Embryonic Development and Prelarva of the Atherinid Fish, *Hypoatherina bleekeri*

Toru Takita and Katsuyuki Nakamura

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Six species of atherinid fishes are distributed in Japanese waters (Ichthyological Society of Japan, 1981). Only a little is known on their spawning, egg morphology and embryonic development (Nakamura, 1936). Nakamura (1936) described the eggs of *Atherion elymus*, spawned in captivity, with the larval and juvenile morphology of those collected from the sea.

Hypoatherina bleekeri (Günther), an atherinid fish common in coastal areas of southern Japan, is presumed to lay eggs with filaments, but there is no evidence for that (Mito, 1965a). The morphology, distribution and food of *H. bleekeri* larvae and juveniles in Omura Bay, Nagasaki Prefecture were reported by Takita and Kondo (1984), but they gave no information on morphology of the egg.

We collected ripe males and femles of *H. bleekeri* with gill nets in Omura Bay in 1984 and were successful in artificial insemination. We also collected the eggs of the fish from the sea. Here we report on the eggs, embryonic development and prelarva of *H. bleekeri*.

Materials and methods

In Omura Bay gill nets were operated every month from April to September in Tokitsu Harbor (33°50′N, 129°51′E) where larvae and juveniles were abundantly caught in a previous study (Takita and Kondo, 1984).

The harbor is about 0.2 km^2, with a muddy, nearly flat bottom that is 2–5 m deep all over the area, except for the peripheral zone of artificially piled rocks, which is 3 m deep, at the most, and about 5 m wide. Tidal influence is small at the innermost area of the bay where the harbor is located, and the tidal range is as low as 50 cm on the average.

Ripe fish with mature eggs or milt were collected from May to August. We artificially inseminated several groups of eggs four times in June. Insemination was carried out on board the ship, with the sperm of several males used on the eggs of one female immediately after the fish were caught. After insemination, the eggs were brought back to the laboratory and embryonic development and larval stages were observed.

On June 12 and August 11 we dove near the place where ripe fish were caught, and collected all kinds of algae and sea grass we saw on the bottom and inspected the plants for eggs.

Results

Eggs. The artificially fertilized eggs (Fig. 1A) are demersal and spherical, measuring 1.08 to 1.20 mm in diameter with around 18 long filaments. The filaments become entangled with each other and it is difficult to measure the exact length, but the longest was about 14 mm. The filaments are generally distributed evenly on surface of the chorion, but some eggs had a small area with no filaments on the chorion. One to three of the filaments on the egg are 1.5 times as thick in diameter as others. There appears to be a tendency for numerous thinner filaments to grow on the chorion away from the thick ones. Each filament is a little thickened at the base. The egg was easily entangled on filamentous structures, but stickiness was not actually recognized. The egg is semitransparent, without color and with a narrow perivitelline space. The yolk sac has no distinctive structure except for the existence of about 80 small oil globules, 0.008 to 0.069 mm in diameter. No change was found in oil globule number during the developmental process.

We dove on June 12 and August 11 at several places in the harbor. Peripheral rocky areas in the harbor were covered with several kinds of algae such as *Ulva pertusa*, *Codium fragile*, *Bryopsis plumosa*, and muddy areas had *Chaetomorpha crassa*, *Cladophora* sp., *Sargassum piluliferum* and the sea grass *Zostera marina*. We collected all types of the plants found on the bottom and inspected to see if the eggs of *H. bleekeri* were attached. The eggs were found only on *Cladophora* sp.

