Japanese Journal of Ichthyology
Vol. 31, No. 3 1984

魚類学雑誌
31巻3号 1984年

Development of Eggs and Larvae of Two Bitterlings, *Rhodeus atremius* and *R. suigensis* (Cyprinidae)

Nobuhiro Suzuki and Takashi Hibiya

(Received December 27, 1983)

Abstract The development of eggs and larvae of *Rhodeus atremius* and *R. suigensis* was observed under controlled water temperature of 22±1°C. The egges of *R. atremius* began to hatch about 36 hours after insemination, and those of *R. suigensis* in about 46 hours. The larvae of both species reached the free-swimming stage about 24 days after hatching. The larval development of both species was so similar that they are considered to be closely related species.

It is well known that bitterlings deposit eggs in the gill cavity of freshwater bivalves. After hatching the larvae stay and develop in the gill cavity, until well-developed larvae swim out from the bivalve.

The development of the bitterling *Rhodeus atremius* (Jordan et Thompson) was described by Nakamura (1969), however that of *Rhodeus suigensis* (Mori) is still unknown.

This paper deals with the comparative development of eggs and larvae of *R. suigensis* and *R. atremius*.

Materials and method

Parental fish of *R. atremius* were collected from the Yabe River, Fukuoka Pref., and those of *R. suigensis* from the Asahi River, Okayama Pref. Artificial insemination was carried out several times from May to July using the same pair from each species. During this period, the ovipositor of both species showed repeatedly growth and reduction. It reached the maximal length (ca. 12 mm) at intervals of 5 to 7 days. The number of eggs obtained from an artificial exploitation ranged from 2 to 12 (mean, 6 eggs) in both species. Eggs and semen were obtained by pushing the belly of fishes. The eggs were transferred into a petri-dish filled with distilled water of 22°C and inseminated by pouring diluted sperm water into the dish. The fertilized eggs and larvae were kept at a water temperature of 22±1°C. Rearing water was changed with fresh water at the same temperature every day. The larvae that reached the free-swimming stage were reared in an aquarium of 30 *l* in volume, and were fed with commericial diets ("Tetramin") for the initial food item.

The development of eggs and larvae was examined under a dissecting microscope. Total length of live larvae was measured with an ocular micrometer.

Observations

Embryonic stages of *Rhodeus atremius*. The ripe unfertilized eggs are nearly pear shaped, opaque yellow in colour, measuring about 3.40 mm in length, 1.45 mm in breadth. (Fig. 1A).

Stage A: Fertilized egg. At fertilization, the cortical alveoli embedded in the cortical protoplasmic layer begin to break down: beginning near the animal pole and ending at the vegetal pole. About 30 minutes after insemination, the chorion is separated from the plasma membrane to form the perivitelline space.

Stage B: Blastodisc (Fig. 1B). About 1 hour after insemination, the protoplasm converges on the animal pole to form a raised blastodisc.

Stage C: Two-celled egg (Fig. 1C). About 2 hours after insemination, the first cleavage plane normally divides the blastodisc into two cells of equal size.

Stage D: Four-celled egg (Fig. 1D). About 2 1/2 hours after insemination, the second cleavage plane is perpendicular to the first and divides the blastodisc into four cells of equal size.

Stage E: Eight-celled egg (Fig. 1E). About 3 hours after insemination, the third cleavage

