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Development of mandibular arch in European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) from the "Cenmar" hatchery, Croatia

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ABSTRACT

The mandibular arch ossification process in *Dicentrarchus labrax* L. is described from day 15 to day 80 post-hatching (DPH). Data on this aspect of larval development were presented for both the DPH and average standard length (Ls). Samples were collected in the "Cenmar" hatchery, near Zadar, Croatia. Preparation techniques included fixation in buffered formalin, trypsin clearing and staining with alcian blue and alizarin red. At 15 DPH (Ls 5.0 mm); there were no bony elements in the mandibular arch. The ethmoid plate represented the roof of the splanchnocranium, while Meckel's cartilage, hyosymplectic and cartilaginous palato-quadrates formed lateral walls. Cartilaginous basibranchial, hyoid bar and branchial basket constituted the bottom of the oral cavity. The first bony elements to be noticed were maxillaries (20 DPH, Ls 5.9 mm), followed by premaxillaries and dentaries (25 DPH, Ls 7.9 mm) - all with dermal (membranous) ossification. Ossification of other mandibular arch elements (angular, quadrate, retroarticular, palatine end ectopterygoid bone) was noticed at 38 DPH, Ls 14.5 mm. Pharyngeal jaws and teeth on premaxillaries and dentaries were noticed at the same time. Ossification process of the above mentioned elements (particularly quadrate bone) appears to be completed at 80 DPH, Ls 32.2 mm. Results are discussed in view of the ontogeny process connected with vital requirements in the environment.

Key words: fish ontogeny, ossification, mandibular arch, European sea bass, *Dicentrarchus labrax*

Introduction

The European sea bass (*Dicentrarchus labrax;* Linnaeus, 1758) is a high quality meat fish found in the Mediterranean and Eastern Atlantic region, with a wide distribution along the Adriatic coast. It can grow to a length of about 1 m and with

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a mass of up to 14 kg. Average dimensions: 30-50 cm, 0.50 kg (MILIŠIĆ, 1994). Estuaries and rivers near the sea, with an abundance of small sea fish, shrimps and human food waste, are their preferred habitats and diet, especially for juveniles. With developments in hatchery and cage culture techniques it became an important commercial mariculture species in the Mediterranean area, including Croatia.

Knowledge of the specific environmental requirements of developmental stages is of primary importance for successful hatching and early larval life. Early espying and reduction of skeletal pathology (KOUMOUNDOUROS et al., 1997; BERALDO et al., 2003) have the same goals. We conclude that there is a need for detailed expertise about ontogenesis and morphology of particular organ systems, primarily skeletal and digestive, because they are closely related in early larval development.

Many studies have focused on post-embryonic development of teleost fish (WAGEMANS et al., 1998; KOUMOUNDOUROS et al., 2000; FAUSTINO and POWER, 2001). A few studies exist which document the development of viscerocranial skeleton with the morphology aspect of feeding and some improvement in feeding ability (MOTEKI, 2002; MOTEKI et al., 2002). One of those papers deals with sea bass postembryonic development of the cephalic skeleton (GLUCKMANN et al., 1999). but none of them concerns the same species in the Adriatic Sea.

Initial stages in skeletal development of the European sea bass (as in other teleosts) were noticed on a number of cephalic elements and are connected with the function of collecting food and the development of the respiratory function. The aim of the present paper is to describe the normal ossification process of *Dicentrarchus labrax* mandibular arch elements: palatine, maxillary, premaxillary, dentary, angular, retroarticular, and quadrate bone. Material originates from the "Cenmar" hatchery, near Zadar, Croatia This study may be utilised for improvements in sea bass hatching and should contribute to a better understanding of abnormalities eventually present in developmental patterns.

Materials and methods

This study was based on an intensively reared population of *Dicentrarchus labrax,* at the "Cenmar" hatchery, located near Zadar, Croatia. The fry were raised in water ranging in temperature from 14-15 °C (at hatching) to 19-20 °C (final stage). The hatchery operates a standard technology procedure for oxygen saturation, salinity, nitrite, pH, NH_3, and photoperiod. Each developmental stage received a specific kind of food, with phyto-and zoo-plankton at the beginning (day 5 - day 25 post-hatching - DPH), improved with protein supplement ("Selco®"- Inve Aquaculture N. V. Belgium at 26-45 DPH). Starter was introduced at 45-59 DPH, and after 59 DPH fry were supplied with ground fish, at 65 DPH with *Copepod nauplii.*

