



**GROWTH AND PHYSIOLOGICAL RESPONSES OF AMUR
CARP, *CYPRINUS CARPIO HAEMATOPTERUS* (MARTENS,
1876) CULTURED IN INLAND SALINE WATER**

Thesis submitted in partial fulfillment

of the requirements

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Ph. D. (Aquaculture)

by

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DECLARATION

I hereby declare that the thesis entitled **“GROWTH AND PHYSIOLOGICAL RESPONSES OF AMUR CARP, *CYPRINUS CARPIO HAEMATOPTERUS* (MARTENS, 1876) CULTURED IN INLAND SALINE WATER”** is an authentic record of work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

Date: 28th Feb., 2019

(Shashank Singh)

Place: Mumbai

Ph.D. Scholar

ICAR- Central Institute of Fisheries Education



देश के वीर शहीदों एवं मत्स्य पालकों को समर्पित

“जय हिन्द”

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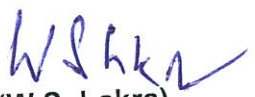
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ABSTRACT

Inland saline area has been identified as one of the new facets of aquaculture with immense potential and plethora of opportunities to be harnessed. India has about 8.7 million ha of inland saline soils and about 40% of these lands are prevailing in North- western parts of the country, where seasonal temperature changes are very high. Amur carp, *Cyprinus carpio haematopterus* is one of the species to tolerate wide range of temperatures. Keeping in view of prevailing environmental conditions, Amur carp was tested in raw inland ground saline water (IGSW). The results indicated that the fish was able to survive up to 15 ppt in IGSW, whereas maximum survival and growth was observed upto 5 ppt. Growth, carcass composition and digestive enzyme activity were significantly ($p < 0.05$) affected by increasing salinity. The results also depicted that increase in salinity influence the osmolality, serum ionic concentrations and $\text{Na}^+ \text{K}^+$ -ATPase activity significantly ($p < 0.05$). Stress and immune responses were significantly ($p < 0.05$) higher at elevated salinities. The histological studies indicated that there was no deleterious effect of salinity up to 5 ppt on the gill tissues, whereas small alterations were observed in fish reared at higher salinities. Potassium deficiency in inland saline water is a common phenomenon, hence an experiment was conducted to evaluate the combined effect of different salinities and potassium ion concentrations on growth and physiological responses of Amur carp. The results indicated that K^+ supplementation had no positive effect on growth and survival at 5 and 10 ppt. However, at 15 ppt higher K^+ supplementation affects on growth and survival adversely. It was also found that the carcass composition and digestive enzyme activity were not significantly influenced by K^+ ion concentration and their interaction with salinity. Stress and immune response of Amur carp inferred an increasing trend with increasing salinity and potassium concentrations. Therefore, Amur carp can be cultured in inland saline waters up to 5 ppt without K^+ fortification, which can act as one of the promising species for inland saline aquaculture in future.

सारांश

अन्तःस्थलीय क्षेत्र में जलकृषि को एक नए संसाधनों के रूप में पहचाना गया है, जिसमें अपार संभावनाएं और अवसरों की अधिकता है। भारत में लगभग 8.7 मिलियन हेक्टेयर अन्तःस्थलीय क्षेत्र है और इनमें से लगभग 40 % उत्तर- पश्चिमी भागों में विद्यमान है, जहाँ मौसमी तापमान में परिवर्तन बहुत अधिक होता है। अमूर कार्प *Cyprinus carpio haematopterus* अत्यधिक तापमान सहन करने वाली मछलियों में से एक है। प्रचलित पर्यावरणीय परिस्थितियों को ध्यान में रखते हुए, अमूर कार्प का परीक्षण अन्तःस्थलीय खारा भू- जल में किया गया। परिणामों ने संकेत दिया कि मछली IGSW में 15 पीपीटी तक जीवित रहने में सक्षम थी, जबकि अधिकतम जीवितता दर और विकास 5 पीपीटी तक देखा गया। वृद्धि, शरीर संघटक अवयव और पाचन एंजाइम गति विधि 5 पीपीटी तक बेहतर पायी गयी जो कि लवणता बढ़ने से प्रभावित हो रही थी। परिणामों ने यह भी संकेत दिया कि लवणता बढ़ने से परासरणता, सीरम आयनिक सांद्रता एवं $\text{Na}^+ \text{K}^+ \text{ATPase}$ प्रभावित हुआ। तनाव एवं प्रतिरक्षा प्रतिक्रिया भी बढ़ती हुई लवणता से बढ़ती गयी। हिस्टोलॉजिकल अध्ययनों से संकेत मिलता है कि गिल के ऊतकों पर 5 पीपीटी तक लवणता का कोई हानिकारक प्रभाव नहीं था, जबकि उच्चतर लवणों में पाली गयी मछलियों में कुछ परिवर्तन देखे गए। चूँकि अन्तःस्थलीय खारे जल में पोटैशियम की कमी होती है, अतः इसके प्रभाव को जानने के लिए विभिन्न लवणों और पोटैशियम सांद्रता के संयुक्त प्रभाव को अमूर कार्प के विकास और शारीरिक प्रतिक्रियाओं पर मूल्यांकन करने के लिए दूसरा प्रयोग किया गया। परिणामों से परिलक्षित होता है कि K^+ पूरकता का 5 एवं 10 पीपीटी पर कोई प्रभाव नहीं है जबकि 15 पीपीटी लवणता पर उच्च K^+ पूरकता प्रतिकूल प्रभाव डालती है। यह भी पाया गया कि शरीर संघटक अवयव और पाचन एंजाइम गतिविधि K^+ पूरकता और उसके लवणता के साथ संयुक्त प्रभाव से प्रभावित नहीं थे। बढ़ते हुए लवणता और पोटैशियम की सांद्रता के साथ अमूर कार्प की तनाव और प्रतिरक्षा प्रतिक्रिया बढ़ती हुयी पायी गयी। इसलिए अमूर कार्प को बिना पोटैशियम सम्पूरित किये अन्तःस्थलीय जल में पालन किया जा सकता है, जो कि भविष्य में अन्तःस्थलीय जल- कृषि के लिए एक आशाजनक प्रजाति हो सकती है।

APPENDICES

ANOVA	Analysis of Variance
CAT	Catalase
dl	Decilitre
DMF	Dimethyl Formamide
DNA	Deoxyribonucleic Acid
DAHDF	Department of Animal Husbandry, Dairying & Fisheries
DO	Dissolved Oxygen
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
g	Gram
h	Hour
ha	Hectare
hrs	Hours
Kcal	Kilo Calorie
Kg	Kilo Gram
L	Litre
Ltd.	Limited
mg	Milli Gram
Min	Minutes
ml	Milli Litre
mM	Milli Molar
mmt	Million Metric Tonnes
m ³	Cubic Metre
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide - Hydrogen
OD	Optical Density
ppm	Parts Per Million

ppt	Parts Per Thousand
Pvt.	Private
rpm	Revolution Per Minute
sec	Seconds
SGR	Specific Growth Rate
SOD	Superoxide Dismutase
<i>Spp</i>	Species
t	Tonnes
TCA	Trichloro acetic Acid
yr	Year
°C	Degree Centigrade
μg	Micro Gram
%	Percentage

5. Discussion

The primary aim of the present study was to delineate the growth and physiological responses of Amur carp reared at different salinities in Inland Ground Saline Water (IGSW). The effect of different potassium concentrations and their interaction with salinity was also evaluated to demonstrate the culture feasibility of Amur carp in the arid and semi-arid regions.

5.1. Experiment- 1

5.2. Physico-chemical Characteristics of Water

Water quality is a vital component of any aquaculture practice and needs to be maintained throughout the culture period as it may affect the growth and physiology of fish. Most of the water quality parameters remained at optimum levels and no significance difference was observed in temperature, pH, dissolved oxygen, $\text{NH}_4^+\text{-N}$ and $\text{NO}_2\text{-N}$ among different treatments throughout the experimental period, which is supported by different workers in different studies (Banerjea, 1967; Stone and Thomforde, 2004; Velasco *et al.*, 2006; Santhosh and Singh, 2007; OATA, 2008; Bhatnagar and Devi, 2013).

Temperature is one of the most important parameters which control other parameters (e.g. dissolved oxygen, ammonia etc.) and thus influence the aquaculture production. Froese and Pauly (2011) reported common carp as eury-thermal which can survive between 3 and 35 °C water temperature range. The optimum water temperature range for growth is 20-25 °C which is supported the average temperature (25.83 °C) obtained during the present experimental period.

The pH is an important factor to ensure better production of fish. The optimum pH range differs among different species while acceptable pH range for fish culture is 6.5 - 9.0 (Zweig *et al.*, 1999). The pH of water in all the experimental groups was ranged between 7.59 ± 0.04 to 7.69 ± 0.07 , which is supported by Heydarnejad

(2012). Similar findings were reported by Ansal *et al.* (2016) during carp culture in different combinations of carp species in inland saline water of Punjab.

The dissolved oxygen content of water play an important role in the growth and other metabolic response in aquaculture and 5 ppm is recommended minimum dissolved oxygen requirements for tropical freshwater fish (Mallya, 2007). The dissolved oxygen concentration of all the experimental groups was recorded within the range of 6.34 ± 0.13 to 6.52 ± 0.11 ppm during the experimental period of 90 days.

$\text{NH}_4^+\text{-N}$ is a less toxic form of Ammonia while unionized NH_3 is more toxic to aquatic organisms (Swingle, 1967). The normal ammonia concentration for aquaculture is <1 ppm (Goran *et al.*, 2016). In the present study, the $\text{NH}_4^+\text{-N}$ ranged from 0.22 ± 0.01 to 0.25 ± 0.01 ppm. The $\text{NO}_2\text{-N}$ content of all the four experimental groups was found to be in the range of 0.03 ± 0.01 to 0.05 ± 0.01 ppm. Stone and Thomforde (2004) recommended a desirable range for $\text{NO}_2\text{-N}$ as 0-1 ppm.

There was a significant difference observed for other parameters like total alkalinity, total hardness, calcium and magnesium hardness, Na^+ , K^+ , Ca^{2+} and Mg^{2+} ion concentrations at different salinities in the present study. Alkalinity is a measure of total concentration of bases in water and higher alkalinity value is an indication of the absence of CO_2 . The total alkalinity was ranged from 113.89 ± 0.22 to 304.38 ± 0.83 at different salinities in the present study and supported by Limhang *et al.*, 2011. According to Stone and Thomforde (2004), an acceptable range for alkalinity is 20 to 400 ppm while Santhosh and Singh (2007) suggested 50-300 ppm as an ideal value for fish culture.

Total hardness was ranged between 169.16 ± 4.73 and 3232.50 ± 0.83 in the present study at different salinities which is supported by several workers at same salinities (Kumar, 2013; Rani, 2015; Upadhyay, 2015). Jahan *et al.* (2018) observed the total hardness range of 2460 to 2793.5 at 10 ppt salinity which is similar to the findings of present study at the same salinity. Limhang *et al.* (2011) inferred that the total hardness as 249.9 ± 12.1 ppm at 0.94 ± 0.16 ppt and 316.0 ± 37.3 ppm at 1.79 ± 0.33 .

Ionic concentrations (Ca^{2+} , Mg^{2+} , Na^+ and K^+) of IGSW were recorded and a significant difference was observed at different salinities in all treatment groups. Ionic concentrations of IGSW was varying from place to place even at same salinity as reported by various authors (Saoud *et al.*, 2003; Mellor and Fotedar, 2005; Pragnell and Fotedar, 2006; Tantulo and Fotedar, 2006; Roy *et al.*, 2007; Saoud *et al.*, 2007; Tantulo and Fotedar, 2007; Lakra *et al.*, 2014). Similar findings for different ion concentration levels were reported by several authors at different salinities which support the findings of present experiment (Ingram *et al.*, 2002; Antony, 2013; Kumar, 2013; Pathak, 2013; Rani, 2015; Upadhyay, 2015; Chitra *et al.*, 2017; Jahan *et al.*, 2018). Low concentration of potassium in ground saline water was also reported by Gurjar and Kaur (2016). All the water quality parameters were in the acceptable range and found suitable for the culture of Amur carp.

5.3. Growth Performance of Amur Carp

The growth performance of Amur carp was evaluated at different salinities (<0.5, 5, 10 and 15 ppt) of IGSW and the findings of the experiment revealed that the growth rate of Amur carp in terms of specific growth rate ($\% \text{ d}^{-1}$), % weight gain and protein efficiency ratio was found better in control while decreased with an increase in salinity and better feed conversion ratio were recorded at <0.5 ppt. Küçük (2013) reported that freshwater fish generally grow well in both freshwater and low salinity environments and growth declines with increasing salinity which is similar in the present study. Decrease in growth may be due to the fact that feed intake and appetite of fish decreased or influenced at higher salinities. The findings related to growth of the present study were supported by findings of other workers on different freshwater fishes (Wang *et al.*, 1997; Jarvis *et al.*, 2001; Lawson and Alake, 2011; Fallah *et al.*, 2013; Rani and Gulia, 2015; Abdullah, 2016). Bœuf and Payan (2001) reported higher growth at 2 ppt in freshwater fish. Konstantinov and Martynova (1993) have also reported higher growth for grass carp, Russian sturgeon and common carp at 2 ppt salinity.

The percentage weight gain of all treatments was varying significantly ($p < 0.05$) with control and observed as 328.03 ± 5.29 , 588.46 ± 6.80 , 670.67 ± 9.87 and

764.68 $\pm$ 8.44 for 15, 10, 5 ppt and control, respectively. Desai and Singh (2009) found approximately similar values when worked on the effects of water temperature and ration size on growth and body composition of fry of common carp (*C. carpio*). In the present study, reduction in growth with increase in salinity was observed which clearly indicates that higher salinity negatively affect the growth of Amur carp. With increase in salinity, decrease in growth was reported in catfish *Mystus vittatus* (Arunachalam and Reddy, 1979) and rainbow trout (Mckay and Gjerde, 1985). The possible reason for reduction in growth is that the amount of available energy is diverted to maintain the intracellular ionic and osmotic regulation (Iwama, 1996).

The specific growth rate (SGR) of Amur carp in the present work showed a decreasing trend with increasing salinity which is similar to the findings of various authors (Wang *et al.*, 1997; Fallah *et al.*, 2013; Islam *et al.*, 2014 and Abdullah, 2016) on different species. The control has significant difference with T₂ and T₃ but did not vary significantly with T₁ while there is no significance difference observed between T₁ and T₂. Fallah *et al.* (2013) reported that one of the species of cyprinidae (*Capoeta damascina*) reared in four different salinities (0.04, 6, 12 and 24 ppt) for 12 months showed better growth performance in freshwater compared to fish at higher salinities which is similar to present experiment. Abdullah (2016) reported negative SGR for silver carp exposed to 12 ppt for 21 days experiment. Küçük (2013) reported negative SGR for crucian carp reared at 20 ppt and in decreasing order (0.53 to 0.06) for Gold fish with increase in salinity from 8 to 20 ppt. Mahon *et al.* (2014) recorded positive SGR for *Nannoperca obscura* juveniles at all salinities varying from 0.3 to 10.1 ppt and mean overall SGR for weight was found 1.10 $\pm$ 0.04%. Basavaraju and Reddy (2013) reported average specific growth rate 2.25 and 2.69 % d⁻¹ under monoculture and polyculture (6 trials for both) respectively at 12 different locations, which is approximately similar for Amur carp reared in IGSW of 5 ppt and 0 ppt (2.27 $\pm$ 0.05 and 2.39 $\pm$ 0.03 % d⁻¹ respectively).

Feed conversion ratio (FCR) and Protein efficiency ratio (PER) of T₃ group varied significantly as compared to other treatment groups and showed highest FCR and lowest PER, while there were no significant difference in FCR and PER of control,

T₁ and T₂ groups. FCR for rohu cultured at different salinities showed an increasing trend with increasing salinities 0, 2, 4, 6 and 8 ppt (Islam *et al.*, 2014). Wang *et al.* (1997) found minimum FCR 1.11 and 1.48 at 2.5 and 0.5 ppt salinity respectively for common carp cultured at different salinities and increased with increasing salinity, while found maximum 5.43 at 8.5 ppt. Markovic *et al.* (2012) showed FCR in the range of 1.49 ± 0.18 to 2.82 ± 0.18 for the *C. carpio* fed with different diets. In present study, PER increased with increase in salinity, which is supported by Al-Khshali (2017) when common carp was exposed to different salt concentrations of 0.1, 5, 10 and 15 ppt. Increase in FCR of Amur carp may be attributed due to acceptance of less feed at higher salinities.

The survival of the fish depends on the ability of the fish to maintain body fluids at least internal osmotic and ionic concentrations (Holliday, 1969). At the end of the experiment, lowest survival percentage was recorded in 15 ppt (85.0 ± 2.89) and significantly higher in control and 5 ppt (100 ± 0.00). The finding was supported by Kumar *et al.* (2017) and concluded that the percentage survival of *Pangasianodon hypophthalmus* was varied between 98.52 ± 0.74 , 97.04 ± 0.74 , 91.86 ± 0.74 and 85.92 ± 0.74 reared in inland saline water with salinities 0, 5, 10 and 15 ppt, respectively. In different studies, 100% survival was recorded for *Labeo rohita* at the salinities of 8 and 6 ppt as reported by Pillai *et al.* (2003) and Islam *et al.* (2014) respectively. Mangat and Hundal (2014) reported 100 % survival of *C. carpio* fingerlings upto 6 ppt for 60 days during different seasons (summer, autumn and winter). Ghosh *et al.* (1973) reported different salinity tolerance limits for Indian major carps at different life stages. With increase in salinity there is decreasing survival rates is reported in *Channa punctata* (Dubey *et al.*, 2016), *C. batrachus* (Sarma *et al.*, 2013). Mortality at higher salinities may be attributed to increasing osmotic maintenance requirements (Kilambi, 1980). Growth response of Amur carp at elevated salinities was decreasing and the possible reason for decreasing weight and survival may be attributed to the increasing energy demand for osmotic maintenance and less acceptance of feed due to stress.

5.4. Carcass Composition of Amur Carp Reared at Different Salinities

The carcass composition of fish is influenced by several factors like seasonal, environmental and biological variations (Desai and Singh, 2009; Tao *et al.*, 2012). The effect of environmental factors like pH, salinity and temperature on carcass composition has been reported for many species and the findings of the present study corroborate with some of the earlier studies that salinity can affect the carcass composition (Fallah *et al.*, 2013; Jalali *et al.*, 2013; Kumar *et al.*, 2016). In the present study, the highest moisture content at higher salinity may be correlated with the fact that the fish start drinking more water to compensate the dehydration as mentioned by Febry and Lutz (1987) that in comparison to freshwater adapted fish, seawater adapted fish have four to nine fold higher drinking rate to maintain the body fluid constant. The present findings are supported by previous research carried on various fishes like *Mugil cephalus* (Barman *et al.*, 2005), *Lateolabrax japonica* (Xu *et al.*, 2010), *Capoeta damascina* (Fallah *et al.*, 2013) and *Barbus grypus* (Jalali *et al.*, 2013). It is well known fact that lipid content in fish is inversely proportional to moisture content (Shearer, 1994) and higher moisture level will result in lower lipid concentrations or vice versa, which is also depicted in similar manner in the present study. Decreasing level of lipid at higher salinities indicate that Amur carp needs more energy for sustaining at higher salinities. Ohta and Watanabe (1996) and Fallah *et al.* (2013) reported that with increase in salinity, percentage of lipid decline which is corroborating with the results of present study. Jarvis and Ballantyne (2003) reported that lipid of shortnose sturgeon decreased with increase in salinity during salinity acclimation and indicate the effective utilization of lipid in the osmoregulation. Crude protein was found lowest at higher salinity which may be due to the less acceptance and utilization of feed provided during culture period. Lower percentage of crude protein at higher salinities may be correlated with the statement of Bhanu and Deepak (2015) that the lower production of protein or impaired protein synthesis in liver could reduce the protein content of tissues which supports the results obtained with Amur carp. Dempson *et al.* (2004) found greater protein and lipid when moisture is less. In the present study, ash content of fish was found to be higher with increase in salinities which might be attributed to the deposition of more ions from

high saline water. Similar finding was reported by Jalali *et al.* (2013) in *Barbus grypus* reared at different salinities (0, 3, 6, 9, 12 and 15 ppt).

Biochemical composition of a fish is an indication of healthy stock, and high protein level in freshwater and 5 ppt support the good health condition of fish. The fish growth was also found better in freshwater and 5 ppt salinity in comparison to other treatment groups. Therefore, Amur carp can be recommended to culture in inland saline water upto 5 ppt with high quality flesh.

5.5. Digestive Enzyme Activities of Amur carp

Breakdown of larger nutrients into smaller subunits in the digestive tract of an animal is mainly depends on the availability of enzymes (Cho, 1987). Digestive enzyme activities are influenced by so many factors and salinity is one of them as stated by Devi and Aravindan (1997). Exposure of fish to different salinities may influence the digestive enzyme activities as it changes the drinking rates (Usher *et al.*, 1988) and salinity of the gut content (Moutou *et al.*, 2004). Fish reared at higher salinities ingest food particles along with surrounding water which is rich in salt content and may alter the ionic concentration in the gastric lumen where enzymes are found (Hamed *et al.*, 2016). Variation in ionic concentration in the gastric lumen may also influence the activities of digestive enzymes. Digestive enzymes are responsible to influence the digestive process and improve the growth of animals (Lemieux *et al.*, 1999). Digestion process is mainly depending on the pH of intestine and stomach and various studies reported that fishes usually maintain acidic pH for stomach and alkaline pH for intestine (Page *et al.*, 1976; Payne, 1978; Anderson, 1991) but increasing salinity makes both of them alkaline (Devi and Aravindan, 1997) which may also affect the digestion process.

In the present study, higher protease, lipase and amylase activity was observed in the fish reared at 0 and 5 ppt salinity while 10 and 15 ppt salinity showed significantly lower activities. Devi and Aravindan (1997) reported higher lipase activities at 0 ppt which is in accordance with present study. Barman *et al.*, 2005 also reported

lower digestive enzyme activities in *Mugil cephalus* exposed to above 10 ppt salinity in inland saline water.

Tsuzuki *et al.* (2007) reported higher nutrient absorption and efficient digestibility in fat snook (*Centropomus parallelus*) at 15 ppt and maximum growth was also observed at the same salinity which is in support with the present study that higher digestive enzyme activities will result in maximum growth. Lower FCR and higher PER indicates the better digestion of food and absorption of nutrients at lower salinities.

Barman *et al.* (2012) stated that intestine plays a major role in osmoregulation which mediates a faster food movement in gut under higher salinities and thus reduced the digestive enzyme activity, time required for complete digestion and absorption of nutrients. It may be the possible reason for lower digestive enzyme activity at 15 ppt reared fishes. So, it has been concluded that increase in salinity negatively affected the digestive enzymes activity which ultimately lowered the growth, PER and increase the FCR.

5.6. Physiological Response

5.6.1. Osmolality and osmoregulatory capacity

The ability of fish to adapt to any medium depends on its ability to maintain body osmoregulation. Serum osmolality is determined by the total concentrations of all dissolved ions including inorganic salts and organic compounds (e.g. sugars and amino acids that are common in biological fluids) (Kültz, 2015). In the present study, there were significant difference ($p < 0.05$) observed in serum and water osmolality among the different treatment groups and an increasing trend of both (serum and water osmolality) was observed with increase in salinity and findings of the present work supported by Mellor and Fotadar (2005). Several authors have worked on similar line and reported a significant increase in serum osmolality of various fishes like Pompano, *Trachinotus marginatus* (Anni *et al.*, 2016), gilthead sea bream, *Sparus aurata* (Laiz-Carrión *et al.*, 2005) and spottedtail goby, *Synechogobius ommaturus* (Shui *et al.*, 2018) with increase in environmental salinity. In their study, juveniles of Murray cod (*Maccullochella peelii*

peelii) reared at different salinities (0, 2.5, 5, 7.5 and 10 ppt) and postulated increase in blood osmolality with increase in salinity and also concluded that osmolality was not influenced by duration of exposure. Sui *et al.* (2016) reported that osmolality of blood parrot fish (*Cichlasoma synspilum* ♀ × *Cichlasoma citrinellum* ♂) is affected by salinity and time when exposed to four different salinities (0.16, 2.5, 5.0 and 7.5 ppt) and evaluated at different time. The serum osmolality was increased after 6 h at all salinities except control group and found maximum at 7.5 ppt, after 12 h the serum osmolality in all treatments returned to the level of the control group and remained stable for 168 h (until the end of experiment). The body fluids of freshwater fishes are hypertonic and Min *et al.* (2005) reported that serum osmolality of freshwater fish is higher than its medium and support the findings of the present study.

Similar findings related to osmolality (water and serum) and osmoregulatory capacity were reported by different authors in different fishes reared at different salinities in IGSW (Upadhyay, 2015; Rani, 2015; Kumar *et al.*, 2017). In the present study, the growth was observed even upto 15 ppt with less survival and osmoregulatory response of Amur carp indicate that fish is a good osmoregulator up to 15 ppt in IGSW.

Osmoregulatory capacity is defined as the difference between the osmotic pressures of internal and external medium (Charmantier *et al.*, 1989). According to Lignot *et al.* (2000) osmoregulatory capacity of fishes at different salinities gives better reflection of changes induced by salinity stress. In the present study, highest value of osmoregulatory capacity was observed in control group after that decreasing trend was recorded in subsequent groups with increasing salinities. The findings of the study showed that survival was slightly influenced by the salinity and positive osmoregulatory capacity indicating the capability of Amur carp to survive upto 15 ppt in IGSW. It was also observed that fish growth was affected with increasing salinity and decrease in growth can be correlated with the fact that amount of available energy is transferred to maintain the ionic and osmotic homeostasis. Lower lipid concentration at higher salinities indicates the role of lipid in osmoregulation.

5.6.2. Serum ionic concentration

Osmoregulation is a function of salinity and associated in the serum osmolality and serum ion concentrations (Na^+ , Cl^- , K^+ , Mg^{2+} and Ca^{2+}). The serum ion concentration is a physiological indicator and used to determine the health status of fish. Freshwater fishes are generally considered as hyper osmotic to their environment and maintain the serum ion concentrations at higher levels for their acclimation and survival at different salinity levels. In the present study, the serum ionic concentration of Amur carp was increased with increase in salinity. The findings of the present study are supported by Morgan and Iwama (1991), Sangiao-Alvarellos *et al.* (2003), Küçük *et al.* (2013), Anni *et al.* (2016) and Soegianto *et al.* (2017). Kulkarni (2015) mentioned an important role of Na^+ , Cl^- and K^+ during osmoregulation and homeostasis maintenance at higher salinities. Ca^{2+} also has a number of vital functions in fish including reproduction whereas role of Ca^{2+} in osmoregulation is also recognized (Wurts and Stickney, 1989). At higher salinities freshwater fishes drinks more water to cope up with water loss and increased drinking rate has help in absorption of ions into blood; and may be the possible reason for elevated serum ionic concentration.

5.6.3. Sodium potassium adenosine triphosphate activity (Na^+K^+ -ATPase)

The transfer of fish from freshwater to seawater or vice- versa, the serum ionic concentrations are usually changed and used to evaluate the osmoregulatory ability of fish (Stewart *et al.*, 2016). ATPase are the membrane-bound enzymes and responsible for the transport of ions through biological membranes. The Na^+K^+ -ATPase activity is the main mechanism for maintaining serum ion and osmotic balance (Stewart *et al.*, 2016; Zhang *et al.*, 2017; Shui *et al.*, 2018). The Na^+K^+ -ATPase is present in specialised ion transport cells known as mitochondrian-rich cells (i.e. chloride cells) in the gills of teleost fish. This enzyme is considered as a good biomarker for osmoregulation (Sui *et al.*, 2016) and varied with variations in salinity (Kang *et al.*, 2008). In the present study, Na^+K^+ -ATPase activity was significantly affected by increasing salinity and found similar with the findings of Salati *et al.* (2011). Lower activity of Na^+K^+ -ATPase is direct related with higher growth which was observed in the

present study and supported by Laiz-Carrión *et al.* (2005). Higher Na⁺ K⁺ -ATPase activity at higher salinities is related to the capability of species to use energy for maintaining ionic homeostasis and associated with lower growth.

5.7. Stress Response

5.7.1. Serum cortisol and glucose

Stress response is the overall physiological reaction of an organism to stress inducing agents (Jumah and Traifalgar, 2015). Serum cortisol is used as a general index for primary stress response and glucose as a secondary stress response (Porchas *et al.*, 2009; Metwally and Wafeek, 2014). Cortisol is the predominant glucosteroid in fish and synthesized by interrenal cells of the head kidney. Cortisol play an important role in many physiological functions and affects carbohydrate metabolism, hydro-mineral balance, energy mobilization, glucose synthesis and plasma protein production (Goss and Wood, 1988; Saravanan *et al.*, 2018). Cortisol also have role in salinity tolerance, proliferation of gill chloride cells and gill Na⁺ K⁺ -ATPase activities (Saravanan *et al.*, 2018). In the present study, higher serum cortisol and glucose levels were observed at increasing salinities. Similar findings were also found in *Carassius auratus* (Al- Khashali and Al- Shawi, 2013) and *Oreochromis niloticus* (Nakano *et al.*, 1997; Jumah and Traifalgar, 2015) at increasing salinities. Glucose is used as energy source necessary to maintain homeostasis in fish and Shahkar *et al.* (2015) inferred that under stressed condition, glucose level increase with increased serum cortisol level. Increased level of serum cortisol and glucose indicates the stress of fish with increasing salinities. The increased cortisol level may be correlated with increased serum osmolality and may be justified the role of cortisol in osmoregulation. Higher glucose in response to higher salinity is in agreement with earlier studies (Lim *et al.*, 2013) and accounts for higher energy demand to maintain osmotic homeostasis.

5.7.2. Muscle and liver AST and ALT enzymes

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the most important aminotransferases and also called as glutamate oxaloacetate

transaminase (GOT) and glutamate pyruvate transaminase (GPT), respectively. Both the aspartate and alanine aminotransferase acts as a link between carbohydrate and protein metabolism by catalyzing the inter-conversion of strategic compounds like α -ketoglutarate and alanine to pyruvic acid and glutamic acid, respectively (Knox and Greengard, 1965). The pyruvate and oxaloacetate thus formed are responsible for the synthesis of non-essential amino acids like alanine, asparagine, glutamine etc. which in turn help in protein synthesis and hence growth of the fish. Apart from this the amino acids are deaminated or transaminated to produce TCA cycle intermediates also helping in energy production in the form of ATP (De Silva and Anderson, 1994). In the present study, the AST and ALT activity was studied in the muscle and liver tissues and significant difference was observed at 10 and 15 ppt in comparison to control and 5 ppt.

Al-Khashali and Al-Shawi (2013) and Jumah and Traifalgar, (2015) inferred serum AST and ALT as a stress indicator and increase with increase in salinity which is in accordance with the findings of present study.

5.7.3. Muscle and liver LDH and MDH enzymes

In the present study, LDH and MDH were increased with increasing salinity levels from freshwater to 15 ppt. Elevation of LDH and MDH activity was exhibited in liver and muscle, this might be due to suppression of oxidative metabolism in fish reared at higher salinities. The changes in LDH and MDH enzyme activity are sensitive to the environmental pollutants and other stressors (Devi, 2003). The findings of present study were supported by several authors where increase in LDH activity after exposing to different salinity, temperature and pesticide levels (Devi, 2003; Das *et al.*, 2004; Akhtar *et al.*, 2014). Akhtar *et al.*, 2014 reported an increased LDH activity in *Labeo rohita* juveniles exposed to salinity and temperature stress as compared to their unexposed counterparts indicated hypoxia in fish resulted in shift in aerobic metabolism to anaerobiosis (Calbreath, 1992) and as the pyruvate (end product of glycolysis) enters into anaerobic pathway, LDH activity increases. It has been also reported that thermal stress causes gill damage (Kumar, 2008) and salinity stress causes impairment of respiratory system (Avella *et al.*, 1993) thus may lead to hypoxia and an increase in

LDH activity in tissues (Das *et al.*, 2004). Malate dehydrogenase activity is important test amongst the several enzymes, carbohydrates play an important role in the cellular process. Under extreme stress conditions, carbohydrate enzyme such as MDH has been known to act as the energy supplier in metabolic pathways and biochemical reactions (Shaikh, 2012). In present study, elevated levels of MDH in higher salinities are might be due to the higher metabolic rate at higher salinities. Shaikh (2012) reported similar trend of elevated MDH upto 4th day in case of freshwater fish, *Anabas testudineus* during post exposure recovery from lead toxicity.

