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Embryonic and Morphological Development of Larval and Juvenile Coral Trout, *Plectropomus leopardus*

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Abstract Embryonic and morphological development of larva and juvenile coral trout, *Plectropomus leopardus*, are described using specimens raised at Yaeyama Station (Ishigaki Island, Okinawa Pref.), Japan Sea Farming Association, from eggs spawned voluntarily by captive brood fish (14 females, 3 males). The eggs were pelagic and spherical, 0.82–0.93 mm in diameter, and possessed single oil droplet and a narrow perivitelline space. Hatching took place 26 h 40 min after spawning, at a temperature of 25.4–26.3°C. The newly-hatched larvae were 1.62 mm in mean total length (TL) with 27 (9+18) myotomes and had an oil droplet in the posterior part of the yolk. Two days after hatching (2.70 mm TL) the mouth opened. Five days after hatching (2.96 mm TL), the pelvic fin spines and 2nd dorsal fin spine emerged. At 4.70 mm TL, a spine formed at the angle of the preoperculum. At 6.10 mm TL, the notochord was slightly flexed, and the hypural bones and caudal fin rays had begun to develop. At 11.8 mm TL the adult complement of spines and soft rays was attained, except in the anal fin. At 18.7 mm TL, the 1st anal fin soft ray had begun to transform into the 3rd anal spine. All fins had the adult complement of rays and spines. By 25.1 mm TL, the body had become red, with some fish sleeping on the bottom at night. By 35.0 mm TL, the fish had become completely demersal and when startled, sought shelter immediately. Two inflections in relative growth were found, at about 7 and 22 mm BL. The first inflection coincided with the development of larval behavior, in which an active movement and feeding on *Artemia* nauplii occurred. The second inflection occurred at transformation, as the larval habitat shifted from the surface and middle layers to the tank bottom.

Fishes of the genus *Plectropomus* are important in commercial and sport fisheries. The coral trout, *Plectropomus leopardus*, is distributed southward from southern Japan and is an important commercial species in Okinawa Prefecture, where it is commonly referred to as Akajin. Kudo et al. (1984) and Goeden (1978) reported on the ecology of mature fish. The early life history of *P. leopardus* is poorly known, there being only a single description of larval development of specimens collected in Australian waters (Leis, 1986).

At the Japan Sea Farming Association, Yaeyama Station, coral trout broodstock have provided fertilized eggs by spontaneous spawning since May 1988. Their spawning and spawning behavior at Yaeyama Station have been described by Teruya et al. (1992).

In this article, the embryonic and morphological development, behavior, and growth of the coral trout from the egg to the juvenile stages are described.

Materials and Methods

Broodstock and egg collection

Adult *Plectropomus leopardus* were collected by fishing around Ishigaki Island (24° 50′N, 124° 20′E) during 1986 and 1987 and kept in an outdoor tank (10×10×2 m). In 1988, fertilized eggs were collected from brood fish comprising 14 females (40.4–60.1 cm fork length [FL]) and three males (59.3–63.3 cm FL) (Teruya et al., 1992).

Observations of embryonic development were made on eggs spawned spontaneously on May 6, 1988, at 18:50. Fertilized eggs were immediately collected using a plankton net to filter overflow water. The eggs were then transferred to a 500 ml

beaker containing filtered seawater. The water temperature was controlled within 23.1–23.7°C.

Larvae and early juveniles

Fertilized eggs collected on May 15, 1991, were transferred to a 60,000*l* indoor tank, and the resulting larvae utilized. The water temperature in the tank was not controlled. The diet sequence was: S-type rotifer (from first feeding), *Artemia* nauplius (from 5 mm TL larvae), *Artemia* cultured to 1–3 mm TL (from 15 mm TL larvae) and commercial diet (from 25 mm TL larvae).

Fish drawings were made with the aid of a camera lucida, using 5–10 individuals sampled every 2–10 days from hatching to 65 days after hatching (1.62–35.0 mm TL). One individual from each sample was chosen for illustration. Larvae and juveniles were described and measured after being anesthetized with MS 222. Measurements of morphometric characters were made to the nearest 0.01 mm, using a profile projector and ruler.

For relative growth assessments, 9–40 individuals were sampled daily until 15 days after hatching and then every 2–13 days (total 325 indiv., 2.42–36.0 mm BL). Total length (TL) and preanal length (AL) were measured from the snout tip to the posterior margin of the caudal fin and anus, respectively. Body length (BL) was measured from the snout tip to the notochord tip until full development of the caudal fin, thereafter to the posterior margin of the hypural plate. Body height (BH) was taken as the vertical distance from the dorsal to ventral body margins at the pectoral fin base. Wet body weights (BW) were also recorded.

During 5–152 days after hatching, 227 specimens (2.5–105 mm BL) were sampled and fixed in 5–10% sea water-buffered formalin. They were subsequently cleared and stained following the method of Dingerkus and Uhler (1977), in order to facilitate observations on spination, serration, and squamation.

