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Morphological Development of Larval and Juvenile Grouper, *Epinephelus fuscoguttatus*

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Abstract The morphological development of larval and juvenile grouper, *Epinephelus fuscoguttatus*, was examined in a hatchery-reared series. By about 4 mm body length (BL), the larvae had developed pigment patterns peculiar to groupers, such as melanophores on the dorsal part of the gut, on the tip of the second dorsal and pelvic-fin spines, and in a cluster on the ventral side of the tail. The spines characteristic of groupers, such as spinelets on the second dorsal and pelvic-fin spines, the preopercular angle spine and the supraocular spine, started to develop by about 5 mm BL. The notochord end was in the process of flexion in larvae of 5 to 6 mm BL, by which time major spines and pigments had started to appear. The fin ray counts attained the adult complement at about 8 mm BL. Major spines disappeared by 15-16 mm BL, from which size, more or less densely-pigmented patches started to appear on the body. In juveniles larger than 20-22 mm BL, the lengths of the second dorsal and pelvic-fin spines in relation to BL became stable.

Groupers have become a challenging target for aquaculture scientists on account of their importance as cultured fish. Although difficulties in larval rearing have impeded their mass seed production, aquaculture scientists, especially those in Asian countries, have succeeded after much effort, in producing larvae of some *Epinephelus* species on experimental scales (Anonymous, 1987; Fukuhara, 1989; Kohno et al., 1990b; Quinitio and Toledo, 1991). The above studies primarily focused on topics concerning larval rearing techniques, rather than detailed morphological development. On the other hand, American and Australian schools have pursued research on grouper larval taxonomy (Johnson and Keener, 1984; Kendall, 1984; Leis, 1986, 1987), following Kendall's (1979) characterization of grouper larvae. Because the materials for the latter studies were, however, wild-caught samples collected by plankton hauls, the study of grouper larval taxonomy has been hindered by a shortage of materials, as pointed out by Leis (1987). These circumstances have necessitated detailed examination of larvae reared in hatcheries, as done recently by Powell and Tucker (1992) for the Nassau grouper, *E. striatus*.

The grouper, *Epinephelus fuscoguttatus*, a species widely distributed in Indo-Pacific tropical and subtropical waters (Randall and Heemstra, 1991) and seen as a promising candidate for culture (Lim et al., 1990; Lim, 1991), has been spawning spontaneously at the Bojonegara Research Station, Research Institute for Coastal Aquaculture, Indonesia, since 1989. This has enabled the accumulation of information on several aspects of larval and juvenile development in the species (see papers in Anonymous, 1990, 1991). In the present study the morphological development of hatchery-reared *E. fuscoguttatus* was examined. Attempts were made to establish ontogenetic intervals for the species, based on the development of the characters examined.

Materials and Methods

The material used in this study came from eggs spawned naturally on 17 August 1990, in a 30-ton concrete tank, in which three males of 5.4-8.6 kg body weight (BW) and seven females of 4.0-6.3 kg BW had been stocked. The number of specimens actually participating in spawning could not be ascertained. The total number of eggs spawned was 2.64×10^6 and the fertilization rate 98.4%. Eggs floating in the tank were transferred into a 3-ton FRP tank at a density of about 100 eggs/*l* for incubation and larval rearing. The estimated hatching rate was 75.0%. The feeding scheme for the resulting larvae and juveniles was as follows: rotifers at a

density of 10 ind./ml, from day 2 (two days after hatching) to day 28; *Artemia* nauplii, 1 ind./ml, from day 15 to day 28; and minced fish, appropriate amount given several times a day, from day 21 until the termination of the experimental rearing period on day 40. During the experiment, water temperatures ranged from 26.0 to 29.5°C and salinity from 30 to 31‰.

Sampling of 1–13 fish was carried out at irregular intervals from day 1 to day 40, a total of 155 specimens (2.30–22.59 mm BL; specimens deposited in the Museum, Tokyo University of Fisheries, MTUF 26896) being fixed and preserved in 5% formalin. In addition, three wild specimens, 46.5, 56.4 and 60.1 mm BL, collected from the shoreline at Bojonegara and preserved in 10% formalin (MTUF 26897) were used as supplemental samples. In this study the size of specimens was expressed as BL *sensu* Leis and Trnski (1989) under preserved conditions.

