

Development Of The Cranial Lateral-Line System And Canal Related Bones In *Salmo Letnica* Kar. (Teleostei: Salmonidae)

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Abstract

In this study the ontogenetic development of the cranial lateral line and the canal related bones of Ohrid trout, Salmo letnica were studied from hatching until the age of 92 days posthatching (PH). Samples at the age of 200 days PH and one-year-old trout's were included in this study. Most of the samples were in toto trypsin cleared and stained; some specimens were used for making serial histological sections. The center and type of ossification was observed using these serial sections. The os dentale, which encloses the rostral part of the praeoperculo-mandibular canal, was the first bone formed. The formation of this bone was observed at the age of one day PH. At the age of 5 days PH, the ossification of the os posttemporale was noticed. Few days later the ossification of the os frontale was noticed. The first sign of the os dermopteroticum was evident at 25 days PH. The formation of the os lacrimale was observed at 29 days. The formation of the os antorbitale, the os infraorbitale II, IV, V, and the os suprapreoperculare was simultaneously at the age of 92 days PH.

Key words: Salmonidae, ontogeny, lateral-line system, osteology, canal bones

Introduction

The lateral line system is a primitive vertebrate sensory system, present in most fishes, as well as in larval and aquatic adult amphibians. The lateral-line system is a fairly important sense organs for fishes, which use it to discover and locate solid bodies situated or moving in water, as well as it plays an important role in a schooling behavior (Halama, 1977; Webb, 1989a). The pattern of this system, especially the position of the sensory canals in particular bones of the skull and the innervations of the neuromast enclosed by these bones, has proven to be of great importance for taxonomic studies, as well as it reflects possible phylogenetic relationships within teleost groups (Andriashev & Jakubowski, 1971; Jakubowski, 1974; Halama, 1978; Webb, 1989a; Arratia & Gayet, 1995). However, ontogenetic evidence of the formation of this lateral-line system as well as the early development of the bony components associated with it, can provide a very important source of information and contributes to the understanding of the adult lateral-line morphology (Jollie, 1975; Webb, 1989b; Adriaens et al, 1997). In this work we present the preliminary results of the early development of the cranial lateral-line system and bones associated with it, focusing on the time of their appearance and their center of ossification in Ohrid trout, an endemic, relict and polymorphic species of Ohrid Lake. This study provides a base for further investigations dealing with the development of the pit organs, pit lines, free neuromasts, which require other methodological approaches.

Material and methods

The samples of the Ohrid trout were taken from the spawning place (artificial habitat) of the Hidrobiological Institute in Ohrid from the moment of hatching. The samples were raised in special incubators with a constant water temperature of 10.5 °C, located in the building, up to an age of 92 days PH. Samples of 150 specimens were taken at intervals of 1, 3, 7, days from hatching to day 92 PH. The total length (TL) was measured in millimeter by calipers for all specimens. A chronological evaluation of the formation of the canal bones and bones related to the head lateral-line system were performed using the following methods: in toto clearing and staining (alcian blue and alizarin red S) and serial histological sections. The material (7-10 samples from every stage) for in toto clearing and staining was fixed for 24-48 hours in a 10% neutral formalin solution. Small specimens were cleared in a trypsin solution, whereas for larger ones a KOH-solution was used. Staining was done according to Balon and Noakes (in Balon 1985). The canal bones and bones associated with the lateral line of the head was studied using a WILD M3C stereomicroscope equipped with a camera Lucida. The

material for histological sectioning was fixed in Bouin's, and embedding was done following Verraes (1974). Serial sections of 10 μ m were cut on a rotation microtome (Microm HM360) and staining was done according to Mangakis & Pikova-Müllerova (1964). The sections were studied using a light microscope Leitz Wetzlar.

Results

1 day after hatching (TL 13.4 -14.4 mm). In the cleared and stained specimens, no bony structure related to the lateral line canal is visible. However, the analysis of the histological sections shows the presence of a dermal bone near the anterior part of Meckel's cartilage. By its location this bone corresponds to the os dentale.

3 days after hatching (TL 13.4 - 15.2 mm). Posterior to the cranium, at the caudal margin of the otic capsule, the presence of an os posttemporale was noticed.

7 days (TL 16.1-16.9 mm) and 9 days after hatching (TL 16.6-17.3 mm). The contour of the os angulare (dermal bone) is visible on the lateral side of the medio-posterior edge of Meckel's cartilage at samples old 7 days PH. In all the cleared specimens examined (9 days PH), the presence of the membranodermal component of the os frontale was observed. The centre of ossification is located dorsally of the taenia marginalis. No sign of neurodermal components was noticed (no serial sections were available of this stage).