5.8. Immune Responses

5.8.1. Gill and liver SOD and catalase activity

Freshwater fish are under continuous pressure to conserve salts, whereas the reverse is true for marine species, which must conserve water (Greenwell *et al.*, 2003).

Superoxide dismutase (SOD) activity and catalase (CAT) activity play an important role to protect cells against H₂O₂ production. SOD is a common antioxidant enzyme that can protect organisms against reactive oxygen species (ROS) induced damage (Stadtman and Levine, 2003; Seifried *et al.*, 2007). Therefore, the antioxidant status in fish can be accurately reflected by SOD and CAT activity. Recent studies have shown that stress responses caused by salinity variations are closely associated with enhanced generation of ROS (Livingstone, 2001). CAT, a very highly conserved enzyme plays an important role in the cell redox equilibrium (Dudley and Klaassen, 1984; Shi *et al.*, 2005). It breaks H₂O₂ into two molecules of water and one of oxygen thus reducing its toxic effect and maintaining cellular redox balance (Rama and Manjabhat, 2014). However, excessive ROS can lead to oxidative stress and cell malfunction, finally resulting in the apoptosis or necrosis (Hermes-Lima and Zenteno-Savin, 2002; Sun *et al.*, 2014). The results of current study showed a significant effect of different salinity levels on SOD and CAT enzyme activity. SOD and CAT activities in gill and liver significantly increased when salinity concentrations were escalated between the treatments (5 ppt, 10 ppt and 15 ppt) as compared to the control group. Salinity has

a major effect on osmoregulation and regulation of fish physiology which can interfere with the integrity of the physiological and biochemical mechanisms in fish (Blanchard and Grosell, 2006; Atli and Canli, 2010). Based on the study, it can be concluded that the higher salinity may cause oxidative stress in the gill tissue of fish and will be reduced, if the salinity decrease. In this work, medium with 10 ppt and 15 ppt salinity could cause ROS on the gills of Amur carp, but it was able to balance the amount of ROS with the antioxidant defense system, the enzyme CAT, thus causing the highest levels on CAT in T₂ and T₃ when compared to control and T₁. The ion balancing process takes place properly so that the ROS induced by the effect of salinity can be offset by the increased levels of CAT at 10 ppt and 15 ppt salinity.

The study on the juvenile silver pomfret, *Pampus argenteus* by Yin *et al.* (2011) showed that salinity change might stimulate SOD activity to some extent, but the activity would recover more or less with the elongation of time. Liu *et al.* (2013) indicated that increased salinity enhances liver SOD enzyme activity. In accordance with the results of present study, adding appropriate salt to water could temporarily enhance the antioxidant ability of fish. However, this strategy is insufficient or harmful for long term culture.

5.9. Histological Studies

Gills is the organ not only for respiration, it is also involved in osmoregulation, excretion of ammonia in fish. Any alteration of morphological structure of gills is the indicator of change in environmental, diet and pollutant exposure to fish. Mucus secreted by gill provides defense mechanism against several substances (Mazon *et al.*, 1999). In present study, hyperplasia with lamellar fusion and epithelial hypertrophy has been reported however, the changed were not showing any deleterious effects. Similarly alternation has been found when fish exposed to DDT and malathion (Walsh and Ribelin, 1975). The most common feature observed in present study was curved secondary gill lamellae.

5.10. Experiment- 2

Salinity of water mainly depends on the presence of dissolved salt in the water and salinity of normal sea water is same around the globe i.e. 35 ppt. The sea water of 35 ppt has 77.8 % of NaCl and 10.9 % of MgCl_2 . It is extremely difficult to measure the average composition of freshwater which is variable whereas it is globally accepted that carbonate and bicarbonate are most abundant in freshwater (Bœuf and Payan, 2001). Inland ground saline water is deficient in potassium (K^+) and contains chlorides and sulphates of sodium, calcium and magnesium salts. Inland saline waters are varying from place to place and at depths, and significant difference can be observed in ionic profiles even in waters derived from the same saline aquifer.

In inland saline regions, various strategies have been proposed and recommended by the researchers in order to enhance the growth and survival of a variety of finfish and shellfish for the aquaculture purposes. However, these strategies may not be generalized to all the species and may vary from species to species. Earlier studies conducted in these areas have proposed the idea of amendment of IGSW through potassium fertilizers for better growth and survival. IGSW fortified with potassium has improved growth and survival in various species,

Keeping all these aspects in mind, the study was designed to evaluate the effect of K^+ fortification (K_1 -25%, K_2 - 50%, K_3 -75% and K_4 - 100%, equivalent seawater of same salinity) on growth, survival and physiological response of Amur carp in IGSW.

5.11. Physico-chemical Characteristics of Water

Most of the water quality parameters were found in the optimum range and considered as vital component of any aquaculture practice. The water quality parameters needs to be maintained throughout the culture period as it may affect the growth and physiology of fish.

Temperature ranged between 25.82 ± 0.12 and 25.86 ± 0.01 °C in different treatment groups. The pH of water was varied between 7.59 ± 0.04 and 7.69 ± 0.02 and suitable for culture. The dissolved oxygen concentration of all the experimental

groups was recorded within the range of 5.96 ± 0.04 to 6.68 ± 0.04 ppm during the experimental period of 90 days. All these parameters were within the range of earlier studies (Banerjee, 1967; Santhosh and Singh, 2007; Bhatnagar and Devi, 2013; Velasco *et al.*, 2006; Ansal *et al.*, 2016). The total alkalinity was ranged from 113.89 ± 0.22 to 252.61 ± 0.40 in different treatment groups in the present study and supported by Limhang *et al.*, 2011. According to Santhosh and Singh (2007) total alkalinity ranged between 50 to 300 ppm considered as an ideal value for fish culture. Total hardness is ranged between 169.16 ± 4.73 and 3462.77 ± 5.74 in the present study in different groups and which is supported by several workers at the same salinities and different potassium groups (Kumar, 2013; Rani, 2015; Upadhyay, 2015). Ammonium and nitrite content of the experimental waters was less than 0.5 ppm in the present study which is in agreement with the findings that normal ammonia and $\text{NO}_2\text{-N}$ concentration for aquaculture is <1 ppm (Stone and Thomforde, 2004; Goran *et al.*, 2016). Ionic concentrations (Ca^{2+} , Mg^{2+} , Na^+ and K^+) of IGSW were recorded in all treatment groups. Ionic concentrations of IGSW was varying from place to place even at same salinity as reported by various authors (Mellor and Fotedar, 2005; Tantulo and Fotedar, 2006; Roy *et al.*, 2007; Saoud *et al.*, 2007; Tantulo and Fotedar, 2007; Lakra *et al.*, 2014). Potassium concentration (K^+) was found increasing with increasing level of potassium fortification which is in accordance with the earlier findings of several authors at different salinities and different potassium level (Partridge and Lymbery, 2008; Kumar, 2013; Pathak, 2013; Rani, 2015; Upadhyay, 2015).

5.12. Growth Performance of Amur Carp

In the present study, salinity and potassium concentration have significant effect on fish growth. The amendment of potassium has shown negative effect on growth and survival of Amur carp which is in contrast with the earlier findings of Doroudi *et al.* (2007). The findings of Doroudi *et al.* (2007) reported that there was no significant difference ($p > 0.05$) in growth and survival of Silver perch, *Bidyanus bidyanus* (a salt-tolerant freshwater species) in three different treatments (Freshwater, raw saline groundwater of 10 ppt and raw saline groundwater of 10 ppt with 100% potassium fortification of equivalent sea salinity). There were several works conducted on various

crustacean species like *Litopenaeus vannamei*, *Penaeus monodon* and *P. semisulcatus* and reported an increase in growth with K⁺ fortification (Saoud *et al.*, 2003; Prangnell and Fotedar, 2005; Antony *et al.*, 2015; Jahan *et al.*, 2018).

Potassium (K⁺) fortification between 40 and 100% of K⁺ concentration of equivalent sea water of 15 ppt showed better result for the growth of *Siganus rivulatus* in comparison to fortified with 15% K⁺ concentration (Mourad *et al.*, 2012) which is not true in the present study and showed opposite result. Mourad *et al.* (2012) also concluded that requirement for K⁺ is species specific. Partridge and Lymbery (2008) reported no significant difference of different potassium concentration (25%, 50%, 75% and 100% of equivalent salinity) on the growth of barramundi (*L. calcarifer*) at 15 ppt and 5 ppt in inland saline groundwater. Authors also suggested for using proportionately more potassium requirement at the salinity of 45 ppt for the culture practice of barramundi, because 50% potassium supplementation at 45 ppt salinity showed negative growth whereas fish reared at 45 ppt with 75% and 100% fortification of equivalent salinity achieved increased weight and survival. Doroudi *et al.* (2006) inferred that combination of salinity (>14 ppt) and potassium concentration (>38% of equivalent salinity sea water) can be recommended for the culture of juvenile mullet (*Argyrosomus japonicus*) in saline groundwater.

All these works were conducted on marine and euryhaline species and positive effect of K⁺ fortification on growth was observed. In the present study, Amur carp (a freshwater fish) showed that potassium did not have positive effect on growth and survival; and findings were corroborated with earlier works conducted in inland saline water of India on different species like *Pangasianodon hypophthalmus* (Kumar, 2013), Red Tilapia (Rani, 2015) and GIFT strain of Tilapia (Upadhyay, 2015).

Findings of the present and the earlier studies of various workers (Doroudi *et al.*, 2007; Kumar, 2013; Upadhyay, 2015) on freshwater fish culture in potassium fortified inland saline water stated that no potassium supplementation is required.

5.13. Carcass Composition of Amur Carp

The carcass composition of Amur carp reared at various salinities along with different potassium concentrations showed that no significant effect of potassium and combined effect of salinity and potassium. The significant effect of salinities on carcass composition observed and discussed earlier in first experiment.

5.14. Digestive Enzyme Activities of Amur carp

The digestive enzyme activities of Amur carp reared at various salinities along with different potassium concentrations showed significant effect of salinities on digestive enzyme activity and discussed earlier in first experiment, whereas no significant effect of potassium and combined effect of salinity and potassium were observed.

5.15. Physiological Response of Amur carp

The combined effect of salinity and potassium concentration was not observed in serum osmolality whereas significant difference was found in water osmolality and osmoregulatory capacity. Mourad *et al.* (2012) reported minute effect of K⁺ fortification on serum osmolality which is found similar to present study for particular salinity and their K⁺ concentration. Partridge and Lymbery (2008) reported that Barramundi, *Lates calcarifer* require more potassium in hyperosmotic (45 ppt) salinities with iso- or hypo-osmotic salinities (15 and 5 ppt, respectively).

In the present study, K⁺ fortification did not show significant effect on serum ionic concentrations while K⁺ ion was affected significantly. K⁺ supplementation did not show significant effect on plasma ionic concentration of Barramundi (Partridge and Lymbery, 2008) upto 5 and 15 ppt whereas there was significant difference was observed in Na⁺ and Cl⁻ of serum ionic level in different K⁺ concentrations. Jahan *et al.* (2018) have reported significant enhancement in serum ionic concentration (K⁺) of *L. vannamei* in fortified water. Laiz-Carrión *et al.* (2005) reported higher growth with lower activity of Na⁺ K⁺ -ATPase which is found similar in case of present experiment where activity was found increased with increasing fortification.

In addition, limited information exists with respect to the effects of salinity and potassium fortification on physiological and immunological response of fish reared in inland saline water. Since, Amur carp is a freshwater fish and no such work has been conducted to evaluate physiological response of freshwater fish. Findings of the present study indicate that K^+ fortification did not show any positive response on growth or physiology of fish. Then, amendment of IGSW with K^+ is not required for the culture of Amur carp.

1. INTRODUCTION

Aquaculture is the fastest growing animal food sector contributing 80 million tonnes towards total global fish production (FAO, 2018) and among it, 64% of the total aquaculture production is shared by the inland sector. With regards to Indian scenario, the total fish production is 11.41 million metric tonnes (MMT) with a contribution of 7.77 MMT from the inland sector which represents a lion share of the production (DAHDF, 2017-18). Although inland sector contributes higher share towards production, salinization of land and groundwater has become a pressing problem leading to significant economic, social and environmental consequences, threatening the viability of numerous rural communities (Beresford *et al.*, 2001). Soil salinization is considered the preliminary stage of environmental destruction harming the ecological harmony (Vengosh, 2003) and about 1000 million ha lands in more than 100 countries are adversely affected by salt (Sandeep *et al.*, 2013). In India salinization of inland waters, particularly in the Indus- Ganga plains of North-west region (Chadha *et al.*, 2018) is increasing at an alarming rate due to both natural and anthropogenic activities (Dhawan *et al.*, 2010). There is about 8.7 million ha of salt-affected lands; prevalent mainly in the arid and semi-arid regions of the country and about 40 % of these lands are contributed by Haryana, Punjab, Rajasthan and Uttar Pradesh (Allan *et al.*, 2009). The lands affected with primary and secondary salinization becomes ill-suited for any worthwhile agriculture activities and the gradual rise in groundwater levels with time, causes secondary salinity.

The use of saline groundwater in the land-based aquaculture is defined as inland saline aquaculture (Allan *et al.*, 2009). Aquaculture system in this region is mushrooming at faster rate and there is an urge among the stakeholders to develop it as a commercial venture in the days to come. Besides this, it offers an additional benefit of being used to reduce the amount of salt in underground water tables which will indeed lead to improvement within the close land usage for agriculture. The increasing demand for aquaculture has led to the development of this system and has been identified as an emerging area for aquaculture research and development. Furthermore, countries like Australia, China, India, Israel and the USA have demonstrated interest in utilizing their saline affected land and water resources for

commercial aquaculture (Ron *et al.*, 2002; McNevin *et al.*, 2004; Zhu *et al.*, 2004; Partridge and Creeper, 2004; Barman *et al.*, 2005). With regards to the Indian context, pioneer research was initiated on fish and prawn culture in ground saline waters of semi-arid regions of Haryana (Dwivedi and Lingaraju, 1986).

The inland ground saline water (IGSW) embraces a different ionic composition which establishes its distinction from marine and brackish water. Among different ions, low concentration of potassium ion (K^+) is the common phenomena of inland saline water around the globe. Potassium is the primary intracellular fluid which plays an essential role in many physiological functions including acid/ base balance, osmo-iono regulation and maintenance of membrane. The inappropriate levels of aqueous K^+ may have an unfavorable effect on osmoregulation (Bursey and Lane, 1971) and $Na^+ K^+$ - ATPase activity (Mantel and Farmer, 1983). The inland saline water at Rohtak, Haryana state, India is also deficient in potassium but has significantly high calcium levels and thus very high hardness (Rahman *et al.*, 2005).

Salinity is an important water quality parameter for the culture of fishes and may influence the growth by controlling the standard metabolic rate, food intake and other physiological parameters. The fluctuations in salinities also disturb digestion by controlling digestive enzyme activities (Sahoo *et al.*, 2003; Barman *et al.*, 2012) and causes salinity stress. The salinity of culture environment differs from the internal osmotic concentration of fish, and at the same time, if salinity exceeds the physiological tolerance of fish, it acts as a stressor. Under stressed conditions, fish represents many responses such as primary response which includes the release of catecholamine and stimulation of hypothalamic- pituitary- interrenal (HPI) axis to release the corticosteroid, and secondary response includes changes in metabolism, respiration, hydro-mineral balance, immune response and cellular responses (Barton, 2002).

Common carp, *Cyprinus carpio* is the important cyprinid species for aquaculture in many Asian and some European countries (Rahman, 2015) and third most widely cultivated freshwater species contributing 8% to the aquaculture in the world (FAO, 2018). It is a hardy fish that thrive in a wide variety of aquatic habitats and also tolerates a wide range of temperatures (3.0 – 35.0 °C) (Froese and Pauly, 2011), but the best growth is obtained at water temperature ranges between 23°C

and 30 °C and can survive salinity up to 9 ppt (Azizi *et al.*, 2011). The optimal pH range for carp culture varies between 6.5 and 9.0. The species can survive at low dissolved oxygen concentration (0.3 - 0.5 mg L⁻¹) as well as at supersaturation of oxygen level.

Inland saline aquaculture has been successfully done around the globe for varieties of finfish, crustaceans and algae (Forsberg *et al.*, 1996; Ingram *et al.*, 1996; Fielder *et al.*, 2001; Raizada *et al.*, 2015; Jahan *et al.*, 2018). In the inland saline region of the country, several experiments had conducted to find suitable brackish and freshwater fish species such as *Mugil cephalus* (Barman *et al.*, 2005), *Chanos chanos* (Raizada *et al.*, 2005), *Lates calcarifer* (Jain *et al.*, 2006), Red Tilapia (Rani, 2015), GIFT Tilapia (Upadhyay, 2015); *Pangasianodon hypophthalmus* (Kumar *et al.*, 2017).

Carps are the mainstay of inland aquaculture practice in India, contributing over 75% of the total aquaculture production (Jena and Das, 2016). Among the carps, Amur carp, *Cyprinus carpio haematopterus* has been identified as one of the fast-growing fish having several characteristics like slender body with low-fat content in the meat, good food conversion ratio, and natural feed selection capacity. As compared to common carp, Amur carp has approximately 29.7% and 40.33% faster growth in monoculture and polyculture systems respectively (Basavaraju and Reddy, 2013).

However, minimal works have been done on the influence of salinity and potassium ion (K⁺) on growth and physiological responses of salt-tolerant freshwater fish in the inland saline region. Therefore, the present study was conducted on Amur carp with the following objectives:

OBJECTIVES

1. To study the growth and survival of *Cyprinus carpio haematopterus* (Martens, 1876) in inland saline water at different salinities
2. To find out optimum ionic concentration with special reference to potassium ion for growth and survival of *Cyprinus carpio haematopterus* (Martens, 1876) at different salinities
3. To observe the effect of salinity on the physiology of fish

3. Materials and Methods

The present work was carried out on Amur carp (*Cyprinus carpio haematopterus*) for studying the effect of salinity at various levels and combinations along with different potassium ion concentrations on growth, survival and physiological response in inland saline water.

3.1. Experimental Site

The experiments were conducted in the wet laboratory at ICAR- Central Institute of Fisheries Education (ICAR-CIFE), Rohtak Centre, Haryana, India. The water quality and some of the physiological parameters were done in the laboratory at ICAR-CIFE, Rohtak Centre and various other parameters were analyzed in several laboratories at ICAR-CIFE, Mumbai, India.

3.2. Experimental Fish

Healthy stocks of Amur carp fry were procured from Instructional Fish Farm, College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India. The fish fry was packed in oxygen filled polythene bags, and each bag contained 1000 numbers (size: 2 to 3cm). The polythene bags were placed in a carton box and transported to the experimental site by road. After proper acclimatization, the fish were stocked carefully in a nursery pond. The fish was raised for 30 days and fed *ad libitum* with commercial floating feed (ABIS Exports India PVT. LTD, Chhattisgarh).

3.3. Preparation of Inland Saline Water at Different Salinities

The 15 ppt Inland ground saline water (IGSW) was collected from a bore-well and allowed to settle for 5 to 7 days in cemented tanks. The water was then pumped into the wet laboratory, filtered with 100 µm filter and stored in properly cleaned, disinfected with KMnO₄ and sun-dried Fiberglass Reinforced Plastic (FRP) tanks (capacity 1200 L). The stored water was diluted with bore-well water (salinity <0.5 ppt) to get desirable salinities (5 and 10 ppt). Then, prepared water was

disinfected with bleach liquor (Sodium Hypochlorite- NaOCl) at the rate of 10 ppm and after 24 hours vigorously aerated for at least 48 hours before use.

3.4. Preparation of Potassium (K⁺) Fortified IGSW at Different Salinities

The water of different salinities (T₁-5 ppt, T₂-10 ppt, and T₃-15 ppt) was amended with different potassium ion concentrations (K₁ - 25%, K₂ - 50%, K₃ - 75% and K₄ - 100%) equivalent to potassium ion concentration in seawater of equal salinity. The potassium ion concentration of IGSW water was measured, and quantity of potassium required for the amendment was calculated by using the formula made with the value given for potassium - 10.7 by Davis *et al.* (2005) and depicted in Table- 2.

Requirement of K⁺ in IGSW = (10.7 × desired salinity) - available K⁺ in IGSW

Table- 2. Availability and requirement of K⁺ ion concentration in inland ground saline water at different salinity

Salinity (ppt)	Available K ⁺ concentration (ppm) in IGSW	Requirement of K ⁺ (ppm) in IGSW
5	5.5	(10.7 × 5) – 5.5 = 48
10	9.7	(10.7 × 10) – 9.7 = 97.3
15	12.8	(10.7 × 15) – 12.8 = 147.7

3.4.1. Requirement of potassium fertilizer for fortification

The IGSW was fortified with Muriate of Potash (KCl) with the required amount at different salinities. Muriate of Potash is the trade name of commercially available potassium fertilizer (Potassium Chloride- KCl) containing 50 % K⁺ was used for fortification. The total quantity of potassium chloride required for the fortification of 1200 L FRP tanks was calculated using the formula below and depicted in Table- 3

The total quantity of KCl (mg/1200 L) = (Requirement of K⁺ in IGSW × 2) × 1200

Table- 3. Amount of KCl required for the fortification (g/1200 L)

Treatments	The quantity of KCl required for the fortification (g/1200 L)			
	K ₁	K ₂	K ₃	K ₄
T ₁	28.8	57.6	86.4	115.2
T ₂	58.38	116.76	175.14	233.52
T ₃	88.62	177.24	265.86	354.48

3.5. Acclimatization of Fish at Different Salinities

Total 1600 fish were randomly selected from nursery pond and equally distributed in four different FRP tanks (capacity 1200 L) filled with freshwater. The sufficient aeration was provided into all the containers and fish were left undisturbed for the whole night. After that, the fish were acclimatized at a desirable level of salinities such as 5, 10 and 15 ppt progressively by adding an appropriate amount of raw IGSW to avoid osmotic shock during the initial experiential period.

3.6. Experimental Design, Set up and Feeding

The present work was carried out in two separate experiments. The freshwater having 0 ppt salinity was used as control (C) for both the experiments. The fish were fed with commercial pelleted feed (crude protein 32% and lipid 4%) at the rate of 4% for first 30 days and 3% for rest of the period in two equal split doses.

3.6.1. Experiment – 1: Effect of different salinity on growth and physiological response of Amur carp cultured in Inland saline water

The experiment-1 was carried out for 90 days to evaluate the effect of different salinities on growth, survival and physiological responses of Amur carp reared in IGSW (Plate- 1). The experiment was conducted in 12 nos. of circular FRP tanks of each 500 L capacity. Each set of three tanks were filled with their respective saline water (0, 5, 10 and 15 ppt) up to 400 L and provided with sufficient aeration from a portable air-blower (Hiblow HP- 60). A total of four groups of experimental tanks named- C, T₁, T₂, and T₃ (Table- 4) were used. The tanks were arranged following Completely Randomized Design (CRD). Two hundred forty fingerlings (avg. wt. 3.48 ± 0.27 g) were equally distributed in 12 FRP tanks of each 20 nos. The fish were fed with a commercial pelleted feed having crude protein 32% and lipid 4% at

the rate of 4% for the first 30 days and at the rate of 3% for rest of the period in two equal split doses. Siphoning of water was carried out daily to remove the excess feed and fecal matters. The fresh medium was added twice a week to maintain a constant water level in all the tanks.

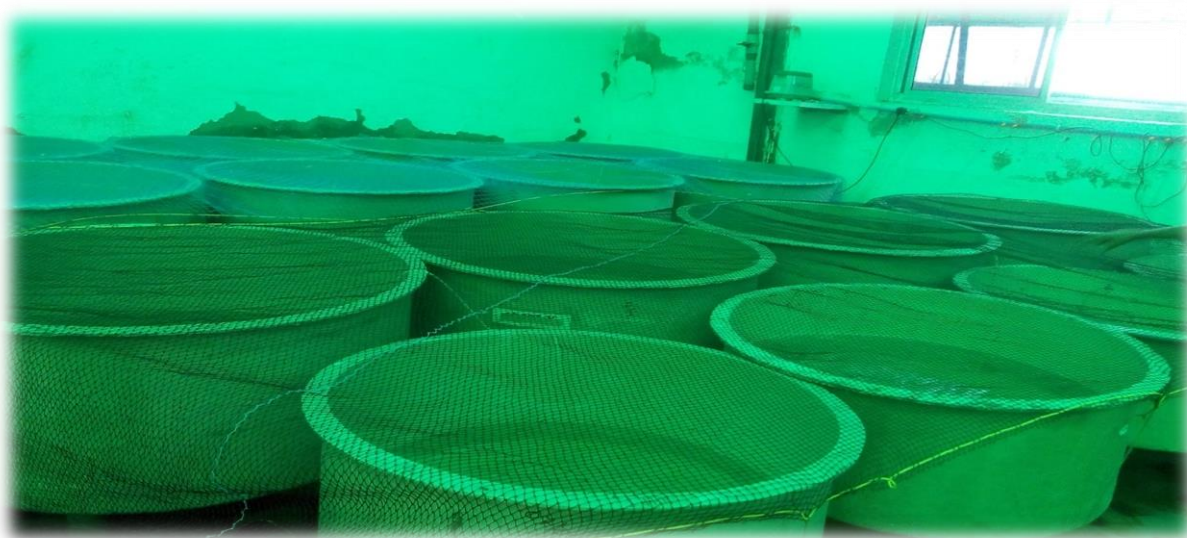
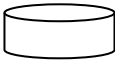
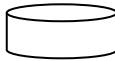
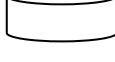


Plate- 1. Experimental Set-up

Table- 4. Experimental design and set up of the first experiment

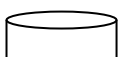
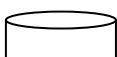
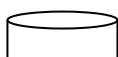
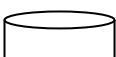
Salinity (ppt)	R ₁	R ₂	R ₃
0 - C			
5- T ₁			
10- T ₂			
15- T ₃			

3.6.2. Experiment – 2: Effect of different salinity in combination with different potassium ion concentrations on growth and physiological response of Amur carp reared in inland saline water

The experiment- 2 was carried out for 90 days to evaluate the effect of different salinities along with different potassium ion concentration on growth, survival and physiological response of Amur carp reared in IGSW. The experiment was conducted in 39 nos. of circular FRP tanks of each 500 L capacity filled up to 400 L,

and round the clock, aeration was provided from a portable air-blower (Hiblow HP-60). Seven hundred eighty fingerlings (avg. wt. 3.51 ± 0.18 g) were equally distributed in all treatments with three replicates (Table- 5). Feeding of fish and siphoning of tanks was done as same as in Experiment- 1.

Table: 5. Experimental design of the second experiment

Salinity (ppt)	K ⁺ concentration (%)	R ₁	R ₂	R ₃
<0.5 - C	-			
5- T₁	25 – K ₁			
	50 – K ₂			
	75 – K ₃			
	100 – K ₄			
10- T₂	25 – K ₁			
	50 – K ₂			
	75 – K ₃			
	100 – K ₄			
15- T₃	25 – K ₁			
	50 – K ₂			
	75 – K ₃			
	100 – K ₄			

3.7. Physico-chemical Characteristics of Water

The water quality parameters were analyzed at the beginning and throughout the experimental period at regular intervals. The water temperature, pH and salinity were recorded daily by using mercury thermometer (0.1 accuracy), pH meter (Hanna Instrument, Italy) and handheld refractometer (Atago, Tokyo, Japan) respectively, while dissolved oxygen, total alkalinity, total hardness, Ca^{2+} and Mg^{2+} were estimated titrimetrically by following the standard protocol (APHA, 2005) twice in a week. The Na^+ and K^+ ions concentration levels were analyzed with microprocessor flame photometer (Model 1382, ESICO International). The ammonium- nitrogen ($\text{NH}_4^+\text{-N}$) and nitrite (NO_2^-) were estimated once in a week using Merck water quality test kits (Spectroquant®, Billerica, MA, USA) and with a photometer (Nova 60, Spectroquant®).

3.8. Sampling and Data Collection for Growth Parameters

The growth parameters were assessed by weighing the fish at a regular interval of every 15 days for the culture period of 90 days (Plate- 2), and the various growth parameters were measured by using the following formula:

3.8.1. Percentage (%) weight gain

$$\text{Percentage (\%)Weight Gain} = \frac{[\text{Final Weight (g)} - \text{Initial Weight (g)}]}{\text{Initial Weight (g)}} \times 100$$

3.8.2. Specific growth rate (SGR)

The values for SGR is expressed as ($\% \text{ d}^{-1}$) by using the following formula

$$\text{SGR} = \frac{[\ln(\text{Final Weight (g)}) - \ln(\text{Initial Weight (g)})]}{\text{Experimental period in days}} \times 100$$

3.8.3. Feed Conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Feed intake [Dry Weight (g)]}}{\text{Body Weight gain [Wet Weight (g)]}}$$

3.8.4. Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Net weight gain [Wet Weight (g)]}}{\text{Protein Fed (g)}}$$

3.8.5. Survival (%)

$$\text{Survival (\%)} = \frac{\text{Total no. of fish on sampling day}}{\text{Total no. of fish stocked}} \times 100$$



Plate- 2. Weight measurement of fish

3.9. Proximate Analysis

Proximate analysis of carcass tissue of experimental fish was done by standard methods (AOAC, 1995) in Fish Nutrition, Biochemistry and Physiology Laboratory, ICAR – CIFE, Mumbai.

3.9.1. Moisture

The moisture content of the experimental fish was determined by taking a known weight of the sample in the petri-dish and drying it in a hot air oven at 106°C till a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula

$$\text{Moisture (\%)} = \frac{[\text{Wet weight of the sample(g)} - \text{Dry weight of the sample(g)}]}{\text{Wet weight of the sample (g)}} \times 100$$

3.9.2. Crude protein

Crude protein (CP) content of experimental fish was estimated quantitatively by micro- Kjeldahl (Kjeldahl, 1883) method (Kjelplus, PELICAN, India) after acid digestion using a fully automatic Kjeltec system. The CP is determined by multiplying the nitrogen percentage by a constant factor (6.25).

$$\text{CP (\%)} = \% \text{ Total nitrogen} \times 6.25$$

3.9.3. Total lipid

Total lipid (TL) of experimental fish was estimated by Soxhlet apparatus using petroleum ether (Boiling point- $55 \pm 5^\circ \text{C}$) as the solvent. The calculation was made as follows:

$$\text{TL (\%)} = \frac{[\text{Initial weight of the sample} - \text{Final weight of the sample}]}{\text{Initial weight of the sample}} \times 100$$

3.9.4. Total ash

Total ash (TA) content of the experimental fish body was estimated by taking a known weight of dried samples in a silica crucible and inserting it in a muffle furnace at 600°C for 5-6 hours. The calculation was done as follows:

$$\text{TA (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.9.5. Total carbohydrate

Total carbohydrate (TC) of the experimental fish was calculated by subtracting the percentage of other nutrients from 100.

$$\text{TC (\%)} = 100 - [\text{Crude protein (\%)} + \text{Ether extract (\%)} + \text{Ash (\%)} + \text{Moisture (\%)}]$$

3.10. Serum Collection

Three fish from each replicate tank were anesthetized with clove oil ($50 \mu\text{l L}^{-1}$), and blood was collected by puncturing caudal vein using a medical syringe (No. 26) and transferred in 1.5 ml Eppendorf tubes without anticoagulant. The blood collected was allowed to clot for an hour and centrifuge at 5000 rpm for 10 minutes. The supernatant (straw colored) was collected with a micropipette and transferred to another Eppendorf tube. The collected serum sample was stored at -20°C until analysis. The serum was used for the estimation of biochemical and immunological parameters.

