Recent Developments On The Aplication Of Artemia In The Ornamental Fish Culture

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Abstract: Production of animals for the aquarium hobbyist trade is a rapidly growing sector of the aquacultural industry, and it will continue to become more important as restrictions are placed on collecting animals for the wild. Improved techniques for marine food-fish larviculture since the early 1980's have greatly enhanced the growth and survival of freshwater ornamental fish larvae largely through improved technology regarding live food culture and larval rearing practices. Research developments in larviculture and early rearing technology have allowed 90% of currently marketed freshwater ornamental fish to be cultured. However, for marine ornamentals, the reverse is true as only a handful of species is produced via aquaculture technology. A major task in devising a protocol for the artificial propagation of a fish species is the development of a feeding regimen for the larvae. Live feeds are a convenient and often essential food source for the larvae of some cultured species, especially those without a fully developed digestive system. In such cases, live food organisms provide digestive enzymes that breakdown the food ingested by larvae and can be described as naturally encapsulated bags of nutrients. Two major concerns among aquaculturists are providing organisms appropriate to the size of the larvae at the first feeding stage and then supplying the large numbers of feed organisms necessary to maintain the larvae. Since no artificial feed formulation is yet available to completely substitute for *Artemia*, feeding live prey to young fish larvae still remains essential in commercial hatchery operations. This paper reports the recent developments in the applications of *Artemia* nauplii, decapsulated *Artemia* cysts and on-grown *Artemia* in the ornamental fish culture.

Key words: *Artemia*, Ornamental Fish, Larvae, Feeding

1. Introduction

The ornamental fish sector is a widespread and global component of international trade, fisheries, aquaculture and socio-economic development. Since 1985, the international trade in exports of ornamentals, which usually takes place in the majority of developing countries, followed an increasing trend with an average growth rate of approximately 14% per year. The entire industry has been estimated to be worth around US$15 billion. This vast industry has the potential to contribute to the economic growth of developing countries which may face future challenges regarding environmental safety (Olivotto et al., 2006). Production of animals for the aquarium hobbyist trade is a rapidly growing sector of the aquacultural industry, and it will continue to become more important as restrictions are placed on collecting animals for the wild. Currently, approximately 90% of freshwater fish traded in the hobbyist industry are captively cultured. While a majority of aquacultural production worldwide is devoted to food production, ornamental fish production is an important component of the aquaculture industry in several nations. In Singapore, ornamental fish accounts for 40% of their total exports. In the United States, ornamental fish production is the fourth largest sector behind catfish, trout, and salmon. Farms in Florida produce 800 varieties of freshwater fish (Tlusty, 2002).

Successful rearing of larval stages of aquatic organisms is a challenge for aquarium hobbyists, an aim and a necessity for the success of the aquaculturist. All these specialists will agree that the primary problem in any type of larval rearing is that of food. Ideally, one would prefer to feed larvae their natural diet, which is characterized by a wide diversity of nutritious live organisms. Live feed is an essential food source for the fry of cultured species, especially those without a fully developed digestive system. In the freshwater ornamental fish culture, *Artemia* nauplii are used as the live feed. Two major concerns of aquaculturists are: (i) providing organisms appropriate to the size of the feed to the first feeding stage and (ii) supplying adequate number of feed organisms to ensure higher survival and faster growth (Arulvasu and Munuswamy, 2009). In nature, zooplankton

