

CULTURE OF FISH FOOD ORGANISMS (1+1)

Overview

Though this field of fish food organisms forms a small part of the aquatic systems but due to increasing commercial interest in the hatchery development of aquatic fin fish and shell fish for human needs lead to increasing urgent need for more development of live feed for providing feed for a larval stages of fish. Fish food organisms are essential for the developmental stages of many aquatic organisms. They are very important in the critical phases of fin fishes and shellfishes for their better survival, growth. They are directly consumed by first feeding phase of fin fishes depending on their mouth size and inclination towards a chosen food. They are rich in proteins, carbohydrates and essential fatty acids. Since cultivable aquatic organisms are fragile and delicate at the hours of first feeding stage, it is very important to provide right feed that they need for proper nourishment for better growth and survival since it is the most critical phase of their life. As a result, the live food organisms are called as living capsules of nutrition for the larvae of fish and prawns. Live feed is one of the main components for the successful operation of aqua-hatcheries mainly in prawns and shrimps. The availability of good quality and quantity of live foods contribute to successful operation of aqua-hatchery both for intensive and small scale operations. These chapters have given a detailed knowledge regarding the plethora of fish food organism available.

Unit 1 - Introduction

Expected Learning Outcome

- It deals with the introduction to zooplanktons as fish food organisms and their culture techniques.

1.1. Introduction to Fish food organisms

Fish food organisms are microscopic organisms naturally present in the aquatic environment as primary food for the larvae of fin fish and shell fish. Their size ranges from a micron to few millimetres. They are tiny forms which suits the mouth size of all kinds of fin fish and shell fish larvae. Eggs feed on yolk to become larvae. In the transitional phase from eggs to larvae they are delicate, they shift in size, gut changes along with digestive apparatus. Much of their protein digestion takes place in the hind gut epithelial cells. To overcome this fish food organisms play a vital role. The major expansion of finfish and shell fish aquaculture around the world is attributed to the development of standard mass production techniques of live feed. Live feeds are able to swim in water column and are thus constantly available to the larvae. Fish food organisms are mainly divided into protozoans, bacteria, phytoplankton, zooplanktons crustaceans and worms. These fish food organisms are cultured based on their biology, reproduction and ecology, nutrition and water requirements. They are obtained by the use of chemical fertilizers, cattle or poultry manure and vermicompost.

1.2. Zooplanktons

Animals which are carried along by the moving waters are known as plankton. Some have minimal independent movement but is negligible compared to water. They are at the mercy of the water currents and drift about passively. Zooplanktons consist of invertebrates and larvae and immature stages of both vertebrates and invertebrates. Important among them being rotifers, cladocerans, copepods, worms larvae, annelids and crustaceans. Some of the important zooplanktons members and their usefulness as fish food organisms are given in detailed sequence below

Unit 2 - Rotifers

Expected Learning Outcome

Rotifers are microorganisms that are readily found in aquatic and semi-aquatic habitats. Some are marine inhabitants, however most species are freshwater organisms. They account for more than 90% of the zooplankton production in some freshwater systems and as such are a vital link in the food chain for most aquatic life. Therefore this chapter gives an insight into the process of culture and its uses.

2.1. Rotifer classification

Kingdom - Animalia
Sub-kingdom - Eumetazoa
Superphylum - Platyzoa
Phylum – Rotifera

2.2. Introduction

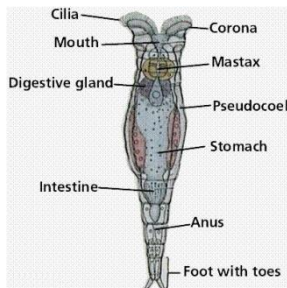
The rotifers (commonly called wheel animalcules) make up a phylum of microscopic and near-microscopic pseudocoelomate animals. Rotifers are microorganisms (100 to 2500 microns) that are readily found in aquatic and semi-aquatic habitats. Some are marine inhabitants, however most species are freshwater organisms. Currently there are over 1000 described species. They account for more than 90% of the zooplankton production in some freshwater systems and as such are a vital link in the food chain for most aquatic life. Rotifers are known to have a very high reproductive rate, reaching population densities of 1000 or more individuals per litre of water. Most rotifers are around 0.1–0.5 mm long (although their size can range from 50µm to over 2 millimeters), and are common in freshwater environments. Some rotifers are free swimming and truly planktonic, others move by inchworming along the substrate, and some are sessile, living inside tubes or gelatinous holdfasts that are attached to a substrate. Rotifers are an important part of the freshwater zooplankton, being a major food source and with many species also contributing to the decomposition of soil organic matter.



Rotifer

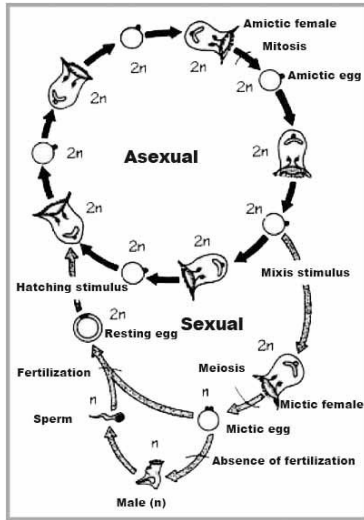
2.3. Biology and life history of Rotifers

The body of rotifers is differentiated into 3 distinct parts head trunk and foot. Head carries the rotary organ corona due to which it gets its name. The retractable corona provides locomotive capabilities and whirling water movement which facilitates their nutrition. The trunk contains the digestive tract, the excretory organs and genital organs. The epidermis contains densely packed keratin proteins known as the lorica. Mastax is the characteristic organ in rotifers which help in grinding of the ingested particles. The shape of the lorica and the profile of the spines help us differentiate the different species and morphotypes. Mass production of rotifers is achieved by by encouraging them to reproduce asexually, since sexual reproduction results in male and resting eggs.



Rotifer morphology and life cycle

Rotifers are dioecious and reproduce sexually or parthenogenetically. They are sexually dimorphic, with the females always being larger than the males. The female reproductive system consists of one or two ovaries, each with a vitellarium gland that supplies the eggs with yolk. Males do not usually possess a functional digestive system, and are therefore short-lived, often already being sexually fertile at birth. Fertilisation is internal. The egg secretes a shell, and is attached either to the substratum, nearby plants, or the female's own body. A few species, such as *Rotaria*, are ovoviviparous, retaining the eggs inside their body until they hatch. Sessile species, however, are born as free-swimming larvae, which closely resemble the adults of related free-swimming species. Females grow rapidly, reaching their adult size within a few days, while males typically do not grow in size at all. The life span of rotifers has been estimated to be between 3.4-4.4 days at 25°C. Generally the larvae becomes adult after 0.5-1.5 days and females there after start to lay eggs approximately every four hours.



2.3.1. Strain differences

Rotifer life history and population dynamics are the driving factors of culture success. Successful mass culture of the marine rotifers *Brachionus plicatilis* (former L-type) and *B. rotundiformis* (former S-type) is one of the milestones of marine fish culture. *B. plicatilis* was first identified as a pest in the pond culture of eels but soon the researchers realized that it could be used suitably as live food.

There are two different strains of *Brachionus* based on lorical length,

	<i>Brachionus plicatilis</i> (S type)	<i>Brachionus plicatilis</i> (Ltype)
Lorica length	100-210 (160 μm)	130-340 (239 μm)
Spines	Points	Angled
Growth temperature (Optimal)*	28-35 °C	18-25 °C

2.3.2. General culture conditions

Intensive production of rotifers is usually performed in batch culture within indoor facilities; the latter being more reliable than outdoor extensive production in countries where climatological constraints do not allow the outdoor production of microalgae. Basically, the production strategy is the same for indoor or outdoor facilities, but higher starting and harvesting densities enable the use of smaller production tanks (generally 1 to 2 m³) within intensive indoor facilities.