European sea bass fry were sampled from 15 to 90 DPH. The specimens were collected every 5 days (15-35 DPH) and, because of the technological possibility, 38, 45, 50, 55, 59, 68, 71, 80, 88 and 90 DPH. Each group was represented with 10 specimens which were fixed in buffered formalin. The DINGERKUS and UHLER (1977) method was used for the staining techniques. Results were clearly apparent: cartilaginous became blue (alcian blue), and bones red (alizarin red). Ontogenesis of mandibular arch elements (with emphasis on palatine, maxillary, premaxillary, dentary, angular, retroarticular and quadrate bone) was observed under Nikon stereoscopic microscope (magnification: 2× and 4×) and Flash Bus FBG computer software. Specimens (10 for each category) were measured and average standard length (Ls) was used for correlation with DPH.

Results will be given, from hatching to older phases. Research commenced on *Dicentrarchus labrax* skeleton (from the fish osteological collection at the Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb) and comparison with those of older fry, and subsequently the structures of older fry with those of younger fry.

Results

Dicentrarchus labrax, as with Percomorphi in general, has a tropibasic skull. It is characteristic by trabecular bars joined in trabecula communis and is recognizable by fish heads which are higher than they are wide.

The sequence of mandibular arch element ossification is as follows: Similar as at hatching, 15 DPH, Ls: 5.0 mm (Fig.1), there are no ossified elements in the mandibular arch. Trabecular bars are joined anteriorly, constituting an ethmoid plate. The ethmoid plate represents the roof of the splanchnocranium, while Meckel's cartilage, cartilaginous palato-quadrates and hyosymplectic form lateral walls. The cartilaginous basibranchial, hyoid bar and branchial basket constitute the bottom of the oral cavity. In a few specimens some osteoid foundations of the maxillary bone were present.

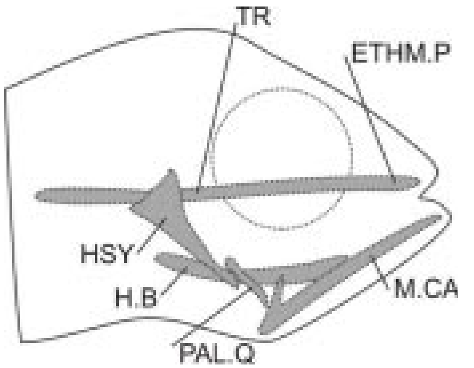

Fig. 1. *Dicentrarchus labrax* L. mandibular arch elements, (15. DPH, Ls: 5.0 mm), lateral view. The dotted lines and greyish colour indicate cartilage. ETHM.P: ethmoid plate; H.B: hyoid bar; HSY: hyosymplectic; M.CA: Meckel's cartilage; PAL.Q: palato-quadrate; TR: trabecula.

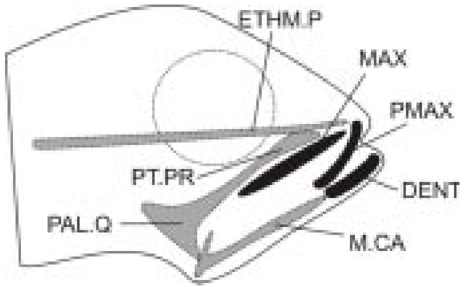

Fig. 2. *Dicentrarchus labrax* L. mandibular arch elements, (25. DPH, Ls: 7.9 mm), lateral view. The dotted lines and greyish colour indicate cartilage. Full lines and black colour represent bony elements. DENT: dentary; ETHM.P: ethmoid plate; MAX: maxillary; M.CA: Meckel's cartilage; PAL.Q: palato-quadrate; PMAX: premaxillary, PT.PR: pterygoid process.

20 DPH, Ls: 5.9 mm. Existing cartilaginous components have enlarged. Meckel's cartilages are curved; left and right elements are in contact with each other anteriorly. Palato-quadrates still appear like small sticks near the hyosymplectic and Meckel's cartilages. First signs of dermal maxillaries are present: two small, curved, osteoid rods.

25 DPH, Ls: 7.9 mm (Fig. 2). Meckel's cartilage starts to resorb in the anterior 1/3, except in the median connecting part. Processus dorsalis is clearly visible. After maxillaries (20 DPH), the first signs of dentaries are present at the same time as the premaxillary. The premaxillary appears as a curved, flat structure in front maxillaries. Pterygoid processes of palato-quadrate extend to the front of the ethmoid plate.

30 DPH, Ls: 9.0 mm (only 4 samples). The splanchnocranium remains practically unchanged. Pterygoid processes of cartilaginous palato-quadrates are clearly visible under the otic capsule. There is no sign of ossification on the palatine or quadrate part.

