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Skeletal Formation of the Fins in the Common Dentex (*Dentex dentex* L., 1758) Under Intensive Culture Condition

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Özet: ***Sinarit (***Dentex dentex ***L., 1758) Larvalarının Kültür Koşullarında Yüzgeç Gelişimleri.*** Bu çalışmada yoğun kültür koşulları altında sinarit (*Dentex dentex*) larvalarının erken dönemlerinde dorsal, anal, pelvik ve pektoral yüzgeç gelişimleri kıkırdak ve kemik yapı oluşumlarına göre incelenmiştir. İlk gelişim gösteren yüzgeç, pektoral yüzgeç olup 3,4 mm total boyda (TB) gözlenmiştir. Bu yüzgeci, dorsal yüzgeç (6,1 mm TB), anal yüzgeç (6,2 mm TB) ve pelvik yüzgeç (7,3 mm TB) oluşumları takip etmiştir. Dorsal yüzgeç yumuşak ışınları (R) ilk olarak 7,0 mm TB'da gözlenmiş ve 9,7 mm TB'da oluşum tamamlanmıştır (10–12 R). Dorsal yüzgeç sert ışınları (S) ilk olarak 7,2 mm TB'da gözlenmiş ve 9,9 mm TB'da tüm oluşumlar tamamlanmıştır (9-11 S). Anal yüzgeç sert ve yumuşak ışınları 7,0mm ile 9,0 mm TB arasında gelişim göstermiş ve bu gelişim 9,2 mm TB'da son bulmuştur (3 S ve 7-9 R). Sparide familyası üyesi olan sinarit larvalarının yüzgeç gelişimleri diğer sparide familyası üyelerinin yüzgeç gelişimlerine benzerdir.

Anahtar Kelimeler: *Dentex dentex*, yüzgeçler, osteoloji, gelişim, larva.

Abstract: Osteological development of dorsal, anal, pelvic, and pectoral fins in the common dentex (*Dentex dentex*) was examined in early stage larvae under intensive culture conditions. The pectoral fin supports started to develop at 3.4 mm total length (TL), followed by those of dorsal fins at 6.1 mm TL, anal fins at 6.2 mm TL and pelvic fins at 7.3 mm TL. Development of dorsal lepidotrichia (R) was firstly observed at 7.0 mm TL and they attained their final number (10-12 R) at 9.7 mm TL. The dorsal spines (S) firstly appeared at 7.2 mm TL and completed all form (9-11 S) at 9.9 mm TL. The anal lepidotrichia appeared during the development phase from 7.0 mm to 9.0 mm TL. They reached their final number (3 S and 7-9 R) at 9.2 mm TL. The pattern of development of fin supports found in *D. dentex* was almost similar to that described in other Sparid species.

Key Words: *Dentex dentex*, fins, osteology, development, fish larvae.

Introduction

Mediterranean aquaculturists are becoming increasingly interested in culturing new species that would complement the production of European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) (Rueada and Martinez 2001). From this point of view, common dentex (*Dentex dentex* Linnaeus, 1758) appears as a credible candidate for marine culture, due to its rapid growth, consumer acceptance and ease of reproduction in captivity; although at present there are some problems with larval production (Espinos et al 2003). *Dentex dentex* is normally found in the Mediterranean basin and in recent years it has been successfully produced in Greece, Turkey, Italy and Spain. It inhabits the Mediterranean, the Atlantic from the Bay of Biscay to Madeira and, rarely, the Black Sea (Espinos 2003).

According to Koumoundouros et al (1999), detailed knowledge of the developmental osteology of a species is important not only from the embryological point of view, but also for fisheries biology and aquaculture. In larviculture, precise developmental knowledge is a prerequisite for the early detection and elimination of skeletal deformities (Koumoundouros et al. 1997; Divanach et al 1997), which are common under rearing conditions (Chatain 1994; Boglione 1995; Divanach et al 1996). Also, development of fins is an important character in the early life stage of fish (Koumoundouros et al 2001a). It is closely correlated with changes in swimming, feeding ability and preferences (Fukuhara 1992). Besides, numerous studies have been carried out on its reproduction and physiology (Chatzifotis et al 2004), embryologic development (Saka et al 2004), larval rearing (Fırat et al 2003), and feeding (Espinos et al 2003). Additionally, there is no literature about its osteological development of the fins in intensive culture conditions, while it is only extensive conditions (Koumoundouros et al 2001a).

