Ornamental Fish

Breeding, Farming and Trade

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Larvi-feed Culture for Seed Production of Ornamentals Fishes

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Abstract

In marine fish breeding, the larval stages of many fishes are critical due to their small mouth gape and changes in feeding habit. As a result survivability is also very less which needs research thrust for augmenting survival rate, and it also heavily relies on the supply of suitable live feed organisms. Among the different live feeds micro algae (the flagellates Isochrysis galbana, Tetraselmis sp., Pavlova lutheri, and chlorococcales Chlorella spp. diatoms Skeletonema costatum, Thalassiosira sp., Chaetoceros gracilis and Chaetoceros calcitrans) and micro zooplanktons (nauplii of the brine shrimp Artemia spp., rotifer Brachionus plicatilis, calanoids and copepods, cladoceran crustaceans (Daphnia spp. and Moina spp.), together plays an important role in larval production system. In the hatchery system, mass scale production of micro algae are being done by various methods viz., Batch culture, semi continuous culture and continuous culture. Rotifers isolated can be cultured in 25 to 30 ppt water using micro algae or yeast as feed and mass scale can be done using rice bran. Artemia nauplii are being produced through decapsulation of artemia cysts and the nauplii thus obtained can be harvested and cultured in seawater using micro algae as feed. Recent findings showed that bioencapsulation of live food organisms with various nutrients have a vital role in larval survivability as the larvae require diets with high protein and sufficient amount of essential fatty acids (eicosapentaenoic acid 20:5n3 and decosa hexaenoic acid 22:6n3), its incorporation is vital for augmentation of larval production. This paper also deals with various aspects of live feed management in clown fish breeding.

Key words: *Larvi-feed culture, seed production, live feed management.*

1. Introduction

Marine ornamental fish production is considered as one of the most important trade in international markets. In the captive production system, the larval rearing of aquatic organisms is an indispensable step, and it heavily relies on the supply of suitable live feed organisms. Micro algae being the predominant component of the first trophic level in the aquatic food chain has got immense value as an aquaculture live feed and as a

result the production of unicellular algae has gained importance in several countries due to their wide use as food in the hatchery seed production of commercially important shell and fin fishes (Benemann, 1992, Muller-Feuga, 2000). The important components of microalgae are the diatoms, dinoflagellates, silicoflagellates (phytoflagellates), coccolithophores, blue green algae and the 'hidden flora'- the nannopiankters. Among these, the diatoms and phytoflagellates are significant organisms since they form the primary link in the food chain of the sea. It is known that the success of any hatchery operation depends mainly on the availability of the basic food, the micro algae and micro zooplanktons.

2. Nutritive value of algae

In the larval feeding systems micro algae are being selected on the basis of their size, nutritional value, culture easiness and absence of negative side effects such as toxicity. Their nutritional value shows a great variability not only among different species, but also in genetically different populations of the same species (strains). Though in wild condition many algae are surviving, very few specie of these planktons are suitable for aquaculture purpose. Therefore, these micro planktons are need to be isolated, identified, and find out the biochemical composition, suitable media, environmental are inevitable to increase the production of aquatic species under captive condition. Thus the culture of micro algae become as an inherent part of aquaculture since these organisms serves as the food source for the larval and juvenile stages of fin and shellfishes. In spite of all efforts to replace micro algae by artificial feeds, aquaculturists are still depending on the production and use of micro algae as live food for the fishes during their different stages of life cycles. Among the different micro algae, only very few species are suitable for fishes and provided better results when fed to organisms and some are reported to be toxic. Some of the microalgae have flagellas (one or two tiny beating hairs) for motility. These microplanktons have been extensively utilized for mass production of zooplanktons such as rotifers, artemia, copepods, etc. and these algae are also used for generating "green water" in many hatcheries using the species such as *Chlorella* sp, *Isochrysis galbana*, *Pavlova lutheri*, *Nannochloropsis occulata* and *Nannochloropsis gaditana*, *Dunaliella tertiolecta* and *Tetraselmis suecica*.

3. Important live feeds

In marine fish breeding, the larval stages of many fishes are critical due to their small mouth gape and changes in feeding habit. As a result survivability is also very less which needs research thrust for augmenting

survival rate. Among the different live feeds micro algae and micro zooplanktons together plays an important role in larval production system. Among the organisms that have been used as live feed, the most important are nauplii of the brine shrimp *Artemia* spp., the rotifer *Brachionus plicatilis*, calanoids and copepods, cladoceran crustaceans (*Daphnia* spp. and *Moina* spp.), and many species of microalgae, diatoms *Skeletonema costatum*, *Thalassiosira* sp., *Chaetoceros gracilis*, *Chaetoceros calcitrans*, the flagellates *Isochrysis galbana*, *Tetraselmis* sp. *Pavlova lutheri*, and the chlorococcales *Chlorella* spp are the most important. The selection of these live feed organisms are based on many factors such as nutritional requirements of the cultured larvae, size of the mouth gape and development of the digestive tract of the cultured larvae, nutritional value, and availability of the live feed and suitability for mass scale production. Though much efforts are been taken world wide to supplement live feed totally or partially with artificial feeds, various study pointed out that supply of suitable live feed organisms fortified with vitamins and fat are essential for the successful completion of the larval stages.

