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Early Development of Cephalic Skeleton in Hatchery-Reared Gilthead Seabream, *Sparus aurata*

Şahin Saka[1], Deniz Çoban[1,*], H. Okan Kamacı[1], Cüneyt Süzer[1], Kürşat Fırat[2]
[1] Ege University, Faculty of Fisheries, Aquaculture Department, 35100 Bornova, Izmir, Turkey.
[2] Ege University, Tire Kutsan Vocational School, 35900 Tire, Izmir, Turkey.

* Corresponding Author: Tel.: +90.232 3434000-5214; Fax: +90.232 3883685;
E-mail: deniz.coban@ege.edu.tr

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Abstract

The development of the cephalic skeleton in gilthead seabream, *Sparus aurata* (L. 1758), larvae was examined from 1 to 41 days post-hatching during the early ontogeny under intensive culture conditions. At hatching, *S. aurata* larvae had 2.68 mm total length (TL) and were devoid of any cephalic elements. At 2.85 mm TL, the trabecular bars and the ethmoid plate appeared. Development of jaws started during the yolk-sac stage. Teeth first appeared at larval stage (7.78 mm TL) and gill filaments formed at size ranging between 8.05 and 8.9 mm TL. Finally, the dentary, maxillary and Meckel's cartilage began to ossify at 9.20 mm TL. It is concluded that cartilaginous elements and structures could be related with vital functions in *S. aurata* larvae. Also, onset time of skull was found different although same developmental pattern of skull has been described for most Sparidae species to date.

Key words: Sparus aurata, skull, ontogeny, osteology, marine fish larvae.

Introduction

The gilthead seabream (*Sparus aurata*) inhabiting temperate waters in the Northeastern Atlantic and the Mediterranean Sea, is a sparid species with high commercial value (Bauchot and Hureau, 1986). It is intensively cultured in the Mediterranean countries and well established technologies for rearing are available (Elbal *et al*., 2004).

Nevertheless not all aspects of their developmental biology are studied. For example, osteological development in fish is important from both fisheries biology and aquaculture aspects. From fisheries perspective, the determination of early life stages based on a developmental series of specimens is necessary for distinguishing families or species with close morphology and overlapping spawning seasons and habitat (Blaxter, 1984). From aquaculture point of view, knowledge on the developmental biology of cultured fish is prerequisite for early detection and prevention of skeletal deformities under culture conditions because such deformities mainly arise in early life stages (Koumoundouros *et al*., 2000).

It is reported that the skull is the first part of skeleton to develop in Sparid larvae (Fausitino and Power, 2001). Additionally, its development represents significant priority to the elements responsible for feeding and respiration (Koumoundouros *et al*., 2000). To perform these functions, the larvae must develop effective means of locomotion, a system for capturing and digesting prey, a new respiratory system, and nervous and sensory systems. Moreover, the prey capture and respiratory systems are located in the skull and consist of skeletal structures and muscle. The development of the skull differs among species in terms of relative sequences of development and relative size, shape etc. of bones (Koumoundouros *et al*., 2000).

Numerous studies have been carried out on reproduction and physiology (Almansa *et al*., 1999), embryologic development and larval rearing (Tandler *et al*., 1989), osteological abnormalities (Andrades *et al*., 1996) and development of digestive system (Elbal *et al*., 2004) and intestine (Calzada *et al*., 1998) of seabream larvae. Additionally, osteological development of its skull although was studied under extensive culture conditions (Faustino and Power, 2001), there is lack of information about it in intensive culture systems. Therefore, the present study aims to describe the ontogenic development of the skull in *S. aurata* larvae under intensive culture conditions as a potential tool in assessing larval quality.

