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Larval and Juvenile Development of Two Dragonets, *Repomucenus richardsonii* and *R. valenciennei*, Reared in a Laboratory

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Abstract *Repomucenus richardsonii* and *R. valenciennei* eggs were obtained from captive spawners, and the larvae were raised to the juvenile stage. Total lengths (TL) of newly-hatched prolarvae were 1.17±0.04 mm and 1.11±0.03 mm, respectively. Each species had 8-9+11-12 myomeres and a large oval yolk sac. Developmental changes in the two species were similar: absorption of the yolk was completed at 5-6 days after hatching at ca. 2.2 mm TL, all fin rays were fully developed at ca. 5.5 mm TL, and the transition to a demersal life style was completed at ca. 10-11 mm TL. The postlarval stage of the two species can be distinguished by the melanophores on the ventral edge of the notochord, on the branchiostegal membrane, and on the ventral finfold. The juvenile stages can be distinguished by the melanophores on the branchiostegal membrane, on the pelvic fins, and just above the lateral line and the infraorbital canal pattern.

Dragonets are common demersal fishes in the sandy-muddy coastal waters of Japan, and their eggs and larvae appear abundantly in plankton samples in coastal areas (Mito, 1965; Takita, 1980, 1983). However, only a few reports on their eggs and young stages have so far appeared. The eggs and prolarvae of four dragonet species, *Repomucenus valenciennei*, *R. richardsonii*, *R. ornatipinnis* and *Paradiplogrammus enneactis calliste* have been described (Takita, 1980, 1983). However, as they have not been raised to later stages, their postlarval and juvenile stages are not known. The larvae and juveniles of *R. beniteguri* have been described by Takai and Yoshioka (1979), but the origin of their materials was not definite. We obtained eggs from *R. beniteguri* that had been kept in a tank, and observed the embryonic, larval and juvenile developments (Eda et al., 1994). Some of our findings were different from those of Takai and Yoshioka (1979).

Repomucenus richardsonii are distributed from southern Japan to the South China Sea, and *R. valenciennei* are distributed in Japan as far north as southern Hokkaido and on the western coast of the Korean Peninsula (Nakabo, 1984). These two species are common in Omura Bay, Nagasaki Prefecture, where we have been conducting surveys of fish eggs and larvae. *R. richardsonii* are generally caught by surf-fishing in shallow water of 1 to 5 m, while *R. valenciennei* are rarely caught. However, *R. richardsonii* often occur in commercial trawl catches at water depth from 15 to 20 m where *R. valenciennei* are abundantly caught, demonstrating an overlapping distribution in deeper areas. Eggs, larvae and juveniles of dragonet species are common in plankton samples from the bay. It is highly probable that larvae and juveniles of the above two species are in those plankton samples. However, information is lacking on how to identify them. In order to elucidate the identification of larvae and juveniles of these two species, eggs were obtained from fish of each species that had been kept in a tank, and larvae and juveniles were raised from the eggs and observed. Here we describe the comparative development of these two species.

Materials and Methods

Mature *Repomucenus richardsonii* and *R. valenciennei* were collected in Omura Bay, Nagasaki Prefecture (lat. 32°50′-33°02′N, long. 129°50′-53′E) in 1992. The former were caught by angling in May

and the latter were caught with a small trawl net in June. The fishes were transported to the Fisheries Experimental Station, Nagasaki University, in Nomo, Nagasaki Prefecture, and held in a concrete tank, 4.8×6.8 m in area and 1.2 m in water depth. Because individual females of each species spawned every day for a certain period, many eggs were collected on a given day. *R. richardsonii* eggs spawned on May 11 and *R. valenciennei* eggs spawned on June 11 were used in this study.

The fishes made an upward spawning trip, as previously reported (Takita and Okamoto, 1979). Eggs released near the water surface were collected with a small dip net immediately after the termination of spawning. The incubation and rearing of the eggs and larvae were described in the previous paper (Eda et al., 1993). As it is well-known that pigmentation in young stages varies with different growing conditions, we tried to keep rearing conditions of larvae and juveniles as uniform as possible. The rearing temperature was 22.6±1.0°C for *R. richardsonii* and 23.4±1.4°C for *R. valenciennei*.

Larvae and juveniles were taken periodically from the rearing tank. The specimens were anesthetized with tricaine methanesulfonate (MS-222), and fixed with 5% formalin (diluted with seawater) for the observation. Some specimens were stained with alizarin red S to observe their fin rays and preopercular spine.

