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The Combined effects of Salinity and Temperature on the survival of Zoeae and postlarvae of *Macrobrachium rosenbergii* at hatchery condition in Odisha, India

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Abstract
Present study was carried out to observe the effect of various ranges of temperature (25 ºC, 31 ºC and 35 ºC) and salinity (6, 9, 12, 15, 18 ppt.) on survival of *Macrobrachium rosenbergii* larvae. The survival rate ranged between 6.54-64.52% at 25 ºC in 9-12 ppt. whereas it was between 2.63-52.38% when reared at 25 ºC in 15-18ppt. Post larvae (PL) showed maximum survivability i.e. 44.29% at 25 ºC, 89.61% at 31 ºC and 42.70% at 35 ºC with the same salinity condition of 12ppt.which were also significantly different from each other (P>0.05).Water quality parameters recorded throughout the study period were found within the tolerable ranges (dissolved oxygen 7.3 ±0.06 mg/L, pH 8.0 ±0.04, ammonia from 0.10 ±0.02 mg/L etc).Present result showed that 12 ppt. salinity and 31 ºC temperature is better for the maximum survivability of larvae (Zoeae) and post larvae of *Macrobrachium rosenbergii* at hatchery condition.

Keywords: *Macrobrachium rosenbergii*, zoeae, post larva, salinity, hatchery.

1. Introduction
The giant fresh water prawn *Macrobrachium rosenbergii* has been acquired global value because of its delicacies and easy culture. Hence many countries of the world are now focusing on freshwater prawn culture and raising foreign exchange of the country through international freshwater prawn trade. In India Odisha state plays a major role in fresh water prawn culture and trade. Besides brackish water shrimp farming, especially *M. rosenbergii* is considered important and valuable for farming. Intensive and extensive culture practices of this giant freshwater prawn are being adopted in some of the regions [1]. of the state which include a hatchery phase performed in tanks stocked in high densities. Although reared in captivity from time immemorial, modern farming of this species originated in the early 1960s when FAO expert Shao-Wen Ling working in Malaysia found that fresh water prawn (*M. rosenbergii*) larvae required brackish conditions for survival. This leads to larval rearing on an experimental basis. Further various workers have experimented with seed production of *Macrobrachium* spp. using brine or salt solution [2]. Aquaculture production in 37 countries is more than 30,000 ton /annum and now production has risen up to $1billion annually. However over 98% of production occurs only in Asia [3]. In India the largest species of interest for aquaculture are *Macrobrachium rosenbergii* and *Macrobrachium malcomsoni* respectively. During the past decade the overall production of giant river prawn *M. rosenbergii* attained 195000 t in 2002 [3]. Early life stages are the most sensitive phase in the complex life cycle of marine invertebrates and to maximize their survival, larvae should reared close to optimal conditions. Both salinity and temperature are the major abiotic factors which affect survival percent and growth of the larvae. The studies on the growth of larvae of fresh water prawn with combined effect of temperature and salinity would lead to a greater understanding of the significance of these factors on survival of larvae. Thus it is important to determine optimum salinity and temperature level for rearing of *Macrobrachium rosenbergii* larvae with the capabilities of withstanding changes during the course of larval development [4-6]. According to Prequeux [7]. optimal salinity level is required for growth, survival and production competence which is also species specific [8, 9]. In tropics fluctuation of salinity and temperature reported frequently where The climate is characterized by wet and dry seasons.

Several studies have reported the effects of salinity and temperature variations on survival and growth of larvae of *Macrobrachium* spp. [10-12]. There are no such information available about its affect when cultured in hatchery condition along the coastal area of Odisha. Thus determining these optimal conditions for culture and development of the larvae of the species may be fundamental for development of rearing protocol to enhance its production. However no serious attempt has been made on the optimum temperature and salinity requirement of the hatched larvae to post larvae of fresh water prawns. The present study was undertaken to determine the survival of larvae (stages of Zoea as $Z_{1,}$- Z_{11} and Post Larva) at different combination of temperatures (25 ºC, 31 ºC and 35 ºC) and salinities (6,9,12,15 and 18 ppt.) in hatchery condition at Chandipur, the coastal area of Odisha, India.

