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Induced Spawning of Striped Mullet by Hormone Injection*

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The striped mullet, *Mugil cephalus* LINNAEUS, is one of the important food fishes of Taiwan. Its annual production amounts to 3,200 metric tons (ANONYMOUS, 1963), of which 61 percents are the so-called sea mullet captured mainly by purse seine along the southwestern coast of the island, and the remaining 39 percents are the so-called pond mullet raised in both fresh and brackish water ponds.

The sea mullet generally school up along the middle part of the western coast and move slowly southwards from November to February with the peak of migration in late December and early January. As they carry eggs, the indication is that this is a spawning migration. At present no spawning has been observed, nor have the eggs been collected in the adjacent seas of Taiwan, but it is believed that spawning possibly occurs off the shore of the southern most part of Taiwan over a broad area extending along the path of the warm Kuroshio Current (OSHIMA, 1921 and TUNG, 1959b). The great majority of the migratory fish are four or five years old with body lengths falling in two peaks of 42 and 45 cm. respectively (TUNG, 1959a). The eggs of these fish are of approximately the same development throughout the ovaries, measuring 0.68±0.015 mm. in diameter. They are massed in the ovary and are non-transparent with a yellowish radiate appearance in their surface. Apparently these eggs have not yet reached a stage of maturity to be fertilizable. The testes of the majority of the males in the fish school have attained the physiological maturation stage and bear motile sperms capable of fertilizing the viable eggs when they are discharged to the sea water by stripping.

The so-called pond mullet is one of the most important species of cultivated fishes in Taiwan. The fingerlings of this fish are customarily used to stock the fresh water ponds in combination with Chinese carps at the rate of 2,000 to 3,000 per hectare. They are also used to stock the brackish water ponds in the central

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western part of the Island where the milkfish, *Chanos chanos* (a very cammon species of brackish-water pond fish in the southwestern part of Taiwan as well as in the countries of Southeast Asia), are unable to survive the winter. These fingerlings are captured in the estuarine waters along the coast in the months from December to March. The total number of the mullet fingerlings required by the fish farmers of Taiwan is estimated at about 10 millions per year. In order to meet this great demand, controlled spawning of this fish and artificial rearing of the fry are considered to be the best means for fish seed production to meet the large need of the fish farmers. Furthermore, controlled reproduction of the fish may also make it possible to develop method for selective breeding of superior strains and for furnishing parasite- and disease-free fish seeds. This paper is a report of the preliminary results obtained from a series of experiments on induced spawning of the striped mullet by hormone injection conducted during the 1963 mullet season in Taiwan. The embryonic development of this fish observed from the external features of the eggs obtained from induced spawning is described in this paper.

Materials and Method

The test fish were collected in the sea on board purse seiners. They were placed in cages suspended in the sea and brought to port by a motor-boat. Immediately after the hormone treatment, they were released into holding boxes made of polythyene-fiber screens attached to a wooden frame. Each box had a capacity of two cubic metres, and several boxes were fastened in rows and anchored in shallow water. The collection and preservation of the pituitaries from the donor mullets, the extraction of the pituitary gonadotropins from the glands, and the techniques employed in administration of the preparation to the test fish were the same as in the previous work on Chinese carps (TANG, *et al*, 1963). The synergistic agent used in combination with the mullet pituitary extract was Synahorin produced by Teikoku Zōki, Tokyo. This preparation is a mixture of chorionic gonadotropin (CG) and mammalian pituitary extract. Artificial fertilization of the hand-stripped eggs was made by the dry method in plastic pans. The fertilized eggs were hatched in aquaria with circulation of sea water as well as in natural condition in shallow sea. The newly hatched larvae were reared under the same conditions as in hatching.

