GONADAL DEVELOPMENT AND INDUCED BREEDING OF CAPTIVE MILKFISH IN TAIWAN

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The induced breeding of milkfish has been attempted by many institutes in the Philippines, Taiwan, Tahiti, Indonesia, and Hawaii. So far, a few successful trials have been achieved only in the Philippines and Taiwan, although different sources of spawners were used. In Taiwan the spawners used were reared from fry to sexual maturity in ponds and concrete tanks. This paper summarizes the gonadal development of captive milkfish at various stages of sexual maturation investigated from 1975 to 1980 and describes three successful trials of induced breeding in 1979, 1982, and 1983 in Taiwan. Finally, the problems that need further study are discussed.

INTRODUCTION

Being tough and highly resistant to diseases, milkfish is easy to culture and is therefore one of the most important cultured fishes in Southeast Asia. It is not only an excellent food fish but also an ideal bait fish for tuna. In the past 2 years it has been shown that milkfish production can be increased three to seven times by using improved culture techniques like deeper ponds and artificial feeds.

There is an increasing demand for milkfish fingerlings owing to the adoption of these improved culture techniques. In the past, 8 000-10 000 fingerlings were stocked in a 1 ha pond; however, 10 000-30 000 are now needed for stocking deeper ponds of the same size. It is now believed that the key point for further development

of the milkfish industry is the adequate supply of fingerlings. Therefore, scientists in areas engaged in milkfish culture such as the Philippines, Taiwan, and Indonesia are trying to develop techniques for artificial propagation. So far, there are only a few cases of successfully induced breeding. Two different sources of spawners have been used, one from the wild (Vanstone et al 1977, Chaudhuri et al 1978, Liao et al 1979) and the other from tanks or ponds (Hsiao and Tseng 1979, Lin 1982) and cages (Juario and Duray 1980, Juario et al 1984).

In Taiwan, wild spawners have also been tried for artificial propagation; however, they are so few that more attention has been focused on tank- or pond-reared spawners since 1970 (Liao and Chang 1976).

This paper summarizes and discusses gonadal development and three successful trials of induced breeding of captive milkfish in Taiwan as well as related problems that need further study.

GONADAL DEVELOPMENT

Results of studies on milkfish reared to maturity in outdoor cement tanks at the Tungkang Marine Laboratory are used in this review. The gonads were examined macroscopically and microscopically for their maturity based on their weight, size, shape, color, and stage of gamete development. Since the external sex characteristics of captive milkfish are not obvious, it was difficult to determine the sex and maturity of first-time spawners. However, the fish were found to be heterosexual, and no incidence of hermaphroditism was present. The six stages of gonadal maturity are summarized in Tables 1 and 2. The gonadal development of milkfish was found to be synchronous. Histological study of the gonads showed that all of the 1 + year old fish contained undifferentiated germ cells, while 2+ year old fish contained spermatogonia in males and previtellogenic oocytes at the oogonium and chromatin-nucleolus stages in females. The sex of dissected fish older than 3 years could easily be distinguished by the presence of ovigerous lamellae in the ovaries of the females. Sperm cells at various stages were present in 4+ year old males. Mature spermatozoa were first observed in a 4 year old male (BW 2.55 kg, TL 70.1 cm, GSI 0.06), and milt oozed out upon pressing the abdomen. Males older than 5 years had running milt during the spawning season. Ovaries of 4+ year old females consisted mostly of oocytes at the chromatin-nucleolus and peri-nucleolus stages. Vitellogenesis first occurred in 5 + year old stock, and oocytes at the yolk vesicle stage were present in these fish. Yolky oocytes were present in most 6+ year old stock and coincided with the increase in gonad weight and gonadosomatic index (GSI). The results indicated that, among tank-reared milkfish, males first attain maturity in 4+ years, females in 5+ years. Seasonal fluctuation of monthly mean GSI values of captive adult milkfish (older than 5 years) over a 5-year period (1975-1980) is shown in Figure 1. The GSI value increased in May and attained a maximum in August, suggesting that the breeding season of captive broodstock is between late May and August. The monthly variation in the percentage of ovarian oocytes at different stages of development confirms the annual cyclic changes of the GSI value (Fig. 2).

