ARTEMIA CULTURE TECHNIQUES FOR MARINE FINFISH LARVAL REARING

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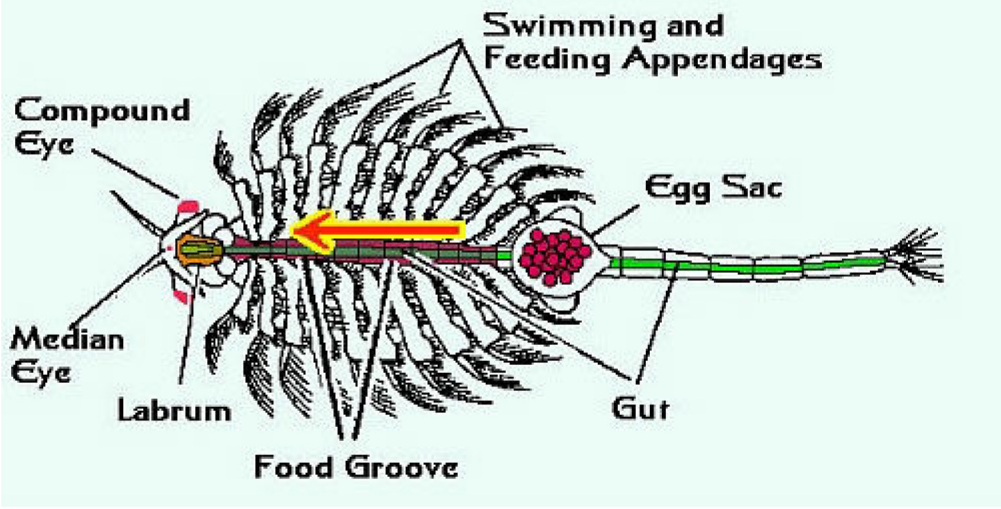
Rotifer is the most commonly used live feed upon transition of the larvae from endogenous (internal energy reserves) to exogenous (external) feeding in larviculture. *Artemia* (Brine shrimp) is another important live feed, most commonly used at completion of the rotifer stage and prior to conversion of the larva to an inert feed. It represents the transitional feed for the larvae, after which artificial feeds can be used. Brine shrimp is typically a primitive crustacean belonging to the class Branchiopoda with a total length of about 7-12 mm. This is a unique marine organism, which can withstand and survive in a wide range of salinity. It is widely distributed and more than 50 strains have so far been recorded across the world. Among the live diets used in aquaculture, *Artemia* nauplii are the most and widely used food item mainly due to its convenience and availability. The unique property of *Artemia* is the formation of dormant embryos, called 'cysts'. Cysts are available year-round in large quantities along the shorelines of hypersaline lakes and coastal lagoons, which can be collected, processed and stored or commercially made available. *Artemia* cysts have remarkable shelf life and can be stored in containers for years and utilized as a "ready-made" live food source. Upon 24-h incubation in seawater, these cysts release free-swimming nauplii that can directly used as a nutritious live food source to the larvae of several varieties of marine organisms. This excellent property of *Artemia* makes them the most convenient and least labour-intensive live food available for aquaculture. Brine shrimp are purchased from commercial suppliers and hatched in tanks. Annually, more than 2000 metric tonnes of dry *Artemia* cysts are marketed worldwide for on-site hatching into 0.4 mm nauplii for feeding finfishes and shellfishes. Like rotifers, brine shrimp must be enriched to increase their nutritional value before they are fed to fish larvae. *Artemia* are nonspecific feeders and will ingest a wide variety of foods. This feeding habit helps in easy nutritional enrichment of *Artemia* to enhance the levels of important marine-based highly unsaturated fatty acids (HUFA).