Cladophora sp. is a fine filamentous green alga growing thickly on the bottom like a mat of up to 10 cm in thickness. This alga is apparently the dominant type on muddy areas in the harbor, alternating here and there with *Sargassum piluliferum* and *Zostera marina*. No vegetation was seen in front of drain mouths. We took several

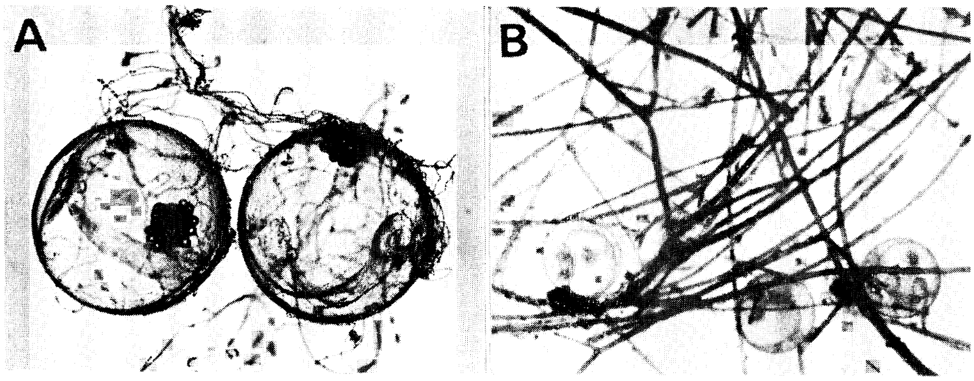

Fig. 1. Eggs of *Hypoatherina bleekeri*. A, artificially fertilized eggs 2.5 days after insemination; B, eggs collected from the sea together with the sea alga, *Cladophora* sp.

handfuls of the *Cladophora* sp. at each place we dove. The eggs were found at every place, entangled in the algae as individuals or in a loose group but not as a clump or cluster (Fig. 1B). We did not find the eggs gathered in a concentrated site, forming an egg mass. But our collections and observations were neither intensive nor farreaching enough to verify that the spawning activity was not concentrated at a limited location, in the form of spawning aggregations such as those observed in other atherinids (Walker, 1952; Middaugh *et al.*, 1981).

Embryonic development. Embryonic development is described from eggs inseminated at 2120 h on June 6. The eggs were taken to the laboratory with in one hour of collection and kept in a holding tank at 21.1–23.1°C, to observe embryonic development and hatching (Table 1 and Fig. 2).

The embryonic shield appeared when the germ ring reached nearly half of the yolk diameter in lateral view. We found the pectoral fins formed in 4.5 days after fertilization, although we failed to note the exact time of the formation. Otocysts were formed when embryo was two-thirds of egg periphery. Melanophores first appeared on the dorsal and lateral sides of head, lateral sides of abdomen and the dorsal areas on yolk sac when the embryo was three-fourths as long as the egg periphery. Large melanophores, typical in some atherinid larvae on the dorsal surface of head (Takita and Kondo, 1984), appeared about 6.5 days after fertilization. Before hatching the body was elongated and reached the whole egg periphery.

Prelarva. Newly hatched larva measured 4.6 mm in total length with a small amount of yolk with oil globules located anteriorly in the yolk sac. The mouth was formed and open and the alimentary canal was already convoluted, as observed in the larvae from the sea (Takita and Kondo, 1984). The anus was located so anteriorly that the trunk was as short as the head in length. The larva had 44 (5+39) myomeres and some cupulae extending from free neuromasts visible on the anterior position of the lateral sides of the tail.

Melanophores were located dorsally and laterally on the head and dorsally on the yolk sac and viscera. There were three conspicuously large melanophores on the dorsal surface of the head, lined up in a longitudinal row on top. Melanophores were lacking on the lateral surface of head in some individuals. Those on the yolk sac and viscera were very thick, and concealed the internal aspects. Xanthophores were located on about the same locations as the melanophores. Neither melanophores nor xanthophores were recognized on the tail. No morphological changes were recognized for 2 days after hatching.

The larvae were kept in a small plastic tank filled with sea water 6 cm deep. Most of them swam actively near the surface in daytime, while at night they floated in the middle of the water column or rested on the bottom. They did not eat brine shrimp nauplii nor artificial foods and died in 3 days, when the yolk had been absorbed.

