Ontogeny of the feeding apparatus of hatchery-reared white margined sole, *Dagetichthys marginatus* (Soleidae): implications for cultivation

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**Summary**

The aim of this study was to describe the ontogeny of the feeding apparatus of hatchery-reared *Dagetichthys marginatus* and to discuss development in relation to generalized early development in (flat)fish and the potential influence of reproduction and/or rearing practices used. Fertilized eggs were obtained from hormone-induced wild females of the white margined sole, *D. marginatus*. Subsequent larvae were reared until shortly after metamorphosis between 35 and 45 dah (days-after-hatching) when mortalities occurred. At 4 dah a small maxilla and two paired branchial arches appeared. Metamorphosis was completed at 22 dah while the left/migrating eye still remained in its initial position at 31 dah. Based on the likely disadvantages for survival it is assumed that the observed development indicates underdevelopment rather than an evolutionary adaptation. The influence of reproduction and rearing practices used on development and mortalities are discussed.

**Introduction**

In Europe the culture of sole, mainly *Solea solea* and *Solea senegalensis*, has attracted research attention over the past three decades (Imsland et al., 2003). The potential for sole aquaculture in southern Africa has only recently been recognized, mainly because of high market value and declining wild stocks of flatfish species (Thompson et al., 2007). The white margined sole, *Dagetichthys marginatus*, has been the focus of several research initiatives. Eggs were first obtained during the 2004/2005 summer spawning season from hormone-induced wild females. In most cases, however, females did not ovulate or fertilization rates were below average. Larvae were obtained from several batches but all died after metamorphosis between 35 and 45 dah (days-after-hatching). The low fertilization rates and the total mortalities may be indicative of poor egg and eleutheroembryo quality, inappropriate culture conditions and/or an inadequate dietary nutrient supply. The objective of this study was to describe the ontogeny of the feeding apparatus of hatchery-reared *Dagetichthys marginatus* and to discuss development in relation to generalized early development in (flat)fish and the potential influence of reproduction and rearing practices used.

**Material and methods**

**Broodstock**

Sole were caught during the 2004/2005 summer spawning season along the south-east coast of South Africa between Port Elizabeth (33°31’S; 27°06’E) and the Great Fish River Point (33°31’S; 27°06’E). Fish were transported to the marine hatchery facilities of the Department of Ichthyology and Fisheries Science in Grahamstown and kept in rectangular tanks (3 × 1.90 × 1.50 m) connected to a recirculation system including mechanical filtration (sandfilter) and biological filtration (trickling filter). Water quality (temperature, salinity, DO, NH4 and NO2-N) was measured every second week. Mean water temperature was 19 ± 1°C and mean salinity 35 ± 1 ppt. Dissolved oxygen (DO) was ≥7.4 mg L⁻¹, TAN was ≤0.014 mg L⁻¹ and NO2-N was ≤0.026 mg L⁻¹. Fish were kept under natural light conditions, varying between 12 and 16 h of daylight depending on the season. About 10% of the total water volume was replaced weekly with mechanically filtered (10 µm) seawater. Sole were fed *ad libitum* levels of shelled sand mussel pieces (*Donax serra*). Uneaten mussel meat was removed daily.

**Spawning, egg incubation and larval rearing**

Larvae and juveniles studied here came from a single batch of eggs of one female and sperm of one male. The female with oocytes in the final stages of maturation were intramuscularly injected with Aquaspawn®, a GnRH analogue (Millar’s Clinical Laboratories, Touws River, South Africa) at 0.5 ml kg⁻¹ body mass. Eggs were stripped approximately 38 h after a single hormone injection. The male was sacrificed, testes removed and homogenized in a small volume (1 ml) of saline solution (0.9 ppt). Eggs were fertilized with the testicular homogenate, washed thoroughly with seawater and divided among 60 L cylindro-conical black upwelling incubators at a density of approximately 20 eggs L⁻¹. Eggs were incubated for about 72 h in complete darkness (light had a negative effect on the hatching rate in previous studies, E. Thompson, pers. comm.). Larvae and juveniles remained in the cylindro-conical incubator tanks. A circular mesh (1000 µm) provided a flat surface for settling larvae. Tanks were connected to a recirculation system including mechanical filtration (sandfilter) and biological filtration (trickling filter). Flow rates were kept at 5 L h⁻¹ during incubation and 30 L h⁻¹ during the larval rearing period. Water quality (temperature, salinity, DO, NH4 and NO2-N) was measured every second week. Mean water temperature was 19 ± 1°C and mean salinity was 35 ± 1 ppt. Dissolved oxygen (DO) was ≥7.4 mg L⁻¹, TAN was ≤0.011 mg L⁻¹ and NO2-N was ≤0.023 mg L⁻¹. Gentle aeration was applied to ensure water circulation and sufficient oxygen supply. About 10% of the total water volume was replaced weekly with mechanically filtered (10 µm) seawater. Tanks were cleaned once a day, uneaten food was siphoned out and dead fish were removed.

