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Growth of the Medaka (II) – Formation of Fins and Fin Appendages

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ABSTRACT

In the present study, we provide information on the development of the structure and composition of fins in the medaka. The pectoral fins, which begin to form in the embryonic stage, first form their fin rays after hatching prior to the formation of other median and pelvic fins. During the growth period from larvae to juveniles, the unpaired anal, caudal and dorsal fins begin to form before the pronounced degeneration or resorption of the median fin fold in the region of the caudal peduncle. The fin rays first appear in the caudal fin fold at the posterior end of the body, and subsequently fin rays of the anal and pectoral fins arise prior to those of the dorsal fin. Paired pelvic fins begin to sprout on the skin of the bilateral sides of a median ventral fin fold, which regresses concomitant with resorption of the fin fold at the dorsal and ventral regions of the caudal peduncle. After complete resorption of those portions of the ventral fin fold in which fin rays do not arise, the pelvic fin rays form.

The pectoral fins are supported by four small radials, the scapulas and the coracoids. Similarly, the pelvic fins are supported by pelvic girdles, the caudal fin by the hypural, and the anal and dorsal fins by distal and proximal pterygiophores. The appearance of these fin appendages closely follows that of fin rays in the fin fold.

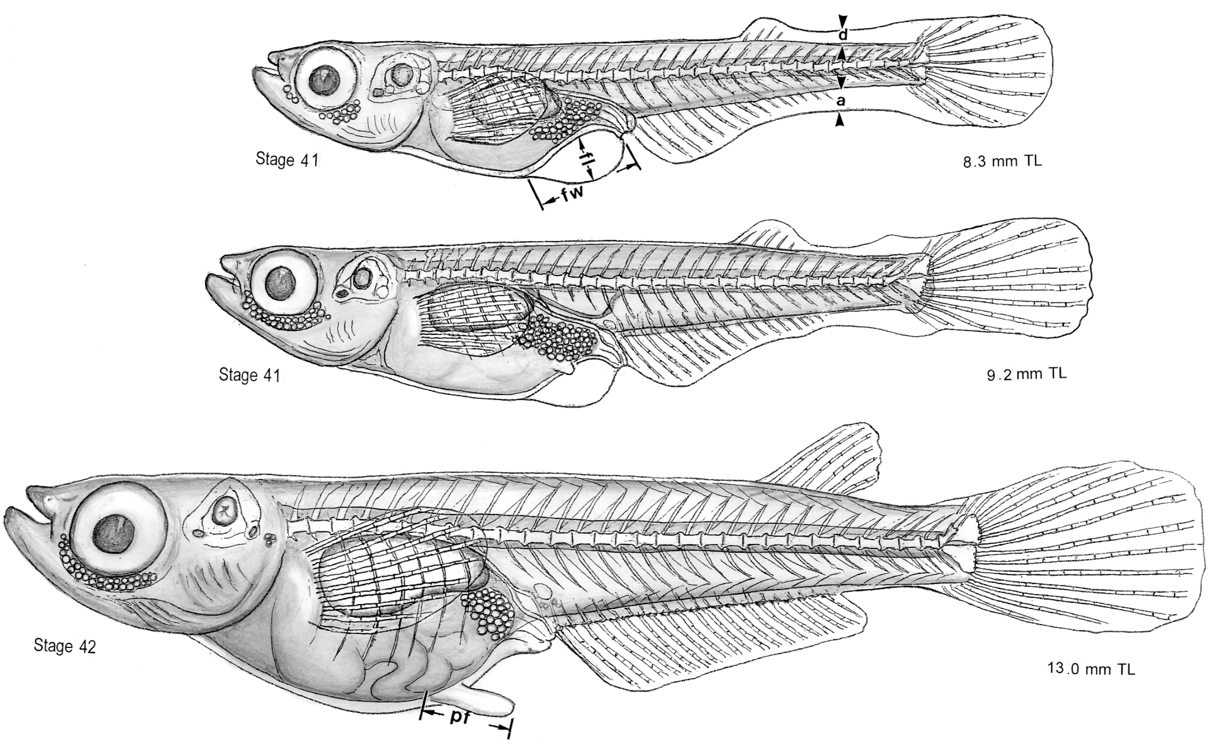
Keywords: fin fold, fin formation, fin appendage, larva, medaka, pterygiophore

INTRODUCTION

The sequence in which ossified structures develop provides evidence pivotal to systematic studies of fish larvae. Between the larval and juvenile stages, the determination of each fin's location and the formation of the fin skeleton are excellent developmental characters for use in systematic inferences. As pointed out by Kendall *et al.* (1983), two ontogenetic processes, loss of specialized larval characters and attainment of juvenile-adult characters, occur during this stage of transition between the larva and the juvenile.

