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Embryonic and Pre-larval Development and Otolith Increments in Two Filefishes, *Rudarius ercodes* and *Paramonacanthus japonicus* (Monacanthidae)

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Abstract Embryonic and pre-larval development, and otolith increments in two monacanthid species, *Rudarius ercodes* and *Paramonacanthus japonicus*, are described. Eggs of both species were adhesive and nearly spherical in shape, measuring 0.53 mm in diameter. Most of the eggs began to hatch 62 h 39 min after fertilization at 20.7-21.3°C in *R. ercodes* and after 29 h at 29.0-29.3°C in *P. japonicus*. Newly hatched larvae, which measured 1.86 and 1.94 mm total length (TL) in *R. ercodes* and *P. japonicus*, respectively, had sagittae and lapilli, but lacked asterisci. It was confirmed that one otolith increment formed before hatching in *R. ercodes* with subsequent increments being formed daily for the next two days. However, increments of *P. japonicus* were so indistinct as to be uncountable.

Monacanthid fishes include commercially important species such as *Stephanolepis cirrhifer*, *Thamnaconus modestus* and *Aluterus monoceros*, which inhabit shallow waters around Japan. In these species, seedling production trials in Japanese prefectural fisheries experimental stations have provided information on embryonic, larval and juvenile development, and growth and feeding in the early developmental stages (Fujita, 1955; Kitajima et al., 1964; Takami and Utsunomiya, 1969; Tsukashima and Kitajima, 1981; Imura et al., 1986). However, little is known about the early life history, such as duration and growth of the planktonic stage and age of settled juveniles, of monacanthid fishes, except in *Parika scaber*, inhabiting rocky reefs around the coast of New Zealand (Kingsford and Milicich, 1987). This paper deals with two further species, *Rudarius ercodes* and *Paramonacanthus japonicus*.

R. ercodes, a relatively small species, attaining 65 mm TL, is commonly seen in shallow bays and rocky reefs in temperate areas around Japan. *P. japonicus*, reaching 100 mm standard length (SL) and also inhabiting shallow rocky reefs, is strongly sexually dimorphic and dichromatic, the males and females at one time having been considered different species (Matsuura, 1984). Aspects of the eggs, and embryonic and prelarval development of *R. ercodes* and *P. japonicus* are described, along with differences in their pigmentation from other monachanthid larvae. In addition, otolith diameter was measured in both species and otolith increment formation in pre-larvae described for *R. ercodes*.

Materials and Methods

Mature male and female *Rudarius ercodes*, measuring 60 mm and 61 mm TL, respectively, were captured by hand net on the rocky reef off Tsuyazaki, northern Kyushu, Japan (33°47′N, 130°29′E) on June 1989, and kept in a 30*l* tank at the Fishery Research Laboratory, Kyushu University. Normal day-night conditions were maintained, the tank being subject to indirect sunlight through the windows. Eggs were spawned on artificial, polyvinyl algae on June 15, 24 and July 2, 1989. Adults were left in the tank after spawning to allow the female to care for the egg mass until hatching (Nakamura, 1942; Kawase and Nakazono, unpubl.). A small number of eggs were sometimes removed from the egg mass spawned on June 15 for microscopic observation of embryonic development. Hatched larvae were reared in a second tank pending developmental studies. Small-type rotifers were given 2 days after hatching, when the yolk of larvae had been absorbed. However, infrequent feeding resulted in the number of larvae decreasing drastically 3 days after hatching, with all larvae having died by the 5th day. Therefore, only larvae up to 2 days post-hatching age, which were considered to have grown under normal

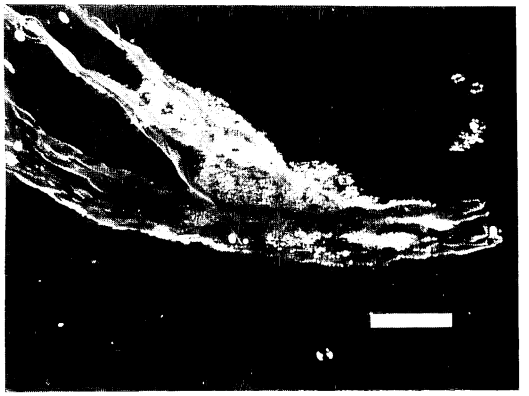

Fig. 1. Egg mass of *Rudarius ercodes* spawned on artificial algae. Scale = 1 cm.