2. Materials and Methods

Sexually matured females of *Macrobrachium rosenbergii* were collected from the Budhabalanga river mouth opening to Bay of Bengal at Chandipur, Balasore, Odisha and observed daily for the presence of eggs and individually transferred in to rearing tank with continuous aeration. After egg laying and hatching newly hatched larvae were siphoned in to a plastic bucket and were gently stirred in a circular motion. Dead larvae were siphoned. Aeration was applied uniformly and number of live ones were estimated volumetrically by taking 50ml samples [13].

The combined effect of salinity and temperature on survival of *Macrobrachium rosenbergii* larval stages (stages of Zoea symbolized as Z_1 - Z_{11} and Post larva symbolized as PL) was determined at hatchery condition on experimental basis. The experiment was carried out in 15 circular fibre tanks (Polyethylene tanks) at the hatchery farm named "Arunodaya Aquatics" situated at costal district Balasore of Odisha, India during July 2010 to August 2010. The tanks were circular, 1000L capacity flat bottomed and known as larval rearing tanks (LRTs). Aeration was done gently at 15-30 mL/sec via four air diffusers. Approximately 300L brackish water (12 ±0.5 ppt) was poured in to LRTs after cleaning, drying and formalin wash. The juveniles were divided in to three groups, and each juvenile was held at one of the constant temperatures 25 ºC, 31 ºC and 35 ºC. Each of the three groups was divided in to 5 subgroups and treated with different salinity levels 6%, 9%, 12%, 15% and 18%. Animals were fed twice a day on a pelletized scampi feed (*Artemia* naupli) based on visual observation of leftover feed and faecal matter to be removed daily from each container (Plastic tanks with a diameter of 70cm.). Water temperature was adjusted daily by using 300W thermostat controlled immersion heater. Salinity was obtained by mixing de-chlorinated tap water with seawater collected from Bay of Bengal and adjusted to the desired levels salinity by measuring with the help of salinometer (TDS-10 Pen type). The rearing period of 20 days was taken in to consideration and survival % was calculated as

$$\text{Survival \%} = \frac{\text{Number of species survived at the end of the experiment}}{\text{Number of species stocked}} \text{X100}$$

2.1. Statistical Analysis

Rate of survival of *M. rosenbergii* larvae at each treatment was analyzed by using two-way ANOVA (Analysis of variance) to determine if there was a significant effect of temperature, salinity and interaction between temperature and salinity on survival of larvae of *M. rosenbergii*. Duncan's Multiple Range Test (DMRT) was then applied to identify which salinity-temperature combinations were significantly different from each other. All statistical analyses were computed using SPSS software version 16.0.

3. Results

100% mortalities were recorded within 48h upon exposure to 6ppt. at any temperature (25 ºC, 31 ºC, 35 ºC). In 9-12 ppt. at 25 ºC survival rates ranged between 6.54-64.52% respectively (Table 1) whereas the rates were between 2.63-52.38% when exposed to 15-18 ppt. in 25 ºC. Exposure of the larvae to different salinity % at 31 ºC showed maximum survivability of 90.50% at 12ppt. (Z_{11}) and minimum 2.01% at 18ppt. In 35 ºC the maximum survivability was observed at 12 ppt. (65.36%) whereas the minimum was 1.12 at 18ppt. (Fig.1a-1f).

Table 1: Average survival % of *M. rosenbergii* cultured in different salinities and temperatures in 20 days.

