CHAPTER 7.
LIVE FEED EXPERIMENT S
7. Live feed experiments

7.1 Introduction

*Penaeus monodon* is the most valuable and profitable commercial species of the shrimp industries in India and other south Asian countries. Decreasing supply of fishery by-products and concerns over its quality, the aquaculture researchers now actively investigating in alternative nutrient source (Naylor *et al.*, 2000). Studies have shown that diets containing fish-based ingredients have generally performed better in terms of growth and feed efficiency than diets containing alternative plant based sources (Moyan *et al.*, 1992; Webster *et al.*, 1992; Kikuchi, 1999; Thompson and Harrison, 1992). Phytoplankton is the main food of larval stages of some crustaceans (Preston *et al.*, 1992) and of the early growth stages of some fishes (Ritan *et al.*, 1994). However, little work has been done on the nutritional requirements of shrimp larvae. There are large differences between the survival and growth of prawn larvae fed different species of algae (Naranjo *et al.*, 1995). Some mixed-algae diets have resulted in higher survival and faster development of larvae than the component species alone (Kurmalay *et al.*, 1989; Liang *et al.*, 2004). Some phototrophic algae are used as live feed for *Penaeus monodon* larvae (Azad, 2002). Hence, it is clear that successful prawn culture still depends to a large extent on live feeds (Liao *et al.*, 1990, 1991).

The following species of algae *Nannochloropsis aculata*, *Nannochloropsis oculata*, *Chlorella* sp., *Chlamydomonas* sp., *Tetraselmis tetrathels* and *T. chuii* are used as live feed for cultured shrimp larvae (Duerr *et al.*, 1998). Fresh algal culture is a major bottleneck in the aquaculture industry (Fuentes *et al.*, 2000; 2001). Algal concentrates made by centrifugation fed to bivalves and prawn larvae resulted a promising results (D’Souza, 1998; D’ Souza *et al.*, 2000; Heasman *et al.*, 2000; Robert *et al.*, 2001). Algae such as *Chactoceros calcitrans* and *Tetraselmis chuii* were fed to *P. monodon* larvae producing the same rate of survival (Millamena *et al.*, 1990). A number of studies have documented the nutritional excellence of dried algal
products in shrimp and fish diet (Wood et al., 1991). Based on the literatures, the
objective of the present study is to carry out the efficacy of micro algal such as,
*Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp. and cyanobacteria such as
*Synechococcus* sp. *Phormidium* sp. on shrimp growth and their survival.

7.2 Materials and methods

7.2.1 Type and source of feed

Three microalgal strains and two cyanobacterial strains were selected for
shrimp growth study. Microalgae (Plate 7.1) such as *Chlorella* sp. (LF-1), *Tetraselmis*
sp. (LF-2), *Isochrysis* sp. (LF-3) were collected from Algal Division, CMFRI,
Mandabam, Ramnad Stock cultures of these microalgae were maintained in 100 mL
of *f₂* medium with vitamin and sodium metasilicate (Guillard 1960; Guillard and
Ryther 1962). Filtered seawater (35 ppt salinity) was used for the preparation of the
media (about 25 μm pore size sand filter). Cyanobacterial cultures (*Synechococcus*
sp. (LF-4) and *Phormidium* sp. (LF-5)) were collected from Cuddalore coastal area.
Microalgal cultures were incubated at 25°C and illuminated from one side with
artificial white light (2000 Lux) under a 12:12 h light-dark cycle. The stock cultures
were subcultured once in 15 days to maintain actively growing cells. Working
cultures were prepared by inoculating one L Erlenmeyer flasks with 25 mL of stock
culture in 700mL of *f₂* medium. Cyanobacterial cultures were maintained and mass
cultivated in one L Erlenmeyer flask containing 750 mL of ASN III medium.

