100.00^{a} \pm 0.00$	$4.47^{a} \pm 0.01$	6.0	$100.00^{a} \pm 0.00$	$6.45^{ab} \pm 0.01$	7.0	$100.00^{b} \pm 0.00$	$8.30^{b} \pm 0.01$	8.0	$98.33^{b} \pm 1.67$	$9.70^{b} \pm 0.01$
T3	4.0	$100.00^{a} \pm 0.00$	$2.43^{a} \pm 0.01$	4.6	$100.00^{a} \pm 0.00$	$4.46^{a} \pm 0.01$	5.3	$100.00^{a} \pm 0.00$	$6.46^{ab} \pm 0.01$	6.0	$100.00^{b} \pm 0.00$	$8.33^{bc} \pm 0.01$	6.6	$100.00^{c} \pm 0.00$	$10.05^{c} \pm 0.01$
T4	4.0	$100.00^{a} \pm 0.00$	$2.44^{a} \pm 0.02$	4.5	$100.00^{a} \pm 0.00$	$4.46^{a} \pm 0.01$	5.0	$100.00^{a} \pm 0.00$	$6.48^{b} \pm 0.01$	5.5	$100.00^{b} \pm 0.00$	$8.36^{c} \pm 0.01$	6.0	$100.00^{c} \pm 0.00$	$10.20^{d} \pm 0.01$
T5	4.0	$100.00^{a} \pm 0.00$	$2.45^{a} \pm 0.01$	4.4	$100.00^{a} \pm 0.00$	$4.46^{a} \pm 0.01$	4.8	$100.00^{a} \pm 0.00$	$6.53^{c} \pm 0.01$	5.2	$100.00^{b} \pm 0.00$	$8.41^{d} \pm 0.01$	5.6	$100.00^{c} \pm 0.00$	$10.31^{e} \pm 0.02$

Treatments	100 days			120 days			140 days			160 days		
	Salinity (‰)	Survival (%)	Weight (g)	Salinity (‰)	Survival (%)	Weight (g)	Salinity (‰)	Survival (%)	Weight (g)	Salinity (‰)	Survival (%)	Weight (g)
C1	0.0	$100.00^{c} \pm 0.00$	$13.39^{f} \pm 0.01$	0.0	$100.00^{d} \pm 0.00$	$15.59^{f} \pm 0.01$	0.0	$100.00^{d} \pm 0.00$	$17.78^{f} \pm 0.01$	0.0	$100.00^{d} \pm 0.00$	$20.02^{f} \pm 0.02$
C2	4.0	$100.00^{c} \pm 0.00$	$13.31^{f} \pm 0.01$	4.0	$100.00^{d} \pm 0.00$	$15.55^{f} \pm 0.02$	4.0	$100.00^{d} \pm 0.00$	$17.76^{f} \pm 0.01$	4.0	$100.00^{d} \pm 0.00$	$20.00^{f} \pm 0.03$
T1	14.0	$93.33^{a} \pm 1.67$	$10.36^{a} \pm 0.01$	16.0	$73.33^{a} \pm 3.33$	$10.53^{a} \pm 0.01$	16.0	$48.33^{a} \pm 1.67$	$10.62^{a} \pm 0.01$	16.0	$5.00^{a} \pm 2.88$	$10.71^{a} \pm 0.01$
T2	9.0	$96.66^{b} \pm 1.67$	$11.08^{b} \pm 0.01$	10.0	$93.33^{b} \pm 1.67$	$12.27^{b} \pm 0.01$	11.0	$86.66^{b} \pm 3.33$	$13.32^{b} \pm 0.01$	12.0	$80.00^{b} \pm 3.33$	$14.38^{b} \pm 0.01$
T3	7.3	$100.00^{c} \pm 0.00$	$11.66^{c} \pm 0.01$	8.0	$98.33^{c} \pm 1.67$	$13.20^{c} \pm 0.01$	8.6	$95.00^{c} \pm 1.67$	$14.71^{c} \pm 0.01$	9.3	$91.66^{c} \pm 1.67$	$16.24^{c} \pm 0.03$
T4	6.5	$100.00^{c} \pm 0.00$	$11.96^{d} \pm 0.01$	7.0	$100.00^{d} \pm 0.00$	$13.70^{d} \pm 0.01$	7.5	$96.66^{c} \pm 1.67$	$15.33^{d} \pm 0.01$	8.0	$93.33^{c} \pm 1.67$	$16.93^{d} \pm 0.01$
T5	6.0	$100.00^{c} \pm 0.00$	$12.24^{e} \pm 0.01$	6.4	$100.00^{d} \pm 0.00$	$14.14^{e} \pm 0.01$	6.8	$100.00^{d} \pm 0.00$	$15.96^{e} \pm 0.01$	7.2	$100.00^{d} \pm 0.00$	$17.79^{e} \pm 0.01$

