In:F Lacanilao, RM Coloso, GF Quinitio (Eds.). Proceedings of the Seminar-Workshop on Aquaculture Development in Southeast Asia and Prospects for Seafarming and Searanching; 19-23 August 1991; Iloilo City, Philippines. SEAFDEC Aquaculture Department, Iloilo, Philippines. 1994. 159 p.

MILKFISH, RABBITFISH, AND MULLET

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ABSTRACT

This paper reviews studies conducted on milkfish (*Chanos chanos*), rabbitfish (*Siganus guttatus*), and mullet (*Mugil cephalus*) at the SEAFDEC Aquaculture Department from 1968 to 1991. Milkfish studies focused on hormonal induction of off-season gonadal maturation, dietary manipulation of milkfish broodstock to improve egg and larval quality, improvement of larval rearing techniques for mass fry production and technology transfer to the private sector, and search for a low-cost, practical diet for milkfish and a supplemental diet to increase pond production. Preliminary success on alternate feed for larval rearing and spontaneous maturation of milkfish in concrete tanks may help alleviate milkfish fry supply in the future. Studies on rabbitfish centered on improvement of larval survival and search for the optimum diet for growth of rabbitfish fry and juveniles reared in ponds. The difficulty in rearing rabbitfish larvae due to high mortality at first week after hatching hinders the development of the rabbitfish industry. Research involving mullet was solely on the establishment of broodstock for fish propagation.

INTRODUCTION

Milkfish *(Chanos chanos* Forsskal), rabbitfish *(Siganus guttatus),* and mullet *(Mugil cephalus)* provide cheap sources of protein for Southeast Asian countries. However, the culture and production of these fishes are hindered primarily by the unpredictable and seasonal seed supply and lack of available practical diet. Since 1976, initial studies were conducted at the Aquaculture

Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) to generate economically feasible seed production and culture techniques for milkfish, rabbitfish, and mullet. From 1988 to 1991, continuing research on these fishes focused on their propagation, nutrition and feed development, pathology, and culture. This review deals with the results of those research studies. It is hoped that these achievements will provide more materials for the generation of a sustainable propagation and culture techniques for these fishes.

MILKFISH

Breeding

Milkfish breeding studies aimed to increase production of good quality eggs through improved maturation and breeding techniques. Implantation of reproductive hormones has been widely used to induce gonadal maturation in teleosts (Crim 1985, Lam 1985). Testosterone implantation with or without luteinizing hormone-releasing hormone analogue (LHRHa) has no marked effect in inducing early sexual maturity (Marte et al. 1989a). In a separate unpublished study, implantation of pelleted methyltestosterone (MT) and LHRHa also fails to induce precocious sexual maturation in contrast to previous report (Lee at al. 1986). A present study using estradiol-17β (E_2) implants produced slightly better results. Milkfish reared in 150- and 200-ton concrete tanks and given E_2 implants alone five times in a year had the most number of maturing and mature females at the end of the spawning season (October) and at the start of the succeeding season (March) than those given E_2 and LHRHa, LHRHa alone, or cholesterol pellets (control. Spontaneous spawning was observed in April a few weeks ahead of the unimplanted controls reared in a separate tank, but since hormone-treated and cholesterol-implanted control fish were reared in the same tank, it was difficult to determine which females spawned.

Recently, spontaneous spawning of milkfish in concrete tanks (Emata and Marte 1990) provides an alternative for milkfish spawning in floating net cages which is site-specific. Furthermore, it allows photoperiodic manipulation to induce off-season maturation.

Induced spawnings of mature milkfish are observed 16-49 hours post-injection in all females given human chorionic gonadotropin (1,000 international units per kilogram body weight) and 87% of females given mammalian gonadotropin-releasing hormone (10 micrograms per kilogram) or salmon gonadatropin-releasing hormone analogue (GnRHa) (100 μg/fish) (Marte et al. 1988b). Induced spawning through pellet implantation of GnRHa are less effective at 100 μg/fish. Induced spawning runs in 1990 indicate that spawning tank size can influence successful embryonic development of spawned eggs resulting in hatched larvae (Emata and Marte 1990).