**ASN III medium composition** (chapter 4)

**f₂ medium composition**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>8.83 x 10⁻⁴</td>
</tr>
<tr>
<td>Na₂HPO₄·H₂O</td>
<td>3.63 x10⁻⁵</td>
</tr>
<tr>
<td>Na₂SiO₃·9H₂O</td>
<td>1.07 x 10⁻⁴</td>
</tr>
<tr>
<td>Trace metal solution</td>
<td>1 mL</td>
</tr>
<tr>
<td>Vitamin solution</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

Make the volume up to one litre with filtered seawater.
Plate 7.1
Live feed organisms

-Chlorella sp.-

-Tetraselmis sp.-

-Isochrysis sp.-

-Synechococcus sp.-

-Phormidium sp.-
**f/2 Trace metal mix solution**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₃. 6H₂O</td>
<td>1 x 10⁻⁵</td>
</tr>
<tr>
<td>Na₂EDTA</td>
<td>1 x 10⁻⁵</td>
</tr>
<tr>
<td>CuSO₄.5H₂O</td>
<td>4 x 10⁻⁸</td>
</tr>
<tr>
<td>Na₂MoO₄.2H₂O</td>
<td>3 x 10⁻⁸</td>
</tr>
<tr>
<td>ZnSO₄.7H₂O</td>
<td>8 x 10⁻⁸</td>
</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>5 x 10⁻⁸</td>
</tr>
<tr>
<td>MnCl₂.4H₂O</td>
<td>9 x 10⁻⁷</td>
</tr>
</tbody>
</table>

Make the final volume up to one litre with distilled water and autoclaved.

**f/2 Vitamin solution**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₁₂</td>
<td>1 x 10⁻¹⁰</td>
</tr>
<tr>
<td>Biotin</td>
<td>2 x 10⁻⁹</td>
</tr>
<tr>
<td>Thiamin HCl</td>
<td>3 x 10⁻⁷</td>
</tr>
</tbody>
</table>

Make the volume up to one litre with distilled water.

### 7.2.2 Experimental setup

The experiments on feeding trials were conducted in plastic troughs of 30-liter capacity. Plastic troughs of uniform size and uniform colour for each experiment were selected and filled with saline water (25 ppt). Each trough was stored with ten juveniles of shrimp and provided aeration continuously. The acclimatized shrimps were starving for 48 hrs period before starting feeding trial. They were weighed accurately after wiping with blotting paper and stocked in each experimental trough. The whole experiment was conducted in the temperature-controlled laboratory. The growth of the shrimp was analyzed by the size, total body weight, mortality ratio and its proximate composition in addition to water quality tests.
7.2.3 Feeding trial

For each type of diet treatment, three replicates were performed. In each replicate tank, shrimps were stocked at a density of 10 juvenile. The culture volume per tank was 20 L. Feed quantity was adjusted according to the body weight of the shrimp and stocking density. Concentrated centrifuged live feed biomass was used as a diet. Equal weight of the live feed biomass was fed to all experimental shrimps. The experiment was conducted 30 days and the shrimp growth performances were accounted carefully.

7.2.4 Sampling method

Shrimp *P. monodon* numbers were counted at every day of the experiment. Percentage of survival (%) was calculated based on the number of surviving shrimp at a particular day of the experiment. Total body length (mm) and the body weight (g) of the shrimp were analyzed periodically in the interval of 10 days up to 30 days of the experiment.

7.2.5 Water quality study

The physico-chemical parameters of seawater of various culture tanks were recorded twice in a week during the whole experimental durations. Water temperature was measured by mercury thermometer having 0.1 °C accuracy, salinity was measured by refractometer, DO was estimated by titrimetric method, pH was measured by digital pH meter and other parameters were estimated by standard estimation methods (APHA, 1998; Golterman *et al.*, 1978).

