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Growth and osmoregulatory response of *Cyprinus carpio haematopterus* (Amur carp) reared in inland saline water

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ABSTRACT

A 90 days experiment was designed to assess the growth and osmoregulatory response of *Cyprinus carpio haematopterus* (Amur carp) in inland saline water of sub-humid and semi-arid/ arid zones of Haryana, India. Two hundred forty fingerlings (avg. wt. 3.48±0.272 g) were equally distributed in 4 treatment groups (salinities; control C, 0 ppt; T_1, 5 ppt; T_2, 10 ppt and T_3, 15 ppt) with 3 replicates in 500 L tanks followed by complete randomized design (CRD). There were no significant differences observed in physico-chemical parameters of water among different treatments and found in optimum range throughout the experimental period. The parameters, viz. total alkalinity, hardness (total, calcium and magnesium) and concentration of ions were varying with the salinity and displayed increasing trend with increase in salinity. At the end of experiment, 100% survival was obtained upto 5 ppt whereas decreasing trend was found with increasing salinity (T_2 and T_3). There was highest% weight gain obtained in control group followed by T_1, T_2 and T_3 groups. The highest specific growth rate (SGR) and protein efficiency ratio (PER) along with lowest food conversion ratio (FCR) was reported in control group followed by T_1 and T_2 groups, while lowest SGR, PER and highest FCR were reported in T_3 group. Serum and water osmolality showed significant increasing trend with increasing salinity, while osmoregulatory capacity was decreasing with increase in salinity. Based on the findings, it is suggested that Amur carp can be cultured with 100% survival up to 5 ppt with slightly lower production rate.

Key words: Amur carp, Growth, Inland saline water, Osmolality, Survival, Water quality parameters

Aquaculture is the fastest growing food sector contributing 80 million tonnes of total global fish production (FAO 2018) and 64% of the total aquaculture production is shared by inland sector. But, salinization of land and ground water in inland sector is an emerging problem and increasing due to both natural and anthropogenic activities (Bennetts *et al.* 2006). About 8.62 million ha area is influenced with salt in the India (Lakra *et al.* 2014) and in this region, several experiment had been conducted to find suitable culturable species to increase aquaculture production (Rani 2015, Upadhyay 2015, Kumar *et al.* 2017, Jahan *et al.* 2018).

Among the cyprinids, Amur carp is one of the fast growing freshwater fish and having several characteristics like slender body with low fat content in the meat, good FCR and natural feed selection capacity. As compared to common carp, Amur carp has approximate 29.7% and 40.33% faster growth in monoculture and polyculture systems, respectively (Basavaraju and Reddy 2013).

However, very little work has been done on influence of inland saline water on growth performance of salt tolerant freshwater fishes. Therefore, with the aim to evaluate the effect of different salinity ranges on growth, survival and osmolality of Amur carp in Inland ground saline water (IGSW) has been assessed to provide a new candidate species to the farmers of the area.

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MATERIALS AND METHODS

Experimental site and fish: A 90 days experiment was conducted at ICAR-CIFE, Rohtak Centre, Haryana, India and healthy fry stock of Amur carp were procured from Instructional Fish Farm, College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Uttrakhand, India.

Preparation of inland saline water with different salinities: The IGSW of 15 ppt was obtained 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 FRP tanks (capacity 1200 L). The stored water was diluted with bore-well water (salinity <0.5 ppt) into desirable salinities (5 and 10 ppt). Then, prepared water was disinfected with bleach liquor (Sodium Hypochlorite- NaOCl) @ 10 ppm and vigorously aerated for 48 h before use.

Experimental setup and design: After proper acclimatization in nursery pond, 1200 fishes were randomly selected and equally distributed in 4 different FRP tanks (capacity 1200 L) having freshwater. The desirable levels of salinity (5, 10 and 15 ppt) were increased progressively by adding the appropriate amount of raw IGSW in tanks (stocked with fish) to avoid osmotic shock during initial experiential period.

