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Strategic manipulation of inland saline groundwater to produce *Macrobrachium rosenbergii* (De Man) post larvae

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A series of four experiments were conducted in inland saline groundwater (ISGW) for successful development of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man) post larvae (PL). The ISGW of 58 ppt was diluted to 12 ppt with freshwater. The concentrations of calcium, potassium and magnesium in ISGW were compared with those of natural seawater (NSW) of equal salinity; they were 340.6, 30.0 and 144.7 mg l^{-1} in ISGW and 175.0, 174.4 and 425.0 mg l^{-1} in NSW, respectively. In the first experiment, newly hatched larvae of *M. rosenbergii* were reared both in ISGW and NSW of 12 ppt. The larvae survived only up to 11 days in ISGW and developed only up to stage IV, whereas they developed normally to PL in NSW within 40-50 days with $6.33 \pm 0.88\%$ survival. In the second experiment, the concentration of calcium in ISGW of 12 ppt was reduced to 168.3 mg l^{-1}. The larvae developed to stage IV, but the survival period increased up to 15 days. In the third experiment, the concentration of potassium in ISGW of 12 ppt was increased separately to 60% (102.2 mg l^{-1}) and 100% (170.5 mg l^{-1}) in addition to reduced calcium (168.3 mg l^{-1}). The larvae became completely metamorphosed to PL with $6.66 \pm 0.76\%$ survival at 60% potassium in a period of 39-50 days but not at 100% potassium. In the fourth experiment, the concentrations of calcium, potassium and magnesium in ISGW were kept the same as those in the case of 60% potassium, but larvae were reared in a recirculating system. The survival of PL increased to $34.56 \pm 1.87\%$ with a rearing period of 23-45 days. The study suggests that ISGW of 12 ppt that contained 168.3 mg l^{-1} calcium, 102.2 mg l^{-1} potassium and 144.7 mg l^{-1} magnesium could be used for the development of *M. rosenbergii* PL.

Key words: *Macrobrachium rosenbergii*, inland saline groundwater, potassium, calcium, magnesium.

INTRODUCTION

The availability of saline groundwater, which is 50% of the total groundwater in the world, is further increasing at alarming rates in many inland regions of the world due to climatic and anthropogenic causes (Joshi & Tyagi, 1994; Forsberg *et al*., 1996; Allan *et al*., 2001). The salinization of inland groundwater resources has exerted serious pressure on the availability of freshwater for drinking, agriculture, industries and fisheries. Therefore, the suitability and potential of inland saline groundwater (ISGW) resources is being evaluated to develop commercial aquaculture in many parts of the world (Dwivedi & Lingaraju, 1986; Forsberg & Neill 1997; Fielder *et al*., 2001; Ingram *et al*., 2002; Jain *et al*., 2002; Partridge & Furey, 2002; Rahman *et al*., 2005).

The ionic composition of ISGW differs from that of natural seawater (NSW) even at similar salinities and also varies from location to location. ISGW of west Texas, USA contains excess of calcium and sulfate but is deficient in potassium (Forsberg *et al*., 1996). In New South Wales, Australia, ISGW contains more calcium and magnesium and less potassium (Fielder *et al*., 2001). ISGW of the Indian sub-continent usually contains more calcium with less magnesium and potassium (Jain *et al*., 2002; Rahman *et al*., 2005). These differences in ionic composition of ISGW affect the survival and growth of fish and shrimp species (Forsberg *et al*., 1996; Fielder *et al*., 2001;

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Rahman *et al*., 2005; Jain *et al*., 2006).

The *Macrobrachium rosenbergii* (De Man) is a freshwater palaemonid prawn. The larval phase of this species is completed in NSW of 12 ppt (Ling, 1967). Larvae pass through 11 developmental stages (Uno & Soo, 1969) over a period of 25-35 days (Correa *et al*., 2000) before being metamorphosed to post larvae (PL). The necessity of NSW for PL production has restricted the setting up of hatchery units in coastal regions only. There is enormous potential to grow *M. rosenbergii* in inland freshwater and saline water of low salinity (Jain *et al*., 2007). The transportation, however, of PL to inland regions involves a high cost and results in high mortality.