7.3 Results

The trial on micro algal and cyanobacterial live feed has a significant effect on size (length in cm) of *P. monodon*. The highest increase in mean length was generally observed after 20 days of shrimp growth, and highest mean length of 4.8 cm achieved on the 30th day of feeding by *Chlorella* sp. (Fig-1). The results show that addition of live algal biomass at optimum proportions has supported the growth of shrimp *P. monodon*. The lowest mean length of 4.1 cm was observed with *Tetraselmis* sp. In the
Fig 7.1 Mean total length of *P. monodon* fed with different live feeds. All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P< 0.01).
study period shrimp fed with *Chlorella* sp. was significantly greater (p<0.01) than that of shrimp fed with other diets. Similarly, weight of the shrimp was also higher at diet *Chlorella* sp. (0.592 g fresh body weight) and lower at diet *Tetraselmis* sp. (0.44 g fresh weight) achieved on 30\textsuperscript{th} day of the feeding experiments. The mean weight of shrimp fed with diet *Chlorella* sp. was significantly greater (P<0.01) than that of shrimp fed with the other diets (Fig 2).

Shrimp fed with diet *Chlorella* sp. which is traditionally used as microalgal feed improves the survival of *P. monodon*. *Tetraselmis* sp. did not support the growth of shrimp better. *Phormidium* sp. when used as the sole cyanobacterial diet gave the highest shrimp survival (Fig. 3). At the end of experimental period (30 days), maximum survival of 83.33% was observed in feed *Phormidium* sp. followed by shrimp fed with diet *Chlorella* sp. survival rate was about 80% and rest of the cultures *Tetraselmis* sp., *Isochrysis* sp. and *Synechococcus* sp. showed poor survival rate.

The range of water quality parameters during the growth period of shrimp (Plate 7.2) is given in Table 7.1. The fluctuation of temperature (°C), pH and salinity was very low in all experimental tanks. Variations in dissolved oxygen (DO), nitrate, ammonia and sulphates were also recorded. Highest dissolved oxygen was observed in *Isochrysis* sp. tank followed by *Phormidium* sp. Low level of DO was recorded in *Tetraselmis* sp. Low level of nitrate in *Chlorella* sp. and higher in *Synechococcus* sp. Higher ammonia in *Isochrysis* sp., lower concentration of sulphate in *Chlorella* sp. and maximum concentration of sulphate in *Tetraselmis* sp. were observed.

The proximate composition of experimental shrimp after the experimental period was given in the Table 7.2. The edible flesh weight, total body protein, total lipid and moisture content were also reported in all groups of shrimps. There was a significant deviation on percentage of edible flesh of shrimp fed with all type of diets, but no variation was recorded in total body protein. Protein and lipid in the shrimp fed with diet *Chlorella* sp. showed significantly higher concentration. Ash content of the shrimp was the
Fig 7.2 Mean weight gain of *P. monodon* fed with different live feeds. All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P< 0.01).
Fig 7.3 Mean survival rate of *P. monodon* fed with different live feeds. All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P<0.01).
Table 7.1 Ranges of water quality parameters in *P. monodon* growth tanks during live feed experimental studies

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quality of water in experimental tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Chlorella</em> sp.</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25.4 – 26.3</td>
</tr>
<tr>
<td>Dissolved oxygen (mgL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.4 – 7.1</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>24.3 – 25.6</td>
</tr>
<tr>
<td>Nitrate (µgL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.7 – 4.7</td>
</tr>
<tr>
<td>Ammonia (µgL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2.1 – 4.0</td>
</tr>
<tr>
<td>Sulphate (µgL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>4.8 – 6.9</td>
</tr>
</tbody>
</table>
Table 7.2 Proximate composition of shrimp fed with different micro algal diet