3.11. Tissue Protein Estimation

Total protein of each tissue sample (Gill, Intestine, Liver, and Muscle) was estimated by the Bradford method (Bradford, 1976). Tissue homogenate ($20 \mu\text{l}$) was collected along with $180 \mu\text{l}$ distilled water and $250 \mu\text{l}$ 1 N NaOH was added into it followed by adding of 5 ml Bradford reagent and kept for 5 minutes. Reading was procured at 595 nm against the blank on the spectrophotometer. The protein content was expressed in mg g^{-1} wet tissue.

3.12. Sample Preparation for Enzyme Assay

At the end of the experiment, three fish were collected from each tank and anesthetized with clove oil ($50 \mu\text{l L}^{-1}$). Fish was then dissected and the tissues viz., gill, intestine, liver, and muscle, were collected immediately and 5% tissue homogenate was prepared in chilled 0.25 M sucrose solution using Teflon coated mechanical homogenizer (REMI Equipment's, Mumbai, India). The whole procedure was followed in cold condition. Homogenized samples were centrifuged at 6000 rpm for 10 minutes at 4°C . The supernatant was collected in glass vials and stored in the deep freezer at -20°C for enzyme assay.

3.13. Digestive Enzyme Activity

3.13.1. Protease activity

Protease activity was ascertained by the casein digestion method (Drapeau, 1974). The reaction mixture consisted of 1% casein in 0.05 M triphosphate

buffer (pH 7.8) and was incubated for 5 minutes at 37 °C followed by the addition of tissue homogenate. After ten minutes, the reaction was stopped by adding 10% TCA and then filtered the whole content. The reagent blank was made by adding tissue homogenate just before ending the reaction, without any incubation. One unit of enzyme activity was defined as the amount of enzyme needed to release acid-soluble fragments equivalent to $\Delta 0.001$ at 280 nm per minute at 37 °C and pH 7.8.

3.13.2. Lipase activity

Lipase activity was determined based on Cherry and Crandall (1932) method. The amount of fatty acid released in unit time was measured by the amount of NaOH required to maintain constant pH. The reaction mixture consisted of distilled water, tissue homogenate, phosphate buffer solution (pH-7) and olive oil emulsion. The mixture was mixed well and incubated at 37 °C for 24 hrs. After incubation, 95% alcohol and two drops of phenolphthalein indicator were added and titrated against 0.05 N NaOH until it showed a permanent pink color. Control was kept by denaturing the enzyme source keeping in boiling water for 5 minutes, before addition of buffer and olive oil emulsion. The milli-equivalent of alkali consumed was taken as a measure of the activity of the enzyme unit/mg protein.

3.13.3. Amylase activity

Amylase activity was estimated as the reducing sugars produced due to the action of gluco-amylase and α -amylase on carbohydrate using di-nitro-salicylic-acid (DNS) method (Rick and Stegbauer, 1974). Specific amylase activity was expressed as the mole of maltose released from starch per minute at 37 °C temperature.

3.14. Physiological Responses

3.14.1. Osmolality and osmoregulatory capacity

The samples (50 μ l) were subjected in triplicate to measure the osmolality (mOsm Kg⁻¹) of serum and water of different treatments by using a cryoscopic osmometer (Osmomat® 030, Gonotec, Germany). Osmoregulatory

capacity (OC) was estimated by subtracting the mean osmolality of rearing water salinities from their corresponding serum osmolality (Lignot *et al.*, 2000).

3.14.2. Serum ionic concentration

For the estimation of serum ionic concentration, the samples were subjected to acid digestion in a microwave digestion system. The nitric acid (HNO₃) and Hydrofluoric acid were added in a 5:1 ratio in the sample for digestion and completely digested samples were allowed to cool at room temperature. Then, the digested samples were filtered with Whatman paper of 0.45 µm pore size and a total volume of 50 ml was made with triple distilled water. The further analysis was done by Inductively Coupled Plasma- Atomic Emission Spectroscopy, ICP-AES (Spectro Analytical Instruments GmbH, Germany) as per the method of (Thompson and Walsh, 1989; Moore, 2012) at sophisticated analytical instrumentation facility, Indian Institute of Technology (SAIF-IIT), Mumbai, India.

3.14.3. Sodium potassium adenosine triphosphatase activity (Na⁺ K⁺-ATPase)

Na⁺K⁺-ATPase activity in gills was determined by the modified method of Post and Sen (1967). Four test tubes were labeled as blank (B), Standard (S), Test (T) and test + ouabain (TO). The reaction mixture consists of 0.1 M Tris HCl buffer (pH 7.8), 100 mM NaCl, 20 mM KCl, 3 mM MgCl₂, 1 mM Ouabain, 5 mM ATP and 0.1 ml of tissue homogenate. 1 ml Tris HCl buffer, 100 mM NaCl, 20 mM KCl, 3 mM MgCl₂, 5 mM ATP and 0.1 ml of tissue homogenate were added in test (T) and test + Ouabain (TO). 0.1 ml distilled water was added in test (T). 0.1 ml Ouabain was added in test tube labeled as test + Ouabain (TO). The mixture was incubated for 15 minutes, and the reaction was terminated by adding of 1 ml 10% trichloroacetic acid in test tube labeled as the test (T) and test + Ouabain (TO). After centrifugation at 3000 rpm for 5 minutes, 1 ml of the supernatant was taken for the estimation of inorganic phosphorus. The inorganic phosphorus was estimated by the method of Fiske and Subbarow (1925) which comprises of 1 ml supernatant, 1 ml of ammonium molybdate, 2.5 ml of distilled water and 0.5 ml of Amino Naphthol Sulfonic Acid (ANSA) which were added in test tube labelled as test (T) and test + Ouabain (TO). 0.1 ml standard, 0.9 ml distilled water, 1 ml ammonium molybdate, 2.5 ml distilled water and ANSA was added in test tube labeled as standard (S). 1 ml distilled water,

1 ml ammonium molybdate, 2.5 ml distilled water and ANSA was added in test tube labeled as blank (B). The reaction mixture was kept back for 5 minutes, and the OD value was observed at 660 nm. The absorbance of all test tubes was measured against the blank (B) in a spectrophotometer at 660 nm. The Na⁺K⁺-ATPase activity was expressed as nanomoles Pi released min⁻¹mg⁻¹ protein at 37 °C.

3.15. Stress Responses

3.15.1. Serum cortisol

Serum cortisol levels were estimated using the Cortisol EIA Kit (Cayman's Chemical, USA). It is a competitive assay, where competition between cortisol and cortisol tracer (Cortisol-Acetylcholine Esterase- AChE) to conjugate for a limited number of cortisol-specific mouse monoclonal antibody binding sites present in the well. Cortisol present in sample limits the binding sites for cortisol tracer (known concentration cortisol tracer was added) in the well. The unbound cortisol tracer was removed from the well by washing buffer. Cortisol tracer bound in the well produces a distinct yellow color which can be measured in 412 nm of microplate ELISA reader. The amount of cortisol tracer bound in the well is inversely proportional to the concentration of cortisol in the sample. The detection limit for this EIA kit was 35 pg ml⁻¹.

3.15.2. Serum glucose

Serum glucose estimation was carried out by Trinder's method using the ERBA kit. Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyzes the oxidative coupling of 4-aminoantipyrine with phenol to produce a coloredquinoneimine complex, with absorbance proportional to the concentration of glucose in the sample.

$$\text{Serum Glucose} = \frac{\text{Conc. of standard} \times \text{Absorbance of Test (T)}}{\text{Absorbance of standard}}$$

where, concentration of standard = 100 mg dl⁻¹

3.15.3. Aspartate aminotransferase (AST)

AST activity from different tissues (muscle and liver) of Amur carp was assayed in different tissue homogenates as described by Wooten (1964). The substrate comprised of 0.2 M DL-aspartic acid and 2 mM α -ketoglutarate in 0.05 M phosphate buffer (pH 7.4). In the treatment and control test tubes, 0.5 ml of substrate was added. The reaction was started by adding 0.1 ml of tissue homogenate in the treatment tube. The assay mixture was incubated at 37 °C for 60 minutes. The reaction was terminated by adding 0.5 ml of 1 mM 2, 4-dinitrophenylhydrazine (DNPH). In the control tubes, the enzyme source was added after the DNPH solution. The tubes were held at room temperature for 20 minutes with occasional shaking. Then 5 ml of 0.4 M NaOH solution was added, and the contents were thoroughly mixed. After 10 minutes, the OD was recorded at 540 nm against blank. The AST activity was expressed as Nanomoles oxaloacetate formed mg^{-1} protein minute^{-1} at 37 °C.

3.15.4. Alanine aminotransferase (ALT)

The procedure adopted for the estimation of ALT activity in muscle and liver of Amur carp was same as that for AST activity estimation except that the substrate comprised of 0.2 M D, L- alanine instead of aspartic acid. The ALT activity was expressed as Nanomoles pyruvate formed mg^{-1} protein minute^{-1} at 37°C.

3.15.5. Lactate dehydrogenase (LDH)

The lactate dehydrogenase (LDH) is a carbohydrate metabolic enzyme and activity was assayed by the method of Wrobleiuski and Laude (1955). The total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1 ml of phosphate buffer solution), 0.1 ml of tissue homogenate and 0.1 ml of sodium pyruvate. The reaction was started after addition of substrate sodium pyruvate. The OD was recorded at 340 nm at 30-sec interval. The specific enzymatic activity was expressed as unit mg^{-1} protein min^{-1} at 25°C where 1 unit was equal to $\Delta 0.01 \text{ OD min}^{-1}$.

3.15.6. Malate dehydrogenase (MDH)

The Malate dehydrogenase (MDH) activity was assayed in different tissues by the method of Ochoa (1955). The total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1 ml of phosphate buffer solution), 0.1 ml of tissue homogenate and 0.1 ml of freshly prepared oxaloacetate solution (2 mg oxaloacetate dissolved in 2 ml chilled distilled water). The reaction was started after addition of oxaloacetate solution as substrate. The OD was recorded at 340 nm at 30-sec interval for 3 min. The specific enzymatic activity was expressed as unit $\text{mg}^{-1} \text{proteinmin}^{-1}$ at 25 °C where 1 unit was equal to $\Delta 0.01 \text{ OD min}^{-1}$.

3.16. Immune Responses

3.16.1. Superoxide dismutase assay

The Superoxide Dismutase (SOD) activity was estimated by the method of Mishra and Fridovich (1972) with slight modifications. Liver tissue was used for the analysis of SOD activity. The assay is based on the oxidation of epinephrine-adrenochrome transition by the enzyme. The reaction mixture comprised of 50 μl of the sample, 1.5 ml of phosphate buffer and 0.5 ml epinephrine (3 mM). The solution was mixed well and the change in OD at 480 nm for 3 minutes was observed in UV spectrophotometer. One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto-oxidation. SOD articulated as unit activity (amount of protein needed to provide 50% inhibition of epinephrine auto-oxidation).

3.16.2. Catalase assay

Catalase activity (CAT) was assessed according to the method of Takahara *et al.* (1960). Gill tissue was used for the analysis of CAT activity. 2.45 ml of phosphate buffer (50 mM, pH 7.0), 50 μl of the tissue homogenate was added, and the reaction was started by the addition of 1.0 ml hydrogen peroxide (H_2O_2) solution. The decrease in absorbance was measured at 240 nm at 30-sec intervals for 2 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of H_2O_2 . The CAT activity was expressed as Nanomoles of H_2O_2 decomposed $\text{min}^{-1} \text{mg}^{-1} \text{protein}$.

3.17. Histological Study

To study the histological changes occurred in Amur carp, as a result of the effect of different salinity and different levels of potassium fortification, the fish from each experimental tank was collected at the end of the experiment and fixed in 70% alcohol at initiation. Fish gill was used for the histological study. Samples were fixed in neutral buffered formalin [In 100 ml: formalin {HCHO (40% v/v), 10 ml; Na₂HPO₄, 0.6 g; NaH₂PO₄, 0.4 g; Double distilled water, 90 ml}] was taken and conceded through the process of dehydration, block making, sectioning, staining and microscopic observations. The tissue was dehydrated in 90% alcohol for an hour and then three times in absolute alcohol for 45 minutes each separately. The tissue was cleared two times in xylene for 30 minutes and embedded in paraffin wax. The tissue was then blocked and allowed to cool. Transverse sections of 4 µm thick were cut on a rotator microtome and mounted on a microscopic slide. Mounted sections were dewaxed in xylene and dehydrated serially in alcohol. The slides were then washed in tap water for a minute, stained in haematoxyline for 12 minutes, washed in running tap water, dipped in 2 % acid alcohol and washed in running tap water for water substitution.

The sections were dehydrated through ascending and descending concentrations of 50%, 70% and 90% alcohol for 2 minutes, stained in eosin for 4 minutes and dipped in absolute alcohol for 1 minute. Finally, the stained sections were cleared in xylene for 5 minutes and mounted with DPX. Slides were examined under a Phase contrast microscope (Model: Zeiss, Scope A1, Germany) and photographs were taken at different magnification like 10x, 40x, etc.

3.18. Statistical Analysis

Data were evaluated using one-way analysis of variance (ANOVA) in the first experiment and by two- way ANOVA in the second experiment using statistical software SPSS (version 22.0). The significant differences between the treatments were determined by Duncan's multiple range test (DMRT), and the statistical significance level was assumed at 5% level of probability ($p < 0.05$) in both the experiments.

2. Review of Literature

Any scientific investigation necessitates an exhaustive review of relevant literature. It embraces the existing knowledge including significant findings, as well as theoretical and methodological contributions to a particular topic. The main idea of conducting it is to provide an insight into the array of methods and procedures used and establish a basis for the interpretation of findings. In this regard, relevant literatures found suitable for the study are incorporated under different sub-headings in this chapter for meaningful use and clear understanding.

2.1. Overview of the Problem

Increase in soil salinity is one of the severe issues affecting the aquatic and terrestrial ecosystem around the globe, particularly in arid and semi-arid regions. Saline soils do not change the physical properties of the soil but are detrimental. Salinization of the fertile agricultural lands and groundwater is a common problem, and there are several factors which pave the way for the formation of inland saline water in different parts of the world. The natural reasons attributed for the above said situation includes intrusion of seawater in to the freshwater aquifers, dissolution of subsurface salts, inadequate rainfall, etc. (Van Weert *et al.*, 2009) while increasing inland salinity due to anthropogenic activities (Williams, 2001) entails pollution, water logging, indiscriminate use of inorganic fertilizers and over irrigation. Salt-affected soils and groundwater salinity conditions prevail either in isolation or in combination with each other (Joshi and Tyagi, 1994). On the basis of electrical conductivity (EC) of the soil solution that detects osmotic problems and exchangeable sodium percentage (ESP) indicative of a physical dispersion, soils can be grouped into three categories as depicted in Table- 1 (Singh *et al.*, 2017). The soil with $\leq 15\%$ ESP and ≤ 4 dSm⁻¹ electrical conductivity is categorised as non- saline or non- sodic soils.

Table: 1 Classification of soil

Salt-affected soils	ESP (%)	EC (dSm ⁻¹)	pH
Saline soil	≤ 15	> 4	< 8.5
Sodic soil	> 15	≤ 4	> 8.5
Saline sodic soil	> 15	> 4	8.5

2.2. Global Status of Inland Saline Aquaculture

Inland saline groundwater distributed in semi-arid and arid regions occur in areas of more abundant rainfall as a result of underground salt deposits, connate water of marine origin and saltwater intrusion in the coastal regions (Boyd *et al.*, 2009). Thus, inland ground saline waters (IGSW) have adversely influenced the agricultural outputs and environment in different parts of the world such as USA, Australia, India, China and Israel (Allan *et al.*, 2001). Characteristics of inland saline water differ from location to location in terms of both salinity and ionic composition and salinity varying from >0.5 to 165 ppt. Generally, the salinity of the inland saline water ranges from 10-25 ppt with a high level of Ca^{2+} and Mg^{2+} , which has resulted in high water hardness (Jana *et al.*, 2004). Chadha *et al.* (2018) mentioned that it is essential to have knowledge about the chemical nature of IGSW water for aquaculture purposes and inferred that saline water contains sodium, calcium, and magnesium along with chlorides and sulphates.

Inland saline aquaculture is land-based aquaculture using ground saline water and identified as a remedy to utilize such water for the culture of many marine, brackish water, and salt- tolerant freshwater fish. Aquaculture in these regions has enormous potential for income generation (Trendall and Pitman, 1998; Beresford *et al.*, 2001). It will help in reducing underground saline water thus preventing secondary salinization and will assist in the development of agricultural land. A variety of aquatic crustaceans, algae, and finfish have been reared in inland saline water around the world (Fielder *et al.*, 2001; Ingram *et al.*, 2002; Saoud *et al.*, 2003; Zhu *et al.*, 2004; Allan *et al.*, 2009). Countries like Australia, China, India, Israel, and the USA have demonstrated an interest in utilizing their saline affected land and water for commercial aquaculture (Ron *et al.*, 2002; McNevin *et al.*, 2004; Partridge and Creeper, 2004; Barman *et al.*, 2005). Allan *et al.* (2008) reported that in Australia, saline groundwater from shallow and deep aquifers has been found suitable for growth and survival of crustaceans (Allan and Fielder, 1997) and euryhaline finfish, such as Australian bass, (*Macquaria nooemaculeata*) Barramundi (*Lates calcarifer*) and Atlantic salmon (*Salmo salar*) (Allan *et al.*, 2001). There are abundant numbers of marine species including: *Sciaenops ocellatus*, red drum (Forsberg *et al.*, 1996; Forsberg and Neil, 1997), *Lates calcarifer*, Barramundi

(Partridge and Lymbery, 2008), *Litopenaeus vannamei*, white leg shrimp (Samocha *et al.*, 1998) and *Penaeus monodon*, tiger prawns (Flaherty and Vandergeest, 1998) have been commercially cultured in moderately saline inland groundwater.

2.3. National Scenario of Inland Saline Aquaculture

Salinization of inland waters is increasing at an alarming rate due to both natural and anthropogenic activities, particularly in the north-west region of the country (Dhawan *et al.*, 2010). There are about 1.93 million square kilometers area facing groundwater salinity (Lakra, 2014; Lakra *et al.*, 2014) prevalent mainly in the arid and semi-arid regions of Andaman & Nicobar, Andhra Pradesh, Delhi, Gujarat, Haryana, Karnataka, Maharashtra, Punjab, Rajasthan, Uttar Pradesh, and Tamil Nadu. In Haryana, Bhiwani, Hisar, Jhajjar, Jind, Mohindergarh, Rohtak and Sirsa districts are mainly affected while south-western districts of Punjab like Bhatinda, Faridkot, Firozpur, Mansa and Muktsar are affected with groundwater salinity (Dhawan *et al.*, 2010). Dhawan *et al.* (2009) reported salinity between 23- 165 ppt in some villages of Muktsar district (Punjab). There are more than 15 districts affected with soil salinization in Uttar Pradesh which includes Aligarh, Azamgarh, Bareilly, Etah, Etawah, Fatehpur, Faizabad, Ghazipur, Hardoi, Kanpur, Lucknow, Mainpuri, Meerut, Pratapgarh, Raibareilly, Sultanpur and Unnao. Soil salinization in Rajasthan persists in the area of Ajmer, Barmer, Bhilwara, Bikaner, Churu, Jaisalmer, Jalore, Jodhpur, and Pali districts. Such types of lands are also found in western Maharashtra primarily confined in Karad, Pune, Raigad, Sangli, Satara and Thane districts (Mandal *et al.*, 2010). The saline soils of Maharashtra are quite distinct than Haryana and Rajasthan; both the states have saline groundwater whereas Maharashtra has rich sulphate soil with fresh groundwater. Saline groundwater is abundant in India, and there is growing interest in developing commercial inland saline aquaculture. Salinity and ionic concentrations of the groundwater gets influenced by the depth of the aquifer and location. Analysis of these areas at Lahli and Baniyani village of Rohtak District, Haryana indicated that the salinity of the groundwater increased at deeper zones. In India, ICAR- Central Institute of Fisheries Education, (Rohtak Centre, Haryana, India) had conducted several experiments on hatchery and grow-out technologies of giant freshwater prawn, *Macrobrachium rosenbergii*, under project entitled 'Developing aquaculture in degraded inland areas

in India and Australia'; funded and collaborated with Australian Centre for International Agricultural Research (ACIAR) during 2004-10. Similarly, niche area project funded by Education Division of ICAR, New Delhi entitled 'Utilization of inland saline and sodic soils for Aquaculture' for providing promising technology and better utilization of unutilized resources was conducted in the similar lines. Under this project, technology was developed for the culture of Tiger shrimp (*Penaeus monodon*) in high saline water (15 ppt) of Baniyani unit of ICAR-CIFE, Rohtak Centre, Haryana. Another project entitled "Upscaling, demonstration, and diversification of technologies for finfish and shellfish in inland saline soils" was in operation during 2012-16 which made significant breakthrough in development of various commercial feasible technologies for culture of Pacific white shrimp, *Litopenaeus vannamei*, *P. monodon*, GIFT, Red Tilapia and Pangasius in low inland ground saline water. Preliminary studies on the pilot scale were also initiated on Amur carp, Pompano and Cobia successfully under this project.

In inland saline regions of the country, several experiments have been conducted to identify suitable marine (Dwivedi, 1984) and brackish water species like *Chanos chanos*, *P. monodon*. The ICAR-CIFE also worked on freshwater species like *M. rosenbergii*. Earlier studies conducted by Dwivedi and Lingaraju (1986) reported good survival of *C. chanos*, *Mugil cephalus*, *Etroplus suratensis* and *P. monodon* in Sultanpur, Distt. Gurgaon (Haryana) at 8-10 ppt. However, later studies reported higher survival of *C. chanos* and complete mortality of *P. monodon* cultured at 23 ppt in saline groundwater of Lahli (Rohtak), Haryana (Jain *et al.*, 2003).

Barman *et al.* (2005) worked on *M. cephalus* at different salinities (0, 10, 15, 20 and 25 ppt) and found the best result at 10 ppt. They also reported that higher salinity affected the growth and metabolism of fish. Jana *et al.* (2006) investigated the effect of IGSW on growth performance and nutritional physiology of *C. chanos* under field and laboratory conditions and found the best result at 25 ppt of salinity. Barman *et al.* (2012) experimented on *C. chanos* and revealed that IGSW of high salinity could be utilized for the culture and maximum growth was observed at 25 ppt salinity. Upadhyay (2015) found that GIFT strain of Tilapia can survive upto 45 ppt in IGSW while most adaptable salinity was upto 5 ppt. The study also concluded that IGSW upto 5 ppt could be utilized for the culture of GIFT and also

found that a decrease in growth with an increase in salinity. Kumar *et al.* (2017) worked on growth and salinity tolerance of *P. hypothalamus* and reported that 10 ppt salinity was optimum for the culture of species while can survive upto 15 ppt in IGSW.

2.4. Importance of Ions and Ionic Manipulation in Inland Saline Aquaculture

Seawater has near uniform salinity and almost a similar ionic composition around the world while in contrast, saline groundwater has varying salinity and ionic composition from place to place. A relative number of studies have been conducted to evaluate the ionic concentrations in inland saline water and identified invariably low potassium ion concentration compared to seawater of equivalent salinity (Boyd and Thunjai, 2003; Gong *et al.*, 2004). Deficiency of potassium in inland ground saline water has been reported from Australia, China, India, and the USA in different studies (Saoud *et al.*, 2003; Partridge *et al.*, 2008; Lakra *et al.*, 2014). It is an accepted fact that inland ground saline water is deficient in potassium, but no such general statement can be made in the case of Ca^{2+} , Mg^{2+} , and SO_4^{2-} . The levels of sodium and chloride were found almost similar to the level of equivalent salinity of seawater in most of the saline groundwater (Boyd *et al.*, 2002; Boyd and Thunjai, 2003). Saline groundwater can contain as low as 5% and as high as 75% K^+ -equivalence of equivalent salinity seawater (Fielder *et al.*, 2001; Partridge and Furey, 2002). However, in a review of saline groundwater sources, Partridge and Creeper (2004) reported that most of those assessed for mariculture contain approximately 20% K^+ -equivalence. In fish, potassium plays an additional critical role in osmo and ionic-regulation and acid/base balance (Marshall and Bryson, 1998; Evans *et al.*, 2005). Partridge and Creeper (2004) suggested that the physiological effects of potassium deficiency are dependent on salinity.

Rahman *et al.* (2005) reported low potassium and magnesium concentration and a high calcium level of IGSW of various salinities (10, 20 and 30 ppt) of equivalent salinity seawater. Jain *et al.* (2006) and Raizada *et al.* (2015) also reported a low concentration of potassium in Rajasthan and Rohtak (Haryana) respectively. Ionic manipulation in inland saline water for commercial shrimp farming is found suitable for better survival. The potassium ion source is the most vital input required for amendment and Muriate of potash is the most widely used agent for the

fortification of inland saline water. Muriate of potash contains approximately 50% potassium and commonly used in the agricultural sector as fertilizer.

In fish, potassium plays critical roles in osmoregulation and acid/base balance (Marshall and Bryson, 1998; Evans *et al.*, 2005). Partridge and Lymbery (2008) reported no significant difference of different potassium concentration (25%, 50%, 75% and 100% of equivalent salinity) on the growth of barramundi (*L. calcarifer*) at 15 ppt and 5 ppt in inland saline groundwater. Authors also suggested for using proportionately more potassium requirement at the salinity of 45 ppt for the culture practice of barramundi, because 50% potassium supplementation at 45 ppt salinity showed negative growth whereas fish reared at 45 ppt with 75% and 100% fortification of equivalent salinity achieved weight and survival.

Doroudi *et al.* (2006) inferred that combination of salinity (>14 ppt) and potassium concentration (>38% of equivalent salinity sea water) could be recommended for the culture of juvenile mullet (*Argyrosomus japonicus*) in saline groundwater. Doroudi *et al.* (2007) worked on Silver perch, *Bidyanus bidyanus* and found no significant difference in absolute growth rate, survival and food conversion ratio between treatments (Freshwater, 10 ppt raw saline groundwater and 10 ppt raw saline groundwater with 100% fortification of equivalent salinity sea water).

Mourad *et al.* (2012) reported that potassium deficient at 15 ppt inland well water is not suitable for long-term culture of rivulatus rabbit fish, *Siganus rivulatus* and also concluded that K^+ does not appear to be the only limiting element in inland low saline groundwater that affects fish osmoregulation. Besides this, Reddy and Harikrishna (2014a and 2014b) has reported that K^+ fortification is essential for commercial farming of *P. monodon* and *L. vannamei* in saline affected soils using inland ground saline water at ICAR- Central Institute of Fisheries Education (ICAR-CIFE), Rohtak Centre (Haryana). However, Kumar (2013) and Upadhyay (2015) worked on *P. hypophthalmus* and GIFT strain of Tilapia, respectively, where they reported that K^+ - fortification does not influence growth in IGSW.

2.5. Biology and Distribution of Amur Carp

Amur carp (*Cyprinus carpio haematopterus*) is an ancient form that originated from the Asian Carp Centre (China) and spread to the water bodies of Western Asia. In Asia, the species is distributed in Amur- Chinese region or far Eastern region. Amur carp inhabit the River Amur, which is located in the northern area between Russia and China and due to its presence in River Amur, the species is commonly known as 'Amur carp' or 'Amur-Chinese carp'. This carp belong to the family Cyprinidae and genus *Cyprinus*. Many distinctive strains of the genus have been identified, and the distinction is mainly made based on differences in morphometric characters, i.e. the number of dorsal fin rays, lateral line scales, gill rakers on the first-gill arch and vertebrae (Komen, 1990). Amur carp have lower counts of dorsal fin rays and gill rakers compared to other carp from Central- Asian regions. Four subspecies of *Cyprinus carpio* has been reported by Jhingran and Pullin (1985) - *C. carpio carpio*, *C. carpio aralensis*, *C. carpio haematopterus*, *C. carpio viridiviolaceus*. In contrast, Balon (1995) mentioned only two subspecies - *C. carpio carpio* and *C. carpio haematopterus*, while Froese and Pauly (2011) did not recognize any sub-species of this genus.

C. carpio haematopterus (Amur carp) was brought into the gene bank of Fish Culture Research Institute (FCRI), Szarvas (Hungary) in the year 1982 from the Russian National Fisheries Research Institute. The Amur carp stock of Fisheries Research and Information Centre (FRIC), Hesaraghatta, Bangalore, Karnataka, India was received from Freshwater Fisheries Research Centre, Hungary for evaluation and breeding programme. This stock has been maintained here for the past ten years improving its purity and quality through genetic management of stocks and improved broodstock management as well as hatchery practices. They are omnivorous, with a high tendency towards the consumption of animal food, like water insects and their larvae, worms, mollusks, and zooplankton. It also has characteristics of late maturity and first spawning occurs at the end of the first year. Komen (1990) mentioned that Amur carp are more resistant to low temperatures. *C. carpio* is generally regarded as a freshwater fish which proliferates in waters of less than 0.5 ppt salinity (Zhang and Tang, 1989) while salinity tolerance of *C. carpio* fingerlings are found to be 10.5 ppt (Wang *et al.*, 1997).

2.6. Effect of Salinity on Growth Performance of Freshwater Fish

Water quality parameters are sine qua non to be maintained within the suitable range for any aquaculture operation, and salinity is a critical ecological factor which is species specific. The salinity tolerance of freshwater fishes varies with the change in species, age, size and acclimating conditions. Dwivedi and Lingaraju (1986) carried out experiments to observe the feasibility of Indian major carps and common carp in IGSW of 8-10 ppt and concluded that Indian major carps could not tolerate beyond 5 ppt whereas common carp had grown to a size of 550 gm in less than a year and also had achieved maturation. Tewari *et al.* (1994) attempted culture of Indian major carps in salt-affected soils of Uttar Pradesh and summarized that salt-affected soils treated with cow dung could be successfully utilized for the culture of these carps. Raizada *et al.*, 2005 reported that monoculture of giant freshwater prawn was found to be suitable in inland saline aquaculture and depicted 1000 kg/ha production in six months with an average weight of 40.66 ± 19.60 g. The seed production of the species was also reported from ICAR- CIFE, Rohtak Centre in the inland saline area. The preliminary studies conducted in Punjab revealed that the freshwater carps and prawn could be cultured in the inland saline area with salinity upto 10 ppt (Dhawan *et al.*, 2009; Dhawan *et al.*, 2010).

Bœuf and Payan (2001) reviewed and stated that salinity has a significant impact on the growth of the majority of freshwater species so far studied. A freshwater fish, *Bidyanus bidyanus*, from Murray- Darling Basin (Australia) showed the highest growth rate at 4 ppt and decreasing growth with increasing salinity (Kibria *et al.*, 1999). The juveniles of *Nannoperca obscura* (Yarra pygmy perch) showed increasing growth for moderate salinities (i.e., 2.5 – 7.5 ppt).

Ghosh *et al.* (1973) reported that common carp could be profitably cultured upto a salinity of 5 ppt and there was no adverse effect on the fish even when the salinity was raised to 7 ppt. Wang *et al.* (1997) evaluated the influence of different salinities (0.5 to 14.5 ppt) on food consumption, growth, and energy conversion efficiency of common carp fingerlings and found highest specific growth rate in freshwater which reduced with an increase in salinity and showed negative percentage at 10.5 ppt. They also reported the highest daily food consumption rate in freshwater.

Maceina *et al.* (1979) showed that the survival rate in grass carp could be increased with proper acclimatization during the transfer of fish from low to high saline water. Pillai *et al.* (2003) worked on *Labeo rohita* under field and laboratory conditions and found that fish can survive upto 8 ppt., the best growth being observed at 0 and 2 ppt. They also reported 100% mortality within 7-8 days at 14 ppt. Islam *et al.* (2014) worked on *L. rohita* to found the effects of salinity (0, 2, 4, 6, 8, 10 and 12 ppt) and reported 100 % survival upto 6 ppt while 100% mortality at 10 and 12 ppt. The findings of the experiment also revealed lower food conversion ratio at 0 ppt. Rani and Gulia (2015) experimented on *C. mrigala* to find out the effect of different salinity (0, 2 and 4 ppt) on growth and reported highest specific growth rate at 0 ppt and lowest at 4 ppt.