Limited work has been done to study the development of PL of *M. rosenbergii* in ISGW. Venugopal (personal communication) who initiated the use ISGW at Lahali (Haryana, India) for PL production was able to grow the larvae up to stage V. Detailed studies on the cause of mortality are not available so far. The variations of the ionic composition between ISGW and NSW could be the major cause of inhibiting the development of *M. rosenbergii* larvae to PL. The brackish water shrimp *Penaeus monodon* (Collins & Russell, 2003; Rahman *et al*., 2005) and a few other marine finfish like *Sciaenops ocellatus* (Forsberg *et al*., 1996), *Pagrus auratus* (Fielder *et al*., 2001) and *Lates calcarifer* (Partridge, 2003) could survive in ISGW only after manipulating its ionic composition.

In the present study, the development of larvae of *M. rosenbergii* was observed in ISGW. The ionic composition of the latter was manipulated to optimize the production of PL. The use of ISGW for PL production will promote the culture of *M. rosenbergii* in inland regions.

MATERIALS AND METHODS

Experiments were conducted to grow the newly hatched *M. rosenbergii* larvae to PL using ISGW and NSW (as control) at the Aquaculture Research Laboratory (Central Institute of Fisheries Education, Mumbai), Udaipur, Rajasthan, India. ISGW of 58 ppt was obtained from an inland shallow open well located at Nimbli, District Pali, Rajasthan, India. NSW was collected off the Versova coast, Mumbai, India. Source water was analyzed for pH, salinity, calcium, magnesium, sodium, potassium and chloride using standard methods (APHA, 1980).

Experiment 1: Culture of larvae in ISGW of 12 ppt

All culture experiments were conducted in Fiberglass Reinforced Plastic (FRP) tanks of 80 l capacity in a closed system. Tanks were filled with ISGW of 12 ppt up to 30 cm. ISGW of 58 ppt (Table 1) was diluted with freshwater (pH 7.0, calcium 40.9 mg l^{-1}, magnesium 16.6 mg l^{-1}, bicarbonate 60 mg l^{-1}, carbonate 5 mg l^{-1}, and negligible amounts of sodium and potassium) to 12 ppt. NSW of 12 ppt was used as a control. Water of all the experimental tanks was constantly aerated through an air pump to maintain dissolved oxygen level at 6.00 ± 0.25 mg l^{-1}. The temperature of the test media was maintained at $27 \pm 1\,^{\circ}C$ by im-

TABLE 1. Abiotic parameters, ionic concentrations and ratios in inland saline groundwater (ISGW) and natural seawater (NSW). SG-Ca: ISGW of 12 ppt of reduced calcium concentration; K-60: ISGW of 12 ppt of reduced calcium concentration and 60% potassium; K-100: ISGW of 12 ppt of reduced calcium concentration and 100% potassium

Parameters	ISGW					NSW		
	58 ppt	12 ppt	SG-Ca	K-60	K-100	35 ppt	12 ppt	Standard*
pH	7.2	7.0	7.0	7.0	7.0	7.0	7.0	7.0
salinity (ppt)	58	12	12	12	12	35	12	12
Ca^{2+} (mg l^{-1})	921.8	340.6	168.3	168.3	168.3	420.8	175.0	139.2
Mg^{2+} (mg l^{-1})	1024.4	174.6	144.7	144.7	144.7	956.3	425.0	469.2
Na^{+} (mg l^{-1})	13768	2440	2440	2440	2440	11673	3825	3654
K^{+} (mg l^{-1})	159.4	30.0	30.0	102.2	170.5	436.8	174.4	128.4
Cl^{-} (mg l^{-1})	39760	6816	6816	6875	6875	18797	7400	6612
K^{+}/Cl^{-}	0.004	0.004	0.004	0.015	0.024	0.023	0.023	0.019
Ca^{2+}/Mg^{2+}	0.009	1.950	1.160	1.160	1.160	0.440	0.411	0.296
K^{+}/Mg^{2+}	0.155	0.172	0.207	0.706	1.178	0.456	0.410	0.273

* Boyd & Thunjai (2003)

mersing a submersible thermostat-controlled heater of 100 W.

