Growth and morphological development of larval and juvenile *Epinephelus bruneus* (Perciformes: Serranidae)

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Abstract The growth and morphological development of larval and juvenile *Epinephelus bruneus* were examined in a hatchery-reared series. Average body length (BL) of newly-hatched larvae was 1.99 mm, the larvae growing to an average of 3.96 mm by day 10, 6.97 mm by day 20, 12.8 mm by day 30, 22.1 mm by day 40 and 24.7 mm by day 45 after hatching. Newly-hatched larvae had many mucous cells in the entire body epidermis. By about 4 mm BL, the larvae had developed pigment patterns peculiar to epinepheline fishes, including melanophores on the dorsal part of the gut, on the tips of the second dorsal and pelvic fin spines, and in a cluster on the ventral surface of the tail. Spinelets on the second dorsal and pelvic fin spines, the preopercular angle spine and the supraocular spine, had started to develop by about 6 mm BL. The notochord tip was in the process of flexion in larvae of 6–8 mm BL, by which time major spines, pigments and jaw teeth had started to appear. Fin ray counts had attained the adult complement at 10 mm BL. After larvae reached 17 mm BL, elements of juvenile coloration in the form of more or less densely-pigmented patches started to appear on the body. Squamation started at 20 mm BL. Major head spines had disappeared or became relatively smaller and lost their serrations by 20–25 mm BL.

Key words. — *Epinephelus bruneus*; growth; morphological development; larva; juvenile.

Epinephelus bruneus (Bloch) occurs from Korea, southern Japan, East China Sea and southern China to the Philippines (Randall and Heemstra, 1991; Senou, 1995). Recently, artificial larval rearing has been carried out because of the value of this species for aquaculture and fisheries (Tsukashima and Yoshida, 1984; Manabe and Kasuga, 1988; Okada et al., 1994, 1996). However, high mortality frequently occurs during the early life stages of artificially-reared larvae, more detailed information on early growth and development being necessary for improving larval rearing techniques.

The development of *E. bruneus* during embryonic and prelarval stages was described by Manabe and Kasuga (1988). However, for postlarval and juvenile stages, morphological development has not yet been reported. In this study the growth and morphological development of hatchery-reared specimens were examined from prelarval to early juvenile stages.

Materials and Methods

Wild-caught broodstocks, maintained in captivity for 8 or 9 years, were used to obtain study materials. On 29 May 1995, 12 ripe females were induced to ovulate by intramuscular injection of salmon pituitary homogenate combined with the genital hormone chorionic gonadotrophin. Eggs from a female (10.7 kg in body weight) were manually stripped and fertilized with milt from a ripe male (25.4 kg in body weight) on 31 May. The fertilization rate was 96.4%. Eggs were kept in a fine mesh net for a day and floating eggs subsequently transferred to a 30 m^3 tank for further rearing. During the experiment, water temperature ranged from 23.4 to 25.8°C (25.0°C average). The feeding scheme for the resulting larvae and juveniles was as follows: small-sized rotifers, *Brachionus rotundiformis*, mean±SD=118.51±8.69 μm in lorica length (n=50), from day 3 to 6 after hatching, large-sized rotifers, *B. plicatilis*, mean±SD=126.42±14.41 μm in lorica length (n=50) from day 7 to 31, *Artemia* nauplii (*Artemia salina*) from day 20, eggs

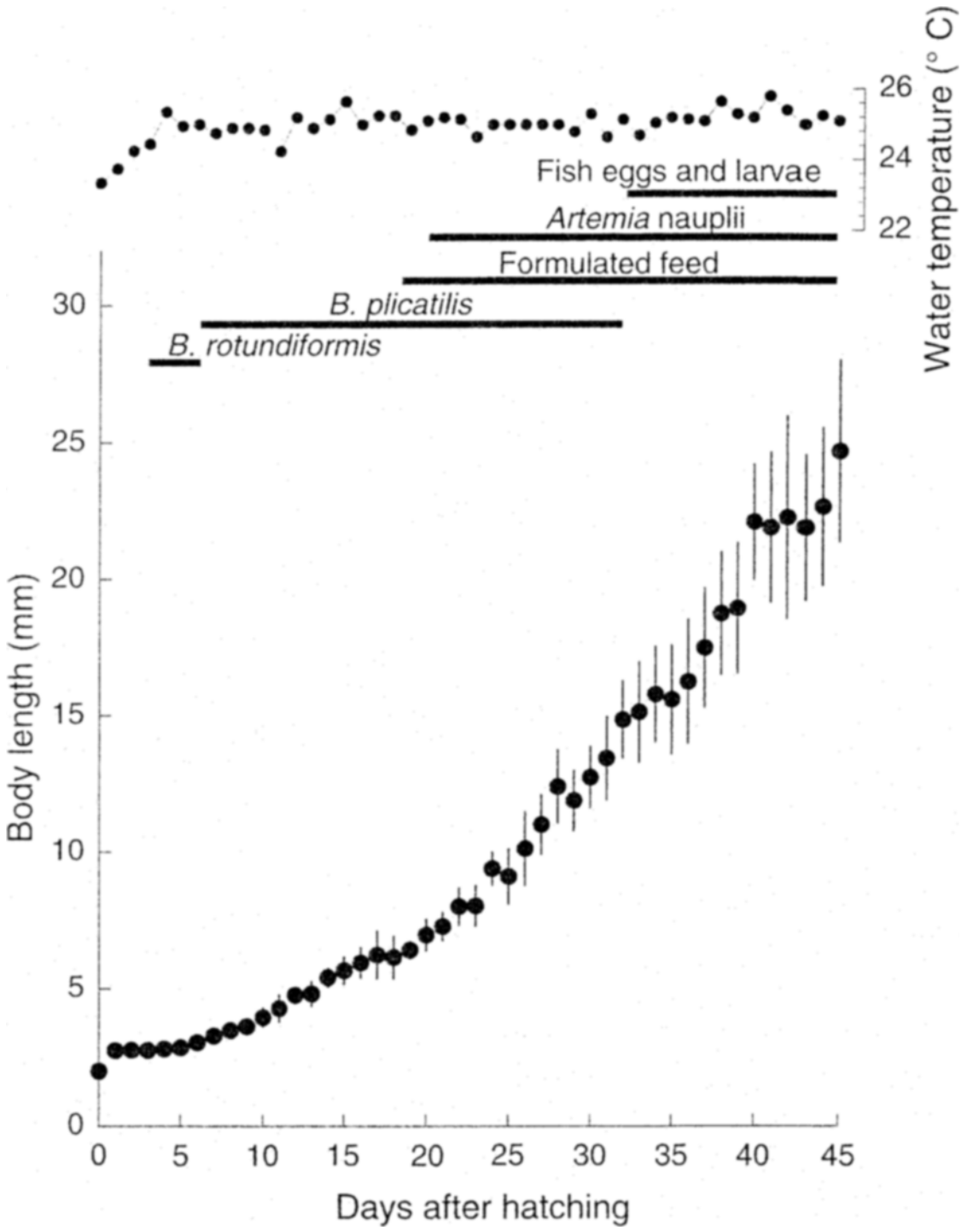

Fig. 1. Body length, diet and water temperature during the rearing of *Epinephelus bruneus*. Vertical lines indicate mean±SD (n=20).

and larvae of *Pagrus major* (Temminck & Schlegel) from day 32, and formulated feed from day 18 until the end of nursery rearing (Fig. 1). *Artemia* nauplii, fish eggs and larvae, and formulated feed were supplied to larvae and juveniles until the end of the experiment.

