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Artemia Culture for Intensive Finfish and Crustacean Larviculture

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Introduction

In Virginia and throughout the United States, freshwater and saltwater finfish and shrimp aquaculture is expanding rapidly. During the cultivation of most marine finfish and shrimp species – as well as some freshwater species – live feeds are an essential component during the larviculture stage. During larviculture, the rotifer is the most commonly used live feed upon transition of the larvae from endogenous (internal energy reserves) to exogenous (external) feeding. Upon completion of the rotifer stage, the most commonly used live feed prior to conversion of the larva to a dry diet is *Artemia* (figure 1).

Figure 1. *Artemia*: just hatched (left), enriched and 24 hours old (right)

As a food source for the larvae, it is imperative that *Artemia* is of high quality, as nutritionally complete as possible, and maintained in this state until consumed by the larvae. There are four distinct stages involved in *Artemia* culture. These stages are: (1) decapsulation, (2) hatching, (3) enrichment, and (4) storage. *Artemia* also represent a potential vector for disease introduction into the larviculture production system. As such, all *Artemia* production and storage procedures must be conducted utilizing hygienic production protocols and proper hatchery sanitation procedures. This document provides the background, rationale, and detailed production protocols for all stages of high-quality *Artemia* culture.

Decapsulation of *Artemia* Cysts

Rationale

Artemia represent one of the few live feeds that can be cultured in sufficient numbers and are of appropriate size for larva to transition to between rotifers and weaning diets. During a portion of their life cycle, *Artemia* hibernate as a desiccated cyst that is capable of withstanding extreme environmental conditions for long periods of time. Cysts are easily shipped and are thus the form purchased by hatcheries.

However, *Artemia* cysts can cause problems during larviculture because:

1. The shell of the cyst is indigestible and may cause intestinal blockage when ingested by larva.
2. Cysts are a potential vector for pathogen introduction to the culture system.
3. *Artemia* consume high levels of endogenous energy reserves when hatching through the cyst shell.
4. Cysts must be physically separated from the live *Artemia* after hatching.

Decapsulation of *Artemia* cyst is a process whereby the external shell or chorion is chemically removed from

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the cyst. This process addresses the concerns noted above and has become standard practice by fish hatcheries looking to produce high quality *Artemia*.

Artemia Decapsulation Requirements

***Artemia* cysts**: 1 kilogram (kg)

Decapsulation vessel: 20 liters (L)

Chlorine bleach (NaOCl; 5.5%): 8 L at 2-10 degrees Celsius (°C)

Sodium hydroxide (NaOH; 40%): 4 L at 2-10°C

Sodium thiosulfate ($Na_2S_2O_3$): 100 g

Harvest bag: 100 micrometer (µm)

Artemia Decapsulation Procedure

Hydration

The first step in the decapsulation procedure is *Artemia* cyst hydration. Hydration of cysts allows for separation of the nauplii from the chorion, facilitating the decapsulation process. For this step, *Artemia* cysts are placed in either fresh or saltwater at room temperature for approximately one hour, using a concentration of 1 g of cysts per 15 milliliters (ml) of water. It is important during this step to maintain sufficient mixing via aeration to keep cysts well suspended. After one hour of hydration, the water and hydrated cysts should be drained through a 100 µm harvest bag; the concentrated cysts are then placed back into the empty decapsulation vessel.

Decapsulation

For decapsulation, pour the chilled sodium hydroxide solution into the decapsulation vessel with hydrated cysts, again making sure there is adequate aeration within the vessel to keep cysts suspended. The chilled bleach should then be added to the cysts to initiate the decapsulation process. Because the chemical reaction during decapsulation is exothermic, it is helpful to begin with chemical solutions chilled to a temperature of 2°C to 10°C. These starting temperatures will prevent the temperature of the chemical solution from exceeding 35°C, which may damage the cysts.

As decapsulation progresses, the chorion is chemically removed, resulting in the cysts gradually changing color from brown to grey, then to orange, and finally to bright orange. This bright orange color indicates that the process is complete. (Cyst buoyancy can also be used as an endpoint indicator: when approximately 90 percent of cysts sink, the process is complete.)

