

## Egg Development, Hatching, and Larval Development of Marble Goby *Oxyeleotris marmoratus* under Artificial Rearing Conditions\*<sup>1</sup>

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To establish a seed production technique for marble goby *Oxyeleotris marmoratus* the eggs and larvae were observed under artificial rearing conditions. Newly ovulated eggs from a female of 255 g in body weight were measured 0.94 and 0.62 mm in the long and short axes, and weighed 1,669.6 eggs/g. After fertilization, the egg had a bundle of adhesive filaments at the basal end of the egg membrane and was elongated after 1 h, when it measured 2.3 and 0.63 mm in its long and short axes, respectively. The eggs hatched during 41–150 h after fertilization (AF) at 27.2–27.5°C. Early and late hatching resulted in high mortality. The optimum hatching stage was considered just before and after the embryonic eyes had become slightly pigmented. All embryonic heads were located at the basal part of the egg and this was taken as normal egg development for *O. marmoratus*.

In spite of their well-developed eyes and mouths, and the fact that they exhibited the active S-posture, the larvae did not feed on artificial powdered feed or *Brachionus* spp. for 4–6 days (d) AF, and the mortality before the feeding of *Brachionus* spp. was much higher than that of the later stages. They shifted their habitat from pelagic to benthic from 30 d AF. About 1,600 seeds (mean total length, 28.5 mm) were produced from 16,000 hatched larvae (mean total length, 3.54 mm) at 70 d AF.

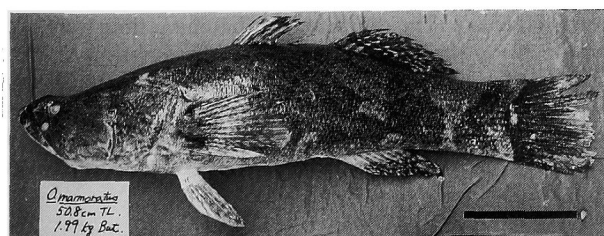
**Key words:** marble goby, egg development, larval development, *Oxyeleotris marmoratus*

Marble goby *Oxyeleotris marmoratus* (Fig. 1) is a freshwater eleotrid fish<sup>1,2)</sup> that grows to more than 50 cm in total length (TL) and 2 kg in body weight (BW). *O. marmoratus* is known as “Ikan Ketutu”, one of the most expensive table fish in Malaysia.<sup>3,4)</sup> Fish farmers wish to develop *O. marmoratus* culture but its seed supply is inadequate. Presently, natural seeds have been overfished and artificial seed production techniques are not yet established.

In an attempt to establish a seed production technique for *O. marmoratus*, the authors observed egg development, hatching, and larval development with behavioral changes following induced breeding and successfully produced a few thousand seeds with a size of 2.85 cm in TL for the first time under tank conditions in Malaysia.

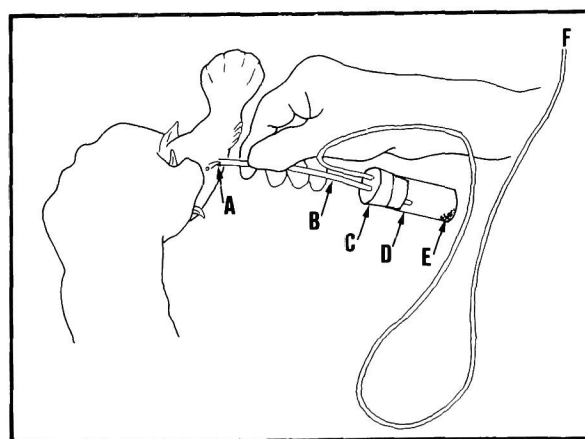
### Materials and Methods

Two experiments were conducted at the Hatchery and Pond Complex



**Fig. 1.** Adult female fish of *Oxyeleotris marmoratus*. 1.99 kg in body weight, 50.8 cm in total length; scale, 10 cm.

