Chapter 6

EVALUATION OF VARIOUS ALGAE IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII* USING MODIFIED STATIC GREEN WATER SYSTEM
6.1 Introduction

Phytoplankton comprises the base of food chain in the aquatic ecosystem and therefore micro algae are indispensable in the commercial rearing of various species of aquatic organisms. The micro algae, besides being used as food source of different growth stages of aquatic organisms, are used to make green water by directly adding into the larval rearing tanks. They are believed to play a role in stabilizing the water quality parameters, nutrition of larvae and microbial control (Lavens and Sorgeloos, 1996).

Controlled low water exchange green water cultures have been used worldwide to rear larvae of fishes and crustaceans. There have been numerous studies from across the world referring to the significant advantages of adding phytoplankton to the larval rearing systems (Howell, 1973; Chiang and Liao, 1985; Naas et al., 1992; Austin et al., 1992; Liao et al., 2001; Regunathan and Wesley, 2004; Faulk and Holt, 2005). Increased survival has been reported in India (Kurup, 2003) as well as Vietnam (Hai, 2003) by using the modified static green water system in the larviculture of M. rosenbergii. However, in all the studies so far conducted using this technology of larval rearing, *Chlorella* sp. is used as the main component of green water (Hein et al., 2000; Oanh et al., 2000; Kurup, 2003). Whereas there are many reports of using other algae as the media in larval rearing of many fishes as well as in other crustaceans. *Tetraselmis* sp has been used widely for rearing marine fish larvae resulting in increased survival and growth. Many studies have been conducted using the micro alga *Nannochloropsis oculata* (Palmer et al., 2007) which is reported to be yielding high hatchery production of many
marine fishes. Live feed *Isochrysis galbana* is extensively used as an organism with a high DHA (12%) content (Liao *et al.*, 2001). When used as the rearing medium, *I. galbana* provided similar growth and survival to live prey, as enriched with commercial fatty acid boosting products (Faulk and Holt, 2005). In Taiwan, presently over 90 species of fin fishes are produced using GWC with *Nannochloropsis* and *Isochrysis* sp. as the micro algae and the results were encouraging in improving hygiene of system and larval survival (Liao *et al.*, 2001).

In this context a study was conducted with three algae other than *Chlorella* as the rearing media in the modified static green water system viz., *Tetraselmis chuii*, *Nannochloropsis occulata* and *Isochrysis galbana* and a mixed culture of these.

Thus the objective of the present study was

- To investigate the efficiency of algal species other than *Chlorella* as a rearing medium in the modified static green water system.

### 6.2 Materials and methods

#### 6.2.1 Brood stock management, algal culture and larval rearing

Berried prawns with black coloured eggs were collected from grow out and transported in black coloured plastic cans to the hatchery at the SIF, disinfected (New, 2002) and transferred to 150 l FRP tanks, one berry each in a tank and maintained in 5 ppt saline water with continuous aeration. The tanks were covered with nets to prevent escapement of prawns and were kept undisturbed at night. The eggs hatched by next day morning.
The algae were cultured using modified Walne's medium (Lavens and Sorgeloos, 1996), the composition of the same is given in Chapter 2 (Table 2.2). Stock cultures (inoculum procured from CMFRI, Cochin and Marine Botany laboratory, CUSAT, Cochin) were maintained at salinity of 20 – 25 ppt. One day prior to introduction of larvae, the inoculum from mass culture along with required quantity of Walne's medium (modified) was added to each rearing tank in order to accomplish an initial density of $3 - 5 \times 10^5$ cells/ml. The algal concentration reached values ranging between $6 - 10 \times 10^5$ cells/ml by the end of the rearing period. Algal culture in exponential growth phase was used as the inoculum. In addition to routine filtration and water treatment, water was sterilized by heating using the immersion heater before inoculation with algae. Moderate to strong aeration was provided in all the tanks to facilitate mixing and keeping waste products in suspension for aerobic bacterial digestion. The larval rearing and feeding (Section 5.2.3 and 5.2.4) were done as explained in Chapter 5 (refer chapter 5 for details). The larvae were initially fed with BSN followed by egg custard from the 5th larval stage.

