Artificial fertilization of eggs and early development of the milkfish *Chanos chanos* (Forskal)

H. Chaudhuri, J. Juario, Jurgenne H. Primavera, R. Mateo, R. Samson, Erlinda Cruz, E. Jarabejo, and J. Canto Jr.

Abstract

Hydrated eggs obtained from a female milkfish were artificially fertilized with the milt collected from a male injected with acetone-dried pituitaries of salmon. The fertilized eggs (1.1 to 1.25 mm in diameter) developed normally in seawater in basins and Petri dishes at a salinity of 30–34 ppt and successfully hatched in 25 to 28½ hours at a temperature of 26.4–29.9°C. The yolk was completely absorbed in about 2½ days and at this period many postlarvae died. A few larvae were reared up to 5 days but all died within 6 days. Effects of feeding the postlarvae from the third day with freshly hatched trochophore larvae of oysters obtained from eggs artificially fertilized in the laboratory could not be ascertained.

Introduction

The milkfish *Chanos chanos* (Forskal) is an important food fish and is extensively cultured in brackishwater ponds in Southeast Asia, particularly in the Philippines, Indonesia, and Taiwan. Since it does not attain maturity or breed in captivity, the only source of its seed is the coastal waters from where milkfish fry are collected during the spawning season. While the milkfish industry is rapidly expanding, the present supply of fry is inadequate to meet the growing demand. Thus, the urgent need to develop a dependable source of seed is now being realized as fishery scientists in a number of countries are engaged in research in inducing maturation and spawning in both pond-grown and wild milkfish by hypophysation.

Our knowledge on milkfish, especially on parameters such as spawning habits and spawning grounds, nature of eggs and the characters of early larvae, is meager. Since Delsman's description (1929) of probable milkfish eggs, there was hardly any information until Senta et al. (1976) reportedly collected a few eggs from a spawning ground near Batbatan island off Antique province in the Philippines.

Preliminary experiments carried out in Taiwan to induce maturation in tank-reared milkfish (Liao and Chang, 1976) have been partially successful. Earlier attempts made in the Philippines to gather information on mature milkfish from the wild and induce them to spawn by hormone injections were not successful (Angeles, 1968; Inland Fisheries Project Technical Report, 1974; Delmendo and Angeles, 1975). More recently, experiments carried out at the Oceanic Institute in Hawaii (Nash and Kuo, 1976) and at the SEAFDEC Aquaculture Department, Philippines (Vanstone, et al., 1976a) to induce spawning in adult milkfish captured from the wild by injections of semipurified salmon gonadotropin (SG-G100) resulted in the release of hydrated eggs from the injected females. These eggs, however, were not fertilized.

* Contribution No. 12 of the Aquaculture Department, Southeast Asian Fisheries Development Center, P.O. Box 256, Iloilo City 5901, Philippines.

The first breakthrough in inducing spawning in wild milkfish by hormone injections and successful hatching of artificially fertilized eggs has recently been achieved at the Pandan and Tigbauan breeding stations of the SEAFDEC Aquaculture Department (SEAFDEC Progress Report, 1977). While the details of the induced breeding experiments conducted by us are being published elsewhere, the present article gives an illustrated account of the artificial fertilization of eggs and the embryonic and larval development of milkfish.

Materials and Methods

Adult milkfish (sabalos) captured from the wild were used for the breeding experiments. Milkfish spawners migrate to the coastal waters of Panay island during the spawning season and are caught in the fish traps and *otoshi ami* (Japanese trap net) operated in the coastal area. Two fish traps in Hamtic supplied most of the sabalos used in the Pandan as well as the Tigbauan breeding stations. A fish trap operating in Tigbauan waters very close to the SEAFDEC Research Station supplied several spawners.

The breeding experiment was initiated at Tigbauan during the second week of April 1977. Milkfish spawners caught in the fish traps were transported in cages and brought ashore. Each fish was then guided into a plastic bag filled with seawater, and carried on a stretcher either to a small canvas tank in a pickup van for transport to the breeding station or carried directly and released into the 12-m diameter canvas holding tanks (Vanstone et al., 1976b). The spawners were immediately sexed (Chaudhuri et al., 1977) and the stage and size of ovarian eggs determined from a few eggs collected by inserting a narrow plastic cannula through the genital pore of the female. Fully ripe males were identified through the milt freely flowing out of the urogenital pore upon slight pressure on the abdomen. In case milt was not freely oozing from the male, the cannula was introduced through its urogenital pore to take a sample of milt to be examined for sperm motility. The dose of hormone to be injected was then determined from the stage of gonadal maturity and size of the spawners.

Artificial fertilization of eggs

On May 13, 1977 at 0100 hr, three sabalos were caught from the fish trap near Tigbauan. These were transferred to the transport cage and brought to the SEAFDEC Research Station at Tigbauan, Iloilo. During sexing, two of them were found to be females and one a male. The male was spent and it was not oozing any milt. One of the females weighing 8.6 kg appeared recently spawned as evidenced by the presence of a few hydrated eggs near the genital pore. A good number of eggs were then collected from the ovaries by inserting a cannula through the genital pore. The eggs thus collected were transferred to moist Petri dishes. Milt was also collected from a running male weighing 7.0 kg by using a cannula. This fish was caught from the same fish trap on May 12 and was injected at 2000 hr of the same night with 3 mL of physiological solution containing 25 mg of acetone-dried salmon pituitary gland. The "dry method" (Chaudhuri et al., 1966) was used in artificially fertilizing the extruded eggs with milt. After about 10 min, the fertilized eggs were diluted with a little water; after another 10 min they were diluted further and the excess milt was washed away. This procedure was repeated several times and the eggs were transferred to a basin with sufficient water. Artificial fertilization was successfully done at 0500 hr. At about 0800 hr, the same female was killed by punching the heart to bleed the fish after which the ovaries were taken out. The ovaries were almost spent and only a few thousand eggs were obtained. These were fertilized by sperm obtained through squeezing the testes excised from a dying male.