The frequency distribution of oocyte diameter showed a distinct spawning pattern in mature captive milkfish (Fig. 3). Examination of ten ovaries revealed that only

Table 1. Macroscopic description of the maturity stages of the gonads of captive milkfish (Liao and Chen 1983).

Maturity stage	Testis	Ovary
Immature virgin	Two small, thread-like elongated bodies with a slightly pinkish-grey coloration suspended by a mesorchium from the ventral side under the ventral column.	As in males, not distinguishable macroscopically; but early oogonia can be recognized histologically.
Developing virgin	Pinkish-grey in color, thicker than thread-like structure.	Reddish-orange in color; increased in width and length; ovigerous lamellae easily discernible; ovarian oocytes not discernible by the naked eye.
Maturing	Pinkish-white in color, flattened and broader in shape, occupying more than one-half the length of the peritoneal cavity.	As in developing virgin stage, but considerably enlarged; ovarian oocytes visible to the naked eye.
Mature	Rosy-white, elongated, and broadest at the middle, occupying about two-thirds the length of the peritoneal cavity. Vas deferens enlarge and milt appears upon pressing the abdomen.	Yellowish-orange or bright yellow in color; occupying large portion of peritoneal cavity; yolk-laden oocytes visible to the naked eye, but not separable.
Gravid and spawning	Fully mature and whitish milt oozes out with pressure.	Abdomen distended, peritoneal cavity filled with the ovaries; eggs translucent golden, with a few opaque; ovulated eggs are released to coelomic cavity and extruded out through genital aperture on pressure.
Spent	Degenerated and reduced in size.	Flaccid and considerably reduced in size with lamellae shrunken and falling apart; reddish-brown in color; a few eggs left in ovaries and coelomic cavity.

Table 2. Histological changes in relation to maturity stages in the gonads of captive milkfish (Liao and Chen 1983).

Maturity stage	Testis	Ovary
Immature virgin	Lobules consisting of primary and secondary spermatogonia; a few primary spermatocytes present.	Ovarian oocytes at oogonium stage located in cysts in the ovigerous lamellae; some early chromatin-nucleolus oocytes present.
Developing virgin	Lobules consisting predominantly of secondary spermatogonia and primary spermatocytes; some lobules at primary spermatogonia.	Ovigerous lamellae containing oocytes at chromatin-nucleolus and peri-nucleolus stages.
Maturing	Testes consisting of various stages of sperm cells, mostly at secondary spermatocyte stage; considerable number of spermatids and spermatozoa present.	Ovarian oocytes mostly at late peri-nucleolus stage and yolk vesicle stage; some primary yolk globule oocytes present. The process of vitellogenesis starts.
Mature	Lobules gorged with spermatozoa, small cysts of developing sperm cells also present.	Oocytes mostly at yolk globule stage, some oocytes at early peri-nucleolus stage occur in the lamellae between the yolk oocytes.
Gravid and spawning	Lobules and spermatic duct are filled with spermatozoa.	Oocytes ripe with finely divided yolk globules, spherical in form, with no covering follicle layer.
Spent	Lobules empty, developing cysts of sperm cells recrudescent.	Empty follicles in each lamella; immature ovarian eggs at peri-nucleolus stage scatter throughout the lamellae; unreleased eggs become atretic and undergo resorption; formation of "corpora atretica."

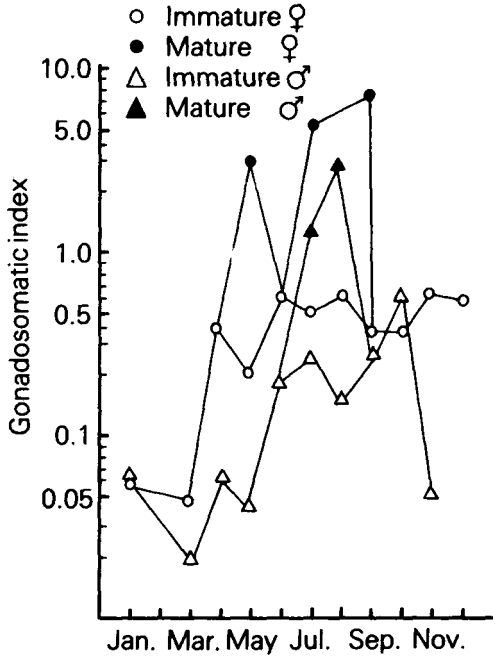

Fig. 1. Annual cyclic changes of gonadosomatic index of captive milkfish (Liao and Chen 1983).