The aim of the present study was to describe development of ontogeny of the fins (pectoral, dorsal, anal, and pelvic fins) in common dentex, *Dentex dentex,* under intensive culture conditions. In addition to this, drawing techniques has commonly used in the osteological studies until so far especially in Sparidae, but this was the first study observation of the osteological development of *D. dentex,* one of the Sparid species, with illustration by colored photographs and compared with other Sparidae.

Materials and Methods

Dentex dentex broodstocks were selected from wild breeders and stock in 10 m^3 tank with a seawater supply of 35 $l.min^{-1}$. Broodstocks were maintained local photoperiod and temperature condition (38°92'N; 27°05'E). Maturation and spawning occurred spontaneously. The water temperatures varied throughout the experimental period between 16-17°C. Eggs were incubated in 0.2 m^3 incubators at an initial density of 1500 $eggs.l^{-1}$ with a gentle flow of seawater of 17.0±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 $mg.l^{-1}$.

After hatching, the larvae were reared in a cylindrical tank (15 m^3), at average density of 100 $ind.l^{-1}$. Larval rearing was carried out in a closed circuit sea water system with UV filters. Water temperature, dissolved oxygen, salinity, pH, ammonia and nitrite levels were monitored daily. Water temperature was maintained between 18.0 and 24.0 °C (temperature increased day by day from 18.0 to 20.0 °C between 0 and 7 days after hatching (DAH), 20.0 to 22.0 °C between 8 and 26 DAH, from 22.0 to 24.0°C between 27 and 46 DAH). During the larval period oxygen, salinity and pH were maintained at >85%, 37‰ and 7.6, respectively. Ammonia and nitrite were kept constant always below 0.01 $mg.l^{-1}$. 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 lx 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 $rotifers.ml^{-1}$ plus green-water composed of *Nannochloropsis* sp., *Chlorella* sp. and *Isochrysis* sp. at a density of $30\text{-}40.10^4$ $cells.ml^{-1}$. From day 15 to day 25, they were fed *Artemia* nauplii (AF 480, INVE Aquaculture, Ghent, Belgium) at 4-6 individuals ml^{-1} and from day 20 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 31 DAH until 46 DAH as 3-8% of biomass per day.

Skeletal formation study was conducted on samples of minimum 30 specimens per 3 day during the larval stages (from days 1 to 46). 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 (15). Specimens were immediately processed or preserved in 100% ethanol for later use. Staining procedures followed by Pothoff (1984). During the study, total 969 larvae were stained individually. Deformed larvae were not analyzed during the study. Measurements of the total length (TL) were carried out from 1 to 46 DAH before fixation of individuals to nearest the 0.01 mm. 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. The anatomical terminology relating to the skeletal structures followed as described by Koumoundourous et al (2001) and Matsuoka (1987) to determine the skeleton systems of the common dentex larvae.

Results

Developmental sequences of all fin supports and rays against total length (TL) in *Dentex dentex* were demonstrated in Figure 1.

