Review of SEAFDEC/AQD Finfish Seed Production Research

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Abstract

Research on seed production of several foodfishes has been a continuing activity of SEAFDEC/AQD since 1976. Fry and juvenile production methods of these fish commodities are in various stages of advancement. For instance, advances in the development of hatchery rearing, particularly feeding and water management schemes, have made mass production of milkfish (*Chanos chanos*) seed a reality, resulting further in the application of the technology in commercial hatcheries. Recent studies now focus on assessing the quality of hatchery seed stocks of milkfish vis-a-vis wild seed during nursery and grow-out culture. Likewise, sea bass (*Lates calcarifer*) seed production has undergone significant improvements since the technology was introduced in the Philippines in 1982. Fatty acid-enrichment of a zooplankton diet can enhance growth and survival of sea bass fry, although other cheaper alternatives and early weaning to formulated diet preparations are currently being tested. Hatchery fry production of grouper (*Epinephelus salmoides* and *E. suillus* syn. *E. coioides*) and snapper is in its infancy, but trials complemented by research on their larval feeding habits and requirements are underway to establish reliable methods of rearing larvae of these species. Although fairly well-established, seed production of rabbitfish (*Siganus guttatus*) requires further improvement in determining an appropriate zooplankton diet to ensure adequate growth and survival of larvae. Hatchery fry production of tilapia (*Oreochromis* sp.), carps (*Aristichthys nobilis, Hypothalmichthys molitrix*) and, to a certain extent, catfish (*Clarias macrocephalus*) can already be categorized as a flourishing industry in some parts of the Philippines. Nonetheless, SEAFDEC/AQD continues to conduct research on these freshwater species, with particular emphasis on nutrition and feed development during the nursery production phase. Together, results of past and on-going research studies ensure that seed supply of these important foodfishes become adequate and sustainable for the grow-out.

Introduction

The availability of quality seed for grow-out culture is a key factor in fish production. The propagation of several important foodfishes such as milkfish, rabbitfish, sea bass through controlled reproduction in captivity and the rearing of

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their resulting larvae in the hatchery may augment and curtail the present dependence on wild seed supply. This paper highlights research studies on fish seed production at SEAFDEC Aquaculture Department (SEAFDEC/AQD).

Milkfish (*Chanos chanos*)

Research on seed production of milkfish has been a continuing activity at SEAFDEC/AQD since 1976. The physiological and nutritional effects of various food types on wild milkfish fry have been the focus of earlier studies. Milkfish fry grew well on rotifer fed *Tetraselmis tetrahele* (61.48 mg weight gain) than *Isochrysis galbana*- (43.56 mg weht gain) and *Chlorella* sp.-fed (11.05 mg weight gain) rotifer (Villegas et al. 1990). Rearing fry on *Chlorella* alone was not effective since fry cannot directly utilize *Chlorella* (Juario and Storch 1984, Segner et al. 1987) nor were *Chlorella*-fed rotifers nutritionally adequate (Segner et al. 1984). Rotifer grown on various species of phytoplankton has been shown to be best for milkfish fry than rotifer fed only one algal species (Acosta and Juario 1983). However, milkfish fry fed the freshwater flea (*Moina macrocopa*) promoted better growth (133.78-213.45 mg final weight) than those fed rotifers (58.90-76.79 mg final weight, Villegas 1990). Under laboratory conditions, acclimated fry grew and survived better when fed *Oscillatoria* alone (2.5×10^6 cells/ml) or in combination with *Chroococcus* (520×10^3 cells/ml) than those fed *Navicula* (15×10^3 cells/ml), *Chlorella* (2.3×10^6 cells/ml), or *Euglena* (575×10^3 cells/ml, Pantastico et al. 1986).

Likewise, fry grew best (134.7 g weight gain) when fed an artificial diet containing 40% protein (Lim et al. 1979). This 40% protein level was then used to fomulate a diet for milkfish fry reared in freshwater (Santiago et al. 1983) and seawater (Alava and Lim 1988). Acclimated fry reared in freshwater earthen ponds showed better growth on formulated feeds (1.31 g weight gain) than on natural food such as *Oscillatoria* (0.616 g weight gain, Santiago et al. 1989). In contrast, starved fry subsequently fed live food can more rapidly re-establish its liver ultrastructure than those reared on artificial diets (Storch et al. 1983).

