Morphological aspects of feeding and improvement in feeding ability in the early larval stages of red sea bream *Pagrus major*

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ABSTRACT: The present study examined the appearance, ossification and growth of the bones that form the oral cavity in early larval stages of laboratory reared red sea bream (*Pagrus major*) for 380 h after hatching. The fundamental elements of the oral cavity appeared 11 h after initial mouth opening (HAMO). Development in the red sea bream, based on the osteological development of the feeding apparatus, was divided into three phases following the first feeding (24 HAMO; mean total body length 3.3 mm). The first phase was the early sucking phase (24 to 80–100 HAMO; approximately 3.9 mm), during which the head and bones increased in size. The intensified sucking phase (to 200–220 HAMO; approximately 4.9 mm) was defined by the appearance of new structural elements and a continued enlargement of the head and bones. Finally, during the transition phase (beyond 300 HAMO; approximately 5.6 mm), larvae used grasping as well as sucking to feed, new elements appeared, ossification began, size increased and teeth were acquired. As the larvae advanced through these three phases, the ability to feed by sucking was enhanced by the appearance and growth of new bones. The developmental phases appear to be linked to the transition from endogenous to exogenous nutrition resources under laboratory rearing conditions and to diversification in the size and components of wild food organisms.

KEY WORDS: feeding mechanism, larval development, osteology, *Pagrus major*, red sea bream.

INTRODUCTION

The red sea bream *Pagrus major*, an important fish in the aquaculture industry of Japan, is mass produced for stock-enhancement purposes. For this reason, the early life history of this species has been studied extensively. Matsuoka1 provided detailed information on osteologic and myologic development, while Kohno et al.2 studied the development of swimming and feeding mechanisms, both in larvae and juveniles, in terms of osteology. The development of the digestive system has been reported for several teleost species, including the red sea bream.3–5 Fukuhara6 studied external morphologic development in relation to larval behavior and Moteki et al.7 investigated the transition from endogenous to exogenous nutritional sources.

Kohno et al.8–10 examined the osteologic development of the feeding apparatus in early stage sea bass *Lates calcarifer*, milkfish *Chanos chanos* and grouper *Epinephelus coioides* and discussed the mechanics of feeding and how they changed with age. Kohno11 summarized larval biology, including the osteologic development, of several marine fish and concluded that, for *Epinephelus*, the delayed development of the feeding apparatus caused difficulties in larval rearing. However, information was insufficiently provided on osteologic development during the change in energy sources by the red sea bream. The transition from endogenous to exogenous feeding is a critical period that often results in high mortality rates.12–15

Matsuoka1 provided detailed information about the osteological development of larval red
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RESULTS

Osteological development of the feeding apparatus

Jaws

The first jaw element to appear in the larvae was Meckel’s cartilage, which appeared at –16 HAMO. The bony dentary, which houses the anterior part of Meckel’s cartilage, was first evident at 85.5 HAMO. An anterodorsally directed projection first formed on the posterodorsal part of Meckel’s cartilage by 109 HAMO (Fig. 1b; 122 HAMO). Of the upper jaw elements, the bony maxilla appeared first, at 0 HAMO (Fig. 1a; 11 HAMO). The short bony premaxilla had appeared in front of the upper part of the maxilla in two of five larvae examined at 220 HAMO.

A single tooth was first observed at 268 HAMO; of the five larvae examined, the tooth was located on the premaxilla in two individuals and on the dentary in three individuals. The number of teeth slowly increased, with one to three (mean (±SD) 2.0 ± 1.0) present on the premaxilla and one to two (1.2 ± 0.5) present on the dentary at 316.5 HAMO.