The pectoral girdle was the first develop of the fin supports in *Dentex dentex*. The pectoral fins and girdle consist of bilateral pairs of membrane and cartilaginous bone. The coracoid, scapula, proximal pterygiophores and distal radials are cartilaginous bones, while all the remaining elements are membrane bones. The ontogeny of the pectoral fins started at almost the end of the yolk-sac stage with formation of the cleithrum (65%, 3.4 mm TL). Coracoid and scapula complex were present at 3.4 mm TL and the cartilaginous fin plate follows them closely, which in the following phases differentiated into proximal pterygiophores. At this period, coracoid-scapula and fin plate were close contact to each other. At 4.5 mm TL, the cartilaginous fin plate was separate from coracoid-scapula complex and a crevice developed in the anterior mid-region of the fin plate. Coracoid and scapula commenced ossifying on the primordial coracoid-scapula cartilage at 7.5-8.8 mm TL, respectively. Second and third crevice of the fin plate was formed at 6.6 and 7.3 mm TL, respectively. Propterygium located above the fin plate cartilage at 6.9 mm TL. First distal radial appeared at 7.0 mm TL, and the last one at 13.4 mm TL. The four proximal pterygiophores (Prx) formed in a ventrad direction (at 7.9, 8.1, 8.5 and 8.7 mm TL, respectively) on the primordial fin plate as a result of the corresponding crevices occurred. The ossification of proximal pterygiophores started at 8.9 mm TL with Prx 1, whereas the last to start ossifying was Prx 4 at 11.9 mm TL. The pectoral lepidotrichia developed ventrad at 6.9 mm TL, simultaneously with appearance of the propterygium cartilage. The lepidotrichia attained a final number of 14-16 at 12.9 mm TL. Hypercleithrum formed at 4.7 mm TL, followed by the posttemporal and the metacleithrum lower at 5.6 mm TL. Metacleithrum upper, extrascapula lower and extrascapula upper appeared at 5.7 mm TL, 11.1 mm TL and 13.1mm TL, respectively. Pectoral fin complex completely formed at 13.3 mm TL and also was not observed fully ossify (Figure 2).

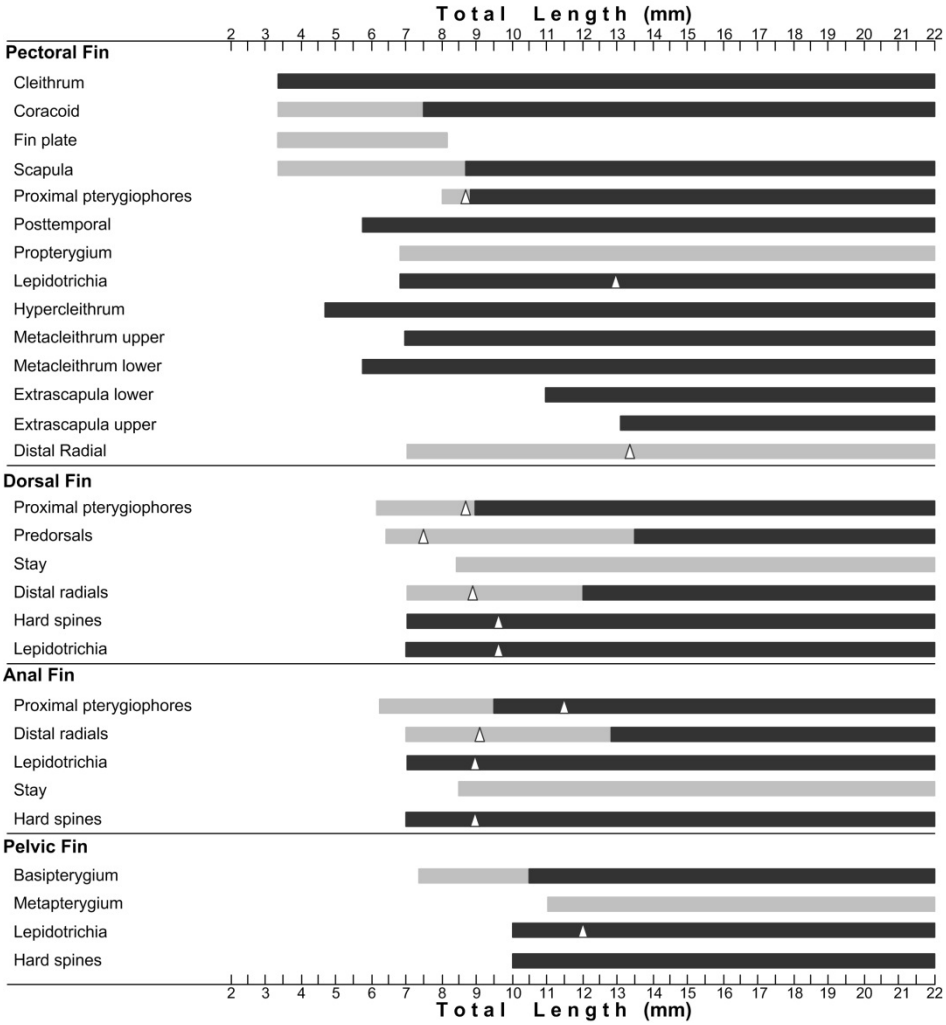

Figure 1. Developmental sequences of fin supports and rays in *Dentex dentex*. Stippled bar, appearance of cartilaginous element. Solid bar, appearance of ossified bone or beginning of ossification of cartilaginous element. ∆ full complement of elements.

