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ORIGINAL ARTICLE

Effect of salinity on growth, survival, haemato-biochemical and antioxidative status of *Anabas testudineus* (Bloch, 1792) juveniles reared in inland saline water

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Abstract

A 60-day feeding trial was conducted to evaluate the growth, survival, physio-metabolic and haemato-biochemical responses of *Anabas testudineus* reared in (ISW) of varying salinity. Fingerlings (initial weight 6.55 ± 0.08 g) were randomly stocked in triplicate in five treatment groups viz., T1 (0 ppt), T2 (3 ppt), T3 (6 ppt), T4 (9 ppt) and T5 (12 ppt) following a completely randomized design (CRD) with 40/m^2 stocking density. No mortality of fish was recorded in any of the treatments during the experimental period. Growth parameters such as weight gain (WG), percentage weight gain (PWG), specific growth rate (SGR) and protein efficiency ratio (PER) were highest ($p < 0.05$) at 3 ppt salinity followed by 6 and 0 ppt and lowest at 9 and 12 ppt, respectively. Lowest ($p < 0.05$) feed conversion ratio (FCR) and highest feed efficiency ratio (FER) were observed at 6 ppt salinity with no significant difference from fish at 0 and 3 ppt, respectively. Lowest ($p < 0.05$) moisture and highest protein and lipid contents were recorded at 3 ppt salinity. Digestive enzyme (protease and amylase) and oxidative stress enzyme (superoxide dismutase, SOD and catalase, CAT) activities were significantly ($p < 0.05$) higher in high saline groups (T4 and T5). Haemoglobin (Hb), haematocrit (Hct), erythrocytes and leukocytes were higher ($p < 0.05$) up to 6 ppt (T3) and reduced thereafter. Highest ($p < 0.05$) serum protein and globulin were recorded at 0 ppt salinity. T5 (12 ppt) group exhibited the highest ($p < 0.05$) serum glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity. The present study concluded that *A. testudineus* can be reared in ISW within an acceptable range of 0–12 ppt salinity. However, salinity above 6 ppt had a negative effect on growth, feed utilization and various physiological parameters related to the well-being of the fish. Therefore, it can be opined that *A. testudineus* can be safely considered as a potential cultivable species for inland saline aquaculture up to a salinity of 6 ppt.

KEYWORDS
Anabas testudineus, growth, inland saline water (ISW), nutrient utilization, salinity, stress

1 | INTRODUCTION

Aquaculture is the fastest growing (5.8% annual growth rate during the period of 2001–2016) (FAO, 2018) food production sector and estimated global production was 114.5 mmt in 2018 (FAO, 2020). Therefore, it contributes a significant share in global animal protein intake to alleviate malnutrition and hunger of the ever-growing population. It was projected that aquaculture will provide 62% of fish for human consumption by 2030 (Kobayashi et al., 2015). Aquaculture being the most diverse food production sector with highest number of cultivable species, the contribution of each species to overall production, also called evenness in aquaculture, is highly skewed and 90% of global aquaculture production is contributed by merely 30 aquatic fish species (FAO, 2020). Similarly, a significant share of Indian aquaculture production is constituted by a limited number of species such as major carps, monosex tilapia, pangasius and *Penaeus vannamei* (DADF, 2019). The two noteworthy factors that play a determining function in the success or failure of aquaculture enterprises are climate change and extreme climatic events (FAO, 2018). Diversification of species and the culture system in the new farming environment will provide the poor with livelihood options. They would also increase the resilience of farmers to external factors such as climatic, socio-economic impacts and emerging markets. Aquaculture sector must devise ways and means for its future expansion amid increasing conflict for resources in the freshwater and coastal areas. In this context, utilization of inland saline areas could be a viable option for the future growth of the industry.

Globally, more than 380 million hectares (ha) land area are salt affected and it is expected to increase by 50% by 2050 (Lambers, 2003) and about 8.62 million ha area is salt affected in India (Allan et al., 2009). Commercial culture of different fish species in these saline areas has been successfully demonstrated by several countries (Barman et al., 2005; Partridge et al., 2008). Although, the ionic composition of (ISW) is one of the major constraints for further expansion of aquaculture in comparison with seawater-based aquaculture (Davis et al., 2002; Ingram et al., 2002), it can nevertheless be harnessed to its potential to support rearing of commercially important fish, especially without amendment. ISW is characteristically rich in calcium (Ca^{+2}) ions and majorly deficient in magnesium (Mg^{+2}) and potassium (K^{+}) ions in comparison with seawater (Jain et al., 2006). Additionally, the high-water hardness index {(Ca+Mg): ($Na^{+}+K^{+}$)}, which interferes with the Ca^{+2} to Mg^{+2} ratio, and the uptake of ions by the cultured animals (Doroudi et al., 2006; Partridge et al., 2008), poses several challenges in usage of this water for sustainable culture of aquatic life forms. Besides, changing pattern of physico-chemical properties over space and time result in lack of productivity of the inland saline soil (spatio-temporal variations), thus making the associated water (ISW) distinctly different from seawater (Aklakur, 2017). Several fish and crustacean species such as amur carp (Singh et al., 2019), *Pangasianodon hypophthalmus* (Kumar et al., 2016), *Mugil cephalus* (Talukdar et al., 2020), *Chanos* (Raizada, Chadha, Javed, et al., 2005), *Trachinotus blochii* (Pathak et al., 2019), GIFT Tilapia (Paul, Sardar, Sahu, Deo, et al., 2022; Paul, Sardar, Sahu, Varghese, et al., 2022; Singha et al., 2020), *Macrobrachium rosenbergii* (Jain et al., 2007), *Penaeus monodon* (Antony et al., 2015; Purushothaman et al., 2014), *Penaeus vannamei* (Jahan et al., 2018; Jana et al., 2021; Talukdar et al., 2021), etc. have been reared for their suitability of culture using ground inland saline water. However, at commercial scale, the technology for the rearing of *P. vannamei* using amended ISW has only been adopted. *P. vannamei* farming, though demonstrated to be highly profitable, has inherent problems such as the requirement of large infrastructure and massive investment, making it difficult for the resource-poor farmers to adopt the technology. This has raised concerns for sustainability of such aquaculture practices for the inland saline regions. These concerns have catapulted research questions prompting research and investigation of an alternative fish species with similar consumer demand, paving the way for hardy air-breathing fish species such as *Anabas testudineus*.