14 days (TL 18.4-19.2 mm) and 16 days after hatching (TL 18.7-19.7 mm). The analysis of the serial sections made from the 14 days (PH) old specimens, shows the presence of a gutter-like, bony formation at the level of a superficial neuromast of the supraorbital canal (is there only one superficial neuromast of the supraorbital canal). This bone corresponds to the neurodermal component of the os frontale. In cleared and stained specimens, the formation of the neurodermal component of this bone was noticed for the first time at 16 days PH. The histological sections of a 14 days (PH) old specimen show the onset of the ossification of the os spleniale, which could not be observed in the cleared and stained specimens of that stage. At 16 days PH old specimens a small gutter-like bone start its formation. It lies near the anterior part of the frontal bone, dorsal of the taenia marginalis anterior. The preopercular bones are visible at the level of the articulation of the postero-ventral margin of palatoquadrate cartilage and the ventral margin of the hyosymplecticum. Around the most posterior part of the processus retroarticularis of Meckel's cartilage, the development of the os retroarticulare could be observed.

19 days after hatching (TL 19.8-20.9 mm). In all cleared and stained specimens the os spleniale was visible. This bone develops as a continuation of the dental bone, around the rostral part of the mandibular canal. An analysis of the serial sections shows the presence of a perichondral ossification, the os mentomeckelium, around the anterior part of the Meckel's cartilage, which is as continuous with the dermal bone.

25 days after hatching (TL 20.7-21.8 mm) and 29 days after hatching (TL 21.6-22.8 mm). At 25 days PH, the formation of the three separate gutter-like bones was noticed at the level of the otic region, lateral to the anterior part of the capsula auditiva (FIG. 1). By their location they correspond to the os dermopteroticum. In all 29 days old specimens, a small tubulous bone, the os lacrimale was observed at the ventral margin of the anterior part of the orbit. The ossification of the os frontale becomes spread caudally.

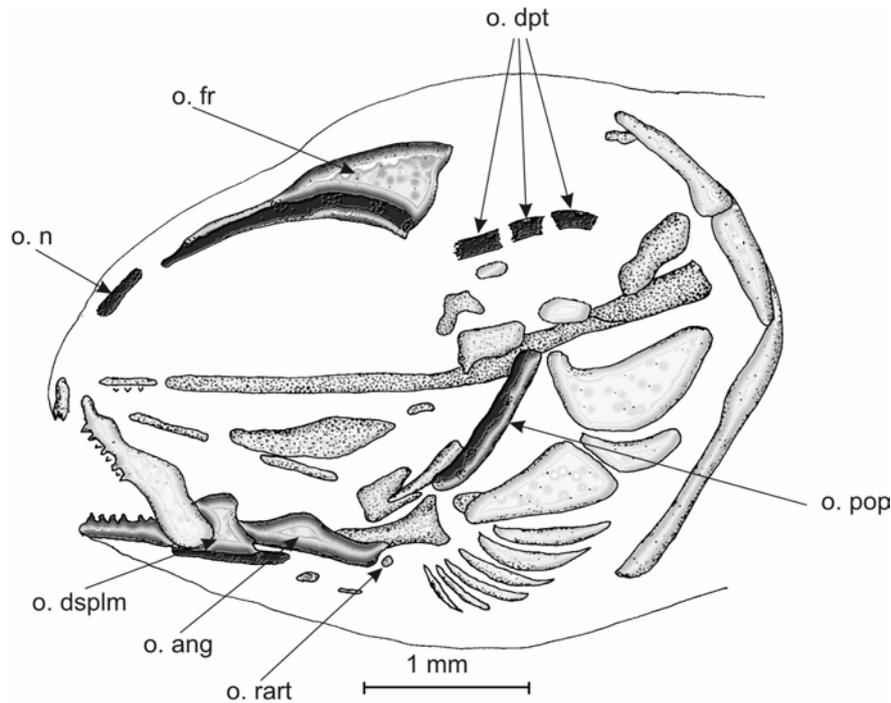


Figure 1. Osteocranium of *Salmo letnica* at 25 days after hatching (lateral view): o. ang, os angulare; o. dsplm, os dento-splenio-mentomeckelium; o. dpt, os dermopteroticum; o. pop, os preoperculum; o. rart, os retroarticulare. Note: only bones which are in correlation with sensory canals are indicated.

39 days after hatching (TL 22.4-23 mm). In comparison with the previous stages, the os dermopteroticum has been added as a single bony tube, encircling the temporal canal. The neurodermal component of the os angulare encloses the lateral part of the mandibular canal.