Table 1. Embryonic development of *Plectropomus leopardus*

Developmental stage	Time after spawning (hrs : mins)
Spawning	00 : 00
One-celled ovum	00 : 25
Two-celled ovum	00 : 45
Four-celled ovum	01 : 00
Eight-celled ovum	01 : 20
Sixteen-celled ovum	01 : 40
Thirty-two-celled ovum	02 : 10
Early morula	02 : 30
Late morula	03 : 00
Early blastula	03 : 30
Middle blastula	05 : 00
Late blastula	06 : 20
Early gastrula	07 : 30
Late gastrula	08 : 30
Appearance of embryo	10 : 10
Formation of optic vesicles	11 : 30
Appearance of Kupffer's vesicle	12 : 30
Closure of blastopore	14 : 20
Formation of auditory vesicles	16 : 00
Disappearance of Kupffer's vesicle	17 : 10
Beginning of heart beat	22 : 30
Hatching	26 : 40

Results

Development of eggs

The development of *Plectropomus leopardus* embryonic stages from fertilization to hatching, are outlined in Table 1.

The eggs were pelagic and spherical, 0.82–0.93 mm in diameter, and possessed single oil droplet and a narrow perivitelline space (Fig. 1A).

Twenty five min after fertilization, a blastodisc developed. The first cleavage occurred after 45 min (Fig. 1B). The early morula stage was reached after 2 h 30 min (Fig. 1C). After 3 h 30 min, five layers of cells were found at the side of the blastoderm and the serrated marginal periblast appeared coincidentally (Fig. 1D). The late blastula stage occurred at 6 h 20 min (Fig. 1E) and epiboly in the blasotoderm, at 7 h 30 min (Fig. 1F). By 8 h 30 min, the blastoderm had attained one third of the longitudinal diameter of the whole egg. The thickened part of the blastoderm bulged from the surface of the egg and the embryonic shield was visible (Fig. 1G). At 10 h 10 min, the embryo appeared, at which point the blastoderm had attained one half of the longitudinal diameter of the whole egg (Fig. 1H). By 10 h 50 min, two somites had formed, the blastoderm covering three-fourths of the longitudinal diameter of the whole egg (Fig. 1I). By 11 h 30 min, optic vesicles had formed and three somites developed. By 12 h 30 min, Kupffer's vesicle had formed and 4 somites appeared. The blastopore had closed and 8 somites were observed at

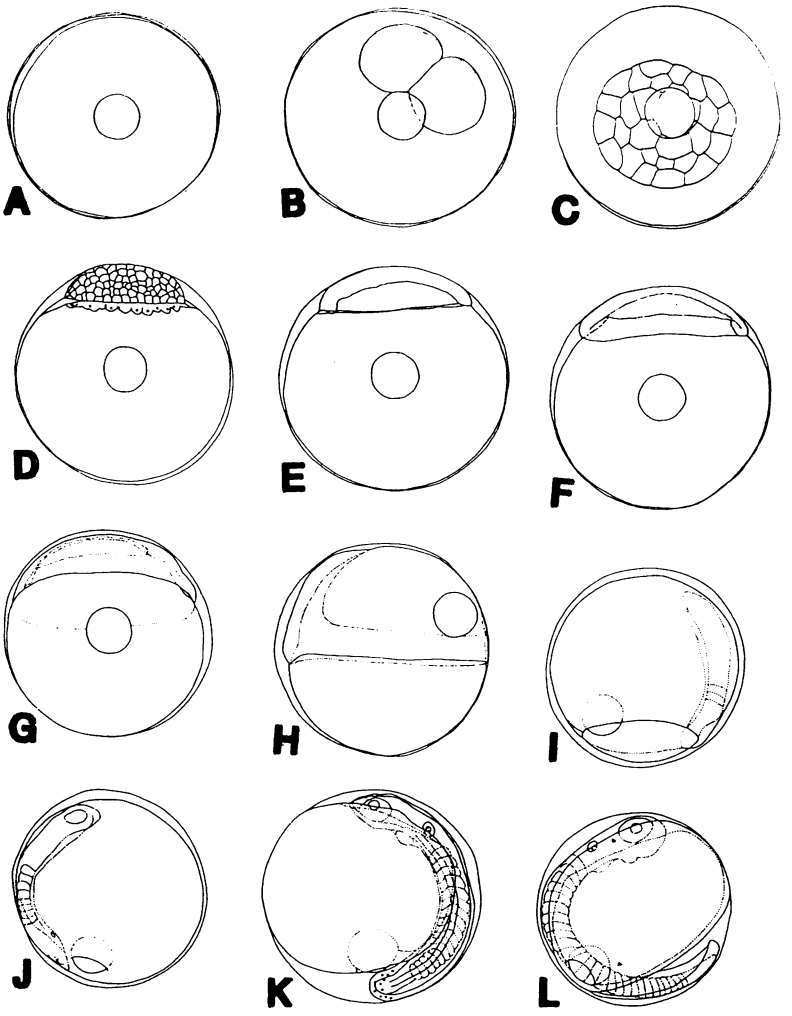


Fig. 1. Embryonic development of *Plectropomus leopardus*. A) Fertilized ovum, 6 min after spawning; B) two-celled ovum, 45 min; C) early morula, 2 hr 30 min; D) early blastula, 3 h 30 min; E) late blastula, 6 h 20 min; F) early gastrula, 7 h 30 min; G) late gastrula, 8 h 30 min; H) formation of embryo, 10 h 10 min; I) appearance of somites, 10 h 50 min; J) closure of blastopore, 14 h 20 min; K) beginning of motility, 23 h 20 min; L) before hatching, 25 h 30 min.

