

Embryonic, Larval and Juvenile Development in Laboratory-reared Dragonets, *Repomucenus beniteguri*

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Abstract *Repomucenus beniteguri* eggs were obtained from spawners reared in a tank, and the larvae raised to the juvenile stage. The eggs measured 0.64–0.72 mm in diameter and shared characteristics in common with those of congeneric species. Hatching took place over 19 hours at 23.3–23.6°C. The newly-hatched prolarvae measured 1.18 ± 0.026 mm in total length (TL) and had a large oval yolk sac. Spinous processes and vacuoles had appeared on the dorsal and ventral finfolds in a 1.92 mm, one-day-old prolarva. Yolk absorption was completed in a 2.39 mm, 6-day-old postlarva. Notochord flexion had started in a 4.42 mm postlarva and was completed in a 5.7 mm juvenile. Initial transition to a demersal habit was observed in a 5.7 mm juvenile and completed in a 12.8 mm juvenile. Relative growth rates changed at ca. 2 mm, 4.5–6.5 mm and 11–13 mm TL, dividing the early developmental stages into 4 phases: prolarval, postlarval, juvenile and adult. Some differences in previously reported embryonic and larval development in this species were found.

Dragonets are common in the sandy-muddy coastal waters of Japan, their eggs and larvae appearing abundantly in plankton samples from coastal areas (Mito, 1965; Takita, 1980, 1983). However, little is known about their eggs and young stages. The eggs and prolarvae of four dragonet species, *Repomucenus valenciennesi*, *R. richardsonii*, *R. ornatipinnis* and *Paradiplogrammus enneactis calliste* have been described from tank-spawned specimens (Takita, 1980, 1983). However, as they were not raised beyond the prolarval stage, their postlarval and juvenile stages were not described.

The dragonet, *Repomucenus beniteguri* (Jordan et Snyder), is endemic to Japan (Nakabo, 1983), being common in the sandy, shallow waters of southern Japan (Nakabo, 1984). Larval and juvenile stages were described by Takai and Yoshioka (1979), who did not clearly indicate the origin of their specimens. A subsequent personal communication from the above authors, indicated that specimens were raised from eggs collected from the sea. Because *R. beniteguri* spawns in the same season with two other sympatric dragonet species, *R. richardsonii* and *R. valenciennesi*, it is highly probable that the eggs of the three species occur together. However, Takai and Yoshioka (1979) did not provide any concrete infor-

mation regarding the identification of the eggs used in their study. Larvae and juveniles of other species so far known from Japanese waters have also been described from specimens collected from the sea without paying strict attention to species identification (Kamiya, 1916; Mito, 1962). In order to elucidate the egg and young stages of *R. beniteguri*, eggs were obtained from adult fish in a tank and observations made on the larvae and juveniles subsequently raised.

Materials and Methods

Mature *R. beniteguri* were caught in October 1992 by hook and line at Kazusa, Nagasaki Prefecture (32°27'N, 130°10'E), which faces Chijiwa Bay. The fish were kept in a square concrete water tank, 4.8 × 6.8 m in area and 1.2 m in depth, at the Fisheries Experimental Station, Nagasaki University, Nomo, Nagasaki Prefecture.

Eggs released at the water surface just behind the mating fish were collected with a small dip net immediately following spawning behavior. The eggs were incubated in a 100 l polycarbonate tank supplied with slow running seawater and constant aeration. For

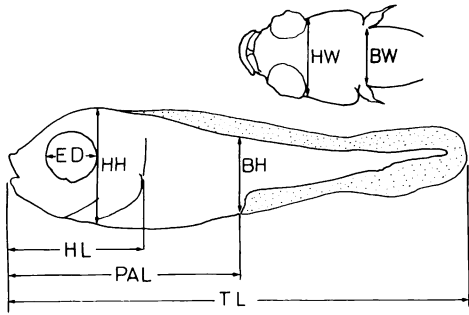


Fig. 1. Illustration of body part measurements. TL—total length; HL—head length; PAL—pre-anal length; ED—eye diameter; HW—head width; BW—body width; HH—head height; BH—body height.

observations of embryonic development, some eggs were kept in 1 l glass beakers at a water temperature of $23.5 \pm 0.1^\circ\text{C}$.

Newly-hatched larvae were maintained at $20.8 \pm 1.4^\circ\text{C}$ in a tank with running seawater and constant aeration. Larvae were initially fed with small S-type rotifers, the food subsequently being altered with fish growth to unselected S-type rotifers, *Artemia* nauplii, and minced krill.