Morphology

Artemia comprises seven to nine species; all these have diverged from the ancestral form of *Artemia* which existed in the Mediterranean area about 5.5 million years ago. Different species name had been assigned to different strains of rotifers based on their reproductive isolation of populations or clusters of populations in different regions. The different species of *Artemia* described till now are *A. salina, A. tunisiana, A. parthenogenetica, A. urmiana, A. sinica, A. persimilis, and A. franciscana. Artemia* is a typical primitive arthropod with a segmented body. The body usually consists of 19 segments, the first 11 of which have pairs of appendages, the next two which are often fused together carry the reproductive organs, and the last segments lead to the tail. The total length is usually about 8–10 mm for the adult male and 10–12 mm for the female, but the width of both sexes, including the legs, is about 4 mm. The body of *Artemia* is divided into head, thorax, and abdomen. The entire body is covered with a thin, flexible exoskeleton of chitin to which muscles are attached internally and shed periodically. Shedding of the shell is by moulting and generally, female *Artemia* moults prior to every ovulation

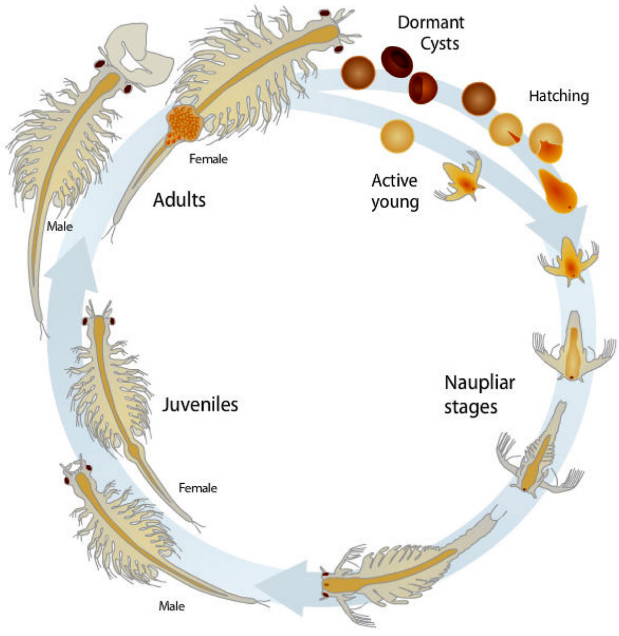


Adult female brine shrimp (Source: http://reefkeeping.com/)

Life cycle

The *Artemia* life cycle begins with the hatching of dormant cysts. The cysts are metabolically inactive embryos that can remain dormant for many years, as long

as they are kept dry and oxygen free which can resume development on re-hydration. The cyst bursts and the embryo leaves the shell after 15 to 20 hrs at 25 ^{o}C of hydration. After which, the embryo hangs underneath the empty shell called as umbrella stage, during which time the development of the nauplius is completed. Within a short period of time the hatching membrane is ruptured (hatching) and the free-swimming nauplius is born. The first larval stage is called Instar-I (400 to 500 µm in length), where the nauplii are a brownish-orange color because of their yolk reserves. Newly hatched *Artemia* do not feed because their mouth and anus are not fully developed. Approximately 12 hours after hatching, the animals moult into the second larval stage called Instar-II. In this stage, small food particles ranging in size from 1 to 50 µm are filtered into the digestive tract. During the next eight days, the nauplii grow and progress through 15 molts before reaching adulthood. Adult *Artemia* size is an average about 8 mm long, but can reach lengths up to 20 mm in optimal environments. An adult brine shrimp is approximately 20 times longer and 500 fold larger in biomass than a nauplius. Male brine shrimp possess a paired penis in the posterior part of their trunk, and adult female *Artemia* can easily be recognized by the brood pouch.