All the preserved specimens, including wild-caught, were used for the studies of relative growth, pigmentation and spine development. Of these, 79 specimens (2.72–8.37 mm BL, from day 7 to day 21) were used for examination of the notochord flexion angle and fin ray development. Some specimens were cleared and stained for easier observation, following Dingerkus and Uhler (1977), or stained solely with alizarin red. Terminology and methods of measurements generally followed Kohno et al. (1983, 1985), Johnson and Keener (1984), Kendall et al. (1984), and Leis and Trnski (1989).

Results

Development of gross morphology of *Epinephelus fuscoguttatus* larvae and juveniles is illustrated in Figure 1.

Notochord flexion

The notochord end had started bending upward at 4.75 mm BL. The largest specimen with a straight notochord was 4.99 mm BL. The largest flexion larva was 6.31 mm BL and the smallest postflexion larva 4.92 mm BL. The size range of 5–6 mm BL was thus considered to be the subdivision of flexion larva.

Development of fin rays

The smallest specimen possessing fin rays was 2.84 mm BL, wherein incipient elements identified as the second dorsal and pelvic-fin spines were observed. However, the smallest specimen with measurable spines was 3.07 mm BL. The first dorsal spine was recognizable at 3.47 mm BL and the third at 4.82 mm BL. Thereafter, the increasingly posterior formation of dorsal spines continued. Caudal fin rays appeared first at 4.86 mm BL and soft dorsal, anal and pectoral fin rays synchronously at 5.62 mm BL. The smallest specimen having an adult complement of fin ray counts (cf., Randall and Heemstra, 1991) was 7.75 mm BL, while the largest one with an incomplete fin ray complement was 8.04 mm BL.

Relative growth

Among six body parts measured in relation to BL, head length, predorsal length, preanal length and body depth at the anus increased rapidly and reached a constant level, whereas snout length and eye diameter increased somewhat parabolically, attaining a peak before subsequently declining. After an initial declining, the snout length maintained a constant level (Fig. 2). The peak in snout length (11–12% BL) was observed at about 6 mm BL. Predorsal length and head length reached their respective constant levels of 40–45% and around 40% BL at about 7 mm BL. At about 10 mm BL, eye diameter reached a peak of about 13% BL, and body depth at the anus reached a constant level of 25–30% BL. Snout length and preanal length maintained a constant level of 8–10% and 65–70% BL, respectively, in larvae beyond about 12 mm BL. The eye diameter proportion decreased continuously after the peak, and no steady level was observed in the specimens examined.

The second dorsal and pelvic-fin spines, both of which were first observed at 3.07 mm BL, increased in length and reached their respective maximum recorded lengths, 5.00 and 4.28 mm, in an 8.30 mm BL specimen. Thereafter, the length of the spines reduced to 3.09 and 3.03 mm, respectively, in the largest reared specimen, 22.59 mm BL. However, the lengths of the second dorsal and pelvic-fin spines were 5.51–6.31 and 6.29–6.60 mm, respectively, in the three wild-caught specimens, 46.5–60.1 mm BL. Lengths of the second dorsal and pelvic-fin spines relative to BL are plotted in Figure 3. Both the spines developed somewhat parabolically and subsequently maintained constant levels. The peaks of the second dorsal and pelvic-fin spines, 70% and 60%

