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Growth and survival of silver pompano *Trachinotus blochii* (Lacepede, 1801) at different salinities in inland saline ground water

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ABSTRACT

Inland saline ground water (ISGW) has attracted great interest for commercial aquaculture of marine species in Haryana and Punjab. The silver pompano (*Trachinotus blochii*), a candidate for mariculture, was investigated for its potential to survive in inland saline water at ICAR-CIFE, Regional Centre, Rohtak, Haryana during 2015–16. The experimental set up comprised ISGW without potassium amendment, ISGW with potassium amendment and artificial sea water of 5 ppt, 10 ppt and 15 ppt salinity. In ISGW without potassium amendment, 100% mortality was observed at 12 h (5 ppt), 24 h (10 ppt) and 48 h (15 ppt). In the second experiment, three treatments (ISW5, ISW10 and ISW15) of ISGW with potassium amendment equivalent to sea water were prepared. A 100% survival was observed at all salinities in ISGW with potassium amendment, similar to the artificial seawater at the end of 90 days. Significantly higher weight gain, specific growth rate (SGR) and average daily growth (ADG) were observed at ISW10 and ISW15 than at ISW5. The plasma osmolality of silver pompano in ISW10 and ISW15 indicated that silver pompano regulates isosmotic condition to maintain the homeostasis of body. The present results indicate that silver pompano can be reared at 10 ppt to 15 ppt salinity with optimum growth and survival in potassium amended ISGW.

Key words: Inland saline aquaculture, Silver pompano, *Trachinotus blochii*

The salinization of agricultural land is a global concern for both developing as well as the developed nations. Saline ground water is not suitable for traditional agriculture (Joshi and Tyagi 1994, Forseberg *et al.* 1996, Allan *et al.* 2001) hence its use for aquaculture of marine water species can be a potential alternative with significant economic gains especially in India because of availability of huge volume of saline ground water resources especially in north-western parts of the country. In India, the salt affected lands are spread over an area of 8.62 m ha with 0.620 m ha in Haryana (Lakra *et al.* 2014). Ionic concentration of inland saline ground water (ISGW) is different from natural sea water in terms of its specific ions, which varies from location to location (Forsberg and Neill 1997). ISGW on the Indian sub-continent usually contains comparatively higher concentration of calcium than magnesium and potassium (Jain *et al.* 2002). The difference in the ionic concentration of ISGW affects the survival and growth of fish and shrimp species (Fielder *et al.* 2001), as potassium plays very important role in osmo- and iono-regulation and acid-base balance in fishes (Marshall and Bryson 1998, Evans *et al.* 2005). India has achieved remarkable success in the farming of *Penaeus monodon* (Lakra *et al.* 2013a, Purushothaman *et al.* 2014) and *Litopenaeus vannamei* (Lakra *et al.* 2013b) at Rohtak, Haryana. Successful experiments were also conducted in the past to evaluate the effects of ionic deficiency and feasibility of marine fish culture in ISGW (Dwivedi and Lingaraju 1986, Jain *et al.* 2002, Raizada *et al.* 2005, Jain *et al.* 2006).

The silver pompano (*Trachinotus blochii*) with its fast growth rate is an important aquaculture species and has a very high market demand due to its good meat quality (Gopakumar *et al.* 2011). In India, ICAR-Central Marine Fisheries Research Institute has successfully carried out broodstock development, induced breeding and larval production of this species in 2011 (Gopakumar *et al.* 2011). The silver pompano is able to acclimatize and grow well even at a low salinity of about 8 ppt (Gopakumar *et al.* 2011). However, the potential of silver pompano culture in inland saline ground water is not known. Therefore, a study was conducted to evaluate the survival, growth and osmolality of the juveniles reared in potassium amended ISGW of varying salinities (5 ppt, 10 ppt and 15 ppt) compared to reconstituted seawater of similar salinities.