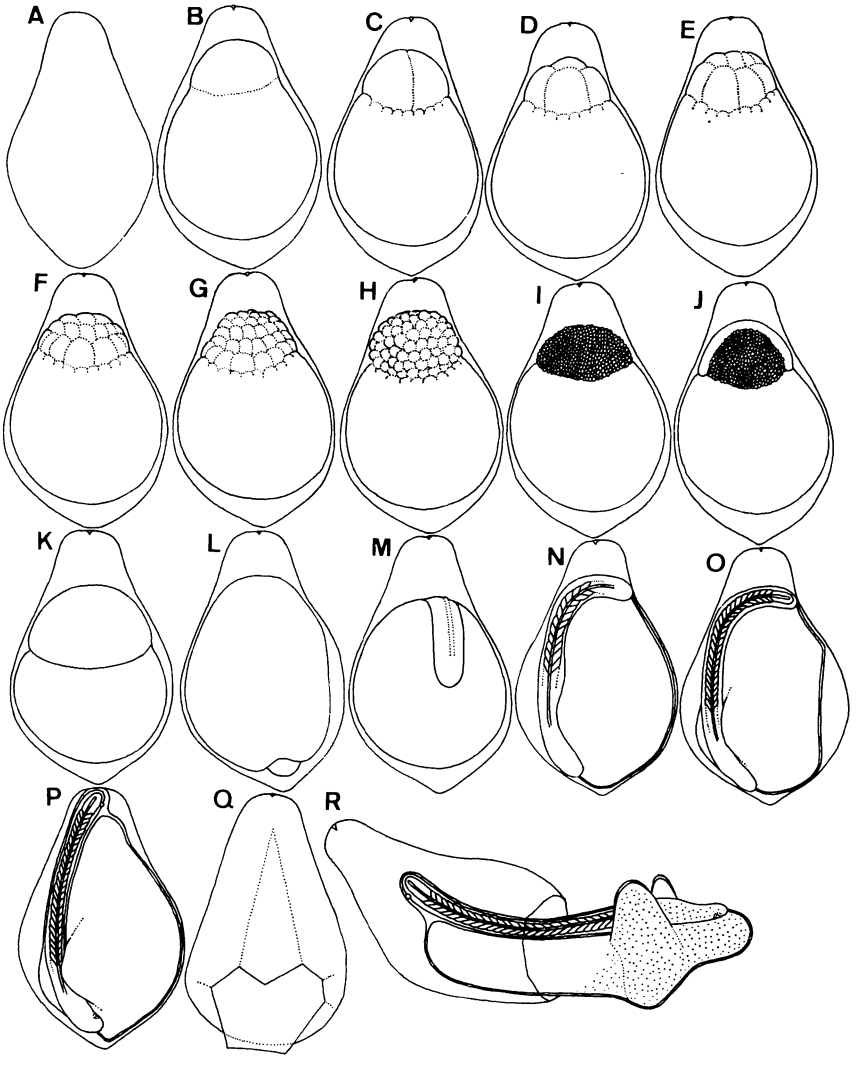

Fig. 1. Egg development of *Rhodeus atremius* at 22±1°C in water temperature. Time required for each developmental stage is shown in Table 1.

plane is parallel symmetrical, elongated in the axis of the second plane of cleavage.

Stage F: Sixteen-celled egg (Fig. 1F). About 3 1/2 hours after insemination, the fourth cleavage plane divides the blastoderm into sixteen cells. The blastodisc becomes flat.

Stage G: Thirty-two-celled egg (Fig. 1G). About 4 hours after insemination, the fifth cleavage plane divides the blastoderm into thirty-two cells. The cleavage furrows among blastomeres at this stage show slightly different orientations.

Stage H: Early morula (Fig. 1H). About 4 1/2 hours after insemination, the sixth and following cleavages are difficult to distinguish.

Stage I: Post morula (Fig. 1I). About 5 hours after insemination, the blastodermal cap at the seventh cleavage consists of cells smaller than those of the previous stages. The number of marginal cells increases.

Stage J: Blastula (Fig. 1J). About 6 hours after insemination, the blastoderm expands over the surface of the yolk sphere.

Stage K: Middle gastrula (Fig. 1K). About

10 hours after insemination, the blastoderm covers about one fourth of the yolk sphere, gastulation begins on the most thickened portion of the germ ring, later forming the embryonic shield. About 15 hours after insemination, the blastoderm covers about half of the yolk sphere, and the embryonic shield increases in size.

Stage L: Blastopore comes near to closing (Fig. 1L). About 18 hours after insemination, the yolk sphere is nearly covered by the blastoderm excepting a small vegetal area. The embryonic shield develops to form the embryo. At this stage, a weak undulating rhythmical movement of the embryo begins.

Stage M: Neurula (Fig. 1M). About 20 hours after insemination, the embryonic shield becomes narrow to form the embryonic body. The embryonic body has a keel-like structure forming the central nervous system.

Stage N: Eleven somites formation (Fig. 1N). About 24 hours after insemination, eleven somites are formed in the central part of the embryonic body. The embryo grows antero-posteriorly and the tail bud appears. A pair of small yolk projections can be seen just posterior to the head region.

Stage O: Embryo formed entirely (Fig. 1O). About 30 hours after insemination. At this stage, the embryo has usually 19 to 27 somites, and its rhythmical contractile movements reach their greatest extent. The posterior tip of the embryo is situated at the animal pole side.

Stage P: The last embryonic stage (Fig. 1P). A few hours before hatching, the tail tip becomes free of the yolk sphere. Kuppffer's vesicle is recognized. Rhythmical contractile movements cease.

Stage R: Hatching (Fig. 1R). About 36 hours after insemination, all embryos hatch from the vegetal pole side (Fig. 1Q). Nineteen to twenty-seven somites are discernible.