Results and Discussion

Many recent reports in medical research indicated that preparations composed of a mixture in a certain ratio of both the anterior lobe of pituitary and CG show stronger action than when they are used independently, and many of these preparations are available on the market. In a preliminary experiment, LIU, demonstrated (1963) that the pituitary of the common carp, *Cyprinus carpio*, combined with a certain amount of Synahorin gave better result in inducing spawning of the Chinese

carps than individual use of the common carp pituitary or Synahorin alone. This may be ascribed to the addition of the luteinizing factor (LH) to the hormonal components of the fish pituitary, as the similarity of CG to LH has long been recognized by endocrinologists. In order to increase the effectiveness of fish pituitary in precipitating ovulation in mullet, various doses of Synahorin have been used in combination with the preparations of fish pituitary extract in the present experiment.

The results of the experiment showed (Table 1) that two out of three female mullet showed positive response to the injections of 2.0 fish pituitaries combined with 40 rabbit units of Synahorin, whereas three fish, each receiving one to three pituitaries alone, did not ovulate at all. Although the data obtained from the present experiments are insufficient to establish that the addition of CG to fish pituitary increases its effectiveness in inducing spawning of mullet, the synergistic effect of

Table 1. The response of female *Mugil cephalus* L. to injection of hormonal materials (Temperature range during the experimental period was from 20.4°C to 24.8°C).

Dosage	Number[1] of injections	Number of fish injected	Response		
			Number ovulating	Number[2] partially ovulating	Number not ovulating
Control	-	-	-	-	3
1.0 Pituitary[3]	1	1	-	-	1
1.0 Pituitary+10 Rabbit Units Synahorin[4]	1	3	-	-	3
1.0 Pituitary+20 Rabbit Units Synahorin	1	3	-	-	3
1.0 Pituitary+40 Rbabit Units Synahorin	1	2	-	-	2
1.0 Pituitary	2	1	-	-	1
1.0 Pituitary+20 Rabbit Units Synahorin	2	3	1	1	1
1.5 Pituitary+20 Rabbit Units Synahorin	2	2	1	1	-
1.0 Pituitary	3	1	-	-	1
0.5 Pituitary+20 Rabbit Units Synahorin	3	2	-	1	1
1.0 Pituitary+20 Rabbit Units Synahorin	3	2	-	2	-

1) Intramuscular injections were made at 12-hours intervals, with the amount of each injection given in the table.
2) The partial ovulation was mostly caused by the death of the recipient fish just when they commenced ovulating.
3) Taken from the fish of the same species of comparable size.
4) A gonadotropic product composed of a mixture of chorionic gonadotropin and hypophysial extract.

this combined preparation on ovulation of fish should not be overlooked. No ovulations occurred when the fish received this combined preparation at dosage of less than 2.0 pituitaries plus 40 rabbit units Synahorin. However, when the combined dosage of pituitary and Synahorin exceeded the above mentioned level, complete or partial ovulations were obtained. From these experiments, the mixture of about 2.0 mullet pituitaries and 40 rabbit units Synahorin might be the threshold dosage for precipitating ovulation of these maturing mullets during their spawning migration

to the southwestern coast of Taiwan. It should be noted, however, that dosages will vary greatly with the physiological conditions of the test fish as well as with the experiment environments.

The greatest difficulty encountered in the course of this experiment was the high mortality of the test fish when they were held in captivity. Most of the hormone treated fish died within 48 hours after they received the initial injection of hormonal materials and while they were in the holding boxes, especially those whose abdominal region was greatly dilated due to the rapid increase of the size of the eggs as a result of the administration of hormonal substances. The test fish were usually of poor condition after they had been held in the boxes for 24 hours, and none of the captive fish including those used for the control were able to withstand confinement of more than 86 hours in the holding box throughout this series of experiment. It is believed that the response of mullets to hormone injection will be greatly improved if the fish are kept in better condition during the time of hormonal treatment. Much work should be done to improve the techniques for collection, transportation and holding of the fish, so that the spawners may adapt themselves to the confined environment and be in a normal physiological condition to receive the hormone substances.