The experiment was conducted in 12 circular FRP tanks (500 L capacity) provided with sufficient aeration from a portable air-blower (Hiblow HP- 60) before the stocking of fish. Fingerlings (240; avg. wt. 3.48±0.272 g) were equally distributed in 4 treatments with 3 replicates following CRD. Fish were fed with commercial floating pellets (ABIS Exports India Pvt. Ltd, Chhattisgarh) having crude protein 32% and lipid 4%, @ 4% for the first 30 days and @ 3% for rest of the period in 2 equal split doses. Siphoning was carried out daily to remove excess feed and fecal matters and fresh medium was added to each tank twice a week.

Physico-chemical parameters of water: The water quality parameters were analyzed at the beginning and throughout the experiment at a regular interval. The temperature, pH and salinity were recorded daily by using mercury thermometer, pH meter (Hanna Instrument, Italy) and hand held refractometer (Atago, Tokyo, Japan) respectively, while dissolved oxygen, total alkalinity, total hardness (EDTA), Ca^{2+} and Mg^{2+} were estimated titrimetrically by following the standard protocol (APHA 2005) twice in a week. The Na^+ and K^+ ion concentration levels were analyzed with microprocessor flame photometer (Model 1382, ESICO International). The ammonium-nitrogen (NH_4^+-N) and nitrite (NO_2^-) were estimated once in a week using Merck water quality test kit (Spectroquant®, Billerica, MA, USA) and the value were recorded using a photometer (Nova 60, Spectroquant®).

Growth parameters: The growth parameters were assessed by weighing the fish at a regular interval of every 15 days during the culture period of 90 days by using the following formulae:

Total weight gain (g) = Final weight (g) – Initial weight (g),

% weight gain = [Final weight (g) – Initial weight (g)] *100/ Initial weight (g),

Specific growth rate (%/d) = [ln (Final weight) (g)– ln (Initial Weight) (g)]*100/Experimental period in day

Feed conversion ratio (FCR) = Feed intake (g) (Dry weight)/ Body weight gain (g) (Wet weight)

Protein efficiency ratio (PER) = Net weight gain (g) (Wet weight)/ Protein Fed (g) and

Survival (%) = Total no. of fish harvested*100/Total no. of fish stocked.

Osmolality and osmoregulatory capacity: Two fish from each replicate tank were anesthetized with clove oil (50 µl/ L) and blood was collected by puncturing caudal vein using medical syringe (No. 26) and transferred in 1.5 ml Eppendorf tubes without anticoagulant. The collected blood was allowed to clot for 1 h and centrifuged at 5,000 rpm for 10 min, after that supernatant was collected and stored at –20°C until analysis. Samples (50 µl) were subjected in triplicate to measure the osmolality (mOsm/kg) of serum and water of different treatments by using a cryoscopic osmometer (Osmomat® 030, Gonotec, Germany). Osmoregulatory capacity (OC) of an aquatic animal is a function of its physiological age (Hart *et al.* 1991) was obtained by subtracting the osmotic concentrations of the internal and external medium at given salinity (Charmantier *et al.* 1989).

Statistical analysis: Data was analyzed by one-way analysis of variance (ANOVA) using SPSS (version 22.0) and the significant difference between the treatments were determined by Duncan's multiple range test (DMRT). Statistical significance level was assumed as P<0.05.

RESULTS AND DISCUSSION

The growth performance of Amur carp was evaluated at different salinities (0, 5, 10 and 15 ppt) of IGSW (Table 1). Statistical analysis of the data revealed significant difference in all the treatments for final weight and mean weight gain. Küçük (2013) reported that freshwater fish generally grow well in both freshwater and low salinity environments and growth declines with increasing salinity which supports the finding of present study. The findings related to growth of the present study were also supported by other workers on different freshwater fishes (Wang *et al.* 1997, Rani and Gulia 2015, Upadhyay 2015, Abdullah 2016). In the present study, the % weight gain of all treatments was varying significantly

Table 1. Growth performance of Amur carp reared at different salinities in IGSW after 90 days