In the morning of June 22, 1977 two sabalos, one male and one female were caught from the same fish trap at Tigbauan. On examination the female (6.8 kg) was found to be recently spawned and was oozing hydrated eggs. The male (6.2 kg) was partially spent. At 0930 hr artificial fertilization of the eggs was done following the method described above. Both fishes were killed to obtain eggs and sperms.

Incubation of eggs

The fertilized eggs were kept in aerated seawater in basins. The salinity and temperature during the incubation period ranged from 30-34 ppt and 26.4 to 29.9°C, respectively. At 30 ppt the developing eggs settled at the bottom of the Petri dishes but when salinity increased to 34 ppt or above, they were bouyed up to the subsurface and the surface.

The stages in the development of eggs starting from early cleavage were examined through a microscope. Photomicrographs of important stages of segmentation, blastulation, differentiation of embryo, and hatching were taken.

Larval rearing

The newly hatched larvae were reared in aquaria, basins and beakers of seawater. These were supplied with feed after 2½ days. The feed consisted mainly of the trochophore larvae of oysters obtained by artificially fertilizing oyster eggs with sperms from ripe males. Occasionally, the milkfish larvae were also supplied with a small quantity of *Chlorella*. Water temperature and salinity were recorded at regular intervals throughout the period of larval and postlarval development. Photomicrographs of the newly hatched larva and subsequent stages were taken.

Results

The development of the fertilized eggs was studied in detail and the characters of the developing embryo, larval and postlarval stages of milkfish were noted (Table 1). The fertilized eggs obtained on June 22, 1977, developed only up to the early gastrula stage after which they started to disintegrate. This may have been due to the delay in the artificial fertilization of the hydrated eggs as these have remained in the abdominal cavity too long.

Fertilized eggs. The fertilized eggs were spherical, nonadhesive and transparent. The yolk had a light yellow tinge and no oil globule. Furthermore, it was granular and appeared to have fine segmentation. The sizes of the fertilized eggs ranged from 1.1 to 1.25 mm with a mean diameter of 1.13 mm. Each egg had a very narrow perivitelline space (Fig. 1). Eggs fertilized on June 22, 1977 had a mean diameter of 1.2 mm.

Embryonic development

Cleavage. The blastodisc appeared in about an hour after fertilization (Fig. 2) and the first cleavage dividing the blastodisc into two blastomeres (Fig. 3) occurred 75 min after fertilization. The second cleavage, perpendicular to the first followed within 10 min (Fig. 4). In another 15 min the 8-cell stage was reached (Fig. 5). The fourth cleavage, which appeared parallel to the second, was effected 10 min later and the 16-cell stage was obtained (Fig. 6). The 32-cell stage followed in the next 15 min (Fig. 7) and the sixth cleavage in another 20 min (Fig. 8). The 128-cell stage was reached in 2 hr and 55 min (Fig. 9). As successive cleavages occurred, the blastomeres further decreased in size (Fig. 10). The morula stage was reached 4 hr and 10 min after fertilization (Fig. 11).

Formation of embryo. The blastula is formed in about 1½ hr. The blastoderm cells start spreading over the yolk mass (Fig. 12) and at about 8 hr after fertilization, yolk invasion progressed considerably (Fig. 13). The egg reached the late gastrula stage 9 hr after fertilization when yolk invasion was two-thirds complete (Fig. 14). In another 45 min, gastrula was completed. The yolk plug and blastophore became visible and the embryonic streak was faintly indicated (Fig. 15).

Differentiation of embryo. In about 10½ hr after fertilization the embryonic rudiment became distinct with 5-6 somites (Fig. 16). Within 40-45 min the somites have increased to 9-10 in number and the Kupfer's vesicle appeared (Fig. 17). Head and tail were clearly differentiated 13 hr after fertilization and 19-20 somites could be counted (Fig. 18).

The optic and auditory vesicles were visible 14½ hours after fertilization and the embryo became C-shaped (Fig. 19). In another hour more myotomes appeared, the embryo developed further and looked like a girdle over the yolk (Fig. 20). Also at this stage brain differentiation progressed considerably. At 18½ hr after fertilization, the embryo further elongated and the tail started to separate from the yolk. The unpaired finfold appeared and the optic lens was indicated (Fig. 21). In another 1½ hr the head separated completely from the yolk and the heart beat was discernible. The tail became further elongated, the yolk was reduced and the optic lens became distinctly visible (Fig. 22). The embryo developed further and twitching movements of the body could be observed approximately 24 hr after fertilization (Fig. 23). In another 4 hours the yolk was further reduced and the tip of the tail extended nearer to the head. At 28 hr after fertilization the embryo was fully differentiated and ready to hatch (Fig. 24).