Samples of 20 individuals were taken from the rearing tank at 4 hour intervals during the first 2 days after hatching, at 12 hour intervals from day 3 to day 5, and every 2 or 3 days thereafter until the end of rearing. Samples were anesthetized with tricaine methanesulfonate (MS-222) and fixed with 5% formalin for observation, or 2.5% glutaraldehyde for measuring, the latter treatment minimizing distortion and shrinkage of specimens (Oozeki and Hirano, 1988). Eight measurements of body parts were made on each glutaraldehyde-fixed specimen: total length (TL), head length (HL), pre-anal length (PAL), eye diameter (ED), head height (HH), body height (BH), head width (HW) and body width (BW) (Fig. 1).

Results

Egg and embryonic development

The fertilized egg of *Repemucenus beniteguri* has identical characteristics to those of other callionymids known to date, being buoyant, spherical and colorless, and having a narrow perivitelline space.

They also have a partially segmented yolk, a hexagonal pattern on the chorion and lack an oil globule (Kamiya, 1916; Mito, 1962; Russell, 1976; Takai and Yoshioka, 1979; Takita, 1980, 1983). The eggs measured 0.64–0.72 mm in diameter with an average of 0.67 mm. The cross axes of the hexagonal pattern on the chorion ranged in size from 0.011 to 0.022 mm with an average of 0.017 mm.

Embryonic development with time elapsed is shown in Table 1 and the developing egg in Figure 2. The segmented yolk granules were located only near the embryo in the early developmental stages, but moved during the epiboly of the blastoderm and were located under the yolk surface after blastopore closure. Melanophores which appeared on the embryonic body were scattered on the dorsal surface. Xanthophores were located on the lateral surface of the embryonic body and the entire yolk sac.

Larval and juvenile development

Newly-hatched prolarvae (Fig. 3A), 1.18 ± 0.026 mm TL, 1.15 ± 0.024 mm in notochord length (NL): The larvae had a large oval yolk sac measuring 0.77–0.81 mm along the major axis with an average of 0.79 mm. The anterior tip of the yolk sac extended beyond the anteriormost part of the head. The anus was located just behind the yolk sac. The number of myomeres was 8–9+11–12. The mouth was closed and pigmentation was lacking on the eyes. A heart beat was not apparent.

One-day-old prolarva, 1.92 mm TL and 1.81 mm NL (Fig. 3B): The pectoral fin buds had formed, along with spinous processes on the edges of the dorsal and ventral finfolds, forming processions. Small vacuoles were located on the dorsal and ventral finfolds between the spinous processes and the body. A heart beat was apparent.

Three-day-old prolarva, 2.06 mm TL and 1.92 mm NL (Fig. 3C): The mouth had opened and the eyes were highly pigmented. The digestive tract was convoluted and the air-bladder well-developed. Little yolk remained. The larvae started to eat rotifers 80 h after hatching.

Six-day-old postlarva, 2.39 mm TL and 2.27 mm NL (Fig. 3D): The spinous processes on the finfolds had increased in number and become sharp. The yolk had been completely absorbed.

Postlarva, 3.16 mm TL and 3.02 mm NL (Fig. 3E): Pelvic fin buds had formed and the olfactory lobes were apparent.

Postlarva, 3.85 mm TL and 3.71 mm NL (Fig. 3F): Caudal fin rays had formed.

Postlarva, 4.42 mm TL and 4.24 mm NL (Fig. 3G): Notochord flexion had started. The pelvic fin rays had formed and eight caudal rays were apparent. An indentation had formed the upper corner of the opercle.

Juvenile, 5.7 mm TL and 4.8 mm in standard length (SL) (Fig. 3H): Notochord flexion was completed and the full complement of fin rays had formed. The head had started to become depressed in shape, with the eyes shifted dorsally. A few spinous processes remained on the dorsal finfold, but none ventrally. Transition to a demersal habit was observed at this stage.

Juvenile, 6.6 mm TL and 5.3 mm SL (Fig. 3I): The indentation on the upper corner of the opercle had become more apparent, but the gill opening was widely gaping. Preopercular projections had formed, protruding horizontally. The finfold between the 1st and 2nd dorsal fins had disappeared.

