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# Early development of hyomandibular in rainbow trout (*Oncorhynchus mykiss*)

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**Abstract:** Hyomandibular is a paired and endochondral bone related to the upper part of the hyoid arch. Since it is present in very primitive to modern fishes, it is highly important in taxonomy. In this research, the early development of hyomandibular from one to 30 dph (day post hatching) has been studied. For this survey, 350 specimens of different ages were sampled from Nowchah, a village near Mashhad, Iran. Samples after anesthesia were fixed using buffered formalin and then the osteology was studied using the double staining protocol of Alcian Blue and Alizarin Red. The qualitative observations were converted to the quantitative data and then were analyzed by MTLAB software. Based on these results, the origin of this bone was endochondral and the time of presence was after its hatching. Finally its existence period was determined, while the ossification started from 13 dph and seems to be continued after 30 dph.

**Keywords**: Hyomandibular, Alizarin red, Alcian blue, development, rainbow trout

## Introduction

The hyomandibula, commonly referred to as hyomandibular [bone] (Latin: *os hyomandibulare*, from Greek: hyoeides, "upsilon-shaped" (υ), and Latin: mandibula, "jawbone") is a set of bones that is found in the hyoid region in most fishes. It usually plays a role in suspending the jaws and/or operculum in teleost fishes (Clack, 1994, Lombard and Bolt, 1979). In jawless fishes a series of gills opened behind the mouth, and these gills became supported by cartilaginous elements. The first set of these elements surrounded the mouth to form the jaw that vertebrate jaws are homologous to the gill arches of jawless fishes. The upper portion of the second embryonic arch supporting the gill became the hyomandibular bone of jawed fishes, which supports the skull and therefore links the jaw to the cranium (Gilbert, 2000). The type of suspensorium helps to determine the flexibility and bracing of the feeding system and thus the basic limitations on feeding mode. The nature of the suspensorium is one of the most important criteria in the determination of relationships among major groups of fish (Lund and Grogan, 2013). Hyomandibula= the upper paired deep bone or cartilage of the hyoid region, sometimes taking part in jaw suspension and which supports the opercle. Dorsally it articulates with the otic capsule at the hyomandibular fossa, ventrally with the quadrate and symplectic. In most Teleostei it has a foramen for the hyomandibular branch of the facial nerve (cranial nerve VII) often spelled hyomandibular (Briancoad, 2015). The hyomadibula is an important element in the total architecture of the head. A structural analysis of the hyomandibula and its relationship function such as the lateral movement of suspensorium and depression of the lower jaw depicts the interrelation between form and function. Although a number of ichthyologists have referred to the hyomandibula while describing the skull of different fishes, few have specifically analyzed the functional significance of its structure. The respiratory function influences certain characteristics of the hyomandibular of different species. To demonstrate that, in Dutta's study, the differences in total shape in hyomandibular in three different species of Anabantoid teleost fishes are explained. The type of operculo-hyomandibular articulation and the kind of hyomandibulo-interhyal articulation demonstrate a form and function relationship. Based on the pervious study, the functions have influenced the structure of the horizontal and the vertical ridges (Dutta, 1980). The functional influence on the hyomandibular structures has a supportive logic in the general statement made by Gans (1989) "Any structure tends to be affected by the influence of multiple functions, and any function will almost

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certainly affect multiple characteristics of the animal". In this study, the origin and the developmental changes during hyomandibular ossification in *O. mykiss* are shown. Besides, the shape of hyomandibular in this species is compared to previous studies.

## Material and methods

The samples of *O. mykiss* were taken from the artificial spawning tanks located in Nowchah, 13 km from Mashhad, Iran. The samples were raised in special incubators, with constant water temperature of 8$^{o}$C corresponding to the lake environment. The material for the analysis was collected from the moment of hatching up to the age of 30 days post-hatching (dph). 350 specimens between the age of 1 day and 30 dph were processed. After hatching, up to the age of 30 dph, specimens were collected every day. The study of the chondrocranial development was conducted by using the following methods: in toto clearing and staining (alcian blue and alizarin red S). The specimens for in toto clearing and staining were anaesthetized, using cloves oil and then fixed in formalin 4% (pH=7). The following is a method for staining for bone and cartilage mainly based on Potthof (1984) and adapted by S. Helland, Nofima Marin (Helland, 2009) based on experience in using this and other staining and clearing methods (Ristovska *et al*., 2006). The double staining protocol for developing rainbow trout was defined based on diverse published protocols. For an optimal staining protocol designed the incubation times of the different solutions were adjusted. The head region of the specimens was studied by using a stereomicroscope (Olympus, SZH 10, Japan) equipped with a digital camera. Then, the qualitative observations were converted to the quantitative data and then were analyzed by MTLAB software.