Conditions for nursery rearing of fry were also investigated in the laboratory. Villaluz and Unggui (1983) observed that, at 20 ppt, wild fry grew faster at higher temperatures (25.8-35.2 °C) than at ambient (23.7-28.9 °C) or low (17.5-22.6 °C) temperature.

Hatchery production of fry became a reality when breeding milkfish broodstock in captivity beginning in 1979 was achieved (Liao et al. 1979, Juario et al. 1984, Marte and Lacanilao 1986). To date, hatchery rearing of milkfish larvae depends entirely on plankton, mainly rotifers (*Brachionus plicatilis,* fed at 10-20 ind/1) and brine shrimp nauplii (*Artemia* sp. fed at 0.5-1.0 ind/ml, Liao et al. 1979, Juario and Duray 1983, Juario et al. 1984, Gapasin and Marte 1990).

Phytoplankton such as *Chlorella, Tetraselmis,* or *Isochrysis* that is added to the rearing water act as conditioner and as food for rotifers.

Under laboratory conditions, the number of rotifers ingested by milkfish larvae increased as the larvae grew (from 4 individuals per 2-day old larvae to about 100 individuals per 21-day old larvae, see Hara et al. 1983, Duray in press a), demonstrating an increase in the capacity of the larvae to take in more rotifers with an increase in mouth size (Duray and Kohno 1990). In addition, the digestive tract of milkfish undergoes significant enzymatic changes during development, which coincides with its dietary and habitat shifts throughout its life history (Ferraris et al. 1987). During the first 14 days of rearing, supplementation with a commercial microparticulate feed (Nosan R-1 at 100 particles/ml fed twice daily) improved growth and survival of larvae, supporting the importance of dietary (n-3) highly unsaturated fatty acid (HUFA) levels in the production of quality seed of marine fish (Marte and Duray 1991). Indeed, pilot production runs have confirmed these results (Duray 1995). During the first 14 days of rearing, larvae have been weaned directly to artificial diets, but poor growth and high mortality have been observed during the first week of introducing these diets (Duray and Bagarinao 1984). Nonetheless, these information are important in the formulation of cost-effective diets for milkfish larvae.

An improved artificial diet is being developed. This formulation was based on available informations on the essential amino and fatty acid requirements of juveniles (Borlongan 1992, Borlongan and Coloso 1993). The particle size of these diets was adjusted to fit the mouth size of the larvae. The formulated diet was tested starting on the fifteenth day of rearing larvae. Initial results showed that survival (18%) and growth (10 mm final total length, TL) of larvae fed artificial diet was comparable to that of Artemia-fed larvae (I. Borlongan personal communication).

Aside from developing appropriate feeding strategies, the development of optimum rearing conditions for milkfish larvae has also been investigated. Fourteen-day old larvae were relatively longer (7.1 mm) and survived better (20.3%) when reared in black-coated tanks than in tan-colored tanks (5.1 mm; 9.4%, respectively) due, perhaps, to the greater visibility of food organisms against a dark background (Duray in press a). The response of larvae to various salinities demonstrated varying degrees of euryhalinity (Dueñas and Young 1984). For instance, newly-hatched (day 0) and 14-day old larvae were moderately euryhaline, having a narrower salinity tolerance range of 8-37 ppt and 6-28 ppt, repectively, than 21-day old larvae (0-70 ppt). Surviving only at salinity of 27-28 ppt, 7-day old are strictly stenohaline.

As SEAFDEC/AQD gradually improved the milkfish hatchery technology over the years, its transfer to commercial operators became its present concern (Duray 1995, Garcia et al. submitted). Commercial production of milkfish fry has been demonstrated to be biotechnically feasible. However, the economic viability of hatchery fry production remained uncertain primarily because of the scarcity of eggs and newly-hatched larvae from a single source. Commercial operators have been encouraged therefore to establish and develop captive breeders to integrate with hatchery fry operation.

Sea Bass (*Lates calcarifer*)

Sea bass was first reared at SEAFDEC/AQD in 1982 using yolk-sac larvae imported from Thailand and adopting the rearing technique developed in that country. The breeding of sea bass spawners at SEAFDEC/AQD (Almendras et al. 1988, Garcia 1989) provided eggs and larvae for further improving the imported technology.

