

ARTIFICIAL PROPAGATION OF MILKFISH: PRESENT STATUS AND PROBLEMS

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Milkfish has been extensively cultured in Indonesia, Taiwan, and the Philippines. At present, the only source of fry for fish farmers is the coastal waters during the spawning season. The supply of fry is therefore often irregular and inadequate. Since the early 1970s attempts have been made to breed milkfish in captivity, particularly in Hawaii, Taiwan, and the Philippines. This paper reviews the progress problems and suggested future research direction for the following areas: induction of ovulation/spawning, sperm preservation, larval rearing, and induction of gonadal maturation.

INTRODUCTION

The extensive culture of milkfish *Chanos chanos* (Forsskal) in the Philippines, Indonesia, and Taiwan has made heavy demands on the supply of fry. At present, milkfish fry come mainly from the wild, and there are already signs of a shortage, particularly in Taiwan and in connection with pen culture in Laguna de Bay, Philippines. There is therefore a need for artificial propagation of milkfish to supplement the fry supply, and research toward this end has been carried out since the early 1970s, particularly in the Philippines, Hawaii, and Taiwan. This paper reviews the progress made and the problems encountered in the following areas: (1) induction of ovulation/spawning, (2) sperm cryopreservation, (3) larval rearing, and (4) induction of gonadal maturation. Other reviews covering one or more of these aspects include Juario et al (1984), Juario and Duray (1983), Kuo (1982), and Liao and Chen (1984).

INDUCTION OF OVULATION/SPAWNING

The available information on successful attempts to induce ovulation in milkfish is summarized in Table 1. The procedures used by the various investigators were variable and lacked standardization. The hormones used were salmon (SPH) or carp (CPH) pituitary homogenate in combination with human chorionic gonadotropin (HCG), or HCG alone. The dosages and other protocols used were also variable, as shown below:

Hormones:	
(a) SPH	6-10 mg/kg
(b) CPH	5-25 mg/kg
(c) HCG	180-2500 IU/kg
Number of injections	1-5 (mostly 2)
Injection interval	6-24 h (mostly 8-12 h)
Time to stripping	6-17 h (12 h appears best)

Part of this variability may have been due to differences in the initial gonadal condition of the fish. It appears that, as the initial egg diameter (eggs sampled by intraovarian cannulation) approaches 0.66 mm, higher dosages and more injections are required; conversely, as the initial egg diameter approaches 0.89 mm, lower dosages and fewer injections are required. However, the procedures have not been standardized and the dose-response relationship has not been studied. Hitherto it has not been possible to obtain enough spawners to conduct such studies, but with the availability of captive broodstock, the situation may change, and studies toward standardization should be carried out.

Partially purified salmon gonadotropin (SG-G100) has also been used (Nash and Kuo 1976, Liao and Chang 1976, Vanstone et al 1976), but the results obtained appear to have been less successful.

The use of HCG alone, shown to be successful in inducing ovulation/spawning in captive milkfish in Taiwan (Liao and Chen 1984), should be further studied. The application of HCG alone may be limited to fish with oocyte diameters of around 0.8 mm or higher (C. M. Kuo, pers. comm.). Fish with oocyte diameters from 0.66 mm to 0.8 mm may still require salmon or carp pituitary homogenate as priming injections. It is also possible that captive fish are more responsive to hormonal treatment than wild fish. HCG may not work as well in wild fish; higher doses and/or more injections may be required.

At the SEAFDEC Aquaculture Department (Philippines), the same dosages of hormones (SPH + HCG) were used to induce ovulation in both wild and captive fish (Table 1). It may be possible to use lower dosages for captive fish.

The ovulatory response of milkfish to hormones appears to be affected by environmental factors. For example, Kuo et al (1979) found that an abrupt change in salinity adversely affected the ovulatory response of milkfish to hormones, more so than gradual salinity adjustment; no salinity change was the best. The salinity level itself may also affect the dose-response relationship. In normal seawater, milkfish appear to require a lower dosage of hormones than at lower salinities (Table 1). Fish reared in

brackish water may require an initial oocyte diameter of 0.72 mm (0.66 mm in seawater) before they can be successfully induced to spawn (C.M. Kuo, pers. comm.).

In determining the time of injection of hormones, the possibility of a circadian rhythm of responsiveness of oocytes to hormones should be considered. In the grey mullet *Mugil cephalus*, the oocytes are more sensitive to hormones at 0900 h and 1800 h than at other times (Kuo and Watanabe 1978). Whether such a circadian rhythm of oocyte responsiveness exists in milkfish should be investigated. Another factor to be considered in this connection is that natural spawning occurs at around midnight (C. Marte, pers. comm.). Injections may be timed such that stripping can be done at midnight; this might give better results.

