Chapter.07
Live feed - Zooplanktons

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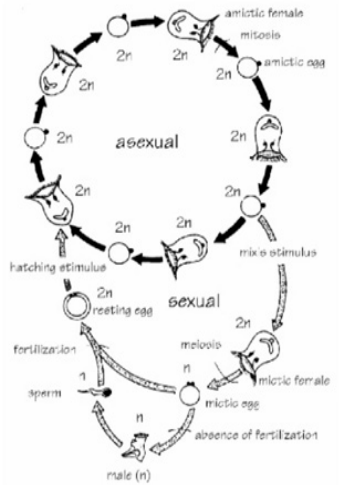
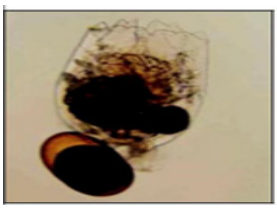
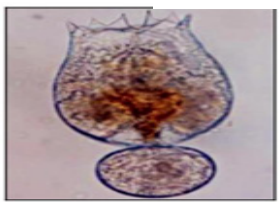
Rotifer culture

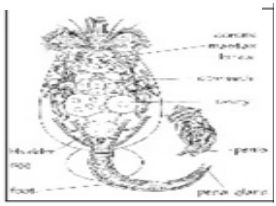
The rotifers, Brachionus plicatilis and B. rotundiformis are widely employed for feeding marine fish larvae. Its body size ranging from 70 - 350 microns, (depending on strain) makes these organisms an appropriate prey to start feeding after the resorption of the yolk. Rotifers are used as first food during a few days or weeks depending on the reared species. The main advantages of rotifers include – 1). High population growth rate, 2). Filtration of particles in suspension, 3). A good tolerance to culture conditions and handling, and 4). Appropriate energy content and reasonable nutritional value. In addition, it is relatively modifiable by dietary manipulation by means of post-culture enrichment.

Rotifers are the smaller size zooplanktons widely used in marine fin fish hatchery operations. The marine fin fish larvae initially feeds on the such smaller size zooplanktons and hence suitable size of rotifers need to be cultured in mass to feed the fish larvae. The important criteria for selecting the rotifer depends on the mouth size of the fish larvae, digestibility, nutritive value of the rotifer and easy for culture and proliferation. Marine and brackish water rotifer species can be artificially propagated in seawater and more popular rotifer species used for marine fin fish hatcheries are









Brachionus plicatilis and *Brachionus rotundiformis.*

Based on the length of lorica, Brachionus is separated into 3 strains,

B. plicatilis as L type (large) with long of lorica 200 – 360 μm

B. rotundiformis as S type (small) with long of lorica 150 – 220 μm

B. rotundiformis as SS type (super small) with long of lorica 70 – 160 μm.

Advantages of rotifers as live feed

- Small body size and round shape.
- Slow swimming speed and habit of staying suspended in the water column.
- Easily enriched with external nutrients resources.
- High reproduction rate and high density cultures.
- Very rich In low molecular weight water soluble proteins.
- Contain a broad spectrum of digestive enzymes such as proteases, peptidases, amylases, lipases and even celluloses.

Pure culture of Rotifer

- Incubated in 500 ml erlenmeyer containing sterile sea water. The culture condition should be equipped with flourescent lamp and aeration to supply oxygen.
- Microalgae such as Chlorella sp, Tetraselmis sp, Dunaliella and Isocrysis sp can be used as feed for rotifer.

Mass culture of Rotifer

- Outdoor mass culture can be carried out in volume range of 5 – 12 m3 (1 tonne, 2 tonne, 5 tonne, 10 tonne).
- Phytoplankton has to be added to rotifer culture tank with density 3-4 million cell/ml for starting the rotifer culture. Rotifers have to be inoculated into tank with initial density 50-100 rotifers/ml.
- The next day, algae has to be added into rotifer culture tank.
- Practically, growth of rotifer can be identified by change of water colour into transparent.