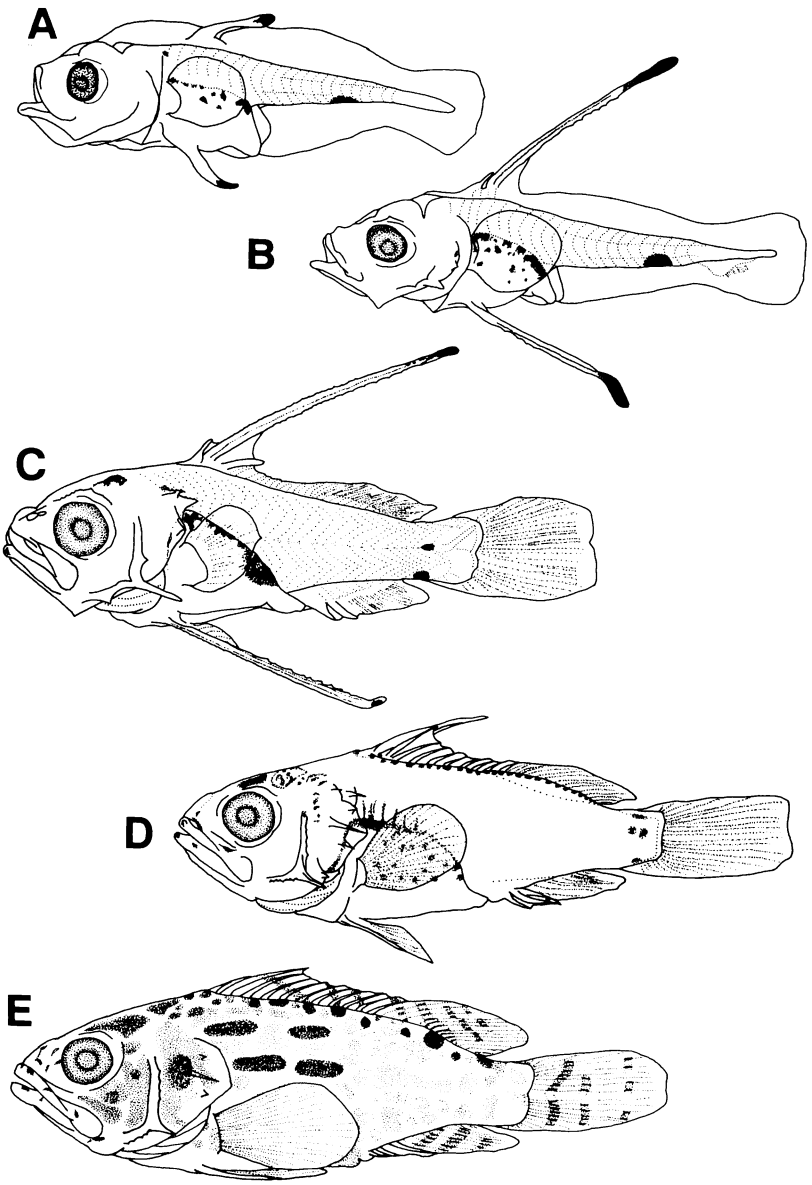

Fig. 1. Development of *Epinephelus fuscoguttatus*. A) 3.28 mm BL; B) 6.31 mm BL; C) 8.04 mm BL; D) 15.82 mm BL; E) 19.80 mm BL.

BL, respectively, occurred at about 6 mm BL, with a constant level of 10–15% BL being maintained in larvae larger than about 20 mm BL.

The preopercular angle spine measured in relation to BL also developed parabolically (Fig. 3), reaching a peak of around 14% BL at about 9 mm BL. Thereafter, the spine length decreased continuously to 1.59% BL in the largest reared specimen, 22.59 mm BL, and to 0.52–0.99% BL in the wild specimens, 46.5–60.1 mm BL.

Pigments

Larvae sampled on day 1, 2.35–2.48 mm BL, had no pigment except for melanophores scattered on the snout. These had disappeared in specimens sampled on and after day 2, irrespective of larval size.