Simultaneously, *A. testudineus* commonly known as koi or climbing perch is a tasty fish with high consumer demand (Nahar, 2015). The fish has shorter life cycle, faster growth rate, omnivorous feeding habit, highly nutritious food value and tolerance towards adverse environmental conditions making this species a good option for aquaculture diversification in inland water areas (Bhaskar et al., 2015). *Anabas* is reported to exhibit faster growth rate in mild saline water compared with freshwater (Widiyati et al., 2019). Several studies have also been reported that growth and survival of *A. testudineus* are not hampered in saline water up to 15 ppt salinity (Chotipuntu & Avakul, 2010; Dubey et al., 2015). Owing to its above potential qualities, the current research was planned and executed to expound the effect of graded levels of salinity on growth performance, nutrient utilization and physiological status of *A. testudineus* fingerlings reared in ISW.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The research undertaken complies with the current animal welfare laws in India, and the use of animals in this study followed the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment & Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research.

2.2 | Acclimatization of experimental fish

Seven hundred (700) numbers of fish (average body weight 6 ± 1.04 g) were collected from the wetlands of Assam, India. After an acclimation period of 2 days at a local hatchery in Assam, fish were carefully transported to ICAR-CIFE, Rohtak centre, Haryana under proper oxygenation. On reaching the laboratory, fish were given mild salt treatment and carefully transferred to aerated rectangular tanks (1000 L) containing freshwater (0 ppt), following which, fish were acclimatized in the same tanks for 15 days. ISW of 12–13 ppt salinity was obtained from borewell located in the high saline zone of Rohtak Farm. Pumped water was filtered with 100 μm filter bag (Raizada et al., 2015) and was stored in a cemented tank (10,000 L, 300 × 200 × 150 cm). After allowing

sedimentation of the collected water for a week, the said ISW (12–13 ppt) was diluted to four different test salinities (3, 6, 9 and 12 ppt) by using freshwater from a borewell located in the freshwater section of the farm. Freshwater (0 ppt) was used as control. Finally, fish were acclimatized in the desired salinity (3, 6, 9 and 12 ppt) of different experimental groups in the following way. Salinity was increased by 1 ppt at every 24 h interval until each experimental units attained the desired salinity. During the period of acclimatization, the fish were fed with commercial-grade fish feed containing 36% crude protein. The tanks were regularly siphoned at every 2 days interval with ISW of equivalent salinity. The stock of ISW (12–13 ppt) salinity from borewell was diluted with freshwater using standard formula to obtain the targeted water salinity.

2.3 | Experimental set up and feeding trial

A. testudineus fingerlings (average body weight 6.55 ± 0.08 g) were randomly distributed into triplicate tanks (500 L capacity, 15 fish/tank, stocking density 40/m^2) in five treatment groups viz., T1 (0 ppt), T2 (3 ppt), T3 (6 ppt), T4 (9 ppt) and T5 (12 ppt) following a completely randomized design (CRD). During the experimental period, the experimental fish were fed to the apparent satiation level twice daily at 9:00 am and 6:00 pm. The particle size of the commercial feed (Growel Feeds Pvt. Ltd.) was 2 mm and it was found to contain 33.89% crude protein, 4.64% crude lipid,10.08% ash and 6.2% crude fibre. To maintain the optimum water quality, tanks were siphoned every day to remove the faecal matter and 30% of water was exchanged at an interval of 3 days with fresh ISW of required salinity.

During the experiment, physico-chemical parameters such as dissolved oxygen, temperature, pH, alkalinity, hardness, ammonia, nitrite, different ions and salinity were recorded daily by following the standard methods (APHA, 2005). Na^+ and K^+ ion concentration was estimated by a flame photometer (Model 1382, ESICO, India).

2.4 | Proximate analysis

Diet and carcass tissues composition were analysed by standard methods (AOAC, 2005). Moisture was analysed by oven drying of sample at 102°C till was reached a constant weight. Nitrogen content of the diet and carcass tissues were analysed by automatic microkjeldahl unit (PELICAN, India) and finally crude protein (CP) was calculated [Crude protein (%) = N_2 (%) × 6.25]. Ether extraction (EE) was done by using Soxhlet unit (PELICAN, India). The total ash (TA) content was analysed by burning the samples in a muffle furnace (WIT; Australia) at 550°C for 6 h. Crude fibre (CF) content was analysed in a fibretec (Tulin, India). The total carbohydrate (TC) content was calculated by the formula:

TC% = 100 − (CP% + EE% + TA% + CF%)

2.5 | Growth and nutrient utilization

The following formulas were used for the calculation:

WG (g) = Body weight final (g) − Body weight initial (g)

PWG (%) = {Body weight final (g)−Body weight initial (g)}/Body weight initial (g) × 100

SGR (%/day) = {Log_e Body weight final (g) − Log_e Body weight initial (g)}/Days of trial × 100

FCR = Dry feed provided (g)/Live wet weight gain (g)

FER = Live wet weight gain (g)/Dry feed provided (g)

PER = Live wet weight gain (g)/Crude protein of fed (g)

Survival (%) = (Live animal harvested/ Animal stocked) × 100

HSI (%) = Liver weight (g)/Body weight (g) × 100

2.6 | Sampling, sample collection and processing

After completion of trial, overnight feed restriction followed by counting of live animal and biomass was carefully taken from each tank for calculation. Six fish from each replicate were anaesthetised with clove oil (50 μl/L) for sample collection (three fish for enzyme analysis and remaining three for whole-body proximate analysis). Fish were dissected and gill, intestine, muscle and liver samples were immediately taken out and homogenized with chilled sucrose solution (0.25 M) using tissue homogenizer (MICCRA D-9, Germany) to prepare 5% tissue homogenate. Tissue homogenates were centrifuged at 10,000 rpm for 15 min at 4°C in centrifuge (ThermoScientific) and collected supernatant were stored in vials at −40°C till further analysis (Talukdar et al., 2020).

2.7 | Tissue protein estimation

Tissue protein was estimated according to Lowry et al. (1951) and used to calculate enzyme activities.