Parameter	Treatment			
	C	T_1	T_2	T_3
Initial wt. (g)	3.58±0.15	3.45±0.06	3.41±0.01	3.51±0.04
Final wt. (g)	30.83^a±0.54	26.56^b±0.73	23.44^c±0.77	15.02^d±0.85
Mean weight gain (g)	27.26^a±0.47	23.11^b±0.77	20.04^c±0.78	11.51^d±0.85
% weight gain	764.68^a±29.93	670.67^b±31.83	588.47^b±23.85	328.03^c±24.03
SGR (%/d)	2.39^a±0.03	2.27^{ab}±0.05	2.14^b±0.04	1.61^c±0.07
FCR	1.69^b±0.07	1.80^b±0.06	1.84^b±0.05	2.20^a±0.07
PER	1.85^a±0.08	1.73^a±0.06	1.70^a±0.05	1.42^b±0.05
Survival (%)	100.0^a±0.00	100.0^a±0.00	91.67^b±1.67	85.0^c±2.89

Value expressed as mean±SE are the means of three replicates (n=3). Different superscripts in same column indicate significant difference (P<0.05). SGR, Specific growth rate; FCR, Food conversion ratio; PER, Protein efficiency ratio.

($P<0.05$) with control but T_1 and T_2 had no significant difference and it showed a decreasing trend with increase in salinity. According to Iwama (1996), the possible reason for reduction in growth is that the amount of available energy is diverted to maintain the intracellular ionic and osmotic regulation. The present study revealed a decreasing trend in SGR with increasing salinity which is similar to the findings of different species (Islam *et al.* 2014, Abdullah 2016). The SGR of control group had significant difference ($P<0.05$) with T_2 and T_3 but did not vary significantly with T_1 while no significant difference was observed between T_1 and T_2. Fallah *et al.* (2013) reported that a species of cyprinidae (*Capoeta damascina*) reared at 4 different salinities (0.04, 6, 12 and 24 ppt) for 12 months showed better growth performance in freshwater compared to fish at higher salinities (similar to present experiment). Basavaraju and Reddy (2013) reported average SGR 2.25 and 2.69%/d 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. FCR and PER of T_3 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, T_1 and T_2 groups. Similar to present study, FCR for rohu cultured at different salinities showed an increasing trend with increasing salinities (Islam *et al.* 2014). Wang *et al.* (1997) found minimum FCR at 2.5 and 0.5 ppt salinity, respectively for common carp cultured at different salinities and increased with increasing salinity. In our study, PER decreased with increase in salinity, which is supported by Upadhyay (2015). At the end of the experiment, survival percentage was lower in T_2 and T_3, and increased significantly in control and T_1. Similar finding was reported in *Pangasianodon hypophthalmus* reared at different salinities in IGSW (Kumar *et al.* 2017). In different studies, the 100% survival was recorded for *Labeo rohita* at the salinity of 8 and 6 ppt as reported by Pillai *et al.* (2003) and Islam *et al.* (2014), respectively. Ansal *et al.* (2016) reported different survival (%) at different salinity and species combination of freshwater carps.

Physico-chemical characteristics of water: Most of the water quality parameters remained at optimum levels and no significant difference was observed in DO, temperature, pH, NH_4^+-N and NO_2-N among different treatments throughout the experimental period (Table 2), which is supported by different workers in different studies (Velasco *et al.* 2006, Santosh and Singh 2007, Bhatnagar and Devi 2013). There was significant difference observed for other parameters like salinity, total alkalinity, total hardness, calcium and magnesium hardness, Na^+, K^+, Ca^{2+} and Mg^{2+} ions concentration at different salinities and the values were increased with increasing salinity (Table 2). Alkalinity is a measure of total concentration of bases in water and higher

