

ARTEMIA: GROWTH AND REPRODUCTION, CYSTS DECAPSULATION, HATCHING TECHNIQUES AND BIOMASS PRODUCTION

Introduction

Among the live diets used in the larviculture of fish and shellfish, the nauplii of the brine shrimp constitute the most widely used food item due to their nutritional value and mobility in water, which makes them perfect prey for young fish. As the mouth size of marine fish larvae increases with age, rotifers are gradually replaced by the larger freshly hatched nauplii of the brine shrimp salina. At present, is being produced and exploited on five continents. Despite this, a large part of the cyst market is still supplied by harvests from one location, the Great Salt Lake. This situation makes the market still extremely vulnerable to climatological and/or ecological changes in this lake.

Systematic position

Phylum: Arthropoda

Class: Crustacea

Subclass: Branchiopoda

Order: Anostraca

Family: Artemiidae

Genus: *Artemia*

Species : *Artemia salina*, *A. tunisiana*, *A. franciscana*, *A. monica*, *A. persimilis*, *A. urmiana*.

Common Name

Popularly called Brine Shrimp or Brine Worm. It is also called Sea Monkey

Morphology

Artemia cysts measure about 200 microns in diameter and are brown in color. It remains covered with a hard shell or chorion. Freshly hatched artemia nauplii are about 0.4 mm in length. Its protein content of it is nearly 50%. Adult Artemia measures about 1-2 cm in length.

Food and feeding

In nature, *Artemia* feeds on algae. It prefers to take *Dunaliella* and *Scenedesmus* but cannot digest Chlorella because of its thick wall. It can take any particulate feed such as bacteria animal manure rice bran etc. Food particles are brought to the mouth by the constant movement of its appendages.

Life History

The use of as a food for the culture of larval organisms began only in the 1930s. In the 1950s commercial supplies of cysts originated from the salt pans in the Bay of San Francisco, California, and the Great Salt Lake, in Utah, U.S.A. They can even survive short periods of time in freshwater, but cannot reproduce in it. are extremely euryhaline and can withstand salinities from 3 ppt to 300 ppt. They can also survive temperatures ranging from 15 to 55°C.

They have two modes of reproduction. Sometimes nauplii (first swimming stage) hatch in the ovisac of the mother and are born live. However, when the waterbody where adults are living, begins to dry up and salinities rise, embryos are encased in a hard capsule, or cyst, so that they are protected and can hatch later when conditions are better.

The cyst is 200 to 300 micrometers in diameter, depending upon the strain. Its external layer is a hard, dark brown shell. Dry conditions cause the encysted embryo to enter a dormant state, which allows it to withstand complete drying, at temperatures over 100 °C. The dehydrated cyst can be stored for months or years without loss of hatchability. Only water and oxygen are required to initiate the normal development of the embryo. The durable, easily hatched cyst makes a convenient, constantly accessible source of live feed for the finfish hatchery operator. Cysts are best stored in a tightly sealed container in a cool, dry environment and, if possible, vacuum-packed.

Within 15 to 20 hours after being placed in seawater at 28°C, the shell breaks, and the pre-nauplius in the E-1 stage appears. For the first few hours, the embryo hangs beneath the cyst shell and this stage is called the umbrella stage. The newly hatched relies on its yolk sac for nutrients because its mouth and anus are not fully developed and its digestive system is not functional yet. The pre-nauplius E-2 stage is then released as a free-swimming nauplius called an Instar 1 nauplius (400 to 500 µm in length) and is orange in color because of its yolk reserves and has a red nauplius eye the head region and three pairs of appendages: i.e., the first antennae (sensorial function), the second antennae (locomotory + filter-feeding function) and the mandibles (food uptake function).

Approximately 8 to 12 hours after hatch, it molts into the second larval stage (Instar II) and starts filter feeding on microalgae, bacteria, and detritus (1 to 50 µm) using the 2nd antennae and ingested into the functional digestive tract. The nauplius can live on yolk and stored reserves for up to 5 days or through the Instar V stage, but its caloric and protein content diminishes during this time.