14 h 20 min (Fig. 1J). Auditory vesicles and 11 to 12 somites were observed at 16 h. Kupffer's vesicle had disappeared and 12 to 13 somites formed at 17 h 10 min. By 23 h 20 min, the tail had elongated and separated from the yolk at the root, and the embryo began to move (Fig. 1K). The tail subsequently became further elongated and the embryo moved more actively (Fig. 1L). A few melanophores appeared on the head. After 26 h 40 min, the embryo began to hatch, the majority having done so by 27 h.

Larval rearing

Two days after hatching (dah), the mouth opened, and the larvae began to feed on rotifers. Survival at this time was 98%, rapidly decreasing to 39% after 6 days. The average size of 12 day-old larvae that were fed *Artemia* nauplii, was 5.5±0.97 mm TL, their survival from hatching being 18%. By 55 dah larvae had reached 22.7±2.37 mm TL, with a survival rate of 3.2%. Cannibalism was seen frequently after the

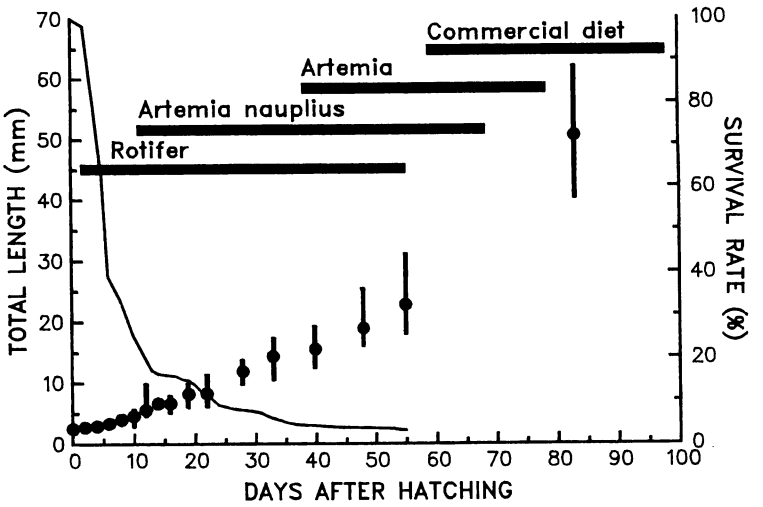


Fig. 2. Growth (as TL), survival rate, and successive diets during rearing of *Plectropomus leopardus*. Vertical lines indicate ranges.

fish became completely demersal at around 65 dah. Growth, survival rate, and diet sequence are described in Figure 2.

Average total length of the coral trout larvae was 22.7 mm (range 18.1–31.0 mm BL) at 55 dah (Fig. 2). Daily growth rates were calculated to average 0.38 mm/day, with a maximum of 0.53 mm/day. The average growth rate from 55 to 83 dah was 1.00 mm/day.

Morphology of larvae and juveniles

The newly-hatched larva was 1.62 mm TL with 27 (9+18) myotomes (Fig. 3A). The elliptical yolk projected anterior to the head. The distance from the tip of the yolk to the end of the larval membrane was 1.80 mm. An oil droplet was situated posteriorly in the yolk. The anus was situated slightly posterior to the middle of the body. Melanophores were found on the trunk and yolk.

12 h after hatching (2.22 mm TL; Fig. 3B): The number of melanophores on the trunk had increased in comparison with the newly-hatched larvae.

30 h after hatching (2.65 mm TL; Fig. 3C): Melanophores were distributed on the head and the yolk surface. The anlagen of the pectoral fins had appeared.

2 dah (2.70 mm TL; Fig. 3D): The mouth and anus had opened. The yolk and oil droplet were almost completely absorbed. The eyes were completely pigmented and the pectoral fins had developed. Melanophores had developed along the dorsal side of the abdominal cavity while those along the ventral tail had increased 17. The anlage of the 2nd spine of the dorsal fin had emerged in the larval membrane.

5 dah (2.96 mm TL; Fig. 3E): The pelvic fin spines and 2nd spine of the dorsal fin had emerged and begun to elongate, melanophores and erythrophores occurring on the tips of the former.

