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Survival and growth of early juveniles of barramundi, *Lates calcarifer* (Bloch, 1790) in inland saline groundwater

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Survival and growth rate of juveniles (35 days of age) of barramundi *Lates calcarifer* were studied in inland saline groundwater (ISGW) at 15 and 25‰ salinities for 30 days and were compared with artificial seawater (ASW) at similar salinities. ISGW of 58‰ was drawn from an open well in Western Rajasthan, India. It was diluted to 15 and 25‰ by adding fresh water. Survival and specific growth rate (SGR) of juveniles were 100% and 4.552 ± 0.126 respectively in ISGW at 15‰. It was similar ($p > 0.05$) to survival (100%) and SGR (4.567 ± 0.165) in ASW at 15‰. Survival and SGR became reduced to 80% and 3.938 ± 0.105 in ISGW at 25‰ and that was significantly ($p < 0.05$) lower to that in ISGW at 15‰. The survival and SGR in ASW at 25‰ was 100% and 4.448 ± 0.416 respectively that was much similar to that in ASW at 15‰. The concentration of potassium was 34.2 and 60.8 mg l^{-1} in ISGW of 15 and 25‰ and 173.8 and 289 mg l^{-1} respectively in ASW of similar salinities. In ISGW at both 15 and 25‰, K:Cl ratio was 0.003, but it was 0.02 in ASW. Potassium deficiency in ISGW did not affect the survival and growth of early juveniles of *L. calcarifer* at 15‰, but it was harmful at 25‰. The present study suggests that ISGW at low salinity (15‰) that was deficient in potassium (34.2 mg l^{-1}) could be used for growing early juveniles of *L. calcarifer*, but it may need potassium supplementation at higher salinities.

Key words: barramundi, *Lates calcarifer*, inland saline groundwater, potassium, ionic ratios.

INTRODUCTION

The suitability of inland saline groundwater (ISGW) has been evaluated for the culture of fish and other aquatic animals (Brune *et al*., 1981; Dwivedi & Lingaraju, 1986; Smith & Lawrence, 1990; Forsberg *et al*., 1996; Sarre *et al*., 1999; Allan *et al*., 2001; Fielder *et al*., 2001; Ingram *et al*., 2002; Samocha *et al*., 2002; Jain *et al*., 2003; Rahman *et al*., 2005) in Australia, USA, India and elsewhere. ISGW, which has formed due to various natural and anthropogenic causes (Joshi & Tyagi, 1994; Forsberg *et al*., 1996; Allan *et al*., 2001), is similar to seawater in ionic constituents but the concentrations and ratios of various ions are different. Most commonly, ISGW is excessive in calcium and deficient in potassium. Salinity of inland saline groundwater is also variable (Forsberg & Neill, 1997; Fielder *et al*., 2001; Rahman, *et al*., 2005). These differences may vary with respect to location and source and may affect the survival and growth of various aquaculture species. Survival and growth of *Sciaenops ocellatus* (Forsberg *et al*., 1996), *Pagrus auratus* (Fielder *et al*., 2001) and *Penaeus monodon* (Collins & Russell, 2003; Rahman *et al*., 2005) were found to be affected by variations of ionic concentrations of ISGW whereas *Acanthopagrus butcheri* (Sarre *et al*., 1999) *Chanos chanos* and *Mugil cephalus* (Jain *et al*., 2003) were not observed to be affected due to changes in ionic concentrations of ISGW.

Lates calcarifer is a euryhaline marine fish that can tolerate salinities of at least 55‰ (Shirgur & Siddiqui, 1998). It is of high commercial importance and is cultured in both brackish and fresh water, but early juveniles are cultured mainly in brackish water. The indoor culture of early juveniles of *L. calcarifer* in brackish water is practiced at hatcheries to grow them to a suitable size of 4-5 g before stocking in

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grow outponds (to avoid cannibalism). Information was available on culture of advanced juveniles in ISGW (Partridge, 2003) but not on early juveniles. In the present study, survival and growth of 35 day old juveniles of *L. calcarifer* were evaluated in ISGW of western India at 15 and 25‰. Success in culture of early juveniles in saline groundwater in inland regions will help saving transportation costs from coastal hatcheries which is very high for larger (4-5 g) juveniles.