Fin growth in teleosts includes the enlargement and elongation of the soft fin rays with multiple segments. The process of segment (joint or node) formation in the elongating fin rays appears to continue throughout the lifetime of the fish. However, basic information regarding morphogenesis of the fins in the medaka remains inadequate (Iwamatsu, 2010). Recently, the genetic control of fin length for other fishes has been reported by many investigators (van Eeden *et al.*, 1996; Mercader, 2007; Parichy *et al.*, 2009). Despite the increasing genetic and developmental information, little detailed fundamental research has been done on the anatomical changes in fin appendages during metamorphosis (van Eeden *et al.*, 1996).

We are curious to determine the common skeletal elements (i.e. the basic bones) of the fin appendages. We have undertaken careful observations on the development of fin appendages of growing medaka fish with the aim of understanding the developmental and evolutionary processes which have led to the fin diversity in teleosts. In the metamorphosis period, the stage assignment should be based on several fundamental features of morphological and morphometric criteria. Events in organ development are expressed in terms of total length, which is a more useful criterion than time for the developmental process. The transition process for each character of the body that defines a growth stage has not yet been observed in detail, although the stages from larva to adult of the medaka have already been described in a previous report (Iwamatsu *et al.*, 2003). The present paper provides data regarding the changes in morphology of the fins and their appendages during the transformation stages (stages 41–43) of the medaka. This information may provide an evolutionary perspective for investigations on limb formation in other vertebrates.



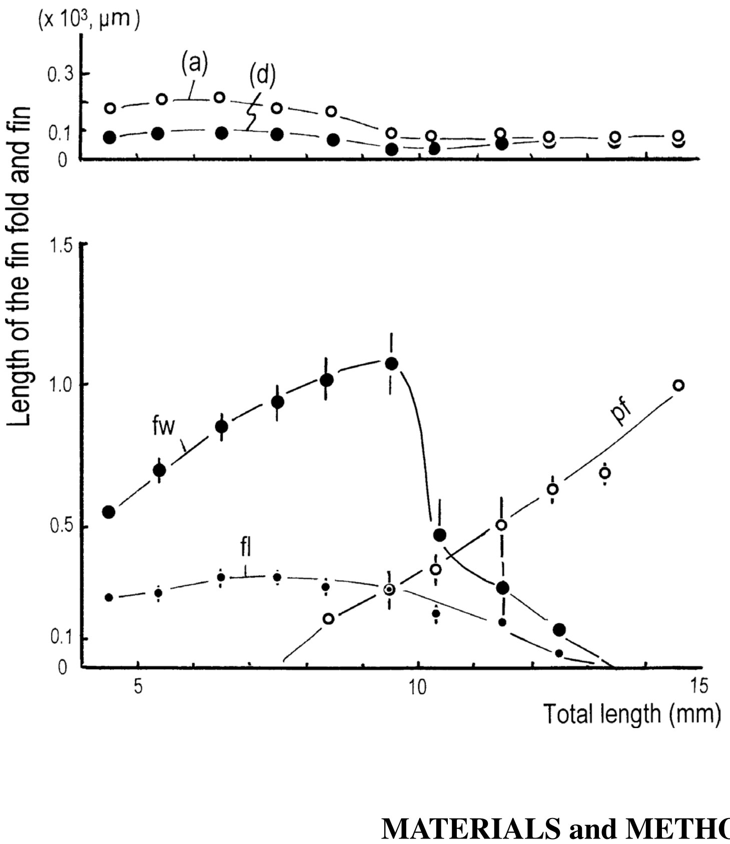


Fig. 1 Changes in the fin folds of the ventral and the caudal peduncle regions and the pelvic fins during metamorphogenesis. a and d, caudal peduncle fin folds at the anal (a) and the dorsal (d) sides; fl, fin fold length; fw, fin fold wide.

MATERIALS and METHODS

The medaka fish, *Oryzias latipes* (d-rR strain), used in the present study were reared in a rectangular glass aquarium (60×35×30 cm, 30–50 individuals per about 60 liters of water) under reproductive conditions (L14: D10, 26–28°C). Under these conditions, females mate with males and spawn every day. After females were netted, fingers were used to pluck off clusters of chorion-hardened eggs that hung from their bellies. During the rearing period, fish were fed a balanced diet containing one part each of shrimp powder, parched barley flour, Tetramin and Otohime no. 1 (Nisshin-seifun).

