ICAR-CIARI/FSD/MARICULTURE/2015/01

Micro algae culture

&

live feed culture for finfish hatchery

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ICAR-CIARI: Microalgae and Live feed culture
Content....

1. Overview --- 1

2. Microalagae --- 3

3. Rotifer --- 9

4. Copepod --- 12

5. Artemia --- 14

6. Moina micrura --- 17

7. Daphnia pulex --- 19

8. Infusoria --- 20

9. Annexure 1 --- 23

10. Annexure 2 --- 24

ICAR-CIARI: Microalgae and Live feed culture
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Andaman & Nicobar Islands
ICAR-CIARI: Microalgae and Live feed culture
1. OVERVIEW

Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplankton’s are generally eaten by zooplankton. Thus, phytoplankton forms the basis of the food chain. Live foods are able to swim in water column and are constantly available to fish and shellfish larvae are likely to stimulate larval feeding response. Most of the fish and shellfish larvae in nature feed on small phytoplanktonic and zooplanktonic organisms. The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food for feeding fish larvae, fry and fingerlings. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids and hence are commonly known as “living capsules of nutrition”. Providing appropriate live food at proper time play a major role in achieving maximum growth and survival of the young ones of finfish and shellfish. Two types of larvae are found in fishes precocial and altricial. Precocial larvae are those that when yolk sac is exhausted, appear as mini-adult exhibiting fully developed fins and mature digestive system. Such fish can ingest and digest formulated diet as first food. But altricial larvae are those when the yolk sac is exhausted remain in a relatively undeveloped state. The digestive system is still rudimentary lacking a stomach. Such digestive system seems to be incapable of processing formulated diet. Live feeds are able to swim in water column and thus constantly available to the larvae. Formulated diets tend to aggregate on the water surface or sink quickly to the bottom and are thus normally less available to the larvae than the live feed. In addition the movement of live feed in the water is likely to stimulate
larval feeding responses since evolutionary history has probably adapted them to attack moving prey in nature.

**SELECTION OF LIVE FEED**

When selecting food to be fed to the larvae, the following points should be considered:

1. The food must be perceived by the larvae
2. The size of food must be such that it can be accommodated by the mouth of the larvae.
3. The feed should have high nutritional quality especially Highly Unsaturated Fatty Acids (HUFA) essential to the growth and survival of the larvae.
4. The feed can be digested by the larvae.
5. Food organisms must be hardy.
6. They must be able to reproduce rapidly and can mass produced under controlled condition.
2. MICROALGAE

Marine microalgae are the floating microscopic unicellular plant of the sea water which is generally free living, pelagic and the size range from 2 to 20μm. The important components of microalgae are the diatoms, dinoflagellates, green algae, blue-green algae and coccolithophores. Most microalgae has got immense value are rich sources of essential fatty acids, pigments, amino acids and vitamins. These micro organisms play a critical role in the coastal and marine aquaculture of fish, molluscs, shrimps and oysters. This phytoplankton plays a vital role in aquaculture to meet the nutritional requirement of the larvae as well as for bioencapsulation. It is an established fact that hatchery operation will depend mainly on the availability of the phytoplankton.

**Media preparation**

For the maintenance and culture of marine microalgae, the sterilized seawater has to be enriched with substances for growth including nutrients, vitamins, trace metals, chelators and buffer compounds. For this purpose, a variety of media are available for enriching the seawater for algal culture. The most common types of media for enrichment are given below

1. **Walne’s Conway Medium**
   **Solution-A**

   |    | Potassium Nitrate (KNO₃) | 100g |

ICAR-CIARI: Microalgae and Live feed culture
<table>
<thead>
<tr>
<th></th>
<th>Chemical</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Sodium di Hydrogen Ortho Phosphate (NaH$_2$PO$_4$·H$_2$O)</td>
<td>20g</td>
</tr>
<tr>
<td>3</td>
<td>EDTA (Na)</td>
<td>45g</td>
</tr>
<tr>
<td>4</td>
<td>Boric Acid</td>
<td>33.6g</td>
</tr>
<tr>
<td>5</td>
<td>Ferric Chloride (FeCl$_3$.6H$_2$O)</td>
<td>1.3g</td>
</tr>
<tr>
<td>6</td>
<td>Manganese Chloride MnCl$_2$</td>
<td>0.36g</td>
</tr>
</tbody>
</table>

Put all the chemicals in a 1000ml standard flask and dissolve it in distilled water to make up to 1000ml. Boric acid should be separately dissolved in little water after heating.