Materials and Methods

Broodstock and Egg Incubation

Gilthead seabream broodstock were selected from wild breeders and stocked in 10 m^3 cylindrical dark-gray tank with a seawater supply of 35 L min^{-1}. Frozen cuttlefish (*Sepia officinalis*), leander squid (*Palaemon elegans*) were provided once a day as primary food source. The fish were subjected to natural photoperiod of rearing season (38º92' N; 27º05' E), and the water temperature varied between

16–18.0°C throughout the breeding period. Eggs spawned by brooders were immediately collected in recuparators. Following the collection, fertilized buoyant eggs were separated from the sinking dead eggs. Eggs were incubated in 1 m^3 incubators at an initial density of 2,000 eggs L^{-1} with a gentle flow of seawater of 18.0±0.5°C. Oxygen saturation was over 85%, salinity was 37 ppt and pH was around 7.65. Ammonia and nitrite components were always <0.012 mg L^{-1}.

Larval Rearing

After hatching, the larvae were reared in a cylindrical tank (30 m^3) at average density of 100 in L^{-1}. The color of the tank was dark-gray. Larval rearing was carried out in a closed circuit sea water system with UV filters and green water technique. Water temperature, dissolved oxygen, salinity, pH, ammonia and nitrite levels were monitored daily. Water temperature was maintained between 17.0 and 22.0°C. Temperature increased day by day from 17.0 to 19.0°C between 0 and 7 DAH (Days After Hatching), 19.0 to 20.0°C between 8 and 26 DAH, from 20.0 to 22.0°C between 27 and 41 DAH. During the larval period, oxygen, salinity and pH were maintained at >85%, 37‰ and 7.6, respectively. Ammonia and nitrite were always kept below 0.01 mg. The water in the tank was static during the first 3 days of the rearing period. From day 4 to 12, the tank water was partially replaced (5-6% daily) by draining through a 160 µm mesh. Water exchange rate was increased gradually with the age of the larvae. The light intensity was 50 lux between 4 and 10 DAH and 250 lx until end of the larval rearing. The daily photoperiod was set at 24 h light until the end of algal addition and then 16 h light and 8 h dark until end of the experiment. Newly hatched larvae were fed from day 4 (when the mouth opened) to day 20 with rotifers (70% *Brachionus rotundiformis* and 30% *Brachionus plicatilis*) cultured with algae and enriched (DHA Protein Selco, Artemia Systems SA, Ghent, Belgium) at a density of 10-15 rotifers ml^{-1}. During this period, *Nannochloropsis* sp., *Chlorella* sp. and *Isochrysis* sp. at a density of 30-40.10^4 cells ml^{-1} were added to the rearing tank to provide green water environment. From day 13 to day 22, they were fed *Artemia* nauplii (AF 480, INVE Aquaculture, Ghent, Belgium) at 4-6 individuals ml^{-1} and from day 16 until the end of the experiment, *Artemia* metanauplii at 2-4 individuals ml^{-1} (EG, Artemia Systems SA), both enriched with Protein Selco (Artemia Systems SA). Extruded microdiet (Proton, INVE Aquaculture) was used from 32 DAH until 41 DAH as 3-8% of biomass per day.

Sampling and Staining

Development of the skull was examined on samples of minimum 30 specimens per 3 day during the larval stages (from days 1 to 41) (Table 1). Larvae were anaesthetized with ethylenglycol-monophenylether (Merck, 0.2-0.5 ml L^{-1}) and fixed in phosphate buffered 10% formalin (pH 7.4) for 24-48 h. Then, the specimens were immediately processed or preserved in 100% ethanol for later use. Double staining method described by Pothoff (1984) was followed. During the study, total of 861 larvae were stained individually. Deformed larvae were not analyzed. Before fixation of individuals, measurements of the TL from the first day after hatching (DAH) to 41 DAH to nearest the 0.01 mm were carried out. The TL at which more than 50% of the individuals presented a certain characteristic was used as the reference point in the description of the ontogeny.

We used the anatomical terminology relating to the skeletal structures given by Matsuoka (1987), Koumoundouros *et al*. (2000) and Faustino and Power (2001) to describe the skull development of seabream larvae.

