

## Effect of Rearing Container's Colour on Metamorphosis and Survival of Larvae of *Macrobrachium rosenbergii* (De Man, 1879).

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### Abstract

*Macrobrachium rosenbergii* larvae (IV stage) were reared in tubs having different colours viz, white, red, blue, dark-violet, grey and green. The time required for the appearance of first post-larvae was observed to be 37.3, 32.0, 29.5, 27.5, 34.0 and 35.8 days and the total amount of time required for obtaining post-larvae was 41.8, 36.3, 33.0, 31.5, 39.0 and 40.0 days in white (T<sub>1</sub>), red (T<sub>2</sub>), blue (T<sub>3</sub>), dark-violet (T<sub>4</sub>), grey (T<sub>5</sub>) and green (T<sub>6</sub>) colours respectively. Significantly less time was required for the metamorphosis of larvae to post-larvae in dark-violet tub as compared to other colours. However, there was no significant difference in grey and green colour treatments. Significantly higher survival (61.38%) was observed in dark-violet colored treatment, followed by 57.88% in blue, 51.63% in red, 43.38% in grey, 35.75% in green and 27.25% in white coloured tubs. The water parameters such as salinity, temperature, pH and dissolved oxygen were observed during the experimental period and were found to be within tolerance limit of *M. rosenbergii* zoeal larvae. Light-intensity in water varied in the range of 33.00 to 94.75 lux, 25.75 to 80.50 lux and 20.00 to 68.00 lux at 11:00, 15:00 and 18:00 hrs respectively in dark-violet, blue, red, grey, green and white colours. A significant difference ( $P < 0.05$ ) in light-intensity was observed in various treatments. Significantly maximum light-intensity was recorded in white tub and minimum in dark-violet tub. Darker internal colour of rearing tubs significantly reduces the metamorphosis period and increases survival rate of larvae of *M. rosenbergii*.

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## Introduction

The awareness of freshwater prawn culture has increased so much, that there is a huge demand for pure hatchery bred prawn seed. However, non-availability of quality seed in quantity at low cost is one of the bottlenecks in the development of scampi farming in the Maharashtra state. The cost of scampi seed as compared to *Penaeus monodon* seed is higher because of the long duration of larval cycle that involves more input. Therefore, it is very essential to minimize the production cost by improving seed rearing techniques.

Several scientists have developed seed production techniques with various extent of success. It is also reported that light intensity, photoperiod, tank type and shape, as well as colour of rearing tank influence the metamorphosis and survival of larvae (Yasharian et al. 2005). Larvae of giant freshwater prawn are sensitive to direct sun light, especially in a "clear water" system. Sebastian and George (1994) found that light intensity and colour of rearing tanks play an important role on feed consumption, larval development and survival of larvae. The impact of tank colouration on metamorphosis and survival of prawn larvae is a controversial topic. Therefore, the present study was undertaken to evaluate the effect of different colours of rearing-containers on metamorphosis, growth and survival of larvae of *M. rosenbergii* aimed at reducing the cost of post-larval production.

## Materials and Methods

*Macrobrachium rosenbergii* berried female with grey colour eggs was obtained from Kharland Research Station Panvel, New Mumbai and brought to Wet-Laboratory of College of Fisheries, Ratnagiri. The female was maintained in a plastic pool containing 12 gL<sup>-1</sup> saline water. The female was fed with chopped clam meat (2-3 times day<sup>-1</sup>). After hatching, the female was separated, shelters were removed and larvae were reared in the same plastic pool having 12 gL<sup>-1</sup> saline water up to IV stage. The IV stage larvae, produced from a single female and from the same batch were used for the experiment.

The pH, salinity, temperature and dissolved oxygen were estimated before initiating the experiment. Water having the required salinity (i.e. 12.5 ± 0.5 gL<sup>-1</sup>) was prepared by mixing the filtered freshwater and seawater (35 gL<sup>-1</sup> salinity).

Six different colours (treatments) such as, white (T<sub>1</sub>), red (T<sub>2</sub>), blue (T<sub>3</sub>), dark-violet (T<sub>4</sub>), grey (T<sub>5</sub>) and green (T<sub>6</sub>) with four replicates of each treatment were evaluated for rearing of zoeal larvae of *M. rosenbergii*. The volume of water maintained in each tub was four litres. Experiment was designed following the standard Completely Randomized Design (CRD) method. Stocking density of *M. rosenbergii* larvae (IV stage) was done at 50 nos. L<sup>-1</sup> (Indulkar 1999). Hence, a total of 200 IV stage larvae were stocked per tub. The rearing of larvae was carried out as suggested by Indulkar et al. (1998). To maintain optimum dissolved oxygen level and to keep feed particles in suspended form, aeration was provided in each tub using a diffuser. Monitoring of water quality was done regularly.