50 days after hatching (TL 22.8-23.4 mm). An analysis of the serial sections shows two ossification centres, which are related to the bones enclosing the mandibular canal. At this stage and by using the mentioned histological methods we found that the first ossification centre arises as a narrow perichondral lamella on the dorsal surface of the Meckel's cartilage, at the place where the tendon of the m. adductor mandibulae is attached dorsally on Meckel's cartilage. This perichondral bone represents the os coronomeckelium. Further investigation should be done on the early development of this bone. The second centre of ossification appeared as a perichondral ossification around the caudal part of Meckel's cartilage, in connection with angular bone. Consequently, this bone ossification corresponds to the os articulare.

61 days after hatching (TL 23.2-24 mm). All cleared and stained specimens from this stage show a perichondral ossification on the lateral wall of the auditory capsule, ventral to the caudal end of the os dermopteroticum. This forms the os autopteroticum. In these specimens studied, a small space seemed to separate these two bones. However, as no serial histological sections were available for this stage, we couldn't verify whether there is a connection between the two bones or not.

82 days (TL 26.6-27.7 mm) and 92 days after hatching (TL 28.5-31.2 mm). In the ethmoid region the presence of the os dermethmoideum and os lateroethmoideum was noticed in 82 days old specimens. At the posterior end of the dermopteroticum, a small inverted T-shaped bone was observed. By its location this bone must correspond with the os extrascapulare lateral, with the posterior lateral line running anteriorly into the temporal canal and branching dorsally into the supratemporal canal. Ventral to the orbit and around the medial part of the infraorbital canal formation of the os infraorbitale I and III was noticed. The formation of the os infraorbitale II, IV and V was only observed at 92 days PH. We should mention that in some of the examples variation in the number of infraorbital bones was noticed. At the same stage the os antorbitale was recognizable.

200 days after hatching (TL 38.3-51 mm). Now the presence of the ossa extrascapularia was observed. These tube-like bones surround the supratemporal canal. In some of the specimens 2

tubular bones on each side of the head and 1 medial bone could be observed, even though in some of the samples there were 3 tubular bones on each side of the head.

Discussion

In generalized teleosts, the cranial lateral-line system comprises seven pairs of cranial lateral-line canals, two cranial lateral-line commissures and several lines of superficial sensory organs (see Adriaens et al, 1997). However, during evolution several canals have become reduced or even have fused with each other (Verraes, 1973; Jollie, 1975; Webb, 1989b). Our investigation shows that in Ohrid trout there is a mandibular, preopercular, infraorbital, supraorbital, temporal and supratemporal (= occipital canal according to the nomenclature of Jollie, 1984; = extrascapular canal of Nelson, 1972).

Supraorbital canal in Ohrid trout runs from the anterior nostril, caudally fusing with the temporal canal (FIGS. 3, 4). A supraorbital groove has invaginated in the middle part above the eye. As a result of this invagination, at 14 days PH the ossification of the neurodermal component of os frontale starts. It is interesting to mention that by using the in toto staining method, we could observe that the membranodermal component ossifies earlier (at 9 days PH) than the neurodermal component (at 14 days PH). The same scheme of formation of these two components is noticed in *Esox americanus* and *E. lucius* (Jollie, 1975), in *Thymallus* the formation of the neurodermal component occurs earlier (Norden, 1961). It seems that the mechanisms of formation of these two components is different (Moy-Thomas, 1941; Orvig, 1972; Verraes, 1974; Adriaens et al, 1997). The nasal part of the supraorbital line invaginates separately 2 days later (16 days PH), initiating the formation of the os nasale. Os dermethmoideum and os lateroethmoideum are not canal bones but we include them here because they support the anterior portion of the supraorbital canal. There is a lot of confusion about the name of the bone that ossifies dorsal to the ethmoid region in salmonids fishes (de Beer, 1937; Norden, 1961; Svetovidov, 1975; Sanford, 2000), as well as in other teleosts (see Adriaens et al, 1997). An analysis of the histological sections of 92 days trout shows that the bone, which develops on the anterodorsal border of the ethmoid cartilage, is a dermal bone, which excludes it from being a supraethmoid (Adriaens et al., 1997).