No.	Parameters	Range
1.	Salinity	27 – 33 ppt
2.	Temperature	26 – 31 °C
3.	Light	2,000 – 3,000 lux
4.	pH	7.5 – 8.3
5.	Dissolved Oxygen	3 – 6.5 ppm

Continuous Culture

- Rotifers can be harvested by flowing out culture water through a plastic hosepipe into a rotifer sieving bag with mesh size of 60micron at the end point of those hosepipe
- Collected rotifers can be transferred into bucket for enrichment with different types of enrichment media.
- Every day, around 30% volume of rotifer culture tank has to be harvested from the total volume of tank
- After repeated use of 3 weeks, the rotifer culture tank should be totally cleaned for fresh culture

Rotifer enrichment

- Rotifers can be enriched with Highly Unsaturated Fatty Acids (HUFA's) which contains high levels of the essential Omega-3 fatty acids, Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA).
- HUFAs are important to maintain fluidity of blood in blood vessel under low temperature condition for the fish larvae
- Especially DHA, an essential fatty acid that accumulates in the brain of fish during early development and it increases neural function
- Feeding with DHA-enriched diet at an early stage of fish larvae has been successful in improving pigmentation
- EFA deficiency creates syndromes like poor vitality, poor growth, low survival and death to simple stress (shock).

Enrichment methods

- Indirect method through feed medium like Nannochloropsis and
- ω – yeast. Rotifers in each culture tank can be fed with concentrated microalgae of Nannochloropsis (1x104) every 8 hrs and yeast once in a day at morning.
- Direct method with emulsified oil (fish oil, etc) for 100 million rotifer with fish oil ± 5 ml, raw egg yolk ± 2 ml and tap water 100– 200 ml

Enrichment with scot emulsion

- Harvested rotifer has to be transferred into a 35 lit tub containing 3/4 part of Nannochloropsis medium and 1/4 part of rotifer (600 to 800 nos / ml)
- 1-5 ml scots emulsion oil can be mixed with freshwater and stirred well.
- This emulsion can be added into the tub containing rotifer and sea water.
- Strong aeration has to be provided for 2 hours.

Harvest rotifers and can be fed to the fish larvae

INVE A1 DHA and SELCO INVE- SELCO

(Self Emulsifying Liquid Concentrates)

- Enrichment is done in buckets containing a volume of 40 L with a
- density of 800-1000 rotifers/ml. Approximately 10 lit of N.
- oculata are added.
- Buckets receive aeration to maintain DO levels above saturation.
- Enrichment media (INVE DHA selco) is added and rotifers are allowed to feed for 4-6hrs.
- Once enrichment is completed, rotifers are filtered to remove residual enrichment diet and placed into a clean bucket containing a final volume of 35 L.
- Enriched rotifers can be fish larvae.

Copepod culture

Copepods are basically classified under the Phylum Arthropoda, Subphylum Crustacea, Class Hexanauplia and Subclass Copepoda. Basically there are nine orders in the Subclass Copepoda: Calanoida, Cyclopoida, Harpacticoida, Platycopioida, Mormonilloida, Misophrioida, Siphonostomatoida, Monstrilloida and Gelyelloida. Among the marine pelagic copepods, calanoid copepods dominate (79.2%) the others. The name copepod is basically derived from the Greek words meaning "animals with oar shaped foot" - *ie, kope* means 'oar' *podos* means 'foot' (Stottrup, 2003).

The basic body structure of Copepods comprises of a large cephalothorax (cephalosome) formed by the fusion of head and thoracic segments and a small segmented abdomen (urosome). The thorax has basically six segments. All segments possess a pair of legs or pleopods which are used for swimming. The 5th and 6th legs are considered to be taxonomically very important and often these are modified or reduced. Cephalic region has a rostrum, a pair of median eyes, a pair of antennule, antennae, mandible, maxilla and maxilliped. Most of the appendages except the antennule are generally biramous. The abdominal segments are reduced and without any limbs except for the caudal furca which form a tail fan with long setae. The sixth thoracic segment unites with first abdominal segment to form a genital double somite. The genital double somite together with abdominal somites form a slender tail like portion called urosome.

Reproduction

Most copepods reproduce sexually. The male deposits a sac containing viable sperm called a spermatophore near the genital aperture of the female. Most calanoids are broadcasters, shedding eggs singly into the water. The number of eggs spawned in a single event may vary from a few eggs to 50 or more eggs, and each spawning event may occur once every 24 hour for extended periods. Free spawning species such as various Acartia species may produce between 11 and 50 eggs per female per day, producing a total of more than 1200 from one single spawning and Calanus species between 15 and 230 eggs per female per day to a total of upto 3800 eggs per female. In most cases, a new mating is necessary for a female to produce eggs again. Other copepods including cyclopoids and harpacticoids, have their eggs contained within one or two egg sacs (ovisac),