is one of the primary foods of larval fish. The brine shrimp *Artemia* is in the phylum Arthropoda, (Crustacea, Anostraca). *Artemia spp.,* are zooplankton, like copepods and *Daphnia*, which are used as live food in the aquarium trade, and for freswater and marine fish larval culture and crustacean larval culture (Lim et.al., 2001). While the adult form of *Artemia* is primarily used as a live, frozen, or freeze-dried food in the aquarium trade, the nauplius stage is used exclusively in fish hatchery operations. It was recognized long ago that freshly hatched *Artemia* nauplii are a high value feed for fish larvae and fry. Because of the size of the nauplius stage, *Artemia* also represent the only practical food source for the early stages of many fish and crustacean larvae. (Tamaru et al., 2001). In Singapore, the top-exporting country of freshwater ornamental fish in the world, *Moina* used to be the most common live food organism used in the industry. As *Moina* is cultured in ponds using pig waste, they are often contaminated with fish pathogens, as well as bacteria of public health concern, such as *Salmonella* and *Vibrio cholera.* To minimize the risk of fish being contaminated with the pathogens, more and more freshwater ornamental fish farmers in Singapore have shifted from *Moina* to the cleaner *Artemia* nauplii for feeding their fish. (Lim et al,. 2002, 2003). Since no artificial feed formulation is yet available to completely substitute for *Artemia*, feeding live prey to young fish larvae still remains essential in commercial hatchery operations. There are more than 50 geographical strains of *Artemia*. Many commercial harvesters and distributors sell brands of various qualities. This paper reports the recent developments in the applications of *Artemia* nauplii, decapsulated *Artemia* cysts and on-grown *Artemia* in the ornamental fish culture.

1.1. Why is Live Feed Necessary?

Fish biologists categories larvae of two types: precocial and altricial. Precocial larvae are those that, when the yolk sac is exhausted, appear as mini-adults, exhibiting fully developed fins and a mature digestive system including a functional stomach. Such fish can ingest and digest formulated diets as a first food and are best exemplified by the salmon and trout raised extensively in hatcheries around the world without the benefit of live food. Altricial larvae are those that, when the yolk sac is exhausted, remain in a relatively undeveloped state. The digestive system is still rudimentary, lacking a stomach, and much of the protein digestion takes place in hindgut epithelial cells (Govoni et al., 1986). Such a digestive system seems (at this point) to be incapable of processing formulated diets in a manner that allows survival and growth of the larvae comparable to those fed on live feed. Altricial larvae therefore appear to require live feed, but there may be other reasons besides the digestibility question. Live feeds are able to swim in the water column and are thus constantly available to the larvae. Formulated diets tend to aggregate on the water surface or, more commonly, sink quickly to the bottom, and are thus normally less available to the larvae than are the live feeds. In addition, the movement of live feed in the water is likely to stimulate larval feeding responses, since evolutionary history has probably adapted them to attack moving prey in nature. Formulated diets are generally capable of moving only in a downward direction, towards the bottom. Finally, live prey, with a thin exoskeleton and high water content, may be more palatable to the larvae once taken into the mouth, compared with the hard, dry formulated diets. This last point is rather critical, especially when considered in light of the fish larva's absence of feeding appendages; any foods must enter the mouth whole (i.e. the larva's mouth gape must be of sufficient size for particle ingestion to occur) and they are quickly either accepted or rejected on the basis of palatability (Stottrup and McEvoy, 2003).

2. *Artemia*

Artemia has several characteristics which make it ideal for aquaculture use. It is easy to handle, adaptable to a wide-range of environmental conditions, non-selective as a filter-feeder which can ingest algae, protozoa and bacteria of the correct size (10–50 μm) and is capable of growing at very high densities (Landau et al. 1985; Lèger et al. 1989). *Artemia* also has a high nutritive value (40–60 percent protein, rich amino acid composition), an unchanging food requirement, high conversion efficiency, short generation time, high fecundity rate and long lifespan. The whole animal (even adult stage) may be consumed without previous processing by many aquaculture organisms. In the food chain the nutritional value of *Artemia* depends on both the macronutrients (proteins, fats and carbohydrates) and micronutrients (vitamins and minerals) it can accumulate from the filtered food. The brine shrimp is considered a continuous, non-selective, obligate phagotrophic filter feeder zooplankton (Fig.1).