Hatching. The elongated tip of the tail struck against the head end of the egg shell causing the latter to break; the head came out first. The larva shook off the shell and emerged completely from the egg case (Figs. 25-28).

Larval development

Newly hatched larva. The milkfish embryo hatched 28½ hours after fertilization at water temperature ranging from 26.4-29.9°C. The second batch of fertilized eggs took only 25 hours to hatch. The newly emerged larva had unpigmented eyes and no fin buds; the mouth was not formed and the anus was situated posterior to the yolk mass. The yolk sac was broad and projected anteriorly near the head end of the larva (Fig. 29 and 30).

The newly hatched larva was 3.2 mm in length (preserved specimens 2.8-2.9 mm.) The yolk was about 2.15 mm long and 0.5 mm wide. Black pigmented cells were present in the finfold except the tip of the caudal region. There were pigments sparsely scattered in the yolk mass and on the head and body of the larva.

The larva swam slowly up to the surface of the water and then gradually dropped down and remained suspended in the column of water in an oblique position with head down and belly up. Occasionally the larva went up and came down in the characteristically inclined posture.

2-hr old larva. The 2-hr old larva (Fig. 31) was about 3.4 mm long. The length and maximum width of the yolk was 2.3 mm and 0.45 mm, respectively. The width of the body was 0.65 mm with preanal length of 2.6 mm. About 34-35 preanal mytomes could be observed. Melanophores were scattered on the yolk and a few could be seen on the head. The distribution of pigment cells on the finfold was almost the same as in the newly hatched larva.

10-hr old larvae. The 10-hr old larva (Fig. 32) was about 3.8 mm in length. Preanal length was 2.8 mm. The greatly reduced yolk was 2.1 mm long and 0.35 mm wide (maximum). The larva still remained suspended in the water column in the oblique position and occasionally went up and down.

24-hour old larva. The day-old larva measured about 4.0 mm in length. The yolk sac was reduced considerably. Faint pigmentation in the eyes commenced. The hindgut was clearly visible. Melanophores were scattered on the head and trunk and also on the dorsal finfold.

30-hour old larva. The 30-hour old larva (Fig. 33) was about 4.3 mm long and its preanal portion was 3.2 mm in length. About 34 myotomes could be counted up to the anus. The mouth was indicated and the yolk further resorbed. The pectoral finbud had also appeared..

48-hour old larva. The two-day old larva (Fig. 34) was about 5.0 mm in length. The yolk was very much reduced. The eyes were fully pigmented and pectoral fins elongated. The mouth was fully formed and the esophagus was distinctly visible. The pigment cells on the unpaired finfold had almost disappeared. There was pigmentation on the dorsal part of the body, and denser on the caudal peduncular region. There were a few dense melanophores over the posterior portion of the gut.

56-hour old larva. The 56-hour old larva (Fig. 35) was similar in size to the 48-hour old larva. The remnant of the yolk sac was seen only as a slender streak. The mouth was open and the intestine fully formed. The optic lens was shiny.

There was a row of melanophores along the dorsal edge of the myotomes and another row over the intestine along the ventral edge of the myotomes. Only a few pigment cells were present in the unpaired finfold near the beginning of the caudal portion of the finfold.

The fry made slow directed movements with occasional jerks and swam on the surface as well as at the bottom of the water column.

Postlarval development

72-hour old larva. The 3-day old larva (Fig. 36) measured the same length of about 5 mm. The larva slowly moved up and down and occasionally rested obliquely in the water column or crept at the bottom. Sometimes it nibbled on the bottom. The larva, photographed and preserved, had a speck of food in the intestine. One row of melanophores was present along the dorsal edge of the myotomes and another row along the ventral edge. Only 32 preanal myotomes could be observed.

81-hour old larva. The 81-hour old larva (Fig. 37) swam slowly on the water surface and occasionally went down. No further differentiation in the body or in the unpaired finfold could be observed. The dorsal as well as ventral rows of melanophores were distinct.

141-hour old larva. The differentiation in the 6-day old larva (Fig. 38) was not appreciable. A faint indication of the anal rays was present. The dorsal row of melanophores was scattered and considerably reduced in number; the pigments were denser posteriorly. The ventral row of melanophores was distinct and very dense over the posterior end of the hindgut.

Table 1. Embryonic and larval development of milkfish in seawater at temperature 26.4–29.9°C and salinity of 30-34 ppt.

Figure Number	Stage of Development	Time After Fertilization/Hatching (hr/min)	Description of Egg/Embryo/Larva
1	Fertilized egg (Diagram)	00:00	Spherical, nonadhesive, transparent, granulated yolk (yellow tinge); no oil globule; average size 1.13 mm.
2	1-cell stage (Diagram)	01:00	Formation of blastodisc.
3	2-cell stage (Diagram)	01:15	1st cleavage; meridional.
4	4-cell stage (Diagram)	01:25	2nd cleavage; perpendicular to 1st.
5	8-cell stage	01:40	3rd cleavage.
6	16-cell stage	01:50	4th cleavage.
7	32-cell stage	02:05	5th cleavage.
8	64-cell stage	02:25	6th cleavage; blastomeres much smaller.
9	128-cell stage	02:55	7th cleavage.
10	Many cell stage	03:35	Late cleavage
11	Morula stage	04:10	Mulberry-like blastodisc consisting of many minute cells.
12	Late Blastula stage	06:00	Blastoderm starts spreading over the yolk.
13	Midyolk invasion stage	08:00	Yolk invasion is about half complete
14	Late-yolk invasion stage	09:00	Gastrulation is in progress; yolk invasion two-thirds complete
15	Gastrula stage	09:45	Yolk invasion complete; yolk plug and blastopore visible; embryonic streak faintly indicated.