MATERIALS AND METHODS

Saline groundwater of 58‰ salinity was obtained from an inland shallow open well located in western India (25° 57' N and 73° 4' E). Hatchery produced 35 day old juveniles of *L. calcarifer* that were first acclimatized to seawater at 20‰ and then transported to the Aquaculture Research Laboratory of CIFE at Udaipur, Rajasthan from the Central Institute of Brackish Water Aquaculture (ICAR), Chennai. Juveniles were maintained in artificial seawater (ASW) at 20‰ until the start of the bioassay experiment. Survival and growth of *L. calcarifer* were evaluated in ISGW at 58‰ diluted to 15 and 25‰ by adding fresh water (pH:7.0, calcium:40.9 mg l^{-1}, magnesium: 16.6 mg l^{-1}, sodium and potassium negligible) and in ASW of similar salinities (control) which was prepared by using marine salts (Dophine Marine Salts, USA). The ionic concentrations and ratios are given in Table 1. All trials (in triplicates) were conducted in glass aquaria (0.60 × 0.30 × 0.30 m) filled with 30L of test water. Each aquarium was stocked with 10 juveniles of an average weight and total length of 0.475 ± 0.094 g and 1.730 ± 0.109 cm, respectively. Juveniles were anesthetized using cod liver oil (50 µl l^{-1}) before being weighed and measured and subsequently allowed to recover in a well aerated medium. Length of juveniles was measured to the nearest millimeter and weight to the nearest milligram. Water in each aquarium was adequately aerated through an air pump. Water temperature was maintained at 27 ± 1 °C in each aquarium by installing a 50 W submersible heater with thermostatic control. Juveniles were fed to satiation on live biomass of *Artemia* twice daily at 08.00 h and 18.00 h. Fifty percent of the aquaria water was exchanged every second day. Water quality (temperature, dissolved oxygen and pH) was monitored twice a week. Concentrations of sodium, potassium, calcium, magnesium and chloride were also estimated (APHA, 1980). The experiment was terminated after 30 days. Juveniles were measured and weighed by applying the method described above. Specific growth rate (SGR) and survival were estimated as follows:

$$\text{SGR} = \frac{\text{Ln Final weight (g)} - \text{Ln Initial weight (g)}}{\text{Culture duration (days)}} \times 100$$

$$\text{Survival} = \frac{\text{Number stocked}}{\text{Number harvested}} \times 100$$

One way analysis of variance (ANOVA) was used to find the treatment effect. The difference of means between any treatments was tested by Duncan's multiple range test (DMRT) at 5% level of significance.

TABLE 1. Concentration of major ions and their ionic ratios in inland saline groundwater (source and treatments) and artificial seawater (control)

Ions (mg l^{-1})	Salinity (‰)						
	Inland saline groundwater					Artificial seawater	
	15	Δ*	25	Δ*	58	15	25
Sodium	2850.000	-40.440	4723.000	-40.780	13768.000	4785.000	7975.000
Calcium	312.200	72.960	535.000	78.150	921.800	180.500	300.300
Magnesium	168.000	-66.270	315.500	-62.000	1024.400	498.000	830.200
Potassium	34.200	-80.330	60.800	-78.970	159.400	173.800	289.000
Chloride	9700.000	16.720	16800.000	21.290	39760.000	8310.000	13850.000
Ca^{2+}/Na^{+}	0.110	194.590	0.110	205.400	0.070	0.037	0.037
Na^{+}/K^{+}	83.333	102.680	77.680	182.160	86.373	27.531	27.595
K^{+}/Cl^{-}	0.003	-85.000	0.003	-85.000	0.004	0.020	0.020

* indicates percentage difference of inland saline groundwater from artificial seawater of same salinity