Unit, Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia. The first experiment on egg development was carried out in August, 1991, and the second experiment on larval development in August to October, 1991. The brood fish were collected from earthen fish ponds in the State of Selangor, Malaysia and were reared in tanks for 8 months. In June to July, 1991, testes were ground with 0.9% saline for fertilization,<sup>5,6)</sup> and valuable males had to be killed. Eggs used were collected using the sperm-egg collectors<sup>7)</sup> shown in Fig. 2. Fresh water for the rearing was well aerated for 24 h. A Nikon V-10 Profile Projector was used for the observations and measurements.



**Fig. 2.** Apparatus of sperm-egg collector used in this study. A, urogenital papilla in the end of a plastic tube; B, plastic tube 5 mm in inner diameter; C, rubber top; D, plastic bottle; E, collected sperm or eggs; F, sucking by mouth.

\*<sup>1</sup> Study of Artificial Seed Production of Marble Goby-I.

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**Table 1.** Sequence of feeds given during the rearing of *O. marmoratus*

Days after fertilization							
5	10	20	30	40	50	60	70
Artificial powdered feed							
<i>Brachionus</i> spp.							
<i>Artemia salina</i> nauplii							
<i>Moina</i> sp.							
<i>Chironomus</i> sp. Larvae							

#### Incubation for Egg Observation

The weight of a mass of newly ovulated eggs from a female of 255 g in BW was measured using an Ohaus E400D Top Pan Balance. Subsequently, the number of eggs was counted with the naked eye. About 1,500 eggs fertilized with the milt of two males, 260 and 235 g in BW, were deposited in a single layer at a density of about 25 eggs/cm<sup>2</sup> in a Petri dish. The eggs were incubated in an aquarium tank (21 × 37 × 28 cm) containing 15 l of water. The water temperature, dissolved oxygen (DO), and pH during egg incubation ranged from 27.2–27.5°C, 7.7–7.8 mg/l, and 7.7–7.9, respectively.

#### Rearing for Larval Observation

The dates for larval rearing were based from the day of fertilization because hatching occurred within 2–5 days after fertilization (AF). The day after fertilization was defined as 1 d AF. Ovulated eggs were collected from two females, 155 and 193 g in BW, and were fertilized with the milt of two males, 195 and 268 g in BW. About 20,000 fertilized eggs were deposited on two rectangular wire-framed plankton nets (21 × 36 cm) of 250 µm mesh size. The nets were then left afloat on the water surface with eggs dangling in the water in a tank (diameter, 153 cm; depth, 60 cm) containing 1 ton of water. About 16,000 larvae measuring 3.54 ± 0.16 mm in TL (mean ± SD) were obtained in total, and the larvae were reared in the same tank.

Different feeds were given at 0800 and 1700 h *ad libitum* in accordance with the schedule in Table 1. The artificial powdered feed (Riken Vitamin & Co., Ltd.) measured about 40–80 µm in diameter. *Brachionus* spp. measured about 150 µm in body length. *Chlorella* sp. were added to the rearing water with 500,000 cells/ml. Live *Chironomus* sp. larvae were chopped into small pieces before feeding.

The rearing water was aerated at 3 positions with 250–500 ml/min. Tank cleaning and water exchanging (30–50% in water volume) were carried out daily from 7 d AF to the end of the experiment. A 20 W fluorescent light above the tank was left on from 0800 to 1800 h with 100–800 lx on the water surface. From 30 d AF, the light was left off because the larvae were negatively phototactic. The water temperature, DO, and pH during the larval rearing ranged from 27.0–29.5°C, 2.1–7.8 mg/l, and 7.5–7.9, respectively. Although the DO dropped to 2.1 mg/l on 15 d AF due to electricity failure, no larval mortality was not observed.

## Results

### Egg Development and Hatching

The morphological changes in the eggs are illustrated in Fig. 3A–Y. Newly ovulated eggs were yellow-orange in color, measured 0.94 ± 0.09 and 0.62 ± 0.05 mm in their long and short axes (Fig. 3A), and numbered 1,669.6 eggs/g. Immediately after fertilization, a bundle of adhesive filaments developed at the basal end of the egg membrane and was strongly attached on the surface of the Petri dish. The eggs then absorbed water and became elongated in their vertical axis.