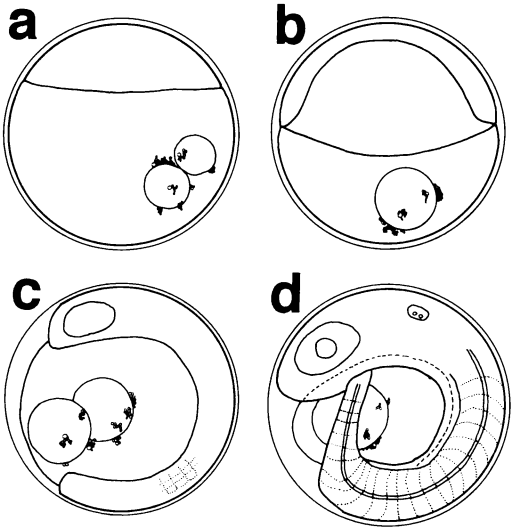

Fig. 2. Embryonic development of *Rudarius ercodes*. a) Appearance of blastodisc, 20 min after fertilization; b) gastrula stage, 9 h 30 min; c) 3-myomere stage, 15 h; d) 22-myomere stage, 36 h.

nutrient conditions, were used. Larvae were removed each day for examination and total length measurement. Water temperatures during the embryonic and pre-larval developmental stages ranged from 20.7 to 21.3°C. In September, 1989, further pairs of *R. ercodes* were kept in 30*l* tanks and the number of eggs spawned on the artificial algae counted.

Male and female *Paramonacanthus japonicus*, each measuring between 70 and 80 mm SL, were captured at Tsuyazaki on August 30, 1990. Mashed testis and extracted eggs were mixed in a petri dish and thus eggs fertilized maintained in an aerated tank. Eggs were sometimes removed subsequently for microscopic observation. Water temperatures ranged from 29.0 to 29.3°C. In addition, an adhesive egg mass cared for by adult *P. japonicus* (Nakazono and Kawase, 1993) was collected from the sandy bottom at Tsuyazaki on August 31, 1993. All the eggs hatched on the way to the laboratory, where the larvae were maintained in a tank for further 3 days without food, at water temperatures ranging from 24.9 to 25.9°C. Larval development between days 0 and 2, before depletion of the york, was described.

For verification of daily formation of otolith increments in *R. ercodes* larvae, daily samples of larvae were preserved in 95% ethanol. Thirty-three specimens were subsequently taken from each sample and cleared in glycerin for one day. For microscopic examination, each specimen was put on a glass slide and slightly pressed with a coverglass to enable the otolith increments to be seen more clearly. Dark bands in the otolith were counted at 1000×. In addition, microscopic examinations of otoliths of 25 *P. japonicus* larvae was also made.

Results

Egg mass of *Rudarius ercodes*

Eggs of *Rudarius ercodes*, spawned on the artificial algae (Fig. 1), were adhesive and attached to the algae in a mass. Females of 37 mm TL, 50 mm TL and 63 mm TL, spawned 3150, 2560 and 8180 eggs, respectively.

Embryonic and pre-larval development of *Rudarius ercodes*

Spawing of *R. ercodes* was observed at 5:01 a.m. on June 15. Fertilized eggs were nearly spherical in shape, measuring 0.53 ± 0.01 mm ($\bar{x} \pm SD$, $n = 5$), and contained 1–3 large oil globules, measuring 0.12–0.17 mm in diameter, and many smaller ones of less than 0.01 mm.

