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FINFISH LARVAL CULTURE

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ABSTRACT

The success of any large-scale finfish culture is mainly dependent upon the continuous and adequate supply of fish seed. Although fish seed may be collected from the wild, natural sources of fish seed are unreliable and mostly seasonal. Here comes the importance of controlled breeding and mass scale larval rearing. In the background of the increased emphasis given by the Government of India to aquaculture, the present status of larval rearing of important cultivable/ ornamental marine finfishes in our country is reviewed in this paper giving stress on the constraints in the progress of the research in this line.

INTRODUCTION

Culture of marine finfishes such as mullets, milkfish and seabass has been practised in India for a very long time. However, most of the culture is done in the traditional pattern along the coastline in the brackishwater systems or on an experimental level to evaluate the feasibility of the culture system (Pillay, 1972). In spite of the availability of finfish seed in the wild, commercial farming of finfish is yet to begin due to the constraint in availability of the wild seed all through the year due to their seasonality and also their inconsistency. Several research establishments are actively involved in the development of hatchery technology related to finfish. The Central Marine Fisheries Research Institute has developed protocols for induced breeding, spawning and experimental larval rearing of some species of mullets, groupers, rabbitfishes and nearly 10 species of ornamental fishes. However, the hatchery technology is still in experimental stages. The Central Institute of Brackishwater Aquaculture has developed hatchery protocols for some species of mullets, pearlspot and sea bass. Of these the hatchery technology for sea bass is now being extended to the Marine Products Export Development Authority. The MPEDA has now opened a hatchery exclusively for sea bass seed production in Tamilnadu, which again is yet to venture seed production in commercial levels. An entrepreneur has initiated attempts to set up a commercial hatchery for groupers in Andaman Islands. From the above account, it could be understood that ventures in seed production technology of marine species of finfishes in our country is still in an experimental level. In this context an attempt is made to evaluate the present status of hatchery technology particularly larval rearing of important cultivable and ornamental marine fishes in our country. Stress has been given to locate constraints imminent in the progress of the hatchery technology and also to point out thrust areas wherein research efforts are needed to ameliorate the constraints, and put our country at par with other nations in relation to hatchery technology development in finfishes.

PRESENT STATUS OF THE TECHNOLOGY

Grey mullets

The candidate species of mullets for culture are *Mugil cephalus*, *Liza parsia, L.macrolepis* and *L.tade*. Mullets are generally used in polyculture with other compatible species of fishes and shrimps in traditional culture systems. *M.cephalus* is the most ideal species achieving maximum growth and production. Induced spawning and experimental larval rearing of *M.cephalus* has been achieved by Anon (1962), Alikunhi *et al.* (1971), Sebastian and Nair (1973), Mohanty (1971), Chaudhuri *et al.* (1977), Krishnan (1989), Rajyalakshmi *et al.* (1991), Krishnan *et al.* (1996) and CIBA (1998). Attempts on the above lines in other species of mullets are those of Alikunhi *et al.* (1971), Sebastian and Nair (1975) Kowtal and Gupta (1986) and James *et al.* (1983) in *L.macrolepis;* Radhakrishnan *et al.* (1976) and CMFRI (1980) in *L.parsia* and Krishnan & George (1985) on a hybrid between *L.parsia* and *L.macrolepis*. In spite of the fact that many of the above workers have been able to complete the larval cycle that is around 40 days, perfection of the protocols, extension of the technology and commercialization of the technique have not been attempted. The procedure followed in larval rearing by all the Indian workers is almost similar. The fertilized eggs measure around 900µ and the larva has a length of 1.5mm. The larvae on hatching are reared mainly in green water using *Chlorella*. Continuous aeration is provided. Rotifers mainly *Brachionus* sp. is fed @ 5-10 nos./ml. to the larvae from the second day to nearly 20 days. Majority of the workers keep the larval rearing system closed in the initial 10 days to retain the green water. By the 15th day in addition to rotifers, copepod nauplii, copepodites (wild collected), other wild collected plankton and *Artemia* nauplii are added as larval feed. Exchange of water is resorted to reduce the salinity of water to brackishwater levels. As the larvae grow bigger *Moina* and even powdered feed pellets or prepared dough are also fed to the larvae. Survival of the larvae is quite low. The low survival rate of larvae is due to natural mortality observed on the 2nd, 3rd day, 7th day and 11th day. These larval mortalities account for nearly 25 to 75 percentage of the total mortality during the larval rearing phase. The mullet larvae show a peculiar downward migration on the second day. Kuo *et al.* (1973) observed that the larvae, sinking to the bottom of the tank always preceded the two critical periods of mortality. The sinking of larvae is thought to be caused due to the change in the specific gravity of the larvae or due to occlusion of the pneumatic duct of the air bladder. The constraints in larval hearing in India are as follows:

