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Title	Breeding and larval rearing of the milkfish <i>Chanos chanos</i> (Pisces: Chanidae).
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(Pisces: Chanidae)**

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**Abstract**

Two sexually maturing female milkfish were captured in April 1977 and induced to spawn by means of acetone-dried Pacific salmon pituitary powder. The eggs were fertilized and incubated and the resultant young reared to 74-day old, 11 cm long fingerlings. Newly fertilized eggs averaged 1.16 mm in diameter and each had a narrow perivitelline space containing several cortical granules which disappeared within a few minutes. The yolk was slightly yellowish, devoid of oil globules and very finely granulated. Embryonic development was very similar to that of other pelagic fish eggs and hatching occurred between 35 to 36 hr at a salinity of 32 ppt and a temperature range of 28.4-29.2°C.

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## Introduction

The milkfish *Chanos chanos* (Forsk.) is the most extensively cultivated fish in coastal ponds in Indonesia, the Philippines and Taiwan. For centuries milkfish larvae have been collected in certain coastal areas and reared to marketable size in fishponds and more recently in fishpens.

While milkfish culture has been well documented, very little is known about the life of the milkfish in the wild. Delsman (1926, 1929) obtained embryonic eggs which he believed to be of milkfish during horizontal surface plankton tows in the Java Sea. The eggs hatched within 12 hr of collection but the larvae died on the 4th day upon reaching a length of about 5 mm. Delsman also obtained several pelagic larvae ranging in size from 6 to 12 mm and confirmed that the larger ones were milkfish by rearing some for 38 days.

Juvenile milkfish are occasionally captured in lagoons and mangrove swamps and there are areas where this species inhabits freshwater lakes for the first few years of life (Delmendo and Angeles, 1969). While adult milkfish occur in the tropical waters of the Indian and Pacific Oceans, from the east coast of Africa to the coasts of southern California and Mexico, their migration at sea is completely unknown and the location of the spawning grounds has been the subject of varied reports (Fowler, 1941; Schuster, 1960).

Senta et al. (1976) suggested that one spawning ground of milkfish in the Philippines is the waters adjacent to Batbatan island off the coast of Antique province, Panay island. This has been confirmed by Kumagai et al. (in preparation). Although milkfish has never been spawned in captivity, Liao and Chang (1976) reported that sexual maturation had occurred in 5-year-old and 6-year-old tank-reared male milkfish. The testes of these males contained mature sperm but little milt. The ovaries of similarly reared females contained eggs at the yolk vesicle stage. Nash and Kuo (1976) and Vanstone et al. (1976) reported that nonhydrated milkfish eggs had a mean diameter of 0.8 mm while eggs which had been induced to hydrate and ovulate were 1.2 mm in diameter. In both cases adult milkfish were obtained from the wild and induced to ovulate with semipurified salmon gonadotropin, SG-G100.\* The hydrated eggs were not fertilized.

Fertilization of milkfish eggs and rearing of resultant larvae to the fry stage were achieved during the 1977 spawning season and are the subject of this report.

## Materials and Methods

### Experimental Fish

Early in the morning of April 14, 1977 several adult milkfish were captured in 1 of the 2 fish corrals located at Hamtic, Antique, on the southwest coast of Panay island (Fig. 1). Groups of 10 to 12 fish were placed in holding transport cages (3x1.5x1.5 m) constructed of bamboo or 5 cm PVC pipe frames and 12.5 or 19 mm stretch nylon netting (Vanstone et al., 1976). Each transport cage was brought to the shore. The fish were guided into plastic bags, carried on a stretcher and released into a 2-m diameter waterproof canvas tank\*\* fitted into a 1.65x1.65 m space in a pickup. The tank contained 30-cm deep seawater supplied with flowing oxygen. The fish were transported at 3 per load to the laboratory at Mag-aba, Pandan, 130 km north of Hamtic, where they were sexed, and placed in a 6-m diameter canvas holding tank containing seawater 1 m deep. The transport from Hamtic to Mag-aba took between 2½ to 3 hr.

\*Prepared by Dr. E. M. Donaldson and donated to SEAFDEC by the Fisheries and Marine Service of Canada.

\*\*Manufactured by Basic Sandusia, Inc., Pasong Tamo, Makati, Metro Manila, per specification by SEAFDEC Aquaculture Department.