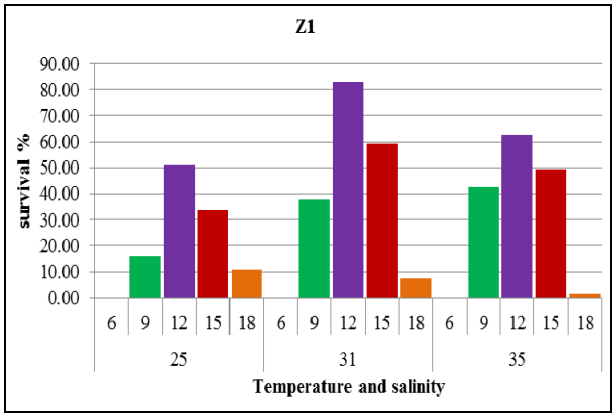
Temp.	**25^0 C**				**31^0 C**				**35^0 C**			
Salinity	9	12	15	18	9	12	15	18	9	12	15	18
Z1	15.96^{de}	51.16^{bc}	33.81^{cd}	10.65^{e}	37.79^{c}	83.16^{a}	59.36^{b}	7.28^{e}	42.49^{bc}	62.67^{b}	49.48^{bc}	1.66^{e}
Z2	12.06^{cd}	45.67^{b}	25.90^{c}	2.63^{d}	44.87^{b}	75.80^{a}	64.00^{a}	4.92^{d}	15.71^{cd}	65.36^{a}	45.98^{b}	2.74^{d}
Z3	13.23^{de}	52.08^{ab}	37.06^{bc}	8.49^{e}	25.98^{cd}	61.84^{a}	39.57^{bc}	4.15^{e}	15.56^{de}	64.38^{a}	57.50^{a}	3.96^{e}
Z4	11.20^{def}	46.39^{b}	33.53^{c}	4.37^{ef}	21.43^{cd}	60.92^{a}	49.76^{ab}	5.78^{ef}	14.22^{de}	49.95^{ab}	30.09^{c}	3.04^{ef}
Z5	15.22^{cde}	42.72^{b}	29.78^{bc}	3.35^{de}	20.30^{cd}	76.30^{a}	63.08^{a}	2.01^{de}	12.47^{cde}	40.42^{b}	26.94^{bc}	2.43^{de}
Z6	10.59^{efg}	49.29^{bc}	23.62^{de}	3.98^{g}	20.36^{def}	67.31^{a}	48.10^{bc}	4.81^{fg}	10.71^{efg}	59.75^{ab}	35.23^{cd}	5.49^{fg}
Z7	14.54^{de}	54.92^{b}	38.87^{bc}	5.04^{de}	21.89^{cd}	78.82^{a}	55.30^{b}	6.63^{de}	23.93^{cd}	50.78^{b}	37.36^{bc}	5.23^{de}
Z8	6.67^{e}	37.94^{bcd}	25.37^{cde}	4.11^{e}	19.50^{cde}	71.85^{a}	56.68^{ab}	11.40^{de}	8.18^{e}	45.06^{bc}	27.37^{cde}	1.12^{e}
Z9	6.54^{ef}	36.46^{bcd}	17.80^{def}	2.89^{ef}	21.15^{cde}	83.31^{a}	75.59^{a}	4.62^{ef}	9.93^{ef}	50.28^{b}	37.70^{bc}	3.04^{ef}
Z10	12.37^{cd}	64.52^{ab}	52.38^{b}	6.21^{d}	19.99^{cd}	85.63^{a}	73.92^{ab}	15.54^{cd}	5.36^{d}	55.12^{b}	30.63^{c}	6.30^{d}
Z11	13.40^{ef}	51.56^{bc}	32.90^{cd}	6.06^{f}	26.16^{de}	90.50^{a}	74.76^{a}	10.05^{ef}	13.56^{ef}	53.62^{b}	41.69^{bcd}	5.10^{f}
PL	12.54^{de}	44.29^{b}	35.11^{bc}	9.59^{de}	28.63^{bcd}	89.61^{a}	82.62^{a}	7.71^{e}	20.14^{cde}	42.70^{b}	34.85^{bc}	6.32^{e}