Larval development of *Rhodeus atremius*. Hatching began 36 hours after insemination and lasted for about 10 consecutive hours at 22 ± 1°C in water temperature.

1) Immediately after hatching, 3.6–3.8 mm in total length (Fig. 2A). Nineteen to twenty-seven myotomes are countable. The yolk sac contains a substantial amount of yolk. A pair of dorsal yolk projections becomes shaped just like an arrow, and the yolk sac is anteroventrally convex. Kupffer's vesicle is present on the tail. The fin-fold at the trunk and caudal portion is small. The larvae at this stage are usually motionless.

2) 2 days after hatching, 5.3–5.8 mm in total length (Fig. 2B). The number of myotomes ranges from 30 to 32 (18–20+12). The tail elongates backward and the caudal fin-fold slightly develops. The anteriormost part of the yolk sac elongates slightly forward. The optic cups without lens and the auditory vesicles with two pairs of otoliths are clearly observed.

3) 4 days after hatching, 5.8–6.0 mm in total length (Fig. 2B). The number of myotomes ranges from 31 to 33 (16–18+15). The larvae at this stage begin to move when the primordial fin-fold becomes well-developed. The larvae, however, usually lay on their back on the bottom of the petri-dish. A pair of dorsal yolk projections is connected with the ventral pair of yolk projections at the mid-yolk sac region (Fig. $2C_1$). The dorsal part of the head is raised slightly and the brain is in the process of further development. The heart begins to pulsate beneath the head region. The anteriormost part of the yolk sac develops downward to form a projection with a conve yolk.

4) 5 days after hatching, 6.5–6.8 mm in total length (Fig. 2D). The circulatory system is already established, blood cells become reddish and increase in number. The dorsal and ventral yolk projections gradually elongate posteriorly. The notochord starts to flex, the cartilaginous hypural elements begin to differentiate, and the incipient fin-rays are not visible. The margin of fin-fold extends.

5) 8 days after hatching, 6.7–7.1 mm in total length (Fig. 2E). The number of myotomes ranges from 31 to 34 (16–19+15). The lenses are already formed. The optic cup envelopes the lens completely, melanin pigment begins to appear on the optic cup. A pair of small nasal sacs become evident in front of the eye cups. Rudiments of the pectoral fins appear as small membranes beneath the auditory vesicles. The vitellocaudal vein is formed, which drains around the median part of the yolk sac and reaches to the heart. A pair of dorsal yolk projections is reduced slightly, while the ventral yolk pair is still well-developed. No pronounced changes of marginal shape in the larval fin-fold can be

