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Development of Laboratory-reared Eggs, Larvae and Juveniles of the Atherinid Fish, *Hypoatherina tsurugae*, and Comparison with Related Species

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Abstract Embryonic, larval and juvenile development of an atherinid fish, *Hypoatherina tsurugae*, are described using a laboratory-reared series. The eggs, measuring 1.60–1.78 mm in diameter, were almost spherical, with numerous chorionic filaments. Hatching occurred between 13 and 17 days after spawning at water temperatures of 18.2–21.8°C. Newly-hatched larvae (5.50–6.70 mm Notochord length) had 5+39 myomeres and the notochord under going flexion. Aggregate numbers of all fin rays were completed at 12.5–14.0 mm Standard length (SL), and squamation at 19.0 mm SL. The early life stages of *H. tsurugae* and related species were compared morphologically, and keys to each provided.

Hypoatherina tsurugae (Jordan et Starks) is a common atherinid fish inhabiting southern Japan and the Korean Peninsula (Yoshino, 1984). Although Uchida (1927) described the larvae and juveniles of this species and its relatives, *Atherion elymus* Jordan et Starks, *H. bleekeri* (Günther) and *Iso flosmaris* Jordan et Starks, and proposed a key to them, his report excluded some developmental stages and lacked sufficient information for identification of early life stages.

A detailed description of the eggs, larvae and juveniles of *A. elymus* has recently been published (Kimura and Tsukamoto, 1990). Furthermore, the availability of a laboratory-reared series of *H. tsurugae* has enabled a detailed description of the latter and clarified methods for identifying the eggs, larvae and juveniles of Japanese atherinids and their relatives.

Materials and Methods

Adult *Hypoatherina tsurugae* caught with a dip net under a fishing torch at Zaga Island in Ago Bay, Mie Prefecture (34°16′15″N, 136°48′30″E), on 23 May–15 June 1985 and 4–5 June 1986, were immediately anesthetized for the purpose of identification with 100 ppm ethylene glycol monophenyl ether. Subsequently, they were treated with 0.05% chlorotetracycline for 10 min, and thereafter kept in a 10 kl concrete tank with running sea water (ca. 75 l/min), at the Fisheries Research Laboratory, Mie University (FRLM). The parental fish comparised 59 males (81.9–138.0 mm Standard length [SL]) and 119 females (85.1–137.3 mm SL) in 1985, and of 57 males (90.4–130.0 mm SL) and 132 females (97.1–145.0 mm SL) in 1986. Two to six artificial spawning beds (45×70 cm wired frames with 1.6 mm and 6 mm mesh polyethylene nets) were set on the bottom and walls of the tank.

Spawning occurred regularly in early morning, from 3:00 to 5:00 at a water temperature of ca. 20°C. In this study, eggs obtained between 24 May to 18 June 1985 and 5 to 7 June 1986 were used for incubation and rearing.

Just after spawning, eggs laid on the spawning beds were transferred to a gauze net cage (30×25×30 cm) suspended in a 500 *l* black polyethylene tank, containing weakly aerated running sea water (260–800 ml/min). Incubating temperatures ranged from 18.2 to 21.8°C.

Just after hatching, the larvae were released from the net cage. Methods of rearing, observation, and measurements followed Kimura and Tsukamoto

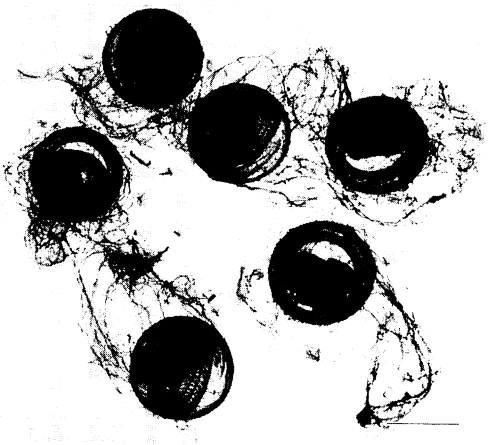

Fig. 1. Photograph of live eggs of *Hypoatherina tsurugae*, FRLM 12040, 9 days after spawning. Scale indicates 1 mm.

(1990). All of the reared specimens considered here were deposited at FRLM (cat. nos. 11863–11907).