The newly hatched larvae were stocked both in test and control groups in triplicate. Each tank containing 50 l water was stocked with 5000 larvae. After 36 hrs of hatching, larvae were fed on newly hatched *Artemia* nauplii. Density of *Artemia* nauplii in the tanks was maintained at 3-4 nauplii ml^{-1} of water. Larvae were also fed on egg custard after stages III-IV of larval development in addition to *Artemia* nauplii. The larvae were fed daily at 08:00 (*Artemia*), 11:00 (egg custard), 13:00 (*Artemia*), 15:00 (egg custard), 18:00 (egg custard) and 20:00 (*Artemia*). Egg custard consisted of an egg (50 g), milk powder (20 g), dry *Acetes* powder (20 g), corn flour (20 g), baker's yeast (1 g), mineral mix (3 g; Nutrimilk, Pfizer Ltd., Mumbai, India) and cod liver oil (0.5 ml; Seacod, Universal Medicare Pvt., Ltd., Mumbai, India). Egg custard was freshly prepared every day. Different developmental stages of larvae (Uno & Soo, 1969) were observed at 10:00 ± 1:00 hr daily. About 30-50% of the test water was exchanged every day and dead PL (if any) were sorted out at the same time. Survival was calculated as the total number of post larvae harvested to the number of larvae stocked at the beginning of the experiment.

Experiment 2: Culture of larvae in ISGW of reduced calcium concentration

The development of newly hatched larvae of *M. rosenbergii* was observed in ISGW of 12 ppt with reduced concentration of calcium (SG-Ca). Calcium was reduced from 340.6 to 168.3 mg l^{-1} by repeatedly passing ISGW through a resin (Softener 220; M/S Ion Exchange, Mumbai) that was calcium-selective. The experimental set up (e.g. feeding and water exchange schedule) was the same as that of Experiment 1.

Experiment 3: Culture of larvae in ISGW of reduced calcium and enhanced potassium concentrations

The development of newly hatched larvae of *M. rosenbergii* was observed in ISGW of 12 ppt with calcium concentration of 168.3 mg l^{-1} and potassium concentration enhanced to about 60% (K-60; 102.2 mg l^{-1}) and 100% (K-100; 170.5 mg l^{-1}) separately in two groups compared with NSW (174.4 mg l^{-1}). The experimental set up (e.g. feeding and water exchange schedule) was the same as that of Experiment 1.

Experiment 4: Culture of larvae in ISGW of reduced calcium and enhanced potassium concentrations in a recirculating system

The development of newly hatched larvae of *M. rosenbergii* was observed in ISGW of 12 ppt having calcium and potassium concentrations of 168.3 and 102.2 mg l^{-1} respectively, as in the case of K-60, which showed a better larval development in Experiment 3, but with a provision of continuous water recirculation. Experiments were conducted in cement tanks of 90 × 60 × 55 cm. Each tank was divided into two separate compartments of 25 and 65 cm in length each. It was provided with two holes (30 cm apart) of 18 mm in diameter about 5 cm above the lower end and a third hole about 5 cm below the upper end. Both the holes at the lower end were fitted with PVC pipes of 15 cm length. The open end of the pipes in the bigger compartment was covered with a nylon cloth of 100 µm mesh. The smaller compartment was filled with small pieces of foam up to a height of 10-15 cm followed by a 20 cm thick layer of charcoal and finally with small pebbles (1-2 mm diameter) up to the upper hole level. Tanks were filled up to 50 cm with water, 24-48 hrs before stocking the larvae. Water in all experimental tanks was constantly aerated through an air pump to maintain dissolved oxygen level at 6.00 ± 0.25 mg l^{-1}. The temperature of the test media was maintained at 27 ± 1 °C by immersing a submersible thermostat-controlled heater of 100 W. A probiotic (Biogreen; M/S J.V. Ent. Co., Ltd., USA) culture was added at 2 g/1000 l of water to improve the efficiency of the biological filter. The water of the bigger compartment was recirculated through the smaller compartment using an airlift system. Each tank (in triplicate) containing 200 l water was stocked with 18000 larvae. Feeding and water exchange schedule and other management protocols were the same as those of Experiment 1. Airlift circulation was turned off for one hour at the time of feeding with *Artemia* nauplii.