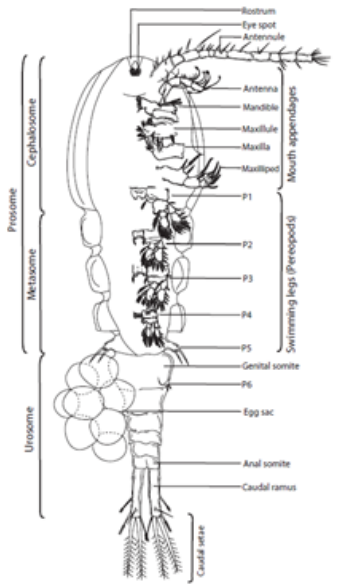
which remain attached to the female genital segment until they hatch. In calanoids, the eggs are not contained in a membrane but adhere to each other as an egg mass and remain attached to the female. Each egg sac or egg mass may contain a few to 50 or more eggs and may be produced at frequent intervals of a few days. Egg production is measured as number of eggs per female per day. Resting eggs are produced by several species of copepod and are the primary mode of dormant state in calanoids. These copepod eggs are laid during development, whereby development is arrested, and possess an additional external envelope of variable thickness. Resting eggs are able to withstand long periods of desiccation, heat or cold. Resting eggs have only been reported for calanoids. Day length and temperature were the principal environmental cues that induce females to switch from active reproductive to a resting reproductive state.

Life Cycle

Once fertilised the eggs pass into the water or into an egg sac. The egg is spherical and protected by a chitinous envelope. The larva that hatches from copepod eggs, the nauplius (NI), develops through five or six moults before passing onto the copepodite stage where they display the general adult features. Most species commence feeding during the third or fourth naupliar stages, although a few species commence feeding during the second and even fewer during the first naupliar stage.

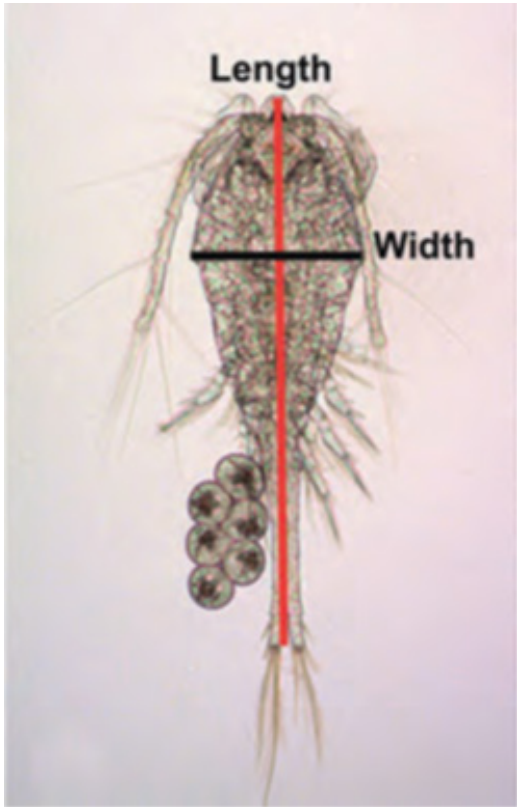
Size

Calanoid eggs produced in egg sacs range in diameter from 70 to around 800μm and are generally larger than freely spawned eggs, which measure around 200μm. Size ranges of newly hatched nauplii also vary. Newly hatched nauplii such as A. tonsa measure less than 100μm in body length whereas as the larger calanoid nauplii measure around 220μm.

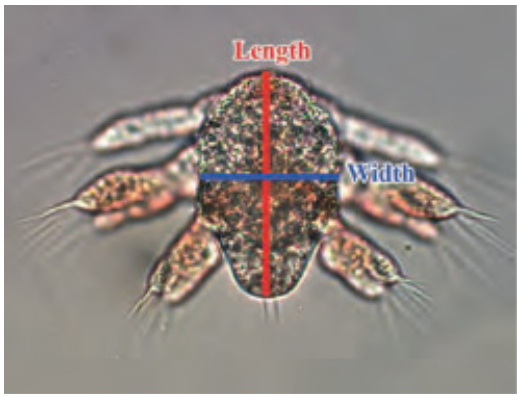


Basic body structure of Copepods (Santhosh et al, 2018)

Size measurement of adult copepod (Santhosh et al, 2018)



Measurement of copepod nauplii (Santhosh et al, 2018)



Generation time

The generation time defined as the time interval between hatching of an individual and the hatching of its progeny which differs from species to species and is positively correlated with increasing temperature. In calanoids reared at different temperatures, generation times varied from around one week in Acartia spp to months. Food supply and salinity may also influence development rates.

