

# Early development of the postcranial axial skeleton in *Salmo letnica* Karaman, 1924 (Teleostei: Salmonidae)

MILICA RISTOVSKA,<sup>1\*</sup> BILJANA KARAMAN,<sup>1</sup> WALTER VERRAES<sup>2</sup> AND DOMINIQUE ADRIAENS<sup>2</sup>

<sup>1</sup>Sv Cyril & Methodius University, Faculty of Natural Science and Mathematics, Institute of Biology, P.O. Box 162, 1000 Skopje, the Republic of Macedonia; <sup>2</sup>Ghent University, Department of Biology, Evolutionary Morphology of Vertebrates, Ledeganckstraat 35, 9000 Gent, Belgium

## Abstract:

*The ontogeny of the axial skeleton of Salmo letnica Karaman, 1924 was investigated, focusing on the formation of the dorsal and anal fins and their pterygiophore supports, the neural and hemal spines, and the formation of the hypural complex. The first appearance and location of these skeletal elements, including the cartilaginous precursors of some of them were observed, and their further ontogeny described, including the ossification sequences. Ontogenetic sequences of the axial skeleton were reconstructed through examination of in toto trypsin cleared and stained ontogenetic series of fishes. At the one dph (days post hatching) old trout the presence of the neural arch from the first to 47<sup>th</sup> vertebra (NA1-NA47) as well as the haemal arches from 29<sup>th</sup> to 47<sup>th</sup> vertebra (HA30-HA47) were noticed. At the same stage, the presence of the cartilaginous hypural 1 and parhypural were noted in the caudal complex. The beginning of the formation of the dorsal and anal fin was observed at 8 dph. Their further development occurred both anteriorly and posteriorly. The rays of the unpaired fins were noted in the 5 dph old trout. Ossification of the hypural complex begins at 16 dph. Ossifications of the vertebral centres start simultaneously with that of the associated haemal and neural arches at 18 dph. A completely ossified vertebral column was observed at 92 days old trout. Baseost from the anal and dorsal fins are the last structures which ossified. Their presence was observed at 184 dph. The results of skeletal ontogeny are compared with the pattern of development of the other teleosts.*

## Introduction

The structural biology of organisms can hardly be understood by considering only their adult forms. Studying their early ontogeny may be crucial for revealing important aspects of the structural basis of the organism Bauplan (Balon, 1986). The ontogeny of fishes and other vertebrates merits attention for various reasons. Description of the ontogeny and ontogenetic transformations is essential for understanding the pattern behind body plan formations. Knowledge of anatomical development is of critical importance in understanding the functional trends and environmental preferences of the different developmental stages (Koumoundouros et al, 2001). On the other hand this knowledge provides information that can be used in reconstructing phylogenies. Both, chondrification and ossification sequences have the potential of being phylogenetically informative (Sanford, 2000), either for detecting heterochronic processes that form the organism as a whole or at the structural level (Grünbaum et al., 2003). Ontogenetic studies in terms of chondrification and ossification sequences are of great interest to understand the evolution of morphological systems. Mabee (1993) and Smith (2001) improved the comprehension on the usefulness of such sequences in understanding the role of ontogeny in evolutionary changes.

Development of fins is an important character in the early life history of fish, as it is closely correlated with changes in swimming mode, swimming velocity and feeding techniques and preferences (Kendall et. al.,

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\* Author to whom correspondence should be addressed: fax: ++38923228141; tel: ++38923117055 e-mail: milica\_ristovska@yahoo.com

1984) and also supports the anatomical and functional needs related to developing skull (Koumoundouros et al., 2001). All these events contribute to the success of differentiation, growth and survival, and leads to a better understanding of the life modes of different species, as well as how the structural ontogeny has been sculpted by evolution. Detailed knowledge on the development is also important for the early detection of skeletal deformities common under rearing conditions, and subsequent efforts for their elimination (Crouch et al., 1973; Koumoundouros et al., 1997; Suzuki et al., 2000). The frequent occurrence of skeletal deformities under rearing conditions necessitates the detailed study of the development of osteological components in all body regions, which, in combination with morphology, ethology, and ecology will contribute to a better understanding of their aquaculture needs (Koumoundouros et al. 1999).