Suspensorium

The symplectic-hyomandibular cartilage first appeared as an element of the suspensorium at –16 HAMO. The small foramen developed on the upper part of the symplectic-hyomandibular cartilage at 99 HAMO (Fig. 1b). At 292 HAMO, ossification began at the lower and upper parts of the symplectic-hyomandibular cartilage (Fig. 1c). The cartilaginous quadrate was found in front of the symplectic-hyomandibular cartilage at –16 HAMO and it started ossifying on the lower part at 292 HAMO (Fig. 1c). The cartilaginous palatine was first visible at 122 HAMO (Fig. 1b) and it became contiguous with the quadrate to form the palatoquadrate cartilage at 174 HAMO. Neither the endopterygoid nor the ectopterygoid was observed during the study period.

Hyoid arch

The ceratohyal-epihyal cartilage first appeared at –16 HAMO. At 220 HAMO, the ceratohyal started ossifying on the anterior part of the ceratohyal-epihyal cartilage. The hypohyal and interhyal cartilages were formed at 0 HAMO (Fig. 1a; 11 HAMO). A single branchiostegal ray was evident at 152

MATERIALS AND METHODS

Fertilized eggs of P. major were collected after they had been naturally spawned in a tank on 22 May 2000 at the Hakata-jima Station of the Japanese Sea Farming Association in Ehime, Japan. The eggs were transferred to a 500 L tank in the rearing facilities of Fukuyama University, Innoshima, Hiroshima, Japan. The eggs began hatching at 12.00 h on 24 May and finished hatching at 18.00 h on the same day. Because peak hatching occurred at 14.00 h, the time of hatching was standardized to be 14.00 h in the present study. Newly hatched larvae were transferred to a 1000 L tank at a density of approximately 40 individuals/L. During the experiment, the water temperature ranged from 18.7 to 21.5°C (mean 20.3°C) and the salinity ranged from 34 to 35 psu. Rotifers (Brachionus plicatilis) were maintained in the tank at a density of 3–10 individuals/L and green algae (Nannochloropsis sp.) were introduced into the water.

From the time of hatching to 380.5 h after hatching, 13–15 larvae were removed one to four times each day and were preserved in 5% neutralized formalin. Total length and body length of freshly collected larvae (i.e. before preservation) were measured under a binocular microscope using an ocular micrometer (for growth in total length, see Moteki et al.7). Body length was considered to decrease by 10% after preservation, as suggested in the literature.18 Five larvae from each sample were cleared and stained approximately 2 months after preservation, following the methods described by Dingerkus and Uhler.17 The cartilage-staining procedure using Alcian blue solution lasted for 2h. Osteologic and morphometric measurements were taken according to Kohno et al.8 All references to time were standardized and expressed as hours after initial mouth opening (HAMO; 0 HAMO = 64 h after hatching; mean (±SD) total length 3.33 ± 0.11 mm).7 All specimens used were deposited in the Museum, Tokyo University of Fisheries (MTUF-P(L) 11700).
Fig. 1 Development of the feeding apparatus and pectoral fin supports in *Pagrus major* (left lateral view). (a) Eleven hours after initial mouth opening (HAMO; mean (±SD) total length (TL) 3.34 ± 0.09 mm); (b) 122 HAMO (mean TL 4.18 ± 0.16 mm); (c) 292 HAMO (mean TL 5.63 ± 0.29 mm). Stippled area, cartilage; open area, ossification. Ac, actinost; An, angular; BrR, branchiostegal ray; C-eC, ceratohyal-epihyal cartilage; Ch, ceratohyal; Cl, cleithrum; C-sC, coraco-scapular cartilage; Dent, dentary; EeB, etethmoid bar; EpT, epiphysial tectum; Hh, hypohyal; Hm, hyomandibular; Ih, interhyal; Iop, interopercle; MC, Meckel’s cartilage; Mx, maxilla; NA, neural arch; OcP, occipital process; Op, opercle; Pal, palatine; Pmx, premaxilla; Pop, pre-opercle; Ps, parapenoid; Qu, quadrate; Scl, supracleithrum; S-hC, symplectic-hyomandibular cartilage; SoB, supra-orbital bar; Sop, subopercle; Sy, symplectic; Tr, trabecula. Bars, 0.5 mm.
Feeding mechanisms in Pagrus major larvae

