Materials and Methods

3.1 Study site description

The East and West coasts that surround the Indian Peninsula are of the longitudinal Pacific type which border mountain chains. The east coast is much broader than the west coast. The east coast of India starts from the edge of the Ganga delta and extends right through to the southern most tip of the Peninsular at Kanyakumari facing the Bay of Bengal. The coast is mostly of the emergent type, that is, it is regular outline and is characterized by offshore bars, fine sea beaches, sand ridges and lagoons. Most of the great rivers of India, barring a few notable exceptions, have their mouths on this coast. The coastal plain on the east is much wider than that on the west. Here the narrow strip of beaches is fronted by rows of sand dunes, broken by a number of lagoons (Fig 1).

In Tamil Nadu, marine sector dominates the entire fishery sector and the state has the coastline of over 1076 Kms. It is divided into 4 zones as given below; (i) The Coramandel coast from Pulicat in the North to Point Calimere in the south extending to about 357.2 kms. (ii) The Palk Bay extending from Point Calimere to Dhanushkodi measuring 293.9 Kms. (iii) The Gulf of Mannar extending from Dhanushkodi to Kanniyyakumari measuring 364.9 Kms. (iv) The tiny western sector with 60 Kms of beach on the Arabian sea from Kanniyyakumari to Neerodi. The country with a long coastline of 8118 Km. has an Exclusive Economic Zone (EEZ) extending to 2.02 million Sq.Km. comprising of 0.86 million Sq.Km. on the West Coast, 0.56 million Sq.Km. on the East Coast and 0.60 million Sq.Km. around Andaman and Nicobar Islands. Tamil Nadu, with it’s 1076 Km of coastline, 0.19 million Sq.Km. of EEZ and a continental shelf of about 41,412 Sq.Km. is a leading State both in culture and capture fisheries.
Broodstocks of *Peneaus monodon* were collected from five different places from east of coast of Tamil Nadu.

1. Chennai,
2. Palayar,
3. Poompuhar
4. Mandapam and
5. Idinthakarai

The present work was partially carried out in George Maijo shrimp hatcheries located at 4/204, MGR Road, Palavakkam, Chennai -600 041, Tamil Nadu, India.
Fig 1

Broodstock collection sites and location of shrimp hatchery in the East coast of Tamil Nadu, India
3.2 Taxonomy of *Penaeus monodon*

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Arthropoda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Crustacea</td>
</tr>
<tr>
<td>Subclass</td>
<td>Malacostraca</td>
</tr>
<tr>
<td>Order</td>
<td>Decapods</td>
</tr>
<tr>
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<td>Natantia</td>
</tr>
<tr>
<td>Infraorder</td>
<td>Penaeidea</td>
</tr>
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<td>Penaeoidea</td>
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<td>Penaeidae Rafinesque, 1815</td>
</tr>
<tr>
<td>Genus</td>
<td>Penaeus Fabricius, 1978</td>
</tr>
<tr>
<td>Subgenus</td>
<td>Penaeus</td>
</tr>
<tr>
<td>Species</td>
<td>monodon</td>
</tr>
</tbody>
</table>

The genus *Penaeus* Fabricius (1978) was placed on the official list of generic Names in Zoology as Name No. 498 upon discovery and description of *Penaeus monodon* by John Christ Fabricius in 1798 (Mohamed, 1970). With the revision of the specific name monodon by Holthuis, the two species have become stabilized and the name *P. monodon* is generally accepted for the present species (Hall, 1961; Mohamed, 1970; Motoh, 1981). No subspecies are currently recognized for this species and *P. monodon manillensis* (Villaluz and Arriola, 1938) was proved to be based on an abnormal specimen of *P. semisulcatus* (Mohamed, 1970; Motoh, 1981). According to FAO, shrimps refer to marine penaeid while prawns refer to fresh water palaemonids.
3.3 Broodstock collection and transport

Broodstocks of *Peneaus monodon* were collected from five different places (Chennai, Palayar, Poompuhar, Mandapam and Idinthakarai) for a period of one year (January to December) on monthly collection. Since they are found in approximately 70 meters depth near river mouth, the broodstocks were captured through Kattamaran and Trawlers. *P. monodon* was identified in the field by the presence of brown, black and white bands on abdominal segments. The female and male were identified by the presence of reproductive organs (Female: external reproductive organs called thelycum at the base of 4th and 5th pair of periopods; Male: external reproductive organs called petasma at the first abdominal segment).

