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Embryonic Development and Larvae of Genus *Eviota* (Pisces : Gobiidae)

I. *Eviota abax* and *E. storthynx*

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Embryonic development and larvae of *Eviotu abm* and *E. storthynx* are described. Eggs are fusiform in both species, measuring 1.16-1.19 ×0.59–0.63 mm in *E. abax* and 1.19-1.31 x0.37-0.42 mm in *E. storthynx*. Numerous minute processes cover the surface of the eggs of both species, Total length of newly hatched larvae is 2.6-2.8 mm in *E. abax* and 1.9-2.1 mm in *E. storthynx*. Cuplae and red pigments are observed in larvae of both species. Larvae of *E. abax*, reared for 16 days after hatching, grew to 4.3-4.5 mm in total length. In these larvae, as they grew, the number of melanophores decreased and the red pigments, which are marked features in the embryo, disappeared.

INTRODUCTION

Eviota abax and *E. storthynx* are small gobies that inhabit rocky reefs and coral reefs. *Eviota abax* is distributed in southern Japan (Yoshino and Shimada, 1985a), and *E. storthynx* occurs northward to Kagoshima Prefecture, Japan and the western Pacific (Yoshino and Shimada, 1985b).

Embryonic development and larvae of *Eviotu abax* and *E. storthynx* were studied by Dotsu *et al.* (1965) and Shinomiya *et al.* (1981a) respectively. In this paper, we redescribe development of eggs and larvae of both species and discuss differences in morphological characteristics of embryos and larvae of these two species of *Eviotu*.

MATERIALS AND METHODS

The specimens of *Eviota abax* (one male, 28.5 mm in standard length (SL) and 3 females, 28.0, 26.2, 26.0 mm SL respectively) were collected by using a hand net at Kuchinoerabu Island, Kagoshima Prefecture, on 14 May 1985. Those of *E. storthynx* (one male, 26.0 mm SL and 2 females, 22.5, 21.7 mm SL respectively) were collected by the same method at Sakurajima Island, Kagoshima Prefecture, on 31 July 1985.

They were brought to the laboratory and kept in a glass aquarium (60×30×28 cm). Water was circulated and filtered through a layer of gravel at the bottom by a air-lift system. An opaque vinyl chloride pipe (2 cm inside diameter and 5 cm long) was put on the bottom as a shelter for spawning and hiding. Minced fresh fish meat was fed once a day.

Spawning of *Eviota abax* was observed on 25 and 28 May, and that of *E. storthynx* on 5, 10 and 17 August. Just after spawning, the opaque vinyl chloride pipe with an egg mass on its ceiling was placed in a plastic vessel (15 × 10 × 8 cm) fixed below the tap of air-lift pipe in the aquarium, so that filtered water ran over the eggs throughout development.

When the larvae hatched, 10 were put in each of several sealed 250 ml glass bottles that were kept in the same aquarium. Half the water was changed daily. After the larvae had absorbed their yolk, rotifers and oyster larvae were put into the bottles. Water temperature, controlled with electric heaters, was kept between 24 and 27°C in both the aquarium and bottles.

Measurements were made through a binocular microscope (Nikon Model S) with a printer display calculater (Texas Instruments TI-5142). Drawings were made by the aid of a camera lucida.

Spawning took about 20-60 minutes in both species. The time when the spawning ended is regarded as that of fertilization.

RESULTS

Embryonic development of *Eviota abax*

Eggs are spawned in a single layer on the ceiling of the shelter. The number of eggs spawned by a female is 250–350 per brood, and a male guards egg masses of one to three females at a time. The fusiform eggs, with a bundle of adherent threads at their base, measure 1.16–1.19 mm (mean 1.18 mm) in length and 0.59-0.63 mm (mean 0.60 mm) in breadth. Numerous minute processes cover the surface of the egg membrane (Fig. 1).