A female specific protein, vitellogenin, with molecular weight of 400 kiloDalton comparable to those found in other teleosts (Hara and Hirai 1982), had been identified in milkfish (Garcia, unpublished results). Further studies on this protein can provide more information on milkfish reproduction.

The breeding technology generated by SEAFDEC/AQD following spontaneous spawning in cages (Marte and Lacanilao 1986) is verified in the National Milkfish Breeding Program. However, in its terminal phase in 1988, spawnings were observed in only 4 out of 13 sites nationwide with very few eggs collected.

A manually-operated sweeper-type egg collector (Garcia et al. 1988) coupled with a fine mesh net lining (Marte 1988) have significantly increased egg collection in floating net cages. Eggs can be transported at a density of 6,000 eggs/1 at 28°C and 28 ppt to maintain high survival (Garcia and Toledo 1988). These techniques can ensure sufficient quantity of viable eggs for hatchery runs.

Ongoing broodstock management studies focused on determining optimum dietary lipid levels to improve egg and larval quality. Several spontaneous spawnings have already occurred in this study but preliminary results appear inconclusive (Marte and Emata, unpublished results). Other broodstock management studies involve determining the influence of several factors (i.e. sex ratio, source, and age) on egg production and egg and larval quality.

Nursery

Nursery studies centered primarily on mass production of hatchery-produced fry. Modified water management and feeding schemes at larval rearing were conducted to test their effectiveness (Gapasin and Marte 1990). Following preliminary trials, heavy mortality occurred at Day 3 in larvae reared in a flow-through system stocked at 90 larvae per liter (Duray, unpublished results). However, resulting larvae were found to be more robust than those reared in a static system (control) stocked at 30 larvae/1.

Continuing tests for an alternate feed to supplement live food *(Brachionus* and *Artemia)* for milkfish larvae initially show that milkfish larvae fed combined microparticulate feed and rotifer, *Brachionus,* are significantly bigger than those fed microparticulate diet or *Brachionus* alone (Marte and Duray 1991). In a subsequent study, milkfish larvae fed microbound diet containing highly unsaturated fatty acid (kappa-carragenan microbound diet) as a supplement to live food and sampled on Day 21 have significantly higher body weight than those of larvae fed another supplemental microbound diet (Nosan R-l) or live food *(Brachionus* and *Artemia)* alone (Marte et al., unpublished results). Further studies are needed to verify these results.

A comprehensive study on feeding biology showed that from Day 3 to Day 21, milkfish larvae initially consumed an average of 4±3 *Brachionus* individuals daily with the number ingested increasing with larval size (Duray, submitted). Day 7, 14, and 21 larvae commenced feeding right before light onset (0400-0600 H) and exhibited 3-4 feeding peaks during the day. These results may avoid mass mortality through improved feeding management.

Hatchery verification of SEAFDEC/AQD-generated technology for milkfish mass fry production (Gapasin and Marte 1990) in collaboration with the private sector is being conducted. Initial hatchery runs revealed heavy mortalities beginning Day 6 probably due to inadequate supply and feeding of live food (Garcia et al., unpublished results).

Physiological studies on metabolism indicated that milkfish weighing 20-50 and 150-250 grams had maintenance ration of 5.2 and 4.9 g $kg^{-0.8}$, respectively, when fed diets containing 41% protein (Schroeder, unpublished results). From oxygen consumption of 20-50 g milkfish in 26-28°C rearing temperature, the standard metabolic rate, routine metabolic rate, and scope for spontaneous activity were 160-250, 260-330, and 200-350 mg O_2 h^{-1} $kg^{-0.8}$, respectively (Schroeder, unpublished results). These values can determine growth potential of milkfish similar to that of tilapia (Becker and Fishelson 1990).