**Flow rates were kept at 5 L h⁻¹ during incubation and 30 L h⁻¹ during the larval rearing period.**
Larvae were fed newly hatched Artemia nauplii (INVE, Belgium) from 4 days after hatch (dah) until 16 dah. From 16 dah until 35 dah larvae and early juveniles were fed Artemia metanauplii enriched with SuperSelco® (Artemia Systems, INVE) and frozen Artemia nauplii. The onset of weaning to an extruded pelleted diet began 25 dah. Larvae were then co-fed on live Artemia metanauplii, frozen Artemia nauplii and the manufactured diet until 35 dah when live Artemia metanauplii and frozen Artemia nauplii were withdrawn. Larvae and juveniles were fed twice a day ad libitum.

Larvae were randomly sampled at 0, 4, 6, 9, 13, 16, 22, 31 and 45 dah. At each date 10 specimens were sampled. The developmental state of each individual was visually examined and compared to other specimens of same age. No considerable differences were observed. Therefore one individual was randomly selected to be drawn to represent the respective developmental stage.

Clearing and staining of samples followed the procedure described by Taylor and Van Dyke (1985). The clearing progress was monitored each hour and lasted between 12 and 24 h for larvae and early juveniles, respectively. Bones were then stained in 1% KOH solution with alizarin red and preserved in a 30% glycerine/70% KOH (1%) for 1 week and subsequently in 60% glycerine/40% KOH (1%). 100% glycerine was used as the final preservative.

Anatomical illustrations were made using a Camera Lucida attached to a binocular microscope (Nikon SMZ-10). The osteological nomenclature followed is that of De Beer (1937).

**Results**

The morphological development of the cranial structures is illustrated in Figs 1–5. Elements of the branchial apparatus referred to in the text are not indicated in Figs 1–4, while the complete branchial apparatus is shown in Fig. 5. Larvae between 4 and 13 dah showed no signs of bilateral asymmetry and Fig. 1a–d therefore only illustrate one side (blind side) of the osteocranium. At 16 dah and subsequent stages larvae and early juveniles developed bilateral asymmetries and the blind and ocular sides are shown.

At 4 and 6 dah the maxilla and two paired branchial arches were present (Fig. 1a,b). On day 9, the Meckel's cartilage appeared, and together with the extended maxilla, comprised the first elements of the buccal region. A small parasphenoid developed between the orbits and was the first neurocranial element to appear. An opercle and two branchiostegal rays were developed (Fig. 1c). Several new cartilaginous elements appeared on day 13 (Fig. 1d). A small and thin edentulous premaxilla became visible anterior to the maxilla and elements of the suspensorium, including the ectopterygoid, quadrate, and the hyomandibulo-symplectic were now present. The symplectic was not yet distinct from the hyomandibula. The ceratohyal appeared ventrally to the quadrate, extending antero-ventrally towards the Meckel's cartilage. Six branchiostegal rays were developed postero-ventrally to the ceratohyal. The parasphenoid extended anteriorly over the orbit and posteriorly to the level of the hyomandibular. The dentary
appeared at the dorsal arch of the Meckel cartilage. The opercle was enlarged, forming a triangle, and a small preopercle appeared posterior to the hyomandibulo-symplectic.