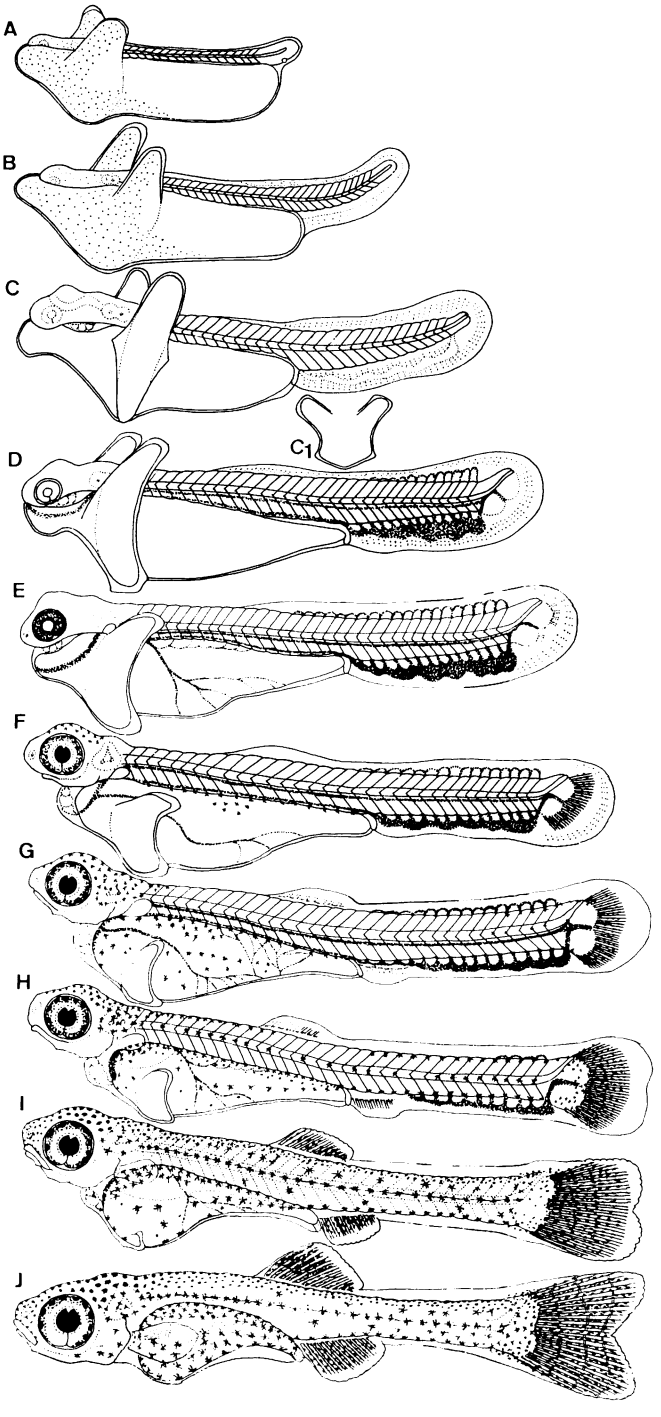

Fig. 2. Larvae of *Rhodeus atremius*. A, immediately after hatching, 3.7 mm in total length (TL). B, 2 days after hatching, 5.5 mm in TL. C, 4 days after hatching, 5.8 mm in TL. D, 5 days after hatching, 6.6 mm in TL. E, 8 days after hatching, 6.9 mm in TL. F, 11 days after hatching, 7.6 mm in TL. G, 13 days after hatching, 7.6 mm in TL. H, 15 days after hatching, 7.6 mm in TL. I, 18 days after hatching, 7.9 mm in TL. J, 24 days after hatching, 8.6 mm in TL.

found. The cartilaginous hypural elements are differentiated completely, and some rays of the caudal fin are formed.

6) 11 days after hatching, 7.0–7.4 mm in total length (Fig. 2F). The number of myotomes ranges from 31 to 34 (16–19+15). Black pigments are developed on the retinal layers. The optic cups are silvery blue because guanine is distributed diffusely on this organ. The mouth sometimes opens and closes. The part of the fin-fold of the future dorsal and anal fins becomes high. The main rays of the caudal fin are formed. The reduced pair of dorsal yolk projections is further reduced considerably in comparison with those of the former stages. Melanophores appear on the dorsal part of both head region and the yolk sac, and on the caudal fin rays. At this stage, the larvae sometimes actively swim upside down.

7) 13 days after hatching, 7.6–7.8 mm in total length (Fig. 2G). The number of myotomes ranges from 32 to 35 (17–20+15). Eye pigments, both melanin and guanine, are heavily concentrated. In the larvae at this stage, no pronounced changes of the distribution of melanophores can be seen, however, melanophores increase in number. The caudal rays are completed in number. About 7 rays are formed in the dorsal and anal fins.

8) 15 days after hatching, 7.6–7.8 mm in total length (Fig. 2H). The upper and lower jaws are approximately equal in size. Melanophores are observed on the head region, the auditory vesicles, the caudal fin-rays, the dorsal, ventral and lateral parts of the body and on the yolk sac. The posterior margin of the caudal fin changes from a rounded to a truncated shape. A small gas bladder and a green gall bladder can be easily seen beneath the pectoral fins. At this stage, the larvae swim with good balanced orientation, however, they cannot continue vigorous swimming for more than a few seconds.

9) 18 days after hatching, 7.8–8.0 mm in total length (Fig. 2I). The dorsal and anal fin rays are completed in number. The caudal fin rays begin to fork into two branches. The gas bladder becomes larger without dividing into two lobes. Melanophores increase in number and newly appear on the dorsal and anal fin rays. Yellow pigments also appear over the melanophores on the head region and on the dorsal part of the body. The yolk projections are barely present. The pectoral fins become functional.

10) 24 days after hatching, 8.2–8.7 mm in total length (Fig. 2J). Free-swimming stage. The gas bladder is divided completely into front and hind lobes. This means that the larvae are able to swim actively with good balanced orientation for hours. Guanine is slightly distributed on the surface of yolk sac. The dorsal and anal fin rays are completed in number. Yellow pigments are widely distributed on the body. Remnants of the larval fin-fold persist: the dorsal and the anal fins are connected with the caudal fin at the anterior portion of the caudal peduncle. The caudal fin becomes emarginated. Rudiments of the ventral fins appear as small membranes on the breast. Some rays are formed in the pectoral fins. The yolk projection on the breast is so reduced that it is difficult to find. Melanophores on the dorsal fin rays are aggregated at its anterior region to form a black spot. Although the yolk still remains, the larvae begin to feed.