Embryonic development is shown in Figure 2 and Table 1. Twenty min after fertilization, a blastodisc appeared (Fig. 2a). The gastrula stage was reached

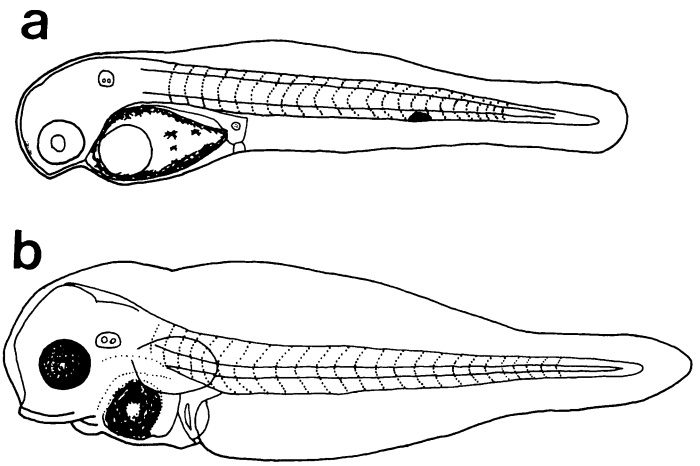

Fig. 3. Pre-larvae of *Rudarius ercodes*. a) Newly hatched larva, 1.85 mm TL; b) 2 days after hatching, 2.11 mm TL.

at 9 h 30 min (Fig. 2b). At 15 h, the blastopore closed and a pair of optic vesicles and 3 myomeres were apparent on the embryonic body (Fig. 2c). At 36 h, 22 myomeres were apparent and a pair of otoliths had appeared in the otic vesicle. At this point the heart was beating and the embryonic body moving frequently (Fig. 2d).

The larvae of *R. ercodes* began to hatch almost simultaneously at 19:40 on June 17, 62 h 39 min after fertilization. The newly hatched larvae measured 1.86 (1.84–1.90) mm TL ($n=4$) and had 5+18 myomeres (Fig. 3a). One brownish-yellow pigment spot occurred on the lower part of the 15th and 16th myomeres, but disappeared when the larvae were fixed in ethanol. The larvae of 1 day old, 24 h after hatching, measured 2.01 (1.90–2.06) mm TL ($n=4$) and had the eyes positioned level with the notochord in lateral view. The larvae of 2 days old, 48 h after hatching (Fig. 3b), measured 2.09 (2.06–2.12) mm TL ($n=5$) and had the mouth and anus open. At this point the larval yolk was completely absorbed and the brownish-yellow pigment spot had disappeared.

Embryonic and pre-larval development of *Paramonacanthus japonicus*

Eggs of *Paramonacanthus japonicus* were adhesive and nearly spherical in shape, measuring 0.53±0.07 mm ($\bar{x}\pm SD$, $n=10$). They contained 12–20 large oil globules, measuring 0.03–0.07 mm in diameter, and many smaller ones of less than 0.01 mm.

Table 1. Embryonic development in *Rudarius ercodes* (water temperature 20.7–21.3°C)

Time after spawning	Developmental stages
20 min	Elevation of blastodisc
1 h 00 min	2-cell stage
1 h 30 min	4-cell stage
2 h 10 min	16-cell stage
5 h 00 min	Early blastula stage
7 h 00 min	Late blastula stage
9 h 30 min	Gastrula stage
12 h 40 min	Rim of germ ring covering 3/4 of yolk
15 h 00 min	3-myomere stage. Formation of optic vesicles
21 h 30 min	12-myomere stage. Formation of Kupffer's vesicles
27 h 00 min	20-myomere stage. Disappearance of Kupffer's vesicles
36 h 00 min	22-myomere stage. Appearance of otoliths
62 h 39 min	Onset of hatching