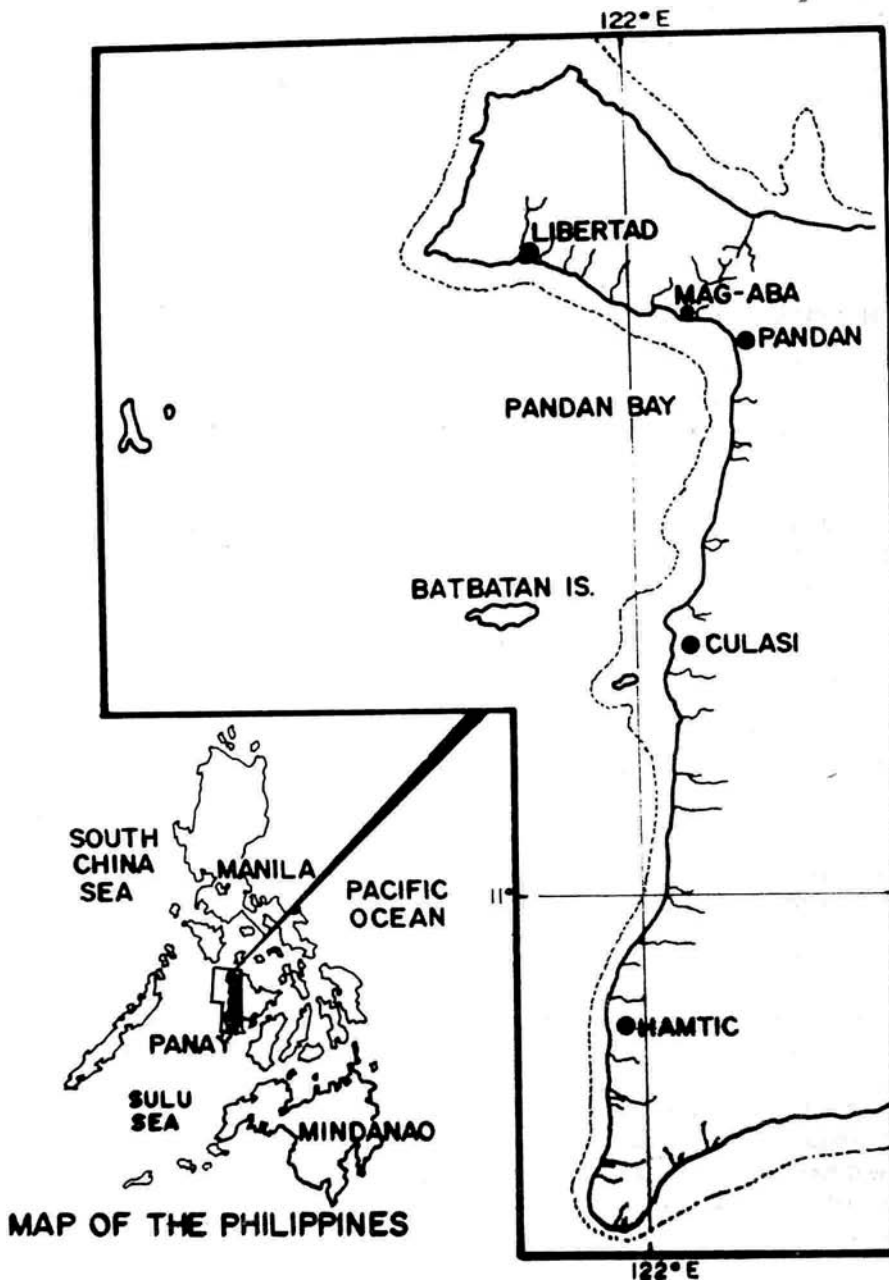


Fig. 1. The western coast of Panay island, Republic of the Philippines, including the shoreline of Antique province, where this study was conducted.

Fourteen freshly spawned milkfish were captured in the April 16 noon catch in the otoshi-ami, a large Japanese-designed set net with a 30-m deep bag, located in 30-m deep water about 500 m from the shore of Mag-aba in Pandan Bay. These fish, 9 females and 5 males, were brought to the laboratory and released into a 10-m diameter canvas holding tank containing seawater 1 m deep.

In the morning of April 17, a gravid female was captured in a fish corral located in Libertad, 17 km west of Mag-aba, brought to the laboratory and released into a 6-m diameter canvas holding tank containing seawater 1 m deep.

