

Development of Eggs and Larvae of the Freshwater Goby, *Rhinogobius brunneus*

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Abstract The development of eggs and larvae of the large-dark type B and that of larvae of the cross-band type of *Rhinogobius brunneus* were observed. Newly hatched larvae of both types were different in total length and also in the position of the anus. Some variations in the pigmentation of melanophores were observed in the larval development of the cross-band type. The larvae of the large-dark type B reached the juvenile stage and wholly abandoned their planktonic mode of life in 32 days after hatching at 21.0~27.5°C. Those of the cross-band type did so in 36 days after hatching at 19.9~22.8°C.

Introduction

Rhinogobius brunneus (Temminck et Schlegel) is a common freshwater goby known to have a very wide range of color variation. The fish has been divided into several types, namely the cross-band type, the dark type, the large-dark type A and B, the cobalt type, the medium egg type and the orange type, based on the differences in color pattern, pectoral ray counts, egg size, etc. (Mizuoka, 1967, 1971, 1974; Nishijima, 1968; Ito and Mizuno, 1972; Mizuno, 1976). No intermediate types, at least among the former four types, are found (Mizuno, 1976), and the identification of each type has been considerably well established in adult fish. However, it is still unknown when and how the color and morphological differentiation is established in the course of ontogeny. Though Kobayashi (1923), Okada and Seishi (1938), and Mizuno (1960) reported on the eggs, larvae and juveniles of this species, they were not aware of the existence of such types in this species at that time and used occasional field samples for their materials.

In the course of our rearing experiments of *R. brunneus*, we were able to observe the development of eggs and larvae of the large-dark type B, and that of larvae of the cross-band type.

Materials and methods

Parental fish of the large-dark type B were collected from the Isumi River, Chiba Pref., and that of the cross-band type from the Obiso

River, Chiba Pref. The identification and naming of each type followed Mizuno (1976). For each type two males and two females were housed in an aquarium of 63 l in capacity with a closed water circulation system. Some curved pieces of plastic plate were set for spawning beds. In the large-dark type B, one of the two females spawned probably with one of the two males. In the cross-band type, both females spawned, but it was unknown whether only one male mated with both females or each male with each female. Immediately after eggs were observed on the spawning bed, the eggs were separated from the guarding male and transferred into another small aquarium of 27 l in capacity for hatching. The development of eggs of the large-dark type B was observed for 42 hours at 25.3~26.7°C in water temperature. Hatched larvae of both types were reared in 5-l glass jars with a closed water system, fed with *Brachionus plicatilis* for the initial food item. Dead larvae and remained foods were removed and the rearing water was partly changed with 1.5 l of clear new water everyday. Four different salinities, 0‰, 33‰, 67‰, and 100‰ (Cl: 18.85‰) sea water, were prepared for rearing the large-dark type B. The development of larvae is described on those reared in 67‰ sea water in which the highest survival rate occurred. The cross-band type was reared in 67‰ sea water only. Water temperature during the rearing period ranged from 21.0 to 27.5°C (mean 25.0°C) for the large-dark type B, and from 19.9 to 22.8°C (mean 21.6°C)

for one of the two spawnings of the cross-band type and from 21.3 to 29.0°C (mean 25.4°C) for the larvae of the other spawning.

Total length of larvae and juveniles were measured immediately after fixation in 5% formaline, using an ocular micrometer attached to a dissecting microscope.

Egg development of the large-dark type B

A female spawned four times at intervals of 7 to 10 days. The number of eggs was not counted. The eggs were elliptic, about 1.9 mm in length and 0.8 mm in breadth, with a round top and a somewhat flat base with glutinous fibers. The eggs were spawned in a one-layer mass, hanging from the underside of the spawning bed and guarded by one male.

The optic vesicle and Kuppfer's vesicle had already appeared and many small to large oil globules were observed when the spawned eggs were recognized (Fig. 1A). The spawning was presumed to have been taken place on the day before, because eggs were not seen on the previous day. The caudal part of the embryo was prolonged and a large oil globule was observed within 6 hours after discovering the eggs (Fig. 1B). Melanophores appeared on the ventral part of the caudal region and on the yolk within 19 hours after discovering the eggs (Fig. 1C). The eye became darker 43 hours after discovering the eggs (Fig. 1D).

