Research on Marine and Freshwater Fishes

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Abstract

Most of the fish research at SEAFDEC AQD in 1992-1994 was on milkfish. Studies were conducted on year-round spawning through hormonal or environmental manipulation; optimum lipid and protein levels and ration size for captive broodstock; and the influence of spawner age on reproductive performance. The economics of hatchery operations, alone or integrated with broodstock as a commercial enterprise, was assessed. Mass production of larvae was refined with the use of commercial or SEAFDEC-formulated larval diets. Alternative rearing schemes in large tanks and ponds were tried. Hatchery-produced and wild-caught larvae were compared in terms of growth and production in experimental nursery and grow-out ponds. Supplemental diets for brackishwater grow-out culture were formulated.

Studies on broodstock management of grouper *Epinephelus* spp. included lipid enrichment of the diet and hormonal induction of sex inversion. Seed production techniques were developed but survival rates were low. Grouper culture was found economically feasible in experimental ponds with 'trash' fish as feed.

The mangrove red snapper *Lutjanus argentimaculatus* was successfully induced to spawn with injection of human chorionic gonadotropin. Initial larval rearing trials were successful but survival rates must be improved.

Hormonal manipulation of spawning of the Asian sea bass *Lates calcarifer* allows seed production during most of the year. Photoperiod manipulation leads to maturation of females, but not males, beyond the natural breeding season (April-November). Nursery rearing of 9 mm juveniles is feasible in floating net cages with night lights that attract food zooplankton. The requirements of sea bass for lipid, protein, carbohydrates, and essential amino acids were determined.

In the rabbitfish *Siganus guttatus,* weekly injections of luteinizing hormone releasing hormone analogue (LHRHa) sustains milt production for three weeks. Thyroid hormones injected into broodstocks improved the growth of larvae to day 7.

Induced spawning techniques for the Asian catfish *Clarias macrocephalus* were refined by determining the seasonal responsiveness to

LHRHa and pimozide injections and testing for pheromonal induction of spontaneous spawning. The optimum insemination rate was determined and egg hatchability was enhanced by removal of the adhesive coat before incubation. Several practical diets for catfish during grow-out culture were tested against 'trash' fish.

The broodstock management for bighead carp *Aristichthys nobilis* was studied. Cage-reared juveniles from cage-reared broodstock showed the best growth. To improve the reproductive performance, the broodstock diets were supplemented with vitamins A, C, and E.

Research on tilapias focused on genetics and strain selection. Several strain testing procedures for Nile tilapia were evaluated in their efficiency to detect economically important strain differences. Reference lines were developed from two existing red tilapia strains to measure and reduce the effects of uncontrolled nongenetic variables in strain evaluation experiments with Nile tilapia. The tolerance of two Nile tilapia strains to heavy metals was similar when gauged by the 24-hour and 96-hour lethal concentration and by fish growth, survival, and reproductive performance. In a separate study, four strains of red tilapia showed generally higher seed production when reared in tanks than in cages. Improvements in the feed and feeding management for Nile tilapia were also studied.

Intensive tilapia farming and feeding have led to oxygen depletion and fish kills in Sampaloc Lake. To rehabilitate the lake, it is imperative to reduce the farming area from 30 to 6 hectares; stop the use of commercial feeds; and remove the water hyacinths and other debris. Fish kills in Laguna de Bay have also become serious in recent years, and a review of the occurrences, losses, and possible causes is currently being conducted. Studies on the epizootic ulcerative syndrome of snakeheads in Laguna de Bay have yet to pinpoint the pathogen. Skin lesions in tilapias in several ponds and lakes in the country were found to be due to bacteria.

Introduction

Growth and expansion of marine and freshwater fish culture is necessary in view of the rapid decline in world fish catch and the degradation of the aquatic environment. The SEAFDEC Aquaculture Department has conducted studies on the artificial propagation, nutrition and feed development, farming and socio-economics, health management, and the environmental impact of culture of several fishes. These studies aim to develop aquaculture techniques that would be sustainable ecologically, economically, and socially. This paper reviews recent (1992-1994) studies on cultured fishes at SEAFDEC AQD.

Studies on Milkfish *Chanos chanos*

Ecology

The systematics, genetics, distribution and life history of milkfish was fully detailed by Bagarinao (1994). The natural life history of milkfish is one of continual migration. Adults are large, long-lived, pelagic, and schooling. They spawn offshore near coral reefs and small islands. The eggs and larvae are pelagic and larger than those of most marine fishes. Larvae over 10 mm long and 2-3 weeks old (the 'fry') move inshore by passive advection and active migration. Passing through shore waters and surf zones, these larvae enter shallow-water depositional habitats such as mangrove swamps, where they metamorphose and settle for a few months as juveniles. Some juveniles may enter freshwater lakes (if available) where they grow into sub-adults but do not mature sexually. Both small juveniles and large sub-adults return to sea when they reach the size limit supportable by the habitat. Little else is known of the population dynamics of wild milkfish. To ensure that milkfish (and other fishery species) continue to support human populations, there must be a conscious effort to protect, manage, and rehabilitate coastal habitats and resource systems.