Deviations in plasma osmolality and chloride concentrations of milkfish juveniles (40-260g BW) taken 0,1,2,3,5,7 and 14 days following abrupt transfer from seawater (32 ppt) to salinities of 0,16,32 (control) and 48 ppt are proportional to the osmotic or ionic gradient but inversely proportional to size (Ferraris et al. 1988). These results suggest that while smaller fish adapts to freshwater better than to seawater, larger fish adapts to seawater better than to freshwater. These osmoregulatory adaptations may reflect natural habitat shifts during development.

Recruitment mechanism of milkfish fry along the coastline remains debatable. Newly-collected milkfish fry had very low feeding incidence in spite of the abundance of plankton (Morioka, unpublished results). In contrast to previous reports (Kumagai 1984), a regular increase in the total length of milkfish fry collected at various moon phases has not been observed (Morioka, unpublished reports).

Milkfish fry given *Tetraselmis tetraheli-* or *Isochrysis* galbana-fed *Brachionus* have higher growth rates than fry given *Chlorella* sp.-fed *Brachionus* (Villegas et al. 1990). The high protein and fat levels of the two algal species may have enhanced the dietary value of *Brachionus.* In a separate study, milkfish fry fed water flea *(Moina macrocopa* Strauss) have better growth than those fed *Brachionus* (Villegas 1990). Fry given frozen *M. macrocopa* has significantly lower growth and survival than those of fry fed live moina (Villegas and Lumasag 1991). But both parameters are unchanged when fry were given live or frozen *B. plicatilis.* The suitability of using frozen rotifers allows short term storage in anticipation to high hatchery demand and overcome any difficulties arising from failure to mass produce *B. plicatilis.* However, milkfish fry dealers presently do not use live food when storing fry (Bagarinao 1991).

Nutrition and Feed Development

Significant achievements on nutritional studies have been obtained to warrant initial formulation of a practical diet for milkfish which has been a major concern of the Feed Development Section. Milkfish fingerlings require a diet of 30-40% protein, 10% fat, and 25% carbohydrates (Alava and Lim 1988, Piedad-Pascual 1989). Energy levels exceeding 3,500 kilo calories per kilogram do not improve weight gain. Amino acid-supplemented diets containing protein-energy ratio to total metabolizable energy ratio of 44% promote highest growth rates of milkfish juveniles compared with any of the other ratios (Coloso et al. 1988).

Based on dry diet, the optimum growth requirements of milkfish juveniles for essential amino acids are: lysine, 2.0%: arginine, 2.1 %; threonine, 1.8%; histidine, 0.8%; isoleucine, 1.8%; leucine, 2.3%; valine, 1.6%; and phenylalanine, 1.9% at tyrosine level of 0.45% and 1.3% at tyrosine level of 1.20% (Borlongan and Benitez 1990, Borlongan 1991, unpublished results). The growth potential of a particular dietary protein source can be determined from the closeness of its amino acid profile to the growth requirements.

Milkfish has a total lipid content of 4.5% to 4.9% (Bautista et al. 1991). Lipids consist mainly of neutral type, primarily triglycerides and cholesterol ester. Essential fatty acid requirements of milkfish indicate that (n-3) fatty acids such as 18:3(n-3) and (n-3) highly unsaturated fatty acids are nutritionally more important than 18:2(n-6) (Borlongan, in press).

Milkfish fingerlings fed diets containing 10% vegetable protein had significantly higher growth rates, protein efficiency ratio, and survival rates than those given animal protein-containing diets. Fingerlings fed mung bean-containing diets also had higher *in vitro* protein digestibility than animal protein-containing diets (de Costa Reis, unpublished results).

Digestive lipases of milkfish have an optimum temperature range of 50-60°C (Borlongan 1990). The depressed lipase activity at 0-25°C, similar to those of amylase and protease, may account for the decreased feeding activity of milkfish cultured during cold months resulting in considerably slower growth.

