GROWTH AND SURVIVAL OF MACROBRACHIUM ROSENBERGII POSTLARVAE (DE MAN, 1879) FED WITH EPA AND DHA ENRICHED ARTEMIA

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Nutritional quality of Artemia used in prawn hatchery determines the quality of seed output and its performance in the culture. The present study aimed at determining the effect of n-3 HUFA enriched Artemia feeding on growth and survival of postlarvae (PL) of M. rosenbergii. The Artemia were fed with n-3 HUFA emulsions at 2 ml L\(^{-1}\) and samples were taken at 0, 12, 24, 36, 48 h for analyzing fatty acid profile. Results exhibited that 24 h enriched Artemia had a significantly higher level of EPA (3.76±0.11%) and DHA (2.29±0.01%) and it declined after 24 h. The PL of M. rosenbergii were fed with freshly hatched (T), 12 h enriched (T\(_2\)), 12 h un-enriched (T\(_3\)), 24 h enriched (T\(_4\)) and 24 h un-enriched (T\(_5\)) Artemia during initial 10 days and reared for 40 days with commercial diet. The highest SGR (3.21% day\(^{-1}\)) and survival (83.33 ± 3.34 %) was observed in T\(_4\) compared to other treatments. Data of the present study suggest that 24 h is an optimum duration for the enrichment of Artemia and feeding it for short duration enhanced the growth and survival of M. rosenbergii.

Keywords: Enrichment, HUFA, live feed, Macrobrachium rosenbergii, hatchery management

INTRODUCTION

The freshwater prawn, Macrobrachium rosenbergii belongs to the Palaemonidae family and is distributed in the Indo-West Pacific region including India. It inhabits in freshwater and brackish water bodies. Several constraints affect the Post Larvae (PL) production in prawn hatcheries (Nhan et al., 2010). The inadequate larval nutrients in larval feed lead to lower survival and growth, and higher mortalities (Das et al., 2007; Roo, Hernandez-Cruz et al., 2019). The significance of essential fatty acids on freshwater prawn juvenile growth performance has been studied extensively (Sandifer and Joseph, 1976). The n-3 highly unsaturated fatty acids are vital components in the cell membrane which regulate the physiological activities. And, the docosahexaenoic acid (DHA) maintains the structural and functional integrity in cell membranes. Further, it is imperative in neural development, and visual function (Bradbury, 2011; Das et al., 2007; Hernández-Cruz et al., 2015; Izquierdo et al., 2000).
To enhance the prawn production, quality seed is very imperative where live feed plays a significant role. About 85% of marine hatcheries worldwide used *Artemia* nauplii as an exclusive diet (Sorgeloos, 1977). Among the live feed, the *Artemia* nauplii are used widely in larviculture of *M. rosenbergii* (New, 1995; Shivananda Murthy and Thanuja, 2006) because it is nutritionally inconsistent and relatively low in EPA (20:5n-3) and DHA (22:6n-3) content (Coutteau and Sorgeloos, 1997; Olsen et al., 2000; Shivananda Murthy and Thanuja, 2006). Further, enriched *Artemia* nauplii increase the quality of larvae Hien et al., 2005 and juveniles of *M. rosenbergii* (Sandifer and Joseph, 1976). A number of enrichment products including self-emulsifying concentrates, microparticulate diets, unicellular algae and omega yeast are used commercially Sorgeloos et al., 2001. Till date, various research has been conducted to assess the effect of EPA and DHA on growth and survival rate of *M. rosenbergii* at different larval developmental stages fed with n-3 HUFA (Das et al., 2007; Hien et al., 2005; Rani et al., 2006) but still, a paucity of information observed on feeding enriched *Artemia* to *M. rosenbergii* PL for a short duration before transit to stocking in the commercial farming. Therefore, the present study aimed to determine optimum *Artemia* enrichment duration and the effect of feeding EPA and DHA of enriched *Artemia* on *M. rosenbergii* PL bio-growth parameters.

**MATERIALS AND METHODS**

This study was carried out at Prawn Hatchery Unit, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India.