**Solution-B**

<table>
<thead>
<tr>
<th></th>
<th>Chemical</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zinc Chloride ZnCl$_2$</td>
<td>2.1g</td>
</tr>
<tr>
<td>2</td>
<td>Cobalt Chloride CoCl$_2$</td>
<td>2.0g</td>
</tr>
<tr>
<td>3</td>
<td>Copper Sulphate CuSO$_4$·6H$_2$O</td>
<td>2.0g</td>
</tr>
<tr>
<td>4</td>
<td>Ammonium Molybdate (NH$_4$)$_6$ Mo$<em>7$O$</em>{24}$.4H$_2$O</td>
<td>0.9g</td>
</tr>
</tbody>
</table>

Put all the chemicals in a 100ml standard flask and dissolve it in distilled water to make up to 100ml.

**Solution-C**

<table>
<thead>
<tr>
<th></th>
<th>Vitamin B$_1$ (Thiamine)</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>200mg</td>
</tr>
</tbody>
</table>

Dissolve in 100ml distilled water

**Solution-D**

<table>
<thead>
<tr>
<th></th>
<th>Vitamin B$_{12}$ (Cyanocobalamine)</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>10mg</td>
</tr>
</tbody>
</table>

Dissolve in 100ml distilled water

**Final media preparation**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
<td>1 ml/L</td>
</tr>
<tr>
<td>Solution B</td>
<td>0.5 ml/L</td>
</tr>
<tr>
<td>Solution C</td>
<td>1 drop</td>
</tr>
<tr>
<td>Solution D</td>
<td>1 drop</td>
</tr>
</tbody>
</table>

ICAR-CIARI: Microalgae and Live feed culture
## 2. f/2 MEDIA

<table>
<thead>
<tr>
<th>Stock solution</th>
<th>Per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NaNO₃</td>
<td>75 g</td>
</tr>
<tr>
<td>2. NaH₂PO₄·2H₂O</td>
<td>5.65 g</td>
</tr>
<tr>
<td>3. Trace elements (chelated)</td>
<td></td>
</tr>
<tr>
<td>Na₂EDTA</td>
<td>4.16 g</td>
</tr>
<tr>
<td>FeCl₃·6H₂O</td>
<td>3.15 g</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.01 g</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.022 g</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>0.01 g</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>0.18 g</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>0.006 g</td>
</tr>
<tr>
<td>4. Vitamin mix</td>
<td></td>
</tr>
<tr>
<td>Cyanocobalamin (Vitamin B12)</td>
<td>0.0005 g</td>
</tr>
<tr>
<td>Thiamine HCl (Vitamin B1)</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.0005 g</td>
</tr>
<tr>
<td>5. Na₂SiO₃·9H₂O (only for diatoms)</td>
<td>30 g</td>
</tr>
</tbody>
</table>

### Final media preparation

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>1 ml</td>
</tr>
<tr>
<td>NaH₂PO₄·2H₂O</td>
<td>1 ml</td>
</tr>
<tr>
<td>Trace elements stock solution (1)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Vitamin mix stock solution (2)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Na₂SiO₃·9H₂O (only for diatoms)</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Make up to 1 litre with filtered natural seawater. Adjust pH to 8.0 with 1M NaOH or HCl.
For agar add 15g per litre Bacteriological Agar. Sterilize by autoclaving for 15 minutes at 15 psi and use when cooled to room temperature.

*Media should be added @ 10% of sterilized seawater used for culture of algae

**Stock culture Maintenance:**

0.1ml of pure culture is inoculated into 20 ml culture tubes or 50 ml culture flasks filled with enriched water and incubated in light intensity of 1000 lux (2 tube lights) with photoperiod of 12 hours to produce one million cells/ml. This can be maintained for 15 days.

**Sub culture:**

Sub-culturing involves inoculating some cells from an old stock culture into fresh culture medium, so that the cells can continue to grow and divide and remain healthy. If sub-culturing is not carried out, the algal cells in the stock culture will eventually die. It is important to take precautions to prevent contaminants from the air entering the stock cultures when sub-culturing. To start a new stock culture, about 20 ml of algae are taken from a stock culture which has been growing for 6 to 7 days and poured into a flask containing 250 ml of fresh culture medium.

**Mass culture:** For aquaculture purposes microalgae are mass produced in three main ways; batch culture, semicontinuous culture and continuous culture.

