

## Artemia Biology and Culture Techniques

A. Mariselvammurugan<sup>1</sup>, M. Anbarasan<sup>2</sup>, M. Santhosh Kumar<sup>3</sup> and S. Manickavasagam<sup>4</sup>

<sup>1</sup>M.F.Sc. Fish nutrition and feed technology Research Scholar, Kerala University of fisheries and Ocean Studies, Panangad, Kerala-682506, India.

<sup>2</sup>M.F.Sc. Aquatic Animal Health Management Research Scholar, SKUAST-K -Faculty of fisheries, Rangil, Jammu and Kashmir-190006, India.

<sup>3</sup>M.F.Sc. Fisheries Extension Research Scholar, Central Institute of Fisheries Education, Panch Marg, Off. Yari Road, Versova, Andheri (West), Mumbai, Maharashtra-400061, India.

<sup>4</sup>Assistant Professor, TNJFU – Directorate of Sustainable Aquaculture, Thanjavur Centre for Sustainable Aquaculture, Thanjavur – 614 904, Tamil Nadu, India

### SUMMARY

Artemia, commonly known as brine shrimp, plays a crucial role in larviculture as a transitional diet for marine larvae before the introduction of artificial nutrients. With a global distribution and exceptional shelf life, Artemia cysts are a convenient and widely used live feed in aquaculture. Their unique morphology, characterized by a tough outer layer and three distinct embryonic layers, contributes to their resilience and adaptability to various environments. The hatching process of Artemia cysts, influenced by factors such as temperature, salinity, and hatching efficiency, marks the beginning of their life cycle. From dormant cysts to free-swimming nauplii, Artemia undergo several developmental stages, ultimately maturing into adult brine shrimp. Understanding the ideal hatching conditions, hatching procedures, and site selection criteria are essential for successful Artemia cultivation. Overall, Artemia cysts serve as a valuable resource in aquaculture, providing nutrient-rich live food for the sustainable growth of marine larvae.

### INTRODUCTION

When larvae in larviculture move from endogenous (internal energy stores) to exogenous (external) feeding, rotifer is the most often employed live feed. Another crucial live feed is Artemia, or brine shrimp, which is often given when the larvae have completed the rotifer stage and are about to turn into an inert feed. It serves as the larvae's transitional diet before artificial nutrients can be introduced. A primitive crustacean of the class Branchiopoda, brine shrimp generally have a length of 7–12 mm. This particular marine creature is resistant to a broad range of salinities and can thrive there. It is extensively dispersed, with over 50 strains identified worldwide to date. Because it is readily available and convenient, Artemia nauplii is the most often employed food item among the live diets used in aquaculture. The development of "cysts," or latent embryos, is Artemia's special characteristic. Large concentrations of cysts are always present near the shorelines of coastal lagoons and hypersaline lakes. Roughly 90% of the Artemia cysts harvested for commercial purposes worldwide come from Utah's Great Salt Lake. This can be gathered, processed, stored, or offered for sale. Artemia cysts can be used as a "ready-made" live food source because of their exceptional shelf life, which allows them to be kept in containers for years. These cysts produce free-swimming nauplii after a 24-hour incubation period in saltwater, which may be immediately used by the larvae of a variety of marine animals as a nutrient-rich live food supply. Because of its great quality, Artemia are the least labor-intensive and most convenient live food for aquaculture. Commercial companies sell brine shrimp, which are hatched in tanks. Worldwide, around 2000 metric tons of dried Artemia cysts are sold each year for on-site nauplii hatching into 0.4 mm size, which are then fed to fin fish and shellfish. Prior to being given to fish larvae, brine shrimp, like rotifers, need to be supplemented to improve their nutritional content. Artemia are general feeders that will take in a broad range of meals. This feeding habit helps in easy nutritional enrichment of Artemia to enhance the levels of important marine-based highly unsaturated fatty acids (HUFA).