On the second day of rearing, sea bass larvae initially fed on rotifers (lorica length: 80-160 µm) when larvae were about 2.6 mm TL and mouth width was 200-240 µm (Duray and Kohno 1990). The number of ingested prey increased with size of the larvae (Kohno and Duray 1990). Growth and survival was improved when the larvae were fed more than 6 *Artemia* nauplii/ml/day 5 times daily starting on the fourteenth day of rearing (Duray 1990a, Parazo et al. 1990). Metamorphosis was enhanced and larval resistance to stress was improved when larvae were fed highly unsaturated fatty acid-enriched *Artemia* (Dhert et al. 1990). Adding freshwater flea (*M. macrocopa*) as a partial supplement to brine shrimp nauplii resulted in better larval survival (7.7%) compared with larvae fed *M. macrocopa* alone (2.6%, Fermin 1991), demonstrating the possibility that partial supplementation of brine shrimp nauplii may reduce fry production cost.

As early as the tenth day of rearing, sea bass larvae can be weaned to feed on a formulated diet although survival was low. Juario et al. (1991) showed that, to be effective, gradual weaning must start when larvae are 20-days old. Abrupt weaning to commercial diets was also attempted but survival rate was again low (40%) compared with *Artemia* nauplii-fed larvae (74%, Duray 1990b). Alkaline phosphatase, esterase, and aminopeptidase have been localized in the brush borders of the pyloric caeca, whereas aminopeptidase and lipase were observed in the brush border of intestinal epithelial cells of 20-day old larvae, indicating that changes in gut enzymes of sea bass larvae coincide with a shift in its feeding habit from a zooplanktivore to a fish carnivore (Minjoyo 1990).

Mortalities due to cannibalism were frequent during the nursery rearing stage. Since sea bass fry devoured prey that were about 67% of its size, size grading of fry stocks has been recommended (Parazo et al. 1991). Fry reared in 1x3x1.5 m net cages set in a pond and fed fish bycatch daily were lighter, shorter, and showed a higher feed conversion ratio (11.9) than those fed daily on a formulated diet (1.5) or a combination of fish bycatch and formulated diet given on alternate days (2.2) (Toledo et al. 1991). These results suggest the feasibility of rearing sea bass in this holding system using formulated diet as partial or total replacement of fish bycatch. Likewise, rearing sea bass in floating net cages provided with illumination to attract zooplankton has also been tested (Fermin et al. 1994). The miminum size of 22- to 29-day old fry stocked in illuminated cages was 11-19 mm TL. Fry stocked at 600-1,200/m^3 in illuminated cages achieved better survival (20-38%) than those stocked at non-illuminated cages supplemented with fish bycatch.

Grouper (*Epinephelus salmoides, E. suillus* syn. *E. coioides*)

Rearing of grouper larvae was first attempted at SEAFDEC/AQD in 1984. The larvae of *E. salmoides* were first fed *Isochrysis* combined with sea urchin eggs until the tenth day of rearing when larvae were subsequently fed rotifers, which resulted in a larval survival rate of 9% on the twentieth day of rearing (Kunvankij et al. 1986).

Hatchery rearing of *E. coioides* larvae has been attempted only recently. Two-day old *E. coioides* larvae (2.6 mm TL) initially fed on rotifers at about 1.3 rotifers/larva. Ingestion rate increased to 62 rotifers/larva among 20-day old larvae. Larval feeding pattern was diurnal. Although older larvae (14- and 21-day old) started to feed early (0500 h), satiation was reached at 1000-1100 h among all larval stages examined (Duray in press b).

Preliminary data indicated an optimum stocking density of 10-20 larvae/1. Oyster eggs and a microparticulate diet (Nosan R-1) in combination with rotifers were tested with little success (Nagai 1990). However, supplementation of rotifers with any of two microparticulate diets (Nosan R-1 or FRIPPAK at 1.0 g/t daily) resulted in significantly higher survival rates among 14-day old larvae (31.9% and 28.6%), respectively) and in comparable or better growth rates (5,41-5.45 mm) than larvae fed *Chlorella*-reared rotifers (5.11 mm, M.N. Duray unpublished data).