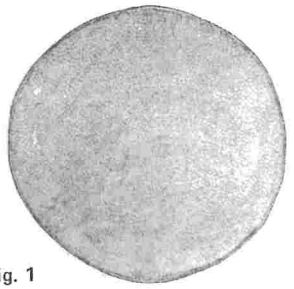


Fig. 1

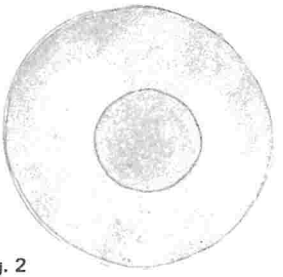


Fig. 2

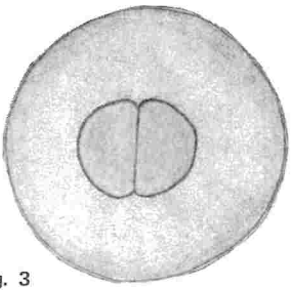


Fig. 3

0.5 mm

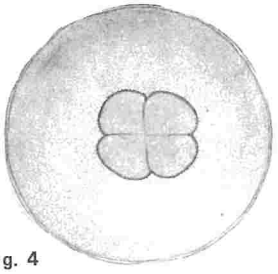


Fig. 4

Fig. 5

Fig. 6

Fig. 7

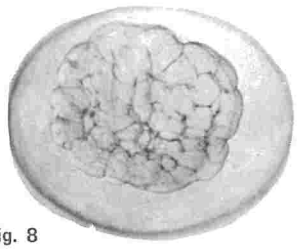


Fig. 8

Fig. 9

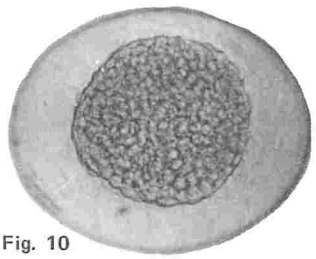


Fig. 10

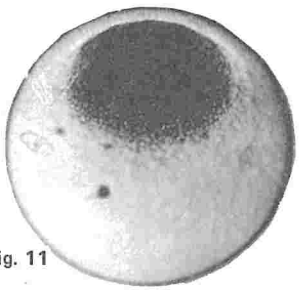


Fig. 11

Fig. 12

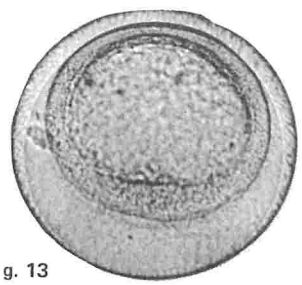


Fig. 13

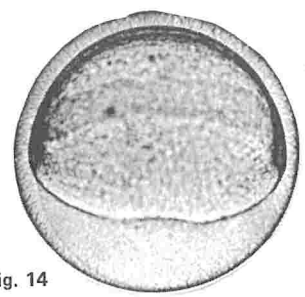


Fig. 14

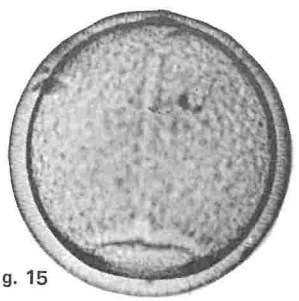


Fig. 15

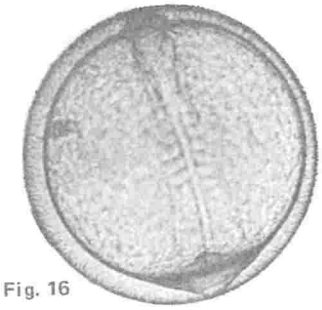


Fig. 16

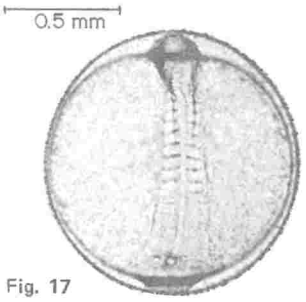


Fig. 17

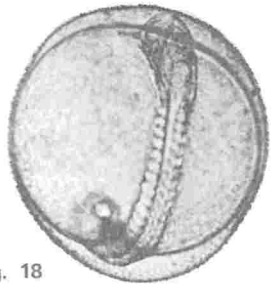


Fig. 18

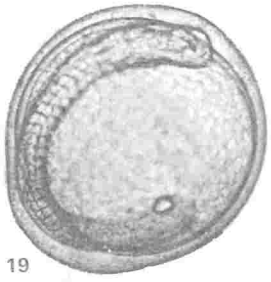


Fig. 19

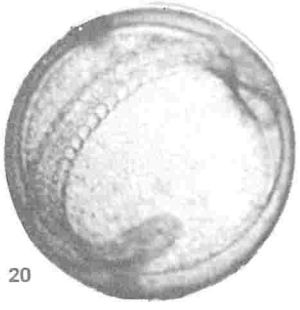


Fig. 20

Fig. 21

Figure Number	Stage of Development	Time After Fertilization/Hatching (hr/min)	Description of Egg/Embryo/Larva
16	Neurula stage	10:35	Embryonic rudiment with 5-6 somites.
17	Differentiation of embryo	11:15	Embryo with 9-10 somites; Kupfer's vesicle visible.
18	–do–	13:00	Embryo with 19-20 somites; head and tail distinctly differentiated.
19	–do–	14:30	C-shaped embryo; optic cups and otic vesicles visible.
20	–do–	15:40	Embryo elongates; forms a girdle over the yolk.
21	–do–	18:30	Unpaired finfold appears; tail starts separating from the yolk.

