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BROODSTOCK MANAGEMENT AND SEED PRODUCTION OF MILKFISH

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ABSTRACT

Milkfish *(Chanos chanos* Forsskal) remains one of the cheapest sources of protein for developing countries in Southeast Asia, particularly in the Philippines. The unpredictable supply of wild fry, the only source of seed for the milkfish farmer, contributed largely to the slow growth of the milkfish industry. Research on the artificial propagation of this fish was, therefore, given emphasis.

Major research achievements in milkfish breeding of the SEAFDEC Aquaculture Department in the last decade include: (1) successful induced spawning of wild and captive breeders using gonadotropin preparations and gonadotropin-releasing hormone analogues (GnRHa); (2) spontaneous maturation and spawning of captive breeders; (3) completion of the life cycle of milkfish in captivity; (4) development of a simple egg-collecting method; and (5) development of techniques for mass production of milkfish fry.

Information on fry ecology and behavior, larval morphology and physiology were also gathered. These published data constitute the bulk of current knowledge on milkfish biology and natural history.

Milkfish breeding technology is currently being pilot-tested in several breeding sites of the Bureau of Fisheries and Aquatic Resources (BFAR). Spontaneous maturation and spawning of milkfish have been verified in four sites which differ in environmental characteristics. The economic feasibility of producing milkfish fry and the socio-economic impact of artificial propagation of milkfish are now being assessed.

INTRODUCTION

Of the finfish species cultured in Southeast Asia, milkfish contributes 56.2% to total fish production from aquaculture (Rabanal 1987, this Proceedings) thus, emphasizing its economic importance. In the

Philippines and Indonesia where milkfish has been cultured for centuries, fry collected from the wild is the only source of seed for the farmer. The relatively slow growth of the milkfish industry has been, in part, due to unpredictable and seasonal supply of fry. Research on the artificial propagation of milkfish was initiated at the SEAFDEC Aquaculture Department in 1976 to ensure an adequate and steady fry supply.

As part of an overall effort to fully understand the biology of milkfish and to document practices related to its culture, ecological studies to identify and characterize milkfish spawning grounds, fry grounds, and nursery grounds were undertaken. The results of these studies have been reviewed by Kumagai (1984) and Bagarinao and Kumagai (in press). Traditional methods used in fry capture, handling and storage have also been documented (Kumagai et al 1980, Villaluz 1984). This paper presents the achievements and results of studies on milkfish breeding and artificial propagation at the SEAFDEC Aquaculture Department.

BROODSTOCK DEVELOPMENT AND MANAGEMENT

The methods of rearing milkfish to maturity are described in Marte et al (1984). Pond-reared stock (BW = 250 g) are collected from brackishwater ponds and transported to the rearing site in canvas holding tanks filled with sea water and provided with aeration. Transport is by van and pumpboat and may take from 2-8 hr.

Broodstock rearing facilities used at SEAFDEC AQD are floating cages, concrete and canvas tanks. The floating net-cage, however, is preferred for reasons of economy and convenience. The fish are initially reared from 1-3 years in smaller net-cages (4-5 m diameter) or stocked directly in 9 or 10 m diameter cages. Stocking densities normally do not exceed 1.5 kg/m^3. Broodstock reared in small cages are transferred to large cages (6-10 m diameter) on the fourth year of rearing.

The floating net cage facilities at Igang Substation, Guimaras Island, are located about 100 m from shore in an area protected from the open sea by small islands. It has a sandy-muddy substratum and a water

depth of 7-10 m. Water transparency is about 5 m; annual salinity and temperature ranges are 26-34 ppt and 25-32°C, respectively. A limited number of broodstock used for various experiments are also reared in canvas or concrete tanks.

One- to three-year old fish are given commercial feed pellets containing 23-32% protein at 1.5%-2% body weight. On the fourth year of rearing or the year before the fish are expected to mature, commercial feed pellet containing 43% protein are fed at 3-5% body weight. The proximate composition of the commercial feed pellets is given in Table 1. The floating net cages are periodically checked for damage and fouling.

Table 1. Proximate composition of milkfish broodstock feed

	Fish Pellet[a]	Crustacean Feed[b] No. 2
Crude protein (%)	35.86	44.26
Crude fiber (%)	6.10	2.26
Crude fat (%)	5.12	4.62
Nitrogen-free Extract (%)	43.18	37.88
Moisture (%)	11.56	8.03
Ash (%)	9.76	10.97

[a]Fed to 1-3 year old milkfish at 1.5-2% body weight

[b]Fed to 4-year old and older milkfish at 3-5% body weight

Reproductive Biology of Captive Milkfish

Captive milkfish sexually mature at 3.5-5 years (Lacanilao and Marte 1980, Marte and Lacanilao 1986). Hatchery-bred fry reared from artificially fertilized eggs from a wild adult female caught in 1978, sexually matured and spawned in 1983 marking the first completion of the life cycle of milkfish in captivity (Marte et al 1983, Marte and Lacanilao 1986). Subsequently, several stocks of milkfish from wild-caught or hatchery-bred fry have matured and spawned at five years.

The results have been verified at four breeding sites of the National Bangus Breeding Program (NBBP) of BFAR located in different regions of the country. Milkfish also matured and spawned at five years in these sites under different environmental conditions.

Captive milkfish undergoes an annual reproductive cycle (Marte and Lacanilao 1986). Sexual maturation occurs during the natural breeding season of wild fish. Gonad development starts in February or March and peaks in April-June (Fig. 1). All fish sampled during these months were maturing or mature (Fig. 2a and 2b). Most fish sampled in July-September were spent. Only regressed and immature fish were obtained in October-December. Sexual maturation appears to coincide with rising seawater temperature and lengthening photoperiod.

Milkfish held in cages smaller than 6 m in diameter have never matured or spawned although fish from the same stock held at the same stocking density but in larger cages spawned (Marte personal observation).

Gonadosomatic index (GSI) of milkfish at different maturity stages is shown in Table 2. GSI for mature females is 1.24-8.12; for mature males, 0.32-3.95. Fecundity is estimated at 250 000-350 000/kg body weight.