Studies of the post-hatching development of salmonids, including developmental sequences and the way adult structures are formed, in for example the skeleton, have been largely neglected despite the large body of literature on early embryological development and relationships among species and populations (Kendall and Behnke, 1984). This paper presents data on the developmental timing of the formation of the unpaired fins in *Salmo letnica* Karaman, 1924, reared under extensive culture conditions. The results of this work give a base for further investigations dealing with the correlation between developmental sequences of the fin structures and other postcranial skeletal elements.

## Material and Methods

The samples of *Salmo letnica* were taken from the artificial spawning tanks located in the Hydrobiological Institute, Ohrid. The samples were raised in special incubators, with constant water temperature of 10, 5° C corresponding to the lake environment. The material for the analysis was collected from the moment of hatching up to the age of 184 days post-hatching (dph). In total, 240 specimens between the age of one and 184 dph were processed. After hatching, up to the age of 37 dph, specimens were collected every day, after which samples were collected on every fifth day up to the age of 92 dph. After 92 dph the samples were collected on every tenth day (seven to 10 fry were taken at each spawning). Specimens were anaesthetized and total length (TL) was measured in mm by callipers (accuracy of 0.05 mm). Specimens were subsequently fixed for 24–48 h in a 10% neutral formalin solution, and stained according to Balon & Flegler-Balon (1985) (bone and cartilage were stained). The postcranial axial structures were studied using a WILD M3C stereomicroscope. Microphotographs were taken by DSC camera-P41.

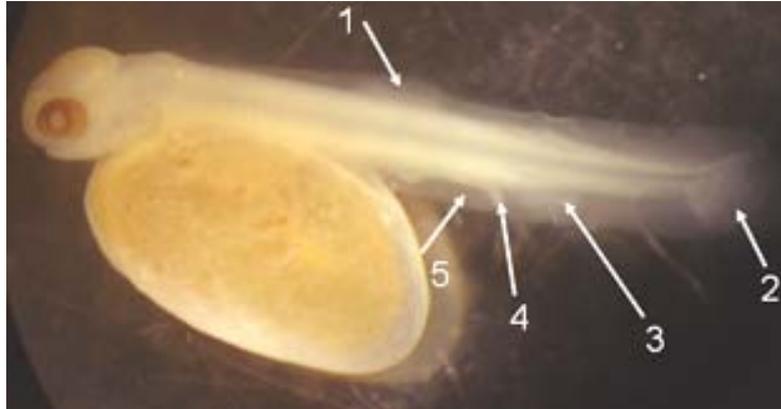
## Results

At the moment of hatching, Ohrid trout possessed a well developed dorsal finfold stretched above the first myomeres and extended to the ventral part of the caudal region from where it is continued as a postanal finfold up to the anus. A preanal finfold extended further up to the posterior border of the yolk sack (Fig. 1). As was mentioned in the introduction, in this paper are represented the developmental sequences of skeletal structures of the dorsal, anal and caudal fin.

### *Dorsal fin*

Both, dorsal and anal fins skeleton consist of the proximal pterygiophores (Pxpt), middle pterygiophores (Mpt) distal pterygiophores (Dpt) and fin rays (FR) (Fig. 2). The development of the proximal pterygiophores of the dorsal fin was noticed for the first time in one of the samples of 8 dph, where already 5 of them were present. Their centres were in close proximity to the neural arches of the 19-23th vertebral centrum. Their subsequent formation continued in a caudad and rostral direction, as well as additional pterygiophores are added (up to 13). However this number was not constant in all samples studied, as it may vary in juvenile trout between 11 and 13. Ossification of the proximal pterygiophores started at 33 dph, and already included nine pterygiophores.

Both, the proximal and middle pterygiophores arise as a single cartilaginous bar that is distally bent. The ossification of the middle pterygiophores was observed for the first time at 184 dph.