Note: All values are expressed as Mean ± SE (n = 3). Mean values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter.
C1 = 0.0‰; C2 = 4‰; T1 = 0.1‰ on daily basis; T2 = 0.1‰ for every 2 days; T3 = 0.1‰ for every 3 days; T4 = 0.1‰ for every 4 days; T5 = 0.1‰ for every 5 days.

Table 4
Metabolic and antioxidative enzyme activity in the liver and Na^+/K^+-ATPase activity in the gill of rohu juveniles reared in different experimental groups at the end of the 160 days of the experiment.

Treatments		ALT	AST	SOD	CAT	Na^+/K^+-ATPase
C1 (0.0‰)		$2.14^a \pm 0.11$	$1.40^a \pm 0.09$	$1.13^a \pm 0.12$	$0.50^a \pm 0.02$	$1.98^a \pm 0.32$
C2 (4‰)		$2.12^a \pm 0.13$	$1.39^a \pm 0.07$	$1.15^a \pm 0.11$	$0.52^a \pm 0.04$	$2.04^a \pm 0.33$
T1 (0.1‰ on daily basis)		$5.12^e \pm 0.09$	$3.98^e \pm 0.06$	$2.72^e \pm 0.07$	$2.50^e \pm 0.02$	$7.79^f \pm 0.16$
T2 (0.1‰ for every 2 days)		$4.78^d \pm 0.10$	$3.77^d \pm 0.02$	$2.66^d \pm 0.13$	$2.41^d \pm 0.03$	$5.71^e \pm 0.01$
T3 (0.1‰ for every 3 days)		$3.66^c \pm 0.14$	$3.06^c \pm 0.07$	$2.59^c \pm 0.11$	$1.68^c \pm 0.06$	$4.04^d \pm 0.09$
T4 (0.1‰ for every 4 days)		$3.32^b \pm 0.12$	$2.37^b \pm 0.03$	$2.11^b \pm 0.09$	$1.15^b \pm 0.10$	$3.03^c \pm 0.27$
T5 (0.1‰ for every 5 days)		$2.09^a \pm 0.10$	$1.34^a \pm 0.06$	$1.18^a \pm 0.10$	$0.55^a \pm 0.02$	$2.16^b \pm 0.31$
Contrast analysis						
P value	*Overall*	0.002	0.004	0.001	0.001	0.002
	Linear	0.001	0.002	0.001	0.001	0.001
	Quadratic	0.002	0.001	0.001	0.001	0.002

Note: All values are expressed as Mean ± SE (n = 3). Mean values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter.
Alanine aminotransferase activity (ALT) expressed as nanomoles of pyruvate formed/mg protein/min at 37 °C (equivalent to 1.67^{-11} katal mg/protein or, 1.00^{-03} U/mg protein).
Aspartate aminotransferase activity (AST) expressed as nanomoles of oxaloacetate formed/mg protein/min at 37 °C (equivalent to 1.67^{-11} katal mg/protein or, 1.00^{-03} U/mg protein).
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, 10^3 U/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/mg protein).
Na^+/K^+-ATPase as nanomoles Pi released/min/mg protein at 37 °C.

Table 5
Serum biochemical response of rohu juveniles reared at different experimental groups at the end of the 160 days of the experiment.