Feeding, Food quality and Food availability

Calanoids are generally herbivorous filter feeders, able to distinguish between particles and selecting between different food particles based on size or taste. The rate of consumption of algal particles is influenced by the size, quantity and quality of the food. However, few species of calanoids have a non-visual, active raptorial mode of feeding, capturing and ingesting a variety of animal prey. Copepod species may also create feeding currents that entrap non-evasive prey. The food concentrations at which egg production commences and at which it attains a maximum level differ between species. Egg production per female per day increases with increasing food concentrations to an asymptotic level. Food quality also influences growth and reproduction.

Harpacticoids are primarily detritivorous, benthic grazers, efficiently utilizing various food sources such as bacteria, microalgae, marsh grass, algal biofilm, diatoms, polychaete meat, etc. Although harpacticoids eat practically anything, their offspring production is not independent of the food quality. Food supply in terms of quality and quantity affects feeding, development and reproduction in harpacticoids.

The use of copepods in marine fish hatcheries for larviculture has started gaining importance recently, due to the development of culture techniques of many species of copepods. The nauplii or copepodite stages of copepods are an ideal feed for fish larvae owing to its small size and rich nutritional profile when compared to rotifers. The most commonly used copepods species in aquaculture belong to the orders – Calanoida, Harpacticoida and Cyclopoida.

Culture of Copepods

Outdoor production in ponds and tanks can be carried out. Filtered seawater is generally used in this system. By using filters of around 20-40 μm, natural phytoplankton can be

transferred to ponds without the accompanying zooplankton or potential predators. The phytoplankton can be fertilized to induce blooms. Filtering devices for selective sieving are used to collect primarily nauplii (80-250 μm), copepodite stages (80-350 μm) or primarily adult stages (250–600 μm) to inoculate the rearing tanks. In Asian countries, copepodites and adult stages of copepods were added in tanks 3 days before stocking of the newly hatched fish larvae. In this system, using wild harvested copepods (*Acartia spp, Pseudodiaptomus spp., Oithona spp.,*and a few harpacticoids) an average survival of 3.4% at harvest of *Epinephelus coioides* was obtained. Regular monitoring of densities of the live prey in these outdoor systems is important for the successful rearing of marine fish larvae.

Calanoids

Most frequently cultured calanoids are coastal species such as Acartia, Centropages, Eutemora and Temora. They are small, relatively of short generation times and a wide thermal and salinity tolerance and are easily adaptable to laboratory condition. Most calanoids require phytoplankton. In many cases the copepods are reared on monoalgal diets, which may not comply with all the requirements for maximum egg production. Somatic growth ceases in adult copepods and growth rate is more or less equivalent to the rate of egg production. The rate of egg production in copepods is dependent on the size, quantity and quality of the algae provided. As a general rule to reach food saturation, high ingestion rates and high egg production rates, cell concentrations of around 103 cells/ml would be sufficient using larger cells and around 104 cells or 105 cells per ml using smaller cells. A combination of at least two algal species with high n-3 polyunsaturated lipid content, and of a size that can be utilized by both the feeding naupliar stages and the copepodite and adult stages comprises an adequate diet for culture.

In one of the culture systems, the eggs are sedimented to the bottom from where they are siphoned daily, simultaneously siphoning out debris, faecal matter and associated ciliates. During the siphoning, the eggs are concentrated on 45μm sieve, allowing most of the debris and ciliates to pass through and get removed from culture. The daily removal of eggs eliminates the potential loss of nauplii through cannibalism by adult population. The presence of ciliates (Euplotes sp.) cause deterioration of water quality and a thorough water

exchange is necessary. Failing to do this will result in a culture crash. The culture is filtered through a 180μm sieve submerged in sea water to retain the adult population and wash out the ciliates. The adults were then used to inoculate a new tank filled with filtered (1μm) sea water.

Light

A photoperiod of 12 hour of light is sufficient. High solar radiation is harmful to copepods; hence adults show negative phototaxis during the day and positive phototaxis during the night.

Aeration and Oxygen

Aeration is required to maintain phytoplankton in suspension and create small turbulence, which helps to distribute the copepods. Too vigorous aeration should be avoided and unnecessary.

Culture tank size and shape

Most calanids require large volumes and the adult density rarely exceeds 100 per litre. Cylindrical tanks with flat bottom (200 litres) are suitable for culture of copepod, Acartia tonsa.

Temperature and Salinity

Temperature has a vital role for copepods. But their adaptability to temperature is remarkable. Coastal species have wider thermal and salinity tolerances than oceanic species.