It is important to feed nauplii to fish larvae as soon as possible after hatching to take full advantage of the yolk and stored reserves found in freshly hatched Instar I nauplii. If there is a delay in feeding, they may also become too fast and too large for the fish larvae to catch and eat. Also, freshly hatched nauplii are dark orange and much easier to see than older nauplii, which are transparent.

The performance of larval fish is directly related to the level of HUFA in being fed and that essential fatty acids are the principal food value of. When containing low levels of HUFA, the survival of larval fish declines. The type of food consumed by the parent greatly influences the fatty acid content of the cysts.

Growth and reproduction

Adults have an elongated body with two stalked complex eyes, a linear digestive tract, sensorial antennulae, and 11 pairs of functional thoracopods. Females can easily be recognized by the brood pouch or uterus situated just behind the 11th pair of thoracopods.

Artemia attains sexual maturity in two weeks and reproduces continuously for up to 30-40 days. It occurs in two strains, one bisexual (presence of male and female) and the other parthenogenetic (only female)

The adult male can be identified by the presence of a pair of large-sized claspers in their anterior portion whereas the female possesses an Ovisac and uterus.

Fertilized eggs normally develop into free-swimming nauplii (= ovoviviparous reproduction) which are released by the mother. The life cycle of Artemia passes through 13 instars. In extreme

conditions (e.g., high salinity, low oxygen levels) the embryos only develop up to the gastrula stage. At this moment they get surrounded by a thick shell, enter a state of metabolic standstill or dormancy (diapause), and are then released by the female (= oviparous reproduction). The cysts usually float in the high salinity waters and are blown ashore where they accumulate and dry. They can resume their further embryonic development when hydrated in optimal hatching conditions. Under optimal conditions, brine shrimp can reproduce at a rate of up to 300 nauplii or cysts every 4 days.

Rearing methods

Artemia rearing for aquaculture purposes follows two ways, naupliar production, and biomass production.

Nauplii and metanauplii are easily produced from hatching cysts in dedicated units. Cyst incubation takes approximately 24 hours at 30°C, in salty water under strong light and with aeration. Fully enriched metanauplii would require an additional 12-24 hours of rearing.

Biomass production takes a few days and adult *Artemia* is harvested. These can be either immediately fed to fry, or frozen to be used later. In recent years, however, this process has been progressively replaced with new and reliable artificial diets.

Conditions for hatching cysts

The suitable conditions for hatching *Artemia* are:

- 1) Temperature (25- 28 °C)
 - 2) Salinity (5 -35ppt)
 - 3) Heavy continuous aeration
 - 4) Constant illumination (two 40- watt fluorescent bulbs) and
 - 5) pH (7.5 -8.0)
- 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. Unhatched cysts, empty shells, and hatched nauplii can be easily removed separately from cone-shaped bottles or tanks.
 - The hatching percentage and density are usually a function of water quality, circulation, and the origin of the cysts.

Requirements and preparation of *Artemia*

The important step to be followed for the preparation of *Artemia* for feeding larval rearing in the hatchery are:

1. Determine the weight of *Artemia* cysts required to feed the larvae in a tank of known volume.
2. Hydration and decapsulation of cysts.
3. Incubation of cysts.
4. Separation of cysts from shells and debris
5. Count the hatched *Artemia*.
6. Calculate the number of *Artemia* remaining in the rearing tank from the previous feeding.

7. Calculate the number of *Artemia* nauplii required by the larvae and transfer them to the rearing tank.

Determine the amount of *Artemia* cysts required

To estimate the amount of *Artemia* required for larval rearing, one must consider both the volume of the tank and the expected number of *Artemia* the larvae will consume.

Artemia nauplii are maintained in the larval culture tank at densities of 2 to 5 nos per ml for most species of finfish.

Each gram of cysts contains approximately 200,000 to 300,000 cysts and generally, *Artemia* has at least 50 percent hatchability.

