Reproductive Behavior, Embryonic Development and Yolk-sac Larva of *Sueviota lachneri* (Pisces: Gobiidae) in the Laboratory

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Abstract Reproductive behavior, embryonic development and yolk-sac larva of the gobiid fish *Sueviota lachneri* are described for the first time. The entire reproductive sequence consisted of nest preparation by a male, courtship behaviors, nest entry by a female, spawning, and paternal care until the hatching of eggs. Motor patterns of courtship sequence were as follows. The male approached a female. The female responded to the male approach with yawning. In leading, the male returned to its nest. Tail-waving was performed during stopping. Eggs were fusiform. Mean length and width of the eggs were 1.62 mm (range=1.52-1.72) and 0.60 mm (0.56-0.67), respectively. The mean number of the eggs per clutch was 91 (34-154). The surface of the eggs was covered with numerous minute processes. It took 132 hrs. after fertilization to hatch. The hatching rate observed was very low, and only one specimen was obtained. The newly hatched larva was in the stage of "yolk-sac larva", measuring 2.54 mm in total length. Melanophores appeared on the optic cups, the gas bladder, the dorsal part of the rectum and the ventral part of the tail. Red pigments were on the gas bladder. There was no difference in the traits of behavioral patterns, eggs and yolk-sac larva between *S. lachneri* and the species of the closely related genus *Eviota*.

Key words: Gobiidae, *Sueviota*, *Eviota*, courtship behavior, embryonic development, yolk-sac larva.

Sueviota is a gobiid genus of small size, phylogenetically close to Eviota (Winterbottom and Hoese, 1988). Both genera are distributed in the tropical and subtropical waters in the Indo-Pacific, inhabiting coral or rocky reefs (Lachner and Karnella, 1980; Winterbottom and Hoese, 1988). Although reproductive behavior, egg development and larvae of Eviota have been reported by Sunobe (1998), Sunobe and Nakazono (1987, 1995, 1999) and Taru and Sunobe (2000), those categories of Sueviota remain unknown. Sueviota lachneri Winterbottom and Hoese was recently recorded from Japanese waters by Randall et al. (1997). The Japanese localities where the species was found included the Ogasawara Islands, Tokunoshima Island and Kagoshima (Randall et al., 1997; Akihito et al., 2000).

In this paper, we first describe the repro-

ductive behavior, embryonic development and yolk-sac larva of *S. lachneri* reared in the laboratory. Then, we compare our observations with the previous reports on the species of *Eviota* (Sunobe and Nakazono, 1987, 1995; Sunobe, 1998).

Materials and Methods

Male and female parents (20.0 and 19.3 mm in standard length, respectively) used in this study were collected at Cape Sata, Kagoshima Pref., Japan on Oct. 12, 1982. They were kept in a glass aquarium $(60\times30\times28\,$ cm) at Faculty of Fisheries, Kagoshima University within one day after collection during the experiments. The holding aquarium was equipped with a typical under gravel filtration system and a single air-lift pipe. An opaque PVC pipe (2 cm inside diameter and 5 cm long) was put on the bottom as

a shelter for spawning and hiding. Fresh minced fish meat was provided once a day as food. Water temperature was maintained between 24–26°C with an electric heater from October 1982 to April 1983 and fluctuated between 25 and 30°C from May to July 1983 in ambient conditions.

As spawning occurred in the morning, observations were made 7:00 to 12:00. The data were recorded visually with a $35 \, \text{mm}$ SLR camera and with a VTR. Just after spawning, the PVC chloride pipe, with an egg mass on its ceiling, was placed in a plastic vessel $(15\times10\times8\,\text{cm})$ fixed below the outflow of the air-lift pipe in the aquarium, so that filtered water ran over the eggs throughout development. Measurements of the egg and larva were made by means of a confocal microscope with an ocular micrometer. Figures were drawn with a camera lucida.

The parental specimens examined in this study were preserved in 70% ethanol and deposited in the National Science Museum, Tokyo (NSMT); male, P41879: female, P41880.

Results

1. Reproductive Behavior

The first spawning was recorded on 10 May 1983, and subsequent 10 spawnings occurred at an interval of 7 to 9 days until July 25, 1983. Reproductive behavior consisted of the following sequence: nest preparation by

the male, courtship behaviors, nest entry by the female, spawning, then parental care by the male until the hatching of eggs.