**Batch culture**

Batch culture is a system where the total culture is harvested. The batch culture
consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. Batch culture systems are widely applied because of their simplicity and flexibility, allowing to change species and to remedy defects in the system rapidly. Although often considered as the most reliable method, batch culture is not necessarily the most efficient method. Batch cultures are harvested just prior to the initiation of the stationary phase and must thus always be maintained for a substantial period of time past the maximum specific growth rate.

**Semi-continuous**

Semi-continuous culture is a system where part of the culture is harvested and is replaced with fresh culture medium (clean sea water and nutrient salts). After allowing 2-3 days for the remaining cells to grow and divide, the process is repeated. Semi-continuous cultures may be operated for up to 7 to 8 weeks. The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment.

**Continuous culture**

The continuous culture method, (i.e. a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out), permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished

a. **Turbidostat culture**, in which fresh medium is delivered only when the cell density of the culture reaches some predetermined point, as measured by the
extinction of light passing through the culture. At this point, fresh medium is added to the culture and an equal volume of culture is removed. The diluted culture increases in cell density until the process is repeated.

b. **Chemostat culture**, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (e.g., nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant. In a chemostat, the medium addition ultimately determines growth rate and cell density.

**Procedure of mass culture:**

Mass culture is done in FRP tanks and are maintained outside in sunlight. The sterilized seawater is enriched with ground nut oil cake @ 250 gm/tone, urea @ 10 gm/tonne and superphosphate @ 5 gm/tone. The microalgal is inoculated as per requirement from indoor cultures.

![Figure 1. Indoor algal facility](image-url)
3. ROTIFER

Rotifers are commonly called as “wheel animals”. They are usually microscopic and size is around 0.1-0.5 mm long, and are common in freshwater throughout the world with a few saltwater species. It occurs in tropical and subtropical waters all over the world. Males are usually smaller than females. The important starter feeds used in larviculture are the marine rotifers; *Brachionus plicatilis* (Large type or L-rotifer) and *Brachionus rotundiformis* (Small type or S-rotifer). It has been most widely used as essential food source in raising marine fish, shrimp and crab larvae due to its tolerance to the marine environment. It thrives best when temperature range is between 22 – 25°C and salinity between 10 – 15 ppt.

**Stock culture:**

For pure culture, stock culture need to be developed. In order to start stock culture, collect *B. plicatilis* from the stagnant salt water with a scoop net having 50 – 100 micron mesh. Dilute the sample by adding fresh clear water having salinity as that of sample. Examine the samples under stereozoom microscope and pickup rotifer with fine dropper and inoculate in a 10ml glass tube containing 5ml water. Feed them with yeast @ 200 ppm or *chlorella* at a cell density of 10 × 10⁶ cells per ml. Serially dilute the test tube cultures daily through several large tube of 20ml containing 10 ml of water. Gradually increase the volume to 50 to 100ml capacity beaker and then to 1 to 2 litre to get a density of 100 to 150 individuals per ml. These cultures are used as inoculums for mass culture.
Mass culture:

There are a large array of culture methods for mass production of rotifer *B. plicatilis*. These can be sorted into three basic methods based on the food and feeding habits.

1) **Daily tank transfer method**

FRP tanks of capacity 200-500 L is inoculated with chlorella @ 10\(\times\)10^6 cells per ml. Introduce rotifer @ 25 – 50 individuals per ml. When the rotifer density reaches to 100 – 150 individuals per ml 50% is harvested. The other half is harvested and transferred to another tank containing chlorella. The vacated first tank is thoroughly cleaned and prepared for chlorella culture for the next. This process is repeated daily.

2) **Continuous culture**

In this system culture can be maintained for about month. First chlorella is cultured using modified Yashima medium which is as follows:

- Ammonium sulphate @100g/t,
- Single super phosphate @10g/t and Urea @10g/t

After fertilization with above medium, the culture tank is inoculated with pure culture of chlorella. As chlorella reaches peak density, tank is re-fertilized with the same medium as used initially and at the same time rotifer is inoculated. When chlorella cells are consumed, Baker’s yeast is introduced @ 1g/million *B. plicatilis*. When it reaches 100 – 150 individual per ml, about 25% of the culture is harvested and transferred to another tank.
4. COPEPODS

The use of copepod nauplii as live prey for first-feeding marine fish larvae is enabling the culture of many marine fish species with small, difficult to rear larvae. Copepods have higher nutritional value than *Artemia* and are better for meeting the nutritional requirements of marine fish larvae. Copepods are good source of proteins, amino acids, fatty acids, vitamins and minerals. Their typical zigzag movement, followed by a short gliding phase, is an important visual stimulus for fish larvae. The males are smaller in size and lower in abundance. Several candidate species belonging to both the calanoid and the harpacticoid groups have been studied for mass production. Calanoids can be easily recognized by their very long first antennae (16-26 segments), while the harpacticoids have only a short first antennae (fewer than 10 segments).