Broodstock development

Reproduction of milkfish broodstock in concrete tanks and floating cages has been fully documented. Milkfish broodstock 9-10 years old spontaneously mature and spawn in concrete tanks 150-200 m^3 in capacity and 2 meters deep (Emata and Marte 1994). From April to November 1991, the different stocks spawned 19-36 times and total egg collection ranged from 8.5 to 12 million (Table 1). As holding facility for milkfish broodstock, concrete tanks are a feasible alternative to floating cages, with no resulting difference in reproductive performance.

Various milkfish stocks in floating cages (24-59 fish per cage, various ages, from either wild-caught or hatchery-bred larvae) had annual egg production ranging from 0.27 to 21.55 million in 1986-1991 (Emata and Marte 1993). The different stocks spawned 5-51 times a year, and averaged from 50,000 to 956,000 eggs per spawning. Older broodstocks from 1978-1981 larvae showed consistently higher annual egg production and number of spawnings. Broodstocks derived from wild-caught or hatchery-bred larvae were not different in reproductive performance (Table 2). Further studies confirmed that broodstock origin (wild or hatchery-bred larvae) does not affect reproductive performance, but that 11-14 year old broodstocks spawned more eggs than 9-year old ones (AC Emata, unpublished).

Milkfish broodstock in floating cages incidentally eat their own spawned eggs (Toledo and Gaitan 1992). The mean number of spawned eggs at the water surface significantly declined one hour after spawning and very few eggs were recovered 4 hours later. Eggs were found in the guts of spawners 5 hours after spawning. Thus, spawned eggs should immediately be collected to prevent egg cannibalism by spawners.

Current studies aim for year-round spawning, increased egg production, and improved egg and larval quality. Off-season spawning was attempted through hormonal implantation. In female milkfish, serum estradiol and testosterone levels are positively correlated with gonadosomatic index and oocyte diameter (Marte and Lam 1992). Testosterone levels were significantly higher in males with moderate amounts of viscous milt than in those with milt that were either scanty and viscous, or abundant and fluid. A single implant of slow-release capsules of estradiol (for females) or 17α-hydroxyprogesterone (for males) resulted in earlier maturation and spawning than of the unimplanted control fish (Marte and Emata, unpublished). Maturation and spawning occurred in March, still within the natural breeding season (March to October). Attempts to induce off-season spawning of tank-reared broodstock through photoperiod manipulation also failed as fish did not spawn during the off-season (AC Emata, unpublished).

Table 1. Reproductive performance of 1981, 1982a, and 1982b milkfish broodstocks in concrete tanks in 1991. Most values are means±standard error. Modified from Emata and Marte (1994).

Broodstocks	Total egg collection (million)	Daily egg collection (×1,000)	Total spawnings	Viable eggs (%)	Hatching rate (%)	Normal larvae (%)	Survival (%) from hatching to 21-23 d	Survival (%) from egg to 21-23 d
1981	10.4	290±40	36	79±3	63±5	81±4 (5 runs)	31±9	20±9
1982a	12.0	230±30	53	81±4	55±5	79±3 (9 runs)	27±5	23±9
1982b	8.5	530±50	19	76±5	59±6	69±5 (3 runs)	30±5	8±5
Overall	31.0	300±30	108	82±2	62±3	81±2 (17 runs)	29±4	17±3

Table 2. Reproductive performance in successive years of milkfish broodstocks raised from 1980 wild-caught or hatchery-produced larvae. Modified from Emata and Marte (1993).

Year	Number of fish	Number of spawnings	Total egg collection (million)	Daily egg collection (×1,000)[d]	Egg viability (%)[d]
From wild-caught larvae 1980					
1986	50	27	13.2	490±100	-
1987	36	24	15.0	730±130	84±3
1988	36	19	7.1	400±60	84±4
1989	39[a]	17	4.1	240±70	83±4
1990	39	23	8.3	380±90	92±3
1991	31[b]	26	16.5	560±150	96±1
From hatchery-bred larvae 1980					
1986	34	27	16.7	640±160	-
1987	34	46	21.6	440±60	84±3
1988	34	33	18.3	540±80	90±2
1989	26	6	5.7	940±330	80±7
1990	26	10	4.7	450±140	97±1
1991	24[c]	4	1.3	310±220	98±1

a Three spawners were added to the stock
b Sampling in Nov 1991 showed 19 females, 9 males, 3 undetermined
c Sampling in Nov 1991 showed 12 females and 12 males
d Mean±standard error

Breeding studies require identification of individual specimens. Visual implant tags were used to tag milkfish at the adipose tissue on the operculum; these tags allowed faster tagging (less than one minute), had higher retention rate and longer retention time, did not cause infections, and were easily readable (Emata and Marte 1992).

