A PRELIMINARY REPORT ON THE GONADAL DEVELOPMENT OF ADULT MILKFISH, CHANOS CHANOS, REARED IN TANK*

by

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Abstract

Milkfish is one of the most important food fishes in Taiwan. There are more than 16,000 ha of culture area and over 160 millions of fry are needed for milkfish farming industry every year. The fry are collected from the sea and also imported from other countries. However, due to several environmental factors, there is unpredictable fluctuations in the occurrence of these wild fry. In recent years, the demand for milkfish fry has gone up considerably owing mainly to the fast-growing populations, the natural resources being so limited that there is insufficient supply of stocking materials of this important foodfish.

To solve the problem of shortage of milkfish fry, Tungkang Marine Laboratory started the preliminary work on artificial propagation of milkfish in 1970. In addition to capturing wild spawners, the Laboratory has also been raising the adult milkfish in tanks for this objective. After being reared for six years, one male and one female were dissected on 11 April 1976. The male had ripe sperms; the testes weighing 4.63 g with the GSI of 0.12. The gonad of the female weighed 21.20 g with the GSI of 0.66 and part of the ovarian oocytes was found to be at the oil droplet (yolk vesicle) stage. Judging from the condition of maturity of the above female, the feasibility of raising tank-reared spawners was ensured. It is believed that this is the first attempt on the world and is the prelude to successful artificial propagation by using tank-reared milkfish as spawner.

Introduction

The milkfish, Chanos chanos is one of the best cultivable fishes in Taiwan, being euryhaline, disease resistant, popular and most economical to grow. There are more than 16,000 ha of area under milkfish culture in Taiwan. At a stocking density of 10,000 per hectare, the annual requirement for milkfish fry exceeds 160 million. In addition, the

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demand for milkfish fry is increasing because young milkfish is found to be an ideal longlining bait in recent years and also because a great many milkfish fry and fingerlings often die of frigid weather during overwintering. Besides, there is unpredictable fluctuations in the occurrence of wild fry due to many environmental factors which further limit the quantity of stocking materials of milkfish.

Usually, only 85% of the total requirements of fry in Taiwan is captured from the wild. The deficiency is made up by importing fry from the Philippines and Indonesia. Recently, supply of seed in both countries are limited. In 1974, only 13% of the demand was collected in the Philippines and most milkfish ponds remained idle (Schmittou, 1975). Such a serious downward tendency will undoubtedly become a big problem in milkfish farming industry.

With a view to supplying an adequate quantity of fry, Tungkang Marine Laboratory initiated the preliminary work on artificial propagation of milkfish in 1970. In addition to capturing wild spawners, the Laboratory has been raising milkfish in cement tanks since then. After being reared for five years, one male and one female adult milkfish were dissected on 13 May 1975. Again on the sixth year of rearing, one male and one female were sacrificed on 11 April 1976. The results were highly encouraging. The male aged five years had mature sperm. The gonadal development of the female of six years of age was more advanced than that of five years. Its gonad weighed 21.20 g with the GSI of 0.66. Part of its ovarian oocytes was found to be at oil droplet (yolk vesicle) stage. Judging from the condition of said gonadal development, the feasibility of raising tank-reared milkfish as spawners and the prospect for their successful artificial propagation are fairly ensured.

Materials and Methods

The materials from the present study were wild milkfish fry collected in the shoreline of Tungkang coast in 1970, and had been reared in cement tanks at Tungkang Marine Laboratory for five and six years.

Two hundred fishes were reared in a rectangular cement tank (10 m x 20 m x 1.5 m deep) for the first three years. Thirty fishes were randomly selected from these and transferred to a round cement tank (8.25 m in diameter, and 1.5 m in depth) (Plate I-1). In the fifth year, one pair of fishes were dissected for investigation on gonadal development. Again, twenty fishes were chosen and continued to be reared in round cement tank of the same size.

The fishes were fed daily in the morning with a compound feed, the composition of which is given in Table 1. The amount given was just sufficient for their satiation.

Aeration was provided throughout the rearing period and water was placed when necessary. The replacement was made more frequently in summer because of the fast growth of phytoplankton. The fluctuation of average water temperature and salinity of rearing water in last one and a half year is shown in Fig. 1. The water temperature ranged from 19.2 to 29.8°C and salinity from 15.78 to 31.18 °/oo.

Table 1. Composition of compound feed.