There are certain behavioral markers which may help to determine both whether the injection given is effective and also the time of stripping. These include the following:

- Color change, due to melanophore stimulating hormone (MSH) in the pituitary homogenate;
- Increased drinking activity, probably to facilitate oocyte hydration;
- Release of calcium deposits, probably with increased drinking; calcium is retained in the gut and then released;
- Distension of abdomen, indicating oocyte hydration; and
- Dribbling of some eggs, indicating that ovulation may be close, consequently a good reference point to determine the time of stripping.

Natural spawning following hormone injections has so far been achieved only in Taiwan using pond-reared milkfish (Liao and Chen 1984). Natural spawning may give better hatchability and survival of the hatched larvae than stripping and artificial fertilization. However, nothing is known of the spawning requirements and behavior of milkfish, and these need to be studied. In the Taiwan experience, a sex ratio of two males to one female was used, but this may not be the ideal ratio.

Spontaneous spawning without hormone treatment has also been achieved with captive broodstock maintained in floating net-cages in the Philippines (Lacanilao and Marte 1980, Marte et al 1984). Again, the environmental triggers have not been identified. Temperature may be important (Wainwright 1982, Marte et al 1984, Lam 1983). It appears that 24°C is the minimum temperature required for milkfish spawning (Wainwright 1982).

PROBLEMS IN INDUCTION OF OVULATION/SPAWNING

When the initial oocyte diameter is less than 0.66 mm (0.72 mm in brackishwater milkfish), it has not been possible to induce ovulation in the fish (Table 2). Many hormone injections are required, and the fish usually dies before any significant advancement of oocyte development is achieved. Histologically, oocytes measuring 0.66 mm are already in the tertiary yolk globule stage (Juario et al 1984), but the maximum diameter of this stage is 0.89 mm. Perhaps at 0.66 mm vitellogenesis has really not been completed. Priming doses of hormones are therefore necessary before induction of spawning can be achieved. The priming hormones for vitellogenesis may include estradiol-17b, pituitary homogenate, and thyroxine (Lam 1982). This

Table 1. Induction of ovulation/spawning and artificial fertilization in milkfish *Chanos chanos* (Forsskal).

Fish	Holding conditions	Body weight (kg)	Initial oocyte diameter (mm)	Injection (IM ^a)		
				Time	Total dose ^b (mg SPH/CPH* + IU HCG)	Specific dose ^b (mg SPH/CPH* + IU HCG per kg body weight)
Wild, injected within 4h of capture	6-m diameter canvas tank, seawater 31 ppt, 28.3-29.0°C, 1 m deep Salinity decreased to 27.6ppt 0630-0800h for 2nd female	?	?	2100	60+4000	?
				0530	90+6000	?
		?	?	1300	60+4000	?
				2215	90+6000	?
				0630	90+6000	?
Wild, injected immediately upon arrival in lab	?	6.5 (7.0 ^c)	0.75	0635	42+2800 +0.5 ml vit. B	6.5+430.8+0.08 ml vit. B
				1840	42+4200 +0.5 ml vit. B	6.5+646.2+0.08 ml vit. B
Captive or wild, injected within 26 h of first handling and sampling	35 ppt (no change)	4.1	0.73	0900 or 1800	100* + 10000	24.4* + 2439.0
				1800 or 0900 ?	as 1st dose	as 1st dose
	35 ppt (no change)	6.5	0.74	0900 or 1800 7	100* + 1000	15.4* + 1533.5
				1800 or 0900 7	100*+ 1500	15.4* + 2307.7
				0900 or 1800 ?	25* + 2500	5.6* + 555.6
38ppt (no change)	4.5	0.81	1800 or 0900 7	as 1st dose	as 1st dose	

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Time interval (h:min)	Diameter hydrated/ ovulated oocytes (mm)	Time to stripping/ spawning (h: min)	Fertilization	Males used	Reference
8:30	1.1-1.23 (fertilized)	15:30	dry and wet	2, similarly injected as female	Vanstone et al 1977
9:15 8:15	1.1-1.23 (fertilized)	8:30 (abdominal cavity exposed, loose eggs removed in both cases)	dry	3, spawned, untreated	
12:05	1.13-1.19	10:50-11:50	dry, 38%	1, ripe untreated	Liao et al 1979
9:00 ?	?	24:00'	?	?	Kuo et al 1979
9:00 ?	?	15:00'	?	?	
9:00 ?	?	23:00'		none available	