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MATERIALS AND METHODS

Experimental design: The experiment was conducted at wet lab complex of CIFE, Rohtak Centre, Haryana, India. Silver pompano (*Trachinotus blochii*) juveniles with an average length and weight of 6 cm and 4.5 g respectively, were procured from Mandapam regional center of Central Marine Fisheries Research Institute. Two sets of experiments were carried out to see the feasibility of silver pompano rearing in inland saline ground water (ISGW). In the first experiment, pompano juveniles were reared in unammended ISGW (RSW) at 5 ppt, 10 ppt and 15 ppt salinity to evaluate the effect of ISGW without any potassium amendment on its survival. Second experiment was conducted to determine the effect of potassium amendment in ISGW on the survival and growth of silver pompano juveniles. To make potassium amendment in ISGW, experimental media were prepared by supplementing commercial grade Muriate of Potash (KCl) with 100% equivalence potassium concentration of seawater (35 ppt) diluted to 5 ppt, 10 ppt and 15 ppt salinity, i.e. ISW5, ISW10 and ISW15 respectively. Reconstituted artificial sea water of 5 ppt, 10 ppt and 15 ppt salinities were prepared as reference media, i.e. ASW5, ASW10 and ASW15 respectively. Ionic profile of treatment and reference medium is given in Table 1. The experimental design used was a completely randomized design (CRD) wherein the reference and treatments were studied in triplicate. A total of 27 FRP tanks of 300 litre capacity were used in the experiment. The tanks were filled with 200 litres of treatment water and fitted with aeration stones to provide continuous aeration. A total of 15 juveniles (~4.5 g) pre-acclimatized to their respective salinities were stocked in each tank. The experiment was carried out for 90 days. Feeding was done using commercial Growel feed crumbles of size 1.8 mm three times a day. Water exchange was carried out once in a day during evening hours wherein 50% of water was exchanged. All the experimental media were prepared 15 days prior to the commencement of the trial. The salinity of the water was tested using a handheld refractometer (Atago, Tokyo, Japan) and was counter checked with titration method (APHA 2005). The Na^{2+} and K^+ levels were analyzed using flame photometry (Microprocessor flame photometer, Model 1382, ESICO, Haryana, India). The total hardness, Ca^{2+} and Mg^{2+} concentrations were analyzed using ethylene diamine tetra-acetic acid (EDTA) titration (APHA 2005).

Survival and growth performance: For the first experiment, survival of juveniles in unammended inland saline ground water (RSW) was recorded at time intervals of 6, 8, 12, 16, 20, 24, 48 and 72 h. During the second experiment, survival and growth were monitored for the juveniles reared in amended ISGW by counting and weighing the fishes every 7th day till the commencement of the experiment.

Percentage survival (PS), weight gain (WG), specific growth rate (SGR) were calculated as per the following formulas:

$$\text{PS (\%)} = \frac{\text{Number of survivors at the end of the experiment}}{\text{Number stocked initially}} \times 100$$

$$\text{WG} = \frac{W_2 - W_1}{W_1}$$

$$\text{SGR} = (\text{\% body weight/day}) = \frac{\ln (W_2 - W_1)}{\Delta t} \times 100$$

$$\text{ADG} = \frac{W_2 \times W_1}{\Delta t}$$

where W_1, initial wet weight of fish at stocking; W_2, final wet weight of fish; t, grow out period.

Plasma and water osmolality: Fishes were anaesthetized using clove oil and blood was extracted from the caudal arch. Blood samples were centrifuged at 3,000 rpm for 3 min and the plasma thus obtained was used for osmolality measurement. Water samples from all the treatments were collected to measure osmolality. The water and plasma osmolality (mOsm/kg) were measured using a cryoscopic osmometer (Osmomat® 030, Gonotec GmbH, Germany). The osmoregulatory capacity (OC) of the juveniles in different treatments was calculated as the difference between the mean osmolality of the fish plasma and mean osmolality of their corresponding rearing media (Greenwell *et al.* 2003).

Statistical analysis: Statistical analysis were performed

Table 1. Ionic profile of treatment and reference medium used for 90 days trial (Mean±SE)

Treatment	Salinity (ppt)	Na^+ (mg/l)	K^+ (mg/l)	Mg^{2+} (mg/l)	Ca^{2+} (mg/l)	Na^+/K^+ ratio	Mg^{2+}/Ca^2 ratio	K^+ (%)
RSW5	5.1±0.10	1393.67	4.5	306.67	156	309.70	1.97	8.4
RSW10	10.2±0.10	2703	8.1	541	314.33	333.70	1.72	7.5
RSW15	15.1±0.10	3712	11.5	767	413	322.78	1.86	7.17
ISW5	5.2±0.10	1394	55.33	396.67	158	25.19	2.51	103.42
ISW10	10.3±0.10	2765	109.43	789.33	320.33	25.27	2.46	102.27
ISW15	15.2±0.10	4066.33	161.83	1016.67	402.67	25.13	2.52	100.83
ASW5	5.1±0.10	1490	54.03	211	59.28	27.58	3.56	100.99
ASW10	10.3±0.10	3043.33	107.89	389.67	110.33	28.21	3.53	100.83
ASW15	15.0±0.20	4467	162.05	554	158.67	27.57	3.49	100.97

using SPSS, version 22. Treatment means were compared with one-way ANOVA and differences evaluated using Duncan's multiple range test. A significance level of P<0.05 was used.