### **Spawning: First Experiment**

Among the adult milkfish captured on April 14 were a sexually mature male and a gravid female whose firm abdomen was distended as far back as a point midway between the pelvic fins and the cloaca. On arrival at Mag-aba at 2040 hr\* these were placed in a 6-m canvas holding tank. At 2100 hr, 4 mL of hormone solution (15 mg acetone-dried Pacific salmon pituitary powder and 1000 I.U. chorionic gonadotropin/mL)\*\* was injected into the dorsal musculature 2-3 cm below the dorsal fin of each fish. A second sexually mature male which arrived at 2135 hr was placed in the same holding tank and similarly injected at 2150 hr. The fish began to imbibe water about 1 hr after the injection.

At 0530 hr on April 15, the fish were recaptured in a plastic bag, gently placed in a 2-m diameter canvas tank suspended within the 6-m diameter canvas tank, and anesthetized with quinaldine sulphate (6 ppm). At this time the abdomen of the female was further distended and softer, and the cloacal region was slightly enlarged and protruding. Each fish was injected with 6 mL of hormone solution and released into the 6-m tank. The 2-m tank and its contents were then removed.

At 1620 hr of April 15 a number of nonhydrated eggs were observed floating in the holding tank. The female was still imbibing water. Her belly was softer to the touch, and further belly distension and protrusion were observed.

At 2100 hr, the female stopped imbibing water. When examined, its cloacal region was larger than it was earlier, and when gentle pressure was applied on it, hydrated eggs were released. The female and the 2 males were killed by a blow to the head; their gill arches were cut and they were left to bleed freely.

The body of the female was opened and all loose eggs within her abdominal cavity were removed. About the same quantities were placed in each of 2 basins, for fertilization by both dry and wet methods with mixed sperms from the 2 males.

The salinity of the water in the holding tank was 31 ppt and the temperature range 28.3-29.0°C.

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\*All times of the day mentioned are Daylight Saving Time, 1 hr in advance of Philippine Standard Time.

\*\*The coho salmon pituitary powder, Code No. 62, containing 17.6 mg gonadotropin/g, was prepared by British Columbia Research Council, Vancouver, Canada, and donated to SEAFDEC by the International Development Research Centre (IDRC). The chorionic gonadotropin, A.P.L. Control No. 1 WWO, was purchased from Ayerst Laboratories Inc., N. Y. The diluent was supplied with the chorionic gonadotropin.

### Spawning: Second Experiment

The gravid female captured in Libertad on April 17 arrived in Mag-aba at 1300 hr the same day. Her abdomen was distended and firm with little enlargement in the cloacal region. She was placed in a 6-m holding tank and immediately injected with 4 mL of hormone solution. She began to imbibe water at 1500 hr. At 2215 hr of the same day, and again at 0630 hr of April 18, she was recaptured and placed gently in a 2-m canvas tank suspended in the holding tank where she was anesthetized with 2-phenoxyethanol at 50 ppm and injected with 6-mL hormone solution. In order to minimize stress on the fish, the salinity of the water was decreased from 31 to 27.6 ppt between 0630 and 0800 hr.

By 1300 hr the fish had stopped imbibing water and at 1430 hr, while being transferred to the 2-m tank for closer examination under anesthetic, she ejected her calcium plug and released a few eggs. She was immediately set free in the larger tank and continuous underwater observation of her behavior was done.

At 1500 hr, the fish was releasing a large number of eggs. At this stage she was recaptured, killed, and bled, and the loose eggs were removed. Three of the spawned males captured at Mag-aba on April 16 were killed. The small amount of motile sperm remaining in the spermatid ducts of each male was mixed and used to fertilize by the dry method.

Approximately 5 min after the first hormone injection in both experiments, the bodies of both male and female changed color from their natural dark bluish green backs and silver sides to pale green. The edges of all fins darkened, and the tips of all fins, the edge of the snout and crown of the head became jet black. Immediately following the cutting of their gill arches and the bleeding, the body colors returned to normal.

### Egg Incubation

Eggs obtained from the spawning of April 15 were placed in a 4-m diameter canvas tank containing seawater 1 m deep, which had been filtered through a 60- $\mu$  mesh screen 5 days earlier. Moderate aeration was supplied through a 4-m diameter ring made of 2.5 cm PVC pipe with 1.5 mm holes spaced at 7.5 cm intervals. Samples of developing eggs were removed periodically for observation. Hatching occurred between 0800 and 0900 hr on April 17. Eggs from the spawning of April 18 were placed in carp egg incubators (Yu et al., 1973) suspended in a 4-m diameter tank and supplied with recirculated water from the same tank. Filtered seawater had been aged in this tank for 8 days prior to beginning of incubation. The salinity of the water during both incubations was 32 ppt and the temperature ranged from 28.4 to 29.2°C.

