Proceedings of the Summer Institute in Recent Advances in Finfish and Shellfish Nutrition

11 TO 30 MAY 1987

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Dr. SALIM ALI ROAD
COCHIN-682 031

Technical Paper-2

SUMMER INSTITUTE IN
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION
11-30 May, 1987

LIVE FOOD ORGANISMS - *ARTEMIA*

PART I - *ARTEMIA* CYSTS AND THEIR UTILIZATION

S.K. PANDIAN

Central Marine Fisheries Research Institute,
Marine Prawn Culture Laboratory
Narakkal-682 505.

INTRODUCTION

Extensive literature reveals that only very few organisms have been utilized as live feed of which the brine shrimp *Artemia*, is the most important one. In the growing aquaculture industry, a great demand exists for *Artemia* cysts which form as dried inert food source. In these cysts embryos, after development upto gastrula stage, are kept in diapause. The cysts are brownish in colour and measure about 0.3 mm in diameter. When dehydrated, they are biconcave in shape.

CYST HARVEST AND CYST PROCESSING

Artemia cysts can be collected from natural saline habitats which are located in coastal as well as inland areas in both temperate and tropical countries. Literature reveals that a good *Artemia* biotope produces 10-20 kg cysts per hectare per season (Persoone and Sorgeloos, 1980). In *Artemia* biotopes, cysts float in saline waters (except that of *Artemia monica*) and are blown to shore by wind action, where they accumulate. Cysts should be harvested as soon as

possible after accumulation. Cysts should preferably be harvested from the water surface rather than from the shores thus assuring less contamination with impurities. The cysts, collected, may be contaminated with sand particles, dirt material, dead Artemia, algae, debris, empty shells, broken shell bits etc. The hatching efficiency of the collected cysts is largely determined by the effective removal of from the cysts and keeping the cyst water content less than 10%. Hence, the cysts have to be cleaned first and then adequately dehydrated.

Cleaning can be done by washing the cysts with freshwater using different mesh-sized screens so that corresponding sized dirt materials can be removed. Generally 400 microns and 100 microns sieves are used for this purpose. When the raw cysts are allowed to pass through 400 microns sieve and collected on 100 microns sieve in the process of washing, debris of above 400 microns and of lesser than 100 microns are removed. Cleaning in freshwater must be very quickly done as prolonged cleaning in freshwater will initiate hydration and subsequent embryological development resulting in energy loss. The dirt materials which are equal in size with the cysts can be removed by the biphase floatation method, in which initially the raw cysts have to be suspended in brine for a period of 24 hours. Cysts and light debris will float while heavy particles such as sand will sink to the bottom. Secondly the floating cysts have to be removed from brine and introduced in freshwater for a short period of 15 minutes. The viable cysts will sink to the bottom whereas the empty shells, non-viable cysts etc float at the surface. The cleaned cysts are to be siphoned off. They have to be spread in uniform thickness over a drying surface and kept for drying in the shade or in a hot air oven at 30-40°C until cyst water content has reached to the level

of 2-9%. Now cysts attain biconcave shape. They have to be packed under vacuum or nitrogen atmosphere and stored in dry place. It is reported that the cysts, thus processed and stored under ideal conditions, retain their viability for at least 12-13 years.