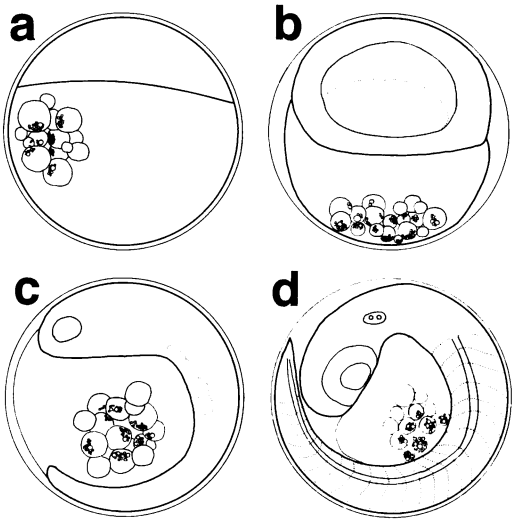

Fig. 4. Embryonic development of *Paramonacanthus japonicus*. a) Appearance of blastodisc, 15 min after fertilization; b) gastrula stage, 5 h 55 min; c) 4-myomere stage, 11 h; d) 23-myomere stage, 21 h 30 min.

Embryonic development is shown in Figure 4 and Table 2. Fifteen min after fertilization, a blastodisc appeared (Fig. 4a). The gastrula stage was reached at 5 h 55 min (Fig. 4b). Four myomeres and a pair of optic vesicles were apparent 11 h after fertilization (Fig. 4c). At 21 h 30 min, 23 myomeres were apparent and a pair of otoliths had appeared in the otic vesicle (Fig. 4d).

The larvae of *P. japonicus* began to hatch 29 h after fertilization. The newly hatched larvae measured 1.94 (1.80–2.06) mm TL ($n=19$) and had 5+18 myomeres (Fig. 5a). A single, large oil globule occurred anterior to the yolk, in addition to brownish-yellow pigments on the myomeres and tail. The pigments disappeared when the larvae were preserved in ethanol. Twenty-four h after hatching, larvae measured 2.14 (2.03–2.24) mm TL ($n=18$) and had brownish-yellow pigments on the head and melanophores along the lower part of the myomeres. Forty-eight h after hatching, larvae measured 2.16 (2.10–2.25) mm TL ($n=10$, Fig. 5b) and had the mouth and anus open.

Otolith increments of pre-larvae

Hatched larvae of both *R. ercodes* and *P. japonicus* already had sagittae and lapilli in the otic vesicles, but asterisci had not appeared by the second day after hatching.

Table 3 shows mean otolith lengths measured along the longest axes of both sagittae and lapilli ($n=22$) of 0, 1 and 2 day-old *R. ercodes*. Otolith growth rates were 4.45 μm/day (sagittae) and 4.40 μm/day (lapilli) for the first 2 days after hatching. In most of the newly-hatched *R. ercodes* larvae, one

Table 3. Otolith length of 0–2 day old *Rudarius ercodes* pre-larvae

Age (day)	Sagitta (μm)*	Lapillus (μm)*	n
0	11.1±0.61	10.3±0.67	7
1	14.6±1.36	14.1±1.17	8
2	19.7±1.97	18.8±1.93	8

* Mean±SD.

Table 2. Embryonic development in *Paramonacanthus japonicus* (water temperature 29.0–29.3°C)

Time after spawning	Developmental stages
15 min	Elevation of blastodisc
40 min	2-cell stage
1 h 00 min	4-cell stage
1 h 40 min	16-cell stage
2 h 00 min	Early blastula stage
4 h 00 min	Late blastula stage
5 h 55 min	Gastrula stage
10 h 00 min	Elevation of embryo
11 h 00 min	4-myomere stage. Formation of optic vesicles
11 h 55 min	8-myomere stage. Formation of Kupffer's vesicles
15 h 00 min	18-myomere stage. Disappearance of Kupffer's vesicles
17 h 00 min	23-myomere stage. Appearance of otic vesicles
21 h 30 min	Appearance of otolith
29 h 00 min	Onset of hatching