### Morphology characters of Brine shrimp

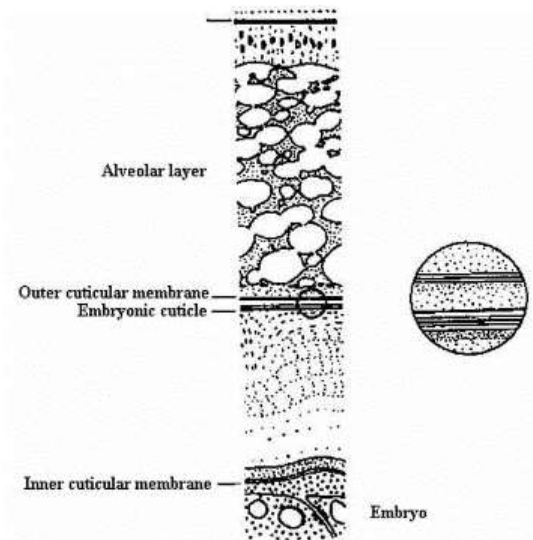
#### Morphology of Artemia cyst

The strains differ in terms of size, dry weight, and energy content. While hatching quality mostly relies on the collecting site, hatching efficiency, percentage hatching rate and quality vary. The effects of salinity and temperature on development and survival are substantial. The strain affects the composition of amino acids and total lipid content.

### A cyst is made up of three layers.

Hematin, chitin, and lipoprotein make up the tough outer layer. The layer is colored a dark brown by the hematin. This layer shields the skin from UV and mechanical damage of any type. Hypochlorite can be used to oxidize, or "decapsulated," this layer. The outer cuticular membrane, a multilayered membrane with a unique filter, is the second layer. This serves as a barrier to permeability and shields the fetus from molecules bigger than CO<sub>2</sub>.

The translucent and very elastic embryonic cuticle makes up the third layer. During hatching, it transforms into the hatching membrane.



### Artemia morphology

Typically, the body is divided into 19 segments: the first 11 include pairs of appendages; the next two, which are sometimes fused together, carry the reproductive organs; the final segment leads to the tail. The mature male's total length is about 8–10 mm, while the female's is 10–12 mm. Both sexes' breadth, including the legs, is around 4 mm. The head, thorax, and abdomen comprise Artemia's body. A thin, flexible chitin exoskeleton covers the whole body, to which muscles are internally linked and regularly shed. Female Artemia typically moults before each ovulation in order to shed her shell.

### Growth of an Artemia cyst from saltwater incubation to the discharge of nauplius

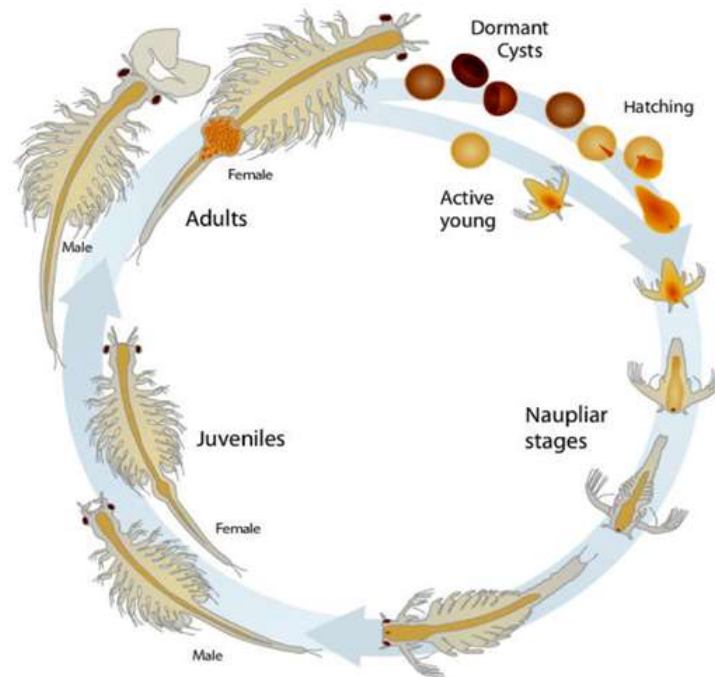
The biconcave cyst grows and becomes spherical in 1 to 2 hours when cultured in seawater. The embryo encircled by the hatching membrane becomes apparent when the cyst shell, including the outer cuticular membrane, ruptures (= breaking stage) after 12 to 20 hours of hydration. The hatching membrane may still be linked to the shell after the embryo fully exits the shell and hangs underneath the empty shell. The development of the pre-nauplius into the instar I nauplius, which begins to move its appendages, may be observed through the transparent hatching membrane. The hatching membrane splits open shortly after, allowing the free-swimming larva to emerge head first. Due to their high hygroscopicity, dry cysts absorb water quickly; in the first few hours, the volume of the hydrated embryo can reach up to 140% water content; however, if the environment is right, active metabolism can begin as soon as the water level reaches 60%. The cyst embryo's aerobic metabolism ensures that the reserve carbohydrate trehalose is converted to glycerol and glycogen, which are used as an energy source.