TABLE 2. Survival, final total length and weight of *L. calcarifer* larvae in inland saline groundwater (ISGW) and artificial seawater (ASW) at 15‰ and 25‰ salinity during the 30-day culture period

Type of water	Salinity (‰)	Survival Mean ± SE (%)	Final length* Mean ± SE (cm)	Final weight** Mean ± SE (g)
ISGW	15	100 ± 0.00[a]	2.422 ± 0.041	1.970 ± 0.147[a]
	25	80 ± 5.77[b]	2.250 ± 0.093	1.616 ± 0.202[b]
ASW	15	100 ± 0.00[a]	2.442 ± 0.042	1.885 ± 0.098[a]
	25	100 ± 0.00[a]	2.375 ± 0.217	1.850 ± 0.239[a]

([a]): $p > 0.05$; ([b]): $p < 0.05$
(*): Average initial total length 1.730 ± 0.109 cm
(**): Average initial weight 0.475 ± 0.094 g

RESULTS

The ionic concentrations and ratios of ISGW at 15‰ and 25‰ were different from those of ASW at similar salinities (Table 1). The concentration of calcium was higher by 72.9% in ISGW at 15‰ and by 78.1% at 25‰ compared with that in ASW at similar salinities, whereas the concentrations of magnesium and potassium were lower in ISGW. The magnesium was lower by 62% at 15‰ and by 66.2% at 25‰. The potassium was lower by 78.9% at 15‰ and by 80.3% at 25‰.

Survival of larvae of *L. calcarifer* was 100% both in ISGW and ASW at 15‰ (Table 2). Survival was reduced to 80% ($p < 0.05$) with increase in salinity of ISGW from 15‰ to 25‰, whereas it remained the same in ASW. SGR in ISGW at 15‰ was 4.552 ± 0.126 which was similar ($p > 0.05$) to ASW of equal salinity where it was 4.567 ± 0.165 (Fig. 1). SGR was reduced to 3.938 ± 0.105 in ISGW at 25‰ that was 4.448 ± 0.416 in ASW of similar salinity. SGR was significantly reduced ($p < 0.05$) with increase in salinity of ISGW to 25‰. SGR was not reduced due to increase of salinity of ASW to 25‰ from 15‰.

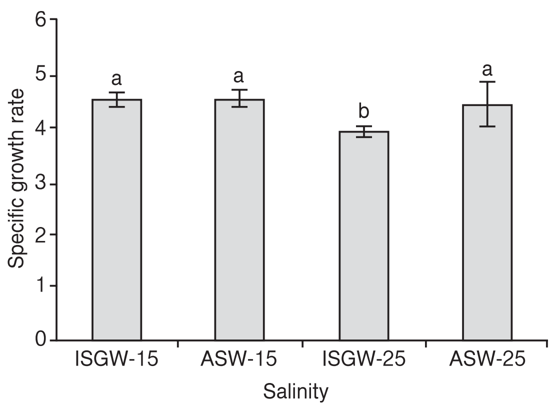

FIG. 1. Specific growth rate of larvae of *L. calcarifer* cultured in inland saline groundwater (ISGW) and artificial seawater (ASW) of different salinities. Statistical significance is shown as a ($p > 0.05$) and b ($p < 0.05$).

DISCUSSION

The survival and SGR of early juveniles of *L. calcarifer* in ISGW at 15‰ was similar to that in ASW but it was lower in ISGW at 25‰. The low survival and SGR in ISGW at 25‰ cannot be attributed to high salinity, as this was not affected in ASW. It could be rather due to the variations in the ionic concentrations and ionic ratios of ISGW than to those of ASW (Forsberg & Neill, 1997; Fielder *et al*., 2001; Rahman *et al*., 2005). The results of the present study were much similar to the findings of Partridge (2003) who evaluated the suitability of ISGW of Toolibin, Western Australia at 45‰ for the culture of advanced juveniles (8.04 ± 0.17 to 41.1 ± 1.5 g) of *L. calcarifer*. The complete mortality was observed at 45‰, but the juveniles survived in ISGW of the same source when diluted to 15‰. The ISGW of Toolibin was excessive in calcium (18%), magnesium (71%) and manganese (99 times high) and deficient in potassium (85%) compared to seawater of similar salinity. These variations of ionic concentrations did not affect the survival of advanced juveniles of *L. calcarifer* at 15‰ but at 45‰. The ionic concentration of ISGW that was used in the present study was very different from that of Toolibin, but the survival and SGR of early juveniles were not affected at 15‰. This suggests that variations due to ionic concentrations of ISGW at low salinity do not affect the survival and growth of juveniles of *L. calcarifer*. Adverse effects were observed at higher salinities.

