Chapter 5
5. Larval rearing of *Penaeus monodon* (Fabricius) larvae with enriched rotifers

5. 1. Introduction

Variation in abundance and distribution of wild penaeid post larvae and the increasing world market for prawn especially tiger prawns has led to the rapid development of commercial larval culture systems for penaeids. Nutritional studies on lipids have demonstrated that crustaceans require essential fatty acids (EPA) for their normal growth (Deshimaru *et al.*, 1979; Kanazawa *et al.*, 1977; Sandifer and Joseph, 1976; Deresse *et al.*, 1990; Sorgeloos and Leger, 1992). Prawn fed diet containing 3% marine shrimp oil had increased accretion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). (Sandifer and Joseph, 1976). Penaeids have a dietary requirement for linoleic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid (Merican and Shim, 1996; D’A bramo 1997; Glencross and Smith 1999, 2001). Marine fish and crustaceans, like all other animals that have been studied to date, lack the ability for de novo synthesis of n-6 and n-3 fatty acids while some aquatic animals, such as salmonids; can synthesize their own highly unsaturated fatty acids. Penaeids either do not have this ability, or it is insufficient for their apparent needs. These families of fatty acids perform essential biological and physiological functions and must be supplied in the diet (Castell *et al.*, 1972; Fujii and Yone; 1976; Kanazawa *et al.*, 1979a).
5.2. Material and Methods

5.2.1. Larval rearing of *Penaeus monodon* with enriched rotifers

The experiment was conducted at Fisheries Research Laboratory of CMFRI at Thoppumpady, Cochin. *Penaeus monodon* (Fabricius) larvae (Plate XI c) were spawned at the Aquaplanza Hatchery at Cheral (Ernakulam Dist.) from wild caught, unablated spawners collected from Munambam harbour (Ernakulam Dist.). Larvae (Mysis-I) were transported in tightly sealed polythene bags, quarter filled with filtered seawater and inflated with oxygen.

Survival percentage and number of days for metamorphosis from Mysis-II to post larvae-I were determined from two trials, each with three replicates for five experimental diets enriched for rotifers. For each trial larvae from different mother prawns were used.

The experimental set up includes 4 litre capacity pearl pets (Plate XI a), which were thoroughly washed with sodium hypochlorite solutions filled with 3 litre of filtered seawater (33±2 ppt), gently aerated by 1-cm diameter air bubbles supplied by blower which is passed through a ciliate filter (Plate XI a). Temperature of the medium was maintained at (31 ± 1.5°C) and pH 8.2. 100 number of larvae (Mysis-II) was counted and introduced slowly into each containers, time in hours was noted and each treatments were named as follows: MNaR (MNaR1, MNaR2, MNaR3); Mysis fed with rotifers enriched by *Nannochloropsis salina*, MCIR (MCIR1, MCIR2, MCIR3); mysis fed with rotifers enriched by *Chlorella marina*, MLgR (MLgR1, MLgR2, MLgR3); mysis fed
Plate – XI

a) Experimental setup for rearing of *Penaeus monodon* larvae with enriched rotifers

b) *P. monodon* larvae feeding on enriched rotifers
with rotifers enriched by *Isochrysis galbana*, MOER (MOER₁, MOER₂, MOER₃); mysis fed with rotifers enriched by shark liver oil emulsion. MYR (MYR₁, MYR₂, MYR₃) and mysis fed with rotifers enriched by yeast.

Rotifer enrichment was carried out in separate containers for duration of 12 hrs, same procedure followed in chapter-4. Enriched rotifers were fed to shrimp larvae in each containers and maintained at 75 nos/ml by feeding in the morning and evening hours (Plate XI b). Seawater was renewed every day morning (50% water exchange) and debris and waste were siphoned out using small tubes through a filter cloth to prevent the escape of live rotifers. Larval growth and survival was assessed every day. Time (hours) taken for the metamorphosis of Mysis-II to postlarvae1 and survival (%) in each treatment was studied. Experiment was repeated with same facility with larvae from different mother prawn is used (Experiment II).

5. 2. 2. Statistical analysis

The transformed survival rates of shrimp larvae were analysed using a two-factor interaction model with feed at five levels and experiments at two levels. The means of five levels of feeds were compared using Duncan's multiple range test, (DMRT). Analysis of variance (ANOVA) was applied on the metamorphosis data (in hours), with feed and experiment being the factors of a two-factor interaction model. The homogenous means among the five levels of feeds were grouped using Duncan's multiple range test (DMRT).
5. 3. Results
5. 3. 1. Larval rearing of *Penaeus monodon* with enriched rotifers

Experiment I

The mean survival and number of days taken for metamorphosis by Mysis II to postlarvae1 of *P. monodon* during the first experiment with different enriched rotifers are given in the Fig. 5. 1 and 5. 2. The highest percentage of survival was attained when *N. salina* was enriched to rotifers (83%) followed by *Isochrysis galbana* enriched rotifers (79%) (Plate XI c). The percentage of larval survival when rotifers fed with shark liver oil emulsion were lower (75.6%) compared with *Nannochloropsis* and *Isochrysis* fed rotifers. *C. marina* and *S. cerevisiae* enriched rotifers exhibited the poor survival rates in the experiments with (63.6%) and (42.5%) respectively. It was observed that the major mortality was during the transformation stage of Mysis III to post larvae. It was also observed that even after the transformation the post larvae became very lethargic and died ultimately. This type of mortality was more prevalent for *chlorella* and yeast enriched rotifers.