Results

Larval and juvenile development of *Repomucenus richardsonii*

Newly-hatched prolarvae (Fig. 1A), 1.17±0.04 mm TL and 1.13±0.04 mm in notochord length (NL): The larvae had a large oval yolk that was segmented peripherally. The anus was located just behind the yolk sac. Myomere counts were 8–9+11–12. The mouth was not formed and the eyes were unpigmented. No heart beat was observed prior to fixing the specimens. The larvae had melanophores dorsally on the head to the tail, except for the tip of the notochord. Melanophores were also on the yolk sac and on the ventral finfold just behind the anus. The larvae bore dense xanthophores laterally on the trunk and on the entire yolk sac. These observations were mainly made on fixed specimens, and thus we did not determine the fate of the xanthophore.

One-day-old prolarva, 1.97 mm TL and 1.85 mm NL (Fig. 1B): The pectoral fin buds were formed, and spinous processes appeared on the edges of the dorsal and ventral finfolds. A small vacuole was located on the dorsal finfold near the trunk, (not illustrated). A heart beat was observed prior to fixing the specimens. The larvae had two layers in the dorsal tissue. Melanophores were scattered over the trunk and tail with a saddle of dense pigment on the mid-dorsal and mid-ventral tail. Melanophores appeared on the digestive tract. Two pigment clusters were located on the dorsal finfold and one on the ventral finfold. Melanophores were also present on the side of the head and on the ventral edge of the notochord.

Three-day-old prolarva, 2.11 mm TL and 1.99 mm NL (Fig. 1C): The mouth opened and the eyes were densely pigmented. The digestive tract was convoluted and hardly any yolk remained. The spinous processes on the finfolds became sharpened and numerous. The gas-bladder was well developed. Melanophores became less dense dorsally and a row of melanophores appeared on the ventral edge of the trunk and tail. Melanophores appeared on the lower jaw. The melanophore saddles on the mid-dorsal and mid-ventral tail in the previous stage were no longer present.

Six-day-old postlarva, 2.23 mm TL and 2.10 mm NL (Fig. 1D): The melanophores disappeared from both the dorsal and ventral finfolds and from the ventral edge of the notochord. A row of melanophores was formed along the lateral mid-line. Melanophores located on the head in the previous satge were found disappeared in this stage exept on the top of the head.

Postlarva, 2.87 mm TL and 2.73 mm NL (Fig. 1E): The pelvic fin buds were formed. The olfactory lobe became apparent. Melanophores increased in number on the body, especially on the top of the head, the posterior half of the trunk and the anterior half of the tail. The melanophores were dense along the lateral mid-line and the ventral edges of the trunk and tail. Melanophores appeared on the base of the pectoral fins and on the side of the head.

Postlarva, 3.70 mm TL and 3.56 mm NL (Fig. 1F): The caudal fin rays were under formation. Two to three melanophores appeared on the basal portion of the branchiostegal membrane.

Postlarva, 4.32 mm TL and 4.15 mm NL (Fig. 1G): The notochord started to flex. The 1st-spines and 2nd dorsal, anal and pelvic fins were under

formation. An indentation was formed on the opercle at the upper corner. Abdominal melanophores increased in number, becoming smaller in size ventrally.

Juvenile, 5.6 mm TL and 4.5 mm in standard length (SL) (Fig. 1H): All fin rays were formed. The head started to become depressed in shape and the eyes were shifting dorsally. Transition to a demersal life style was observed at this stage. Tiny melanophores appeared dorsally on the body.

Juvenile, 6.3 mm TL and 5.2 mm SL (Fig. 1I, J): The indentation on the opercle becoming more apparent at the upper corner. The preopercular projections were formed, protruding horizontally. The tiny melanophores increased in density dorsally on the body. In contrast, melanophores became less dense on the ventral side, especially on the abdomen. One melanophore appeared on each pelvic fin.

Juvenile, 11.2 mm TL and 9.0 mm SL (Fig. 1K, L): The head was well depressed with the eyes protruding above the dorsal head profile. The gill opening started to close from the isthmus upward and from the indentation downward. On the preopercular projection, an antrorse spine was formed and the formation of the third upwardly directed spine was started by bifurcation of the projection tip. Dorso-cephalic lateral lines appeared. The fish completed the transition to a demersal life style, and acquired the adult body form. The melanophores on the dorsal body extended to the ventral side, forming several vertical bands. Abdominal melanophores disappeared in this stage. Melanophores appeared on the 1st and 2nd dorsal and caudal fins.

Juvenile, 15.8 mm TL and 12.9 mm SL (Fig. 1M): Gill openings closed, leaving a pore-like gill opening where the indentation had been formed at the larval stage. Five upwardly directed spines appeared on the preopercular projection. A downward branch of the infraorbital canal was formed on the cephalic lateral line pattern, and the pattern became close to that in the adult. Several vertical melanophores bands formed on the side of the body, contrasting strongly with the whitish ventral surface. On the branchiostegal membrane, melanophores disappeared in this stage. Four to six melanophores were present on each pelvic fin.