Embryonic stages of *Rhodeus suigensis*. The

Table 1. Comparison of time required for embryonic stages in *R. atremius* and *R. suigensis* at 22±1°C in water temperature.

Stage*	*R. atremius*	*R. suigenis*
	Time after insemination (hr : min)	
A	—	—
B	1 : 00	1 : 00
C	2 : 00	2 : 00
D	2 : 30	2 : 30
E	3 : 00	3 : 00
F	3 : 30	3 : 30
G	4 : 00	4 : 00
H	4 : 30	4 : 30
I	5 : 00	5 : 00
J	6 : 00	6 : 00
K	15 : 00	26 : 00
L	18 : 00	28 : 00
M	20 : 00	30 : 00
N	24 : 00	33 : 00
O	30 : 00	40 : 00
P	34 : 00	44 : 00
Q	—	—
R	36 : 00	46 : 00

* Stages A to R correspond to those in Fig. 1 and Fig. 3, respectively.

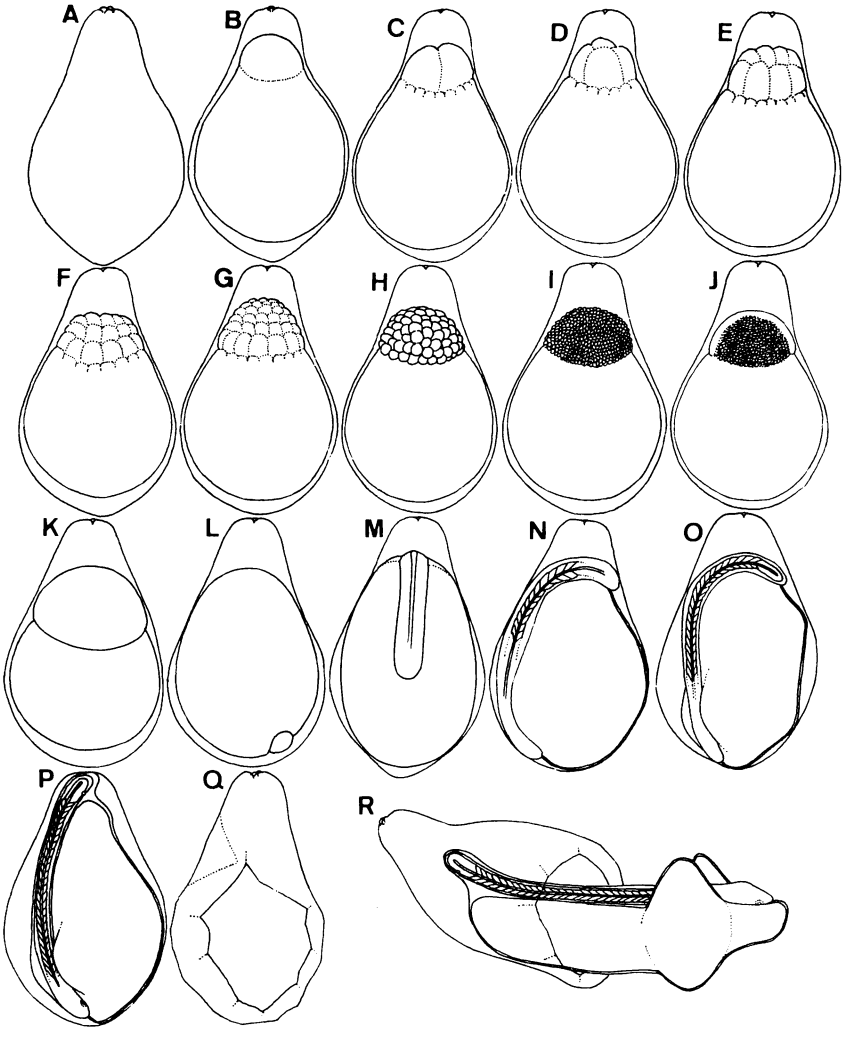

Fig. 3. Egg development of *Rhodeus suigensis* at 22±1°C in water temperature. Time required for each developmental stage is shown in Table 1.

ripe unfertilized eggs are nearly pear shaped, opaque yellow coloured like those of *R. atremius*, measuring about 3.00 mm in length, 1.80 mm in breadth (Fig. 3A). The embryonic stages of this species are the same as those of *R. atremius* (Fig. 3). The time for embryonic development of both species is approximately equal until Stage J, however, after Stage K the development of *R. suigensis* progresses more slowly than that of *R. atremius* (Table. 1). Therefore, the time required for hatching of *R. suigensis* is about 10 hours longer than that of *R. atremius*.