Embryos in the eggs used for observation directed their heads towards the egg base. This was not true in eggs used for rearing. This might be due to the difference in the posture of eggs; eggs observed under the microscope were laid on their side on a horizontal floor all the time, whereas those used for rearing were left hanging down from the spawning bed. Hatching began 4 days after the discovery of the spawned eggs and lasted for three consecutive days.

Development of larvae

In both types, larvae hatched out earlier were in less advanced stages than in those emerging later. The following description is made of larvae from the latest hatching.

Large-dark type B

1) Immediately after hatching, 3.5~3.8 mm (30 individuals) in total length (Fig. 2A):

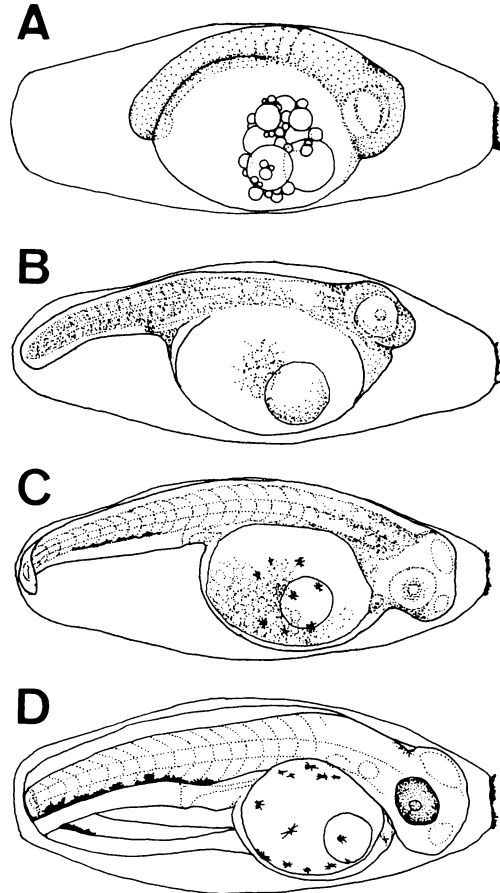


Fig. 1. Egg development of the large-dark type B of *R. brunneus*. A: Egg when first observed, not more than one day after spawning. B: 6 hours after A. C: 19 hours after A. D: 42 hours after A.

Myotomes numbered 25~26. The preanus length was 42.9~48.0% (mean 45.5%) of the total length. The mouth was opened, but a yolk with an oil globule somewhat larger than the eye in diameter still remained. Melanophores were observed on the belly, on the gas bladder, around the anus, and on the ventral part of the caudal region. Yellow pigments were also observed over melanophores on the ventral part of the caudal region and also on the dorsal part of the caudal region where no melanophores appeared. The yellow pigments faded away after fixation. The larvae tended to swim upwards and showed positive phototaxis.

2) 2 days after hatching, 3.8 mm in total length (Fig. 2B): The gas bladder was expanded. The oil globule was reduced in size, while the yolk was absorbed. Larvae were kept in 67% sea water and fed with *Brachionus plicatilis* at this stage. The larvae tended to float in the surface layer of the water.

3) 9 days after hatching, 5.3 mm in total length (Fig. 2C): The oil globule was already absorbed. Five rays were formed in the second dorsal fin and in the anal fin. The end of the caudal part bent slightly upwards, and rays of the caudal fin also began to appear.

4) 13 days after hatching, 6.1 mm in total length (Fig. 2D): The adult complement of 9 rays was completed in the second dorsal fin and in the anal fin. The main rays of the caudal fin were formed. A melanophore appeared in the suborbital region, under the otic vesicle and immediately behind the second dorsal fin.

5) 19 days after hatching, 6.8 mm in total length (Fig. 2E): All fin folds disappeared except for one in the region where the first dorsal fin is destined to be formed. Rudiments of the ventral fins in the form of small membranes appeared on the breast.