Contamination

Contamination of copepod cultures by bacterial blooms, ciliate infections, other copepods or rotifers may pose a problem. Use of same siphon/sampling devices, etc. for all copepod tanks should be avoided. In commercial facilities, contamination by rotifers is the most likely cause of the collapse of copepod culture, since the rotifers with their higher reproductive rate would quickly outcompete the copepods. It is therefore important to keep these cultures strictly apart.

Ciliates are utilized by copepods and may in periods of low phytoplankton concentrations constitute the major dietary source. In intensive cultures, the presence of certain ciliates such as *Euplotes sp.* is often an indication of overfeeding and should be avoided.

For eliminating ciliates it is advisable to empty the culture using a 60 or 80μm mesh which retains the adult copepods, but allows the ciliates to be washed out. Cultures may succumb to uncontrolled proliferation of bacteria, Eventhough bacteria often constitute a part of the diet of copepods. Calanoids are sensitive to high ammonia concentrations.

Harvest and Storage

Copepods can survive for short periods in gauze as they are transferred from one tank to another. They can also survive for an extended time at very high densities, provided that there is sufficient oxygen. Freely spawned calanoid eggs sink to the bottom and can be harvested by siphoning the bottom once daily. The day's production can be transferred to individual hatching tanks to be used as live feed.

Harpacticoids

Harpacticoids have been cultured in batch and continuous systems to provided food for marine fish larvae

Advantages

i. High tolerance to a wide range of environmental conditions

ii. Ability to feed on a wide range of live or inert diets

iii. High reproductive capacity

iv. Relatively short life cycles (7-29 days)

v. Ability to be cultured in high densities

vi. Requirement for surface area rather than volume

vii. Planktonic naupliar stages

viii. Can be used as tank cleaners in rotifer cultures, other copepod cultures or larval tanks.

ix. The culture conditions for harpacticoids are less demanding than those for calanoids. Filtered seawater may be used and a whole range of inert food is acceptable to many harpacticoid species. This simplifies the culture method and eliminates the need for culture of phytoplankton.

Food and feeding:

If algae are readily available, a mixture of two algal species would be the preferred choice. Algae which quickly sediment are very appropriate for benthic copepods, possibly because bacteria colonise these cells, and the mixture of algae and bacteria may be a superior combination for harpacticoids.

Light: A photoperiod of 12L/12D was shown to be most favourable for offspring production.

Aeration: Aeration may be applied to maintain an even distribution of food

Culture tank size and shape: The mass culture of benthic harpacticoids is dependent on the availability of surface area rather than culture volume.

Temperature and Salinity: Most harpacticoids have wide thermal and salinity tolerances.

Contaminants: Rotifers and ciliates are the major contaminants

Harvest, storage and Transport

Since harpacticoids are not free spawners, harvest methods for collecting nauplii need to be developed. Concentrating nauplii by light can be practised for harvesting. Harpacticoids are relatively tolerant to high stocking densities and can be transported for a period of upto 2-3 days, kept cool in blood transfusion bags (2 litres) at densities of 200,000 individuals per litre. Excess nauplii can also be stored at 4°C for upto one week and used on days when production output is below the required amount.

Isolation and Identification

Collection from the wild – filter with particular mesh size (100micro to 500 micron)

100 nos individual (1:1 M/ F) isolate to 50 ml test tube Add microalgae as feed (5 ml / 2 days once)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31^{0}C

Generation time – 7 days

Harvest nauplii / copepodite (harvest continuously for 3 months)

Intermediate culture

Nauplii (1000 nos) transfer to 1000 ml beaker Add microalge as feed (25 ml / 2 days)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31^{0}C

Generation time – 7 days

Collect nauplii / copepodite

Mass culture

Nauplii transfer to 1 to 2 tonn tank

Add microalgae as feed (500 lit algae / week)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31^{0}C

Generation time – 7 days

Harvest nauplii / copepodite (3-5 nos / ml) Total drain and harvest the tank after 1 month.

Culture Protocol

- Population counts should be done weekly for feeding adjustments.
- Count algae concentrations and compute required feed volumes to be added.

- Siphon detritus from tank bottom daily.
- Remove sufficient water volume to allow addition of new feed volume(s), while maintaining 200 L total volume. As the population matures this volume can be increased to 300 L. Adjust feed computations accordingly.
- Use 40 micron will retain faeces, nauplii, and eggs. Rinse sieve gently into separate collection bucket. Buckets are allowed to stand for 10 minutes to allow settling. Adults and nauplii are then attracted to the upper layer of the bucket with light and decanted for return to the tank.