Juvenile, 12.8 mm TL and 10.2 mm SL (Fig. 3J): The head had become well depressed, the dorsalmost part being below the upper part of the eye. The gill opening had started to close. On the preopercular projection, an antrorse spine and three upwardly directed spines had formed. The adult body form acquired, the fish had completed its transition to a demersal habit.

Juvenile, 17.3 mm TL and 13.8 mm SL (Fig. 3K): The gill opening had closed, leaving a pore-like gill opening at the position of the initial formation of the indentation in the larval stage. Five upwardly

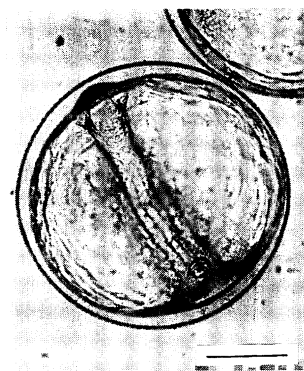


Fig. 2. Developing egg of *Repomucenus beniteguri*, 11 h 30 min after spawning. Bar indicates 0.2 mm.

directed spines were present on the preopercular projection.

Pigmentation

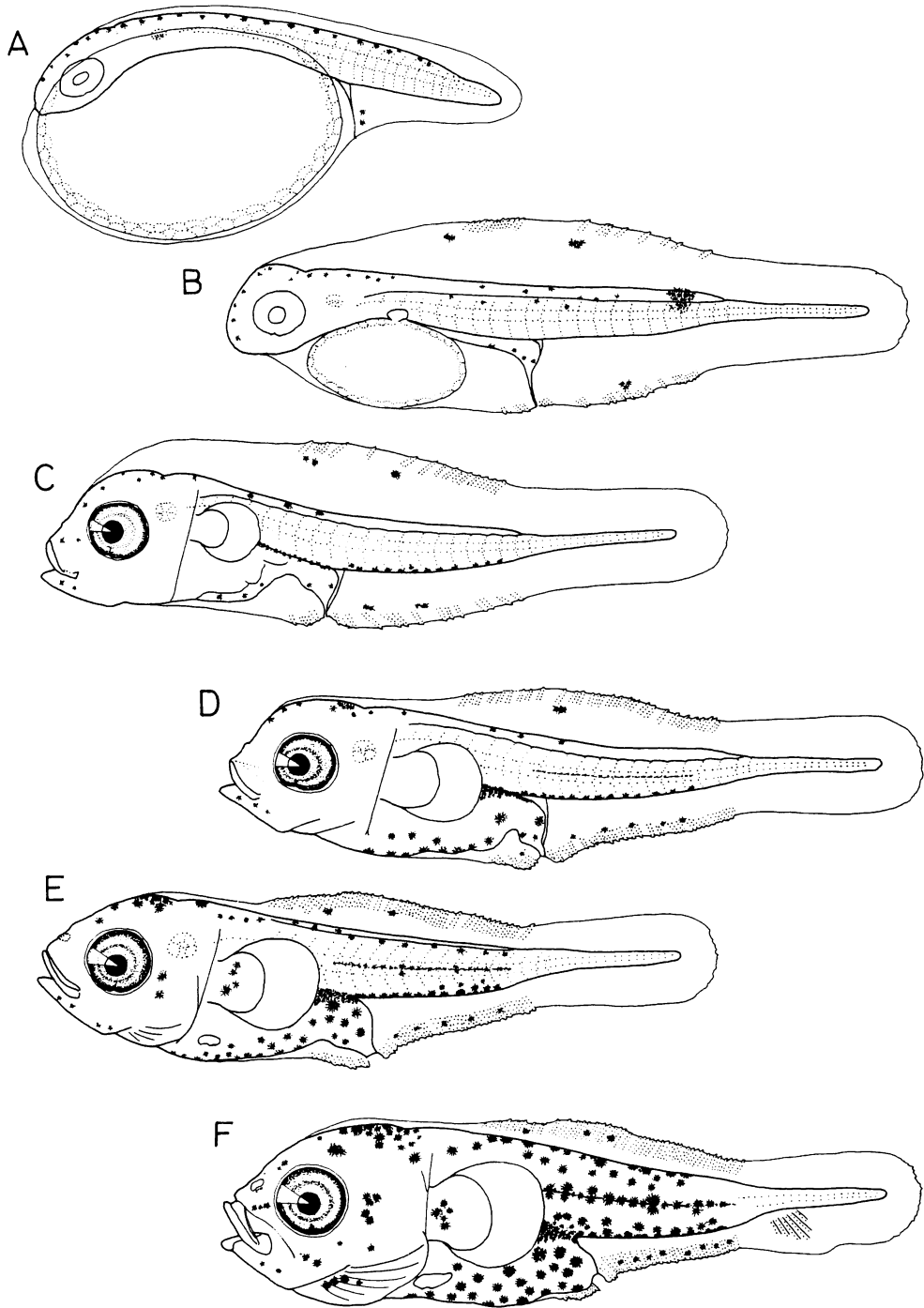
Newly-hatched prolarvae had a row of dorsal melanophores from the head to the middle of the tail (Fig. 3A). Some remained dorsally on the body, but others shifted ventrally, being located laterally in 1-day-old prolarvae (Fig. 3B) and ventrally in 3-day-old prolarvae (Fig. 3C). In 1-day-old prolarvae, some melanophores appeared on the digestive tract and were densely assembled mid-dorsally on the tail (Fig.3B), but the latter disappeared in 3-day-old prolarvae (Fig.3C).