<table>
<thead>
<tr>
<th>Calanoid</th>
<th>Harpacticoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acartia tonsa</em></td>
<td><em>Tisbe holothuriae</em></td>
</tr>
<tr>
<td><em>Eurytemora affinis</em></td>
<td><em>Tigriopus japonicus</em></td>
</tr>
<tr>
<td><em>Calanus finmarchicus &amp; C. helgolandicus</em></td>
<td><em>Tisbenta elongata</em></td>
</tr>
<tr>
<td><em>Pseudocalanus elongates</em></td>
<td><em>Schizopera elatensis</em></td>
</tr>
</tbody>
</table>

**Collection from wild**

There are several bottlenecks in the culture of copepods. The collection of copepods from natural waters may be the easiest method by sieving wild-plankton from natural waters. These organisms can be collected using zooplankton net (75 μm and 100 μm)
during early morning hours from the coastal waters and wild collection can be obtained during the early morning hours. The collected copepods contain adult copepods, copepodites and copepod nauplii etc. These can be separated into different size groups using mesh net of different sizes in the descending order (500, 350, 200, 100 and 50 μm). By using a light source in darkness, the differenciated copepod stages can be separated from other planktonic and non planktonic. The collected sample can be kept in a glass container which is placed in a dark room. The phototactic movement of copepod makes it to concentrate near the light source. They may be isolated from near the light source using pipette for further culture in larger tanks using algae.
5. ARTEMIA

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp \textit{Artemia} constitute the most widely used food item. It is also called as brine shrimp or sea monkey. The widely used species of \textit{Artemia} is \textit{A. salina}. The females can produce eggs either as a result of mating or via parthenogenesis. The thin shelled eggs hatches immediately and thick shelled eggs can remain in dormant state and forms cysts that float at the water surface and that are thrown ashore by wind and waves. These cysts are metabolically inactive and do not further develop as long as they are kept dry. Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo resumes its interrupted metabolism. After about 20 h the outer membrane of the cyst bursts and the embryo appears, surrounded by the hatching membrane. While the embryo hangs underneath the empty shell, the development of the nauplii is completed and within a short period of time the hatching membrane is ruptured and the free-swimming nauplii is born.

Procedure for hatching \textit{Artemia} cysts in hatchery

\textit{Artemia} cysts are hatched into nauplii following the standard procedure involving the following steps

1. **Hydration of cyst**

\textit{Artemia} cysts are put into container containing low salinity water or freshwater at 25\textdegree C for 1 hour with aeration. After an hour, hydrated cysts are filtered through 100-
125µm blotting silk cloth.

2. Decapsulation

a. Using Sodium hypochlorite

Hydrated cysts are kept in a beaker filled with 5% sodium hypochlorite solution @ 15 ml for every one gram cyst. In order to prevent the temperature rising above 30°C, the beaker containing the hydrated cyst and sodium hypochlorite are kept in a trough containing cool water or ice. In about 10 minutes, chorion of hydrated cysts gets dissolved and is then filtered through a 100 µm blotting silk cloth.

b. Using Calcium hypochlorite

The scoop net containing hydrated cysts are kept in a bucket filled with water containing calcium hypochlorite having 200-250 ppm of chlorine. In about 10 minutes, chorion of hydrated cysts gets dissolved and is then filtered through a 100 µm blotting silk cloth.

Note: The decapsulated cysts should be thoroughly washed in tap water or seawater for about 10 minutes until no chlorine smell can be detected. To ensure complete removal of chlorine, the cysts are dipped in 0.1% sodiumthiosulphate solution.