7 dah (3.22 mm TL; Fig. 3F): Pelvic and 2nd dorsal fin spine lengths had reached 0.72 and 0.60 mm, respectively. A spinelet had developed on the apex ridge of the pelvic spine. No spinelet was visible on the 2nd dorsal spine. Nostrils had appeared and teeth had begun to develop on the upper jaw. The number of ventral melanophores on the tail had decreased rapidly to 7. The 2nd dorsal and pelvic fin spines both bore melanophores.

10 dah (4.70 mm TL; Fig. 3G): Pelvic and 2nd dorsal fin spine lengths had reached 1.84 and 1.92 mm, respectively. The spines were triangular in cross-section, with serrations beginning to develop on each ridge. Melanophores and erythrophores were spread over the spines, erythrophores also being scattered on the abdomen. A spine had formed at the angle of the preoperculum.

13 dah (6.10 mm TL; Fig. 3H): The tip of the 2nd dorsal fin spine reached beyond the end of the tail. The posterolateral wings of the 2nd dorsal spine had

enlarged and recurved, and bore a small number of spinelets, the apex ridge of the spine also bearing a number of small spinelets. The pelvic fin spines were armed with three differently sized spinelets on a triangular pyramid. The apex ridge of the spine had the smallest and greatest number of spinelets compared to the others. The dorsomedial ridge had the largest and least number of spinelets. The 1st and 3 rd dorsal fin spines had appeared, and the notochord was slightly flexed. Hypural bones and the soft rays of the caudal fin had begun to develop. Pectoral fin soft rays were not apparent. Seven melanophores occurred on the tail. Teeth were present on the upper jaw, but not on the lower jaw. The posttemporal spine had emerged and the supraocular ridge bore spinelets.

18 dah (7.30 mm TL; Fig. 3I): Each fin had begun to develop and the 2nd anal spine had emerged. The fin membrane had begun to recede ventrally and dorsally in the caudal peduncle region. The preoperculum spines numbered four including the angle spine. The supracleithral spine had appeared, along with teeth on the lower jaw. Melanophores had spread to the occipital region and the top of the head. With the development of each fin, the larvae showed active movement and had begun to feed actively on *Artemia* nauplii. When larvae were drifting in water, they spread the 2nd dorsal and pelvic fin spines, but when moving quickly, the fin spines were recessed.

28 dah (11.8 mm TL; Fig. 3J): The 2nd dorsal and pelvic fin spines had become more elongated. The fin membrane had receded completely at the caudal peduncle. The overall adult number of spines and soft rays was complete, but the 3rd anal spine had still to transform from a ray. A small spine had emerged before the 1st anal spine (see below). The 1st to 8th spines of the dorsal fin were serrated. The number of preopercular spines had increased to 5, including the largest angle spine. The angle spine of the preoperculum was armed with small spinelets. The supraocular ridge had two spine points and weak serrations. The three edges of the supracleithral spine were armed with spinelets.

48 dah (18.7 mm TL; Fig. 3K): In some specimens the entire body had become a light red color, but the majority of larvae remained translucent. The 2nd dorsal and pelvic spine lengths had decreased (2.7 and 2.8 mm, respectively). The 1st to 3rd dorsal, pelvic, and 2nd anal fin spines were still serrated. The 1st anal soft ray, which had been unsegmented, had begun to transform into the 3rd anal spine, thus completing the adult complement of rays and spines for all fins. In some specimens examined, the small spine previously noted anterior to the 1st anal spine was absent. The nostrils had become separated into two pores. Squamation had begun from around the supracleithral spine.

55 dah (25.1 mm TL; Fig. 3L): The body had become red, and serrations on the dorsal, pelvic, and anal fin spines reduced. The squamated area of the body extended along the midline of the trunk from the posterior of the operculum to the caudal peduncle. Except for the ventral abdomen, melanophores were spread over the whole body. In daylight, the fish inhabited all depths of the tank. At night, some fish slept on the bottom.

65 dah (35.0 mm TL; Fig. 3M): The wet body weight was 0.56 mg. Melanophores on the caudal fin were in two lines. The fish had become completely demersal and when startled, sought shelter immediately.

Relative growth

Total length, body height, preanal length, and body weight in relation to body length were calculated (Figs. 4, 5). Inflections in all four proportional measurements occurred at about 7 and 22 mm BL. Relative growth was determined from the following regression equations:

$$TL = 1.10 \times BL - 0.129 \quad (r^2 = 0.994,\ n = 122)$$
$$BL < 7\ \text{mm}$$
$$TL = 1.23 \times BL - 0.195 \quad (r^2 = 0.996,\ n = 146)$$
$$7 \leqq BL < 22\ \text{mm}$$
$$TL = 1.07 \times BL + 3.34 \quad (r^2 = 0.960,\ n = 57)$$
$$BL \geqq 22\ \text{mm}$$
$$AL = 0.498 \times BL - 0.187 \quad (r^2 = 0.979,\ n = 122)$$
$$BL < 7\ \text{mm}$$
$$AL = 0.666 \times BL - 0.657 \quad (r^2 = 0.987,\ n = 145)$$
$$7 \leqq BL < 22\ \text{mm}$$
$$AL = 0.541 \times BL + 2.19 \quad (r^2 = 0.883,\ n = 57)$$
$$BL \geqq 22\ \text{mm}$$
$$BH = 0.257 \times BL - 0.104 \quad (r^2 = 0.946,\ n = 122)$$
$$BL < 7\ \text{mm}$$
$$BH = 0.304 \times BL - 0.146 \quad (r^2 = 0.975,\ n = 145)$$
$$7 \leqq BL < 22\ \text{mm}$$
$$BH = 0.261 \times BL + 0.647 \quad (r^2 = 0.902,\ n = 57)$$
$$BL \geqq 22\ \text{mm}$$
$$BW = 0.00338 BL^{3.65} \quad (r^2 = 0.954,\ n = 116)$$
$$BL < 7\ \text{mm}$$
$$BW = 0.00684 BL^{3.35} \quad (r^2 = 0.986,\ n = 145)$$