3.3.1 Broodstock selection criteria

Broodstocks were selected based on the weight of individual: Female weight between 100 gm-120 gm and male weight between 80 gm-90 gm for male were considered as the criteria for broodstock selection. The animals with opaque white and bulging Thelycum as these indicates the presence of spermatophore were selected, feel the shell of the shrimp lower portion of the carapace was felt hard, when the broodstock held with both hand. Those animals with soft and injured shell with dull red colour, melanosis on the body parts and black spot on the Thelycum were rejected. All broodstocks rostrum and telson were covered with rubber cap to prevent puncturing of polyethylene bags. For ideal stocking density in each bag, one female per bag and two male per bag were followed.

3.3.2 Packing and Transport

The collected broodstocks were packed in polyethylene bag with 6 litres of filtered sea water and 6 liters oxygen filled for transport. Polyethylene bags with brood stock were
Plate 1

Broodstock collection and identification

A. Trawler Collection

B. Catamaran Collection

C. *Penaeus monodon* Broodstock

D. Female Broodstock

E. Male Broodstock
Plate 2

Broodstock packing and transport

A. Broodstock Holding
B. Broodstock Packing
C. Broodstock Transport – Car
D. Broodstock Transport - Train
E. Broodstock Quarantine
F. DNA Thermal Cycler
Plate 3

Broodstock screening and eye stalk ablation

A. Gel Electrophoresis

B. UV Transluminator

C. PCR based detection of MBV - AMPLI-MBV Kit

D. PCR based detection of WSSV - AMPLI-WSSV Kit

E. Eye Stock Ablation

F. Ablated Broodstock
kept in insulated box to avoid increase in temperature during train transportation but in car transport polyethylene bags were kept directly inside the car. Throughout the collection and packing process, animals handled only with proper brood stock collection net, not with hands. Styrofoam boxes with brood stocks were carefully transported to hatchery through train. Upon arrival at hatchery, brood stock collection net was used to remove the animals from insulated box.

3.4 Screening of broodstock

Broodstock were checked at quarantine for the viruses namely MBV (Monodon Baculo Virus) and WSSV (White Spot Syndrome Virus). Polymerase chain reaction (PCR) was used to diagnose the MBV & WSSV at pathology laboratory. Mangalore Biotech Kits (AMPLI- MBV Kit, AMPLI- WSSV Kit) were used to detect WSSV and MBV in broodstock. Pleopod -swimming leg of male and female broodstock were taken for PCR analysis.

3.5 Experimental chemicals and Probiotics used for maturation and larval rearing

The two Antibiotics namely Oxytetracyclin and Chloramphenicol and one Probiotics were selected for the present investigation. These two Antibiotics were purchased from local supplier in Chennai and Probiotics (BACIMOR Hi-Spore Hatchery) purchased from Hi-Line Aqua, Chennai, India for experimental analysis.

3.5.1 Conditioning of Broodstock and Experimental design of Maturation

Four concrete tanks (5 m x 5 m x 1 m) were used for conditioning of broodstock. One tank was kept as control and each similar tank was kept for Oxytetracyclin, Chloramphenicol s Probiotics treatment. In each tank, 40 females and 20 males were introduced from pool of screened broodstock. The 2 ppm of Antibiotics such as Oxytetracyclin, Chloramphenicol and Probiotics were added daily in the respective holding
tanks after water exchange to maintain the concentration. This was maintained for 3-5 days for acclimation to the local condition. The mixed feed (Crab, Marine Polychaet worms Squid, Green mussel) were given to each tank daily at 10-30% body weight. Water exchange was given at 100% flow through per day. During these 3-4 days selected females and males were acclimated to hatchery conditions. Before going for eyestalk ablation four maturation tanks were kept ready for experimental purpose.