RESULTS AND DISCUSSION

During the experimental period of 90 days, all the water quality parameters did not differ among the treatments (Table 2). Mean values of all water quality parameters in treatment medium with potassium amendment (ISW) and reference medium reconstituted sea water (ASW) at 5 ppt, 10 ppt and 15 ppt were within the acceptable ranges for pompano culture (Jayakumar *et al.* 2014, Hamed *et al.* 2016). Except potassium concentration in ISGW, all the major ions were at par with sea water of equivalent salinity.

Inland saline ground water (ISGW) without any potassium amendment (RSW) was not suitable for the growth and survival of silver pompano (*T. blochii*), as the first experiment of feasibility study in RSW of all the experimental salinities resulted in 100% mortality. In 5 ppt RSW, juveniles became motionless in first 3 h, abnormal swimming was observed until 6 h and all the juveniles died before 12 h. At 10 ppt RSW, fishes exhibited normal swimming until 8 h. After 12 h juveniles lost the balance and complete mortality was observed in 24 h. However in 15 ppt RSW, survival was recorded for 48 h. During the current study, mortality of juveniles in RSW at different experimental salinities could not be only as a result of salinity change alone, as *T. Blochii* is a euryhaline fish (Kalidas *et al.* 2012). The difference in survival period in RSW could be attributed to osmotic stress caused by variations in ionic concentration of ISGW as compared with sea water of equivalent salinities. Several authors have also reported that, ion deficiency is a kind of stress which can have several effects on the fish including; changes in metabolic rate, health, behaviour, growth, survival and reproductive success (Brett 1958, Wedemeyer *et al.* 1976, Esch and Hazen 1980, Barton *et al.* 1990). It is also assumed that animals will grow best in low salinity water with ion ratios similar to that of diluted seawater (Boyd 2006). Potassium plays an important role in many physiological processes by maintaining the electrolyte and acid base balance (Wilson and El Naggar 1992). Deficient potassium in inland saline water caused mortality in a number of cultured marine finfish species. Fielder *et al.* (2001) reported mortality of Australian snapper (*Pagrus auratus*) juveniles in 4 days when stocked in 19 ppt raw saline groundwater to the low concentration of potassium (5% K equivalence). Partridge and Creeper (2004) also stated mortality caused by hypokalemic muscle myopathy in barramundi, *Lates calcarifer*, reared in 45 ppt groundwater containing 25% of the potassium found in equivalent salinity seawater (25% K equivalence). Doroudi *et al.* (2006) observed the failure of juvenile mulloway to survive at a low potassium concentration in inland saline groundwater. Saoud *et al.* (2012) reported that potassium-deficient low salinity well water is also not suitable for long-term culture of *Siganus rivulatus*. In case of crustaceans, it's a very well established fact that potassium ion deficiency in ISGW has caused total mortality in *P. monodon* (Raizada *et al.* 2003, Rahman *et al.* 2005) and *Litopenaeus vannamei* (Lakra *et al.* 2013, Pathak *et al.* 2013). This problem was solved with ionic supplementation of potassium for *P. monodon* (Raizada *et al.* 2003a, Rahman *et al.* 2005, Tantulo and Fotedar 2006) and other marine shrimps (Saoud *et al.* 2003, McNevin *et al.* 2004, Pragnell and Fotedar 2005, Bartholomew 2008, Pathak *et al.* 2013). However, initial experiments of rearing brackish water fishes in subsoil water indicated that culture of *Etroplus suratensis* and *Mugil cephalus* is suitable without potassium supplementation (Dwivedi and Lingaraju 1986). Raizada *et al.* (2005) also reported that the milkfish *Chanos chanos* was also grown well in inland saline ground water of 18–25 ppt. Even in some freshwater fishes such as *Pangasius hypophthalamus*, Red tilapia, a potassium amendment does not have much influence on survival and growth as reported by Kumar *et al.* (2013) and Poonam *et al.* (2015) respectively. The experiment conducted by Ingram *et al.* (2002) achieved 97% survival of silver perch, *Bidyanus bidyanus*, in 10 ppt ground water with 22% K equivalence, highlights that tolerance to potassium deficiency is species specific. It was therefore necessary to amend potassium in ISGW for survival and growth of silver pompano.