Table. 2 Physico-chemical parameters of water

Parameter	Treatments			
	C	T_1	T_2	T_3
Salinity (ppt)	0.13^d±0.00	5.08^c±0.01	10.04^b±0.01	15.04^a±0.01
Temperature (°C)	25.82±0.12	25.78±0.12	25.88±0.10	25.79±0.10
DO (ppm)	6.48±0.12	6.52±0.11	6.46±0.10	6.34±0.13
pH	7.59±0.04	7.60±0.05	7.63±0.05	7.69±0.07
NH_4^+-N (ppm)	0.22±0.01	0.24±0.01	0.24±0.02	0.25±0.02
NO_2-N (ppm)	0.03±0.01	0.04±0.01	0.04±0.01	0.05±0.01
Total alkalinity (ppm)	113.89^d±0.22	163.22^c±0.78	214.11^b±0.78	304.38^a±0.83
Total hardness (ppm)	169.16^d±4.73	1398.88^c±1.46	2678.33^{bc}±5.46	3232.50^a±0.83
Ca hardness (ppm)	101.94^d±2.37	401.66^c±0.96	533.88^b±5.28	830.00 ^{a}±7.12
Mg hardness (ppm)	67.22^d±2.64	997.22^c±2.00	2144.44^b±0.56	2402.50^a±6.82
Ca^{2+} (ppm)	40.78^d±0.95	160.67^c±0.39	213.56^b±2.11	332.00^a±2.85
Mg^{2+} (ppm)	16.34^d±0.64	242.33^c±0.49	521.10^b±0.14	583.81^a±1.66
Na^+ (ppm)	9.67^d±0.75	1586.11^c±10.59	3177.78^b±7.01	4464.44^a±13.31
K^+ (ppm)	2.48^d±0.06	5.54^c±0.02	9.69^b±0.04	12.77^a±0.01

Data expressed as the means of three replicates (n=3). Different superscripts in same column indicate significant difference ($P<0.05$). DO; Dissolved oxygen.

Table 3. Osmoregulatory response of Amur Carp reared at different salinities in the IGSW

Parameter (mOsm/kg)	Treatment			
	C	T_1	T_2	T_3
Serum osmolality	305.00^d±8.54	350.00^c±2.88	384.00^b±1.16	454.33^a±15.30
Water osmolality	7.00^d±1.73	108.00^c±2.31	268.67^b±4.33	380.00^a±12.70
Osmoregulatory capacity	298.00^a±7.00	242.00^b±2.65	115.33^c±5.49	74.33^d±2.60

Data expressed as Mean±SE. Different superscripts in same column indicate significant difference ($P<0.05$).

alkalinity value is an indication of absence of CO_2. In present study, varying level of total alkalinity was at different salinities as supported by Limhang *et al.* (2011). Santosh and Singh (2007) found 50–300 ppm as ideal alkalinity value for fish culture. Total hardness was observed maximum at T_3 followed by T_2, T_1 and control. Jahan *et al.* (2018) observed the total hardness range of 2460 to 2793.5 at 10 ppt salinity which is similar to the finding of present study at the same salinity. 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 (Mellor and Fotedar 2005, 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 (Rani 2015, Upadhyay 2015, Chitra *et al.* 2017, Jahan *et al.* 2018).

Osmolality and osmoregulatory capacity: There were significant difference ($P<0.05$) observed in serum and water osmolality among the different treatment groups. The findings of the present work were supported by Mellor and Fotedar (2005), who reported increase in blood osmolality in juveniles of Murray cod (*Maccullochella peelii peelii*) reared at different salinities with increase in salinity and also concluded that osmolality is not influenced by duration of exposure. Similar findings related to osmolality (water and serum) and osmoregulatory capacity were reported by different authors in different fishes reared at different salinity in IGSW (Rani 2015, Upadhyay 2015, Kumar *et al.* 2017). In the present study, the continuous 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.

It can be concluded that freshwater (control) and IGSW of 5 ppt salinity showed better growth performance without significant difference in SGR, FCR, PER and survival. The growth was slightly affected at 5 ppt salinity and strongly influenced at 10 and 15 ppt salinity. At salinity of 10 and 15 ppt, growth reduction may be due to altering energetic cost for maintaining the ionic and osmotic homeostasis. It is suggested that the Amur carp can be reared at 5 ppt and can tolerate upto 15 ppt salinity.

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