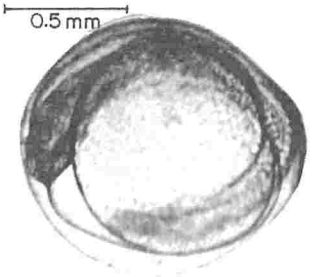


Fig. 22

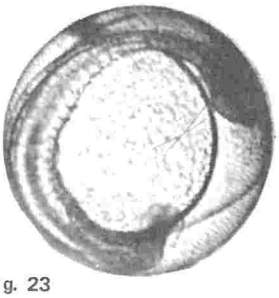


Fig. 23

Fig. 24

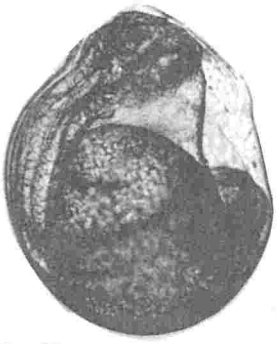


Fig. 25

Fig. 26

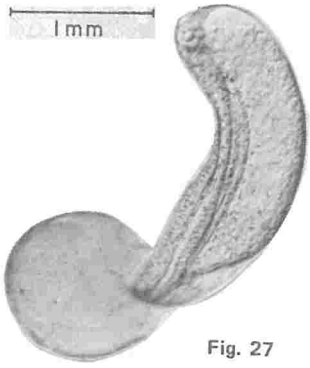


Fig. 27

Figure Number	Stage of Development	Time After Fertilization/Hatching (hr/min)	Description of Egg/Embryo/Larva
22	Differentiation of embryo	20:00	Tail elongates; heart pulsation observed.
23	–do–	24:00	Embryo starts twitching movement of the body.
24	–do–	28:00	Embryo fully formed; ready to hatch.
25	Hatching of embryo	28:20	Head end of the eggshell breaking off.
26	–do–	28:28	Head is first to emerge out of the egg shell.
27	–do–	28:29	Embryo emerges out of egg shell; tail end still inside.

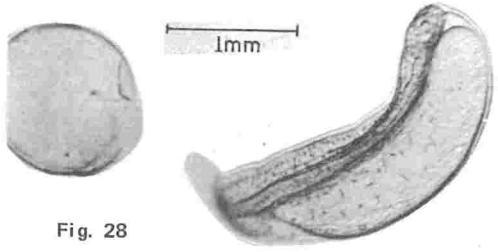


Fig. 28

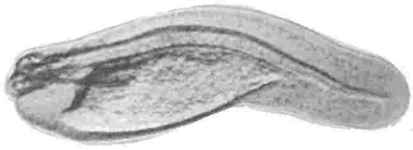


Fig. 29

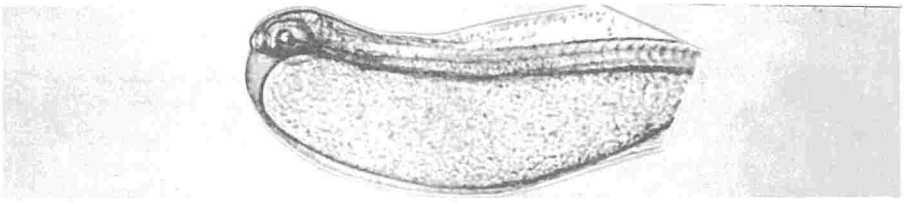


Fig. 30 0.5mm

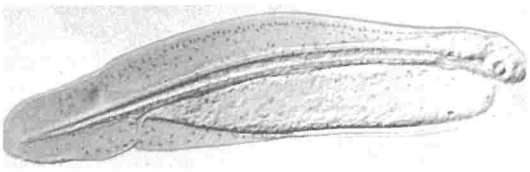


Fig. 31 1mm

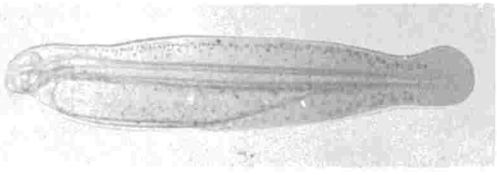


Fig. 32 1mm

Figure Number	Stage of Development	Time After Fertilization/Hatching (hr/min)	Description of Egg/Embryo/Larva
28	Hatching of embryo	28:29	Larva is completely out of the egg case.
29	Newly hatched larva	28:30	Hatchling with broad yolk mass; eyes transparent; mouth not formed; pigment cells on finfold.
30	Newly hatched larva	28:30	Anterior portion of larva enlarged.
Incubation period: 25 to 28½ hr after fertilization			
31	Larval stage	2-hr old larva	Body and yolk elongates; pigment cells on the body, yolk and finfold.
32	–do–	10-hr old larva	Yolk much reduced; 34 preanal myotomes.