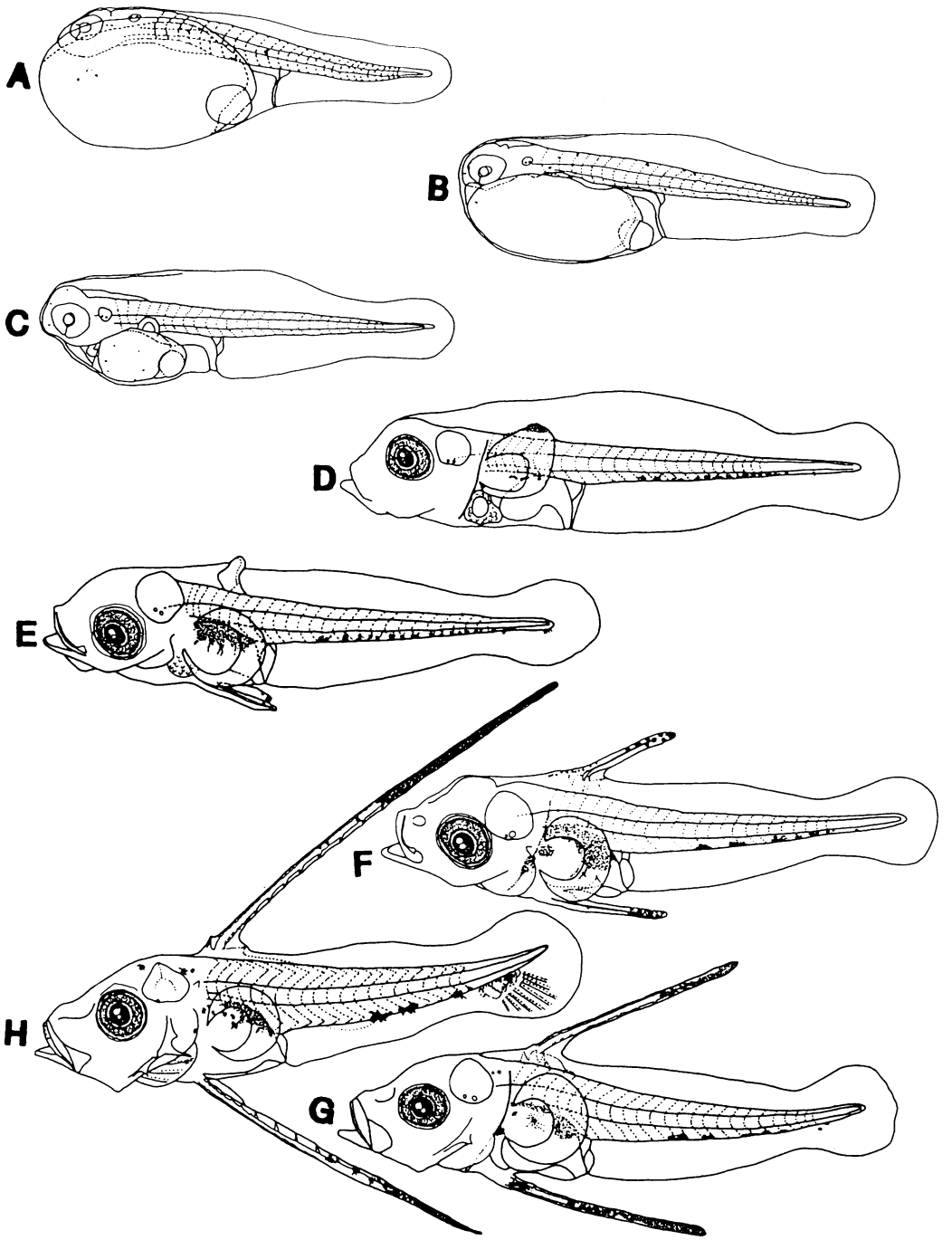
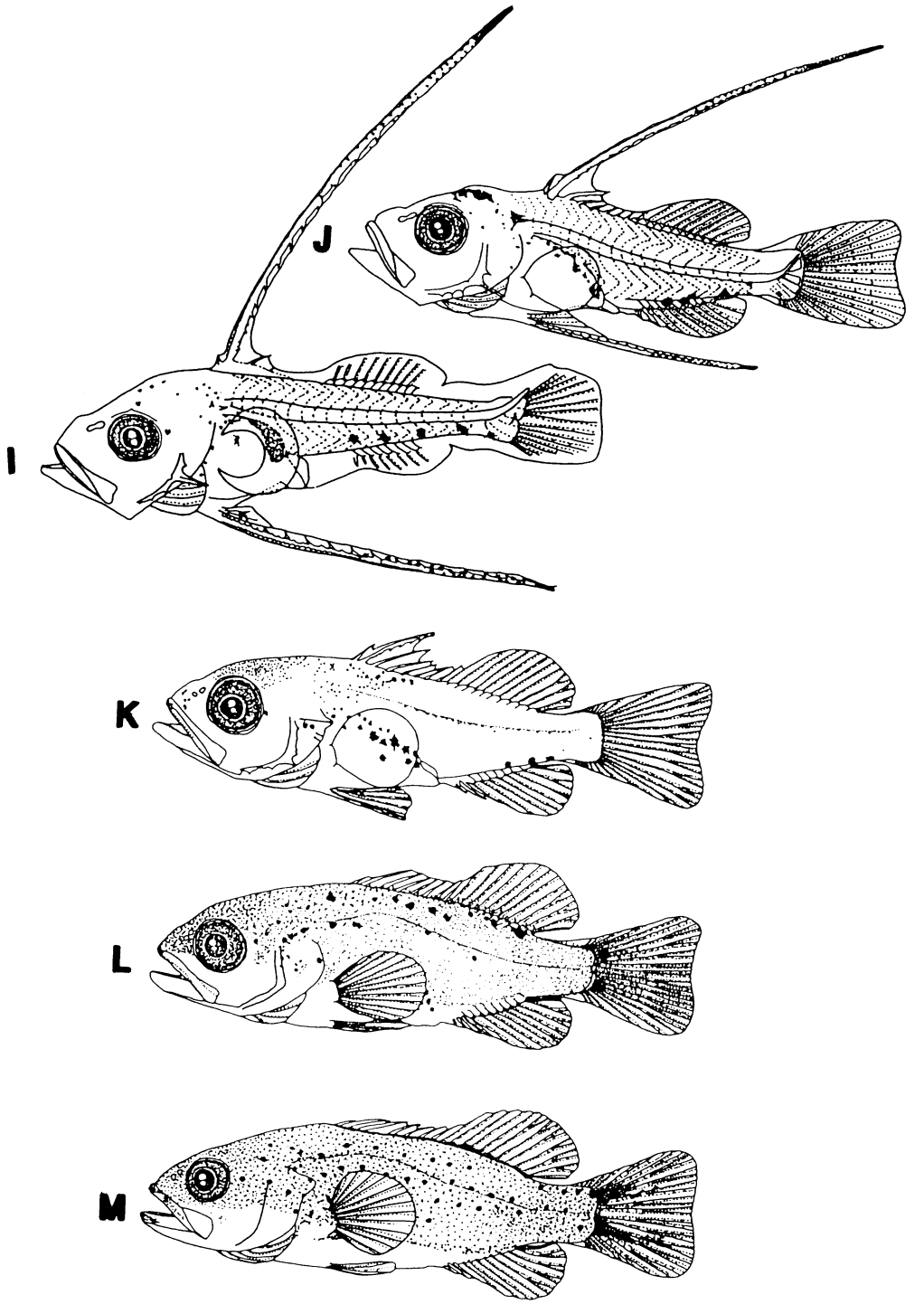