6) 25 days after hatching, 10.3 mm in total length (Fig. 2F): The adult complement of 6 rays was completed in the first dorsal fin. The ventral fins were united together to form a sucking disc, but its posterior margin was still concave. Scales appeared on the caudal peduncle. Melanophores increased in number, and appeared on the head and both the lateral and dorsal parts of the body. While the large gas bladder still remained, the larvae sometimes crawled on the floor of the glass jar, when they were disturbed. Some of them died after such disturbances.

7) 32 days after hatching, 13.0 mm in total length (Fig. 2G): Twenty rays were completed in the pectoral fins. The posterior margin of the ventral fins became round. Scaly part extended to the pectoral axilla. Melanophores on the lateral part of the body were bunched, forming 7 to 8 colonies of melanophores. The anterior and posterior nostrils were clearly observed. The larvae reached the juvenile stage and wholly abandoned the planktonic mode of life. They

crawled on the floor or wall of the jar, and tended to hide themselves behind stones or other shelters. The rearing water was changed gradually to fresh water from this stage.

Cross-band type from one spawning

1) Immediately after hatching, 3.8~4.3 mm (181 individuals) in total length (Fig. 3A): Myotomes numbered 25~26. The preanus length was 43.9~48.7% (mean 46.2%) of the total length. The mouth was opened. An oil globule somewhat smaller than the eye in diameter was observed. The pattern of pigment formation resembled that of the large-dark type B but no melanophore was seen on the posteriormost ventral part of the caudal region. Six of 181 individuals had a small melanophore on the dorsal part of the caudal region. The larvae showed positive phototaxis as in the large-dark type B. They were kept in 67% sea water and fed with *Brachionus plicatilis* at this stage.

2) 3 days after hatching, 4.3~4.6 mm (5 individuals) in total length (Fig. 3B): The oil globule was absorbed. A small melanophore appeared on the posteriormost ventral part of the caudal region.

3) 14 days after hatching, 5.4~6.8 mm (3 individuals) in total length (Fig. 3C): The adult complement of 9 rays was completed in both the second dorsal and anal fins. The main rays of the caudal fin were formed. A melanophore appeared on the suborbital region.

4) 20 days after hatching, 7.5~9.0 mm (3 individuals) in total length (Fig. 3D): All fin folds disappeared except for one in the region where the first dorsal fin is destined to be formed. Rudiments of the ventral fins in the form of small membranes appeared on the breast. A melanophore appeared under the otic vesicle.

5) 28 days after hatching, 10.6~11.8 mm (2 individuals) in total length (Fig. 3E): The adult complement of 6 rays was completed in the first dorsal fin. The ventral fins became larger. Scales had not yet appeared. The larvae sometimes crawled on the floor of the glass jar, when they were disturbed.

6) 36 days after hatching, 13.5 mm in total length (Fig. 3F): Nineteen rays were completed in the pectoral fins. The ventral fins were already united together to form a sucking