At 0.25 h AF, the blastodisk rose and the egg diameters were 1.56 ± 0.10 and 0.66 ± 0.02 mm in their long and short

axes (Fig. 3B). At 1 h AF, the first cleavage occurred, water absorption was completed (Fig. 3C) and the egg diameters were 2.25 ± 0.10 and 0.63 ± 0.01 mm in their long and short axes. The fertilization rate at this stage was 90.5%. At 2.5, 3.5, and 5–7 h AF, the morula, blastula, and gastrula stages were observed in that order (Fig. 3G–J). At 14 and 17 h AF, the optic and otocyst vesicles appeared respectively (Fig. 3O, Q). At 21 h AF, the heart and lens on the optic vesicles formed and the embryos commenced to move actively (Fig. 3S).

Hatching began 41 h AF before eye pigmentation (Fig. 3U). Hatching peaked around 60–70 h AF (Fig. 3V, W) while the eyes were slightly pigmented. Some larvae that hatched before this peak could not swim and died on the tank bottom. At the peak, hatching was easily induced by using a pipette to create a gentle water flow. The egg diameters of unhatched eggs at 72 h AF were 2.13 ± 0.12 and 0.77 ± 0.05 mm in their long and short axes (Fig. 4). After the peak, the mechanical stimuli of the pipette were not effective to induce hatching.

At 96 h AF, the embryonic head became more developed and filled the basal part of the egg entirely (Fig. 3X). Thereafter, the embryos did not move as actively as in the earlier stages and some of them died before hatching. At 120 h AF, the embryonic head fitted more tightly into the basal part of the egg and consequently the membrane lost its original shape (Fig. 3Y). At this stage, some of the newly hatched larvae were deformed and died, and the eggs could not hatch by the stimulus of the water flow.

Sizes of the yolk sac for unhatched embryos and hatched larvae are shown in Fig. 5. There were not many developmental differences in on eye pigmentation or yolk sac absorption between unhatched embryos and hatched larvae of the same age. The differences in hatching time were not dependent on the stage of embryonic development.

In all developmental stages, the embryonic head was located at the basal part of the egg. The hatching glands were not observed under the profile projector. Five hundred and thirty one larvae hatched from the 1,500 fertilized eggs by 150 h AF.

### Larval Development

The morphological changes in the larvae are illustrated in Fig. 6A–M. The correlation between the morphological features and behavioral changes is shown in Table 2.