4. Micro algal culture

Suitable micro algae can be isolated through various techniques viz. pipette method, centrifuge or washing method, phototactic movements, agar plating method and serial dilution and the isolated species can be cultured in mass quantity using suitable culture media. Although most algae are photoautotrophic and can grow in purely inorganic media, many other required organic compounds, the requirements of which may be either absolute or stimulatory. While most of the microalgae can be successfully cultured on synthetic inorganic media, a few genera require organic compounds for their rapid growth, and therefore the culture are supplemented with soil extracts, yeast extracts or organic salts. Since the microalgae in any water body require the nutrients such as nitrates and phosphates roughly in a ratio of 10:1 (N:P) for its normal growth and reproduction, the culture media used in the laboratory should have sufficient quantities of these elements besides other growth promoting agents would definitely reflect on the growth of microalgae especially in a culture system. The most widely used culture media are 'Conway' or Walne's medium Erd-Schreiber's and Miquel's TMRL, Suto, PM, SEAFDEC, Gulliard f, f/2,f/4, Johnsons (J/1) ASW, MN, ASPW, etc. and fresh water micro algal medium are Bold basal, BG-11, PHM-1, Botoryococus and Zarrouk media.

5. Stock culture of micro algae

Maintenance of isolated algae in good growth condition as inoculum is the back bone of any algal production system. Stock or starter cultures are need to be frequently sub cultured to maintain the culture in the exponential growth phase which is the key factor for the successful and efficient algal production system. The major methods are indoor culture in which the isolated species are maintained as stock in small container under controlled condition in an aseptic algal culture laboratory whereas mass scale can be carried out indoor as well as out door in which production relies on natural conditions. In all the methods, the culture must be inoculated and allowed to grow and divide. The rate of growth varies depend upon the type of algae and its culture condition.

6. Mass culture

Since stock cultures are not sufficient for the requirements of zooplanktons, larvae fishes, crustacean and molluscs in hatcheries, algae are need to be multiplied in large quantity in minimum period of time. This methods can be done either indoor or out door with suitable culture media. Indoor with transparent roofing is the ideal situation for getting non contaminated algae especially for the feeding the larvae of the fishes. Fully-grown culture from the stock culture can be used as inoculum to avoid contamination in mass culture. Mass culture can be carried out 10 l. polythene bags or 20 l. glass carbuoys or 100 and 250 l. perspex and glass tanks or 500 l. capacity FRP tanks kept in wooden or cement racks or elevated platform and in polyethylene bags with light and aeration and suitable environmental parameters . The mass culture can be carried out in three major methods viz. Batch culture in which the total culture is harvested and used as food and a fresh culture of the same species is set up to replace it. In Semi-continuous culture system a part of the culture is harvested and used as food, and the amount taken is replaced with fresh culture medium. The continuous culture system includes Turbidostat culture in which the number of algal cells in the culture is monitored and, as the cells divide and grow, an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium whereas in Chemostat culture a flow of fresh medium is introduced into the culture at a steady, pre-determined rate. In both the above methods (Turbidostat culture and Chemostat culture) the surplus culture overflows into a collecting container, from which it can be taken and used as food.

7. Micro zooplanktion culture

Micro zooplanktons (the rotifer *Brachionus plicatilis*, calanoids and copepods, cladoceran crustaceans (*Daphnia* spp. and *Moina* spp.), can be isolated from their natural environment, and after isolation and purification the required organisms can be subjected to mass scale production through feeding algae and rice bran. *Artemia* nauplii can be produced through decapsulation of *Artemia* cysts. The tiny size and enormous reproductive potential of rotifers makes them a popular choice as a food item among breeders of marine fishes and invertebrates.