RESULTS

Experiment 1: Culture of larvae in ISGW

The larvae started developing into stage II both in ISGW and NSW from the 2^{nd} day onwards. On average, 52 ± 4% larvae developed into stage III on the 4^{th} day in NSW, but only 31 ± 2% by the 5^{th} day in ISGW. Mortality of larvae started from stage III onwards in ISGW. A percentage of 65 ± 5% larvae developed into

TABLE 2. Development and survival rates of larvae of *M. rosenbergii* in inland saline groundwater (ISGW) with different ionic concentrations and natural seawater (NSW). Numbers indicate time period in days. SG-Ca: ISGW of reduced calcium concentration; K-60: ISGW of reduced calcium concentration and 60% potassium; K-100: ISGW of reduced calcium concentration and 100% potassium

Stages	Culture medium (12 ppt)					
	NSW	ISGW	SG-Ca	K-60	K-100	K-60[1]
I	1	1	1	1	1	1
II	2-3	2-5	2-7	2-5	2-5	2
III	6-8	5-10	5-12	5-7	4-13	3-4
IV	7-10	10-11*	7-15*	7-10	5-15*	4-5
V	10-17			9-13		5-9
VI	16-22			10-20		7-11
VII	19-25			16-27		10-12
VIII	23-27			23-29		11-13
IX	25-30			28-32		12-15
X	26-36			30-36		14-19
XI	30-42			33-40		17-22
PL	40-50			39-50		23-45
Survival (%)	6.33 ± 0.88	0	0	6.66 ± 0.76		34.56 ± 1.87

*: Total mortality

[1]: Recirculatory rearing system

stage IV on the 7th day in NSW, but only 8 ± 2% larvae could reach stage IV in ISGW. Larvae became moribund and started settling at the bottom of the tank in ISGW and total mortality was observed before 11 days. Development of the larvae remained continuous in NSW and the first PL was observed on the 40th day while 95% of individuals reached PL stage by the 50th day with 6.33 ± 0.88% survival.

Experiment 2: Culture of larvae in ISGW of reduced calcium concentration

The larvae started developing into stage II from the 2nd day in SG-Ca as in ISGW and NSW, but it took 7 days for all the larvae to develop into stage II. About 38 ± 4% of the larvae developed into stage III on the 5th day and 56 ± 3% into stage IV on the 7th day (Table 2). The larvae started settling down at the bottom of the tank after stage IV. No larvae were observed to develop into stage V and total mortality was observed by the 15th day.

Experiment 3: Culture of larvae in ISGW of reduced calcium and enhanced potassium concentrations

The larvae developed into stage II both in K-60 and K-100 similarly to ISGW and SG-Ca. The stage III started appearing from the 5th day in K-60 (48 ± 5%), but from the 7th day in K-100 (35 ± 5%). On average 68 ± 4% of all larvae developed into stage IV in K-60 on the 7th day and 100% on the 10th day, but stage IV was completed by the 15th day in K-100. None of the larvae developed into stage V in K-100 and total mortality of larvae was observed by the 15th day. The larvae developed into stage V from the 9th day in K-60. The development of larvae continued in K-60 much similarly to NSW with the appearance of the first PL on the 39th day and complete metamorphosis to PL by the 50th day. The total survival to PL in K-60 was 6.66 ± 0.76% that was similar ($p > 0.05$) to the control group (Table 2).