For observations, live fish were anesthetized in saline containing a mixture of 7 parts of phenylurethane and 3 parts of ethanol. The size of the whole body and the internal morphology were examined in anesthetized fish using a stereoscopic microscope

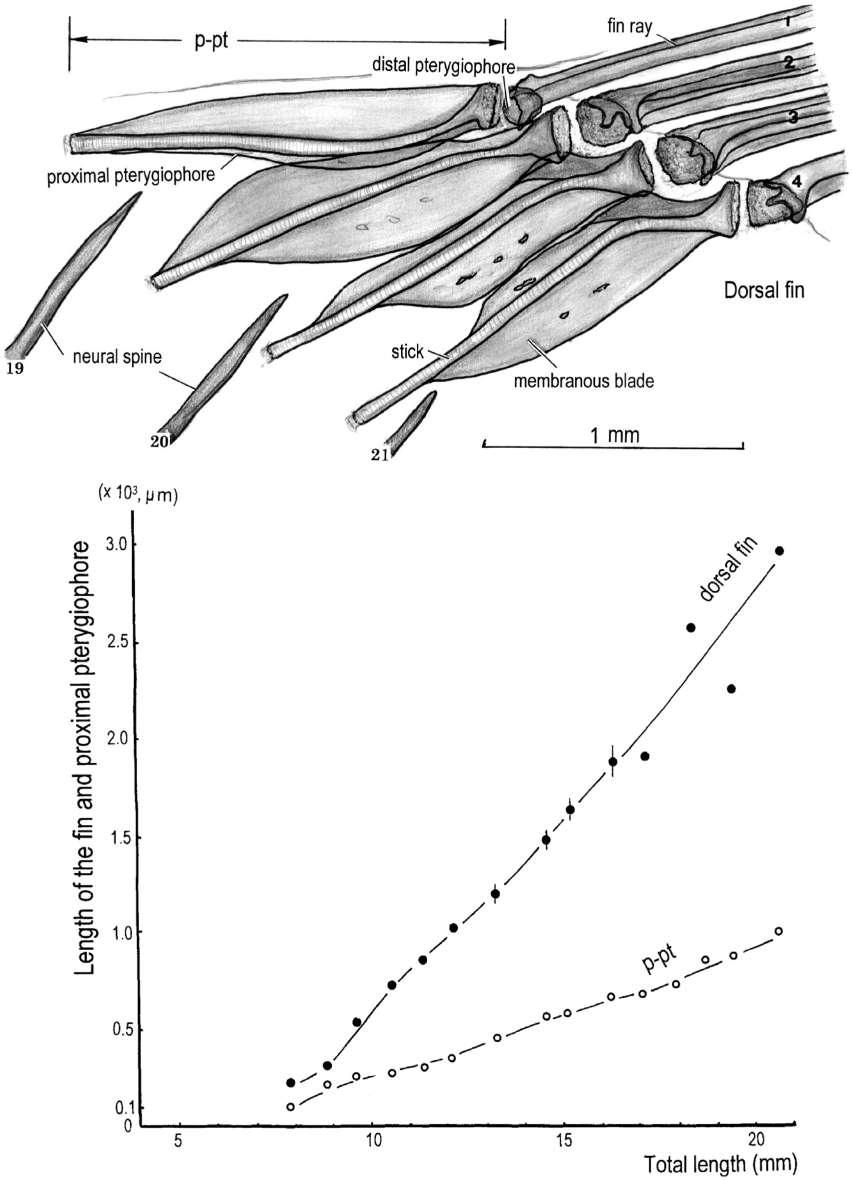


Fig. 2 Changes in the dorsal fin and its proximal pterygiophore in growth period. p-pt: proximal pterygiophore.

(×20, Olympus SZX12) equipped with a calibrated ocular micrometer. The width of the median fin fold at the restricted region of the caudal peduncle was measured between the distal ends of the haemal spines of the 23rd and 24th vertebrae. The length of the fin and its pterygiophores was measured using the 5th fin ray of the anal fin and the 2nd fin ray of the dorsal fin. Body size was represented by total length (TL, from the snout to the extremity of the caudal fin) by which the stage of fish development was determined.

For examinations of the skeleton, deeply anesthetized fish were fixed in 2% glutaraldehyde-saline for one hr. After they were rinsed in distilled water, the specimens were treated for one min in 0.5 N NaOH and then stained with 0.5% alizarin red S (phosphate buffered to pH 7.0: Nakarai Chemicals Ltd.) and alcian blue 8GX (Wako-junyaku) for several hrs. The stained specimens were again treated with 0.5 N NaOH for a few hrs, completely rinsed in tap water and finally cleared in 50% glycerol for observation. Growth stages of fish were assigned following the author's developmental criteria (Iwamatsu, 1994, 2004, Iwamatsu *et al.*, 2003). Names used for skeletal structures are the traditional ones provided by Owen (1984) and Yabumoto and Ueno (1984).

OBSERVATIONAL RESULTS and DISCUSSION

At hatching the medaka still has a large yolk sphere but begins to feed freely (about 4.5 mm TL). These yolk-larvae already possess most organs in a rudimentary or incomplete state. In the medaka, the larval stage corresponds to the period from stage 40 to stage 42 during which complete fin ray number is established and ray node segments appear in all fin rays. These stages are characterized by a loss of specialized larval characters and attainment of adult characters. The juvenile fish in stage 43 attain additional adult structures such as dichotomous branching at the tips of fin rays, increase in scale ridges and the secondary sex characters until stage 44 (Iwamatsu *et al.*, 2003), although they are superficially similar to the adult.