Salinity:

Although *Brachionus plicatilis* can withstand a wide salinity range from 1 to 97 ppt, optimal reproduction can only take place at salinities below 35 ppt.

Temperature:

Rearing rotifers at high temperature enhances the cost for food. This is essential for the maintenance of good water quality, and to avoid periods of overfeeding or starvation which are not tolerated at suboptimal temperature levels. For example, at high temperatures starving animals consume their lipid and carbohydrate reserves very fast. Rearing rotifers below their optimal temperature slows down the population growth considerably.

Dissolved oxygen:

Rotifers can survive in water containing as low as 2 mg.l⁻¹ of dissolved oxygen. The level of dissolved oxygen in the culture water depends on temperature, salinity, rotifer density, and the type of the food.

pH:

Rotifers live at pH-levels above 6.6, although in their natural environment under culture conditions the best results are obtained at a pH above 7.5.

Ammonia (NH₃):

The NH₃/NH₄⁺ ratio is influenced by the temperature and the pH of the water. High levels of un-ionized ammonia are toxic for rotifers but rearing conditions with NH₃ concentrations below 1 mg.l⁻¹ appear to be safe

2.3.3. Culture procedures

2.3.3.1. Stock culture of rotifers

Culturing large volumes of rotifers on algae, baker's yeast or artificial diets always involves some risks for sudden mortality of the population. Technical or human failures but also contamination with pathogens or competitive filter feeders is the main cause for lower reproduction which can eventually result in a complete crash of the population. Relying only on mass cultures of rotifers for re inoculating new tanks is too risky an approach. In order to minimize this risk, small stock cultures are generally kept in closed vials in an isolated room to prevent contamination with bacteria and/or ciliates. These stock cultures which need to generate large populations of rotifers as fast as possible are generally maintained on algae.

The rotifers for stock cultures can be obtained from the wild, or from research institutes or commercial hatcheries. However, before being used in the production cycle the inoculum should first be disinfected. The most drastic disinfection consists of killing the free-swimming rotifers but not the eggs with a cocktail of antibiotics (e.g. erythromycin 10 mg.l⁻¹, chloramphenicol 10 mg.l⁻¹, sodium oxolinat 10 mg.l⁻¹, penicillin 100 mg.l⁻¹, streptomycin 20 mg.l⁻¹) or a disinfectant. The eggs are then separated from the dead bodies on a 50 µm sieve and incubated for hatching and the offspring used for starting the stock cultures. However, if the rotifers do not contain many eggs (as can be the case after a long shipment) the risk of losing the complete initial stock is too big and in these instances the rotifer should be disinfected at sublethal doses; the water of the rotifers being completely renewed and the rotifers treated with either antibiotics or disinfectants. The treatment is repeated after 24 h in order to be sure that any pathogens which might have survived the passage of the intestinal tract of the rotifers are killed as well. The concentration of the disinfection products differs according to their toxicity and the initial condition of the rotifers. Orientating concentrations for this type of disinfection are 7.5 mg.l⁻¹ furazolidone, 10 mg.l⁻¹ oxytetracycline, 30 mg.l⁻¹ sarafloxacin, or 30 mg.l⁻¹ linco-spectin.

2.3.3.2. Upscaling of stock cultures to starter cultures

The upscaling of rotifers is carried out in static systems consisting of erlenmeyers of 500 ml placed 2 cm from fluorescent light tubes (5000 lux). The temperature in the erlenmeyers should not be more than 30°C. The rotifers are stocked at a density of 50 individuals.ml⁻¹ and fed 400 ml freshly-harvested algae (Chlorella 1.6.10⁶ cells.ml⁻¹); approximately 50 ml of algae being added every day to supply enough food. Within 3 days the rotifer concentration can increase to 200 rotifers.ml⁻¹ (Fig. 3.5.). During this short rearing period no aeration is applied.

Once the rotifers have reached a density of 200-300 individuals.ml⁻¹ they are rinsed on a submerged filter consisting of 2 filter screens. The upper mesh size (200 µm) retains large waste particles, while the lower sieve (50 µm) collects the rotifers. If only single strainers are available this handling can be carried out with two separate filters. Moreover, if rinsing is performed under water the rotifers will not clog and losses will be limited to less than 1%.

The concentrated rotifers are then distributed in several 15 l bottles filled with 2 l water at a density of 50 individuals.ml⁻¹ and a mild tube aeration provided. In order to avoid contamination with ciliates the air should be filtered by a cartridge or activated carbon filters. Fresh algae (Chlorella 1.6 × 10⁶ cells.ml⁻¹) are supplied daily. Every other day the cultures are cleaned (double-screen filtration) and restocked at densities of 200 rotifers.ml⁻¹. After adding algae for approximately one week the 15 l bottles are completely full and the cultures can be used for inoculation of mass cultures.

2.3.3.3. Mass production on algae

Undoubtedly, marine microalgae are the best diet for rotifers and very high yields can be obtained if sufficient algae are available and an appropriate management is followed. Unfortunately in most places it is not possible to cope with the fast filtration capacity of the rotifers which require continuous algal blooms. If the infrastructure and labor is not limiting, a procedure of continuous (daily) harvest and transfer to algal tanks can be considered. In most places, however, pure algae are only given for starting up rotifer cultures or to enrich rotifers.

Batch cultivation is probably the most common method of rotifer production in marine fish hatcheries. The culture strategy consists of either the maintenance of a constant culture volume with an increasing rotifer density or the maintenance of a constant rotifer density by increasing the culture volume. Extensive culture techniques (using large tanks of more than 50 m³) as well as intensive methods (using tanks with a volume of 200-2000 l) are applied. In both cases large amounts of cultured microalgae, usually the marine alga Nannochloropsis, are usually inoculated in the tanks together with a starter population containing 50 to 150 rotifers.ml⁻¹.

2.3.4. Enhancement of nutritional value of cultured rotifers

- Techniques for (n-3) HUFA enrichment
- Techniques for vitamin C enrichment
- Techniques for protein enrichment

2.3.4.1. Techniques for (n-3) HUFA enrichment

Algae:

The high content of the essential fatty acid eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3) in some microalgae (e.g. 20:5n-3 in *Nannochloropsis occulata* and 22:6n-3 in *Isochrysis galbana*) have made them excellent live food diets for boosting the fatty acid content of the rotifers. Rotifers submerged in these algae (approximately 5.106 algae.ml⁻¹) are incorporating the essential fatty acids in a few hours time and come to an equilibrium with a DHA/EPA level above 2 for rotifers submerged in *Isochrysis* and below 0.5 for *Tetraselmis*

Formulated feeds:

Oil emulsions: One of the cheapest ways to enrich rotifers is by using oil emulsions. Although home-made emulsions can be prepared with egg lecithin and fish oils Commercial emulsions are generally more stable and have a selected HUFA composition.

2.3.4.2. Techniques for vitamin C enrichment

The vitamin C content of rotifers reflects the dietary ascorbic acid (AA) levels both after culture and enrichment Enrichment of rotifers with AA is carried out using ascorbyl palmitate (AP) as a source of vitamin C to supplement the boosters. AP is converted by the rotifers into active AA up to 1700 mg.g⁻¹ DW after 24 h enrichment using a 5% AP (w/w) emulsion.

2.3.4.3. Techniques for protein enrichment

The high levels of proteins allow the cultures to continue to grow and to develop during the enrichment period. Normally it is used in the same way as an oil emulsion (blended in a kitchen blender) and distributed in the tank at a concentration of 125 mg.l⁻¹ seawater at two time intervals of 3 to 4 hours.