6.2.2 Evaluation of results

The results of different treatments were ascertained based on the time required for larvae to reach each stage and their relative survival. Thirty larvae from each tank were randomly sampled every fifth day and the larval stages were identified following the descriptions of Uno and Kwon (1969). Mean larval stage (MLS) was used for determining the development of larvae and calculated using the formula given by Lovett and Felder (1988). 

$$\text{MLS} = \sum (S \times P_s)$$

where $S$ is the larval stage number and $P_s$ is the proportion of larvae at
stage S. Survival of the larvae were estimated by taking ten 1 liter samples from the rearing medium under strong aeration. The estimation of percentage composition of the different larval stages and survival continued till 20th and 25th day respectively. Since it was not possible to draw uniform samples, due to the benthic habit of post larvae neither staging nor estimation of survival was continued after appearance of considerable number of post larvae (Alam et al., 1993a). However, all the tanks were observed for appearance of post larvae after 15 days from the start of the experiment and the day on which more than 95 % of larvae metamorphosed was noted and the experiment terminated.

Temperature, pH and salinity were measured daily using mercury thermometer, pH meter (Eutech Cyberscan model 510), and ATAGO refractometer. Dissolved oxygen was estimated twice a week by Winkler's method (APHA, 1995). The water samples were filtered through GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrite-N and total ammonia nitrogen (TAN) (Phenol hypochlorite method) (Grasshoff et al., 1983). Total heterotrophic bacteria (THB) were estimated following standard methods and the results are expressed as colony forming units (cfu) / ml (APHA, 1995).

On the completion of the experiment, 50 post larvae from each treatment were randomly selected for measuring the total length from tip of rostrum to end of the telson and the wet weight of the body. Data were analysed by Analysis of variance using SPSS 16 statistical software. Duncan's multiple range test (DMRT) was used to analyse the significant difference
among means at 5 % probability level. Percentage data were normalised by arcsine transformations. Nonetheless non transformed data is presented in the Table 6.3 and Fig 6.5.

6.3 Results

Physico chemical parameters like temperature, pH, salinity and dissolved oxygen were found to be optimum for larval rearing (New, 2002) of *M. rosenbergii*. The average values of the water quality parameters recorded are given in Table 6.2.

However, the nutrients like TAN and nitrite nitrogen showed a gradual increase towards the completion of the rearing period. The larvae were not found to be affected by the increase in the nitrogenous toxicants, as there was no specific increase in the mortality or retardation of growth (Fig 6.3 and 6.4) at any of the sampling days. Fig. 6.1 and 6.2 depicts the TAN and nitrite nitrogen variation in the rearing tanks during the larviculture. Although significant variation (P<0.05) existed between the tanks on different sampling days with respect to these parameters (Fig 6.1 and 6.2), no specific pattern of increase or decrease in any of the treatments was observed. However, a gradual build up of these toxicants could be observed in all the tanks towards the termination of the experiment. The highest TAN and nitrite nitrogen values recorded during the larval rearing period were 1.04 ± 0.21 mg/l and 0.76 ± 0.12 mg/l respectively.

The number of colony forming units of total heterotrophic bacteria was also found to increase with time and it ranged between 1.3 ± 2.65 x 10^5 to 79.67 ± 2.08 x 10^5 cfu/ml. However no significant difference was observed
with respect to THB among any of the treatments throughout the larval rearing period.