Treatment		AST (IU/L)	ALT (IU/L)	Glucose (mg/dL)	Cortisol (ng/mL)	Serum lysozyme activity (μg/mL)
C1 (0.0‰)		$20.98^a \pm 0.32$	$38.61^a \pm 0.27$	$22.93^a \pm 0.40$	$18.10^a \pm 0.42$	$17.16^e \pm 0.32$
C2 (4‰)		$20.96^a \pm 0.29$	$38.90^a \pm 0.40$	$22.92^a \pm 0.41$	$18.75^a \pm 0.39$	$16.85^e \pm 0.38$
T1 (0.1‰ on daily basis)		$34.16^e \pm 0.23$	$64.42^e \pm 0.12$	$48.76^d \pm 0.28$	$45.80^d \pm 0.31$	$7.88^a \pm 0.39$
T2 (0.1‰ for every 2 days)		$32.82^d \pm 0.37$	$58.61^d \pm 0.11$	$45.28^c \pm 0.05$	$42.91^c \pm 0.27$	$11.66^b \pm 0.61$
T3 (0.1‰ for every 3 days)		$32.12^c \pm 0.36$	$47.25^c \pm 0.10$	$37.11^b \pm 0.41$	$32.32^b \pm 0.20$	$13.08^c \pm 0.28$
T4 (0.1‰ for every 4 days)		$23.06^b \pm 0.34$	$45.42^b \pm 0.17$	$37.06^b \pm 0.42$	$31.61^b \pm 0.27$	$13.99^d \pm 0.42$
T5 (0.1‰ for every 5 days)		$20.91^a \pm 0.28$	$38.98^a \pm 0.40$	$22.93^a \pm 0.33$	$19.04^a \pm 0.42$	$16.78^e \pm 0.38$
Contrast analysis						
P value	*Overall*	0.003	0.004	0.001	0.002	0.003
	Linear	0.001	0.001	0.001	0.003	0.001
	Quadratic	0.004	0.001	0.001	0.002	0.001

Note: All values are expressed as Mean ± SE (n = 3). Mean values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter.

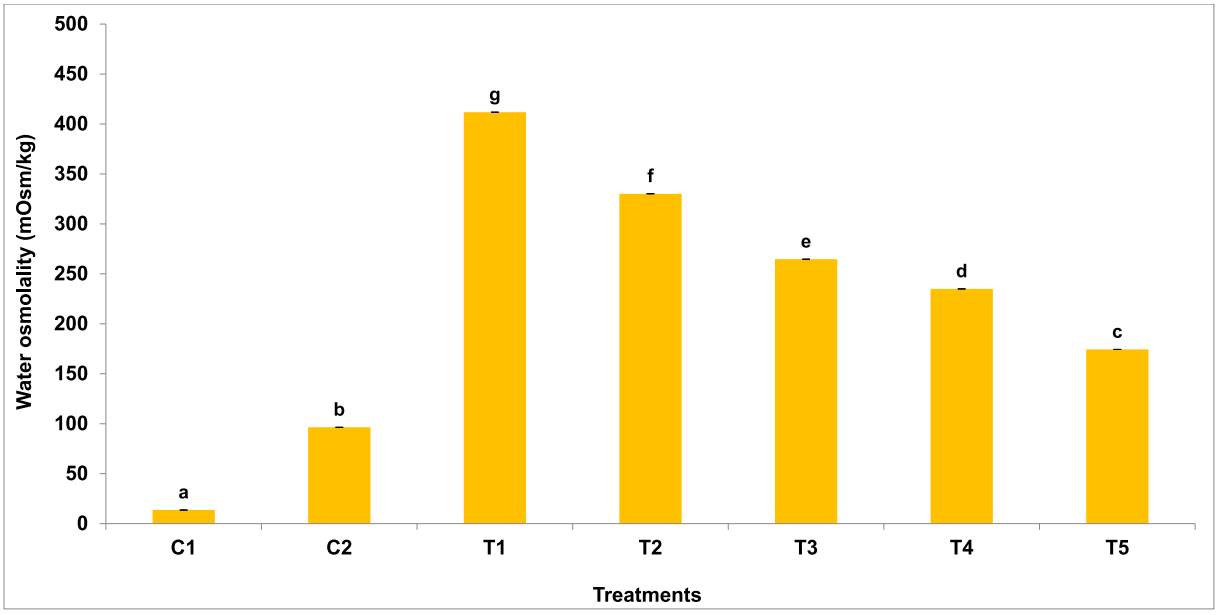

Fig. 4. Water osmolality in different treatments. All values are expressed as Mean ± SE (n = 3) and the bars with different superscripts differ significantly ($p < 0.05$).