By about 3 mm BL, pigment patterns characteris-

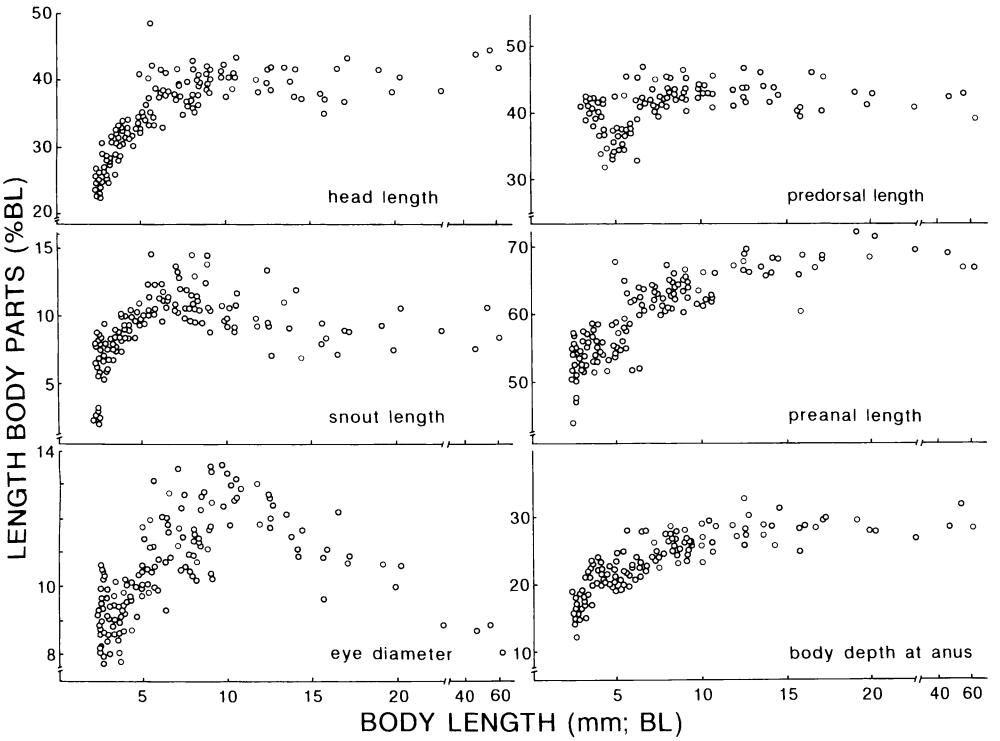

Fig. 2. Some body proportions of *Epinephelus fuscoguttatus*, shown as percentages of body length.

tic of grouper larvae had appeared as follows: melanophores scattered on the dorsal part of the gut, which developed into a dorsal cap of melanophores over the gut in later developmental stages, in specimens larger than 2.30 mm BL; four out of 28 specimens (2.30–2.74 mm BL) had a small melanophore on the ventral side of the tail, with a cluster of external melanophores appearing on the ventral side of the tail at 2.80 mm BL; the tips of the second dorsal and pelvic-fin spines, which were covered with swollen membranous sheaths, had become heavily pigmented by 3.07 mm BL. All larvae up to about 5 mm BL had these characteristic pigments. In addition, small internal melanophores appeared dorsally on the anterior part of the notochord in larvae larger than 2.72 mm BL. However, the presence of the latter pigment was subject to considerable individual variation (48.0% in 100 specimens, 2.72–9.95 mm BL). The pigment became invisible when the larvae reached about 10 mm BL.

Pigment started to develop in several other body parts in larvae larger than about 5 mm BL: the upper and lower jaw tips at 4.75 mm BL; the cleithral symphysis at 4.92 mm BL; the midbrain at 5.62 mm BL; the upper margin of the maxillary at 6.22 mm BL; the base of the 1st and 2nd dorsal spines and the membrane between the 2nd and 3rd anal spines at 7.06 mm BL; the forebrain at 7.12 mm BL; the operculum at 7.60 mm BL; and the soft dorsal fin base at 9.04 mm BL. However, the presence of pigment in some of these body parts was subject to considerable individual variation, e.g. the cleithral symphysis pigment appeared in 35 out of 75 specimens (46.7%) ranging from 4.92 to 15.60 mm BL.

Some melanophores forming the dorsal cap of the gut extended upward along myosepta in some specimens larger than 4.92 mm BL. In the cluster on the ventral side of the tail, some melanophores began to migrate upward at 4.92 mm BL, forming an internal cluster at the midlateral part of the tail. Some of the midlateral melanophores then extended laterally to form external melanophores. All specimens from 5.62–7.06 mm BL had both ventral and midlateral melanophores on the tail. In larvae larger than 7.12 mm BL, whereas the midlateral pigment was always observed, the presence of pigment on the ventral and dorsal sides of the tail varied individually as follows: out of the 49 specimens examined (7.12–15.82 mm

BL), only the ventral melanophore was present in 36 specimens (73.5%), only the dorsal melanophore in three (6.1%), both were present in two (4.1%), and neither were present in eight (16.3%). In addition to the melanophores covering the sheath of the second dorsal and pelvic-fin spine tips, some melanophores forming a single row on the distal part of the spines appeared as the larvae grew. However, the melanophores on both spines became scarce as the larvae developed further.

The largest specimen showing larval pigment traits was 15.82 mm BL, while the smallest one with pigments considered to be transitional to the juvenile state, i.e., more or less densely pigmented patches appearing on the body, was 12.44 mm BL. More distinct body patches appeared and increased in number as the larvae grew. In three wild-caught specimens, 46.5–60.1 mm BL, the body was covered by densely-pigmented, polygonal aggregations, separated by a thin whitish meshwork. In addition, two densely-pigmented blotches were positioned on the anterolateral part of the body, five along the dorsal fin base and one on the dorsal part of the caudal peduncle.