### Larval Rearing

Six larvae obtained from the spawning of April 15 and the 7 surviving larvae obtained from the spawning of April 18 were reared in glass aquaria (30.8 x 15.7 x 20.5 cm) containing filtered seawater which was gently aerated with a stream of fine bubbles. The larvae were fed various mixtures: 1) *Chlorella* with contaminating diatoms and protozoa, 2) immature *Brachionus plicatilis* (40-60  $\mu$ ), and 3) mixed zooplankton between 40 to 60  $\mu$  in size which were collected by means of an airlift pump (Novotny, 1971) from the lagoon adjacent to the laboratory. During larval rearing in the aquaria, the salinity ranged from 32 to 34 ppt and the temperature from 27.5 to 29.7°C.



An unknown number of almost invisible larvae and dead eggs were left in the 4-m diameter tank used for incubating the eggs obtained from the spawning of April 15. *Chlorella*, at a final density of 150,000 cells/mL, was added at 1600 hr on April 17. Immature *Brachionus* (40-60  $\mu$ ) and mixed zooplankton (40-60  $\mu$ ) were added the following day. A bloom of *Chlorella*, diatoms and protozoa occurred. On May 13 the bloom had declined to a level where between 40 to 50 26-day old milkfish larvae, together with *Enteromorpha* and mixed copepods, could be observed.

Due to evaporation between April 17 and May 13, salinity in the incubation tank increased to 36 ppt. Half of the water volume was siphoned off on May 14 and a mixture of freshwater and filtered seawater was added over the next 24 hours to decrease the salinity to 31 ppt. Mixtures of *Chlorella*, *Brachionus* and cultured mixed diatoms were added daily from May 14. Starting May 23 a supplement of mixed wild zooplankton (40-191  $\mu$ ) was added daily.

Larvae were examined and measured after anesthetizing them with MS-222, or shortly after they died in the aquaria or after 7 days preservation in 5% seawater formalin. A preserved 28-day old larva was cleared and stained with alizarin red (Hollister, 1934) to determine its degree of vertebral ossification.

## Results and Discussion

The fertilized egg (Fig. 2) was spherical and contained no special structures on the egg membrane. Egg sizes ranged from 1.1 to 1.23 mm, with a mean diameter of 1.16. The perivitelline space was approximately 20  $\mu$  in width and contained scattered cortical granules which disappeared between 20 and 45 min after fertilization. The yolk was slightly yellowish, devoid of oil globules and finely granulated. Yolk granules ranged in size from 12 to 20  $\mu$ . The egg had a slightly negative buoyancy in water with salinity of 31 ppt, at 28-29°C.

Embryonic development (Figs. 3-18) which was very similar to that described for other pelagic fish eggs, is tabulated in Table 1. First cleavage occurred 70 min after fertilization with subsequent cleavages occurring at intervals of 5 to 26 min. Immediately prior to hatching, the tail extended forward of the head. The embryo contained approximately 34 well-defined myotomes in front of the posterior end of the anus with small pigmented melanophores distributed along the medial dorsal line. The egg at this stage was buoyant in water of 34 ppt salinity and hatching occurred 35 to 36 hr afterward.

There was one exception observed in the above sequence of embryonic development and hatching. One of the eggs hatched at 30.5 hr just as the tail of the embryo had reached its head. This newly hatched larva had a total length of 2.89 mm (2.54 mm, preserved).

Newly hatched larvae (Figs. 19 and 20) measured 3.5 mm in total length (3.1 mm, preserved). The yolk was relatively large and tapered posteriorly and the head projected in front of it. Melanophores were distributed on the finfolds but were not present in the tail region. A few melanophores were present on the head, body and yolk surface.

Twelve hr after hatching, the larvae (Fig. 21) had total lengths ranging from 4.7 to 4.8 mm (4.2 mm, preserved) and the yolk had now become spindle-shaped. Thirty-four myotomes were present before the posterior end of the anus.