Results

In newly hatched *S. aurata*, no cartilaginous or bony viscerocranial elements were noticeable (Figure 1-A). The development of the neurocranium started almost at the end of yolk-sac absorption with the formation of trabecular bars and the ethmoid plate at 2.85 mm TL. The trabecula was positioned between the eyes. The sclerotics which are cartilaginous rings around the eyes appeared toward the end of sac fry stage (Figure 1-B). Hyosypletic cartilage appeared as a common cartilage at 3.20 mm TL. Development of the jaws was initiated during the yolk-sac stage. Meckel's cartilage was positioned anterolaterally in a plane ventral to the trabeculae (Figure 1-B). As the larvae grow, the posterior end of Meckel's cartilage extended and articulated in a shallow socket together

Table 1. Means total length (±SD) of larvae in between 1 to 41 days after hatching (DAH) in *Sparus aurata*

DAH	n	TL (Mean+SD)(mm)	Range (mm)
1	64	2.68±0.27	2.41–3.06
4	69	3.05±0.32	2.76–3.52
7	72	3.65±0.22	3.24–4.14
10	57	4.09±0.50	3.51–4.43
13	63	4.51±0.20	4.15–4.83
16	67	5.13±0.31	4.09–5.74
20	61	6.14±0.54	4.80–7.61
23	66	6.46±0.52	5.17–7.49
26	56	6.57±0.59	5.15–7.93
29	63	7.48±0.58	6.13–9.49
32	54	7.95±0.80	6.54–10.07
35	65	8.15±0.97	6.70–10.51
38	51	9.48±1.12	7.68–12.01
41	53	10.80±1.24	8.26–14.10

with the anteroventral tip of quadrate cartilage. All elements of branchial arches were cartilaginous bones. Their development was evident at the end of yolk-sac larval stage with the anterior pair of ceratobranchials forming first, followed by the three pairs of ceratobranchials 2 to 4 (Figure 1-C). The coraco-scapular cartilage and the cleithrum formed posterior of the skull at 3.59 mm TL. The ceratohyal and the epihyal appeared at the base of the splanchnocranium in the form of a common cartilaginous hyoid bar. The cartilaginous glossohyal formed at the larval stage (7.09 mm TL), between the two limbs of the hyoid arch and rostrally to the branchial skeleton (Figure 1-D). Branchiostegal rays were membranous bones that articulated on to ceratoyal and epihyal. They followed a rostral direction of development with full bilateral symmetry. The secondary upper jaw was developed when the premaxillaries formed by fibrous elements. The rostral cartilage formed between the premaxillaries at 7.4 mm TL (Figure 1-E). The premaxillary and dentary teeth were observed in jaw at 7.78 mm TL. The ossification of the elements of cephalic skeleton was apparent at the beginning of metamorphosis at 9.35 mm TL (Figure 1-F). During skull development, first ossification started in dentary, maxillary and Meckel's cartilage of seabream larvae.