Unit 3 - Cladocerans

Expected Learning Outcome

Cladocera or cladocerans are small crustaceans commonly called water fleas, part of the Class Branchiopoda. In contrast to the prosperity of cladocerans in freshwater systems, with more than 600 recorded species, marine cladocerans show a very low diversity, with only eight truly marine species. Therefore this notes is expected to give detailed understanding of the process involved in the production of Cladocerans.

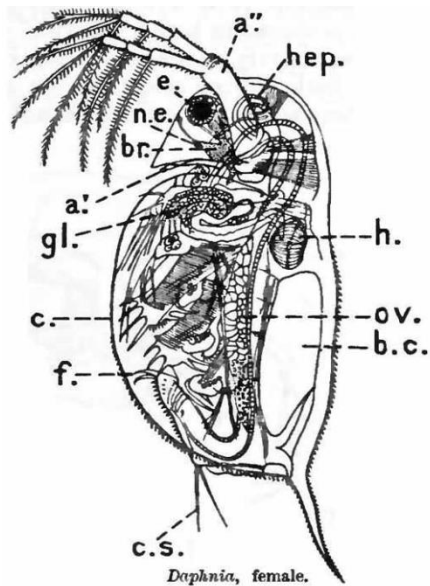
3.1. Cladocerans classification

Kingdom - Animalia
Phylum - Arthropoda
Subphylum - Crustacea
Class - Branchiopoda
Subclass – Phyllopoda
Order – Cladocera

3.2. Biology and nutritional value of cladocerans

Cladocera or cladocerans are small crustaceans commonly called water fleas, part of the Class Branchiopoda. In contrast to the prosperity of cladocerans in freshwater systems, with more than 600 recorded species, marine cladocerans show a very low diversity, with only eight truly marine species. These 8 species can be divided in two distinct groups, the Podonidae (represented by the genera *Evadne*, *Pleopis*, *Podon* and *Pseudevadne*), and the Sidaidae with only one species, *Penilia avirostris*).





- Daphnia*, female.
- a', antennule ;
 - a'', antenna ;
 - b,c, brood-chamber ;
 - br, brain ;
 - c, margin of carapace ;
 - c.s, caudal setae ;
 - e, compound eyes coalesced into one ;
 - f, furca ;
 - gl, maxillary gland ;
 - h, heart ;
 - hep, hepatic diverticulum of gut ;
 - n.e, nauplius eye ;
 - ov, ovary.

3.2.1.Daphnia

The most commonly known genus is *Daphnia* (freshwater fleas), which is the most researched in this group, and *Moina*. Cladocerans are free-swimming organisms, and most orientate themselves with dorsal side up. The head is typically separated from the body by a deep indentation, but also may not be separated. It projects forward as a beak or rostrum. On the forehead is an unpaired compound eye, a result of two fused eyes, and, in most species, a simple naupliar eye.

3.2.1.1.Daphnia classification

Kingdom: Animalia
 Phylum: Arthropoda
 Subphylum: Crustacea
 Class: Branchiopoda
 Order: Cladocera
 Family: Daphniidae
 Genus : *Daphnia*

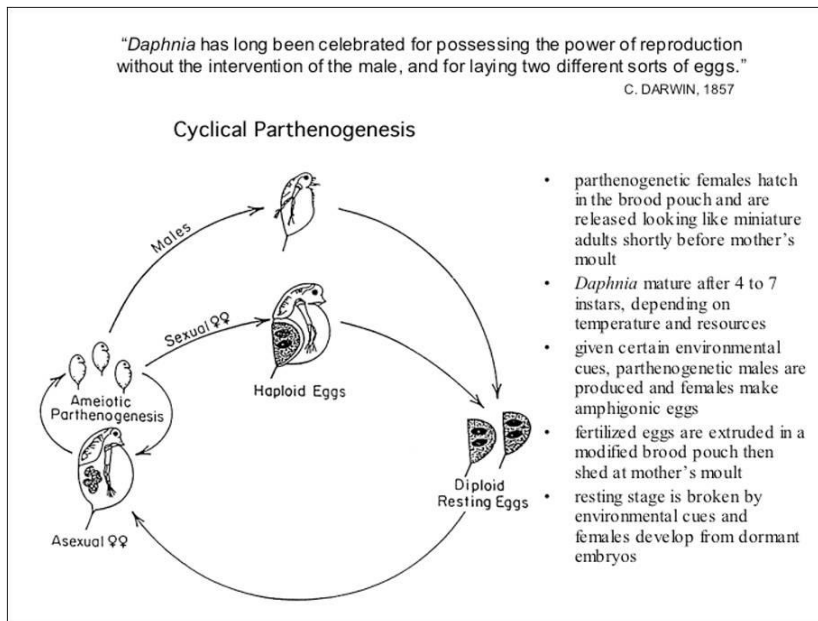
3.2.1.2.Nutritional value of Daphnia

The nutritional value of *Daphnia* depends strongly on the chemical composition of their food source. However, since *Daphnia* is a freshwater species, it is not a suitable prey organism for marine organisms, because of its low content of essential fatty acids, and in particular (n-3) HUFA. Furthermore, *Daphnia* contains a broad spectrum of digestive enzymes such, as proteinases, peptidases, amylases, lipases and even cellulase, that can serve as exoenzymes in the gut of the fish larvae.

3.2.1.4.Biology and life cycle of Daphnia

Daphnia is a frequently used food source in the freshwater larviculture (i.e. for different fish species) *Daphnia* are

small crustaceans that are almost exclusively living in freshwater. The head projects ventrally and somewhat posteriorly in a beak-like snout. The trunk appendages (five or six pairs) are flattened, leaf-like structures that serve for suspension feeding (filter feeders) and for locomotion. Species of the genus *Daphnia* are found from the tropics to the arctic, in habitats varying in size from small ponds to large freshwater lakes. The adult size is subjected to large variations; when food is abundant, growth continues throughout life and large adults may have a carapace length twice that of newly-mature individuals. Normally there are 4 to 6 Instar stages; *Daphnia* growing from nauplius to maturation through a series of 4-5 molts, with the period depending primarily on temperature (11 days at 10°C to 2 days at 25°C) and the availability of food. *Daphnia* species reproduce either by cyclical or obligate parthenogenesis and populations are almost exclusively female. Eggs are produced in clutches with parthenogenetic eggs produced asexualy and result in females, but in some cases males can appear. Factors, such as change in water temperature or food deprivation as a result of population increase, may induce the production of males. The fertilized eggs are large, and only two are produced in a single clutch (one from each ovary), and are thick-shelled: these resting or dormant eggs being enclosed by several protective membranes, the ephippium.



3.2.2.Moina

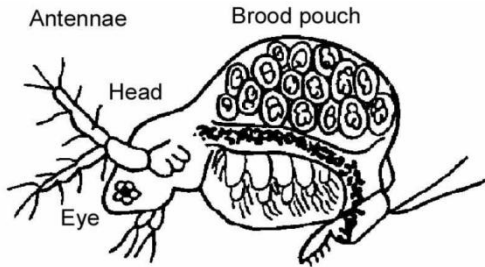


Various species includes *Moina affinis*, *M. australiensis*, *M. belli*, *M. brachiata*, *M. brachycephala*, *M. flexuosa*, *M. hartwigi*, *M. hutchinsoni*, *M. macrocopa*, *M. micrura*, *M. minuta*, *M. mongolica*, *M. rectirostris*, *M. reticulata*, *M. salina*, *M. tenuicornis*. Adult *Moina* have an average size (700-1,000 µm) and are approximately the same size or only slightly larger than adult rotifers and smaller than newly-hatched brine shrimp. *Moina* are ideally suited for feeding freshwater fish fry as they have a longer life span. *Moina micrura* grown in ponds, fertilized with mostly chicken manure or, less frequently, with pig manure, are used as the sole food for fry of many ornamental tropical fish species, with a 95-99% survival rate.