Table 1. continued

Fish	Holding conditions	Body weight (kg)	Initial oocyte diameter (mm)	Time	Injection (IM')	
					Total dose' (mg SPH/CPH* + IU HCG)	Specific dose' (mg SPH/CPH* + IU HCG per kg body weight)
	35 ppt (2 ppt increase per h from 7 ppt)	3.5	0.67	0900	25* +	7.1* +
or				2500	714.4	
?						
1800				as 1st	as 1st	
or				dose	dose	
0900						
?						
0900				25* +	7.1* +	
or	20000	45714.3				
1800						
?						
1800	as 3rd	as 3rd				
or	dose	dose				
0900						
?						
0900	as 3rd	as 3rd				
or	dose	dose				
1800						
?						
Wild, injected	34 ppt, 26-30°C	7.0 ^e	0.67 ±0.06 (n=68)	70+1250	0933	10+178.6
2-4 h after release into holding tank				70+10000	1745	10+1428.6
				as 2nd dose	0245	as 2nd dose
				as 2nd dose	0900	as 2nd dose
		7.0 ^e	0.77 ±0.04 (n=113)	70+10000	0830	10+1428.6
				70+10000	1800	10+1428.6

Time interval (h:min)	Diameter hydrated/ovulated oocytes (mm)	Time to stripping/spawning (h: min)	Fertilization	Males used	Reference
9:00 ?					
9:00 ?	?	73:00			
9:00 ?					
9:00 ?					
8:12	1.08±0.05	6:10	wet, 0%	2, injected with 5000 IU HCG and placed with female after her 2nd injection; insufficient milt	Juario et al 1979, 1984
9:00					
6:15					
9:30		10:30	dry, 59.4%	3, newly caught, ripe; placed with female after 1st injection; injected with 5000 IU HCG when female received 2nd injection	

Table 1. continued

Fish	Holding conditions	Body weight (kg)	Initial oocyte diameter (mm)	Time	Injection (IM ^a)	
					Total dose ^b (mg SPH/CPH* + IU HCG)	Specific dose ^c (mg SPH/CPH* + IU HCG per kg body weight)
		10.0d	0.74	?	100+10000	10+1000
				?	100+20000	10+2000
Captive (net-cage)	34ppt, 26-30°C		0.76	7	40+5000	10+1250
				?	40+10000	10+2500
8-year old pond-reared	indoor tank, 30.5°C	(66 cm total length)	0.6 to 0.8 (n=10)	1800	100 mg phenobarbital	7
				soon after ?	0+3500	

^a IM = intramuscular.

^b SPH = acetone-dried salmon pituitary homogenate, CPH* = acetone-dried carp pituitary homogenate, HCG = human chorionic gonadotropin.

^c Time from last injection.

^d Estimated body weight.

^e Time after cannulation (intraovarian oocyte sampling).

hormone combination was tried on mullet (*Crenimugil* sp.) with oocytes less than 0.6 mm in diameter. The results were encouraging, and when tried on milkfish the response was likewise good, but the milkfish could not tolerate more than three injections (Table 3).

Milkfish is highly sensitive to stress. Atresia of oocytes soon occurs in wild spawners (*sabalo*) if they are not given hormone injections within a few hours after capture. However, the fish usually die if given more than three injections. Handling the wild *sabalo* therefore poses a problem. The problem may be less for captive broodstock but is not absent. The use of valium (diazepam) as a tranquilizer was tried with success in wild milkfish (Kuo 1982); repeated injections of valium at 8- or 12-h intervals at 0.7 mg/kg did not interfere with oocyte maturation but did tranquilize the fish.

Another problem is egg dribbling after hormone injections. The eggs that are dribbled out are hydrated or hydrating but may not be ovulated and fertilizable. They therefore constitute a loss. One solution is the use of a plug (Juario and Duray 1983), but this problem should be studied. Egg dribbling may be a natural phenomenon; it may serve to release pheromone(s) for stimulation of males.