35 DPH, Ls: 11.2 mm. Maxillaries, premaxillaries and dentaries: ossification has developed, while the cartilaginous structures continue to regress. The maxillary's posteroventral end is in contact with the posterodorsal edge of the dentary.

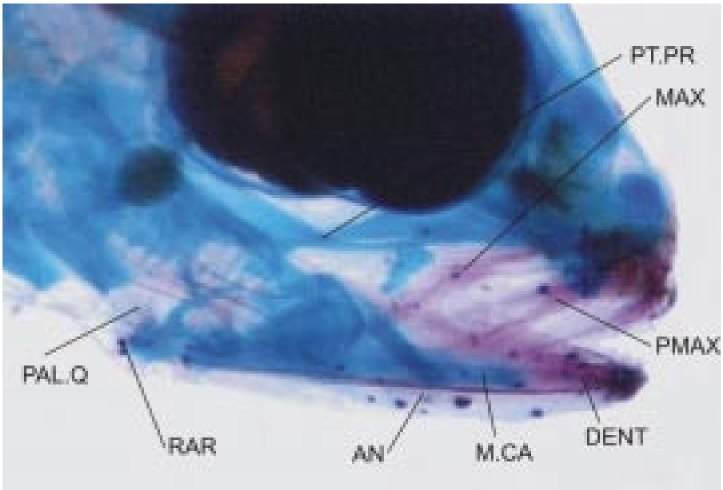

Fig. 3. *Dicentrarchus labrax* L., (38. DPH, Ls: 14.5 mm), lateral view. AN: angular; DENT: dentary; MAX: maxillary; M.CA: Meckel's cartilage; PAL.Q: palato-quadrate; PMAX: premaxillary; PT.PR: pterygoid process; RAR: retroarticular.

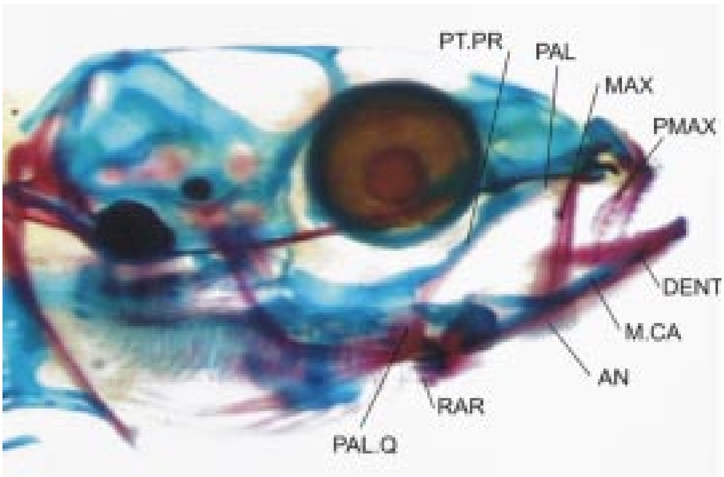

Fig. 4. *Dicentrarchus labrax* L., (50. DPH, Ls: 18.9 mm), lateral view. AN: angular; DENT: dentary; MAX: maxillary; M.CA: Meckel's cartilage; PAL: palatine; PAL.Q: palato-quadrate; PMAX: premaxillary; PT.PR: pterygoid process; RAR: retroarticular.

38 DPH, Ls: 14.5 mm (Fig. 3). Premaxillaries and dentaries are well ossified, and teeth are visible. Toothless maxillaries are enlarged and are still in the process of ossification. The osteocranium has progressed. First chondral ossification appeared: the articulation of angular and quadrate, the same as the palatine bone. An indication of membranous retroarticular is present; the ectopterygoid bone is visible between the quadrate and palatine. The retroarticular bone is attached to caudal margin of the angular. Bony pharyngeal jaws are visible.

45 DPH, Ls: 14.6 mm. Meckel's cartilage is reduced in its permanent size. Angular, quadrate and retroarticular are in the middle of the ossification process. Ossification spreads anteriorly towards dentary, and posterodorsally towards quadrate. The bony splanchnocranium now displays maxillaries, premaxillaries, paltine, and dentaries.

50 DPH, Ls: 18.9 mm (Fig. 4) - 55 DPH, Ls: 20.5 mm. Ossification of cartilaginous-origin bones (palatine, angular, and quadrate) is still in progress. Maxillaries, premaxillaries, dentaries and retroarticular bone have become enlarged.