A few larvae hatched accidentally when the wire-framed

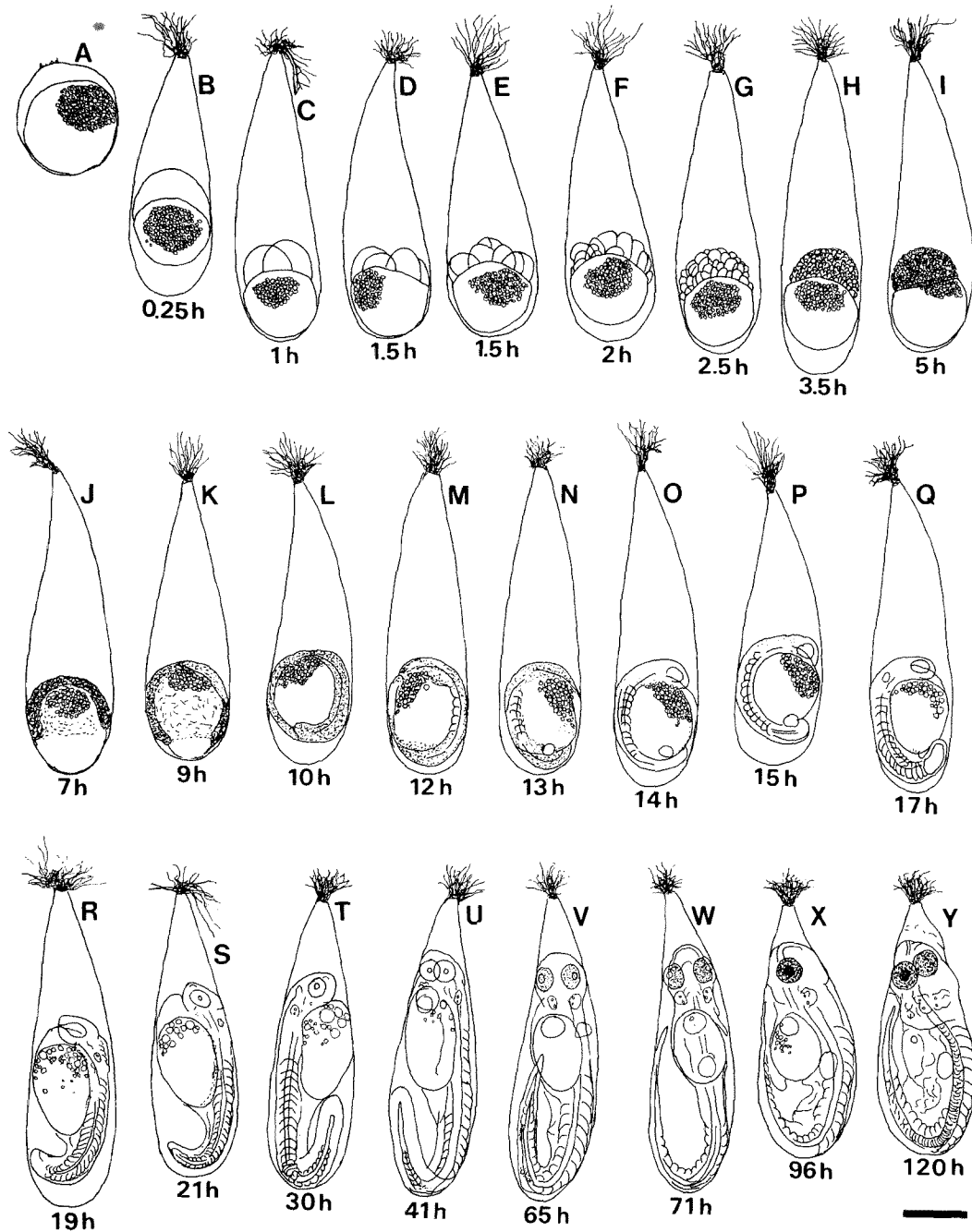


Fig. 3. Egg development of *O. marmoratus*.

A, unfertilized egg; B, fertilized egg; C, 2-celled stage; D, 4-celled stage; E, 8-celled stage; F, 16-celled stage; G, morula stage; H, blastula stage; I, early gastrula stage; J, gastrula stage; K, blastopore nearly closed; L, embryo formation, blastopore completely closed; M, 5-myomere formed; N, Kupffer's vesicle appeared; O, optic vesicles appeared; P, tail separated from the yolk sac; Q, otocyst vesicles appeared; R, head formed; S, lens and heart formed and embryo commenced moving; T, tail elongated to the yolk sac; U, hatching started; V, eyes pigmented; W, vesicle of air bladder formed (hatching peak); X, tail elongated to the head; Y, egg membrane transformed by developed embryonic head; scale, 0.5 mm.

plankton net was picked up from the tank for observation at 25 h AF (Fig. 6A). Such larvae could not swim and died on the tank bottom. In newly hatched larvae at 49 h AF, the mouth and anus were not formed, and the eyes were not pigmented (Fig. 6B). Most of the larvae hatched during

2–3 d AF; newly hatched larvae lay on the tank bottom and gradually exhibited the “swim up, sink down” behavior (Fig. 6B–D). On 3 d AF, the mouth was opened, and the pectoral fins were formed (Fig. 6D). On 4 d AF, the eyes were deeply pigmented, and the lower jaw and in-

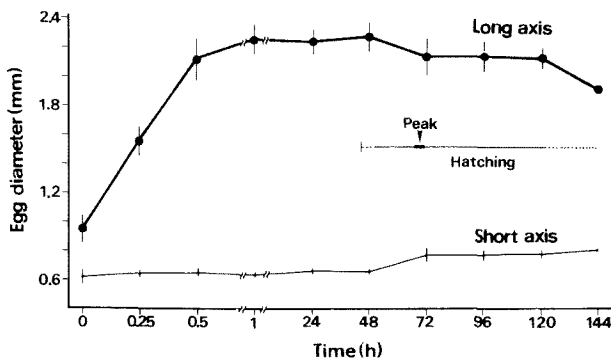


Fig. 4. Changes in egg diameters in the long and short axes of *O. marmoratus*.