In order to compare early life stages of *H. tsurugae* with related species, recourse was made to both previous reports (Uchida, 1927; Takita and Kondo, 1984; Takita and Nakamura, 1986a, b; Kimura and Tsukamoto, 1990) and wild specimens, i.e. *Hypoatherina tsurugae*, FRLM 11908–11910, 11968, 11981–11983, Zaga Is., June–August, 1985–1987, and FRLM 11911–11913, Wagu, Pacific coast of Sakishima Peninsula, Mie Prefecture (34°15′5″N, 136°48′30″E), 2–9 July 1987; *H. bleekeri*, FRLM 11856, 11859–11862, 11984, Zaga Is., 7 and 17 July 1986, FRLM 11857–11858, Wagu, 2 and 9 July 1987, and FRLM 11841–11851, Omura Bay, Nagasaki Prefecture (33°50′N, 129°51′E), 11 June–12 October 1981; *Atherion elymus*, FRLM 11966–11967, Wagu, 7 and 6 July 1987; *Iso* sp., FRLM 11973–11978, Wagu, 2 and 9 July 1987. The latter species, commonly found along the Pacific coast of Sakishima Peninsula, is not *I. flosmaris*, which name has been sometimes used in the past, instead possibly being referable to *I. rhothophilus* (Ogilby). Because of the nomenclatural uncertainty, examples were simply referred to as *Iso* sp. in this paper.

The system of higher taxonomy of the atherinids and relatives follows Nelson (1984) in this paper.

Development

Eggs.—The eggs were demersal and almost spherical, measuring 1.60–1.78 mm in diameter, with a colorless transparent chorion, slightly yellowish yolk and narrow perivitelline space. The chorion had 50–65 long filaments (2.0–3.0 mm) evenly distributed over its surface (Fig. 1). Embryonic development is shown in Table 1 and Figure 2. Numerous tiny oil globules (0.03–0.10 mm in diameter), which were initially scattered (just after spawning), gathered around the vegetal pole before the early blastula stage, and eventually moved to the front of the embryonic head during olfactory vesicle formation. Embryonic afferent and efferent circulation was completely separated by the development of the heart, anterior to the head. The first vitelline circulation

Table 1. Embryonic development of *Hypoatherina tsurugae* at water temperatures of 18.2–21.8°C

Time elapsed after spawning	Developmental stages observed
1 h	Elevation of blastodisc (Fig. 2A)
1 h 30 min	2-cell stage
2 h 30 min	8-cell stage (Fig. 2B)
20 h	Blastula stage
30 h	Beginning of embryo formation (Fig. 2C)
36 h	Closure of blastopore. Formation of optic and Kupffer's vesicles. 4 myomeres (Fig. 2D)
60 h	Disappearance of Kupffer's vesicle. Formation of optic lens
61 h	Formation of otocyst
65 h	Beginning of heart pulse. 19–21 myomeres
4 days	Beginning of embryo movement (Fig. 2E)
6–7 days	Formation of pectoral fin bud
9–11 days	Mouth opening formed. Appearance of iridophores on eye and hatching glands on head (Fig. 2F)
13–15 days	Hatching