Sahoo *et al.* (2003) inferred that *Clarias batrachus* fingerlings can be reared in 2 ppt salinity without affecting growth, survival and biomass production adversely. The study revealed that at higher salinity, weight loss occurred due to low feed intake and increased osmotic stress. Sarma *et al.* (2013) found better growth and survival of *C. batrachus* at the freshwater and decreasing trend with an increase in salinity (4 and 8 ppt).

Dubey *et al.* (2016) studied the effect of salinity on survival and growth of *Channa punctata* and reported good tolerance up to 10 ppt while maximum growth and survival was observed at 0 and 5 ppt respectively, without significant difference between both the treatments. Chowdhury *et al.* (2014) stated that *Anabas testudineus* (Thai strain) could tolerate a wide range of salinity (0, 8 and 10 ppt) and better growth and survival was observed at higher salinity in comparison to freshwater. Mangat and Hundal (2014) worked on salinity tolerance of common carp fingerlings reared at different salinities (0, 1.5, 3, 6 and 12 ppt) during different seasons for 60 days and reported 100% survival rate in all the seasons (summer, autumn and winter) up to 6 ppt, while 100% mortality was observed within 3- 4 days during summer and autumn at 12 ppt salinity whereas 50% survival rate was found during winter at the same salinity.

2.7. Effect of Salinity on Carcass Composition of Fish

Carcass composition is the right indicator of the physiological condition of a fish and includes the analysis of moisture, protein, fat, ash and carbohydrate. It is used as an indicator to assess the nutritional status of fish. Various studies have been made on body compositions of various species like *Cyprinus carpio* (Geri *et al.*, 1995), Atlantic salmon (Dempson *et al.*, 2004), *Rutilus frisii* (Gholampoor *et al.*, 2011), *Barbus grypus* (Jalali *et al.*, 2013), *Capoeta damascina* (Fallah *et al.*, 2013), *Pangasianodon hypophthalmus* (Kumar *et al.*, 2016).

Freshwater fish species are an important source of polyunsaturated fatty acids (PUFA) like marine water fishes, and these species can maintain the high level of EPA and DHA levels even when they are fed with a diet deficient in EPA and DHA (Guillou *et al.*, 1995). Trbović *et al.* (2013) evaluated two different diets (maize and extruded feed) on proximate composition and fatty acid profile in common carp and postulated that diet could influence proximate composition, i.e. protein, lipid and ash were higher in the extruded feed.

2.8. Effect of Salinity on Digestive Enzymes Activity of Fish

Digestive enzyme activities are influenced by the biotic, abiotic and water content of gut (Devi and Aravindan, 1997; Bœuf and Payan, 2001). As reported by Haliloglu *et al.* (2004) salinity affects the digestibility of protein, lipid and some dietary fatty acids in trout species. Devi and Aravindan (1997) observed the effect of different salinity (0, 8.7 and 25 ppt) on the digestive activity of *Oreochromis mossambicus* (Peters) and concluded highest lipase and amylase activity at 8.7 ppt, due to the isotonicity of the medium and body fluid of the fish.

Amylase is a vital carbohydrate digestive enzyme in fish, which converts the polysaccharide starch into disaccharide maltose by its hydrolysis action (Abowei and Ekubo, 2011). In general, amylase activity is higher in herbivorous and omnivorous rather than in carnivorous fishes. Tsuzuki *et al.* (2007) reported higher digestive enzyme activity at 15 ppt when *Centropomus parallelus* was reared at different salinities (5, 15 and 35 ppt). Lipase activity in fishes may differ from higher vertebrate since fish is a lower vertebrate and maintaining acidic stomach is more expensive for their bodies (Achionye-Nzeh *et al.*, 2005).

M. cephalus showed higher digestive enzyme activity maintained at 10 ppt in comparison to fish reared at different salinities (10, 15, 20, 25 and 30 ppt) (Barman *et al.*, 2005). Barman *et al.* (2012) found higher enzyme activity (Protease, Amylase, Cellulose, and Lipase) in milkfish reared at 25 ppt compared to fish reared at other salinities (0, 5, 10, 15, 20 and 30 ppt). Senlin (1998) reported higher digestive enzyme activity of *Pagrosomus major* at 25 ppt and decreased with increasing salinity.

2.9. Effect of Salinity on Physiological Response of Fish

Fish displayed different physiological response when exposed to various salinities and based on the reaction showed; fish can be grouped into two types, i.e., stenohaline and euryhaline. Stenohaline freshwater fish hyper-regulate to allow a continuous influx of water. When exposed to saltwater, euryhaline freshwater fish are hypo-regulators that prevent net water loss by drinking water from the external environment to keep the composition of body fluids at a constant level (Assem and Hankf, 1979). Teleost exposed to hyperosmotic environments showed osmotic water loss coupled with diffusive ion gains and to compensate the water loss, an increase in drinking rate becomes necessary for fish (Brocksen and Cole, 1972; Foskett *et al.*, 1981).

Osmoregulation is an essential tool in environmental adaptation in the majority of fishes (Pequeux, 1995) and gill, intestine and kidney are the prime organs for osmoregulation in fishes. Exposure of freshwater fish to high saline water (salinity stress) is capable of changing the serum osmolality and serum protein (Peyghan *et al.*, 2014). Grau *et al.* (1994) reported that Prolactin (PRL) cells present in pseudobranch of gills act as a chemoreceptor to provide information about water salinity. Chloride cells present in pseudobranch of gills of the fishes play a significant role in osmoregulation. In freshwater fishes, chloride cells pump the salt ions from the external environment into fish body fluid; seawater fishes, chloride cells pump the salt ions from body fluid into the external atmosphere (Zadunaisky, 1996). The body fluids of freshwater fishes are hypertonic, and they osmoregulate by producing copious urine as well as by active uptake of ions through gills (Sahoo *et al.*, 2003). Fashina-Bombata and Busari (2003) stated that the regulation of plasma ions to maintain internal osmotic pressure depends on the salt tolerance of fish, regulating

capacity and environmental salinity. The intestine also has an important role in osmoregulation and in higher salinity movement of food in intestine may become faster rate which leads to incomplete digestion and lower absorption of nutrients (Barman *et al.*, 2012).

The energetic cost for ionic regulation of fishes increases with alteration of salinity away from their iso-osmotic conditions. In the iso-osmotic medium, fishes spend less energy to maintain the osmoregulation, and this saving energy is substantial enough to increase growth (Arnason *et al.*, 2013). The higher salinity can cause an imbalance in osmoregulation which leads to the death of the fishes. Bøeuf and Payan (2001) stated that in critical situations growth is stopped because osmoregulation is not controlled entirely and under such severe conditions fish can survive, but it cannot grow further. In higher salinities, fishes spend more energy to maintain an osmotic balance between the body fluid and external environment. The blood electrolytes (Na^+ , K^+ , Ca^{2+} and P^+) are commonly used to determine the physiological characteristics, toxicity and health status of fish (Percin *et al.*, 2010). Stress caused by salinity can change the blood composition and immune mechanisms. Na^+ and Cl^- form the significant ions contributing at least 90% in the blood of most of the teleost fishes and plays a vital role in osmoregulation and homeostasis; and rest 10% are met by K^+ , Ca^{2+} , proteins and small organic molecules (Gilles and Delpire, 1997).

$\text{Na}^+ \text{K}^+$ -ATPase activity in the gill of teleost fishes play an essential role in regulating the serum ionic concentrations (Martinez-Alvarez *et al.*, 2005) and involved in the mechanism of freshwater to seawater acclimation. Higher levels of salinity results in the augmentation of $\text{Na}^+ \text{K}^+$ -ATPase activity as well as morphological changes in chloride cells (mitochondrial rich cells) (Uchida *et al.*, 1996). The changes in environmental salinity are known to alter the number, size, and location of branchial cells. Changes in morphology of chloride cells the following transfer to saltwater and freshwater suggest that filament and lamellar chloride cells are essential in seawater and freshwater osmoregulation respectively.

2.10. Effect of Salinity on Stress Response of Fish

The stress response in teleost fish shows many similarities to that of the terrestrial vertebrates. Cortisol and Glucose are considered as a stress indicator because in stressed situation fish show the plasmatic rise of these chemicals (Barcellos *et al.*, 1999). Cortisol is the major corticosteroid in teleost fish, and during stress conditions, its concentration in the blood rises gradually (Mommsen *et al.* 1999). Morgan *et al.* (1997) reported that Tilapia (*O. mosambicus*) raised in seawater showing a lower blood cortisol and glucose level compared to freshwater and 12 ppt reared tilapia.

Measurement of metabolic enzyme activity can also serve as a valuable stress indicator in organisms like marine invertebrates and fishes (Dahlhoff, 2004). The gluconeogenic pathway is active in fishes since they prefer to utilize the protein and lipid rather than carbohydrate for energy requirements and under stress glucose is mainly derived by gluconeogenesis (Vijayan *et al.*, 1993). The stress response in fish can be manifested through crucial gluconeogenic enzymes, enzymes of protein metabolism mainly transaminases, the terminal enzyme of the glycolytic pathway (i.e., lactate and malate dehydrogenase- LDH and MDH). Tsuzuki *et al.* (2001) reported that moderate salinities reduce the stress responses and ionic/osmotic imbalances in adult *Odontesthes bonariensis* (Pejerrey). Cortisol is also known to increase the activity of amino acid metabolizing enzymes (Aspartate and Alanine amino transferase- AST and ALT) and Glutamate dehydrogenase (GDH) in different animal tissues (Mommsen *et al.*, 1999). Jumah and Traifalgar (2015) reported an elevated level of cortisol, glucose, AST, and ALT at higher salinities.

2.11. Effect of Salinity on Immune Response of Fish

Martínez-Álvarez *et al.* (2005) reported that increased environmental salinity strengthened the antioxidant defense activities like superoxide dismutase (SOD) and catalase (CAT) in sturgeon fish (*Acipenser naccarii*). Shin *et al.* (2010) studied the immune responses like SOD and CAT of olive flounder (*Paralichthys olivaceus*) on oxidative stress induced by hypo-osmotic condition. Yanjiao *et al.* (2011) investigated the non-specific immune response and disease resistance of

juvenile turbot in different salinities challenged with *V. anguillarum*. Arnason *et al.* (2013) reported that reduction of salinity was not showing any detrimental effect on immune parameters of Atlantic cod (*Gadus morhua*).

2.12. Effect of Salinity on Histology of Fish

Fish gills are one of the most sensitive organs, which reacts first in the changed environment since respiration, excretion and osmoregulation is performed through the gills (Mallatt, 1985; Lease *et al.*, 2003). The gill has a delicate structure and responds to environmental changes by structural alteration. Fishes exposed to contaminants both in the laboratory (Thophon *et al.*, 2003) and field studies (Hinton *et al.*, 1988) have been evaluated through investigating the histopathological changes. An added benefit of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney, and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al.*, 2001). The alterations found in these organs are relatively easier to identify than the functional ones (Fanta *et al.*, 2003), and serve as warning signs of damage to animal health (Hinton *et al.*, 1988).

Ghahremanzadeh *et al.* (2014) studied the size and number of chloride cells in Kutum (*Rutilus frisii*) from brackish (Caspian Sea) and freshwater (Khoshkrood River) environments for a better understanding of the osmoregulation of the species and found larger average size and number of chloride cells in the stocks from the Caspian Sea. Moghddam *et al.* (2013) reported that different types of chloride cells and gill structure are affected in *Liza aurata*, when 12 ppt adapted fry were directly transferred to higher salinities (40, 45, 55, 60, 65 and 70 ppt). 100% mortality was observed in salinity more than 60 ppt. Gill alterations showed edema of the epithelial cells along with the breakdown of pillar cells, hypertrophy and hyperplasia of epithelial and chloride cells.

2.13. Effect of K⁺ Fortification in IGSW on Enzymes

Jahan *et al.* (2017) reported significant difference in enzymatic activity (SOD, Catalase, AST and ALT) of haemolymph of *L. vannamei* reared at 10 ppt (IGSW and 100% K⁺- Mg²⁺ fortified IGSW of equivalent sea water) and fed with three

different gelatin coated diets (various combinations of K^+ - Mg^{2+}). Digestive enzyme activity was significantly higher in the fortified IGSW group. The findings also revealed that there is stress in treatments where lack of dietary minerals in IGSW compared to fortified IGSW.

Based on the above review, the present experiment was planned to assess the feasibility of Amur carp culture in IGSW. However, a limited number of information was available on the effect of K^+ concentration on growth and physiological response of the fish.

4. RESULTS

Two separate experiments of 90 days each were carried out to find out the feasibility of IGSW with and without K⁺ amendment for the culture of Amur carp.

4.1. Experiment – 1: Effect of different salinity on growth and physiological response of Amur carp cultured in Inland saline water

The first experiment was conducted in the raw IGSW of varying salinities (5, 10 and 15 ppt) to find out the growth and physiological response of Amur carp in comparison to freshwater (<0.5 ppt) as control.

4.2. Physico-chemical Parameters of Water

The physico-chemical parameters of water such as salinity (ppt), temperature (°C), pH, dissolved oxygen (ppm), NH₄⁺-N (ppm), NO₂-N (ppm), total alkalinity (ppm), total hardness (ppm), calcium and magnesium hardness (ppm), and ionic concentrations (Na⁺, K⁺, Ca²⁺ and Mg²⁺) were recorded and average values of all the parameters in accordance with treatments are depicted in Table- 6. In addition to this, value given in the bracket is depicting the range of particular water quality parameter varied during the culture period.

The salinities of all the treatments were estimated daily and mean values observed were 0.13 ± 0.00 , 5.08 ± 0.01 , 10.04 ± 0.01 and 15.04 ± 0.01 in control, T₁, T₂, and T₃ respectively. The water temperature of all the four experimental groups were recorded throughout the experimental period and were found in the range of 25.78 ± 0.12 to 25.88 ± 0.10 °C. The pH remained at optimum level and values were recorded within the range of 7.59 ± 0.04 to 7.69 ± 0.07 . The dissolved oxygen concentration of all the experimental groups was varied between 6.34 ± 0.13 and 6.52 ± 0.11 ppm during the experimental period of 90 days.

The NH₄⁺- N content of all the four experimental groups was recorded once in a week, before water exchange. It was found in the range of 0.22 ± 0.01 to 0.25 ± 0.01 ppm. The NO₂ - N content of all the four experimental groups was found between 0.03 ± 0.01 and 0.05 ± 0.01 ppm. Total alkalinity ranged between $113.89 \pm$

0.22 and 304.38 ± 0.83 ppm in all the experimental groups. Total hardness varied between 169.16 ± 4.73 to 3232.50 ± 9.83 ppm among the treatment groups according to their salinity. The calcium and magnesium hardness obtained in the range of 101.94 ± 2.37 to 830.00 ± 7.12 and 67.22 ± 2.64 to 2402.50 ± 6.82 ppm, respectively.

The ionic concentration of Ca^{2+} , Mg^{2+} , Na^+ and K^+ were measured twice a week in IGSW and significantly varied between all the treatment groups. The ionic concentration of all the ions was increasing with increase in salinity. Ca^{2+} concentration varied from 40.78 ± 0.95 to 332.00 ± 2.85 ppm, Mg^{2+} from 16.34 ± 0.64 to 583.81 ± 1.66 , Na^+ varied between 9.67 ± 0.75 and 4464.44 ± 13.31 and K^+ ranged from 2.48 ± 0.06 to 12.77 ± 0.01 .

Table: 6. Physico-chemical parameters of IGSW used in the experiment

Parameters	Treatments			
	Control	T ₁	T ₂	T ₃
Salinity (ppt)	$0.13^d \pm 0.00$ (0.00-0.20)	$5.08^c \pm 0.01$ (5.00-5.20)	$10.04^b \pm 0.01$ (10.00-10.10)	$15.04^a \pm 0.01$ (15.00-15.20)
Temperature (°C)	25.82 ± 0.12 (22.40-29.30)	25.78 ± 0.12 (22.40-29.40)	25.88 ± 0.10 (22.40-29.30)	25.79 ± 0.10 (22.50-29.30)
pH	7.59 ± 0.04 (7.40-7.80)	7.60 ± 0.05 (7.40-7.80)	7.63 ± 0.05 (7.40-7.90)	7.69 ± 0.07 (7.40-7.90)
Dissolved Oxygen (ppm)	6.48 ± 0.12 (6.40-6.80)	6.52 ± 0.11 (6.00-7.20)	6.46 ± 0.10 (6.00-6.80)	6.34 ± 0.13 (5.80-6.80)
NH₄⁺-N (ppm)	0.22 ± 0.01 (0.01-0.26)	0.24 ± 0.01 (0.01-0.36)	0.24 ± 0.02 (0.01-0.32)	0.25 ± 0.02 (0.01-0.36)
NO₂-N (ppm)	0.03 ± 0.01 (0.01-0.05)	0.04 ± 0.01 (0.01-0.08)	0.04 ± 0.01 (0.01-0.08)	0.05 ± 0.01 (0.01-0.09)
Total Alkalinity (ppm)	$113.89^d \pm 0.22$ (96-126)	$163.22^c \pm 0.78$ (150-172)	$214.11^b \pm 0.78$ (196-248)	$304.38^a \pm 0.83$ (288-324)
Total Hardness (ppm)	$169.16^d \pm 4.73$ (140-210)	$1398.88^c \pm 1.46$ (1240-1560)	$2678.33^{bc} \pm 5.46$ (2630-2750)	$3232.50^a \pm 9.83$ (3200-3260)
Ca Hardness (ppm)	$101.94^d \pm 2.37$ (80-140)	$401.66^c \pm 0.96$ (350-470)	$533.88^b \pm 5.28$ (490-620)	$830.00^a \pm 7.12$ (800-890)
Mg Hardness (ppm)	$67.22^d \pm 2.64$ (40-90)	$997.22^c \pm 2.00$ (880-1090)	$2144.44^b \pm 0.56$ (2090-2190)	$2402.50^a \pm 6.82$ (2310-2460)

Ca²⁺ (ppm)	40.78 ^d ± 0.95 (32-56)	160.67 ^c ± 0.39 (140-188)	213.56 ^b ± 2.11 (196-248)	332.00 ^a ± 2.85 (320-356)
Mg²⁺ (ppm)	16.34 ^d ± 0.64 (9.72-21.87)	242.33 ^c ± 0.49 (213.84-264.70)	521.10 ^b ± 0.14 (507.87-532.70)	583.81 ^a ± 1.66 (561.00-597.20)
Na⁺ (ppm)	9.67 ^d ± 0.75 (7-12)	1586.11 ^c ± 10.59 (1460-1730)	3177.78 ^b ± 7.01 (3020-3290)	4464.44 ^a ± 13.31 (4320-4620)
K⁺ (ppm)	2.48 ^d ± 0.06 (2.20-2.80)	5.54 ^c ± 0.02 (5.40-5.80)	9.69 ^b ± 0.04 (9.40-11.00)	12.77 ^a ± 0.01 (12.50-13.00)

Data expressed as the means of three replicates (n=3), mean ± SE. Different superscripts in same row indicate significant difference (p <0.05). Value given in the bracket depicting the range during the culture period

4.3. Growth Performance of Amur Carp

Initial weight (g) of the Amur carp in all the treatments had no significant difference and observed as 3.58 ± 0.15 , 3.45 ± 0.06 , 3.41 ± 0.01 and 3.51 ± 0.04 in C, T₁, T₂ and T₃, respectively. Growth parameters such as % Weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival were recorded for all the treatment groups over and depicted in Table -7.

4.3.1. Percentage weight gain (%WG)

The %WG of all the treatment groups was recorded at an interval of 15 days for the total experimental period of 90 days. There was significant difference observed (p<0.05) in the %WG among different treatment groups at the end of the experimental period (Table- 7 and Fig.-1). At the end of the experiment, the final %WG of Amur carp was significantly higher in control groups (764.68 ± 8.44) than the other treatments. There was decreasing trend in %WG of Amur carp with increased salinity and recorded lowest in T₃ (328.03 ± 5.29).

4.3.2. Specific growth rate (SGR)

The SGR of Amur carp in different treatments were found to be significantly different (p <0.05) and depicted in (Table- 7 and Fig.-2). The highest SGR was recorded in the control group C (2.39 ± 0.01) followed by T₁ (2.27 ± 0.01). The lowest SGR was recorded in T₃ (1.61 ± 0.02).

4.3.3. Feed conversion ratio (FCR)

The FCR of various treatment groups was found to be significantly different ($p < 0.05$). The highest FCR was recorded in the T_3 group (2.20 ± 0.07). There was no significant difference between C, T_1 and T_2 (Table- 7 and Fig.-3).

4.3.4. Protein efficiency ratio (PER)

The highest PER was recorded in the control group (1.85 ± 0.12) and no significant difference was observed with T_1 and T_2 (Table- 7 and Fig.-4), whereas the lowest PER was recorded in the T_3 group (1.42 ± 0.04).

4.3.5. Survival rate (%)

The survival rate of Amur carp in different treatment groups are depicted in Table- 7 and Fig.- 5. There was 100% survival in both control and T_1 groups whereas lowest survival was observed in T_3 (85.00 ± 2.89).

Table: 7. Growth performance of Amur carp reared in IGSW at different salinities

T	% WG	SGR	FCR	PER	Survival Rate (%)
Control	764.68 ^a ± 8.44	2.39 ^a ± 0.01	1.70 ^b ± 0.11	1.85 ^a ± 0.12	100.00 ^a ± 0.00
T_1	670.67 ^b ± 9.87	2.27 ^{ab} ± 0.01	1.80 ^b ± 0.01	1.73 ^a ± 0.01	100.00 ^a ± 0.00
T_2	588.46 ^c ± 6.80	2.14 ^b ± 0.03	1.83 ^b ± 0.01	1.70 ^a ± 0.01	91.66 ^b ± 1.66
T_3	328.03 ^d ± 5.29	1.61 ^c ± 0.02	2.20 ^a ± 0.07	1.42 ^b ± 0.04	85.00 ^c ± 2.88
P Value	0.001	0.000	0.006	0.013	0.000

*Data are presented as Mean ± SE (n=3), values with different superscripts in the same column differ significantly ($p < 0.05$); T: Treatments; WG: Weight gain; SGR: Specific growth rate; FCR: Food conversion ratio; PER: Protein efficiency ratio.

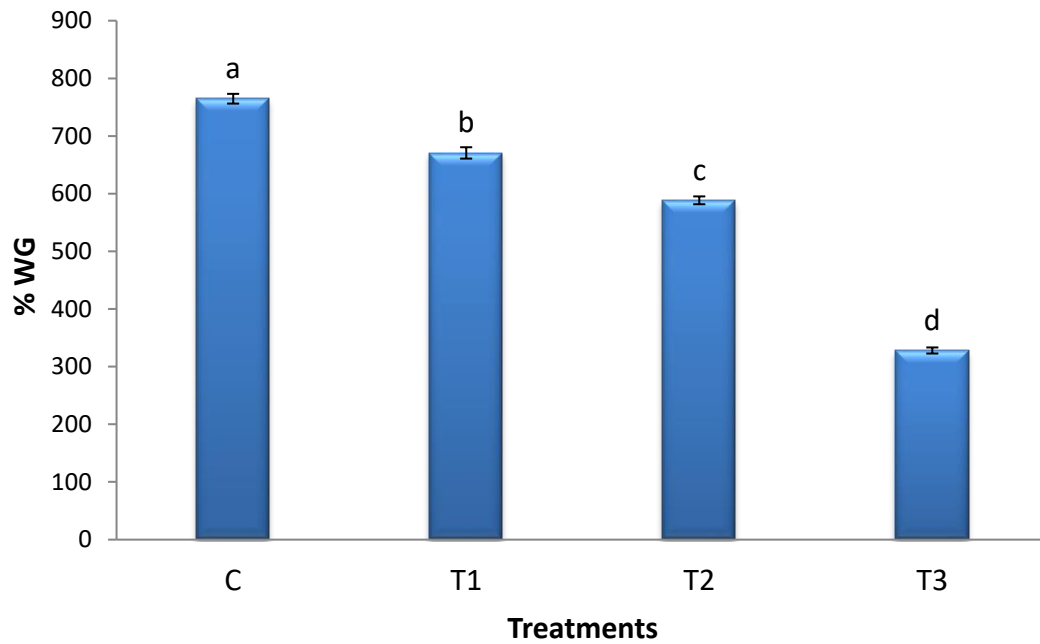


Fig. 1: Percentage weight gain of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

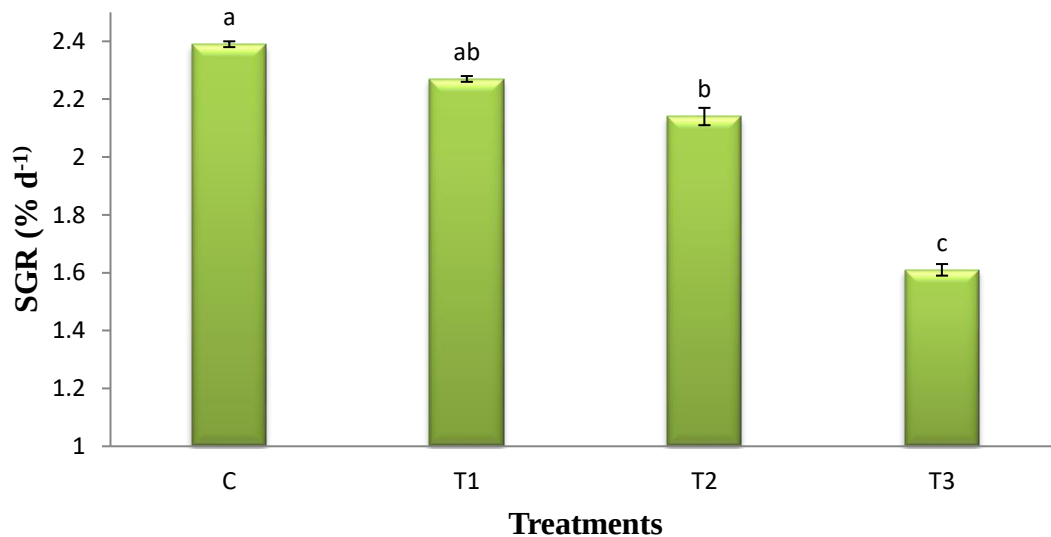


Fig. 2: Specific growth rate (SGR) of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

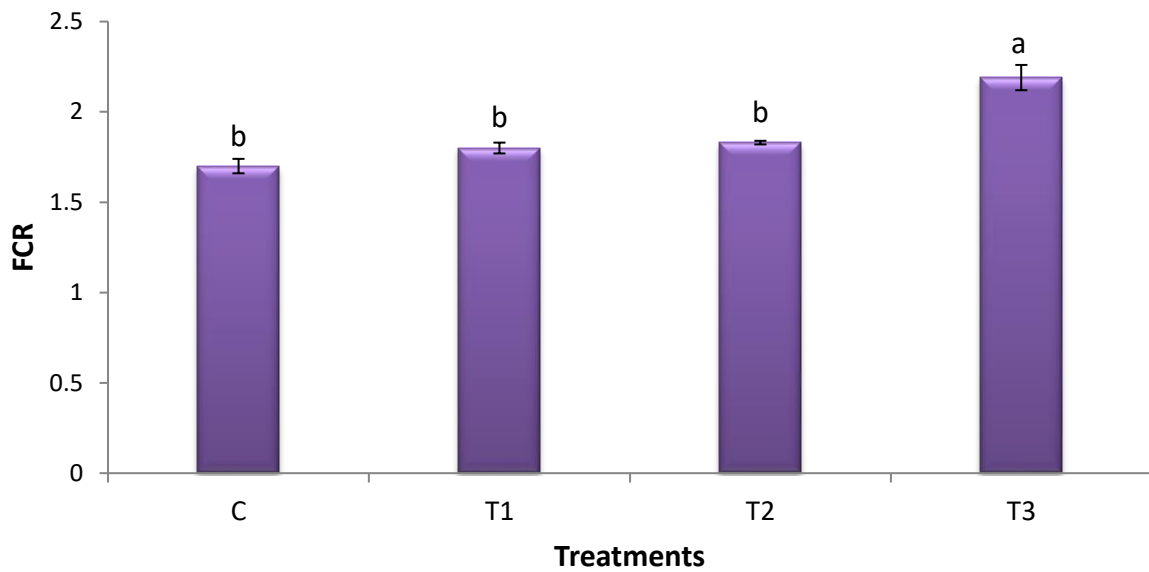


Fig. 3: Feed conversion ratio (FCR) of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

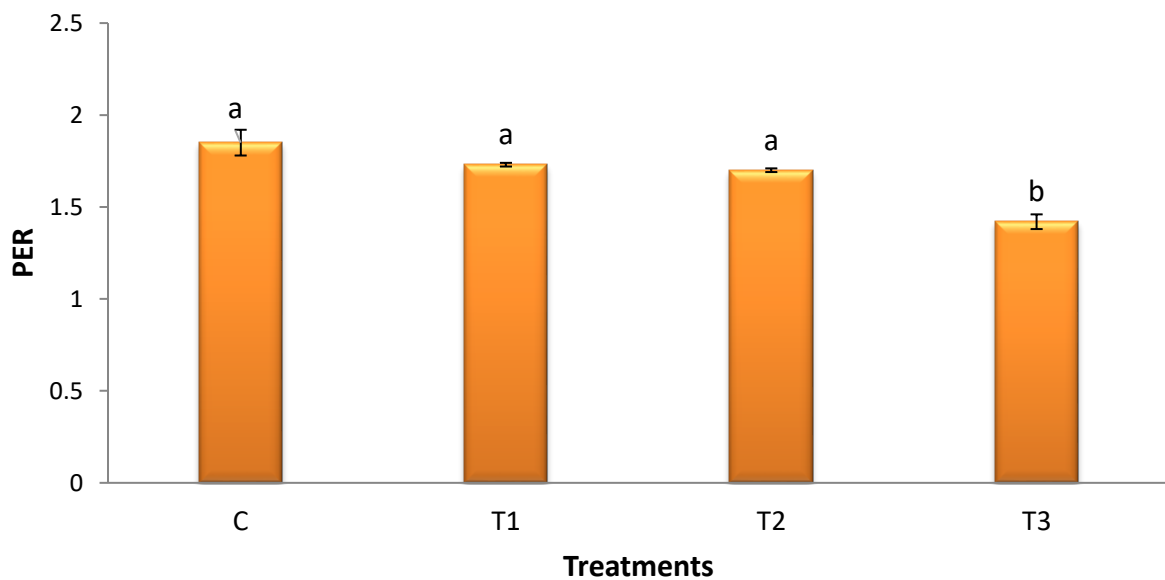


Fig. 4: Protein efficiency ratio (PER) of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

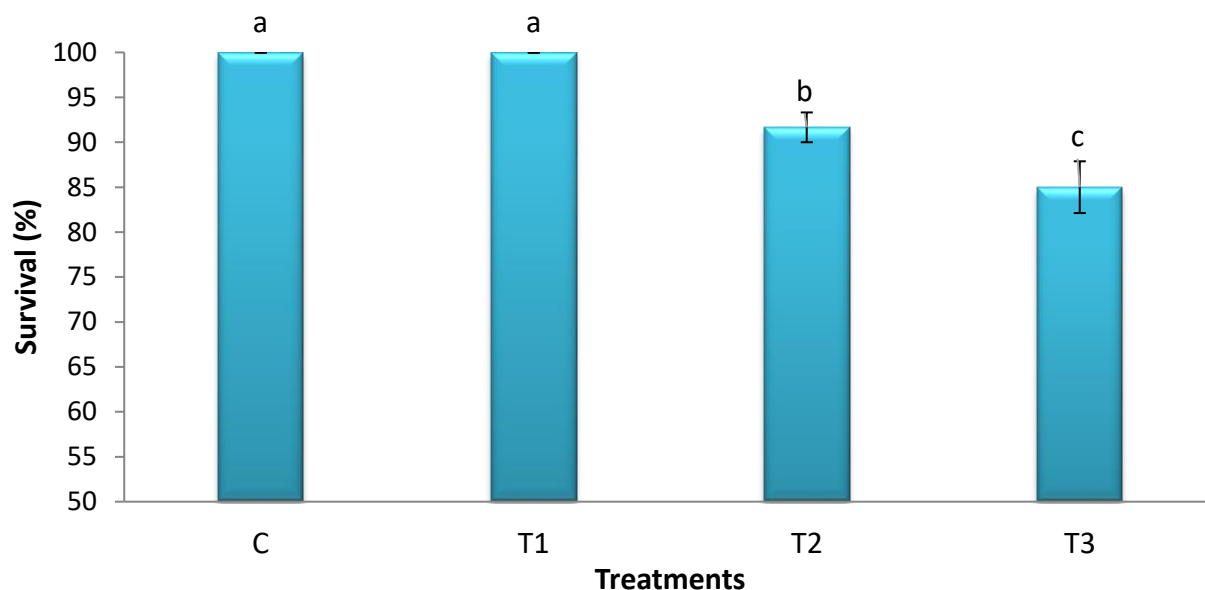


Fig. 5: Amur carp Survival in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.4. Carcass Composition of Amur Carp Reared at Different Salinities

The biochemical composition in terms of moisture, crude protein, total lipid, total carbohydrate, and total ash of all the experimental fish in different treatment groups were analyzed at the end of the experiment and represented in Table- 8.