Horizontal broken and solid lines show the hatching period; during the period with the broken line, high mortality of newly hatched larvae was observed; closed circle, mean; vertical bar, standard deviation ( $n=10$ ).

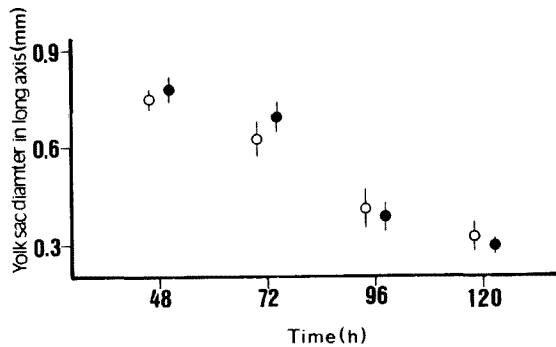


Fig. 5. Comparison of yolk sac diameter in long axis between unhatched embryos and hatched larvae.

Unclosed circle, mean of yolk sac diameters in the long axis of unhatched embryos; closed circle, mean of yolk sac diameters in the long axis of newly hatched larvae; vertical bar, standard deviation ( $n=10$ ).

testinal tracts began to move (Fig. 6E). The artificial powdered feed and *Brachionus* spp. were not observed in the intestinal tracts in spite of the developed eyes, mouths, and intestinal tracts, and the fact that they exhibited the horizontal swimming and active S-posture during 4–6 d AF (Fig. 6E–G). At 6 d AF, the distal part of the intestinal tract near the anus was stained green in color (Fig. 6G); this could be due to the ingestion of *Chlorella* sp. cells.

*Brachionus* spp. were observed in the intestinal tracts of some larvae on 7 d AF. The upper jaw length of larvae that fed well on *Brachionus* spp. measured 250  $\mu\text{m}$ . The larvae that did not feed on *Brachionus* spp. could not swim actively and most of them eventually died. The growth increment was only 0.18 mm in TL during 3–8 d AF (Fig. 7).

The larvae of *O. marmoratus* preferred *Artemia salina* nauplii to *Brachionus* spp., *Moina* sp., and *Chironomus* sp. larvae during the period in which the *Artemia* was given (Fig. 6I–M). On 17 d AF, the rudiments of second dorsal, anal, and caudal fins appeared, and the spinal cord flexion was observed (Fig. 6J). On 35 d AF, all fins were formed, and dark pigmentations appeared from the base of the second dorsal fin to the anal fin, and another part of the

caudal fin (Fig. 6M). From 60 d AF, only the *Chironomus* were given and most of the juveniles became used to feeding on it within a few days. The juveniles fed on the *Chironomus* inactively and slowly at the tank bottom.

The survival during 5–10 d AF was 31.5%, much lower than that during 10–70 d AF in which 69.0% was recorded (Fig. 8). The larval mortality peaked during 7–9 d AF. Mortalities due to cannibalism and disease were not observed. The survival during 5–70 d AF was 10.1% and 1,610 seeds measuring  $28.5 \pm 1.38$  mm (mean  $\pm$  SD) in TL were produced.

## Discussion

### Ontogenetic Features

1. Position of the embryonic head: In many gobies, the embryonic head is located at the distal part of the egg before hatching.<sup>5,6,8–22</sup> An embryonic head located at the basal part of the egg has been referred to as agrippa egg.<sup>5,6,14,16,22</sup> Tan and Lam considered that the agrippa egg condition of *O. marmoratus* resulted in high mortality. In this study and our unpublished experiments, the embryonic head was located at the basal part in all stages and showed normal embryonic development with high survival of hatched larvae. Therefore, the agrippa egg is considered to be a normal characteristic of this goby.