Time interval (h:min)	Diameter hydrated/ ovulated oocytes (mm)	Time to stripping/ spawning (h: min)	Fertilization	Males used	Reference
12:00	?	10 11:30	dry?, 25% 28%		
24:00	?	10 12 14	dry?, 9% 32% 10%		
	1.0-1.1	16:45	wet (several ml 20% glucose solution)	1, injected with 25 mg testosterone propionate + 1500 IU HCG at 1800 h	Tseng and Hsiao 1979

Other problems include: (1) limited availability of ripe males, (2) insufficiency of milt from one male, (3) milt resorption after 2-3 days in captivity, and (4) limited availability of gravid females. One solution to the first three of these problems is to use hormones such as HCG and androgens to induce spermiation; a long-lasting mixture of androgens, Durandron Forte "250" (Organon), has been used by Juario et al (1980) with success. Another solution is cryogenic sperm preservation, which is discussed in the following section. The problem of insufficiency of female broodstock is discussed in the section on induction of gonadal maturation.

SPERM CRYOPRESERVATION

Attempts have been made by Hara et al (1982) and Kuo (1982) to preserve milkfish sperm at near-zero temperatures (0-4°C) and in liquid nitrogen (— 196°C). Various extenders were used, but milkfish serum was found to give the best results in terms of motility and fertilizing capacity of the preserved sperm (Hara et al 1982). Cryopreservation was superior to near-zero liquid preservation. Nevertheless, the near-zero-preserved sperm still showed appreciable fertilizing capacity after 5 days.

Table 2. Unsuccessful attempts to induce ovulation in milkfish with oocytes of less than 0.66 mm in diameter.

Fish	Holding conditions	Body weight (kg)	Initial oocyte diameter (mm)	Injection (IM ^a)				Remarks	Reference
				Time	Total dose ^b (mg SPH/CPH* + IU HCG)	Specific dose ^c (mg SPH/CPH* + IU HCG per kg body weight)	Time interval (h:min)		
Wild, injected immediately upon arrival in lab.	?	9.8 (7.5)	0.63	1600	45 + 1500 + 0.5 ml vit. B	4.6 + 153.1 + 0.05 ml vit. B		fish died; final oocyte diameter 0.71 mm	Liao et al 1979
				0445	45 + 3000 + 0.5 ml vit. B	4.6 + 306.1 + 0.05 ml vit. B	12:45		
				1600	45 + 3000 + 1.0 ml vit. B	4.6 + 306.1 + 0.1 ml vit. B	11:15		
Wild or captive, injected within 26 h of first handling and sampling	35 ppt (1 ppt increase per h from 7 ppt)	4.8	0.60	0400	180 + 6000 + 1.0 ml vit. B	18.4 + 612.2 + 0.1 ml vit. B	12:00		
				0900 or 1800?	50* + 5000	10.4* + 1041.7		no response in 3 days	Kuo et al 1979
				0900 or 1800?	50* + 5000	10.4* + 1041.7	24:00?		
				0900 or 1800?	50* + 5000	10.4* + 1041.7	24:00?		

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Table 2. continued

	5.0	0.62	0900 or 1800?	50* + 5000	10* + 1000	no response in 3 days	
			0900 or 1800	50* + 5000	10* + 1000	24:00!	
			0900 or 1800?	50* + 5000	10* + 1000	24:00?	
	4.5	0.62	0900	50* + 5000	11.1* + 1111.1	no response in 4 days	
			1800	100* + 10000	22.2* + 2222.2	9:00	
			0900	100* + 10000	22.2* + 2222.2	9:00	
			1800	100* + 10000	22.2* + 2222.2	9:00	
			0900	200* + 20000	44.4* + 4444.4	9:00	
			1800	200* + 20000	44.4* + 4444.4	9:00	
			0900	200* + 20000	44.4* + 4444.4	9:00	
Wild	?	6.5 (7.0)	0835	70 + 10000	10.8 + 1538.5	one oocyte group reached	Juario et al 1979
			1745	140 + 10000	21.5 + 1538.5	9:10	
			0430	140 + 15000	21.5 + 2307.7	10:45	
			1645	140 + 15000	21.5 + 2307.7	12:15	
						mm	
						in diameter;	
						fish died	
Captive	35 ppt (4 ppt increase per h from 7 ppt)	2.25	0900 or 1800 ?	(mg SG-G100) 5	2.2	no response	Kuo et al 1979
		0.625		5	2.2		
				10	4.4	?	
				10	4.4		
				10	4.4		

* IM = intramuscular.

^bSPH = acetone-dried salmon pituitary homogenate,

CPH* = acetone-dried carp pituitary homogenate,

HCG = human chorionic gonadotropin.

^c Estimated body weight.

Table 3. Use of estradiol-17 β and thyroxine in addition to salmon pituitary homogenate and HCG to induce ovulation in fish with oocytes of less than 0.66 mm in diameter.

