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Embryonic Development and Newly Hatched Larvae of the Little Dragon Sculpin *Blepsias cirrhosus*

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Abstract This paper describes both embryonic development and newly hatched larval morphology of the little dragon sculpin *Blepsias cirrhosus*. The eggs of *B. cirrhosus* are almost spherical, 3.0–3.2 mm in diameter, and have a yolk color of burnt orange. Development is very slow, being especially sluggish once the embryo appears. The embryo begins forming from the 10th day. In size, the early embryo is less than 1/6 of the yolk's circumference. Incubation at 10℃ takes about 200 days, 50 days shorter than the incubation period in a natural environment, with a mean water temperature of 11℃. The notochord length of newly-hatched larvae averages 11.1 mm. The larvae are developed so fully that the notochord is already flexing and the caudal and pectoral rays are forming.

The little dragon sculpin, *Blepsias cirrhosus* (Pallas), inhabits north Pacific coastal waters (Hart, 1973). A few records of larval and juvenile stages have been reported (Watanabe, 1958; Richardson, 1981; Shiogaki, 1988), but there is no published data on the pre-hatching stages. In this study, we observed the development of eggs from fertilization to hatching, and report on the embryonic development and the newly-hatched larval morphology of the species.

Materials and methods

Eggs were collected by dissection from mature females that were caught by trammel net in the coastal waters of Usujiri (41°57′N, 140°58′E), southern Hokkaido, Japan, on July 21, 1986.

Because this species is a copulating fish, fertilization occurs without artificial insemination when the eggs come into contact with seawater (Munehara et al., 1991). About 100 eggs which had been taken from a female fish were incubated in a 1 *l* capacity glass beaker at a water temperature of approximately 10℃. The seawater in the beaker was always circulated by bubbling. Half of the sea water was changed once every week.

Measurements and observations of embryonic development were carried out on the living organisms every two hours of the first 24 hours after immersion in seawater. These were subsequently continued at intervals of 1–5 days.

Results

The eggs of *B. cirrhosus* were demersal, weakly adhesive, and almost spherical in shape (Fig. 1A). The egg diameter ranged from 3.0–3.2 mm. The egg envelope was about 75 μm in thickness. The yolk was burnt orange in color. Many oil droplets were observed. In addition, small, numerous, whitish granular materials formed a mass within the yolk.

Embryonic development was as follows. At the 6th hr following immersion in seawater, the blastodisk began to elevate. The blastodisk of this species was very small in proportion to the egg size. The first cleavage took place at the 18th hr (Fig. 1B). The second cleavage followed approximately at the 24th hr, and the 32-cell stage was reached on the 2nd day. It took 3 and 6 days from fertilization to the morula and blastula stage, respectively.

On the 9th day, the semi-sphere of the yolk was covered with blastoderm. The embryo began forming from the 10th day, and was clearly observable on the 13th day, at which time its length was less than 1/6 of the yolk's circumference (Fig. 1C). A pair of optic vesicles appeared on the 15th day. A pair of optic lenses and auditory vesicles, Kupffer's vesicle and 15–22 myomeres were formed by the 17th day (Fig. 1D). By this time the embryo had grown to 1/4 of the yolk's circumference. By the 20th day, the