A row of melanophores was formed along the

Table 1. Embryonic development of *Repomucenus beniteguri* at a water temperature of $23.5 \pm 0.1^\circ\text{C}$

Time elapsed from spawning*	Developmental stages observed
30 min	Two cell stage
1 h 55 min	Early morula stage
4 h 50 min	Early gastrula stage
8 h 15 min	Beginning of embryonic body formation. Germ ring equal to half yolk diameter in lateral view
10 h 15 min	Optic vesicle formation
10 h 25 min	Formation of Kupffer's vesicle and two or three myomeres
11 h 10 min	Appearance of xanthophores on embryo and yolk sac
11 h 20 min	Closure of blastopore
11 h 50 min	Appearance of melanophores on embryo
14 h 55 min	Differentiation of tail
15 h 20 min	Optic lens and otocyst formation
16 h 50 min	Disappearance of Kupffer's vesicle
19 h 00 min	Hatching

* The eggs were spawned at 6:18 p.m. on October 14, 1992.



Early Development of *Repomucenus beniteguri*

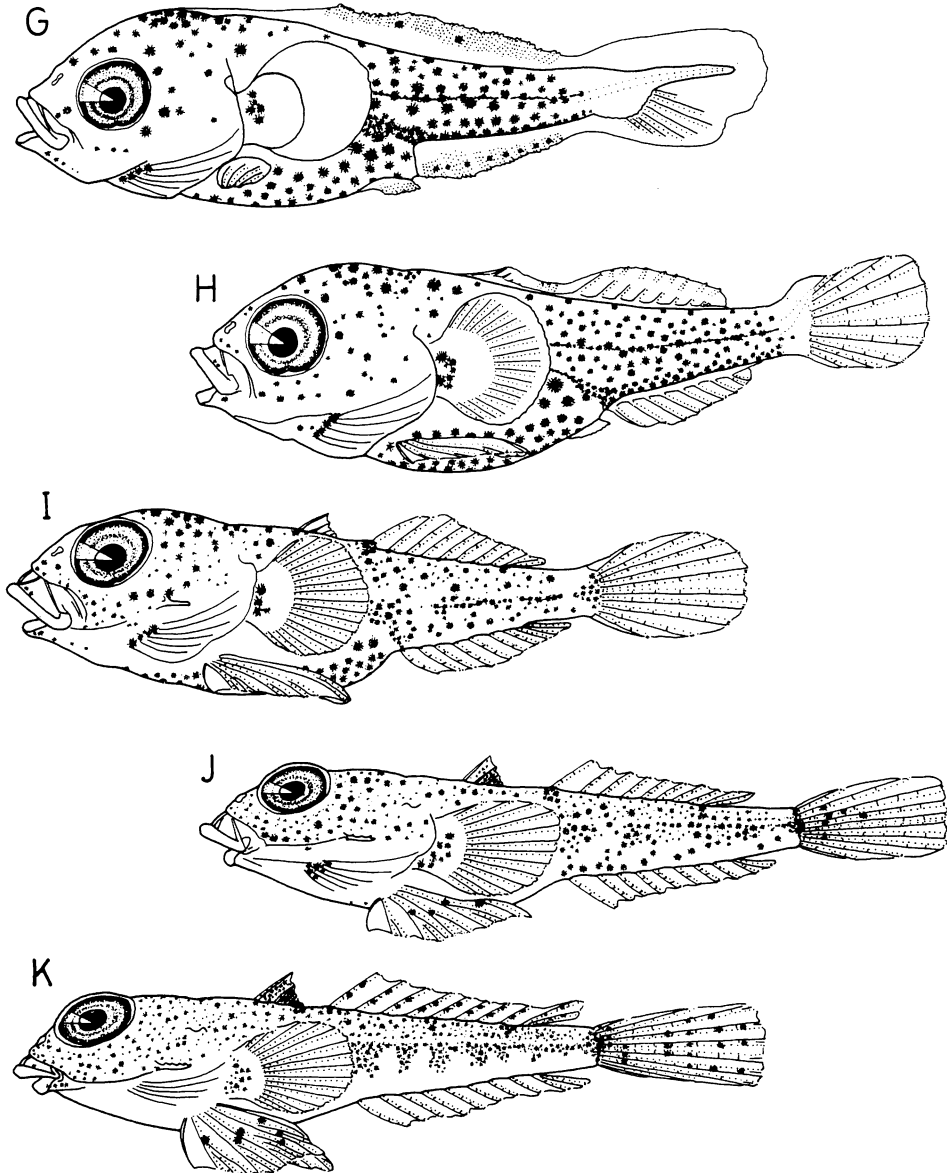


Fig. 3. Larvae and juveniles of *Repomucenus beniteguri*. A) Newly-hatched prolarva, 1.16 mm TL; B) one-day-old, 1.92 mm; C) three-day-old, 2.06 mm; D) six-day-old, 2.39 mm; E) 10-day-old, 3.16 mm; F) 16-day-old, 3.85 mm; G) 18-day-old, 4.42 mm; H) 22-day-old, 5.7 mm; I) 25-day-old, 6.6 mm; J) 32-day-old, 12.8 mm; K) 40-day-old, 17.3 mm. Vacuoles on the dorsal and ventral finfolds are eliminated.