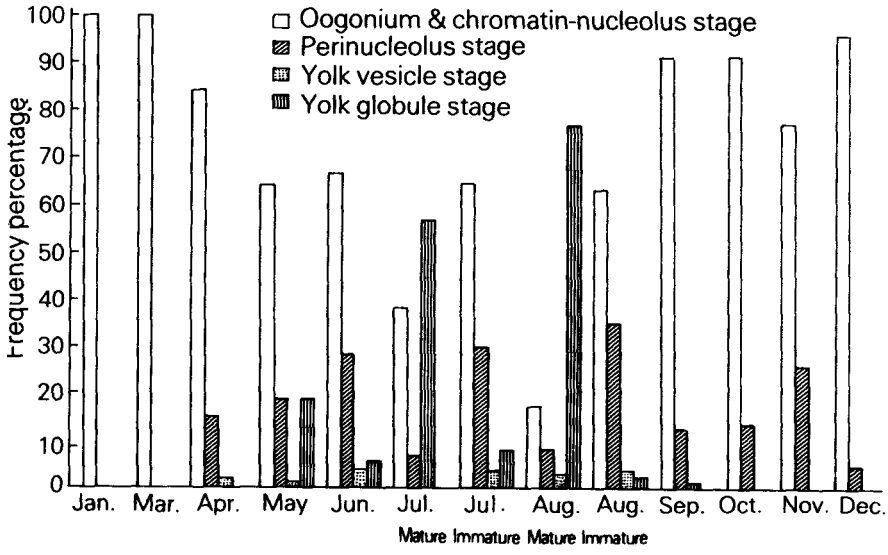

Fig. 2. Annual cyclic change in the frequency percentage of ovarian oocytes of captive milkfish during the period 1975-1980 (Liao and Chen 1983).

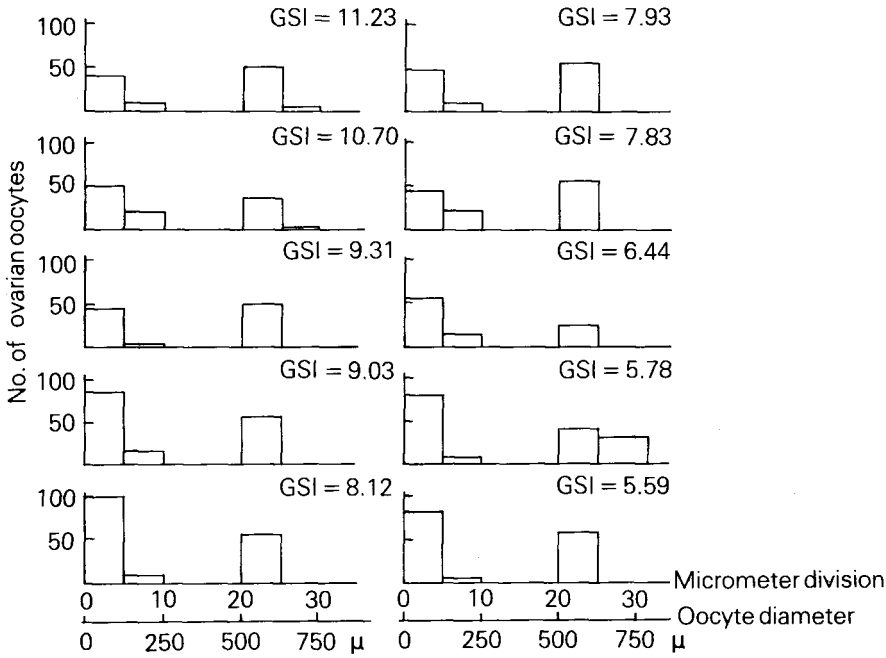

Fig. 3. Frequency distribution of oocyte diameter of ten mature captive milkfish (Liao and Chen 1983).

yolk-laden oocytes ranging from 500 to 750 mm and immature oocytes ranging from 10 to 250 mm in diameter were present. The intermediate group of maturing oocytes was not found. The frequency distribution of oocyte diameter and different stages of development suggest that captive milkfish have a synchronous spawning pattern. The fish spawns once a year during June-August.