Dietary factors (proteins, lipids, vitamins) known to influence egg and larval production and quality of fish broodstock were also evaluated. Cage-reared broodstock fed diets with 36% protein at 4% body weight daily had higher egg production, mean number of eggs per spawning, and spawning frequency than broodstocks fed diets with 36% protein at 2% ration or 42% protein at 2 or 4% ration (CL Marte, personal communication). In a later study, the same broodstock fed diets with 6% lipid at a 4% ration produced the highest total number of eggs and the highest mean number of eggs per spawning. However, they did not differ from broodstocks fed diets with 6% lipid at 2% ration nor 10% lipid at 2 or 4% ration in terms of the frequency of spawning, the quality and sizes of eggs and larvae, and larval survival (CL Marte, personal communication). Present and future studies will test lower dietary protein (24 and 30%) and vitamin supplements to further refine the broodstock feed.

Seed production

Agbayani et al. (1991) showed that an integrated milkfish broodstock and hatchery enterprise is not economically attractive (i.e., has negative net present value and negative internal rate of return) up to the 15th year. These results were based mostly on theoretical Figures. The economics of milkfish hatchery operations, alone or integrated with broodstock, was reassessed in collaboration with operators of commercial hatcheries in Panay Island (LMB Garcia, personal communication). A milkfish hatchery would be profitable if the cost of milkfish eggs or newly hatched larvae does not exceed P6,000 per million (US$1=P25). However, an integrated broodstock and hatchery operation has low profit margins. If the hatchery depends on just a small number of broodstock that can not produce enough eggs, the facilities become underutilized and the operation fails.

Morphological abnormalities occur in 2-17% of hatchery-produced milkfish larvae and become obvious at day 35 (GH Garcia, personal communication). These deformities of the opercular bones (exposing the gills) and of the branchiostegal rays and membrane are similar to the shortened operculum of teleosts fed diets deficient in vitamin C (Halver et al. 1975). The causes of these deformities in milkfish are being studied. Trials were made to enrich food organisms with vitamin C and highly unsaturated fatty acid, or their combination, but these enriched foods did not enhance growth and survival nor get rid of the deformities of milkfish larvae (RSJ Gapasin, personal communication). Another study will look at vitamin C supplementation of the broodstock diet as a way to minimize if not eliminate the deformities in the offspring.

Mass production of milkfish larvae was refined with the use of commercial or SEAFDEC-formulated diets for shrimp larvae (MN Duray, personal communication). The larval feed Nosan R-1 (Nosan Kogyo, Japan) could replace up to 50% of the rotifer requirement. Either Lansy A-2 (Artemia Systems, Belgium) or the carageenan-microbound diet (c-MBD, SEAFDEC AQD) could replace half of the requirement for *Artemia* nauplii. Total replacement of the natural food organisms with formulated larval diets gave poor results.

Practical diets were formulated and tested for milkfish larvae (IG Borlongan, personal communication). Two diets were found effective as supplement or partial replacement of natural food organisms. These practical diets could reduce the capital requirements for natural food production in hatcheries.

Whole-body concentrations of the thyroid hormones thyroxine T_4 and triiodothyronine T_3 were measured in different stages of milkfish larvae to determine their role in early development (Jesus 1994). The hormone levels surged during metamorphosis. Information like this may lead to a design of an appropriate protocol for thyroid hormone treatment to improve growth rates and yields of milkfish.

The bacterial load in the hatchery system during routine operations was assessed in order to develop health management techniques for milkfish and other fishes in the hatchery (R Duremdez-Fernandez, personal communication). The chemotherapeutant nifurpirinol was shown to be toxic to milkfish (Tamse and Gacutan 1994).

Alternative rearing schemes for milkfish larvae were tested. Larval rearing in large concrete tanks and earthen ponds with fertilizers applied to encourage plankton blooms) were tried for milkfish, but survival rates were only 2-5% (CL Marte, personal communication).

The concept and practicalities of a multi-species hatchery were described by Duray (1994) to guide shrimp hatchery owners who want to venture into seed production of milkfish and other fish species.

Grow-out culture

Hatchery-produced milkfish larvae reared in brackishwater nursery ponds for 45 days had higher mean body weight but lower survival than wild-caught larvae (EM Rodriguez, personal communication). However, no such difference was found after another 45 days in grow-out ponds. In this study, 13% of the hatchery-produced larvae had opercular deformities.

Semi-intensive milkfish culture requires supplemental diets. The energy and protein requirements, food intake, and the effects of dietary protein and ration size have been determined (Sumagaysay 1993, 1994). Growth of milkfish is correlated with food consumption and is higher in the presence of supplemental feed than on natural food alone (Table 3). Among fish grown on natural food alone, food consumption and growth during the wet season (average salinity 22 ppt) are higher than during the dry season (32 ppt) (Sumagaysay 1994). A supplemental diet with 25% protein was as good as one with 36% protein. A further study showed that a supplemental diet with 24% protein given at 4% of body weight per day is optimal for milkfish growth, production, and profitability in ponds (Sumagaysay, in press).

Table 3. Weights, growth, and daily ration (mean±standard error) of milkfish fed natural food and supplemental diet. Initial weight of fish, 5.5 grams. Modified from Sumagaysay (1993).