lateral mid-line in 6-day-old postlarvae (Fig. 3D). Melanophores gradually increased thereafter on the body, especially on the top of the head, the posterior half of the trunk and the anterior half of the tail. They were dense along the lateral mid-line and ventral edges of the trunk and tail in the 3.85 to 5.7 mm TL larvae. Melanophores were absent on the poste-

rior half of the tail of larvae, but had appeared on the base of the pectoral fins in the 3.16 mm TL postlarva.

On the ventral finfold, minute melanophores were located just behind the anus in newly-hatched prolarvae (Fig. 3A). In 1 to 3-day-old prolarvae, a few clusters of melanophores were located sporadically on the dorsal and ventral finfolds (Fig. 3B). On the

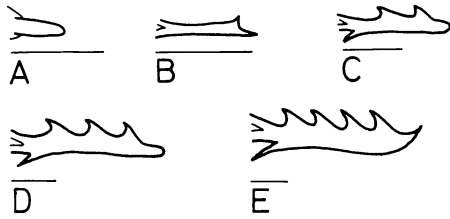


Fig. 4. Formation process of the left preopercular projection of *Repomucenus beniteguri*. A) 5.9 mm TL; B) 7.2 mm; C) 9.0 mm; D) 12.8 mm; E) 17.3 mm. Bars indicate 0.2 mm.

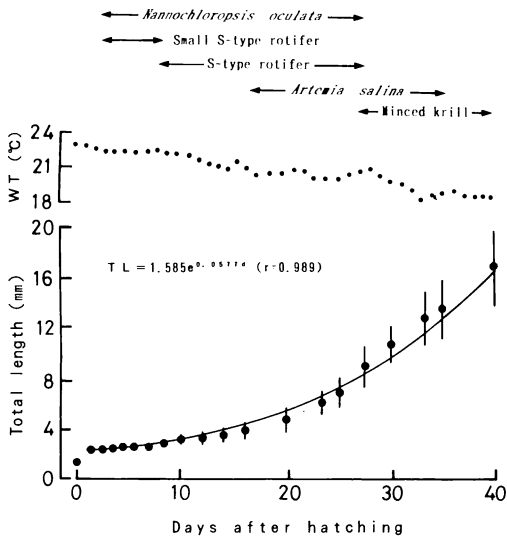


Fig. 5. Growth of *Repomucenus beniteguri* larvae and juveniles with water temperature and diet scheme. Closed circles and bars represent averages from 20 individuals and standard deviations, respectively.

ventral finfold, melanophores increased in number with growth, forming a row (Fig. 3D–G), but disappeared in the juvenile stage (Fig. 3H).