Discussion

Among atherinid eggs, those of *Leuresthes*

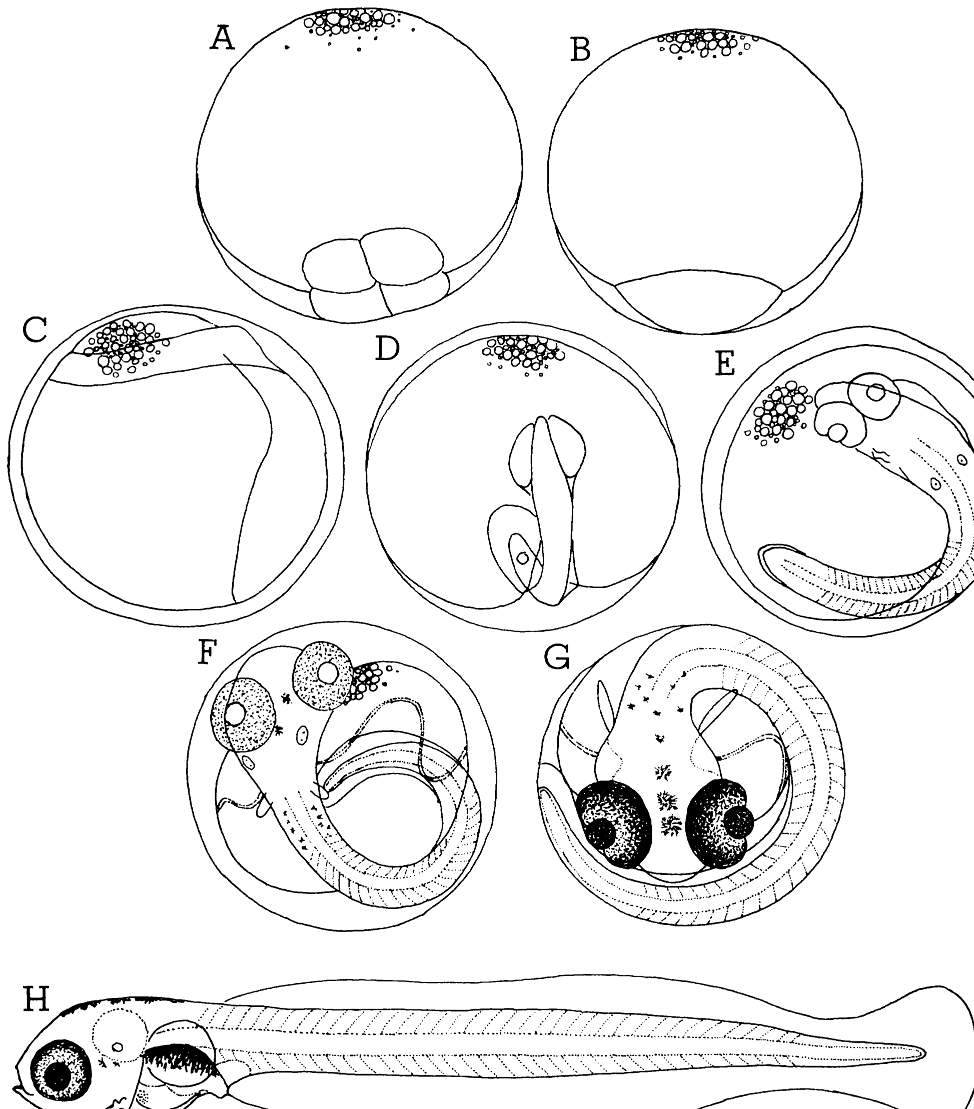

Fig. 2. Eggs and larva of *H. bleekeri*. A, 2 hr 40 min after fertilization; B, 7 hr 40 min; C, 1 day 3 hr; D, 1 day 12 hr; E, 2 days 14 hr; F, 4 days 13 hr; G, 6 days 23 hr; H, Newly hatched larva, 4.9 mm in total length.

tenuis are nonadhesive and lack filaments (Hubbs, 1965; Breder and Rosen, 1966), and those of *Atherion elymus* have an adhesive chorion and lack filaments (Nakamura, 1936). However, many atherinid eggs are known to have adhesive filaments (Breder and Rosen, 1966). In *Hypoatherina bleekeri*, adhesion was not observed on the filaments but since tiny debris was seen attached to the filaments during incubation, the filaments may be slightly adhesive.