**Hatching of *Artemia* cysts**

*Artemia* nauplii were produced using commercially *Artemia* cysts (O.S.I. PRO 80, USA). The hydrated cyst (1 h) were decapsulated using 5% sodium hypochlorite about 10 minutes then it dipped into sodium thiosulphate (0.1%) to remove the chlorine residue and washed in water. After that, the cysts were transferred to cylinder conical tank having 35‰ seawater for incubation. Dissolved oxygen (DO) was maintained more than 5 mg L⁻¹ through aeration. The newly hatched *Artemia* nauplii were collected after 24 h of hatching for further use.

**Emulsion preparation and Enrichment**

A gelatin capsule containing fish lipid oil equivalent to EPA and DHA content of 180 and 120 mg respectively was used to prepare the emulsion using the emulsifier Tween 80 by following a standard protocol (Tamaru et al., 1999). The emulsion of EPA and DHA was added at 2 ml L⁻¹ to the cylindroconical tank having instar I nauplii of *Artemia* at the density of 100-150 nauplii ml⁻¹. For analysis of fatty acid, samples were obtained at 0, 12, 24, 36, and 48 h.

**Fatty acid analysis**

Fatty acid composition of *Artemia* was analyzed using Gas-Liquid Chromatography (Chemito, GC 8610). The sodium hydroxide (5 N) in methanol and methanol (5 drops and 50 ml respectively) were added into the 1 g *Artemia* nauplii sample and were refluxed about 30
mins. The mixture was acidified with H₂SO₄ (5 ml) using methyl orange indicator and refluxed for 30 mins. The petroleum ether (40-60 °C) was used to extract the Esterified sample and collected through sodium sulfate. The ether layer was evaporated and diluted with acetone 1:25 (ratio) and then injected (1µl) in GLC. The nitrogen was used as a carrier gas and the injector was set at 250°C and detector at 260 °C.

**Experimental design**

A total of two hundred PL was used in this study. The 20 individuals of PL of *M. rosenbergii* (1.72 cm in length; 0.0453 g in weight) were stocked randomly in plastic troughs (40 L capacity) into five treatments (newly hatched (T₁), 12 h enriched (T₂), 12 h un-enriched (T₃), 24 h enriched (T₄) and 24 h un-enriched (T₅) *Artemia* nauplii), in duplicate by following completely randomized design. The round clock aeration was provided to maintain the water quality parameters at an optimum level. The cleaning of excess feed and animal excreta were done daily before feeding and they fed with the experimental diet for initial 10 days and further reared 40 days with commercial diet in order to find out the influence of enrichment diet of artemia. The initial and final weight of larvae for each treatment was measured using a digital balance. However, the weight of *M. rosenbergii* PL at the end of 10 days with experimental diet was considered as an initial weight to calculate the bio-growth parameters. And, the formula given below were used to calculating bio-growth parameters.

- Weight gain, g = final weight (g) − initial weight (g)
- Daily weight gain(g) = \(\frac{\text{final weight (g)} - \text{initial weight(g)}}{\text{duration of rearing period (days)}}\)
- Weight gain percent (%) = \(\frac{\text{final weight (g) - initial weight(g)}}{\text{initial weight (g)}} \times 100\)
- Specific growth rate (%day⁻¹) = \(\frac{[\text{Ln (final weight (g))} - \text{Ln (initial weight (g))}]}{\text{duration of rearing period (days)}} \times 100\)
- Survival rate (%) = \(\frac{\text{final number of larvae}}{\text{initial number of larvae}} \times 100\)

**Statistical analysis**

Data are presented as mean ± SD. The data normality and homogeneity of variance were analyzed by K–S and the Levene tests respectively. One-way analysis of variance (ANOVA) was used to compare the mean value of different treatments and Duncan’s multiple range tests (DMRT) was performed to find the significant difference between treatment mean. The data were analyzed using SPSS.
RESULTS

