Japanese Journal of Ichthyology
Vol. 34, No. 3 1987

魚類学雑誌
34巻3号 1987年

Development of Eggs, Larvae and Juveniles of the Cottid Fish, *Pseudoblennius cottoides*, Reared in the Laboratory

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(Received February 2, 1987)

Abstract Embryonic, larval and juvenile development of the cottid fish, *Pseudoblennius cottoides*, were described based on a series of laboratory-reared specimens. The eggs were demersal, adhesive, almost spherical in shape, measuring 1.60–1.98 mm in diameter, and with numerous various-sized oil globules. Neighboring eggs adhered to each other to form an egg mass. Hatching occurred between 13 and 15 days after spawning at a water temperature of 15.2 to 17.0°C. Newly hatched larvae measured from 6.3 to 7.1 mm, averaging 6.7 mm TL, and possessed 40 myomeres. Absorption of the yolk was completed at about 7.5 mm TL. Flexion of the notochord started and finished at about 10 mm TL and about 14 mm TL, respectively. Aggregate numbers of all fin rays were completed at over 16 mm TL, at which time the larvae reached the juvenile stage. The pigment pattern became the same as that of adults in juveniles longer than 20 mm TL. Lateral lines were completed at over 37 mm TL, at which time the juveniles attained to the young stage.

Pseudoblennius cottoides (Richardson) is a very common marine cottid fish inhabiting *Zostera marina* or *Sargassum* beds in shallow waters along the coast from southern Hokkaido to Kyushu in Japan. Despite common occurrence of the larvae and juveniles (Kimura et al., 1983, 1984), little is known about their development or early life history because of the difficulty in distinguishing them from other cottid larvae. The embryonic development and the hatched larvae of this species were described briefly by Watanabe (1958, 1976) and Shiogaki and Dotsu (1974). In this report, embryonic, larval and juvenile development were described from a series of reared specimens to provide more information on the identification of the larvae and juvenile of this species.

Materials and methods

Ten females (85.0–127.8 mm in total length (TL)) were caught by angling around Zaga Island in Ago Bay, Mie Prefecture (lat. 34°16′15″N, long. 136°48′30″E) in November and December, 1984. They were maintained in a 500 *l*-capacity black polyethylene tank containing diluted sea water (25–28‰ salinity) at a water temperature (WT) of about 16°C.

Just after spawning was occurred, egg masses deposited on the bottom of the tank were transferred into a guze net cage (30×25×30 cm) which was suspended in the parental fish tank. The hatched larvae were transferred into a 500 *l*-capacity black polyethylene tank containing diluted sea water with weak aeration. The larvae were reared in still water until they developed to the flexion stage. Subsequently, the water circulation system was put into operation. The larvae were fed with rotifers, *Branchionus plicatilis*, for the first five days. Subsequently, *Artemia salina* nauplii, wild zooplanktons, and larval *Sebastiscus marmoratus* were fed to the larvae in accordance with their development. The juveniles were fed with minced *Spratelloides gracilis*.

Larvae and juveniles were removed periodically from the larval rearing tank, and anesthetized by 10–100 ppm ethylene glycol monophenyl ether for subsequent morphological observation and measurements. Specimens were preserved in 10% buffered formalin (diluted with sea water) after morphological observations, and stained with Alizarin red S to observe the spines on head and fin rays. The measurements of body parts followed the methods of Leis and Rennis (1983).

Results

Spawning. The females spawned fertilized eggs, proving that they had already copulated before our collection. Fertilization took place entosomatically maybe just before spawning. Spawn-