a) Lack of a properly planned and scientifically designed finfish hatchery with a good running water facility.

b) Most of the workers depended on wild broodstock and did not think of developing a good broodstock before venturing to breeding.

c) Lack of adequate facilities to rear mass culture of live feed organisms / inability to rear smaller strains of rotifers or copepod nauplii and copepodites less than 100µ size to feed the mullet larvae in the earlier critical periods. It has been reported that most marine fish larvae start feeding on organisms of 50-100µ width. Similar is the case with mullets (Kuo *et al.*, 1973).

d) Prioritizing on hatchery technology related to shellfishes due to their foreign exchange potential.

Milk fish

The milkfish *Chanos chanos* has been used for monoculture as well as polyculture with compatible species of shrimps and fishes by the traditional farmers of our coasts. Majority collect wild fry of 10-15 mm, allow them to grow to 100 mm or so in separate enclosures before releasing them to the culture system. Unlike in other countries hatchery technology of milkfish has not been successful in India so far. Researchers of CMFRI strived for the initial build up of a brood stock in Narakkal and Mandapam. Researchers of CMFRI at Mandapam tried on the experimental transport of live, ripe females and holding them in a brood stock pond in 1978. But the efforts failed (Nammalwar and Mohanraj, 1990). The constraints in development of a hatchery technology for the species are the same as mentioned for the mullet above. Availability of milkfish brood stock is far less compared to mullets in India. Compared to many cultured species, milkfish rearing is easy due to their bigger egg size (1.2mm), bigger size of larvae (3.7 mm) and bigger mouth width at opening (214µ) as reported by SEAFDEC. Duration of larval rearing period is from 18-24 days.

Sea bass

The sea bass *Lates calcarifer* popularly called as bhekti is a highly potential candidate species for farming. The species has been traditionally cultured in the Northern west Bengal using wild collected seed. This species has now attracted the attention of culturists even as an alternate candidate species to shrimp. An initial trial to breed the species was recorded by Mathew *et al.* (1997) in Madras using a collected and monitored brood stock. Successful spawning and fertilization and successful larval rearing of *L.calcarifer* was achieved in 1997 by the Central Institute of Brackishwater Aquaculture (CIBA, 1998). Larval rearing duration is 18-25 days. Successful larval rearing procedures are as follows. Larvae are stocked at 15-60 numbers /l. Water exchange and feeding are done from the 3rd day onwards. SEAFDEC reports the mouth width of newly hatched larvae to be 170-220µ. The larvae are fed with rotifers *Brachionus plicatilis* and *B.rotundiformes* in sizes from 50 to 175µ. The rotifers are also enriched before feeding to the larvae till the 8th day. A high density of rotifers @ 20 to 30 nos. /ml is maintained. The rearing water also has algae dominated by *Chlorella* @ 8000 to 15,000 cells /ml. From the 9th to the 15th day *Artemia* nauplii @ 20 to 40 number /l are supplemented along with rotifers. During 16 to 25th day the larvae are extensively fed with *Artemia* nauplii @ 30-50 nos/l. The *Artemia* nauplii are enriched with cod liver oil. A survival rate of 0.2 to 32% has been obtained at a stocking density of 35 numbers of larvae /l. In nursery rearing beyond 25 days, larvae are reared @ 200 to 2000 nos/m^2 fed ad *libitum* with *Artemia* biomass or cooked minced fish meat. Survival of 3-96% with an average of 53% is reported at an optimum density of 1000 nos/l (CIBA, 2002).

