



Effects of Salinity and Ca^{2+} : Mg^{2+} Ratio on Growth Performance and Survival of *Penaeus vannamei* (Boone, 1931) Reared in Inland Saline Ground Water

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ABSTRACT

Background: A 60-day experiment was conducted to evaluate the combined effect of salinity and Ca^{2+} : Mg^{2+} ratio on the growth performance and survival of *Penaeus vannamei* reared in inland saline ground water (ISGW) at ICAR-Central Institute of Fisheries Education (CIFE), Rohtak Centre, Haryana, India.

Methods: The study used three different salinities viz. T_1 (5 ppt), T_2 (10 ppt) and T_3 (15 ppt) with four different level of Ca^{2+} : Mg^{2+} ratios such as 1:1, 1:2, 1:3 and 1:4. juveniles of *P. vannamei* with average body weight of 3.70 ± 0.02 g, were stocked in 100 L capacity of circular plastic tanks. Total 36 experimental tanks were used as experimental unit. The stocking densities were 20 animals in each tank and each treatment were kept in triplicate followed the factorial design (3×4).

Result: *P. vannamei* expressed better growth performance in terms of SGR, FCR, FER and PER in the T_2 and T_3 fortified Ca^{2+} : Mg^{2+} ratio of 1:3. Lowest growth observed in T_1 fortified with Ca^{2+} : Mg^{2+} ratio 1:1. The study found 100% survival in T_2 and T_3 fortified with Ca^{2+} : Mg^{2+} ratio of 1:3 compared with other treatment groups, which showed <80% survival. Based on the results, it can be concluded that the better growth performance, survival of *P. vannamei* observed in 10 and 15 ppt salinities of ISGW fortified with 1:3 ratio of Ca^{2+} : Mg^{2+} .

Key words: Growth performance, ISGW, *Penaeus vannamei*, Survival.

INTRODUCTION

Salinization is a serious environmental damage which highly affected an area of about one billion hectares, almost 7% of world total area (Shrivastava and Kumar, 2015). Generally, it seen in arid and semi-arid zones, where primary source of water is extracted from ground (Re and Sacchi, 2017). Further, due to several anthropogenic activities in the inland areas, salinity had significantly intimidated the livelihood of countless rural communities which in turn causing major economic, social and environmental consequences (Chand *et al.*, 2012). India has an estimated inland saline water resource around 8.62. million ha and most of them were situated in the north-western parts of India (Lakra *et al.*, 2014). These ISGW area affected by the salinization is not suitable for human livelihood or other agricultural purposes.

In general, wide range of aquatic animals, including fish in euryhaline nature, are reared using various aquaculture production systems in extensive environmental conditions. Therefore, it can play a major role to utilize these unproductive ISGW resource by the aquaculture and alleviate the pressure on fresh water resources (Jamil *et al.*, 2011). The imbalanced ionic composition in ISGW is the major drawback, which varies place to place. The ionic composition of ISGW shows similarities with sea water of equivalent salinity, however, it is either deficient in K^+ or Mg^{2+} or high in calcium. The lack of important ionic ratio such as Na^+/K^+ and $\text{Ca}^{2+}/\text{Mg}^{2+}$ significantly affects the normal growth and survival for shrimps (Antony *et al.*, 2015).

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Calcium is essential for hard tissue structure, muscle construction, blood clotting, nerve transmission, act as cofactor for enzymatic procession and osmoregulation (Lovell, 1989) and majority of aquatic animals absorbs calcium directly from surrounding environment (Roy *et al.*, 2006). The major portion of crustacean exoskeleton are constituted by Mg^{2+} which significantly contributes to molting

process (Wilder *et al.*, 2009). Mg^{2+} also act as cofactor for Na-K-ATPase enzyme involved in osmoregulatory activity important for mineralization, growth and survival (Brown *et al.*, 1991). Therefore, fortification of inland saline water with essential minerals is being experimented to overcome the deficiency of minerals for white leg shrimp (Hernandez *et al.*, 2023).