One-day old larvae (Fig. 22) were 5.1 mm long (preserved). At this stage melanophores were present at the edges of the eyes and along the dorsal and ventral margins of the myotomes. The buds of the pectoral fins were present.

In 2-day old larvae 5.1 mm in length (preserved) (Fig. 23) there were more melanophores on both the dorsal and ventral margins of the myotomes, but there were fewer on the finfolds. At this stage the eyes had become black and the mouth appeared to be forming. A little yolk was still present.

Table 1. Embryonic development of milkfish *Chanos chanos*, at a temperature range of 28.4-29.2<sup>0</sup>C, and salinity of 31 ppt.

Time from Fertilization (hr/min)	Stage	Remarks
01:10	2-cell	
01:15-20	4-cell	
01:30-35	8-cell	
01:40-50	16-cell	
02:10-15	32-cell	
02:30	64-cell	
03:30	Many-cell	
05:10 to 09:25	Blastula	Blastulation in progress
09:30 to 12:10	Gastrula	Gastrulation in progress
12:50	Neurula	Appearance of neural groove
13:50		Beginning of optic vesicle differentiation
14:27		Start of notochord formation
15:30		Somite segmentation begins
18:30		Embryo 1/2 of egg circumference
22:50		Embryo 3/4 of egg circumference, heart beating, tail free from yolk and occasional body movement
24:10		Frequent body movement
30:00		Tip of tail reaches the head
34:00		Tip of tail past the head
35:00	Hatching	
36:00		Hatching in progress



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7

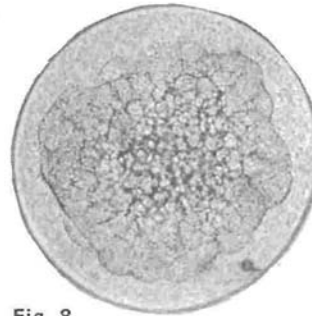


Fig. 8

Figure Number	Stage of Development	Time after Hatching or Fertilization (hr/min)	Description of Egg/Embryo/Larva
2	Newly fertilized	00:00	3 cortical granules present; disappeared in 20-45 min
3	2-cell	01:00	
4	4-cell	01:15-20	
5	8-cell	01:30-35	
6	16-cell	01:40-50	
7	32-cell	02:10-15	
8	64-cell	02:30	

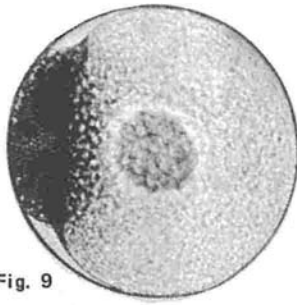


Fig. 9



Fig. 10

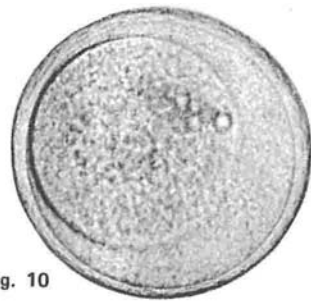


Fig. 11



Fig. 12



Fig. 13

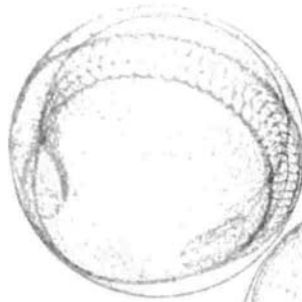


Fig. 14

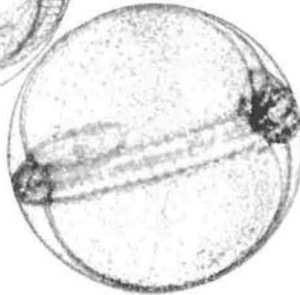


Figure Number	Stage of Development	Time after Hatching or Fertilization (hr/min)	Description of Egg/Embryo/Larva
9	Many-cell	04:15	
10	Blastula	07:25	
11	Gastrula	10:00	
12	Neurula	16:00	
13	-do-	19:00	Embryo more than 1/2 the egg circumference
14	-do-	22:00	Embryo 2/3 of the egg circumference; heart beating; tail not free from yolk