INDUCED BREEDING

Work on the artificial propagation of captive milkfish in Taiwan has involved maintenance of broodstock, induction of maturation and spawning, artificial insemination, and larval rearing. Broodstock has been reared in cement tanks or earthen ponds which are in either low or highly productive condition. The pond area covers 150-750m^2. Salinity ranges from l4 to 34 ppt and temperature from 21 to 31°C. The stocking density ranges from 20 to 200 fish in each pond. The adult stock is fed daily with formulated diets, occasionally supplemented with natural food. For example, a high-protein diet (CP 45.7%) together with shrimp meal or adult *Artemia* is used at the Tungkang Marine Laboratory (Liao and Chen 1979); compound food for shrimp and/or freshwater fish plus seaweeds is used at the Shin-Li Fish and Shrimp Hatchery

(Hsiao and Tseng 1979); a diet containing rice bran, cereal soybean cake, eel feed, and some trash fish is fed to the broodstock at the Tung-Hsing Fish and Shrimp Hatchery (Lin 1982). During the breeding season, the adult stock shows an acceleration in gonadal development, with vitellogenic oocytes appearing and increasing in number. Some fish may become fully mature. At present, the percentage of broodstock which can reach full maturity and can be used for induced spawning trials is still quite low compared with that of other captive fish species. In general, about 8 out of 20 captive fish attain maturity, and of the 8 only 1 or 2 females are fully mature.

In Taiwan, captive milkfish were first successfully induced to spawn in 1979 (Hsiao and Tseng 1979). Much progress was made in 1982 (Lin 1982) and 1983 (Lin, pers. comm.). The results of successful trials to induce spawning are summarized in Table 3. Apparently, captive spawners can be used for induced spawning and mass production of fingerlings. So far, the best results of induced spawning among captive stock were obtained from females with egg diameters ranging from 0.6 to 0.8 mm which were injected with 1000-1300 IU human chorionic gonadotropin (HCG)/kg body weight. The suitable time to spawn/strip the fish was 15-20 h after injection. Males were injected with 750-1500 IU HCG/fish to improve milt quality. Eggs collected were artificially fertilized and incubated at a salinity of 31-34 ppt at 29-32°C.

The larvae started to hatch 20-25 h after fertilization. One day after hatching, *Chlorella* sp. was added to the rearing tanks and fertilized oyster eggs, rotifers, and *Artemia* nauplii were given subsequently as feed. Twenty-one days after hatching, the larvae attained a total length of 1.2-1.8 cm, which is the size of milkfish fry used for stocking ponds. At present, the survival rate of the larvae ranges between 11 and 43%. Compared with other marine fish, it seems relatively easy to rear milkfish larvae to fry. Further refinements of the technique will improve the survival rate.

DISCUSSION

Both wild and captive milkfish undergo regular, cyclic gonadal changes yearly. In Taiwan, captive milkfish do not spawn spontaneously without hormonal treatment. The annual breeding season occurs from late May through August, during which the GSI and the percentage of viable gametes increase. September is supposed to be the postspawning or regressive period, and the remaining months of the year are the resting or recovering period of gonadal development. Mature wild milkfish spawners were captured from April to June (Liao 1971), and the fry could be caught from April through October in the coastal waters of southern Taiwan (Liao et al. 1977). Thus, the breeding season of captive milkfish occurs later and is shorter than that of milkfish in their natural habitat. Although most of the captive stock first attain maturity at the age of 4 in males and 5 in females, females older than 6 years undergo rapid development from the time yolk is first deposited in the oocytes, i.e., in late May; some females reach full maturity in August. Males older than 5 years can attain full maturity and produce viable sperm. The captive 6+ year old adults can attain sexual maturity and be used for induced breeding regardless of their body length and weight, suggesting that age is a more crucial factor for sexual maturity than other parameters. In addition, as shown in Table 3, the probability of successful induced breeding increases if the spawners are more than 8 years old.

Table 3. Records of successfully induced breeding of captive milkfish in Taiwan.