2. Hatching: Hatching usually occurred after eye pigmentation in many gobies.<sup>5,6,8–22</sup> In the present study and an earlier one,<sup>23</sup> *O. marmoratus* eggs hatched before, during, and after eye pigmentation. Fish egg membrane consists of the internal and external layers, and hatching occurs when the embryonic hatching enzyme degrades the internal layer and embryonic muscular motion mechanically breaks the external layer.<sup>24</sup> When *O. marmoratus* eggs were hatched in running and continuously filtered water, the hatching time was no longer variable.<sup>23</sup> In another of our experiments on natural spawning of *O. marmoratus* (unpublished), the eggs hatched just before and during eye pigmentation under protection through fanning by the male brood fish, and thereafter the mortality of hatched larvae was low. The optimum hatching stage of *O. marmoratus* was considered just before and after the embryonic eyes became slightly pigmented.

3. Egg shape: Two morphological types of *O. marmoratus* eggs have been observed (Fig. 9). Eggs reported by Tan and Lam<sup>23</sup> were pear-shaped and shorter in the long axis (Fig. 9A) as compared to those of the present study (Fig. 9B). The differences in the shape and size of the eggs could not clearly be attributed to genealogical differences and/or the egg quality, which depends on the rearing conditions and hormone treatments on brood fish.<sup>24</sup>

4. Larval development and behavior: Gobiidae has a large variation in the early development. *Odontobutis obscura* and *Rhinogobius flumineus* undergo larval development in large eggs and hatch at a juvenile stage.<sup>25</sup> *Eleotris oxycephala* and *Sicyopterus japonicus* hatch from small eggs in very undifferentiated conditions.<sup>25</sup> *O. marmoratus* showed intermediate characteristics of development at the time of hatching; the anus is located near the middle of the body, the eye diameters are 1/2–1/3 of the head length, and the air bladder is relatively big and pigmented. At the time of yolk sac absorption, *Glossogobius*