3.5.2 Induced Maturation by Unilateral Eye Stalk Ablation

After conditioning of all females in four batches were unilaterally eyestalk ablated using a pair of flamed surgical scissors to enhance ovarian maturation. The method adapted for eyestalk ablation was cutting and cautery with surgical scissors. It was observed that cautery prevented bleeding of hemolymph and infection. Hard shelled females were taken for eyestalk ablation. Selected hard shelled female from conditioning tank was taken one by one with help of brood stock collection net. The brood stocks were helded firmly and kept the body inside the palm, only head was outside the hand with antenna. Forceps was used to hold one eye of the animal and cauterized with surgical scissors heated with blow lamp. After eyestalk ablation, the females were released into the respective maturation tanks along with males. The maturation tanks were closed with black plastic sheet to avoid the light. After ablation mild aeration was provided. In maturation tank water level was maintained at 60 cm. This low level was allowed both male and female to recognize and chase very easily for mating. Ablated female were kept in maturation tank at a density of 5-7/m² and sex ratio of one male and two female (1:2). Fresh and fresh frozen feeds were given at right time to four experimental tanks with mixed diet. Antibiotics such as Oxytetracycllin and Chloramphenicol and Probiotics were added in
the respective tank daily after water exchange to maintain the 2 ppm concentration. This experiment was maintained for 10-15 days for naupli production.

3.5.3 Broodstock Feeds during maturation

Fresh frozen feeds were given to broodstocks at control and all the experimental maturation tanks to induce egg development after eyestalk ablation. The availability of an optimal diet was identified as a crucial factor for the sexual maturation and reproduction of shrimp. Fresh and fresh frozen feeds were collected from local suppliers. Four type of fresh frozen feeds were used (Crab, Marine polychaet worms Squid and Green mussel) to brood stock during the entire operation of maturation. Fresh feeds were calculated based on the shrimp biomass. About 10-30% of the body weight (Primavera, 1985) was distributed daily as per given time schedule.

<table>
<thead>
<tr>
<th>Time</th>
<th>Feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>06.00 Hrs</td>
<td>Crab</td>
</tr>
<tr>
<td>12.00 Hrs</td>
<td>Marine polychaet worms</td>
</tr>
<tr>
<td>18.00 Hrs</td>
<td>Green mussel</td>
</tr>
<tr>
<td>24.00 Hrs</td>
<td>Squid</td>
</tr>
</tbody>
</table>

In captivity, the breeding season was simulated, in an attempt to trigger the hormonal activity that control reproduction.

3.6 Spawning and Hatching

3.6.1 Sampling

The gonadal development of an ablated female was monitored after 3 to 5 days after ablation. Checking and sampling were done at evening time between 16:00 to 18:00 hrs. Water in the maturation tank lowered to 30cm to facilitate close observation. During the sampling an under water flash light was held close to the shrimp so that the light strikes
Plate 4

Maturation feeds, sampling and spawning

A. Marine polychaet Worms
B. Squid Tubes
C. Mud Crab
D. Green Mussel
E. Completely ripped, Stage IV – Ovaries (Sampling)
F. Spawning Tanks
perpendicularly on the dorsal part of the body where the ovaries are located. Only gravid females with stage IV ovaries (looks butterfly in shape) were collected from each experimental tank and transferred to separate circular spawning tanks treated with 2 ppm of Oxytetracyclin, Chloromphenicol and Probiotics. Control spawning tank was kept without any treatment.

3.6.2 Spawning

Spawning tank made circular to stream line swimming activity of spawner while spawning and capacity of the tanks were 500 liters. Only one gravid female from experimental tanks was introduced in each tank for spawning. Gentle aeration was provided during spawning. Spawning was taken place between 22:00 and 24:00 hrs. After spawning animals were removed from the tank with long handled collection net and culled. Eggs were siphoned out from spawning tanks to egg collection buckets with 100 micron mesh. Before siphoning out the eggs, scum was removed by using scoop net with 500 micron mesh size. The collected eggs were disinfected by the following procedure to avoid bacterial and fungal infection during hatching (Chen et al., 1996).

\[
\begin{align*}
\text{Eggs} & \rightarrow \text{Running} \rightarrow \text{Formalin} \rightarrow \text{Iodophore} \rightarrow \text{Running} \\
\text{Sea water} & \quad (200\text{ppm}) \quad (50\text{ppm}) \quad \text{Sea water} \\
1 - 2\text{min} & \quad 30\text{sec} \quad 30\text{sec} \quad 1 - 2\text{min}
\end{align*}
\]

3.6.3 Hatching

After disinfection, the eggs were transferred to hatching tank with sterilized sea water treated with 2 ppm of Oxytetracyclin, Chloromphenicol and Probiotics. Control incubation tank was kept without any treatment for further incubation and subsequent hatching. From the incubation tank, 10 ml of water sample taken and eggs were counted to determine the number of eggs spawned per female. The density of naupli was estimated a day after hatching eggs. Three times 10ml water samples taken from the naupli holding
Plate 5