Every day from hatch out day (day 0) to day 45, 20 fish were fixed and preserved in 5% formalin. 920

specimens, 1.90 mm≤BL≤37.0 mm, were used for studies of absolute and relative growth, pigmentation and spine development. Among these, 60 specimens, 4.58 mm≤BL≤28.7 mm, were cleared and stained for easier observation of spine development and squamation, following the method of Kawamura and Hosoya (1991). In addition to daily sampling, three larvae at a time were sampled 0, 2 and 6 days after hatching and used to study mucous cell development. These fish were fixed in Bouin's solution and preserved in 80% ethanol for histological preparation. Examination by light microscopy followed staining of the mucus by the following method. Whole bodies of each larva were embedded in paraffin, longitudinally sectioned to 4 μm thickness, and stained with alcian blue (AB) at pH 2.5 and by Periodic Acid Schiff (PAS) reaction.

Measurements were made with computer-captured video images with a camera attached to a binocular microscope. Lengths in the video image were measured using NIH Image, developed at National Institutes of Health (U.S.A.), to an accuracy of ±1%. In this study specimen size was expressed as body length (BL). Before and during notochord flexion, BL was taken from the tip of the upper jaw to the end of the notochord. After notochord flexion, BL was taken from the tip of the upper jaw to the posterior margin of the hypurals. Terminology generally follows Leis and Rennis (1983), Johnson and Keener (1984) and Kendall et al. (1984). A representative series of specimens used in this study were deposited in the Aquatic Natural History Museum of Fisheries Research Station, Kyoto University (FAKU 128829–128857).

Results

Gross morphology. Development of gross morphology of *Epinephelus bruneus* larvae and juveniles is illustrated in Figure 2. Epinepheline larvae are typically kite-shaped with elongated, serrated second dorsal and pelvic fin spines. The head is large with a moderate to large mouth reaching to or beyond mid-eye (Leis and Rennis, 1983). Also characteristic of epinepheline larvae are a long serrated spine at the angle of the preopercle and a large pigment spot on the caudal peduncle, which moves from the ventral midline to a mid-lateral position during early development (Johnson and Keener, 1984).

Absolute growth. The diameter of fertilized eggs was 0.91±0.17 mm (mean±SD, n=20) and that of oil globules 0.19±0.04 mm. The body length of newly-hatched larvae was 1.99±0.04 mm (mean± SD, n=20, Fig. 1). On day 3 after hatching, the mouth opened and larvae commenced feeding (2.76±0.11 mm BL, mean±SD, n=20). The yolk was present until day 5 (2.86±0.10 mm BL). The larvae grew to 3.96±0.35 mm BL by day 10, 6.97±0.56 mm BL by day 20, 12.8±1.1 mm BL by day 30, 22.1±2.1 mm BL by day 40 and 24.7±3.3 mm BL by day 45. From hatching to day 20, the growth rate was 0.25 mm·day^{-1}. The rate was accelerated to 0.71 mm·day^{-1} from day 20 to 45 (from about 7 mm BL). From about day 40 (about 22 mm BL), juveniles began to settle on the tank walls and bottom or under cover of objects such as water pipes and netting.

Notochord flexion. The notochord tip started bending upward at about 6 mm BL (Fig. 2E), the largest specimen with a straight notochord being 6.24 mm BL. The smallest postflexion larva measured 6.70 mm BL and the largest flexion larva 8.68 mm (Fig. 2F), the size range 6–8 mm BL thus being considered as the flexion larva stage.

Relative growth. Among 11 body parts measured in relation to BL, relative head height, eye diameter and snout length increased before subsequent declining (Fig. 3). These proportions reached maximum ratios at 8–13 mm BL. At sizes smaller than about 7 mm BL, the ratio of body height changed considerably, but thereafter ranged from 30 to 35%. Head length, upper jaw length and caudal peduncle depth proportions increased initially in larvae to about 12 mm BL, but changed little subsequently, ratios ranging from 35 to 42%, 15 to 20% and 10 to 12%, respectively. Total length increased from the initial ratio (106% between 2.5 and 5.5 mm BL), reaching a maximum at 15 mm BL before decreasing. The ratio of preanal length decreased between 2.5 mm and 5.5 mm BL (from about 60% to 50%), but increased thereafter.

Lengths of the second dorsal and pelvic fin spines relative to BL are plotted in Figure 4. Both spines developed somewhat parabolically and declined subsequently, their lengths attaining maximum ratios of 70% for the second dorsal fin spine at 7.0 mm BL and of 60% BL for the pelvic fin spine at 6.1 mm BL. Thereafter, the relative lengths of the spines decreased to 19.1% and 18.0%, respectively, in the largest specimen (37.0 mm BL).

Pigmentation. Newly-hatched larvae, 1.89–2.06 mm BL, had no melanophores (Fig. 2A), but acquired several dendritic melanophores on the snout and ante-