For nest preparation, the male removed sand in the nest by scooping with its mouth and cleaned the ceiling by rubbing material off with its abdomen. During this stage, the male often chased away the female. After nest preparation, the male repeatedly courted the female.

The observed courtship sequence included three basic components: approach by the male, female response and male leading of the female to the nest.

Approaching. The male approached the female by simultaneously moving its pectoral fins with both dorsal fins erect (Fig. 1A). Just before reaching the female, the male advanced bit by bit, the head held horizontally and the dorsal fins erect (Fig. 1A).

Female response. The female responded to the male approach with yawning (Fig. 1B).

Leading. The male returned to its nest by simultaneously moving its pectoral fins with erecting both of dorsal fins (Fig. 1B). Tailwaving was performed during stopping.

After the male's leading, the female entered the nest. During the female's entering, the male push its body on the female. Both the male and female adopted upside-down postures on the ceiling of the pipe, simultaneously moving back and forth within the nest. Within 2 or 3 minutes, the female began to

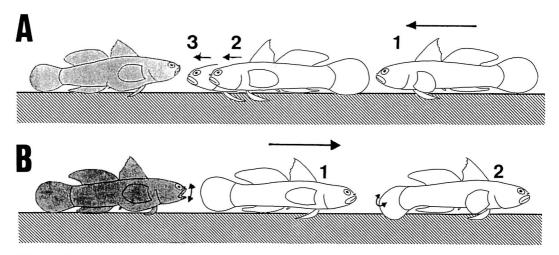


Fig. 1. Motor patterns of courtship behavior. A, approaching; B, female response and leading. Male, unshaded; female, shaded. Numerals indicate the order of male positions. Arrows mean moving.

spawn eggs, the body quivering simultaneously. Eggs were attached in a single layer on the ceiling of the nest. Spawning bouts started between 7:00 and 10:30, lasting for 30 min. to 1 hr. After spawning, the female left the nest and the male guarded the eggs until hatching.

2. Embryonic Development and Yolk-sac

The shape of the eggs was fusiform with a bundle of adherent threads at the base (Fig. 2). Mean length (mm) and width (mm) of the eggs were 1.62 ± 0.07 SD (range = 1.52-1.72, n=8) and 0.60 ± 0.04 (0.56-0.67, n=8). The mean number of the eggs per clutch was $91\pm$

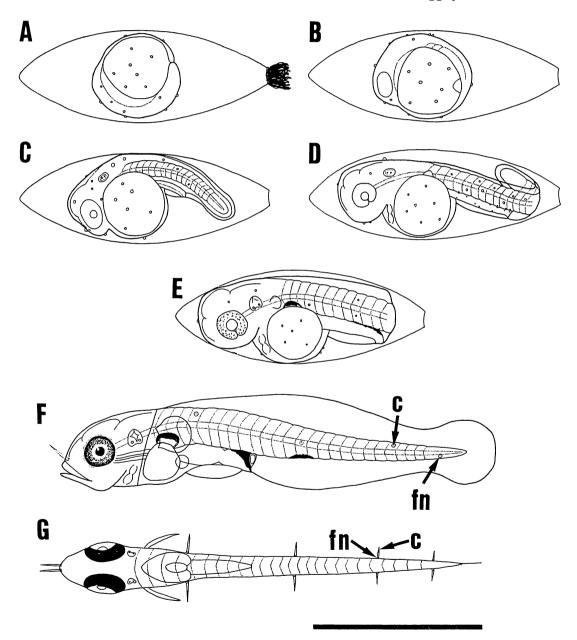


Fig. 2. Embryonic development and yolk-sac larva. A, 15 hrs. after fertilization; B, 17 hrs.; C, 33 hrs.; D, 47 hrs.; E, 70 hrs.; F, yolk-sac larva; G, dorsal view of the same specimen. c, cupla; fn, free neuromast. Scale indicates 1 mm.

44 (34–154, n=8). The surface of the eggs was covered with numerous minute processes as observed in the species of *Eviota* (Sunobe and Nakazono, 1987, 1995).