(Different superscripts indicate significantly differences from DMRT $P < 0.05$)

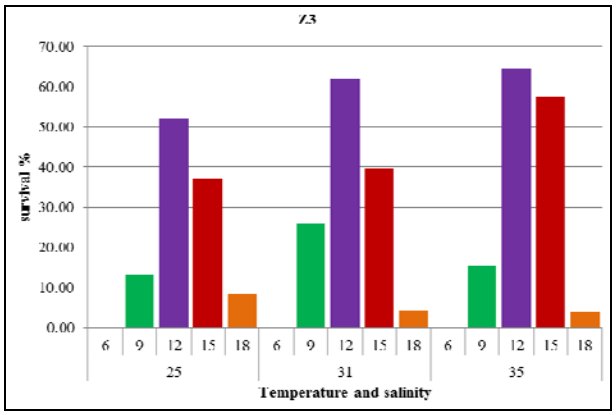
The effect of salinity and temperature-salinity interaction was significant at 5% level. The best survival of PL (post larvae) was found at 31 ºC for all ranges of salinities except 6 ppt. while lowest (7.71%) was observed at 18 ppt. The lower value of survival % was observed for all salinity ranges at 25 ºC (Fig.2).

Temperature and salinity were extremely important parameters affecting growth of the larvae of freshwater prawn

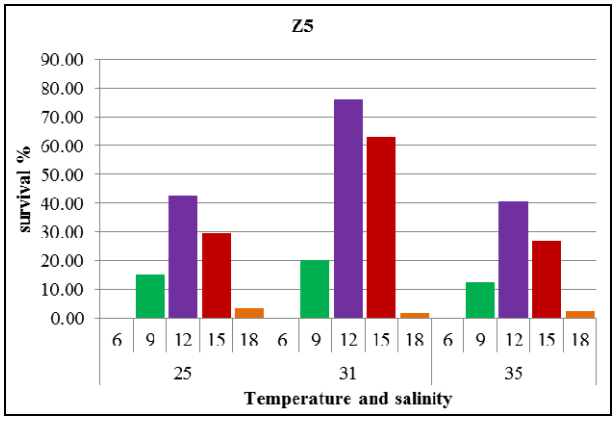
Macrobrachium rosenbergii. Zoeae of T_1 tank under 25 ºC & 31 ºC having body length 1.90mm were observed to be transparent body with horizontal rostrum having no rostral teeth. The second larval stage Z_2 was measured 1.92mm in body length, eye became stalked with appearance of supraorbital spines. Body length of Zoeae stages Z_3, Z_4 and Z_5 was found to be 2.0-2.5mm with uropod and development of rostral teeth. Only the Zoeae of tank T_2rearing at 31 ºC developed broad telson. Zoeae of stages 6,7 and 8 were found to have 3.0-3.5mm body length and of stages 9,10,and 11 were found to be 4.0-4.5mm.The other physic-chemical conditions of culture media was maintained with dissolved oxygen of 6.2 to 7.3mg/L.



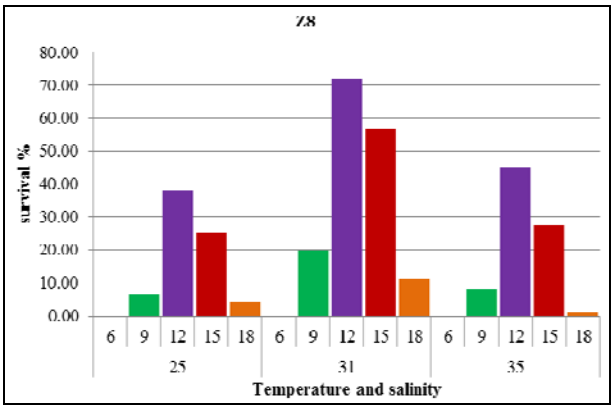
(A)



(B)



(C)