The ovulated eggs are transparent, non-adhesive and spherical in shape, measuring 0.93 ± 0.032 in diameter. It has a single oil globule measuring 0.38 ± 0.020 in diameter and light yellow in color. These eggs are suspended in the circulating water in the aquarium, but sink to the bottom in standing water having a specific gravity of $\sigma 15=1.022$.

The external features of embryonic development of the striped mullet as observed are described in Table 2 and shown in photomicrographs from living specimens in Plates No. 1 to 22. Hatching took place in 59 to 64 hours at water temperatures ranging from 20.0° to 24.5°C and salinity from 24.39 to 35.29‰. The fertility of the eggs was extremely low. A rough estimate made by four sample examinations indicated that the percentage of fertilization was 32 and the rate of hatching was less than 10 percent.

The newly hatched larva measured 2.2 mm. in total length, with an oil globule situated in the posterior part of the yolk. The number of myotomes in this growing stage was very difficult to determine because of the dense distribution of melanophores throughout the entire body. These larvae were usually suspended in the surface layer of the water upside down. This is much the same as in other pelagic fish during this growing stage. Most of the larvae died two days after hatching, and none of them remained alive for over four days and grew beyond the prelarval stage. The dath may be caused by the rapid fluctuation of water temperature during rearing and/or by lack of appropriate food in their growing stage.

Table 2. The embryonic development of *Mugil cephalus* L.
(Water temperatures ranging from 20.0°C to 24.5°C from the beginning to the end of the hatching period)

Stage	Time after fertilization	Description of the external features
Two-Cell Stage	1 hour 30 minutes	1st cleavage (Pl. IV, No. 1, surface view).
Four-Cell Stage	1 hour 50 minutes	2nd cleavage (Pl. IV, No. 2, surface view).
Eight-Cell Stage	2 hours 10 minutes	3rd cleavage (Pl. IV, No. 3, surface view).
Sixteen-Cell Stage	2 hours 30 minutes	4th cleavage (Pl. IV, No. 4, surface view).
Thirty-Three-Cell Stage	2 hours 50 minutes	5th cleavage (Pl. IV, No. 5, surface view).
Late Segmentation Stage	3 hours 50 minutes	The blastodisc had become three-storied blastomeres (Pl. IV, No. 6, surface view).
	5 hours	The blastodisc was composed of many small blastomeres (Pl. IV, No. 7, surface view).
Blastula Stage	8 hours	The periblast had become distinct. The appearance of blastocoel could not be asertained without observation made by sectioning, but this was not done (Pl. IV, No. 8, surface view).
Early Gastrula Stage	9 hours 30 minutes	The blastodisc became larger and began to expand over the yolk sphere (Pl. IV, No. 9, side view).
Late Gastrula Stage	11 hours	The germ ring had been formed as the rim of the blastodisc had become thick and the central area had become thin (Pl. IV, No. 10, surface view).
	14 hours 30 minutes	The blastodisc had grown over about three-fourths of the yolk sphere. The embryonic shield had raised conspicuously (Pl. IV, No. 11, surface view).
Embryonic Body Formation Stage	15 hours	The embryonic body had become distinct in front part, but obscure in rear part. The KUPFFER's vesicles appeared (Pl. IV, No. 12, surface view).
Optic Vesicle and Myotome Formation Stage	16 hours 30 minutes	A pair of optic vesicles appeared and the myotomes numbered 3 to 4 (Pl. V, No. 13, surface view).
Otic Vesicle Formation Stage	17 hours 10 minutes	A pair of otic vesicles appeared. The number of myotomes were 6 to 7 (Pl. V, No. 14, surface view).
	32 hours 40 minutes	The melanophores appeared on the oil globule (Pl. V, No. 15).
Brain Differentiation and Pulsation Commencement Stage	33 hours 40 minutes	The fore brain, mid brain and hind brain were clearly differentiated. The heart had commenced pulsation. The optic cups and lenses appeared. The rear end of the embryo had separated from the yolk (Pl. V, No. 16).
	48 hours	The embryo had grown over about three-fourths of the yolk sphere and moved vigorously (Pl. V, No. 17).
Membranous Fin Formation Stage	50 hours	The embryo body covered approximately the whole yolk sphere. The membranous fin appeared in rear part of the body (Pl. V, No. 18).
	54 hours	The tail end of the embryo had grown to the optic cups (Pl. V, No. 19).
Hatching Stage	59 hours	Hatching with tail ahead from the chorion (Pl. V, No. 20). The embryo just emerged from the chorion (Pl. V, No. 21). Six hours after hatching (Pl. V, No. 22).