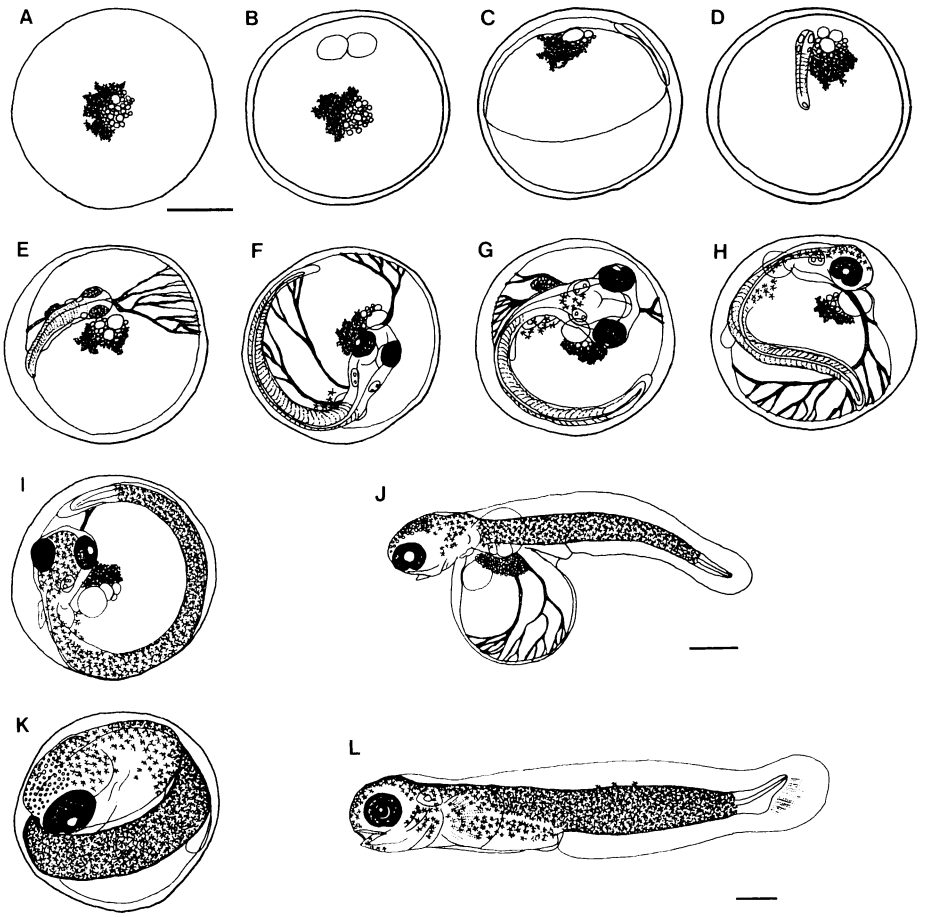

Fig. 1. Embryonic development and newly hatched larvae of *Blepsias cirrhosus* kept at 10℃. A, Ripe egg; B, 2-cell stage, 18 hrs after immersion in seawater; C, Formation of embryo, 13 days; D, Formation of optic lenses, auditory vesicles and Kupffer's vesicle, 17 days; E, Appearance of melanophores on eyes and of blood vessels, 24 days; F, Appearance of melanophores on the marginal region between embryo and yolk, and formation of pectoral fins, 42 days; G, Appearance of melanophores around nape, 61 days; H, Melanophores appearance on the top of head, 92 days; I, Appearance of melanophores on snout, opercles and anterior trunk, 138 days; J, Embryo extracted out of the egg at the same stage as I; K, Just before hatching, 199 days; L, Newly hatched larva, 199 days. Scales are 1 mm.

myomeres had increased in number to 26–32, a pair of otoliths had been found, the heart was pulsating, and Kupffer's vesicle had disappeared. At this point the embryo was observed to move occasionally. On the 22nd day, the tail started to grow free from the yolk, and the embryo extended to about 1/3 of the yolk's circumference. On the 24th day, the eyes began blackening, the intestine and the liver were observed to differentiate, and simultaneously blood vessels appeared along the yolk below the thoracic region of the embryo (Fig. 1E). On the 26th and the 30th days the embryos had extended to approximately 1/2 and 2/3 of the yolk's circumference, respectively. On the 42nd day, melanophores were seen on the peritoneal wall, and a small pair of pectoral fins had formed (Fig. 1F).

On the 55th day, the embryo measured 3/4 of the yolk's circumference. On the 61st day, some melanophores appeared on and around the nape, and the mouth was observed to open spasmodically (Fig. 1G). On the 92nd day, melanophores also appeared on the top of the head. In addition, minute projections, which may have been hatching glands, were observed arranged regularly on the head, snout and

upper jaw (Fig. 1H). By the 138th day, the embryo had extended, enabling the tip of the tail to nearly reach the snout. At this stage the embryo completely encircled the yolk. Melanophores were located on the snout, opercles and whole trunk, with the exception of the posterior portion of the tail (Fig. 1I, J).