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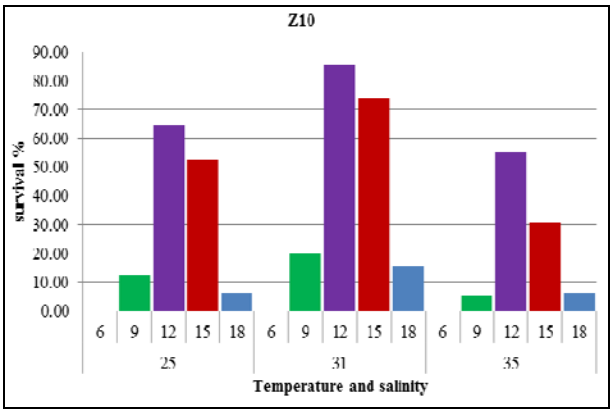
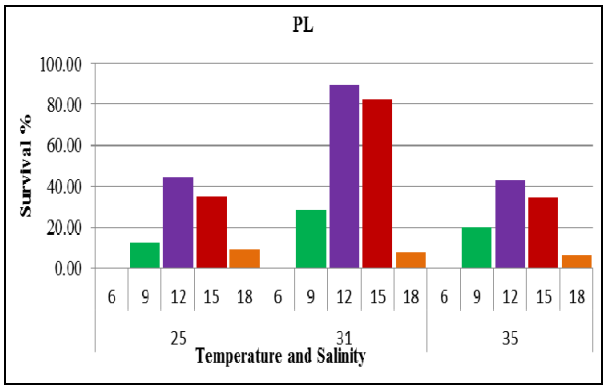


(E)



(f)

Fig 1: (**a-f**) Percent survival of each stage of *M.rosenbergii* zoea and PL reared at various combinations of salinity and temperature.

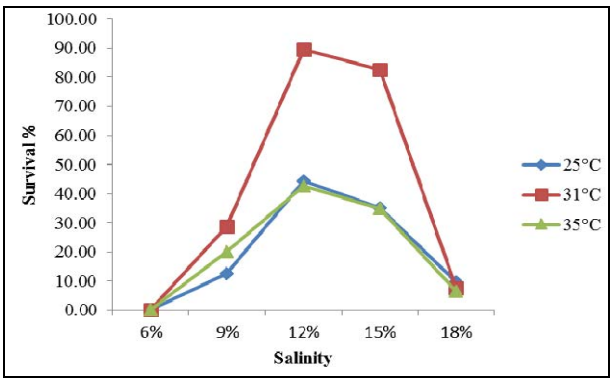


Fig 2: Average survival % of larvae of *M. rosenbergii* at various temperatures and salinity.

Instar duration at each larval stage varied markedly in 4 different salinity conditions (9, 12, 15 &18ppt.). Lesser instar duration was observed in the 12 and 15 ppt. in comparison to 9 and 18 ppt. Larvae grew from stage 1 to 5 within 6.7 days under salinity condition 12 ppt. with temperature 31 ºC whereas the growth from stage 1 to 5 under salinity of 9 and 15 ppt. was taken 20.5 days and 7.5 days respectively. Regression analysis between temperature with survival % and salinity with survival % (Fig.3 & 4) showed positive relation with increased value ($R^2 = 0.090$, $R^2 = 0.050$).

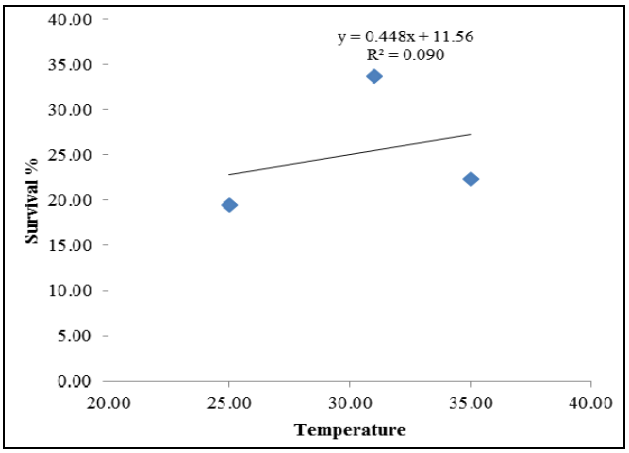


Fig 3: Regression analysis of survival % with different temperatures.