The involvement of the pituitary gland in gonad development is well documented in many fish species. In milkfish, the changes in the pituitary during gonad development have been described (Tan 1985), providing a morphological basis to assess the effects of hormonal manipulation on gonad maturation ana spawning.

The number of natural spawnings from various broodstock observed from 1980-1987 has increased (Table 3). Spawnings of a hatchery-bred stock from 1985-1987 indicate that the month of peak spawning varies and may be influenced by the condition of the broodstock. In 1985, peak spawning from Cage 43 occurred in June and July while in 1986 the spawning peak was in September and October. Some fish in this cage were transferred to another cage in March 1986 when gonad development was in progress. The disturbance caused by the transfer may have induced gonadal regression of maturing fish resulting in delayed maturation of the stock. The number of spawnings from Cage 43 (Fig. 3) with 10-16 females from July-October 1986 (23) and June-August 1987 (30) also indicates that milkfish spawns at least twice during the breeding season. Data from fish sampled from various

cages since 1980 indicate that a sex ratio of 1 female to 1 male is the norm.

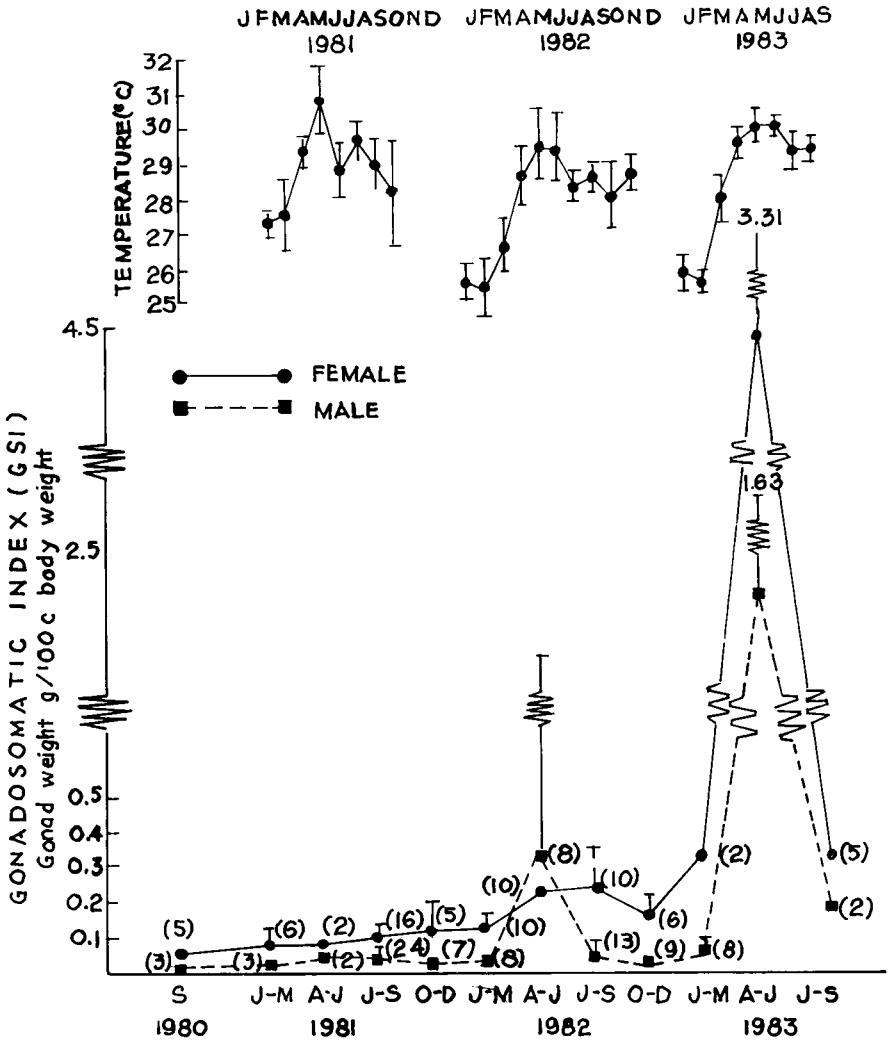

Fig. 1. Mean gonadosomatic index (GSI) of hatchery-bred milkfish sampled in 1980-1983. Points are means, vertical lines represent standard deviation, bracketed numbers denote sample size. Mean surface temperatures are shown on top of figure (From Marte and Lacanilao 1986)

Natural spawning of captive broodstock occurs around midnight although occasional spawnings have been observed at daytime. Wild milkfish was also reported to spawn at around midnight (Senta et al

Table 2. Fork length, body weight, gonad weight and gonadosomatic index (GSI) of Hatchery-bred milkfish at different maturity stages. Values are means ± standard error (From Marte and Lacanilao 1986)

Maturity Stage	N	Folk Length (cm)	Body Weight (kg)	Gonad Weight (g)	Gonadosomatic Index (%)
			FEMALE		
Immature	34	53.0 ± 0.47	2.6 ± 0.07	5.7 ± 0.58	0.21 ± 0.02
Maturing	3	54.8 ± 1.56	2.8 ± 0.10	20.8 ± 8.22	0.74 ± 0.29
Mature	7	55.8 ± 0.62	3.5 ± 0.10	193.6 ± 30.64	5.58 ± 0.94
Spent	5	56.3 ± 1.04	3.0 ± 0.10	9.7 ± 0.04	0.33 ± 0.01
			MALE		
Immature	27	51.2 ± 0.49	2.5 ± 0.09	0.9 ± 0.12	0.03 ± 0.004
Maturing	6	53.7 ± 0.78	2.7 ± 0.11	12.9 ± 5.31	0.51 ± 0.23
Mature	6	56.8 ± 2.09	3.3 ± 0.20	105.9 ± 12.95	3.23 ± 0.32
Spent	7	55.4 ± 0.86	2.9 ± 0.10	4.2 ± 0.77	0.14 ± 0.02