Larval and juvenile development of *Repomucenus valenciennei*

Newly-hatched prolarvae (Fig. 2A), 1.11±0.03 mm TL and 1.07±0.03 mm NL: The condition of the yolk, the location of the anus, the absence of pigmentation in the eyes, and the incomplete formation of the mouth and heart were the same as in newly-hatched *Repomucenus richardsonii*. Myomere counts were 8–9+11–12. No differences were found between *R. valenciennei* and *R. richardsonii*, either in the melanophore patterns distributed dorsally on the head to the tail, on the yolk sac or on the ventral finfold, or in the xanthophore patterns that were densely distributed laterally on the trunk and over the entire yolk sac.

One-day-old prolarva, 1.73 mm TL and 1.64 mm NL (Fig. 2B): The formations of the pectoral fin buds and the spinous processes on the edges of the dorsal and ventral finfolds were seen. A small vacuole on the dorsal finfold was found near the trunk, (not illustrated). A heart beat was observed prior to the fixing specimens. The doubled dorsal tissue was present. Melanophores were scattered over the trunk and tail with a saddle of dense pigment on the mid-dorsal and mid-ventral tail. Melanophores appeared on the digestive tract. Melanophores also were obseved in sporadic clusters in the dorsal and ventral finfolds. Near the end of the notochord, melanophores were located on both dorsal and ventral edges. Melanophores were also present on the side of the head.

Three-day-old prolarva, 1.84 mm TL and 1.73 mm NL (Fig. 2C): The mouth opened and the eyes become pigmented. The digestive tract was convoluted and the gas-bladder was well developed. The spinous processes on the finfolds became sharpened and numerous. Melanophores became less dense dorsally, and a row of melanophores appeared on the ventral edge of the trunk and tail. Melanophores also appeared on the lower jaw. The melanophores on the mid-dorsal and mid-ventral tail disappeared.

Five-day-old postlarva, 2.13 mm and 2.00 mm NL (Fig. 2D): The yolk was completely absorbed. The melanophores disappeared from the dorsal finfold but remained in two rows on the ventral finfold. The melanophores also disappeared dorsally from the notochord, but not ventrally. A row of melanophores was formed along the lateral mid-line. Melanophores located on the head in the previous satge were found disappeared in this stage exept on the top of the head.

Postlarva, 2.72 mm TL and 2.59 mm NL (Fig. 2E): The pelvic fin buds were formed and the olfactory lobe became apparent. Melanophores