Diet	Day	Weight (g)	SGR[a] (g/d)	Daily ration per fish (g/d)	(kcal/d)
Natural Food	42	35.2 ± 2.0^{a}	3.8 ± 0.2^{c}	0.4 ± 0.3^{a}	0.6 ± 0.4^{a}
	76	57.0 ± 0.4^{b}	1.4 ± 0.1^{ab}	0.7 ± 0.1^{ab}	1.4 ± 0^{a}
	96	76.6 ± 0.8^{b}	1.2 ± 0.4^{a}	0.7 ± 0.2^{ab}	1.4 ± 0.4^{a}
Supplemental feed	42	32.3			
	76	71.6 ± 3.4^{b}	2.4 ± 0.4^{bc}	1.4 ± 0.5^{a}	5.9 ± 2.2^{a}
	96	116.4 ± 5.4^{c}	3.1 ± 0.3^{c}	4.8 ± 0.4^{b}	19.7 ± 0.7^{b}

SGR, specific growth rate

For each column, values with the same superscripts are not significantly different. Daily ration was calculated from gastric evacuation rates.

Snails like *Cerithium* and *Telescopium* compete with milkfish for food in brackishwater ponds. Organotin pesticides like Aquatin and Brestan have been banned in the Philippines as in many other countries, and environment-friendly ways to eliminate pond snails must be found. Rice straw piled up 15 cm thick on pond bottoms with snails can be burned to kill the snails (Triño et al. 1993).

Occasional mass kills of milkfish in brackishwater ponds have been attributed to low dissolved oxygen in the morning, or to acid pond water due to sulfates washed from the dikes by rains. Sulfide, a toxin produced by sulfate-reducing bacteria in sediments, was also examined as a possible cause of mass kills. In bioassays in flow-through seawater with different sulfide concentrations, 200 μM (=6.4 mg/l) total sulfide was found lethal for 50% of juvenile milkfish after 8-24 hours (TU Bagarinao, personal communication).

Feed development

The requirements of juvenile milkfish for fatty acids and essential amino acids are now known (Borlongan 1992a, 1992b, Borlongan and Coloso 1993, Coloso et al. 1992). Milkfish grown in sea water differ in the lipid and fatty acid composition of the tissues than those grown in fresh water (Borlongan and Benitez 1992). The total polyunsaturated fatty acids, especially ω-3, were higher in seawater than freshwater milkfish. The nutrient requirements of milkfish are shown in Table 4 as summarized by FDS (1994).

Inexpensive and locally available leaf meals of swamp cabbage *Ipomoea aquatica,* sweet potato *I. batatas,* ipil-ipil *Leucaena leucocephala,* cassava *Manihot esculenta,* or their combination were tested as protein sources of diets for milkfish (Borlongan and Coloso 1994). About 15% of fish meal protein may be replaced with proteins from all leaf meals, except ipil-ipil, without any change in the growth, feed conversion ratio, and survival of milkfish in ponds.

The levels of several minerals in milkfish tissues and in sea water were measured as a step toward determining the dietary mineral requirements (GG Miñoso, personal communication).

Studies on the Grouper *Epinephelus coioides*

Editors' note: The grouper that has been called *Epinephelus suillus* in many studies and publications of the SEAFDEC Aquaculture Department and that called *Epinephelus tauvina* in other reports from Kuwait, Singapore, and Thailand should be correctly referred to as *Epinephelus coioides* (Doi et al. 1991; Heemstra and Randall 1993).

Broodstock development

Spontaneous spawning of wild-caught grouper in concrete tanks produced large numbers of eggs; however, egg and larval quality varied widely (Toledo et al. 1993). Efforts were made to improve egg quality and hatchability by enriching the 'trash' fish with cod liver oil or a commercial emulsion of highly unsaturated fatty acids. However, broodstock fed lipid-enriched diets had lower egg production, spawning frequency, fertilization rates, and hatchability (GF Quinitio, personal communication).

Table 4. Summary of known nutrient requirements of milkfish *Chanos chanos,* Nile tilapia *Oreochromis niloticus,* and Asian sea bass *Lates calcarifer.* Modified from Feed Development Section (1994; see original references therein).

Nutrient	Requirement (% of dry diet or % of protein)		
	Milkfish	Nile tilapia	Asian sea bass
Protein[a]			
For larvae	40	35	43
For juveniles	30-40	25-30	
Protein:energy ratio	44	-	50
Essential amino acids[b]			
Arginine	5.2%	2.2	3.6
Histidine	2.0	1.7	
Isoleucine	4.0	3.1	
Leucine	5.1	3.4	
Lysine	4.0	5.1	4.5
Methionine + Cysteine	2.5 (cys 0.8)	3.2 (cys 0.5)	2.4 (cys 0.7)
Phenylalanine +Tyrosine	4.2 (tyr 1.0)	5.5 (tyr 1.8)	
Threonine	4.5		3.8
Tryptophan	0.6	1.0	0.5
Valine	3.6	2.8	
Lipid[a]	7-10	6-10	10
Essential fatty acids[a]			
PUFA (ω-3)	1-1.5%	-	0.5
PUFA (ω-6)	-	0.5	0.5
Carbohydrate[a]	25	25	20-25
Digestible energy (kcal/kg)	2,500-3,500	2,500-4,300	

[a] Requirement as % of dry diet; [b] Requirement as % of protein
PUFA, polyunsaturated fatty acids

Among cage-reared groupers, spermiating males were observed in February-September and females with mature oocytes were found in July-November (GF Quinitio, personal communication). Natural sex inversion was observed when a 7.8 kg fish had mature oocytes in July but spermiated in August. Differences in body size appeared to induce sex inversion: the larger of two females in a 2x2x3 meter deep cage became male and the smaller one remained female Further studies will determine if this practical sex inversion technique can be used widely in aquaculture.