Enrichment of Artemia

The EPA (20:5n-3) and DHA (22:6n-3) composition (% of total fatty acid) of enriched Artemia nauplii at different hours are presented in Fig. 1. Among different enrichment groups, 24 h enriched Artemia showed significantly (P<0.05) higher EPA and DHA content of 3.76±0.11% and 2.29±0.01% respectively than those of other treatments. The total EPA and DHA were 0 in freshly hatched artemia nauplii (0 h), 4.09±0.04% in 12 h, 6.05±0.12% in 24 h, 2.48±0.07% in 36 h and 2.43±0.06 % in 48h. No significant differences (P<0.05) were observed in the n-HUFA content at 36 and 48 h enriched Artemia.

Growth and survival rate of M. rosenbergii

The growth and survival rate of M. rosenbergii fed with EPA and DHA enriched, and un-enriched Artemia nauplii at different hours are portrayed in Table 1. There was a significant difference (P<0.05) found in the final weight, weight gain, weight gain percent, specific growth rate and survival rate between the enriched and un-enriched Artemia fed M. rosenbergii. The M. rosenbergii fed 24 h enriched Artemia nauplii had the significantly (P<0.05) higher weight gain (0.405 ± 0.02) (Fig. 2) as compared to the un-enriched and freshly hatched Artemia nauplii. Significantly higher (P<0.05) SGR of 3.21% day\(^{-1}\) was observed in experimental animal fed with 24 h enriched artemia nauplii whereas the lower SGR of 2.75% day\(^{-1}\) in experimental animal fed with 24 h un-enriched artemia. The survival was found to be significantly greater (P<0.05) in experimental animal fed with 24 h enriched artemia nauplii (83.33 ± 3.34 %) compared to other treatments. Fig. 3 and 4 shows the trend line of the SGR and survival rate of M. rosenbergii in different treatments. The SGR and survival rate of PL was influenced by total EPA+DHA content of enriched Artemia nauplii (Fig. 5). The SGR trend line showed a good positive relationship to the total EPA +DHA and similar trend line was also observed in survival rate. The SGR was remarkably increased with increasing the total EPA + DHA. The SGR and survival rate of the M. rosenbergii can be described through y = 2.9025 + 0.0556x: R\(^2\) = 0.9444 and y = 63.722 + 3.4354x: R\(^2\) = 0.9667 respectively.
Table 1: Production parameters of the *M. rosenbergii* PL fed with enriched and un-enriched *Artemia* nauplii diet

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
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<tbody>
<tr>
<td></td>
<td>T₁</td>
</tr>
<tr>
<td>Initial Weight (g)</td>
<td>0.145 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final Weight (g)</td>
<td>0.460 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>0.315 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight gain percent (%)</td>
<td>217.24 ± 23.57&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>2.89 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>63.33 ± 3.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given in mean ± SD. Different superscript in lower case letter in the same row indicates significant difference among the different treatment of a given parameter (P<0.05, One-way ANOVA, Duncan Post-Hoc). T₁ - newly hatched *Artemia* fed group; T₂ - 12 h enriched *Artemia* fed group; T₃ - 12 h un-enriched *Artemia* fed group; T₄ - 24 h enriched *Artemia* fed group; T₅ - 24 h un-enriched *Artemia* fed group.

**Fig. 2.** Weight gain of the *M. rosenbergii* at a different time interval. Values in the clustered bar with different superscripts differ significantly (P<0.05, One-way ANOVA, Duncan Post-Hoc). T₁ - newly hatched *Artemia* fed group; T₂ - 12 h enriched *Artemia* fed group; T₃ - 12 h un-enriched *Artemia* fed group; T₄ - 24 h enriched *Artemia* fed group; T₅ - 24 h un-enriched *Artemia* fed group.