Feedstuff	Fish meal	Rice bran	Yeast	Soy bean	Wheat flour	Soy bean oil	Multiple vitamins
%	25	20	20	15	19	0.5 ~ 1.0	0.1 ~ 0.2

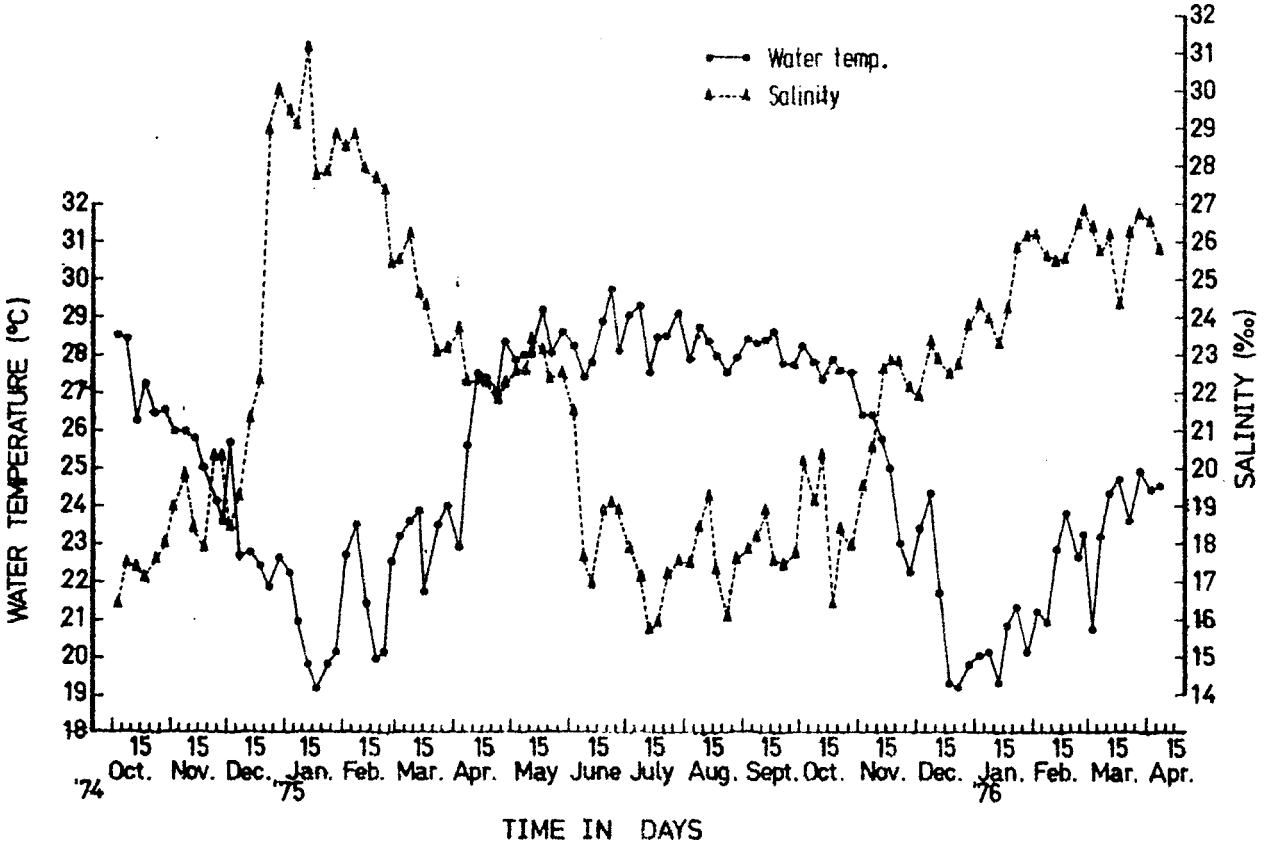


Fig. 1 The fluctuation of average water temperature and salinity of rearing water in late one and a half year.

Two pairs of milkfish were harvested respectively on 13 May 1975 and 11 April 1976 (Plate P-1). The external morphology and genital pores were carefully observed to distinguish the male from the female. They were then sacrificed. Measurement of body weight and body length was made before dissection and gonadal weight was also determined. The gonad was fixed in Bouin's fluid and stained with Delafield's hematoxylin and eosin for histological study.

Results

The sex of milkfish was fairly hard to be distinguished by their external characteristics as reported by Tampi (1957). Both during 1975 and 1976 a pair of milkfish could be selected with great difficulty and confirmation made only after the dissection.

The ovaries or testes of milkfish are more or less symmetrically developed, being suspended in coelomic cavity by mesovarium or mesorchium which connects their dorsal inner side with the peritoneal wall.