The process should take from one to three minutes, but time may differ due to temperature variations. Cysts can easily be damaged by overexposure to the decapsulation solution, adversely affecting the resulting hatch rate. It is imperative to closely monitor the process and standardize it for your particular conditions.

Harvest

When it is determined that the cysts are adequately decapsulated, add 75 g of sodium thiosulfate to the decapsulation vessel to neutralize the chlorine, then immediately begin to drain cysts into the 100 µm harvest bag. During the harvest process (figure 2), rinse with ample amounts of water (fresh or salt) while providing ample aeration via an air stone to keep decapsulated cysts in suspension. When all decapsulated cysts have been collected, the remaining sodium thiosulfate should be added to the harvest bag. Continue rinsing the bag until water runs clear and no presence of chlorine can be detected.

Figure 2. Harvesting decapsulated *Artemia*: note harvest bag and rinse water leaving bucket

Storage

Decapsulated cysts can be drained of excess water and stored in an airtight container in a refrigerator for up

to two weeks. For longer-term storage (two months or more), cysts must be dehydrated by placing them in aerated brine (330 g of sodium chloride [NaCl] per liter of water) at the concentration of 1 g of cysts per 20 ml of brine for 24 hours. They can then be drained and placed into a suitable container, topped with fresh brine, and placed in a refrigerator.

Hatching of *Artemia* Cysts

Rationale

While it is a straightforward process, proper hatching and harvesting of *Artemia* nauplii is vital to maximizing quality. Standardization of protocols is important, as slight deviations in the process pro foundly 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. As a result, every attempt must be made to maximize hatch rate and quality. Furthermore, because the risk of pathogenic contamination is high, biosecurity measures should be in place to minimize this risk.

Artemia Hatching Requirements

Temperature: 26-30°C

pH: 8.0-9.0

Dissolved oxygen: > 4 mg/L

Light level: ~2000 lux

Salinity: 25-35 parts per thousand (ppt)

Hatching density: ≤ 2 g dry cysts/L
(up to 5 g/L with supplemental O_2)

Sodium bicarbonate ($NaHCO_3$)**:** 0.5 g/L

Antifoam (silicone based)**:** 1 ml/100 L

Artemia Hatching Procedure

Fill a clean, cone-bottomed hatching tank with warm, filtered seawater. If warm seawater is not available, allow enough lead time for water to be warmed to 26°C to 30°C in the hatching tank via submersible heaters. Add 0.5 g of sodium bicarbonate per liter of water in order to maintain the pH between 8.0 and 9.0 throughout the entire hatching process. The use of antimicrobial products such as INVE's Hatch Controller (INVE, Salt Lake City, Utah) can be used to help minimize growth of pathogenic bacteria in the hatching tank. The proper stocking density for nondecapsulated cysts is approximately 2 g per liter.

When using decapsulated cysts, approximately 5 g per liter can be stocked. These numbers can be doubled through the use of pure oxygen supplementation, which is needed to maintain dissolved oxygen levels greater than 4 milligrams per liter. Attempting to hatch at higher stocking densities can result in physical damage to the nauplii and reduced quality.

It is important to maintain sufficient aeration at the bottom of the cone to keep cysts suspended (figure 3). When hatching large volumes of cysts, it is advantageous to use a food-grade antifoam product to minimize excessive foaming in the culture. Hatching times will vary based on strain and age of cysts, temperature and salinity of water, etc. Thus, it is important to minimize variation between hatches for consistency.

Generally, *Artemia* require 18 to 24 hours of incubation to hatch. Decapsulated cysts, however, may be ready to harvest after only 16 hours of incubation. When feeding nauplii directly to fish, timing of the hatch is very important. If nauplii remain in the hatching tank for too long, they will grow too large and their nutritional quality will decrease. Determining the endpoint of the hatch should be made through microscopic observation of the relative numbers of hatched nauplii, prehatched nauplii, and unhatched cysts.

Figure 3. *Artemia* hatching cone: note pure oxygen injection regulators on wall and wire from submersible heater on front edge of tank

Artemia Harvesting

The harvesting procedure varies depending upon whether decapsulated or nondecapsulated cysts were hatched.