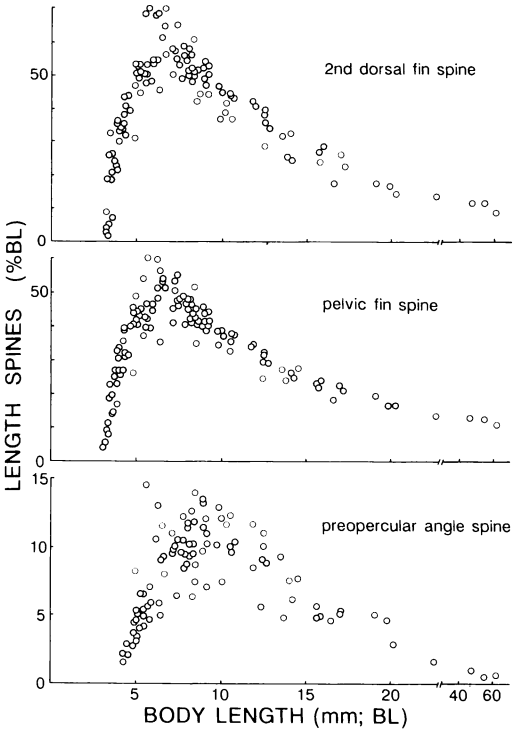

Fig. 3. Proportions of the second dorsal and pelvic-fin spines, and the preopercular angle spine of *Epinephelus fuscoguttatus*, shown as percentages of body length.

Spines

Major spines first appeared at a size range of 4–5 mm BL: spinelets on the second dorsal and pelvic-fin spines at 3.85 mm BL; the inner preopercular spine at 4.00 mm BL; the supraocular and preopercular angle spines at 4.27 mm BL; the posttemporal spine at 4.86 mm BL; and the supracleithral spine at 4.92 mm BL. An opercular spine was first observed at 5.48 mm BL and the interopercular and subopercular spines at 6.22 and 9.09 mm BL, respectively.

The number of opercular spines had increased to three, as in adults, by 11.80 mm BL. The supraocular spine disappeared by 11.87 mm BL, at which size some specimens had a serrate supraocular bone with a spine. Six specimens, 12.45–15.82 mm BL,

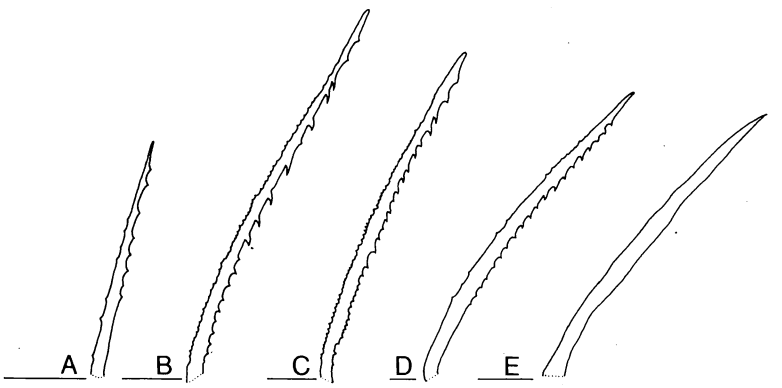

Fig. 4. The second dorsal spine (left lateral view) of *Epinephelus fuscoguttatus*. A) 4.00 mm BL; B) 5.42 mm BL; C) 8.01 mm BL; D) 15.60 mm BL; E) 22.59 mm BL. Scale bar indicates 0.5 mm.

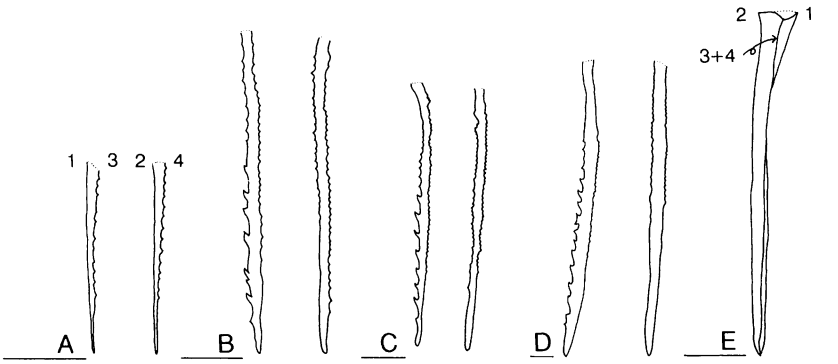

Fig. 5. The left pelvic fin spine (ventromedial and ventrolateral views) of *Epinephelus fuscoguttatus*. *1*—dorsomedial ridge; *2*—ventromedial ridge; *3*—ventrolateral ridge; *4*—dorsolateral ridge. A) 4.00 mm BL; B) 5.42 mm BL; C) 8.01 mm BL; D) 15.60 mm BL; E) 22.59 mm BL. Scale bar indicates 0.5 mm.