Invaginations of the infraorbital canal in Ohrid trout start from front (29 days PH) to back (92 days PH). 7 bones surround the canal: os lacrimale, and the ossa infraorbitalia I-V (FIG. 4). Here we include the os antorbital, even though this bone doesn't surround this canal. According to Sanford (2000) this bone is plesiomorphically associated with the laterosensory canal and in salmonids it has been lost this association. As we mention in the results in some of the samples of Ohrid trout the number of infraorbital bones was 8. The variation in the number of ossicles is also noticed in other salmonids fish (Norden, 1961). The last infraorbital bone terminates at the junction between the supraorbital and temporal canals. Much confusion stems from authors referring to this bone as the dermosphenotic. By Sanford (2000) the homology of the dermosphenotic in relation to the infraorbital bones has not been resolved, thus it will be referred to as the last infraorbital. The last infraorbital has 3 different conditions within Salmonidae (Sanford, 2000). In Ohrid trout the infraorbital canal enters this bone ventrally and runs dorsally to connect with the supraorbital and temporal canal, thus forming a T-junction at the dorsal margin of the bone. This configuration can also be found in *Salmo*, *Oncorhynchus* and *Salvelinus* (Sanford, 2000).

The mandibular groove start to invaginated at the same day as the invaginations of the supraorbital canal and the preopercular canal. Simultaneous invaginations of these two canals were also observed in *O. kisutch* (Jollie, 1984). This invaginations start at the middle part of the mandibular canal and during ontogeny of Ohrid trout, it spreads anteriorly and caudally. The bones that surround the anterior part of this canal make a complex dento-spleno-mentomeckelium, as well as the bones surrounding the posterior part of that canal comprise an angulo-spleno-articular complex (FIG. 2). The preopercular canal is the dorsal part of the preoperculo-mandibular canal. The invaginations of its groove start at the same day as that of the mandibular and supraorbital canals. This invagination in Ohrid trout starts at the anterior (lower) end of the preopercular canal, similar as in *Amia* and *Esox* (Jollie, 1975), but opposite of *O. kisutch* where the invaginations start at the top (Jollie, 1984). In one-year-old Ohrid trout, five branches radiate out from primary preopercular canal. The suprapreopercular bone is a small, tubular ossification surrounding the sensory canal lying between the dorsal tip of the preopercle and the temporal canal. This bone is present in *Salmo*, *Oncorhynchus*, *Salvelinus*, *Branchymystax* and *Hucho*, but is absent in all other salmonids (Sanford, 2000). According to the same author, in some specimens of *O. masou* a second suprapreopercular bone was observed.

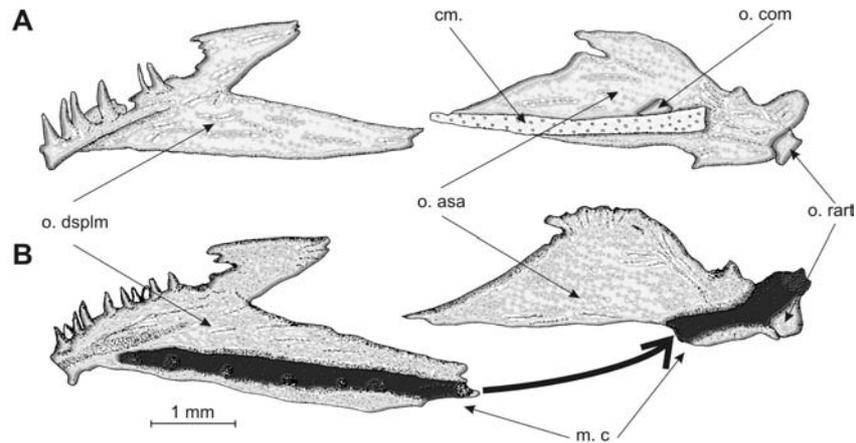


Figure 2. Lower jaw of *Salmo letnica* at 200 days after hatching . A. medial view; B. lateral view (the dark arrow indicates the mandibular canal): cm, cartilago Meckelio; o. asa, os angulo-splenio-articalre; o. dsplm, os dento-splenio-mentomeckelium; o. com, os coronomeckelium; o. rart, os retroarticulare

The pterotic bone encloses the temporal canal. This bone in Ohrid trout, as well as in other salmonids fishes, comprises two parts: the dermo- and autopterotic. There is a lot of disagreement on whether a "fusion" occurs between these two bones (de Beer, 1937; Patterson, 1977; Verraes, 1973; Jollie, 1984). Further investigation should be done on the ontogenetic development of this bone in Ohrid trout, using histological sections.

In comparison with other cranial lateral-line canals, the invaginations of the supratemporal canal starts very late in Ohrid trout. The first evidence was only observed at 82 days PH. The canal bone that surrounds the supratemporal canal, was observed much later, i.e. at 200 days PH. Variation in the number of the extrascapular bones occurs in this trout (FIG. 3), which is also reported for other salmonids fishes (Norden, 1961; Jollie, 1984; Sanford, 2000). The os extrascapulare laterale encloses the anastomosis of the temporal, the supraoccipital and the posttemporal branches. The posttemporal canal, surrounded by the neurodermal component of the posttemporal bone and the supracleithrum, forms a connection between the cranial lateral line and the postcranial lateral line. During the ontogenetic development of the trout, ossification of the membranodermal component of these two bones occurs earlier than that of the neurodermal part.