	1st trial (Hsiao and Tseng 1979)	2nd trial (Lin 1982)	3rd trial (Lin, pers. comm.)				
No. of fish	1♀ 3♂	1♀ 2♂	1 ♀2♂	1♀ 1♂	1♀ 1♂	1♀ 1♂	1♀ 1♂
Age	8	8-9	9-10	9-10	9-10	9-10	9-10
Body weight ♀ Body length	3.5 kg 66 cm	5.0 kg 76 cm	7.0 kg 80 cm	6.0 kg 72 cm	6.0 kg 73 cm	6.0 kg 71 cm	6.5 kg 75 cm
Egg diameter (before injection)	0.6-0.8 mm	—	—	—	—	—	—
Date and time of injection	14 July 1979 1800 h	4 Aug 1982 1120 h	20 July 1983 1600 h	1 Aug 1983 1600 h	2 Aug 1983 0800 h	16 Aug 1983 1630 h	18 Aug 1983 1800 h
Dosage of hormone injection	3500 IUHCG 100mg phenorbarbital	6000 IUHCG	10000 IUHCG	8000 IUHCG	8000 IUHCG	8000 IUHCG	8000 IUHCG
Date and time of spawning egg collection	15 July 1979 1033 h 1st 1045 h 2nd 1307 h	5 Aug 1982 — 1st 0220 h 2nd 0250 h	21 July 1983 0000 h — —	2 Aug 1983 1100 h 1100 h —	3 Aug 1983 — 0800 h —	17 Aug 1983 — 1130 h —	19 Aug 1983 0900h 1st 0900 h 2nd 0930 h

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Table 3. continued

Duration between injection & spawning	16 h 33 min	15 h 20 min	8 h	19 h	24 h	19 h	15 h
Estimated no. of eggs collected (total)	—	1st 300 000 2nd 500 000 (800 000)	(800 000)	(300 000)	(200 000)	– (250 000)	(600 000)
Incubation condition	29.0-31.0°C 33.2 ppt	29.5-30.0°C 29.5 ppt	31.0.32.0°C 31.2 ppt	30.0-31.5°C 31.2 ppt	30.5-31.5-°C 31.2 ppt	31°C 31.2 ppt	31°C 31.2 ppt
Date and time of hatching (time after fertilization)	16 July 1979 1645 h (24.0-32.0 h)	6 Aug 1982 0420 h (25.5-32.0 h)	21 July 1983 2030-2130 h (20.5-22.0 h)	3 Aug 1983 0900-1100 h (22.0-24.0 h)	4 Aug 1983 0700-0900 h (23.0-25.0 h)	18 Aug 1983 1000-1130 h (22.5-24.0 h)	20 Aug 1983 0700-0830 h (22.0-23.5 h)
Estimated no. of hatched larvae	120	120000	300 000	30 000	2 000	20 000	150 000
No. of fingerlings harvested (day after hatching)	32 (52nd)	13 266 (22nd)	1 500[b] (21st)	8 479[c] 21st-22nd		7600 (20th)	64 140 (21st)

[a] Spawned and fertilized spontaneously. At 0530 h, 21 July 1983 fertilized eggs in gastrula stage with germ ring formed were found.
[b] Due to overfeeding and poor condition of rearing water, mass mortality occurred 11 days after hatching.
[c] Larvae were cultured together, and fingerlings were counted on 25 Aug 1983.

Although captive milkfish can attain full maturity, the percentage of fully mature ones is still low, and individual variations in gonadal development are remarkable. This may be attributed to inadequate environmental and nutritional conditions. Environmental factors (such as size, shape, depth, and substratum of the pond, and circulation, turbidity, temperature, and salinity of the water), rearing conditions (such as stocking density and feeding rate), and type of feed seem to influence gonadal development and spawning, but the key factors have not been identified. However, it is recommended that for maintenance of spawners the holding pond should not be too small and the stocking density not too high, since insufficient exercise owing to limited space may cause obesity of the spawners and probably retard their gonadal development. Furthermore, the water quality should be kept as similar as possible to the natural habitat of wild spawners.

Development of an optimal diet for both female and male spawners to reduce the accumulation of visceral fat, stimulate gonadal development, and improve quality and quantity of gametes is essential for the establishment of captive broodstock. According to the experience of Tseng and Lin (pers. comm.), a high protein diet does not seem indispensable to induce gonadal development. However, gonadal development may require specific levels of some dietary components such as polyunsaturated fatty acids, which are generally found in some natural foods.