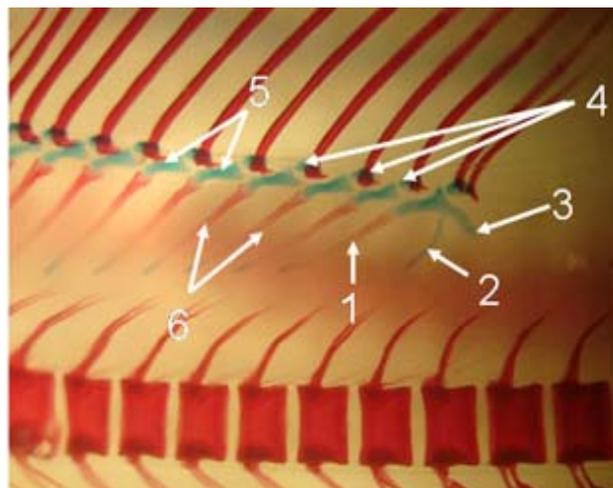


**Figure 1.** *Salmo letnica* old one day posthatching. 1. dorsal fin fold; 2. caudal finfold; 3 anal finfold; 4. anus; 5. preanal finfold

The stay cartilage was formed at 90 dph, at the posterior end of the dorsal fin, being fused with the posterior medial pterygiophore. In some of the samples, an accessory proximal pterygiophore was observed as a narrow, cartilaginous strut (Fig. 2).

Although the distal pterygiophores developed later than the proximal ones, they followed the same sequential pattern. They were first noticed at 17 dph, where already three distal pterygiophores were present dorsal to the proximal ones 2 to 4. During further development their number increased up to 12 (in some of the samples this number ranged from 9 to 12). Each of these cartilaginous elements articulates with the two halves of the dorsal fin rays. The last distal pterygiophore articulates with the last two dorsal fin rays.

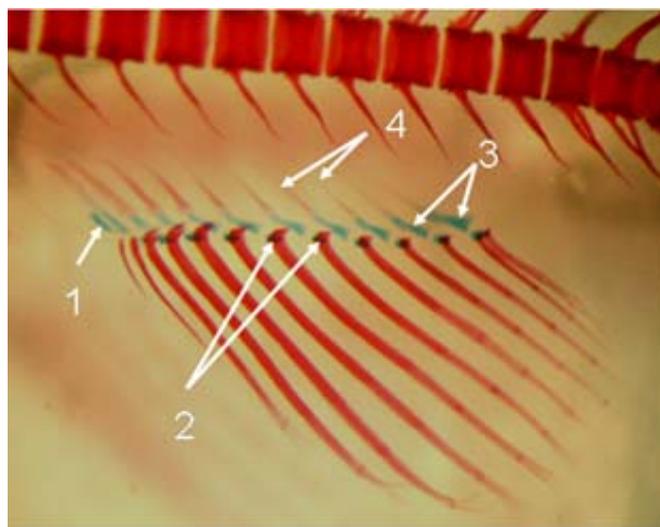
The number of the dorsal fin rays can be 15 or 16. Their first ossification was observed in some samples at 5 dph, and their presence was noticed at all examine samples at 10 dph. The sequential development of the fin rays followed that of the pterygiophores.



**Figure 2.** Posterior region of the dorsal fin in *Salmo letnica* old 90 dph. 1. last proximal pterygiophore; 2. accessory proximal pterygiophore. 3. stay; 4. distal pterygiophores; 5. middle pterygiophores; 6. proximal pterygiophores