1) Fin development

Fin fold appearance:

In embryonic stages (St. 27; Iwamatsu, 1994, 2004), the paired fan-shaped fin folds of the pectoral fins first arise from fin buds (mesenchymal proliferation) . A caudal fin fold is recognizable in the tail of embryos at stage 29. The anterior point of the dorsal fin fold lies between the distal ends of the neural spines of the 18th and 19th vertebrae. The pectoral fin fold is well developed near the time of hatching.

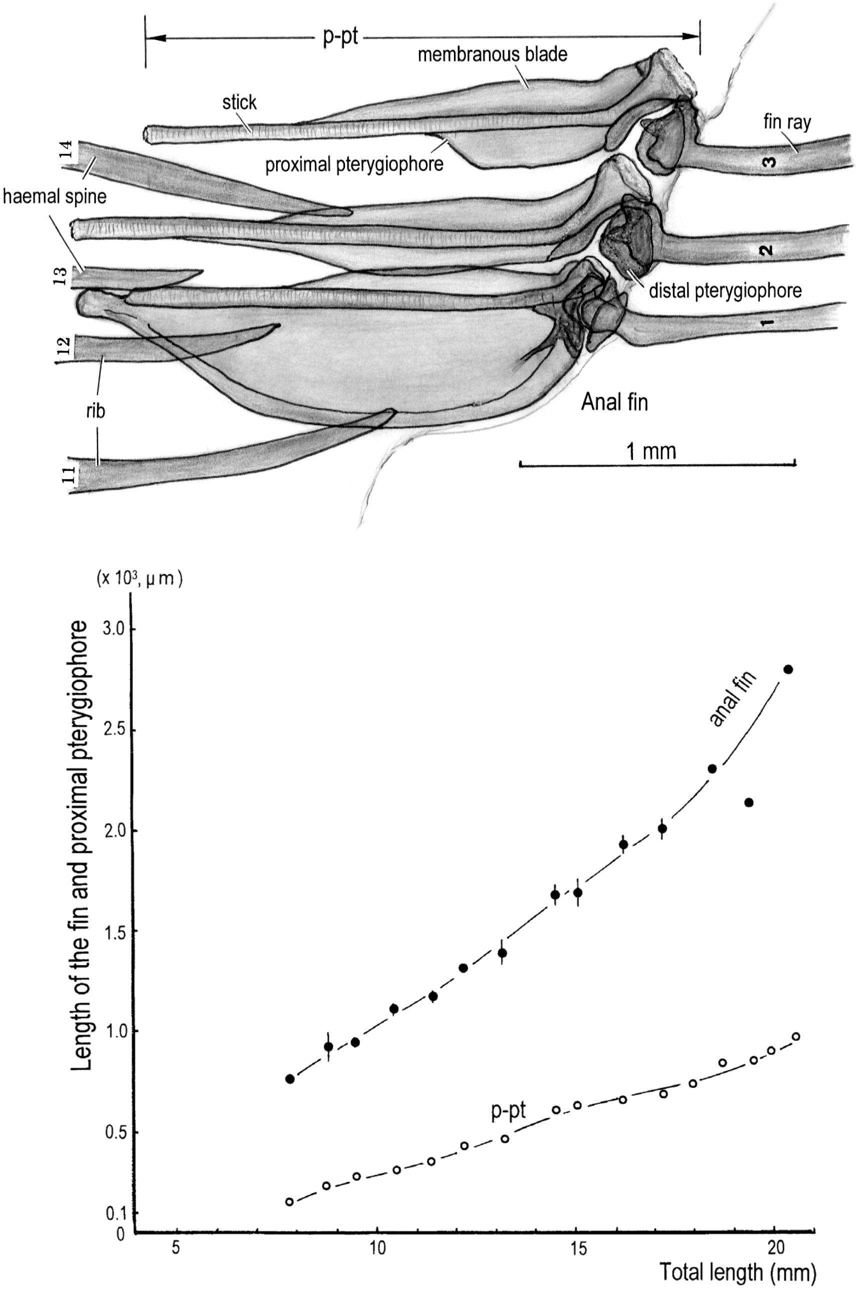


Fig. 3 Changes in the anal fin and its proximal pterygiophore in growth period. p-pt: proximal pterygiophore.

Fin formation:

The rudiments of the fin rays first appear in the caudal fin fold at the posterior end of the tail (St. 38). In stage 40, after hatching, fin rays first appear in the caudal fin. Adult fins consist of a fin fold and soft fin rays. A single soft ray is composed of an apposed pair of two long half fin ossicles which are segmented along their proximodistal axis, as seen in the zebrafish (Grandel and Schulte-Merker, 1998).