4.4.1. Moisture

The moisture content (%) of Amur carp significantly varied among all the treatments (Table- 8 and Fig. 6). The highest moisture content was in T_3 group (75.22 ± 0.32) fish.

4.4.2. Crude protein

The crude protein (%) of Amur carp was ranged between 13.17 ± 0.06 and 16.32 ± 0.28 (Table- 8 and Fig.-7) with highest in control followed by T_1 (16.19 ± 0.22), T_2 (14.50 ± 0.18) and T_3 (13.17 ± 0.06). The crude protein content in control and T_1 were not significantly varied ($p > 0.05$). However, it was significantly different ($p < 0.05$) in T_1 , T_2 and T_3 .

4.4.3. Total lipid

The total lipid (%) of Amur carp was significantly varied ($p < 0.05$) and decreasing trend was observed with increase in salinity. The total lipid was varied from 5.49 ± 0.04 to 7.65 ± 0.003 (Table- 8 and Fig. 8).

4.4.4. Total ash

The total ash content (%) of Amur carp was found highest (4.06 ± 0.30) in T₃ group, the lowest being in control group (Table-8 and Fig. 9).

4.4.5. Total carbohydrate

Total carbohydrate (%) of Amur carp reared at different salinities in IGSW ranged between 2.05 ± 0.09 and 3.82 ± 0.22 (Table- 8 and Fig.10).

Table: 8. Carcass composition of Amur carp reared in IGSW at different salinities (% wet weight basis)

Treatments	Moisture	Crude Protein	Total Lipid	Total Ash	Total Carbohydrate
Control	$70.96^d \pm 0.08$	$16.32^a \pm 0.28$	$7.65^a \pm 0.003$	$1.24^d \pm 0.03$	$3.82^a \pm 0.22$
T ₁	$71.86^c \pm 0.07$	$16.19^a \pm 0.22$	$6.67^b \pm 0.09$	$1.95^c \pm 0.17$	$3.33^a \pm 0.11$
T ₂	$74.13^b \pm 0.32$	$14.50^b \pm 0.18$	$6.15^c \pm 0.07$	$2.73^b \pm 0.09$	$2.48^b \pm 0.37$
T ₃	$75.22^a \pm 0.32$	$13.17^c \pm 0.06$	$5.49^d \pm 0.04$	$4.06^a \pm 0.30$	$2.05^b \pm 0.09$
P Value	0.002	0.001	0.000	0.000	0.002

*Data expressed as Mean $\pm$ SE (n=3), values with different superscripts in the same column differ significantly ($p < 0.05$).

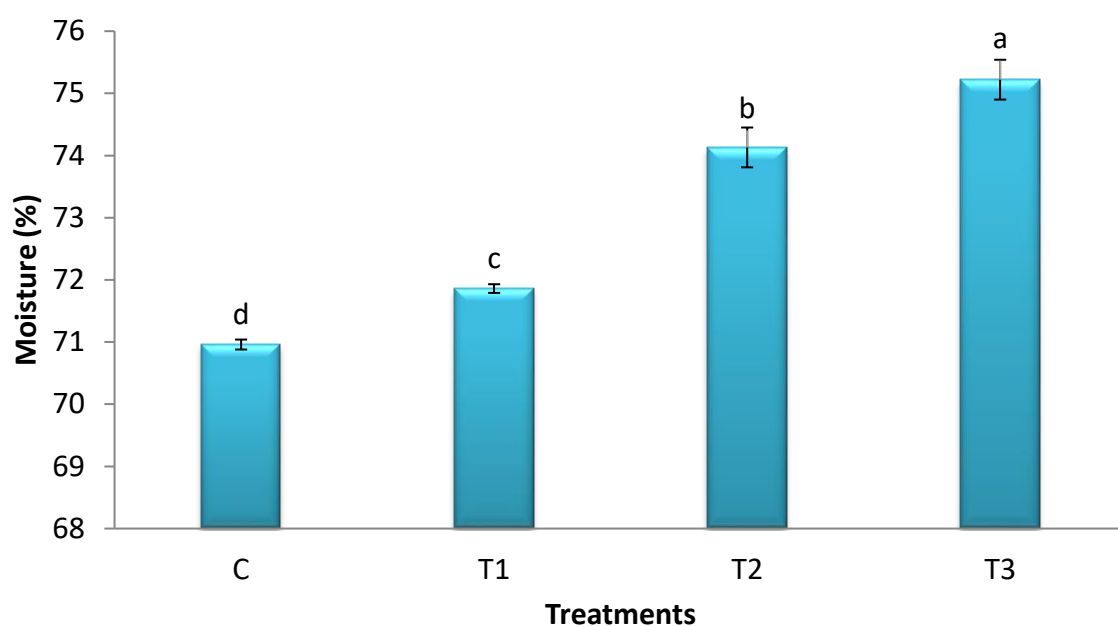


Fig. 6: Moisture content of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

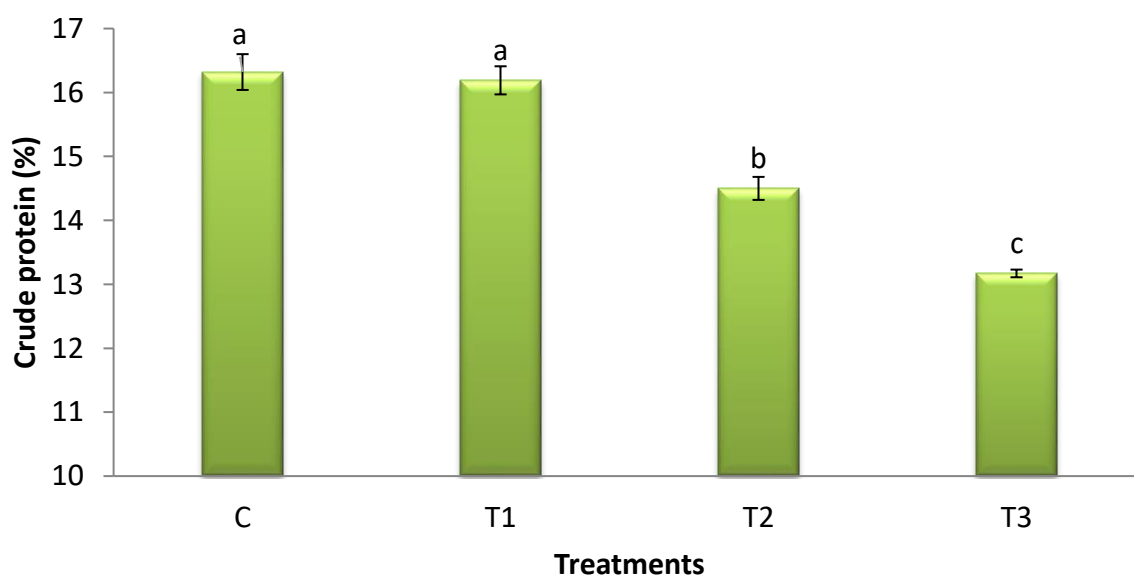


Fig. 7: Crude protein content of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

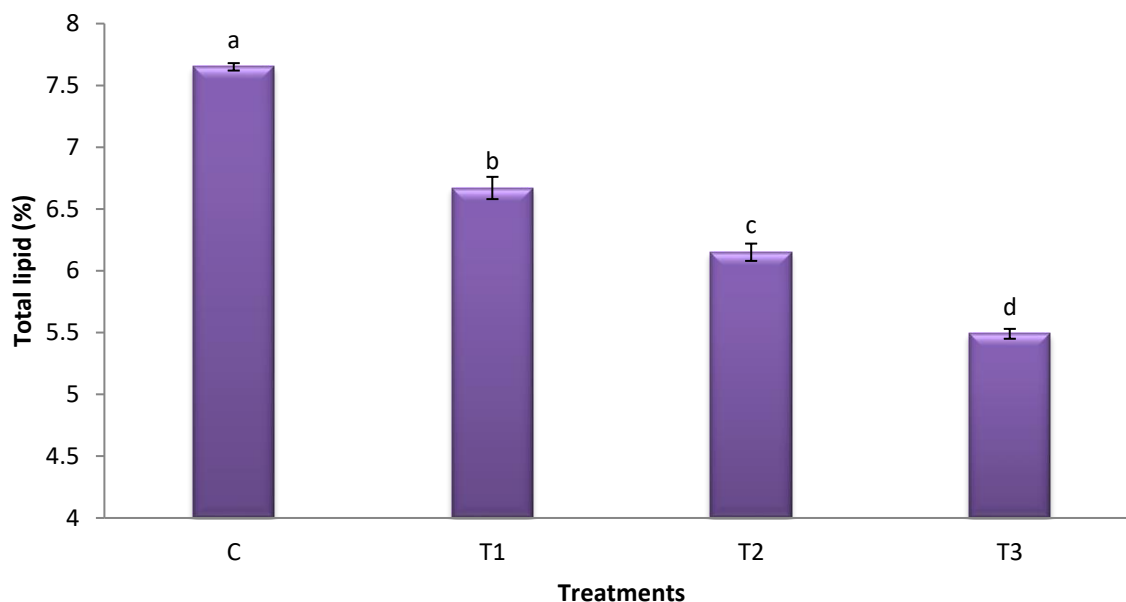


Fig. 8: Total lipid content of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p<0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

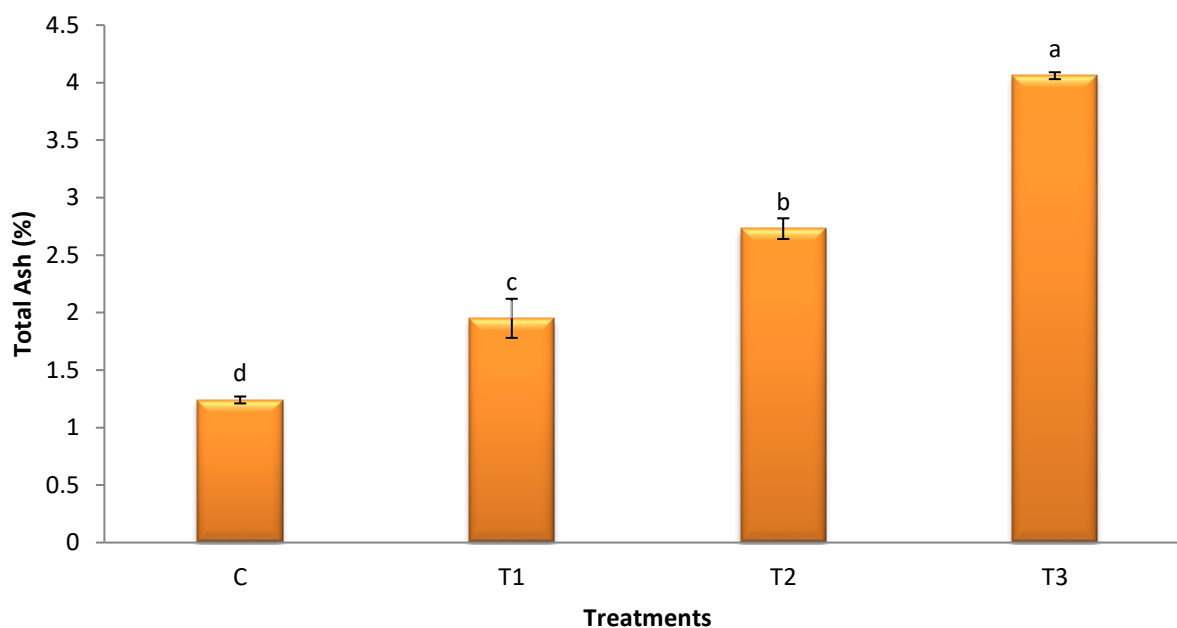


Fig. 9: Total Ash content of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p<0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

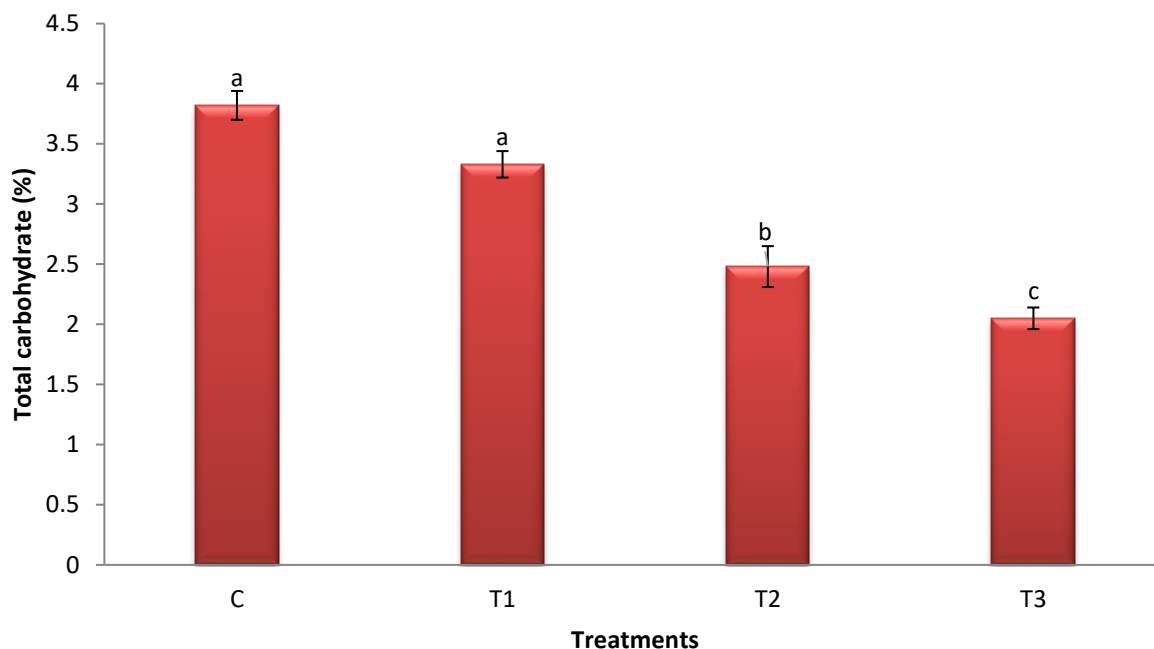


Fig. 10: Total carbohydrate content of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.5. Digestive Enzymes Activity

4.5.1. Protease

Protease activity in the intestine of Amur carp was analyzed for all the treatments and depicted in Table- 9 and Fig. 11. The protease activity in the intestine of Amur carp reared in control group had no significant ($p > 0.05$) difference with T₁ group. It was higher in C (17.23 ± 0.43) followed by T₁ (16.16 ± 0.50), T₂ (13.42 ± 0.34) and T₃ ($9.93^c \pm 0.23$) treatment groups.

4.5.2. Lipase

Lipase activity of different treatment groups has been analyzed and given in Table- 9 and Fig. 12. Its activity had no significant difference ($p > 0.05$) with control and T₁ treatment groups. The lipase activity was highest in C (12.08 ± 0.30) followed by T₁ (11.35 ± 0.17) and T₂ (8.48 ± 0.16) treatment groups and lowest in T₃ (6.74 ± 0.19).

4.5.3. Amylase

Amylase activity of different treatment groups has been analyzed and presented in Table- 9 and Fig. 13. The amylase activity was showing no significant difference ($p > 0.05$) between control and T_1 treatment groups. However, it was significantly ($p < 0.05$) different between T_2 and T_3 (Table-9). The amylase activity was higher in C (8.55 ± 0.12) followed by T_1 (8.41 ± 0.18), T_2 (5.29 ± 0.19) and T_3 (3.08 ± 0.14).

Table: 9. Digestive enzyme activity in the intestine of Amur carp reared in IGSW at different salinities

Treatments	Protease	Lipase	Amylase
Control	$17.23^a \pm 0.43$	$12.08^a \pm 0.30$	$8.55^a \pm 0.12$
T₁	$16.16^a \pm 0.50$	$11.35^a \pm 0.17$	$8.41^a \pm 0.18$
T₂	$13.42^b \pm 0.34$	$8.48^b \pm 0.16$	$5.29^b \pm 0.19$
T₃	$9.93^c \pm 0.23$	$6.74^c \pm 0.19$	$3.08^c \pm 0.14$
P Value	0.001	0.001	0.002

Activities are expressed as follows: Protease as nano mole of tyrosine released/min/g protein; Lipase as units/mg protein; Amylase as micromole of maltose released/min/g protein; Values are Means $\pm$ SE ($n = 3$). Different superscript in the same column differ significantly ($p < 0.05$).

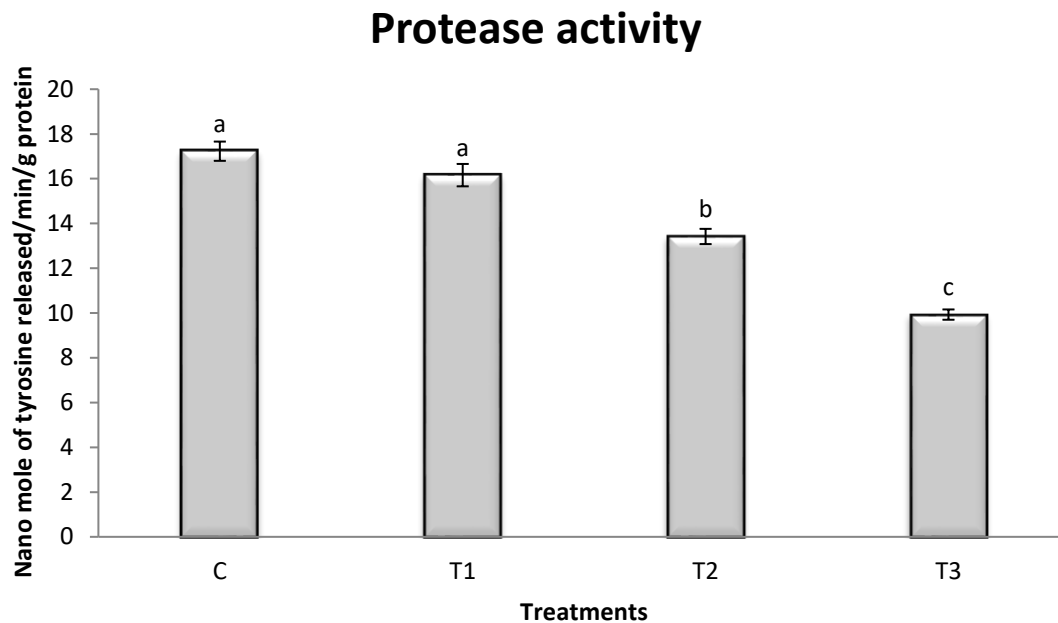


Fig. 11: Protease activity of Amur carp reared in IGSW at different salinities. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

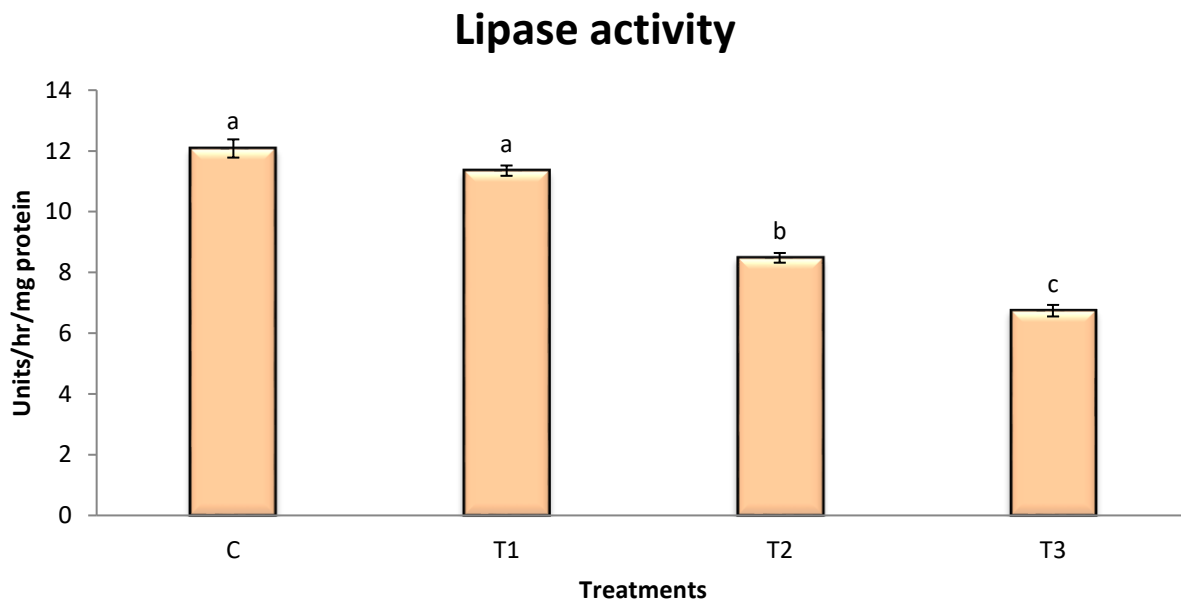


Fig. 12: Lipase activity of Amur carp reared in IGSW at different salinities. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

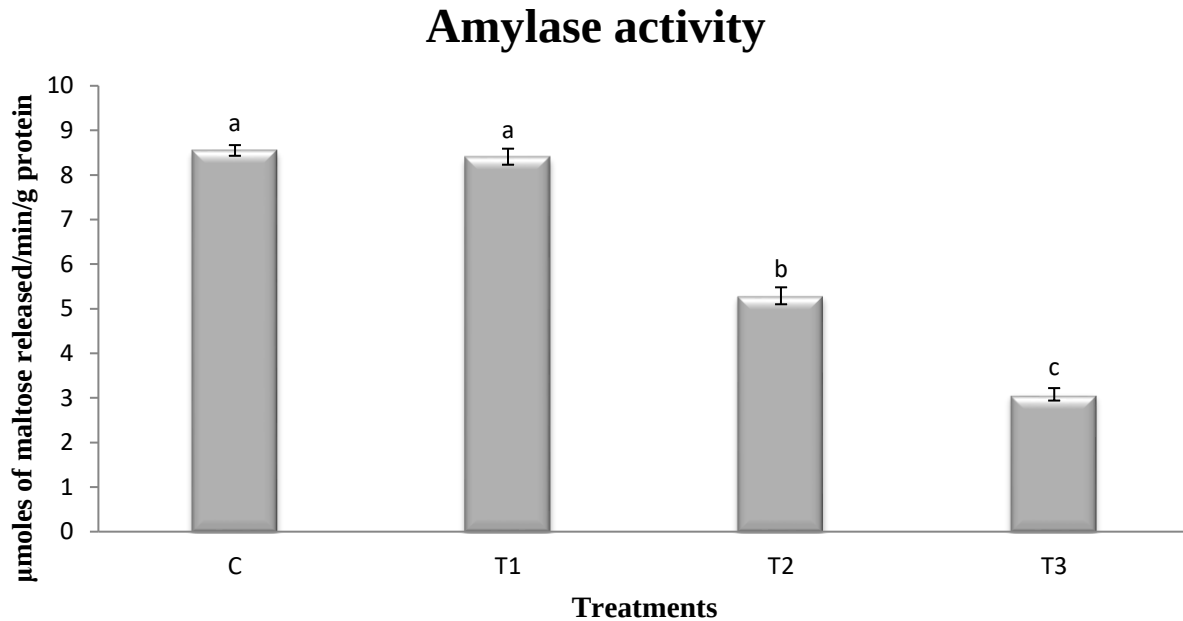


Fig. 13: Amylase activity of Amur carp reared in IGSW at different salinities. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.6. Physiological Response

4.6.1. Osmolality and osmoregulatory capacity

At the end of the experiment, serum and water osmolality (mOsmol/Kg) was recorded in different treatment groups and osmoregulatory capacity was obtained by subtracting water osmolality with serum osmolality of respective group (Table- 10 and Fig. 14).

There was significant difference ($p < 0.05$) observed in the serum osmolality, water osmolality and osmoregulatory capacity among all treatment groups. Highest serum osmolality was recorded in T_3 treatment group (454.33 ± 15.30) followed by T_2 (384.00 ± 7.54) and T_1 (350.00 ± 2.88) groups and the lowest osmolality was recorded in the control groups (305.00 ± 8.54). Similar trend was observed in water osmolality. The highest osmoregulatory capacity was recorded in control groups and decreased with increase in salinity.

4.6.2. Serum ionic concentration

Ionic concentration of serum is depicted in Table- 11. In the present study, the serum ionic concentration of Amur carp was increasing with elevated salinities. The serum Na^+ concentration were recorded highest in T_3 (300.98 ± 4.06) and lowest in control (135.33 ± 3.05) group (Fig. 15). Similarly, serum Cl^- was recorded lowest in control (107.29 ± 1.21) and highest in T_3 (241.46 ± 2.26) treatment groups (Fig. 15). The serum ionic concentration of Mg^{2+} , K^+ and Ca^{2+} varied significantly ($p < 0.05$) and depicted in Table-11 and Fig.16. The serum ionic concentration of Mg^{2+} , K^+ and Ca^{2+} were recorded lowest in control (c) group and shown increasing trend with increase in salinity.

Table: 10. Serum osmolality, water osmolality and osmoregulatory capacity of Amur carp reared in IGSW at different salinities

Treatments	Serum Osmolality	Water Osmolality	Osmoregulatory Capacity
Control	$305.00^d \pm 8.54$	$7.00^d \pm 0.43$	$298.00^a \pm 7.00$
T_1	$350.00^c \pm 2.88$	$108.00^c \pm 2.30$	$242.00^b \pm 4.65$
T_2	$384.00^b \pm 7.54$	$268.66^b \pm 4.33$	$115.33^c \pm 5.48$
T_3	$454.33^a \pm 15.30$	$380.00^a \pm 8.70$	$74.33^d \pm 2.60$
P Value	0.000	0.003	0.002

Serum osmolality, water osmolality and osmoregulatory capacity are expressed as mOsmol/Kg; Data expressed as Mean $\pm$ SE (n=3), values with different superscripts in the same column differ significantly ($p < 0.05$).

Table: 11. Serum Ionic concentration of Amur carp reared in IGSW at different salinities

Treatments	Sodium	Chloride	Magnesium	Potassium	Calcium
Control	135.33 ^d ± 3.05	107.29 ^d ± 1.21	0.61 ^d ± 0.01	0.38 ^d ± 0.02	0.90 ^d ± 0.03
T₁	163.30 ^c ± 3.95	123.43 ^c ± 1.83	0.83 ^c ± 0.01	0.50 ^c ± 0.01	1.29 ^c ± 0.03
T₂	213.25 ^b ± 4.98	188.26 ^b ± 2.37	1.07 ^b ± 0.06	0.61 ^b ± 0.01	1.66 ^b ± 0.06
T₃	300.98 ^a ± 4.06	241.46 ^a ± 2.26	1.22 ^a ± 0.02	0.72 ^a ± 0.01	2.48 ^a ± 0.14
P Value	0.001	0.003	0.001	0.002	0.001

Serum sodium, chloride, potassium, magnesium and calcium ion concentrations are expressed as mmol/l. Data expressed as Mean ± SE (n=3), values with different superscripts in the same column differ significantly (p <0.05).

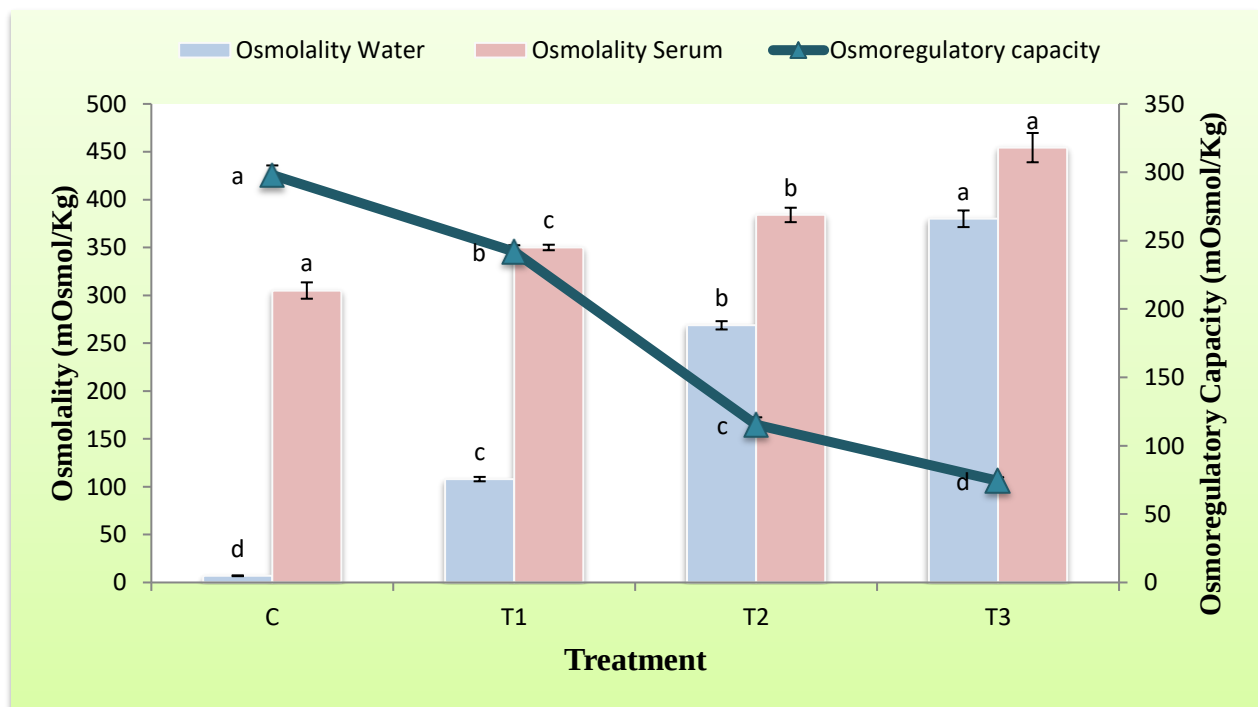


Fig. 14: Serum osmolality, water osmolality and osmoregulatory capacity of Amur carp reared in IGSW at different salinities. The bar bearing of different superscript differ significantly (p <0.05). Data expressed as Mean ± SE (n=3).