Morphometrical development of the feeding apparatus

Head length increased linearly until approximately 200 HAMO, after which growth stabilized. Head length (mean ± SD) was 0.54 ± 0.09, 1.20 ± 0.15 and 1.32 ± 0.06 mm at 0, 197 and 316 HAMO, respectively (Fig. 3). The length of the suspensorium increased linearly with age until approximately 200 HAMO and then increased rapidly (Fig. 3). Asymptotic growth curves were recorded for ceratohyal-epiophyal cartilage length and basibranchial cartilage length until approximately 200 HAMO. The length

Branchial arch

The cartilaginous basibranchial and the two anterior-most ceratobranchials appeared as lower arch elements at −16 HAMO. The ceratobranchial-3 and -4 were added at 0 and 8 HAMO, respectively. The two anterior-most hypobranchial were observed at 8 HAMO and the hypobranchial-3 was evident at 11 HAMO (Fig. 2a). Isolated basibranchial cartilage appeared at 102 HAMO (Fig. 2b; 122 HAMO). The ceratobranchial-1 to -5 started ossifying at 292 HAMO (Fig. 2c) and the basihyal appeared at 292 HAMO (Fig. 2c). A single tooth appeared on the ceratobranchial-5 at 244.5 HAMO. At 292 HAMO, two to three gill rakers were observed on the ceratobranchial-1 and a single gill raker was observed on ceratobranchial-2.

At 102 HAMO, the first upper arch element, the cartilaginous pharyngobranchial-3, appeared, bearing a single tooth (Fig. 2b). The pharyngobranchial-2 and -4 were added at 197.5 and 268 HAMO, respectively, with pharyngobranchial-4 bearing a single tooth. The three anterior-most epibranchials appeared at 122 HAMO (Fig. 2b). The upper pharyngobranchial teeth increased in number with age; pharyngobranchial-3 was equipped with one to two teeth at 220 HAMO. Ossification of the epibranchial-3 and pharyngobranchial-3 and -4 was evident at 292 HAMO.

Opercular bones

The pre-opercle and opercle were first formed at 72.5 and 77.5 HAMO, respectively, and both the subopercle and interopercle were added at 220 HAMO (Fig. 1b,c).

Neurocranium

The cartilaginous trabecula, ethmoid plate, parasymphal and auditory capsule appeared at −16 HAMO. The cartilaginous occipital process was observed at 94 HAMO, followed by the cartilaginous supraorbital bar and epiphysial tectum at 122 HAMO (Fig. 1b). The bony parasphenoid was evident on the ventral side of the posterior part of the trabecula at 220 HAMO. The cartilaginous ectethmoid bar appeared and was contiguous with the supraorbital bar at 174 HAMO.

Fig. 2 Development of branchial arches in Pagrus major. A dorsal view of the lower branchial arches and a ventral view of the upper branchial arches is shown. Stippled area, cartilage; open area, ossification. (a) Eleven hours after initial mouth opening (HAMO); (b) 122 HAMO; (c) 292 HAMO. Bb, basibranchial; Bh, basihyal; Cb1, ceratobranchial-1; Eb1, epibranchial-1; Hb1, hypobranchial-1; ICBr, isolated cartilaginous basibranchial; Pb1, pharyngobranchial-1; Pb3, pharyngobranchial-3. Bars, 0.5 mm.
of both components stabilized from approximately 130 to 200 HAMO (ceratohyal-epihyal cartilage $0.51 \pm 0.02$ mm and basibranchial cartilage $0.38 \pm 0.03$ mm at 132 and 197.5 HAMO, respectively). After 200 HAMO, both values started increasing again with lengths of $0.67 \pm 0.03$ mm (ceratohyal-epihyal) and $0.48 \pm 0.03$ mm (basibranchial) at 292 HAMO (Fig. 3).