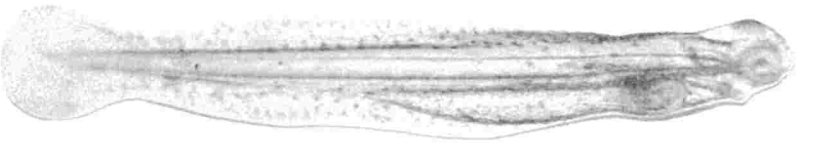


Fig. 33

1 mm

Fig. 34

1 mm

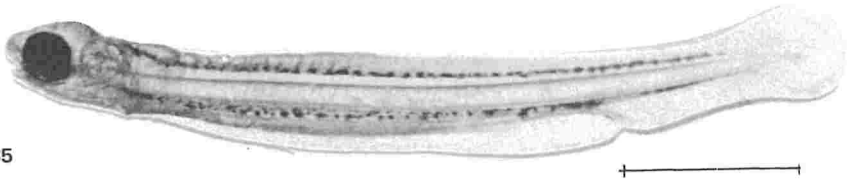


Fig. 35

1 mm

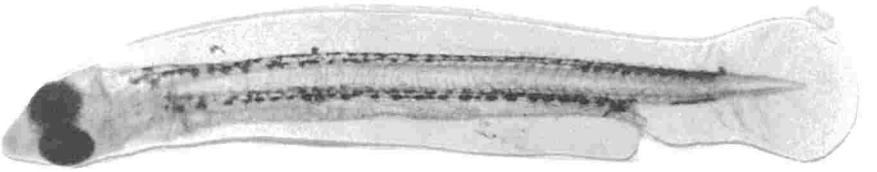


Fig. 36

1 mm

Figure Number	Stage of Development	Time After Fertilization/Hatching	Description of Egg/Embryo/Larva
33	Larval stage	30-hr old larva	Eye pigmentation commence; mouth indicated; pectoral finbud appears.
34	–do–	48-hr old larva	Yolk further absorbed; eyes fully pigmented; pectoral fins elongate.
35	–do–	56-hr old larva	Yolk sac almost absorbed · only a slender streak left; mouth opens.
36	Postlarval stage	72-hr old larva	Yolk completely absorbed; started feeding; a speck of food item seen in the gut.

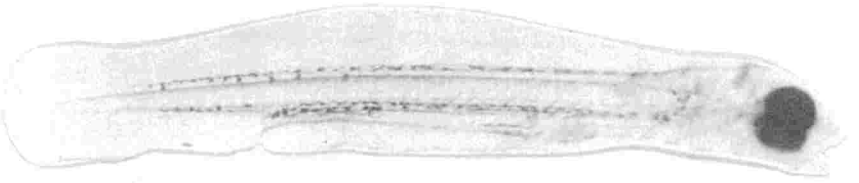
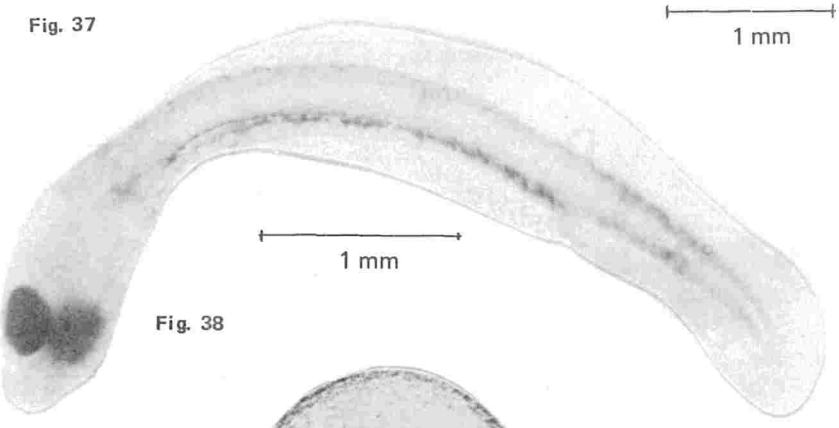


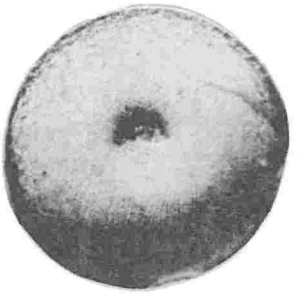
Fig. 37

1 mm



1 mm

Fig. 38



1 mm

Fig. 39

Figure Number	Stage of Development	Time After Fertilization/Hatching	Description of Egg/Embryo/Larva
37	Postlarval stage	18-hr old larva	A dorsal row and a ventral row of melanophores are present; dense pigmentation over the posterior portion of the hindgut.
38	—do—	141-hr old larva	Dorsal row of melanophores reduced considerably in number; ventral row prominent.
39	Patterns marking the egg shell give it a segmented appearance. Similar markings were observed in eggs preserved in Bouin's fluid.		

Larval rearing

Since the mouth and the intestine were fully formed in the 56-hour old larvae and the yolk sac was almost absorbed, these were given newly hatched trochophore larvae of oysters as feed. The eggs of oysters were artificially fertilized in the laboratory by sperms obtained from male oysters. A few mL of *Chlorella* were also introduced along with the trochophore larvae. However, there were larval mortalities during this critical period when the yolk was completely absorbed and the larvae started to feed.