Fish	Body Weight (kg)	Day	Holding conditions	Oocyte diameter (mm)	Injection (IM ^a)			Time interval (h:min)	Remarks
					Time	Total dose ^b (mg + IU HCG)	Specific dose ^c (mg + IU HCG per kg body weight)		
Wild mullet <i>Crenimuga</i> sp.	3	1	16 ppt, 30°C	0.64	1700	3 mg E, +	1 mg E, +	1:00	
					1800	30 mg SPH + 5000 IU HCG + 3 mg T ₁	10 mg SPH + 1666.7 IU HCG + 1 mg T ₁		
	2	2	25 ppt, 28°C	0.66	0645	30 mg SPH + 10000 IU HCG + 1 ml vit. B	10 mg SPH + 3333.3 IU HCG + 0.3 ml vit. B	12:45	
					1730	30 mg SPH + 20000 IU HCG + 1 ml vit. B	10 mg SPH + 6666.7 IU HCG + 0.3 ml vit. B	10:45	
	3	3	32 ppt, 27°C	0.97	0530				released a few ovulated eggs, stripped but no male
					0645				

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Table 3. continued

Wild milkfish <i>Chanos chanos</i> (Forsskal)	8	1	0.57	1022	8 mg E ₂ + 80 mg SPH + 5000 IU HCG + 8 mg T ₄	1 mg E ₂ + 10 mg SPH + 625 IU HCG + 1 mg T ₄	24.28
		2	0.62	1050	as 1st dose	as 1st dose	
		3	0.66	1115	40 mg E ₂ + 80 mg SPH + 10000 IU HCG + 8 mg T ₄	5 mg E ₂ + 10 mg SPH + 1250 IU HCG + 1 mg T ₄	
		3		1710			fish died

^aIM = intramuscular.

^bSPH = acetone-dried salmon pituitary homogenate,

HCG = human chorionic gonadotropin,

E₂ = estradiol-17 β ,

T₄ = thyroxine.

Subsequent work (Hara and Tiro 1984) showed that dimethyl sulfoxide (DMSO) is a better cryoprotectant than glycerine in the cryopreservation of milkfish sperm. In fact, sperm preserved for 10 days in milkfish serum containing 12.5% DMSO even yielded apparently higher fertilization and hatching rates, as well as a higher survival rate of larvae at day 21, than fresh semen.

LARVAL REARING

Available information on successful rearing of milkfish larvae is summarized in Table 4. Feeding of larvae was consistently observed by the different workers to begin on the 4th day after hatching, and the critical period was between the 4th and 6th days.

Juario et al (1984) reviewed several years' experience in rearing milkfish larvae. Foods given were *Chlorella virginica*, *Isochrysis galbana*, *Tetraselmis chuii*, *Brachionus plicatilis*, *Tisbintra elongata* (copepod), and *Artemia salina*. A significant improvement in larval survival was found when *I. galbana* and *Tetraselmis chuii* were added to the rearing tanks in addition to *C. virginica*, perhaps related to the possibility that the latter is deficient in 20:5 w3 fatty acids, which are essential for marine fish (Watanabe et al 1983).

Larval survival rates as high as 71% were obtained (Juario et al 1984). Larvae from artificially fertilized eggs showed highly variable survival rates, whereas those from naturally spawned eggs showed consistently high survival rates. Concomitantly, there were more abnormal larvae from artificially fertilized eggs than from naturally spawned eggs.

Recent studies on the salinity tolerance of milkfish larvae (Dueñas and Young 1984) showed that, while larvae at day 0 and day 14 are fairly euryhaline and at day 21 highly euryhaline (0-70 ppt), those at day 7 are markedly stenohaline (27-28 ppt). This suggests that rearing milkfish larvae at a constant salinity of 27-28 ppt may improve their survival rate. Further studies are necessary.

INDUCTION OF GONADAL MATURATION

Milkfish are able to undergo sexual maturation in concrete tanks, ponds, enclosed lagoons, and floating net-cages. Some of the associated factors are summarized in Table 5. It appears that milkfish attain sexual maturity at around 5 years of age; males may reach maturity earlier, at around 4 years of age. It is difficult to pinpoint the important factor(s) for milkfish gonadal development. However, salinity does not seem important, since milkfish can mature in salinities ranging from 8 to over 100 ppt, although salinity affects ovulation and may exert some influence on gonadal development when it is extremely high (Crear 1980). Temperature and photoperiod may be important (Lee 1984, Marte et al 1984, Wainwright 1982, Lam 1983), but there is not enough information available. In the Philippines, the rapid gonadal development of milkfish prior to the spawning season seems to coincide with rising water temperatures from 25° to 32°C and with increasing photoperiod from 11 to 14 h of light (Marte et al 1984).