The ovary consists of numerous ovigerous lamellae where ovarian oocytes are present. The inner side of ovaries is surrounded with fibrous ovarian wall whereas ovigerous lamellae on outer side are exposed in coelom. As shown in Table 2 and Plates I-3 and I-5, the ovary of adult milkfish aged five years weighed 6.99 g with the GSI of 0.23 and most of the ovarian oocytes observed from the histological section of the ovary were found at the primary stage. Only 0.54% of the ovarian oocytes in the present study were at oil droplet (yolk vesicle) stage (Yamamoto et al., 1965; Hayashi, 1975) and the rest (99.46% were still at peri-nucleolus stage or even at the chromatin-nucleolus stage. The ovary of the tank-reared milkfish aged six years weighed 21.20 g with the GSI of 0.66. The ovary was yellowish orange incolor with distinctly separate eggs. The biggest ones measured 0.4 mm in diameter. Histological study of ovarian section showed that 8.41% of the ovarian oocytes were at oil droplet (yolk vesicle) stage and with some extra small eosinophilic granules while the rest (91.59%) were at the peri-nucleolus stage or even at the earlier stage. In other words, the ovary of milkfish aged six years is found comparatively more mature than that of five-year old female.

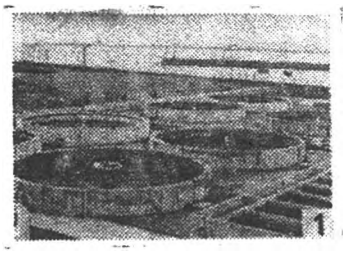
Table 2. Gonadal development of tank-reared adult milkfish.

Fish No.	Date	Sex	Age (yr)	B.W. (kg)	T.L. (cm)	G.W. (g)	GSI	Gonadal development
1	13-5-75	♀	5	2.99	71.95	6.99	0.23	Immature; 99.46% of oocytes at earlier and peri-nucleolus stage; 0.54% at oil droplet (yolk vesicle) stage.
2	13-5-75	♂	5	2.34	64.55	0.94	0.04	Mature; spermatogenesis in process with spermatocytes at various stages.
3	11-4-75	♀	6	3.20	70.04	21.20	0.66	Immature; 91.59% of oocytes at earlier and peri-nucleolus stage; 8.41% at oil droplet (yolk vesicle) stage.
4	11-4-76	♂	6	3.89	72.67	4.63	0.12	Mature; spermatogenesis in process with spermatocytes at various stages.

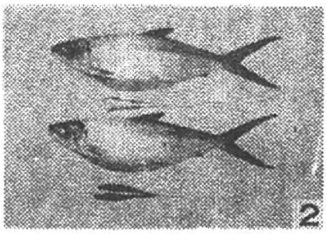
The testes were covered with a layer of tunica forming the smooth surface. The testes appeared tough, flat and greyish white in color. Each testes consists of numerous testicular lobules that have lumen inside and have spermatogonia in peripheral zone. As shown in Plates I-4 and I-6, there were mature spermatozoa and many spermatocytes at various stages of development in testicular lobules of milkfish aged five or six years. In fact, there was no distinct difference in the stages of maturity of testes between five years and six years old milkfish. The only difference was that the testes of the latter weighed heavier than those of the former.

Discussion

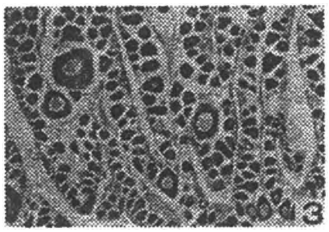
Tampi (1957) was the first scientist to report on this subject. He made a detailed and clear description of the structure of milkfish gonad. Comparing with the more popular classification of gonadal maturity stages as described by Yamamoto et al. (1965) and Hayashi (1972), the one



The round cement tank
for rearing the adult milkfish
(8.25 m in diameter, 1.5 m in depth)

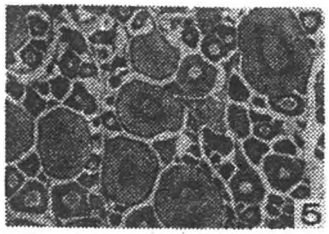


The adult tank-reared milkfish
(above, male; below, female)
and their gonads.
(Fish No. 3 and 4 in Table 2)

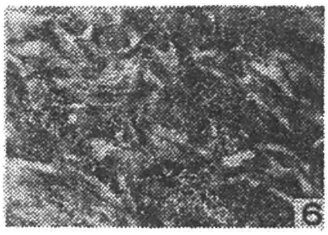


Section of the ovary of Fish no. 1
in Table 2 showing the small oocytes
at primary stage.