When harvesting previously **decapsulated cysts**, simply drain the entire water column into a 125 harvest bag. An air stone should be placed in the bag to maintain oxygen levels while keeping nauplii in suspension. After all nauplii have been collected, rinse them in the bag with clean water for at least five minutes.

After rinsing, attempt to separate the hatching membranes (which remain intact after the decapsulation process) from the nauplii. To do this, place *Artemia* into a cooler, enrichment tank, or other clean container at a density less than 5 million per liter. Using a micropore oxygen diffuser (Point Four Systems, British Columbia, Canada), oxygen should be injected into the cooler. The tiny oxygen bubbles will adhere to the membranes and they will begin to float after a few minutes, where they can be skimmed from the top. After removing membranes, nauplii are ready to be fed to the larva, transferred to subsequent enrichment, or placed into cold storage.

If harvesting **nondecapsulated cysts**, turn off the air for 10 to 15 minutes. This will allow nauplii – as well as any unhatched cysts – to settle to the bottom of the cone, while hatched cysts will float to the surface. Keeping the air off for more than 15 minutes may result in *Artemia* suffocation. *Artemia* move toward light, so covering the tank and/or placing a light source at the bottom of the cone will aid in separation. After settling, slowly open the bottom drain and purge off the unhatched cysts that will come out first.

Conversely, a standpipe can be placed in the tank prior to settling to allow the bottom 1 inch of settled material to remain undisturbed. Newly hatched nauplii should then be collected in the harvest bag and rinsed for at least five minutes. If nauplii have settled properly, only 75 percent of the water column will need to be drained. While harvesting, check on the relative ratio of nauplii to cysts by transferring a sample to a glass beaker. This will help determine when the harvesting process is finished or if more time is needed to allow *Artemia* to settle. The nauplii are now ready to be fed to your fish, transferred to subsequent enrichment, or placed into cold storage.

Artemia Counting

Counting of harvested *Artemia* is necessary to determine accurate dosage rates for feeding and enriching, and as quality control for the hatch. Hatch rates will vary depending on strain and age of cysts, but generally speaking, experienced users should see hatch rates with GSL cysts of 200,000 or more nauplii per gram of nondecapsulated cysts. While there are a number of methods used for counting hatched *Artemia* (two are presented below), it is important to choose the best one for your situation and stick with it to develop standardized counting protocols for your facility in order to minimize variation.

Method 1:

After harvesting and rinsing *Artemia*, store in a clean, well-aerated container at a density no greater than 5 million per liter. To count *Artemia*, a small subsample should be collected from the well-mixed storage container and diluted 10-fold. Load a Sedgwick-Rafter slide (figure 4) with 1 ml of the diluted sample and add one to two drops of formalin or Lugol's solution to immobilize the *Artemia*. Count under low magnification and record the number of intact, healthy-looking nauplii. Counts should be conducted two to three times to determine an average. Multiply the average by 10 (rate of dilution) to determine the number of *Artemia* per milliliter in the storage container.

Figure 4. Newly hatched *Artemia* on Sedgwick rafter counting slide; round object is the cyst the Artemia just hatched from

Method 2:

Concentrate the rinsed *Artemia* in 10 L of saltwater using vigorous aeration. Collect 10 ml of *Artemia* and add to 990 ml of saltwater. Collect 1 ml of sample with

a pipette and count the number of live *Artemia* within the pipette. Return the sample to the container, stir, and repeat the process five to 10 times; then determine an average. The average of these counts conducted in this fashion multiplied by 1 million (rate of dilution) equals the total number of *Artemia*.

Enrichment of *Artemia*

Rationale

Before being fed to larvae, *Artemia* nauplii are usually fed a specialized diet in order to increase their size and nutritional profile. While freshly hatched *Artemia* nauplii are rich in protein and can serve as a bridge between rotifers and enriched *Artemia* for many species, they are largely void of the beneficial fatty acids required for proper growth and development of most larvae. For the purpose of the following *Artemia* enrichment procedure, the protocol developed for the use of the INVE product, DC DHA SELCO, will be utilized.