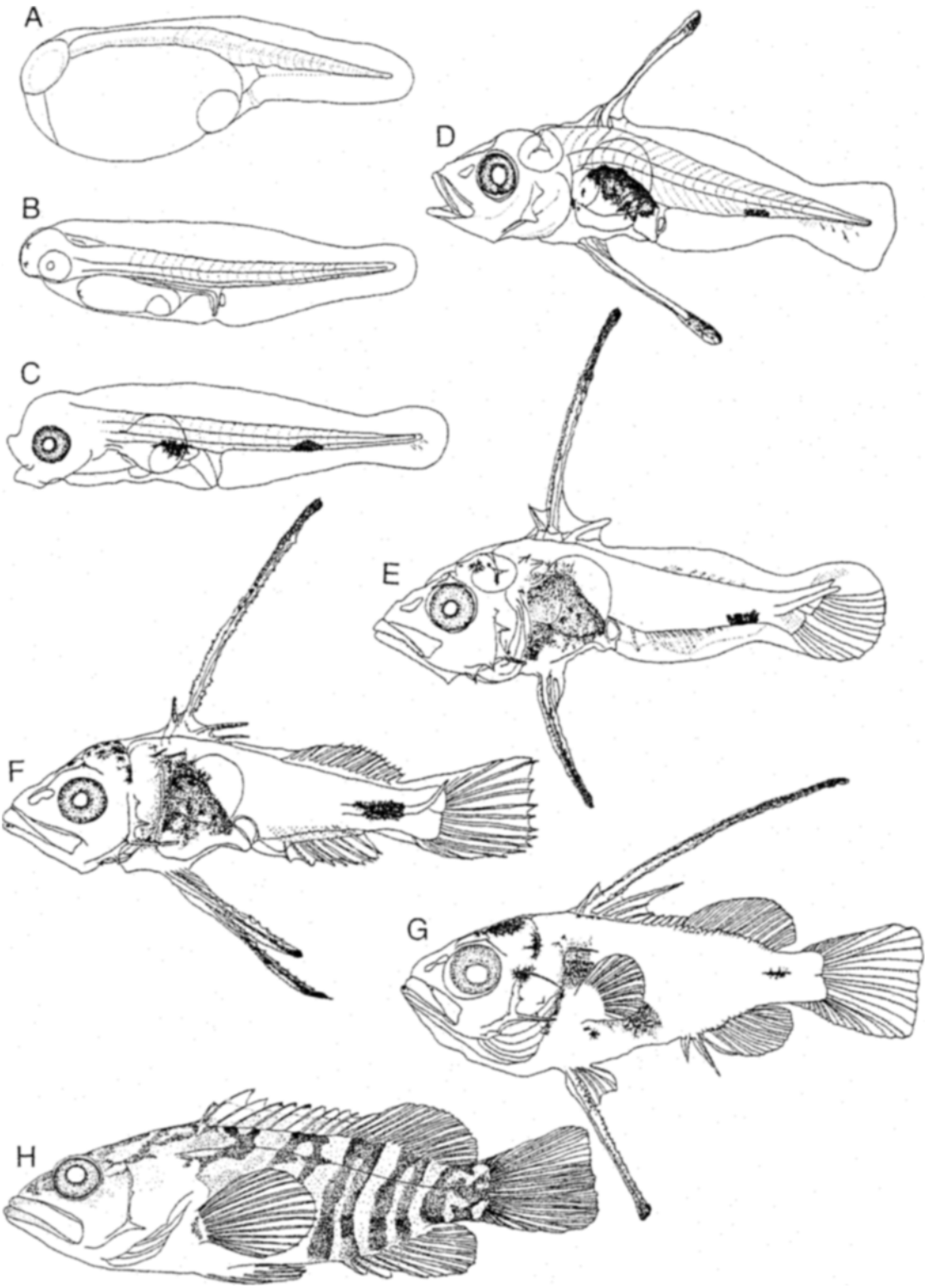

Fig. 2. Development of *Epinephelus bruneus*. A) Newly-hatched larva, 1.91 mm BL; B) 1-day-old larva, 2.74 mm BL; C) 4-day-old larva, 2.83 mm BL; D) 12-day-old larva, 4.60 mm BL; E) 17-day-old larva, 6.03 mm BL; F) 23-day-old larva, 9.02 mm BL; G) 35-day-old juvenile, 10.4 mm BL; H) 45-day-old juvenile, 31.0 mm BL.

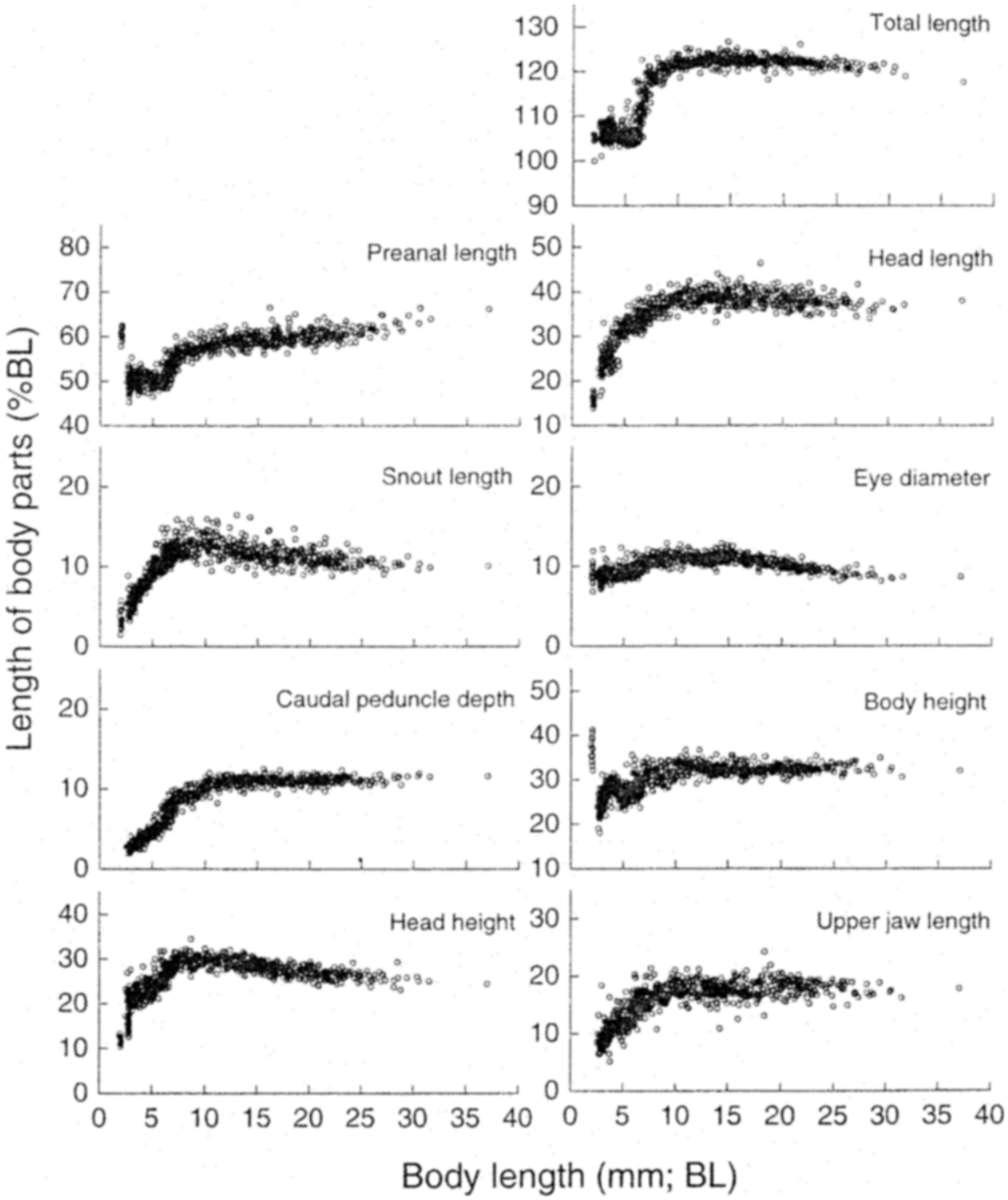

Fig. 3. Some body proportions of *Epinephelus bruneus*, shown as percentages of body length.