At 16 dah two to four larval teeth appeared on either side of the dentaries postero-laterally to the Meckel's cartilage (Fig. 2). The dentary on the right side appeared small in structure, while the one on the left side was well pronounced and triangular in structure. These differences between the right and left dentaries were the first morphological asymmetries to be observed. No asymmetries were observed between the maxillae and the premaxillae of the right and the left side. At this stage no teeth were developed on either side of the premaxilla. The opercular apparatus was complete with the appearance of the subopercle and the interopercle. All existing cranial structures increased in length and diameter leading to two major articulations, the first between the Meckel's cartilage and the quadrate and the second between the hyomandibulo-symplectic and the cranium (otic capsule). The interhyal linked the ceratohyal with the dorsal suspensorium, whose elements the hyomandibular and symplectic appeared as single bones. No asymmetries were observed between the elements of the suspensorium and the opercular bones between the future blind and ocular side. The left prospective migrating eye remained in its initial position, slightly more dorsal than the future ocular eye. All elements were cartilaginous at this stage.
At 22 dah the left / migrating eye still remained in its initial position (Fig. 3). Except for the mesopterygoid all elements of the splanchocranium were developed and were completely or partially ossified. Pronounced asymmetries were observed among elements of the oral jaws which prefigured the structures observed in the adult. The premaxillae and maxillae appeared ossified on either side of the head. The concave shaped premaxilla of the left side was covered with one to two rows of villiform teeth, while its counterpart on the right side was smaller and edentulous. The maxilla on the left side appeared concavely curved, robust and almost entirely excluded from the mouth gape by a large premaxilla. The maxilla of the right side appeared curved with a roughly ‘Y-shaped’ anterior end, bearing the socket of the premaxilla. The dentaries and the anguloarticulars appeared well ossified with the left side lower jaw being quadrangular in shape and its dentary bearing numerous inwardly-curved conical teeth, while the lower jaw on the right side was an edentulous bar-like structure. The hyomandibula and the symplectic appeared as single structures on either side of the head, separated from each other at the level of the preopercular arch. The 'Y-shaped' dorsal edge of the hyomandibula showed some degree of ossification at the level of the neurocranial articulation. The symplectic appeared lightly ossified at the anterior and posterior ends fitting into the posterior slit of the quadrate. No differences in the degree of ossification were observed among the elements of the hyomandibula and the symplectic between the left and right side. The ectopterygoids were ossified on either side of the head and palatines had formed at their dorso-anterior arm. The palate on the left side appeared ossified, while the one on the right side remained cartilaginous. Both quadrates appeared to be well ossified at the level of the joints with the degree of ossification decreasing posteriorly. The quadrate of the left side was larger than its counterpart on the right side. The metapterygoid appeared postero-dorsally to the quadrate on either side of the head. These rhombic-like, at this stage translucent, structures were connected to the bordering suspensorial structures by a cartilaginous complex, which extended towards the palatine anteriorly on the left side of the head. Two halves of the basioccipital appeared along with paired exoccipitals and pterotics comprising the first parts of the cranial vault. These elements were lightly ossified on either side of the head. The parasphenoid appeared well ossified and extended towards the basioccipital posteriorly and towards the ethmoidal region antero-dorsally. The opercles and subopercles were similar in shape and size on either side of the head, while the preopercle and interopercle were larger on the future blind side. The preopercles appeared well ossified on either side of the head. The opercle were only ossified on their anterior margin. The subopercle and interopercle remained unossified at this stage. No significant differences in the degree of ossification were observed between the blind and ocular side opercle, subopercle and interopercle. The ceratohyals were similar in shape and size on both sides of the head. The anterior extended process and the posterior triangular parts were ossified, while the middle section, best described as y-shaped, was cartilaginous. Four ossified branchiostegal rays were articularated to the middle section and three ossified branchiostegal rays were connected to the anterior arm. The interhyal was situated vertically between the posterior part of the suspensorium and the dorso-posterior end of the ceratohyal. The middle section
appeared ossified, while the articulating dorsal and ventral ends were cartilaginous. The posterior bar-like extension of the urohyal was slightly curved downwards and connected to the ceratothy on anteriorly.

At 31 dah the left migrating eye still remained in its initial position (Fig. 4). Most existing structures of the splanchnocranium had advanced in size and degree of ossification. Only the subopercle and interopercle remained unossified. The mesopterygoids were still absent on either side of the head. The cranial vault was almost completely enclosed by cartilaginous structures, though only the basioccipital, the exoccipital, the prootic and pterotic appeared ossified at this stage. Due to the light ossification it remained difficult to distinguish between the single elements of the cranial vault.