had the serrate bone only. The inner preopercular and posttemporal spines had disappeared by 14.00 mm BL. Out of 57 specimens, 6.13–14.00 mm BL, 42 had a serrate ridge at the center of the preopercular angle spine. The largest specimens having the interopercular, supracleithral and subopercular spines were 17.00, 17.21 and 21.18 mm BL, respectively.

The second dorsal spine developed three ridges, comprising an apex ridge and posterolateral wings, by 3.28 mm BL, but no spinelets were observed on the ridges at that time. They subsequently appeared on both the apex and posterolateral wings by 3.85 mm BL (Fig. 4A), although spinelets were observed on the posterolateral wings only in two specimens, 4.17 and 4.26 mm BL. The spinelets on the posterolateral wings became enlarged and recurved, larvae having them ranging from 4.51 to 15.82 mm BL (Fig. 4B–D). However, the enlarged spinelets decreased in size as the larvae grew. In a 17.00 mm BL specimen, spinelets were seen on the posterolateral wings, but not on the apex ridge. Larvae larger than 16.57 mm BL, except for a single 17.00 mm BL specimen, lacked spinelets on the apex ridge and posterolateral wings (Fig. 4D).

An incipient pelvic fin spine, covered with a swollen membranous sheath, developed three ridges formed from ridges 3 (ventrolateral) and 4 (dorsolateral) and the posterior membranous sheath keel by 3.28 mm BL. At 3.85 mm BL, ridges 1 (dorsomedial) and 2 (ventromedial) appeared to form four ridges (plus the membranous sheath keel), with spinelets being first seen on ridges 3 and 4 (Fig. 5A). All the ridges had spinelets in two specimens, 4.51 and 4.91 mm BL. Specimens ranging from 4.75 to 15.82 mm BL developed spinelets on ridges 2, 3 and 4 and enlarged, recurved spinelets on ridge 1 (Fig. 5 B, C). The enlarged spinelets became more widely spaced and bifurcate with growth (Fig. 5D). The lesser spinelets had disappeared in three specimens, 13.72–17.00 mm BL, although the enlarged spinelets remained. In specimens larger than 16.57 mm BL, the number of ridges decreased to three, comprising 1, 2 and 3+4 ridges, as in adults (Fig. 5E).

Discussion

Ontogenetic intervals

The development of the characters examined in this study is schematically represented in Figure 6. Based on the developmental events, *Epinephelus fuscoguttatus* larvae and juvenies were divided into seven intervals, as below (Fig. 6) (yolk-sac larvae were beyond the scope of the present study; see Kohno et al. [1990a, c] for the development of larvae from hatching to about 3 mm BL).

Interval A (to about 4 mm BL): larvae start to develop pigment patterns characteristic of grouper larvae, i.e. melanophores on the dorsal part of the gut, which develop to a dorsal cap of melanophores over the gut in further developmental stages, melanophores on the tip of the second dorsal and pelvic-fin spines, and a cluster of melanophores on the ventral side of the tail; no spines are seen; the notochord end is straight. Interval B (from 4 to about 5 mm BL): the major spines characterizing grouper larvae, i.e.