Advantages of Copepods

i. The three main copepod orders viz., Calanoida, Harpacticoida and Cyclopoida have been investigated for their suitability as feeds for larval and juvenile fish. While each copepod order has its advantages and disadvantages, it is generally agreed that the following are the benefits of using copepods for larviculture.

ii. Copepods have a larger size range from first nauplii to adult copepodites and offer good size ranges for the entire hatchery phases for certain species of finfish

iii. They have superior nutritive value in comparison to rotifers and Artemia

iv. Copepod nauplii may be more easily and completely digested than either rotifers or Artemia

v. Copepods are natural sources of antioxidant astaxanthin and Vitamins C and E

vi. The movement of copepods and their nauplii triggers the feeding responses in fish larvae. The 'jerking' swimming action of most copepod nauplii and adults is an important stimulus for initiating feeding by fish larvae

vii. Use of copepods in larval fish diets have been associated with a decrease in fish malpigmentation and deformity rates

Artemia nauplii

It is used in marine aquaculture worldwide. Although Artemia is not a natural part of marine larvae, it has been favoured due to its convenience for use and high nutritional value. One of its most interesting features of this organisms is its ability to form dormant cysts that are highly resistant to adverse environmental conditions and can be kept viable for years. They are normally stored under dry and cool conditions. The ease and simplicity of hatching brine shrimp nauplii makes them the most convenient and least labour intensive live food available for aquaculture. However, the only negative aspect is its high cost, hence now a days the practical strategy adopted in larviculture of marine species has been to attempt early weaning in conjunction with a prolonged rotifer feeding period to eliminate the use of Artemia. However, this is not always possible and in some species whose larvae are relatively larger at hatching, Artemia nauplii might even be the only live prey used in larviculture.

Artemia cyst strains

Having a larger size than rotifers, the brine shrimp Artemia salina are used as the second (after rotifers), live feed organisms to feed fish larvae. Artemia is not cultivated in the hatchery as in the case for algae and rotifers, but their larval stages are obtained by incubating and hatching their resting eggs, which are available commercially as dry storable cysts. The first Artemia larval form is the nauplii, which are smaller in size and richest in yolk, and followed by a larger size metanauplius, whose nutritional value has to be boosted by feeding them with special enrichment diets 12 to 24 hours before feeding to the fish larvae.

Cysts of different strains can be used as per the requirement:-

i. Cysts giving small Instar I-nauplii (with a length of around 430 μm at yolk- sac stage) with high levels of the essential highly unsaturated fatty acids (n-3 HUFA). An example is the AF strain of the Belgian producer INVE. Such nauplii allow an early switch from rotifers to Artemia in larval feeding.

ii. Cysts producing medium-sized Instar I-nauplii (around 480 μm) with high levels of n-3 HUFA, such as the AF 480 strain of INVE. These nauplii are useful to switch from the smaller nauplii to the bigger metanauplii.

iii. Cysts hatching large Instar I-nauplii (around 520 μm)

with low levels of n-3 HUFA, such as the EG strain of INVE. These cysts, which are the commonest and cheaper, are widely used to produce metanauplii which represent the bulk of live feeds in larval fish rearing.

Their enrichment is mandatory to increase the essential fatty acids content to meet the nutritional requirements of young fish. Two additional parameters characterize the Artemia batches: the number of cyst per gram and their hatching rate (the number of nauplii produced per gram of cysts). The best strains can give about 290 000 - 300 000 nauplii per gram of cyst hatched, with a hatching rate close to 95%. In a hatchery the use of good quality cysts allows a synchronization of the production cycle on a 24-h period, with the harvest of freshly hatched nauplii coinciding with the start of the incubation of new batches.

Disinfection and decapsulation of brine shrimp cysts

Artemia cyst shells are usually contaminated with bacteria, spores of fungi and other microorganisms. Fish larvae can be infected when untreated empty shells, unhatched cysts or cyst hatching medium residues are introduced into the larval rearing tank. Before incubation, cysts should therefore be disinfected. This process also improves hatching by reducing the bacterial load of the hatching medium. Disinfection is done by keeping the cysts for a few minutes in a hypochlorite solution at a maximum density of 50 g/litre. This product is easily available as commercial grade bleach. The duration of the treatment varies according to the active chlorine concentration of the disinfecting solution.

Typical duration is :

- 1 minute in a 10 000 ppm solution,
- 20 minutes in a 200 ppm solution.