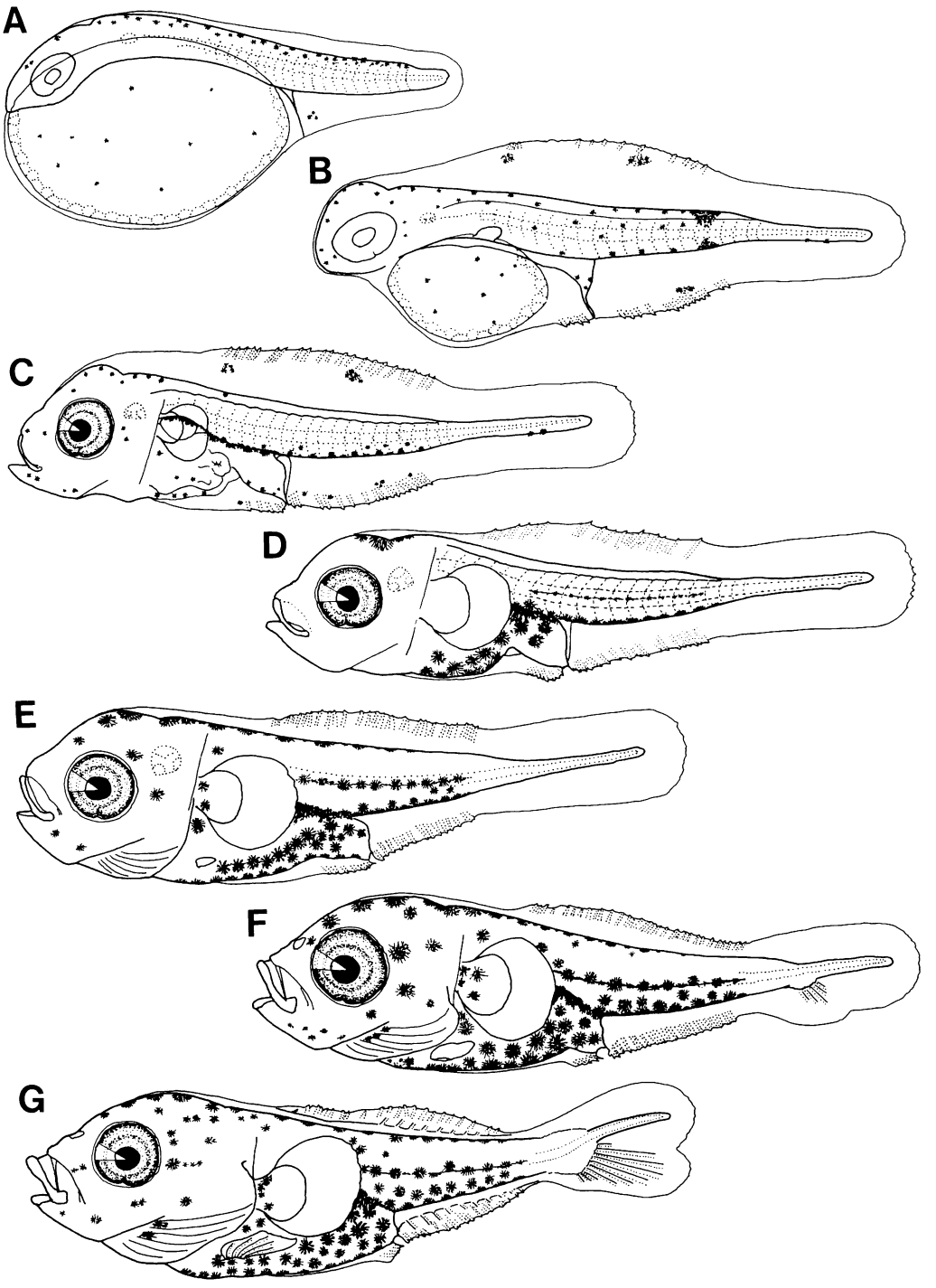

Fig. 1

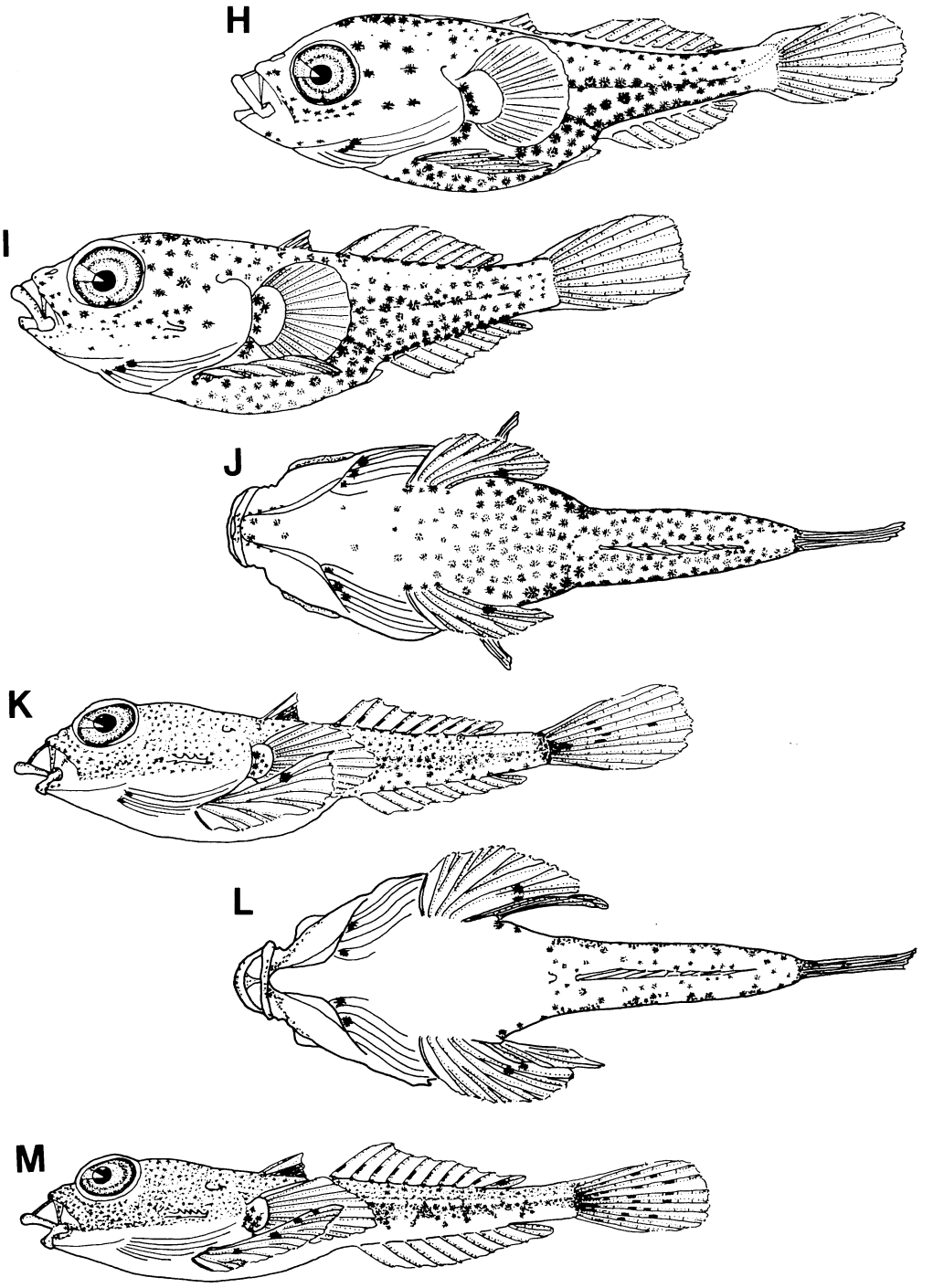

Fig. 1. Larvae and juveniles of *Repomucenus richardsonii*. A) Newly hatched prolarva, 1.18 mm TL; B) 1-day-old, 1.97 mm TL; C) 3-day-old, 2.11 mm TL; D) 6-day-old, 2.23 mm TL; E) 12-day-old, 2.87 mm TL; F) 16-day-old, 3.70 mm TL; G) 18-day-old, 4.32 mm TL; H) 20-day-old, 5.6 mm TL; I) 22-day-old, 6.3 mm TL; J) ventral view of I; K) 27-day-old, 11.2 mm TL; L) ventral view of K; M) 35-day-old, 15.8 mm TL.