rior surface of the yolk-sac on day 1, 2.49–2.83 mm BL (Fig. 2B). These had disappeared in specimens sampled on day 3 (2.66–2.94 mm BL) and thereafter (Fig. 2C). By about 4 mm BL, pigment patterns characteristic of epinepheline larvae had appeared as follows: melanophores scattered on the dorsal part of the gut, subsequently formed a dorsal cap of melanophores over the gut in later developmental stages, in specimens larger than 2.50 mm BL; a single cluster of external melanophores appeared on the ventral side of the tail at 2.73 mm BL; and the tips of the second dorsal and pelvic fin spines, which were covered with swollen membranous sheaths, had become pigmented by 3.82 and 3.62 mm BL, respec-

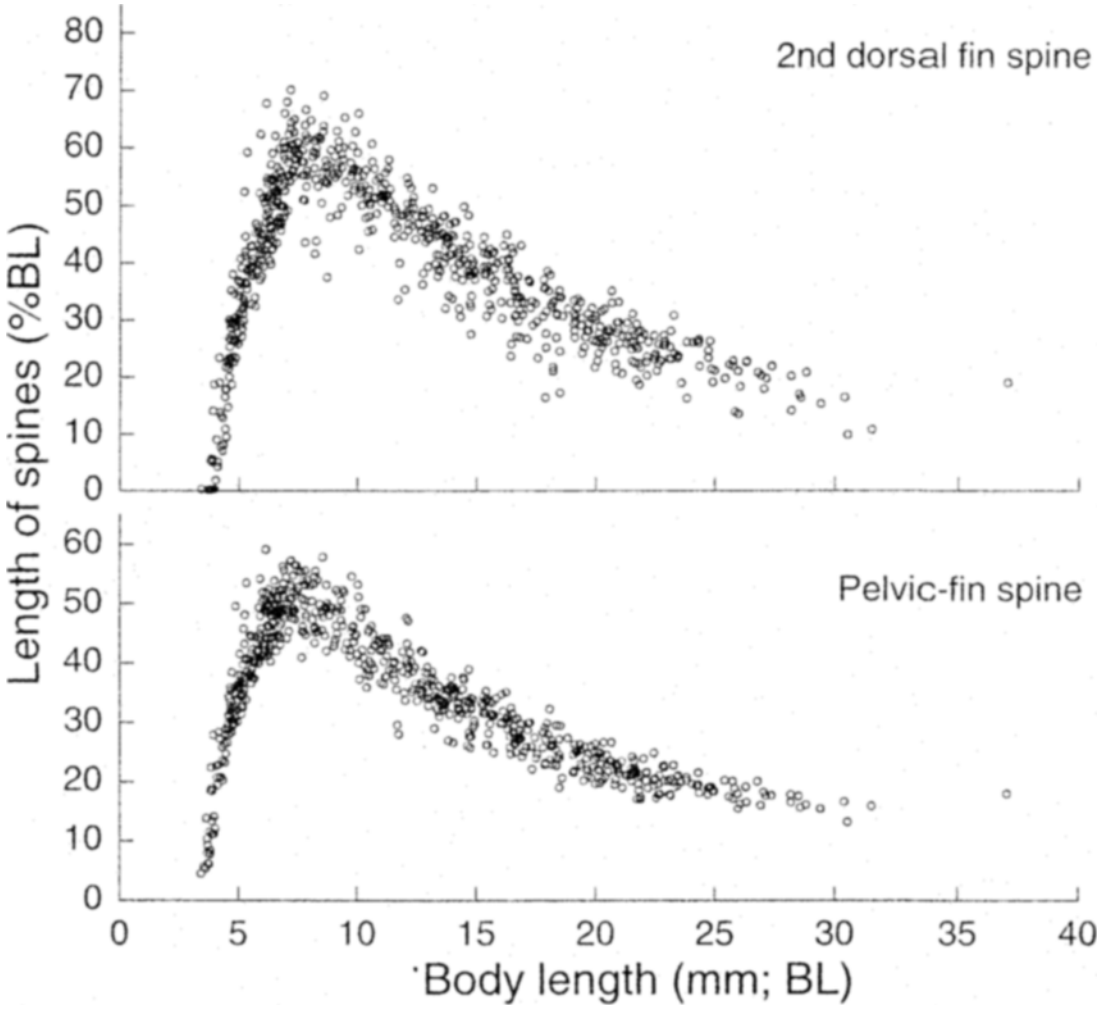

Fig. 4. Proportions of the second dorsal and pelvic fin spines of *Epinephelus bruneus*, shown as percentages of body length.

tively (Fig. 2D).

Melanophores started to develop on several other body parts in larvae larger than about 6 mm BL, the size of the smallest specimen having such melanophores (largest specimen lacking such in parentheses): on the midbrain – 5.68 mm BL (6.51), on the cleithral symphysis – 5.85 mm BL (7.00) (Fig. 2E), on the lower jaw tip – 6.76 mm BL (14.3) (Fig. 2F), on the forebrain – 7.89 mm BL (8.83), at the base of the 1st and 2nd dorsal fin spines – 9.62 mm BL (11.4), on the operculum – 10.2 mm BL (11.2), on the soft dorsal fin base – 10.4 mm BL (12.8), on the maxillary – 10.4 mm BL (10.5) (Fig. 2G), on the upper jaw tip – 11.4 mm BL (12.9), on the membrane between the 2nd and 3rd anal-fin spines – 11.9 mm BL (21.2).

Although melanophores forming the dorsal cap of the gut extended upward along the myosepta in specimens larger than 2.74 mm BL (Fig. 2C), the extension had much individual variation, especially in specimens smaller than about 4 mm BL. From about 5 to 6 mm BL, the area of gut pigmentation showed maximal extension (Fig. 2E); the dorsal cap of melanophores extended downward on the gut surface (to the ventral surface in a few specimens). Thereafter, melanohpores on the ventral part became scarce but enlarged (Fig. 2G). In the cluster on the ventral surface of the tail, some melanophores began to extend upward at 6.53 mm BL, forming a cluster extending both internally and externally at the midlateral part of the tail. Internal melanophores surrounded the notochord. Specimens between 6.62 and 7.94 mm BL had both ventral and midlateral clusters of melanophores on the tail. These clusters never existed separately, although melanophores in the ven-

tral cluster began to disappear in specimens larger than about 8 mm BL (Fig. 2F). All specimens larger than 10.1 mm BL had only the midlateral cluster.

In addition to the melanophores covering the sheath of the second dorsal and pelvic fin spine tips, melanophores appeared progressively toward the base of the spines (from the tips) from about 4.4 mm BL (Fig. 2D). The extent of melanophore appearance reached a maximum at about two-thirds of the spine length, at 9–11 mm BL for the second dorsal fin spine (Fig. 2G) and 12–14 mm BL for the pelvic fin spine (almost the entire length of the spine). Melanophores on both spines became scarce as the larvae developed further.