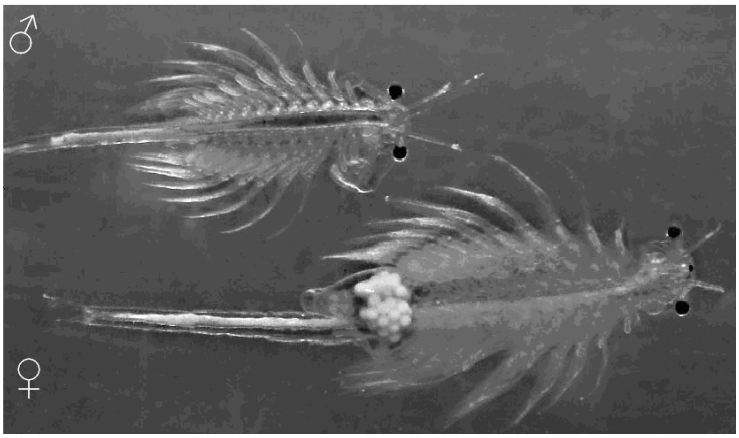

Figure 1. Adults *Artemia* sp.

Artemia are extremely euryhaline, withstanding salinities from 3 ppt to 300 ppt. They can even survive short periods of time in freshwater, but cannot reproduce in it. *Artemia* survive temperatures ranging from 15 to 55 ^{o}C. They have two modes of reproduction. Sometimes nauplii (first *Artemia* swimming stage) hatch in the ovisac of the mother and are born live. However, when the body of water where adult *Artemia* 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 (Fig 2). Dry conditions cause the encysted embryo to enter a dormant state, which allows it to withstand complete drying, temperatures over 100 ^{o}C or near absolute zero, high energy radiation, and a variety of organic solvents. The dehydrated cyst can be stored for months or years without loss of hatchability.

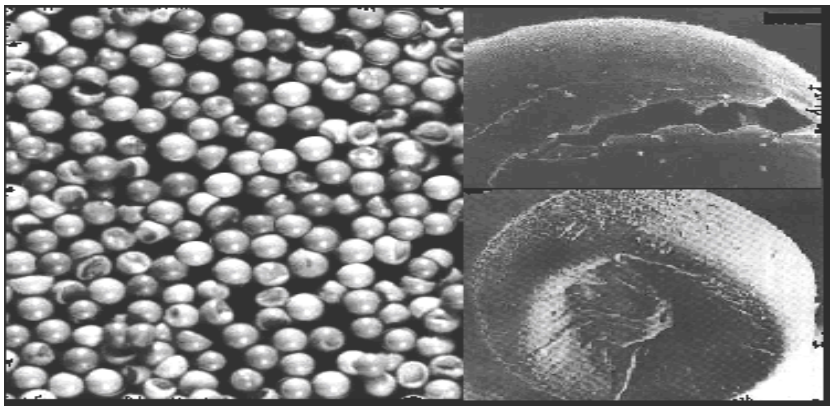

Figure 2. *Artemia* Cysts.

Only water and oxygen are required to initiate the normal development of the *Artemia* embryo, but it does help the hatch rate to maintain the temperature above 25 ^{o}C and place a light near the eggs. The durable, easily hatched cyst makes *Artemia* a convenient, constantly accessible source of live feed for the finfish hatchery operator. *Artemia* 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 ^{o} the shell breaks and the prenauplius in E-1 stage appears (Fig. 3). For the first few hours, the embryo hangs beneath the cyst shell in what is called the umbrella stage. The newly hatched *Artemia* relies on its yolk sac for nutrients because its mouth and anus are not fully developed. The pre-nauplius E-2 stage is then released as a free-swimming nauplius called an Instar 1 nauplius. In this stage it is brownish orange because of its yolk reserves. It uses specially modified antennae for locomotion and later for food filtering. Approximately 12 hours after hatch it molts into the second larval stage (Instar II) and starts filter feeding on microalgae, bacteria and detritus. The *Artemia* nauplius can live on yolk and stored re-serves for up to 5 days or through the Instar V stage (Fig. 3), but its caloric and protein content diminish during this time (Briksi et.al., 2008).