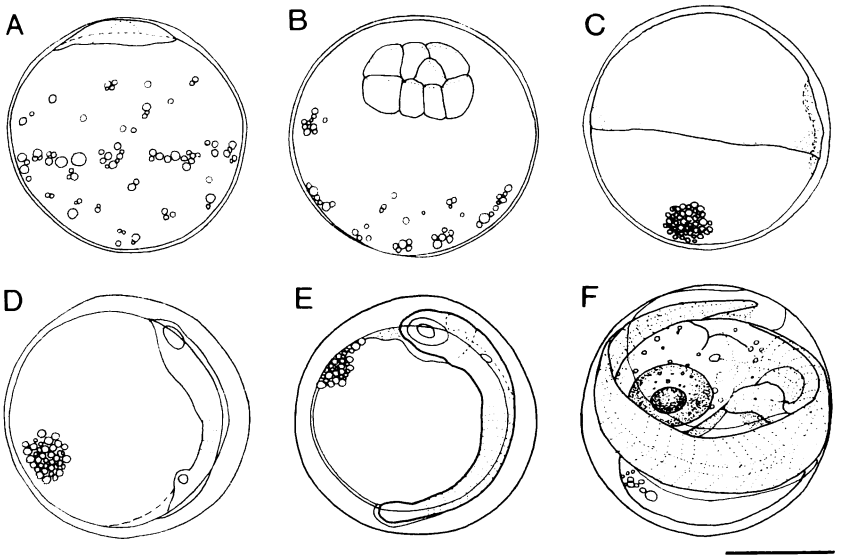

Fig. 2. Embryonic development of *Hypoatherina tsurugae*. Chorionic filaments are omitted. A) 1 h after spawning; B) 2 h 30 min; C) 30 h; D) 36 h; E) 4 days; F) 11 days. Scale indicates 1 mm.

system was simple, being unbranched and looping. Hatching occurred between 13 and 15 days after spawning.

Yolk-sac and flexion larvae.—The newly hatched larvae, measuring 5.5–6.7 mm notocord length (NL) and with 4+37=41 myomeres, had a round head, fully developed mouth, short trunk, and long tail. The notochord was under going flexion, but the yolk-sac remained, just anterior to the anus (Fig. 3A). Three large conspicuous melanophores were present dorsally on the head (Fig. 3B). Branched melanophores were distributed as follows: 0–1 cell on the opercle, 0–10 cells along the dorsal contour of the trunk and tail, 4–8 cells on the dorsal surface of the abdominal cavity, 0–2 cells along the mid-lateral surface of the tail, and 0–3 cells along the ventral contour of the tail. These melanophore numbers increased with growth. The larvae swam actively in the surface layer of the rearing tank, and showed weak phototaxis. After complete absorption of the yolk at ca. 6.5 mm NL, 2–3 days after hatching, caudal rays started to form and branched melanophores appeared in the otic capsule (Fig. 3C).

Postflexion larvae.—Flexion of the notochord was completed by the time larvae attained ca. 8.6 mm SL. From this point the second dorsal and anal fin rays started to form and caudal rays begin to segment. Branched melanophores appeared on the snout, opercular region, posterior corner of the lower jaw, and dorsally and ventrally on the notochord in the caudal peduncle (Fig. 3D). These melanophore numbers increased with growth. Branched melanophores formed a single row along the dorsal contour between the head and caudal peduncle, except along the second dorsal fin base, where they occured in two rows. A single row of branched melanophores was located on the ventral contour anterior to the caudal peduncle, forming a double row on the latter (Fig. 3 E, F). Pelvic fin rays started to form in larvae over 10.5 mm SL, with the second dorsal and anal fin rays beginning to segment in larvae over 11.0 mm SL. The pelvic fin rays started to form in larvae over 11.5 mm SL. Melanophores on the mid-lateral surface were arranged in a broken line (see Fig. 5B). In larvae of 11.5–12.5 mm SL, the first dorsal fin spines started to form.

Juveniles.—Aggregate numbers of all fin rays, including the pelvic rays, were completed at 12.5–14.0 mm SL, the ventral fin fold origin being positioned just behind the anus. Branched melanophores appeared on the caudal base, and a series of punctuate melanophores on the mid-lateral surface appeared as a single black line from the pectoral base to the caudal peduncle. Subsequently, branched melanophores appearing just above the former, resulted in parallel black lines (Fig. 4A). Melanophores on the dorsum and ventral contour of the tail increased in number, and, in juveniles over 14.5 mm SL, the first