The largest specimen retaining characteristic larval pigmentation was 16.5 mm BL, the smallest with pigmentation considered to indicate a transition to the juvenile state, i.e., somewhat densely pigmented patches appearing on the body, being 15.5 mm BL. More distinct body patches appeared and increased in number as the larvae grew. From about 30 mm BL, six densely pigmented bands, which is the pattern characteristic of *E. bruneus*, appeared on the body.

Fin development. The smallest specimen possessing fin rays, in which incipient elements identified as the second dorsal and pelvic fin spines were observed, was 2.86 mm BL. However, the smallest specimen with a measurable spine was 3.32 mm BL (pelvic fin spine) and 3.57 mm BL (second dorsal fin spine). The first dorsal fin spine was recognizable at 4.30 mm BL (Fig. 2D) and the third at 4.53 mm BL (Fig. 2E). Thereafter, the increasingly posterior formation of dorsal spines continued (Fig. 2F–H).

The second dorsal fin spine developed three ridges, comprising an apex ridge and posterolateral wings, by 4.05 mm BL, but no spinelets were observed on the ridges at that time (Fig. 5A, for spine ridge terminology see Johnson and Keener, 1984). Spinelets first appeared on the apex ridge at 4.62 mm BL, subsequently appearing on the posterolateral wings as well by 5.39 mm BL. Distal spinelets on the posterolateral wings became enlarged and recurved, a series of smaller spinelets occurring proximally, in larvae ranging from 5.43 to 19.2 mm BL (Fig. 5B, C). By 6.5 mm BL, the second dorsal spine was well-developed and elongated, and bore diagnostic spinelets. With further growth, the enlarged spinelets of the apex ridge decreased and distal spinelets disappeared at the distal part (Fig. 5D). In a 25.4 mm BL specimen, spinelets were apparent on the posterolateral wings, but not on the apex ridge. A specimen of 30.1 mm BL lacked spinelets on both the apex ridge and the posterolateral wings (Fig. 5E).

Pelvic fin spine buds appeared at 3.14 mm BL. An incipient pelvic fin spine, covered with a swollen membranous sheath, developed three ridges from ridges 3 (ventrolateral) and 4 (dorsolateral), and the posterior membranous sheath keel by 3.56 mm BL. At 4.40 mm BL, ridges 1 (dorsomedial) and 2 (ventromedial) began to form four ridges (plus the membranous sheath keel), with spinelets first being seen on ridges 1 and 2 (Fig. 6A). All the ridges in three specimens, 5.54, 5.58 and 5.69 mm BL, had spinelets. Specimens ranging from 5.32 to 16.2 mm BL had spinelets on ridges 3 and 4 and enlarged, recurved spinelets on ridges 1 and 2 (Fig. 6B, C). Spinelets of ridge 3 were small and straight, whereas those of ridge 4 were enlarged near the base and tip when fully developed. The enlarged spinelets became more widely spaced and bifurcated with growth (Fig. 6D). In specimens larger than about 22 mm BL, the number of ridges decreased to three, comprising ridges 1, 2, and 3+4 (the same complement as in adults) (Fig. 6E), and the spinelets began to disappear.

Caudal fin rays first appeared at 5.10 mm BL and soft dorsal, anal and pectoral fin rays synchronously at 5.85 mm BL. The first and second anal fin spine buds appeared at 6.03 mm BL. The smallest specimen having an adult complement of fin ray counts (cf., Senou, 1995; Randall and Heemstra, 1991) was 9.21 mm BL, the largest with an incomplete fin ray complement being 9.66 mm BL (Fig. 2G).

Head spination. Major head spines first appeared in specimens 4–5 mm BL: the inner preopercular spine at 4.43 mm BL, the preopercular angle spine at 4.52 mm BL (Fig. 2D), the supracleithral spine at 4.82 mm BL, the post-temporal at 5.00 mm BL and the supraocular spine at 5.17 mm BL (Fig. 2E). An opercular spine was first observed at 6.68 mm BL, and the interopercular and subopercular spines at 6.12 mm BL and 12.4 mm BL, respectively. However, the presence of an interopercular spine was subject to individual variation.

The number of opercular spines had increased to three (adult number) by 16.5 mm BL. The single supraocular spine had disappeared by 13.2 mm BL. Specimens between 6.00 and 11.6 mm BL had a serrate supraocular bone with a spine (Fig. 2E, F, G), wherears specimens larger than 13.2 mm BL had only the serrate bone. All the inner preopercular and post-temporal spines had disappeared by 23.7 mm BL (Fig. 2H). The largest specimens still possessing the interopercular and supracleithral spines were 18.1 and 25.4 mm BL, respectively. A specimen of 31.5 mm BL