Larval development of *Rhodeus suigensis*. Hatching began 46 hours after insemination and lasted for about 10 consecutive hours at 22±1°C in water temperature.

1) Immediately after hatching, 3.5–3.7 mm in total length (Fig. 4A). Nineteen to twenty-five myotomes are countable. A pair of small yolk projections just posterior to the head region appear at the embryonic Stage N (Fig. 3N) and is arrow shaped. The ventral yolk projection of the larvae also becomes more developed. Kupffer's vesicle is still recognized on the tail. The larvae at this stage usually show no motion.

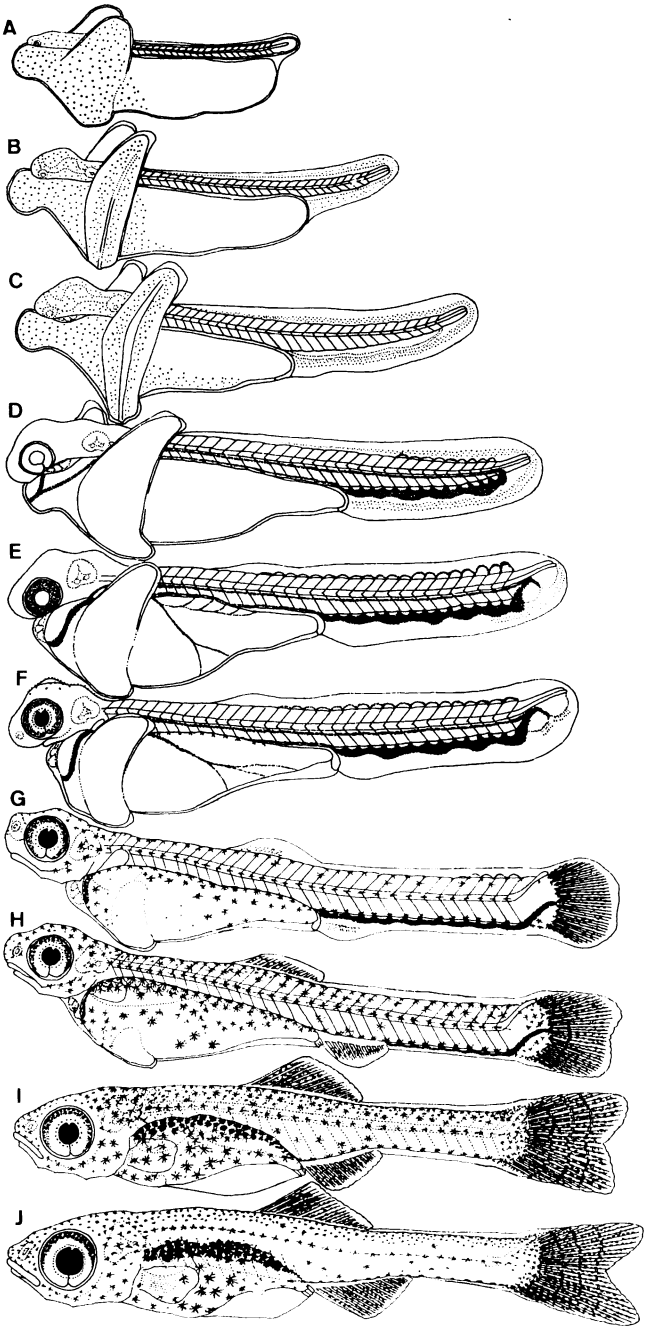

Fig. 4. Larvae of *Rhodeus suigenisis*. A, immediately after hatching, 3.5 mm in total length (TL). B, 2 days after hatching, 4.5 mm in TL. C, 3 days after hatching, 5.2 mm in TL. D, 6 days after hatching, 5.7 mm in TL. E, 8 days after hatching, 6.6 mm in TL. F, 10 days after hatching, 6.9 mm in TL. G, 15 days after hatching, 7.3 mm in TL. H, 18 days after hatching, 7.5 mm in TL. I, 24 days after hatching, 8.1 mm in TL. J, 28 days after hatching, 9.3 mm in TL.

2) 2 days after hatching, 4.3–4.5 mm in total length (Fig. 4B). The number of myotomes ranges from 29 to 32 (21–23+8–9). The tail elongates backward and the caudal fin-fold develops slightly. The anteriormost part of yolk sac develops downward to form a projection with a convex yolk. A pair of dorsal yolk projections is connected with the ventral yolk projections at the mid-yolk sac region. In this stage, the larval yolk projections of this species are more developed than those of *R. atremius*. The optic cups without lens and the auditory vesicles with two pairs of otoliths are clearly observed. The dorsal part of the head raises slightly and the brain has undergone further development.