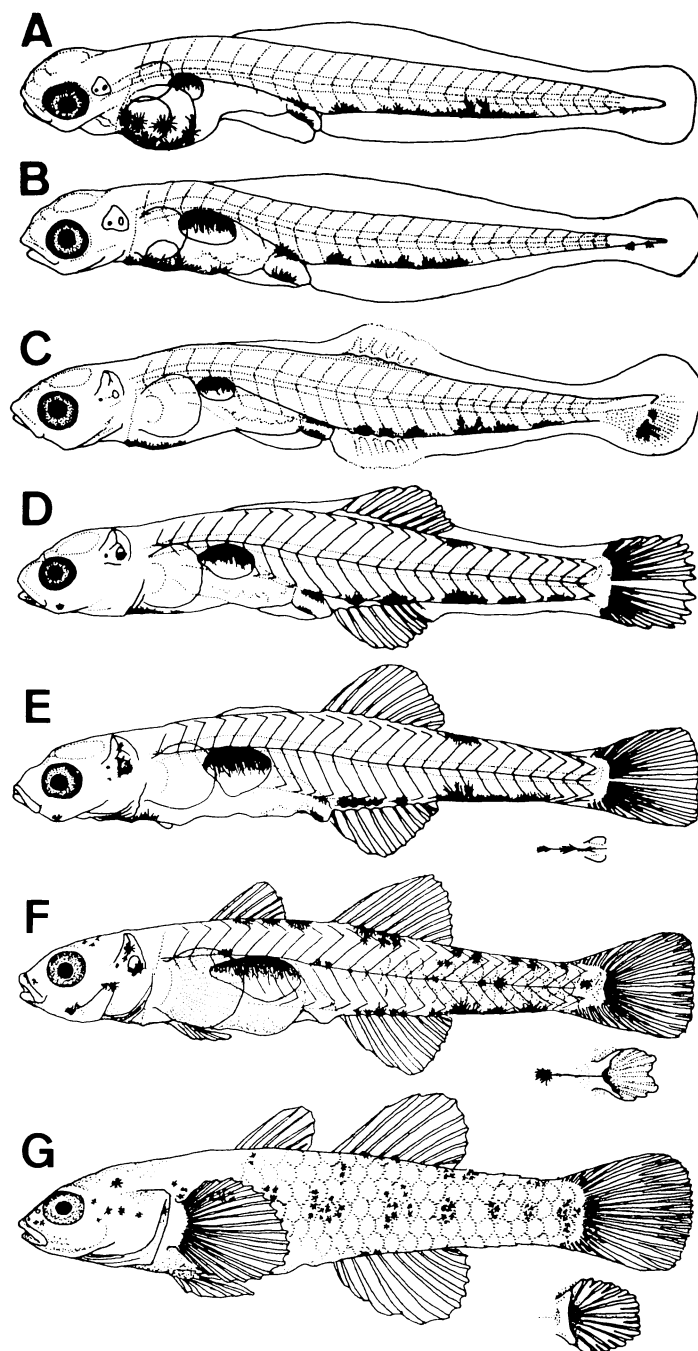


Fig. 2. Larvae and juvenile of the large-dark type B of *R. brunneus*. A: Prelarva immediately after hatching, 3.7 mm in total length (TL). B: Prelarva 2 days after hatching, 3.8 mm TL. C: Postlarva 9 days after hatching, 5.3 mm TL. D: Postlarva 13 days after hatching, 6.1 mm TL. E: Postlarva 19 days after hatching, 6.8 mm TL. F: Late postlarva 25 days after hatching, 10.3 mm TL. G: Juvenile 32 days after hatching, 13.0 mm TL.