<table>
<thead>
<tr>
<th>Contents</th>
<th>Proximate contents of shrimp fed with different diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Chlorella</em> sp.</td>
</tr>
<tr>
<td><strong>Moist weight basis (%) g</strong></td>
<td></td>
</tr>
<tr>
<td>Edible flesh ((%)</td>
<td>59.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>± 2.45</td>
<td>± 3.15</td>
</tr>
<tr>
<td>Waste</td>
<td>40.65</td>
</tr>
<tr>
<td>± 2.45</td>
<td>± 3.15</td>
</tr>
<tr>
<td>Moisture content</td>
<td>70.25</td>
</tr>
<tr>
<td>± 4.25</td>
<td>± 3.14</td>
</tr>
<tr>
<td><strong>Dry weight basis (%) g</strong></td>
<td></td>
</tr>
<tr>
<td>Dry weight</td>
<td>29.98</td>
</tr>
<tr>
<td>± 4.55</td>
<td>± 3.14</td>
</tr>
<tr>
<td>Protein</td>
<td>55.85</td>
</tr>
<tr>
<td>± 1.65</td>
<td>± 2.5</td>
</tr>
<tr>
<td>Lipid</td>
<td>5.3</td>
</tr>
<tr>
<td>± 0.1</td>
<td>± 0.05</td>
</tr>
<tr>
<td>Ash</td>
<td>11.1</td>
</tr>
<tr>
<td>± 0.2</td>
<td>± 0.45</td>
</tr>
<tr>
<td>NFE</td>
<td>15.7</td>
</tr>
<tr>
<td>± 2.3</td>
<td>± 2.4</td>
</tr>
</tbody>
</table>

All values are mean for triplicates with SD. Values with the same superscript letters in the same row are not significantly different (P<0.01) from each other.
Plate 7.2
Growth of *Penaeus monodon* fed with Microalgal and cyanobacterial live feed

LF 1 = *Chlorella* sp.
LF 2 = *Isochrysis* sp.
LF 3 = *Tetraselmis* sp.
LF 4 = *Synechococcus* sp.
LF 5 = *Phormidium* sp.
lowest in shrimp fed with diet *Chlorella* sp. Higher total lipid content was found in shrimp fed with *Phormidium* sp. diet and lower lipid was found in *Isochrysis* sp. diet.

**Microbial study**

Total bacterial count of shrimp growth tank water is in Fig 7.4. Bacterial abundance was reduced during the culture days. Higher bacterial load recorded in feed *Isochrysis* sp. (LF3) that was 29.3 x 10^3 CFU mL^-1 during initial day; it was reduced to 10.3 x 10^2 CFU mL^-1 after 30^th^ day. Fig 7.5 revealed the total bacterial count of shrimp *P. monodon* intestinal tract samples. Maximum total viable count was resulted in *Phormidium* sp. feed (19.1 x 10^4 CFU g^-1) after 30 days sample. But the bacterial count of shrimp intestinal tract was decreased in *Isochrysis* sp. followed by *Tetraselmis* sp. and *Chlorella* sp.

Total fungal count of shrimp culture tank water was estimated using PDA plates. The result showed the fungal count ranged from 13 CFU mL^-1 to 137 CFU mL^-1. Highest fungal count was accounted in tank LF3 on 30 day and it was lower in *Chlorella* sp.

Bacterial population in intestinal tract of *P. monodon*, seven different bacterial genera were identified (Table 7.3). Bacteria such as *Vibrio*, *Pseudomonas*, *Aeromonas*, *Flavobacterium*, *Staphylococcus*, *Bacillus* and *Micrococcus* and some unidentified gram positive and Gram-negative rods are also analyzed. Among the bacterial genera *Pseudomonas*, *Aeromonas* and *Vibrio* are frequently present in all water samples. *Vibrio* species such as *V. alginolyticus*, *V. marinus* and *V. parahaemolyticus* were commonly occurred. Abundance of *V. alginolyticus* was reduced in all live feed tanks and *V. parahaemolyticus* was not recorded in LF5 tank water. *Aspergillus*, *Penicillium*, *Fusarium* and *Mucor* are the fungal group identified from the shrimp culture tank. Fungal abundance was increased during the culture period (Table 7.4).
Fig 7.4 Total bacterial load of shrimp *P. monodon* culture tank water (live feeds). All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P< 0.01).
Fig 7.5 Total bacterial load of shrimp *P. monodon* intestinal tract (live feeds). All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P< 0.01).
Fig 7.6 Total fungal load of shrimp *P. monodon* Culture tank water (live feeds). All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P< 0.01).
<table>
<thead>
<tr>
<th>Organisms</th>
<th>Chlorella sp.</th>
<th>Tetraselmis sp.</th>
<th>Synechococcus sp.</th>
<th>Phorutium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
<td>Days</td>
<td>Days</td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td>0 10 20 30</td>
<td>0 10 20 30</td>
<td>0 10 20 30</td>
<td>0 10 20 30</td>
</tr>
<tr>
<td>V. alginolyticus</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Vibrio sp.</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
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<tr>
<td>Pseudomonas sp.</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
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<tr>
<td>Flavobacterium sp.</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
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<tr>
<td>Staphylococcus sp.</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