Under grow-out conditions, a fish meal-based formulated diet promotes higher growth rate of fingerlings than those given *Oscillatoria* that were inoculated and grown in the ponds and with or without formulated diets (Santiago et al. 1989). Fingerlings fed formulated diet also have higher growth rate than those given *Spirulina* powder and formulated diet or rice bran alone.

Pathology

Tolerance tests showed milkfish fingerlings have median lethal concentrations of 332, 260, 241, and 232 parts per million formalin at 24, 48, 72, and 96 hours bioassays, respectively (Cruz and Pitogo 1989). Milkfish fingerlings exhibit median lethal concentrations of 1.48 and 1.49 milligrams per liter potassium permanganate at 24 and 48 h bioassays, respectively (Cruz and Tamse 1989).

Aeromonas hydrophila infection have incurred heavy mortalities of milkfish juveniles freshly stocked in freshwater pens. Milkfish vaccinated with antigens obtained from a pool of 5 strains of *A. hydrophila* screened from naturally occurring milkfish did not develop protective immunity (Po, unpublished results). Current research will test immunogenecity of antigens from a single strain of *A. hydrophila.*

Farming Systems

Economic analysis of the modular pond system, which can increase croppings up to 7 times annually, shows higher profitability levels compared to the straight-run method. This system has a return of investment (ROD of 69% and payback period of 1.25 years (Agbayani et al. 1989) compared with the 56% ROI and 1.37 years payback period (Bombeo-Tuburan et al. 1989) of the straight-run method.

Economic analysis of an integrated milkfish broodstock and hatchery operation as a public enterprise shows negative figures on economic indicators (net present value and internal rate on return) up to the fifteenth year (Agbayani et al. 1991). However, both indicators show upward trends beginning on the seventh year. The high investment in hatchery facilities and low utilization of hatchery facilities because of short spawning season (April to October) may have caused the poor economic indicators. The discounted cash flow computations were based on an annual stocking of 100 milkfish juveniles for 4 years, egg production beginning on the fifth year, and revenues solely coming from the sales of fry. However, increased survival in the mass production of milkfish fry resulting from better broodstock nutrition and effective larval management may shorten the payback period.

At higher stocking densities (6,000 and 9,000 per hectare) of milkfish juveniles, supplemental feeding at the last month of culture increases yield than feeding natural food alone (Sumagaysay et al. 1990). Supplemental feeds containing 22.0% and 27.4% protein increase growth, yield, and net profit than those of fish given rice bran or unfed (Sumagaysay et al. 1991). Survival of milkfish given supplemental feeds or none are similar. Following 4 months of culture, the concentration of metabolites in the culture water is low in all treatments but the level of total carbon dioxide and nitrite-nitrogen increase significantly as biomass increases. The organic matter in supplemental feeds can be replaced with rice straw compost of up to 50% without affecting growth and yield (668-725 kilograms per hectare) but doubling the net income (Sumagaysay 1991). Growth and yield decrease significantly with 75% replacement. The compost is not a satisfactory feed substitute as fish derive most of its nutrition from formulated feeds. However, addition of rice straw compost enhances fertilization rate in ponds leading to higher production of autotrophic and heterotrophic microorganisms which will compensate for decreasing nutrient output. At a feeding rate of 1.75% BW using low protein (23.8%) supplemental feed, the 50% replacement of dietary organic matter with rice straw compost is an innovative way of reducing feed cost and increasing net profit. Even the addition of dietary fiber (33%, mostly from rice hull), a low protein supplemental diet (14%) given 25 days after stocking can be an economical way of increasing milkfish production in brackishwater ponds as it greatly reduced cost of feed input (Sumagaysay and Chiu-Chern 1991). Further studies are needed to formulate a suitable supplemental feed at certain pond productivity and environmental condition.