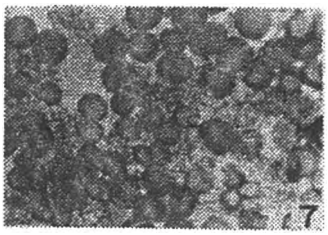
Section of the testis of Fish
no. 4 in Table 2.



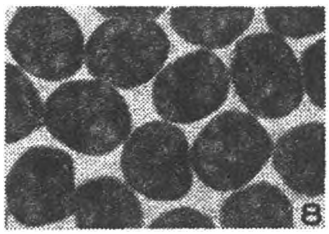
Oocytes at oil droplet (yolk vesicle)
stage and peri-nucleolus stage in the
ovary of Fish no. 3 in Table 2.



Section of the testis from Fish
no. 2 in Table 2.



Immature eggs (0.3 0.4 mm
in diameter) of Fish no. 3 in Table 2.



Nearly mature eggs (0.89 x 0.98
1.32 x 1.50 mm in diameter) from
Fish no. 1 in Table 3.

Plate 1

described by Tampi (1957) is somewhat broad; he classified it into four stages depending on the degree of development, for his convenience. Tampi's description is not complete probably because he did not come across the intermediate stages. Since there is no other paper that gives more confirmative and complete information on the gonadal development of milkfish, the classification of Yamamoto et al. and Hayashi has been followed in the present study.

Ovary of milkfish is of "gymnovarian type" but not "cystovarian type" as in most teleosts, while its testes is just like that of other teleosts,

The biggest ovarian oocytes of fish aged six years measured 0.4 mm in diameter and were found already at oil droplet (yolk vesicle) stage when vitellogenesis was ongoing as the important step towards attaining maturity. As shown in Table 2, the gonadal development of the six-year old female milkfish was found more advanced than that of five-year old one. This observation, supported by the GSI study by Kuo et al. (1975), suggests the likelihood of the oocytes reaching secondary yolk stage or tertiary yolk stage if they are reared for several more months in captivity. By that time, hormore treatment can be given based on the information obtained in an induced breeding of grey mullet done in this Laboaatory. Kuo et al. (1975) mentioned that receptive females should have the oocytes of the tertiary yolk stage between 0.7 and 0.8 mm in diameter. For inducing breeding of milkfish by hormone treatment, six milkfish of both sexes aged six years are being treated with hormone*. The feasibility of raising tank-reared spawners will be fully ascertained if these treated females reach fully ripe condition. The success will be a big breakthrough in artificial propagation of milkfish.

Liao (1971) observed that in coastal area of southern Taiwan, wild mature spawners of milkfish are captured during the period from April to June. However, there is no record so far of capturing them in the fall months. According to the occurrence of wild milkfish fry, there should be two spawning seasons, one in spring and the other in fall. Tampi (1957) reported that each milkfish normally spawns only once a year. Therefore, it might be conjectured that oocytes at oil droplet (yolk vesicle) stage in April of the six-year old tank- reared milkfish would reach secondary yolk stage or even tertiary yolk stage in fall of the same year. It is important to investigate the monthly gonadal development of milkfish reared in captivity.

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*No significant response was observed after the fifth injection on 24 April 1976. Fish are healthy and are continued to be treated.

Tampi (1957) pointed out that the age of wild female milkfish at first maturity is roughly between four and five years. However, our unpublished data on the gonadal development of tank-reared milkfish at different age, showed that the ovarian oocytes of fish under five years developed to peri-nucleolus stage only and oocytes at oil droplet (yolk vesicle) stage started to appear at the age of five years. As noted previously by Liao (1971), the minimum age of wild female milkfish at first maturity was five years in Taiwan. Age of tank-reared milkfish can be correctly ascertained based on the date of stocking. However, age of captured fish was determined from scale rings although some parts are not clear enough for reading. Therefore, further study in this respect is needed.

Tampi (1957) observed that the size of captured female fish at its sexual maturity is about 110 cm total length and body weight about 11.0 kg, which is larger than that observed in wild spawner in Taiwan (Table 3). Liao (1971) attributed the reason that probably different spawning stocks are there in Mandapam area and Taiwan area. As listed in Tables 2 and 3, the measurements of tank-reared spawners were smaller than those of wild ones. These data correspond more to that recorded by Kuo et al. (1975). As listed in Table 4, the fish being reared in a big pond for two to three years measured almost the same in total length and body weight as the fish of five years of the present study, but gonadal development of the former was far behind that of the latter. It is concluded that age of fish is the more important factor to sexual maturity than its size.