In the second experiment, survival of juveniles in amended inland saline ground water was not affected by experimental salinities, which was close to 100% in all

Table 2. Water quality parameters of treatment and reference medium holding *Trachinotus blochii* (silver pompano) juveniles at different salinities during 90 days trial, expressed as Mean±SE

Water quality parameter	Treatment medium			Reference medium		
	ISW 10	ISW 15	ISW 15	ASW 5	ASW 10	ASW 15
Dissolved oxygen (mg/l)	7.10±0.21	6.83±0.33	6.77±0.20	7.00±0.12	7.03±0.07	7.10±0.21
Temperature (°C)	27.83±0.19	27.67±0.27	28.53±0.43	28.80±0.31	26.75±9.15	28.17±0.15
Free CO_2 (mg/l)	0.85±0.20	0.91±0.23	0.64±0.17	0.78±0.21	0.85±0.31	0.91±0.21
pH	8.20±0.06	8.23±0.15	8.20±0.06	8.33±0.12	8.37±0.09	8.30±0.17
Alkalinity (mg/l)	195.33±9.33	218.00±4.36	242.33±4.48	182.33±9.91	228.00±6.81	249.67±19.10
Hardness (mg/l)	1342±10.12	2398.33±49.69	3239.67±72.13	1517.67±47.42	2351±72	3357.67±116
NH_3-N (mg/l)	0.03±0.00	0.06±0.01	0.08±0.01	0.07±0.01	0.05±0.01	0.04±0.01
NO_2-N (mg/l)	0.004±0.001	0.003±0.001	0.003±0.001	0.002±0.001	0.003±0.000	0.002±0.001

treatments (P>0.05) where potassium was amended at 100% equivalence to sea water of similar salinity. This was in agreement with the previous silver pompano research conducted by Kalidas *et al.* (2012) who reported minimum salinity required for survival of juveniles as 4 ppt. Sampaio *et al.* (2003) observed that pompano tolerate wide range of salinity, between 7 and 58 ppt on acute exposure of individuals acclimated to seawater (35 ppt), and on gradual exposure to diluted lower salinity sea water. The experiments conducted by Allen and Avault (1970) and Kumpf (1971) also reported that juvenile pompano were able to grow at a salinity of 5 ppt and were able to tolerate salinities as low as 2 ppt and as high as 45 ppt respectively. In the present study, the initial average body weight of silver pompano juveniles was not significantly different (P<0.05) at the commencement of the experiment. However, final mean individual weight, weight gain and SGR were significantly different towards the end of the trial (Table 3). Silver pompano juveniles exhibited significantly lower final mean body weight of juveniles in ISW 5 (14.31 g) and ASW 5 (15.59 g) than those of their counterparts in other treatments and reference media respectively. Highest final mean body weight was observed in ISW10 (36.12 g) followed by ISW 15 (35.85 g), but did not differ significantly to each other (P>0.05), similar trend was observed for weight gain, SGR and ADG. The growth of silver pompano juveniles in 10 and 15 ppt ISW and ASW showed no significant difference though numerically higher values were obtained at 10 ppt ISW and 15 ASW. Highest growth of pompano in 15 ASW medium was observed similar to the trend reported by Kalidas *et al.* (2012), who recommended salinity range for silver pompano in low saline water as 15–25 ppt.

Growth is the net positive result from the energy provided by food ingestion and the metabolic expenditure (Jobbling 1994) hence, the growth of cultured fish would be maximum when they are reared in salinity near the isosmotic condition as the osmoregulatory cost is proportional to the osmotic gradient existing between the fish body fluids and the external medium (Handeland *et al.* 1998). Brown (2007) evaluated growth of Southern flounder, *Paralichthys lethostigma* in inland low saline water and reported that at lower salinities (2.7 ppt and 3.4 ppt), the growth was very low as less energy is available for growth of fish. Instead of expending energy on somatic growth, fishes are constantly adjusting to their external surrounding environments and maintaining the proper balance of salt solutions within their bodies (isosmotic) by osmoregulation (Lisboa *et al.* 2015). Osmolality of silver pompano at 5 ppt exhibited a stressful condition as plasma osmolality of silver pompano juveniles in ISW 5 and ASW 5 (158.67 mmol/kg and 197.33 mmol/kg respectively) was significantly different than their medium osmolality (251.67 mmol/kg and 246 mmol/kg) respectively after 90 days. The lower growth observed in silver pompano juvenile maintained at 5 ppt ISGW in the present study seems to be related to a higher energy cost associated with osmoregulation. Fishes reared in hypo-osmotic environments would show additional energy requirements for osmoregulation that could hamper growth when compared to the fishes kept at an isosmotic environment (Boeuf and Payan 2001, Tsuzuki *et al.* 2007, Herrera *et al.* 2009, Perez-Robles *et al.* 2012). Laiz-Carrion *et al.* (2005) also reported that gilthead sea bream exhibited osmoregulatory difficulties when reared at 4 ppt due to the lower plasma osmolality after 42 and 84 days and in European sea bass after 24 h of transfer to salinities less than 20 ppt (Venturini *et al.* 1992). Low salinity has been reported to cause difficulties in homeostasis in other euryhaline teleost species, such as the sheepshead minnow *Cyprinodon variegates* (Nordlie, 1985) and flounder *Paralichthys orbignyanus* (Sampaio and Bianchini 2002). Osmoregulatory imbalance indicates the salinity stress (Franklin *et al.* 1992), and negative effects of stress on growth are well documented in teleosts (WendelaarBonga 1997). Plasma osmolality of silver pompano juveniles in ISW 10 and ISW 15 at the end of experimental period (332.2 and 413 mmol/kg respectively) indicated that silver pompano regulated osmotic balance with treatment medium salinities. Similar trend was observed in plasma osmolalities of juveniles stocked in ASW 10 and ASW 15 (352.6 and 412.6 mmol/kg respectively). Plasma osmolality level of a marine fish is