3.2.2.1.Moina classification

Kingdom:Animalia
Phylum:Arthropoda
Subphylum:Crustacea
Class:Branchiopoda
Order:Cladocera
Family:Moinidae
Genus:Moina

.2.2.2.Life Cycles of Moina



The reproductive cycle of Moina has both a sexual and asexual phase. Normally, the population consists of all females that are reproducing asexually. Under optimum conditions, Moina reproduce at only 4-7 days of age, with a brood size of 4-22 per female. Broods are produced every 1.5-2.0 days, with most females producing 2-6 broods during their lifetime. Under adverse environmental conditions, males are produced and sexual reproduction occurs resulting in resting eggs (ephippia). The stimuli for the switch from asexual to sexual reproduction in populations of Moina is an abrupt reduction in the food supply, resulting in an increase in resting egg production. However, it is advantageous to keep the population well fed and in the asexual mode of reproduction, since fewer progeny are produced with resting eggs.

Unit 4 - Copepods

Expected Learning Outcome

This gives an exposure to detail study of copepods. Copepods are common zooplankton both in freshwater and in brackishwater. Their advantages being wide range of body size within and between species. They are natural feeds for larvae and juveniles of many finfish and crustaceans. The early stage nauplii and copepodites can be extremely useful as initial prey for species that have very small mouth gape at first feeding.

4.1.Introduction

Copepods are common zooplankton both in freshwater and in brackishwater. Their advantages being wide range of body size within and between species. They are natural feeds for larvae and juveniles of many finfish and crustaceans. The early stage nauplii and copepodites can be extremely useful as initial prey for species that have very small mouth gape at first feeding. In the wild, most marine larvae feed on copepod eggs and nauplii during the first few weeks of life. Since a number of larval fish cannot be reared using rotifers as the first feed but have to be reared on either laboratory reared or wild caught copepod nauplii. Adult copepods range from 0.5 to 5.0 mm. The larval stages consist of six naupliar and six copepodite stages.



Copepod

4.2.Copepod classification

Kingdom :Animalia
Phylum :Arthropoda

Sub-phylum : Crustacea
Class : Maxillopoda
Sub-class : Copepoda

4.3. Biology and life cycle of copepods

Most adult copepods have a length between 1 and 5 mm. The body of most copepods is cylindrical in shape, with a wider anterior part. The trunk consists of two distinct parts, the cephalothorax (the head being fused with the first of the six thoracic segments) and the abdomen, which is narrower than the cephalothorax. The head has a central naupliar eye and uniramous first antennae that are generally very long. Planktonic copepods are mainly suspension feeders on phytoplankton and/or bacteria; the food items being collected by the second maxillae. The male copepods are commonly smaller than the females and appear in lower abundance than the latter. The eggs hatch as nauplii and after five to six naupliar stages (moltings), the larvae become copepodites. After five copepodite moltings the adult stage is reached and molting is ceased. A diapause stage is present in the development of the copepods so as to survive adverse environmental conditions, such as freezing. The major diapause habitat is the sediment, although a minor part of the diapausing individuals may stay in the planktonic fraction, the so-called "active diapause". Harpacticoid copepods are less sensitive and more tolerant to extreme changes in environmental conditions (i.e. salinity: 15-70 g/l; temperature: 17-30°C) than calanoids and thus are easier to rear under intensive conditions. Moreover, harpacticoids have a higher productivity than calanoids and can be fed on a wide variety of food items, such as microalgae, bacteria, detritus and even artificial diets. However, as mentioned previously, care should be taken in this respect as the lipid and (n-3) HUFA composition of the copepods is largely dependent on that of the diet fed.

4.4. Candidate species and culture techniques

The main suborders of copepods found in brackishwater are calanoids (*Acartia*, *Calanus* and *Pseudocalanus* spp.), harpacticoids and cyclopoids. Herbivorous copepods are primarily filter feeders and typically feed on very small particles. But they can feed on larger particles, which gives them an advantage over the rotifers. Copepods can also eat detritus.

Calanoida is an important order of copepods, a kind of zooplankton. They include 43 families with about 2000 species of both marine and freshwater copepods. Calanoid copepods are important in many food webs, taking in energy from phytoplankton and algae and 'repackaging' it for consumption by higher trophic level predators like birds, fishes and mammals. Many commercial fishes are dependent on calanoid copepods for diet in either their larval or adult forms. Baleen whales such as the bowhead whale eat copepods of the genera *Calanus* and *Neocalanus*.

4.5. Culture techniques

A continuous production system for the calanoid copepod *Acartia tonsa* consists of three culture units: basis tanks, growth tanks and harvest tanks. The *Acartia tonsa* are isolated from natural plankton samples or reared from resting eggs onwards. The basis tanks (200 l grey PVC tanks: 1500 x 50cm) are run continuously and the eggs produced are used to adjust population stocks. These tanks are very well controlled and kept under optimal hygienic conditions: using filtered (1 µm) seawater (salinity 35 g/l) and fed with *Rhodomonas* algae. Temperatures are kept at 16-18°C and a gentle aeration from the bottom is provided. Adult concentrations with a ratio of 1:1 males to females are maintained at less than 100 per l by adjusting once a week with stage IV-V copepodites. Approximately 10 l of the culture water is siphoned daily from the bottom of the tanks (containing the eggs), and replaced by new, clean seawater. Eggs are collected from the effluent waters by the use of a 40 µm sieve; production averaging 95,000 eggs/day, and corresponding to a fecundity rate of 25 eggs/ female per day. The basis cultures are emptied and cleaned two to three times per year, by collecting the adults on a 180 µm sieve and transferring them to cleaned and disinfected tanks. Collected eggs are transferred to the growth tanks where maximal densities reach 6000/l. The nauplii start to hatch after 24 h with hatching percentages averaging 50% after 48 h incubation. Initially *Isochrysis* is given at a concentration of 1000 cells.ml/1 and after 10 days a mixture of *Isochrysis* and *Rhodomonas* administered at a concentration of 570 and 900 cells.ml/1, respectively. The generation time (period needed to reach 50% fertilized females) is about 20 days with a constant mortality rate of about 5%/day. After 21 days, the adults are collected using a 180 µm sieve and added either to the basis or harvest tanks. Harvesting tanks are only in use once the fish hatchery starts to operate. Cultures are maintained in 450 l black tanks under the same conditions as described above. Each tank receives a daily amount of 16X10⁸ *Rhodomonas* cells, harvested from bloom cultures. These tanks are emptied and cleaned more regularly than stock tanks. To facilitate the harvesting of solely nauplii or copepodites of a specific stage (depending on the requirements), eggs are harvested daily and transferred to the hatching tanks; the aeration levels within these tanks being increased to maintain 80% oxygen saturation. Nauplii of appropriate size (and fed on *Isochrysis*) are harvested on a 45 µm screen and by so doing cannibalism by the copepod adults is also minimized.

4.5.1. Use of resting eggs

Many temperate copepods produce resting eggs as a common life-cycle strategy to survive adverse environmental conditions. Resting eggs can tolerate drying at 25°C or freezing down to - 25°C and that they are able to resist low temperatures (3-5°C) for as long as 9 to 15 months. These characteristics make the eggs very attractive as inoculum for copepod cultures. Samples of

sediments rich in resting eggs can be stored in a refrigerator at 2-4°C for several months. When needed, the sediment containing the resting eggs is brought in suspension and sieved through 150 µm and 60 µm sieves. The resting eggs is then immersed in the disinfectant, (i.e. FAM-30 or Buffodine); surface-disinfection being needed to eliminate contaminating epibiotic micro-organisms. After disinfection, the eggs are then washed with 0.2 µm filtered sterile seawater and transferred to disinfected culture tanks or stored under dark, dry and cool conditions. Before starting the surface-disinfection procedure attention must be paid to the physiological type of resting eggs. Some marine calanoids are able to produce two kinds of resting eggs, i.e. subitaneous and diapause eggs. Since subitaneous eggs only have a thin vitelline coat covering the plasma membrane, they are more susceptible to disinfectants than the diapause eggs which are enveloped by a complex four-layer structure.