8. Bio-encpasulation

Live food constitute the main diet for marine fish larvae but a single live food species is often unable to fortify the complete nutritional requirements of the species under captive condition. Successful rearing of marine fish larvae is partially dependent upon the proper availability of lipids, proteins, carbohydrates, vitamins, and minerals via the diet (Watanabe and Kiron, 1994 and Kanazawa, 2003). One important aspect of larval nutrition is providing adequate levels of highly unsaturated fatty acids (HUFAs) including eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) since deficiencies in these lipids result in poor growth, low feed efficiency, anemia and high mortality (Sargent et al., 1999; Olivotto et al., 2003). As a result recent findings showed that bio-encapsulation of live food organisms with various nutrients have a vital role in larval survivability as the larvae require diets with high protein and sufficient amount of essential fatty acids, its incorporation is vital for augmentation of larval production. The live food that have been most intensively investigated with respect to their nutritive stability are brine shrimp (*Artemia* spp.) and rotifer (*Brachionus* spp). Though artemia is low in the essential fatty acids eicosapentaenoic acid (20:5n3 EPA) and decosa hexaenoic acid (22:6n3 DHA), the simple methods of bioencapsulation have been developed to incorporate these fatty acids in to the nauplii. The nauplii are offered to the larvae after being enriched with these biomolecules (Watanabe et al.,1982). The nutritive value of rotifer is made suitable by culturing them with a suitable medium by feeding with mixed marine micro algae (*Chlorella* spp. and *Nannochloropsis* spp.) all of which are rich in n-3 poly unsaturated fatty acids. It is generally considered that EPA and DHA are the important fatty acids in the nutrition of larval fish though its specific fatty acid requirements vary amoung species. It is recommended that adequate nutrition has an important role in the reproductive success, and it has been shown that essential fatty acids, vitamin (A,D E and C), trace minerals and other carotenoids can affect fecundity, egg quality, hatchability and larval

quality. Broodstocks fed with EPA (n-3PUFA) deficient diets produced eggs with significantly lower survival and high level of larval deformities. Finfish nutrition during the embryonic stage is provided by the yolk sac and oil globules. The transition from an endogenous to an exogenous food supply, which marks the onset of larval stage is one of the most critical phases of the life cycle and is the period when much of the mortality of hatchery reared stocks occur.

9. Feed management in larval rearing

The difficulties in larval rearing of marine fishes are primarily related to nutrition. The small eggs and correspondingly small mouth gape of the larvae at first hatching makes feeding over the first few days crucial (Dhert et al.,1998). For the clown fish larval rearing, on the day of expected hatching the eggs were transferred to larval rearing tank and provided complete darkness. Soon after hatching the larvae were provided with a mixed culture of *N. occulata* and *C. marina* (1.5 x106 cells/ml) and the larvae were allowed to remain in this green water systems and provided 24 hrs light up to 15 days of post hatch. As many of the larvae had only little quantity of yolk material, it starts feeding within few hour after hatching and more over successful feeding strikes is also low at first feeding but rises rapidly during early development. At this stage provision of suitable size and nutritionally adequate enriched feed in high density is one of the important factor for their survival as the larvae could able to accept a small size organism due to the small mouth gape. If they do not encounter and successfully capture food before depleting their energy reserves, the larvae may starve and it will eventually leads to the mass mortality. In the case of *A. percula* as the mouth gape of the larvae is between 80-123 µ, the larvae were fed with live feeds measuring less than 100µ for its active feeding. Feeding of larvae was performed in two stages: Stage 1 covered the rotifer with algae feeding phase from Day 1st to 8th day and the Artemia and rotifer with algae feeding phase from 9th to 20th days. The successful feeding strikes is low at first feeding but rises rapidly during early development in *A. percula* and this can be attributed to improved ability to maneuver, feeding experience, changes in mouth size and structure as also reported in other fish larvae (Colgan et al., 1986; Meyer, 1986; Coughlin, 1991 and Liem, 1991). For the successful prey capture of larvae, 50-100 numbers ml^{-1} supper small rotifer (*B. plicatilis*) having size 60 to 100 µ were provided after enriched with vitamins and fatty acids. As the larvae attains successful prey capture within two days, the density of rotifer in the larval rearing tank is reduced to 30-50 nos. ml^{-1} from 3rd to 8th day. From

9th day onwards the larvae were weaned onto newly hatched *Artemia* nauplii (10 - 15 nos. ml^{-1}) along with rotifer (SS and S type) (50-60 nos/ml) and micro algae (1.5 x106 cells/ml). Liem (1991) reported that clown fish larvae of *A. frenatus* capture prey using ram feeding during the first 10 days after hatching whereas Coughlin et al. (1992) and Coughlin (1994) reported that the success of feeding strikes on prey *B. plicatilis* in *A. perideraion* larvae was 100% only on 3 to 7 days after hatching. The clownfish have a larval period of between 10 and 20 days.

10. Juvenile rearing

The rearing was carried out in the same tank for period of 40 days and then after transferred to different juvenile rearing tanks. Water was changed initially on the fourth day and everyday thereafter with an initial exchange rate of 50% per day. On completion of metamorphosis, the juveniles were graded into several groups and stocked in separate tanks in which biological filtrations system was provided. The juveniles were fed with wet feeds (prawn, clam and mussel meat) to satiation four times during the light period. Through these feeding schedule the larvae attained 10to 12 mm within 30 days of post hatch and the juveniles reached 25 to 35 mm within 60 days and reaches marketable size within 6 months after post hatch.

11. Conclusion

The pivotal part of the ornamental seed production is production of live feeds in which the nutritive quality of live feeds are further enhanced through bio-encapsulation with EPA, DHA and micro algae. High survivability and healthy larval production are the baseline factors for the seed production of ornamental fishes.

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