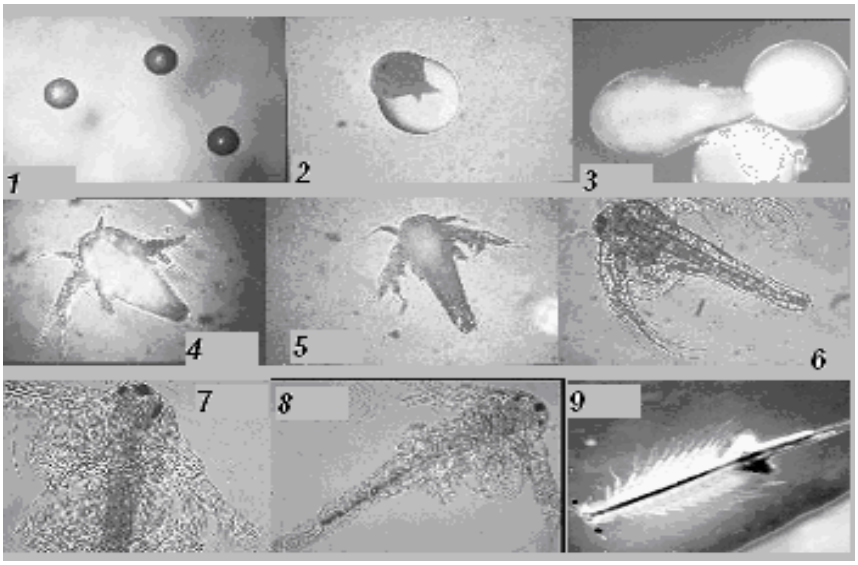

Figure 3. Steps in Life Cycle of *Artemia*
1: Cysts, 2: Breaking stage, 3: Umbrella stage: emerging embryo, 4: Instar I(E-1) newly hatched nauplii (with yolk), 5: Instar II(E-2), 6: Differeatiation (molting) stage, Instar III-IV, 7: Instar VI-VIII, 8: Instar IX-X, Sub-adult stage, 9: Adult stage.

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) storage, (4) enrichment, (5) harvestin and usage. *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 to developments on the aplication of *Artemia* in the ornamental fish culture.

2.1. Decapsulation of *Artemia* Cysts

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

The fry of all the five common ornamental fish species tested (guppy *Poecilia reticulata*, molly *Poecilia sphenops*, platy *Xiphophorus maculatus*, swordtail *Xiphophorus helleri* and neon tetra *Hyphessobrycon herbertaxelrodi*) could readily feed on the decapsulated cysts, and their performances in terms of stress resistance, growth and survival are comparable to or better than those fed on *Artemia* nauplii or *Moina*. A culture system for production of on-grown *Artemia* has also been developed specifically for the use in freshwater ornamental fish farms (Lim et al. 2003).

2.1.1. *Artemia* Decapsulation Procedure and 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)

2.1.2. 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.

2.1.3. 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 % 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 (Fig. 4).

2.1.4. Decapsulated Cysts 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 (Fig. 4), 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.

2.1.5 Decapsulated Cysts Storage

Decapsulated cysts can be drained of excess water and stored in an airtight container in a refrigerator (+ 4 ºC) for up to 5-6 days. For longer-term storage (two weeks 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 (Fig. 5).

Figure 4. Harvesting Decapsulated *Artemia*

Figure 5. Decapsulated *Artemia* Cysts Hatching Storage

2.2. Hatching of *Artemia* Cysts and 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

Fill a clean, cone-bottomed hatching tank with warm, filtered seawater or fresh water addet 30-35 g salt. 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 can be used to help minimize growth of pathogenic bacteria in the hatching tank. The proper stocking density for nondecapsulated cysts is approximately 2-3 g (max. 5 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 4-5 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. 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 (Fig 6).

Figure 6. *Artemia* Hatching Cone
(pure oxygen injection regulators on wall and wire from submersible heater on front edge of tank)

The harvesting procedure varies depending upon whether decapsulated or nondecapsulated cysts were hatched. Harvesting of *Artemia* nauplii is done after 5 to 10 minutes interruption of the aeration and remove the airstone. Wait approximately 5 minutes for the empty casings to float to the surface of the water. Empty cyst shells float to the surface, while the nauplii concentrate in the lower part of the tank and the unhatched cysts accumulate underneath the nauplii. Since most nauplii are positively phototactic, their concentration can be hastened and increased by shading the upper part of the hatching container with a black plastic sheet so that light reaches the lower part of the container only. Remove the unhatched cysts for the second hatching, after which the nauplii can be collected. A second collection of nauplii may be done 5 to 10 minutes after the first. 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. Remove the unhatched cysts for the second hatching, after which the nauplii can be collected. A second collection of nauplii may be done 5 to 10 minutes after the first. The nauplii are now ready to be fed to your fish, transferred to subsequent enrichment, or placed into cold storage.