Domestication of captive broodstock can contribute greatly to success in the artificial propagation of milkfish. Captive fish are rather small, generally healthy, and easy to handle. It is possible to control gonadal development and induce breeding through environmental, nutritional, social/behavioral, and hormonal manipulation, and thus to minimize stress or physical injury.

With regard to induced spawning by hormone injection, pond-reared spawners apparently need a dosage lower than wild spawners. Only one injection of 1000-1300 IU HCG/kg was needed to induce spawning; however, Juario et al (1984) used 10 mg salmon pituitary homogenate (SPH)/kg + 1000 IU HCG/kg body weight for the first injection and 10 mg SPH/kg + 2000 IU HCG/kg body weight for the second injection to induce spawning in wild and captive stock reared to maturity in floating cages. The difference in response to hormone treatment can be attributed to the degree of domestication, stage of maturation, and physical condition of the fish. According to Liao and Chen (1979), fecundity of captive milkfish ranges from 620 000 to 1 300 000. However, Table 3 shows that the total number of eggs collected after hormone treatment is relatively low (ranging from 200 000 to 800 000). It seems, therefore, that the technique to induce ovulation or spawning is still unsatisfactory. Besides, the gamete quality of spawners should be taken into consideration. It is well known that poor egg quality causes low fertilization, hatching, and larval survival rates. Although the techniques of induced breeding for captive spawners have been preliminarily established, more reliable procedures to improve egg quality and fertilization, hatching, and larval survival rates remain to be developed and standardized.

Because of the difficulty in determining sex and maturity of virgin spawners from external characteristics, gametes have to be exteriorized through a cannula; however, handling of spawners during the breeding season easily causes atresia and resorption of the gonads. Prospective spawners should therefore be distributed to as many

ponds as possible long before the breeding season. Techniques of determining sex and oocyte maturity which minimize stress, such as ultrasonography or immunological tests, should be developed. Furthermore, the milt of ripe males can be squeezed out on pressure, but the quantity produced from one male at one time is usually insufficient to fertilize the eggs of one female spawner, or the milt is not available when needed. Therefore, techniques to cryopreserve sperm effectively should also be developed.

To recapitulate, the ideal way to induce gonadal development and to breed milkfish is to allow them to mature and spawn spontaneously by a combination of hormonal, environmental, nutritional, and social/behavioral manipulations, and then to collect fertilized eggs or larvae for rearing. More research efforts must be devoted toward further improvement of techniques in these particular phases of milkfish culture.

LITERATURE CITED

Chaudhuri, 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.

Hsiao, S. M., and L. C. Tseng. 1979. Induced spawning of pond reared milkfish, *Chanos chanos* Forsskal. China Fish. Monthly 330:7-13 (in Chinese with English abstract).

Juario, J. V., and M. N. Duray. 1980. The induced spawning of captive milkfish. Asian Aquaculture 3:4.

Juario, J. V., and M. N. Duray. 1982. A guide to induced spawning and larval rearing of milkfish, *Chanos chanos* (Forsskal). Aquaculture Department, SEAFDEC, Tech. Rept. No. 10. 27 p.

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.

Liao, I. C. 1971. Note on some adult milkfish from the coast of southern Taiwan. Aquiculture 1(3):1-10 (in Chinese with English abstract).

Liao, I. C, and Y. S. Chang. 1976. A preliminary report on the gonadal development of adult milkfish, *Chanos chanos* reared in tanks. Proc. Int. Milkfish Workshop-Conf., Tigbauan, Iloilo, Philippines, Working paper no. 9.

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. Soc. 10:317-331.

Liao, I. C, and T. I. Chen. 1983. Report on the gonadal development of milkfish, *Chanos chanos,* reared in tank. Aquiculture 2 (in press).

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.

Liao, I. C., H. H. Yan, and M. S. Su. 1977. On morphology and its related problems of milkfish fry from the coast of Tungkang, Taiwan. J. Fish. Soc. Taiwan 6:73-83 (in Chinese with English abstract).

Lin, L. T. 1982. Further success in induced spawning of pond-reared milkfish. China Fish. Monthly 357:17-19 (in Chinese with English abstract).

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). Aquaculture Department, SEAFDEC, Tech. Rept. 3:3-17.