The milkfish larvae were also fed with trochophore on the fourth day. But the majority of the 4-day old larvae died. The dying ones were found to be infested with ciliates which got into the aquaria and basins where the larvae were reared along with the trochophore larvae. A few milkfish larvae still alive on the fifth day were transferred to culture dishes and Petri dishes.

On the sixth day only 2 larvae were alive. These were kept at a salinity of 29-30 ppt and 24-26^{o}C temperatures. However, both died when they were 141 hours old.

Discussion

The success in artificial fertilization of milkfish eggs has bridged a big gap in our knowledge on the nature of its eggs and larvae. The study supports the observations made by Delsman (1926; 1929) about 50 years ago on the characters of milkfish eggs and larvae.

Available information on the maturity, spawning habits, eggs and larval development of milkfish is very scanty. Tampi (1957) was the first to make detailed observation on the milkfish gonads. He found the average diameter of a mature intraovarian egg to be 0.8 mm. Delsman (1929) found 0.7 mm as the average diameter of a mature milkfish ovarian egg. Vanstone et al. (1976a) and Nash and Kuo (1976) injected mature females with ovarian eggs 0.8 mm in size and obtained hydrated eggs 1.2 mm in diameter. In our experiments on induced breeding of milkfish, routine examination of the ovarian eggs of female spawners was done by collecting egg samples by a cannula inserted through the genital pore. Females having ovarian eggs of 0.7 mm in diameter or more were ordinarily selected for hormone injection.

Fertilized eggs

Our observations on the fertilized milkfish eggs agree mostly with those described by Delsman (1926) and Chacko (1950). Delsman (1926, 1929) in his description of milkfish eggs, however, did not mention any perivitelline space. We observed a very narrow perivitelline space which is hardly discernible in many eggs. Another interesting feature that we observed, especially, in the second experiment was the prominent marking patterns visible in the egg shell itself giving it a segmented appearance (Fig. 39). At first, we thought the segmented appearance was due to abnormalities in the hydrated eggs left in the abdominal cavity for a relatively long time. However, similar markings were also observed in the hydrated (preserved in Bouin's fluid) and developing eggs in the first experiment (Figs. 11-16 and 23). The markings were very faint in most of the developing eggs and could not be clearly observed in the shells of embryos about to hatch. Such characteristic markings are also present on the surface of the egg shells of the silver mullet *Mugil curema* (Anderson, 1957), yellow fin menhaden (Reintjes, 1962) and *Mugil cephalus* and *Liza troschelii* as observed by one of us (Chaudhuri).

Bouyancy of eggs

Our observations on the bouyancy of milkfish eggs were not conclusive. While the unfertilized eggs quickly settled at the bottom of the containers, the fertilized eggs were observed to go down slowly at a salinity of 30 ppt. When salinity was increased to 34 ppt or above, however, the eggs either stayed at the subsurface or floated at the surface of the water.

Delsman (1929) described the milkfish eggs as pelagic. Senta et al. (1976) made similar observations. According to the latter, live milkfish eggs floated to the water surface when placed in a glass jar. The observations of different authors, however, are varied with regard to the bouyancy of marine fish eggs. The grey mullet *Mugil cephalus* has pelagic eggs, slightly smaller (0.93-0.95 mm) than milkfish eggs and having a big oil globule (0.38 mm). Liao (1975) mentioned that the fertilized grey mullet egg is bouyant and stays afloat near the surface of water with slight aeration but settles down slowly in still water. Tang (1964) observed that the eggs were suspended in the circulating water but sank to the bottom in standing water, while Yashouv (1969) reported the sinking of mullet eggs towards the end of incubation. According to Kuo et al. (1973), the majority of eggs which sink are unfertilized. The bouyancy of eggs, however, depends on several factors. May (1974), for example, found that the eggs of the sciaenid fish *Bairdiella icistia* fertilized in lower salinities were larger and more bouyant than eggs fertilized in higher salinities. Furthermore, Blaxter (1969) observed that the pelagic eggs of most marine fishes sink when put in salinities below a certain threshold.

Incubation period

The incubation period for eggs depends largely on temperature. According to our observations, milkfish eggs hatched in 25 to 28½ hours at a temperature range of 26.4-29.9°C. Delsman (1929) observed that milkfish eggs from catches made in the morning hatched in the evening of the same day. Taking into account the short incubation period of the pelagic eggs of related species, he concluded that the eggs had been shed 24 hours prior to hatching (Schuster, 1960). Delsman and Hardenburg (1934; cf Schuster, 1960), stated that spawning took place in the evening from 8 to 10 hours and the eggs developed in about 24 hours. Senta et al. (1976) likewise found that most of their collected eggs hatched in the evening. The two recently spawned females observed by us appear to have spawned sometime before midnight since they were caught in the trap shortly after midnight. May (1970) stated that the incubation period in *Bairdiella icistia* eggs depended both on temperature and salinity. The duration of hatching tended to be greater at lower salinities and temperatures. Grey mullet eggs hatched in 59 to 64 hours at 20.0-24.0°C (Tang, 1964). Liao (1975) reported that the hatching of grey mullet eggs took place in 34 to 38 hours at 23-24.5°C temperature and 49 to 54 hours at 22.5-23.7°C at the salinity of 30.1-33.8 ppt. Chaudhuri (1968) mentioned that the incubation period of *Mugil cephalus* in India is 48 hours at 22.5°-23.5°C. Kuo et al. (1973) hatched grey mullet eggs in 36 to 38 hours at 24°C and in 48 to 50 hours at 22°C. Compared to that of the mullet, the period of incubation in milkfish is generally much shorter.