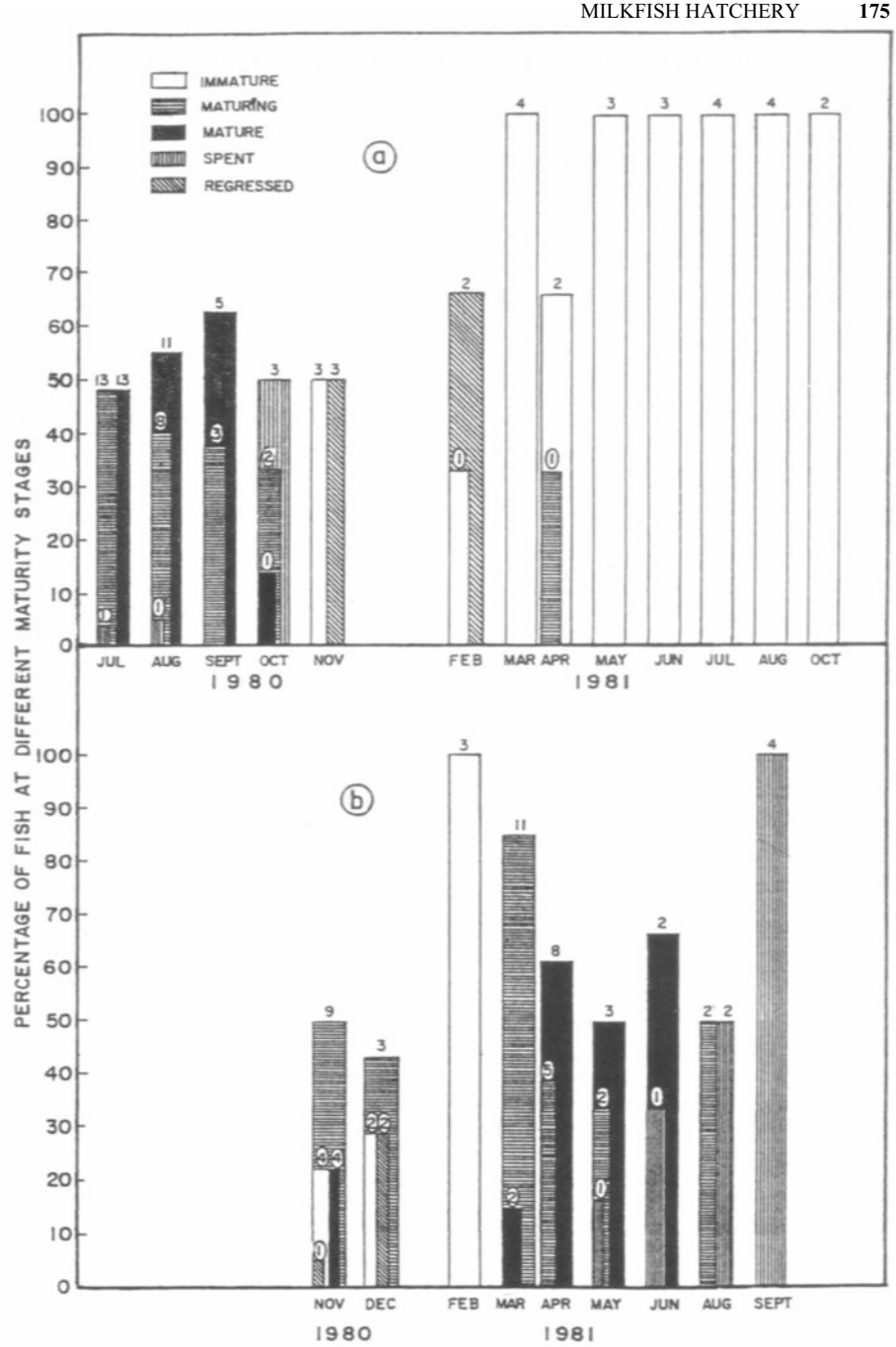

Fig. 2. Milkfish broodstock from wild-caught fry at different maturity stages: a. fish from Cage 5 sampled July 1980-October 1981; b. fish from Cage 6 sampled November 1980-September 1981. *'Maturing or mature gonads in atresia. Numbers denote sample size (From Marte and Lacanilao 1986)

Table 3. Natural spawnings of milkfish 1980-1987 in floating net cages

Year	No. of Spawnings	No. of Cages	No. of Eggs Collected	Range (In Thousands)
1980	2	1	1 400	0. 50- 0.90
1981	8	1	15 765	0.342- 6.293
1983	14	1	315 421	0.770- 114.00
1985	41	4	6 317 132	1. 50- 143.00
1986	64	6	29 196 500	1. 20-2 226.00
1987 (May to Aug.)	61	4	49 279 801	21. 00-2 928.00