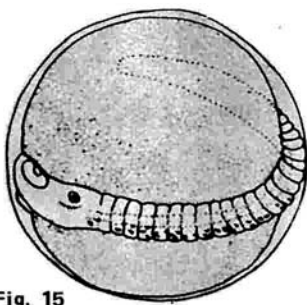


Fig. 15

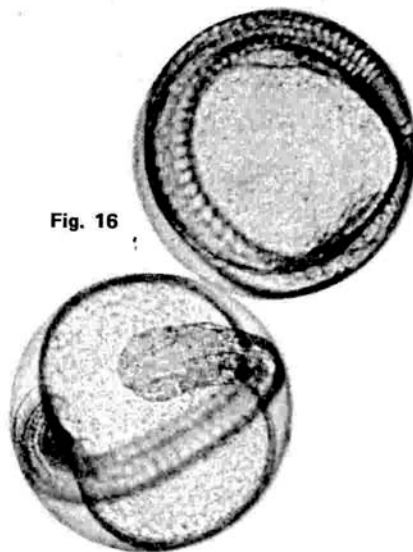


Fig. 16



Fig. 17

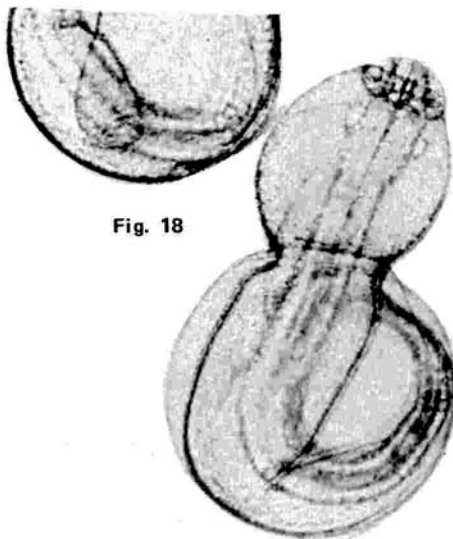


Fig. 18

Figure Number	Stage of Development	Time after Hatching or Fertilization (hr/min)	Description of Egg/Embryo/Larva
15	Neurula	22:00	(Diagram)
16	-do-	25:00	
17	-do-	35:00	Immediately prior to hatching
18	Hatching	35-36:00	
12			