2.8 | Digestive enzyme analysis

Protease and amylase activities were determined using the method of Drapeau (1974) and Rick and Stegbauer (1974), respectively. Lipase activity was determined according to Cherry and Crandall Jr (1932).

2.9 | Antioxidant enzymes

The catalase (CAT) and superoxide dismutase (SOD) activities were determined according to Takahara et al. (1960) and Misra and Fridovich (1972), respectively.

2.10 | Haemato-biochemical parameters

2.10.1 | Blood and serum collection

Three fish were collected from each replicate and anaesthetised with clove oil (50 μl/L) followed by puncturing of caudal vein by hypodermic syringe (without anticoagulant) to collect blood samples. The samples were kept in Eppendorf tubes and left undisturbed for

an hour for clotting and centrifugation was done at 5000 rpm for 8 min at 4°C to obtain serum and stored at −40°C. Some amount of blood was drawn following the same method and was kept in tubes with anticoagulant (2.7% EDTA coated) for haematological analysis (Paul, Sardar, Sahu, Deo, et al., 2022).

2.10.2 | Haematological assay

Counting of red blood cells (RBC) was done in a Neubauer's haemocytometer using the method described by Hendricks (1952). White blood cells (WBC) count was determined according to Shaw (1930). The haemoglobin (Hb) content was determined using the cyanmethemoglobin method (Van Kampen & Zijlstra, 1961). Haematocrit was determined using a haematocrit centrifuge at 1000 g for 10 min (Boon et al., 1990).

2.10.3 | Haemato-biochemical assay

Serum glucose, total protein and albumin were estimated using commercials kits from ERBA, India. Serum globulin and albumin to globulin ratio (A/G) were determined by the formulae:

Globulin (g/dl) = Total protein (g/dl)−Total albumin (g/dl)

A/G = Total albumin (g/dl)/Total globulin (g/dl)

2.10.4 | Protein metabolic enzymes

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using colorimetric assay kit procured from SIGMA-ALDRICH, USA (MAK055 and MAK052).

2.11 | Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test was used to evaluate the significant differences between treatment means at a 5% probability level ($p < 0.05$).

3 | RESULT

3.1 | Physico-chemical parameters ISW

Different physico-chemical parameters of experimental ISW such as temperature, dissolved oxygen, total alkalinity, pH, salinity, hardness, ammonia-N, nitrite-N, calcium, magnesium, sodium and potassium ions were found in the range of 28.5–30.0°C, 5.5–6.4 mg/L, 126.0–213.5 mg/L, 7.39–7.84, 0.03–12.38 ppt, 120–3260 mg/L, 0.52–1.52 mg/L, 0.10–0.65 mg/L, 39.75–299.00 mg/L, 8–556 mg/L, 19.70–3540.00 mg/L and 3.72–16.73 mg/L, respectively (Table 1).

3.2 | Growth, nutrient utilization and survival

In the present study, the effect of varying salinity levels on the growth, survival and nutrient utilization of *A. testudineus* fingerlings have been presented in Table 2. Considering the mean of weight gain (WG), percentage weight gain (PWG), specific growth rate (SGR) and protein efficiency ratio (PER) of fish, significant differences were found in all the treatments at the end of 60 days. Whereas, no significant differences were observed in T1 (0 ppt), T2 (3 ppt) and T3 (6 ppt). WG was recorded higher ($p < 0.05$) in T2 (156.79 ± 5.02) followed by T3 and T1, respectively. The lowest WG (109.09 ± 1.38) was observed in T5 and WG exhibited decreasing trend in T4 (115.39 ± 6.27) followed by T1 (144.09 ± 12.17) < T3 (149.47 ± 11.24) < T2 (156.79 ± 5.02). Similar trends were also observed for the PWG, SGR and PER of the fish. Lowest values of FCR and highest FER were found in T3 group, followed by T2 and T1, respectively. Moreover, FCR was found to be directly proportional with salinity (as recorded in T5 and T4 groups). No mortality was observed in all treatments throughout the experimental period.

3.3 | Body indices and carcass composition

The proximate composition of experimented fish differed significantly ($p < 0.05$) among the treatments (Table 3). The T3 and T5 groups showed a higher ($p < 0.05$) moisture content than the T1 and T2 groups, without showing any difference ($p > 0.05$) with the T4 groups. The crude protein (CP) content of the T1 and T2 groups were significantly higher ($p < 0.05$) than in the T3 group and were comparable ($p > 0.05$) with the T4 and T5 groups. The whole-body lipid (EE) content was significantly ($p < 0.05$) lower in the T1, T4 and T5 groups than in all other groups. A higher ($p < 0.05$) total ash (TA) content was recorded in the T4 and T5 groups in comparison with the T2 and T3 groups. While the T3 group showed a significantly lower value than all other treatment groups. Further, among the treatment groups, highest total carbohydrate (TC) content was recorded in freshwater reared fish (T1). On the other hand, hepato-somatic index (HSI) values of *A. testudineus* did not differ significantly ($p > 0.05$) among the groups.

3.4 | Digestive enzyme assay

The intestinal lipase activity did not vary significantly ($p > 0.05$) among the groups but amylase and protease activities increased significantly with the increasing levels of salinity (Table 4). Higher ($p < 0.05$) amylase and protease activity were recorded in higher salinity group (T5 and T4) in comparison with low salinity groups (T1, T2 and T3).