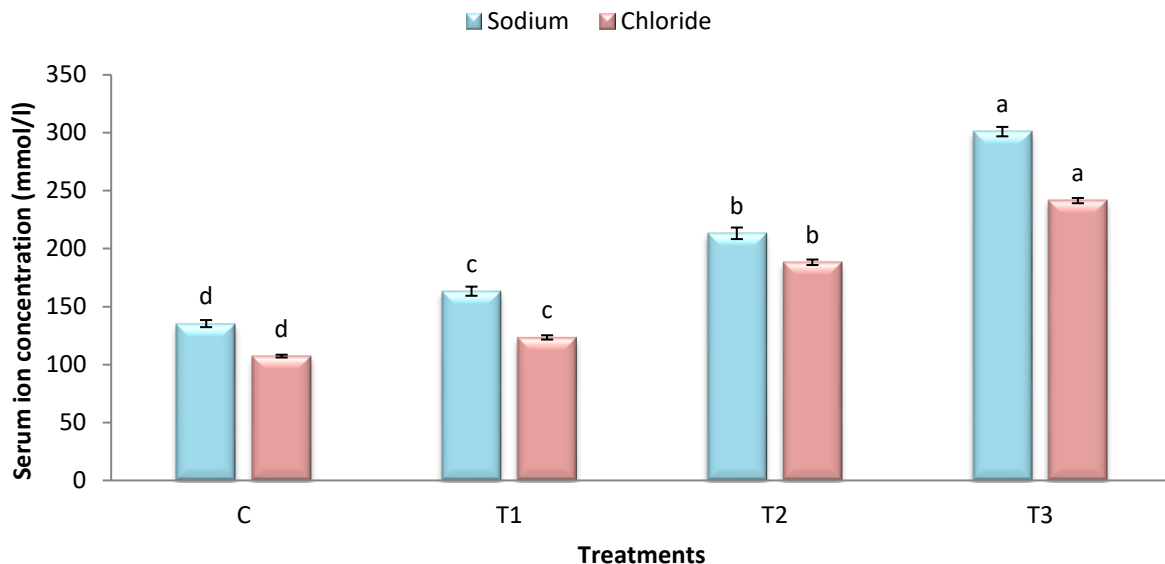


Fig. 15: Serum Sodium and Chloride ion concentration of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

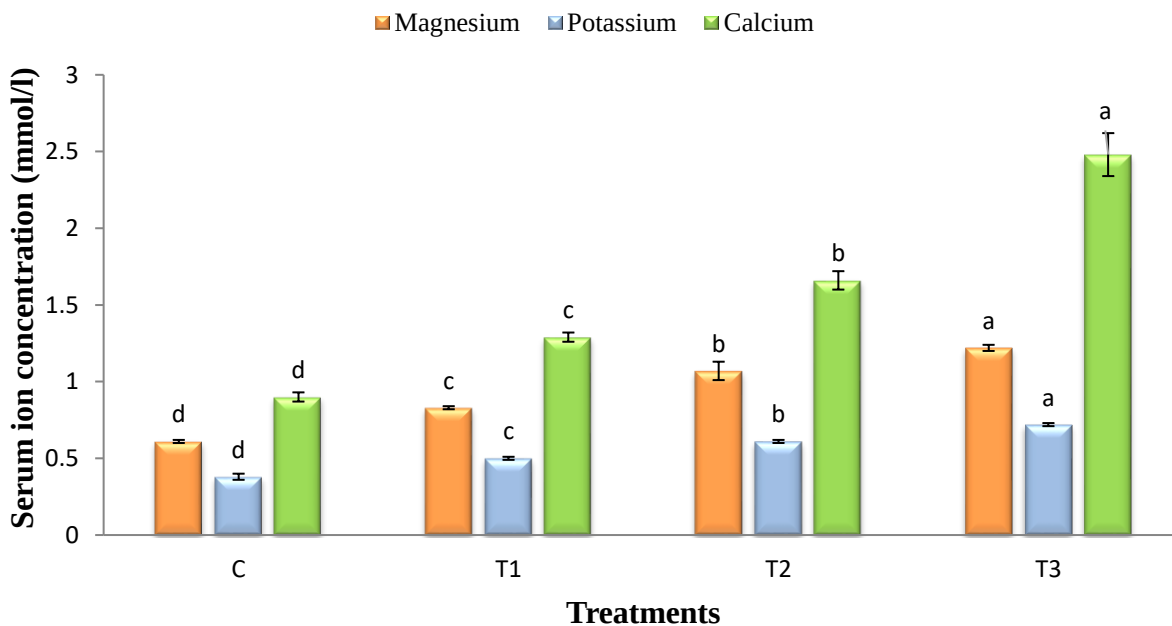


Fig. 16: Serum Magnesium, Potassium and Calcium ion concentration of Amur carp reared in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.6.3. Sodium potassium adenosine triphosphate activity (Na⁺K⁺ -ATPase)

Na⁺ K⁺ -ATPase activity in gills of Amur carp reared at different treatment groups has been analyzed and results are illustrated in Table- 12 and Fig. 17. When compared among treatment groups significantly higher values were observed in T₃ (10.50 ± 0.32) group and the lowest activity in control groups (2.92 ± 0.05).

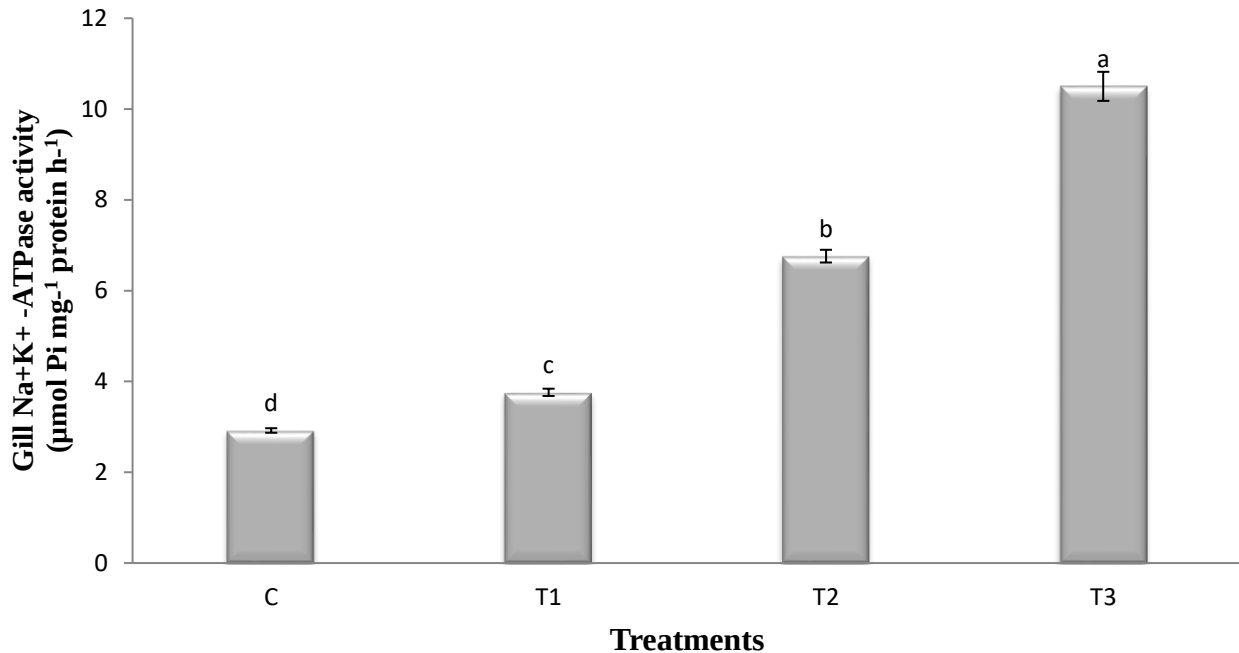


Fig. 17: Gill Na⁺ K⁺ -ATPase activity of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.7. Stress Response

4.7.1. Serum cortisol

Cortisol concentrations of different groups had been analyzed from fish serum and results are depicted in Table- 12 and Fig. 18. Cortisol was showing significant difference ($p < 0.05$) among all the treatments. Cortisol level was highest in T₃ (85.29 ± 1.18) group and lowest in C (57.81 ± 0.92).

4.7.2. Serum glucose

Serum glucose observed in various experimental groups and depicted in Table- 12 and Fig. 18. Significantly higher serum glucose was exhibited by T₃ (85.29 ± 1.18) while control and T₁ groups showed no significant difference.

Table: 12. Gill Na⁺ K⁺ -ATPase activity, serum cortisol and serum glucose level of Amur carp reared in IGSW at different salinities

Treatments	Gill Na ⁺ K ⁺ -ATPase activity	Serum Cortisol	Serum Glucose
Control	2.92 ^d ± 0.05	57.81 ^d ± 0.92	64.48 ^c ± 2.12
T ₁	3.76 ^c ± 0.08	69.79 ^c ± 1.35	68.12 ^c ± 2.01
T ₂	6.76 ^b ± 0.14	76.82 ^b ± 1.12	80.36 ^b ± 1.28
T ₃	10.50 ^a ± 0.32	85.29 ^a ± 1.18	90.96 ^a ± 1.20
P Value	0.001	0.001	0.003

Gill Na⁺ K⁺ -ATPase activity expressed as µmol Pi mg⁻¹ protein h⁻¹; Serum cortisol are expressed as ng/ml; Serum glucose are expressed as mg/dl. Data expressed as Mean ± SE (n=3), values with different superscripts in the same column differ significantly (p < 0.05)

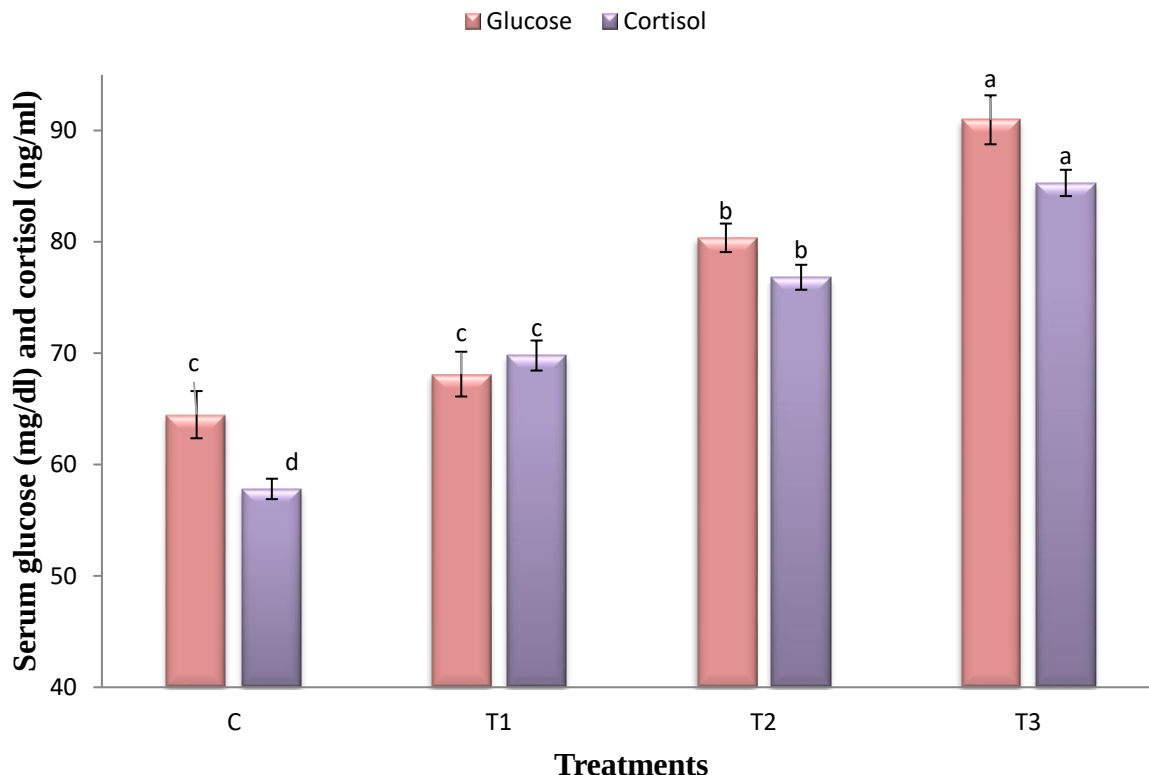


Fig. 18: Serum glucose and cortisol levels of Amur carp reared in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$)

4.7.3. Aspartate aminotransferase (AST)

AST activity from the liver and muscle of different treatment groups had been analyzed and represented in Table-13 and Fig. 19. The mean AST level of liver and muscle in T_3 was 7.16 ± 0.19 and 5.61 ± 0.16 respectively. Control showed lower value in both liver (2.86 ± 0.04) and muscle (1.87 ± 0.06). The AST level in T_3 was significantly ($p < 0.05$) higher than control and other treatment groups.

4.7.4. Alanine aminotransferase (ALT)

ALT activity from the liver and muscle of different treatment groups had been analyzed and depicted in Table- 13 and Fig. 20. The mean value of ALT of liver and muscle in T_3 (5.33 ± 0.17 and 3.76 ± 0.09 respectively) group was significantly

higher compared to other groups. The lowest value of ALT was found in control for both liver (1.30 ± 0.11) and muscle (1.07 ± 0.05).

Table: 13. AST and ALT activities in the liver and muscle tissue of Amur carp reared in IGSW at different salinities

Treatments	AST (Liver)	AST (Muscle)	ALT (Liver)	ALT (Muscle)
Control	$2.86^c \pm 0.04$	$1.87^c \pm 0.06$	$1.30^c \pm 0.11$	$1.07^c \pm 0.05$
T₁	$3.25^c \pm 0.14$	$2.23^c \pm 0.12$	$1.77^c \pm 0.08$	$1.53^c \pm 0.05$
T₂	$5.18^b \pm 0.17$	$4.47^b \pm 0.18$	$3.51^b \pm 0.12$	$2.22^b \pm 0.08$
T₃	$7.16^a \pm 0.19$	$5.61^a \pm 0.16$	$5.33^a \pm 0.17$	$3.76^a \pm 0.09$
P Value	0.001	0.002	0.001	0.001

Aspartate aminotransaminase (AST) - nano moles oxalo acetate released/min/mg protein at 37°C; Alanine aminotransferase (ALT)- nano moles Na pyruvate released /min/mg protein at 37°C. Values are Means $\pm$ SE (n= 3). Values with different superscript in the same column are differ significantly (p <0.05).

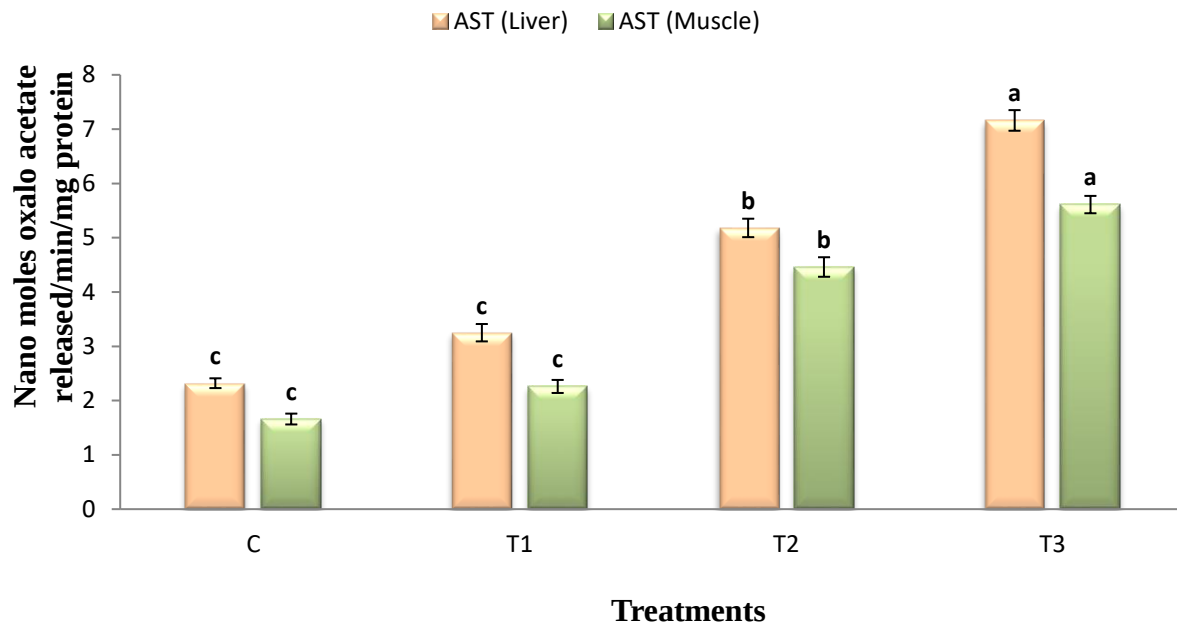


Fig. 19: AST activity in the liver and muscle of Amur carp reared in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

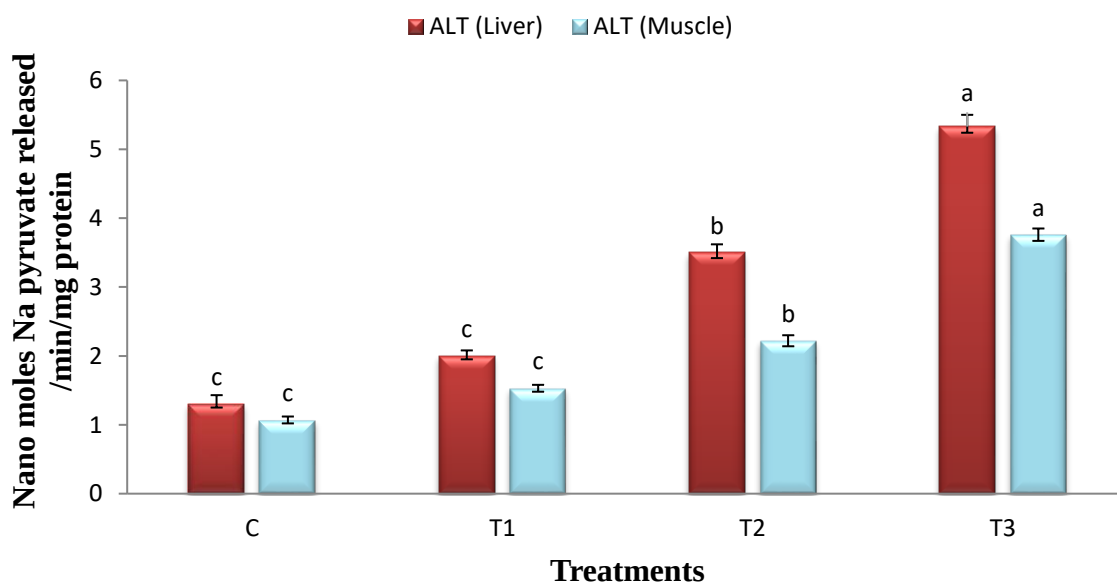


Fig. 20: ALT activity in the liver and muscle of Amur carp reared in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.7.5. Lactate dehydrogenase (LDH)

LDH activity from the liver and muscle of different treatment groups had been analyzed and given in Table-14 and Fig. 21. The mean LDH value of liver and muscle in T₃ (21.70 ± 0.42 and 21.32 ± 0.85 , respectively) group was significantly higher compared to other groups. Lowest values were found in control both in liver (10.04 ± 0.19) and muscle (10.12 ± 0.44).

4.7.6. Malate dehydrogenase (MDH)

MDH activity from the liver and muscle of different treatment groups had been analyzed and illustrated in Table-14 and Fig. 22. The mean MDH value of liver and muscle in T₃ (5.02 ± 0.04 and 4.88 ± 0.16 respectively) treatment groups were significantly higher compared to other groups. Lowest values were found in control both in liver (1.04 ± 0.02) and muscle (1.29 ± 0.06).

Table: 14. LDH and MDH activities in the liver and muscle tissue of Amur carp reared in IGSW at different salinities

Treatments	LDH (Liver)	LDH (Muscle)	MDH (Liver)	MDH (Muscle)
Control	$10.04^d \pm 0.19$	$10.12^d \pm 0.44$	$1.04^d \pm 0.02$	$1.29^c \pm 0.06$
T ₁	$12.67^c \pm 0.23$	$12.53^c \pm 0.39$	$1.60^c \pm 0.01$	$1.59^c \pm 0.07$
T ₂	$16.59^b \pm 0.31$	$16.16^b \pm 0.57$	$3.05^b \pm 0.03$	$3.00^b \pm 0.12$
T ₃	$21.70^a \pm 0.42$	$21.32^a \pm 0.85$	$5.02^a \pm 0.04$	$4.88^a \pm 0.16$
P Value	0.000	0.001	0.001	0.003

LDH-Lactate dehydrogenase; MDH-Malate dehydrogenase, LDH activities are expressed as: Units/min/mg protein at 37°C; MDH: specific activity expressed as Units /min/ mg protein at 37 °C, Values are Means $\pm$ SE (n = 3). Different superscript in the same column differ significantly (p<0.05).

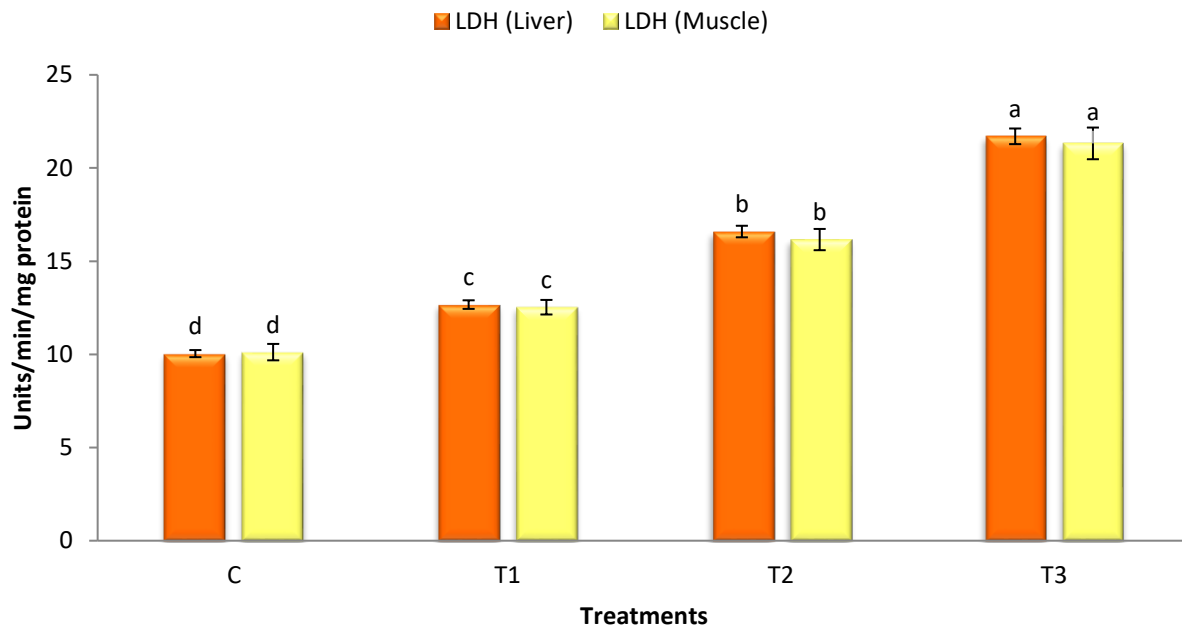


Fig. 21: LDH activity in the liver and muscle of Amur carp reared in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

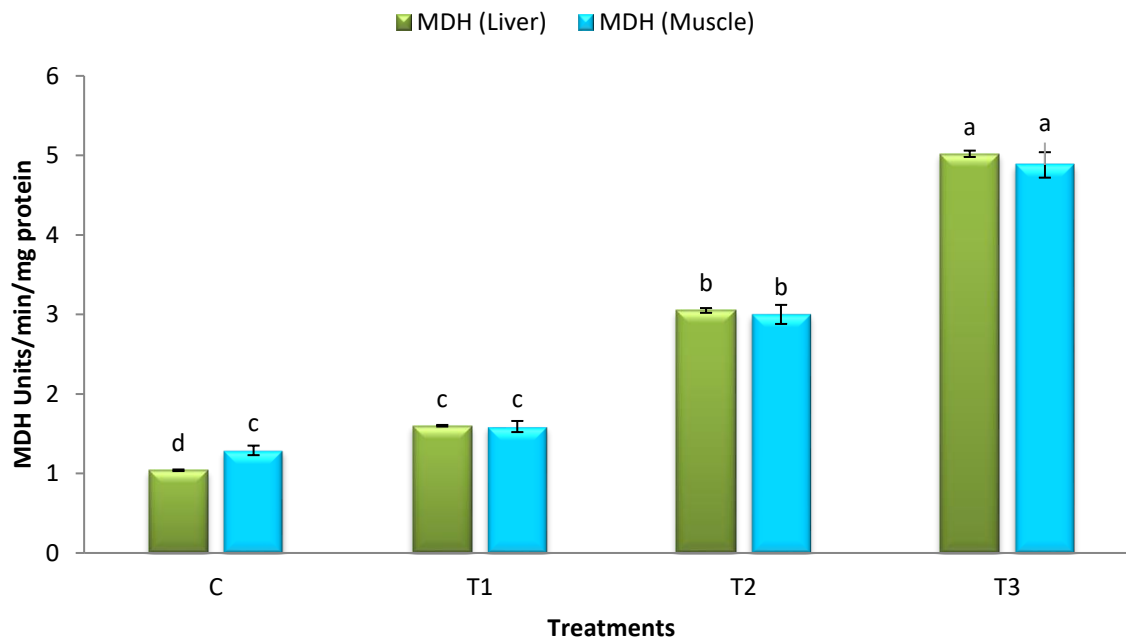


Fig. 22: MDH activity in the liver and muscle of Amur carp in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.8. Immune Response

4.8.1. Superoxide dismutase (SOD) assay

SOD activity of the gills and liver of Amur carp reared at different salinity had been analysed and depicted in Table- 15 and Fig. 23. There was a significant difference ($p < 0.05$) between control and treatments. The mean SOD value of gills and liver in T_3 (4.50 ± 0.12 and 4.11 ± 0.054 , respectively) treatment group was significantly higher compared to other groups. Lowest value was recorded in control group in both gills (0.67 ± 0.07) and liver (1.06 ± 0.06).

4.8.2. Catalase (CAT) activity

CAT activity of the gills and liver of different treatment groups had been analysed and depicted in Table- 15 and Fig. 24. There was a significant difference ($p < 0.05$) between control and treatments. The mean CAT value of gills and liver in T_3 (4.02 ± 0.16 and 3.72 ± 0.12 , respectively) treatment group was significantly higher compared to other groups. Lowest value was found in control both in gills (0.31 ± 0.01) and liver (0.39 ± 0.01).

Table: 15. SOD and CAT activities in the Gill and liver of Amur carp reared in IGSW at different salinities

Treatments	SOD (Gill)	SOD (Liver)	CAT (Gill)	CAT (Liver)
Control	0.67 ^c ± 0.07	1.06 ^c ± 0.06	0.31 ^d ± 0.01	0.39 ^d ± 0.01
T₁	0.96 ^c ± 0.05	1.31 ^c ± 0.07	0.77 ^b ± 0.01	0.79 ^c ± 0.02
T₂	2.41 ^b ± 0.14	2.18 ^b ± 0.11	2.50 ^b ± 0.09	2.16 ^b ± 0.07
T₃	4.50 ^a ± 0.12	4.11 ^a ± 0.054	4.02 ^a ± 0.16	3.72 ^a ± 0.12
P Value	0.001	0.002	0.000	0.001

SOD- Superoxide dismutase; SOD activity is expressed as 50% inhibition of epinephrine auto oxidation/mg protein/min; CAT- Catalase; Catalase activity expressed as Nanomoles H₂O₂ decomposed/min/mg protein. Values are Means ± SE (n = 3). Different superscript in the same column differ significantly (p <0.05).

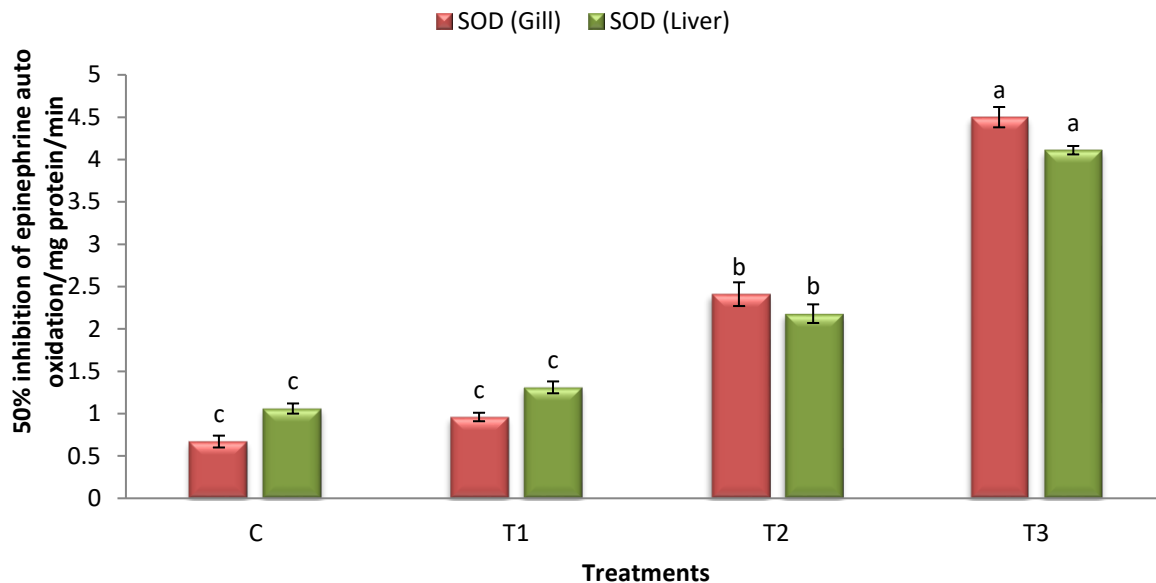


Fig. 23: SOD activity in the gill and liver of Amur carp reared in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

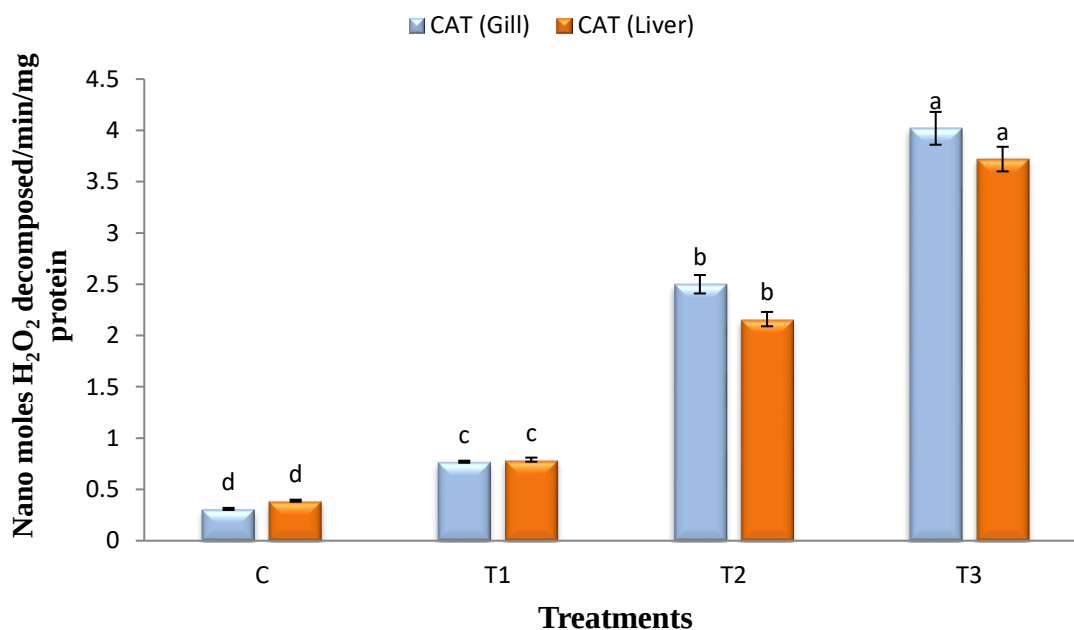


Fig. 24: CAT activity in the gill and liver of Amur carp reared in different experimental groups. The bar bearing of different superscript differ significantly ($p < 0.05$). Data expressed as Mean $\pm$ SE ($n=3$).