### Artemia life cycle

Dormant cysts hatch to initiate the life cycle of Artemia. The cysts are metabolically inert embryos that, given enough time and dry conditions, can stay dormant for many years. Rehydrating them will allow them to begin the process of developing again. At 25°C of hydration, the cyst breaks and the embryo emerges from the shell after 15 to 20 hours. The nauplius growth is then finished as the embryo hangs underneath the empty shell known as the umbrella stage. The hatching membrane ruptures (hatching) after a short while, resulting in the birth of the free-swimming nauplius. Due to their yolk reserves, the nauplii in the first larval stage, known as Instar-I (400 to 500 µm in length), are brownish-orange in hue. The mouth and anus of newly born Artemia are not fully grown, therefore they cannot eat. The individuals molt into Instar-II, the second larval stage, around 12 hours after hatching. Small food particles, sized between 1 and 50 µm, are filtered into the digestive system at this stage. The nauplii develop throughout the course of the following eight days, going through 15 molts to become adults. In ideal conditions, adult Artemia may grow to lengths of up to 20 mm, with an average length of 8 mm. 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. 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. Unfavorable environmental factors, such as excessive salinity, prolonged starvation, or cyclic oxygen stress, are typically responsible for inducing cyst formation. In these situations, embryos grow to the gastrula stage before being encased in a thick shell. When this shell forms, the organism enters a condition of metabolic slumber known as diapause. The female releases cysts during oviparous reproduction, which float to the coast and dry up.



### The ideal environment for Artemia cysts to hatch

- Temperatures over 25°C, with 28° C being the ideal temperature, are ideal for the hatching of artemia.
- 5 ppt salinity
- Constant, intense aeration.
- Continuous lighting (two 40-watt fluorescent lights, for instance, to light four 1-liter hatching cones).
- A pH of around eight.
- A liter of water must contain no more than 5 grams of cysts to achieve the desired stocking density. Maintaining good circulation is necessary to keep the cysts suspended.
- The ideal container is a cone or a V-shaped one (two-liter bottles work nicely; just glue a valve to the bottle top and flip it over). The best container is a separation column, which costs more but may be found in any lab supply.
- 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.

### Procedures used in the hatching and decapsulation process

#### Hydration

Fill the desiccated cysts with fresh saltwater. Employ a clear conical tank or funnel-shaped container (such as a glass or plastic cylinder, thick plastic bags fashioned into the required shape), and aerate from the apparatus's bottom for an hour to maintain the cysts in continuous suspension. The dry cysts, which collapse like bean seeds, take on a spherical form when they are hydrated. To guarantee that the inside of the indented dry cyst shell will be fully revealed with the addition of the decapsulation solution, full hydration is required.

#### Decapsulation (hypochlorite)

Utilizing 1N NaOH, sodium hypochlorite (NaOCl), and saltwater, prepare the decapsulation solution. Give the hydrated cysts seven to fifteen minutes to react with the hypochlorite decapsulation solution. Keep the temperature below 40°C by utilizing a water bath or adding ice cubes to the solution to prevent embryo harm. The response is generally finished when the cysts' color changes from brown to white to orange. If you can, look under a microscope.