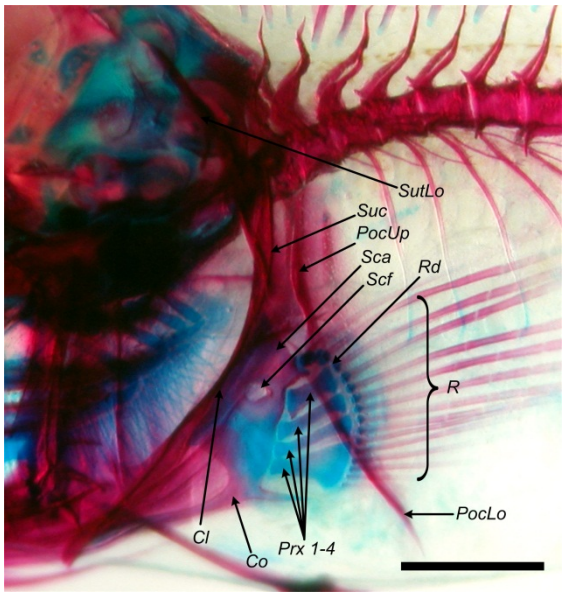

Figure 2. Osteological features of pectoral fin at 13.3 mm TL. *SutLo*, Extrascapula upper; *Suc*, Hypercleithrum; *PocUp*, Metacleithrum upper; *Sca*, Scapula; *Scf*, Scapular foramen; *PocLo*, Metacleithrum lower; *Cl*, Cleithrum; *Co*, Coracoid; *Prx*, Proximal radial; *Rd*, Distal radial; *R*, Lepidotrichia. Scale bars indicate 1.0 mm.

The pelvic fins consist of basipterygium, which is cartilaginous bone, and one hard spine and 5 lepidotrichia on each side of the trunk, which are membranous bone. Basipterygium located behind the pectoral girdle, gradually elongated in an anterior direction towards the cleithrum at 7.3 mm TL. The metapterygium developed as a small cartilaginous structure at 11.0 mm TL. Ossification of basipterygium started at 10.5 mm TL. The lepidotrichia and spine firstly appeared at 7.0 mm TL. Development of pelvic fin completed at 12.1 mm TL (Figure 3A).

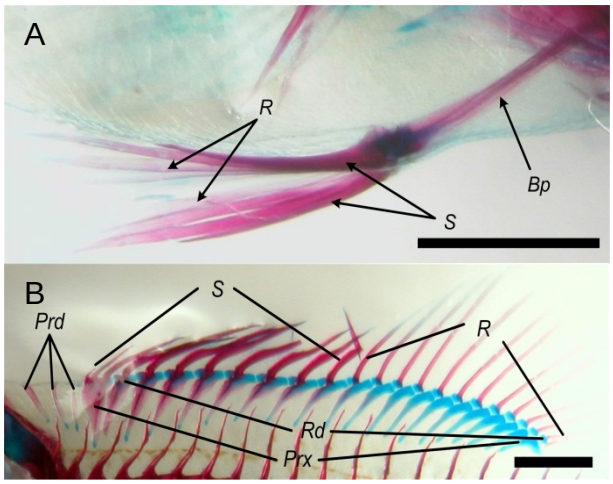

Figure 3. Osteological features of (A) pelvic fin at 12.1 mm TL and (B) dorsal fin at 9.7 mm TL. *Prx*, Proximal radial; *Rd*, Distal radial; *S*, Hard spine; *R*, Lepidotrichia; *Prd*, Predorsal; *Bp*, Basipterygium. Scale bars indicate 1.0 mm.