## Results

The appearance of forming hyomandibluar was seen from the 1 dph, but it was clearly seen from 2 dph. Figure 1 shows the lateral view of the head in rainbow trout. The specimen was stained by Alcian blue to detect the chondrocytes. As the picture demonstrates, hyomandibular is obviously seen as a chondral element. According to this figure, the hyomandibular in this species is dorsally articulated to the otic capsule and ventrally articulated to symplectic. The anterior rim of the hyomandibular is almost triangular in shape, and its dorsal margin has a pointed process. Also a large pore presents in its middle part of the hyomandibular. The dorsal margin of hyomandibular is wider than its ventral part. The hyomandibular possesses two condyles on its dorsal edge (anterior and posterior hyomandibular condyles) which are articulated to the neurocranium. Figure 2 and 3 show the lateral view of the head stained by Alizarin red to detect the ossified elements in two different developmental stages. As the figures show, the signs of hyomandibular ossification are seen in 9 dph and then from 13 dph we could definitely define it as an ossified hyomandibular. In other words, the ossification started from 9 dph gradually and in 13 dph the chondrocytes almost disappeared. To prove our observation, a double staining was done, as Figure 4 demonstrates, the red colors show the ossified parts stained by Alizarin red and the blue colors show the chondral parts stained by Alcian blue. In this figure, we can obviously see that the hyomandibular in 28 dph is almost ossified, but comparing with its shape in 2 dph we can assume that the ossification is not complete yet. Figure 5 shows the quantitative data demonstrated by MATLAB software. It is a developmental diagram to show the developmental stage of hyomandibular during 30 days. According to figure 5 and all mentioned above, the hyomandibular is chondral from 2dph to 13 dph and after that the ossification started. According to this result, we can recognize the origin of this bone as endochondral ossification.

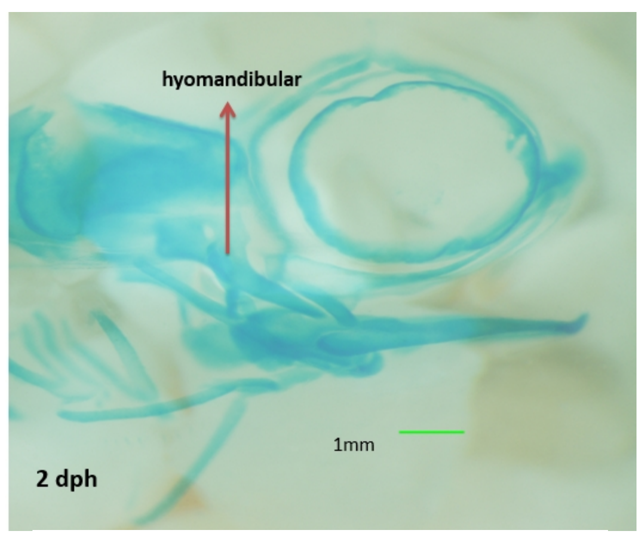


**Fig. 1: Lateral view of 2 dph rainbow trout (Alcian blue).**

## Discussion

The bones origin, the time of presence and its existence period of hyomandibular in rainbow trout