Fig. 3. Development of reared larvae and juveniles of *Plectropomus leopardus*. A) Newly-hatched larva, 1.62 mm TL; B) prelarva, 12 h after hatching, 2.22 mm TL; C) prelarva, 30 h after hatching, 2.65 mm TL; D) postlarva, 2 dah, 2.7 mm TL; E) postlarva, 5 dah, 2.96 mm TL; F) postlarva, 7 dah, 3.22 mm TL; G) postlarva, 10 dah, 4.70 mm TL; H) postlarva, 13 dah, 6.10 mm TL; I) postlarva, 18 dah, 7.30 mm TL; J) juvenile, 28 dah, 11.8 mm TL; K) juvenile, 48 dah, 18.7 mm TL; L) juvenile, 55 dah, 25.1 mm TL; M) juvenile, 65 dah, 35.0 mm TL.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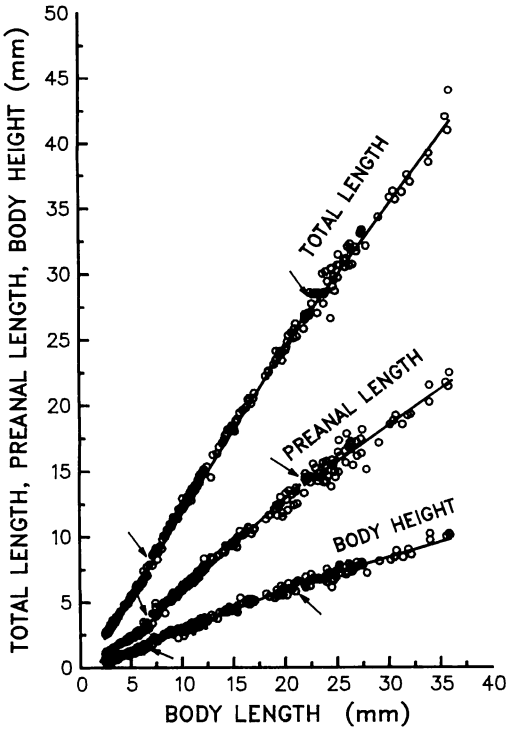