3.5 | Oxidative stress enzymes

The SOD and CAT activities increased significantly in the higher salinity groups (Table 5). The fish of T1, T2 and T3 groups exhibited

TABLE 1 Physico-chemical parameters of experimental ISW of different salinity for 60 days

Parameters	[a]Treatments				
	T1	T2	T3	T4	T5
Temperature (°C)	28.53–29.87	28.50–29.93	28.60–29.73	28.63–29.97	28.67–30.00
[b]DO (mg l^{-1})	5.85–6.30	5.50–6.05	5.75–6.15	6.15–6.40	5.75–6.05
pH	7.61–7.84	7.39–7.55	7.57–7.60	7.57–7.59	7.52–7.72
Salinity (ppt)	0.03–0.13	2.91–3.12	5.98–6.35	9.12–9.56	11.56–12.38
Hardness (mg l^{-1})	120–140	950–1010	1760–1880	2230–2300	3080–3260
Total alkalinity (mg $CaCO_3$ l^{-1})	126.00–128.25	144.00–146.25	161.50–165.75	177.75–183.75	211.25–213.50
[c]TA-N (mg l^{-1})	0.50–0.67	0.83–0.89	0.98–1.20	1.25–1.27	1.20–1.52
[d]NO_2^--N (mg l^{-1})	0.10–0.55	0.28–0.43	0.47–0.65	0.44–0.49	0.47–0.59
[e]Ca^{2+} (mg l^{-1})	39.75–51.00	98.00–100.50	157.50–164.00	216.00–220.50	281.50–299.00
[f]Mg^{2+} (mg l^{-1})	8–12	155–170	298–330	391–405	510–556
[g]Na^+ (mg l^{-1})	19.70–26	800–860	1580–1670	2740–2800	3480–3540
[h]K^+ (mg l^{-1})	3.72–4.17	6.33–6.44	10.61–10.77	12.65–12.85	15.71–16.73

[a]Treatment groups having graded levels of salinity i.e., T1, 0 ppt; T2, 3 ppt; T3, 6 ppt; T4, 9 ppt; T5, 12 ppt ISW.
[b]DO, Dissolved oxygen;
[c]TA-N, Total ammonia nitrogen;
[d]NO_2-N, Nitrite nitrogen;
[e]Ca^{2+}, Calcium ion;
[f]Mg^{2+}, Magnesium ion;
[g]Na^+, Sodium ion;
[h]K^+, potassium ion.

TABLE 2 Growth performance, nutrient utilization and survival of *Anabas testudineus* juveniles reared in ISW of different salinity for 60 days

Parameters	Treatments[a]					
	T1	T2	T3	T4	T5	*p*-value
IBW[b] (g)	6.21 ± 0.07	6.02 ± 0.02	6.05 ± 0.02	6.18 ± 0.10	6.22 ± 0.08	0.166
FBW[c] (g)	15.81 ± 0.72[b]	16.48 ± 0.83[b]	16.01 ± 0.94[b]	13.87 ± 0.68[a]	13.50 ± 0.73[a]	0.12
WG[d] (g)	144.09 ± 12.17[b]	156.79 ± 5.02[b]	149.47 ± 11.24[b]	115.39 ± 6.27[a]	109.09 ± 1.38[a]	0.003
PWG[e] (%)	154.55 ± 11.64[b]	173.54 ± 4.90[b]	164.83 ± 12.72[b]	124.64 ± 7.57[a]	116.88 ± 0.50[a]	0.003
SGR[f] (%/day)	1.55 ± 0.08[b]	1.68 ± 0.03[b]	1.62 ± 0.08[b]	1.35 ± 0.06[a]	1.29 ± 0.06[a]	0.003
FCR[g]	1.63 ± 0.07[a]	1.58 ± 0.03[a]	1.51 ± 0.02[a]	1.81 ± 0.04[b]	1.98 ± 0.06[c]	0.001
FER[h]	0.62 ± 0.03[b]	0.63 ± 0.01[b]	0.66 ± 0.01[b]	0.55 ± 0.01[a]	0.50 ± 0.02[a]	0.001
PER[i]	4.25 ± 0.36[b]	4.63 ± 0.15[b]	4.41 ± 0.33[b]	3.40 ± 0.18[a]	3.22 ± 0.04[a]	0.003
Survival (%)	100	100	100	100	100	-

Note: Values are expressed as Mean ± SE (n = 3); Mean values in each row with different superscripts differ significantly ($p < 0.05$).
[a]Treatment groups having graded levels of salinity, i.e. T1, 0 ppt; T2, 3 ppt; T3, 6 ppt; T4, 9 ppt; T5, 12 ppt ISW.
[b]IBW, Initial body weight;
[c]FBW, Final body weight;
[d]WG, Weight gain;
[e]PWG, Percentage weight gain;
[f]SGR, Specific growth rate;
[g]FCR, Feed conversion ratio;
[h]FER, Feed efficiency ratio;
[i]PER, Protein efficiency ratio.

TABLE 3 Whole body proximate composition (on % wet weight basis) and HSI of *Anabas testudineus* juveniles reared in ISW of different salinity for 60 days

	Proximate composition					
Treatments[a]	Moisture (%)	CP[b] (%)	EE[c] (%)	TA[d] (%)	TC[e] (%)	HSI[f] (%)
T1	75.41 ± 0.61[a,b]	13.32 ± 0.36[b]	4.66 ± 0.06[a]	3.88 ± 0.09[b,c]	2.73 ± 0.13[b]	2.31 ± 0.06
T2	75.21 ± 0.54[a]	13.17 ± 0.29[b]	6.20 ± 0.17[c]	3.58 ± 0.08[b]	1.84 ± 0.10[a]	2.35 ± 0.04
T3	78.57 ± 0.40[c]	11.29 ± 0.33[a]	5.49 ± 0.08[b]	2.95 ± 0.05[a]	1.70 ± 0.07[a]	2.25 ± 0.05
T4	77.48 ± 0.41[c]	12.30 ± 0.22[a,b]	4.51 ± 0.15[a]	4.17 ± 0.10[c]	1.55 ± 0.11[a]	2.24 ± 0.04
T5	77.67 ± 0.06[c]	12.46 ± 0.06[a,b]	4.44 ± 0.07[a]	4.13 ± 0.02[c]	1.55 ± 0.09[a]	2.22 ± 0.05
p-value	0.001	0.001	0.001	0.001	0.001	0.865

Note: Values are expressed as Mean ± SE (n = 3); Mean values in each column with different superscripts differ significantly ($p < 0.05$).

[a]Treatment groups having graded levels of salinity i.e., T1, 0 ppt; T2, 3 ppt; T3, 6 ppt; T4, 9 ppt; T5, 12 ppt ISW.

[b]CP, Crude protein;

[c]EE, Ether extract;

[d]TA, Total ash;

[e]TC, Total carbohydrate;

[f]HSI, Hepato-somatic index.