In India, after the outbreak of WSSV, introduction of *P. vannamei* was started in 2003 and later in 2009, commercial culture of *P. vannamei* was permitted (Kumaran *et al.*, 2012). Because of its wide range of salinity tolerance, the farmers were started culture in low salinity (McGraw *et al.*, 2002). The positive result of *P. vannamei* farming in inland saline states encouraged, the farmers to take a great leap within a short period. However, still many farmers are facing problems regarding growth and survival of *P. vannamei* in salt affected areas because of less knowledge of ISGW ionic composition and its regional ionic variability. To consider this challenge, the present study was performed to assess the effect of salinity and Ca^{2+} : Mg^{2+} ratio on growth and survival of *P. vannamei* juveniles in ISGW.

MATERIALS AND METHODS

A 60 days experiment was carried out at ICAR-Central Institute of Fisheries Education, Mumbai, Rohtak centre, Haryana, India. A total 720 juveniles of *P. vannamei* with average weight 3.70 ± 0.02 g were used as experimental animal. The juvenile shrimps were collected from a grow out shrimp pond of the experimental site and kept for 7 days to acclimatization in same salinity source water in 300 L FRP tanks. ISGW obtained from the experimental site had 15 ppt which was further diluted with freshwater for maintaining 10 ppt and 5 ppt. The study prepared three treatments of ISGW salinities as 5, 10 and 15 ppt and named as T_1 , T_2 and T_3 respectively. In each treatment group with four different ratios of Ca^{2+} : Mg^{2+} ratios 1:1, 1:2, 1:3 and 1:4 was prepared by fortifying Ca (CaCO_3) and Mg (MgCl_2), (Goldberg, 1963). Commercial grade potassium ion was fortified in all treatment groups to maintain the K^+ levels as of sea water. The acclimatized shrimp were carefully transferred to the experimental tanks of different salinities by decreasing 1 ppt at every 18 hrs until they reached a required level of salinity of 5 ppt, 10 ppt (Nunes and Lopez, 2001).

A total 36 experimental plastic tanks (100 L capacity) were used and filled (80 L) with prepared salinities and ions manipulations. In each tank 20 shrimps were stocked and each treatment were kept in triplicate following the factorial design (3×4). Shrimp were fed 7% of body weight as the experiment progressed it was reduced to 3%. A commercial shrimp feed (Avanti) having crude protein (35%), moisture (11%) crude fibres (4%) and crude fat (5%) fed twice in a day (09.00 hrs and 21.00 hrs). The aeration was provided with air stone and regulator to control the air pressure in all the experimental units. Water physico-

chemical parameters like salinity and temperature were observed daily basis and dissolved oxygen, pH, hardness, total alkalinity was estimated once in ten days (APHA, 2005). The experimental mediums of different salinity waters were prepared (5, 10 and 15 ppt) and kept in 12 FRP tanks of 1-ton capacity.

Once 10 days growth sampling (length and weight) were recorded and each time 50% (10 shrimp/tank and 30 animals/treatment) of the stocks were sampled. The growth parameters such as Weight gain (%), specific growth rate (SGR, %/day), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and survival rate (%) were calculated using standard formulae.

The collected data of growth parameters and survival were subjected to two-way analysis of variance (ANOVA) using SAS Institute 2010, SAS/STAT user guide, VERSION 9.3. SAS INSTITUTE, CARY, NC, USA (1-940) for windows. Post comparison of different treatment groups mean values was carried out using Duncan's multiple range tests. All the data were expressed as mean $\pm$ standard error with a statistical significance value of $p < 0.05$.

RESULTS AND DISCUSSION

Water quality parameters

All the water quality parameters were within the optimum level to support the growth and survival of *P. vannamei* (Table 1). The present study recorded a temperature range of 26 to 30°C. In any aquatic organisms, temperature plays a vital role in maintaining the physiology and the shrimp maintained at the temperature of 20 to 32°C had shown better growth rate (Jiang *et al.*, 2000). Shrimp culture prefers the pH value of 7.5 to 8.5 and alkalinity values of ≥ 100 mg/L CaCO_3 for optimal production (Cohen *et al.*, 2005), similar range of pH and alkalinity values were observed in the present study.