3) 3 days after hatching, 5.0–5.2 mm in total length (Fig. 4C). The number of myotomes ranges from 30 to 32 (18–19+14–15). The heart begins to pulsate beneath the head region. The larvae at this stage begin to move when the primordial fin-fold becomes well-developed. The larvae, however, usually lay on their back on the bottom of the petri-dish.

4) 6 days after hatching, 5.5–5.8 mm in total length (Fig. 4D). The number of myotomes ranges from 30 to 32 (15–17+15). The dorsal and ventral yolk sac projections gradually elongate posteriorly. As for the rate of development of the larval yolk projections, *R. atremius* and *R. suigensis* are the same in this stage. The circulatory system is already established, blood cells become reddish and increase in number. The notochord begins to bend upward.

5) 8 days after hatching, 6.5–6.7 mm in total length (Fig. 4E). The number of myotomes ranges from 32 to 34 (17–19+15). The lenses are already formed. The optic cup envelops the lens completely, melanin pigment begins to appear on the optic cup. The vitello-caudal vein is formed and reaches to the heart. The cartilaginous hypural elements are differentiated completely, but the caudal fin rays are not yet seen.

6) 10 days after hatching, 6.7–6.9 mm in total length (Fig. 4F). The number of myotomes ranges from 32 to 34 (18–19+14–15). A pair of small nasal sacs becomes evident in front of the eye cups. Some rays of the caudal fin are formed. A pair of dorsal yolk projections is reduced slightly, while the ventral yolk pair is still well-developed. Rudiments of the pectoral fins are small membranes and also appear beneath the auditory vesicles. Black pigments are developed on the retinal layers. The optic cups are silvery blue because guanine is distributed diffusely on this organ. The mouth is sometimes opened and closed. Melanophores appear on the dorsal part of both the head region and the yolk sac. The part of the fin-fold of the future dorsal and anal fins becomes high. The larvae at this stage sometimes swim actively lying trunk down on the bottom of the petri-dish.

7) 15 days after hatching, 7.1–7.3 mm in total length (Fig. 4G). The number of myotomes ranges from 32 to 34 (17–19+15). The pair of reduced dorsal yolk projections is further reduced considerably compared with those of the former stages. Eye pigments, both melanin and guanine, are heavily concentrated. About 5 rays are formed in the dorsal and anal fins. The main rays of the caudal fin are formed. The small gas bladder and a green gall bladder can be easily seen beneath the pectoral fins. Melanophores are observed on the head region, the auditory vesicles, the caudal fin rays, the dorsal, ventral and lateral parts of the body and on the surface of the yolk sac.

8) 18 days after hatching, 7.5–7.7 mm in total length (Fig. 4H). The number of mytomes ranges from 32 to 34 (17–19+15). The gas bladder becomes larger without dividing into two lobes. The caudal fin rays are completed in number and they begin to fork into branches. The dorsal and anal fin rays are also completed in number. Melanophores increase in number and newly appear on the dorsal and anal fin rays. Yellow pigments also appear over the melanophores on the head region and the dorsal part of the body. The yolk projections are barely present. The pectoral fins become functional. The posterior margin of the caudal fin changes from a rounded to truncated shape. The upper and lower jaws are approximately equal in size. At this stage, the larvae swim with good balanced orientation, however, they can not continue vigorous swimming for more than a few second.s

9) 24 days after hatching, 8.0–8.3 mm in total length (Fig. 2I). Free-swimming stage. The gas bladder completely divides into front and hind lobes. This means that the larvae are

able to swim actively with good balanced orientation for hours. Yellow pigments are widely distributed on the body. Remnants of the larval fin-fold persist: the dorsal and the anal fins are connected with the caudal fin at the anterior portion of the caudal peduncle. The caudal fin becomes emarginated. The yolk projection on the breast is so reduced that it is difficult to find. Although the yolk still remains, the larvae at this stage begin to feed.

10) 28 days after hatching, 9.0–9.4 mm in total length (Fig. 4J). Rudiments of the ventral fins appear as small membranes and also can be seen on the breast. Some rays are formed in the pectoral fins. Guanine is slightly distributed on the belly. Melanophores on the dorsal fin rays aggregate at its anterior region to form a black spot, which is similar to that of *R. atremius*.

Discussion

It was reported that *R. atremius* is distributed in the northeastern part of Kyushu, Japan, and *R. suigensis* in Korea (Mori, 1935; Uchida, 1939). Thereafter, Nakamura and Motonobu (1965) stated that *R. suigensis* is also distributed on the Okayama Plain, Japan.