TABLE 4 Digestive enzyme activities of *Anabas testudineus* juveniles reared in ISW of different salinity for 60 days

	Digestive enzymes		
Treatments[a]	Amylase[b]	Protease[c]	Lipase[d]
T1	1.53 ± 0.01[a]	1.53 ± 0.07[a]	0.23 ± 0.003
T2	1.54 ± 0.01[a]	1.53 ± 0.02[a]	0.23 ± 0.003
T3	1.54 ± 0.17[a]	1.54 ± 0.05[a]	0.23 ± 0.003
T4	2.07 ± 0.05[b]	1.89 ± 0.08[b]	0.23 ± 0.003
T5	2.09 ± 0.09[b]	1.87 ± 0.03[b]	0.24 ± 0.003
p-value	0.003	0.001	0.858

Note: Values are expressed as Mean ± SE ($n = 3$); Mean values in each column with different superscripts differ significantly ($p < 0.05$).

[a]Treatment groups having graded levels of salinity i.e., T1, 0 ppt; T2, 3 ppt; T3, 6 ppt; T4, 9 ppt; T5, 12 ppt ISW.

[b]Amylase activity is expressed as the micromole of maltose released/min/mg protein.

[c]Protease activity is expressed as millimole of tyrosine released/min/mg protein.

[d]Lipase activity is expressed as units/min/mg protein.

lower ($p < 0.05$) SOD and CAT activities as compared with other treatment groups (T4 and T5) of higher salinity.

3.6 | Haematological parameters

The Hb, Hct, RBC and WBC contents of fish showed a significantly ($p < 0.05$) decreasing trend with the increasing salinity levels (Table 6). The higher ($p < 0.05$) Hb content was found in the T1, T2, and T3 groups than in the T4 and T5 groups. A similar trend was also observed for the values of Hct. Although, the Hct values of T2 were significantly higher than in all other groups except T3. The RBC count of T2 was significantly ($p < 0.05$) higher than in all other groups except T1. Inversely, the T5 group showed lower count than the rest of the groups except T4 group. The highest ($p < 0.05$) WBC counts were recorded in lower salinity groups (T1, T2 and T3), and conversely, the lowest recorded in higher salinity groups (T4 and T5).

3.7 | Haemato-biochemical indices

Serum total protein and globulin contents were significantly ($p < 0.05$) affected due to increasing levels of salinity (Table 7). However, total albumin and A/G found non-significant ($p > 0.05$) among the treatments. Higher ($p < 0.05$) serum total protein content was found in the T1, T2 and T3 groups than in the T5 group, while T4 group did not differ significantly with T3 and T5 groups but showed a lower value than the T1 and T2 groups. A similar trend was also observed for the values of globulin. However, the globulin content of the T3 group was significantly lower than T1 and T2 groups, and higher than T4 and T5 groups. The serum glucose values of the T2, T3 and T4 groups did not show significant differences with T1 and T5 groups. Again, the value of the T1 group was significantly lower than the T5 group. The serum AST and ALT levels increased significantly with the increasing levels of salinity, showing a direct correlation of these with the latter. Among the treatment groups, significantly highest and lowest values of ALT were recorded in T5 and T1, respectively. The AST values of the T4 and T5 groups were significantly higher than in all other groups.

4 | DISCUSSION

4.1 | Water quality parameters

Physio-chemical parameters play a dynamic role in regulating physiological homeostasis in animals (Singha et al., 2020). Among the different physico-chemical parameters of water, temperature

TABLE 5 Oxidative stress enzymes of *Anabas testudineus* juveniles reared in ISW of different salinity for the period of 60 days

Treatments[a]	Oxidative stress enzymes			
	SOD[b]		CAT[c]	
	Liver	Gill	Liver	Gill
T1	4.02 ± 0.11[a]	4.69 ± 0.04[a]	7.53 ± 0.12[a]	1.53 ± 0.08[a]
T2	4.03 ± 0.10[a]	4.73 ± 0.13[a]	7.56 ± 0.01[a]	1.54 ± 0.05[a]
T3	4.07 ± 0.09[a]	4.77 ± 0.01[a]	7.59 ± 0.02[a]	1.55 ± 0.06[a]
T4	4.86 ± 0.11[b]	5.49 ± 0.17[b]	8.05 ± 0.01[b]	2.11 ± 0.11[b]
T5	4.90 ± 0.10[b]	5.53 ± 0.14[b]	8.15 ± 0.04[b]	2.16 ± 0.06[b]
p-value	0.001	0.001	0.001	0.001

Note: Values are expressed as Mean ± SE ($n = 3$); Mean values in each column with different superscripts differ significantly ($p < 0.05$).

[a]Treatment groups having graded levels of salinity i.e., T1, 0 ppt; T2, 3 ppt; T3, 6 ppt; T4, 9 ppt; T5, 12 ppt ISW.

[b]SOD, Superoxide dismutase activity is expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min.

[c]CAT, Catalase activity is expressed as nanomoles H_2O_2 decomposed/min/mg protein.

TABLE 6 Haematological indices of *Anabas testudineus* juveniles reared in ISW of different salinity for 60 days

Treatments[a]	Parameters			
	Hb[b] (g/dl)	Hct[c] (%)	RBC count[d] ($\times 10^6$/mm[c])	WBC count[e] ($\times 10^c$/mm[c])
T1	10.37 ± 0.20[b]	32.43 ± 1.36[b]	3.16 ± 0.10[c,d]	146.43 ± 4.56[b]
T2	11.00 ± 0.31[b]	36.53 ± 0.68[c]	3.36 ± 0.11[d]	151.03 ± 3.32[b]
T3	10.40 ± 0.15[b]	33.33 ± 1.10[b,c]	2.92 ± 0.07[b,c]	148.47 ± 0.72[b]
T4	8.60 ± 0.17[a]	27.60 ± 0.15[a]	2.64 ± 0.06[a,b]	126.20 ± 2.12[a]
T5	8.27 ± 0.09[a]	27.13 ± 1.01[a]	2.52 ± 0.03[a]	119.27 ± 4.44[a]
p-value	0.001	0.001	0.001	0.001

Note: Values are expressed as Mean ± SE ($n = 3$); Mean values in each row with different superscripts differ significantly ($p < 0.05$).

[a]Treatment groups having graded levels of salinity i.e., T1, 0 ppt; T2, 3 ppt; T3, 6 ppt; T4, 9 ppt; T5, 12 ppt ISW.