Based on this, the requirements of *Artemia* cysts need to be hatched daily can be calculated as follows :

$$\text{Weight of Artemia cysts required} = \frac{\text{Total volume of all rearing tanks (in ml) X No. of Artemia required per ml}}{\text{Percentage hatch rate X No. of cysts per gram}}$$

Hydration and decapsulation of cysts

Decapsulating, or removing the shell from *Artemia* cysts helps to disinfect the cysts, makes nauplii more digestible, improves hatchability, and makes it easier for the nauplii to emerge.

Moreover, acceleration or speeding up the hatching process, allows the nauplii to not use much energy for emerging, thereby making them a better food source.

Separating nauplii from their shells is essential as the shells are indigestible and can lodge in the gut of larvae, causing fatal obstructions and may eventually cause mortality.

The process of decapsulation is accomplished in three steps:

1. Hydrating the cysts
2. Decapsulation and
3. Washing and deactivating the residual chlorine.

Hydration

- Dry cysts have a thick shell, which makes it hard to remove the complete inner membrane. Hence the cysts are first hydrated in distilled freshwater or low salinity water (less than 10 ppt) at 25°C for 1 hour with aeration.
- Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell, the embryo resumes its interrupted metabolism.
- The lower the temperature, the longer it takes to hydrate them.
- Cysts should be filtered on a 100 to 125µm bolting silk cloth.

Decapsulation

The hydrated cysts need to be decapsulated immediately using a decapsulation solution that dissolves the shell.

Two types of chemicals are available for the purpose.

One chemical is commercial-grade sodium hypochlorite (NaOCl) which is commonly available in any laboratory reagents shop. In this method, the hydrated cysts are kept in 5% Sodium hypochlorite solution @15 ml for every 1 gm cyst. The oxidation process starts immediately as a result of which heat is produced and the temperature rises beyond 40°C. In order to prevent damage to the embryo from the rising temperature the container containing the hydrated cyst is placed inside a trough containing cool water or ice cubes. During this period the cysts are constantly stirred using a glass rod to facilitate uniform cooling. As the chorion dissolves a gradual change in the color of cysts can be noticed from dark brown to white. In about 5-10 mins, the chorion gets dissolved and the decapsulated cysts are filtered on a 100-micron mesh bolting silk cloth. The decapsulated cysts are then thoroughly washed in freshwater or seawater to remove the traces of toxic chlorine. In order to ensure the complete removal of chlorine, the decapsulated cysts are given a dip in 0.1% sodium Thiosulphate Solution.

Another chemical used for dissolving the chorion of the hydrated artemia cysts is the commercial bleaching powder, containing 30-33% active chlorine concentration. In this method, the hydrated cysts are filtered into a scoop net made of 100-micron bolting silk cloth. The scoop net containing the cysts is then kept in a bucket filled with water containing 200-250 ppm of Chlorine prepared by using commercial bleaching powder. After having been kept in chlorinated water like this for 15-20 mins the chorion of cysts are get dissolved and the color of cysts turn white. The scoop net along with the decapsulated cysts is now removed from the water. The traces of Chlorine are then removed in the same way as stated above.

Incubation of cysts

The washed decapsulated cysts are then transferred to seawater.

Optimum water quality required for hatching:

Temperature: 27-30°C

pH:7.5 – 8.5

Salinity: 25 – 30 ppt

Light: 1000 lux

Dissolved Oxygen: Saturation point

The decapsulated cysts are stocked in the jar @ 0.5 – 1.0 g liter. The cysts hatch into nauplii in about 12- 24 hours.

Harvesting: Separation of cysts from shells

After 15 to 20 hours of incubation, most of the cysts will be hatched and there will be a noticeable color change in the culture from brown to orange. At this time stop the aeration and the pinkish-orange nauplii will be seen swimming.

Any empty or undissolved shells tend to float, while the unhatched cysts and debris sink. If the container is clear, a black cloth can be placed on top of the container to speed up the concentration of nauplii toward the bottom.