Experiment 4: Culture of larvae in ISGW of reduced calcium and enhanced potassium concentrations in a recirculatory system

The development of larvae was faster in a recirculatory system. The PL started appearing only after 23-24 days. About 81 ± 5% of the total larvae became metamorphosed to PL by the 35th day and 100% by the 45th day with a total survival of 34.56 ± 1.87% (Table 2).

DISCUSSION

The larvae of *M. rosenbergii* did not develop to PL in ISGW of 12 ppt. The mortality of larvae at early developmental stages could be mainly attributed to

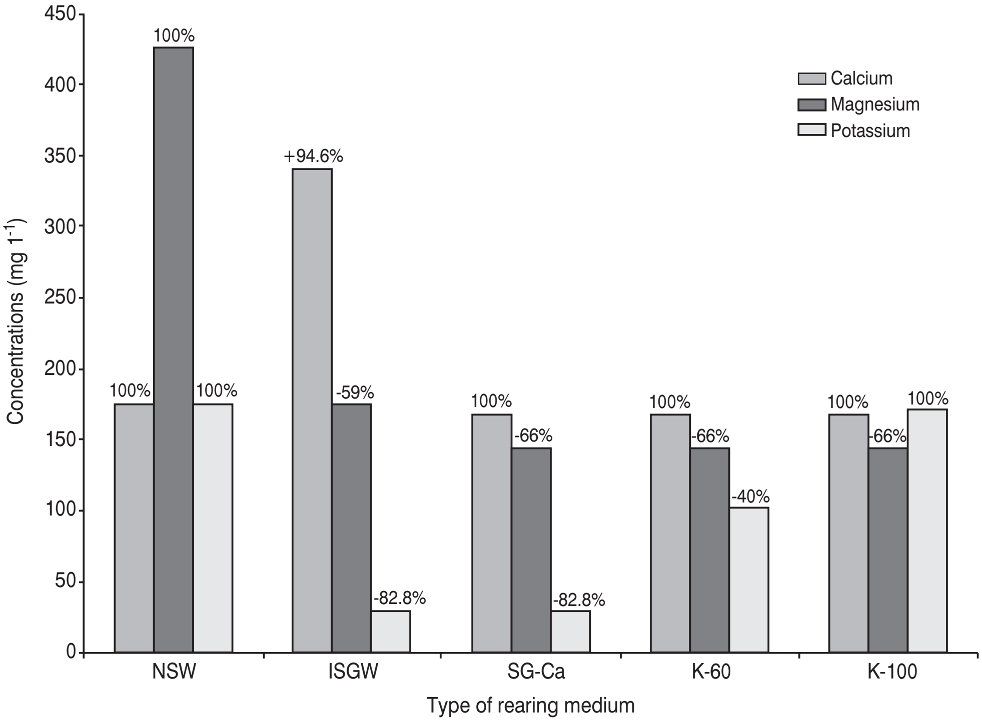

FIG. 1. Percentage increase or decrease of the concentrations (in parentheses) of calcium, magnesium and potassium in ISGW (inland saline groundwater) relative to NSW (natural seawater) of 12 ppt. SG-Ca: ISGW of reduced calcium concentration; K-60: ISGW of reduced calcium concentration and 60% potassium; K-100: ISGW of reduced calcium concentration and 100% potassium.

variations in the ionic concentrations of ISGW compared with NSW of equal salinity (Forsberg *et al.*, 1996; Fielder *et al.*, 2001; Rahman *et al.*, 2005). The concentration of calcium was higher by 94.6%, while concentrations of magnesium and potassium were lower by 59% and 82.8%, respectively in ISGW of 12 ppt compared with NSW of similar salinity (Fig. 1). The variation of the ionic concentrations in the external medium relatively shifts the intracellular and extracellular concentrations of the respective ions and other dependent mechanisms that affect the survival and growth of aquatic species (Ketola *et al.*, 1988; Partridge & Creeper, 2004).