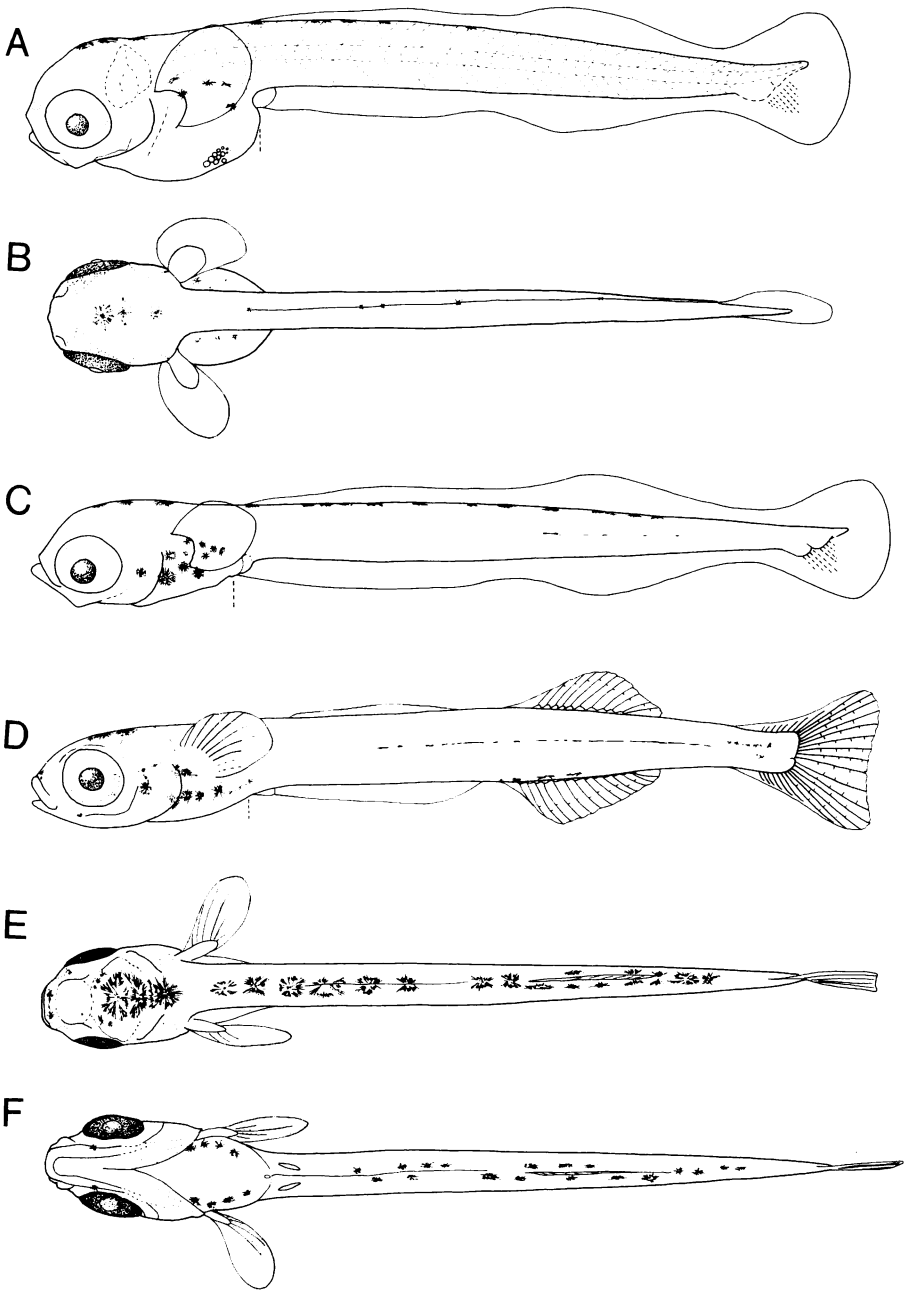

Fig. 3. Larvae of *Hypoatherina tsurugae*. A–B) newly hatched larva, FRLM 8163, 5.9 mm NL; C) flexion larva, FRLM 8164, 6.4 mm NL; D–F) postflexion larva, FRLM 8165, 11.5 mm SL. A, C and D) lateral view; B and E) dorsal view; F) ventral view.

scales appeared, mid-laterally just before the caudal peduncle. The anus began to migrate posteriorly at ca. 15.0 mm SL, with branched melanophores appearing on the pelvic fin base. In juveniles over 18.0 mm SL, the pelvic fin rays started to segment and the melanophores on the lateral surface started to diverge (Fig. 4B). In juveniles of 18.5–19.5 mm SL, the second dorsal and anal fin rays started to branch.