Table 4. Larval rearing in milkfish.

Incubation	Newly hatched		Food given		Day feeding first observed	Critical period (days)	Survival rate	Reference
	Period	Total length (mm)	Yolk sac (mm)	Day				
32 ppt, 28.4-29.2°C	30-35	3.5	large	?	?	?	one to day 7	Vanstone et al 1977
30-34 ppt, 26.4-29.2°C	25-28.5	3.2	2.15 long, 0.58 wide	2.5	Ch + TL	4th-6th	two to day 6	Chaudhuri et al 1978
34 ppt, 27-32°C	25.75-32	3.4	2.20 long, 0.28 wide	1-21 2-6 2-21 14-21	Ch E + TL R C + AN + F + PF	4th-6th	8.8-46.8% to day 21	Liao et al 1979
34 ppt, 26-29°C	24-35	?	?	1-21 2-10 2-21 2-21 7-21 10-21	Ch I T R C AN	4th-7th	8-71% (artificially fertilized), 19-50% (naturally fertilized to day 21)	Juario et al 1984

*AN = *Artemia* nauplii
C = copepods
F = flour
I = *Isochrysis galbana* (2.5×10^6 cells/ml)
PF = prepared feed
R = rotifers (*Brachionus plicatilis*)
Ch = *Chlorella virginica*
5-3.5 x 10⁶ cells/liter (Liao et al 1979)
2-5 x 10⁶ cells/ml (Juario et al 1984)
E = fertilized eggs of oysters
TL = trochophore larvae of oysters

20-30/ml for days 2-10, 10-20/ml for days 10-21 (Juario et al 1984)

Table 5. Factors associated with sexual maturation in milkfish.

Factors	Concrete tank			Pond (Hawaii)			Lagoon (Christmas Island)			Floating net cage (Philippines)
	Taiwan	Oceanic Institute (Hawaii)	Kauai	Kona	Oceanic Institute	Isles	Pelican	Azur		
Age(yr)	♂ 4+ ♀ 5+	6-7	?	?	?	4-5	4-5	6	5	
Diameter or area	8.5 - 12 m	5.2 × 4.8 m	10 ha	1.4 ha	0.05 ha	~4 × 1 mile	~0.5 × 1.0 mile	~0.5 × 1.0 mile	9 or 10m	
Depth (m)	1.2	1.3	?	1-2	1	~3	~3	~3	3	
Temperature (°C)	21-31	26	24-30	24-30	24-30	27-32	27-32	27-32	25-32	
Salinity (ppt)	14-30	32	38-42	8-12	32	102-130	60-76	80-100	25-38	
Photoperiod (h light)	?	artificial ^a	10.5 - 13.5	10.5 - 13.5	10.5 - 13.5	?	?	?	11 - 14	
Food (% protein)	3.7-4.6	Ralston Catfish Chow	?	?	Ralston Catfish Chow	benthic mat ^a + <i>Artemia salina</i>	benthic mat ^a	benthic mat ^a	42	
Feeding rate	once to satiation	?	?	?	?	intense ^a	intense ^a	intense ^a	2% body wt	
Stocking density	2.0-3.0 per tank	9 - 12 per tank	?	700/pond	12/pond (+100-200 mullet)	low	high	high?	80 - 100/cage (0.5kg/m)	
Flushing rate	flow-through	4.9 gal/min	low	moderate (upwelling)	moderate	no flushing	no flushing	no flushing	good	
Reference	Liao and Chen-1979, 1984, and pers. comm.	Lee 1984	Nash and Kuo 1976, Kuo et al 1979, Kuo 1982 and pers. comm.	?	Kuo 1982, Vanstone 1982, Crear 1980 and pers. comm.	?	?	?	Lacamilao and Marte 1980, Marte et al 1984 and pers. comm.	

^a Fish subjected to 6, 12, and 18 h of light at different times. ^b Composed of halophilic bacteria, blue-green algae, diatoms, and fungi.