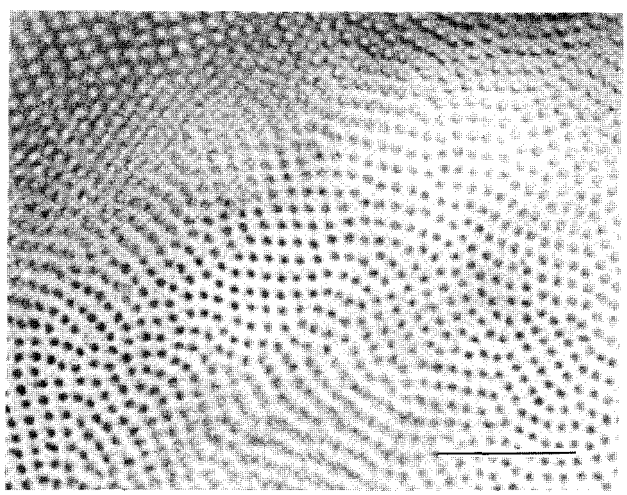


Fig. 1. Surface of the egg membrane of *Eviotu abm*. Scale indicates 20 pm

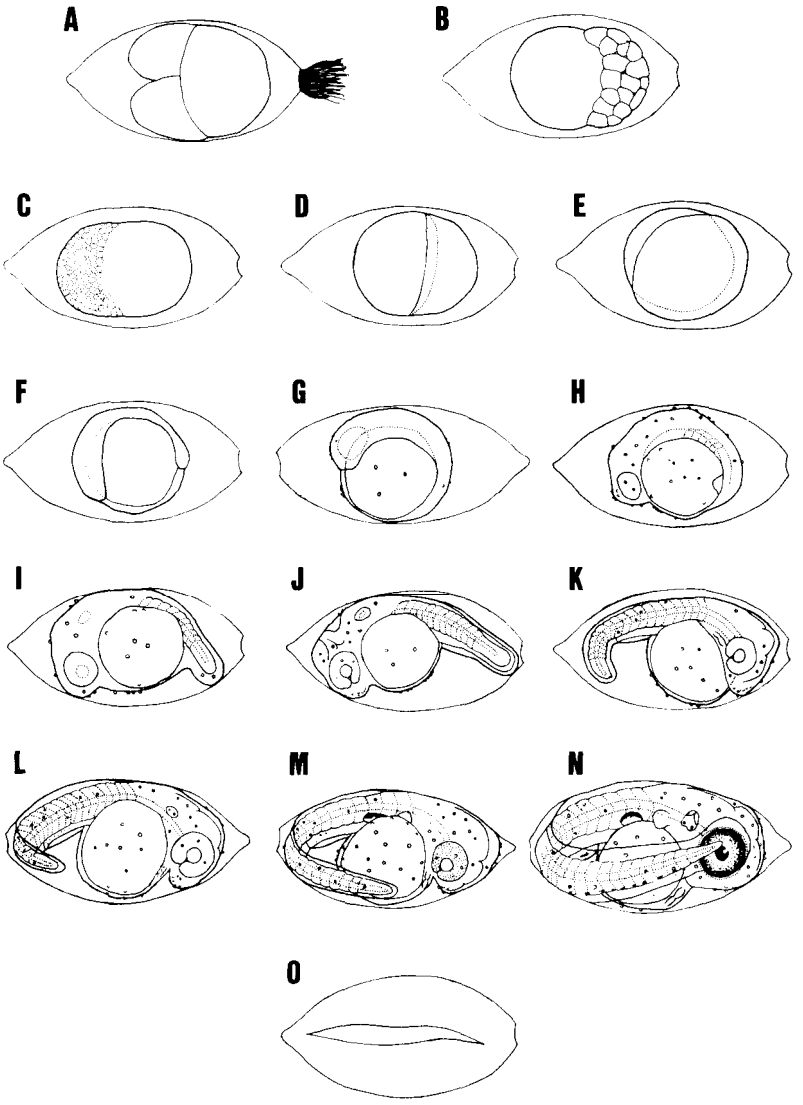


Fig. 2. Embryonic development of *Eviota abax.* A, 40 min after fertilization. B, 2 hr. 40 min after fertilization. C, 4 hr. after fertilization. D, 5 hr. after fertilization. E, 12 hr. after fertilization. F, 17hr. after fertilization. G, 19 hr. after fertilization. H, 21 hr. after fertilization. I, 29 hr. after fertilization. J, 34 hr. after fertilization. K, 42 hr. after fertilization. L, 48 hr. after fertilization. M, 62 hr. after fertilization. N, 80 hr. after fertilization. 0, Empty egg capsule with a hatching cleft.