Unit 5 - Infusorians

Expected Learning Outcome

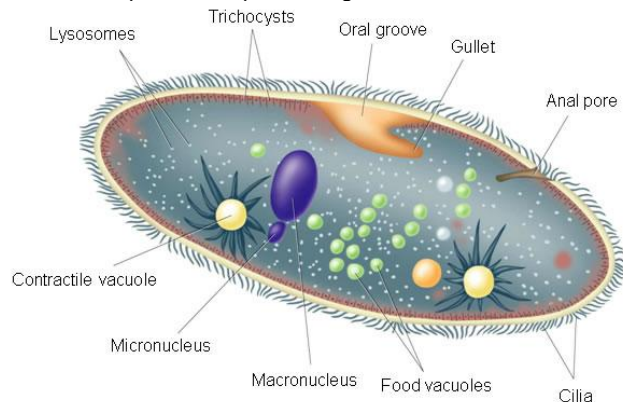
- The student can understand the details regarding Infusorians which can shed light into in depth understanding regarding their culture aspects.

5.1.Introduction to Infusorians

These "animalcules" were first described by Antony Van Leeuwenhoek in his Philosophical Transactions of 1677. The term "infusoria" comes from the practice of steeping substances (usually hay) in water by soaking at temperatures less than the boiling point. Only the largest of the protozoans (infusoria) are large enough to be seen by the naked eye. Most species require a microscope to view. The "green water" or cloudiness of aquarium water is sometimes an indication of an overabundance of infusoria which can be caused by overfeeding. The importance of infusoria to the pisciculturists is with their use as fry food. Their small size, ranging from 25 µm to 300 µm makes them an ideal live food for young fry which have just consumed their yolk sac. This includes ciliates and protozoan's. They obtain holozoic nutrition from bacteria, algae and detritus. They reproduce by binary fission or conjugation. Freshwater species includes Paramecium and Stylonichia and marine water species includes Euplotes (20-35 ppt).

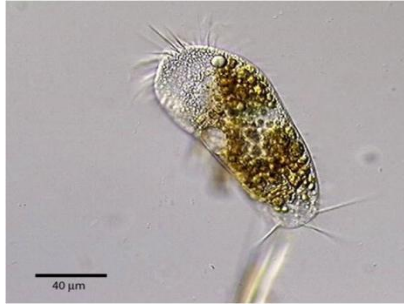
5.1.1.Paramecium

These unicellular organisms are among the most advanced protozoans and are characterized by the presence of many surface cilia (hairs) which are used for swimming and collecting food. These hairs are very fine and are only clearly seen under an electron microscope. Paramecia also have a very distinctive slipper-like appearance and range between 60 µm to 300 µm in length, with most around 150 µm.



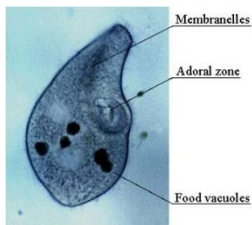
5.1.2.Stylonichia

It is a genus of ciliate, included among the stichotrichs. It is very common in fresh water and soil, found on filamentous algae, surface films, and among particles of sediment. Like its relatives, Stylonychia has cilia grouped into membranelles alongside the mouth and cirri over the body. It is distinguished partly by long cirri at the posterior, usually a cluster of three.



5.1.3. Fabrea salina

The heterotrichous ciliate, Fabrea salina had been reported from diverse environments such as salt marshes and hypersaline lakes.



Culture method of Fabrea salina

Culture can be done using hay, milk, yeast and banana peels (2-3 peels per 50l of mixture). This generates a film of bacteria followed by ciliates. It can be harvested with filter paper.

5.1.4. Euplotes

It is a common, bacteriovorous, hypotrichous ciliate of fresh or salt water.



5.2. General Culture techniques of Infusorians

- Lettuce culture
- Potato culture
- Banana peel culture

5.2.1. Collecting Infusoria

Infusoria can be collected at almost any stagnant pond where plants and/or algae are growing in excess. Jars of the decaying vegetable matter can be collected and brought home along with some of the pond water collected from various places in the pond, i.e. the surface, the middle, and the bottom of the pond. Let it stand for a day or so before transferring small amounts to containers along with the collected pond water which can be stored. Other sources of infusoria are old aquarium filter pads and the water in which cut flowers have been standing for several days.

5.2.2. Culturing Infusoria

Culturing infusoria can be accomplished by soaking substances in previously boiled water for several weeks. Substances that

can be used include hay, banana peels, potato peels, dried beans, lettuce, cabbage, egg yolks, dried blood, spinach, tree leaves and dried aquarium plants. Unchlorinated water like distilled, rain, boiled pond water, and spring water works best for the culture.

5.2.3.Lettuce culture

Place brown, rotting lettuce leaves are placed in a widemouth glass jar. Put in enough to cover the bottom of the jar. Add water that is almost boiling to fill the jar about 3/4 of the way full. Leave this standing, uncovered for 24 hours. Then add 50-100 ml of old aquarium water (from the surface of the aquarium) and cover the jar. After about one week or ten days, the water in the jar should have a heavy growth of infusoria. Once in every ten days a piece of scalded lettuce should be added to keep the culture growing.

5.2.4.Potato culture

Cut a raw white potato into quarter-inch squares and wash thoroughly for use as a medium. Add about sixty of these squares to a gallon of spring water and allow them to stand overnight. Then inoculate the mixture with about one ounce of old culture material and let stand for about ten days. As the culture water is removed for use it can be replaced with boiled pond or spring water.

5.2.5.Banana peel culture

Fill a gallon jar with clean, filtered pond water and add a dried banana skin. After two days the skin will have sunk to the bottom and a heavy bacterial scum should cover the water's surface. At this time a very small quantity of old culture water or old aquarium water is added. After two weeks infusoria will normally be in evidence as the water clears up, and the culture water can then be used as a source of food.

Unit 6 - Microworms

Expected Learning Outcome

- This can give the student a chance to differentiate in detail regarding regular worms and microworms and their necessity in aquaculture.

6.1.Chironomid larvae

The chironomid larvae is known as blood worms due to the presence of hemoglobin in their bodies. Chironomus genus is the largest members among them. The larvae sustain on the organic material contained in the mud and are found in all kinds of still water where there is a deposit of mud and organic debris. The larvae attain a size suitable for feeding purposes in 16-20 Days. Its growth and development can be influenced by numerous environmental factors including temperature and photoperiod, food availability and food quality and quantity. Chironomid larvae are excellent source of protein lipid, vitamins and minerals.