Regarding the metamorphosis of larvae from Mysis II to post larvae, larvae fed with rotifers enriched with *Nannochloropsis*, shark liver oil emulsion and *Isochrysis galbana* had taken only less than three days to transfer from Mysis II to Mysis III and to post larvae 1 (60 hrs, 50.6 hrs, 73.3 hrs). Mysis III had taken only less than 1.5 days to transform further to post
larvae in all the above three treatments with variation only in few hours. Larvae fed with yeast enriched rotifers taken more than 4 days (97.3 hrs) for the first transformed post larvae. In this treatment about 30% mortality was observed due to the delay of transformation of Mysis II to Mysis III. Mysis II had taken more than two days to transform to Mysis III and also taken more than two days to transform to post larvae. In the case of larvae fed with Chlorella enriched rotifers, Mysis II had transformed with in 4 days (95.3 hrs) to PL-I without heavy mortality but the transformation from Mysis III to post larvae was delayed to more than two days. During this transformation heavy mortality of larvae has observed in the rearing containers.

![Fig. 5. 2. Metamorphosis of shrimp larvae fed with enriched rotifer](image)

(time in days)

![Bar chart showing metamorphosis of shrimp larvae fed with different enriched rotifers.](chart)
Experiment II

The mean survival and number of days for the transformation of Mysis II to post larva 1 of *P. monodon* with various enriched rotifers are given in the Fig. 5.3 and 5.4. Larvae fed with *N. salina* and *I. galbana* enriched rotifers produced a better survival rate with a mean of 82% and 79% respectively compared with larvae fed on shark liver oil emulsion enriched rotifers which gave a mean survival rate of 71%. In all the above-mentioned treatments maximum mortality up to 30% was observed during the transformation from Mysis III to post larvae I. The larval survival for *Chlorella* and yeast enriched rotifers were poor with 60% and 40% respectively. In both the treatments more than 20% mortality was observed even during the Mysis II to Mysis III transformation stage, but higher mortality observed during the transformation during Mysis III to post larvae I.
larvae fed with *N. salina*, *I. galbana* and oil emulsion enriched rotifers had taken less than three days (66 hrs, 73.3 hrs, 52 hrs) for all the three treatments respectively. Larvae fed with *C. marina* and *S. cerevisiae* had taken 4 days (94 hrs,) and 5 days (96.6 hrs) respectively. Mysis II had taken less that 1.5 days for *N. salina; I. galbana* and oil emulsion enriched rotifers to transform to Mysis III and further 1.5 days to post larvae in all the three treatments. In the treatments like yeast and *C. marina* fed rotifers the larval Mysis II had taken 4 days and 5 days respectively to transform into post larvae with 2 days each for first transformation i.e., into Mysis III and further the yeast fed rotifer larvae had taken 3 more days to post larvae I while *C. marina* fed rotifers had taken 2 more days to post larvae I. In both the treatments high mortality was observed during the Mysis III to post larvae I transformation period.
Fig. 5.3. Survival of shrimp larvae fed with enriched rotifer

Fig. 5.4. Metamorphosis of shrimp larvae fed with enriched rotifer
5.3.2. Statistical analysis

The two factor (with interaction) ANOVA of the transformed survival rate of shrimp larvae (post larvae) given in the Table 5.1. It can be observed that the different feed has a significant contribution \((P< 0.05)\) to the total variation in the data whereas the experiment levels and their interaction with the different levels of feed were not significant. The post Hoc Duncan’s multiple range test (DMRT) revealed the grouping of the five levels of feeds into four subsets. (Mean of the transformed values is given in the Table 5.2.

### Table 5.1. ANOVA for transformed survival percentage of post larvae fed with enriched rotifers

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Experiment</td>
<td>0.006</td>
<td>1</td>
<td>0.006</td>
<td>2.905</td>
<td>0.104</td>
</tr>
<tr>
<td>Feed</td>
<td>0.769</td>
<td>4</td>
<td>0.192</td>
<td>101.608</td>
<td>0.000</td>
</tr>
<tr>
<td>Exp*Feed</td>
<td>0.002</td>
<td>4</td>
<td>0.001</td>
<td>0.315</td>
<td>0.865</td>
</tr>
<tr>
<td>Error</td>
<td>0.038</td>
<td>20</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 5.2. DMRT of transformed value for post larval survival