Induction of sex inversion through hormonal implants is also important because male groupers are scarce and natural sex inversion takes time. In one study, 17a-methyltestosterone (MT) induced spermiation of larger groupers (1.2-1.6 kg) 5 months after treatment (Tan-Fermin et al. 1994); however, these fish reverted back to being female 8 months after MT withdrawal (Table 5; Tan-Fermin 1992a). In another study, sex inversion of 3-year old cage-reared females was also induced by MT (4 mg/kg) or MT+LHRHa (20 μg/kg) implanted bimonthly, or MT injected biweekly (CL Marte, personal communication). Fish left untreated, or given only bimonthly LHRHa implants, or the hormone vehicle, remained female. The optimum MT dose and duration of treatment and the fertilizing capacity of milt from sex-inversed fish have yet to be determined.

Seed production

Larval rearing trials initially examined the food and feeding biology of grouper larvae (MN Duray, personal communication). Then mass production trials were made. Larvae fed small rotifers (screened with a 90 μm mesh net) had higher growth and survival than larvae fed unscreened rotifers. Survival and growth after three weeks were improved by a high *Anemia* ration (3/ml each day) and by the commercial diet Lansy A-2. Further studies will define hatchery production techniques that ensure high survival.

Grow-out culture

Wild-caught juvenile groupers stocked in brackishwater ponds at a density of 6,000 per hectare and fed 'trash' fish *ad libitum* for 5 months grew to mean body weights of 400 grams with 88% survival (I Bombeo-Tuburan, personal communication).

The fishery for juvenile grouper at Sapian Bay, Capiz was studied. Most of the juveniles were *Epinephelus sexfaciatus* and *E. coioides* collected with artificial shelters ('bonbon') in July-September (NB Solis, personal communication).

Health management

Bacterial infections due to *Vibrio* sp. occur among wild-caught juvenile and adult groupers held in concrete tanks (Lavilla-Pitogo et al. 1992). It is recommended that methods of grouper collection, handling, and transport be improved such that injuries, crowding, and stress are avoided and the fish do not succumb secondarily to bacterial infections.

Studies on the Snapper *Lutjanus argentimaculatus*

Thirteen species of snappers of the genus *Lutjanus* were found year round (with a peak in May) in the markets in lloilo (Cheong et al. 1992). The most common and abundant were *Lutjanus vitta, L. gibbus,* and *L. argentimaculatus.* Juveniles of the mangrove red snapper *L. argentimaculatus* are commonly found in mangrove areas and thus probably amenable to pond culture (TU Bagarinao, personal communication).

Broodstock development

Mangrove red snapper broodstock (1.8-4.9 kg BW) raised from wild-caught juveniles in floating net cages were found sexually mature from March to November (AC Emata, unpublished data). Broodstock held in concrete tanks were sexually mature only in May-October. A mature male and a female (2.5-4.9 kg) given a single intramuscular injection of human chorionic gonadotropin (1,500 IU/kg body weight) spawned 0.5-1.2 million eggs within 32-40 hours (Emata

Table 5. Stage of gonad development in the protogynous grouper *Epinephelus suillus* (20 individuals) during treatment with various doses of 17α-methytestosterone and 8-9 months after withdrawal of treatment. Modified from Tan-Fermin (1992).

Methyltestosterone (mg/kg fish)	Fish weight (kg)	Stage of gonad development During treatment 3 months	6 months	After treatment 8-9 months
0	0.7	primary oocytes, gonial cells		
0	1.4	spermatogenesis		
0	1.5	not sacrificed	milt at 5 months	with cannulated eggs
0	1.3	not sacrificed	primary oocytes, gonial cells	
0		not sacrificed	not sacrificed	with cannulated eggs
0		not sacrificed	not sacrificed	with cannulated eggs
0.5	0.8	primary oocytes, gonial cells		
0.5	1.5	spermatogenesis		
0.5	1.5	not sacrificed	milt at 5 months	with cannulated eggs
0.5	0.8	not sacrificed	primary oocytes, gonial cells	
1.0	1.0	primary oocytes, gonial cells		
1.0	1.2	spermatogenesis		
1.0	1.5	not sacrificed	cannulated milt	with cannulated eggs
1.0	0.6	not sacrificed	primary oocytes, gonial cells	
1.0		not sacrificed	not sacrificed	with cannulated eggs
5.0	1.2	spermatogenesis		
5.0	1.6	spermatogenesis		
5.0	0.8	not sacrificed	spermatogenesis	
5.0		not sacrificed	not sacrificed	with cannulated eggs
5.0		not sacrificed	not sacrificed	with cannulated eggs

et al. 1994). Egg viability ranged 65-80%. Rematuration of cage-reared broodstock occurred monthly for 5-6 consecutive months.