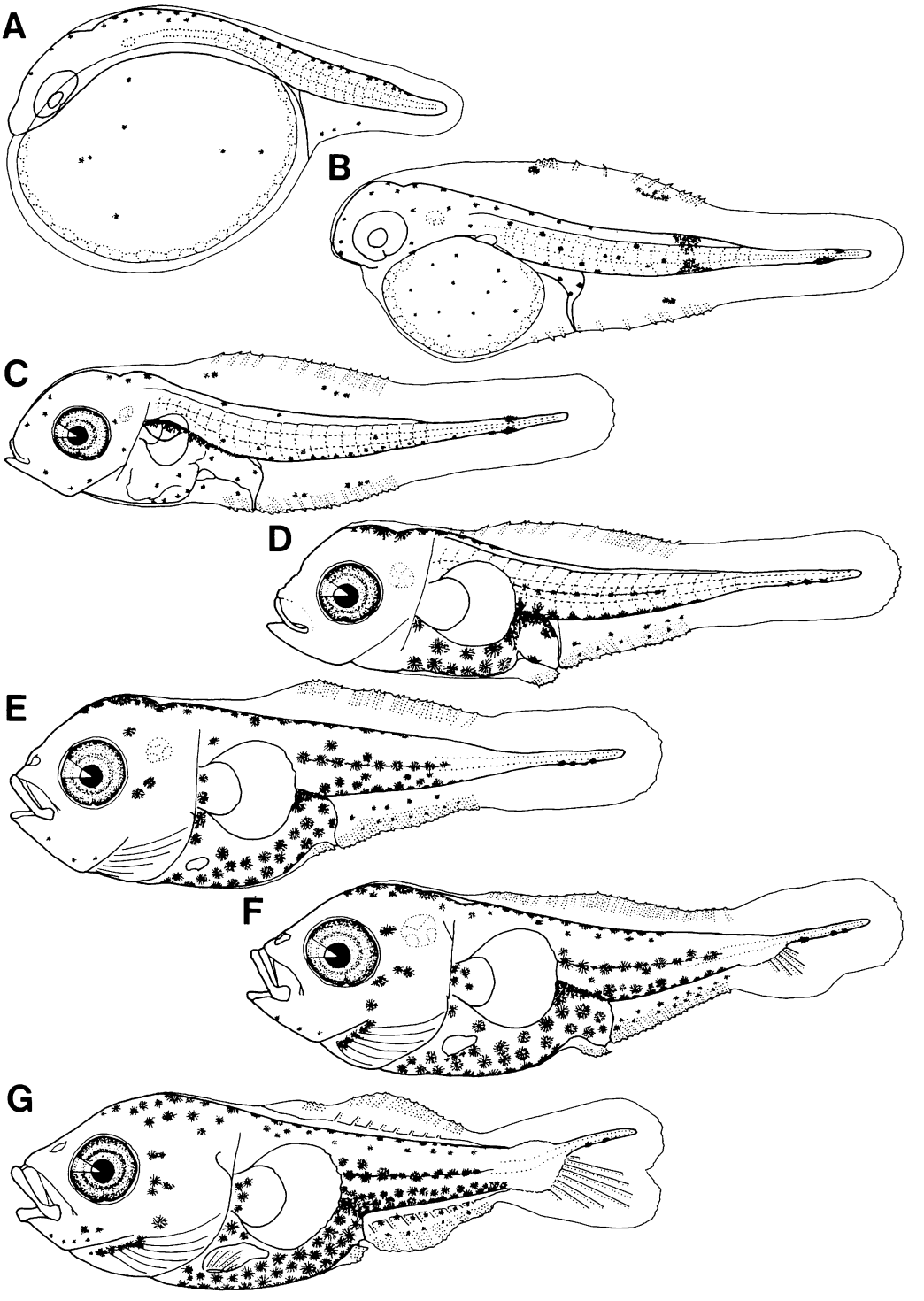

Fig. 2

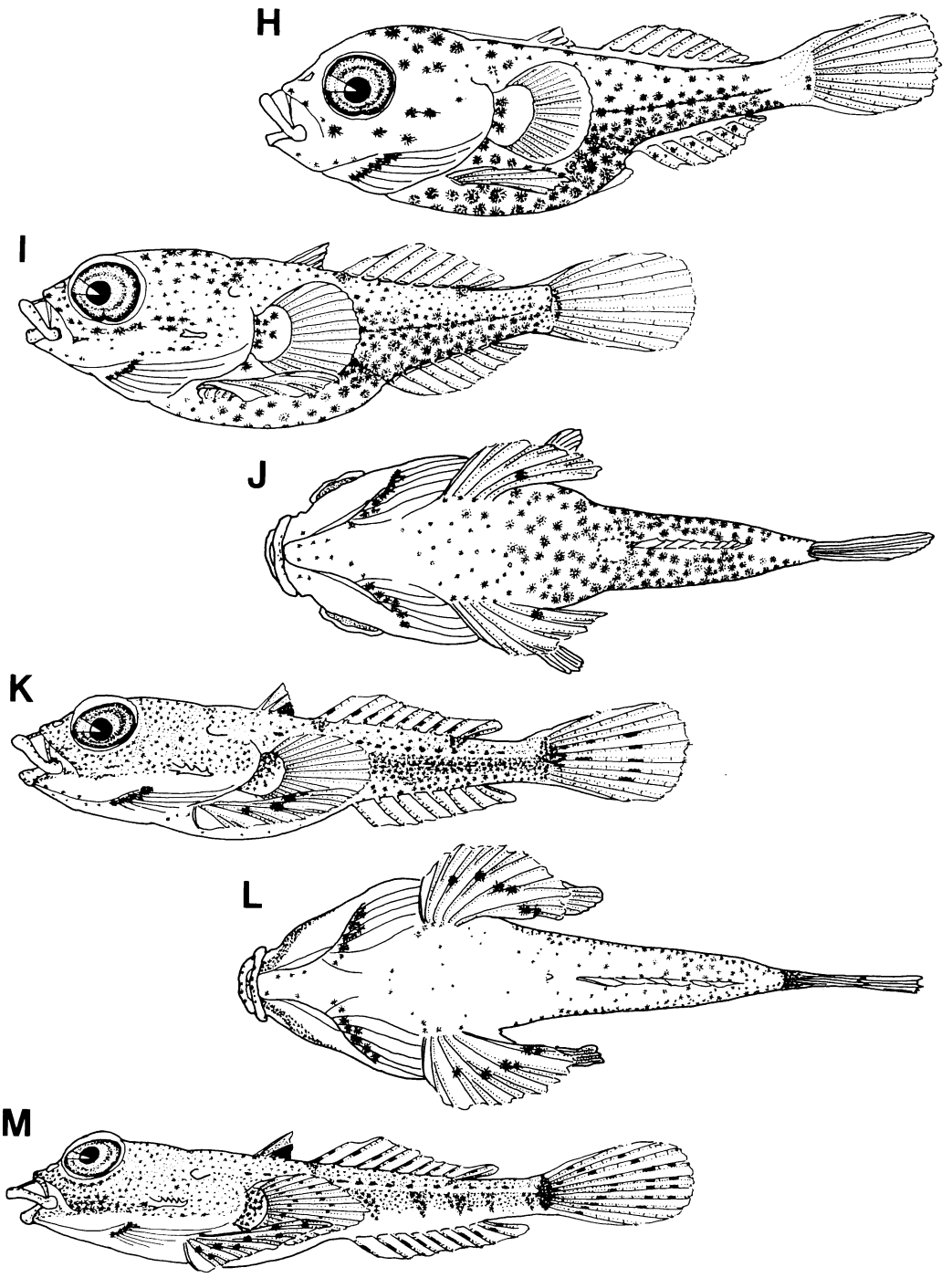

Fig. 2. Larvae and juveniles of *Repomucenus valenciennei*. A) Newly hatched prolarva, 1.12 mm TL; B) 1-day-old, 1.73 mm TL; C) 3-day-old, 1.84 mm TL; D) 5-day-old, 2.13 mm TL; E) 10-day-old, 2.72 mm TL; F) 14-day-old, 3.56 mm TL; G) 16-day-old, 4.20 mm TL; H) 18-day-old, 5.4 mm TL; I) 20-day-old, 6.4 mm TL; J) ventral view of I; K) 25-day-old, 10.2 mm TL; L) ventral view of K; M) 34-day-old, 15.4 mm TL.

increased dorsally on the head, the posterior half of the trunk and the anterior half of the tail. Melanophores also appeared on the base of the pectoral fins and on the side of the head.

Postlarva, 3.56 mm TL and 3.42 mm NL (Fig. 2F): The caudal fin rays were under formation. Five to six melanophores appeared on the basal portion of the branchiostegal membrane.

Postlarva, 4.20 mm TL and 4.03 mm NL (Fig. 2G): The notochord started to flex. The 2nd dorsal, anal and pelvic fin rays were under formation. The indentation was formed on the opercle at the upper corner. Abdominal melanophores increased in number, becoming smaller in size on the ventral side.

Juvenile, 5.4 mm TL and 4.6 mm SL (Fig. 2H): All fin rays were formed. The head starts to become depressed and the eyes begin to move dorsally, accompanied by the transition to a demersal life style. The melanophore row along the lateral mid-line of the body became less dense. The tiny melanophores appeared dorsally on the body. Six to eight melanophores were present on the branchiostegal membrane.

Juvenile, 6.4 mm TL and 5.0 mm SL (Fig. 2I, J): The indentation on the opercle became more apparent. The horizontal preopercular projections were formed. An increase of the tiny melanophores on the dorsal body and the gradual reduction of pigment on the ventral side of the abdomen occurred at this stage. One melanophore appeared on each pelvic fin.