The MPEDA has started hatchery production of sea bass at Sirkazhi in Tamilnadu and in the last quarter of 2001, about 2 lakh larvae were sold to the fish farmers (MPEDA, 2002).

Grouper

Many species of groupers are widely distributed along the coastal waters of our country.

Availability of wild seed of groupers has also been reported near Vellapati in North of Tuticorin, Gulf of Mannar (Rengasamy *et al.*, 1999). CMFRI has developed of a viable brood stock, big achieving sex reversal of female to male and in obtaining natural spawning in *Epinephelus tauvina* (Grace Mathew *et al.*, 2002). The larval rearing is yet to be perfected. The constraints are difficulties in production of mass culture of suitable algae such as *Tetraselmis* and *Chlorella,* suitable smaller sized rotifers, and copepods etc. The smaller mouth of the grouper larva in the initial period, that is critical, demand the supply of copepodites and copepod nauplii in sufficient numbers. Groupers have the disadvantage of smaller egg size (0.8mm), smaller larva (1.3mm) and smaller mouth gape (193µ) and extended larval cycle (55 to 60 days). Larvae are very sensitive to environmental disturbances and there is also the major problem of cannibalism of larvae and juveniles.

Other species

Success has been achieved in the experimental breeding of *Siganus canaliculatus* at Mandapam Regional Centre of CMFRI (Nammalwar and Mohanraj, 1990). Induced spawning and larval rearing has also been attempted on the sea bream, *Sparus datnia* (Rajyalakshmi *et al.*, 1991).

Ornamental fishes

India has a vast potential in marine ornamental fish trade. The natural stock of ornamentals in Lakshadweep and Andaman Islands and also Gulf of Mannar may get depleted once the export trade takes off (CMFRI, 2002). The only way to protect the natural stock is to resort to hatchery technology. CMFRI has already initiated steps in this direction. Nearly 10 species of ornamental fishes mainly clowns, damsels, seahorse and pipefish have been bred in captivity and larvae reared to juvenile. The rearing medium is green water using *Chlorella.* Diffuse lighting is provided. Larvae are fed with a variety of live feed organisms such as rotifers, *Artemia* nauplii, mixed zooplankton, *Daphnia, Moina,* infusorians, copepods, bloodworms, earthworms, *Tubifex* and also ciliates grown on micro algal culture.

Many constraints are imminent in the larval culture of marine ornamentals (Gopakumar, 2001).

a) Brood stock development is difficult in captive conditions.
b) Complex sex change patterns determined by social structure.
c) Variations in egg laying habits - some are egg scatterers such as the angelfish, butterfly fishes; some are egg anchorers such as gobies, damselfishes; some are mouth brooders such as jewfishes and cardinalfishes and some are live bearers such as the seahorse.
d) Hatched larvae need very small sized live feed.
e) Unique conditions to be maintained during planktonic life of larvae of each species.
f) Larvae exhibit the peculiar head butting syndrome i.e., swimming towards reflected light and bashing themselves against the sides of the tank until they die.
g) Gapes of mouth in the majority are very small.

A good hatchery specially designed for ornamentals is lacking and CMFRI has taken necessary steps for establishing a marine ornamental hatchery at Mandapam.