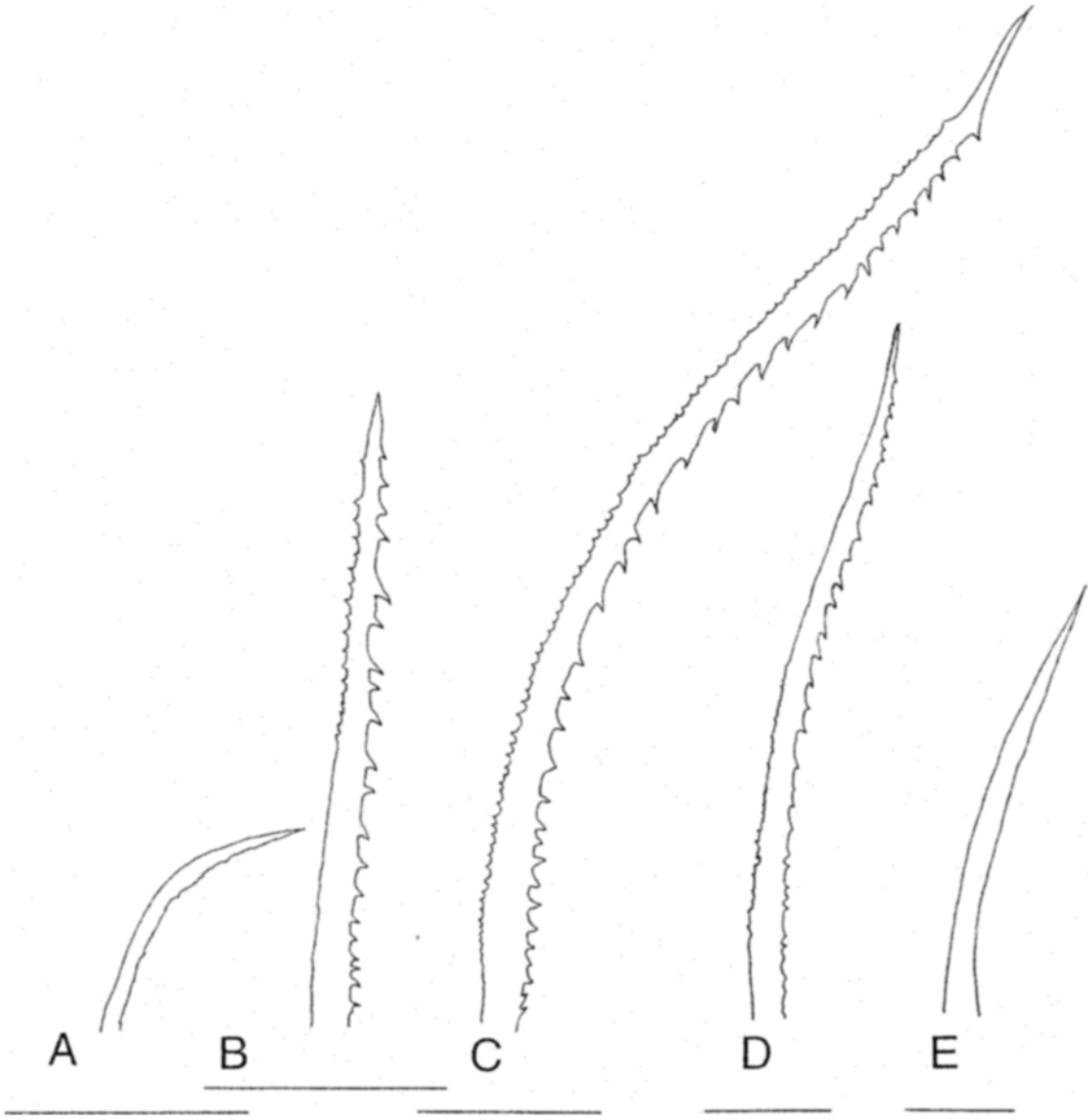

Fig. 5. The second dorsal fin spine (left lateral view) of *Epinephelus bruneus*. A) 4.40 mm BL; B) 7.06 mm BL; C) 16.0 mm BL; D) 20. 8 mm BL; E) 30.1 mm BL. Scale bars indicate 1.00 mm.

still had the opercular, preopercular angle and subopercular spines, but the largest specimen examined, 37.0 mm BL, had lost the subopercular spine.

Jaw teeth. Upper and lower jaw teeth first appeared at 6.01 mm BL and 6.56 mm BL, respectively. The largest specimens not having upper and lower jaw teeth were 7.01 mm BL and 7.32 mm BL, respectively.

Squamation. Lateral line scales first appeared near the operculum at about 20 mm BL (the smallest specimen with lateral line scales was 19.5 mm BL). From about 22 mm BL, scales formed sparsely on the central part of the trunk in addition to the lateral line scales. Scale distribution become dense and the squamated area extended antero-posteriorly and dorsoventrally as the larvae grew. Scales first appeared on the operculum at about 27 mm BL. The largest specimen examined, 30.2 mm BL, was still incompletely squamated, lacking scales near the dorsal fin base and on the abdomen.

Mucous cells. Large granular cells were distri-

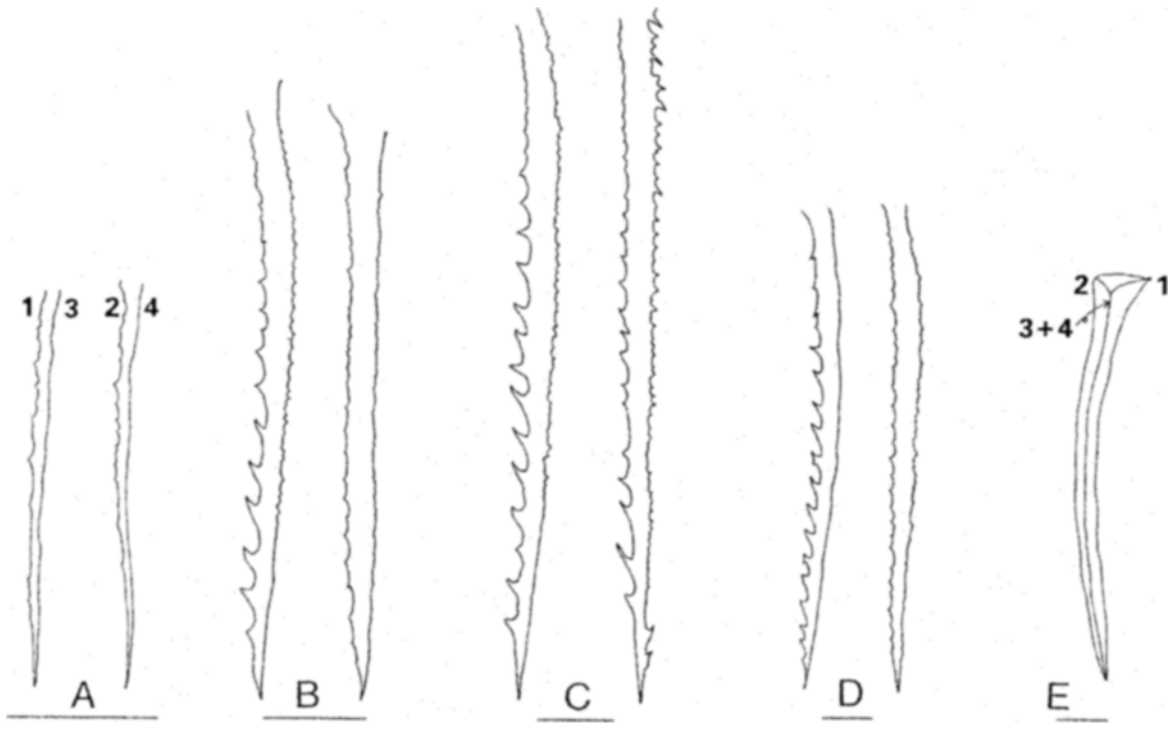

Fig. 6. The left pelvic fin spine (ventromedial and ventrolateral views) of *Epinephelus bruneus*. 1—dorsomedial ridge; 2—ventromedial ridge; 3—ventrolateral ridge; 4—dorsolateral ridge. A) 4.40 mm BL; B) 7.06 mm BL; C) 16.0 mm BL; D) 20.8 mm BL; E) 30.1 mm BL. Scale bars indicate 1.00 mm.

buted in the epidermis over the entire body of newly-hatched larvae (1.98–2.01 mm BL). The epithelium of these larvae consisted of a single cell layer. About 10% of the epithelial cells stained with AB and about 73% of cells showed weak PAS reaction. Diameters of the AB-positive and weakly PAS-positive cells were 12.7±1.8 μm (mean±SD, n=30) and 11.3±2.2 μm (mean±SD, n=30), respectively, the sizes of the two cell types were not differing significantly.

In 2-day-old larvae (2.52–2.83 mm BL), large granular cells were distributed all over the newly-appeared pectoral fins. In the epidermis of these larvae, an additional type of PAS-positive cells was observed. Such cells containing a small PAS-positive spot and accounting for 10% of the epidermal cells overall, were 20.2±2.8 μm (n=30) in diameter. Additionally, AB-positive and weakly PAS-positive cells had become significantly larger than those of newly-hatched larvae (p<0.01, Student's t-test), 19.0±2.1 μm and 17.0±2.5 μm in diameter (n=30), respectively, and accounted for 10% and 27%, respectively, of all epidermal cells.