A siphon also can be used to remove first the debris and then the nauplii from the bottom. The nauplii should be collected on a 100 to 120-micrometer screen, washed with clean seawater, and placed in a small volume of water.

Washing removes contaminants and hatching metabolites. The phototactic method also can be used to harvest the newly hatched nauplii. Wash the harvested nauplii for feeding.

Counting of hatched *Artemia*

As the hatch rates vary under different conditions, it is important to quantify the number of *Artemia* that hatched.

For this bring the level of seawater in the beaker holding the concentrated freshly hatched nauplii to 100 ml. The nauplii should be mixed continuously while a 1-ml sample is drawn off with a pipet and placed in another beaker.

The contents of this beaker should be increased to 100 ml with distilled water (1:100 dilution). Add full-strength Lugol's stain or iodine to the beaker to slow down or kill the nauplii if high concentrations are present.

Mix the contents of the beaker well and draw a 1-ml sample with a pipet for counting. If the numbers are high, count the nauplii in 0.1 ml. This count gives the concentration of nauplii in 0.1 ml of the dilution (H). Multiply the number counted by ten to get the number of *Artemia* in 1 ml, then multiply by the dilution factor (100) to calculate the number of *Artemia* per ml. To get the total number of *Artemia* use the formula:

Number of *Artemia*/ml concentrated in 100 ml beaker (G) = H x 1000 (where H = number in 0.1 ml)

Calculation of the number of *Artemia* in the rearing tank

Scoop 100 ml of sample from the rearing tank into a beaker and add Lugol's stain or iodine to kill the *Artemia*. Use the above procedure, but do not dilute the sample. The concentration of *Artemia* in the rearing tank can be calculated using the following formula

Concentration of *Artemia* per ml (J) = number per 0.1 ml (H) x 10

Calculate the number of *Artemia* to be fed

The quantity of *Artemia* added to the larval system must be adjusted on the basis of the number remaining from the previous feeding. Each day, the stage of the fish larvae is assessed and used to determine the total quantity of *Artemia* required per ml of the rearing tank (K). From this subtract the number of *Artemia* per ml present in the tank (J) from the total you need to find the quantity per ml to be added. The quantity to be added (L) equals (K) minus (J). Multiply this number by the volume of the tank in ml to find the total number you require in the tank (M). Divide this number by the number of nauplii per ml in the fresh batch of *Artemia* (G) to determine the number of ml to be transferred to the fish tank.

Production of *Artemia* Biomass

Artemia juveniles and adults are used as a nursery diet not only for their optimal nutritional value but also for energetic advantages as well. This improved energy balance may result in better growth, a faster developmental rate, or an improved physiological condition.

The nutritional quality of *Artemia* biomass produced in semi-intensive or super-intensive systems is analogous to naturally produced biomass except for the lipid content. The protein content of ingrown *Artemia* is independent of its rearing conditions or food. Though the lipid profile showed variation with respect to the diet offered to the *Artemia* cultures, high levels of essential fatty acids can easily and very quickly be attained in the *Artemia* biomass by applying simple bio-encapsulation with a HUFA enrichment product. Less than one hour of boosting the (n-3) HUFA content in *Artemia* raised from a low level of 3 mg.g⁻¹ DW up to levels of more than 50 mg.g⁻¹.

Culture techniques

Culture of *Artemia* are mainly two types :

- Production in saltwater ponds and
- Production in controlled systems (Tanks, raceways, and flow-through systems).

Production in saltwater ponds

Large-scale culture of *Artemia* can be carried out in manmade saltwater ponds for biomass and cysts production. Such a system is termed an extensive culture.

Several thousands of hectares of salterns unsuited for classical agriculture due to salinity problems and having a salinity of 30 to 200 ppt and water temperatures 25 to 35°C in Brazil, China, Philippines Thailand, and Mexico, etc have been used for biomass and cyst production.