3. Hatching/ Incubation of decapsulated cyst

Decapsulated artemia cysts are hatched in a cylindroconical FRP jar having transparent bottom with continuous aeration. Decapsulated cysts are stocked @ 0.5 to 1 gram per litre of seawater. The optimum water quality parameters required
for hatching of cysts is 27-30°C temperature, 7.5-8.5 pH, 25-35 ppt salinity, 1000-2000 lux light and saturated dissolved oxygen concentration

4. **Harvesting of nauplii**

The decapsulated cysts hatch out after a period of 12-24 hrs. Harvesting is done by taking advantage of phototactic movement of *Artemia* nauplii. The top of the cylinder is closed with a lid and illumination is provided at the bottom transparent part. After 5 minutes, the nauplii gets accumulated at the bottom. The outlet of the tank at the bottom is opened and water is sieved through 100-120 µm net to harvest the hatched out nauplii.
6. MOINA MICRURA

Moina is a transparent zooplankton with thin, flexible and collapsible exoskeleton. The length of the female body varies from 0.4 mm to 1.65 mm. The body consists of a large head, trunk and post abdomen. The head is usually smoothly rounded dorsally with no rostrum. A single large spherical compound eye is located at the apex of the head. The ventral part of the head bears snot like labrum, dorsal to which is a pair of mandibles. Antennules are fairly long. The trunk is enclosed in a two valved carapace and the abdomen is slightly protruding posteriorly beyond the carapace. The large second antenna which is the main swimming organ is a biramous structure with a stout basal joint from which the two branches arise. The five pairs of trunk limbs are enclosed within a ventral filter chamber of the carapace. The post abdomen bears a pair of long abdominal setae. The anus is not terminal and the conical portion beyond anus ends in a strong anal claws. Moina can be collected from brackishwater channels, pools and freshwater ponds. A stock culture can be built up from a single parthenogenetic female. The adult releases young ones at the rate of 10-12 nos./day and the young ones become adult in 18-24 hours and begin to reproduce. It is possible to obtain 42,000 nos. within 12 days from a single female.

Stock culture:

- Collect Moina from freshwater ponds with help of 250-500 micron mesh scoop net. Dilute the sample by adding clear water and examine under a microscope to pick up Moina with help of a dropper
- Inoculate Moina @ 1no/ 10 ml of filtered water in a 20 ml glass tube
• Feeding can be done with yeast @ 200 no/ml or Chlorella @ $10 \times 10^6$ cells/ml
• Each gravid Moina produces 8 to 10 offsprings in 18-24 hrs

**Mass culture:**

• Fertilize the tank (1 tonne) with single super phosphate, groundnut oil cake and urea @ 20 ppm, 75 ppm and 8 ppm respectively
• Freshwater Chlorella sp. is inoculated on the same day with a cell concentration of 30-50 million/ml (5-10 litre/tonne)
• Inoculate Moina @ 40-50 no/litre once algal bloom develops
• Within a week it will reach a density of 20000-25000 no/litre
• At this stage 30 to 50% of the population can be harvested and replaced by freshwater with proportional amount of the above fertilizer or with Chlorella from a separate tank
• Harvesting can be done daily morning or evening, when they swarm at the surface by a zooplankton net
7. DAPHNIA PULEX

Adult measures about 0.2-3 mm in length. Body is not distinctly segmented and is enclosed in a shell like structure referred to as Carapace. Head is single with a compounded eye. Sometimes the anterior portion of head is pointed to form a helmet. Two antennas present. One is attached to head and the longer one is used for swimming. Mouth is located in the junction between head and body. Mandibles present. Intestine ends at the anus region. Thorax forms the central portion and bears 4 to 6 pairs of flattened legs. Brood chamber is located between the body wall and dorsal surface of the carapace. It reproduces parthenogenetically. Young ones are released in small batches. Daphnia feeds on algae, bacteria, fungi and organic debris.

Culture:

- Stock culture of Daphnia is fed with yeast @ 200 mg/litre or Chlorella at a cell density of $10^6$ cells/ml
- The culture tanks are first fertilized with groundnut oilcake, single super phosphate and urea @ of 75, 20 and 8 mg/litre respectively
- After fertilization, chlorella is added to the tank @ of $10^6$ cells/ml
- Inoculate Daphnia @ 40-50 individuals/litre after 3-4 days
- After 6-7 days of inoculation, a density of 20000-25000 individuals per litre is reached
8. INFUSORIA

Infusoria is a collective term for minute aquatic creatures such as ciliates, euglenoids, protozoa, unicellular algae and small invertebrates. It is generally found in green water ponds, freshwater bodies and brackishwater bodies. It mainly feeds on bacteria, algae, flagellates and on debris. It reproduces by sexually by conjugation and asexually by binary fission. The freshwater infusorians are used for the early stages of freshwater ornamental fishes.