Chironomid larvae of microworms

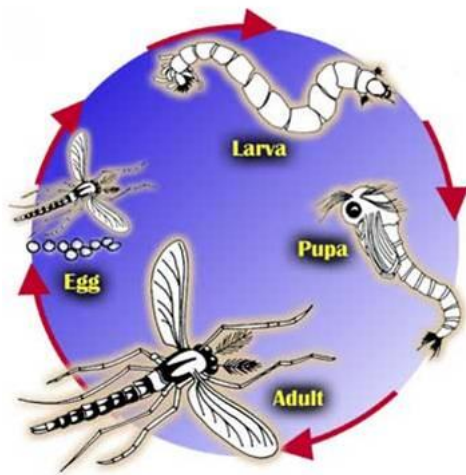
6.1.1.Classification of Chironomid larvae

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Suborder: Nematocera
Infraorder: Culicomorpha
Superfamily: Chironomoidea
Family: Chironomidae
Subfamily: Chironominae

Tribe: Chironomini
Genus: Chironomus

6.1.2. Biology and culture of chironomid larvae

After mating in flight the female releases the eggs while skimming the water surface. Their eggs are laid in gelatinous strings with some species having red larvae. Egg numbers can range from 50 to 700. The eggs sink to the bottom where, under tropical conditions, they hatch in 24~48 hours into the next stage - the larva or aquatic stage. The newly hatched larvae are not more than 1 mm long but they can measure up to 10~25 mm when they reach the last stage of the larva period. The larva stage can last from less than 2 weeks up to 7 weeks depending on temperature. Each larva moults four times before it reaches the pupal stage. The pupa is usually formed in the mud tube and it protrudes its head from one end of the tube so that the breathing filaments can be waved about in the water. The pupae emerge to the surface before swimming. They have high reproductive capacity, each female lay about 2300 eggs in one batch which hatch in about three days at temperature (18-22°C). Those that reach the surface emerge into flying adults after a few hours and immediately fly off to mate, living only a few hours or days. The adults do not feed during their adult existence and mating normally occurs during the night. The entire life cycle can be completed in 2 weeks, although it is common for the life cycle to take longer to complete.



6.1.2.1. Culture techniques

Preparation of the site

Can be cultured on a vegetable field 700-1000m², with 10-13 cm top mud. Clear the weeds on a 30 cm bund and sun dry so as to kill other eggs. Spread poultry manure evenly @1200-1500kg/1000m². Fill with water upto 12 cm. Adults lay eggs at edge of the pond after 8-10 days they hatch to 0.3cm larvae. One cycle consists of 50 day culture. A week before harvest weedicide (mild) and tea seed cake and algal stimulator is used. Water exchange is done to one third.

Harvesting

Starts after 20-25 days at temperature of 25°C. Lower temperature causes slow growth in 30-40 days. Harvesting is done in the morning to avoid high temperature.

6.2. Tubifex classification

Kingdom: Animalia
Phylum: Annelida
Class: Clitellata
Order: Oligochaeta
Family: Naididae
Subfamily: Tubificinae
Genus: Tubifex



6.2.1. Food And Feeding

Tubifex worms feed on decaying organic matter, detritus, vegetable matter which commonly available in segment drains.

6.2.2. Reproduction

Tubifex worm is a hermaphrodite, because it has both male (testes) and female (ovaries) organs in the same animals. These minute reproductive organs attached to the ventral side of the body wall in the celomic cavity. In mature specimens, the reproductive organs are clearly found on the ventral side of the body.

6.2.3. Copulation and cocoon formation

Although the Tubifex worm is a hermaphrodite, the male and female organs become mature at different times; and thus self-fertilization is avoided, and cross fertilization is encouraged. Two mature Tubifex worms undergo copulation by joining their ventral and anterior surfaces together with their anterior ends pointing opposite directions. Thus the spermathecal openings of each Tubifex worm be nearer to the male apertures of another worm. The penial setae of one Tubifex worm penetrate into the tissues of other worm and thus the conjugants are held together. At this stage the sperms of one worm are passed into the spermathecae of the other worm. After copulation the Tubifex worm separate and begin to produce egg cases containing eggs. These egg cases are called cocoons. The cocoon is formed around the clitellum as a soft box-like structure into which the ova and the sperms are deposited. Soon the Tubifex worm withdraws its body from the egg case by its backward wriggling movements. As the worm withdraws, the cocoon completely closes and become a round shaped case. The case contains both sperms and ova. After fertilization several eggs are formed in the cocoon. The fertilized eggs undergo complete development in the cocoon by utilizing the albuminous nutritive fluid for growth. The period of development varies with temperature and lasts for two to three weeks. After complete development the young worms come out of the cocoon.

6.2.4. Culture of Tubifex

Tubifex can be easily cultured on mass scale in containers with 50 to 75 mm thick pond mud at the bottom, blended with decaying vegetable matter and masses of bran and bread. Continuous mild water flow is to be maintained in the container, with a suitable drainage system. After the arrangement of the system, the container is inoculated with Tubifex worm which can be obtained from nearby muddy canals or sewage canals. Within 15 days, clusters of Tubifex worm develop and this can be removed with mud in masses by means of a spade and kept in large wide mouth plastic container. When worms will come to the surface due to lack of oxygen, they are collected and washed under brisk stream of water to remove residual mud attached to the body. Since their guts still may contain mud which they have eaten it is necessary that they are kept long enough under stream of water for the mud to be evacuated from the intestine. After proper cleaning only, the worms are fed to the fish.

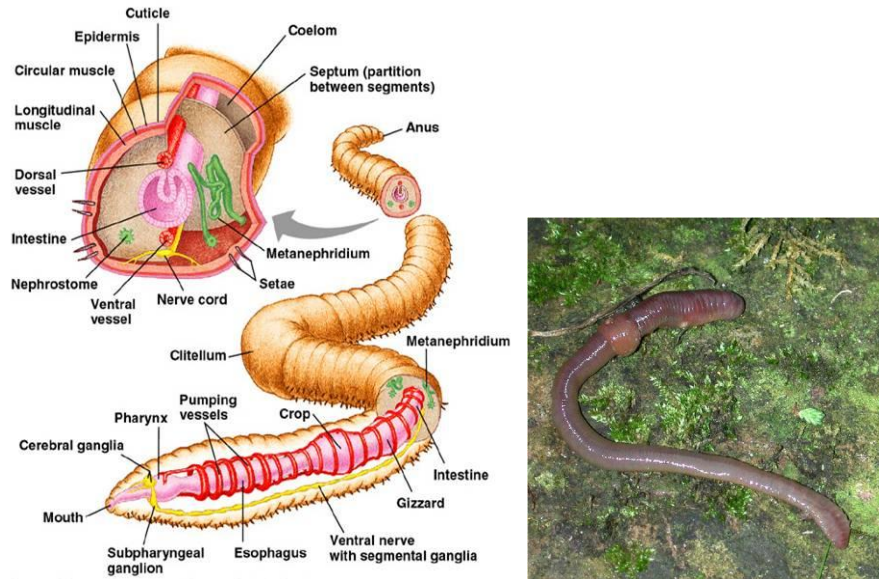
Unit 7 - Earthworms

Expected Learning Outcome

The earth worms are natural tillers of the soil. Apart from helping the agriculture they serve as fish food also. Here the student can have a first hand information regarding its suitability in culture.

7.1. Earthworm

Total 500 species and 13 species are found in India. The cultivable species includes *Edulis engeniae*, *Eisenia fetida*, *Peryonx excuvatus*.



7.1.1. Classification

Kingdom: Animalia
 Phylum: Annelida
 Class: Clitellata
 Order: Haplotaxida
 Suborder: Lumbricina

7.1.2. Culture method

It can be cultured using box made from wood cement bricks or plastic. Care should be taken that there are no holes in the box and they must be placed in a cool place with plenty of shade. Box dimension should be 1mX1mX0.3m depth. Prepare a layer of saw dust, sand and top soil each of 3cm depth. Add about 6 l water to keep them moist. Add cattle dung: poultry: veg waste: cereal waste in the ratio 10:1:1:1. One kilogram worms feed on 5-10kg feed. Inoculate 100 small worms per box @25 kg wet weight.

7.1.3. Maintenance

Remove worms waste daily and maintain water content of less than or equal to 50%. Maintain a temperature of 20-30°C and keep control over ants and other worms.

7.1.4. Harvesting

Arrange soil with worms in conical heap for 24 hours. This causes the worm to settle at the bottom therefore collect them and store them in closed vessel with moisture. Worms will shed all the waste in the gut in 2-3 days. Wash and use them fresh or live or as worm meal (drying at 60°C. First harvest can be done after 3 months and in 6 months 2 more harvest can be done. As continuation add more manure, change the soil and retain young ones.