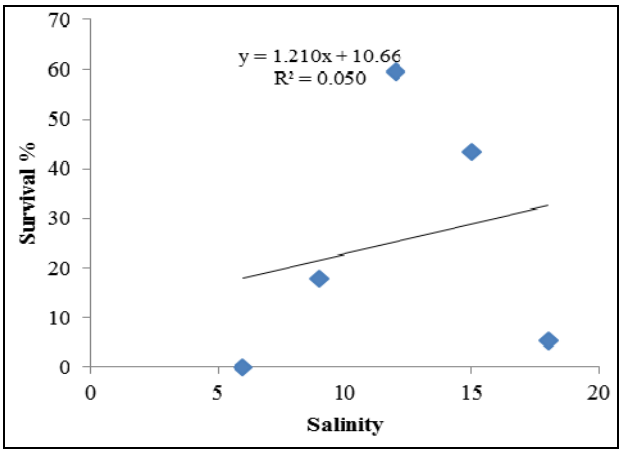


Fig 4: Regression analysis of survival % with different salinity.

4. Discussion

Salinity played a vital role on rearing and survivability of larvae of freshwater prawn (*Macrobrachium rosenbergii*).Optimum level of salinity was an important factor for the growth and rearing of this species [14]. Results of the present study indicated that highest % of survivability of larvae and post larvae was found at 12 ppt. salinity level and then at 15 ppt. level. Thus it supports that the species exhibits a wide range of tolerance to abrupt salinity changes. In an earlier study also stated that larvae and adults of *M. rosenbergii* are euryhaline to a considerable degree and tolerated salinities up to 21ppt [15, 16]. The water quality parameters like pH, dissolved oxygen, alkalinity, unionic ammonia during rearing period were within acceptable range for freshwater prawn rearing [17, 18]. Temperature also had a greater influence on the growth and survivability of larvae. The growth was increased from 25 ºC to 31 ºC. This might be due to the increased calorific intake at higher temperature. Staples and Heales [19, 20] have confirmed that high temperature to a certain point increases the molting frequency and growth of the prawn. Further they indicated that juveniles of *M. rosenbergii* grows more rapidly in slightly brackish water when compared to more brackish water of salinity above 15%. The optimum salinity-temperature combinations that supported development of juveniles had been reported in other crustacean larvae. Prequeux [7] explained that the osmoregulatory process is an important adaptation of many crustaceans to overcome changes in salinity. In this study the larvae of *Macrobrachium rosenbergii* hyper-osmoregulated when salinity was above iso-osmotic point and hypo-smoregulated when salinity was below this point. Several studies confirmed that *M. rosenbergii* is an osmoregulator in freshwater up to salinities at the iso-osmotic point (14-15ppt) [21-23]. In fresh water condition aquatic species spend a certain amount of energy to compensate the salt lost through passive diffusion, providing mild brackish water (≤10-12ppt) reduces energy expenditure and consequently promotes growth [24]. The results of the present study indicated that salinity plays a significant role in the culture of larvae of *M. rosenbergii* and the species showed satisfactory growth and survival at salinity 12-15ppt.

5. Conclusion

The result of the present findings indicated that salinity and temperature plays a significant role in the culture of larval conditions of *M.rosenbergii* and the species showed satisfactory survival and moulting at optimal range. Thus in views of future climatic conditions of the coastal areas of Odisha, India are going to be vulnerable as specially the brackish water condition. Under such condition *M.rosenbergii* can be considered as an ideal shrimp species as it can be reared in farm site selection and salinity maintenance to maximize the commercial production.

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