From the 4.42 mm TL stage (Fig. 3G), the number of melanophores on the abdomen increased with growth, becoming smaller in size ventrally. The melanophores had started to decrease in number and become less conspicuous on the ventral side, in the 6.6 mm TL juvenile (Fig. 3I), and had disappeared in the 12.8 mm TL juvenile (Fig. 3J).

The melanophore row along the lateral mid-line of the body also became less conspicuous with growth in the juvenile stage (Fig. 3I). From the 6.6 mm TL stage (Fig. 3I), tiny melanophores appeared dorsally on the body, becoming dense with growth, whereas

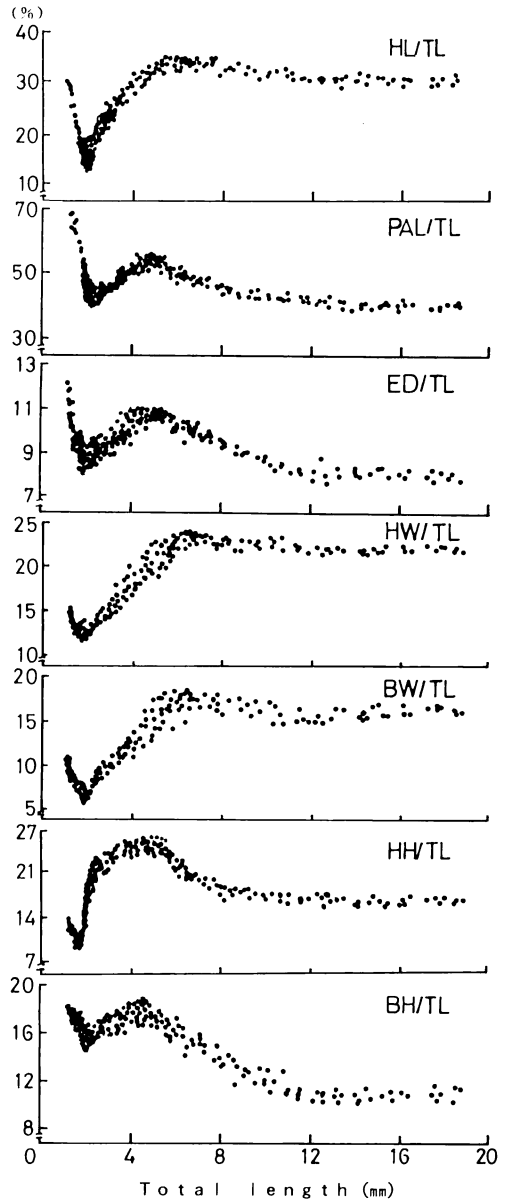


Fig. 6. Changes in body proportions with growth. Abbreviations as in Figure 1.

the ventral body melanophores decreased. Subsequently, the dorsal melanophores grew more dense, extending ventrally in five vertical bands, which contrasted strongly with the whitish ventral surface (Fig. 3K).

Melanophores had appeared on the 1st dorsal, pelvic and caudal fins in the 12.8 mm TL juvenile (Fig. 3J), and on the 2nd dorsal fin in the 17.3 mm

TL juvenile (Fig. 3K).

Newly-hatched live prolarvae bore dense xanthophores laterally on the trunk and on the entire yolk sac, but they became inconspicuous around 5 days after hatching. Because most observations were made on fixed specimens, subsequent details regarding xanthophores were not recorded.

Characteristics of dragonet morphology

The indentation on the opercle in postlarvae (Fig. 3G) was the start of the formation of the pore-like gill opening characteristic of dragonets. Membranes were formed gradually, extending upward from the isthmus and downward from the indentation in juveniles about 11–13 mm TL (Fig. 3J), finally leaving a pore-like opening. The latter had been completed in the 17.3 mm TL juvenile (Fig. 3K).

The formation process of the preopercular projec-

tion is shown in Figure 4. The projection was first apparent in a 5.9 mm TL juvenile as a horizontally protruded process (Fig. 4A). An upwardly directed spine had started to form following the bifurcation of the projection tip in a 7.2 mm TL juvenile (Fig. 4B), with an antrorse spine being apparent in a 9.0 mm TL juvenile (Fig. 4C). The upwardly directed spines increased in number with growth (Fig. 4D), which had acquired the same number in the 17.3 mm TL juvenile (Fig. 4E) as that in the adult, but the form was still different from adult condition.