The cartilagious hypural elements are seen in the caudal fin fold at the distal end of larvae 4.7 mm TL. The development of the hypural complex that supports the rays of the caudal fin was described by Fujita (1992). The earliest segmentation is recognizable in the caudal fin rays of growing larvae 5.0 mm TL a few days after hatching. In the pectoral fins, fin rays begin to appear in larvae between 8.0 mm and 8.5 mm TL, and the first ray segment appears between 6.8 and 7.0 mm TL. The anal and dorsal fins differentiate from a single median fin fold of the tail, with ray formation occurring prior to the time when definite resorption of the fin fold at the margin of the caudal peduncle portion begins at about 9.0 mm TL. Conspicuous resorption of the fin fold in the ventral region commences in concert with that occurring at the caudal peduncle margin between the distal ends of the 23rd and 24th haemal spines (Fig. 1). However, the pelvic fins never arise from the ventral fin fold; instead the fin buds appear and develop to pelvic fins with fin rays beginning in larvae about 8.0 mm TL. Prior to the initiation of striking resorption of the ventral fin fold, the pelvic fins begin to sprout as rounded protuberances from the abdominal body wall at the bilateral sides of the ventral fin fold in larvae about 9.0 mm TL (Fig. 1). The lobed buds subsequently elongate as resorption of the ventral fin fold accelerates. The maximum ray number of the pelvic fins is achieved by 13.0 mm TL. The segmentation of the fin rays commences in larvae 12.0–12.5 mm TL. The ray number of pelvic fins ranges from five to seven in rice fishes, and six is the plesiomorphic number (Parenti, 2008).

Maximum fin ray number is achieved by about 12.0 mm TL for the caudal fin, and 18.0 mm TL and 18.5 mm TL for the dorsal and anal fins, respectively. Branching of the distal tips of the fin rays commences long after segmentation occurs, and dichotomous fin rays in all fins are observed in juveniles 16.0 to 24.4 mm TL (St. 43). There are usually 21 fin rays in the caudal fin, 6 fin rays in the dorsal fin and 18–20 fin rays in the anal fin (Fig. 3 in Iwamatsu *et al.*, 2003). The number of caudal ray segments that first form in larvae 5.3 mm TL shows a relatively linear increase for the longest fin rays as total length of the body increases. All fins complete segmentation of their rays in larvae of about 11.0 mm TL. As previously reported (Table 1 in Iwamatsu *et al.*, 2003), branching of the fin rays is completed at 17.4–17.8 mm TL in the pectoral fins, 16.0–16.2 mm TL in the