Newly hatched larva

Our observations on the characters of newly hatched milkfish larva agree well with the description of Delsman (1929) except on one point. While he stated that the head does not project in front of the yolk in the newly hatched larva, we, on the contrary found that the yolk mass does not reach as far anteriorly as the tip of the head. The length of the newly hatched larva

is 3.2 mm as compared to that of Delsman's which is about 3 mm. A great variation in the size of newly hatched larvae have been observed in many fish species. In the grey mullet the size of newly hatched larvae varies from 2.2 to 3.5 mm. While Tang (1964) obtained 2.2 mm long larva, Kuo et al. (1973) reported the size as 2.65 mm, and according to Liao (1974) newly hatched grey mullet larvae measure from 2.8 to 3.5 mm in length. The newly hatched larvae of milkfish as well as the grey mullet are more or less at the same stage of development, having unpigmented eyes, scattered melanophores and without findbuds or mouth parts. Kuo et al. (1973) observed that newly hatched mullet larva when inactive remains suspended in water column in an inclined position with the ventral side oriented towards the surface; when in motion, it jerks, rights its position, darts rapidly upward then sinks to its resting position. We have observed similar movements in the newly hatched milkfish larva.

Larval rearing

The larval development of milkfish that we have observed is generally in agreement with those described by Delsman (1929). The growth of the larvae was initially very fast and continued for 2 days during which the yolk was steadily resorbed. From the 3rd day until the 6th day when all larvae died, there was practically no further growth. Similar observations were made by Kuo et al. (1973) on mullet. They also observed that the 2nd growth period coincided with the onset of larval feed (5th-8th day) and intensification of feeding (9th-12th day).

The mouth was fully formed in 2-day old milkfish larva and complete resorption of yolk was effected in 2½ days. Delsman (1929) had also made similar observations. In grey mullet, formation of the mouth occurred on the 3rd day (Liao et al., 1971) and 2nd to 3rd day (Kuo et al., 1973) and the yolk was completely absorbed by the 5th day. Both Kuo et al. (1973) and Liao et al. (1971) mentioned two "critical" periods in mullet larvae when very high mortalities occur. While the former indicated the 2nd to 3rd day coinciding with the opening of the mouth and the 8th to 12th day preceding its second growth phase, the latter reported the two critical periods as the 3rd day and 11th to 13th day, respectively. We observed the first critical period to be the 2nd to 3rd day which coincided with the complete resorption of yolk and full formation of mouth and intestine. Majority of the larvae died during this period. Senta et al. (1976) could rear only one larva beyond the critical period from the collection of eggs which hatched.

This problem of heavy mortality in rearing marine fish larvae has baffled fishery scientists for decades. Attempts were made to overcome this difficulty by providing suitable food for the larvae in adequate quantities. May (1970) made a review of various available larval food and discussed the major food types used by fish breeders. Mullet breeders had to face the difficult task of feeding mullet larvae to obtain higher rates of survival. Concerted efforts are being made along this line during the current decade. Liao et al. (1971) mentioned the quality, size, density and mobility of the food as the important factors for developing larval rearing techniques. They used eggs and trochophore larvae of oysters for feeding the mullet larvae during the first few days. The density was maintained as high as 400-500 organisms/mL. This was followed by rotifers and copepods as the larvae grew and *Artemia salina* was provided to the bigger larvae. "Green water" containing *Chlorella* and other algae was tried initially without much success. Kuo et al. (1973) used veliger larvae of both oysters and sea urchin followed by day-old nauplii of *Artemia salina*. The survival rate was improved by supplementary feeding with marine plankton from the 3rd day onward. Prepared feeds such as cooked egg yolk have been successfully fed to many marine fish larvae but these generate problems of water fouling due to decay of leftover food. In our

experiments, the milkfish larvae were provided with trochophore larvae of oysters from the 3rd day but how far they utilized these feeds could not be ascertained.

Besides the nonavailability of the right type of feed, there are other causes of mortality such as high temperatures and high salinities which should be carefully avoided while rearing larvae. Higher temperatures generally increase the metabolic rate and accelerate yolk abosorption, and hasten death by starvation while high salinities may increase larval mortality by causing osmotic or ionic changes in the interior milieu (May, 1975).

Due to wholesale mortality of the larvae when about 6 days old, it was not be possible to rear them to the stage (10-15 mm) when similar larvae are collected by fry gatherers from the coastal waters. However, one interesting point about the milkfish larvae is that, unlike their nearest relatives, namely, the 10-pounder *Elops saurus* (Family: Elopidae) and the tarpon *Megalops cyprinoides* (Family: Megalopidae), which have the characteristic ribbon-shaped leptocephalus stage (Alikunhi and Rao, 1951), the milkfish larvae do not pass through such stage (Delsman, 1929).

In the foregoing, we have discussed our observations on milkfish eggs and larvae and compared these with the observations of other workers. In the absence of adequate information on milkfish eggs and larvae, comparison has been made with the other important food fish, namely, the grey mullet *Mugil cephalus* whose eggs and larvae are akin to those of *Chanos chanos* and about which considerable information is available. Mullet rearing has been a problem of the last decade. It is hoped that the information available on the rearing of mullet larvae will be of great help in solving the problem of feeding milkfish larvae and developing the technique of commercial production of milkfish seed.

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