AN OVERVIEW OF THE ESSENTIAL PREREQUISITES FOR SUCCESSFUL LARVAL REARING OF FINFISHES

Live feed

Live feed organisms play a vital role in the dietary regime of finfish larvae. Successful rearing of larvae in fish hatcheries mainly depends on feeding the larvae with appropriate, nutritionally balanced, nonpolluted, economically viable and readily acceptable feed in order to get optimum growth and survival (Kulasekarapandian and Radhakrishnan, 1989). Traditionally, algae, rotifers, cladocerans, copepods and brine shrimp have been utilized as live feed in the hatcheries throughout the world. Watanabe (1985) summarized the most suitable diets for various developmental stages of some finfishes in Japan that in a broad way can be taken as an ideal package for all finfish. For finfish larvae greater than 2-3mm, rotifers are the ideal initial diet and can be continued for 30 days. When the larvae are around 7mm, marine copepods such as *Tigriopus, Acartia, Pseudodiaptomus, Harpacticoids, Paracalanus* and cladocerans such as *Moina, Daphnia* are also fed to the larvae along with the rotifers. Copepods possess a high content of PUFA essential for the growth of marine fish larvae. *Tigriopus* contains relatively high amounts of 20:5 ω3 regardless of its culture medium. Unfortunately, copepod large-scale culture has not yet been perfected. The few works on such lines are as those of Zillioux and Wilson (1966); Zillioux (1969) in the continuous culture of *Acartia clausii and Omori (1973). Hippocampus kuda* juveniles showed better survival when fed with a combined diet of rotifers and copepods. *Acartia* has the highest n-3 and HUFA content compared to many other copepods. In SEAFDEC, larvae of the grouper *Epinephelus coioides* fed initially with copepodites (60 to 80/l) showed better survival compared to those fed with rotifers (Masanori Doi *et al.*, 1997). *Tigriopus* has been used as feed for marine fish larvae for a long time (May, 1970) and is one of the several copepods considered for mass cultivation in Japan (Omori, 1973).

Nutritional quality of live feed

It is well known that rotifers cultured in yeast are low in ω3 highly unsaturated fatty acids which is essential for marine finfish. So rotifer should be enriched using marine *Chlorella*, microencapsulated diets, yeast and emulsified lipids rich in ω3 highly unsaturated fatty acids. In the larval rearing of malloway and snappers in Australia, high mortality occurring around 30-40th day was observed to be lessened when the larvae were fed with nutritionally enriched rotifers/*Artemia*. Sulaiman *et al.* (2000) working on the larvae of silver pomfret used enriched rotifers and *Artemia* with super selco and DHA protein selco. Usage of enriched rotifers also led to the success in larval rearing of sea bass in India (CIBA, 2002).

Nutrition of larvae has attracted the attention in recent times since survival is related to nutrition. Since live feed mass culture is cost oriented, weaning marine fish larvae at around 25 days to accept artificial diet like pellets is also helpful. Microbound diets (MBD) have been tried for larvae of many marine fish such as barramundi larvae. Usage of liposomes as nutrient supplement in first feeding of the larvae of the seabream *Sparus aurata* and *E.aeneus* has also been tried with success. In SEAFDEC, feeds with exogenous enzymes gave encouraging results in survival of milkfish larvae. Studies on the requirements of larvae related to fatty acids, vitamin C, vitamin, E, phospholipids etc. in larval feeds are in progress. Usage of enrichment diets, immunostimulants, feed additives are also in progress in recent times. The recent innovation is using biochemical agents (BCA) in culture medium of larvae that promote fish growth and repress the growth of the pathogens. SEAFDEC has initiated such studies using *Caranx delicatissimus* and *P.major*. Research on the above aspects are progressing in sea bass, turbot, breams, halibut, cod, milk fish, grouper and mahi mahi in Laboratory of Aquaculture-Artemia Reference Centre, Ghent, Belgium.

From very early times many researchers used *Chlorella* to enrich the rotifers before being fed to the larvae. It was observed that the EPA (20:5n3) content eicosapentaenoic acid content was high in *Tetraselmis* and very low in *Chlorella*. Survival rate and growth of sea bass larvae *Dicentrarchus labrax* were improved as EPA content increased in their feed (El-Daker *et al.*, 2001). In another find, feeding spray dried *Schizochytrium* sp. (a heterotrophic micro algae) to rotifers or *Artemia* was observed to increase the HUFA in live feed because *Schizochytrium* has unique n-3 & n-6 HUFA. The alga was also observed to enrich the live feed with DHA and EPA (William Barclay and Sam Zeller, 1996). Many workers feel that success in larval rearing is achieved by a combination of live natural feeds and artificial feeds.