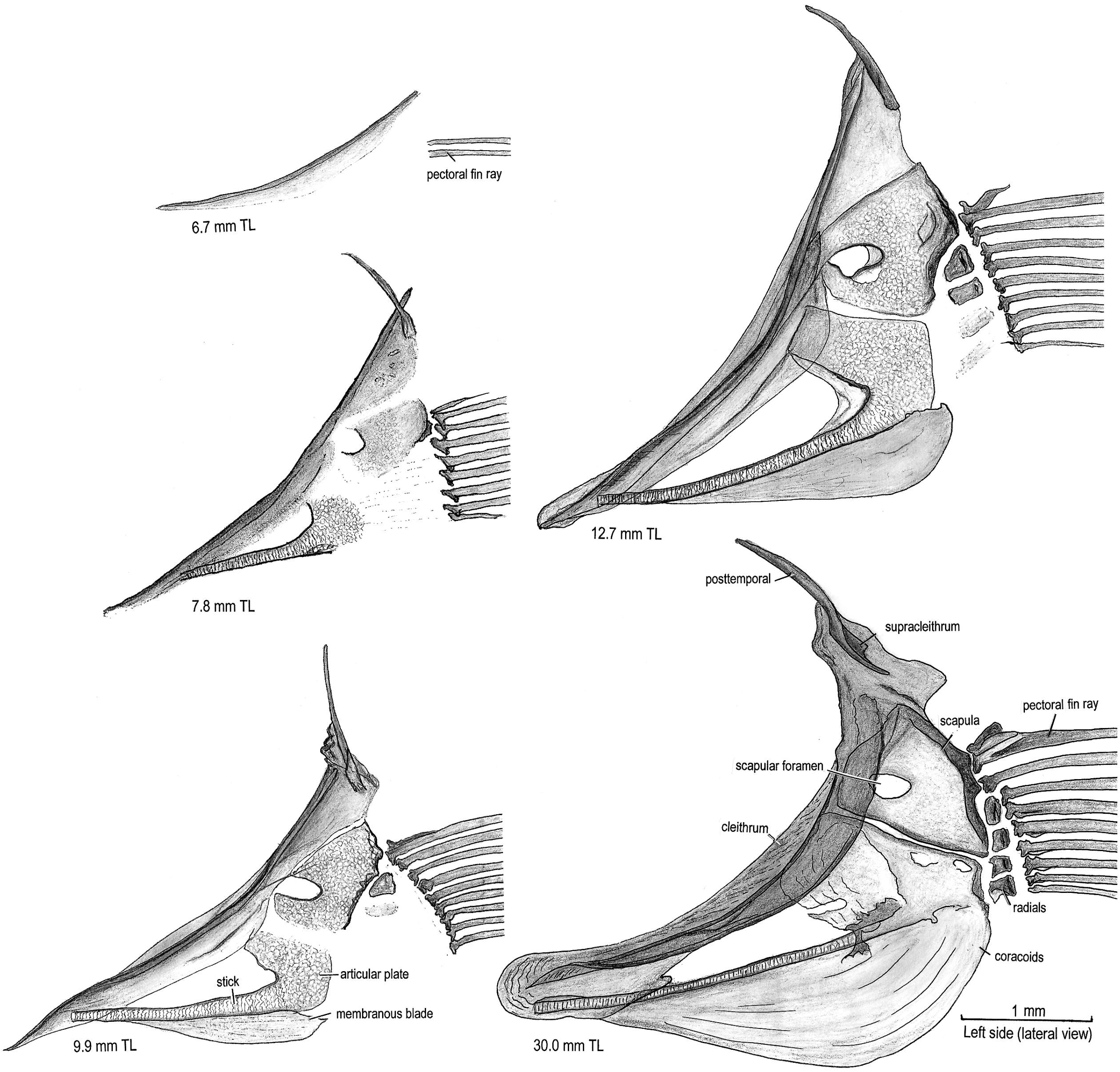


Fig. 4 Development of coracoid and scapula during metamorphogenesis.

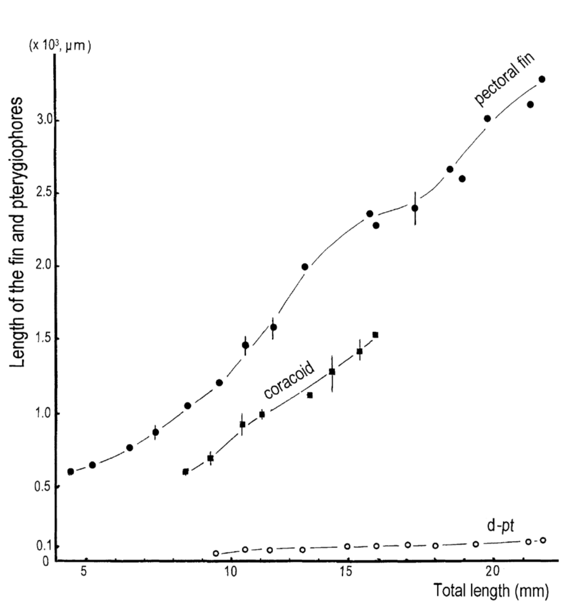


Fig. 5 Changes in the pectoral fins and their distal pterygiophore in growth period.

caudal fin, 21.0–24.0 mm TL in the anal fin, 18.0–18.3 mm TL in the dorsal fin and 17.4–17.8 mm TL in the pelvic fins. All fins complete the second ray branching by about 30 mm TL.

2) Development of median fin appendages

The ossification of rays in the unpaired fins begins prior to the initiation of fin appendage formation. In these early fins without any ray segments, the rounded distal pterygiophores lie between the paired bases of two apposed half fin rays (Figs. 2 and 3).

Appendages of the anal and the dorsal fins:

There is a small, rounded distal pterygiophore and an elongate proximal pterygiophore in each ray of the dorsal and anal fins (Fig. 3). In the anal and the dorsal fins, the proximal pterygiophores interdigitate between the distal ends of the haemal and the neural spines, respectively. In most dorsal fins, the tip of the first proximal pterygiophore lies between the 18th and

19th (Fig. 2) vertebrae, and the last one between the 21st and 22nd vertebrae.

The proximal pterygiophore is composed of an ossified stick with a broad, bent triangular articular plate and a membranous blade (vane) (Figs. 2 and 3). In the anal and the dorsal fins, the ossified stick appears first, and subsequently the cartilage of the membranous blade becomes ossified around the stick (13.5–14.0 mm TL). In the anal fin, the length of the stick (about 220 μm in 10 mm TL) increases linearly with the increase in total length (Fig. 3). The distal pterygiophore that lies between the bases of the paired half fin rays first ossifies at the two sides in contact with the half fin rays. The first small spine and the second soft fin ray are supported by the first proximal pterygiophore, which has a better developed membranous blade than the other pterygiophores. The first proximal pterygiophore makes contact with the distal portions of two ribs connecting with the parapophyses of the 11th and 13th vertebrae in adult anal fins. The second pterygiophore wedges between the tips of the first (13th vertebra) and the second (14th vertebra) haemal spines. In most anal fins, the last proximal pterygiophore lies between the 21st and 22nd vertebrae.