Life cycle of brine shrimp (Source: http://wildaboututah.org/)

In nature, fertilized females usually produce free-swimming nauplii at a rate of up to 75 nauplii per day (ovoviviparous reproduction) under suitable environment. Normally, the average life span of the female is about 50 days. But under ideal conditions, an adult *Artemia* can live as long as three months and produce up to 300 nauplii or cysts every 4 days. Normally the cyst production is induced under unfavorable environmental conditions, such as high salinity, chronic food shortages and/or cyclic oxygen stress, where embryos develop up to the gastrula stage, and then get surrounded by a thick shell. Formation of this shell initiates a state of metabolic dormancy (diapause), and the cysts released by the female (oviparous reproduction), float to the shoreline and dehydrate.

Hatching/Production

Proper hatching and harvesting of *Artemia* nauplii are very important to maximizing quality. Standardization of protocols is important, as slight deviations in the process will extremely affect the hatching rate, nutritional makeup, and final size of the harvested nauplii. *Artemia* cysts are expensive, making them one of the largest variable costs for a hatchery. Therefore, every attempt must be made to maximize hatch rate and quality. In the hatching process, cysts are hatched out after decapsulation.

Decapsulation

The hard egg shell that covers cyst is to be completely removed by short exposure to a bleach solution and this procedure is called decapsulation. Decapsulation of the cyst is necessary for the following reasons:

i. When normal cysts (non-decaptulated) hatch out, the separation of nauplii from their shells is not always possible. Un-hatched cysts and empty shells can cause deleterious effects in the larval tanks when they are ingested by the fry. The egg casing cannot be digested and may obstruct the gut.

ii. Nauplii produced from decapsulated cysts have a higher energy content and individual weight (30-55% depending on strain) than the nauplii from the normal cyst.

iii. Decapsulation results in disinfection of the cysts. Therefore, infection due to contamination by dirty brine shrimp eggs is avoided.

iv. The illumination requirements for hatching decapsulated cysts are lower.

Decapsulation process

In this process, the cysts are hydrated and become ready for decapsulation when the cysts are spherical in shape. After hydration, decapsulation starts with removal of the brown shell in a bleach solution, followed by washing and neutralization of the remaining bleach. These decapsulated cysts can be hatched into nauplii immediately, or dehydrated in concentrated brine solution and then stored for hatching later. Decapsulated *Artemia* cysts can be stored for a few days in the refrigerator without any noted decrease in hatching rate.

i. Hydrate the cysts by placing them for 1 h in water (< 100 gm/l), with aeration at 25-32 °C.

ii. Collect cysts on a 125 µm mesh sieve, rinse, and transfer to the hypochlorite solution. (The hypochlorite solution can be made up of either liquid bleach NaOCl (fresh product; activity normally =11-13% w/w) or bleaching powder $Ca(OCl)_2$ (activity normally ± 70%).

iii. Add the hydrated cysts and then keep them in suspension using aeration tube for 5-15 min. Check the temperature regularly, since the reaction is exothermic; never exceed 40 °C (if needed add ice to decapsulation solution).

iv. During the process, the cysts turn grey (with powder bleach) or orange (with liquid bleach), after 3-15 min. Remove the cysts from decapsulation suspension and rinsed with water on a 125 µm screen until no chlorine smell is detected. It is very important that, embryos should not be kept for long time in the decapsulation solution, since this will affect their viability.

v. Deactivate all traces of hypochlorite by dipping the cysts (< 1 min.) either in 0.1 N HCl or in a 0.1% $Na_2S_2O_3$ solution, then rinse again with water. Hypochlorite residues can be detected by putting some decapsulated cysts in a small amount of starch-iodine indicator. When the reagent turns blue, washing and deactivation has to be continued.