Juvenile, 10.2 mm TL and 7.9 mm SL (Fig. 2K, L): The body was close to that of the adult form, having the well-depressed head and the eyes extending above the top of the head. The gill opening started to close from the isthmus upward and from the indentation downward. On the preopercular projection, an antrorse spine and the third upwardly directed spine were formed. Dorso-cephalic lateral lines appeared. The transition to a demersal life style was completed. The melanophores that had originated dorsally on the body extended to the ventral side. Melanophores appeared on the 1st and 2nd dorsal fins and on the caudal fin, and those on the pelvic fins increased in number to 5 or 6. Melanophores were densely formed in a row just above the lateral line. This melanophores row was not found in *R. richardsonii*.

Juvenile, 15.4 mm TL and 12.0 mm SL (Fig. 2M): The pore-like gill opening was complete and five upwardly directed spines on the preopercular projection were formed. The cephalic lateral line pattern had three downward branches on the infraorbital canal, being close to that in the adult. The melanophore bands were formed on the whitish ventral surface of the body side. Melanophores appeared on the pectoral and anal fins, and 8 to 10 melanophores were on each pelvic fin.

Discussion

Newly-hatched prolarvae of dragonet species are very poorly developed (Takita, 1980, 1983; Eda et al., 1994). They have no pigmentation in the eyes, and the mouth and the heart are still under formation. The heart beat has been reported to begin a few hours prior to hatching in *Repomucenus beniteguri* (Kashiwagi et al., 1993). However, we observed no

Table 1. Comparison of the melanophore pattern in the postlarval and juvenile stages of three dragonet species in different size ranges. Species are compared either by the presence (+) or absence (−) of melanophores, or by the number of melanophores

	Range in TL (mm)	*R. richardsonii*	*R. valenciennei*	*R. beniteguri*
Ventral edge near the end of the tail	ca. 2.2–4.5	−	+	−
Dorsal finfold	ca. 2.2–4.5	−	−	+
Ventral finfold	ca. 2.2–4.5	−	+	+
Branchiostegal membrane	ca. 3.5–4.5	2–3	5–6	3–4*
	ca. 5.5–12	2–4	6–8	4–6*
	ca. 15–17	0	6–8	0*
Pelvic fin	ca. 6.0–6.5	1	1	1*
	ca. 10–12	2–3	5–6	3–4*
	ca. 15–17	4–6	8–10	5–7*
2nd dorsal fin	ca. 10–12	+	+	−
Above the lateral line	ca. 10–17	−	+	−

*H. Eda, unpublished data.

heart beat either in embryos or in newly hatched prolarvae in either dragonet species, as reported by Takita (1980, 1983) and Eda et al. (1994).