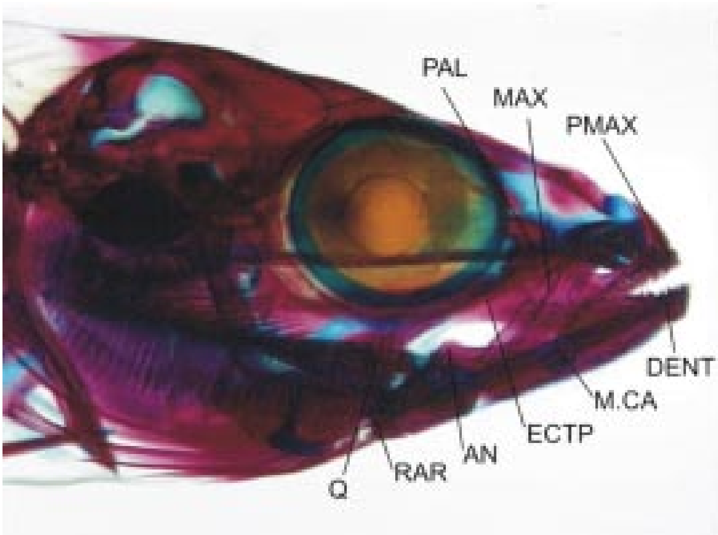

Fig. 5. *Dicentrarchus labrax* L., (80. DPH, Ls: 32.2 mm), lateral view. AN: angular; DENT: dentary; MAX: maxillary; M.CA: Meckel's cartilage; PAL: palatine; Q: quadrate; PMAX: premaxillary; ECTP: ectopterygoid; RAR: retroarticular.

59 DPH, Ls: 22.1 mm - 71 DPH, Ls: 27.3 mm. - Different calcification is visible through the red coloured intensity of specific mandibular arch elements. At the end of this period, ossification of almost all mandibular arch elements is complete. This includes fusion of each angular with the corresponding dentary and fusion retroarticular with the rest of the mandible. At day 71 DPH, only angular and quadrate had not completed the ossification process.

80 DPH, LS: 32.2 mm (Fig. 5). All bone elements of mandibular arch are present. The upper jaw is represented by toothless maxillary (paired) and tooth-bearing premaxillaries (paired). The lower jaw (mandible) is composed of the tooth-bearing dentary (paired, firmly connected), angular (paired, bears articulatory surface for the quadrate), retroarticular (paired) bone and Meckel's cartilage. Meckel's cartilage still exists but is maximally reduced. The mandible, across the angular bone, appears to articulate perfectly with the quadrates. Quadrates are connected through the ectopterygoid to the palatine bone.

During the ossification process the researched material displayed no pathological modification on cephalic region elements.

Discussion

Different style and description beginnings, different living and farming conditions makes for difficulties in comparing stages of development of cephalic bony elements in teleosts (VANDEWALLE et al., 1995). Therefore, we discussed bony development in *Dicentrarchus labrax* L. showing two parameters: DPH (day post-hatching) and standard length (Ls). The fry receive their initial nutritional supplement at 5-6 DPH. Yolk sac reduction and its disappearance in combination with mixed-type feeding occur between 8-10 DPH. (ŠARUŠIĆ, personal comm.). At 15 DPH, European sea bass juveniles demonstrate some kind of exogenous feeding (bigger *Artemia* sp.), but there are no bony elements in the oral cavity. As was evidenced by the current study, the oral cavity of juveniles is formed by cartilaginous elements: ethmoid plate, Meckel's cartilage, palato-quadrates, hyosymplectic, basibranchial, hyoid bar and branchial basket. In other researches involving the same species, GLUCKMANN et al. (1999) reports a different situation. They noticed onset of ossification of maxillaries and lower jaw ossification before disappearance of the yolk sac (in larvae Ls = 5.2 and 5.7 mm). However, the maxillaries are the first bone in the cephalic region. The same applies to *Dentex*

dentex L. (KOUMOUNDOUROS et al., 2000), while VANDEWALLE et al. (1995) mention dentary as a first osifficate bone (2 DPH) in *Chrysichthys auratus* (Geoff. Saint-Hilaire, 1808).

Regression of cartilaginous parts exists in many teleosts, although it commences at different developmental sizes in different species (GLUCKMANN et al., 1999). In the mandibular arch of *Dicentrarchus labrax* L., the regression process begins first at Meckel's cartilage. This process commences soon after maxillary appearance (20 DPH), and before dentary indication (25 DPH).