+ present; - absent
Table 7.4 Occurrence of different bacteria in shrimp intestinal tract (live feed study 30 days)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Chlorella sp.</th>
<th>Tetraselmis sp.</th>
<th>Isochrysis sp.</th>
<th>Synechococcus sp.</th>
<th>Phormidium sp.</th>
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<tbody>
<tr>
<td></td>
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<td>Days</td>
<td>Days</td>
<td>Days</td>
<td>Days</td>
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<td>10  20  30</td>
<td>10  20  30</td>
<td>10  20  30</td>
<td>10  20  30</td>
<td>10  20  30</td>
</tr>
<tr>
<td>V. alginolyticus</td>
<td>+  +  +</td>
<td>+  +  +</td>
<td>+  +  +</td>
<td>+  +  +</td>
<td>+  +  +</td>
</tr>
<tr>
<td>V. marinus</td>
<td>+  -  -</td>
<td>+  -  -</td>
<td>+  -  -</td>
<td>+  +  +</td>
<td>+  -  +</td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>+  -  -</td>
<td>+  -  -</td>
<td>+  -  -</td>
<td>+  -  -</td>
<td>+  -  -</td>
</tr>
<tr>
<td>Vibrio sp.</td>
<td>+  +  -</td>
<td>+  +  -</td>
<td>+  +  -</td>
<td>+  +  +</td>
<td>+  +  +</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>+  +  +</td>
<td>+  +  +</td>
<td>+  +  -</td>
<td>+  +  +</td>
<td>+  +  +</td>
</tr>
<tr>
<td>Aeromonas sp.</td>
<td>+  +  -</td>
<td>+  +  -</td>
<td>+  +  -</td>
<td>+  +  +</td>
<td>+  -  -</td>
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<tr>
<td>Flavobacterium sp.</td>
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<td>-  -  +</td>
<td>-  -  -</td>
<td>-  -  -</td>
<td>-  -  +</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
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<td>+  -  -</td>
<td>-  -  +</td>
<td>-  -  +</td>
<td>-  -  +</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>+  +  +</td>
<td>+  -  -</td>
<td>+  -  +</td>
<td>+  -  -</td>
<td>+  -  -</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>-  -  -</td>
<td>-  -  -</td>
<td>-  -  -</td>
<td>-  -  +</td>
<td>-  +  -</td>
</tr>
</tbody>
</table>

+ present; - absent
Table 7.5 Occurrence of different fungus in shrimp growth tank water (live feed study 30 days)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Chlorella sp.</th>
<th>Tetraselmis sp.</th>
<th>Isochrysis sp.</th>
<th>Synechococcus sp.</th>
<th>Phormidium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
<td>Days</td>
<td>Days</td>
<td>Days</td>
<td>Days</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. terreus</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Other fungi</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ present; - absent
7.4 Discussion