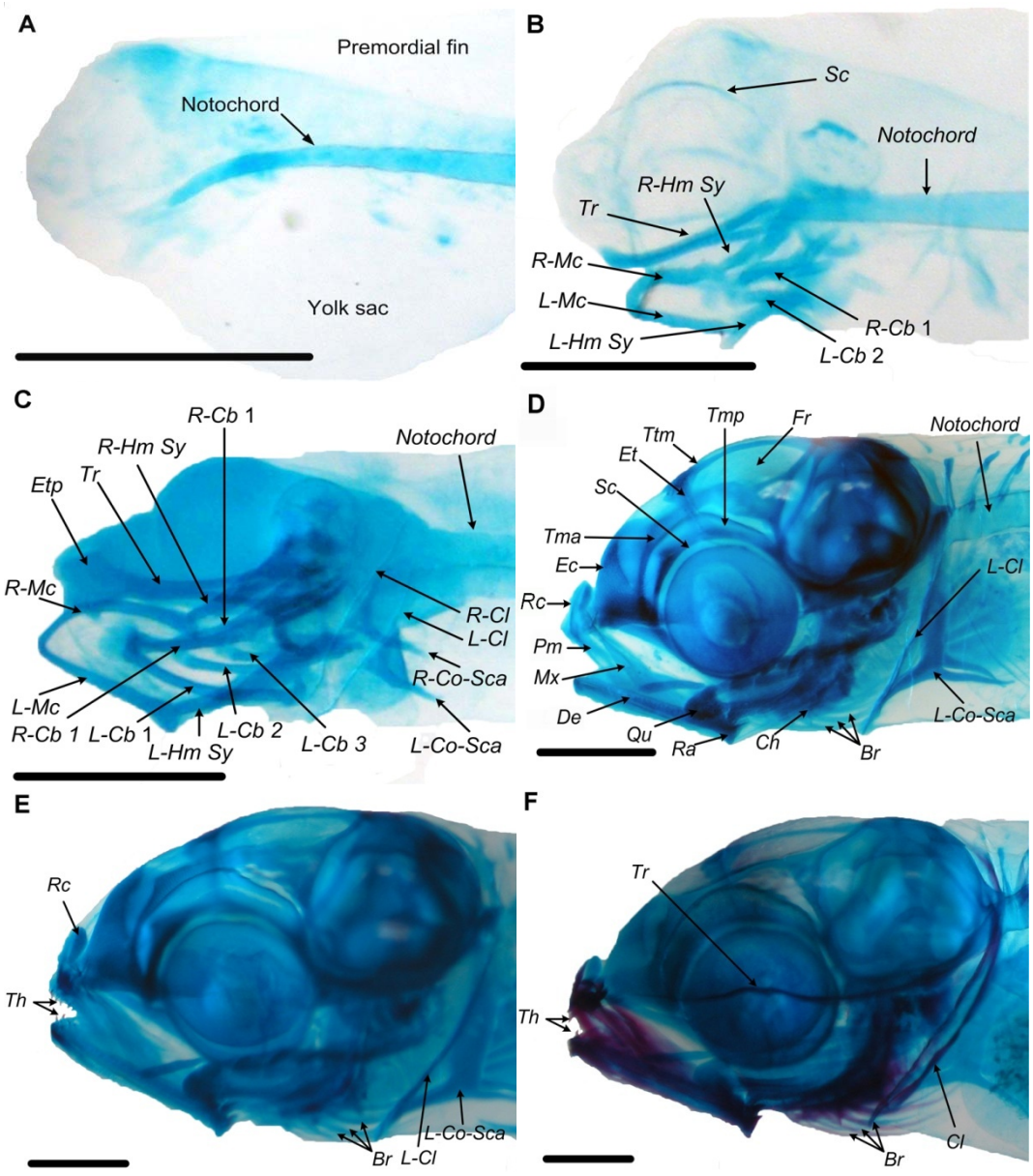

Figure 1. Development of skull in *Sparus aurata* larvae. A. 2.70 mm TL; B. 3.22 mm TL; C. 3.68 mm TL; D. 7.49 mm TL; E. 7.96 mm TL; F. 9.50 mm TL.

Br, Branchiostegal rays; *Cb*, Ceratobranchial; *Ch*, Ceratohyal; *Cl*, Cletihrum; *Co-Sca*, Coraco scapular cartilage; *De*, Dentary; *Ec*, Lamina precerebralis; *Et*, Epiphysial tectum; *Etp*, Ethmoid plate; *Fr*, Frontal; *HmSy*, Hyosypletic cartilage; *Mc*, Meckel's cartilage; *Mx*, Maxillary; *Pm*, Premaxillary; *Qu*, Quadrate; *Ra*, Retroarticular; *Rc*, Rostral cartilage; *Sc*, Sclerotic ; *Th*, Theet.*Tma*, Taenia marginalis anterior; *Tmp*, Taenia marginalis posterior; *Tr*, Trabecula; *Ttm*, Taenia tecti medialis. *R*, right; *L*, left. Sclae bars indicate 1.0 mm.