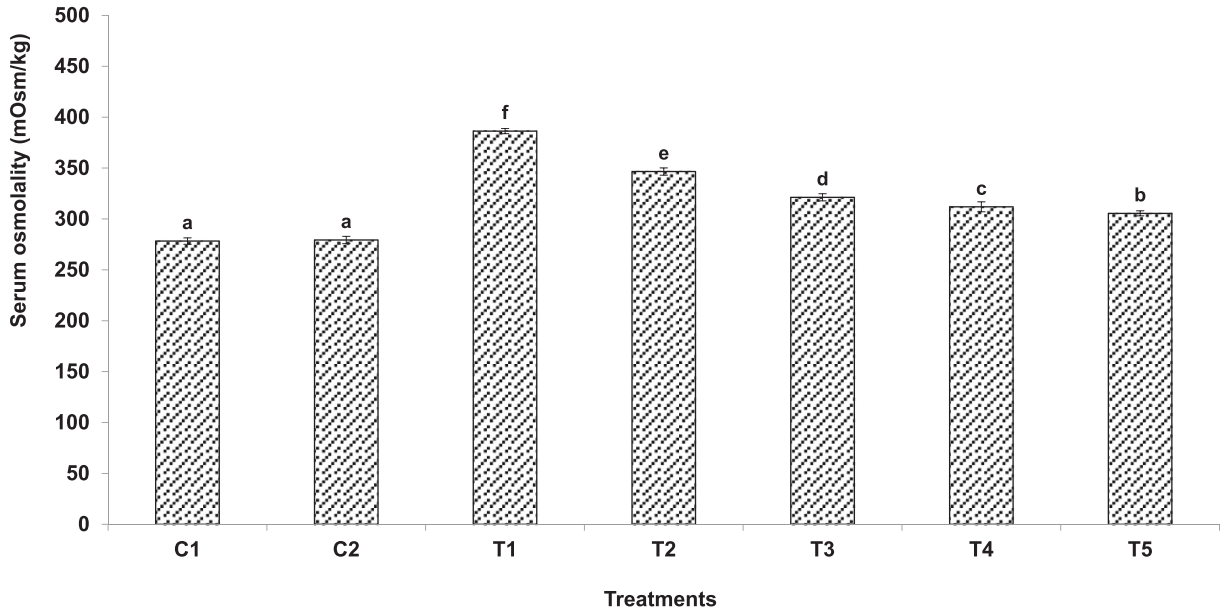

Fig. 5. Serum osmolality of L. *rohita* in different treatments. All values are expressed as Mean ± SE (n = 3) and the bars with different superscripts differ significantly ($p < 0.05$).