4.9. Histological Analysis of Gills

In the present study, the histological changes occurred in the gills was studied at the end of the experimental period and depicted in Plates 3 - 6. The histological section of gills of Amur carp showed normal structure of primary lamella (PL) and secondary lamellae (SL) in control group, whereas gills of the fish reared in IGSW of 5, 10 and 15 ppt, depicted some histological alterations. In the present study, the hyperplasia condition occurred in T₁ and epithelial lifting was observed. In addition, T₂ group showed atrophied gills and increased RBC infiltration. However, T₃ displayed atrophied gills, RBC infiltration and fusion of gills. Pillar cells were found to be normal upto 10 ppt.

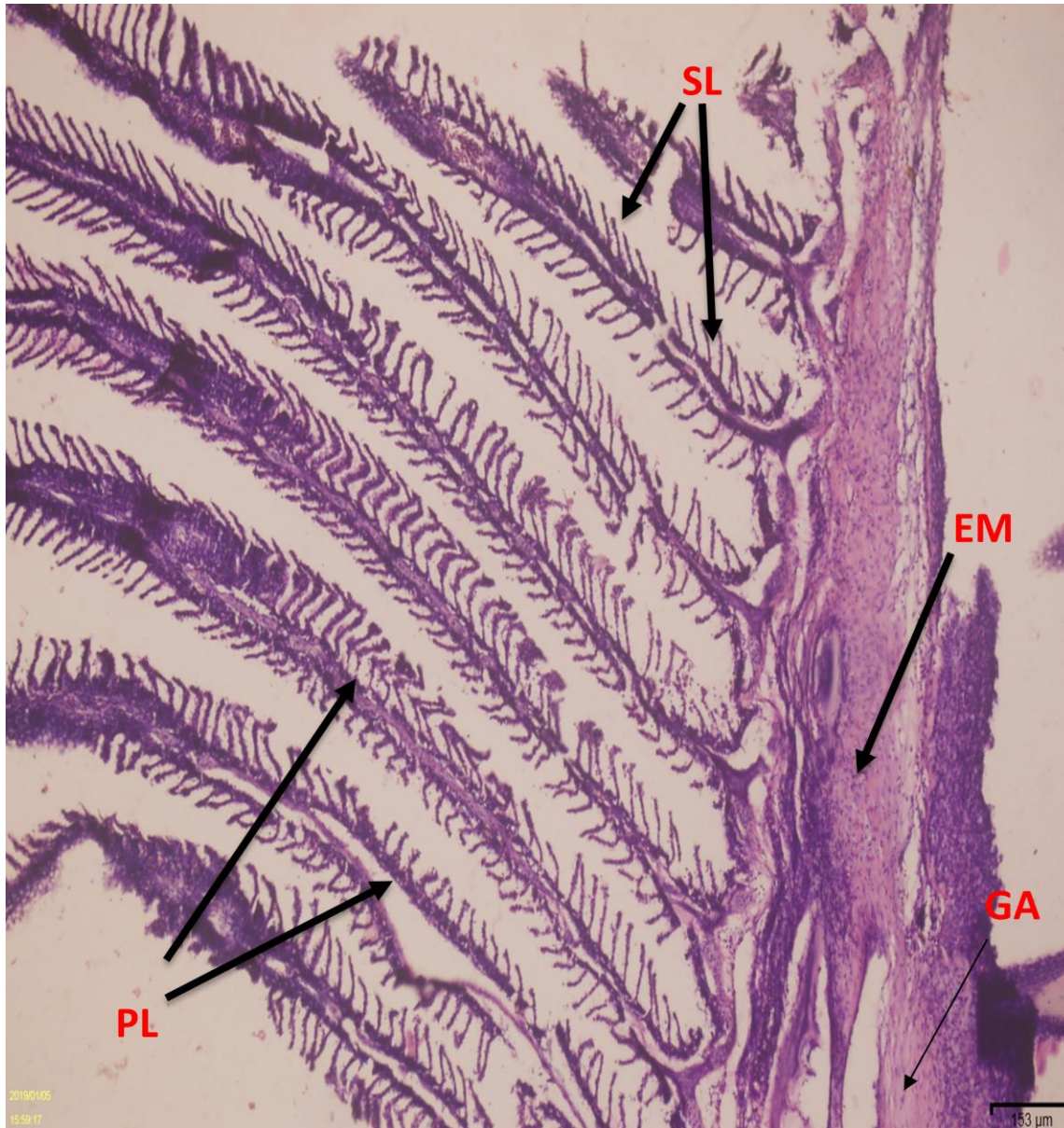


Plate 3. Histological section of Gill of Amur carp reared in freshwater at 4.2 X, Scale- 153 μm; EM: Extracellular Matrix; GA- Gill Arch; PL- Primary lamellae; SL- secondary lamellae.

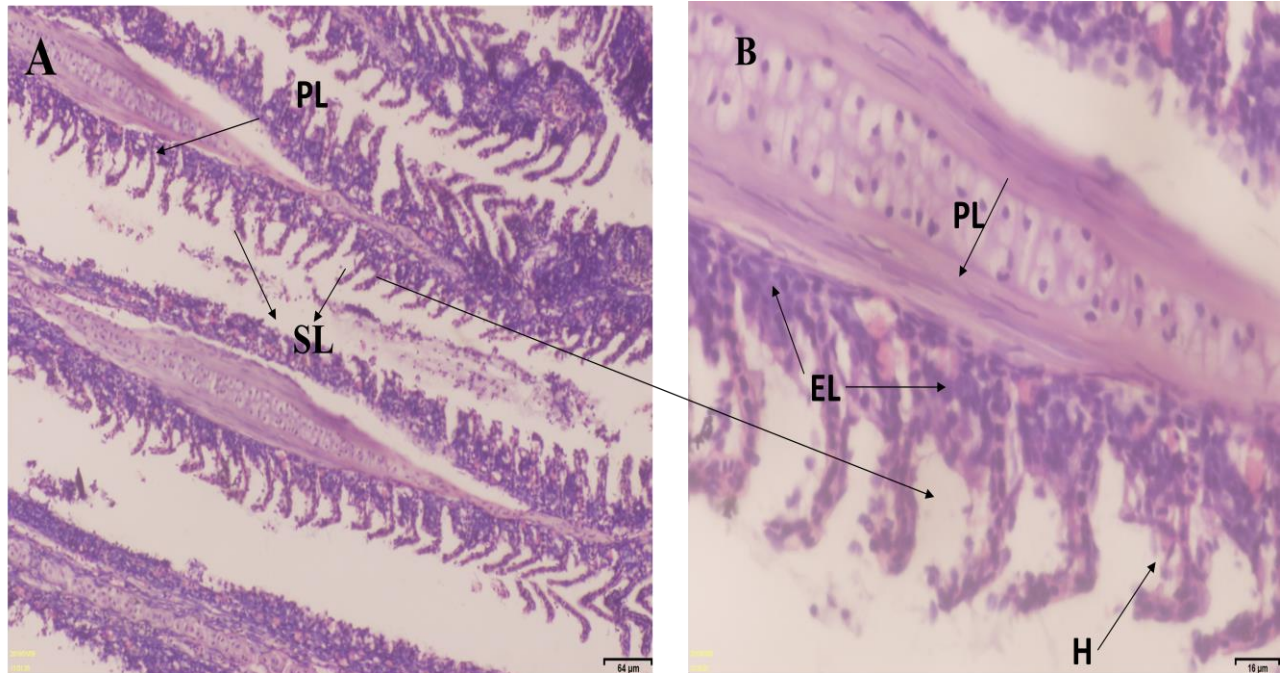


Plate 4. Histological section of Gill of Amur carp reared at 5 ppt; (A) Gill of fish exposed to 5 ppt salinity at 10 X, Scale- 64μm (B) Gill of fish exposed to 5 ppt salinity at 40 X, Scale- 16 μm; H-Hyperplasia, EL- Epithelial Lifting, PL-Primary lamellae, SL- secondary lamellae

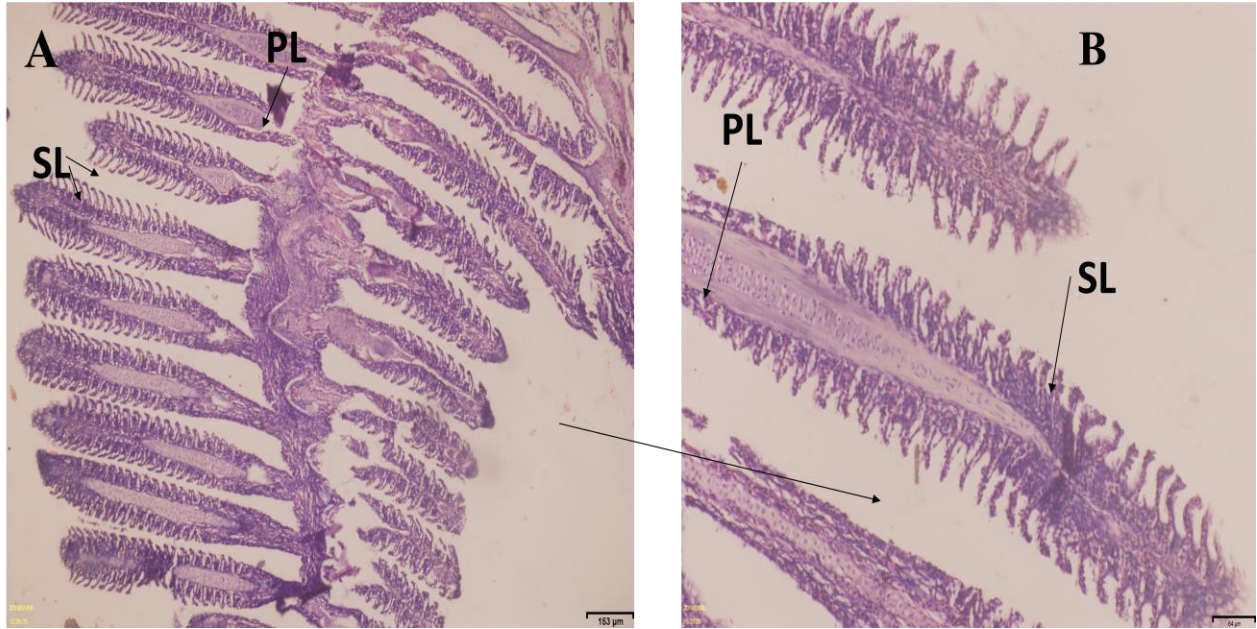


Plate 5. Histological section of Gill of Amur carp reared at 10 ppt; (A) Gill of fish exposed to 10 ppt salinity at 4.2 X (Scale- 153 μ m) (B) Gill of fish exposed to 10 ppt salinity at 10 X (Scale- 64 μ m); PL-Primary lamellae; SL- secondary lamellae.

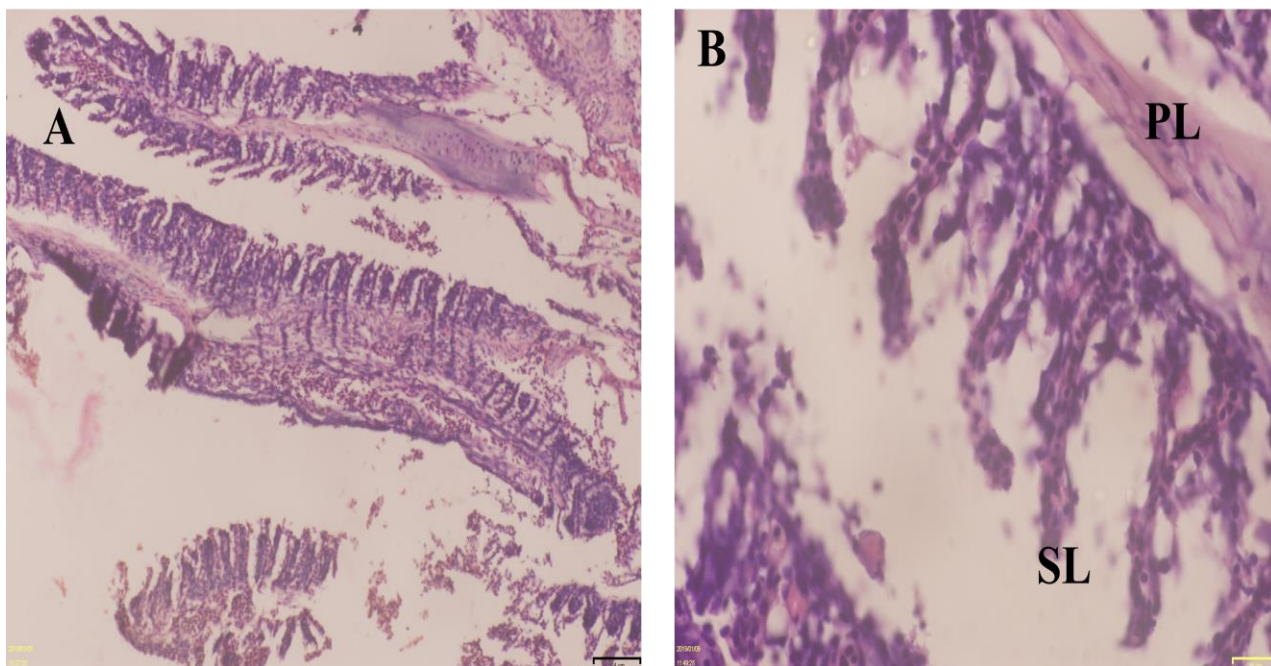


Plate 6. Histological section of Gill of Amur carp reared at 15 ppt; (A) Gill of fish exposed to 15 ppt salinity at 10 X (Scale- 64 μ m) (B) Gill of fish exposed to 15 ppt salinity at 40 X (Scale- 16 μ m) , PL-Primary lamellae; SL- Secondary lamellae;

4.10. Experiment – 2: Effect of different salinity in combination with different potassium ion concentrations on growth and physiological response of Amur carp reared in inland saline water

The second experiment was conducted to evaluate the effect of different potassium concentrations (K_1 - 25%, K_2 - 50%, K_3 - 75% and K_4 - 100%) at different salinities (T_1 -5 ppt, T_2 -10 ppt and T_3 -15 ppt) and their combinations with various salinities on growth and physiological response of Amur carp.

4.11. Physico-chemical Parameters of Water

The physico-chemical parameters of water such as salinity (ppt), temperature ($^{\circ}$ C), pH, dissolved oxygen and total alkalinity (ppm) were recorded and mean values of all the parameters in accordance with treatments are depicted in Table-

16. The water temperature of all experimental groups were recorded throughout the experimental period and were found to be in the range of 25.82 ± 0.12 to 25.86 ± 0.01 °C. The pH remained at optimum level and values were recorded within the range of 7.59 ± 0.04 to 7.69 ± 0.02 . The dissolved oxygen concentration of all the experimental groups was recorded within the range of 5.96 ± 0.04 to 6.68 ± 0.04 ppm during the experimental period of 90 days. Total alkalinity ranged between 113.89 ± 0.22 and 252.61 ± 0.40 ppm among all the experimental groups and found increasing value with increase in salinity.

Total hardness, calcium and magnesium hardness were recorded and average values in accordance with treatments are depicted in Table- 17. Total hardness varied between 169.16 ± 4.73 and 3462.77 ± 5.74 ppm among the treatment groups and found increasing with increase to salinity. The calcium and magnesium hardness obtained in the range of 101.94 ± 2.37 to 854.72 ± 8.55 and 67.22 ± 2.64 to 2614.44 ± 3.20 , respectively. The NH_4^+ -N content of all the treatment groups was recorded once in a week, before water exchange. It was found to be in the range of 0.22 ± 0.01 to 0.51 ± 0.01 ppm. The NO_2 -N content of all the groups was found in the range of 0.03 ± 0.01 to 0.04 ± 0.01 ppm.

The ionic concentration (Ca^{2+} , Mg^{2+} , Na^+ and K^+) of potassium fortified IGSW were measured twice a week and found increasing with increase in salinity (Table- 18). Ca^{2+} concentration varies from 40.78 ± 0.95 to 341.89 ± 3.42 ppm, Mg^{2+} from 16.34 ± 0.64 to 635.31 ± 0.78 , Na^+ concentration from 9.67 ± 0.75 to 4566.94 ± 5.05 and K^+ concentrations ranged from 2.48 ± 0.06 to 206.67 ± 0.80 . All the ions showed increasing trend with increase in salinity and potassium fortification.

Table: 16. Physico-chemical Parameters of Water (Salinity, Temperature, pH, Dissolved Oxygen and Total Alkalinity) used in various experiments

Treatments	Salinity (ppt)	Temperature (°C)	pH	Dissolved Oxygen (ppm)	Total Alkalinity (ppm)
Control	0.13±0.00	25.82±0.12	7.59±0.04	6.48±0.12	113.89±0.22
T ₁ K ₁	5.11±0.01	25.83 ±0.03	7.65 ±0.05	6.66 ±0.12	169.89± 2.08
T ₁ K ₂	5.13±0.02	25.84 ±0.01	7.66 ±0.05	6.68 ±0.04	179.89± 1.49
T ₁ K ₃	5.12±0.01	25.85 ±0.01	7.69 ±0.02	6.61 ±0.11	180.67 ±0.42
T ₁ K ₄	5.14 ± 0.01	25.86 ± 0.01	7.69±0.02	6.42 ± 0.08	188.83 ± 0.88
T ₂ K ₁	10.09±0.03	25.83 ±0.03	7.67 ±0.06	6.63 ±0.08	215.39± 1.31
T ₂ K ₂	10.11±0.04	25.84 ±0.01	7.69 ±0.02	6.67 ± 0.02	221.72±0.78
T ₂ K ₃	10.12±0.04	25.85 ±0.01	7.67 ±0.06	6.60 ±0.08	232.00 ±0.82
T ₂ K ₄	10.11 ± 0.03	25.86 ± 0.01	7.68 ±0.06	6.52 ± 0.09	242.22 ± 3.39
T ₃ K ₁	15.08 ±0.01	25.83 ±0.13	7.69 ±0.02	6.34 ±0.11	242.61± 0.83
T ₃ K ₂	15.10±0.03	25.84 ±0.01	7.68 ±0.06	6.07 ±0.07	251.67± 1.78
T ₃ K ₃	15.11±0.03	25.85 ±0.02	7.65 ±0.05	6.19 ±0.11	248.61±0.40
T ₃ K ₄	15.11 ± 0.04	25.85 ± 0.01	7.66 ± 0.05	5.96 ± 0.04	252.61 ± 0.40

Table: 17. Physico-chemical Parameters of Water (Total Hardness, Ca Hardness, Mg Hardness, NH₄⁺-N and NO₂⁻-N) used in various experiments

Treatments	Total Hardness (ppm)	Calcium Hardness (ppm)	Magnesium Hardness (ppm)	NH ₄ ⁺ -N (ppm)	NO ₂ ⁻ -N (ppm)
Control	169.16±4.73	101.94±2.37	67.22±2.64	0.22±0.01	0.03±0.01
T ₁ K ₁	1432.50± 4.81	392.50± 1.73	1040.00± 3.82	0.29 ±0.04	0.03±0.01

T₁K₂	1446.94± 9.75	401.39 ± 4.20	1042.77± 8.64	0.26 ±0.04	0.03±0.01
T₁K₃	1413.61 ± 2.17	398.33 ±1.27	1034.72± 4.55	0.43 ±0.01	0.04 ± 0.01
T₁K₄	1420.83 ± 1.27	395.83 ± 4.11	1025.00 ± 2.89	0.36 ± 0.05	0.04 ± 0.01
T₂K₁	2436.38 ±10.00	690.56± 8.74	1745.83± 8.78	0.39 ±0.07	0.03 ±0.01
T₂K₂	2484.72± 1.47	741.67 ± 4.28	1743.06± 3.13	0.46 ±0.05	0.04 ± 0.01
T₂K₃	25.85 ± 10.84	664.44 ±13.46	1921.39± 24.13	0.32 ±0.05	0.04 ± 0.01
T₂K₄	2578.33 ± 14.29	698.33 ± 12.44	1880.00 ± 24.49	0.47 ± 0.05	0.04 ± 0.01
T₃K₁	3260.00 ±1.27	825.83 ± 4.64	2434.16± 4.11	0.51 ±0.01	0.04±0.01
T₃K₂	3269.44± 7.99	854.72 ± 8.55	2414.72± 2.22	0.35 ±0.05	0.04±0.01
T₃K₃	3392.50± 11.49	821.39 ±3.13	2571.11 ± 14.60	0.39 ±0.07	0.04 ±0.01
T₃K₄	3462.77 ± 5.74	848.33 ± 3.47	2614.44 ± 3.20	0.51 ± 0.01	0.04 ± 0.01

Table: 18. Physico-chemical Parameters of Water (Ionic concentrations) used in various experiments

Treatments	Ca²⁺ (ppm)	Mg²⁺ (ppm)	Na⁺ (ppm)	K⁺ (ppm)
Control	40.78 ± 0.95	16.34 ± 0.64	9.67± 0.75	2.48 ± 0.06
T₁K₁	157.00 ± 0.69	252.72 ± 0.93	1578.22 ± 9.49	24.68 ± 0.02
T₁K₂	160.56 ± 1.68	253.39 ± 2.09	1603.75 ± 16.01	41.84 ± 0.04
T₁K₃	159.33 ± 0.51	251.44 ± 1.11	1608.89 ±13.96	63.93 ± 0.19
T₁K₄	158.33 ± 1.64	249.08 ± 0.70	1601.38 ± 14.23	79.34 ± 0.26
T₂K₁	276.22 ± 3.49	424.24 ± 2.13	3268.89 ± 7.78	46.79 ± 0.07
T₂K₂	296.67 ± 1.71	423.56± 0.76	3051.11 ± 14.02	81.87 ± 0.08
T₂K₃	265.78± 5.38	466.89 ± 5.86	2993.61± 5.19	122.53 ± 0.52
T₂K₄	279.33 ± 4.98	456.84 ± 5.95	2933.06 ± 3.67	145.31 ± 0.39
T₃K₁	330.33± 1.86	591.50 ± 0.99	4531.67 ± 3.63	68.72 ± 0.18

T₃K₂	341.89± 3.42	586.78± 0.54	4551.39 ± 2.94	126.61 ± 0.45
T₃K₃	328.56 ± 1.25	624.78± 3.55	4566.94 ± 5.05	165.17 ± 0.68
T₃K₄	339.33 ± 1.38	635.31 ± 0.78	4480.00 ± 6.03	206.67 ± 0.80

4.12. Growth Performance

Initial weight (3.51 ± 0.18 g) of the Amur carp in all the treatments had no significant difference and mean initial weight was observed as. Growth parameters such as % Weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival were recorded for all the treatment groups and represented in Table- 19.

4.12.1. Percentage weight gain (%WG)

The %WG of all the treatment groups was recorded at an interval of 15 days for the total experimental period of 90 days. Based on two way ANOVA, the different salinity and different potassium concentrations along with their interaction had significant difference ($p < 0.05$) on %WG among various treatment groups. At the end of the experiment, the final %WG of Amur carp was significantly higher in control group (764.68 ± 8.44). Combination of salinity and potassium fortification did not show significant increment in growth and %WG was decreased with increase in salinity and potassium fortification. Individual effect of salinity showed significant difference ($p < 0.05$) on %WG and decreased with increase in salinity. Individual effect of potassium inferred that there was significant difference ($p < 0.05$) at each level of fortification and IGSW fortified with 25% potassium (equivalent to same salinity of seawater) had maximum growth (514.56 ± 2.97) while decreased with increase in level of fortification.

4.12.2. Specific growth rate (SGR)

The SGR of all the treatment groups was recorded at an interval of 15 days for the total experimental period of 90 days. Based on Two way ANOVA, the different salinity and different potassium concentration along with their combination had

significant difference ($p < 0.05$) on SGR among various treatment groups. The highest SGR was recorded in the control group C (2.39 ± 0.01) followed by T_1K_1 (2.31 ± 0.01), T_1K_2 (2.26 ± 0.01) and T_1K_3 (2.26 ± 0.01). The lowest SGR was recorded in the treatment group T_3K_3 (1.28 ± 0.01) and T_3K_4 (1.28 ± 0.02). Individual effect of salinity showed significant difference ($p < 0.05$) on SGR and decreased with increase in salinity. Individual effect of potassium inferred that there was significant difference ($p < 0.05$) at IGSW fortified with 25% potassium (equivalent to same salinity of seawater) and had maximum growth while decreased with increasing level of fortification.

4.12.3. Feed conversion ratio (FCR)

The interaction effect of salinity and potassium on FCR showed significant difference ($p < 0.05$) and lowest FCR was recorded in the control group C (1.70 ± 0.12). There was no significant difference ($p > 0.05$) observed among T_1K_1 (1.83 ± 0.02), T_1K_2 (1.84 ± 0.02) and T_1K_3 (1.85 ± 0.02). The highest FCR was recorded in the treatment group T_3K_3 (3.03 ± 0.01) and T_3K_4 (3.04 ± 0.02). Individual effect of salinity showed significant difference ($p < 0.05$) on FCR and increase with decrease in salinity. Individual effect of potassium inferred that there was no significant difference ($p > 0.05$) between 25% and 50% potassium fortified IGSW of equivalent salinity of seawater. FCR increased with 75% and 100% potassium fortification.

4.12.4. Protein efficiency ratio (PER)

The interaction of salinity and potassium had no significant effect on PER. The salinity has individual effect on PER and found decreasing with increase in salinity. The fish reared in control had maximum (1.85 ± 0.02) whereas minimum (1.08 ± 0.02) PER was observed at 15 ppt. The different potassium concentration had significant ($p < 0.05$) effect and 100% potassium fortification had lowest (1.36 ± 0.02) PER.

4.12.5. Survival (%)

The survival rate of Amur carp indicated that there was significant ($p < 0.05$) effect of salinities, potassium concentrations and their interaction. There were 100% fish survived at 0 ppt and 5 ppt (at each level of potassium fortification) while

lowest survival (60 %) was observed in T₃K₄. Individual effect of salinity showed significant difference and lowest survival at 15 ppt (78.33 ± 0.89). Different potassium concentrations had significant effect on survival and 100 % fortified water showed lowest survival.

Table: 19. Growth performance of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	% WG	SGR	FCR	PER	Survival
Salinity × K⁺ ion conc.	Control	764.68 ^a ±8.45	2.39 ^a ±0.01	1.70 ^f ±0.12	1.85±0.13	100.00 ^a ±0.00
	T₁K₁	696.52 ^b ±4.52	2.31 ^b ±0.01	1.83 ^e ±0.02	1.71±0.01	100.00 ^a ±0.00
	T₁K₂	663.91 ^c ±2.71	2.26 ^c ±0.01	1.84 ^e ±0.02	1.70±0.02	100.00 ^a ±0.00
	T₁K₃	663.59 ^c ±7.43	2.26 ^c ±0.01	1.85 ^e ±0.02	1.69±0.02	100.00 ^a ±0.00
	T₁K₄	600.69 ^d ±4.42	2.16 ^d ±0.01	1.97 ^d ±0.02	1.59±0.01	100.00 ^a ±0.00
	T₂K₁	601.47 ^d ±9.27	2.16 ^d ±0.01	2.01 ^d ±0.04	1.56±0.03	98.33 ^a ±1.67
	T₂K₂	576.16 ^e ±2.34	2.12 ^e ± 0.03	1.98 ^d ±0.03	1.58±0.02	98.33 ^a ±1.67
	T₂K₃	567.05 ^{ef} ±4.66	2.11 ^{ef} ±0.01	1.99 ^d ±0.04	1.57±0.03	91.67 ^b ±4.41
	T₂K₄	553.31 ^f ±1.08	2.09 ^f ± 0.03	2.13 ^c ±0.01	1.47±0.01	91.67 ^b ±1.67
	T₃K₁	245.68 ^g ±1.86	1.38 ^g ±0.01	2.73 ^b ±0.01	1.14±0.01	91.67 ^b ±1.67
	T₃K₂	238.54 ^g ±1.67	1.35 ^h ± 0.03	2.83 ^b ±0.01	1.11±0.03	81.67 ^c ±1.67
	T₃K₃	216.77 ^h ±1.22	1.28 ⁱ ±0.01	3.03 ^a ±0.01	1.03±0.03	80.00 ^c ±2.89
	T₃K₄	216.88 ^h ±5.24	1.28 ⁱ ±0.02	3.04 ^a ±0.01	1.03± 0.02	60.00 ^d ±0.00
	P value	0.00	0.00	0.01	0.75	0.00
Salinity	Control	764.68 ^a	2.39 ^a	1.70 ^d	1.85 ^a	100.00 ^a
	T₁	656.17 ^b	2.25 ^b	1.87 ^c	1.67 ^b	100.00 ^a
	T₂	574.49 ^c	2.12 ^c	2.03 ^b	1.54 ^c	95.00 ^b
	T₃	229.47 ^d	1.32 ^d	2.91 ^a	1.08 ^d	78.33 ^c
	SEM	2.57	0.01	0.02	0.02	0.89
	P value	0.00	0.00	0.00	0.00	0.00
K⁺ ion conc.	K₁	514.56 ^a	1.95 ^a	2.19 ^c	1.47 ^a	96.67 ^a
	K₂	492.87 ^b	1.91 ^b	2.21 ^c	1.46 ^a	93.33 ^{ab}
	K₃	482.47 ^c	1.88 ^c	2.29 ^b	1.43 ^{ab}	90.56 ^b
	K₄	456.96 ^d	1.84 ^d	2.38 ^a	1.36 ^b	83.89 ^c
	SEM	2.97	0.01	0.02	0.02	1.03
	P value	0.00	0.00	0.00	0.01	0.00

Data are presented as Mean $\pm$ SE (n=3), Mean values with different superscripts in the same column under each category differ significantly ($p < 0.05$); %WG: %Weight Gain; SGR: Specific Growth Rate; FCR: Food Conversion Ratio; PER: Protein Efficiency Ratio.

4.13. Carcass Composition of Amur Carp

The carcass composition of Amur carp showed no significant difference of potassium concentration while salinity significantly affected carcass composition of the fish (Table- 20).

The individual effect of salinity showed that the moisture content (%) of fish significantly affected in all the salinities and found increasing with increase in salinity. As such the highest (75.16 ± 0.12) moisture content was in fish of T₃ group. The crude protein (%) was ranged between 13.19 ± 0.09 and 16.32 ± 0.09 and found highest in control followed by T₁ group and had no significant ($p > 0.05$) difference while significantly varied ($p < 0.05$) with other two treatments (T₂ and T₃). The total lipid (%) was significantly varied ($p < 0.05$) and decreasing trend was observed with increase in salinity. The total lipid was found lowest in T₃ (5.36 ± 0.07) and highest in control (7.65 ± 0.07). The total ash content (%) of T₃ group was found highest and it showed lower range for fish reared at control group. Total carbohydrate (%) of Amur carp reared at different salinities in IGSW ranged between 1.24 ± 0.09 and 4.02 ± 0.09 .

4.14. Digestive Enzymes Activity

Digestive enzyme activities of Amur carp revealed that salinities had significant effect ($p < 0.05$) on Amur carp reared at IGSW of different salinities and potassium concentrations while the interaction of potassium concentrations along with salinity and different potassium (K⁺) concentrations did not show any significant effect on digestive enzyme activities (Table- 21).

Protease activity was found highest in control group (17.23) followed by T₁ (16.50), T₂ (14.12) and T₃ (10.31) treatment groups. Lipase and Amylase activities was also found higher in control and there was no significant difference ($p > 0.05$) observed between C and T₁. However, a significant ($p < 0.05$) difference was noticed between T₂ and T₃.