Results of the study revealed that the fresh Chlorella sp. and Phormidium sp. have significant increase in growth of shrimp P. monodon. The physiochemical characters of the tank water were analyzed at the interval of 10 days for total of 30 days. The physicochemical characters of all experimental tanks, water is within the normal limit. The present study, indicates that growth of shrimp depends on the species used as a live feed. There were no significant differences in the mean total length of the juvenile fed with the experimental diets, Chlorella sp and Phormidium sp. Significantly higher growth was observed when fed with Chlorella sp. followed by Phormidium sp., but the survival and development of the larvae was affected by other algal diets. Larvae fed with mixed diet of Chaetoceros muelleri and Thalassiosira suecica performed as well (D’Souza and Loneragan, 1999). Survival and development of P. monodon may be better on a mixed diet of C. muelleri and T. suecica (Kurmalv et al., 1989). Some authors have correlated the poor performance of prawn larvae to the large size of the algal cells in the diet (Tobias-Quinitio and Villegas, 1982: Sanchez, 1986). The present study shrimp fed with Chlorella sp. showed higher body weight. But lower body weight showed in shrimp fed with Tetraselmis sp.

Microalgae such as Chlorella sp., Tetraselmis tetratheca and T. chuii were used as a feed for shrimp and fish.Survivals of P. monodon fed with the five diets were showed differences in their performance. Survival rates of shrimps were observed to be higher in diets mixed with Phormidium sp. (83.33%) followed by Chlorella sp. (76.67%). Unlike microalgae, the Phormidium sp. cells were easily ingested and assimilated by shrimps. Larvae fed with fresh microalgae had high survival and development of P. monodon larvae (D’Souza et al., 2000, 2002). The survival and development of prawn larvae varied greatly. Survival and development of spawning feed with algal diet have been reported for Metapenaeus ensis larvae (Chu and Lui, 1990). Crocos and Coman (1997) have shown that when the diet of Penaeus semisulcatus at protozoea (PZ1) stage varies with such factors as age of the brood stock and season of spawning. Fresh microalgae Tetraselmis chuii is used as a...
diet for *P. monodon* (Heasman *et al.*, 2000). *T. suecica* grown in the higher nitrogen medium was the better diet for growth of shrimp (Jackson *et al.*, 1992; D’Souza and Kelly, 2000). The survival rate of larvae was not affected by the algal diet. The survival rate of the prawn larvae fed on both algae was high compared with that of sole feeding trials (Okauchi and Tokuda, 2004). In the present study, shrimp mortality was observed to be lower in shrimp fed with *Phormidium* sp. compared with other micro algal feed.

Survival alone is not a suitable measure of the nutritional value of diets for prawn larvae. Earlier studies revealed, some species of algae with high nutritive value and growth capacity such as *Isochrysis* sp. (Okauchi *et al.*, 1997), *T. tetrathele* (Okauchi and Hirano, 1986) and *T. chuii* (Tobias-Qunitro and Villegas, 1982). The shrimp that performed best had significantly more lipid and carbohydrate than those fed with micro algal diet. Similar finding was reported when *P. monodon* larvae were fed micro algal feed (Tobias-Quinitio and Villegas, 1982; D’Souza and Loneragan, 1999). The biochemical compositions of the larva fed with microalgal diet were also measured. The carbohydrate content was increased three fold in the lower nitrogen algae, while protein and lipid were reduced slightly compared to the control (D’Souza and Kelly, 2000). In this study, maximum protein was accumulated in shrimp fed with *Phormidium* sp. and lowered in shrimp fed with *Synechococcus* sp.