### *Anal fin*

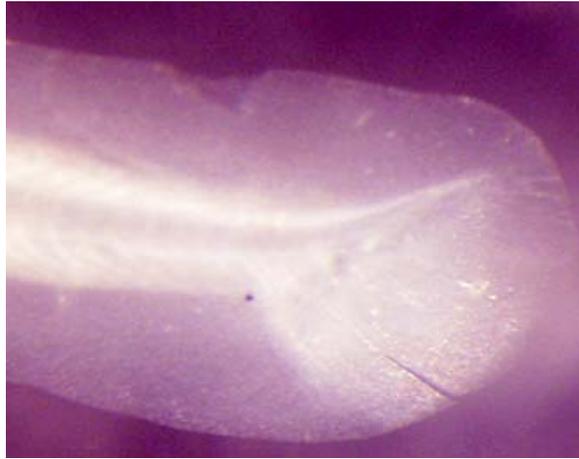
Anal fin development starts at 5 dph with the formation of the fin rays, followed by the formation of four cartilaginous proximal pterygiophores (first one under the haemal arches of the centrum C38). The subsequent development of the proximal pterygiophores followed in a caudad and rostrad direction. Pxp1 was the last one to be formed (Fig. 3). Its presence was noticed at 78 dph old trout. Their ossification started at 12 dph and followed in same direction as their cartilaginous precursors. The distal pterygiophores developed after the proximal ones, following the same sequential pattern. The presence of the stay was noticeable at 90 dph, posteriorly to the last PxPT. Compared to the dorsal fin, the ossification of the middle pterygiophores wasn't as pronounced at 184 dph. The numbers of the anal rays varied from 13-14.



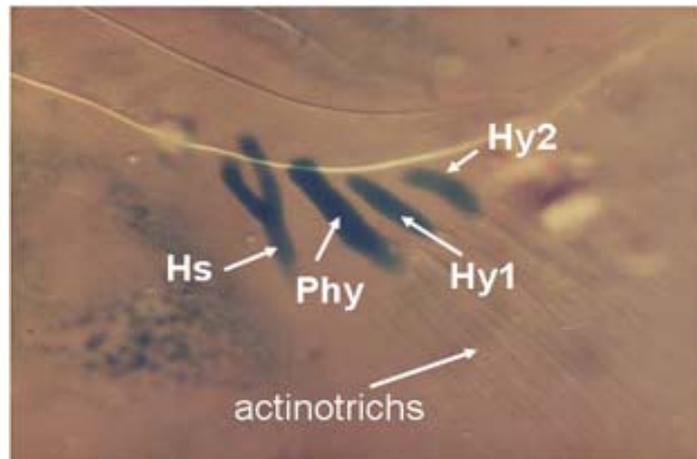
**Figure 3.** Anal fin of 78 dph old *Salmo letnica*. 1. first proximal pterygiophore; 2. distal pterygiophores; 3. middle pterygiophores; 4. proximal pterygiophores

### *Caudal fin*

The majority of the elements of the caudal fin complex are endochondral bones and only the uroneurals (UN2-3), preural centra 1-4 (PU1-4), ural centra 1-2 (U2-1), and the caudal fin rays (principal and secondary) are dermal bones. At the ventral distal portion of the notochord, which is slightly flexed at the moment of hatching (Fig. 4), the hypural 1 (Hy1) and parhypural (Phy) were the first cartilaginous elements to be formed, subsequently followed by the Hy2 and haemal spine of the preural 2 (PU2) (4 dph; Fig. 5); Hy3, Hy4 and hemal arche and spines of the PU3 and PU4 (6 dph); Hy5 (12 dph), and lastly Hy6 (16 dph). At the dorsal part of the notochord, the formation of the neural arch and spine of the PU3-PU4 started simultaneously, while the formation of the neural arch and spine of the PU2 was noticed at 12 dph.



**Figure 4.** *Salmo letnica* one day post hatching. Flexion of the notochord at the caudal finfold



**Figure 5.** Caudal fin of *Salmo letnica* at 4 dph. Hy1-2, hypural 1,2; Phy, parhypural; Ha, haemal spine of the preural 2

In some of the samples, the formation of neural arch and spine above the PU1 was registered. Dorsal to the notochord, the formation of the cartilaginous uroneural 1 (UN1) was noticed at 12 dph and epural elements were the last cartilaginous caudal structures to be formed. Their presence were noticed in all examined species at 24 dph.