The spawning season of *R. atremius* in northeastern Kyushu is from March to June (Nakamura, 1969). On the other hand, there is only one report on the spawning season of *R. suigensis*: it begins to spawn in early summer in the Okayama Plain (Hosoya, 1982). Judging from our present study, the spawning period of both species continues from early May to the middle of July, and for this short duration they spawn at least 5 times at intervals of about 7 days.

Rhodeus suigensis is characterized by having a more slender body than *R. atremius* and is less intensively pigmented on both sides of the body (Nakamura and Motonobu, 1965). However, as regards morphological characters in embryonic and larval development, we could not find differences between *R. atremius* and *R. suigensis*, except for the following two characters: the time required for hatching is ca. 36 hours in *R. atremius* and ca. 46 hours in *R. suigensis* and the rudiments of the ventral fins in *R. atremius* are formed earlier than in *R. suigensis* under controlled water temperature of $22\pm1^{\circ}C$. Such differences are also seen between *R. ocellatus ocellatus* (Kner) and *R. ocellatus smithi* (Regan) at the same water temperature (Nagata and Miyabe, 1978; Suzuki, unpublished).

On the other hand, *R. atremius* and *R. suigensis* share the following two characters which are not shared by two subspecies of *R. ocellatus*, i.e., (1) fertilized eggs are nearly pear shaped, and (2) the anteriormost part of the yolk sac develops to form a projection with a convex yolk during larval development (Nakamura, 1969). Moreover, the fertile F_1 hybrids between *R. suigensis* and *R. atremius* were obtained (unpublished data).

These facts suggest that these two forms should not be separated at species level and should be regarded as two subspecies of *R. atremius*.

Acknowledgments

We are grateful to Mr. Yoshiteru Kimizuka, Mr. Yutaka Akai of Nihon University and Mr. Hisashi Katayama, Okayama Research Group of Freshwater Fishes, for supplying specimens. We also thank Dr. Ryoichi Arai of the Department of Zoology, National Science Museum, Tokyo, for critical reading of the manuscript.

Literature cited

Hosoya, K. 1982. Freshwater fish Fauna of the Yoshii River, Okayama Prefecture. Bull. Biogeogr. Soc. Japan, 37 (5): 23–35.

Mori, T. 1935. Descriptions of three new cyprinoids (Rhodeina) from Chosen. Zool. Mag. (Tokyo), 47 (562/563): 547–559, 1 pl. (In Japanese with English summary).

Nagata, Y. and H. Miyabe. 1978. Developmental stages of the bitterling, *Rhodeus ocellatus ocellatus* (Cyprinidae). Mem. Osaka Kyoiku Univ., Ser. III, 26 (3): 171–181.

Nakamura, M. 1969. Cyprinid fishes of Japan. Spec. Publ. Res. Inst. Nat. Resources, (4): 1–8+1–455, 2 cols., pls. 1–149. (In Japanese).

Nakamura, M. and T. Motonobu. 1965. *Pseudoperilampus suigensis* Mori, Korean bitterling, collected in a stream of Okayama Prefecture. Misc. Rep. Inst. Resources, (64): 59–60. (In Japanese with English summary).

Uchida, K. 1939. The fishes of Tyōsen (Korea). Part 1. Nematognathi, and Eventognathi. Bull.

Fish. Exp. Stn. Goverment-General of Tyōsen, (Husan), (6): i–viii＋1–458., pls. 1–47. (In Japanese).

(College of Agriculture and Veterinary Medicine, Nihon University, Simouma 3–34–1, Setagaya-ku, Tokyo 154, Japan)

カゼトゲタナゴとスイゲンゼニタナゴの初期発育過程

鈴木伸洋・日比谷　京

カゼトゲタナゴとスイゲンゼニタナゴの卵発生ならびに前期仔魚の発育について経時的に観察し併せて，両種の個体発生の比較を行った．カゼトゲタナゴの卵発生ならびに前期仔魚の発育経過および発育形態は中村 (1969) の報告にほぼ一致した．スイゲンゼニタナゴのそれもカゼトゲタナゴに酷似し，22°C の飼育下では受精後約 46 時間から孵化を開始し，浮上期に達するのにほぼ 24 日を要した．この期間の本種の個体発生は卵黄嚢の変化した 1 対の突起（翼状突起）が発達することと，卵黄嚢の前端部が下方へ突出して突起を形成することが特徴である．このことから本種は，カゼトゲタナゴに極めて近縁な種類であると判断された．

(鈴木・日比谷: 154 東京都世田谷区下馬 3-34-1　日本大学農獣医学部水産学科)