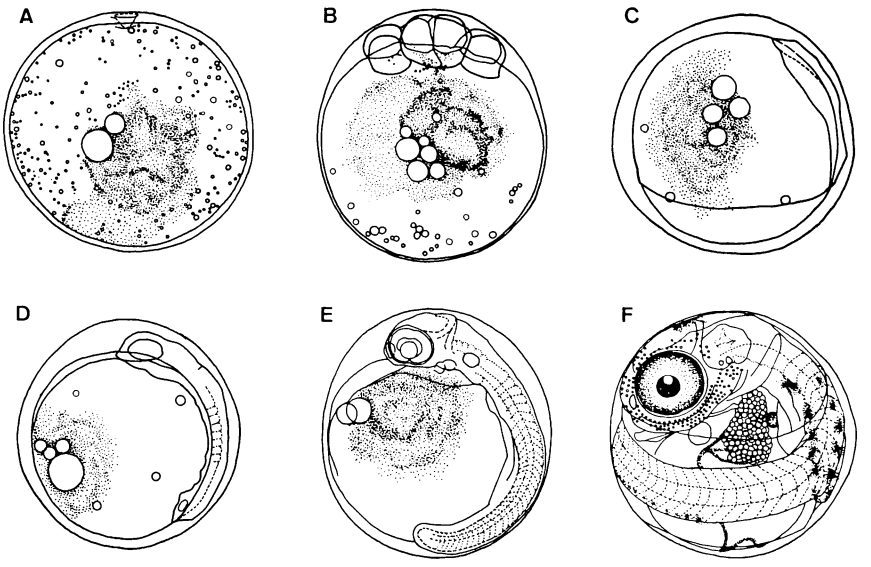

Fig. 1. Development of eggs of *Pseudoblennius cottoides*. A, fertilized egg newly spawned; B, 8-cell stage, 7 h 50 min after spawning; C, formation of embryo, 2 d 9 h; D, formation of Kupffer's vesicle, 3 d; E, 33-myomere stage, 5 d; F, just before hatching, 13 d.

ing occurred continuously from December 19, 1984 to Febeuary 13, 1985. After mid-January, the eggs spawned reduced in number, and the hatching rate also decreased. The spawning time was estimated at 7:00 to 8:00 and 17:00 to 18:00 from the embryonic development.

Embryonic development. The eggs were demersal, adhesive, and almost spherical in shape, measuring 1.60–1.98 mm in diameter (Fig. 1A). Neighboring eggs adhered to each other to form an egg mass. The egg mass separated easily into some small masses. The chorion was colorless and transparent. The yolk was pale yellow or pale greenish yellow in color. There were numerous and various-sized oil globules in the yolk just after spawning. The perivitelline space was narrow.

Embryonic development of the eggs was as follows: The blastodisk began to elevate at 2h 20 min after spawning. 2-cell stage at 4h 20 min, 4-cell stage at 6h, 8-cell stage at 7h 30 min (Fig. 1B), blastula stage in 1d 7h, gastrula stage in 2d after spawning. Two days and 9 hours after spawning, 3/4 of the yolk was covered by blastoderm and the embryonal body appeared (Fig. 1C). Optic vesicles were observed in 2d 13h after spawning. Three days after spawning, the blastopore was closed, Kupffer's vesicle appeared, and seven myomeres were present (Fig. 1D). Auditory vesicles, optic lenses, and 14 myomeres were observed in 3d 12h after spawning. Four days after spawning, 20 myomeres were observed and Kupffer's vesicle disappeared. Five days after spawning, the myomeres increased in number to 33, the heart pulsated, and the embryonal body moved intermittently (Fig. 1E). The eyes became blackish in color in 6d, and the pectoral fins were formed in 7d after spawning. Nine days after spawning, the mouth was open, numerous hatching glands were present on the head, and melanophores were located on the top of the head, on the dorsal surface of the yolk, on the yolk beneath the pectoral base, and along the ventral contour of the tail. Hatching occurred between 13 and 15 days after spawning.

During embryonic development, the oil globules decreased in number, and became a single droplet about 7 days after spawning. No xanthophores were located on the embryonal body. The perivitelline circulation was observed before hatching.