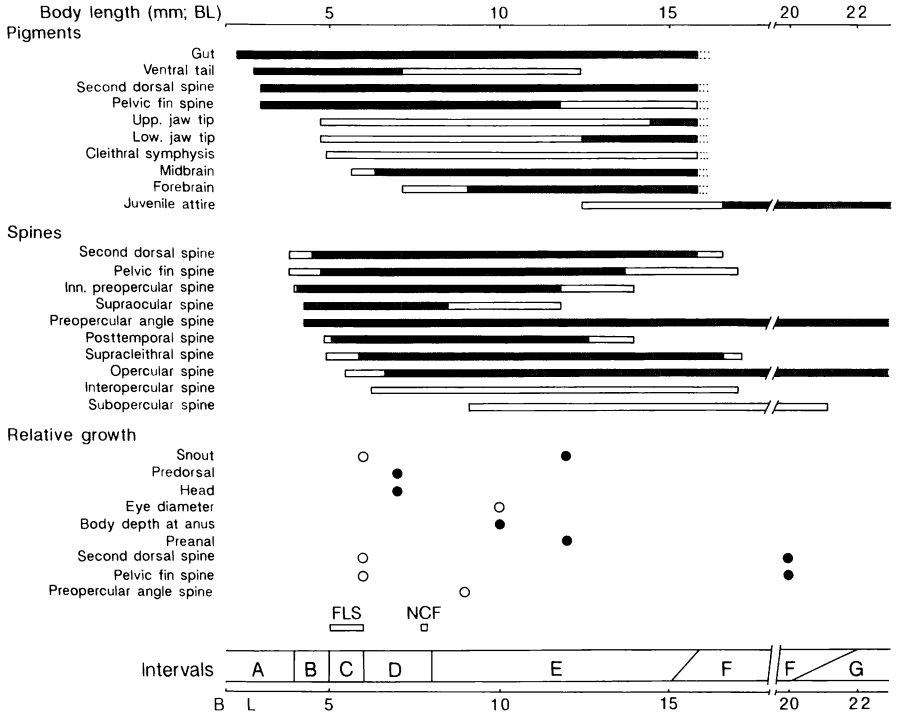

Fig. 6. Schematic representation of the development of pigment, spines and relative growth (length of body parts in relation to body length) in hatchery-reared *Epinephelus fuscoguttatus*. Flexion larva subdivision (*FLS*) and numerical complement of fin-rays (*NCF*) are also shown. Appearance of pigment and spines subject to individual variation (□). Pigment and spines present in all specimens examined (■). Peak value of body proportion (relative to BL) (○). Attainmnet of constant value by body proportion (relative to BL) (●). For each interval, see text.

spinelets on the second dorsal and pelvic-fin spines, preopercular angle spine and supraocular spine start to develop; the notochord end is still straight. Interval C (from 5 to about 6 mm BL): the notochord undergoes flexion; major pigments and spines start to form. Interval D (from 6 to about 8 mm BL): notochord flexion is complete; all larval pigments and spines appear; some body proportions relative to BL peak or reach constant levels. Interval E (from 8 to 15–16 mm BL): the fin ray counts attain an adult complement; some body proportions reach peaks or constant levels. Interval F (from 15–16 to 20–22 mm BL): in addition to the larval pigments, more or less densely-pigmented patches start to appear on the body; major spines disappear. Interval G (beyond 20–22 mm BL): lengths of the second dorsal and pelvic-fin spines relative to BL attain a constant level, probably representing juvenile proportions.

Sakai (1990) recommended using the peaks in histograms of developmental event numbers as thresholds for establishing intervals in his study on morphological development related to feeding and swimming functions in the cyprinid *Triborodon hakonensis*. The histogram method is considered useful, as shown by Sakai (1990), for characters related to larval functions (and hence survival), because such characters are hardly subject to individual variation of presence or absence. However, it appears difficult to apply the method to characters such as those examined in the present study. Therefore, the characters used as "threshold-indicators" in this study are some key characters such as the appearance of pigment and spines, notochord flexion, fin-ray counts, pigment distribution patterns and certain body proportions.

Whether or not the intervals established in this study reflect any changes in larval/juvenile habits of *E. fuscoguttatus* is unknown, since little data have thus far been obtained on their habits. According to Slamet et al. (1991), digestion time and rotifer consumption in *E. fuscoguttatus* larvae shortened and increased, respectively, at about 5 mm BL, which

corresponds to the boundary of Intervals B and C in this study. At this boundary, about 5 mm BL, *E. fuscoguttatus* larvae started to ingest *Artemia* nauplii, when the larvae were fed with rotifers and *Artemia* nauplii as food (Supriatna and Slamet, 1991). The consumption of *Artemia* nauplii increased rapidly at a size range of 5–6 mm BL (Supriatna and Slamet, 1991), corresponding to Interval C.

Preliminary grouping of Indo-Pacific *Epinephelus* larvae

Although few attempts have been made to compare Indo-Pacific *Epinephelus* larvae due to the limited information available, a preliminary grouping of some Indo-Pacific *Epinephelus* larvae is attempted below, based on pigment data obtained from this study and from published accounts.