The performances of the algae in all the treatments were comparable except the significantly lower mean survival (45 ± 2.71%) recorded in the T1 with T. chuii as the rearing medium. No significant difference was observed among the treatments with the control (Chlorella vulgaris) with respect to the larval progression (Fig. 6. 4), appearance of the first PL, duration of the experiment, total length and wet weight of the post larvae. However, the highest mean survival of 49.03 ± 2 %, (very narrow difference was recorded between the treatments except T1) was recorded in the I. galbana medium. The appearance of the first PL was earliest (average of 19.7 ± 0.6 days) in the same medium. Same is the case with duration of the experiment which was lowest in T2 with an average value of 29.7 ± 0.6 days. However, the highest mean total length and wet weight (9.84 ± 0.36 and 10.1 ± 0.34) were observed in T4 with a mixed culture of all the four algal species. The production details of the post larvae are given in Table 6.3. The Figs. 6.4 and 6.5 show the MLS and survival respectively recorded at every 5 days interval during the experimental period.

6.4 Discussion

There are many reports of utilizing the algal species used in the present experiment for larval rearing of fish and crustaceans around the world, both as food and as well as rearing medium and for enriching the live feed (Liao et al., 1983; Naas et al., 1992; Hernandez-Cruz et al., 1994; Gulbrandsen et al., 1996; Liao et al., 2001; Papandroulakis et al., 2001, 2002; Faulk and Holt,
By using algae in larval rearing of *M. rosenbergii*, Maddox and Manzi (1976) obtained 30% greater survival than the control without algae.

Among the various algae used in the present study, two are motile (*I. galabana* and *T. chuii*) and are widely used for developing green water cultures. *Tetraselmis suecica* was found to provide antibacterial benefits in the shrimp larviculture (Regunathan and Wesley, 2004) by inhibiting pathogens. The alga was also found to inhibit fish pathogens (Austin *et al.*, 1992). The beneficial effects like contrast enhancement and chemical stimulation provided by the microalga *T. suecica* resulted in higher rotifer consumption rates rather than clean water in the larval rearing of green back flounder, *Rhombosolea tapirina* (Shaw *et al.*, 2006). However, a significantly lower survival of 45% (45 PL/l) was obtained when *T. chuii* was used as the medium in the present study. This was due to the increased mortality that occurred in one of the rearing tanks with *T. chuii* as the medium before the initial sampling. No reason could be attributed for the same where as the other two tanks among the triplicate showed no such larval mortalities. However though the survival recorded was significantly low in the treatment with the alga *T. chuii*, the other growth indicators like length and weight of post larvae, appearance of PL and duration of the rearing period did not differ significantly with the other treatments.

*Isochrysis galbana* which was found to be ingested by the larvae of sea bass *Dicentrarchus labrax* while drinking, is thought to beneficially trigger the production of digestive enzymes in the pancreas and intestine (Cahu *et al.*, 1996).
1998). The studies conducted on the larval rearing of Cobia, Rachycentron canadum showed that larval survival was significantly improved in the presence of Nannochloropsis occulata and Isochrysis galbana (Faulk and Hault, 2005). The beneficial effects of using I. galbana in the larval rearing of M. rosenbergii have also been reported by Thresiamma et al. (2006) and Devika et al. (2006). The highest post larval production, best larval progression and the earliest settlement of post larvae in the present study was recorded in the treatment with I. galbana as the rearing medium.

Nannochloropsis occulata has been used to create green water culture system for larval rearing of marine fishes like Asian seabass (Lates calcarifer), Australian bass (Macquaria nomenculata), snapper (Pagrus auratus), dusky flathead (Platyccephalus fuxus), sand whiting (Sillago ciliata) and zoo plankton like Brachionus plicatilis (Palmer et al., 2007). The author has reported a considerable increase in the production of fry and fingerlings of the important cultivable finfish species by using the green water culture when compared to the clear water. Survival rates ranging from 80% to as high as 100% have been reported and in most cases it was above 95% and the entire larvae were found to be very healthy by the same authors. Better survival for larval Sparus auratus (Hernandez-cruz et al., 1994) and Mugil cephalus (Tamura et al., 1994) have also been reported by the use of green water. The first -feed larvae of Cod (Gadus morhua) were also found to ingest cells of the alga Nannochloropsis atomus at lower rates consistent with larval drinking of water. A recent study using the Australian strain of M. rosenbergii has demonstrated improved survival and growth of the prawn larvae by using the micro alga.
Nannochloropsis sp. at higher concentration of $12.5 \times 10^5$ cells/ml (Lober and Zeng, 2009). In the present study also, the post larval production recorded by using *N. occulata* as the rearing medium was comparable with all the other treatments of the present study and well above the average survival of 30% (Murthy, 2006) reported from commercial hatcheries in India. Thus, in the present study also, in comparison to the conventionally used *Chlorella* sp. for larval rearing in modified static green water system, the use of other algal species have also resulted in equally competent results.