[b]Hb, Haemoglobin;

[c]Hct, Haematocrit;

[d]RBC, Red blood cell;

[e]WBC, White blood cell.

TABLE 7 Haemato-biochemical parameters of *Anabas testudineus* juveniles reared in ISW of different salinity for 60 days

Treatments[a]	Parameters					
	T1	T2	T3	T4	T5	*p*-value
Serum total protein (g/dl)	3.66 ± 0.05[c]	3.71 ± 0.06[c]	3.52 ± 0.05[b,c]	3.38 ± 0.05[a,b]	3.22 ± 0.04[a]	0.001
Albumin(g/dl)	0.79 ± 0.04	0.83 ± 0.03	0.82 ± 0.05	0.86 ± 0.06	0.80 ± 0.04	0.562
Globulin(g/dl)	2.87 ± 0.03[d]	2.88 ± 0.04[d]	2.70 ± 0.02[c]	2.52 ± 0.04[a,b]	2.42 ± 0.05[a]	0.002
A/G[b]	0.28 ± 0.03	0.29 ± 0.02	0.30 ± 0.03	0.34 ± 0.03	0.33 ± 0.02	0.634
Serum glucose (mg/dl)	88.35 ± 2.39[b]	87.63 ± 2.65[a,b]	89.37 ± 1.63[a,b]	84.12 ± 1.24[a,b]	80.00 ± 0.83[a]	0.001
ALT[c]	70.96 ± 0.83[a]	78.66 ± 0.33[b]	84.21 ± 0.55[c]	92.78 ± 2.19[d]	100.31 ± 0.59[e]	0.001
AST[d]	28.54 ± 0.80[a]	30.57 ± 1.41[a]	30.93 ± 0.96[a]	35.68 ± 0.28[b]	38.22 ± 0.46[b]	0.001

Note: Values are expressed as Mean ± SE ($n = 3$); Mean values in each row with different superscripts differ significantly ($p < 0.05$).

[a]Treatment groups having graded levels of salinity i.e., T1, 0 ppt; T2, 3 ppt; T3, 6 ppt; T4, 9 ppt; T5, 12 ppt ISW.

[b]A/G, Albumin to globulin ratio.

[c]ALT Alanine aminotransferase activity is expressed as nmol/min/ml.

[d]AST, Aspartate aminotransferase activity is expressed as nmol/min/ml.

plays a vital role (optimum 26–30°C) in maintaining the metabolic activity in poikilothermic fish (Boyd, 1990; Widiyati et al., 2019). During trial, temperature was maintained within the optimal range of 28.5–30.0°C. The optimum range of pH for koi culture ranges from 6 to 9 (Widiyati et al., 2019) and pH value during the trial ranged from 7.39 to 7.84. Total alkalinity (mg $CaCO_3$/l) value in the present experiment ranged from 126.0 to 213.50, with an increasing trend with rising salinity. This might be due to the rising concentration of bicarbonate anions in the water with an increase in salinity of the water. Santhosh and Singh (2007) reported that the ideal value of total alkalinity for fish culture is in the range of 50–300 mg/L. Total hardness is the concentration of divalent cations in the form of calcium and magnesium which are the most dominant cations in natural waters (Boyd et al., 2016). Calcium is probably the most important component of hardness due to its ability to reduce water flux across the gills. A reduced water flux lowers the amount of energy required to maintain proper ionic balance during stress (Seals et al., 1994). Many fish species can adapt to a wide range of hardness, particularly if it is changed gradually (Adey & Loveland, 2007). However, morbidity and mortality occur when animals experience sudden changes from hard to soft water. In the present study, the total hardness of the water varied between 178.75 mg/L and 3093.75 mg/L. The higher hardness values of the T3, T4, and T5 groups did not affect the survival of fish. These may be due to the stress mitigating role of the higher calcium levels of these groups. Again, experimental fish were acclimatized gradually towards the higher hardness and salinity level. This might mitigate the ill effects of higher hardness on survival as evident by the previous studies in tilapia (Singha et al., 2020), *Pangasianodon hypophthalmus* (Kumar et al., 2016) and koi carp (Sharma et al., 2017) reared in ISW. Furthermore, Buentello and Gatlin III (2002) reported that Channel catfish at a higher level of water hardness accumulates taurine and other amino acids in the muscle-free pool as a strategy for elevating osmolality in hard water without raising the ionic concentration of the body fluids. TAN was found to range 0.52–1.52 mg/L. *A. testudineus* can excrete ammonia very actively; hence it can tolerate high ammonia levels in water (Tay et al., 2006). It has been reported earlier that after immediate salinity stress, *A. testudineus* tends to accumulate amino acids to regulate cell volume, thus reducing the ammonia excretion. However, after acclimation to high salinity, amino acid concentrations in the body tissues returned to normal baseline concomitant with higher excretion of ammonia (Chang et al., 2007). Stone and Thomforde (2004) depicted the desirable limit of nitrite is 0–1 mg/L for fish culture. Nitrite value falls within the desirable range. The water ions viz., Ca^{2+} (39.75–299.00 mg/L), Mg^{2+} (8–556 mg/L), Na^{+} (19.70–3540 mg/L) and K^{+} (3.72–16.73 mg/L) concentrations were recorded within a range depicted as characteristic of ISW as described earlier by various authors (Rahman et al., 2005; Raizada, Chadha, Ali, et al., 2005; Raizada, Chadha, Javed, et al., 2005 Jain et al., 2007; Reddy & Harikrishna, 2014; Raizada et al., 2015).