In 6-day-old larvae (2.92–3.24 mm BL, Fig. 7), AB-positive cells still accounted for 10% of epidermal cells, the percentages of weakly PAS-positive cells and those with a small PAS-positive spot being 20% and 27%, respectively. The diameters of these cells were 20.0±2.4 μm, 17.9±2.5 μm, and 21.9±2.4 μm, respectively and were not significantly different from those of 2-day-old larvae.

Morphological abnormalities. The morphological abnormality observed most frequently during this study was deformation of the lower jaw. In deformed specimens, the lower jaw was much shorter than normal owing to the deformation of both the dentary and angular. Although the incidence of lower jaw deformation was not determined precisely in this study, it was usually not more than 10% (maximum) in the seedstock production of *E. bruneus*.

Discussion

Morphological development of *Epinephelus bruneus* is summerized in Figure 8. There were a few morphological events during early development, which allow comparison among *Epinephelus* species,

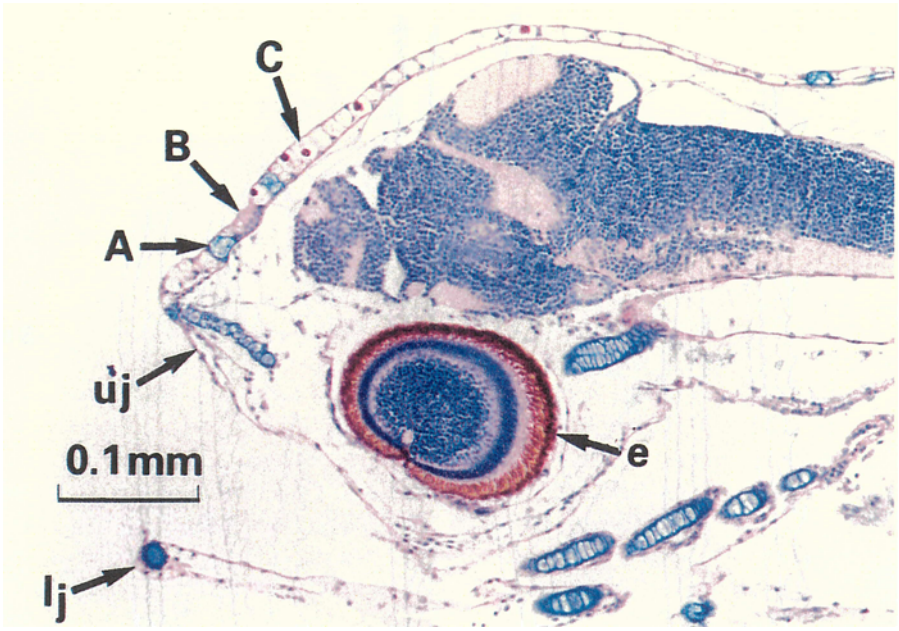

Fig. 7. Mucous cells in the epidermis of 6-day-old *Epinephelus bruneus*. Photograph shows longitudinal section of head, A—AB-positive cell; B—weakly PAS-positive cell; C—cell with a small PAS-positive spot; e—eye; uj upper jaw; lj—lower jaw.

although the available information is limited, especially regarding the individual development variations. Notochord flexion and numerical complements of fin rays are important landmarks, dividing the larval stage into preflexion, flexion and postflexion, signifying the postflexion/juvenile transition (Kendall et al., 1984). *E. bruneus* reached these landmarks at larger sizes than other *Epinephelus* species: the size of flexion larva was 6–8 mm BL in *E. bruneus*, compared with 5–6 mm SL in *E. fuscoguttatus* (Forsskål) (see Kohno et al., 1993) and *E. striatus* (Bloch) (Powell and Tucker, 1992); the adult complement of fin ray counts was attained at 9.21–9.66 mm BL in *E. bruneus*, 6.8 mm BL (minimum) in *E. striatus*, at 7.75–8.04 mm BL in *E. fuscoguttatus* and 7.3–9.6 mm BL in *E. akaara* (Temminck et Schlegel) (see Fukuhara and Fushimi, 1988). These differences may indicate a greater potential for culture of *E. bruneus* than for other *Epinephelus* species owing to the more rapid growth relative to developmental stage, in the former. In order to confirm this, more detailed culture experiments involving these epinepheline fishes is needed.

The melanophore cap covering the gut dorsally is one of the pigmentation characteristics of epinepheline larvae. Kohno et al. (1993) divided early larval Indo-Pacific *Epinephelus* into three groups according to the distribution modes of gut (and trunk) melanophores. *E. bruneus* examined in this study included two types; specimens with melanophores on the ventral surface of the gut in addition to the dorsal cap (belonging to group 2 or intermediate between groups 1 and 2, according to Kohno et al., 1993) and specimens without (belonging to group 1). Kohno et al. (1993) included *E. bruneus* (as *E. moara*; synonymized under *E. bruneus* by Randall and Heemstra [1991]) in group 2, on the basis of a specimen (4.7 mm TL) reported by Manabe and Kasuga (1988). However, many more specimens examined in the present study belonged to group 1 than group 2.

On the basis of tail pigmentation, *Epinephelus* larvae were divided into four groups by Kohno et al. (1993). *E. bruneus* larvae examined in this study belonged to their group 3, along with *E. fuscoguttatus*, *E. malabaricus* (Bloch & Schneider) and *E. tauvina* (Forsskål), which all have a large cluster of melanophores on the ventral surface of the tail.

Like other larvae of the tribe Epinephelini, *E. bruneus* larvae have elongated, serrated dorsal and pelvic fin spines. Although the function of serrations on these spines is not known, Johnson and Keener (1984) showed that spinelet morphology, in conjunction with the frequency distributions of meristic characters and geographic distribution, can be an aid to identification of American epinepheline larvae. However, the fact of spine configuration changing ontoge-