2.3. Enrichment of *Artemia* and 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

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 daphnia, rotifer 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.

Olivotto et al. (2006) studied on growth and metamorhosis larvae of Sunrise Dottyback, *Pseudochromis flavivertex*. Larvae were divided into different experimental groups and fed on different feeding combinations in order to test the importance of food enrichment on larval survival, growth and metamorphosis timing. A first group (Group A) was fed on enriched *Brachionus plicatilis* and enriched *Artemia nauplii*; a second one (Group B) on enriched *B. plicatilis* and not enriched *Artemia nauplii* and a third one fed on not enriched live preys (Group C) used as control group. Live prey enrichment was essential for rearing this species. In fact, larvae fed on not enriched live preys did not past day 7. Highest survival rates (39% juveniles) were observed in Group A with respect to Group B (11% juveniles). Moreover, evidences of the importance of enrichment on growth and metamorphosis timing were observed since larvae reared using enriched live preys grew faster and completed metamorphosis earlier than those fed on not enriched *Artemia nauplii*. The results presented here provide additional evidence of the importance of live prey enrichment in ornamental larval fish rearing.

2.3.1. *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 (Fig. 7). 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 (Delbos, 2009).

Figure 7. Multiple *Artemia* Enrichment Cones (heavy aeration)

2.4. Harvest and Cold Storage

At the end of the enrichment process, the entire volume of water should be drained into a 100-125 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. *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 (Fig. 8). Under these conditions, *Artemia* can be concentrated as high as 5,000 per milliliter and stored for up to 24 hours (Delbos, 2009).

Figure 8. Cold-Banked *Artemia*
(ice jugs for temperature control and air line for aeration to keep *Artemia* suspended)

3. Conclusions

The ornamental fish producer would have no problem to assign such a small area for setting up the culture system in their aquarium or farms. While the use of a batch culture system instead of a flow-through system would cut down the volume of seawater required for *Artemia* culture, the use of artificial seawater would enable farms that have no access to seawater to operate the system. To cut down the cost of salts required for

preparation of artificial seawater, the present system, for the first time in commercial *Artemia* production, used diluted artificial seawater (salinity 30-40 ppt) instead of full strength seawater for the culture. Change of water was not necessary during the 14-16 day culture period. These characteristics made the system suitable for operation in freshwater ornamental fish farms, and would allow existing ornamental fish farmers to integrate the *Artemia* production system in their farm operation. The present system did not use expensive mechanical and biological water treatment equipment such as bio-filter, mechanical filter, plate separator, sensors etc. and hence the cost of setting up the system was € 90,000-100,000 only. Bioencapsulation to enhance the nutritional quality of on-grown *Artemia* was conducted only when the *Artemia* failed to meet the fish requirement. The same applied to all other live food organisms such as rotifers and *Artemia* nauplii which might also require bioencapsulation due to their nutritional deficiency (Leger and Sorgeloos 1992; Sorgeloos and Leger 1992; Sorgeloos et al. 1995, Sorgeloos et. al., 2001). It was performed by fish farmers just before feeding the *Artemia* to fish, and not by producer of the organism. Hence the cost of bioencapsulation was not included in the production cost of the *Artemia.* Nevertheless, the cost of the enrichment media (€ 80-90/kg) used in bioencapsulation was estimated to be € 3-5/kg of on-grown *Artemia* (in 50 liter of water at 0.6 g/L.). The present *Artemia* culture system is a cheap alternative to the more sophisticated intensive system used in sectoral aplications. Compared to the complex automated system, the present system is cost effective, simple and easy to set up and operate. As the system occupies only a small land area and uses diluted artificial seawater for culture, the freshwater ornamental farmers will have no problem to integrate *Artemia* production using the culture system into their farm operation to increase farm profitability. By varying the time of harvesting, farmers may harvest any specific size of on-grown *Artemia* of up to 5 mm from the culture system to suit the age and size of their fish. The use of the right size of on-grown *Artemia* for feeding would ensure a better energy balance in food uptake and assimilation, thereby improving the performance of the fish. These characteristics, coupled with the use of bioencapsulation technique to enhance the quality of the on-grown *Artemia,* would make the organism an ideal nursery diet for freshwater ornamental fish. The availability of on-grown *Artemia* and the application of bioencapsulation techniques using the organism are likely to have a positive impact to the ornamental fish industry.