The first cleavage divides the blastodisc into two cells of equal size 40 min after fertilization (Fig. 2A). The 32-cells stage is achieved 1 hr. 40 min after fertilization (Fig. 2B), and the morula stage 4 hr. after fertilization (Fig. 2C). In the early gastrula stage, 5 hr. after fertilization (Fig. 2D), the blastodermal cup begins to spread over the surface of the yolk. By 12 hr. after fertilization (Fig. 2E), the rim of the germ ring covers about three-quarters of the yolk. Seventeen hr. after fertilization (Fig. 2F), the blastopore closes, and an embryonic body is formed. A pair of optic vesicles appears at 19 hr. (Fig. 2G), and granules are visible on the surface of the embryo. A Kupffer's vesicle appears and five pairs of myotomes can be recognized 21 hr. after fertilization (Fig. 2H). The number of the granules on the surface of the embryo increases.

Lenses are observed in the optic cup and a pair of ear vesicles appear at 29 hr. on the lateral sides of the nape (Fig. 21). A slight tail bud projects. The Kupffer's vesicle had disappeared. Nine pairs of myotomes are recognized, and the notochord is visible.

Brain differentiation is recognized and the tail become longer by 34 hr. (Fig. 2J).

Forty-two hr. after fertilization (Fig. 2K), a heart appears anterior to the yolk, the intestine is distinguished as a straight tube, and 18 pairs of myotomes are recognized.

The heart exhibits a rhythmical beat 48 hr. after fertilization (Fig. 2L). Two pairs of otoliths are recognized in the ear vesicles. Melanophores appear on the ventral part of the tail.

A gas bladder appears and pectoral fin buds are recognized 62 hr. after fertilization (Fig. 2M). Melanophores and red pigments appear on the gas bladder.

Red pigments appear on the anus and melanophores on the optic cups at 80 hr. (Fig. 2N). The tip of the looped tail reaches to the level of the eye.

Larvae hatch after sunset, 129 hr. after fertilization. An empty egg cupsule with a hatching cleft is shown in Fig. 20. The small oil globules in the yolk never merged to form larger ones.

Larvae of *Eviota abax*

The newly hatched larvae measure 2.6-2.8 mm in total length(TL)(Figs. 3A-1, A-2), and have 9+16= 25 myotomes (adult :10+16= 26). The mouth has opened, although the yolk still remains. Peristalsis of the digestive tract can be seen. The larvae show positive phototaxis. Eight pairs of cuplae and free neuromasts are observed (Fig. 3A-2). The cuplae easily fall off after fixation with 5 % formalin. Four to eight small dendritic melanophores are observed on the ventral part of the tail. Others are recognized on the optic cups, the dorsal surface of the gas bladder and the dorsal part of the rectum. Red pigments are on the dorsal surface of the gas bladder, the anus, ventral part of the abdomen, and the tail region.

One day after hatching, when the larvae are 2.9-3.0 mm TL (Fig. 3B), the yolk has been absorbed, and a few rotifers are recognized in the digestive tract.

The myotomes attain the same number as adult (10 + 16) seven days after hatching, when the larvae are 3.4-3.6 mm TL (Fig. 3C). The melanophores and the red pigments decrease in number. However, a melanophore newly appears on the otolith. The cuplae and the free neuromasts disappear. Primordiums of hypurals and caudal fin rays appear.

Fifteen days after hatching, at 4.3-4.5 mm TL (Fig. 3D), the end of the notochord