It has been reported that *R. valenciennei* has melanophores in the prolarval stage on both dorsal and ventral edges of the notochord, while *R. richardsonii* has pigment only on the ventral edge (Takita, 1980). This difference was reconfirmed in the present study, although some individual variations were found.

Postlarval and juvenile morphologies of *R. richardsonii* and *R. valenciennei* were not only similar to each other but they were also similar to those of *R. beniteguri* described by Eda et al. (1994). Although some differences were observed in the melanophore pattern between species (Table 1), because melanophore development is affected by growing conditions, further study with natural specimens is needed to confirm whether the differences in the melanophore patterns observed in the larval and juvenile stages can distinguish these species.

In the postlarval stage, the melanophores on the ventral edge of the notochord, the branchiostegal membrane and the ventral finfold in *R. valenciennei* may distinguish it from *R. richardsonii*. *R. richardsonii* and *R. valenciennei* may be distinguished from *R. beniteguri* which have some melanophores on the dorsal finfold.

In the juvenile stage, the melanophores on the branchiostegal membrane, the pelvic fins and just above the lateral line in *R. valenciennei* may distinguish it from *R. richardsonii*. The absence of melanophores of the 2nd dorsal fin of *R. beniteguri* may distinguish this species from the other two.

The cephalic lateral lines are one of the keys used to define the species of the genus *Repomucenus* (Nakabo, 1983). They were observed to develop dorsally in ca. 10–11 mm TL juveniles. From the ca. 16 mm TL stage, the infraorbital canal pattern might be used as a key to define species. The canals gradually extend downward with growth, forming a single downward branch in *R. richardsonii*, and three downward branches in *R. valenciennei*. A downward branch was also observed in juveniles of *R. beniteguri* (H. Eda, unpublished data).

The formation of the pore-like gill opening that is characteristic of dragonets has been reported in *R. beniteguri* (Eda et al., 1994). We observed no difference in this process in either *R. richardsonii* or *R. valenciennei*.

The development of preopercular spines in dragonets has been described in *Callionymus lyra*, *C. maculatus* and *C. reticulatus* by Demir (1972) and in *R. beniteguri* by Eda et al. (1994). The process of preopercular spine formation was similar in *C. maculatus*, *R. beniteguri*, *R. richardsonii* and *R. valenciennei*.

In conclusion, *R. valenciennei* differs to some extent from *R. richardsonii* and *R. beniteguri* only in pigmentation and cephalic lateral line formation, but in these respects, the latter two species are very similar to each other. In Omura Bay where we are conducting fish egg and larva surveys, *R. richardsonii* and *R. valenciennei* are dominant, and thus, most of the collected dragonet larvae may be these two species, which are distinguishable. *R. beniteguri* is restricted to small areas in Omura Bay, and thus, its occurrence might be negligible.

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ネズミゴチとハタタテヌメリの仔稚魚

枝　浩樹・田北　徹・宇野ゆか子

ネズミゴチとハタタテヌメリを水槽内で産卵させ，仔稚魚の観察を行った．ふ化直後の仔魚の全長は，ネズミゴチでは 1.17±0.04 mm，ハタタテヌメリでは 1.11±0.03 mm であった．2 種ともに，筋節数は 8–9＋11–12 で，楕円形の大きな卵黄をもつ．ふ化後の発育過程に 2 種の相違はほとんどみられず，卵黄はふ化後 5–6 日，全長約 2.2 mm で吸収された．鰭条数は全長 5.5 mm 前後で定数に達し，底生生活への移行は 10–11 mm で完了した．後期仔魚では脊索末端と膜鰭上の黒色素胞の有無及び鰓膜上の黒色素胞の数が，稚魚では鰓膜と腹鰭上の黒色素胞の数，側線背側の黒色素胞列の有無及び眼下管の分枝が種の識別点となりうる．

（枝：〒852 長崎市文教町 1–14　長崎大学海洋生産科学研究科；田北：同住所　長崎大学水産学部；宇野：〒857 佐世保市広田 4–5–9　西部環境（株））