In the dorsal fins of larvae about 8 mm TL, the cartilaginous stick (length 250 μm in about 10 mm TL larvae) is detectable but lacks the membranous blade. The membranous blade first forms in larvae about 14.8 mm TL. The length of the proximal pterygiophore increases in proportion to the total length the same as for the anal fin.

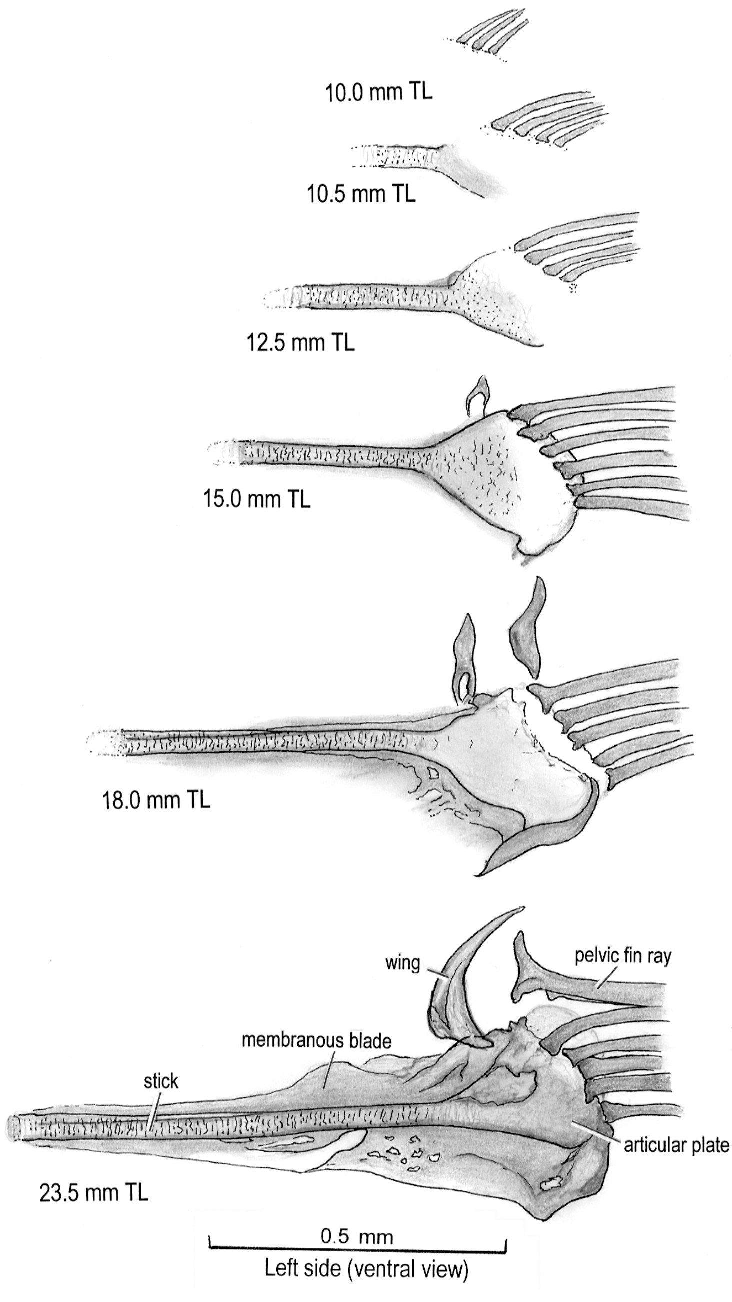


Fig. 6 Development of the pelvic girdles during metamorphogenesis.

The caudal fin has two hypurals instead of the triangular articular plate of the proximal pterygiophores.

3) Development of paired fin appendages

The paired fin appendages, the pectoral and the pelvic fins, are supported by the bones of the pectoral and the pelvic girdles.

Pectoral fin appendages:

The bones of the pectoral girdle consist of the supracleithrum, cleithrum, postcleithrum, scapula, coracoid, and four proximal radials (Fig. 4). The distal pterygiophore between the proximal ends of the paired half fin rays is not observed in the pelvic and pectoral fins.