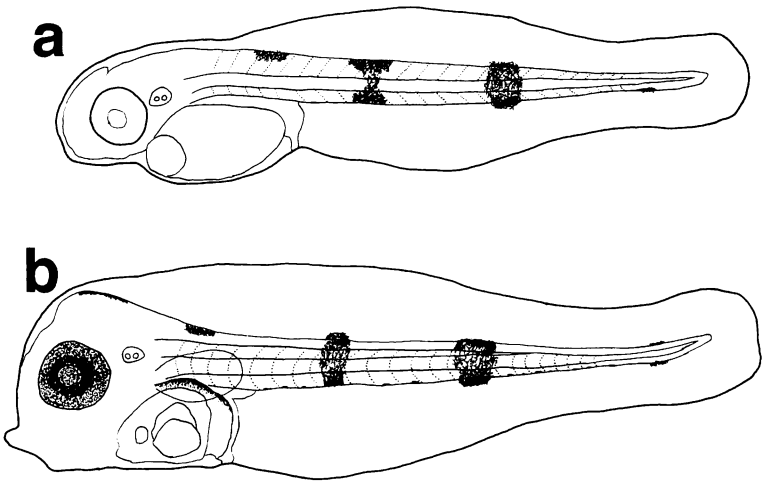

Fig. 5. Pre-larvae of *Paramonacanthus japonicus*. a) Newly hatched larva, 1.97 mm TL; b) 2 days after hatching, 2.15 mm TL.

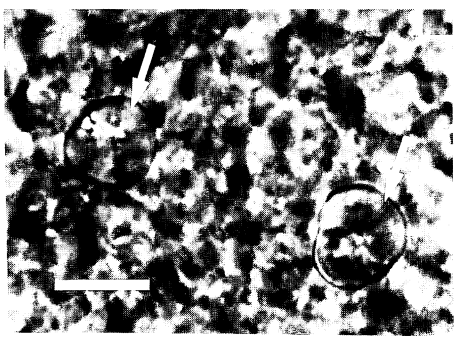

Fig. 6. Sagitta (*right*) and lapillus (*left*) of newly hatched larva of *Rudarius ercodes*. White arrow indicates first increment. Scale indicates 10 μm.

increment was observed in both the sagittae and lapilli (Fig. 6). The number of otolith increments (N) and the age in days (D) after hatching are shown in Figure 7. Linear regressions between N and D were fitted as follows:

Sagittae; $N=1.106+0.955D$ ($n=33$, $r^2=0.775$)
Lapilli; $N=1.045+0.864D$ ($n=33$, $r^2=0.792$)

Furthermore, the slopes and intercepts of the linear regressions did not differ significantly from 1 in both sagittae and lapilli (t-test, $p>0.05$). These results indicate that one increment had already formed by the time of hatching, with a further increment being formed daily at least for 2 days after hatching, in

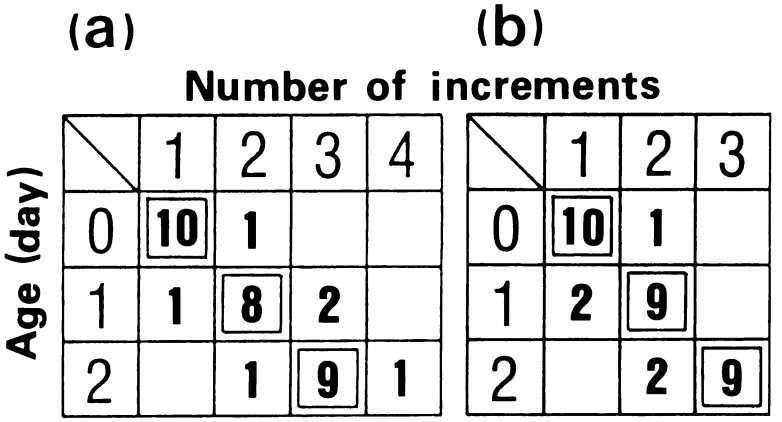

Fig. 7. Number of increments in sagittae (a) and lapilli (b) of 0–2 day-old *Rudarius ercodes* larvae.

both sagittae and lapilli of *R. ercodes*. Accordingly, the relationship between N and D can be simplified as $N=D+1$.