The dissolved oxygen level (7.8 - 8.9 mg/L) was also above optimal level in the present study. More than 1 mg/L of total ammonia-N in culture water is lethal for shrimp (Jhingran, 1982), however, the present study recorded lower range of ammonia. Similarly, nitrite level was recorded as 0.001-0.005 mg/L range which was well in the acceptable limit for pond aquaculture (Boyd and Tucker, 1998). The total hardness values of the present study were highly varied among the treatment groups and it was increased with the increasing salinity and Ca^{2+} : Mg^{2+} fortification. The ionic composition of ISGW varies both locally and regionally and, in general, ISGW has low potassium in relation to equal salinity of seawater. Marine shrimps, mostly lives in the coastal waters, inhabits in the water which are having major cations and anions as that of seawater (Fielder *et al.*, 2001). Moreover, the ionic concentration of water affects the animal physiological activities than the salinity. Mostly, potassium and magnesium ions are limited in the ISGW which in turn affects the growth and survival of fish (Fielder *et al.*, 2001). Shortages of these two ions can be overcome by additions of mineral amendments (Boyd *et al.*, 2007) such as muriate

of potash (KCl), potassium magnesium sulphate and magnesium chloride.

Growth performance parameters

There was no significant difference in the mean body weight of the shrimp at the time of stocking. However, at the end of the experiment, significant difference was observed in the average body weight, weight gain percentage and SGR of *P. vannamei* reared in different treatment groups (Table 2). A significant interaction effect ($P < 0.05$) was noticed between salinity and Ca²⁺: Mg²⁺ ratio. On the other side, Ca²⁺: Mg²⁺ and salinity had also significantly affected the growth performance of *P. vannamei*. Growth parameters such as

body weight, weight gain percentage and SGR were significantly higher in 10 and 15 ppt salinity groups fortified with Ca²⁺: Mg²⁺ ratio of 1:2, 1:3 and 1:4.

Feed utilization parameters such as FCR, FER and PER were significantly differed among various treatment groups (Table 2). However, no interaction effect was found between salinity and Ca²⁺: Mg²⁺ ratio in different groups. Experimental group maintained with 10 and 15 ppt salinity and Ca²⁺: Mg²⁺ ratio of 1:2 and 1:3 had display highest FER and PER and lowest FCR values. On the other hand, poor feed utilization in terms of low FER and PER and high FCR was observed in 5 ppt salinity with 1:1 Ca²⁺: Mg²⁺ ratio. Significant difference ($p < 0.05$) was noticed in the survival

Table 1: Water quality parameters and ionic composition recorded during the culture trial.

Treatments (Salinity)	Ca ²⁺ : Mg ²⁺	Temp. (°C)	CO ₂ (ppm)	pH	Oxygen (ppm)	Hardness (ppm)	Ca ²⁺ (ppm)	Mg ²⁺ (ppm)	Ca ²⁺ : Mg ²⁺	K ⁺ con. (ppm)	Alkalinity (ppm)
5 ppt	1:1	26-30	Nil	8.5-8.6	7.1-7.2	1010	142	148	1.04	46	206-210
	1:2	26-30	Nil	7.2-8.3	6.7-7.6	1220	143	285	1.99	48	207-212
	1:3	26-30	Nil	8.1-8.3	6.8-7.1	1880	143	416	2.90	49	220-230
	1:4	26-30	Nil	8.2-8.5	6.9-6.9	3270	143	562	3.93	50	233-250
10 ppt	1:1	26-30	Nil	7.7-8.4	6.4-7.4	2300	374	372	0.99	49	272-280
	1:2	26-30	Nil	8.3-8.6	6.5-7.3	2800	371	732	1.97	52	270-284
	1:3	26-30	Nil	8.2-8.5	6.6-7.6	3700	370	1050	2.91	54	270-276
	1:4	26-30	Nil	7.8-8.5	6.5-7.2	5200	372	1462	3.93	56	285-300
15 ppt	1:1	26-30	Nil	7.8-8.3	6.4-7.3	3200	412	422	1.02	56	334-346
	1:2	26-30	Nil	7.8-8.5	6.5-7.6	3600	430	900	2.09	58	335-340
	1:3	26-30	Nil	7.8-8.5	7.1-7.3	5700	484	1450	2.99	59	342-350
	1:4	26-30	Nil	8.1-8.3	6.5-7.6	7000	510	2080	4.07	60	340-343