In *Dicentrarchus labrax* L., shortly after maxillaries (20 DPH, Ls 5.9 mm), ossification begins on the premaxillaries (25 DPH, Ls 7.9 mm) and on dentaries at the same time. A similar situation, despite ossification order (neither time nor Ls), is noticed in *Dentex dentex* L. (KOUMOUNDOUROS et al., 2000) and *Xenistius californiensis* (WATSON and WALKER, 1992). GLUCKMANN et al. (1999) establish a different order for *Dicentrarchus labrax* L.: first maxillaries (Ls 5.2 mm), then dentaries (Ls 5.7) and ten premaxillaries (Ls 7.6 mm).

KATAVIĆ (1984) divides postembryonic development in *Dicentrarchus labrax* L. into three phases: 0-7 DPH larvae ("yolk sac"), 7-40 DPH post larvae (exogenous feeding), after 40 DPH juveniles. According to that division, the present research confirmed the initiating process of ossification on all elements of the mandibular arch in the larval phase (before 40 DPH) and is not completed until juvenile phases, respectively, at 80 DPH, although the first bony elements of the mandibular arch exist before the introduction of protein supplement in food (26 DPH). At the same time, all mandibular arch elements were initiating the ossification process prior to the introduction of starter food. In *Dentex dentex* L., all elements of the mandibular arch are formed during the larval stage (KOUMOUNDOUROS et al., 2000), while in *Chrysichthys auratus,* the ossification process is not completed until day 28 post-hatching (VANDEWALLE et al., 1995).

Obtained data suggest that successful mariculture is possible only if the rearing process is successful, with maximal food adjustment to different fish stages and diminishing food loss at the same time, with an increasing degree of juvenile survival. A strong correlation exists between all the above mentioned facts, as well as knowledge of the ontogeny process and specific requirements of different fish species or category. This is particularly important at that point in time when fry switch from endogenous to exogenous feeding. In *Dicentrarchus labrax* L., during that specific time, no bony elements are noticed in the mandibular arch. If we know that all bone elements in mandibular arch have been in the process of ossification at 38 DPH, it can be concluded that all food which needs to be consumed is maximally profitable after that point, or even after (80 DPH) when all process are completed.

Different data have been obtained about the correlation of DPH and Ls (KATAVIĆ, 1984; GLUCKMANN et al., 1999). There is also a possible difference in ossification process order, or at the time when it occurs. These results may have an influence on the ichthyoplancton identification process or on establishing a method for determination of DPH based on Ls. This may be partly explained by different environmental conditions, or perhaps by a different population of the same species.

Acknowledgments

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KUŽIR, S., Z. KOZARIĆ, S. NEJEDLI: Razvoj donjočeljusnog luka u brancina, *Dicentrarchus labrax* L. iz uzgajališta "Cenmar", Hrvatska. Vet. arhiv 74, 321-330, 2004.

SAŽETAK

Proces okoštavanja donjočeljusnog luka u brancina, *Dicentrarchus labrax* L. opisan je od 15. dana poslije valenja (DPV) do 80. DPV. Navedeni razvoj je prikazan istodobno prema dobi ličinke izraženoj u danima poslije valenja i srednjoj standardnoj dužini ličinke. Uzorci su bili podrijetlom iz uzgajališta "Cenmar" pokraj Zadra, Hrvatska. Postupak pripreme uzoraka obuhvatio je njihovo držanje u formalinu, uklanjanje pigmenta tkiva tripsinom te bojanje alcijanskim modrilom i alizarinskim crvenilom. Na uzorcima uzetim 15. DPV, (Ls 5,00 mm) nisu zamijećeni koštani dijelovi donjočeljusnog luka. Etmoidna ploča činila je gornji dio, a Mekelova hrskavica, nepčano-kvadratna i hiosimpletična hrskavica postrane osnove usne šupljine. Bazibranhialna hrskavica zajedno s drugim branhialnim hrskavicama predstavljala je dno iste šupljine. Prvi uočeni koštani dijelovi bile su maksile (20. DPV, Ls 5,9 mm), zatim premaksile te dentalne kosti (25. DPV, Ls 7,9 mm), sve nastale membranoznim okoštavanjem. Okoštavanje drugih dijelova viličnog luka (angularne, kvadratne, retroartikularne, nepčane i ektopterigoidalne kosti) su uočene 38. DPV, Ls 14,5 mm. Proces okoštavanja na navedenim dijelovima, čini se završenim 80. DPV, Ls 32,2 mm. Rezultati su razmatrani u smislu povezanosti ontogeneze i odgovora ličinke na okolišne uvjete.

Ključne riječi: ontogeneza riba, donjočeljusni luk, brancin, *Dicentrarchus labrax*