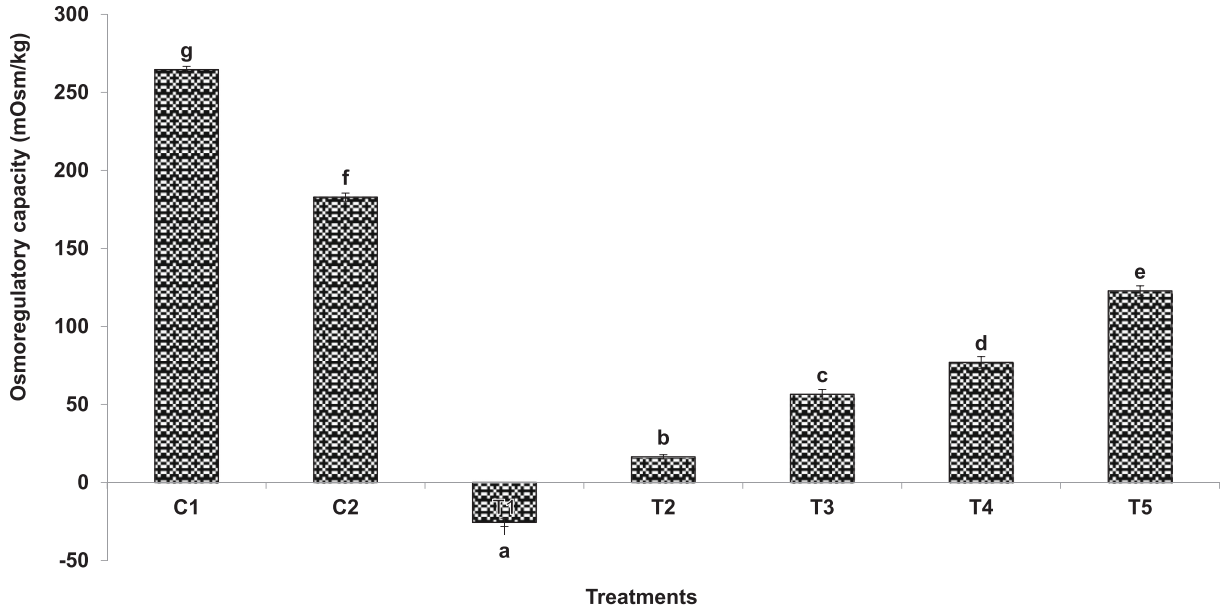

Fig. 6. Osmoregulatory capacity of L. *rohita* in different treatments. All values are expressed as Mean ± SE (n = 3) and the bars with different superscripts differ significantly ($p < 0.05$).

very low environmental salinity (5‰). However, Moniruzzaman et al. (2022) noticed a significant decline of Na^+/K^+-ATPase in the gill of *Notopterus chitala* exposed to different salinity levels compared to the controls.

4.4. Serum biochemical response

Increasing ALT and AST enzyme activity are symptomatic of hepatic tissue injury, necrosis, and degeneration in response to environmental stressors (Bruslé and Anadon, 1996). Our study revealed that fish from the treatment groups with high salinity exhibited considerably higher ALT and AST levels than T5 and both the controls. This finding further corroborates the study on chum salmon juveniles, which showed increased AST activity in groups with high salinity compared to low-saline groups (Liu et al., 2013). Luo et al. (2017) have reported that AST activity was increased with increasing salinity in genetically improved tilapia (*Oreochromis niloticus*) raised in biofloc systems with varying levels of salinity (0, 10, and 20). Increasing patterns of ALT and AST in *Oreochromis aureus* in response to an increase in salinity levels were reported by AlKatrani et al. (2018).

Determining the glucose levels in the blood is a valuable technique to assess the health status because blood glucose level is a suitable biomarker for studying the secondary stress responses on direct sympathetic and humoral pathways (Wedemeyer and Mcleay, 1981). Stress may cause fish to acutely increase their plasma levels of catecholamines like adrenaline and noradrenaline as well as corticosteroids and cortisol (Sumpter, 1997). The feedback elicited by the glucose mobilizing effects of both types of hormones is frequently an increase in the plasma level of

glucose in response to increases in catecholamine and corticosteroid levels (Barton and Iwama, 1991; Wendelaar Bonga, 1997). In the present investigation, the blood glucose level was maximum in T1, where the salinity was the highest. As a result, the fish showed limited adaption to this treatment, resulting in higher mortality. The blood glucose level remained unchanged in T4, T5 and both controls, indicating that the animals are well adapted. In our study, serum glucose levels were increased with increasing salinity levels among the treatments. Tsui et al. (2012) and Ahmmed et al. (2017) have also reported similar findings in *Epinephelus malabaricus* and *Heteropneustes fossilis* respectively.