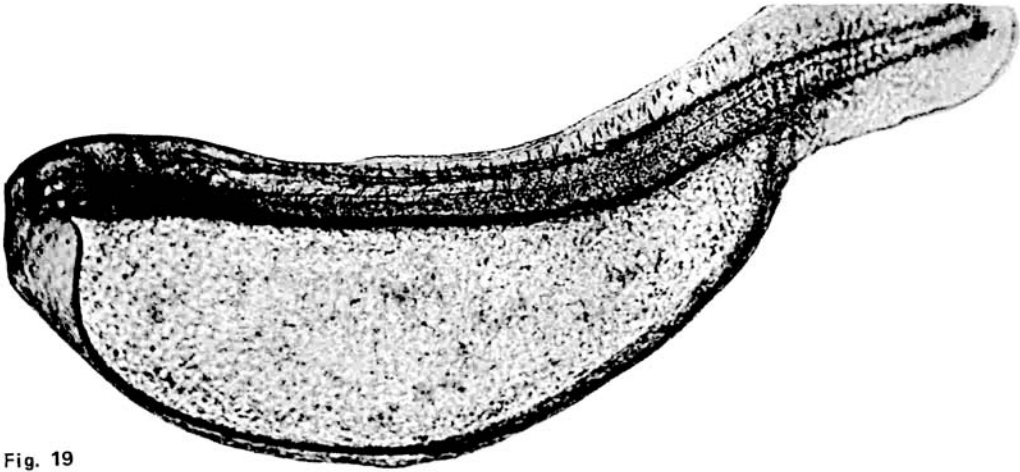


Fig. 19

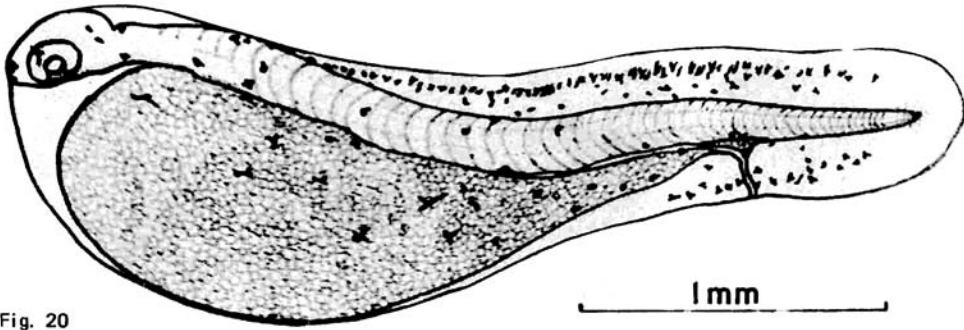


Fig. 20

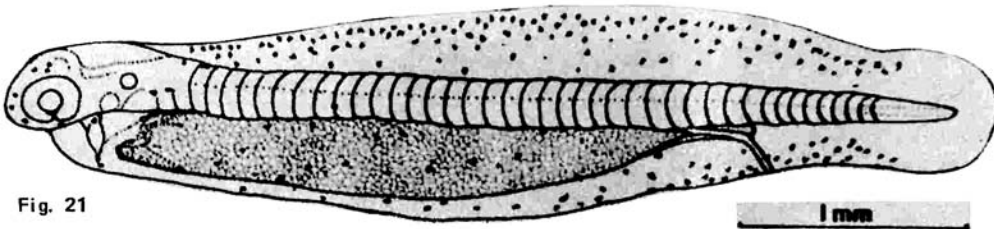


Fig. 21

Figure Number	Stage of Development	Time after Hatching or Fertilization (hr/min)	Description of Egg/Embryo/Larva
19	Newly hatched	35-36:00	Live; total length 3.5 mm
20	Newly hatched	—do—	(Diagram of larva, preserved; total length 3.1 mm)
21	Diagram of 12-hr-old larva, preserved, total length 4.2 mm		

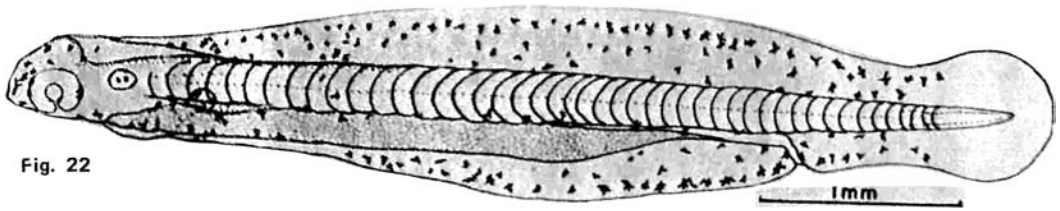


Fig. 22

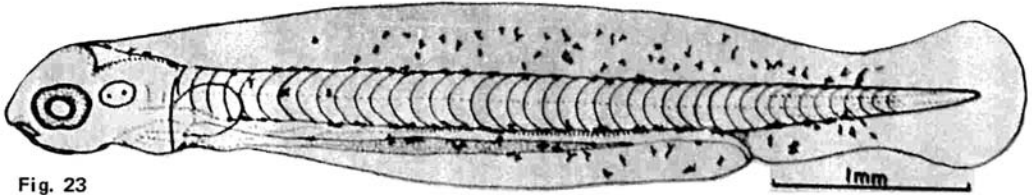


Fig. 23

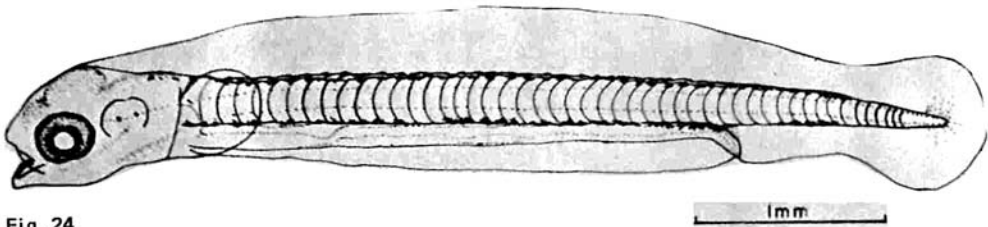


Fig. 24



Fig. 25

Figure Number	Stage of Development	Time after Hatching or Fertilization	Description of Egg/Embryo/Larva
22			Diagram of 24-hr-old larva, preserved, total length 5.1 mm
23			Diagram of 2-day-old larva, preserved, total length 5.1 mm
24			Diagram of 3-day-old larva, preserved, total length 5.2 mm
25			Diagram of 4-day-old larva, preserved, total length 5.2 mm