As in commercial bleach, the chlorine content may range from 5 to 15%, it is mandatory to check the actual chlorine concentration in the bleach that is going to be used. This can be done either by titration or by determination of the refractive index. The following example shows how to disinfect one kg of cysts in a 200 ppm hypochlorite solution obtained from a household bleach with 5% active chlorine:

- One kg of cysts needs 20 l of fresh water for the disinfecting solution.

- If this solution is going to be used for a 20 minutes bath you will need 20 l x 200 mg/l = 4 000 mg = 4 g active chlorine.
- The quantity of 5% bleach required to give 4 g active chlorine is: (1 000/50) x 4 = 80 ml pour 80 ml of 5% bleach in 20 l of fresh water.
- Add one kg of cysts; place an airstone for continuous aeration to keep cysts in suspension, and keep the cyst in the solution for 20 minutes.
- Harvest cysts on a sieve (125 ìm mesh size) and rinse thoroughly with plenty of tap water.
- Transfer the rinsed cysts to the incubation tank.

A more effective way to obtain completely contaminant-free cysts is decapsulation, which implies the elimination of the cysts thick external layer, the chorion, by chemical oxidation. This process, which requires greater attention, has additional advantages. As they spend less energy to hatch after the removal of the chorion, the hatching nauplii have better nutritional value. Moreover, fish do not risk suffocating by gulping empty or unhatched cysts offered together with the nauplii, as it may happen when using disinfected cysts. The decapsulation process consists in four steps: hydration, treatment in a chlorine solution, washing and deactivation of the residual chlorine. The example described below refers to the decapsulation procedure of one kg of cysts. The hydration, a necessary step as the complete removal of the chlorine may only happen when cysts are spherical, proceeds as follows:

- Water volume required: around 6 l per kg (maximum amount: 200 g/l); both fresh and sea water can be used; water temperature should be between 20-25°C;duration: 45 minutes;
- aeration: sufficiently strong to keep cysts in constant suspension; use an open end pipe in a 10 l bucket.
- Collect the hydrated cysts on a sieve and treat them immediately with the decapsulation solution.

The decapsulation solution requires a source of hypochlorite, usually liquid bleach (NaOCl), and an alkaline product to increase pH level of the decapsulation solution above pH10. Usually technical grade caustic soda (sodium hydroxideNaOH) is used. The first product is added at 0.5 g active chlorine per gram of cysts, and the second as 0.15 g of sodium hydroxide per gram of cysts. For hydrated cysts the procedure is as

follows (figures refers to one kg of cysts):

- Prepare 0.5 g Cl x 1 000 g cysts = 500 g of active chlorine, equal to 3 33 l of a 15% bleach.
- Prepare 0.15 g NaOH x 1 000 g cysts = 150 g of NaOH, equal to 0.375 l of a 40% NaOH solution.
- Put the bleach and NaOH in a suitable container (e.g.: a 20 l plastic bucket) and fill with seawater to 14 litres (14 – 3.33 – 0.375 = about 10.3 l of seawater) provide a strong aeration and eventually if available add antifoam.
- Place the hydrated cysts in the bucket; control the temperature: it should remain within 25°-30°C. In case of higher temperatures, add ice to prevent that it reaches 40°C which are lethal for the cysts.
- Verify cyst colour changes. The change in cyst colour confirms that decapsulation is in progress.
- The cyst colour shifts from dark brown to grey and finally to orange, which is the colour of the nauplius body seen by transparency through its outer cuticular membrane, left exposed by the dissolution of the chorion. The process usually lasts 5 to 15 minutes.
- Using a pipette or a graduated cylinder, check floatability: non decapsulated cysts will float and decapsulated cysts will sink; as soon as all cysts have turned orange, stop the process by harvesting them on a sieve and rinse thoroughly with plenty of tap water and rinse well until no more chlorine smell is noticed
- The residual hypochlorite adsorbed by the decapsulated cysts has to be neutralised by dipping them in a 0.1% solution of sodium thiosulfate (Na2S2O3 . 5H2O) for 5 minutes; then, after a final rinsing, they were transferred to the incubation tank.

Figure A. Small scale Artemia cyst hatching system and B. Microscopic observation of hatched Artemia nauplii

Counting and evaluating *Artemia nauplii*

To assess the hatching results and to feed the larval rearing tanks at the established densities you have to count the Artemia nauplii. Three methods are described below, first for high nauplii densities, such as after harvesting and in a cold storage tank, second for counting the nauplii when they are in the incubation tank and the third for low nauplii densities, similar to those which can be found in fish tanks.