The dorsal fin and supports consist of membrane and cartilaginous bones. The lepidotrichia and the hard spines are the only membrane bones, while all the remaining elements are cartilaginous bones. Three cartilaginous proximal pterygiophore (Prx) were visible dorsally (Prx2, Prx3 andPrx4) at 6.1 mm TL. The proximal pterygiophores developed to caudal direction and by 8.8 mm TL, all of them formed (counting 18-21). Onset of their ossification observed at 9.0 mm TL and developed from anterior to posterior. Predorsal (Prd 1) cartilage appeared in front of the proximal pterygiophores at 6.4 mm TL. The Prd 2 formed at 6.8 mm TL and followed by Prd 3 (6.9 mm TL). Their ossification started at 13.5 mm TL. Distal radial (Rd) firstly observed at 7.0 mm TL (Rd 1-3) and its formation completed at 8.9 mm TL. The cartilaginous distal radial began to ossify at 12.0 mm TL (Rd 1-4). Development of lepidotrichia (R) firstly occurred at 7.0 mm TL and they attained their final number (10-12 R) at 9.7 mm TL. Dorsal spines (S) firstly appeared at 7.0 mm TL and completed all form at 9.7 mm TL (9-11 S). Only first proximal pterygiophore was support two hard spines. A cartilaginous process formed anterior to Prx 1 at 8.8 mm TL and fused to it forming one continuous element at 10.6 mm TL. The stay cartilage formed as a separate element posterior to the last Prx at 8.5 mm TL. These two elements fused at 10.1 mm TL. At 8.2 mm TL, other separate cartilaginous element formed dorsal to Prx 1 and fused with it at 8.7 mm TL. Development of dorsal fin completed at 9.7 mm TL and also was not observed fully ossify (Figure 3B).

Anal fin consist of the proximal pterygiophores (Prx), the distal radials, the predorsals, the hard spines and the lepidotrichia. The hard spines and the lepidotrichia are only the membrane bones, while all the remaining elements are cartilaginous bones. Prx3-Prx5 of the anal fin appeared first during the developmental range of 6.2-6.7 mm TL and all of them formed at 11.5 mm TL (Figure 4). The ossification started with Prx 1 at 9.5 mm TL and followed caudally. The distal radial was firstly observed at 7.0 mm TL. Full formation of the distal radial completed at 9.2 mm TL and their ossification began at 12.8 mm TL with first distal radial. Development of lepidotrichia appeared during the development phase from 7.0 mm to 9.0 mm TL whereas the hard spines followed them. They attained their final number (3 S and 7-9 R) at 9.2 mm TL. The Prx 1 had two spines at 8.2 mm TL. The stay cartilage formed at 8.5 mm TL and fused to the last Prx at 10.4 mm TL.

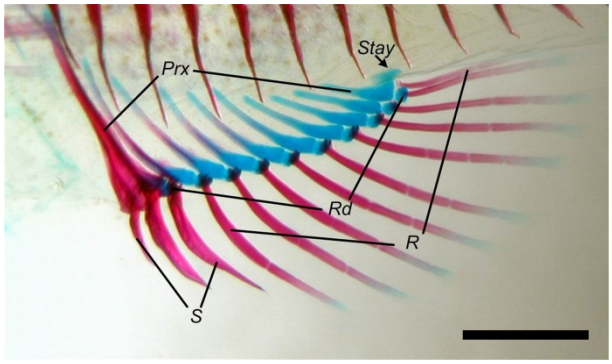

Figure 4. Osteological features of anal fin at 11.5 mm TL. *Prx*, Proximal radial; *Rd*, Distal radial; *R*, Lepidotrichia; *S*, Hard spine. Scale bars indicate 1.0 mm.