Table 3. Gonad weight and GSI of four adult milkfish captured from the coast of Southern Taiwan.

Fish No.	Date	Sex	Age (yr)	B.W. (kg)	T.L. (cm)	G.W. (g)	GSI	Remarks
1	5-6-70	♀	6	8.20	104.0	950.0	11.59	Almost mature with eggs 0.89 x 0.98 ~ 1.32 x 1.50 mm in diameter, captured from Heng-Chun.
2	22-4-71	♀	5	6.03	87.0	201.2	3.34	Immature with eggs 0.54 x 0.59 mm in average diameter, captured from Heng-Chun.
3	9-6-72	♂	6	7.69	98.7	482.1	6.27	Mature, captured from Nan-Jen Wan.
4	10-4-74	♂	6	9.50	100.0	416.5	4.38	Mature, captured from Nan-Jen Wan.

Table 4. Gonad weight and GSI of four milkfish cultured in pond near Tungkang.

Fish No.	Date	Sex	Age (yr)	B.W. (kg)	T.L. (cm)	G.W. (g)	GSI
1	3-7-75	♀	2	2.62	68.2	1.23	0.05
2	2-8-75	♀	3	2.18	-	1.97	0.09
3	2-9-75	♂	3	2.91	71.34	0.47	0.02
4	2-9-75	♂	2	2.42	-	0.44	0.02

Both measurement of body length, body weight and gonad weight and gonadal development of tank-reared spawners (Table 2) were less than that of wild milkfish of same age (Table 3). Development of the ovarian oocytes in the same tank-reared spawner were rather diverse just as in captive grey mullet (Pien, 1975) and this may vary the egg production. It might be caused by either ecological or physiological factors. Further study on the reproductive ecology of wild milkfish is also needed in order to improve the rearing techniques to accelerate their growth and gonadal development.

As shown in Table 2, both the male milkfish aged five years and six years had mature sperms, but little milt was obtained on stripping the testes of the latter only. However, their GSI was much lower than that of wild spawner (Table 3) and there was very limited amount of milt probably even not enough for the eggs of one female. Consequently, hormone treatment is needed to induce proper development as well as maturation of gonad that has reached the developmental stage as the experimental fish in the present study.

In recapitulation, male tank-reared milkfish is mature at the age above five years and may be stripped for milt through the help of hormone treatment. As for female milkfish of six years age, they are not ripe enough to be stripped but the gonadal development is encouraging. The smaller size of tank-reared spawners than the wild ones may cause some effect on their fecundity but is advantageous to handle and operate in artificial propagation. In addition, the domestication of tank-reared spawners is also an advantage in the process of hormone treatment. On the contrary, wild adult milkfish are highly excitable and extremely difficult to capture and handle without causing stress and shock which usually result in injury and death. It is concluded that the idea to start raising milkfish in captivity six years ago is of significance. There still exist many

unsolved problems such as improving the composition of compound feed lest the milkfish should be too fatty and finding out a dependable method to distinguish the male from the female adult milkfish. It is hoped to establish and complete the process of artificial propagation of the economically important milkfish through continuous effort in the near future.

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References

Hayashi, I. 1972. On the ovarian maturation of the Japanese sea bass, Lateolabrax japonicus. Japan J. Ichthyol., 19(4) : 243-254.

Kuo, C.M., C.E. Nash and D. Lin. 1975. Reproductive physiology of important finfish. The Oceanic Institute Annual Report 1975, 20-22.

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).

Pien, P.C. 1974. Histological studies on the gonadal development of the grey mullet, Mugil cephalus Linnaeus. Master's Thesis, Institute of Oceanography, College of Science, National Taiwan University (in Chinese).

Schmittou, H.R. 1975. Sabalo spawning. Proceedings of the National Bangos Symposium, Punta Baluarte, Philippines, July 25-28, 1975, 56-61.

Tampi, P.R.S. 1957. Some observations on the reproduction of the milkfish Chanos chanos (Forskal). Proc. Indian Acad. Sci. B46 : 254-276.

Yamamoto, K., I. Oota, K. Takano, and T. Ishikawa. 1965. Studies on the maturing process of the rainbow trout, Salmo gairdnerii irideus -- I. Maturation of the ovary of a one-year old fish. Bull. Japan. Soc. Sci. Fish., 31(2) : 123-132 (in Japanese with English abstract).