Artemia Enrichment Requirements

Temperature: 25°C

pH: 8.0-8.5

Dissolved oxygen: > 4 mg/L

Salinity: 20-30 ppt

Density: ≤ 300 nauplii/ml

DC DHA dosage: 0.6 g/L

Enrichment duration: 20-24 hours

Artemia Enrichment Procedure

There are a number of commercially available *Artemia* enrichment products. Because these products have different ingredients, nutritional profiles, and enrichment protocols, it is up to hatchery managers to decide which product is most suitable for their conditions and species. Once an enrichment product is chosen, it is important that standardized protocols be developed and strictly followed. Slight changes in temperature or enrichment time, for example, can have significant effects upon the size and nutritional quality of the final product.

Preparation of enriched *Artemia* requires a two-day lead time: one day is required for hatching of *Artemia* (see *Artemia* hatching protocol) and a second day for the enrichment process. Having a second, dedicated enrichment tank is necessary to facilitate this process. As with hatching, a cone-bottomed tank is ideal for enrichment and helps to ensure adequate mixing and complete draining during harvest. Prior to stocking, the enrichment tank should be filled with a suitable amount of water, and water-quality parameters (salinity, temperature, and pH) must be adjusted to match the requirements listed above.

It is important to begin the enrichment process with healthy, high-quality nauplii. Nauplii that are damaged or sluggish prior to enrichment will result in suboptimal nutrient uptake. Care should be taken to remove hatched cysts (nondecapsulated cysts) or hatching membranes (from decapsulated cysts) as described in the *Artemia* hatching section. *Artemia* nauplii should also be rinsed well prior to stocking into the enrichment tank. This is especially important when using an additive such as INVE's Hatch Controller or antifoam during the hatching process, as ingredients in these products can interfere with enrichment uptake.

During enrichment, vigorous aeration should be applied through the bottom of the enrichment vessel, and dissolved oxygen levels should be closely monitored throughout the process (figure 5). The use of supplemental oxygen during this stage will likely be necessary to maintain oxygen levels above 4 milligrams per liter. Temperature must also be maintained at 25°C through the use of submersible heaters or ice packs, as dictated by ambient conditions.

Figure 5. Multiple *Artemia* enrichment cones: note heavy aeration

Harvest

At the end of the enrichment process, the entire volume of water should be drained into a 125 µm harvest bag with sufficient aeration to keep enriched *Artemia* in suspension. Oxygen levels should be closely monitored in the harvest bag. The bag containing the *Artemia* should be rinsed well for five minutes or until the water runs clear. Thereafter, *Artemia* should be transferred into a container containing clean water of a known volume, aerated vigorously, and enumerated as discussed above. If *Artemia* will not be fed to larvae immediately, it should be placed directly into cold storage, as described below.

Cold Storage

Artemia not fed to larvae or enriched immediately needs to be stored under cold conditions. Cold storage of *Artemia* dramatically decreases its metabolism, which directly reduces further growth and metabolism of their protein and lipid stores. *Artemia* should be transferred to a cooler or suitable container and stored at 2°C to 10°C, with adequate aeration to prevent settling (figure 6). Under these conditions, *Artemia* can be concentrated as high as 5,000 per milliliter and stored for up to 24 hours.

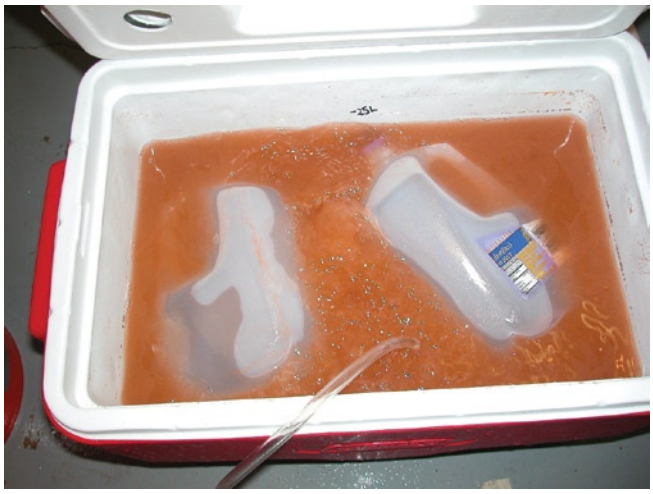

Figure 6. Cold-banked *Artemia*: ice jugs for temperature control and air line for aeration to keep *Artemia* suspended

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