The blastopore was closed and an embryo developed 15 hrs. after fertilization. Granules were visible on the surface of the embryo. The yolk was filled with small oil globules (Fig. 2A). By 17 hrs. after fertilization (Fig. 2B), the embryo was been formed. A pair of optic vesicles and a Kuppffer's ves-Two myomeres were also icle appeared. counted. Thirty-three hrs. after fertilization (Fig. 2C), lenses were formed in the optic cup and a pair of ear vesicles appeared. Brain differentiation was also observed, number of myomeres was 16, and the Kupffer's vesicle had disappeared. After 47 hrs. (Fig. 2D), a pair of pectoral fin buds was recognized, and a heart appeared in front of the yolk. Melanophores were found on the abdominal region. Gas bladder was formed 70 hrs. after fertilization (Fig. 2E). Melanophores were visible on the optic cups, the gas bladder and the rectum. Red pigments appeared on the gas bladder.

Larvae hatched at 22:00, 132 hrs. after fertilization. The hatching rate observed was very low, and only one specimen was obtained. The newly hatched larva was on the stage of "yolk-sac larva", as the yolk still remained. Its total length was 2.54 mm. The mouth had opened, and peristalsis of the digestive tract was seen. It also had 9+16 myomeres (in adult 10+16). Melanophores appeared on the optic cups, the dorsal part of the gas bladder, the dorsal part of the rectum and the ventral part of the tail. Red pigments were present on the gas bladder. The small oil globules in the yolk never merged throughout the development (Fig. 2E). Five pairs of cuplae and freeneuromasts were observed on the lateral sides of the body (Fig. 2F). The cuplae readily fell off after fixation in 5% formalin.

Discussion

Sunobe (1998) described courtship behavior of six *Eviota* species, showing that classification of behavioral patterns of each species corresponds to the species grouping by a combination of morphological characters

(Lachner and Karnella, 1980). The male behavioral features of *Sueviota lachneri* are very similar to that of *E. storthynx*, and the female response, yawning, was also observed in *E. queenslandica*.

Numerous minute processes on the surface of the eggs are a diagnostic character for the *Eviota* species (Sunobe and Nakazono, 1995). Red pigments on the gas bladder and melanophores on the optic cups, the gas bladder, the dorsal part of the rectum and the ventral part of the tail are characters, which can distinguish the yolk-sac larvae of the *Eviota* species from the other gobiid fishes (Sunobe and Nakazono, 1987; 1995). The eggs and yolk-sac larva of *S. lachneri* also share these same characters with the species of *Eviota*.

The hatching rate was very low in *S. lachneri*. Egg development seemed to be normal, comparing with those of *Eviota* species (Sunobe and Nakazono, 1987; 1995). While newly hatched larvae of the *Eviota* species hatch without any aid of the parental males, behavioral action by the male may be needed to hatch in *S. lachneri*.

Winterbottom and Hoese (1988) described *Sueviota* as a new genus, forming the sister group of the *Eviota* species, and the former is distinguished from the latter by the presence of a branched fifth pelvic-fin ray. However, our results show that there are no distinguishable characters of this species from the species of *Eviota* in the courtship behavioral patterns, eggs and yolk-sac larva.

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ハゼ科アワセイソハゼの飼育下における 繁殖行動, 卵発生および前期仔魚

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ハゼ科アワセイソハゼの飼育下における繁殖行動, 卵発生および前期仔魚を初めて記載する.本種の繁殖行動は雄による巣の準備,求愛行動,雌の入巣,産卵,雄の卵保護から成っていた.求愛行動は雄が雌に接近するところから始まる.雌がそれに対し口を開けて応答すると、雄は巣に戻った.途中,停止すると尾部を左右に振った.卵は紡錘形で長径 1.62 mm (範囲 1.52-1.72),短径 0.60 (0.56-0.67).一卵塊の卵数は平均 91 個 (34-154).表面は細かい突起物で覆われていた.孵化には 132 時間を要した.孵化率は低く,唯一得られた前期仔魚の全長は 2.54 mm で,黒色素胞は眼,鰾,直腸上部,尾部下縁部に,赤色胞は鰾に出現した.近縁のイソハゼ属と比較すると,繁殖行動,卵および前期仔魚の特徴にはいずれも大きな違いは見られなかった.