Yolk-sac larva. The newly hatched larvae measured 6.3 to 7.1 mm, averaging 6.7 mm TL, and contained a large amount of yolk with a single oil globule (Fig. 2A). The head length (HL) and pre-anal length (PAL) were 17% and 38% of TL, respectively. The eye diameter (ED) was 59% of

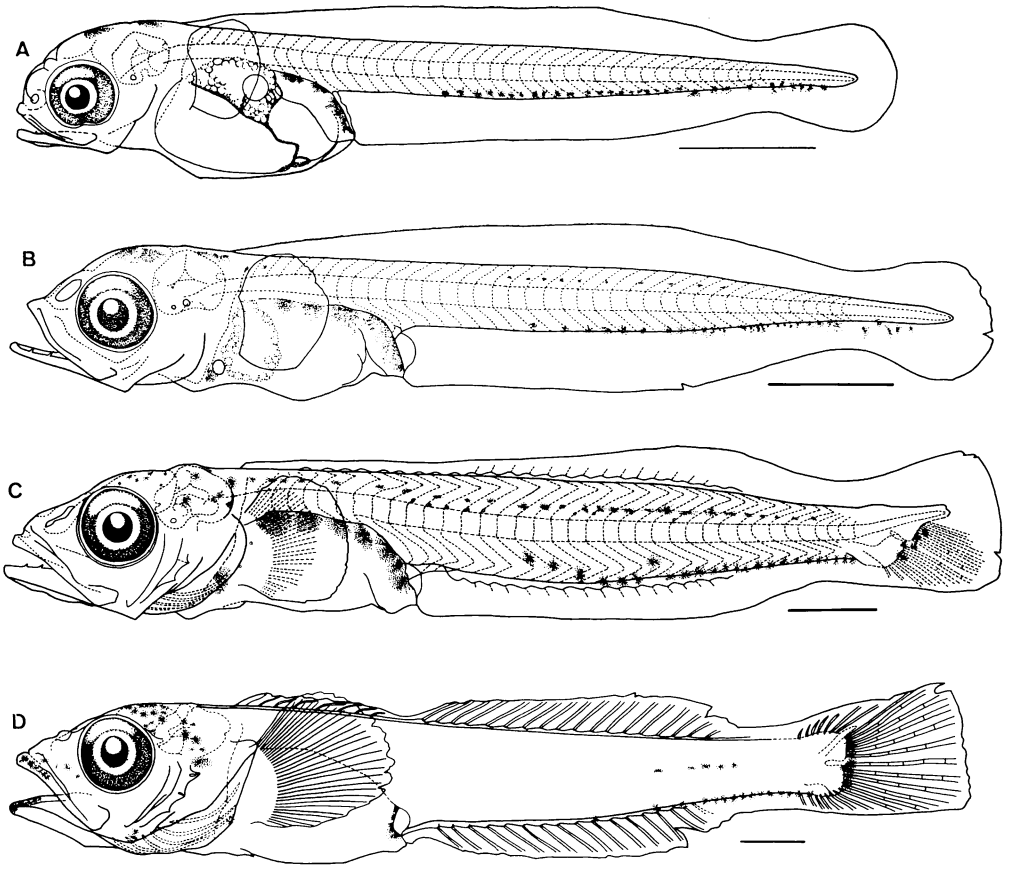

Fig. 2. Development of larvae of *Pseudoblennius cottoides*. A, yolk-sac larva newly hatched, 6.5 mm TL; B, preflexion larva, 7.5 mm TL; C, flexion larva, 10.6 mm TL; D, postflexion larva, 14.2 mm TL, entosomatic melanophores were omitted. Scales indicate 1 mm.

HL. The alimentary canal was already convoluted. A pair of conical teeth was present on both sides of the lower jaw. Melanophores were distributed on the top of the head (1–5 cells), on the nape (0–2), on the dorsal surface of the visceral cavity (6–12), at the anterior tip of the yolk (0–1), and along the ventral contour of the tail (20–33). No xanthophores were present. The larvae possessed 9+31=40 myomeres. The larvae swam actively in the surface layer of the rearing tank, and showed strong phototaxis.