Spawning, Algae culture and Artemia hatching

A. Egg & Nauplii Washing Bucket

B. Egg Hatching Tanks

C. Algae – Indoor Culture
   Inside – Chaetocerous calcitrion

D. Algae – Outdoor Culture

E. Artemia Hatching Tank

F. Freshly Hatched Artemia
tank were counted and average was taken to determine the quantity of naupli. The following formula was employed to determine hatching rate

\[
\text{Hatching rate} (\%) = \frac{\text{No of naupli counted} \times 100}{\text{No of eggs count}}
\]

Naupli were then transferred to disinfection buckets to take preventive treatment against fungus, bacteria and virus by dipping naupli as per the following method

**Running** → **Formalin** → **Iodophore** → **Running**

<table>
<thead>
<tr>
<th></th>
<th>Running</th>
<th>Formalin</th>
<th>Iodophore</th>
<th>Running</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea water</td>
<td>Sea water (200ppm)</td>
<td>(50ppm)</td>
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<tr>
<td>1 – 2min</td>
<td>30sec</td>
<td>30sec</td>
<td>1 – 2min</td>
<td></td>
</tr>
</tbody>
</table>

After disinfection, naupli were kept in sterilized sea water for further photo taxis screening. Naupli demonstrate positive photo taxis from hatching onward. Naupli collection scheme was used to attract by light to help concentration and to transfer larvae to larval rearing tanks. Naupli collected from control, Oxytetracyclin, Chloromphenicol and Probiotics treated hatching tanks were kept separately for further larval rearing purpose.

**3.7 Algal culture (Indoor and Outdoor mass culture)**

The most common Diatom (*Chaetoceros calcitr翁*) was used at experimental hatchery to rear larvae as feed from Zeea to Mysis stages. The process of diatom culture involved three main aspects, namely a) Preparation of a suitable medium for its growth and multiplication, b) Obtaining a starter or inoculums by isolating it from the wild collection and c) Scaling up the operation to optimum level. Sea water was sterilized for every time to remove all living organisms and then enriched with required nutrient. *Chaetoceros calcitr翁* were collected by towing special towing material through seawater. The fine mesh about 50 micron size made of nylon material was used for collecting algae from wild. The isolation of a specific phytoplankton from the collected crude sample was prerequisite for the establishment of unialgae culture. Serial dilution method was employed to isolate
*Chaetoceros calcitrion.* The following two medium were used for Chaetoceros calcitrion culture. 1. Guillard and Rhyther Modified F medium (1975) and 2. Liao and Huang’s (1973) modified TMRL medium.

Axenic or pure algal culture was referred to population of a single algal species as indoor culture. The absence of sunlight in controlled room was provided with cool-white daylight fluorescent tubes. One to two tubes (1000 to 2000 lux) depending upon the Chaetoceros calcitrion growth. For outdoor algal culture, tanks were located to area that is exposed to morning sunlight. Incubation temperature for algal culture ranged between 24 and 25°C. The scaling up of outdoor culture was usually done early in the morning to avoid temperature shock. Aeration was provided to keep the algae cells in suspension, disperse dissolved materials and to avoid adherence of cells to the wall of culture vessel. Maintenance and production of algae was a routine job in a shrimp hatchery. Stock culture was kept at refrigerator in agar slants or unaerated smaller volumes of culture (50-100 ml). Scaling-up of algae was done according to daily demand at larval rearing tanks. Before scaling up, stock cultures were monitored for contamination and to select a new starter for the next batch based on the cell quality. Transfer of algae was done when cultures at log phase of growth. During renewal, duplicate culture was done to allow the technician to choose inoculum. For starter/stock culture, a smaller volume about 10% of inoculum used while large scale culture 10-20% of the total volume was used. The continuous culture system was used at experimental hatchery, scales upward volume at indoor culture starts from 250 ml foil capped conical flask to 1 liter aerated conical flask and to 10 lits carboys. Used 500 lits FRP tanks at intermediate culture to 5 tons concrete tanks for outdoor mass culture from where algae used for larval feeding. All indoor cultures were grown in Modified F medium and outdoor culture grown in TMRL medium.
Cell counts were made daily from all algae culture tanks and carboys in the indoor culture room. Algae cell density was measured and expressed as cell number based on the count of algae cells in an aliquot of the sample (Density = No. of cells/ml of sample). Algal cell count was done everyday as described by Fox (1983). Algal culture regularly started at 7.00 Hrs when there is sunrise and completed at the earliest so that the new culture ready for next day feeding from morning to evening. Algae were fed three times a day 8:00, 16:00 and 24:00 Hrs and maintained cell density of 50,000-100,000 cells/ml at larval rearing tanks from Zoea to Mysis stage. Outdoor culture planned according to the number of tanks and quantity of nauplii stocked in each tank. When the diatom in the exponential phase of growth attained a maximum density then were harvested and utilized as feed for larvae at larval rearing tanks. Appropriate quantity of diatom culture was added to larval rearing tank with the help of 0.5 hp submersible electrical pumps.