Résumé

Preliminary results obtained from a series of experiments conducted during the striped mullet (*Mugil cephalus* LINNAEUS) fishing season of 1963 indicated that the ripe striped mullets during their spawning migration to the southwestern coast of Taiwan could be induced to spawn by injection of hormonal materials.

The threshold dosage for precipitating ovulation of the female mullets was approximately at the level of 2.0 pituitaries taken from fish of the same species and of comparable size combined with 40 rabbit units of Synahorin (a gonadotropic product composed of a mixture of chorionic gonadotropin and hypophysial extract). The great majority of the males, however, did not require administration of hormones to furnish sperms for the ovulated eggs.

The embryonic development of this species observed from the external features is described and shown in photomicrographs of living specimens in this paper. The hatching of these eggs took place in 59 to 64 hours at water temperatures ranging from 20.0° to 24.0°C.

Most of the newly hatched larvae died two days after hatching, and none of them survived beyond the prelarval stage when reared either in aquaria or holding boxes in shallow sea.

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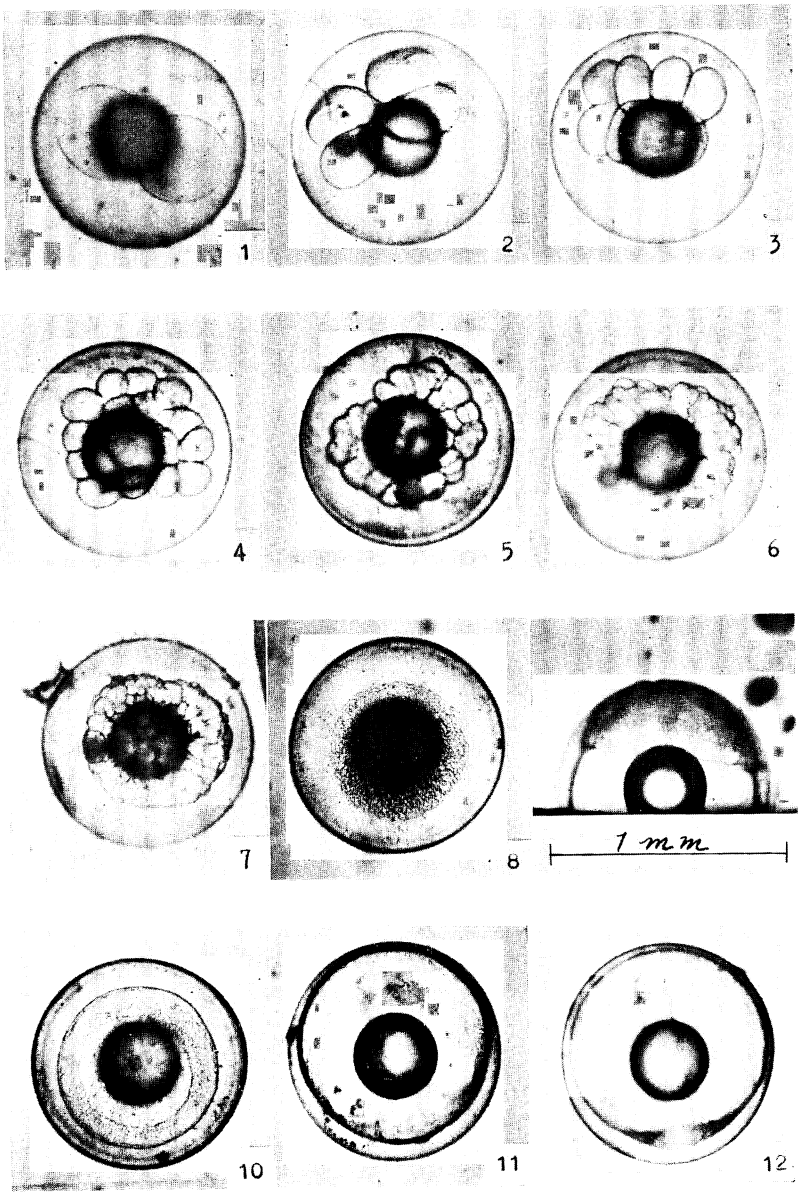
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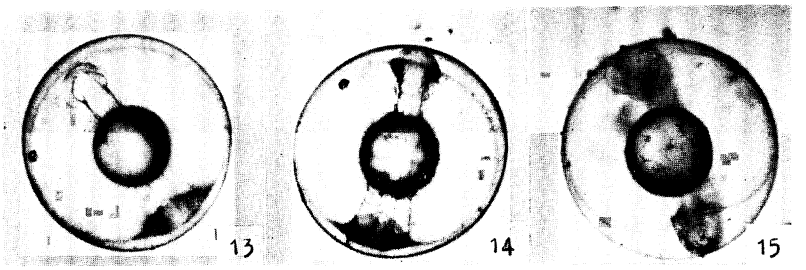
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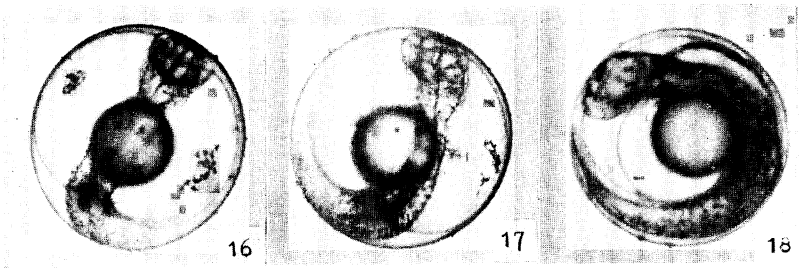
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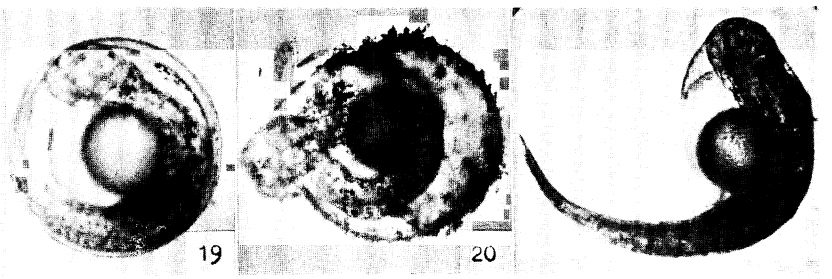
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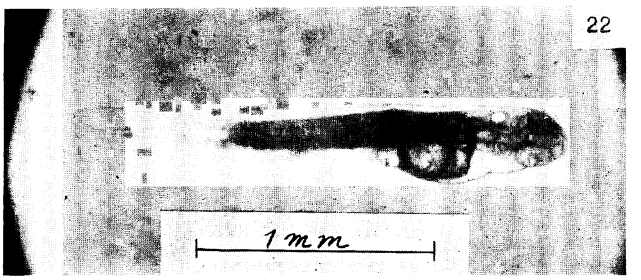
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