Preflexion larvae. Absorption of the yolk was completed when the larvae attained to about 7.5 mm TL (Fig. 2B). HL and PAL were 20% and 40% of TL, respectively. ED was 38% of HL. A series of melanophores was present along the spinal chord posterior to the anus. The snout was elongated, and the larvae began to feed on the rotifers. In larvae over 8.5 mm TL, distinctive xanthophores appeared on the head, on the dorsal surface of the visceral cavity, and along the dorsal and ventral sides of the notochord. Melanophores were present along the entire length of the spinal chord. The rudiment of the caudal fin appeared ventrally at the posterior tip of the notochord. Both jaws became strong, and the food preference changed from rotifers to planktonic crustaceans.

Flexion larvae. The notochord started to flex when larvae attained to about 10 mm TL (Fig. 2C). HL and PAL were 24% and 41% TL, respectively. ED was 32% of HL. The second dorsal and pectoral soft rays appeared. Pelvic buds and segmentation of the caudal rays were observed. In a larva of 12.6 mm TL, the dorsal spines were formed, and melanophores appeared at

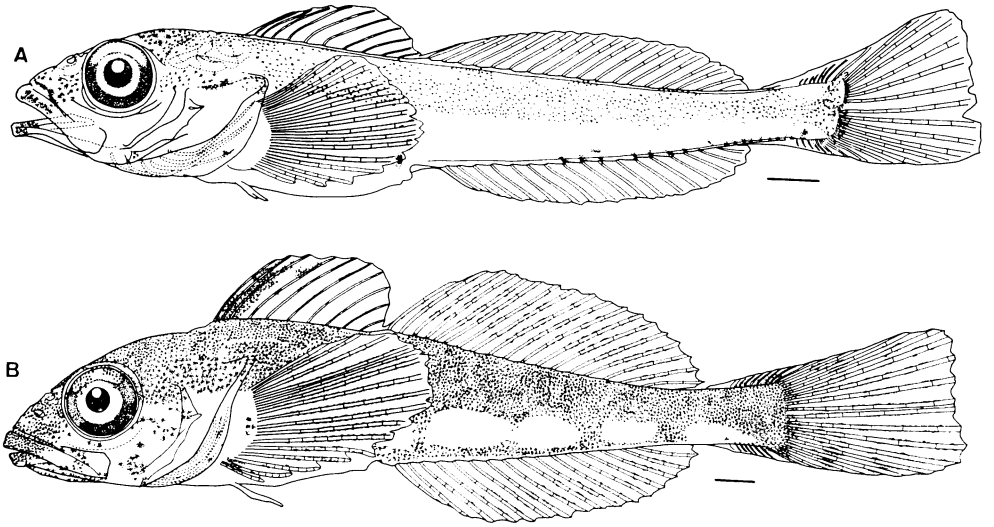

Fig. 3. Development of juveniles of *Pseudoblennius cottoides*. A, early juvenile, 17.7 mm TL; B, juvenile, 23.9 mm TL. Scales indicate 1 mm.

the anterior tips of both jaws and on the opercle. Remarkable cannibalism occurred, and the larvae began to feed intensively on the larval *Sebastiscus marmoratus*.

Postflexion larvae. Flexion of the notochord was completed when larvae attained to about 14 mm TL (Fig. 2D). HL, PAL and the depth of caudal penduncle (CPD) were 26%, 41% and 5.4% of TL, respectively. ED was 30% HL. Though the dorsal and anal fins were still connected with the caudal fin by remnants of the finfold at the caudal peduncle, the fin rays were completed in number except the pelvic. Three preopercular spines were present. Melanophores newly appeared on the snout, at the posterior end of the angular, and along the lateral median of the tail, and increased in number at the anterior tips of both jaws, on the top of the head, and at the base of caudal fin.