Abbreviation: K⁺= potassium; Mg²⁺= magnesium; Ca²⁺= calcium; Ca²⁺: Mg²⁺=calcium: magnesium ratio.

T₁- 5 ppt; Ca²⁺: Mg²⁺ (1:1, 1:2, 1:3 and 1:4), T₂- 10 ppt; Ca²⁺: Mg²⁺ (1:1, 1:2, 1:3 and 1:4) and T₃- 15 ppt; Ca²⁺: Mg²⁺ (1:1, 1:2, 1:3 and 1:4). Values in the same column with different superscripts differ significantly ($P < 0.05$) for each parameter. Data expressed as means±S.E.M (n=3). Two-way ANOVA was used following Duncan multiple range test in SAS-9.3.

Table 2: Growth parameters of different experimental groups of *P. vannamei* reared in ISGW fortified with different levels of Ca²⁺: Mg²⁺ ratio.

Treatments	Ca ²⁺ : Mg ²⁺	Ave weight (gm)	Weight gain (%)	FCR	PER	FER	SGR	Survival (%)
5 ppt	1:1	5.73 ^d ±0.14	54.87 ^c ±2.91	2.525 ^a ±0.07	1.56 ^c ±0.08	0.20 ^d ±0.01	0.73 ^c ±0.02	10 [±] 0.15
	1:2	6.86 ^c ±0.19	62.28 ^c ±3.01	2.073 ^a ±0.04	1.78 ^c ±0.05	0.26 ^d ±0.03	0.80 ^c ±0.01	40 [±] 2.15
	1:3	7.39 ^{bc} ±0.20	103.31 ^b ±2.33	1.668 ^c ±0.03	2.95 ^b ±0.07	0.42 ^c ±0.02	1.18 ^b ±0.02	60 [±] 0.82
	1:4	7.37 ^{bc} ±0.11	98.51 ^b ±2.13	1.905 ^c ±0.08	2.81 ^b ±0.20	0.40 ^c ±0.03	1.14 ^b ±0.03	45 [±] 1.56
10 ppt	1:1	7.78 ^b ±0.25	109.64 ^b ±3.50	1.801 ^b ±0.02	3.13 ^b ±0.06	0.45 ^c ±0.04	1.23 ^b ±0.03	30 [±] 2.29
	1:2	8.72 ^a ±0.16	133.32 ^a ±4.20	1.683 ^c ±0.00	3.80 ^a ±0.05	0.55 ^b ±0.03	1.41 ^a ±0.03	39 [±] 1.94
	1:3	9.02 ^a ±0.30	142.57 ^a ±3.11	1.765 ^c ±0.02	4.07 ^a ±0.100	0.59 ^{ab} ±0.02	1.48 ^a ±0.01	100 [±] 1.47
	1:4	8.78 ^a ±0.28	135.99 ^a ±1.89	2.221 ^c ±0.05	3.88 ^a ±0.09	0.56 ^{ab} ±0.03	1.43 ^a ±0.012	50 [±] 1.39
15 ppt	1:1	7.73 ^b ±0.23	1.08.15 ^b ±3.33	2.193 ^a ±0.07	3.09 ^b ±0.01	0.45 ^c ±0.05	1.22 ^b ±0.03	40 [±] 1.26
	1:2	8.83 ^a ±0.26	137.06 ^a ± 4.11	1.752 ^c ±0.03	3.91 ^a ±0.05	0.57 ^{ab} ±0.04	1.44 ^a ±0.02	80 [±] 1.37
	1:3	9.20 ^a ±0.31	147.63 ^a ±3.59	1.626 ^c ±0.01	4.22 ^a ±0.15	0.61 ^a ±0.03	1.51 ^a ±0.04	100 [±] 1.21
	1:4	9.00 ^a ±0.25	141.89 ^a ±3.21	1.691 ^{ab} ±0.01	4.05 ^a ±0.12	0.59 ^{ab} ±0.01	1.47 ^a ±0.03	49 [±] 1.4