Discussion

It is commonly known that, teleost larvae show a remarkable variability with respect to developmental stage of the skull at hatching. For instance, in *Salmo trutta* (De Beer, 1937) and *Barbus barbus* (Vandewalle *et al*. 1992) development of the skull starts well before hatching, without however any ossified elements been present, in contrast to what was presented in *Galeichthys feliceps* (Tilney and Hecht 1993). In other species such as *Clarias gariepinus* (Surlemont and Vandewalle, 1991), *Heterobranchus longifilis* (Vandewalle *et al*., 1997), *Scophthalmus maximus* (Wagemans *et al*., 1998), *S. aurata* (Faustino and Power, 2001; the present study) and *Dentex dentex* (Koumoundouros *et al*., 2000) the skull starts developing after hatching. According to Koumoundouros *et al*. (2000), ontogeny of the cephalic skeleton in *D. dentex* occurs in three stages. The first one is the yolk-sac (during the phase of no growth) and the early larval stage (up to 6.9 mm TL) in which the majority of the skull elements form, while the second is ontogenies of the skull develop the late larval period (up to 11.3 mm TL). Finally, the metamorphosis stage (up to 24.0 mm TL) during which the remaining infraorbitals form, the cartilaginous areas of the neuroskull regress and the sclerotics start to ossify. In this study, almost the same developmental patterns were observed. Similar developmental stages in skull ontogeny also appear in other teleostean species such as *Pagrus major* (Matsuoka, 1987), *Barbus barbus* (Vandewalle *et al*., 1992) and *Heterobranchus longifilis* (Vandewalle *et al*., 1997), but it is not a common trend among all teleosts (Vandewalle *et al*., 1997).

It is strongly pointed out that absorption of the vitelline reserves is the critical step for the onset of feeding and the development of elements which serve in the function of food ingestion (Divanach, 1985; Koumoundouros *et al*., 1998). The importance of this process for the survival of the larvae is further underlined by the pathological state called “point of no return”, which is induced by the incapability of early larvae to eat or prey (O'Connell, 1976; McGurk, 1984; Clemmesen, 1987). In *S. aurata*, neurocranium and splanchnocranium form during the yolk-sac stage well before the onset of exogenous feeding. Also, sense capsules and brain develop during or at the same period. These results were in agreement with Faustino and Power (2001) for seabream, but main differences were observed in other Sparid larvae at the timing of onset.

In many Sparid species, the biting function presupposes the appearance of teeth which is called as ontogenical progress (Koumoundouros *et al*., 2000). This phase of switching to biting function in *S. aurata* is similar to many other teleosts (Vandewalle *et al*., 1997; Wagemans *et al*., 1998). The other priority in skull development of *S. aurata* is the formation of the arches. Also, it serves not only in feeding function, but also in respiration function by supporting the gill filaments (Koumoundouros *et al*., 2000). Besides, the replacement of the initially cutaneous respiration through the primordial fin fold by gill respiration is imperative for the growing larvae (Oozeki and Hirano, 1994; van Snik *et al*., 1997). According to Koumoundouros *et al*. (2000), based on anatomical criteria, this replacement is initiated by *D. dentex* larvae during the first stage of skull development. In this study, there is an agreement with these findings, but main differences were observed in other Sparid larvae at the timing of onset. The ontogenic differences could probably be related to both rearing temperature and requirements for survival of the early life stages of these species. It can be concluded that the ontogeny of the osteological development of the skull in *S. aurata* larvae followed the same general pattern that has been described for most Sparidae species to date. According to Fukuhara (1992), in the scientific fields of aquaculture, osteological process serves in understanding the functional trends and the environmental needs of different developmental stages. Additionally, osteological malformations are undesirable in fish culture. Hence, it would be beneficial to study osteological developmental process in this species. The avoidance of skeletal deformities when rearing fish requires detailed knowledge of their ontogeny (Divanach *et al*., 1997; Koumoundouros *et al*., 1997), which contributes to a better understanding of the species under aquacultural, systematic and ecological considerations (Koumoundouros *et al*., 2001).

As described by Faustino and Power (2001), there are no differences in skull development of *S. aurata* under extensive culture conditions, although some differences during ontogenic development of skull were recorded in different times under intensive culture conditions for this species. Similar findings were reported for *D. dentex, Pagrus pagrus* and *Pagrus major* larvae (Koumoundouros *et al*., 2000; Çoban *et al*., 2008; Matsuoka, 1987).

Further studies on chondrification and ossification sequences will be required at both basic and applied levels to determine how the development of the skull skeleton is regulated in fish and the role of environmental factors in this process. Also, it will be useful to understand how development and function interact to influence a morphological program and create morphological diversity.

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