Seed production

Larval rearing trials on mangrove red snapper concentrated on feeding management (MN Duray, personal communication). The larvae increased rotifer consumption during growth and began to feed on *Artemia* nauplii on day 22. Rotifers may be supplemented with the commercial diets Nosan R-1 or Frippak, but survival at day 21 was only 3% on average. Larvae fed *Artemia* nauplii at a higher ration of 3-4/ml per day had better growth and survival.

Studies on the Asian Sea Bass *Lates calcarifer*

Broodstock development

Wild-caught juvenile sea bass grown in floating cages mature spontaneously, the males at 2-2.5 years and the females at 3-4 years of age (Toledo et al. 1991). A stock of 13 females and 28 males in a floating cage spawned 26 times from June to October, with monthly egg collection ranging from 393,000 to 60 million. Spawnings were mostly during the first or the last quarter moon periods.

Manipulation of the reproductive cycle with LHRHa treatment was studied further. Spontaneous spawning of sea bass in floating net cages followed a semilunar cycle, but LHRHa induced spawning any day during the lunar cycle (Garcia 1992). Alternative LHRHa delivery through rectal intubation or oral administration turned out unsatisfactory (LMB Garcia, personal communication).

Milt dilution may be induced by LHRHa treatment; a single LHRHa (40 μg/kg BW) injection reduces the sperm count 12-36 hours after injection (GH Garcia, personal communication). However, such injection must be given to males not later than 24 hours after injection into females.

Tank-reared broodstock kept under constant 8, 12, or 16 hours light all spawned spontaneously throughout the natural spawning season (May-November) but not during the off season (AC Emata, unpublished). However, females with mature oocytes and males with scanty milt were observed in December-March under the short and normal photoperiod. Females with mature oocytes were found in January-March under the long photoperiod.

Seed **production**

Nursery rearing of sea bass in illuminated cages was found feasible for juveniles of initial size about 9 mm but not for the smaller nor larger ones (Fermin et al. 1994a, 1994b). Among the natural plankton attracted to the cages by the night light, copepods comprised 67-90% of the diet of the test juveniles. Growth and survival increased with zooplankton density, which was highest at a light intensity of 300 lux. This promising nursery technique will be further refined.

The cladoceran *Moina macrocopa* was tested as an alternative live food for sea bass in the nursery (Fermin and Bolivar 1994, Ganzon-Naret and Fermin 1994). Conditions for the mass production of the cladoceran *Diaphanosoma celebensis* were determined. This cladoceran had higher reproductive rate when fed *Tetraselmis tetrahele* than when fed rice bran, rice straw extract, bagasse extract, and baker's yeast (MR de la Peña, personal communication). Actual production and use of cladocerans as food for larger sea bass (and other fish) larvae have to be conducted under hatchery conditions.

Sea bass (30 days old) stocked at $5/m^2$ in brackishwater nursery ponds grew well on a ration of 'trash' fish alone or in combination with commercial feeds (Triño and Bolivar 1993).

The weight-specific ammonia excretion rate in sea bass is higher in fresh water than in sea water and is largely unaffected by prolonged starvation (Almendras 1994).

Feed development

A practical diet for sea bass is in the making (Table 4). Sea bass juveniles fed diets with 20% carbohydrates and 12% lipid, and 42.5% protein level showed the highest weight gain of 6.4x and feed conversion ratio of 1.22 after 12 weeks (MR Catacutan, personal communication). The *in vitro* protein digestibilities of raw or processed leguminous seeds (white and black cowpeas, green and yellow mungbeans, rice bean, and soybean) were determined preparatory to their possible use as protein and energy sources in seabass diets (PS Eusebio, personal communication).

Studies on the Rabbitfish *Siganus guttatus*

Broodstock development

Male rabbitfish given weekly LHRHa injections showed greater sperm production and greater amounts of expressible milt up to 3-4 weeks than control males injected with saline (Garcia 1993).

Seed production

The physiological role of thyroid hormones in early development was studied by Ayson and Lam (1993). Rabbitfish spawners were given T_4 injection. Levels of thyroxine T_4 and triiodothyronine T_3 in maternal plasma, eggs, and yolk-sac larvae increased following the injection. Apparently T_4 was converted into T_3 by the spawner. At day 7, larvae from spawners given T_4 at doses of 10 and 100 μg/g body weight survived and grew better than those from spawners given 1 μg/g.

The salinity tolerance of rabbitfish eggs and larvae indicates that 14-37 ppt sea water is suitable for the period from spawning to 24 hours after hatching (Young and Dueñas 1993).