Alternate live feed organisms

Fermin (1991) advocates usage of *Moina macrocopa* instead of *Artemia* in larval rearing of seabass as well as milk fish larva. He reports that *Moina* has a high nutrient content and contains high levels of 20:5ω3 highly unsaturated fatty acid needed by most marine fish larvae. The brackishwater cladoceran *Diaphanosoma* sp. is given from 10^{th} to 33rd day in Philippines to the larvae of *E.tauvina*. Live mysids *Mesopodopsis* sp. is also given a few days before metamorphosis (Chen, 1979). Parazo *et al.* (1998) also recommend giving *Moina* to sea bass larvae. Nutritionally enriched free living nematode *Panagrellus redivivus* have been tried as feed for the grouper *E.coioides* larvae by SEAFDEC. It is well known that SS and S strain of rotifers have revolutionized the field of larviculture. Such strains of rotifers are small enough to be fed to the newly hatched larvae of many cultivable fish with a minimal mouth gape such as the grouper. Survival rate as high as 29.4% has been obtained in rearing trials at the Gondol Research Institute for Marine Culture with *Cromileptes altivelis* using a combination of SS and S strain rotifers, brine shrimp and artificial larval diets. Studies on genetic variations among *Brachionus* strain is on to find out an appropriate sized live feed organism. Semimass culture of the dinoflagellate *Gymnodinium splendens* as live feed has been tried in initial feeding of marine finfish larvae. Grouper post larvae of 96 hours have also been fed successfully with oyster trochophore larvae and later with rotifers, copepods, *Artemia nauplii*, eel meat and frozen mysids. To improve the survival of cultured juveniles it is also important to understand the prey preferences and predator behavior of fish larvae.

Other factors controlling larval rearing

Many other factors apart from feed also control the success of survival of marine finfish larvae. Tank colour influenced the rotifer intake, early growth and survival of *E.suillus* larvae. Tank bottom cleanliness helps the survival of the larvae of *M.cephalus* (Syd Kraul, 1983). Light also helps in the ability of larvae to see the prey. It also affects the productivity and water quality of algal-based systems. Optimal light intensity that provides adequate prey illumination is ideal. Also diffused natural light had good influence on the larval survival in mullet larvae. Studies conducted by SEAFDEC in grouper showed that a light intensity of 200 lux is required to initiate successful feeding. Aeration also had an influence on the larvae. Low aeration led to better hatchability and production of normal larvae. Mild aeration was good for larval feeding also. Addition of oil ($0.3 ml/m^2$) on water surface of larval rearing tanks significantly reduced grouper larval mortality. Excessive horizontal flow at the surface can impede swim bladder inflation in grouper larvae. Regular water exchange has many desirable qualities. This helps in dilution of concentration of toxic metabolites developed in water due to concentration of faeces, decomposition of uneaten food and dead larvae. Hatching of grouper eggs was higher at higher salinities of 32-40 ppt.

METHODS TO OVERCOME THE PROBLEM

In spite of the fact that development of hatchery technology related to marine finfishes has imminent problems and constraints which are diverse and species related, development of protocols for hatchery seed production of selected and potentially important marine fin fishes can be turned to realities once we have facilities such as:

a) A properly planned and scientifically designed hatchery with a good running water facility for marine finfish, wherein brood stock of potential species both cultivable and ornamental could be maintained, as a National facility-at least one on each of our coasts and one each in the islands of Lakshadweep and Andamans.

b) Prioritization of research programmes oriented to development of protocols in development of hatchery technology at the national level. Consistent efforts in development of the hatchery technology work by involving all available experts in the country under one leadership.

c) Strengthening research programmes related to rearing and mass culture of live feed organisms / smaller strains of rotifers or copepods; copepod nauplii and copepodites less than 100μ size and other alternate species of live feed organisms to feed the marine finfish larvae in the early critical periods.

d) Focusing research on nutritional requirements of larvae, nutritive values of live feeds and development of formulated feed for all types of marine fish larvae.

e) Concentrating research on i) species which can cater to the domestic demand, and. ii) species exclusively for export and earning of foreign exchange.

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