The response of newly-hatched *E. suillus* larvae to abrupt transfer from full-strength seawater (32 ppt) to test salinities ranging from 0 to 56 ppt was investigated (Parado-Estepa 1991). Median lethal time (LT_{50}) was significantly higher at 8, 16, and 24 ppt than at other salinities, suggesting that larvae adapted well to brackishwater after abrupt transfer. The response of older larvae to various salinities has not been tested.

Under simulated transport conditions, survival of yolk-sac larvae loaded at 8,000-32,000 larvae/1 for 2 h at 29.5-30.3 °C and 33 ppt were significantly higher (76-91%) than larvae transported at 64,000 larvae/1 (Quinitio et al. 1991).

Rabbitfish (*Siganus guttatus*)

Hatchery seed production of rabbitfish began in 1980. Rabbitfish larvae were reared on rotifers, newly-hatched *Artemia,* and artificial diet (Juario et al. 1985, Hara et al. 1986a). Two-day old larvae fed on rotifer when larvae were 2.6 mm TL and on *Artemia* nauplii when larvae were 4.4 mm TL. Bigger larvae (8-9 mm TL) preferred *Artemia* nauplii (Hara et al. 1986b). High mortalities among rabbitfish larvae occurred during hatchery rearing possibly because of the small mouth size (90-110 µm) of larvae (Duray and Kohno 1990). However, survival of larvae can be improved (22%) if small-sized rotifer (less than 90 µm) are fed to larvae at 10-20 rotifer/ml (Duray 1986). Unfed larvae die within 80 hours after hatching (Bagarinao 1986), which coincided with the complete resorption of yolk (Kohno et al. 1988). Compared to those reared under natural daylight conditions, larvae survived better (21.15-40.50%) and are bigger (3.31-3.49 mm TL) during the first feeding period (7 days of rearing) when reared under continuous light perhaps since larvae can feed throughout the day (Duray and Kohno 1988).

Artemia-fed larvae had lower growth (188 mg weight gain) and survival (51%) than those fed formulated diet larvae (418-479 mg and 59.9-70.3%, respectively (Parazo 1991). However, a formulated diet containing 40% protein and a metabolizable energy of 3,971 kcal/kg was found suitable for hatchery production of rabbitfish.

Under transport conditions, all rabbitfish fry (47-days old) packed at 100 fry/1 survived up to 8 h at 28 °C and 32 ppt (Ayson et al. 1990). Loading densities greater than 100 fry/1 resulted in lower survival.

Snapper (*Lutjanus argentimaculatus*)

The mangrove red snapper has been successfully spawned in captivity since 1992 (Emata et al. 1994). Following feeding and water management schemes already established in milkfish, snapper larvae were reared in the hatchery until larvae were only 28-days old (A. Emata unpublished data). But, when hatchery rearing techniques for rabbitfish and grouper were followed, mean survival rate of 24- and 55-day old larvae was 27% and 10.6%, respectively (M.N. Duray unpublished data).

Tilapia (*Oreochromis* sp.)

Tilapia fry (*Oreochromis niloticus*) are successfully reared in tanks and in hapa net cages (Fermin 1988) set in a freshwater lake, Laguna de Bay. They are fed a diet containing 35% crude protein at 15% of fish biomass daily (Santiago et al. 1982). The diet of cage-reared fry have been supplemented with chicken egg yolk, blended shrimp by-catch or formulated feed, but supplemental feeding was terminated as Laguna Lake's primary productivity reached 3 g C/m^2/day (Bautista 1986). Red tilapia fry grew best (7.5 g weight gain) when fed for 8 weeks a 40%

protein diet with a protein-to-energy ratio (P/E) of 111 mg/kcal (Santiago and Laron 1991). Santiago and Lovell (1988) found that the essential dietary amino acid requirement of Nile tilapia highly correlated with the essential amino acid composition of its muscle.

Alternative dietary protein sources such as leaf meals have also been explored to lessen the cost of tilapia feeds. *Leucaena leucocephala* and *Azolla pinnata* have been therefore tested as protein sources of formulated diets for tilapia fry. After 7 weeks of culture, high growth rates (296-643 mg weight gain) were attained by fry fed a diet with *A. pinnata.* As protein source than that of *L. leucocephala.* Growth rates increased further as the level of dietary *Azolla* meal increased (Santiago et al. 1988).