Studies on the Asian Catfish *Clarias macrocephalus*

Broodstock development

The Asian catfish spontaneously matures but does not spawn in captivity. Hormonal manipulation of reproduction is necessary to ensure a steady seed supply. In one study, oocyte maturation and ovulation was induced 15-16 hours after injection of 0.01-0.10 μg LHRHa + 1 μg pimozide per gram body weight (Tan-Fermin 1992b). Injection of 0.05 μg LHRHa + 1 μg pimozide per gram body weight and stripping of eggs 16-20 hours later is a reliable method to obtain high rates of ovulation, fertilization, and hatching (Table 6).

To further refine the breeding technique, the standard LHRHa-pimozide treatment was tested at different times of the year. Ovulation was 100% before (Apr-May) and at the peak of the natural spawning season (Jul-Sep), but only 80% at the end (Oct-Dec) and 60% during the off season (Jan-Mar) (JD Tan-Fermin, personal communication).

Table 6. Effect of a fixed dose of pimozide with various doses of LHRHa on egg production, fertilization, and hatching rates (mean±standard error) of gravid catfish *Clarias macrocephalus*. Modified from Tan and Emata (1993).

Date of experiment	Treatment LHRHa (μg/g)	Treatment Pimozide (μg/g)	Egg production (eggs/g fish)	Fertilization rate (%)	Hatching rate (%)
Jun 1990	0.10	1	47.4 ± 5^{a}	90 ± 4^{a}	39 ± 3^{a}
	0.05	1	67 ± 10^{a}	84 ± 7^{a}	51 ± 22^{a}
	0.025	1	53 ± 3^{a}	75 ± 12^{b}	48 ± 45^{a}
	0	1	65	39	0
	0	0	-	38	1
	0	-	40	36 ± 10^{c}	1b
	-	0	40 ± 4^{a}	39	0b
Aug 1990	0.10	1	82 ± 4^{a}	87 ± 4^{a}	58 ± 4^{a}
	0.05	1	77 ± 4^{a}	90 ± 4^{a}	79 ± 1^{b}
	0.025	1	79	71	46
	0	1	-	-	-
	0	0	-	-	-
	0	-	-	-	-
	-	0	-	-	-
Sep 1990	0.05	1	96±6	97±1	69±5
	0	-	-	-	-

For LHRHa, the 0 dose means 0.9% sodium chloride. For pimozide, the 0 dose means 1:9 dimethylsulfoxide and propylene glycol.
For each experiment, means with the same superscript under each column are not significantly different.

The pheromones etiocholan-3α-ol-17-one glucuronide, 11β-hydroxyetiocholanolone glucuronide, or their combination were used to induce spontaneous spawning of hormone-treated catfish. Release of gametes was not observed even 30 hours after injection (LMB Garcia, personal communication).

Artificial fertilization techniques were also refined. Fertilization and hatching rates were highest when 25-50 μl of milt was diluted 3.5x in saline solution and mixed with 2.5-10 grams of stripped eggs (MV Tambasen-Cheong, personal communication). The variability in hatchability of eggs was also addressed by testing chemical washes to remove the adhesive egg coat. Eggs washed with saline, tannin, or their combination had higher hatching rates (17-23%) than those washed with water alone (control, 10%) (JD Tan-Fermin, personal communication).

Feed development

Juvenile catfish fed *ad libitum* four practical diets with 38% protein had mean weight gains of 120-200% after 36 weeks, whereas those fed 'trash' fish and practical diets had a weight gain of 61% (CB Santiago, personal communication). Although the catfish were relatively small, some females already had mature gonads at harvest.

Studies on the Bighead Carp *Aristichthys nobilis*

Cage-reared broodstock were fed diets with or without supplemental vitamins A, E, and C (CB Santiago, personal communication). Assessment of reproductive performance was inconclusive as fertilization and hatching rates and larval production from three spawning trials were variable within and among treatments.

The commercial production of bighead carp in hatcheries around Laguna de Bay relies only on several broodstocks. Purchasing and exchanging breeders among hatcheries is a common industry practice that can lead to problems like inbreeding and negative selection. Thus, the breeding management techniques of three commercial hatcheries were assessed based on growth performance of juveniles. Juveniles were obtained from spawns of broodstocks grown in either ponds, cages, or reared in cages and conditioned in ponds prior to induced spawning. These juveniles were then reared in either cages or laboratory tanks. Growth after 90 days was best among cage-reared juveniles from cage-reared broodstock (AE Gonzal, personal communication).

Mass production of the freshwater rotifer *Brachionus calyciflorus* for culture of bighead larvae was studied. Mean population density and intrinsic growth rate was highest when the rotifer was cultured in *Scenedesmus* + chicken manure extract (SF Baldia, personal communication). The rotifer did not survive in filtered lakewater, green water, yeast, and chicken manure extract.

Studies on the Nile Tilapia *Oreochromis niloticus* and Red Tilapia

Genetics

Several strain testing procedures for Nile tilapia in small to medium-size experimental facilities were evaluated for their efficiency to detect economically important strain differences

(ZU Basiao, personal communication). Size grading or having a common starting size among genotypes detected true strain differences better than mixed-size grading. Initial size differences resulted in apparent growth depensation under experimental conditions but growth compensation in rice-fish farms.