Ossification of the caudal fin skeleton started with the ossification of the principal rays at 5 dph. At 16 dph, ossification of the Hy 1, 3, 4 started. Ossification of Hy2 was observed at 21dph, while ossification of the Hy 6 started at 35 dph. Simultaneously ossification of the haemal spine of the PU1-4 was noticed at 16 dph and completely ossification of their neural spine was registered at 33dph. On the posterodorsal part of the notochord ossification of UN1 started at 20 dph, UN2 at 33dph and UN3 at 46 dph. Dorsal to the UN1, an ossification of EP1-2 was observed at 35 dph. The caudal centrum complex began to ossify at 24 dph, and was completed at 92 dph.

## Discussion

At hatching, Teleostei present a remarkable variability in the developmental staging of the skeleton (Koumoundouros et al., 2001). Most of this variability depends of the size of the yolk. Larvae from eggs with small yolks are less developed at hatching than those that hatch from eggs with larger yolks (Kendall et al., 1984; Adriaens and Vandewalle, 2003). In *Salvelinus namaicush* (Balon, 1980) and *Sebastes schegeli* (Omori et al., 1996) the development of the skull and fins starts well before hatching while in *Dentex dentex* (Koumoundouros et al., 2001) or *Scophtalmus maximus* L. (Chanet and Wagemans, 2001) development of the skeleton starts a few days after hatching. However, morphological structures and their development are strongly correlated with functional requirements (Mabee et al. 2002). The skeletal system of a fish must ossify and grow to provide leverage for feeding and swimming as the fish becomes active in prey capture and predator avoidance (Green and McCormick, 2001).

The various locomotory functions can arise at different stages during larval ontogeny, depending on the presence of involved musculo-skeletal elements, and is related to different functional requirements of the fishes in their environment. This is one of the reasons why the skeletal elements of the fins develop in different sequences and directions in different fishes.

Previous studies showed that the caudal fin is the first fin to differentiate from the median finfold, (Omori et al., 1996; Kohno et al. 1996; Sfakianakis et al., 2004). This is due to the fact that the fish is first endowed with the ability to produce propulsive forces and transmit it along the body axis (Koumoundouros et al., 2001). Also, the fact that the caudal fin is part of the central vertebral column and other unpaired fins are formed as appendices to this column, may explain this timing consistency.

In *S. letnica* development of the finfold starts well before hatching, with the presence of the first cartilaginous structures (Hy1 and Phy) noticed along the ventral side of the posterior end of the notochord. Immediately thereafter, the first caudal fin rays appear. The first principal rays are located in the middle, with additional rays being added progressively above and below, following the pattern of the formation of the caudal skeleton. Dorsal and ventral secondary rays of the caudal fin begin forming sequentially in an anterior direction, immediately after all principal caudal fin rays have formed. During the ontogeny of *S. letnica*, they are the last group of fin rays among all fins to form, despite the early onset of caudal fin formation. Similar results have been reported for other Teleostei (Arratia and Schultze, 1992; Bird and Mabee, 2003).

During the early development of *S. letnica*, dorsal and anal fin were the next fins to develop and their formation starts simultaneously. The proximal pterygiophores and the fin rays are the first skeletal elements to be formed, with their further development in a caudad and rostrad direction. The first Pxpt1 and the stay were the last cartilaginous structure formed during the early development of Ohrid trout. This corresponds with the investigation of Potthoff (1986); Crotwell et al. (2001) and Sfakianakis et al; (2004). Within teleosts, it appears that a bidirectional wave of development of dorsal and anal fins is predominant, with some exceptions when dorsal and anal fins can differentiate from anterior to posterior direction or posterior to anterior (Mabee et al., 2002). A complete and detailed survey of the developmental sequences for all components of the postcranial skeleton of the Ohrid trout, as well as their detailed morphology, will contribute to a better understanding of the pattern behind postcranial ontogeny of this endemic and economic value specimen and will be useful to understand the connection between development of the structures and their functions.

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