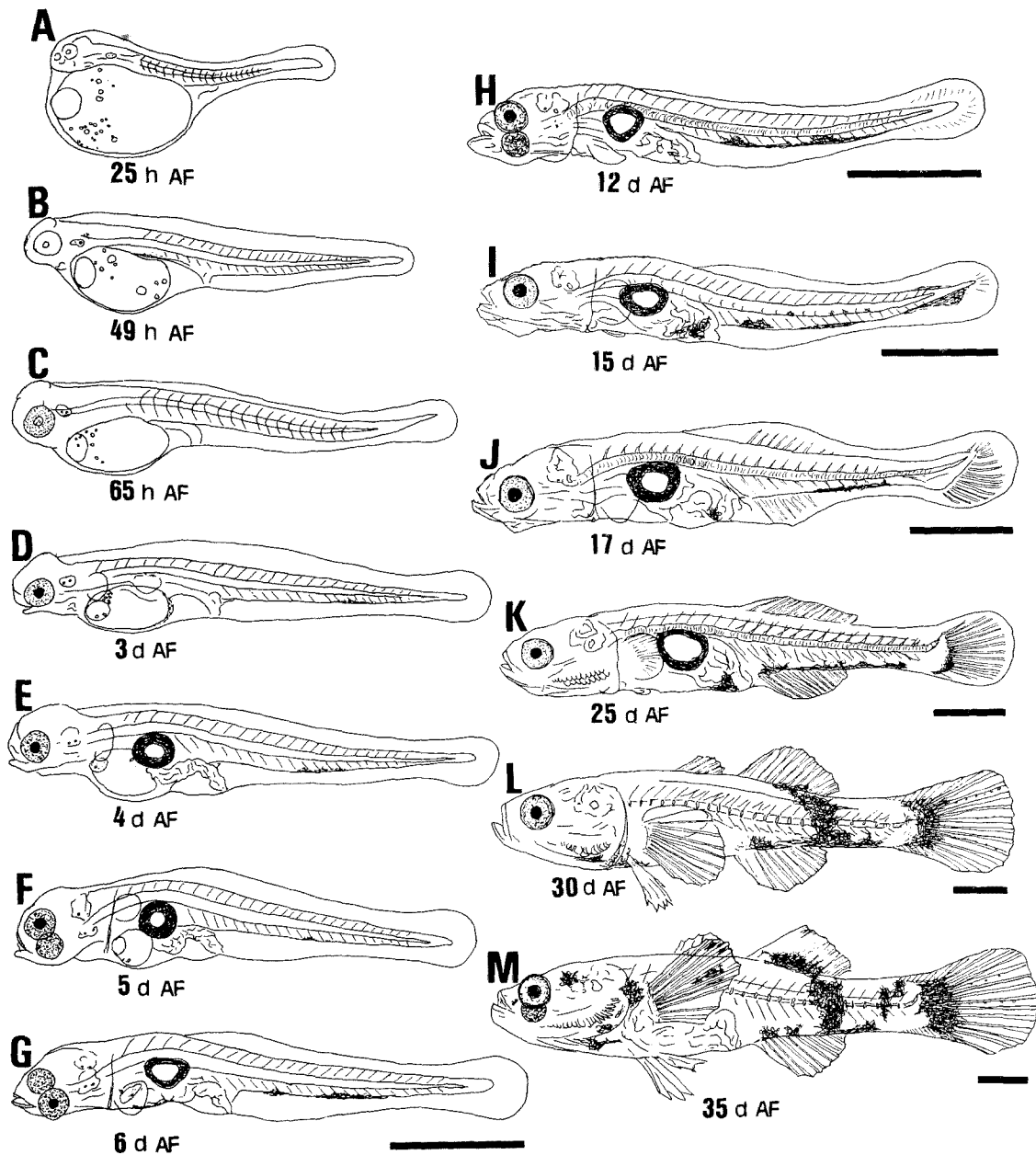


Fig. 6. Development of *O. marmoratus* larvae. Larval age is shown by time after fertilization (AF); scale, 1 mm.

*olivaceus*<sup>21)</sup> larvae smaller than *O. marmoratus* larvae fed on *Brachionus* spp., but *O. marmoratus* larvae did not, in spite of having well-developed eyes, jaws, and intestinal tracts. This might be due to the smaller mobility of *O. marmoratus*. In many marine fish larvae, the eyes, jaws, and intestinal tracts are functional just before yolk sac absorption and larvae have small mouths, but their locomotory organs are not well developed.<sup>26)</sup> Therefore, the diet for the first feeding of larvae are limited to small micro-plankton which are slow in locomotion.<sup>26)</sup>

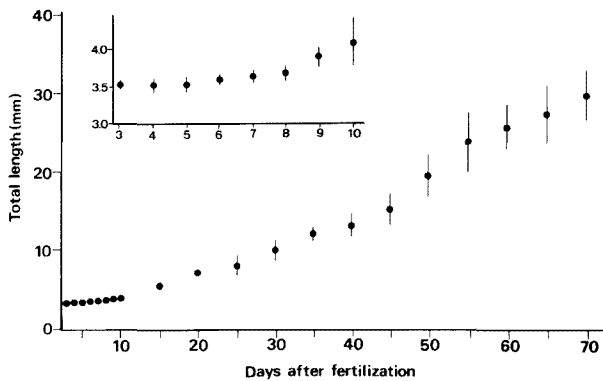
*O. marmoratus* preferred the *Artemia* to the other feeds as well as the goby *Lubricogobius exiguus*.<sup>14)</sup> In fish larvae,

the chemical selection of food commences after the formation of the taste buds.<sup>27)</sup> Many freshwater fish larvae develop taste buds before their first feeding.<sup>28)</sup> The preference for *Artemia* by *O. marmoratus* may not be due to the level of development of the taste buds and mouth size.