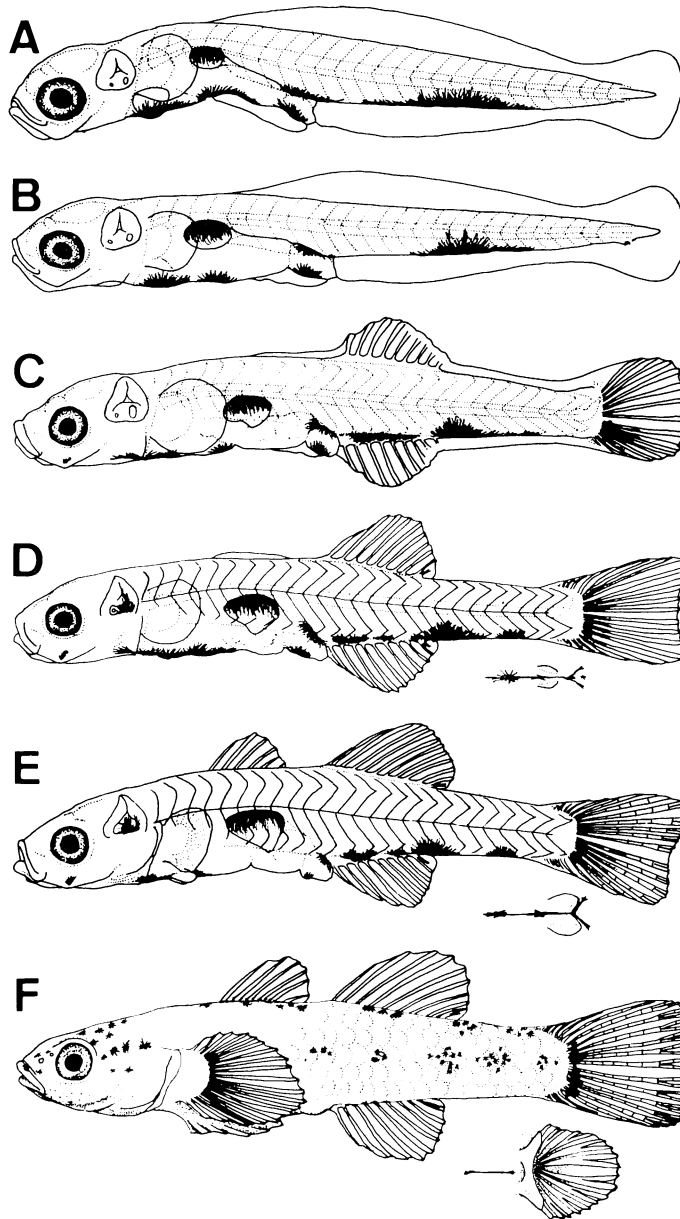


Fig. 3. Larvae and juvenile of the cross-band type in one of two spawnings of *R. brunneus*. A: Prelarva immediately after hatching, 4.2 mm in total length (TL). B: Postlarva 3 days after hatching, 4.6 mm TL. C: Postlarva 14 days after hatching, 6.8 mm TL. D: Postlarva 20 days after hatching, 9.0 mm TL. E: Late postlarva 28 days after hatching, 10.6 mm TL. F: Juvenile 36 days after hatching, 13.5 mm TL.

disc. Scales already appeared. Melanophores increased in number, and appeared on the head and both the lateral and dorsal parts of the body. Those on the lateral part of the body were bunched, forming some colonies of

melanophores. The anterior and posterior nostrils were clearly observed. The larvae reached the juvenile stage and wholly abandoned the planktonic mode of life. The rearing water was changed gradually to fresh water.

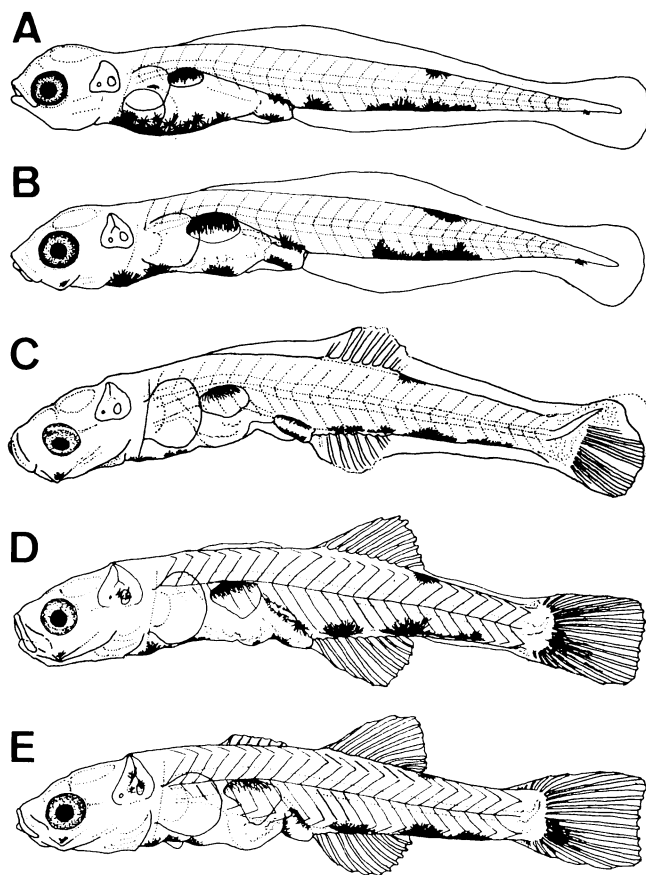


Fig. 4. Larvae of the cross-band type in another spawning of *R. brunneus*. A: Prelarva 1 day after hatching, 3.9 mm in total length (TL). B: Postlarva 7 days after hatching, 4.4 mm TL. C: Postlarva 13 days after hatching, 5.5 mm TL. D: Postlarva 19 days after hatching, 6.6 mm TL. E: Late postlarva 25 days after hatching, 8.9 mm TL.