The maxillary length was $0.16 \pm 0.02$ mm at 0 HAMO and this increased linearly with age until approximately 200 HAMO ($0.33 \pm 0.08$ mm at 197.5 HAMO), after which time growth became more rapid ($0.54 \pm 0.06$ mm at 316.5 HAMO; Fig. 4). The premaxillary length, and its ratio to maxillary length, increased rapidly from its appearance at 220 HAMO to 316.5 HAMO. The distance from the posterior end of Meckel’s cartilage to the anterior tip of the hyoid arch (M-H distance) was positive in most larvae, indicating that the latter was positioned ahead of the former. The M-H distance showed an asymptotic curve with age until approximately 200 HAMO ($0.03 \pm 0.04, 0.14 \pm 0.03$ and $0.14 \pm 0.06$ mm at 0, 99 and 197.5 HAMO, respectively)

**Fig. 3** Changes in head and bone lengths with time after initial mouth opening in *Pagrus major*.

**Fig. 4** Changes in maxillary (Max.) and premaxillary (Premax.) lengths, ratios of premaxillary length to maxillary length and distance between the posterior end of Meckel’s cartilage and the anterior tip of the hyoid arch (M-H distance) with time after initial mouth opening in *Pagrus major*. 
and then increased to 0.27 ± 0.01 mm at 220 HAMO and to 0.31 ± 0.05 mm at 316.5 HAMO.

Fin development

The bony cleithrum appeared at –16 HAMO. The coraco-Scapular cartilage, bearing both the dorsal and posterior processes, was first evident at 0 HAMO. A small anterior-directed process developed on the anterodorsal ventral part of the coraco-Scapular cartilage at 11 HAMO (Fig. 1a). The bony supracleithrum appeared at 268 HAMO, with a posterior-directed spine evident at 292 HAMO (Fig. 1c). The post-temporal appeared at 316.5 HAMO. In the caudal fin, the parhypural and the two anterior-most cartilaginous hypurals were first observed at 220 HAMO. Notochord flexion started at 292 HAMO and the dorsal and anal pterygiophores were visible at 316.5 HAMO.

DISCUSSION

Most teleosts ingest their food by suction feeding, wherein food is drawn into the oral cavity through negative pressure created by a suction pump that is supported by bones. Based on osteological development, Kohno et al. described the shift in feeding mechanisms from 'sucking' to 'grasping' in larval sea bass L. calcarifer. Sucking and grasping feeding mechanisms have also been reported in grouper E. coioides larvae. However, milkfish C. chanos larvae, which have a long, cylindrical oral cavity, were found to use a 'straining' mechanism. Based on the development of the oral cavity, the present study shows that red sea bream larvae feed by using sucking and grasping mechanisms.

Within 11 h of initial mouth opening (mean (± SD) total length 3.33 ± 0.10 mm), red sea bream larvae are equipped with the fundamental components of the oral cavity (Fig. 5). At this developmental stage, larvae have a large levator arcus palatini (peripheral head muscle), which expands the oral and branchial cavities to allow the sucking motion for acquiring food. This indicates that the necessary development for feeding by sucking occurs before initial feeding starts at 24 HAMO.

In the early sucking phase, from initial feeding to 80–100 HAMO (3.93 ± 0.11 mm at 97 HAMO), new bone elements are not added, but the lengths of the head and some oral cavity elements increase rapidly. Of these, the suspensorium, hyoid arch and lower branchial arch work as levers to expand the oral cavity. During this phase, larvae feed more as they age and they feed primarily by suction. Thus, the improvement in their sucking ability seems to depend mainly on size development.