Hatching

For hatching, the decapsulated cysts are to be transferred to the hatching container and suitable environment needs to be provided. The best hatching results are achieved in conical bottom containers, aerated from the center bottom. Cylindrical or square-bottomed tanks will have dead spots in which *Artemia* cysts

and nauplii accumulate, so these shapes need to be used. During hatching the intensity of aeration should be sufficient to maintain high oxygen levels, because increased hatching has been reported with increasing oxygen level. As for temperature, optimal hatching occurs in the range of 25-28 °C; below 25 °C cysts hatch more slowly and above 33 °C the cyst metabolism is stopped. Reports from commercial hatcheries suggest that strong illumination (approximately 2000 Lux at the water surface) is essential for maximal hatching, and that this lighting is essential during the first hours after complete hydration, in order to initiate embryonic development. The optimal conditions for hatching *Artemia* are: Temperature: 26-30 °C, salinity: 25-35 ppt, pH range 8-9, Illumination: 2000 Lux at water surface, Aeration: heavy continuous aeration with open end (DO : > 4 mg/L)

Harvesting of nauplii

After hatching, the nauplii are harvested by simply turning off the air, and allowing the culture to settle for approximately 10 minutes. Hatched, empty shells float to the surface, and unhatched cysts will sink to the bottom. The newly hatched nauplii will concentrate just above the unhatched cysts on the bottom. Since the newly hatched nauplii are attracted to light, using flashlight at the center of the container will concentrate the nauplii where it is easy to siphon them off.

Feeding and enrichment

Artemia are non-selective filter feeders and therefore will ingest a wide range of foods. The main criteria for food selection are particle size, digestibility, and nutrient levels. The best feeds for *Artemia* are live microalgae such as *Nannochloropsis, Tetraselmis*, *Isochrysis,* and *Pavlova*. Moreover, a combination of live phytoplankton fed to *Artemia* cultures have demonstrated superior enrichment characteristics (i.e., increased HUFAs) over feeding single phytoplankton species. But some unicellular algae are not appropriate for sustaining *Artemia* growth. For example, *Chlorella,* and *Stichococcus* have a thick cell wall that cannot be digested by *Artemia*. In addition to live algae, *Artemia* cultures can be enriched by feeding a wide variety of processed foods, including yeasts, fish meal, soybean powder, egg yolk, and micronized rice bran. Like rotifers, the inherent nutritional value of *Artemia* is low, resulting in similar enrichment requirements. *Artemia* are enriched with commercially available

enrichment medium (DC DHA Selco, algamag). Once enriched, *Artemia* are rinsed, concentrated, enumerated, and then fed to larvae.

Farming of *Artemia*

Farming of *Artemia* is practiced in different place for large scale production of *Artemia* biomass and cysts to meet the feed requirement of the globally expanding fish farming industry. Controlled *Artemia* production is carried out in coastal salt pans where seawater is concentrated by evaporation until crystallization. *Artemia* can be cultured in permanent solar salt operations and seasonal artisanal units. There are different culture methods being practiced for mass scale production of *Artemia* including extensive, semi intensive and highly intensive methods of culture. These methods are classified based on the sophistications used for culture, yield and productivity from a limited area. In many cases, salt ponds are modified for *Artemia* production by deepening the ponds to 40-50 cm with high temperatures. The size range of the suitable pond for *Artemia* production is from 0.05 to 0.5 ha. Generally, two different systems used for the production of *Artemia.*

i. Seasonal unit

Seasonal units are small traditional salt pans in the tropical & subtropical belt, which are only operational during the dry season. The size of the pond is only a few 100 m^2 with depths of 0.1 to 0.6 m and normally these types of ponds are operated for a few months during summer, when the balance between evaporation/precipitation is positive. During rainy seasons, these ponds are used for fish culture purpose.

ii. Permanent unit

Permanent units are established especially for production of *Artemia* alone. This unit consist of several interconnected evaporation ponds and crystallizers. Size of each pond in this system varies from few to several hundred hectares with depths of 0.5 to 1.5 m. Seawater is pumped into the first pond and flows through the successive evaporation ponds; in the meantime salinity levels gradually increases as a result of evaporation and this high salinity water is used for *Artemia* farming.