Table 3. Performance of *Trachinotus blochii* (silver pompano) juveniles at different salinities of treatment and reference medium during 90 days trial, expressed as Mean±SE (n=3)

Parameter	Treatment medium			Reference medium		
	ISW 5	ISW 10	ISW 15	ASW 5	ASW 10	ASW 15
Initial body weight (g)	1.48±0.02	1.46±0.04	1.53±0.06	1.49±0.06	1.51±0.07	1.51±0.04
Final body weight (g)	14.31±2.55^{a}	36.12±2.87^{b}	35.85±2.35^{b}	15.59±1.64^{a}	37.42±3.66^{b}	39.13±1.18^{b}
Weight gain (g)	12.83±2.54^{a}	34.65±2.83^{b}	34.32±2.31^{b}	14.10±1.71^{a}	35.91±3.71^{b}	37.62±1.22^{b}
SGR (%/day)	2.48±0.19^{a}	3.55±0.06^{b}	3.50±0.06^{b}	2.60±0.17^{a}	3.56±0.15^{b}	3.62±0.06^{b}
ADG (g/f/d)	0.143±0.03^{a}	0.383±0.03^{b}	0.380±0.02^{b}	0.16±0.02^{a}	0.40±0.04^{b}	0.42±0.01^{b}
Survival (%)	100	100	100	100	100	100
Water osmolality (mOsm/kg)	251.67±19.6^{a}	345±10.2^{b}	446.67±13.3^{c}	246±21.7^{a}	372.33±12.5^{b}	433.67±10.4^{c}
Plasma osmolality (mOsm/kg)	158.67±9.68^{a}	332.33±4.91^{c}	413±5.69^{d}	197.33±4.06^{b}	352.67±9.53^{c}	412.67±13.1^{c}

^{a,b,c}Values marked with superscript letters show significant differences in the growth parameters among three treatments compared to reference medium (P<0.05).

typically maintained in between 380–450 mmol/kg (Conte 1969, Johnson 1973, Bond 1991, Kirschner 1991) and in teleosts, the plasma isosmotic point generally corresponds to the water salinity of 12 ppt (Boeuf and Payan 2001, Tsuzuki *et al.* 2007, Herrera *et al.* 2009, Nordlie 2009). Lowest osmoregularity capacity was observed at ISW 10 and ASW 10 (12.67 and 19.66 mmol/kg respectively) followed by ASW 15 and ISW 15 (21 and 33.67 mmol/kg respectively). Sarwono (2005) also reported in juvenile sea bass that the differences between blood and culture media osmolality in 10 and 15 ppt salinity were minimum, exhibiting the iso-osmotic point at 10 and 15 ppt. These results were in agreement with the fact that optimum growth of fish can be achieved when a particular range of salinity have the isotonic condition as the isotonic salinity has an effect on metabolic rates (Morgan and Iwana 1991). This suggests that silver pompano might have maintained isosmotic conditions at 10 ppt and 15 ppt ISW, thereby energetic loss for osmoregulation is lower associated with minimum gradients between water and blood. This enhanced the growth of silver pompano juveniles in these two treatments than at 5 ppt ISW.

Inland saline ground water is deficient in potassium and unsuitable for rearing silver pompano juveniles without potassium amendment. It has been observed that after potassium amendment, the growth was lower at 5 ppt with high osmotic capacity. However, the culture of silver pompano (*T. Blochi*) is feasible at 10 to 15 ppt inland saline ground water provided potassium amendment is done at par with that of sea water of equivalent salinity. Further studies should be oriented on physiological changes in silver pompano at different levels of potassium amendment.

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