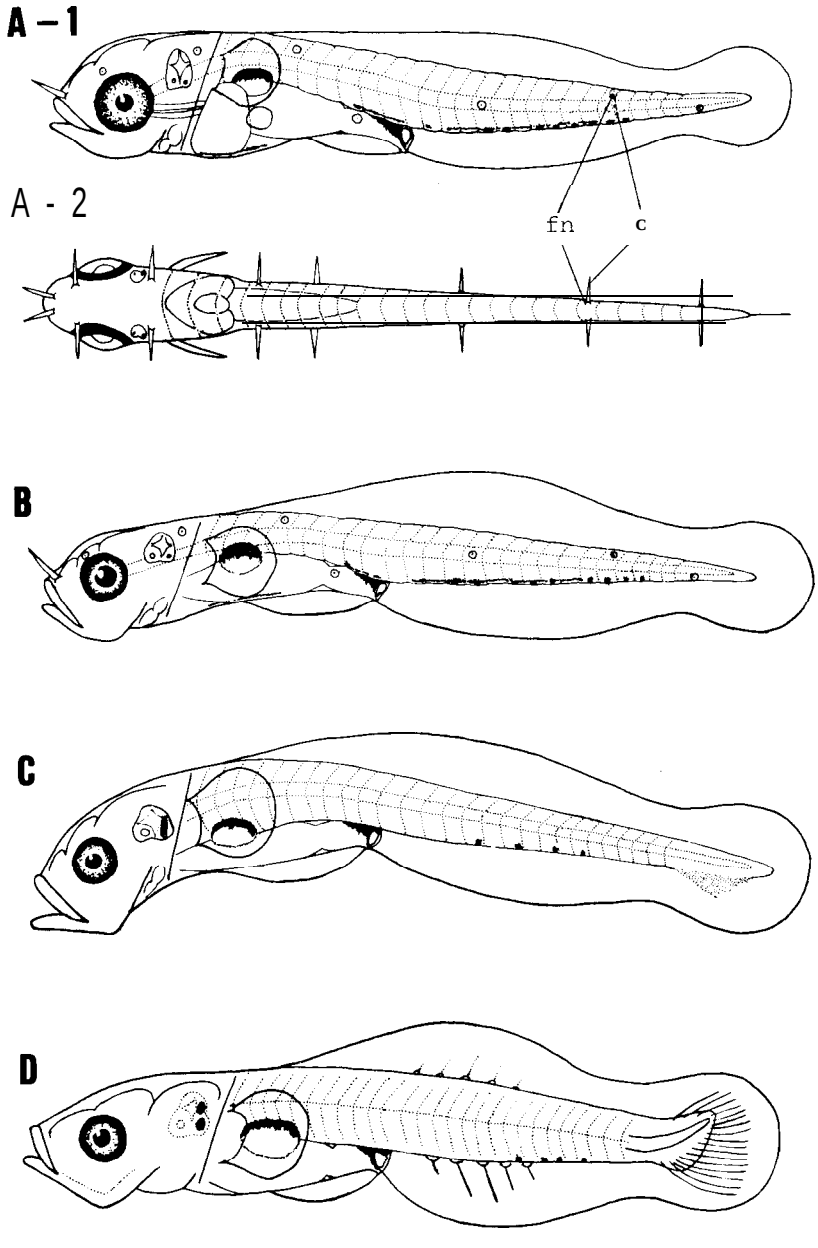


Fig. 3. Larvae of *Eviota abax*. A-l, newly hatched larvae, 2.62 mm in total length (TL). A-2, Dorsal view of the same specimen. B, 1 day old after hatching, 2.90 mm TL. C, 7 days after hatching, 3.55 mm TL. D, 15 days after hatching, 4.36 mm TL. c, cupla; fn, free neuromast.

bends upward and urostyle is formed; buds of dorsal and anal fins disappear; a melanophore on the otolith is divided into two ; the red pigments disappear.

By the 17th day after hatching, all the larvae had died.

Embryonic development and larvae of *Eviota storthynx*

We will breafly describe embryonic development and larvae of *E. storthynx,* as Shinomiya *et al.* (1981a) reported it in detail.

Eggs are spawned in a single layer on the ceiling of the shelter. They are fusiform with a bundle of adherent threads at their base, measuring 1.19-1.31 mm (mean 1.24 mm) in length and 0.37-0.42 mm (mean 0.40 mm) in breadth, and have numerous minute processes and large ones of irregular size scattered all over the egg membrane (Fig. 4). The number of eggs spawned by a female is 200-250 per brood.

The 2-cells stage is achieved 30 min after fertilization (Fig. 5A), and the morula stage 4 hr. after fertilization (Fig. 5B).

Eighteen hr. after fertilization (Fig. 5C), a Kupffer's vesicle appears three pairs of myotomes are recognized. Some granules are observed on the surface of the embryo.