The food value of a live food organism for a particular fish species was primarily determined by its size and form. While a small food organism was desirable for fish larvae in term of ingestibility, the use of larger organisms was more beneficial as long as the size of the food organism did not interfere with the ingestion mechanism of the predator (Merchie 1996). Fish would take a long time to attain satiation if fed with smaller live food organism, and this would result in poor growth due to inefficient feeding and waste of energy. The on-grown *Artemia* in the culture system grew from 0.45 mm at inoculation to an average length of about 5 mm in 12 days. This size range was considered suitable for all sizes of freshwater ornamental fish species of up to 10 cm total length. By varying the harvesting time during the 12-day cycle, it was possible to obtain *Artemia* of any specific size within the size range for feeding, which would ensure a better energy balance in food uptake and assimilation. The nutritional quality of on-grown *Artemia* was comparable or superior to the common food organisms being used by the freshwater ornamental fish industry, such as *Artemia* nauplii, *Moina* and bloodworms. The on-grown *Artemia* was rich in protein (67 %) and low in crude fat (4 %). It was reported to have superior nutritional digestibility and a thin exoskeleton rich in essential amino acids (Leger et al. 1989). The latter was consistent with our amino acids analyses, which showed that the essential amino acids in the on-grown *Artemia* were comparable to *Moina* and richer than *Artemia* nauplii and bloodworms. An important dietary characteristic of live food organism was its composition of essential fatty acids. Watanabe (1987) reviewed the essential fatty acid requirement of freshwater and marine fish and concluded that freshwater species required mainly LLA (linolenic) or LNA (linolenic acid) or both. Although the on-grown *Artemia* obtained from the present study was deficient in LNA, its LLA was the highest among all the four diets tested. The DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), which were widely considered as essential for marine organisms (Dhont and Lavens, 1996), were also highest in on-grown *Artemia.* Due to lack of published data, it was not known whether the levels of LLA, LNA, EPA and DHA in food organisms would be important to freshwater ornamental fish. Recent study on the fatty acid profiles of common feed items used by the industry for maturation such as beef heart and tubifex worms found unusually high ADA (arachidonic acid) levels (Ako et al. 1999). Availability of the on-grown *Artemia* would offer our farmers and exporters the possibility to apply the bioencapsulation technique to improve their fish performance and quality. In addition, the effective bioencapsulation characteristics of on-grown *Artemia* also make the organism a useful tool for larval nutrition study on freshwater ornamental fish. The present *Artemia* culture system is a cheap alternative to the more sophisticated superintensive system. By varying the time of harvesting, aquarists may harvest any specific size of on-grown *Artemia* of up to 5 mm from the culture system to suit the age and size of their fish. The use of the right size of on-grown *Artemia* for feeding would ensure a better energy balance in food uptake and assimilation, thereby improving the performance of the fish. These characteristics, coupled with the use of bioencapsulation technique to enhance the quality of the on-grown *Artemia,* would make the organism an ideal nursery diet for freshwater ornamental fish. The availability of on-grown *Artemia* and the application of bioencapsulation

techniques using the organism are likely to have a positive impact to the ornamental fish industry. Finally, demonstrated that the commercial production of on-grown *Artemia* using the present culture system was highly viable for freshwater ornamental fish applications.

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