7.1.5. Production

100 worms of 25g in 3 months become 2kg (wet weight) which give s 500 gm worm meal. In span of 6 months 2 more harvests can be done.

Unit 8 - Brine Shrimp or Artemia

Expected Learning Outcome

This will give an exposure to the details regarding Artemia. Even though this information is sufficient, more details regarding this can be referred from various publications.

8.1. Brine shrimp classification

Kingdom: Animalia
 Phylum: Arthropoda
 Subphylum: Crustacea

Class: Branchiopoda
Order: Anostraca
Family: Artemiidae
Genus: Artemia



8.2. Brine shrimp-Introduction

History and present status of brine shrimp in aquaculture

Brine shrimp is the English name of the genus *Artemia* of aquatic crustaceans. *Artemia*, the only genus in the family Artemiidae, have evolved little since the Triassic period. The historical record of existence of *Artemia* dates back to 982, more than one thousand years ago, from Lake Urmia, Iran, while Schlösser was the first person to give drawings of *Artemia* in 1756. *Artemia* are found worldwide in inland saltwater lakes, but not in oceans. *Artemia* are zooplankton, like copepods and *Daphnia*, which are used as live food in the aquarium trade and for marine finfish and crustacean larval culture. The cost of good quality cysts fluctuates with supply and demand. Normally 200,000 to 300,000 nauplii might hatch from each gram of high quality cysts.



Biology, ecology and natural distribution

There are more than 50 geographical strains of *Artemia*. The most described *Artemia* species include *Artemia salina*, *Artemia tunisiana*, *Artemia monica*, *Artemia persimilis* and *Artemia urmiana*. Many commercial harvesters and distributors sell brands of various qualities. Approximately 90 percent of the world's commercial harvest of brine shrimp cysts (the dormant stage) comes from the Great Salt Lake in Utah. However, the lake's cyst production is heavily influenced by freshwater inflow, and the supply varies dramatically.

8.3. Brine shrimp-morphology and physiology

Morphology of *Artemia* cyst

There is a variation in the size, dry weight and energy content of the strains. Hatching quality, percentage hatching rate and efficiency varies although hatching quality mainly depends on collection site. Temperature and salinity significantly affects survival and growth. Total lipid content and amino acid composition also varies depending on the strain.

Cyst consists of three layers

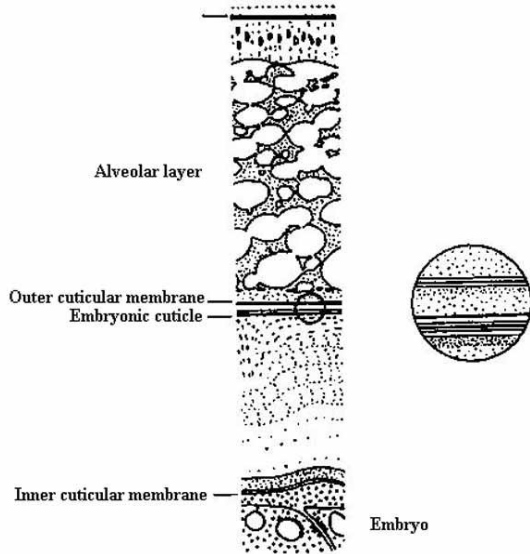
The first layer is hard with lipoprotein, chitin and hematin. The hematin imparts dark brown colour to the layer. This layer provides protection against any kind of mechanical and UV radiation. This layer can be removed by oxidation (decapsulation) by hypochlorite.

The Second layer is the outer cuticular membrane which is a multilayered membrane with special filter. This protects the

embryo from molecule larger than CO₂ and acts as permeability barrier.

The Third layer is the embryonic cuticle which is transparent, highly elastic. It develops into hatching membrane during hatching.

4.Schematic diagram of the ultrastructure of an Artemia cyst

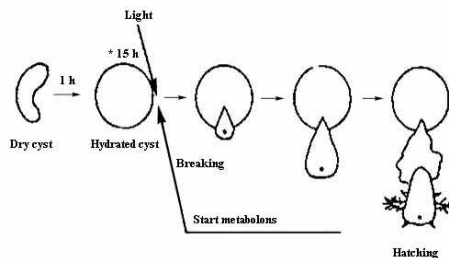


8.4.1.Embryo

Undifferentiated gastrula which is a metabolic at <10% H₂O in the absence of oxygen. Presence of oxygen or cosmic radiation results in formation of free radicals which destroy specific enzyme system in the ametabolic artemia cyst.

8.4.2.Physiology of the hatching process

The development of an Artemia cyst from incubation in the hatching medium till nauplius release is shown in the figure.



8.4.3.Development of an Artemia cyst from incubation in seawater until nauplius release

When incubated in seawater the biconcave cyst swells up and becomes spherical within 1 to 2 h. After 12 to 20 h hydration, the cyst shell (including the outer cuticular membrane) bursts (= breaking stage) and the embryo surrounded by the hatching membrane becomes visible. The embryo then leaves the shell completely and hangs underneath the empty shell (the hatching membrane may still be attached to the shell). Through the transparent hatching membrane one can follow the differentiation of the pre-nauplius into the instar I nauplius which starts to move its appendages. Shortly thereafter the hatching membrane breaks open (= hatching) and the free-swimming larva (head first) is born. Dry cysts are very hygroscopic and take up water at a fast rate i.e. within the first hours the volume of the hydrated embryo increases to a maximum of 140% water content; However, the active metabolism starts from a 60% water content onwards, provided environmental conditions are favourable. The aerobic metabolism in the cyst embryo assures the conversion of the carbohydrate reserve trehalose into glycogen (as an energy source) and glycerol.

8.4.4.Ideal conditions for hatching Artemia cysts

The optimal conditions for hatching Artemia are:

- temperature above 25°C with 28°C being optimum;
- salinity of 5 ppt (1.030 density);

- heavy, continuous aeration;
- constant illumination (example: two 40- watt fluorescent bulbs for a series of four 1-liter hatching cones); and
- a pH of about 8.

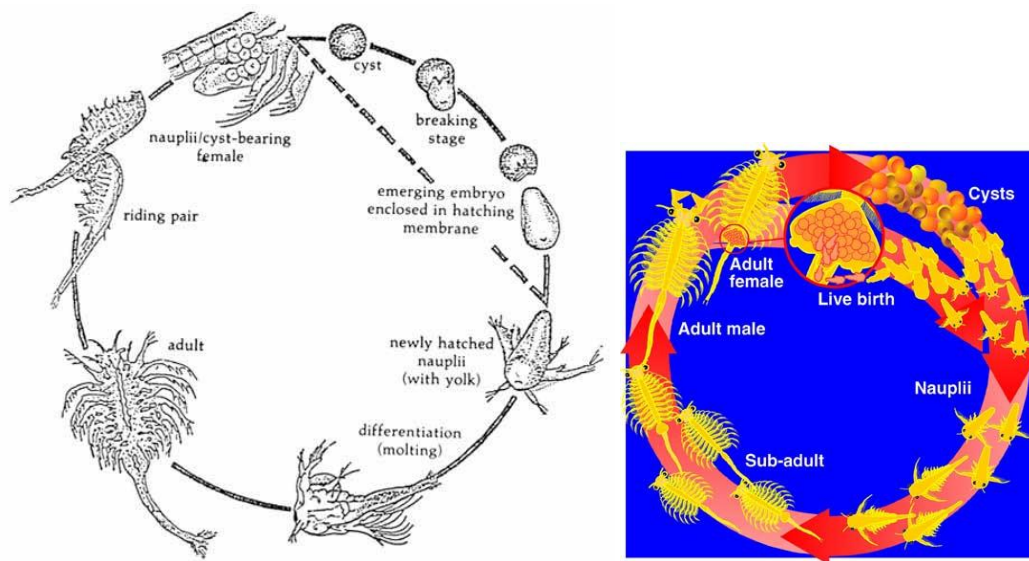
Stocking density is set by adding no more than 5 grams of cysts per liter of water. Good circulation is needed to keep the cysts in suspension. A container that is V-shaped or cone-shaped is best (2-liter bottles work well; glue a valve on the bottle cap and invert it). The best container is a separation column, found in any lab supply, although it is more expensive. Unhatched cysts, empty shells and hatched nauplii can be easily removed separately. The hatching percentage and density are usually a function of water quality, circulation, and the origin of the cysts

8.4.5. Brine shrimp-Decapsulation and hatching of cyst

Process involved in decapsulation and hatching of cysts and its direct usage

To produce nauplii, Artemia cysts are either hatched naturally by incubation in seawater for 24–48 hours or hatched after decapsulation.