3.8 Artemia hatching

Artemia nauplii in instar I stage with stored yolk which is nutritionally suitable for the shrimp Post Larvae. At experimental hatchery Artemia was given to Mysis-III - PL 14. Each gram of cysts contained average 2,50,000 cysts to 3,00,000 cysts with different hatching rate. Artemia cysts with 90% hatching rate were used to avoid fungal infection at larval rearing tanks due to unhatched artemia cysts. There was three times Artemia feedings (8:00, 16:00, 24:00 Hrs) at shrimp hatchery when they reached post larvae stage. Three times Artemia and three times formulated diets were in regular feeding schedule. In order to avoid bacterial contamination, routine disinfection of the cyst with sodium hypochlorite was used. Cysts were dispersed in fresh water at 50gms/lit of water with 200ppm hypochlorite solution for 30 minutes. After disinfection, the cysts were collected in a nylon
net (100 micro size) and washed with fresh water. The washed cysts were dispersed in at 5gms/lits of sea water in hatching containers containing fresh and filtered sea water. Every batch of artemia was checked for hatching efficiency. Artemia cysts with hatching rate of 90% and above was normally selected for feeding. The basic procedures and physical conditions required for hatching cysts followed by artemia section as suggested by Sorgeloos (1980). The disinfectTed Artemia naupli collected and kept in 100 lits plastic container for feeding at larval rearing tanks. Number of naupli/ml of 100 lits plastic container was counted every day after the harvest. Number of post larvae was counted in each post larvae tank. Feeding was done according to the stage and demand. The Artemia density was increased from 0.25 to 8 individuals/ml during rearing period (Treece, 1985).
3.9 Larval rearing

The larval rearing tank was stocked with correct number of larvae and exchanged sufficient water to maintain optimum water quality throughout the entire larval rearing process. Feeding regime and water exchange protocol has given in Table.1. Naupli were examined for the quality (yolk size, degree of response to light and other behaviour) and quantity prior to stocking in the larval rearing tank at 100 ind/lit. Naupli were acclimated to the physico-chemical condition of the water (e.g. temperature and salinity) in the larval rearing tanks.

3.9.1 Experimental design of Antibiotics and Probiotics for larval rearing

Twelve tanks (3 m x 3 m x 1.4 m) were used for experiment. The treatments as follows:

T1- without Antibiotics
T2- 2 ppm Oxytetracyclin
T3- 2 ppm Chloromphenicol
T4- 2 ppm Probiotics

There were three replicates for each treatment.

3.9.2 Larval Feeding

Optimization of feeding regime was done based on live feeds which help to maintain good water quality, promoting excellent growth and good survival rate of the larvae. Formulated Microencapsulated feed (less than 50 micron size) was given to Zoea I-III stages for three times per day and brown algae Chaetoceros calcitron was given three times per day. For Mysis I-III stages, Formulated Microencapsulated feed (50 to 150 micron size particle) was fed every 4 hrs on demand. Artemia naupli was given at 1-3 individuals/ml of rearing water to Mysis III stage. Early post larvae were fed with
Plate 6
Larval stages, larval rearing tank and formulated feeds

A. Egg
B. Naupli
C. Zoea
D. Mysis

E. Early Post Larvae
F. Late Post Larvae

G. Larval Rearing Tank
H. Formulated Feeds
microparticulate feed (100 to 150 micron particles) three times a day and artemia naupli 4-8 individuals/ml /day of rearing water, devided equally for three times feeding per day. Late post larvae fed a dry micro-particulate feed (300 microns size) for three times per day and artemia naupli 9 to 22 individuals/ml /day of rearing water, devided by three times feeding per day. Uneaten food and fecal matter were siphoned from bottom of the tanks periodically. It was done by turning off the air and allowing the larvae to come to the surface of the tank. Debris from the bottom of the tank was siphoned in to a net and the contents put in to a plastic basin to separate and return any larvae siphoned from the tank. Water exchange was given from 20 to 100% flow through per day depending on the water quality.
### 3.9.3 Table showing feeding Regime and Water Exchange