Three tilapia strains (Israel, NIFI, CLSU) were evaluated for growth performance under restricted feeding (Romana-Eguia and Eguia 1993). Growth was retarded by restricted feeding in all three strains but it differed significantly among strains. The Israel strain was the best.

Two Nile tilapia strains (NOT, CLSU) were evaluated for their resistance to heavy metal exposure. In one study, the growth, survival, and reproductive performance of two Nile tilapia strains were not affected by a two-month exposure to a sublethal mixture of zinc, cadmium, and inorganic mercury (Cuvin-Aralar and Aralar 1993). In both strains, 88-99% of the metal burdens were eliminated after two months in metal-free water (Cuvin-Aralar 1994). Full-sib juveniles (one month old) from nine families of the NIFI and commercial Nile tilapia strains showed similar tolerance to inorganic mercury (ML Cuvin-Aralar, personal communication).

Two hybrid reference lines were developed from existing red tilapia strains to measure and reduce the effects of uncontrolled nongenetic (environmental) variables in experiments that evaluate Nile tilapia strains (MR Romana-Eguia, personal communication). Variation in the growth data of Nile tilapia strains was indeed lowered when either of the two red tilapia hybrid reference lines was used as a covariate.

The reproductive performance of four red tilapia strains held in cages or in tanks at a stocking density of 12 females and 4 males per cage or tank was also assessed. Cage- or tank-reared broodstocks of the NOT red tilapia had higher egg and larval production than the other strains (MR Romana-Eguia, personal communication). In all strains, seed production was generally lower among cage- than tank-reared broodstock.

Feed development

A tilapia diet has been developed by AQD (Table 4). This diet supplemented with 5% soybean oil as lipid source resulted in better reproductive performance of Nile tilapia broodstock than diets with corn oil, cod liver oil, or a cooking oil made from coconut oil (Table 7; Santiago and Reyes 1993). It is not necessary to add oil to tilapia diets, but if desired, soybean oil is the best among the vegetable oils tested. In another study, it was found that free essential amino acids in muscle did not consistently confirm amino acid requirements (Santiago and Lovell 1994).

Feeding management methods for Nile tilapia are being developed to minimize feed wastes. In one study, juvenile tilapia fed diets with 25% protein for 7 weeks had a higher weight gain than those given an 18% protein diet throughout, or alternate feeding of 18% and 25% protein diets (CB Santiago, personal communication).

Fish health

Skin lesions have recently been observed among Nile tilapia. In a brackishwater pond in Oriental Mindoro, incidence of skin lesions was highest (51%) in July when chloride, alkalinity, and water hardness were highest (FP Palisoc, personal communication). *Acinetobacter* was the

Table 7. Spawning and juvenile ('fry') production of Nile tilapia fed diets with supplemental oils for 24 weeks. Modified from Santiago and Reyes (1993).

Dietary treatment	Number fish that spawned	Spawning frequency per female[a]	Number of juveniles per spawning[b]	Total juveniles per spawner[c]
Control diet (no oil added)	2/5	1.8	526	1,078
+ Cod liver oil	1/5	0.4	18	18
+ Corn oil	3/5	1.6	646	1,586
+ Soybean oil	5/5	3.4	603	1,865
+ Coconut oil-based cooking oil	3/5	1.8	647	1,856
+ Cod liver oil and corn oil	3/5	1.2	594	1,593
Soybean meal diet	4/5	1.4	539	775

[a] Mean for all females
[b] Mean for spawnings with viable juveniles
[c] Mean for females that spawned

most dominant bacteria isolated from infected fish. Tilapias in Lake Sebu in South Cotabato also developed skin lesions in January to March. These skin lesions must be studied to develop appropriate health management methods.

Studies in Lake Ecology

Monitoring of the dissolved oxygen in Sampaloc Lake in Laguna indicated a progressive depletion of oxygen in the subsurface waters due mainly to the wasted feeds from intensive tilapia farming (Santiago and Arcilla 1993). Only the top one meter of the lake water has oxygen at 3 mg/l or greater and can presently support fish. The current levels of total ammonia (3 mg/l) and sulfide (5 mg/1) are already toxic. Fish kills in Sampaloc Lake have become frequent. To save the lake from imminent biological death, it is imperative to: (1) reduce the farming area from 30 to 6 hectares, (2) stop the use of commercial feeds, and (3) remove the water hyacinths and other debris.

Fish kills in Laguna de Bay have been recorded since 1932 but have become more serious in recent years. The SEAFDEC Aquaculture Department is presently conducting a review of the occurrences, losses, and possible causes of fish kills (AE Santiago, personal communication).

Ecological and microbiological studies on the epizootic ulcerative syndrome of snakeheads in Laguna de Bay indicate that low temperature is a predisposing environmental factor, but have yet to pinpoint the primary causative pathogen (Cruz-Lacierda and Torres 1994, FP Palisoc and GD Lio-Po, personal communication).

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