### Sieving and washing

Once the decapsulated cyst suspension has been drained into a fine-mesh screen immediately rinse with saltwater six to ten times, or until the hypochlorite odor is eliminated. Decapsulated cysts can be kept in a saturated brine solution at a low temperature for later use or they can be supplied straight to the cultured fish and crustaceans.

### Incubation

The decapsulated cysts should be incubated in natural saltwater for 24 to 48 hours, with a maximum density of 5 grams of cysts per liter of incubation media. Maintain a pH of 8–9 and a temperature of 30°C for the best hatching conditions. At least for the first two hours, provide enough light, or better yet, ideally constant lighting of around 1000 lux (achieved with a 40-watt fluorescent light bulb placed 20 cm away from the hatching container). Throughout the incubation phase, keep the dissolved oxygen near saturation and the cysts suspended. Feed the Artemia to the shrimp or fish larvae as soon as they hatch to take advantage of the yolk in the Nauplii if cultivation to the adult stage is not required.

### Harvesting processing and packaging of artemia cyst

Most of the cysts will hatch after 15 to 20 hours of incubation, and the culture's color will noticeably shift from brown to orange. You may observe the pinkish-orange nauplii swimming at this time, along with gentle aeration. Undissolved or empty shells tend to float, whereas unhatched cysts and detritus sink. To remove the nauplii from the bottom, first the trash may also be done using a siphon. The next step is to gather the nauplii on a screen measuring 100–120 micrometers, wash them in clean water, and then submerge them in a tiny amount of water. Contaminants and hatching metabolites are eliminated by washing. For feeding, wash the gathered nauplii.

### Basic criteria for site selection

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

- Water ought to have a high salinity: The pond should be made of high salinity water from fish ponds in late summer or a salt pan with a minimum salinity of 80 parts per thousand.
- For optimal results, the pond's depth should be at least 30 to 40 cm. The temperature needed to produce cysts and for culture.
- Regular water intake facilities are necessary for the pond area. Water has to be filled once or twice a week at the very least.
- Water that is free from contaminants and pollutants is necessary.

### CONCLUSION

Artemia cysts, with their unique morphological characteristics and adaptable life cycle, emerge as indispensable components in larviculture and aquaculture practices worldwide. Their ease of availability, long shelf life, and nutritional value make them a preferred choice for feeding marine larvae. Proper understanding of Artemia morphology, hatching processes, and cultivation techniques is essential for maximizing their utility in aquaculture operations. By adhering to recommended procedures and selecting suitable cultivation sites, aqua culturists can harness the potential of Artemia cysts to support the healthy growth and development of various marine species, contributing to sustainable aquaculture practices and biodiversity conservation efforts.

### REFERENCES

- Das, P., Mandal, S. C., Bhagabati, S. K., Akhtar, M. S., & Singh, S. K. (2012). Important live food organisms and their role in aquaculture. *Frontiers in aquaculture*, 5(4), 69-86.
- Ogello, E.O., Kembanya, 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.
- Radhakrishnan, D.K.; AkbarAli, I.; Schmidt, B.V.; John, E.M.; Sivanpillai, S.; Vasunambesan, S.T. Improvement of nutritional quality of live feed for aquaculture: An overview. *Aquac. Res.* **2020**, *51*, 1–17.
- 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.
- Van Stappen, G. 2011. In: Cultured aquatic species information programme Artemia spp. In: FAO Fisheries and Aquaculture department. Rome.

Xavier, B., Ranjan, R., Megarajan, S., Loka, J., & Kizhakudan, J. K. (2023). Training Manual on Livefeed culture techniques for Mariculture applications.

ICAR E-Course Fish Food Organism Manual

<http://www.brineshrimpdirect.com/brineshirmparticles1.html>

[http://www.angelfire.com/wa/AquariaWeb/\\_Artemia\\_.html](http://www.angelfire.com/wa/AquariaWeb/_Artemia_.html)

<http://www.fao.org/docrep/003/w3732e/w3732e0t.html>