According to White *et al.* (1984) who have summarized the various conditions of filament placement on atherinid chorion, the filaments can be scattered over the surface in *Atherinops* and *Atherinopsis* as was observed in *H. bleekeri.*

The youngest larva of *H. bleekeri* previously shown (Takita and Kondo, 1984) had the same features as presented here. Since the yolk sac and viscera are covered by thick melanophores, remnants of yolk are apt to be overlooked in prelarvae. The previously described stage (Takita and Kondo, 1984) may be a prelarva.

From the observations on the postlarvae of *H. bleekeri*, the rows of melanophores on the lateral sides are presumed to be one specific aspect (Takita and Kondo, 1984) that did not appear on the larva observed in this study. Careful rearing is needed to prove this presumption.

Behavior of the larvae in the tank suggests a probable shift of their distribution to lower layers at night, whereas a surface oriented distribution, in both light or dark conditions, has previously been suggested (Mito, 1965b).

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Table 1. Embryonic development of *Hypoatherina bleekeri*.

Time elapsed after fertilization			Water temp. (°C)	Developmental stages observed
day	hr	min		
0	1	25	23.1	Two cell stage.
0	2	20	22.3	Four cell stage. Fig. 2A.
0	5	20	22.0	Morula stage.
0	7	40	21.9	Blastula stage. Fig. 2B.
0	14	30	21.8	Early gastrula stage.
0	23	50	21.8	Beginning of embryo formation. Germ ring is two-thirds of yolk diameter in lateral view. A little before Fig. 2C.
1	4	50	21.6	Closure of blastopore and eye vesicle formation.
1	8	40	21.4	Some have Kupffer's vesicle and three or four myomeres. A little before Fig. 2D.
1	20	40	21.1	Optic lens formation. Eleven myomeres.
2	3	45	21.3	Otocyst formation. Embryo is two-thirds of yolk diameter.
2	8	50	21.2	Eighteen myomeres. Kupffer's vesicle has disappeared.
2	14	10	21.3	Beginning of tail formation and heart beat. Fig. 2E.
3	18	0	21.6	Appearance of melanophores on embryo and yolk sac.
4	11	50	21.7	Appearances of xanthophores on embryo and yolk sac and melanophores on the eyes. A little before Fig. 2F.
6	12	20	21.3	Appearance of a row of large melanophores with fine branches on dorsal region of the head. Eleven hr before Fig. 2G.
10	2	40	22.4	Hatching.

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(Note: Titles in parentheses are originally given in Japanese, and put into English by the authors.)

(Faculty of Fisheries, Nagasaki University, 1–14, Bunkyo-machi, Nagasaki 852, Japan)

トウゴロウイワシの卵内発生と前期仔魚

田北　徹・中村勝行

トウゴロウイワシの人工授精を行って，卵内発生と卵・前期仔魚の形態を明らかにし，潜水調査で卵が海底に分布する状態を観察した．卵は直径 1.08–1.20 mm のてん絡卵で，卵膜のほぼ全面に 18 本前後の長いてん絡糸を持ち，卵黄内に多数の小油球を持つ．海中では卵は水深 2–5 m の砂泥底に繁茂する糸状の緑藻，*Cladophora* sp. にからまってまばらに産みつけられていた．人工授精した卵は受精後 21.1–23.1°C において約 10 日でふ化した．ふ化直後の仔魚は，全長 4.6 mm で，頭部背面に 3 つの大型の星状黒色素胞がある．

(852 長崎市文教町 1-14 長崎大学水産学部)