Table: 20. Carcass composition of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	Moisture	Crude Protein	Total Lipid	Total Carbohydrate	Total Ash
Salinity × K⁺ ion conc.	Control	70.96±0.24	16.32±0.19	7.65±0.14	3.82±0.19	1.24±0.17
	T₁K₁	71.87±0.24	16.18±0.19	6.60±0.14	3.39±0.19	1.96±0.17
	T₁K₂	71.88±0.24	16.17±0.19	6.59±0.14	3.39±0.19	1.97±0.17
	T₁K₃	71.90±0.24	16.14±0.19	6.59±0.14	3.39±0.19	1.98±0.17
	T₁K₄	71.90±0.24	16.13±0.19	6.58±0.14	3.38±0.19	2.01±0.17
	T₂K₁	74.09±0.24	14.48±0.19	6.07±0.14	2.64±0.19	2.70±0.17
	T₂K₂	74.10±0.24	14.45±0.19	5.94±0.14	2.79±0.19	2.72±0.17
	T₂K₃	74.10±0.24	14.44±0.19	5.92±0.14	2.81±0.19	2.72±0.17
	T₂K₄	74.12±0.24	14.42±0.19	5.90±0.14	2.81±0.19	2.75±0.17
	T₃K₁	75.14±0.24	13.21±0.19	5.39±0.14	2.28±0.19	3.99±0.17
	T₃K₂	75.14±0.24	13.19±0.19	5.36±0.14	2.30±0.19	4.00±0.17
	T₃K₃	75.14±0.24	13.18±0.19	5.36±0.14	2.28±0.19	4.03±0.17
	T₃K₄	75.19±0.24	13.17±0.19	5.33±0.14	2.27±0.19	4.05±0.17
	P value	1.00	1.00	0.99	0.99	1.00
Salinity	Control	70.96 ^d	16.32 ^a	7.65 ^a	3.82 ^a	1.24 ^d
	T₁	71.89 ^c	16.15 ^a	6.59 ^b	3.39 ^b	1.98 ^c
	T₂	74.10 ^b	14.45 ^b	5.96 ^c	2.77 ^c	2.72 ^b
	T₃	75.16 ^a	13.19 ^c	5.36 ^d	2.28 ^d	4.02 ^a
	SEM	0.12	0.09	0.07	0.09	0.09
	P value	0.01	0.00	0.02	0.00	0.01
K⁺ ion conc.	K₁	73.70	14.65	6.02	2.77	2.89
	K₂	73.71	14.61	5.97	2.83	2.90
	K₃	73.72	14.59	5.96	2.83	2.91
	K₄	73.74	14.57	5.93	2.82	2.93
	SEM	0.14	0.11	0.08	0.11	0.10
	P value	0.99	0.99	0.89	0.98	0.99

Data expressed as Mean ± SE (n=3), mean values with different superscripts in the same column under each category differ significantly ($p < 0.05$)

Table: 21. Digestive Enzyme Activity of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	Protease	Lipase	Amylase
Salinity × K⁺ ion conc.	Control	17.23±0.44	12.08±0.40	8.56±0.12
	T₁K₁	16.84±0.19	11.59±0.39	8.36±0.14
	T₁K₂	16.61±0.29	11.55±0.36	8.29±0.09
	T₁K₃	16.50±0.39	11.49±0.31	8.23±0.07
	T₁K₄	16.05±0.69	11.36±0.19	8.16±0.10
	T₂K₁	14.27±0.29	8.71±0.38	5.09±0.09
	T₂K₂	14.18±0.32	8.68±0.41	4.98±0.09
	T₂K₃	14.08±0.38	8.50±0.38	4.84±0.22
	T₂K₄	13.96±0.28	8.35±0.69	4.58±0.09
	T₃K₁	10.63±0.35	6.67±0.32	3.23±0.34
	T₃K₂	10.52±0.27	6.64±0.44	3.17±0.34
	T₃K₃	10.31±0.02	6.46±0.16	3.10±0.28
	T₃K₄	9.78±0.06	6.30±0.16	2.92±0.10
	P Value	0.99	1.00	0.99
Salinity	Control	17.23 ^a	12.08 ^a	8.56 ^a
	T₁	16.50 ^b	11.49 ^a	8.26 ^a
	T₂	14.12 ^c	8.56 ^b	4.87 ^b
	T₃	10.31 ^d	6.52 ^c	3.11 ^c
	SEM	0.17	0.19	0.09
	P Value	0.00	0.00	0.00
K⁺ ion conc.	K₁	13.91	8.99	5.56
	K₂	13.77	8.96	5.48
	K₃	13.63	8.82	5.39
	K₄	13.26	8.67	5.22
	SEM	0.19	0.22	0.11
	P Value	0.14	0.72	0.17

Activities are expressed as follows: protease as nano mole of tyrosine released/min/g protein; Amylase as micromole of maltose released/min/g protein; Lipase as units/mg protein; Values are Means ± SE (n = 3). Different superscript in the same column under each category differ significantly (p <0.05).

4.15. Physiological Response

4.15.1. Osmolality and osmoregulatory capacity

There is no significant ($p > 0.05$) interaction effect of salinity and potassium concentration on serum osmolality while interaction significantly ($p < 0.05$) affected, water osmolality and osmoregulatory capacity (Table- 22). Water osmolality was found increasing with increase in salinity and potassium concentrations while it was reverse in case of osmoregulatory capacity. Salinity and potassium concentrations had individual effect on osmolality and osmoregulatory capacity. Serum and water osmolality was found increasing significantly ($p < 0.05$) with increase in salinity and potassium concentrations, whereas osmoregulatory capacity was significantly ($p < 0.05$) decreasing with increasing salinity.

4.15.2. Serum ionic concentration

Ionic concentrations of serum are depicted in Table- 23. There was no significant ($p > 0.05$) effect of K^+ fortification and their interaction with salinity on sodium, chloride, calcium and magnesium concentrations. Whereas it was significantly ($p < 0.05$) affect potassium ion concentration. Salinity had significant effect ($p < 0.05$) on serum ionic concentrations and showed increasing trend with increase in salinity.

Table: 22. Serum osmolality, water osmolality and osmoregulatory capacity of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	Serum osmolality	Water osmolality	Osmoregulatory capacity
Salinity × K⁺ ion conc.	Control	305.00±8.54	7.00 ^k ±1.73	298.00 ^k ±7.00
	T₁K₁	355.00±1.16	144.33 ^j ±6.89	210.67 ^k ±6.69
	T₁K₂	358.00±0.58	158.67 ^j ±1.20	199.33 ^k ±1.20
	T₁K₃	361.33±1.45	163.00 ^j ±5.00	198.33 ^k ±3.71
	T₁K₄	362.33±2.03	175.00 ^h ±3.79	187.33 ^k ±5.36
	T₂K₁	390.33±0.88	250.00 ^g ±5.29	140.33 ^k ±5.78
	T₂K₂	391.67±0.88	303.67 ^f ±1.20	88.00 ^k ±0.58
	T₂K₃	393.67±1.20	313.67 ^e ±1.20	80.00 ^k ±2.31
	T₂K₄	396.33±1.76	327.67 ^d ±2.91	68.67 ^k ±1.33
	T₃K₁	459.00±1.53	419.00 ^c ±1.53	40.00 ^k ±3.06
	T₃K₂	462.00±4.00	427.67 ^{bc} ±2.33	34.33 ^k ±0.33
	T₃K₃	464.33±2.08	435.33 ^b ±1.33	29.00 ^k ±2.52
	T₃K₄	467.67±3.79	447.67 ^a ±0.33	20.00 ^k ±2.00
	P Value	0.99	0.00	0.00
Salinity	Control	305.00 ^d	7.00 ^d	298.00 ^a
	T₁	359.17 ^c	160.25 ^c	198.92 ^b
	T₂	393.00 ^b	298.75 ^b	94.25 ^c
	T₃	463.25 ^a	432.42 ^a	30.83 ^d
	SEM	1.39	1.64	1.95
	P Value	0.00	0.01	0.00
K⁺ ion conc.	K₁	401.44 ^b	271.11 ^d	130.33 ^a
	K₂	403.89 ^{ab}	296.67 ^c	107.22 ^b
	K₃	406.44 ^{ab}	304.00 ^b	102.44 ^b
	K₄	408.78 ^a	316.78 ^a	92.00 ^c
	SEM	1.61	1.89	2.26
	P Value	0.02	0.00	0.01

Data expressed as Mean ± SE (n=3), Value expressed as mOsmol/Kg. Mean values with different superscripts in the same column under each category differ significantly (p <0.05).

Table: 23. Serum ionic concentrations of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	Sodium	Potassium	Chloride	Calcium	Magnesium
Salinity × K⁺ ion conc.	Control	135.33±1.76	0.38 ^k ±0.02	107.29±1.22	0.90±0.04	0.62±0.01
	T₁K₁	164.63±2.02	0.52 ^j ±0.003	121.10±1.42	1.28±0.03	0.85±0.01
	T₁K₂	164.97±1.77	0.54 ⁱ ±0.002	121.17±1.41	1.28±0.04	0.86±0.003
	T₁K₃	165.30±1.56	0.55 ^{hi} ±0.001	121.83±0.99	1.30±0.04	0.88±0.01
	T₁K₄	165.63±1.89	0.57 ^h ±0.002	121.87±0.97	1.33±0.02	0.88±0.01
	T₂K₁	214.26±1.71	0.62 ^g ±0.003	189.93±1.87	1.68±0.05	1.11±0.04
	T₂K₂	214.59±1.72	0.63 ^{fg} ±0.003	190.27±1.99	1.71±0.04	1.12±0.03
	T₂K₃	215.26±1.14	0.65 ^{ef} ±0.003	190.33±1.92	1.71±0.03	1.14±0.03
	T₂K₄	216.22±1.74	0.66 ^e ±0.001	191.33±2.62	1.74±0.03	1.15±0.02
	T₃K₁	302.32±1.63	0.74 ^d ±0.006	243.80±1.10	2.50±0.13	1.24±0.02
	T₃K₂	302.65±1.58	0.77 ^c ±0.006	243.93±1.21	2.52±0.12	1.24±0.03
	T₃K₃	303.65±2.51	0.79 ^b ±0.002	244.27±1.39	2.53±0.11	1.25±0.02
	T₃K₄	304.32±2.44	0.83 ^a ±0.002	244.33±1.44	2.57±0.09	1.27±0.02
	P Value	1.00	0.04	1.00	1.00	1.00
Salinity	Control	135.33 ^d	0.38 ^d	107.29 ^d	0.90 ^d	0.62 ^d
	T₁	165.13 ^c	0.54 ^c	121.49 ^c	1.30 ^c	0.87 ^c
	T₂	215.08 ^b	0.64 ^b	190.47 ^b	1.71 ^b	1.13 ^b
	T₃	303.23 ^a	0.78 ^a	244.08 ^a	2.53 ^a	1.25 ^a
	SEM	0.92	0.003	0.78	0.04	0.01
	P Value	0.00	0.01	0.00	0.00	0.02
K⁺ ion conc.	K₁	227.07	0.63 ^d	184.94	1.82	1.06
	K₂	227.40	0.65 ^c	185.12	1.83	1.07
	K₃	228.07	0.66 ^b	185.48	1.85	1.09
	K₄	228.72	0.68 ^a	185.84	1.88	1.10
	SEM	1.06	0.04	0.90	0.04	0.01
	P value	0.69	0.00	0.89	0.77	0.24

Serum sodium, potassium, chloride, calcium and magnesium ion concentrations are expressed as mmol/l. Data expressed as Mean ± SE (n=3), values with different superscripts in the same column under each category differ significantly (p <0.05).

4.15.3. Sodium potassium adenosine triphosphate activity (Na^+K^+ -ATPase)

Interaction of salinity and potassium had no significant effect ($p > 0.05$) on Na^+K^+ -ATPase activity in gills of Amur carp (Table- 24). Individual effect of salinity and potassium fortification had showed significant difference ($p < 0.05$) on the activity. The activity was observed to be increased with increase in salinity and potassium levels.

4.16. Stress Response

4.16.1. Serum cortisol and glucose

Interaction of salinity and potassium had no significant effect ($p > 0.05$) on cortisol and glucose level of the Amur carp, whereas salinity and potassium have individual effect on both the parameters. A significant increasing trend was observed for cortisol and glucose levels with increase in salinity and potassium concentrations.

4.16.2. AST and ALT activity

There was no significant ($p > 0.05$) interaction effect of salinity and potassium on AST and ALT activity in liver and muscle of different treatment groups. Salinity and potassium had individual effect on AST and ALT activity. It also observed that activity was significantly ($p < 0.05$) increasing with increase in salinity and increasing level of potassium (Table- 25).

4.16.3. LDH and MDH activity

LDH and MDH activities in the liver was significantly ($p < 0.05$) affected by different salinities, potassium concentrations and their interaction (Table- 26) and showed increasing trend with increase in salinities. LDH activity in the muscle tissue of Amur carp was significantly ($p < 0.05$) affected by salinity and potassium concentrations while MDH activity in the muscle did not show significant ($p > 0.05$) effect of potassium concentration. Interaction of salinity and potassium had no significant ($p > 0.05$) effect on LDH and MDH activity of muscle.

Table: 24. Gill Na⁺ K⁺ -ATPase Activity, Serum Cortisol and Serum Glucose of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	Gill Na ⁺ K ⁺ -ATPase Activity	Serum Cortisol	Serum Glucose
Salinity × K⁺ ion conc.	Control	2.93 ± 0.13	57.81±1.36	64.48±0.97
	T₁K₁	3.64 ± 0.13	68.46±0.57	68.12±0.97
	T₁K₂	3.74 ± 0.13	68.79±0.36	68.47±0.97
	T₁K₃	3.86 ± 0.13	69.46±0.63	69.09±0.97
	T₁K₄	4.00 ± 0.13	71.29±0.13	70.96±0.97
	T₂K₁	6.73 ± 0.13	76.64±0.12	81.83±0.97
	T₂K₂	6.71 ± 0.13	77.01±0.23	81.98±0.97
	T₂K₃	6.98 ± 0.13	78.13±0.35	82.31±0.97
	T₂K₄	7.24 ± 0.13	80.14±0.20	84.20±0.97
	T₃K₁	10.66 ± 0.13	86.30±0.61	92.30±0.97
	T₃K₂	10.79 ± 0.13	86.46±0.41	93.13±0.97
	T₃K₃	10.94 ± 0.13	87.99±0.75	94.90±0.97
	T₃K₄	11.23 ± 0.13	90.23±0.32	96.12±0.97
	P Value	0.96	0.94	0.96
Salinity	Control	2.93 ^d	57.81 ^d	64.48 ^d
	T₁	3.81 ^c	69.50 ^c	69.16 ^c
	T₂	6.91 ^b	77.98 ^b	82.58 ^b
	T₃	10.91 ^a	87.75 ^a	94.11 ^a
	SEM	0.06	0.28	0.49
	P Value	0.02	0.00	0.00
K⁺ ion conc.	K₁	7.01 ^b	77.13 ^c	80.75 ^b
	K₂	7.08 ^b	77.42 ^{bc}	81.19 ^b
	K₃	7.26 ^{ab}	78.53 ^b	82.10 ^{ab}
	K₄	7.49 ^a	80.56 ^a	83.76 ^a
	SEM	0.07	0.33	0.56
	P value	0.00	0.00	0.004

Gill Na⁺ K⁺ -ATPase Activity expressed as μmol Pi mg⁻¹ protein h⁻¹; Serum cortisol are expressed as ng/ml; Serum glucose expressed as mg/dl. Data expressed as Mean ± SE (n=3), values with different superscripts in the same column under each category differ significantly (p <0.05).

Table: 25. AST and ALT activities in the liver and muscle tissue of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	AST (Liver)	AST (Muscle)	ALT (Liver)	ALT (Muscle)
Salinity × K⁺ ion conc.	Control	2.86± 0.04	1.87±0.06	1.35±0.08	1.07±0.06
	T₁K₁	3.07±0.13	2.30±0.09	1.84±0.03	1.29±0.05
	T₁K₂	3.09±0.05	2.37±0.09	2.03±0.14	1.38±0.01
	T₁K₃	3.28±0.06	2.59±0.07	2.24±0.03	1.56±0.03
	T₁K₄	3.95±0.05	3.19±0.02	2.81±0.05	1.97±0.03
	T₂K₁	4.83±0.01	4.33±0.14	3.61±0.19	2.18±0.04
	T₂K₂	4.88±0.03	4.34±0.18	3.64±0.07	2.31±0.09
	T₂K₃	5.03±0.09	4.44±0.19	4.04±0.10	2.39±0.02
	T₂K₄	5.66±0.09	4.67±0.20	4.09±0.14	2.85±0.07
	T₃K₁	7.22±0.33	5.46±0.04	5.09±0.09	3.86±0.09
	T₃K₂	7.24 ±0.22	5.64±0.02	5.32±0.01	3.94±0.06
	T₃K₃	7.36±0.13	5.94±0.01	5.49±0.03	4.22±0.08
	T₃K₄	7.60±0.03	6.07±0.09	5.59±0.04	4.33±0.11
	P Value	0.45	0.40	0.37	0.21
Salinity	Control	2.86 ^d	1.87 ^d	1.35 ^d	1.07 ^d
	T₁	3.35 ^c	2.61 ^c	2.23 ^c	1.55 ^c
	T₂	5.10 ^b	4.44 ^b	3.84 ^b	2.43 ^b
	T₃	7.35 ^a	5.78 ^a	5.38 ^a	4.09 ^a
	SEM	0.07	0.07	0.07	0.03
	P Value	0.00	0.00	0.00	0.00
K⁺ ion conc.	K₁	5.04 ^b	4.03 ^c	3.51 ^c	2.44 ^c
	K₂	5.07 ^b	4.12 ^{bc}	3.66 ^{bc}	2.55 ^c
	K₃	5.22 ^b	4.33 ^b	3.92 ^{ab}	2.72 ^b
	K₄	5.74 ^a	4.64 ^a	4.16 ^a	3.05 ^a
	SEM	0.08	0.08	0.08	0.04
	P Value	0.00	0.00	0.00	0.00

Aspartate aminotransaminase (AST) - nano moles oxalo acetate released/min/mg protein at 37°C; Alanine aminotransferase (ALT)-nano moles Na pyruvate released /min/mg protein at 37°C. Values are Means ± SE (n= 3). Values with different superscript in the same column are significantly (p <0.05).

Table: 26. LDH and MDH activities in the liver and muscle tissue of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	LDH (Liver)	LDH (Muscle)	MDH (Liver)	MDH (Muscle)
Salinity × K⁺ ion conc.	Control	10.04 ^m ±0.02	10.13±0.44	1.05 ⁱ ±0.03	1.29±0.14
	T₁K₁	12.56 ^j ±0.01	11.39±0.04	1.68 ^h ±0.02	1.61±0.14
	T₁K₂	12.71 ^k ±0.01	11.68±0.06	1.63 ^h ±0.01	1.67±0.09
	T₁K₃	12.80 ^j ±0.01	11.82±0.06	1.67 ^h ±0.03	1.71±0.12
	T₁K₄	13.00 ⁱ ±0.01	12.64±0.32	1.82 ^g ±0.01	1.93±0.01
	T₂K₁	16.82 ^h ±0.01	15.53±0.21	3.12 ^f ±0.01	3.11±0.03
	T₂K₂	17.00 ^g ±0.03	15.98±0.04	3.20 ^e ±0.01	3.19±0.11
	T₂K₃	17.28 ^f ±0.01	16.45±0.17	3.23 ^e ±0.01	3.27±0.08
	T₂K₄	17.52 ^e ±0.01	17.31±0.16	3.64 ^d ±0.02	3.45±0.17
	T₃K₁	21.74 ^d ±0.01	20.47±0.44	5.07 ^c ±0.04	4.93±0.14
	T₃K₂	21.94 ^c ±0.02	20.85±0.24	5.12 ^c ±0.05	5.05±0.10
	T₃K₃	22.11 ^b ±0.01	21.56±0.44	5.28 ^b ±0.01	5.11±0.04
	T₃K₄	22.58 ^a ±0.01	22.01±0.18	5.37 ^a ±0.02	5.24±0.05
	P value	0.00	0.85	0.00	0.99
Salinity	Control	10.04 ^d	10.13 ^d	1.05 ^d	1.29 ^d
	T₁	12.77 ^c	11.89 ^c	1.70 ^c	1.73 ^c
	T₂	17.16 ^b	16.32 ^b	3.29 ^b	3.26 ^b
	T₃	22.09 ^a	21.22 ^a	5.21 ^a	5.08 ^a
	SEM	0.07	0.13	0.01	0.05
	P value	0.00	0.00	0.00	0.00
K⁺ ion conc.	K₁	17.04 ^d	15.80 ^c	3.29 ^c	3.22
	K₂	17.22 ^c	16.17 ^{bc}	3.32 ^c	3.30
	K₃	17.40 ^b	16.61 ^b	3.39 ^b	3.36
	K₄	17.70 ^a	17.32 ^a	3.61 ^a	3.54
	SEM	0.01	0.15	0.01	0.06
	P value	0.00	0.00	0.00	0.06

LDH: Lactate dehydrogenase; MDH: Malate dehydrogenase, LDH activities are expressed as: Units/min/mg protein at 37°C; MDH: specific activity expressed as Units /min/ mg protein at 37 °C, Values are Means ± SE (n = 3). Different superscript in the same column differ significantly (p <0.05).

4.17. Immune Response

4.17.1. SOD and Catalase (CAT) activity

SOD and CAT activity of the gills and liver of Amur carp reared at different treatment groups showed that salinity had significant ($p < 0.05$) effect whereas potassium concentration and interaction had no significant effect ($p > 0.05$). It was observed that activity increased with increase in salinity. Maximum activity of SOD and CAT was observed at T₃.

Table: 27. SOD and CAT activities in the Gill and liver of Amur carp reared in IGSW at different salinities and potassium (K⁺) concentration

	Treatments	SOD (Gill)	SOD (Liver)	Catalase (Gill)	Catalase (Liver)
Salinity × K⁺ ion conc.	Control	0.68±0.08	1.07±0.07	0.31±0.01	0.39±0.01
	T₁K₁	0.97±0.06	1.23±0.11	0.76±0.08	0.79±0.06
	T₁K₂	1.00±0.07	1.24±0.10	0.77±0.07	0.83±0.09
	T₁K₃	1.07±0.04	1.27±0.09	0.80±0.05	0.87±0.08
	T₁K₄	1.12±0.02	1.06±0.36	0.81±0.05	0.93±0.08
	T₂K₁	2.31±0.17	2.25±0.05	2.39±0.25	2.25±0.05
	T₂K₂	2.35±0.15	2.28±0.07	2.46±0.22	2.30±0.01
	T₂K₃	2.42±0.21	2.35±0.13	2.57±0.26	2.33±0.03
	T₂K₄	2.58±0.19	2.47±0.08	2.65±0.23	2.40±0.04
	T₃K₁	4.61±0.12	4.04±0.09	4.12±0.15	3.83±0.13
	T₃K₂	4.72±0.08	4.07±0.07	4.22±0.25	3.87±0.16
	T₃K₃	4.76±0.04	4.16±0.07	4.28±0.23	4.00±0.04
	T₃K₄	4.91±0.07	4.28±0.18	4.46±0.26	4.11±0.09
	P value	0.99	0.71	0.99	0.93
Salinity	Control	0.68 ^d	1.07 ^c	0.31 ^d	0.39 ^d
	T₁	1.04 ^c	1.20 ^c	0.79 ^c	0.85 ^c
	T₂	2.42 ^b	2.34 ^b	2.51 ^b	2.32 ^b
	T₃	4.75 ^a	4.14 ^a	4.27 ^a	3.95 ^a
	SEM	0.06	0.07	0.09	0.04
	P value	0.00	0.00	0.00	0.00
K⁺ ion conc.	K₁	2.63	2.51	2.42	2.29
	K₂	2.69	2.53	2.48	2.33
	K₃	2.75	2.59	2.55	2.39
	K₄	2.87	2.60	2.64	2.48
	SEM	0.07	0.08	0.11	0.05
	P value	0.11	0.79	0.53	0.06

SOD- super oxide dismutase; SOD activity is expressed as 50% inhibition of epinephrine auto oxidation/mg protein/min; Catalase activity expressed as Nano moles H₂O₂ decomposed/min/mg protein. Values are Means ± SE (n = 3). Different superscript in the same column differ significantly (p <0.05).

6. SUMMARY

Inland saline area has been identified as one of the new facets of aquaculture with immense potential and plethora of opportunities to be harnessed. There is about 8.7 million ha of salt-affected lands and about 40 % of these lands are distributed in the areas of Haryana, Punjab, Rajasthan and Uttar Pradesh. The lands affected with primary and secondary salinization becomes ill-suited for any worthwhile agriculture activities and the gradual rise in groundwater levels with time, causes secondary salinity. These demands a new system to respond to the problem and inland saline aquaculture is the perfect blend to utilise this opportunity. Inland saline aquaculture is mushrooming at a faster rate and there is an urge among the stakeholders to develop it as a commercial venture in the present days to come. Besides this, it offers an additional benefit of being used to reduce the amount of salt in underground water tables which will indeed lead to an improvement within the close land usage for agriculture. The inland ground saline water (IGSW) embraces a different ionic composition as compared to other water resources like fresh and brackish water and involves a low concentration of potassium ion (K^+). The lack of adequate levels of aqueous K^+ may have unfavourable effect on osmoregulation and $Na^+ K^+$ -ATPase activity. The inland saline water at Rohtak, Haryana state, India is also deficient in K^+ but has significantly high calcium levels and thus has very high hardness. Salinity being a key important factor for the culture of fishes may influence the growth by controlling the standard metabolic rate, food intake and other physiological parameters. Against this background, the present work was carried out to study the growth and survival of Amur carp (*Cyprinus carpio haematopterus*) in inland saline water at different salinities.

The present work was carried out on Amur carp (*Cyprinus carpio haematopterus*) for studying the effect of salinity at various levels and combinations along with different potassium ion concentrations on growth, survival and physiological response in inland saline water. The experiments were conducted in the wet laboratory at ICAR- Central Institute of Fisheries Education (ICAR-CIFE), Rohtak Centre, Haryana and most of the parameters were analysed in the laboratories at ICAR-CIFE, Mumbai, India. Healthy stocks of Amur carp fry were

procured from Instructional Fish Farm, College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India. The inland saline water required for the experiment was prepared at different salinities following the standard protocols. The work was carried out in two separate experiments. The freshwater was used as control (C) for both the experiments.

The experiment-1 was carried out for a period of 90 days to evaluate the effect of different salinities on growth, survival and physiological responses of Amur carp reared in IGSW and it was conducted in 12 circular FRP tanks of each 500 L capacity. The tanks were arranged following Completely Randomized Design (CRD). Two hundred forty fingerlings (avg. wt. 3.48 ± 0.27 g) were equally distributed in 12 FRP tanks of each 20 nos. The fish were fed with commercial pelleted feed having crude protein 32% and lipid 4% at the rate of 4% for the first 30 days and at the rate of 3% for rest of the period in two equal split doses. The experiment- 2 was carried out for a period of 90 days to evaluate the effect of different salinities along with different K^+ ion concentration on growth, survival and physiological response of Amur carp reared in IGSW. The experiment was conducted in 39 circular FRP tanks of each having 500 L capacity filled up to 400 L and round the clock aeration was provided from a portable air-blower. Seven hundred eighty fingerlings (avg. wt. 3.51 ± 0.18 g) were equally distributed in all treatments with three replicates. Feeding of fish and siphoning of tanks was done following the similar procedures as in experiment -1.

The water quality parameters were analysed at the beginning and throughout the experiment at regular intervals. The water temperature, pH and salinity were recorded daily by using mercury thermometer (0.1 accuracy), pH meter (Hanna Instrument, Italy) and hand-held refractometer (Atago, Tokyo, Japan) respectively, while dissolved oxygen, total alkalinity, total hardness, Ca^{2+} and Mg^{2+} were estimated titrimetrically by following the standard protocol twice in a week. The Na^+ and K^+ ions concentration levels were analysed with microprocessor flame photometer (Model 1382, ESICO International). The ammonium- nitrogen (NH_4^+-N) and nitrite (NO_2^-N) were estimated once in a week using Merck water quality test kits (Spectroquant®, Billerica, MA, USA) and with photometer (Nova 60, Spectroquant®).

The growth parameters were assessed by weighing the fish at a regular interval of every 15 days during the culture period of 90 days and the various growth parameters were assessed using standard formulas such as weight gain percent (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (%) . Besides this, an analysis of carcass composition was also done. In addition to these, digestive enzyme activity (protease, lipase, and amylase), physiological responses (osmolality and osmoregulatory capacity, serum ionic concentrations and Sodium Potassium Adenosine Tri-phosphatase Activity, stress responses (serum cortisol, serum glucose, Aspartate amino transferase, Alanine amino transferase, Lactate dehydrogenase, Malate dehydrogenase) and immune responses (Superoxide Dismutase Assay, Catalase Assay) were also measured. The histology of gills was also performed and it depicted histological changes in Amur carp due to exposure at different salinities. Data were analysed by one-way analysis of variance (ANOVA) in the first experiment and by two- way ANOVA in the second experiment using statistical software SPSS (version 22.0). The significant differences between the treatments were determined by Duncan's multiple range test (DMRT).

The results of the study depicted that the initial weight (g) of the Amur carp in all the treatments had no significant difference. However, a significant difference was observed in the %WG among different treatment groups at the end of the experimental period and the final %WG of Amur carp was significantly higher in control groups than that of other treatments. A decreasing trend in %WG of Amur carp with increasing salinity was also reported. The SGR of various treatments were found to be significantly different and it was highest in the control group and the lowest was recorded in the T₃. The moisture content (%) of fish was found to be significantly varying across all the treatments. The total lipid (%) significantly varied and depicted a decreasing trend with increase in salinity. There was significant difference ($p < 0.05$) in the serum osmolality, water osmolality and osmoregulatory capacity across all treatment groups. The serum ionic concentration of Amur carp was found to increase with elevated salinities. Cortisol also showed significant difference among all the treatments. The findings of the present study also indicated that increase in salinity influences the growth as well as the osmolality, ion concentration and Na^+K^+ -ATPase activity. Higher levels of Cortisol, Glucose, AST,

ALT and other metabolic enzymes indicated that fish were in stress condition at higher salinity. It was recorded that at low concentration of ISGW (5 ppt), no deleterious effects were found on the gill tissues. However, there were small changes in histological structures in the gill, recorded at higher salinity (10 and 15 ppt).

The second experiment was conducted to evaluate the combined effect of different salinities and potassium concentration on growth and physiological response of Amur carp. The result obtained in the study indicated that K⁺ supplementation have no positive effect on growth and survival while at 15 ppt higher supplementation affects growth and survival adversely. The proximate composition of fish also exhibited significant difference with increase in salinity. The study also reported that the fish can perform better up to 5 ppt salinity without affecting much growth, carcass composition and stress parameters. Significant differences were observed at different salinities, different K⁺ concentrations and their interaction, while there was no significant difference of salinity and K⁺ interaction on PER. There was no effect of salinity and K⁺ interaction on serum osmolality while individual effect of salinity and K⁺ concentration showed significant difference on serum and water osmolality. The activity of digestive enzyme was not influenced by K⁺ concentration and their interaction. Beside this, the study reveals no evidence on combined effect of different salinity and K⁺ ion concentration on digestive enzymes, activity of carbohydrate and protein metabolic enzymes and other stress parameters. Physiological parameters like SOD, Catalase, Glucose, AST, ALT, LDH, MDH and N⁺ K⁺-ATPase activity and osmolality was not varying much in control and 5 ppt salinity thus it can be attributed for better survival of the species.

In view of the results and above discussion, it can be concluded that Amur carp can be cultured in low inland saline water upto 5 ppt without affecting survival, stress enzyme, osmoregulatory capacity. Apart from this, there was no positive effect of K⁺ fortification on growth and survival of Amur carp, *C. carpio haematopterus* which can be promising fish in inland saline aquaculture in future, as it doesn't require fortification of inland water with K⁺ for the culture of this fish. In addition, as a lead recommendation of this study, we can infer that commercial culture of Amur carp can be done upto 5 ppt salinity in the inland saline region for

better prospects. Further research may be performed to measure the exact salinity for obtaining an optimum growth of Amur carp in inland saline water.

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