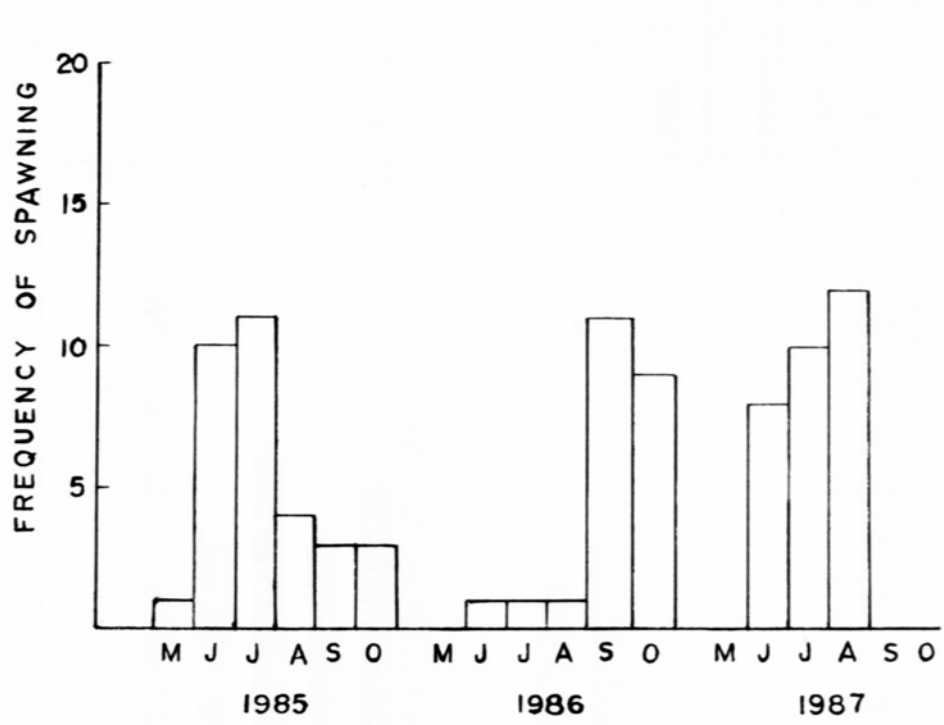

Fig. 3. Monthly spawning of 1980 hatchery-bred milkfish from Cage 43

1980). This observation was based on the developmental stage of milkfish eggs obtained by plankton tows in ecological studies done to identify and characterize milkfish spawning grounds. From the number of eggs collected from the wild and the frequency of fry occurrence, spawnings of wild milkfish were reported to be influenced by lunar phase (Kumagai 1984). The observed spawnings of captive milkfish

from floating cages, however, do not indicate a similar lunar periodicity (Table 4).

Table 4. Moon phase and frequency of spawning of milkfish reared in floating cages

	New Moon	First Quarter	Full Moon	Last Quarter
1985	6	11	15	9
1986	6	14	14	17
1987	19	13	12	17
	31	38	41	43

Egg Collection

Although milkfish spawns spontaneously in net-cages, collection of spawned eggs was a technical problem. Various methods of collecting eggs were tried from 1980-1983 with only limited improvement in the number of eggs collected (Table 5, Marte et al 1987b). Eggs were lost through the cage bottom and from predation by other fishes that had easy access into the cage. Recently, a nylon net-cage of 1 mm mesh (hapa) installed inside the broodstock cage partially solved the problem (Marte in press). The number of eggs collected by manual seining in the

Table 5. Egg collector type, spawning frequency, and egg collection (1980-1985) (From Marte et al 1987b)

Egg Collector Type	Year	Cage No.	Number of Spawnings	Number of Eggs Collected
Type A stationary plankton net	1980	C-5	2	500 - 900
Type A stationary plankton net	1981	C-6	8	342 - 6 293
Type B Stationary plankton net	1983	C-2	14	770 - 114 000
Type C stationary plankton net	1985	C-5	1	1 050
Seine net	1985	C43	35	1 500-769 000
Egg sweeper	1985	C-42	5	4 280 - 212 000

hapa enclosed cage increased more than tenfold (Table 6). Manual seining, however, is laborious and requires at least two persons to do the collection at dawn. Recently, a manually operated "egg sweeper" has considerably facilitated egg collection (Garcia et al 1988). The total number of eggs collected from May-August 1987 in four cages holding about 120 fish was 45 115 000. This is a marked improvement over the number of eggs collected in 1986 from 5 cages during the same period (Table 7).

Egg Transport

Milkfish eggs are transported from the spawning site at Igang Substation to the hatchery at Tigbauan Research Station. Duration of transport is from 2-5 hr. Estimate of hatching rates of eggs after transport was consistently lower compared to hatching rates of eggs from the same batch retained at Igang. In addition, a high percentage of abnormal larvae was observed from several batches of eggs indicating that conditions during transport may have adversely affected early embryonic development. Eggs are transported at gastrula to neurula stages. Preliminary results of simulated egg transport experiments indicated that eggs may be transported: (1) at densities of 7 000/1, (2) at salinities of 20-32 ppt, and (3) at ambient temperature of 28°C (Garcia and Toledo in press). Lowering the temperature of the transport water resulted in significantly higher egg mortality and decreased hatching rate. Eggs are routinely transported in oxygenated plastic bags inside *pandan* bags at densities of 100 000 eggs/15 1 of sea water.

Induced Spawning

Initial efforts to breed milkfish focused on the development of techniques to spawn wild adults or sabalo. Methods of capturing and techniques of handling wild adults have been developed (Vanstone et al 1976). A method for sexing wild spawners based on the external appearance of the urogenital apertures has also been reported (Chaudhuri et al 1976). To confirm the sex of breeders, gamete samples are obtained by inserting a polyethylene cannula (Clay-Adams PE 100) through the abdominal or urogenital pore. Yolky eggs obtained in this manner are measured to determine readiness of the female for induced spawning. Salmon pituitary homogenate (SPH) alone or in combination with human chorionic gonadotropin (hCG) were used for spawning wild adults or captive breeders (Vanstone et al 1977, Liao et al

Table 6. Number of eggs collected from two milkfish broodstock cages with and without "hapa" net cage (From Marte in press)

	Without "Hapa"			With "Hapa"		
	No. of Spawns	Range of Eggs Collected	Total Eggs Collected	No. of Spawns	Range of Eggs Collected	Total Eggs Collected
Cage 42	17	3 340 - 668 000	2 141 000	14	330 000 - 2 040 000	10 902 000
Cage 43	9	6 400 - 200 000	329 500	14	292 000 - 2 942 000	15 355 000
TOTAL	26		2 470 500	28		26 237 000

Table 7. Monthly spawning from different milkfish broodstock cages from 11 May to 24 October 1986 (From Marte in press)

Cage Number	34*	38*	41	42	43	44*	
Number of Fish	23	31	60	39	35	30	
Age of Fish (Yrs)	8	10	5	6	6	6	
Month							No. of Eggs Collected
May	2	3		4			1 484 400
June		2		10	1	1	1 135 200
July				2	1		31 500
August				2	1		810 000
September			1	8	11		11 976 800
October			1	5	9		13 758 500
TOTAL	2	5	2	31	23	1	29 196 500

*Fish in these cages were used for induced spawning and rematuration experiments in May-September 1986.