Food seems to be an important factor. High protein diets were used in Taiwan, the Oceanic Institute (Hawaii), and the Philippines, and abundant food is available in the lagoons of Christmas Island. It is interesting that in the Isles Lagoon, where brine shrimp *A. salina* was introduced and has flourished, milkfish showed reproductive readiness throughout the year; the males were spermiating while the females showed maturing if not ripe gonads throughout the year (Crear 1980). Analysis of gut contents of the fish revealed that brine shrimp constituted about 25% (by volume) of their diet. Thus brine shrimp may be a good broodstock diet.

Obviously, experimental studies are needed to confirm the importance of temperature, photoperiod, and food in milkfish gonadal development. Other factors such as swimming space, stocking density, and feeding rate should also be investigated. Such studies are most worthwhile because manipulation of environmental and nutritional factors represents the most practical approach to induction of gonadal maturation in fish (Lam 1983).

Attempts have been made to use hormones to induce gonadal maturation in milkfish (Lam 1982, Lacanilao et al 1984, Lee et al 1984). The results obtained were not encouraging, except perhaps for the male (Lee et al 1984). The problems encountered were as follows:

- Stress due to frequent handling seemed to negate the stimulatory effect of hormones. Administration of hormones (salmon pituitary, estradiol-17b, and thyroxine) by pellet implantation was tried in order to reduce frequency of handling, but with little success (Lacanilao et al 1984, Marte and Crim 1983). The only handling-free method of hormone administration is through feeding. However, not all hormones can be orally administered; protein hormones such as gonadotropins cannot be given this way. Lee et al (1984) managed to obtain some stimulation of spermatogenesis in 4-year old milkfish through feeding of 17 a-methyl testosterone.
- Milkfish less than 4 or 5 years old may not have developed the receptors to respond to hormone treatments.
- Similarly, spent fish may lack hormone receptors. It is not known how long it takes spent fish to undergo recrudescence (rematuration) or whether they remature at all in captivity. It is also not certain whether milkfish are total or intermittent spawners.

CONCLUSION

It is clear from the above brief review that, although much has been done, much more needs to be done before artificial milkfish propagation can be carried out on a standardized routine basis.

LITERATURE CITED

- Chudhuri, H., J. V. Juario, J.H. Primavera, R. Samson, and R. Mateo. 1978. Observations on artificial fertilization of eggs and the embryonic and larval development of milkfish, *Chanos chanos* (Forsskal). *Aquaculture* 13:95-113.
- Crear, D. 1980. Observations on the reproductive state of milkfish populations *Chanos chanos*

- from hypersaline ponds on Christmas Island (Pacific Ocean). Proc. World Maricul. Soc. 11:548-556.
- Dueñas, C.E., and P.S. Young. 1984. Salinity tolerance and resistance of milkfish larvae. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 Oct, 1983.
- Hara, S., and L.B. Tiro, Jr. 1984. The effect of cryoprotectant on the cryogenic preservation of the sperm of milkfish, *Chanos chanos*. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 Oct. 1983.
- Hara, S., J.T. Canto, Jr., and J.M.E. Almendras. 1982. A comparative study of various extenders for milkfish, *Chanos chanos* (Forsskal), sperm preservation. Aquaculture 28:339-346.
- Juario, J.V., and M.N. Duray. 1983. A guide to induced spawning and larval rearing of milkfish, *Chanos chanos* (Forsskal). Tech. Rep. 10, SEAFDEC Aquaculture Department and IDRC. 22p.
- Juario, J.V., M. Natividad, G. Qunitio, and J. Banno. 1979. Experiments on the induced spawning and larval rearing of the milkfish, *Chanos chanos* (Forsskal) in 1979. SEAFDEC Aquaculture Dept. Quarterly Res. Rep. 3:1-3.
- Juario, J. V., G.F. Qunitio, J.E. Banno, and M.R. Natividad. 1980. The effect of exogenous hormone injections on milt consistency in newly caught wild mature milkfish. Kalikasan, Philipp. J. Biol. 9:321-326.
- Juario, J.V., M.N. Duray, V.M. Duray, J.F. Nacario, and J.M.E. Almendras. 1984. Induced breeding and larval rearing experiments with milkfish *Chanos chanos* (Forsskal) in the Philippines. Aquaculture 36:61-70.
- Kuo, C.M. 1982. Progress on artificial propagation of milkfish. ICLARM Newsletter 5:8-10.
- Kuo, C.M., and C.E. Nash. 1979. Annual reproductive cycle of milkfish, *Chanos chanos* (Forsskal) in Hawaiian waters. Aquaculture 16:247-252.
- Kuo, C.M., and W.O. Watanabe. 1978. Circadian responses of teleostean oocytes to gonadotropins and prostaglandins determined by cyclic AMP concentration. Ann. Biol. Anim. Biophys. 18:949-956.
- Kuo, C.M., C.E. Nash, and W.D. Watanabe. 1979. Induced breeding experiments with milkfish, *Chanos chanos* (Forsskal), in Hawaii. Aquaculture 18:95-105.
- Lacanilao, F.L., and C.L. Marte. 1980. Sexual maturation of milkfish in floating cages. Asian Aquaculture 3:4-6.
- Lacanilao, F., C.L. Marte, and T.J. Lam. 1984. Problems associated with hormonal induction of gonad development in milkfish *Chanos chanos* (Forsskal). Proc. 9th Int. Symp. Comp. Endocrinol., Hongkong, 1981. In press.
- Lam, T.J. 1982. Applications of endocrinology to fish culture. Can. J. Fish Aquat. Sci. 39:111-137.
- Lam, T.J. 1983. Environmental influences on gonadal activity in fish. Pages 65-116 in W.S. Hoar, D.J. Randall, and E.M. Donaldson, eds. Fish Physiology, Vol. 9B, Academic Press, New York.
- Lee, C.S. 1984. Preliminary studies on the maturation of milkfish *Chanos chanos* (Forsskal) in an environmentally controlled system. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 Oct. 1983.
- Lee, C.S., G.M. Weber, and J. Thomas. 1984. Effect of 17 α -methyl testosterone on gonad development in immature milkfish, *Chanos chanos* (Forsskal). Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 Oct. 1983.
- Liao, I.C., and Y.S. Chang. 1976. A preliminary report on the gonadal development of adult milkfish, *Chanos chanos*, reared in tanks. Pages 121-132 in Proc. Int. Milkfish Workshop