Just before hatching, the embryo actively twisted within the egg envelope and occasionally moved its opercles. These embryos extended to one and 1/3 of the yolk's circumference (Fig. 1K). Hatching began on the 199th day and was completed within seven days.

The newly-hatched larvae averaged 11.1 mm in notochord length (range 10.8–11.4 mm) (Fig. 1L). The body was slender, brownish in color and heavily pigmented. In some specimens, a few melanophores were observed on the base of the posterior dorsal fin. The snout was round. At the time of hatching, preopercular spines were not conspicuous. On the other hand, the notochord was already flexing and the caudal and pectoral rays had begun developing. At this stage larvae were swimming powerfully under the surface of the water, and displayed strong phototaxis.

Discussion

In the coastal waters of Usujiri, the spawning season of *B. cirrhosus* is during June and July, with larvae developing within the March-May period (Fukuda, unpubl. data). Therefore, this species has an embryonic stage of more than 250 days in the natural environment. On the other hand, this study demonstrates that incubation at 10℃ takes about 200 days. The mean water temperature in the region from July-March is 11℃ with the range of 2–19℃ (daily survey in the coastal waters of Usujiri by the Usujiri Fisheries Laboratory, Hokkaido University). This suggests that the seawater temperature is lower than is effective for embryonic development in winter and/or out of the required range for proportional development in summer.

The incubation period of *B. cirrhosus* is extremely long among teleosts. It was also noticed that the newly-hatched larvae are well-developed. Such slow embryonic growth seems to be caused by the large egg size of this species in conjunction with the fact that the early embryos are exceedingly small relative to yolk volume. Hatching is delayed by this embryo/yolk volume relationship as well as by the necessity for the embryos to be fully developed before hatching.

Regarding cottid fishes, the eggs and embryonic development of *Hemitripterus americanus* (see Warfel and Merriman, 1944; Fuiman, 1976) and *H. villosus* (see Kyushin, 1968; Okiyama and Sando, 1976) have been reported. In these species, the egg sizes were more than 4 mm in diameter, incubation periods were about 100 days, and flexing of notochord and formation of caudal rays had already begun by the time of hatching. Examples of the longest incubating periods known among teleosts are seen in the agonid fish, *Agonus cataphractus* (see Breder and Rosen, 1966) and sandfish, *Trichodon trichodon* (see Okiyama, 1990). These species needed approximately one year for their embryonic development. In addition, the agonid fishes, *Agonomalus proboscidalis* (see Iioka and Gunji, 1979) and *Brachyopsis rostratus* (see Sugimoto, 1987), were reported to have embryonic stages of approximately 100 days and 300 days, respectively. The newly-hatched larvae of these fishes were also fully developed, as is the case in *B. cirrhosus*.

It is considered that embryonic development is organized in association with the early life history of fishes (Balon, 1986). Precocial characters enable the fast swimming of larvae, and facilitates successful feeding and skillful escape from predators (Balon, 1986; Okiyama, 1990). These faculties probably contribute to a lowering of mortality during the larval period of *B. cirrhosus*.

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イソバテングの卵内発生とふ化仔魚

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イソバテング *Blepsias cirrhosus* (Pallas) の卵内発生過程およびふ化仔魚の外部形態を観察した．卵はほぼ球形で，卵膜径が 3.0–3.2 mm，卵膜の厚さが 75 μm あり，卵黄は柿色であった．胚発生が極めて遅いこと，初期の胚が卵黄の大きさと比較して相対的に小さいこと，およびふ化仔魚がよく発育していることが特異的であった．水温 10℃ でふ化まで約 200 日を要し，初期胚は卵黄周の 6 分の 1 を占めるに過ぎなかった．ふ化仔魚は生時で平均脊索長 11.1 mm あり，脊索は上屈を開始し，胸鰭および尾鰭軟条の形成も始まっていた．

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