CYST HATCHING

At least five conditions are essential for restarting the arrested embryological development in cysts leading to hatching (Sorgeloos and Kulasekarapandian, 1984). They are hydration of the cysts, oxygenation of the medium, illumination of the hydrated cysts, temperature of 26-35°C and pH above 8.0. Hatching can be carried out in salinities ranging from 5 to 75 ppt. Artemia cysts never hatch at high salinities because they cannot hydrate enough which is one of the pre-requisite for the onset of hatching mechanism. For practical convenience, seawater (enriched with 2 gm $NaHCO_3$ per litre) is used for hatching. Continuous moderate aeration, which keeps the cysts in suspension, is beneficial in hatching. Hatching efficiency decreases when dissolved oxygen content goes below 2 ppm. It is completely inhibited at 0.6 to 0.8 ppm in California strain. The light triggers the "biological clock" to start again in the hydrated cysts. Illumination for 10 minutes at an intensity of 1000 lux is sufficient in California strain. Eventhough several types of hatching containers have been used by different workers (Shelbourne et al., 1963; Jones, 1972), funnel shaped glass or plastic containers are better for hatching. Because of transparency, adequate illumination is ensured and as the bottom is funnel-shaped moderate aeration is sufficient to aerate the medium and simultaneously keep the cysts in adequate suspension. In the cylindrical containers, 7-10 gm cysts per litre of medium, can be subjected for hatching.

SEPARATION OF HATCHED NAUPLII FROM THE HATCHING DEBRIS

Consumption of empty shells block the gurt of the predator when an uncleaned hatching mixture is fed. Moreover, unhatched and empty cysts have a very high bacterial load and their introduction will spoil the predatory medium. Hence hatched nauplii alone have to be collected from the hatching debris and utilized as feed.

The positive phototactic behaviour of the nauplii is exploited for separating the nauplii from the empty and unhatched cysts. Directing a light beam on the transparent hatching device results in the larvae swimming towards the light as soon as the aeration has been turned off. They can be siphoned off from that particular place. However, this rough separation technique, although commonly used, requires skill to remove the nauplii without siphoning off the debris, accumulated in the bottom and surface. These problems can be solved by using a separator box which has a central dark compartment and an outer bright compartment both being separated by a partition. When the mixture of nauplii and debris is introduced in the central dark compartment of the box, the nauplii swim through holes or slits from the dark compartment to the brighter side. Once separation is completed, the partition can be closed and the larvae can be siphoned off from the brighter outer compartment, while unhatched cysts and empty shells being retained in the dark, central compartment. Shelbourne et al. (1963) have used rectangular separator boxes in which separation is poor because the light stimulus is not uniform in all directions. However, Persoone and Sorgeloos (1972) have used cylindrical separator boxes in which the light stimulus is uniform for all the nauplii from any direction.

CYST DECAPSULATION

The hard shell or chorion of the cyst can be removed without affecting the viability of the embryo by short exposure of the hydrated cysts to a hypochlorite solution and this process is known as decapsulation, which eliminates the cumbersome process of separating nauplii from the hatching debris. Treatment with hypochlorite solution disinfects the cysts and the decapsulated cysts can be directly used as food to predators. For example, the larvae of Lebistes, Macrobrachium, Penaeus, Portunus, Scylla and Xiphonophorus have been successfully reared by giving a diet of decapsulated cysts.

In the decapsulation process, complete removal of the chorion will be possible when the cysts are spherical in shape and to obtain this desirable stage, the cysts, as an initial step, have to be hydrated by keeping them for required period (1 to 2 hours for most of the strains) either in freshwater or seawater at about 25°C. Prolonged hydration has to be avoided as it will induce embryological development and consequent loss of energy. Subsequent to hydration, cysts have to be transferred to decapsulation solution which can be prepared with either liquid bleach (Sodium hypochlorite), NaOCl or bleaching powder, $Ca(OCl)_2$ and NaOH. 0.5 gm active product and 14 ml of decapsulation solution are required to decapsulate one gram of cyst. Decapsulation solution has to be made up with 35 ppt seawater. When the cysts are in the decapsulation solution, a gradual colour change in the cysts will be observed from dark brown to grey and then to orange. During decapsulation the temperature should not be allowed to raise above 40°C. Prolonged immersion in decapsulation solution will kill the embryo and hence cysts have to be removed from the solution as soon as