Growth and relative growth

Changes in total length are shown in Figure 5, along with changes in water temperature and diet scheme during the rearing experiment. Larval growth was initially slow, although the rate later increased, the growth curve being expressed by the

Table 2. Summary of regression parameters ($P=aTL+b$) for proportional changes and correlation coefficients (r) in *Repomucenus beniteguri*. Abbreviations as in Figure 1

Proportion (P%)	Range in TL (mm)	Parameters		r
		a	b	
HL/TL	1.19–1.99	–15.298	43.870	0.920
HL/TL	1.99–5.51	1.985	9.947	0.840
HL/TL	5.51–11.6	–0.342	34.555	0.570
HL/TL	11.6–19.2	–0.020	30.295	0.102
PAL/TL	1.19–2.03	–25.938	95.552	0.977
PAL/TL	2.03–4.92	5.151	32.046	0.920
PAL/TL	4.92–12.6	–1.172	56.913	0.906
PAL/TL	12.6–19.2	–0.076	43.344	0.294
ED/TL	1.19–2.01	–2.749	13.984	0.857
ED/TL	2.01–4.92	6.893	0.840	0.828
ED/TL	4.92–11.7	–0.367	12.543	0.960
ED/TL	11.7–19.2	0.002	8.349	0.020
HW/TL	1.19–1.92	–4.555	20.230	0.857
HW/TL	1.92–5.86	2.321	8.461	0.951
HW/TL	5.89–19.2	–0.046	22.749	0.229
BW/TL	1.19–1.94	–5.465	16.528	0.910
BW/TL	1.94–5.40	2.145	3.533	0.912
BW/TL	5.40–10.8	–0.351	19.634	0.662
BW/TL	10.8–19.2	–0.030	16.905	0.125
HH/TL	1.19–1.81	–4.645	17.914	0.804
HH/TL	1.81–2.13	29.594	–41.899	0.863
HH/TL	2.13–4.55	2.260	15.199	0.863
HH/TL	4.55–8.04	–2.023	34.136	0.925
HH/TL	8.04–11.4	–0.381	21.130	0.694
HH/TL	11.4–19.2	–0.060	17.756	0.465
BH/TL	1.19–2.07	–3.027	21.928	0.605
BH/TL	2.07–4.92	1.254	12.898	0.594
BH/TL	4.92–11.7	–0.794	20.194	0.859
BH/TL	11.7–19.2	0.123	8.770	0.365

following equation:

$$TL = 1.585 e^{0.0577d} \quad (r = 0.989),$$

where TL is total length (mm) and d is the number of days after hatching. Throughout the rearing period, no cannibalism was observed.

Changes in body proportions with growth against total length are shown in Figure 6, being approximated by $P = aTL + b$, where P is the percentage of each body part length (mm) in the total length (TL, mm). Parameters a and b are given in Table 2. Inflections in body proportion changes occurred at ca. 2 mm, 4.5–6.5 mm and 11–13 mm TL, corresponding to morphological transitions to the post-larval stage, juvenile stage and adult form, respectively.

Discussion

Dragonet eggs are distinguishable from other pelagic teleost eggs by the hexagonal pattern on the chorion, partially segmented yolk and absence of an oil globule. However, these features are similar among dragonet species, making specific identifications of eggs difficult (Mito, 1962). Eggs of *Repomucenus valencienni* are reported to differ from those of two other Japanese dragonet species, *R. richardsonii* and *R. ornatipinnis*, in having a larger hexagonal pattern (Takita, 1980). The eggs of *R. beniteguri* not only have a similarly-sized hexagonal pattern to those of the latter two species, but also to those of another Japanese dragonet, *Paradiplogrammus enneactis calliste*, described by Takita (1983). *R. beniteguri* occurs sympatrically with *R. richardsonii*, the two species spawning concurrently. As yet, their eggs are not able to be distinguished.

According to the descriptions of embryonic development in *R. beniteguri* by Takai and Yoshioka (1979), the embryonic body, Kupffer's vesicle and eye vesicles are formed in this species after blastopore closure. However, the present study revealed that these were formed before blastopore closure, as described by Mito (1962) and Takita (1980, 1983) in other species. Although Kashiwagi et al. (1993) recorded the onset of heart beat a few hours prior to hatching of *R. beniteguri* eggs, such was observed neither in embryos nor in newly-hatched prolarvae during the present study, in accordance with reports for other species (Takita, 1980, 1983).