At 45 dah the transforming stage was completed (Fig. 5). Both eyes were now located on the right side of the head. The neurocranium was almost completely ossified, though cartilage remained along the borders of some elements. The least degree of ossification was observed among the elements of the sphenotic, parietal and the posterior parts of the frontals. The supraoccipital and the epiparietal were clearly recognizable. Pronounced asymmetries were evident among the anterior elements of the neurocranium. The frontals on the eyed side appeared ventrally curved making contact with the eyeside lateral ethmoid.

The lateral ethmoid of the blind side extended dorso-posteriorly and fused with the frontals to form a wall for the migrated eye's orbit. The ethmoid was curved towards the blind side while the vomer was curved towards the eyed side. Asymmetries between suspensorial and oral elements became very distinct with the elements of the blind side generally being broader than those on the eyed side. Differences in length and shape were observed between the oral jaw elements of the blind side and eyed side. The metapterygoids on either side remained unossified and similar in size and shape. The mesopterygoids were absent on either side of the head.

The subopercle and interopercle remained unossified. All elements of the branchial arch (basibranchials, hypobranchials, ceratobranchials, epibranchials, and infrapharyngobranchials) were ossified, with cartilaginous elements between articulation points. The fifth pair of ceratobranchials was dentulous and together with the dermal toothed plates on infrapharyngobranchials 2, 3 and 4 formed the pharyngeal jaws. The branchial apparatus showed no significant asymmetries and no deflection to either side of the head.

Discussion

Teleosts, including *S. solea* generally form oral jaws, branchial arches and a paraphenoid prior to or simultaneously with the onset of exogenous feeding, suggesting their particular importance for fish larval survival at first feeding (Otten, 1982; Liem, 1991; Van der Meeran, 1991; Hunt von Herbing et al., 1996a; Wagemans and Vandewalle, 2001). In *D. marginatus* the only cranial structures formed at the onset of exogenous feeding were a small maxilla and two paired branchial arches, while further key structures like the Meckel's cartilage and the paraphenoid appeared at 9 dah, about 6 days after complete absorption of the yolk sac. The absence of these cranial structures at the beginning of exogenous feeding may negatively influence feed intake and survival (Verraes, 1974; Potthoff et al., 1987, 1988; Vandewalle et al., 1992, 1995, 1997; Wagemans and Vandewalle, 2001) which is unlikely to be an evolutionary adaptation but rather indicates underdevelopment. The unique adaptation of some flatfish species to benthic life is associated with the development of cranial asymmetries and translocation of one eye resulting in location of both eyes on the upper side (Brewster, 1987, Wagemans et al., 1998). In *D. marginatus* metamorphosis was completed at 22 dah, while the left migrating eye remained in its initial position at 31 dah. Translocation of the migrating eye was completed some time before 45 dah (no samples were taken between 31 and 45 dah). Having settled with the prospective migrating eye remaining in its initial position may cause eye damage and bacterial infection. Impaired vision by one eye only makes the fish more susceptible to predation. The observed eye migration seems more likely to be delayed rather than an evolutionary adaptation.

The development of the feeding apparatus in the present study is based purely on descriptive anatomy and not supported by statistical procedures. The extent to which applied reproduction procedures or rearing conditions have influenced the development of the feeding apparatus cannot be judged, since this was not the specific aim of the study. Present results indicate, however, that the cranial development did not meet the functional demands required to survive. All reproductive efforts resulted in total mortalities shortly after settlement during the period of eye migration (35–45 dah). Subsequent mortalities may be explained by increased energy requirements during metamorphosis that could not be met by the underdeveloped larvae (Brewster, 1987). Another explanation is bacterial infection-caused eye damage. As early larvae already appeared underdeveloped, poor egg quality as a consequence of hormone-induced spawning may be the origin of subsequent mortalities. This is supported by the low fertilization rates obtained during all reproductive efforts. Similarly, although not comprehensively studied, hormone-induced spawning in *S. solea* and *S. senegalensis* resulted in low fertilization rates and poor egg quality (Immsland et al., 2003). Consequently, future efforts should focus on conditioning mature broodstock populations of this species to achieve natural spawning.

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References


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