Cross-band type from another spawning

1) 1 day after hatching, 3.9 mm in total length (Fig. 4A): Myotomes numbered 26. An oil globule somewhat larger than the eye in diameter was observed. A melanophore was already seen in both the dorsal and posteriormost ventral parts of the caudal region. They were kept in 67% sea water and fed with *Brachionus plicatilis* at this stage.

2) 7 days after hatching, 4.4 mm in total length (Fig. 4B): A melanophore appeared on the suborbital region. Fin folds in the area destined to form the second dorsal fin and the anal fin became high.

3) 13 days after hatching, 5.5 mm in total length (Fig. 4C): Eight rays appeared in both the second dorsal and anal fins. The

end of the caudal part bent upwards and 10 caudal fin rays were formed.

4) 19 days after hatching, 6.6 mm in total length (Fig. 4D): The adult complement of 9 rays was completed in both the second dorsal and anal fins. The main rays of the caudal fin were formed. A melanophore appeared under the otic vesicle.

5) 25 days after hatching, 8.9 mm in total length (Fig. 4E): Fin folds totally disappeared. The adult complement of 6 rays was completed in the first dorsal fin. Rudiments of the ventral fins in the form of small membranes appeared on the breast.

By 35 days after hatching, all individuals died from the abrupt fall of water temperature and deterioration of water conditions.

Adults of two types

Large-dark type B (Fig. 5A, B)

This type can be characterized by the combination of the following characters: no marking on cheek, cross-bands on the side of body indistinct, the end of caudal peduncle with a dark triangular blotch, the base of pectoral fin with a diamond-shaped dark blotch, vertical fins white-fringed, rays darker than

membranes. This type is generally smaller in size and more indistinct in color pattern than the large-dark type A. Mizuno (1976) considers it to be a dwarfed type of the large-dark type A. Mizuoka (1976), on the basis of electrophoretic comparison, is in the opinion that the large-dark type B is not a dwarf form of the large-dark type A.

Cross-band type (Fig. 5C, D)