The appearance of new elements in the oral cavity characterized the intensified sucking phase from 80–100 to 200–220 HAMO (4.85 ± 0.23 mm at 197 HAMO); all elements were formed by 220 HAMO. Feeding during this phase was by sucking only, because the elements that facilitate the grasping of food, such as the premaxilla and jaw teeth, had not yet appeared. By 220 HAMO, the head, suspensorium and maxilla lengths had all increased, but growth had stabilized for the ceratohyal-epiphyal cartilage, basibranchial cartilage and the M-H distance. However, after 110 HAMO, the mouth widened, allowing feeding to increase rapidly (intensified feeding phase in Fig. 5). At this stage of development, larvae are thought to swallow larger food items, because the size of a food item is limited by the width of the mouth.

Hence, the appearance of new elements in the oral cavity and overall growth, including increased mouth width, are the main factors that improve suction feeding in larvae during the secondary sucking phase.

In the transition phase, from 200 to 220 HAMO to the end of the experiment (5.63 ± 0.29 mm at 292 HAMO), several developments occurred. These included the appearance of the jaw, lower pharyngeal teeth (ceratobranchial teeth) and premaxilla, which enables grasping of food items. Ossification of existing elements and a rapid increase in bone length were also observed, suggesting an enhancement of the sucking mechanism. The M-H distance began to increase at 220 HAMO and continued until the end of the experiment. A larger M-H distance generates a stronger negative pressure in the oral cavity. Matsuoka observed thickened protractor hyoidei (geniohyoideus) and sternohyoideus muscles at approximately 280 HAMO (14.5 days after hatching), which allowed for wide opening of the mouth. Thus, the improved ability of larvae to feed during the transition phase depends on their acquisition of grasping and the enhancement of sucking.

No distinct change in swimming was inferred from the osteologic development during the experiment, despite the appearance of some components involved in fin support and the beginning of notochord flexion at 220–315 HAMO. Swimming changes from 'less active' to a 'transitional stage' of caudal propulsion occur at approximately 300 HAMO in red sea bream larvae.

Moteki et al. studied the change in nutritional sources for larval Pagrus major under laboratory rearing conditions and divided the transitional process into four phases (Fig. 5; the first 'primordial' phase before –20 HAMO is not shown).
Fig. 5  Schematic representation of the development of elements comprising the oral cavity and fins in *Pagrus major*.  

- (○) appearance of cartilaginous elements;  
- (●) appearance of ossified bone or the beginning of ossification of cartilaginous elements.  

Developmental events regarding the enhancement of feeding ability and changes of feeding mode are summarized in a horizontal column and arrows at the bottom. For feeding mode, the boldness of the arrows indicates the degree of ability in each mode. Developmental phases and food organisms are summarized in the lower-most two columns.
Changes in the principal food organisms found in natural waters (western Wakasa Bay, facing the Japan Sea) during the larval period of red sea bream are shown in Fig. 5 (based on Suzuki and Kuwahara23). Under laboratory conditions, larvae began feeding at 24 HAMO and feeding increased rapidly after 110 HAMO (intensified feeding period).7 However, larvae in natural waters began feeding on copepod nauplii at approximately 100 HAMO (body length approximately 3.5 mm).23 Before 100 HAMO, diatoms were the only food items consumed by the larvae and the feeding incidence was low (one of 10 larvae examined). The improved feeding at approximately 100 HAMO coincides with the appearance of new oral cavity elements, as shown in the present study. Furthermore, in natural waters, the proportion of larger copepods and cladocerans in the diet of larvae increases after approximately 270 HAMO (body length approximately 5 mm).23 This diversification in size and species of food organisms seems to be supported by the acquisition of grasping and the enhancement of sucking in red sea bream larvae.

ACKNOWLEDGMENTS

This work would not have been possible without the technical assistance and invaluable support of T Ishikawa, N Teraoka, T Miyamukai and H Fushimi, all of whom are in the Department of Marine Biotechnology, Fukuyama University. I thank H Kohno, at the Laboratory of Ichthyology, Tokyo University of Fisheries, for critically reading the manuscript and for providing valuable advice during the course of this study. My gratitude also goes to T Fukunaga and the staff at the Hakata-jima Station of the Japan Sea-Farming Association for kindly providing the Pagrus major eggs.

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