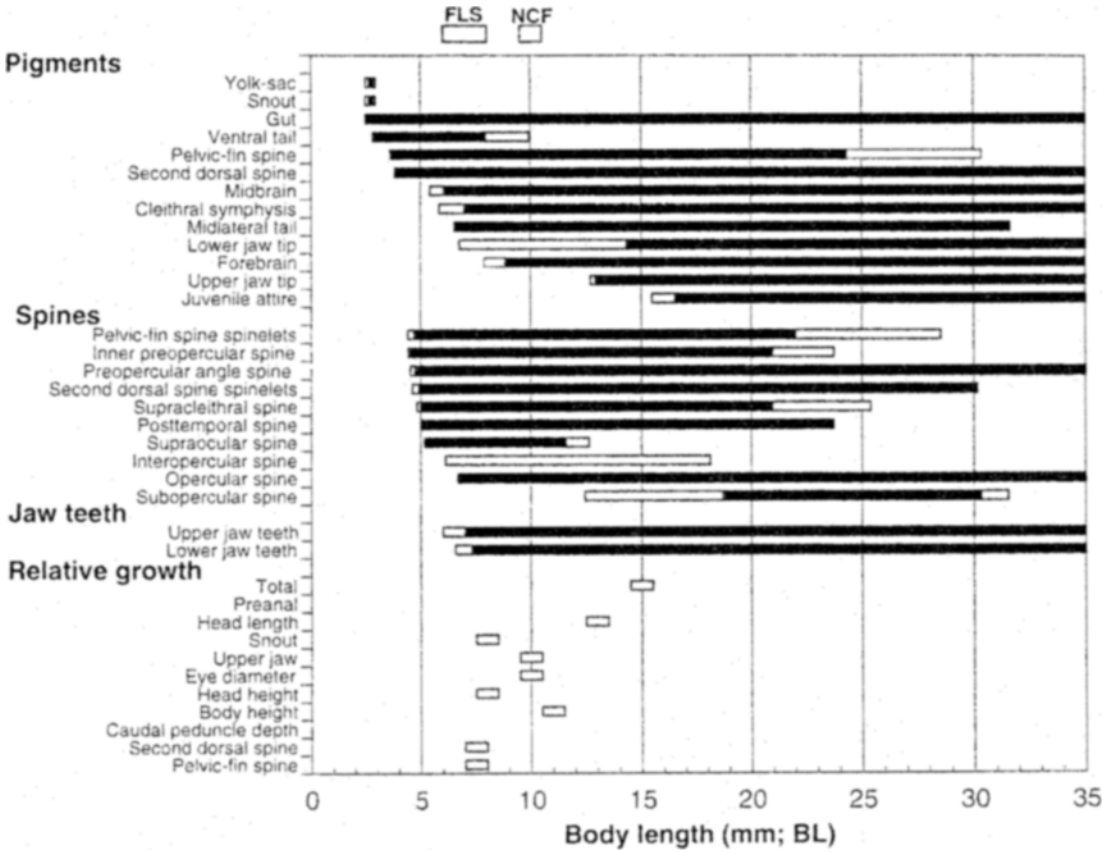

Fig. 8. Schematic representation of the development of pigment, spines and relative growth in hatchery-reared *Epinephelus bruneus*. Flexion larva subdivision (FLS) and numerical complements of fin rays (NCF) are also shown. Appearance of melanophores, spines and jaw teeth subject to individual variation (□). Melanophores, spines and jaw teeth present in all specimens examined (■). Under relative growth, peak values of body proportions (relative to BL) (□).

netically must be considered when making comparisons among species. For Indo-Pacific *Epinephelus*, no detailed descriptions are available, except for *E. fuscoguttatus* (see Kohno et al., 1993). More data are needed before comparisons of spine morphology among Indo-Pacific *Epinephelus* species can be made.

As Kitajima et al. (1991) pointed out, relative lengths of the second dorsal and pelvic fin spines differ significantly among species. The maximum spine length ratios of *E. bruneus* were around the average of those reported for Indo-Pacific *Epinephelus* species (see Table 1 of Kitajima et al., 1991).

In the aquaculture of epinepheline fishes, heavy mortalities during early developmental stages are obstacles to progress. The small mouth size has been suggested as one of the causes of such heavy mortalities (Hussain and Higuchi, 1980; Kayano, 1988; Kohno et al., 1997), the mouth size of larvae at the time of commencement of feeding being an important factor in choosing appropriate food for the achievement of a high survival rate. Shirota (1970) examined the mouth size of larvae of 33 species (*Epinephelus* species not included) at the time of commencement of feeding and concluded that the quicker the growth of fish, the greater the mouth size at the time of commencement of feeding. The mouth size of first feeding larvae of *E. bruneus* in this study averaged 233 μm, according to the Shirota's calculation of mouth size (mouth size=[upper jaw length]$\times 2^{0.5}$). This belongs to the smallest size group, together with *Hypomesus olidus* (Pallas), *Ammodytes personatus* (Girard) and *Platycephalus indicus* (Linnaeus), but is larger than that of *E. akaara*, which ranged from 156 to 204 μm (Kayano, 1988).

First appearance of *E. bruneus* jaw teeth (6.01 mm BL) was at a larger body size than representative mass-cultured species such as *Pagrus major* (4.40

mm SL, Kohno et al., 1983), *Lates calcarifer* (Bloch) (2.62 mm TL, Kohno et al., 1996) and *Seriola quinqueradiata* (Temminck & Schlegel) (4.6 mm SL, Fukuhara et al., 1986), but at a smaller size than *E. akaara* (6.8 mm SL, Fukuhara and Fushimi, 1988). Kohno et al. (1997) pointed out that the delayed development of feeding-related bony elements was a cause of the difficulty in rearing early-stage epinepheline larvae. This is also considered to apply to *E. bruneus*.

Large granular cells were densely distributed in the epidermis of *E. bruneus* larvae and other epinepheline larvae, such as *E. akaara* (see, Kaji et al., 1995) and *E. septemfasciatus* (Thunberg) (Sawada, unpubl. data). These larvae are often observed to have a thick epidermal mucus coat. Staining showed that the large granular cells in the epidermis of *E. bruneus* were in large part mucous cells. Altogether, three types of mucous cells, characterized by different mucus composition, occur. Cells stained with AB contain acidic glycoprotein in the mucus, whereas those showing PAS-positive staining contain neutral carboxylated and sulfated glycoprotein (Sano, 1985). PAS-positive cells of *E. bruneus* were divided into two types: cells with a weak PAS-positive reaction throughout, and those containing a small PAS-positive spot. The appearance and sizes of these cells changed with growth.

A number of functions have been ascribed to the mucus layer, such as protection against mechanical injury (Pickering and Richards, 1977), provision of a primary barrier against infection by microorganisms (Ingram, 1980) and reduction of friction (Rosen and Conford, 1971). On the other hand, mucous cell development in *E. akaara* has been examined in order to understand its relationship to and the mechanism of high mortality in the prelarval stage around the time of mouth-opening (Kaji et al., 1995). At this stage, *E. akaara* larvae die from becoming dried-out when they are attached to the water surface and their body surface exposed to the air (Takaya, 1987). This has also been observed in newly-hatched larvae of *E. bruneus* and *E. septemfasciatus* and sometimes causes heavy mortality (Sawada, unpubl. data). *E. septemfasciatus* has been reported to have a thick mucus coat on the larval body surface and large granular cells in the epidermis (Kitajima et al., 1991), as in *E. bruneus* in this study. The thick mucus coat is probably responsible for the death of epinepheline larvae when they become attached to the water surface under rearing conditions. While the existence of mucous cells in the epidermis is thought to be one of the morphological characteristics of grouper larvae, more research is needed for an understanding of the function of the mucus coat and for clarifying the mechanism of mucus coat-related deaths.

Lower jaw deformation has often been observed in reared specimens of *E. septemfasciatus* (Sawada, unpubl. data) as well as *E. bruneus*. The cause of such deformation is unknown, but apparently stems from a common factor, such as a shortage of some nutrients in the food supply.

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