**Microbiological enumeration**

Bacterial coexistence is the major problem in shrimp culture system. Several microbial pathogens are known to causes diseases in shrimps. There is a very limited literature available on the micro flora associated with live feed studies. Few studies have been made on bacteriology of cultured shrimps and the environment (Fonsek, 1990). Lightner (1993) described about some species of bacteria associated with shrimps cause disease under stress conditions. In this study, total bacterial count of the experimental tank water was considerably increased during the culture period. The bacteria ranged from $9.7 \times 10^3$ to $39 \times 10^3$ CFU mL$^{-1}$. But the bacterial load of the water samples were decreased during the culture period mainly in LF3 (*Isochrysis*
sp.). More number of total bacteria was recorded in the LF1 (Chlorella sp.) on 10th day. The present study supported the earlier results on total viable count of bacteria in the shrimp pond water ranging from $10^3$ to $10^5$ CFU mL$^{-1}$ (Scott and Thune, 1998; Fonseka, 1990). In this study, shrimp intestinal tract samples, bacterial load ranged from $13.8 \times 10^4$ to $7.8 \times 10^3$ CFU mL$^{-1}$. Total bacterial load was drastically reduced in shrimp fed with live feed LF3 (Isochrysis sp.). But the bacterial count in the intestinal tract of shrimp was significantly higher in LF4 (Synechococcus sp.) and LF5 (Phormidium sp.). The total bacterial count reduction may be due to the antibacterial property of microalgae or other stressful conditions. Many authors have found antibacterial activity of microalgae due to fatty acids (Cooper et al., 1983; Findlay and Patil, 1984; Viso et al., 1987; Kellam et al., 1988; Naviner et al., 1999). Unsaturated and saturated long chain fatty acids of diatoms have bacteriocidal activity (Galbранity and Miller, 1973). Chlorella vulgaris, Skeletonema costatum and T. succiesa have antibacterial properties (Naviner et al., 1999).

In the present investigation, seven most common bacterial genera were recorded. Bacteria such as Vibrio, Pseudomonas, Aeromonas, Flavobacterium, Staphylococcus, Bacillus and Micrococcus were identified. Among these species, Pseudomonas, Vibrio and Aeromonas were most frequently appeared. Sharmila et al. (1996) found fourteen bacterial genera from the P. indicus culture ponds, such as Vibrio spp., Staphylococcus spp., Enterobacteria spp., Micrococcus sp., Bacillus spp., Acinetobacter spp., Cornebacterium spp., Flavobacterium spp., Moraxella spp. and Pseudomonas spp. Aeromonas and Vibrio species were dominant on shrimps that showed poor growth (Scott and Thune, 1998; Leano et al., 1998). In this study, three Vibrio species were predominant such as V. alginolyticus, V. marinus, and V. parahaemolyticus. Leano et al. (1998) reported V. parahaemolyticus, V. alginolyticus, and V. anguillarum were responsible for bacterial septicemia in cultured shrimps.

Total fungal load of the shrimp culture tank water were significantly differed (P<0.01). This variation was observed at 20th day and 30th day samples. Higher fungal count was recorded in LF3 (Isochrysis sp.), LF4 (Synechococcus sp.) and LF5.
(Phormidium sp.). Very low fungal count was recorded in initial day samples. *Aspergillus, Penicillium, Fusarium* and *Mucor* were the common fungal genera identified. *Aspergillus niger* and *A. terreus* are the two *Aspergillus* species frequently identified.

### 7.5 Summary

- Efficacy of cyanobacterial live feed was compared to micro algal live feed. Fresh biomass of *Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp., *Synechococcus* sp. and *Phormidium* sp. were used as feed for shrimp *P. monodon*.
- Mean total length of shrimp was higher when fed with *Chlorella* sp. (4.8 cm) followed by *Phormidium* sp. (4.4 cm), mean total weight was also higher in shrimp fed with *Chlorella* diet (0.59 g) followed by *Phormidium* diet (0.569 g).
- The survival rate of the shrimp was improved in shrimp fed *Phormidium* diet (83.33 %) and it was lowered in microalgae *Isochrysis* diet (36.67 %).
- The shrimp that perform best had significantly more edible flesh (59.35 %) in *Chlorella* diet, protein and lipid content in *Phormidium* diet, carbohydrate in *Tetraselmis* diet.
- Water quality of the tank was better in shrimp fed with *Chlorella* diet in term of microbiologically.
- *Chlorella* sp. and *Phormidium* sp. promotes the growth performance of *P. monodon* by providing good nutrients and other conditions for better growth.