Abbreviation: K⁺= potassium; Mg²⁺= magnesium; Ca²⁺= calcium; Ca²⁺: Mg²⁺=calcium: magnesium ratio.

T₁- 5 ppt; Ca²⁺: Mg²⁺ (1:1, 1:2, 1:3 and 1:4), T₂- 10 ppt; Ca²⁺: Mg²⁺ (1:1, 1:2, 1:3 and 1:4) and T₃- 15 ppt; Ca²⁺: Mg²⁺ (1:1, 1:2, 1:3 and 1:4). Values in the same column with different superscripts differ significantly ($P < 0.05$) for each parameter. Data expressed as means±S.E.M (n=3). Two-way ANOVA was used following Duncan multiple range test in SAS-9.3.

rate (%) of shrimp and 100% survival was recorded in 10 ppt and 15 ppt having Ca^{2+} : Mg^{2+} ratio of 1:3. Significantly lower (10%) survival was observed in 5 ppt salinity at Ca^{2+} : Mg^{2+} ratio of 1:1. Interestingly, in each salinity group treatment tanks with Ca^{2+} : Mg^{2+} ratio of 1:1 had lower survival rate.

In the present study, Ca^{2+} : Mg^{2+} ratio and the salinity had significantly affected the survival rate of *P. vannamei*. The survival rate was lower in treatment groups fortified with Ca^{2+} : Mg^{2+} ratio of 1:1 which may be due to low Mg^{2+} level in the culture medium. Ca^{2+} require for the absorption of phosphorus, cell signalling, muscle contraction, scale and bone formation, cofactor, etc. (Lovell, 1989). In shrimp culture, the requirement of Ca^{2+} was increased during moulting phase, as the shrimp do not have any internal calcium reserves (McWhinnie, 1962). Similarly, magnesium, a divalent cationic ion, plays an important role in shrimps' metabolism, Na^+ - K^+ -ATPase (NKA) synthesis and osmoregulation in fish (Romano and Zeng, 2012). To maintain the sufficient level of Mg^{2+} in to the cells, animal expend lot of energy to maintain the physiological homeostasis. Among the treatment salinities, survival rate of shrimp was significantly increased with the fortification of Mg^{2+} and Ca^{2+} : Mg^{2+} ratio which signifies the dependency of shrimp on Ca^{2+} : Mg^{2+} ratio for its survival.

The better survival rate was observed in Ca^{2+} : Mg^{2+} ratio of 1:3 which is due to similar Ca^{2+} : Mg^{2+} (1:3.6) ratio of sea water. The present study survival rate was similar with the earlier reports where they found an inverse relationship between salinity and survival rate of shrimp in raw ISGW of (Jain *et al.*, 2005).

CONCLUSION

Better growth performance was observed in salinities fortified with Ca^{2+} : Mg^{2+} ratio of 1:3. On the other side, poor growth pattern was noticed in salinity treatments fortified with Ca^{2+} : Mg^{2+} ratio of 1:1, which clearly indicate that shrimp requires Ca^{2+} : Mg^{2+} ratio closer to 1:3 for optimal growth and survival. Overall, the study found better survival in higher salinities, 10 and 15 ppt, compared to low salinity, 5 ppt. therefore, the study suggests rearing of *P. vannamei* in ISGW is possible with Ca^{2+} : Mg^{2+} ratio closer to 1:3 for better production.

Conflict of interest

The authors declare that there is no conflict of interest.

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