1979 and Juario et al 1984). The results of these early experiments are summarized in Table 8. The hormone solution was injected intramuscularly (IM) from two to four times at intervals of 6-24 hr. Ovulation occurred 6-15 hr after the last hormone injection. The ovulated eggs were stripped and artificially fertilized by the "wet" or "dry" method from milt obtained from 1 to 3 males. Mature males were induced to spermiate with injections of SPH or testosterone. In these experiments, females with oocyte diameter of 0.66 mm or greater were found to respond to the hormone injections. Often, wild adult females and males were not caught together. To partially solve this problem, experiments to prolong spermiation in males by administration of long-acting hormone preparations were done. Durandron forte (Organon), a long-acting testosterone preparation, was found to prolong spermiation for as long as seven days (Juario et al 1980). Experiments to cryopreserve sperm were also carried out. Milkfish serum was reported to be a good extender for cryopreserving milkfish sperm (Hara et al 1982). Fertilization rate of ovulated eggs from a hormone-treated wild female was comparable with eggs fertilized with fresh sperm.

Difficulty in obtaining wild adults and the few available captive broodstock hampered efforts to define an effective spawning regime using fish gonadotropins or hCG. Wild fish were always in a badly damaged condition after capture and handling, and this greatly affected response to the induced spawning treatment.

With the availability of captive broodstock, recent methods for inducing ovulation and spawning fish using synthetic analogues of luteinizing hormone releasing hormone (LHRH-a) and salmon gonadotropin releasing hormone (sGnRH-a) were tried. A single injection, pellet implantation, or osmotic pump implant of LHRH-a and sGnRH-a were effective in inducing ovulation and spawning in maturing milkfish (Fig. 4, Marte et al 1987a). A single injection of 1000 IU/kg hCG was also as effective as the LHRH-a treatments (Marte et al in press). Spontaneous spawning occurred from 16-32 hr after treatment with the analogues of LHRH. Fertilization rate ranged from 20-88%.

Induction of Gonad Development

Early attempts to induce gonad development in fish younger than 5 years old and in refractory fish were unsuccessful. Chronic administration of gonadotropins in cholesterol pellets (SPH, SG-G100 or hCG)

Table 8. Successful spawning attempts of wild adult and captive milkfish

BW (kg)	Initial oocyte diameter (mm)	Total Dose SPH (mg) + HCG (IU)	Weight-Specific Dose	Fertilization (%)	Source
?	?	60 + 4000			Vanstone 1977
		90+6000		ND*	
?	?	60 + 4000			
		90 + 6000			
		90 + 6000		ND	
6.5	0.75	42 + 2600	6.5+ 430.8	38	Liao et al 1979
		42 + 4200	6.5+ 646.2		
7.0	0.77	70+ 10000	10+1428.6	59.0	Juario et al 1984
		70+ 10000	10+1428.6	36	
7.0	0.66	70+10000	10+1428.6		
10.0	0.74	100+ 10000	10+1000	25	
		100+20000	10+2000	28	
4.0	0.76	40+5000	10+ 1250	9	
		40+10000	10+2500	32	
				10	

*ND — not determined

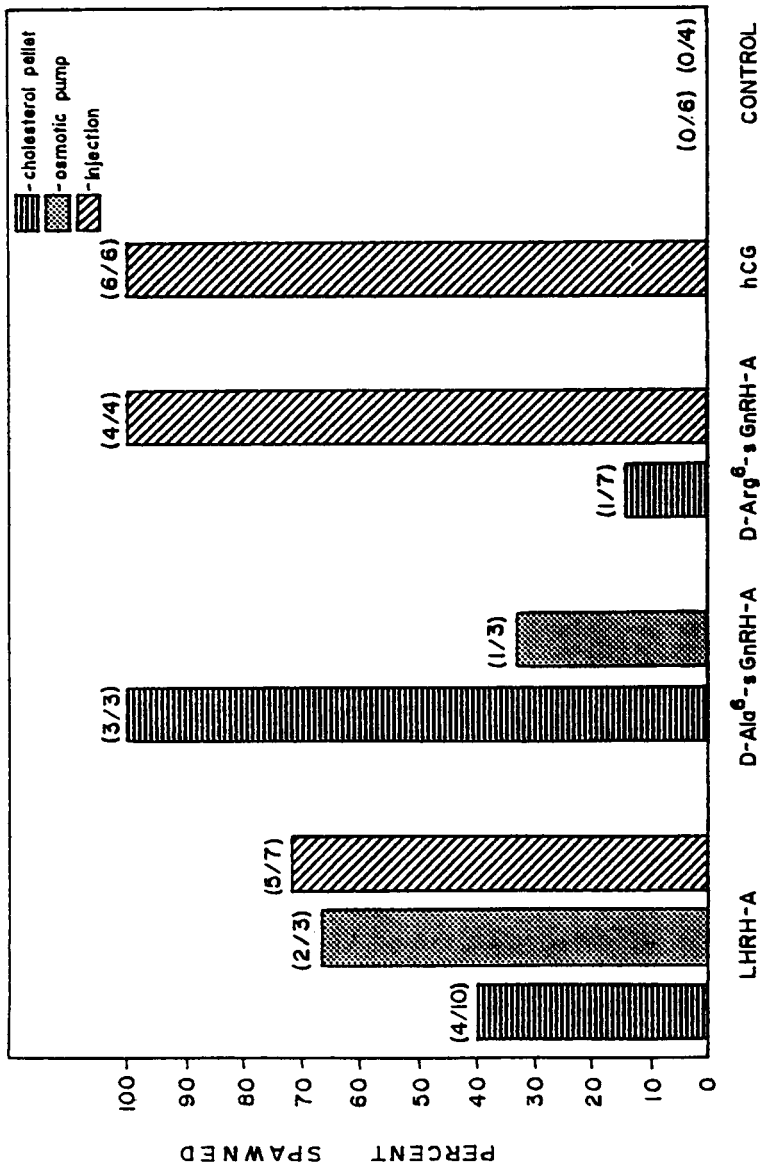

Fig. 4. Percentage of maturing milkfish that spawned following a single treatment of gonadotropin releasing hormone analogues (GnRH-A) and human chorionic gonadotropin (hCG). Hormone dosages were: Chlolesterol pellet-100 µg/pellet; LHRH-A and sGnRH-A Osmotic pump; LHRH-A and sGnRH-A injection-10 µg/kg; hCG injection-1000 IU/kg (From Marte et al 1987)

alone or in combination with estradiol or oral administration of thyroxine failed to induce gonad development in 3-5 years old fish or wild-regressed adults (Lacanilao et al 1985). Factors which may have contributed to the negative response include stress, unfavorable holding conditions, and ineffective hormone preparations. The expected slow and sustained release from the gonadotropin-cholesterol pellet was not obtained even at the highest dose given. Gonadotropin levels were elevated in hormone-implanted fish one day after treatment but dropped to control levels after 2 or 3 days (Marte and Crim 1983).