Decapsulation is the removal of the outer membrane of a cyst called the chorion by dissolution in hypochlorite, without affecting the viability of the embryo. The outer shell often causes problems when not removed since it can harbour bacteria and other organisms which may be harmful to the species feeding on Artemia. Also, non-hatched cysts and their shells cannot be digested and may cause blockage of the gut in fish and crustaceans.



8.5. Processes involved in decapsulation/hatching process

8.5.1. Hydration

Hydrate the dry cysts in natural seawater. Use a transparent conical tank or funnel-shaped container (e.g., glass or plastic cylinder, thick plastic bags formed into the desired shape) and keep the cysts in continuous suspension by aerating from the bottom of the apparatus for one hour.

Upon hydration, the dry cysts which are deflated like bean seeds become round-shaped. Full hydration is necessary to insure that the inner part of the indented dry cyst shell will be completely exposed when the decapsulation solution is added.

8.5.2. Reaction with decapsulation solution (hypochlorite)

Prepare the decapsulation solution using 1N NaOH, sodium hypochlorite (NaOCl) and seawater.

Allow the hydrated cysts to react with the decapsulation solution (hypochlorite) for 7–15 minutes. To prevent damage of embryo, keep the temperature below 40°C by adding ice cubes to the suspension or by using a water bath. A change in colour of the cysts from brown to white to orange usually indicates that the reaction is complete. Check under the microscope if possible.

8.5.3. Sieving and washing

Drain the suspension of decapsulated cysts into a fine-mesh sieve and rinse immediately with seawater 6–10 times or until the smell of the hypochlorite is removed.

Decapsulated cysts may be fed directly to the cultured fish/ crustacean or stored in saturated brine solution at low temperature for future use.

8.5.4. Incubation

Incubate the decapsulated cysts for 24–48 hours in natural seawater at a density not greater than 5 grams cysts per liter of incubation medium. For optimum hatching, keep the temperature at 30°C and the pH at 8–9. Provide sufficient light at least during the first two hours or preferably continuous illumination of about 1000 lux (attained with 40-watt fluorescent light tube, 20 cm away from the hatching container). Maintain the dissolved oxygen at levels close to saturation, with cysts kept in suspension throughout the incubation period. If culture to the adult stage is not intended, it is preferable to feed the Artemia to the fish or shrimp larvae immediately upon hatching to take advantage of the yolk in the nauplii.

8.5.4.1. Harvesting processing and packaging of artemia cyst

After 15-20 hours of incubation, most of the cysts will be hatched and there will be noticeable colour change in the culture from brown to orange. At this time soft aeration and the pinkish orange nauplii will be seen swimming. An empty or undissolved shell tends to float, while the unhatched cyst and debris sink. A siphon also can be used to remove first the debris and then the nauplii from the bottom. The nauplii should then be collected on a 100-120 micrometer screen, washed with clean water, and placed in a small volume of water. Washing removes contaminants and hatching metabolites. Wash harvested nauplii for feeding.

8.5.4.2. Enrichment of artemia with HUFA

Artemia juveniles can be enriched in the same way as nauplii, but due to higher filtration efficiency, this results in higher n-3 HUFA levels in much shorter enrichment periods (1-4) hrs. It is assumed that these short enrichment periods may result in higher final DHA/EPA ratios in the biomass compared to the nauplii because there is insufficient time to break down the DHA as in the case of nauplii

Unit 9 - Bait fish

Expected Learning Outcome

This will give awareness to the details regarding bait fishes, their importance in the industry, and their utility in Tuna fisheries. This will provide information regarding the kinds of bait fishes and their utilities in the fishing industry. Since bait fishes is not much used in all parts of India apart from Lakshadweep Islands and Andaman's, this will give them preliminary information regarding them.

9.1. Bait fish and forage fish

Importance of bait fish and forage fish in aquaculture

Bait fish are small fishes caught for use as bait to attract large predatory fish, particularly game fishes. Small fish produced specifically for anglers to attract desirable food or game fishes are referred to as baitfish. Baitfish is the common term given to a multitude of small, schooling fish as they are an important food source for other fish. Species used are typically those that are common and breed rapidly, making them easy to catch and in regular supply. This large and diverse group of fishes is an integral part of the complex, interconnected marine food web. Live bait fishes are used for chumming and attracting the tuna shoals. Live baits are essential for operation of the pole and line fishery. These fishes caught from the reefs and lagoons and kept in live condition in special region of the boats for use at the time of capture. Bait fish, by contrast to forage fishes are fishes that are caught by humans to use as bait for other fish. The terms also overlap in the sense that most bait fish are also forage fish, and most forage fish can also be used as bait fish. Forage fishes are small fishes that are preyed upon in the wild by larger predators for food. The predators can be other larger fish, seabirds and marine mammals. Typical ocean forage fish feed near the base of the food chain on plankton, often by filter feeding. Forage fish compensate for their small size by forming schools. Some swim in synchronised grids with their mouths open so they can efficiently filter plankton. These schools can become immense shoals which move along coastlines and migrate across open oceans.

9.1.1.Types of bait in various fish types

Types of bait	Common Species it Will Catch
Prawns & Shrimps (marine)	Bream, whiting, flathead, snapper, cod, yellowtail, flounder, goatfish, marine perch, redfish, barramundi.
Crabs	Reef fish such as cod, drummer, snapper, bream, grouper, marine perch, parrot fish, wrasse.
Saltwater Yabbies (Nippers)	Bream, whiting, flathead, flounder, snapper.
Shellfish (Pipies, mussels, abalone, clams, oysters)	Whiting, dart, leatherjackets, bream, drummer, snapper, Australian salmon.
Squids & Octopus	Snapper, kingfish, goatfish, parrot fish, bream, coral trout, boarfish, marine perch, emperors, leather jackets.
Marine Worms (beach, blood)	Whiting, bream, flathead, flounder, dart, salmon.
Live Fish (Yellowtail, mackerel and mullet etc)	Snapper, tuna, mackerel, barramundi, wahoo, cobia, cobia, cods, sharks.
Whole Dead Fish (pilchards, whitebait etc)	Tunas, Salmon, kingfish, mackerel, snapper, coral trout, emperors, sharks.
Cut Fish Flesh (strips, cubes, fillets)	Bream, Snapper, flathead, dart, cod, flounder, mangrove jack, goatfish, redfish, coral trout, emperor, marine perch, sharks.
Marine Seaweeds	Drummer, mullet, bream

9.1.2.Candidate species

Examples of marine bait fish are *Spratelloides delicatulus*, *S.japonicus*, *Apogon sangiensis*, *A. savayensis*, *Chromis tematensis*, anchovies, halfbeaks and scad. Freshwater bait fish include any fish of the minnow or carp family (Cyprinidae), sucker family (Catostomidae), top minnows or killifish family (Cyprinodontidae), shad family (Clupeidae), sculpin of the order Osteichthyes or sunfish family (Centrarchidae). Forage fishes include the fishes of the family Clupeids as well as anchovies, capelin and halfbeaks.

THE END