Partridge (2003) could improve the survival and growth of advanced juveniles of *L. calcarifer* in ISGW at 45‰ by enriching it with potassium to increase the K:Cl ratio from 0.005 to 0.010-0.025 in ISGW. In seawater of similar salinity, K:Cl ratio is 0.025. However, the SGR was lower in potassium-enriched saline groundwater at 45‰ compared to the control treatment. This was possibly attributed to the high concentration of manganese that was not studied. The concentration of manganese in ISGW of western Rajasthan (personal observation) that was used in the present study was 0.01 mg l^{-1}. It is similar to the concentration of manganese in seawater. Therefore, in the present study, the lower growth of early juveniles of *L. calcarifer* in ISGW at 25‰ could be mainly attributed to potassium deficiency (Partridge, 2003) and not to any other ion. Partridge & Creeper (2004) have described that the deficiency of potassium in saline groundwater caused the hyperplasia of chloride cells and vasoconstriction of skeletal muscles in juveniles of *L. calcarifer*, which finally lead to mortality. The survival and growth of *Pagrus auratus* and *Argyrosomus japonicus* were also found to be affected by deficient potassium in ISGW, which could be improved by increasing the concentration of potassium to $>40\%$ in the seawater in order to obtain a K:Cl ratio of 0.010-0.018 (Fielder *et al*., 2001; Doroudi *et al*., 2004).

The potassium requirements of stenohaline and euryhaline fishes have been found different in ISGW culture. The stenohaline fishes viz; *A. japonicus* and *P. auratus* (Fielder & Bardsley, 1999; Fielder *et al*., 2001; Doroudi *et al*., 2004) were observed to need potassium enrichment in ISGW at low salinities of 5-12‰, but in the euryhaline fishes viz; *A. butcheri* and *L. calcarifer* (Sarre *et al*., 1999; Partridge, 2003), potassium enrichment was needed only at higher salinities. Other euryhaline species viz; *C.chanos* and *M. cephalus* were also reported to grow normally in ISGW at 15-25‰ (Jain *et al*., 2003), but not at 55‰ (Jain, 2005), whereas the optimum growth of *C. chanos* has been reported at 55‰ in brackish water (Swanson, 1998). The cause of less growth of *C. chanos* in ISGW at 55‰ (Jain, 2005) could be due to the deficiency of potassium in ISGW that was not investigated.

The potassium is needed to maintain the electrolyte and acid base balance by regulating the activities of Na^+, K^+-ATPase and $Na^+/K^+/2Cl^-$ co-transporter (Marshall, 1995; McCormick, 1995; Karnaky, 1998) in a hyperosmotic environment. Fish maintains plasma potassium concentrations at specific levels probably by active uptake in low potassium environments and diffusional uptake in high potassium environments (David Evnas; pers.comm.). It does not explain the reasons of different requirement of potassium in stenohaline and euryhaline fishes. It warrants detailed studies on the role of potassium in ionic regulation in stenohaline and euryhaline species and also on its uptake mechanism (Eddy & Bath, 1979; Sanders & Kirschner, 1983; Eddy, 1985; Gardaire *et al*., 1991; Wilson & El Nagar, 1992).

The present study suggests that the inland saline groundwater of low salinity that is different from the seawater in ionic concentration could be used to grow early juveniles of *L. calcarifer* to a size suitable for stocking in grow-out ponds, but potassium enrichment may be needed in saline groundwater of high salinities, as suggested by Partridge (2003).

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