Recently, maturation of 4-year old fish was enhanced with chronic administration of testosterone and LHRH-a (Marte et al in press). Testosterone (1 mg) and LHRH-a (100 µg) incorporated in cholesterol pellets were implanted intramuscularly to 4-year old fish held in tanks. More mature 4-year old males were obtained in the hormone-implanted groups than in the controls and the two hormone-implanted females that matured were induced to spawn with LHRH-a. The females (BW= 1.8 and 2.5 kg) were about half the size of captive spawners. This is the first report of maturation in fish younger than 5 years. A single intraperitoneal implant of 1 mg testosterone together with monthly implants of 100 µg LHRH-a pellet also induced rematuration from 2-4 times in three regressed females.

Diseases of Broodstock

Good water exchange in the floating cage site and low stocking densities have ensured healthy rearing conditions for milkfish broodstock. A few cases of localized infections at hormone implantation sites in fish repeatedly implanted with hormones have been reported (Lio-Po et al 1986).The causative organism was tentatively identified as *Vibrio parahaemolyticus,* a normal bacterial flora of seawater. The same organism was associated with the occurrence of opaque eyes in transport-stressed milkfish and in some fish cultured in earthen ponds (Muroga et al 1984). Milkfish broodstock often develop opaque eyes after capture from the net cage during hormone induction experiments or whenever they are transferred or disturbed. The histological changes found in the cornea ("adipose membrane"), iris, retina, and lens in fish with the opaque eye syndrome were described (Tamse et al 1983).

Broodstock held in canvas or concrete tanks have occasionally been infected by the parasitic copepod *Caligus.* The recommended treatment is a gradual change from sea water to fresh water (Lio-Po,

pers. comm.). *Caligus* can also be controlled by a dilute solution of 90 ppm formalin (Lio-Po pers. comm.) or 0.25 ppm Neguvon (Laviña 1978).

SEED PRODUCTION

The fish hatchery facilities at SEAFDEC AQD include 250-1 to 1.5- ton fiberglass tanks, 2.5-5-ton canvas tanks and 3.0-12.0-ton concrete tanks. These are used for rearing larvae and for production of natural food such as *Chlorella* and *Brachionus.* The layout of a pilot hatchery for the NBBP now currently used for verification trials is shown in Fig. 5.

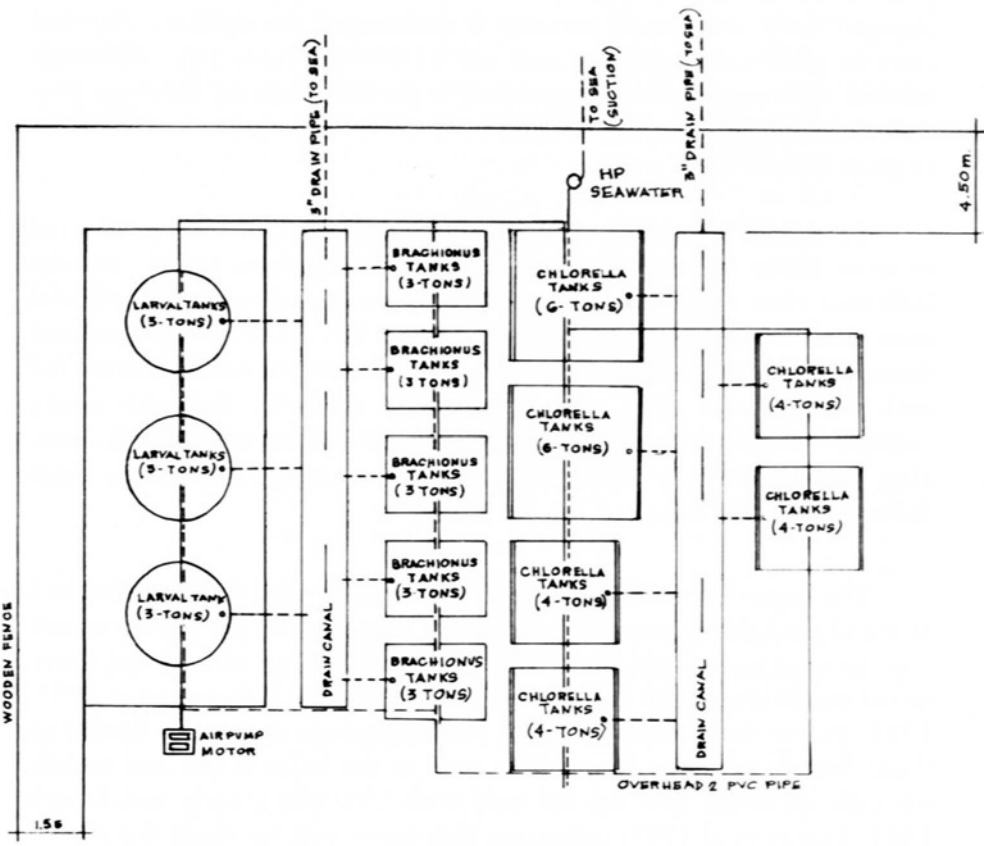

Fig. 5. Lay-out of the National Bangus Breeding Project Pilot hatchery at SEAFDEC, Aquaculture Department, Tigbauan, Iloilo