Mean otolith length in newly hatched *P. japonicus* was $13.1\pm0.9\,\mu m$ (sagittae) and $13.7\pm1.1\,\mu m$ (lapilli) ($\bar{x}\pm SD$, $n=5$). As the otolith increments were indistinct, they could not be accurately counted in the larvae of 0–2 days old.

Discussion

Eggs of *Rudarius ercodes* and *Paramonacanthus japonicus* are small and adhesive. They are similar to those of other monacanthids: *Thamnaconus modestus*, 0.60–0.64 mm; *T. septentrionalis*, 0.63 mm; *Stephanolepis cirrhifer*, 0.61–0.66 mm; *Aluterus monoceros*, 0.68 mm; and *Oxymonacanthus longirostris* 0.7 mm (Fujita, 1955; Kitajima et al., 1964; Zhang et al., 1985; Imura et al., 1986; Barlow, 1987). Thus, adhesiveness and small size seem to be common features of monacanthid eggs.

Pigmentation varies among monacanthid prelarvae. *R. ercodes* have a brownish-yellow pigmental spot on the lower part of the 15th and 16th myomeres, and *P. japonicus* have brownish-yellow pigments on the head, myomeres, and tail. By comparison, yellow pigment bands occur on the tail in *S. cirrhifer* and melanophores on the ventral part of the body in *T. modestus* (Fujita, 1955; Kitajima et al., 1964). The number of myomeres in newly hatched larvae also varies: 5+18 in *R. ercodes* and in *P. japonicus*, 6+14 in *S. cirrhifer* and 4+17 in *T. modestus* (Fujita, 1955; Kitajima et al., 1964; present study).

The present study revealed that one otolith increment had already been formed in both sagittae and lapilli of *R. ercodes* by the time of hatching, with subsequent increments formed daily. However, the time of first otolith increment formation differs among teleosts. Newly hatched *Chromis notatus notatus* larvae have one or two otolith increment (Kawase et al., 1993), whereas such form only after hatching, the number of increments corresponding to age (days after hatching), in *Parika scaber* and 4 species of damselfishes (Kingsford and Milicich, 1987; Thresher et al., 1989). The first increment is formed 6 days after hatching in *Engraulis mordax* (Brothers et al., 1976). Clearly, the time of formation of the first increment is essential information for accurate age determination from otolith increment analysis.

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アミメハギとヨソギの卵発生と前期仔魚の形態および耳石輪紋の形成

川瀬裕司・中園明信

水槽内で自然産卵させたアミメハギの卵と，人工授精した卵と野外で採集したヨソギの卵を用いて，卵発生と前期仔魚の形態，および耳石の輪紋形成周期を観察した．両種の卵は，ほぼ球形で粘着性があり，卵径はともに 0.53 mm であった．アミメハギの仔魚は，水温 20.7–21.3°C で受精から 62 時間 39 分後に，ヨソギの仔魚は，水温 29.0–29.3°C で受精から 29 時間後に孵化し始めた．孵化直後の仔魚の全長は，それぞれ 1.86 mm, 1.94 mm で，耳石は sagitta と lapillus が観察された．アミメハギの孵化直後の仔魚の耳石には既に 1 本の輪紋が形成されており，孵化後 2 日まで日周輪の形成されることを確認したが，ヨソギでは輪紋が不鮮明で日周輪の確認は出来なかった．

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