Fig. 4. Relative growth of total length, preanal length, and body height in relation to body length in larval and juvenile *Plectropomus leopardus*. Arrows indicate inflections.

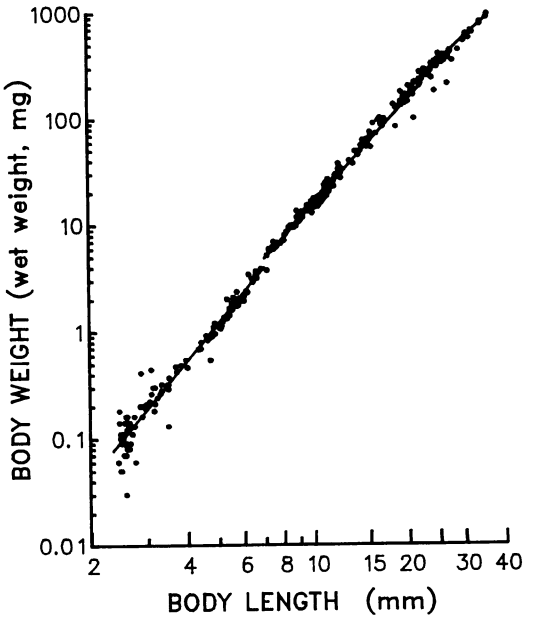

Fig. 5. Relationship between body length and body weight in larval and juvenile *Plectropomus leopardus*.

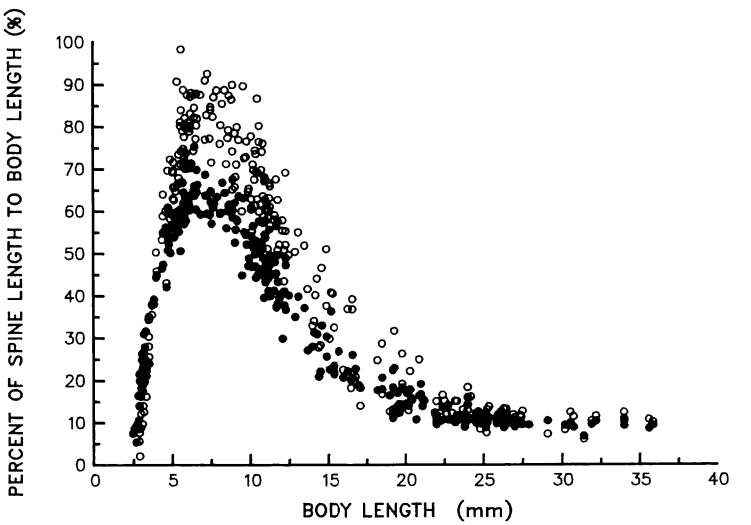

Fig. 6. Changes in lengths of the 2nd dorsal and pelvic fin spines as a percentage of the body length during larval and juvenile development of *Plectropomus leopardus*. 2nd dorsal spine (○); the pelvic spine (●).

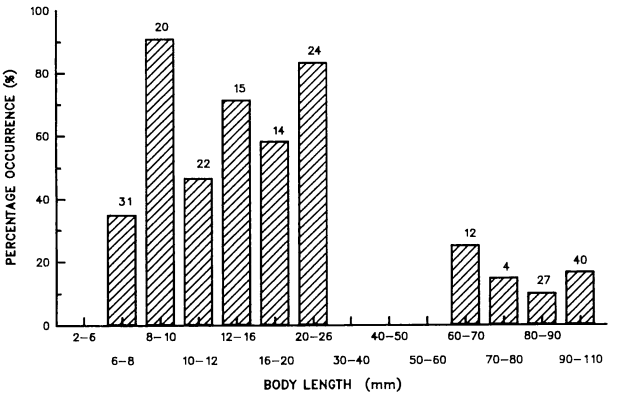

Fig. 7. Percentage occurrence of a small spine anterior to 1st anal spine of *Plectropomus leopardus* in relation to body length ranges. Number of specimens is indicated above each bar.

$$BW = 0.0483BL^{2.73} \quad (r^2 = 0.869,\ n = 56)$$

$7 \leqq BL < 22$ mm

$BL \geqq 22$ mm

The ratios of the 2nd dorsal and pelvic fin spine lengths to body length changed with growth (Fig. 6). The smallest larva found with pelvic fin spines was 2.46 mm BL and the largest without, 2.70 mm BL. The smallest larva with an elongated 2nd dorsal fin spine was 2.82 mm BL and the largest without, 2.86 mm BL. At around 7 mm BL, the 2nd dorsal and pelvic fin spines attained their maximum ratios of about 70–90% and 60–70%, respectively, of body length. The ratios rapidly decreased between 7 and 20 mm BL, and were constant at about 10% for larvae >20 mm BL.