Counting high density nauplii samples

- Take a 10-ml sample of the population to be estimated.
- Dilute the nauplii concentration by adding 90 ml of sea water to obtain a total sample volume of 100 ml.
- Take three sub-samples with a 1-ml pipette, avoiding sucking air bubbles.
- Transfer each sub-sample to a Petri dish.
- Add a few drops of the fixative staining solution Lugol to each of
- the Petri dishes and wait until all nauplii are immobile and deeply stained.
- Add water so as to distribute the nauplii over the whole surface of the three Petri dishes.
- Put each Petri dish on a grid and count the nauplii present in each 1 ml sub-sample.
- Calculate the average number of nauplii per ml by dividing the sum of the three counts by three.
- The total nauplii density per litre is given by the average number of nauplii per ml multiplied by 10.
- To give the number per undiluted ml and finally by 1 000 to get the final density per liter.

Counting nauplii samples from the incubation tank

- Take with a test tube a 50-ml sample of the population to be estimated.
- Take three sub-samples with an automatic 0.1-ml pipette.
- Transfer each sub-sample to a 3 cm-wide Petri dish, whose bottom has been subdivided in a 5mm-grid.
- Add a few drops of the fixative staining solution Lugol to each of the Petri dishes and wait until all nauplii are immobile and deeply stained.

- Add water so as to evenly distribute the nauplii over the whole surface of the three Petri dishes.
- Count the nauplii present in each 0.1 ml sample.
- Calculate the average number of nauplii per ml by dividing the sum of the three counts by three.
- The total nauplii density per litre is given by the average number of nauplii per ml multiplied by 10 to give the number per ml and finally by 1000 to get the final density per liter.

Counting low densities nauplii samples

- Take three 50 or 100 ml samples, using a graduated cylinder which has been cut at the 50 or 100 ml mark.
- Transfer each sample to a large Petri dish.
- Add several drops of Lugol to each of them and wait until all nauplii are immobile and deeply stained.
- Put each Petri dish on a grid and count the nauplii present in each sample.
- Calculate the average number of nauplii present in each sample.
- Calculate the nauplii density per litre by multiplying this count either by 20 (50 ml sample) or by 10 (100 ml sample).
- Hatching rate: the number of nauplii hatched per 100 cysts; good batches have a hatching rate around 90-95%.
- Hatching efficiency: the number of nauplii produced per gram of cysts; top quality cysts yield about 300 000 nauplii/g.

Artemia enrichment

- Prepare culture vessel for the enrichment process using fresh seawater.
- Salinity 35 - 45 (ppt); temperature: 27 -28 C and vigorous aeration.
- Harvest the newly hatched nauplii.
- Gently rinse with fresh water with suitable filter.
- Transfer to a clean culture vessel.
- Stock at the rate of 8,000 -10,000 newly hatched brine shrimp per liter
- Do not feed nauplii at this time - they are absorbing their attached yolk sac.

The two main criteria to evaluate hatching results are:

- Approximately 8 to 10 hours from time of transfer to clean culture vessel, the nauplii will have molted into the Instar II feeding stage.
- Add **SELCO 0.2 g per 1,00,000** Artemia nauplii per liter.
- Ensure that the aeration is vigorously mixing the water column.
- After approximately 12 hours, the intestinal tract of the nauplii should be fully enriched with SELCO.
- Harvest enriched nauplii and feed immediately maintaining a nauplii concentration of 2-3 nos. per ml.
- Unfed enriched nauplii can be stored in the refrigerator for later feedings .

Enrichment using Instar-II-nauplii :

Add **0.6g (in a minimum of 2 rations (0 hrs – 12 hrs)** of A1 DHA SELCO per liter of seawater containing up to 3,00,000 Artemia nauplii, in the enrichment tank. Enrich for a period of 24 hours while maintaining min, 4 ppm DO and p H of 7.5-8.5.

Optimal enrichment conditions

- For water quality and container design, see previous section; initial nauplii density: between 150,000 and 300,000 nauplii/l.
- Vigorous water agitation to keep the nauplii in suspension and pure oxygen to keep dissolved oxygen above 4 ppm throughout the enrichment period: use one open PVC pipe for air and a micro-bubbles diffuser for oxygen.
- Lighting: not required.

Prepare the enrichment meal as specified by the producer, and make sure to prepare a new enrichment emulsion for each meal. At the end of the enrichment time harvest the metanauplii as usual, rinsing them thoroughly with seawater until no oily emulsion is noticed in the outflowing water.