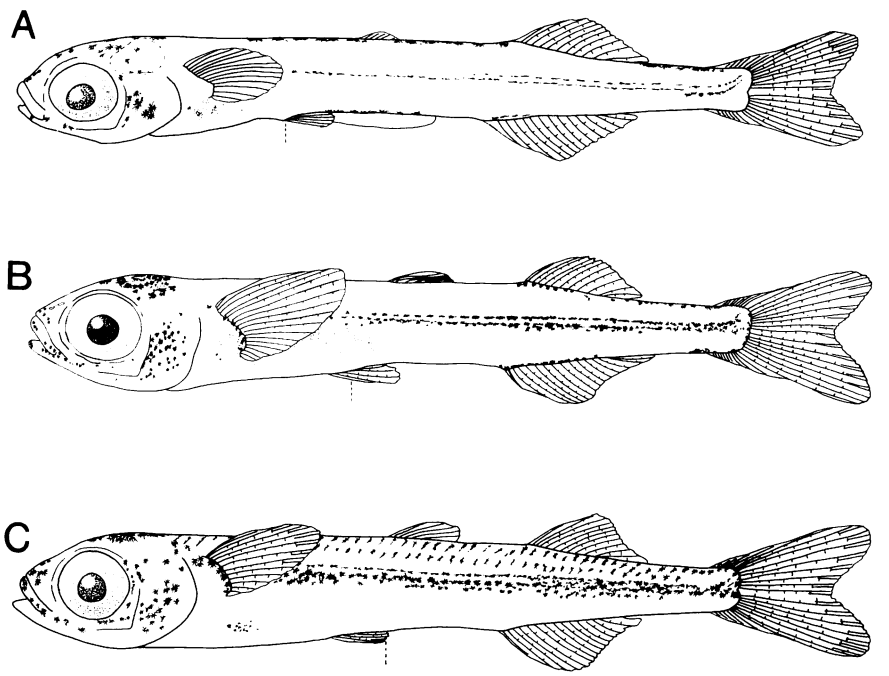

Fig. 4. Juveniles and young of *Hypoatherina tsurugae*. Scales are omitted. A) juvenile, FRLM 8166, 14.3 mm SL; B) juvenile, FRLM 8167, 17.6 mm SL; C) young, FRLM 8168, 22.0 mm SL.

Although by this stage, squamation was almost finished, the ventral finfold origin remained just behind the anus. Branched melanophores appeared on the dorsolateral body scale margins, increasing in number with growth.

Migration of the anus and disappearance of the ventral fin fold was completed in specimens of ca. 21.0 mm SL, which had an adult appearance. Branched melanophores on the scale margins were well developed (Fig. 4C).

Identification of Four Species of Atherinoidea during Early Life Stages

Eggs.—Eggs of four Atherinoidea, *Atherion elymus*, *Hypoatherina bleekeri*, *H. tsurugae*, and *Iso* sp., are demersal and almost spherical, with a slightly yellowish yolk and chorionic filaments (Takita and Nakamura, 1986a; Kimura and Tsukamoto, 1990). However, they can be distinguished from earch other by diameter, and the number and arrangement of

Table 2. Comparison of eggs of Atherinoidea found around southern Japan

Species	Diameter (mm)	Chorionic filaments		Sources
		Arrangement	Number	
Atherinidae				
Atherion elymus	0.87–1.02	Bipolar	ca. 30–50/tuft	Takita and Nakamura (1986b)
	0.90–1.05	Bipolar	ca. 40–55/tuft	Kimura and Tsukamoto (1990)
Hypoatherina bleekeri	1.08–1.20	Uniform	ca. 18	Takita and Nakamura (1986a)
H. tsurugae	1.60–1.78	Uniform	50–65	Present study
Nothocheiridae				
Iso sp.	1.13–1.23	Uniform	ca. 200	Data from unfertilized eggs (Tsukamoto and Kimura, unpubl.)

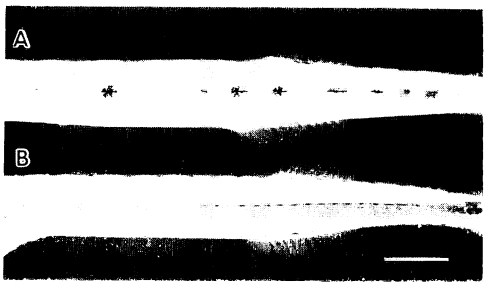

Fig. 5. Comparison of pigmentation on the mid-lateral surface of the tail between *Hypoatherina bleekeri* and *H. tsurugae*. A) flexion larva of *H. bleekeri*, 7.6 mm SL; B) flexion larva of *H. tsurugae*, 7.4 mm SL. Scale indicates 1 mm. (*Atherion elymus* and *Iso* sp. resemble *H. tsurugae* and *H. bleekeri*, respectively.)