The pectoral girdle components arise connecting with each other by cartilage. However, the postcleithrum in the medaka, as described in Yabumoto and Ueno (1983), does not make contact with the other pectoral girdle elements or the 10 fin rays. It appears concurrently with the ribs, which form the coelom. The dorsal portion of the triangular scapula is overlapped by the cleithrum, and its ventral portion is connected to the coracoid by connective tissue, sometimes completely fusing with it. The coracoid that is first recognizable in larvae about 7.0 mm TL is comprised of a stick, articular plate and membranous blade, probably corresponding to the proximal pterygiophores of the anal and dorsal fin appendages. In the coracoid, a stick-like process with a cartilaginous articular plate begins to grow from the scapulocoracoid region towards the ventral midline underneath the cleithrum in conjunction with the elongation of the pectoral fins (Fig. 5). In stage 41 (6.7 mm TL), there are unsegmented fin rays in the pectoral fin fold, and the cleithrum first appears as a small arc-shaped cartilage (Fig. 4). In advanced stage 41, the early cartilaginous coracoid (about 0.4 mm in length) consists of a stick-like process and expanded articular plate before the radials appear in larvae 7.2 mm TL. A scapular foramen forms near the dorsal side of the cartilage near its contact with the cleithrum. The cartilaginous radials (distal pterygiophores) are first visible as a single squarish rod on the posterior margin of the scapular cartilage (9.5 mm TL). In this stage, the membranous blade first forms in contact with the ventral surface of the stick bone (Fig. 4). The second radial cartilage becomes ossified when the scapula is almost completely formed (12.7 mm TL). The third and 4th radials are ossified in larvae 15–18 mm TL. Four small squarish radials support the pectoral fin rays in adults. The first and second radials articulate with the scapula by connective tissue. The third and fourth radials are in contact with the articular plate of the coracoid.

Pelvic fin appendages:

For the pelvic fins, a cartilaginous endoskeleton comprises the pelvic girdle. The pelvic girdle is composed of a long stick process, its articular plate, a membranous blade, and wing (rod-like process) (Figs. 6 and 7). The rate of elongation of the proximal pterygiophore (stick bone) coincides with those of the proximal pterygiophores in the anal and dorsal fins. The membranous blade around the stick process arises in contact with the wing extending towards the lateral side. The morphology of paired pelvic girdles was not symmetrical. The basal portion (articular plate) of the pelvic girdles makes direct contact with 6 fin rays in the absence of pterygiophores or cartilaginous radials, unlike the situation in the trout *Salmo gairdneri* (Geraudie, 1981) and the zebrafish *Danio rerio* (Grandel and Schulte-Merker, 1998). The paired pelvic girdle is located in the abdominal area between the 3rd and 5th ribs (between 6th and 7th somites) in most individuals. The 3rd to 5th pleural ribs connect with the parapophyses of the 5th–7th vertebrae.

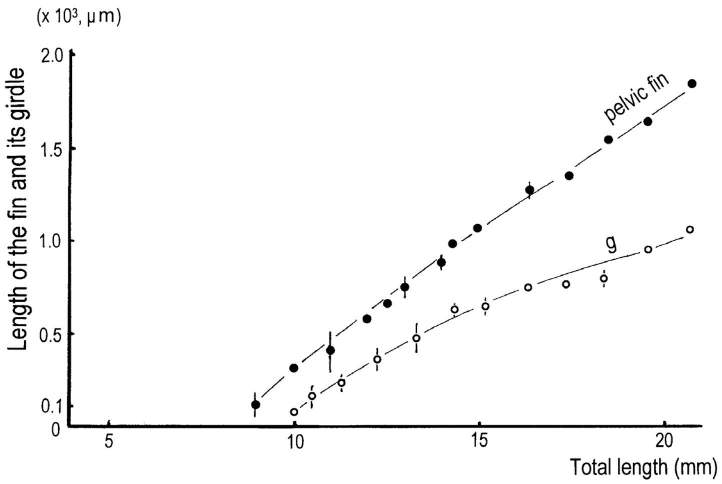


Fig. 7 Changes in the pelvic fins and their proximal pterygiophore in growth period. g, pelvic girdle.

The sequence of ossification in the pelvic girdle and fins proceeds from the fin rays, the stick process, the articular plate, the wing and finally the membranous blade (Fig. 7). Pelvic girdle formation is preceded by the appearance of pelvic fin rays (Fig. 6). A few ossified fin rays are first visible within the elongating pelvic fin at the bilateral sides of the ventral fin fold (about 10 mm TL). The first ossification in the pelvic girdles is recognized in the stick and articular plate with additional formation of fin rays around these bones (12.5 mm TL). The rod-like stick process elongates along the cranio-caudal axis before the ventral fin fold is completely resorbed, concurrently with a subtle resorption of the fin fold in the peduncle region (Fig. 1). The paired wings (about 250 μm in length) appear as small, faint bones towards each bilateral side of the posterior articular plates (15.0 mm TL), concomitantly with ossification of the articular plate. The membranous blade is faintly recognizable around the stick near the articular plate when the fish is about 15 mm TL. The long wing expands laterally as ossification of the thin membranous blades around the ossified stick and articulate plate progresses (about 15 mm TL). In this individual, the number of pelvic fin rays is 6.

As described above, the triangular articular plate with a stick bone seems to be a common skeletal component in all fin appendages. It will therefore be interesting to infer whether the articular plate of the coracoid in the pectoral fins possibly corresponds to the hypural in the caudal fin and the articular plate of the proximal pterygiophores in the anal and dorsal fins. A subsequent investigation is planned to study expression of the secondary sexual characteristics that are recognized in the fins.

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