**Fig. 3.** Specific growth rate of the *M. rosenbergii* fed with different treatment diet. T₁ - newly hatched *Artemia* fed group; T₂ - 12 h enriched *Artemia* fed group; T₃ - 12 h un-enriched *Artemia* fed group; T₄ - 24 h enriched *Artemia* fed group; T₅ - 24 h un-enriched *Artemia* fed group.
DISCUSSION

Enrichment of Artemia

The Artemia nauplii enriched with the emulsion of n-3 HUFA differed significantly with enrichment hours. The newly hatched nauplii of Artemia had no DHA (Evjemo et al., 1997; Shivananda Murthy and Thanuja, 2006; Tamaru et al., 1999); further crustaceans cannot synthesise HUFA (Sargent et al., 1999). The total content of EPA and DHA in Artemia nauplii was influenced by the duration of n-3 HUFA enrichment (Tamaru et al., 1999). In the present study, the total EPA and DHA content were increased into 4.09 and 6.05% after 12 and 24 h of enrichment respectively. However, it started declining into 2.43% at 48 h owing to the metabolic fate of HUFA in Artemia nauplii. The Artemia franciscana was also rapidly metabolizing DHA for energy production (Evjemo et al., 1997). The duration of Artemia enrichment should also be considered when using it as food for the larvae (Tamaru et al., 1999). There is a minimum need of 12 h enrichment for instar II nauplii to fully pack the gut (Shivananda Murthy and Thanuja, 2006) but present study result demonstrates that there was a reduction in EPA and DHA content of artemia after 24 h of enrichment. Therefore, 24 h enrichment in the present study was taken as the upper limit due to thereafter decline of EPA and DHA. We evaluated the production parameters of M. rosenbergii PL fed with freshly hatched, enriched (12 and 24 h), and un-enriched (12 and 24 h) Artemia nauplii.

Growth and survival rate

The present study found that the PL of M. rosenbergii fed with enriched Artemia nauplii had higher growth performance and survival rate than that of un-enriched and freshly hatched Artemia nauplii. The EPA and DHA enriched Artemia nauplii showed a significantly
higher survival than that of receiving un-enriched and freshly hatched nauplii of *Artemia*. The highest body weight gain of 0.405 and 0.360 g was noticed in T₄ and T₂ respectively which attained may be due to the presence of a greater proportion of EPA and DHA. The highest survival rate (83.33% at 24 h) was found in *M. rosenbergii* fed enriched *Artemia* and the lowest in newly hatched *Artemia* (63.33% at 0 h). The better growth, stress resistance, and survival were observed in freshwater prawns fed with a diet of enriched *Artemia* with n-3 HUFA (Devresse *et al.*, 1990). The growth and survival of *M. rosenbergii* juvenile were improved while supplementing lipid extract from shrimp head through diet which had rich n-3 HUFA (Sandifer and Joseph, 1976). When the *M. rosenbergii* larvae fed with dietary squid oil with high n-3 HUFA, particularly EPA and DHA, had the better post-larval metamorphosis, survival and growth (Hien *et al.*, 2005). Further, the high n3-HUFA levels in the feed enhance the tolerance to the stress in the freshwater prawn (Devresse *et al.*, 1990; Romdhane *et al.*, 1995). The higher survival of freshwater prawn larvae fed by n-3 HUFA-enriched Moina due to an improved ability of the PL to withstand stressful condition (Das *et al.*, 2007). Similarly, When *M. rosenbergii* PL fed HUFA-enriched *Artemia* resisted osmotic stress better as compared to control PL (Kontara *et al.*, 1995). Here, in the present study, a higher percentage of survival rate was found in *M. rosenbergii* PL fed with n-3 HUFA enriched *Artemia* compared to unenriched *Artemia* which might be owing to the ability of PL to withstand adverse environmental condition.

**CONCLUSION**

The *M. rosenbergii* PL fed with *Artemia* enriched with the emulsion of n-3 HUFA showed that there was a significant increase in growth performance and survival rate compared to unenriched and newly hatched *Artemia*. Further, this study suggests that 24 h n-3 HUFA enriched *Artemia* feeding to *M. rosenbergii* PL for a short duration, approximately 10 days in commercial hatcheries, before supplying to farmers in order to enhance the growth and survival of *M. rosenbergii* for commercial farming.

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**REFERENCES**