Larval feed was prepared by using a combination of various ingredients such as, ribbonfish powder (28%), milk powder (20%), agar-agar (2.0%), poultry egg (wet weight 40%), corn flour (8.0%), vitamin mineral mixture (1.0%) and dry yeast (1.0%) in the form of wet cake.

In addition to prepared larval feed, newly hatched *Artemia* nauplii were used as live food during the experiment. Upto IV stage, freshly hatched *Artemia* nauplii were given twice a day i. e. morning 08:00 hrs and evening 20:00 hrs. After IV stage, prepared feed combined with *Artemia* nauplii was given. The feeding rate and feeding schedule were followed as described by Indulkar et al. (2003). The experiment was carried out until the last post-larva was observed from each replicate of treatments.

During the experiment, stage of larvae, period required for each stage, total period required for post-larval formation and larval mortality in each replicate of treatment were recorded every day. The larval stages were identified under a microscope using a standard larval key (New and Singholka 1985) during the period of larval rearing. When larvae metamorphosed into postlarvae, the total numbers of post-larvae were counted and the percentage survival from each replicate was recorded. During the experimental period, regular water parameters such as salinity, water temperature, pH and dissolved oxygen were recorded daily following the standard methods (APHA 1998). Light intensity in the larval rearing tubs was measured using a digital Lux Meter. Inspections on left over feed, attack of external parasites on larvae, etc. were made regularly.

Standard statistical methods were adopted for testing significance. One-way analysis of variance (ANOVA) and Student-Newman-Keuls test (SNK) (Zar 2005) were applied to find out the differences among the mean period required to pass each larval stage and period required for the appearance of first post-larva, total period required for entire post-larval formation and mean value of mortality.

## Results

Total period required for the post-larval formation and survival in different colours of rearing tub viz, white (T<sub>1</sub>), red (T<sub>2</sub>), blue (T<sub>3</sub>), dark-violet (T<sub>4</sub>), grey (T<sub>5</sub>) and green (T<sub>6</sub>) are shown in [table 1](#).

Total period required varied from 108 to 144 hours (4.5 to 6.0 days); 180 to 234 hours (7.5 to 9.8 days); 228 to 324 hours (9.5 to 13.5 days); 342 to 456 hours (14.3 to 19.0 days); 414 to 558 hours (17.3 to 23.3 days); 474 to 678 hours (19.8 to 28.3 days) and 564 to 792 hours (23.5 to 33.0 days) for V, VI, VII, VIII, IX, X and XI stage formation respectively. Minimum period required was recorded in T<sub>4</sub> treatment and maximum in T<sub>1</sub> treatment. ANOVA revealed significant difference (P<0.05) in period required among the treatments. The Student-Newman-Keuls analysis showed significant differences among all treatments with each other.

Total period required for first post-larvae formation varied from 660 to 894 hours (27.5 to 37.3 days). Minimum period required was recorded in T<sub>4</sub> treatment and maximum in T<sub>1</sub> treatment. ANOVA revealed significant difference (P<0.05) in period required for first post-larvae formation among the treatments. The Student-Newman-Keuls analysis showed significant differences among all treatments with each other.

**Table 1.** Total period required (hrs) to pass each larval stage and survival of *M. rosenbergii* reared in plastic tubs with different colours