Important infusoria in aquaculture- Paramecium and Stylonychia: Freshwater species

Fabrea salina and Euplotes spp.: Marine species

Culture methods for freshwater Infusoria

Culture by banana peelings:

Keep 2 to 3 banana peelings in a big jar or glass aquarium filled with filtered freshwater. For an aquarium containing about 50 litre of water. Cover the container with cloth. It will prevent the entry of mosquitoes and files, but will allow entry of air. Keep the container in a cool place where natural light is available. In a day or two, the water will turn milky and may also emit foul smell. This is due to the multiplication of a large number of bacteria causing decay of banana peelings. A film of slime will be formed on the water surface. In about 4 to 5 days, the water will turn clear, becoming transparent with light yellowish colour. This is because of the floating spores of infusoria in the air, which have
settled on the water and are feeding upon the bacteria and multiply in large numbers. Subsequently, the film of the slime on the water surface breaks up and disintegrate. The culture is now ready for feeding the early stages of fish larvae. Once the culture reaches its peak density, it must be harvested, if not, the density will suddenly fall due to lack of space and oxygen depletion. The culture will sustain upto 2 to 3 weeks if regular harvesting is done and a few drops of milk is added to it regularly.

**Culture by using hay infusion:**

Take dry hay (straw) into a pan and pour boiling water over it. Transfer the hay together with water to a jar or aquarium. After this, repeat the process as described under banana pealing method above. By using lettuce leaves Aquarium set-up with banana peelings for culture of infusoria. In place of hay, lettuce leaves can also be used for the culture of infusoria. But in this case, pouring of boiling water will not be required, plain water will do. After this, follow the same process as stated for banana peelings above.

**Culture by using milk:**

Add a teaspoon of skimmed milk or two pinches of milk powder to the culture tank filled with 50 litre of water. Thereafter, follow the same process as described for using banana peelings.

**Culture by using apple snail:**

Keep the snail, Ampullaria globosa in an aquarium and feed with lettuce leaves or water plants such as Hydrilla. The droppings (faeces) of snail will contain half digested leaves and will give rise to a large population of infusoria, when added to the water.
Marine infusorians

*Fabrea salina*

It is found in stagnant marine water conditions. It is a floating pelagic form. It is very nutritious and delicious food for early stages of fish and crustacean. Size ranges from 40-60 microns. It reproduces in wild at temperature of 5-40 °C and a salinity upto 100 ppt. It can also be cultured using yeast, fermented wheat flour, fermented rice extract. It has been found that a temperature of 37-40°C and salinity of 45-60 ppt is suitable for culture under controlled conditions. The young ones reproduce after 20-22 hrs. A maximum density of 100 individuals/ml can be obtained in 6-7 days.

*Euplotes* spp.

It is a saline benthic tiny organism found in stagnant water where there is accumulation of decaying organic matter. It feeds on bacteria and can be used to feed early stages of fish. It can be cultured in controlled conditions to obtain a density of 15000 individuals/ml after 5-6 days. Temperature between 20-25°C and salinity of 20-35 ppt is suitable for its culture.
Annexure 1. Estimation of microalgae

Haemocytometer method

- Clean the slide before you load the sample by rinsing the slide and cover slip with 70-95% ethanol. Air dry or gently wipe the slide and cover slip with lens paper.
- Using a micropipette, add 10 μl (or 1 drop) to the v-shaped groove of the hemacytometer.
- Place the hemacytometer under a compound microscope to visualize the grid.
- Count the number of cells in the 4 outer squares (Squares indicated by arrows in Figure 1).
- Count the cells in a square following the procedure shown in Figure 2.

Cell concentration per milliliter = Total cell count in 4 squares x 2500 x dilution factor

If 1ml of sample is diluted with 10ml of distilled water, dilution factor is 10.
Annexure 2. Estimation of Zooplankton

Sedgwick-Rafter method

- Filter water sample through a plankton net of bolting silk no 25
- Transfer the sample to centrifuge tube and add a few drops of 5% of formalin
- Centrifuge sample for 15 minutes and place the sample into Sedgwick rafter counting cell
- Observe under microscope

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N = \frac{a \times c \times 1000}{L}
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$N$, the number of plankton per litre of water

$a$, the average of plankton in all counts in a counting cell of 1 mm capacity

$c$, the volume of original plankton filtered in ml

$L$, the volume of water sample filtered in Litres

Sedgwick rafter counting cell