The basic principle for *Artemia* production in saltern is the inoculation of a small quantity of freshly hatched nauplii (1 to 10gm/l) after ensuring that predators are excluded. The ponds are fertilized to augment enough food production to maintain a continuous ovoviviparous mode of reproduction to facilitate a fast increase in population density to harvest biomass and further expose *Artemia* to higher salinity levels (above150ppt) giving stress to the population thereby helping in the induction of oviparity to produce cysts.

The liberated cysts will float and due to wind action they will accumulate on shore and from there they can be harvested. A production of 15gmliveweight/m³/day and 60kg dry weight/ha/5 months is usually achieved from salt pans.

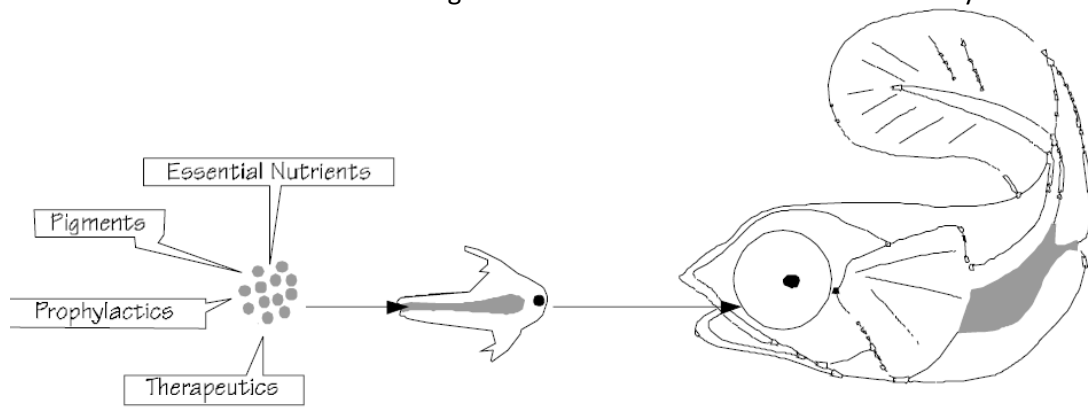
Artemia production in controlled systems

Biomass production of *Artemia* under controlled conditions (intensive) can be carried out in **batch** or in **flowthrough culture systems** with provisions for maximum oxygenation and ensuring continuous food availability.

Enrichment with nutrients

An important factor affecting the nutritional value of *Artemia* as a food source for marine larval organisms is the content of essential fatty acids, eicosapentaenoic acid (EPA: 20:5n-3), and even more importantly docosahexaenoic acid (DHA: 22:6n-3). In contrast to freshwater species, most marine organisms do not have the capacity to biosynthesize these EFA from lower-chain unsaturated fatty acids, such as linolenic acid (18:3n-3). In view of the fatty acid deficiency, research has been conducted to improve its lipid composition by prefeeding with (n-3) highly unsaturated fatty acid (HUFA)-rich diets. It is fortunate in this respect that, because of its primitive feeding characteristics, allows a very convenient way to manipulate its biochemical composition. Thus, since molting to the second larval stage (*i.e.*, instar II stage about 8 hours following hatching), is non-selective in taking up particulate matter, simple methods have been developed to incorporate lipid products into the brine shrimp nauplii prior to offering them as a prey to the predator larvae. This method of

bioencapsulation, also called enrichment or boosting is widely applied at marine fish and crustacean hatcheries all over the world for enhancing the nutritional value of with essential fatty acids.



Schematic diagram of the use of as vector for transfer of specific components into the cultured larvae.

There are many enrichment products, including unicellular algae, ω -yeast and/or emulsified preparations, compound diets, micro-particulate diets or self-emulsifying concentrates. The Selco diet is a self-dispersing complex of selected marine oil sources, vitamins, and carotenoids. Upon dilution in seawater, finely dispersed stable microglobules are formed which are readily ingested by and which bring up EFA-enrichment levels.