Lenses are observed in the optic cups and a pair of ear vesicles appears on the lateral sides of the nape at 33 hr. (Fig. 5D). The slight tail bud projects. The Kupffer's vesicle had disappeared. Seven pairs of myotomes are recognized. The notochord is visible.

Pectoral fin buds are recognized, and melanophoes appear on the optic cups 70 hr. after fertilization (Fig. 5E).

Larvae hatch after sunset, 110 hr. after fertilization. An empty egg capsule with a hatching cleft is shown in Fig. 5F. The small oil globules in the yolk did not merge to form larger ones throughout development.

The newly hatched larvae measure 1.9-2.1 mm TL (Figs. 5G-1, G-2), and have 8+

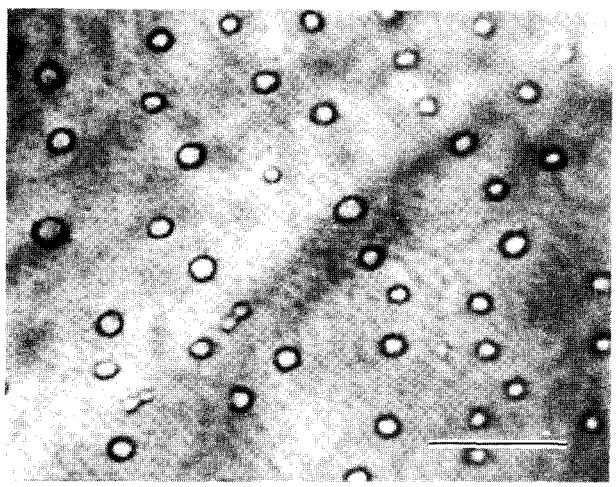
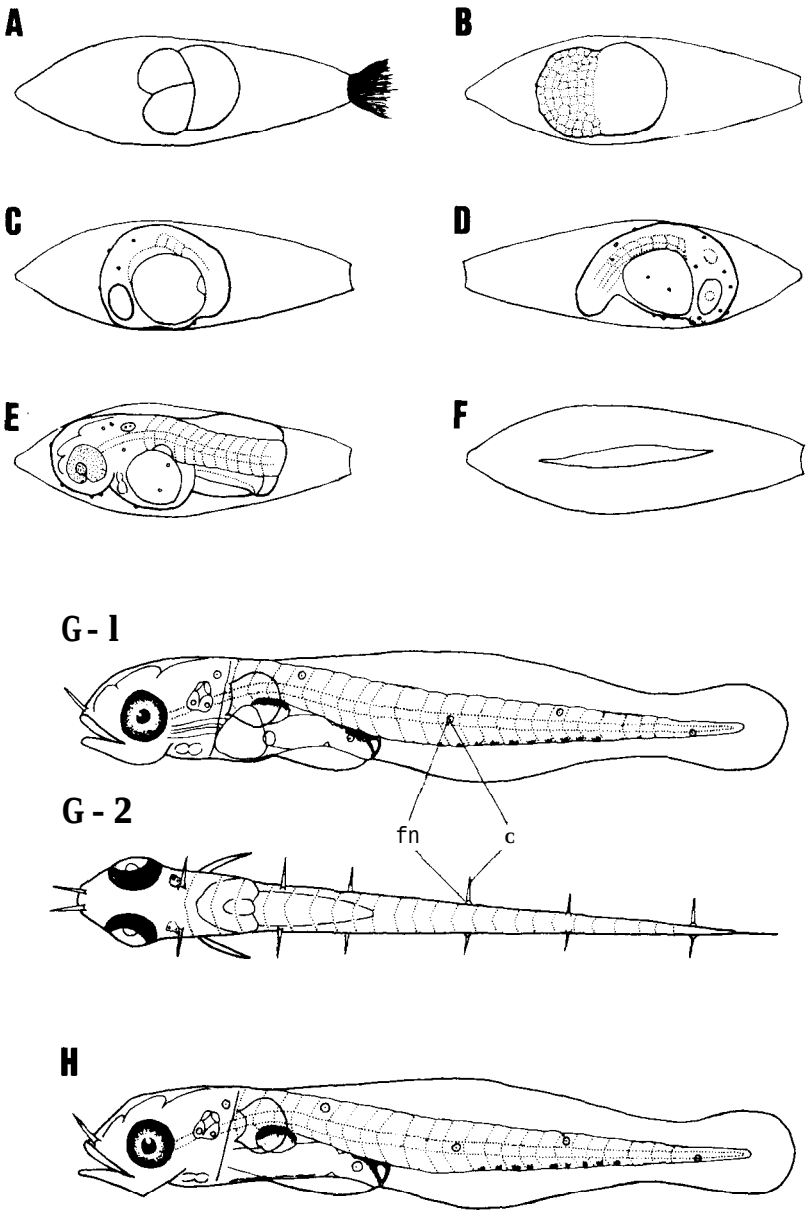