Food and Feeding

Larval rearing procedures for milkfish were described by Juario and Duray (1983). Several feeding schemes have been tried with success (Table 9). Survival rates of up to 71% in larvae fed with a combination of the algae *Chlorella virginica, Isochrysis galbana,* and *Tetraselmis chuii* together with the zooplankton *Brachionus plicatilis* (BP) and *Artemia salina* (AS) were reported (Juario et al 1984). Stocking densities used, however, were relatively low. The feeding scheme currently adapted is shown Fig. 6. This involved feeding larvae with *Chlorella*-fed *Brachionus* at 15 individuals/ml from day 2 to day 15 and at 5 individuals/ml from day 16 to day 21. Green water or *Chlorella* is maintained in the larval rearing tank to condition the water and maintain *Brachionus.* Brine shrimp nauplii is introduced only at day 15. Water is changed daily and gentle aeration is maintained throughout. Survival rates in pilot-scale hatchery runs are 11-90.6% (Table 10). Although survival rates are relatively lower than in previous reports, hatchery production using this feeding scheme is simpler and requires less facilities to grow natural food.

To reduce dependence on live feed, experiments were conducted to wean larvae to artificial diets (Duray and Bagarinao 1984). Results indicated that 14-day old larvae may be weaned abruptly to artificial diets such as the artificial plankton AS and BP, SEAFDEC-formulated diets, commercial feed, and moist egg diets. Survival rates of larvae fed with the different diets were comparable to larvae fed with newly hatched *Artemia* nauplii and was higher in BP-fed larvae. Growth, however, was significantly higher for *Artemia*-fed larvae compared to those fed with artificial diets.

The hepatocyte ultrastructure of milkfish fry fed different live and artificial feed differ markedly indicating that hepatocyte ultrastructure may be used to evaluate the quality and acceptability of feed and nutritional condition of the fry (Storch and Juario 1983; Storch et al 1983, 1984; Juario and Storch 1984). Histopathological changes similar to those found in starved larvae were seen in the hepatocytes and intestinal cells of larvae and fry fed only with *Chlorella* (Juario and Storch 1984, Segner et al 1987) indicating that larvae and fry could not directly utilize *Chlorella.* Milkfish, however, could utilize *Tetraselmis* and *Isochrysis* but these algae when fed alone were nutritionally inadequate to support growth (Juario and Storch 1984).

Table 9. Larval survival of milkfish under varying conditions of density and using different live food organisms, 1978-1980 (after Juario et al 1984)

Year	Live Food[a] Used	Tank Vol. (l)	Mean Stocking Density Larvae/l	Mean Survival Rate (%)	No. of Tanks
1978	C+O+R+BS	600	20	18.0 (7-30)	6
1979	C+R+BS	600	27.7	7 (3-17)	6
1980	C+Iso+T +R+BS	600	22.3	43 (8-71)	6

[a]C: *Chlorella virginica,* 2-5 × 10^5 cells/ml
Iso: *Isochrysis galbana,* 2-5 × 10 cells/ml
T: *Tetraselmis chuii,* 2-5 × 10^4 cells/ml
O: Oyster trocophore, 30-300 ind/ml (Dl-6)
R: *Brachionus plicatilis,* 20-30 ind/ml (Dl-10), 10-20 ind/ml (Dll-21)
BS: *Artemia salina* nauplii

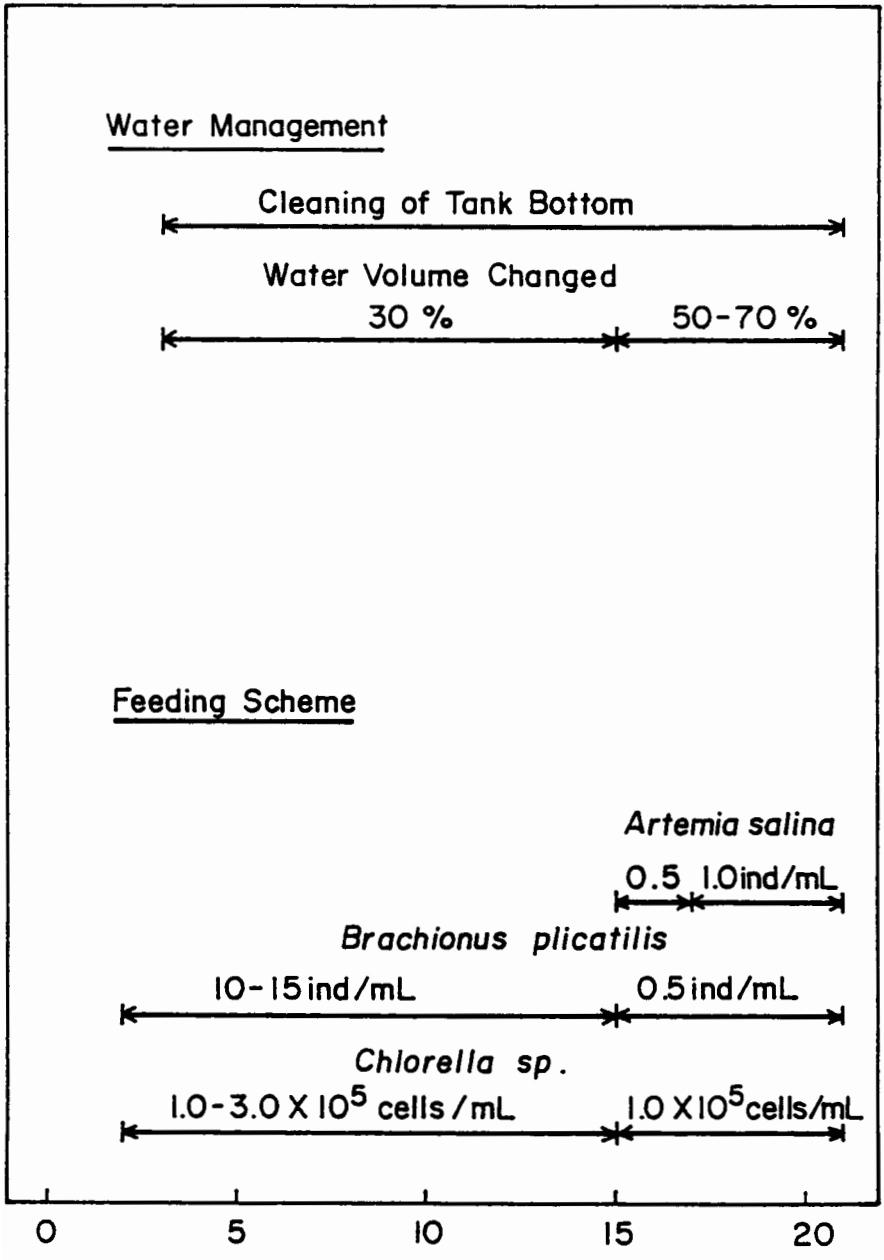

Fig. 6. Feeding and water management scheme followed in the larval rearing of milkfish (Juario and Duray, personal communication)