Discussion

In current study, we investigated that osteological development of fins (pectoral, pelvic, anal, and dorsal) were examined in *Dentex dentex* during the early life development under intensive culture conditions. It is well known that intensive fish culture has many advantages over the extensive rearing conditions (Wedemeyer 1996). For instance, the water volume is required to provide only living for the fish. Its flow through the tanks is used to deliver the required amount of dissolved oxygen. Metabolic wastes are simple remove from rearing units. Degree of control in intensive condition is easier than in extensive condition. Feeding in intensive condition can easily be mechanized and automated. Under intensive culture condition, fish density in per liter or m^3 is higher than extensive. For these reasons, the trend in fish culture worldwide has been toward more intensive conditions (Tucker and Robinson 1996). The mesocosms have been used with success for rearing a variety of species, among which *Sparus aurata* and *Pagrus pagrus* (Khemis 1997), *Diplodus puntazzo*, *Diplodus sargus* and *Seriola dumerili* (Papandroulakis et al 2004; 2005). Also, osteological development of the fins in *D. dentex* was previously studied by Koumoundouros et al. (2001a) under extensive conditions. Moreover, the intensive method of larval rearing in this study was chosen because these species of Sparidae has reared commercial in intensive conditions.

It is commonly known that teleostei is presented a remarkable variability in respect to the developmental stage of the skeleton at hatching (Koumoundouros et al 2001b). Additionally, skull and fin development was observed after

hatching in Sparidae family (Matsuoka 1987; Koumoundouros et al 2001b; Faustino and Power 2001; Sfakianakis et al 2004; 2005), while, those were determined before hatching in Salmonidae (Kendal et al 1984). Also, skeletal development in *Dentex dentex* larvae was observed after hatching at 3.4 TL related with formation of cleithrum and coracoid-scapula such as *Pagellus erythrinus* (Sfakianakis et al 2004), *Sparus aurata* (Faustino and Power 2001) and *Diplodus sargus* (Koumoundouros et al 2001b).

As usual known, the cleithrum contributes to feeding functions, as it supports the sternohyoideus muscle, which is involved in opening the mouth (Matsuoka, 1987). Exploration of the food is mainly depended on both prey localization and predator avoidance presupposes the development of locomotive ability (Koumoundouros et al 2001a and 2001b). Also, the swimming of the larvae is initially carried out by movements of the primordial marginal finfold, assisted by the early-developed pectoral fin plates (Webb and Weish 1986). The propulsive forces by the caudal part of the body increase as a result of the development of the corresponding fin (Matsuoka 1987). These two developmental stages caused to non-complex larval movements, thus, carried out vital functions for survival and feeding of larvae in early life development. While caudal fin was formed for the first time in *D. dentex* larvae, similar findings were reported in previous studies for Sparid species such as *Pagrus major* (Matsuoka 1987), *Pagellus erythrinus* (Sfakianakis et al 2004), *Diplodus puntazzo* (Sfakianakis et al 2005), *Sparus aurata* (Koumoundouros et al 1997; Faustino and Power 2001), and *Diplodus sargus* (Koumoundouros et al 2001b).

In teleost larvae, dorsal and anal fins and their fin supports were similar in ontogenic development (Faustino and Power 2001). The proximal radials were the first structures to develop, followed by distal radials, predorsals and then rays and spines (Kohno and Taki 1983). Nevertheless, these developmental stages were different both in Sparidae family and among the same species. Moreover, in *D. dentex*, predorsals development was observed after formation of proximal radials, same results were determined in previous studies for *Pagrus major* (Matsuoka 1987), *Pagellus erythrinus* (Sfakianakis et al 2004), *Diplodus puntazzo* (Sfakianakis et al 2005), *Sparus aurata* (Koumoundouros et al 1997; Faustino and Power 2001), and *Diplodus sargus* (Koumoundouros et al 2001b).

It can be concluded that the ontogeny of the osteological developments of the fins in *D. dentex* larvae followed the same general pattern that has been described for most Sparidae species to date. Also, in both fisheries biology and aquaculture, osteological ontogeny serves to promote understanding of functional trends and environmental preferences of the different developmental stages (Fukuhara 1992). Hence, osteological malformations were undesirable factors in fish culture. 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 (Koumoundouros et al 1997; Divanach et al 1996; 1997), which contributes to a better understanding of the species under aquacultural, systematic and ecological consideration (Boglione et al 1995; Webb and Weish 1986). Further studies on chondrification and ossification sequences will be useful to understand how development and function interacts to influence a morphological program and create morphological diversity.

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