The use of different algae was not found to influence the fluctuation (probably due to the instability in phytoplankton population) or the built up of the nitrogenous toxicants like TAN or nitrite nitrogen. The elevated levels of TAN or nitrite nitrogen in various treatments especially towards the end of the rearing period, however, was not found to have any specific negative impact on the larval growth and survival. TAN levels up to 2 mg/l has been recorded by Kurup (2003) in the modified static green water system for rearing *M. rosenbergii* larva, without any harmful effects on the growth and survival of the larvae. A similar observation was also made by Phuong *et al.* (2000) who recorded TAN values ranging between 0.923 and 1.778 mg/l, but did not observe any deleterious effects on the larvae.

Thresiamma *et al.* (2006) reported a survival as high as 59.7% while using *I. galbana* in the rearing of *M. rosenbergii* in comparison to the control (survival 46.7%) without alga. The *Artemia* nauplii concentration used by the authors, was however higher in comparison to that used in the present study. Moreover, the survival of 49.07% recorded for the larvae reared in *I. galbana*
In the present study was comparable to 47.3 % reported by Thresiamma *et al.* (2006) by feeding about 4 nos /ml of BSN, which was the concentration of *Artemia* nauplii used for feeding the prawn larvae in the present experiment. The mean survival achieved by feeding *N. occulata* (48.47 %) in the present experiment is slightly lower when compared to 63.3% reported by Lober and Zeng (2009) using the alga *Nannochloropsis* sp. at concentration of $12.5 \times 10^5$ cells /ml in the larval rearing of Australian strain of *M. rosenbergii*; but higher than 35% reported by the same authors for an algal density of $6.25 \times 10^5$ cells/ml. However, both Thresiamma *et al.* (2006) and Lober and Zeng (2009) used lower initial stocking densities (50 and 30 larvae /l respectively) where in the larvae were stocked @ 100 / l in the present study.

High survival and growth were also observed in the mixed culture of algae and the length and weight of the larvae were also found to be the highest in the treatment T4 in which a combination of all the algae was used as the rearing medium. The use of a combination of two or more of complementary algal species like *N. occulata* and *l. galbana* has been recommended for enhancing the nutritional benefits of the algae to the larval fishes (Liao *et al.*, 2001). A combination of algae at different densities are also used in the feeding of penaeid larvae (Lavens and Sorgeloos, 1996; FAO, 2007). The use of extensive mesocosms utilizing wild plankton (consisting of different micro algae) has been found to be beneficial for rearing marine fish larvae (Naas *et al.*, 1992). Likewise, the use of a mixture of algae as the rearing medium resulted in the production of larger postlarvae with an equally
comparable postlarval production when compared to control with Chlorella vulgaris as the rearing medium.

6.5 Conclusion

No difference was observed in the overall performance of the prawn larvae in any of the algal medium or when their combinations were used except T. chuii in which a lower post larval survival was recorded in one of the triplicate tanks. Hence, use of any of the algae viz. Isochrysis galbana, Nannochloropsis oculata and Tetraselmis chuii or their combination can be recommended along with Chlorella vulgaris as the rearing medium in the modified static green water system of larval rearing of M. rosenbergii.
**Table 6.1** Algal species used in different treatments in the larviculture of *M. rosenbergii* adopting MSGWS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Algal species</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td><em>Tetraselmis chuia</em></td>
</tr>
<tr>
<td>T2</td>
<td><em>Isochrysis galabana</em></td>
</tr>
<tr>
<td>T3</td>
<td><em>Nannochloropsis occulata</em></td>
</tr>
<tr>
<td>T4</td>
<td>Mixed culture</td>
</tr>
<tr>
<td>T5 (Control)</td>
<td><em>Chlorella vulgaris</em></td>
</tr>
</tbody>
</table>