Stern *et al.* (1987) observed a high degree of osmoregulatory ability of PL of *M. rosenbergii* in ISGW that differed from NSW in concentrations and ratios of various ions, which affect the growth and development of PL. The osmoregualtory ability and ionic requirements of larvae of *M. rosenbergii* could be different from those of PL, as larval phase is essentially completed in seawater of 12 ppt (Ling, 1967). Therefore, the larvae were not able to develop in ISGW that was different from the seawater in ionic concentrations.

The larvae of *M. rosenbergii* did not survive in ISGW of 12 ppt after lowering the concentration of calcium to 168.3 mg l^{-1} that was much similar to 175.0 mg l^{-1} in NSW of equal salinity. It could only help to slightly prolong the survival period at stage IV, but did not improve any further development. The survival time of juveniles of *P. monodon* could also be increased by seven days only by lowering the concentration of calcium of ISGW (12.5 ppt) to the level of NSW (Rahman *et al.*, 2005). This suggests that high calcium alone is not the only limiting factor for the development of larvae in ISGW. It is possible that low potassium (Fielder *et al.*, 2001; Partridge & Creeper, 2004; Rahman *et al.*, 2005) and magnesium in addition to high calcium may cause mortality of larvae in ISGW.

The potassium enrichment (to 60%) in calcium reduced ISGW medium (K-60) contributed to the advancement of the development of larvae to PL. This suggests that in addition to high calcium, low potassium (but not magnesium) is the limiting factor for the development of the larvae of *M. rosenbergii* in ISGW. Survival and growth of juveniles of *P. monodon* could also be possible in ISGW of 12.5 ppt by only manipulating the concentration of calcium and potassium, but not of magnesium that was only 49% (Rahman *et al*., 2005). The potassium requirements in ISGW have been found to vary in different aquatic animals (Fielder *et al*., 2001; Collins & Russell 2003; Partridge & Creeper, 2004; Rahman *et al*., 2005). The mortality of larvae in the potassium saturated ISGW could be due to the higher change in the ionic ratio compared with the seawater. The balance of ionic ratios in addition to the minimum requirement of a specific ion is important for the survival and growth of marine aquatic animals. In the present study, K^+/Mg^{2+} ratio was 0.70 at 60% and 1.17 at 100% potassium concentrations respectively in ISGW that was 0.41 in NSW. The K^+/Mg^{2+} ratio of 1.17 at 100% potassium in the external medium is possibly too high to maintain the internal homeostasis of the body fluid of an aquatic animal (Maetz, 1969; Marshall, 1995; McCormick, 1995; Karnaky, 1998).

The larvae of *M. rosenbergii* metamorphosed to PL in ISGW of 12 ppt having 100% calcium, 60% potassium and 34% magnesium compared to seawater of equal salinity with a very low survival of 6.66 ± 0.76%. The total duration of the larval phase was also 39-50 days that was much longer than that of 25-35 days in the seawater (Correa *et al*., 2000). However, survival and duration were much similar to those of the control conditions, but they could not be considered as commercially viable. This could be due to the static rearing system (Valenti & Daniels, 2000). The use of the recirculatory system compressed the larval period to 23-45 days and enhanced the survival to 34.56 ± 1.87%.

The present study suggests that ISGW of 168.3 mg l^{-1} calcium, 102.2 mg l^{-1} potassium and 144.5 mg l^{-1} magnesium in a recirculatory system could be used for the successful production of PL of *M. rosenbergii*. Further studies may be useful to determine the minimum and optimum requirements of these ions and their ratios.

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