4.2 | Growth, nutrient utilization and survival

Salinity had no pronounced effect on the final body weight of the fish, indicating fair ability of *A. testudineus* to tolerate salinity up to 12 ppt. Earlier some studies reported *A. testudineus* to be naturally present in brackish water, hence this species might have developed evolutionary adaptation to deal with mild saline conditions (Jayaram, 1981). However, our results are in contrast with the findings of Nahar (2015), who demonstrated a positive effect of salinity on the growth of *A. testudineus* in normal seawater. The differences in growth may be attributed to differences in the salinity acclimation regime as well as the size of the fish selected for experiment. WG, PWG and SGR were observed maximum in T2 followed by T3 and T1 groups. In freshwater, fish tends to compensate loss of salts by spending some energy during passive diffusion, therefore exposing fish to low salinity can decrease the expenditure of energy to promote growth (Kelly & Woo, 1999). Altinokand and Grizzle (2001) also concluded improved growth performance and feed conversion for Channel catfish and goldfish (freshwater stenohaline fish) when reared in low saline water. Dubey et al. (2015) reported that *A. testudineus* shows the highest SGR, WG and PWG at 5 ppt salinity and different indices of growth were not significantly affected in normal saline water condition. Imanpoor et al. (2012) also found that salinity up to 6 ppt did not affect biometric indices of goldfish, that is, SGR, WG, and FCR. The highest FCE and lowest FCR values were recorded at T3 group (6 ppt), which was found similar with T1 and T2 groups. Otto (1971) reported that *Oncorhynchus kisutch* growth rate, feed intake, and feed conversion efficiency were higher at the salinity of 5–10 ppt. Higher FCR in high saline groups (T4 and T5) may be due to increased energetic cost for osmoregulation following salinity exposure, inducing fish to consume more feed to derive energy for maintaining other physiological functions (Rahman, 2020). This is in consonance with the report of Nahar (2015) for *A. testudineus*. Stickney (1979) stated that the survival rate of fish against salinity stress entirely depends on the quick adaptive ability of fish body fluid ionic concentration with the surrounding environment. During stressed condition fish try to tolerate the changes of plasma osmolality and body fluid ionic concentrations with respect to the surrounding environment and mitigate the stress. In the present study, 100% survival was observed up to 12 ppt (T5), which is supported by the findings of Bersa (1997) who reported that *A. testudineus* fingerlings (6–10 g) could withstand 2.5–10 ppt saltwater without mortality. Zahari et al. (2018) reported that *A. testudineus* could tolerate the salinity up to 15 ppt. Chang et al. (2007) have also reported the ability of *A. testudineus* to tolerate saline water of 30 ppt in a short-term study of 6 days.

4.3 | Whole-body carcass composition

Environmental, nutritional and biological factors can directly influence the carcass composition of fish (Tao et al., 2012). Several

ecological factors like salinity, temperature and pH directly or indirectly affect the whole-body carcass composition of many fish. In our study, moisture content increase with increasing salinity levels which are in agreement with previous studies (Fallah et al., 2013; Jalali et al., 2013; Singh et al., 2019). Moisture content of fish range from 75.21 ± 0.54 to 78.57 ± 0.40, which is more or less similar to the observations of Chowdhury et al. (2014); where they found the moisture content of *A. testudineus* ranged from 65.28 ± 0.002 to 79.29 ± 0.005. Higher moisture content was found in higher salinities (6, 9 and 12 ppt), which might be correlated with the fact that *A. testudineus* starts drinking more water to compensate the body water loss due to dehydration at higher salinities. When freshwater fish are acclimated to salt water, they counteract the water loss, and intestine plays a crucial osmoregulatory response for reabsorption of water to maintain the balance. Singh et al. (2019) and Barman et al. (2005) depicted similar report on Amur carp and *Mugil cephalus*, respectively. The lipid content ranged from 4.44 ± 0.07 to 6.20 ± 0.17, which also supported by the observation of Chowdhury et al. (2014) in *A. testudineus*. In the present study, lipid content decreased in higher salinities. It is well-established fact that moisture and lipid contents of fish body have an inversely proportional relationship (Shearer, 1994). Furthermore, higher moisture content results in lower lipid deposition in the body muscle or vice versa. Decreasing level of lipid at higher salinities (6, 9 and 12 ppt) indicates that *A. testudineus* need more energy for sustaining at higher salinities. Singh et al. (2019) reported that at higher salinities the lipid content of Amur carp showed a declining trend. Jarvis and Ballantyne (2003) also reported that the crude lipid content of fish sturgeon decreased with increasing salinity, indicating that at higher salinities, fish utilize lipid as a source of energy for maintaining the internal body homeostasis compared with the surrounding environment. In this present study, the protein content ranged from 11.29 ± 0.33 to 13.32 ± 0.36, which was found similar to the findings of Chowdhury et al. (2014) and Paul et al. (2017). At high salinity exposure, the degradation rate of body protein far exceeds amino acid catabolism rate in *A. testudineus* (Chang et al., 2007). Authors also depicted that salinity exposure greatly increases the body energy demand resulting into increased catabolism of body amino acids, which can produce ATP through tricarboxylic acid (TCA) cycle to satiate the metabolic energy needs of fish. Amino acid accumulated in the process serves as osmolytes for dealing with a hyper-osmotic environment. This explains the low crude protein content at higher salinities in this experiment. Bhanu and Deepak (2015) reported that in higher salinities, fish liver suffers damage and hence the rate of synthesis of protein is affected, which supports the results observed in our study. Further, the ash content of fish ranges from 2.95 ± 0.05 to 4.17 ± 0.10, Paul et al. (2017) and Chowdhury et al. (2014) reported similar observations. Ash content was higher in *A. testudineus* at higher salinities attributed to higher deposition of ions and minerals in the muscle and bones of fish reared at high salinity. Singh et al. (2019) reported a similar trend of total ash content in amur carp raised at different salinities (0, 5, 10 and 15 ppt).

4.4 | Digestive enzyme assay

Digestive enzymes depict the overall digestion process and nutritional status of fish (Bolasina et al., 2006; Haque et al., 2021). In teleosts, several studies demonstrated that intestinal enzyme activity changes with salinity (Bolasina et al., 2007; Gheisvandi et al., 2015). In the present study, high digestive enzyme activity observed at higher salinities (9 and 12 ppt) compared with lower ones (T2 and T3) and freshwater T1 (0 ppt) group. This increase in digestive enzyme activities at higher salinities may possibly be due to increased osmoregulatory adaptations to break energy dense nutrients by fish (Bœuf & Payan, 2001). Furthermore, the increased energy requirement for osmotic regulation following stress in the environment causes changes in the physiological process for maintaining energy, such as an increase digestive enzyme activity for food digestion and absorption thereof (Psochiou et al., 2007). Similar findings were also reported by Gheisvandi et al. (2015) in *Caspian kutum*.