- Conf., Tigbauan, Iloilo, Philippines, 19-22 May 1976.
- Liao, I.C., and T.I. Chen. 1979. Report on the induced maturation and ovulation of milkfish *Chanos chanos* reared in tanks. Proc. World Maricul. 10:317-331.
- Liao, I.C., and T.I. Chen. 1984. Gonadal development and induced breeding of captive milkfish in Taiwan. Pages 41-51 in Proc. 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, 4-8 Oct. 1983.
- Liao I.C., J.V. Juario, S. Kumagai, H. Nakajima, M. Natividad, and P. Buri. 1979. On the induced spawning and larval rearing of milkfish, *Chanos chanos* (Forsskal). Aquaculture 18:75-93.
- Marte, C.L., F.J. Lacanilao, and J.V. Juario. 1984. Completion of the life cycle of milkfish, *Chanos chanos* (Forsskal) in captivity. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 Oct. 1983.
- Nash, C.E., and C.M. Kuo. 1976. Preliminary capture, husbandry, and induced breeding with the milkfish, *Chanos chanos* (Forsskal). Pages 139-159 in Proc. Int. Milkfish Workshop Conf., Tigbauan, Iloilo, Philippines, 19-22 May 1976.
- Tseng, L.C., and S.M. Hsiao. 1979. First successful case of artificial propagation of pond-reared milkfish. China Fish. 320:9-10 (in Chinese).
- Vanstone, W.E. 1982. Attempted breeding of milkfish on Kiribati (Christmas Island). Republic of Kiribati, 25 June-12 Sept. 1982. Report to UNDP and Govt. of Kiribati.
- Vanstone, W.E., A.C. Villaluz, and L.B. Tiro, Jr. 1976. Spawning of milkfish, *Chanos chanos* in captivity. Pages 206-221 in Proc. Int. Milkfish Workshop Conf., Tigbauan, Iloilo, Philippines, 19-22 May 1976.
- Vanstone, W.E., L.B. Tiro, Jr., A.C. Villaluz, D.C. Ramsingh, S. Kumagai, P.J. Dulduco, M.M.L. Barnes, and C.E. Dueñas. 1977. Breeding and larval rearing of the milkfish *Chanos chanos* (Pisces: Chanidae). SEAFDEC Aquaculture Dept., Tech. Rep. 3:3-17.
- Wainwright, T. 1982. Milkfish fry seasonality on Tarawa, Kiribati, its relationship to fry seasons elsewhere, and to sea surface temperatures (SST). Aquaculture 26:265-271.
- Watanabe, T., C. Kitajima, and S. Fujita. 1983. Nutritional values of live organisms used in Japan for mass propagation offish: A review. Aquaculture 34:115-143.