This type can be characterized by the com-

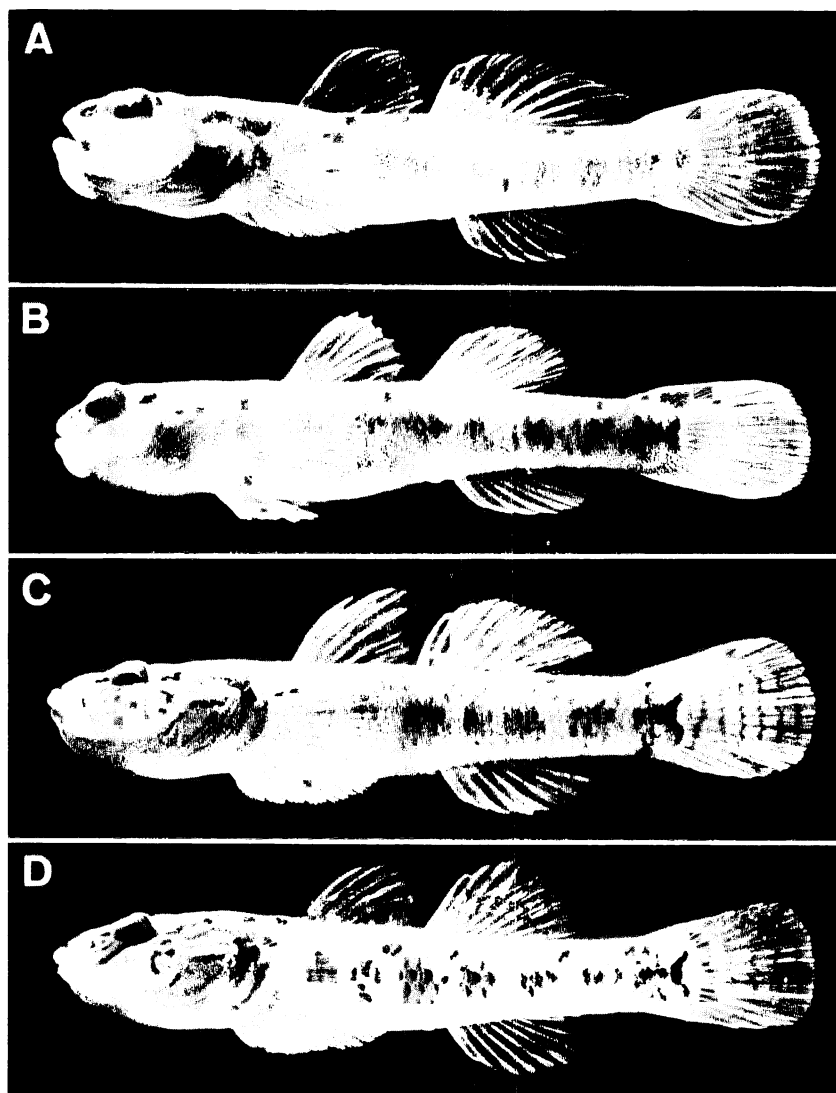


Fig. 5. Adults of the two types of *R. brunneus*. A: Large-dark type B after 9 months' rearing. Male, 47.5 mm in total length (TL). B: Large-dark type B after 9 months' rearing. Female, 45.0 mm TL. C: Cross-band type from the Tagoe River, Kanagawa Pref. Male, 46.1 mm TL. D: Cross-band type from the Tagoe River, Kanagawa Pref. Female, 41.7 mm TL.

bination of the following characters: cheek with red vermiculate markings, cross-bands on the side of body obvious, the end of caudal peduncle with a slightly wavy vertical band, belly with a broad blue longitudinal band, the base of pectoral fin with 2 to 3 reddish crescent vertical bands, vertical fins with several rows of small spots.

Notes

Newly hatched larvae of the large-dark and cross-band types of *R. brunneus* slightly differed in the position of the anus. Namely, the anus was situated slightly anterior in the large-dark type B compared with the cross-band type. The length from the tip of the snout to the anus was 42.9~48.0% (mean 45.5%) of total length in the former, and 43.9~48.7% (mean 46.2%) in the latter. As far as the results of this study are concerned, these two types were further distinguishable in the length of the prelarvae. Total length of newly hatched larvae ranged from 3.5 to 3.8 mm (mean 3.7 mm) in the large-dark type B, and from 3.8 to 4.3 mm (mean 4.2 mm) in the cross-band type.

In the cross-band type, while the process of pigmentation was nearly constant in almost all parts of the body, that in the dorsal part of the caudal region showed a variation to a great extent. In larvae from one spawning, no melanophore was observed in this part of the body in 175 of 181 samples of newly hatched larvae. A small melanophore was seen in the remaining six larvae. All of the random-sampled specimens of more advanced stages up to 28 days after hatching had no pigment in this area. In larvae from another spawning, a melanophore was present from the earliest stage observed, though only one specimen was sampled in each stage.

In the large-dark type B, all of the 30 newly hatched larvae observed had no melanophore in this area. Each individual sampled at 13, 19 and 25 days after hatching had a melanophore in the dorsal part of the caudal region.

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ヨシノボリ *Rhinogobius brunneus* の卵発生と仔魚の発育

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ヨシノボリ *Rhinogobius brunneus* の黒色大型Bについて卵および仔魚，横斑型について仔魚の発育を観察した。両型の孵化仔魚を比較した結果，全長および肛門の位置に差異が見られた。横斑型の仔魚では，黒色素胞の出現に変異が見られた。飼育した仔魚は，それぞれ黒色大型Bでは孵化後 32 日頃に（水温 21.0~27.5°C），横斑型では 36 日頃に（19.9~22.8°C）各鰭条が定数に達し，底棲生活に移行した。

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