the completion of the process which can be judged by periodical observation oc systs under microscope. Cysts, after treatment in decapsulation solution, have to be washed with tap water and afterwards dipped a couple of times in 0.1 N Hcl. Subsequently, the cysts have to be washed again with tap water or seawater. The hydrated, decapsulated cysts can be offered directly as food to the predator. If needed they can be stored for a few days in the refrigerator at 0-4°C. When used directly as food, it is essential to keep the cysts in suspension, by sufficient aeration and circulation, for better utilization by predators since the hydrated decapsulated cysts sink in seawater or freshwater. Decapsulated cysts can be stored for a short period of six months by keeping them for dehydration in NaCl saturated brine (± 330 g/l). The decapsulated cysts sink in brine and become coffee-bean shaped as a result of dehydration. Brine will not help for long term storage for which the hydrated decapsulated cysts have to be dried and packed in dry and oxygen free containers.

NUTRITIVE VALUE AND QUALITY IMPROVEMENT IN ARTEMIA NAUPLII

Quality of Artemia cysts as naupliar food source, is evaluated on the basis of its nutritive value. It is profitable to use decapsulated cysts as feed instead of freshly hatched nauplii as the former contain 30-40% more energy when compared to the latter. In other words, hatching mechanism consumes 30-40% energy which can otherwise be utilized by directly giving decapsulated cysts as feed. The energy contents and individual dry weights decrease with 22-37% and 16-34% respectively while the freshly hatched nauplii (instar I larvae) develop into instar II larvae. Hence it must be taken into account that Artemia nauplii have to be fed as soon as the hatching is over to avoid the energy loss.

Marine predators need high levels of the fatty acids $20:5w^3$ and $22:6w^3$, the latter being seldom present in detectable levels in Artemia nauplii. The fatty acid pattern; more particularly the content in polyunsaturated fatty acids (PUFA), appears to vary in the Artemia as a function of the source of the cysts and even within a particular strain from cyst harvest to harvest. With PUFA poor Artemia nauplii, low survival is reported in marine fish and shrimp hatcheries. The reason for the poor PUFA-profile in some Artemia cyst sources, is related to the biochemical composition (i.e. PUFA content) of natural food of Artemia. In fact, in the high salinity environment where Artemia live, those species of diatoms and flagellates known to be rich in PUFA, do not or only seldom occur. Correspondingly the cysts produced by the Artemia of these types of biotopes, are also poor in PUFA content.

These nutritively poor 1-2 days old Artemia nauplii can be enriched with PUFA via their diet. Different particulate products can be used for enrichment of the instar II Artemia nauplii through bioencapsulation and metabolisation (accumulation). Microencapsulated diets and micronized particles respectively coated with PUFA-rich oils such as cod liver oil and PUFA-rich emulsions or micro algae rich in PUFA such as Isochrysis, Chaetoceros, Chlorella can be used as enrichment diets for instar II Artemia nauplii (Sorgeloos and Kulasekarapandian, 1984). Optimum enrichment can be achieved by feeding the Artemia nauplii, which are stocked at the rate of 25000-50000 nauplii per litre during 24 to 48 hours post-hatching with dense concentration of PUFA-rich algae or particle suspensions (100000-500000 cells/ml).

REFERENCES

Jones, A.J. 1972. An inexpensive apparatus for the large scale hatching of Artemia salina L. J.Cons.Int.Explor.Mer., 34(3): 351-356.

Persoone, G. and P. Sorgeloos, 1972. An improved separator box for Artemia nauplii and other phototactic invertebrates. Helgolander wiss Meeresunters., 23: 243-247.

Persoone, G. and P. Sorgeloos, 1980. General aspects of the ecology and biogeography of Artemia. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Eds.). The brine shrimp ARTEMIA, Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren (Belgium), pp. 3-24.

Shelbourne, J.E., J.D. Riley and G.T. Thacker 1963. Marine fish culture in Britain. I. Plaice rearing in closed circulation at Lowestoft, 1957-1960. J.Cons.int.Explor.Mer., 28(2): 50-69.

Sorgeloos, P. and S. Kulasekarapandian 1984. Production and use of Artemia in aquaculture. CMFRI Special Publication No. 15, pp.74.