<table>
<thead>
<tr>
<th>LARVAL STAGES</th>
<th>Inve (Thailand) feeds</th>
<th>DIATOMS</th>
<th>Artemia</th>
<th>Water exchange</th>
<th>Survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the feed</td>
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<td>MYYSIS</td>
<td>EPL</td>
<td>LPL</td>
<td>PL300+ (Cells/ml)</td>
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<tr>
<td>N6</td>
<td>CAR#1</td>
<td>2CD</td>
<td>PL150+</td>
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<tr>
<td>Z1</td>
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<td></td>
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### 3.9.4 Larval Health Monitoring

Shrimp health was analyzed daily to ensure that problems were identified early and corrective measures were taken to increase productivity. Assessment of larval health condition was done every morning before water exchange after that feed adjustment and other management activities were followed. The larvae in each tank were monitored every day after feeding. Initially activity of larvae, the condition of the water in the rearing tank and feed consumption were observed visually. 500 ml glass beaker was used to take larvae from the tank and observed under microscope at observation room located in
between larval and post larval rearing sections. Observations were made on the larval stages, health, digestion and physical deformities.

3.10 Post Larvae health analysis

Samples brought to the pathology laboratory at 14 days old post larvae (PL14) to screen viral diseases (WSSV and MBV) and post larvae quality assessment. This post larvae quality assessment was involved in three main areas,

1. Gross examination
2. Microscopic examination
3. PCR screening

3.10.1 Gross Examination

A preliminary examination of post larvae in the tank made to assess the

- Size distribution : at PL 14 to be 12 mm.
- Colour : must be dark or clear, not white / red
- Swimming activity : larvae to swim against water current in a basin or in a bowl.
- Behaviour : jump when bowl is tapped.
- Gut content : feeding and gut fullness.

3.10.2 Microscopic examination

For this closer examination a sample of 100 post larvae are randomly selected and examined at 10 to 40 x magnification.

- Gut condition: hind gut lining must be smooth and there should not be any swelling
- Gills condition: there should not be filamentous or protozoan attachments.
- Rostrum number : at PL 15 to 20 the rostrum number should be 5-7
- Necrosis: appendages and body muscles must be free from necrosis.
Plate 7
Post Larvae Harvest and packing

A. PL Harvest
B. PL Holding Tank
C. Packing of PLs
D. Packed PLs
E. Transportation to Farm
Plate 8

Viral and Bacterial Diseases

A. WSSV
(White Spot on Carapace)

B. MBV
(Occlusion bodies in Hepatopancreas)

C. Swollen Hindgut

D. Filamentous Bacteria

E. Bolitus Syndrome

F. Appendages Necrosis
Plate 9

Bacterial, fungal and protozoan diseases

A. *Vibrio harveyi*

B. Larval Mycosis

C. Vorticella

D. Zoothamnium
Gut muscle ratio: at PL 15 to PL20 ratio should be 1:4 (muscle 80% width, gut
20% width)

3.10.3 PCR Screening

A sample of 100 post larvae, preferably weakest one was collected randomly from
post larval rearing tank and preserved in 90% alcohol. The samples were detected for
MBV and WSSV by PCR tool. Mangalore Biotech Kits (AMPLI- MBV Kit, AMPLI- WSSV
Kit) were used to detect WSSV and MBV.

3.10.4 Scoring of Larval Health Analysis

The Larval health analysis summary report (Appendix I) maximum score is 100%
and the pass mark is usually set at 80%. Any batch with less than 80% of the score was
considered as fail and the larvae were not fit for stocking in the farm. Post larval density at
PL14 and fry health analysis scores from each experimental tanks (Control, Oxytetracyclin,
Chloramphenicol & Probiotics) were recorded for future reference and interpretations.

3.11 Statistical analyses

Measures of central tendency and dispersion were used to describe the data collected
from different sites. F-test and Chi-square test were used to detect the significance of total
number of eggs and naupli production. Student t test was used to find out the variables on
significance. One way ANOVA was used to calculate variance between different larval
stages treated with Antibiotics and Probiotics.