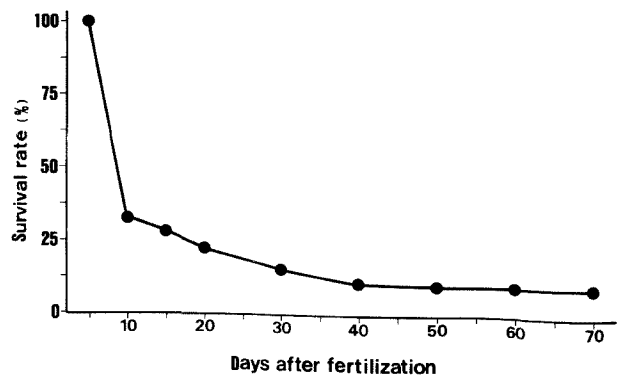
Usually gobies change their habitat from pelagic to benthic as they change from the larval to the juvenile stages. They are scared to shift into the benthic habitat immediately after hatching, as reported in *Mogurnda obscura*.<sup>15)</sup> Many gobies show positive phototaxis during the early larval stages.<sup>6,9,17,19,21)</sup> In the case of *O. marmoratus*, its behavior with regard to habitat preference and phototaxis was

**Table 2.** Correlation between morphological and behavioral changes in *O. marmoratus* Larvae

Morphological changes	Days after fertilization	Behavioral changes
Hatching commenced	2	Laid down on the tank bottom
Mouth not formed, Anus closed, 24-myomere	2-3	Vertical swimming
Eyes lightly pigmented, Peak of hatching		
Vesicle of air bladder appeared		
Mouth opened, Eyes more pigmented	3	First S-posture
Pectoral fins formed, 25-26-myomere		
Air bladder inflated		
Eyes deeply pigmented	4	Active S-posture Horizontal swimming Positively phototactic
Jaw and intestinal tract movement		
Hatching completed	5	Schooling under the fluorescent light Strong phototaxis
Eye movement		
Intestinal tract active movement	6	First S-strike First feeding on <i>Brachionus</i> spp.
Yolk sac not apparent		
Intestinal tract stained green		
Jaw active movement	7	Active feeding on <i>Brachionus</i> spp.
Lower jaw elongated		
Rudiments of second dorsal fin anal fin } appeared caudal fin }	12	First feeding on <i>Artemia salina</i> nauplii Active and strong S-strike Schooling in the middle water column
	15	
	17	
Rudiment of pelvic fin } appeared first dorsal fin }	25	Schooling in the bottom water column Negatively phototactic
All fins formed	30	Benthic habitat Active swimming on the tank bottom
Dark pigmentation appeared from base of second dorsal fin to anal fin, and another part of caudal fin		
Yellow-ocher pigmentation appeared on body Juvenile stage	35	Not active swimming
	40	



**Fig. 7.** Growth of *O. marmoratus* in artificial conditions. Inserted figure with magnified scales shows early growth; closed circle, mean; vertical bar, standard deviation ( $n=10$ ).



**Fig. 8.** Change in survival rate of *O. marmoratus*.

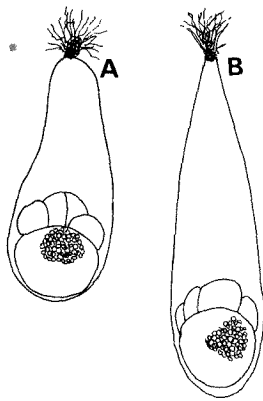


Fig. 9. Short (A) and long (B) type eggs of *O. marmoratus* at the 4-celled stage.

similar to that of other gobies.

#### For Artificial Propagation

The females used in the present study were of nearly half or of the same size as those used in previous studies<sup>23,29,30</sup> and the working fecundity, fertilization, and hatching rates were almost the same or better than those reported. Egg collection of *O. marmoratus* can be carried out for seed production all year round using the present technique.

Hatching can be induced by the stimulus of water flow in other gobies.<sup>6,15,21,22</sup> Induced hatching by mechanical stimulus such as controlling the aeration rate to regulate water flow at the optimum hatching stage is recommended for the higher survival of *O. marmoratus* larvae.

In the previous<sup>23,30</sup> and present studies, the mortality in the early larval stages 5–10 d AF were much higher than those in the later larval stages. Tan and Lam<sup>23</sup> discussed that the causes of high larval mortality were due to attacks by ciliates, starvation, poor water quality, and the detrimental effects of hormone treatment. The authors felt that the major cause of mortality was starvation during the early larval stages. With improvements to the first feeding method for the larvae, higher production can be anticipated.

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