Some differences in larval development from the descriptions of Takai and Yoshioka (1979) were also found. Newly-hatched prolarvae in their study lacked melanophores on the ventral finfold, contrary to the present finding. Nor did they find melanophores ventrally on the tail until the larvae had attained 10-days of age (2.7 mm TL), whereas such were present in 3-day-old prolarva (2.06 mm TL) in the present study. Other differences included the formation of pectoral fin buds and spinous finfold processes 5 days after hatching (present study—observed in 1-day-old prolarva, 1.92 mm TL) and complete absorption of yolk 10 days after hatching (present study—6 days). Moreover, no description was given of the finfold vacuoles. Although the larval development might have been influenced by temperature, Takai and Yoshioka (1979) gave no detailed description of the rearing conditions employed. Because their rearing experiments took place in September (present study—October), marked differences in egg and larval development between the two studies are unlikely. It is very likely that specimens used in the rearing experiment by Takai and Yoshioka (1979) contained numerous dragonet species, rather than just *R. beniteguri*.

Some specific differences in dragonet larvae have been found in the distribution of melanophores on the posterior end of the tail (Takita, 1980). *R. beniteguri* did not have melanophores as in *R. ornatipinnis*. No melanophores were found in *Paradiplogrammus enneactis calliste* either (Takita, 1983). These three species may be distinguished from *R. valencienni* and *R. richardsonii* which have some melanophores at the posterior end of the tail. This study failed to find characters differentiating between the larvae of *R. beniteguri* and *R. ornatipinnis*. The dorsal melanophore pattern and stuffed melanophores along the ventral finfold edge distinguish *Paradiplogrammus enneactis calliste* larvae (Takita, 1983) from *R. beniteguri*.

The larva and juvenile of unidentified dragonet species were reported from Japanese waters by Mito (1966). The lack of melanophores on the larval finfolds and dense melanophores on the ventral body of the juvenile, as described by Mito were not seen in *R. beniteguri*, although pigmentation may have been influenced by rearing conditions.

Demir (1972) described the development of preopercular spines in three dragonet species, *Callionymus lyra*, *C. maculatus* and *C. reticulatus*. The shape of the preopercular spine of *R. beniteguri* is closest to

that of *C. maculatus*. The process of spine formation, which is initiated by the bifurcation of the projection tip, being almost the same in the four species.

Dragonet larvae hatch with an undeveloped appearance, the morphological transition from that stage being reflected in the considerable changes in body proportions during the 1st growth phase. In the postlarval stage, the head and trunk portions are initially conspicuous. Subsequently, the body becomes depressed until the fish reaches the juvenile stage. The 2nd growth phase reflects the morphological transition to the juvenile stage. The gradual changes of the 3rd growth phase stabilise when the fish takes on a demersal habit, the attainment of the adult body form being consistent with the behavioral shift.

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トビヌメリの卵発生と仔稚魚

枝 浩樹・藤原隆典・田北 徹

トビヌメリを水槽内で産卵させ、卵、卵内発生と仔稚魚の観察を行った。卵は直径 0.64-0.72 mm で、その形態はこれまでに知られている同属の卵によく似ている。水温 23.3-23.6°C で受精後約 19 時間でふ化した。ふ化直後の仔魚は平均全長 1.18±0.026 mm で、楕円形の大きな卵黄をもつ。ふ化後 1 日目の仔魚は背・腹側膜鱗上に泡状組織と棘状突起を持つことが特徴的である。卵黄はふ化 6 日後に完全に吸収された。脊索の屈曲は全長 4.42 mm で開始し、5.7 mm で完了した。底生生活への移行は 5.7 mm の稚魚で観察され、12.8 mm で完了した。相対成長は 2 mm, 4.5-6.5 mm と 11-13 mm に大きく変化する。すなわち、各々の変化は仔魚後期、稚魚期に達する時期および成魚の体型へ移行する時期にあたる。本種仔稚魚の形態として報告された既往の記載は海から採集した卵の飼育によるもので、本研究の結果とかなり食い違っており、卵を誤同定している可能性が考えられた。

(枝: 〒852 長崎市文教町 1-14 長崎大学海洋生産科学研究科; 藤原・田北: 同住所 長崎大学水産学部)