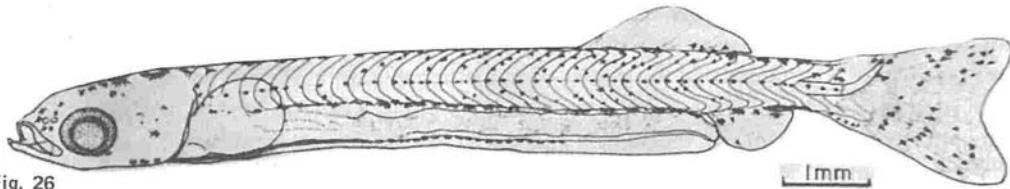


Fig. 26



Fig. 27(a)



Fig. 27(b)



Fig. 28(a)

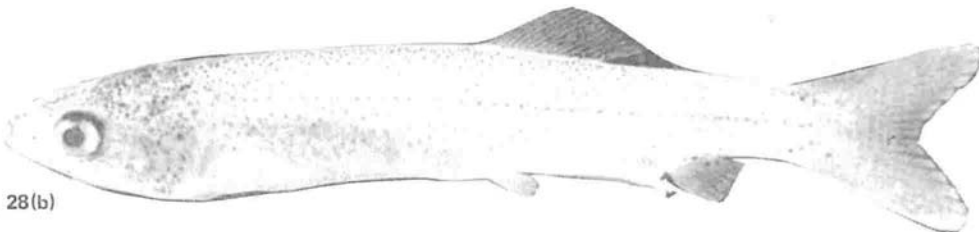


Fig. 28(b)

Figure Number	Stage of Development	Time after Hatching or Fertilization	Description of Egg/Embryo/Larva
26			Diagram of 28-day-old larva, preserved, total length 11.2 mm
27			(a) Laboratory-hatched and reared 31-day-old larva, total length 14.13 mm; (b) larva of similar length caught from the shore.
28			(a) Laboratory-hatched and reared 35-day-old fry, total length 22.6 mm; (b) wild fry (caught from the shore) of similar length, captured 9 days previously and reared in captivity.



The yolk was completely absorbed in 3-day old larvae (Fig. 24) which also measured 5.1 mm (preserved). Melanophores were absent from the finfolds but had become numerous along the ventral margin of the myotomes. Two lines of melanophores were present on the dorsal edge of the myotomes and these merged into one prominent line near the tail. A prominent line of melanophores was present on the ventral margin of the myotomes between the anus and the caudal dip. The mouth was fully formed and functioning and the gut was well developed.

Two lines of melanophores on the dorsal edge of the body had fused into a single line in 4-day old larvae (Fig. 25) which were 5.2 mm in total length (preserved).

The preceding observations, with one exception, agree with those obtained by Delsman (1926, 1929). The one exception reported here is that in the newly hatched larvae described by Delsman the yolk projected in front of the head.

All attempts to rear the larvae in the glass aquaria were unsuccessful and the larvae began to die 24 hr after hatching. The longest surviving larva died on its 7th day. The 2-, 3- and 4-day old larvae described here, and probably those described by Delsman, were either weak and dying or already dead when sampled. There is the possibility that Delsman's and the above descriptions of larval development will be modified when healthy specimens are examined.

Most larvae caught along the shores of Antique by fry collectors for eventual stocking in ponds and pens range in total length from 13 to 16 mm. However, shorter wild larvae are occasionally obtained. Two laboratory-reared, 28-day old larvae (Fig. 26) were of the same total length (11.93 and 12.66 mm) as small wild larvae; however, ossification of their vertebrae was more advanced and was comparable to that of 14-15 mm wild larvae.

Laboratory-reared, 31-day old larvae 14-15 mm long contained numerous melanophores on the dorsal and lateral parts of the body with several present on the head and both the dorsal and anal fins (Fig. 27). In comparison, larvae of similar size from the wild possess a very simple pigmentation pattern, and with the exception of their black eyes, are almost invisible.

Pelvic fins were fully formed in 35-day old fry of total length 20.1 to 22.6 mm, as well as in fry of similar size which had been caught at the shoreline and reared in captivity for 9 days (Fig. 28).

Thirty-two 74-day old fingerlings from the spawning of April 15 were alive on June 30 when they were transferred from the canvas incubator to a 12 m diameter canvas rearing tank containing flowing seawater 31-32 ppt in salinity. Nine of these fingerlings, anesthetized with 2-phenoxyethanol and measured prior to being transferred, had an average fork length of 108 mm (S.D. = 5 mm) and an average total length of 121 mm (S.D. = 7 mm).

### Acknowledgements

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