**Table 6.2** Average ± s.d of various water quality parameters recorded during the experimental period in the larviculture of *M. rosenbergii* using MSGWS with different algae as the rearing medium

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td>27.37 ± 0.55</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>8.13 ± 0.14</td>
</tr>
<tr>
<td><strong>Salinity (ppt)</strong></td>
<td>13.09 ± 0.54</td>
</tr>
<tr>
<td><strong>Dissolved oxygen (mg/l)</strong></td>
<td>6.99 ± 0.18</td>
</tr>
</tbody>
</table>
Table 6.3 Effect of different algae as the rearing medium on survival, appearance of first post larvae, duration of rearing period, length and wet weight of the post larvae of *M. rosenbergii* in the larviculture using MSGWS

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Stocking density(larvae/litre)</th>
<th>% survival</th>
<th>Appearance of first post larvae(days)</th>
<th>Duration(Number of days for more than 95% post larval settlement)</th>
<th>Total length(mm)</th>
<th>Wet weight(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>100</td>
<td>45 ± 2.71b</td>
<td>20.7 ± 6a</td>
<td>30.3 ± 6a</td>
<td>9.74 ± 0.32a</td>
<td>9.96 ± 0.36a</td>
</tr>
<tr>
<td>T2</td>
<td>100</td>
<td>49.07 ± 2a</td>
<td>19.7 ± 6a</td>
<td>29.7 ± 6a</td>
<td>9.81 ± 0.28c</td>
<td>10.08 ± 0.27a</td>
</tr>
<tr>
<td>T3</td>
<td>100</td>
<td>48.47 ± 1.7a</td>
<td>20.3 ± 6a</td>
<td>30a</td>
<td>9.77 ± 0.42a</td>
<td>10.03 ± 0.46a</td>
</tr>
<tr>
<td>T4</td>
<td>100</td>
<td>48.6 ± 1.2a</td>
<td>20a</td>
<td>30a</td>
<td>9.84 ± 0.36a</td>
<td>10.1 ± 0.34a</td>
</tr>
<tr>
<td>T5 (Control)</td>
<td>100</td>
<td>48.93 ± 1.8a</td>
<td>20.3 ± 6a</td>
<td>30a</td>
<td>9.73 ± 0.41a</td>
<td>9.96 ± 0.37a</td>
</tr>
</tbody>
</table>

Mean ± s.d of three triplicate groups; Means sharing same same superscript letter in each column doesnot differ significantly.
Fig 6.1 Effect of using different algae as the rearing medium on TAN in different treatments in the larviculture of *M. rosenbergii* using MSGWS

![Graph showing TAN levels over days for different algae](image)

Means in each day with identical letters or represented by a single letter (a) are not significantly different.

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Fig 6.2 Effect of using different algae as the rearing medium on nitrite nitrogen in different treatments in the larviculture of *M. rosenbergii* using MSGWS

![Graph showing nitrite nitrogen levels over days for different algae](image)

Means in each day with identical letters or represented by a single letter (a) are not significantly different.
**Fig 6.3** Effect of using different algae as the rearing medium on THB in different treatments in the larviculture of *M. rosenbergii* using MSGWS

![Graph showing THB (x10^5 chl/ml) over days](image)

Means in each day with identical letters are not significantly different.

**Fig 6.4** Mean Larval Stages in treatments with different algae as the rearing medium in the larviculture of *M. rosenbergii* using MSGWS

![Graph showing MLS variation over days](image)

MLS did not differ significantly between treatments on any of the days.
Fig 6.5 Effect of using different algae as the rearing medium on survival of larvae and post larvae in different treatments in the larviculture of M. rosenbergii using MSGWS

Vertical lines represent s.d. Means in each day with identical letters are not significantly different.