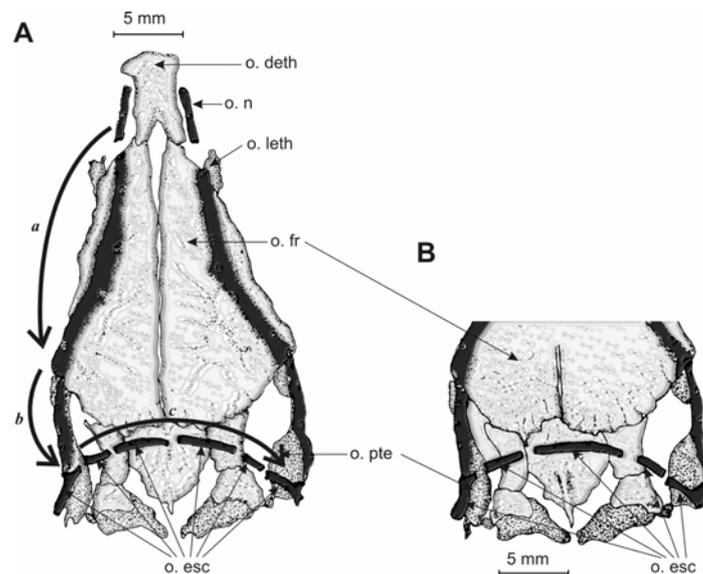


Figure 3. Neurocranium of *Salmo letnica* at one-year-old after hatching. A. dorsal view of all neurocranium (dark arrow indicates: a. the bones which enclosed the supraorbital canal, b. bone which enclosed the temopral canal; c. the bones which enclosed the supratemopral canal), B. dorsal view of posterior region of neurocranium (variation in the numbers of the extrascapular bones are indicated on A. and B.): o. deth, os dermoethoideum; o. esc, ossa extrascapularia; o. fr, os frontale; o. leth, os lateroethmoideumo. o. pte, os pteroticum.

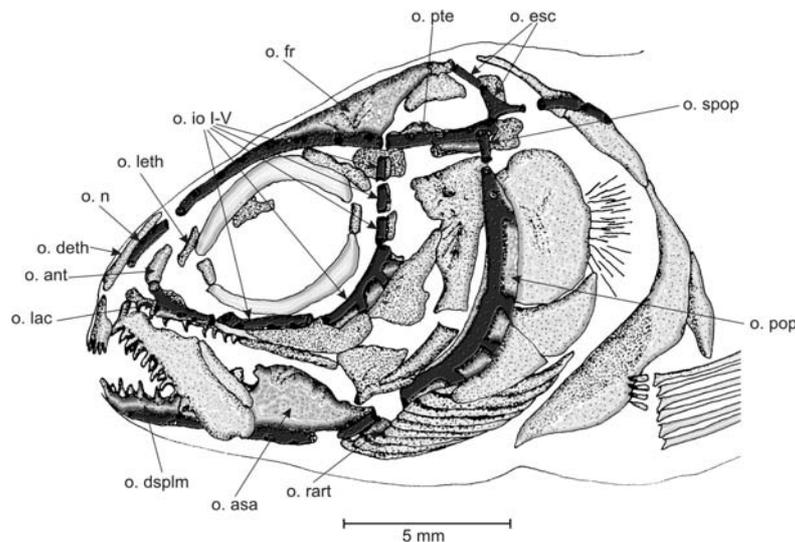


Figure 4. Osteocranium of *Salmo letnica* one-year-old after hatching. (lateral view): o. ang, os angulare; o. ant, os antorbitale; o. asa, os angulo-splenio-articalre; o. deth, os dermoethoideum; o. dspim, os dento-splenio-mentomeckelium; o. esc, ossa extrascapularia; o. lac, os lacrymale; o. leth, os lateroethmoideum; o. io I-V, os infraorbitale I-V; o. pop, os preoperculum; o. pte, os pteroticum; o. rart, os retroarticulare; os spop, os suprapreoperculare. Note: only bones which are in correlation with cranial laterl-line canals are indicated.

Acknowledgments

Successful collections of material were realised thanks to the support of the Ohrid Hidrobiological Institute. Special thanks to M.Sc. Zoran Spirkovski for his great assistance in the fieldwork.

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