Larval Stage	Period required (hrs)					
	Treatments					
	White (T <sub>1</sub> )	Red (T <sub>2</sub> )	Blue (T <sub>3</sub> )	Dark violet (T <sub>4</sub> )	Grey (T <sub>5</sub> )	Green (T <sub>6</sub> )
V	144 ±09.7 <sup>b</sup>	126 ±06.0 <sup>ab</sup>	126 ±06.0 <sup>ab</sup>	108 ±06.9 <sup>a</sup>	132 ±06.9 <sup>ab</sup>	138 ±06.0 <sup>ab</sup>
VI	234 ±06.0 <sup>b</sup>	210 ±11.5 <sup>b</sup>	222 ±06.0 <sup>b</sup>	180 ±06.9 <sup>a</sup>	228 ±06.9 <sup>b</sup>	228 ±06.9 <sup>b</sup>
VII	324 ±06.9 <sup>c</sup>	276 ±06.9 <sup>b</sup>	270 ±06.0 <sup>b</sup>	228 ±06.9 <sup>a</sup>	294 ±06.0 <sup>b</sup>	300 ±12.0 <sup>b</sup>
VIII	456 ±13.9 <sup>d</sup>	384 ±09.8 <sup>b</sup>	372 ±06.9 <sup>ab</sup>	342 ±15.0 <sup>a</sup>	402 ±06.0 <sup>bc</sup>	426 ±11.5 <sup>cd</sup>
IX	558 ±11.5 <sup>d</sup>	468 ±15.5 <sup>b</sup>	444 ±06.9 <sup>b</sup>	414 ±06.0 <sup>a</sup>	498 ±06.0 <sup>c</sup>	522 ±06.0 <sup>c</sup>
X	678 ±11.5 <sup>f</sup>	558 ±06.0 <sup>c</sup>	510 ±11.5 <sup>b</sup>	474 ±06.0 <sup>a</sup>	594 ±11.5 <sup>d</sup>	636 ±06.9 <sup>e</sup>
XI	792 ±09.8 <sup>f</sup>	666 ±11.5 <sup>c</sup>	606 ±06.0 <sup>b</sup>	564 ±06.9 <sup>a</sup>	708 ±15.5 <sup>d</sup>	744 ±09.8 <sup>e</sup>
1 <sup>st</sup> PL	894 ±11.5 <sup>f</sup>	768 ±09.8 <sup>c</sup>	708 ±06.9 <sup>b</sup>	660 ±06.9 <sup>a</sup>	816 ±09.8 <sup>d</sup>	858 ±15.0 <sup>e</sup>
Entire cycle	1002 ±06.0 <sup>e</sup>	870 ±11.5 <sup>c</sup>	792 ±09.8 <sup>b</sup>	756 ±06.9 <sup>a</sup>	936 ±09.8 <sup>d</sup>	960 ±17.0 <sup>d</sup>
Entire cycle (days)	41.8 ±0.25 <sup>e</sup>	36.3 ±0.47 <sup>c</sup>	33.0 ±0.40 <sup>b</sup>	31.5 ±0.28 <sup>a</sup>	39.0 ±0.40 <sup>d</sup>	40.0 ±0.70 <sup>d</sup>
Avg. survival (%)	27.25 ±1.68 <sup>e</sup>	51.63 ±1.43 <sup>b</sup>	57.88 ±1.23 <sup>a</sup>	61.38 ±1.81 <sup>a</sup>	43.38 ±1.46 <sup>c</sup>	35.75 ±1.71 <sup>d</sup>

Total period required for completion of larval cycle or entire post-larvae formation varied from 756 to 1002 hours (31.5 to 41.8 days). Minimum period required was recorded in T<sub>4</sub> treatment and maximum in T<sub>1</sub> treatment. ANOVA revealed significant differences (P<0.05) in period required for entire post-larval formation among the treatments. The Student-Newman-

Keuls analysis showed no significant difference ( $P>0.05$ ) among treatments  $T_5$  and  $T_6$ . All the remaining treatments significantly differed ( $P<0.05$ ) from each other.

Maximum survival (61.38%) was observed in dark-violet tub, followed by blue tub (57.88%), red tub (51.63%), grey tub (43.38%), green tub (37.75%) and minimum in white tub (27.25%). ANOVA showed significant difference ( $P<0.05$ ) in zoel larval mortality reared in different colours of tub. The Student-Newman-Keuls analysis showed no significant difference ( $P>0.05$ ) in mortalities among treatments  $T_3$  and  $T_4$ . All the remaining treatments significantly differed ( $P<0.05$ ) from each other.

The light-intensity in water was measured during larval rearing at 11:00, 15:00 and 18:00 hrs from each replicate of treatments. The average light-intensity varied in the range of 33.00 to 94.75 lux, 25.75 to 80.50 lux and 20.00 to 68.00 lux at 11:00, 15:00 and 18:00 hrs respectively (Table 2). ANOVA revealed significant difference in light-intensity observed in various treatments. A significantly maximum light-intensity was recorded in  $T_1$  and minimum in  $T_4$  treatment. The Student-Newman-Keuls analysis showed significant ( $P<0.05$ ) difference among all treatments at 11:00, 15:00 and 18:00 hrs.