Fig. 4. Surface of the egg membrane of *Eviota storthynx*. Scale indicates 20 μm.


A
B
C
D
E
F
G-1
G-2
fn
c
H

15 = 23 myotomes (adult: 10 + 15 = 25). The yolk sac still remaines, but the mouth had opened and peristalsis of the digeastive tract can be seen. The larvae show positive phototaxis. Seven pairs of cuplae and free neuromasts are observed (Fig. 5G-2). The cuplae easily fall off when the animal is fixed with 5 % formalin. Eight to twelve small dendritic melanophores are observed on ventral part of the tail. Others are recognized on the optic cups, the dorsal surface of the gas bladder, and the dorsal part of the rectum. Red pigments are on the dorsal surface of the gas bladder and the ventral part of the abdomen.

One day after hatching, the larvae measure 2.0-2.2 mm in TL (Fig. 5H), the yolk has been absorbed, but no food is recognized in the digestive tract. All larvae had died of starvation by the 4th day after hatching.

DISCUSSION

Dotsu *et al. (1965)* reported that diameter of eggs and total length of newly hatched larvae of *Eviota abax* were 2.3-2.5 *x* 1.0-1.1 mm and 4.4-4.5 mm, respectively at Tsuyazaki, Fukuoka Prefecture. This is nearly twice as large as those of the present results. On the other hand, those from Nagashima, Kagoshima Prefecture, are 1.4-1.5 $\times$ 0.7-0.9 mm and 3.1-3.3 mm (Sunobe, unpublished). It seemes to be intraspecific variations of egg diameter and total length of newly hatched larvae of *E. abax*.

It is known that small oil globules in the yolk merge to large ones through egg development in the most of gobiid fishes (Tanaka *et al.*, 1982). However, those of *Eviota abax* and *E. storthynx* did not merge. The few other gobiid species with the same character are as follows : *Bathygobius fuscus* (*Gobius poecilichthys* in original paper), *Parioglossus dotui* (*taeniatus*), *Silhouettea* (*Ctenogobius*) *dotui, Odontobutis obscura obscura (Mogurnda obscura), Periophthalmus cantonensis, Gobiosoma oceanops, Acentrogobius pflaumi, Favonigobius* (*Acentrogobius*) *gymnauchen, Parioglossus formosus (taeniatus)* and *Valenciennea (Eleotriodes) helsdingeni* (Dotsu, 1955 ; Dotsu, 1956 ; Dotsu, 1958; Dotsu and Tsukahara, 1964 ; Kobayashi *et al.*, 1972 ; Valenti, 1972 ; Uchida and Dotsu, 1980 ; Uchida and Dotsu, 1980 ; Shinomiya *et al.*, 1981b; Tanaka *et al.*, 1982).

Dotsu *et al. (1965)* and Shinomiya *et al.* (1981a) did not recognize any Kupffer's vesicle in *Eviota abax* and *E. storthynx*. However, the present study revealed the existence of the Kupffer's vesicle as is usual in other gobiid fishes. Morphological characteristics of eggs and embryonic development of *E. abax* and *E. storthynx* include numerous minute processes on the surface of the egg membrane, granules on the surface of the embryo, and unmerged oil globules. Larvae of both species have red pigments, which is rarely known in other gobiid fishes except for *Acanthogobius lactipes* (Uchida and Dotsu, 1980). Further comparative study is needed to determine whether these characteristics are specific to the present species or not.

Larvae of *Eviota abax* and *E. storthynx* resemble each other in the distribution of melanophores (Figs. 3A-1; 5G-1). Total length of larvae can be used to distinguish *E. abax* from *E. storthynx*.

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