filaments, as shown in Table 2. Eggs of Beloniformes also have chorionic filaments, but most of those found in southern Japan can also be discriminated on the basis of egg diameter, and the number and arrangement of the filaments (Nakamura, 1944; Uchida et al., 1958; Imai, 1959, 1960; Collette et al. 1984). Egg characters of the exocoetids, *Cypselurus hiraii*, *C. starksi*, and *C. heterurus doederleini*, are similar to those of *H. tsurugae* (see Uchida et al., 1958; Imai, 1959), but the former have much longer filaments (Imai, 1959).

Larvae and juveniles.—Although the larvae of the above Atherinoidea resemble each other closely, having a round head, short trunk and long tail, being common characteristics of Atherinoidea larvae (White et al., 1984), some differences occur in the arrangement of melanophores (e.g., pigmentation on the mid-lateral surface, see Fig. 5) and body proportions. These morphological differences can be utilized in keys for each developmental stage of the four Atherinoidea species, as given below.

Key to Yolk-sac and Preflexion Larvae

1a. Three large melanophores present on the top of the head 2
1b. Two large melanophores present on the top of the head ..... *Iso* sp(p).
2a. Melanophores just before notocord tip absent or present; when present, branched melanophores present on the mid-lateral surface ..... *Hypoatherina bleekeri*
2b. Melanophores present just before notocord tip. Melanophores on mid-lateral surface absent or present; when present, arranged in a broken or dotted line ..... *Atherion elymus*

H. tsurugae is excluded from this key because notocord flexion has already started by the time of hatching.

Key to Flexion Larvae

1a. Head length (HL) > body depth (BD) 2
1b. HL < BD ..... *I.* sp(p).
2a. Melanophores present or absent on mid-lateral surface; when present, arranged in a broken line and unbranched 3
2b. Branched melanophores present on mid-lateral surface, not arranged in a broken line ..... *H. bleekeri*
3a. Melanophores present just before notochord tip ..... *A. elymus*
3b. Melanophores absent just before notochord tip ..... *H. tsurugae*

Key to Postflexion Larvae

1a. HL > BD 2
1b. HL < BD ..... *I.* sp(p).
2a. Melanophores present or absent on the mid-lateral surface; when present, arranged in a broken line 3
2b. Melanophores on mid-lateral surface well spreaded, not arranged in a broken line ..... *H. bleekeri*
3a. Melanophores present between anus and anal fin origin ..... *H. tsurugae*
3b. Melanophores absent between anus and anal fin origin ..... *A. elymus*

Key to Juveniles

1a. HL > BD 2
1b. HL < BD ..... *I.* sp(p).
2a. A. I, 10–13 3
2b. A. I, 15–16. Second dorsal fin origin posterior to base of 6th anal fin ray ..... *A. elymus*
3a. Second dorsal fin origin anterior to base of second anal fin ray ..... *H. tsurugae*
3b. Second dorsal fin origin almost above base of 4

th anal fin ray .....*H. bleekeri*

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ギンイソイワシの卵および仔稚魚

塚本洋一・木村清志

ギンイソイワシの水槽内自然産出卵を飼育し，卵および仔稚魚の形態を観察，記載した．さらに南日本に分布するトウゴロウイワシ上科4種（ムギイワシ，ギンイソイワシ，トウゴロウイワシ，ナミノハナ属の1種）の卵，仔稚魚の形態を比較し，これらの識別方法を明らかにした．ギンイソイワシの受精卵は卵膜の全面にわたり50–65本の纏絡糸を有した沈性卵で，直径は1.60–1.78 mm，卵黄は淡黄色を呈している．水温18.2–21.8°Cでは，13日後に孵化が始まった．孵化仔魚は脊索長5.5–6.7 mmで頭部背面に3個の樹枝状黒色素胞が存在する．孵化時にすでに脊索は屈曲を開始しており，体長12.5–14.0 mmですべての鰭条が定数に達し稚魚に，体長約21 mmで若魚になった．トウゴロウイワシ上科4種の卵は卵径や纏絡糸の分布状態から，仔魚は黒色素胞の分布状態などによって，稚魚は体型や鰭の位置関係などによってそれぞれ識別可能である．

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