In teleost fish, cortisol is the principal glucocorticoid released by the interrenal tissue (steroidogenic cells) in the head kidney (Iwama et al., 1999), and increased cortisol levels were observed when fish were exposed to an environmental stressor (Fiúza et al., 2015; Luz et al., 2008). According to Patel et al. (2022), increased salinity considerably changed fish's physiological status as a significant stressor, as observed by the elevated cortisol level in rohu. Similar findings on the response of cortisol to increasing salinity levels and acclimation period were reported by Fiúza et al. (2015) in *C. macropomum*, Mohamed et al. (2021) in *Oreochromis niloticus* and Moniruzzaman et al. (2022) in *Notopterus chitala*.

It has been reported that extreme stressors, such as hypersalinity and heat stress suppress lysozyme activity (Simide et al., 2016). Several studies have shown that hypersalinity stress has a deleterious influence on antioxidative and immunological responses in finfish species (Chen et al., 2021; Dawood et al., 2022). Our results also indicate suppressed serum lysozyme activity in rohu exposed to higher salinity with a low acclimatization period. However, when exposed to salinity (7.2‰) in T5 treatment with a higher acclimatization period, animals showed no impacts of salinity. These results corroborated the finding of Kim et al. (2017) where sable fish subjected to hypersalinity and temperature stresses displayed poor lysozyme activity. Similarly, common carp exposed to hypersalinity before heat stress showed declined lysozyme activity (Dawood et al., 2022).

4.5. Osmolality response

The level of osmolality in teleost fish blood was observed within the range of 280–360 mmol/kg, and it is strongly maintained in a species-dependent range of salinities (Varsamos et al., 2005). The present study indicates that rohu juveniles require a certain acclimation period to sustain the ion osmoregulatory system to adapt to environmental salinity changes. The occurrence of mortality in rohu was observed in treatment with less acclimatization period (T1). The absence of mortality observed in T5 indicated its strong ion-regulatory ability due to the longer acclimatization period provided to fish. It has been reported that the transfer of fish from freshwater to saline water resulted in a rise in the osmotic concentration of blood serum which led to a decline in the daily growth rate of the animals (Nahar et al., 2016). The present study indicated osmoregulatory dysfunction in treatments with higher and lower salinity levels as observed by other researchers (Anni et al., 2016; Galkanda-Arachchige et al., 2021; Mylonas et al., 2009; Saoud et al., 2007).

5. Conclusion

In our previous study, we found that transferring fish directly from lower salinity to different levels of higher salinities hampered the growth and other health status indices of the animals at salinities above 4‰. Complete mortality of the animals was observed at 14‰ during the experimental period of 120 days (Patel et al., 2022). The present study was conducted to determine whether the temporal increase in salinity with different acclimation periods affects the animals' growth parameters and physiological status. It was found that after the temporal increment in salinity, >60% survival was observed at 16‰ on the 120th day of the experiment. During the entire 160 days of the investigation, the salinity reached 16‰, where survival of 5% was noticed. The present study indicated that at a higher acclimatization period (T5), where the salinity was 7.2‰ after 160 days, improved growth with no physiological changes was observed compared to the other treatments. The present study demonstrated that the temporal increment in salinity with a higher acclimation period causes minimal impairment in growth and other physiological parameters of rohu. From the current study, it may also be concluded that rohu could be cultured in ISGW up to 7.2‰ (with temporal increment in salinity) in place of freshwater, thereby, enhancing the productivity in agriculturally barren lands, subsequently improving the livelihood of farmers.

Conflicts of interest statement

The authors whose names are listed below certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

CRediT author statement

Ravi Kumar Patel: Investigation, Methodology, Formal analysis, Writing-Original draft. **Ajit Kumar Verma:** Conceptualization, Supervision, Writing-review and editing. **Kishore Kumar Krishnani:** Methodology, Formal analysis. **Sreedharan Krishnan:** Methodology, Supervision, Formal analysis, Writing-review and editing. **Chandrakant Mallikarjun Hittinahalli:** Formal analysis, Writing-review and editing. **Angom Lenin Singh:** Methodology, Formal analysis. **Ramjanul Haque:** Methodology, Formal analysis, Writing-Original draft.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

No data was used for the research described in the article.

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