Modifications of culture practices to increase production and profitability were conducted. Stunting of milkfish fingerlings does not affect growth, survival, and net production, but the practice provides year round supply of fingerlings (Bombeo-Tuburan 1988). The silo method of fertilizer application, where fertilizer is placed in a sack and submerged in the pond for gradual release, does not increase fish production and growth over the broadcast method (Gerochi et al. 1988), but the former method is cheaper, less laborious, and produces consistent growth of *lablab* (matrix of micro and macrobenthic organisms) than the latter method. Application of 50 kg organic fertilizer/ha and biweekly water replenishment are recommended for better growth, survival, and gross production (Bombeo-Tuburan 1989). In a separate study, 50 kg organic fertilizer/ha and 15 kg inorganic fertilizer/ha do not significantly increase yield but give more profit than any of the other fertilizer and dosage combination tested (Bombeo-Tuburan 1989).

RABBITFISH

Breeding and Nursery

Two injections of 2 international units human chorionic gonadotropin per gram body weight given 24 hours apart induce spawning of female rabbitfish (Ayson 1991). Hormone injections are given to females with average oocyte diameters less than 0.46 mm which can normally spawn naturally. The number of eggs spawned (424,000 per female), fertilization rate (96%), and hatching rate (59%) are similar between artificial and natural spawning.

Broodstock management studies on rabbitfish initially attempted to enhance survival of rabbitfish larvae. Stress due to handling and repeated injections significantly enhances spawning but has no effect on larval survival (Ayson 1989). Rabbitfish given diets containing 18% lipid have significantly higher number of normal larvae with increased survival rate than those of fish given diets containing 12 or 15% lipid (Hara et al. 1986). The significance of dietary lipid was also demonstrated in rabbitfish fed diets enriched with cod liver and soybean oils. All rabbitfish females given lipid-enriched diets produce mature eggs monthly for five consecutive months, while females given lipid-poor diets intermittently produced mature eggs (Emata, unpublished results).

Hormonal manipulation of sexually mature rabbitfish to enhance larval quality and improve survival was also tested. A single thyroxine injection given to broodfish a day or two prior to spawning significantly increased thyroid hormone concentrations in the plasma of female rabbitfish, in spawned eggs, and in newly-hatched larvae but did not enhance larval development and survival rates (Ayson and Lam, submitted) in contrast to previous reports (Lam 1980, Brown et al. 1988).

Mass mortality of rabbitfish larvae within days following hatching occurs at the transition from endogenous to exogenous feeding. Kohno et al. (1988) indicated that the larvae must be able to start feeding in order to survive.

However, the conditions for successful feeding transition are still unknown. Rabbitfish larvae reared at continuous light have higher mean survival (31%) and bigger larval size at first feeding than those reared under natural daylight (17%) (Duray and Kohno 1988).

Under simulated transport conditions, survival of rabbitfish fry at 100 fry/1 under ambient conditions of 28°C and 32 ppt is not adversely affected by up to 8 hours of transport (Ayson et al. 1990). Stocking densities greater than l00 fry/1 shortens transport time to maintain survival rate of 96%.

Nutrition and Feed Development

Nutrition studies focused on determining the optimum protein requirements for growth of rabbitfish larvae, fry, and juveniles. Diets containing 39, 41, 51, or 56% protein promote better growth of 25-day old larvae than *Artemia* alone (Parazo 1991). However, weight gain, metamorphosis, and survival rates do not vary among protein levels. A diet of 35% protein and 3,832 kilocalories per kilogram-gave highest growth rate and protein efficiency of rabbitfish fry (Parazo, unpublished). A supplemental diet containing 26% protein gave higher average body weight (81.6 g) of rabbitfish juveniles in ponds than those given diets containing 21% protein (555 g) or fed *lumut* (48.8 g) (Bombeo-Tuburan, unpublished results).

MULLET

Breeding

A captive broodstock for mullet was established with 30 wild spawners in a 6-meter diameter by 3-meter deep floating net cage. Sexually maturing fish were observed in November, but spontaneous spawning was never observed probably due to stress following monthly samplings (Quinitio, unpublished results).

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