Carp (*Aristichthys nobilis, Hypopthalmichthys molitrix*)

The availability of spawns by lake-reared bighead (*A. nobilis*) and silver (*H. molitrix*) carp in 1983 initiated seed production studies (Fermin 1988).

Silver carp eggs have been effectively incubated in well water having a total hardness of 300-500 ppm (Gonzal et al. 1987). Eggs prematurely burst when incubated for 5-8 h in lake or underground water having a total hardness of 75-150 ppm. Under normal conditions, silver carp eggs hatch after 13-18 h at 28-30 °C and hatching rates usually varied from 7 to 36% (Fermin 1988). In the bighead carp, growth of larvae was high (0.75%/day) when fed ad libitum a combination of *M. macrocopa* and an artificial diet and then followed by artificial diet alone (Fermin and Recometa 1988). Larvae fed *M. macrocopa* and artificial diet together with phytoplankton (green water) enhanced survival rates (53%).

A. nobilis and *H. molitrix* fry have also been reared in tanks or in fine-meshed nylon net cages set in a pond. Tank-reared fry have been fed rotifers, ad libitum, in combination with an artificial diet. Fermin (1985) recommended an artificial diet with 40% protein. Fry were then slowly weaned to nauplii and small-sized adults of *M. macrocopa* followed by the introduction of mixed-sizes of *M. macrocopa* in combination with an artificial diet. Although survival rates (94%) were high among fry fed rotifer only, the best feeding scheme consisted of a combination of rotifers and an artificial diet with 41.5% protein (Santiago and Reyes 1989). A diet consisting of 30% protein was shown to be optimum for growth of fry (Carlos and Santiago 1988, Santiago and Reyes 1991). Bighead carp fry grew best (618-651%) weight gain) when fed artificial diets with 3,130-3,470 kcal metabolizable energy/kg and P/E ratios of 92 and 100 mg protein/kcal (Trono-Legiralde 1990).

Catfish (*Clarias macrocephalus*)

To augment the declining population of the native catfish in the Philippines, SEAFDEC/AQD initiated work on breeding and seed production of this species in 1988 (Tan-Fermin 1992).

In a preliminary study, Fermin and Bolivar (1991) determined growth and survival of catfish larvae using live zooplankton (*Artemia* or *M. macrocopa*) and/or a dry artificial diet. Although zooplankton was required for successful rearing, catfish larvae may directly accept artificial diets during the initial stages of exogenous feeding. Further improvements of this feeding scheme will be tested.

Recommendations

Although seed production of milkfish has been a continuing concern, the present hatchery and nursery techniques developed over these years require constant improvement to ensure sustainable mass fry production. Of particular concern will be to demonstrate the quality of milkfish seed stocks produced in the hatchery vis-a-vis wild seed. Likewise, continuing assessment of the financial and socioeconomic viability of seed production technology is needed to its commercial application. Although hatchery fry production of sea bass is fairly established, nursery production of sea bass juveniles remains costly due to the carnivorous and cannibalistic habit of this species. Cheap alternatives to the fish bycatch diet, early weaning to artificial diet preparations, and effective grading methods of culturing sea bass juveniles must therefore be explored. Unlike milkfish and sea bass, techniques for the production of grouper seed is not as well-developed. Hence, further studies are required to find suitable and alternative live food organisms for the larvae, and to develop hatchery systems and appropriate water management schemes. An extensive larval rearing technique also needs to be developed as a cost-efficient alternative to the usual hatchery rearing of grouper and other fish species. A major concern in rearing larvae of grouper, snapper, and rabbitfish is the persistent difficulty of overcoming transitory mass-mortalities commonly observed at various stages of post-yolk sac development. These mortalities may reflect inadequate rearing techniques, which may likely be incompatible with the changing food and environmental requirements of the developing larvae. Research on the food and feeding biology of these species must therefore be continued. Pioneering investigations on hatchery seed production of the snapper and the catfish will need to focus on the development of reliable rearing techniques. Cost-effective diets suitable for nursery rearing of tilapia and bighead carp must be continued.

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