The distribution mode of gut (and trunk) pigments divides early larval *Epinephelus* into three groups: 1) a cap of melanophores covers the gut dorsally, the melanophores sometimes extending upward along the myosepta in *E. akaara* (*sensu* Ukawa et al., 1966; Mito et al., 1967), *E. fuscoguttatus* (present study) and *E. septemfasciatus* (Kitajima et al., 1991); 2) in addition to the dorsal cap, melanophores appear on the ventral part of the gut and extend upward on the gut surface in *E. akaara* (*sensu* Tseng and Ho, 1979), *E. amblycephalus* (Tseng and Chan, 1985), *E. malabaricus* (*sensu* Hamamoto et al., 1986; Lin et al., 1986), *E. moara* (Manabe and Kasuga, 1988) and *E. tauvina* (*sensu* Hussain and Higuchi, 1980); and 3) melanophores develop on both the dorsal and ventral sides of the gut and trunk, producing a transverse band of melanophores, in *E. malabaricus* (*sensu* Maneewong et al., 1986; Predalumpaburt and Tanvilai, 1988), *E. tauvina* (*sensu* Chen et al., 1977) and *E. suillus* (Doi et al., 1991).

On the other hand, *Epinephelus* larvae are divided into four groups on the basis of pigments on the tail (data sources are the same as above unless otherwise noted): 1) no melanophores appear on the tail in *E. amblycephalus* (this is an atypical grouper pigmentation, see Powell and Tucker, 1992); 2) a melanophore appears on both the dorsal and ventral sides of the tail in early stages, the former subsequently disappearing and the latter developing to a cluster of melanophores in *E. septemfasciatus*; 3) a large cluster of melanophores appears on the ventral side of the tail in *E. fuscoguttatus*, *E. malabaricus* (*sensu* Hamamoto et al., 1986; Lin et al., 1986; Predalumpaburt and Tanvilai, 1988), *E. moara* and *E. tauvina*; and 4) a row of several small melanophores appears initially, and develops into a cluster on the ventral side of the tail in *E. akaara*, *E. malabaricus* (*sensu* Maneewong et al., 1986) and *E. suillus*.

Johnson and Keener (1984) examined in detail the structure of the second dorsal and pelvic-fin spines of American groupers. However, no detailed descriptions are available on Indo-Pacific *Epinephelus* species, although Kitajima et al. (1991) summarized the maximum ratio of second dorsal and pelvic-fin spine lengths to BL in some Indo-Pacific *Epinephelus* species.

Further comments and concluding notes

On the structure of the pelvic fin spine, Leis (1986) stated that in *Cephalopholis* ridges 3 and 4 arose from a combined proto-ridge, later than the other two ridges. Although detailed histological examination was not carried out, the present study showed that in *Epinephelus*, ridges 3 and 4 were formed prior to ridges 1 and 2. This difference suggests a large gap between the two genera, although a close relationship has been recognized between the two on the basis of adult characters (Leis, 1986). Further detailed studies are needed on the osteological development of the pelvic fin spine.

In his analysis of relationships between six genera of the tribe Epinephelini, Leis (1986) considered the series of ventral pigment on the tail to be a primitive character state within the tribe. Johnson (1988) disagreed with Leis' polarity assessment and mentioned that the pigment series is less general among the epinephelins, with which the present study concurs.

Clearly, more detailed studies are needed on the morphological development of grouper larvae, especially on hatchery-reared series. Grouper studies thus far made in Asian countries have focused on larval-rearing techniques, with mass seed production of some *Epinephelus* species having been achieved at some experimental institutions. However, no studies have dealt with detailed morphological development of grouper larvae obtained from hatcheries. The present study, as well as the work of Powell and Tucker (1992), demonstrated that such larval series can provide good material for the study of morphological development in groupers. Studies of such material will also provide useful information on the

early life history of groupers.

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アカマダラハタ仔稚魚の形態発育

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アカマダラハタ飼育仔稚魚の形態発育を観察した．体長4mmまでにハタ類に特徴的な黒色素胞，すなわち腹腔背面，第2背鰭棘と腹鰭棘の先端および尾柄腹面の黒色素胞が出現した．一方，第2背鰭棘と腹鰭棘上の小棘，前鰓蓋骨隅角棘および眼上棘は体長5mmまでに出現した．体長5–6mmの仔魚では脊索末端部が上屈中であり，また主な黒色素胞と頭部棘の発現が観察された．鰭条数が定数に達したのは体長8mmであった．主な頭部棘は体長15–16mmまでに消失する一方で，やや分布密度の濃い黒色素斑が体側に出現しはじめた．体長20–22mm以上の稚魚では体各部の体長比が一定となった．

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