Table 10. Larval survival of milkfish, pilot-scale production runs, 1987

Live Food	Tank Vol. (l)	Stocking Density ((Larvae/1)	Survival Rate (%)	No. of Runs
C+ R + BS	2 500[b]	30	16 - 90.6	9
	3 000	30	11 -39	5
	5 000	30	12-30	5

[a]C: *Chlorella,* 1-3 × 10^5 cells/ml (Dl - D15), 0.5 × 10^5 cells/ml (D16 -D20)

R: *Brachionus,* 15 ind/ml (Dl - D15), 5 ind/ml (D16 - D21)

BS: *Artemia* nauplii, 0.5 - 1.0 ind/ml (D15 - D21)

[b]NBBP pilot-hatchery

Histochemical and ultrastructural observations on fry fed with different artificial diets support previous observations on survival and nutritional effects of these diets. Degenerative changes in the hepatocytes were seen in fry fed with egg yolk and rice bran, indicating that these feeds are nutritionally inferior (Segner and Juario 1986). Egg yolk is commonly used as feed for fry by milkfish concessionaires while rice bran is given as supplemental feed in nursery ponds. From growth and hepatocyte ultrastructure, mixed diet consisting of a SEAFDEC artificial diet (43.2% crude protein, 9.4% crude fat, 32.8% carbohydrate, 7.1% ash and 7.5% water) fed together with *Artemia* gave the best results (Storch et al 1984). The fry, however, can be successfully reared on the artificial diets.

Larval Development

Artificially fertilized or naturally spawned eggs hatch 25-32 hr after fertilization at a water temperature between 26.4 and 29.9°C (Liao et al 1979, Chaudhuri et al 1978). The embryonic and post larval development of milkfish was described by Chaudhuri et al (1978) and Liao et al (1979).

In unfed larvae, yolk is completely resorbed at 138.8 hr, time after hatching (TAH), while first feeding occurs as early as 77.0 hr TAH. All larvae start to feed by 125.0 hr TAH (Bagarinao 1986).

Five phases of larval development are recognized based on early morphological changes and transition from endogenous to exogenous feeding (Kohno et al in press). These are:

1. rapid early growth due to rapid resorption of yolk (hatching at about 10 hr TAH),

2. rapid growth with a relatively slow slope and organogenesis based on utilization of yolk (to about 75 hr TAH),

3. slow growth and organogenesis owing to yolk (to about 75 hr TAH),

4. slow growth based on yolk and exogenous food (to about 120 hr TAH), and

5. accelerated growth from exogenous food only (beyond 120 hr TAH).

Development of fin supports and branchial system were described and correlated with mode of swimming and feeding in larvae and fry (Taki et al 1986, 1987). Milkfish larvae acquire efficient swimming ability at around 10.5 mm standard length (SL) when major components of the caudal skeleton develop and ossify. This supports the view of active shoreward migration of milkfish larvae in the wild and corroborates earlier drift card experiments which indicated that surface currents are not the major factor in the shore-ward transport of milkfish larvae (Kumagai and Bagarinao 1979).

Fry gathering is an important industry in many coastal villages. These are caught with a variety of catching gears (Kumagai et al 1980, Villaluz 1984). Behavioral studies of milkfish larvae were conducted to explain phenomena of fry distribution, recruitment, and mass appearance near the shore of fairly uniform-sized larvae (10-17 mm). Milkfish larvae initially exhibits strong rheotactic but weak optomotor response. Positive optomotor reaction becomes pronounced as larvae transforms into the juvenile phase ("metamorphic stage" .) The well-developed optomotor response and the histological structure of the larval eye support the view that milkfish larvae feed primarily by vision (Kawamura and Hara 1980). Abrupt changes in optical behavior during the "metamorphic stage" is also thought to be related to the abrupt disappearance of larvae from the shore.

Diseases and Parasites

Incidences of larval mass mortalities have been frequently observed. Low hatching rates and mortalities during the early rearing stage seem to be related to unfavorable conditions during egg transport. Some mortalities are due to the presence of gas bubbles in the abdominal cavity or within the digestive tract (Lio-Po et al 1983). Gas bubble disease appears to be associated with high level of oxygen in the rearing tanks during periods of plankton blooms. These observations emphasize the need to improve methods of transporting eggs including water and tank management procedures during larval rearing.

Milkfish Seed Production Constraints

With proper management and adequate feeding, milkfish matures and spawns at five years. Care for the broodstock entails huge investments which can discourage most fish farmers from rearing milkfish for hatchery production of fry. Various means to reduce the cost of rearing broodstock are possible but still have to be tried. Recent results in the hatchery indicate that low survival is, in part, due to inadequate nutrition of broodstock. The nutritional requirement of milkfish broodstock is not known. This needs to be investigated to obtain the information needed to formulate a practical diet which can provide the nutrients for better-quality eggs and fry.

The hatchery technique developed for rearing milkfish larvae is adequate for small quantities of eggs. Mass production techniques to accommodate the large number of eggs spawned by a few females still need to be tested. Improvements in hatchery rearing such as increasing stocking densities of larvae and adopting more efficient water management procedures are being investigated.

Artemia is still an important component of larval diet. Artificial diets, however, are being developed and tested to replace or at least reduce the dependence on this costly imported larval food.

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