Others

Two hundred and twenty-seven specimens, 2.5–105 mm BL, were examined for the development of a small spine in front of the 1st anal fin spine, the spine point on the supraocular ridge, and squamation.

The fishes with a small spine occurring in front of the 1st anal fin spine ranged from 6.86–98 mm BL. The smallest specimen examined with the 1st anal fin spine was 5.90 mm BL. The percentage occurrence of the small spine in relation to body length is shown in Figure 7. Occurrence of the spine was 30–90% for fishes smaller than 26 mm BL and <30% for those larger than 60 mm BL. Although specimens from 30 to 50 mm BL were lacking, it is believed that the percentage occurrence might decrease with growth.

Spine points on the serrated supraocular ridge numbered 1 or 2 in fishes from 4.68–13.5 mm BL. Fishes with more than 2 such spines were not found. The serrations on the supraocular ridge became smooth in fishes larger than about 15 mm BL.

Squamation began to develop from about 15 mm BL, covering the entire body in fishes larger than 25 mm BL.

Discussion

The two inflections in proportional measurements of total length, body height, preanal length and body weight found at 7 and 22 mm BL (8.4 and 27 mm TL), coincided with the development of larval behavior. At about 7 mm BL, the larvae began to show active movement and to feed actively on *Artemia* nauplii. The larval habitat shifted from the surface or middle layers to the tank bottom at about 22 mm BL. Beyond 25 mm BL, when squamation covered the entire body, the larval habitat shifted completely to the bottom.

Kitajima et al. (1991) compared the maximum ratios of the 2nd dorsal and pelvic fin spine lengths to BL and/or TL among 5 species of *Epinephelus* and showed that *E. septemfasciatus* had the longest 2nd dorsal (90%) and pelvic fin spine (65%) lengths relative to BL. The equivalent ratios in *Plectropomus leopardus* in the present study were 90 and 70%, respectively, of BL, the ratio of pelvic spine lengths to BL being longer than in *E. septemfasciatus*. Leis (1986), on the other hand, examined the ratio of the 2nd dorsal fin spine length to BL for 4 species of

Plectropomus and showed that *P. leopardus* and *P. maculatus* were intermediate between *P. laevis* and *P. areolatus*. According to Leis (1986), the ratio of 4–8 mm BL ranged from 100–140% BL in *P. leopardus*. However, the ratio obtained in the present study for the latter was less than 100% BL, even allowing for body shrinkage affecting BL measurements (Fukuhara, 1979). This result was rather similar to that for *P. laevis*, in which the 2nd dorsal spine was the shortest among the 4 species (Leis, 1986).

The number of spine points on the supraocular ridge is one of the important characters for the classification of the genus *Plectropomus* (Leis ,1986). All larvae examined here had 2 points only on the supraocular ridge, whereas those of *P. leopardus* examined by Leis (1986) had 3–4 points. This difference might be due to geographical variation or abnormalities caused by captive rearing. Examination of wild *P. leopardus* larvae from around Ishigaki Island will be necessary in order to determine whether or not geographical differences occur.

A small spine occurring in front of the 1st anal fin spine was observed in many fixed specimens. Such a spine has not been found in wild *Plectropomus* larvae (Leis, 1986), and is probably an abnormality of the laboratory-reared fish, which might diminish with growth as the elongated spines become shorter owing to erosion (Leis, 1986). Further study is necessary to determine if the appearance of the spine is a function of growth.

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スジアラの卵発生と仔稚魚の形態

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水槽内で飼育したスジアラ親魚から自然産卵によって得た受精卵の発生を記載した．さらに，その卵より得た孵化仔魚を飼育し，孵化仔魚から稚魚期までの形態の記載と形態変化に伴う行動の変化について観察した．スジアラの卵は分離浮遊卵で1個の油球を有し，卵径は0.82–0.93 mmであった．受精後26時間40分 (25.4–26.3°C) で孵化した．孵化直後の全長は1.62 mmで，孵化後2日目（全長2.70 mm）に開口し摂餌が始まった．孵化後5日（全長2.96 mm）に腹鰭棘と第2背鰭棘が出現し，28日（全長11.8 mm）で鰭条数は定数に達したが，未だ腎鰭第3棘の棘条化は始まっていなかった．全長約25 mm位から徐々に底棲性が現われ，体色も赤化し，全長35 mmで完全な底棲生活に入った．相対成長における屈折点は体長7 mmと22 mmに認められ，行動の変化する時期と一致した．

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