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Name of the feed fed to rotifers</th>
<th>*Mean of transformed value of post larval survival.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>N. salina</em></td>
<td>1.143(^a)</td>
</tr>
<tr>
<td>2</td>
<td><em>I. galbana</em></td>
<td>1.095(^a)</td>
</tr>
<tr>
<td>3</td>
<td><em>C. marina</em></td>
<td>0.905(^d)</td>
</tr>
<tr>
<td>4</td>
<td><em>S. cerevisiae</em></td>
<td>0.696(^c)</td>
</tr>
<tr>
<td>5</td>
<td>Shark oil emulsion</td>
<td>1.03(^b)</td>
</tr>
</tbody>
</table>

* Means with similar superscripts do not differ significantly.
<table>
<thead>
<tr>
<th></th>
<th>Shark oil emulsion</th>
<th>51.33&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
</table>

* The means with similar superscripts do not differ significantly.
5.4. Discussion

Rotifers are fed to a number of larval stages of penaeid prawn and it is an established fact that rotifers are given to protozoea II onwards were often seen to clutch and eat the rotifers. Although Brachionus was taken during PZ- II it was considered that the larva were unlikely to consume much until M1 when the pereopod endopods were formed (Emmerson, 1984). In the present study Brachionus rotundoformis is fed to M II to PL I without supplementing any other live or artificial feeds.

Studies on the nutritional requirements of marine fish and crustaceans have shown that fatty acids of the n-3 family have higher EFA values than the fatty acids of the n-6 family and have also demonstrated that marine fish and crustacean lack the ability for de novo synthesis of n-6 and n-3 fatty acids (Sargent et al., 1990). Growth (moulting) and the survival by shrimps larvae fed with different enriched diets proved that particular dietary essential fatty acids have considerable growth promoting effects and the interaction dietary n-3 and the n-6 fatty acids also has considerable impact on the growth of the prawns. Kanazawa and Teshima (1977) demonstrated that penaeid shrimps are not capable of synthesizing PUFA’s such as linolenic and (18:2n-6) linolenic acid (18:3n-3), Eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). They inferred that these fatty acids are essential for shrimp and should be supplied through their diet. In the present experiment rotifers enriched with shark oil emulsion and Nannochloropsis salina with a very good profile of EFA’s produced better survival and growth against other rotifers enrichment feeds like Chlorella marina, Isochrysis
galbana and yeast which having low EFA profile. The moulting of M II had shortened and survival percentage increased when HUFA enriched feeds are given to larvae. This is in conjunction with the findings made by Immanuel et al., 2003. Apart from accelerating survival of candidate species, dietary lipids may also influence the somatic growth. Also the growth in turn determined by moulting which is an indispensable and an important phenomenon in crustaceans. The involvement of lipid during moulting has been well established by Forster (1976) and Read (1977). Briggs et al., (1988) have reported that 9.5% of lipid in the diet registered 56% of survival and 12.5% of lipid in the diet showed only 45% of the survival rate in P. monodon. In the present study survival percentage had increased to 72-83% when feed containing all the n-3 HUFA’s were fed to shrimp larvae i.e., N salina, shark liver oil and I galbana enriched rotifers.

Moultng is largely influenced by fatty acids of the n-3 family (Guary et al., 1976). Glencross et al., (2002) stated that prawns have some capacity for synthesis n-3 HUFA, inclusion of the n-3 HUFA in the diet doubt alleviates some of the resources required for the synthesis of HUFA and subsequently allows for improved growth potential. In the present study the moulting time between successive larval stages reduced when enriched rotifers especially shark liver oil and N salina was fed to larvae. This breakthrough is a very important in the feeding protocols of shrimp hatcheries by reducing the high priced Artemia nauplii and some of the inert feeds which deteriorates the water quality. It has been documented that P. monodon cannot synthesis
either 18:2n-6 or 18:3n-3 (Kanazawa et al., 1979 a, b). However, limited conversion of these shorter chains polyunsaturated (PUFA) to the longer HUFA (20:n-6, 20:5n-3 or 22:6n-3) was observed (Kanazawa et al. 1979a, b). Elongation and desaturation of 18:3n-3 to HUFA n-3 (20:5n-3 and/or 22:6n-3) by *M. japonicus* was observed to be about 20% of that achieved by rainbow trout (Watanabe, 1982). Kanazawa et al., (1985) observed that survival of *M. japonicus* increased with increasing n-3 HUFA levels from 0% to 1% but decreased at 2% suggesting a possible over done or contamination by oxidation product of HUFA. Xu et al., (1994) suggested that the relatively high levels of HUFA such as AA, EPA and DHA in the body lipids of *F. chinensis* fed essential fatty acid free diets were probably. The result of preferential utilization of short and medium chained fatty acid as energy source for metabolism rather than on increase in absolute content of the HUFA.

The present study demonstrated this, as previously seen for other penaeid shrimp. The n-3 is essential for normal growth and survival of larval *Penaeus monodon*. It is clear from the experiments that feeds lacking HUFA's (yeast, *Chlorella marina*) had poor survival and growth. Rotifers enriched with shark liver oil emulsion, *Isochrysis galbana* and *Nannochloropsis salina*, had good survival and growth.