**Table 2.** Average light-intensity (Lux) in water of rearing containers having different colours

Time (hrs)	Particulars	Light-intensity (Lux)					
		Treatments					
		White ( $T_1$ )	Red ( $T_2$ )	Blue ( $T_3$ )	Dark violet ( $T_4$ )	Grey ( $T_5$ )	Green ( $T_6$ )
11:00	Range	92 to 98	50 to 57	38 to 42	31 to 35	64 to 71	69 to 75
	Mean	94.75 ± 1.37 <sup>f</sup>	53.50 ± 1.55 <sup>c</sup>	39.75 ± 1.31 <sup>b</sup>	33.00 ± 0.91 <sup>a</sup>	67.50 ± 1.55 <sup>d</sup>	72.25 ± 1.37 <sup>e</sup>
15:00	Range	79 to 84	42 to 47	31 to 35	24 to 28	57 to 61	64 to 66
	Mean	80.50 ± 1.19 <sup>f</sup>	44.50 ± 1.04 <sup>c</sup>	33.00 ± 0.91 <sup>b</sup>	25.75 ± 0.85 <sup>a</sup>	58.75 ± 0.85 <sup>d</sup>	64.75 ± 0.47 <sup>e</sup>
18:00	Range	65 to 71	30 to 32	24 to 26	18 to 22	41 to 45	47 to 51
	Mean	68.00 ± 1.29 <sup>f</sup>	31.25 ± 0.47 <sup>c</sup>	25.00 ± 0.40 <sup>b</sup>	20.00 ± 0.91 <sup>a</sup>	42.75 ± 0.85 <sup>d</sup>	49.00 ± 0.91 <sup>e</sup>

Daily observations on water parameters were done during the experimental period. Temperature varied in the range of 26.0 to 31.0 °C, dissolved oxygen 5.8 to 7.2 mgL<sup>-1</sup>, pH 7.5 - 7.8 and salinity was maintained between 12 to 13 gL<sup>-1</sup>. All the parameters were found to be within the tolerance limits of *M. rosenbergii* larvae (New and Singholka 1985).

The above result indicated that, the total period required to complete the entire cycle was 41.8, 36.3, 33.0, 31.5, 39.0 and 40.0 days in white ( $T_1$ ), red ( $T_2$ ), blue ( $T_3$ ), dark-violet ( $T_4$ ), grey ( $T_5$ ) and green ( $T_6$ ) colours respectively. The total period required for a complete metamorphosis

of larvae to post-larvae was reduced by ten days in dark-violet tub as compared to the white tub treatments. The period required for the first post-larvae formation was significantly less in dark-violet tub as compared to blue, red, grey, green and white colour tub. However, there was a significant difference in survival and total period required for post-larvae formation in larvae reared in dark-violet tub having minimum light-intensity as compared to the remaining colour treatments. The *M. rosenbergii* larvae reared in dark-violet tub resulted in maximum survival (61.38%).

## Discussion

Several external and internal factors have been observed to play a role in the metamorphosis and survival of freshwater prawns. The factors, particularly temperature, water quality, larval feed etc. have been observed to play a greater role in *M. rosenbergii* seed production techniques (Raje and Joshi 1992; Reddy 1997; Rao 1998; Indulkar and Belsare 2001; Kovaleno et al. 2002; Chandra Prakash and Reddy 2003; Cheng et al. 2003). It is also reported that light intensity, photoperiod, tank type and shape, colour of rearing tank influence the metamorphosis and survival of larvae (Menasveta and Piyatiratitvokul 1982; Lin and Omori 1993; Correia et al. 2000; Yasharian et al. 2005). Larvae of giant freshwater prawn are sensitive to direct sunlight, especially in a “clear water” system.

Generally, animals depend on light for orientation, diurnal migration and synchrony of rhythmic activities. The light reception is probably the most important sensory modality in the exploration of the environment. Response to light is generally considered to be one of the most important factors to enable the free-swimming organisms find a favourable place in the water column. It stimulates locomotion at certain intensity irrespective of the direction of light (Vernberg and Vernberg 1983). It has been observed that light can influence molt initiation in some crustaceans. Illumination may act on the heart rate through several mechanisms. The heart rate of shrimp is about 30% lower when the animal is on the dark back ground or when the eyes and eyestalks have been removed, than when the eyes are present and the animal is illuminated (Maynard 1960). Some scientist found that light had negligible effect on respiration of vertically migrating crustaceans (Vernberg 1983). Withyachumnarnkul et al. (1970) observed that, continuous darkness stimulates growth of *M. rosenbergii* juveniles.