4.5 | Oxidative stress-related enzymes

SOD and CAT activities play a significant role in protecting cells against H_2O_2 production (Karadag et al., 2014). Higher SOD and CAT activities were observed at higher salinities (9 and 12 ppt), which indicates that some physiological disorders occurred in the *A. testudineus* at higher salinity. However, fish of T3 group (6 ppt) showed no difference in SOD and CAT activities as compared with T1 (0 ppt) and T2 (3 ppt) groups, suggesting that *A. testudineus* has the potential to tolerate a certain level of salinity (Nahar, 2015). To support our result, higher SOD and CAT activities were recorded in 16 and 24 ppt compared with 8 and 0 ppt salinity in tilapia (Gan et al., 2016; Wang et al., 2008).

4.6 | Haematological parameters

Haematological parameters can be considered as valuable indicators for identifying fish health (Paul, Sardar, Sahu, Deo, et al., 2022) and also guide stakeholders as well as biologists to interpret the physiological stress due to sudden changes in environmental parameters such as temperature, salinity, etc. (Valenzuela et al., 2007). Haematological parameters such as Hct, Hb, RBC and WBC decreased in higher salinities (9 and 12 ppt). Authors reported a significant effect of salinity exposure on different haematological parameters in several fish, which may be attributed to salinity-induced damage of osmoregulatory responses in fish (Elarabany et al., 2017; Fazio et al., 2013; Soltanian et al., 2016). Low Hct percentage value in fish under salinity stress could be attributed to reduced volume of red blood cells, which is due to osmotic changes caused by ion leakage from the plasma (Alwan et al., 2009). WBC count in fish is a reliable biomarker of physiological status (Paul, Sardar, Sahu, Deo, et al., 2022; Svobodová et al., 2001). Lower WBC count in higher

salinities could be attributed to the weaker defence mechanisms (immuno-suppression) of fish in the altered environment. Fazio et al. (2013) demonstrated similar observation in mullet and Al-Hilali and Al-Khshali (2016) in common carp, wherein, increasing salinity caused drastic reduction in WBC count.

4.7 | Haemato-biochemical parameters

Serum protein profile is a nonspecific immunological variable and gives a fair idea about the immune potential of the fish in different environmental conditions (Yılmaz & Ergün, 2012). Serum total protein and globulin were lower at 9 (T4) and 12 ppt (T5) salinity group compared with the T1 (0 ppt), which could be related to fish using plasma protein as a source of energy to mitigate the osmoregulatory stress. Chang et al. (2007) reported that *A. testudineus* gradually adopt higher ambient salinity by the process of argininolysis or purine catabolism followed with uricolysis. Carbon chains generated during the process would enter the citric acid cycle leading to the production of ATP, as reported in nile tilapia and sea bream (*Sparus sarba*) by Elarabany et al. (2017) and Kelly and Woo (1999), respectively.

The blood glucose level is an important stress indicator in fish and gives an idea about the adaptability of fish in changing environmental conditions (Yin et al., 1995). Serum glucose level decreased with higher salinity (9 and 12 ppt), which might be correlated with an elevated glycogenolysis pathway to compensate higher energy demand for osmoregulation (Kavya et al., 2016; Vijayan et al., 1996). Similarly, glycogen/glucose turnover mainly occurs in the liver of fish. During osmotic adaptation at higher salinities, increased metabolism in the liver ensures the sufficient availability of glucose to serve as fuel for other physio-metabolic and osmoregulatory adaptations in kidney and gill tissues (Vijayan et al., 1996). Similar findings were reported by Fazio et al. (2013) for mullet and Imanpoor et al. (2012) for goldfish.

ALT and AST, mainly found in hepatocytes and cardiomyocytes of fish, are known to play a significant role in protein metabolism (Fazio et al., 2013). Plasma ALT and AST concentrations increased significantly with gradual increase in salinity. Liver tissue destruction correlated with increased ALT and AST concentration happens due to oxidative damage caused by increased metabolic rates under stress. This increase in ALT and AST can be a testimony to the role of these enzymes in initial amino acids compensation that the body needs due to changing physiological needs and energy demands (Ebeid et al., 2005). Stress-induced husbandry processes may cause an elevation of the internal oxidizing effort towards membrane permeability which in turn increases the fluxes of ALT and AST enzymes into the bloodstream (Bahjat & Shaban, 1985). Küçük (2013) reported that ALT and AST activity of goldfish increase when fish is transferred from freshwater to saline water. Akhtar et al. (2014) also reported enhanced AST and ALT levels in muscle and liver of *Labeo rohita* due to salinity exposure. Similarly, salinity challenge to tilapia resulted in elevated activities AST and ALT in the blood, which was explained to be associated with the destruction of liver protein (Vijayan et al., 1996). Sultan (2007) reported increased plasma ALT and AST concentration of *Acanthopagrus latus* juveniles in relation to increasing salinity levels.

5 | CONCLUSION

It has been elucidated from the present study that salinity plays a significant role for the culture of the freshwater climbing perch, *A. testudineus*, which can be reared in ISW within an acceptable range of 0–12 ppt salinity without affecting its survival, although, better growth responses may be achieved by rearing the *A. testudineus* in ISW of up to 6 ppt. In view of increase in magnitude of salt-affected inland areas vulnerable to salinity variation in India, *A. testudineus* can be proposed as an ideal species for utilization of such otherwise unproductive soils (and water bodies) for production of valuable animal protein. This study will help farmers to make a decision on species selection that can facilitate risk management in such disadvantaged regions of our country both in terms of time, space and money. Moreover, further studies may be conducted to enhance the growth of *A. testudineus* in higher salinities through dietary interventions (supplementation/inclusion) of osmolytes and nutraceuticals.

AUTHOR CONTRIBUTIONS

Subam debroy investigated the study, validated data, implemented software programs and prepared the original draft. Narinder Kumar Chadha conceptualized the study, supervised the study, and reviewed and edited the manuscript. Satya Prakash designed methodology, involved in visualization and validation, and reviewed and edited the manuscript. Paramita Banerjee Sawant and Vungurala Harikrishna involved in visualization and validation, and edited the manuscript. Mujahidkhan A Pathan performed data curation and validation. Ramjanul Haque, Prasanta Jana and Udipta Roy performed formal analysis, software implementation and data curation.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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