For *Artemia* culture, pond should be prepared as similar to shrimp culture. Pond preparation should begin at the end of the rainy season with ploughing. For optimal

productivity, proper pond management must be ensured by maintaining optimal salinity (80-100 ppt) and temperature (< 33 °C) through regulation of water supply and pond modification. During culture, management and feeding procedures should be optimum and it should target the development of suitable phytoplankton and avoid proliferation of benthic macro-algal mats. In a typical *Artemia* pond, 'greenwater' (phytoplankton rich water) is pumped every two days with water level increments of 2-5 cm for compensating seepage and evaporation loss of water; this provides the *Artemia* with food. Regular physical disturbance of pond bottom is necessary to retard the development of algal mats, known as 'lab-lab'; filamentous algal mats may develop and these will trap cysts, thus affecting *Artemia* populations. Cysts released by females float on the water surface, cysts floating in the corner are collected by dip nets of appropriate mesh size (e.g. 150 ìm), normally every morning depending on production level. For collecting adult *Artemia*, a 1 mm mesh net is used after three weeks of culture, especially in the morning or afternoon when most individuals are found near the water surface. Harvesting frequency may depend on need, it can be done daily or twice a week with biomass harvests of 25 to 50 kg ww/ha/day, with production levels as high as 0.7 to 1 tonnes/month/ha over a culture period of four to five months.

Basic criteria for site selection

The following parameters should be present as a minimum requirement for selecting a site for *Artemia* farming.

i. Water salinity should be high: The pond should have salinity of at least 80 ppt, The pond should be either salt pan or high saline water from fish ponds in late summer.

ii. Depth of pond should be at least 30-40 cm to maintain optimum required temperature for culture and cyst production.

iii. Pond area should have regular water intake facilities – Water should be filled at least once or twice in a week.

iv. Pollution and contamination free water is required.

References

Browne, R. A. and Bowen, S.T. 1991. Taxonomy and population genetics of *Artemia*. In: *Artemia* Biology: Browne, R. A., Sorgeloos, P and Trotman, C.N.A. (Eds), CRC Press, Boca Raton Ann, Arbor Boston, USA, pp 221235.

Lavens P. and Sorgeloos, P. 1987. Design, operation, and potential of a culture system for the continuous production of *Artemia* nauplii. In: *Artemia* Research and its Applications. Vol. 3. Ecology, Culturing, Use in Aquaculture P. Sorgeloos, D. Bengtson, W. Decleir and E. Jaspers (Eds) Universa Press, Wetteren, Belgium: 339-345.

Lavens P. and Sorgeloos, P. 1991. Chapter XIII : Production of *Artemia* in culture tanks. In: *Artemia* Biology. R.A. Browne, P. Sorgeloos and C.N.A. Trotman (Eds). CRC Press, Inc. Boca Raton, Florida, USA, 317-350.

Lavens, P. and Sorgeloos, P. 1999. Manual on the production and use of live food for aquaculture. FAO Fisheries Techni-cal Paper No. 361. FAO, Rome, Italy. 305 pp.

Ogello, E.O., Kembenya, E., Githukiya, C.M., Nyonje, B.M., Munguti, J.M. 2014. The occurrence of the brine shrimp, Artemia franciscana (Kellog 1906) in Kenya and the potential economic impacts among Kenyan coastal communities. International journal of fisheries and aquatic studies, 1(5): 151-156.

Pilla, E.J.S and Beardmore, J.A. 1994. Genetic and morphometric differentiation in old

world bisexual species of the brine shrimp (*Artemia*). Heredity, 72: 4756.

Van Stappen, G. 2011. In: Cultured aquatic species information programme *Artemia spp.* In: FAO Fisheries and Aquaculture department. Rome.

Van Stappen, G., Litvinenko, L.I., Litvinenko, A.I., Boyko, E.G., Marden, B. & Sorgeloos, P. 2009. A survey of Artemia resources of Southwest Siberia (Russian Federation). Reviews in Fisheries Science, 17:117-148.

Online sites:

- http://www.brineshrimpdirect.com/brineshirmparticles1.html
- http://www.angelfire.com/wa/AquariaWeb/_Artemia_.html