Several scientists attempted to find out the role of colour variations of container on the growth and survival of *M. rosenbergii* larvae (Sebastian and George 1994; Yasharian et al. 2005). In this experiment, *Macrobrachium rosenbergii* larvae were reared in tubs with different internal colours such as, white, red, blue, dark-violet, grey and green. The period required for the appearance of first post-larvae was observed to be 37.3, 32.0, 29.5, 27.5, 34.0 and 35.8 days and the total period required for obtaining post-larvae was 41.8, 36.3, 33.0, 31.5, 39.0 and 40.0 days in white (T<sub>1</sub>), red (T<sub>2</sub>), blue (T<sub>3</sub>), dark-violet (T<sub>4</sub>), grey (T<sub>5</sub>) and green (T<sub>6</sub>) colours respectively. Significantly shorter period (31.5 days) was observed to be required for larvae to metamorphose into post-larvae in dark-violet coloured tubs followed by blue coloured tubs (33.0 days). However, there was no significant difference in total period observed in grey and green colours, but significantly longer period was observed to be required for larvae to metamorphose into post-

larvae in white coloured tubs (41.8 days). It was also observed that there was significant difference in mortality from IV stage to post-larvae. A significantly high mortality was observed in white coloured tubs (72.75%), whereas lower in dark-violet coloured tubs (35.75%). However, no significant difference was observed in mortality of dark-violet and blue coloured tubs. Higher survival (61.38%) was recorded in dark-violet coloured tubs, followed by 57.88% in blue, 51.63% in red, 43.38% in grey, 35.75% in green and 27.25% in white coloured tubs.

During this experiment, larval feeding regime, water quality management and other parameters such as salinity, pH, temperature, etc. were the same in all treatments except light-intensity in water which varied in the range of 20.00 to 94.75 lux. Light-intensity in water was observed at 11:00, 15:00 and 18:00 hours, which significantly differed in different coloured tubs. A significantly maximum light-intensity was recorded in white coloured tubs whereas it was minimum in dark-violet tubs, even though all tubs were exposed to identical light and water sources. This suggests that the internal colour of rearing tubs have an influence on larval metamorphosis and survival. The internal colour of rearing tubs affects light-intensity in water, which was responsible for the overall larval development. It seems that, the role of light-intensity and its interaction with colour appears to be important in the metamorphosis and survival of *M. rosenbergii* larvae. The result of this experiment indicated that the colour of rearing tub and light-intensity in water had a great influence on the metamorphosis and survival of *M. rosenbergii* larvae. The result indicated that a darker internal colour of rearing container significantly reduces metamorphosis period and increases the survival of larvae of *M. rosenbergii*. The result of this study agrees with the results reported by Sebastian and George (1994). They have observed that brown colour water gives a slightly higher production than clear water. They have also reported that the feed, general physico-chemical parameters of larval rearing water and light intensity play an important role in larval development and survival. They have likewise reported that the colour of larval rearing tank appeared to have a definite bearing on food consumption and survival of larvae and further emphasized the need for detailed study.

However, the result reported by Yasharian et al. (2005) did not support the present study. They have reported that the tank colour had no significant impact on metamorphosis and growth of larvae where, survival was significantly ( $P > 0.05$ ) impacted by tank colour. Lin and Omori (1993) reported that feeding rates decreased with the increase in the lightness of colour of the rearing container. The highest feeding rate was recorded for the larvae placed in black containers and the lowest was for those kept in white and shiny tin containers. Additional experiment indicated that the swimming behaviour of the larvae also differed significantly with the colouration of the container. Correia et al. (2000) reported that dark interior gives the best results, because animals can detect their food more easily against a dark background and suggested the use of tungsten lamp or blue-black fluorescent tubes.

Daniels et al. (1992) suggested the use of indirect light source to give good colour contrast to *Artemia* so as to feed more efficiently. Rodrigues et al. (1998) did not find any evidence that, either sunlight or tank colour affected the capture or ingestion of food by freshwater prawn larvae. However, these authors found greater numbers of post-larvae in black coloured tanks as opposed to white tanks, irrespective of the incidence of the sunlight. Furthermore, they have reported that larger and heavier post-larvae were produced when black tanks with sunlight were used. Similar observations were also made during the present study.

The role of colour of rearing tubs and its interaction with light-intensity appears to be important in yielding higher survival of larvae of *M. rosenbergii*. Darker coloured rearing containers (dark-violet in the present study), recorded a short metamorphosis period and high survival of larvae of *M. rosenbergii*.

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