Juveniles. Aggregate numbers of all fin rays including the pelvic were completed in specimens larger than 16 mm TL (Fig. 3A). HL, PAL and CPD were 26%, 42% and 4.5% of TL, respectively. ED was 28% of HL. The first dorsal fin well developed. Segmentation of the dorsal and pectoral soft rays started. The preopercular spines diminished in size except the uppermost one. Melanophores on the head increased in number, and those along the lateral median of the body spread upwards to the dorsal median. The juveniles with pigmented dorsal surface of the body changed from swimming at the surface to swimming at the bottom of the tank. In juveniles of about 18 mm TL, melanophores appeared on the first dorsal fin membrane between the first and fourth spines. In juveniles over 20 mm TL (Fig. 3B), HL, PAL and CPD were 26%, 40% and 6.0% of TL, respectively. ED was 32% of HL. Melanophores were distributed on the ventral surface of the lower jaw, beneath the eye, on the cheek, at the pectoral base, and on the soft rays of the fins except pelvic. The patches of melanophores below the lateral median of the body were connected partially with those above the anal base, subsequently the pigment pattern became the same as that of adults. Iridophores appeared on the cheek, on the opecular bones, at the pectoral base, and on the lateral surface of the body. Lateral lines began to form. In juveniles of about 25 mm TL, supraocular cirri and axillary scales were formed.

Young. In specimens over 37 mm TL, formation of lateral lines was completed and the caudal rays started to branch.

Discussion

Embryonic development and morphology of the yolk-sac and preflexion larvae described by Shiogaki and Dotsu (1974) agree with those obtained

here in general. But there are some discrepancies concerning the color of yolk (light greenish yellow in their descriptions), the incubation period (17 to 20 days at 12°C WT), and number of melanophores on the larval body (2–6 cells on the top of the head, 1 or 2 on the nape, 5 or 6 on the dorsal surface of the visceral cavity, and 26 to 32 along the ventral contour of the tail).

Watanabe (1958, 1976) described that the eggs varied from red-orange to yellow in color, hatching occurred 11 or 12 days after spawning at 9 to 12°C WT, and the sizes of newly hatched and preflexion larvae were about 8.0 and 12.5 mm in standard length, respectively. There are considerable differences between Watanabe's descriptions and those of ours and Shiogaki and Dotsu's (1974). Accordingly, it seems that Watanabe (1958, 1976) are based on another cottid fish but not *Pseudoblennius*.

Two wild juveniles of 12.6 and 29.0 mm TL described as *P. cottoides* by Nakamura (1934) can be identified with *P. marmoratus* (Döderlein) because their pigment patterns differ from those of our juveniles and Nakamura's specimens have 12 pectoral rays (16–19 in *P. cottoides*).

We succeeded in rearing of *P. percoides* (Günther) from the eggs to juveniles; detailed comparison between *P. cottoides* and *P. percoides* will be published elsewhere.

Acknowledgments

We wish to express our sincere gratitude to Dr. Kiyoshi Suzuki, Faculty of Fisheries, Mie University, for his helpful suggestions and critical reading of the manuscript. Thanks are also due to Dr. Kunihiko Fukusho, Dr. Teruyuki Nakanishi and Dr. Masanori Okauchi, National Research Institute of Aquaculture, for their advice in culture of food organisms. Messrs. Masato Aritaki, Takaharu Okazawa, Atsushi Sato and Toyoharu Ito, the students of our university, are thanked for their help in the present study.

This work was supported partially by a Grant-in-Aid from the Itoh Foundation for Advancement of Ichthyology.

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アサヒアナハゼの卵および仔稚魚

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水槽内で自然産卵させたアサヒアナハゼ卵を飼育し，卵内発生および孵化仔魚から若魚までの外部形態の形成過程を観察した．本種は体内受精を行い，卵は産出直前に受精する．卵は球形の沈性凝集卵で，卵径 1.60–1.98 mm，卵黄は淡黄色から淡緑黄色を呈し，多数の油球が存在する．水温約 16°C で受精 13–15 日後に孵化する．孵化仔魚は全長 6.3–7.1 mm，黄色素胞はない．全長約 7.5 mm で卵黄が完全に吸収される．脊索末端の屈曲は全長約 10 mm で開始し，約 14 mm で終了する．全長 16 mm 以上になると，各鰭条数が定数に達し，稚魚になる．側線は全長 37 mm 以上で完成する．

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