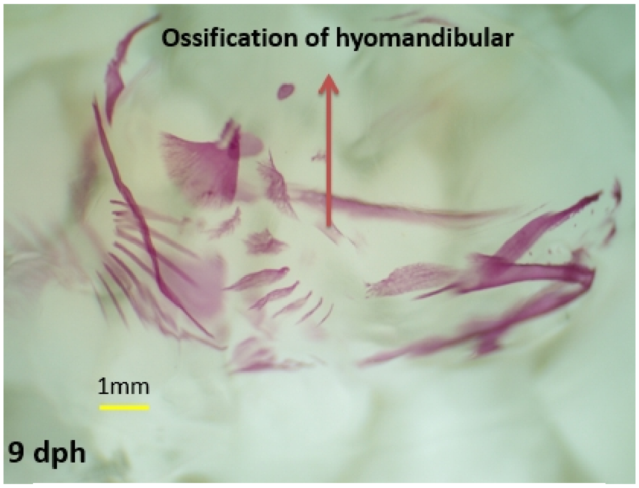


**Fig. 2: Lateral view of 9 dph rainbow trout (Alizarin red)**

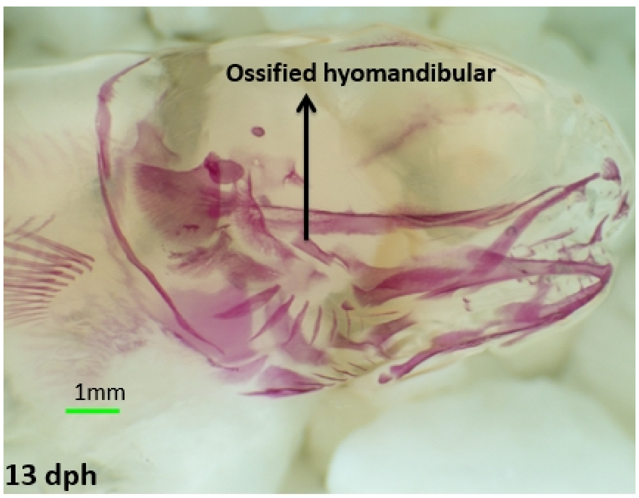


**Fig. 3: Lateral view of 13 dph rainbow trout (Alizarin red)**

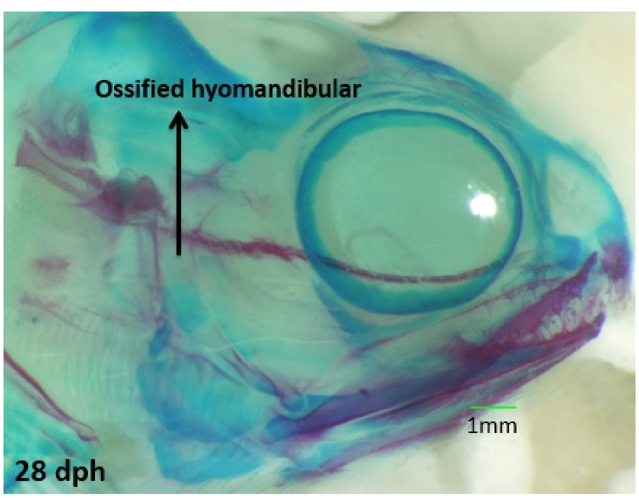


**Fig. 4: Lateral view of 28 dph rainbow trout (Alcian blue/Alizarin red double staining).**

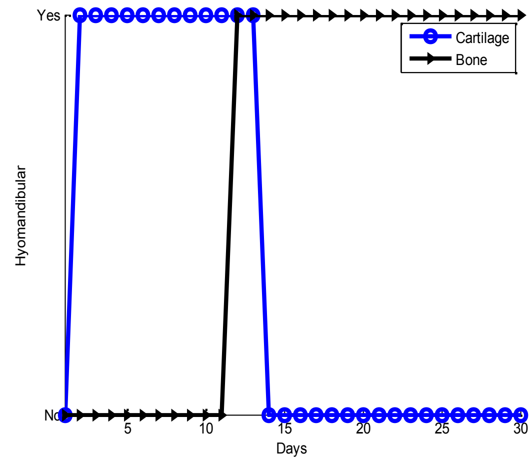


**Fig. 5: The development diagram of hyomandibular during 30 days in rainbow trout.**
**The blue circular curve shows the presence of chondrocytes and the oriented black curve shows the bone. The X axis demonstrates different developmental stages from 1 to 30 dph. In this diagram two horizontal axis are seen, one of them is named "yes" and the other one is "No". The former and the latter express the presence and absences of chondrocytes or bone respectively.**

(*Oncorhynchus myikss*) were determined. The ossification in the last samples of this study (30 dph) did not complete yet. So its period should be longer than 30 days. Besides, the chondral period was about 13 days and after that the chondral hyomandibular was disappearing, in which this period is longer in turbot, occurred in 18 dph and in 25 dph the upper part of each chondral hyomandibular was reduced which is longer comparing to *O. mykiss* (Wagemans *et al*., 1998). In contrast, these structures, namely: the hyomandibular, symplectic, quadrate and ceratohyal begins to ossify at 131 h which is sooner than *O.mykis* (Vandewalle *et al*., 2005). As a result, this bone has an endochondral ossification which ossification substitute for chondrocytes. As Wagemans *et al.* (1998) mentioned, the developmental time of hyomandibular with other suspensorium elements might be simultaneous or different. In *O.mykiss*, the hyomandibular and the quadrate are the first elements of suspensorium which appears sooner and simultaneously. This is in contrast with *Barbus barbus* in which the hymandibular ossifies later than the quadrat. So, it can be concluded that, its articulation with the braincase becomes functional in the same period as the quadratomandibular; the same seems be true for the hyomandibular opercular joint. In most teleost, the ossification of the quadrate and hyomandibular begins at the joint (similar to *O.mykiss*) and other element such as endopterygoid, ectopterygoid, palatine, metapterygoid and symplectic begins at the center. This ossification pattern suggested that the joints are functionally important at an early stage (Wagemans *et al*., 1998). Besides, this bone is dorsally articulated to the otic capsule and ventrally articulated to symplectic. Similar to turbot (Wagemans *et al*., 1998), the ossified part of each hyomandibular corresponds with the still cartinoginous joint region connected to the neurocranium and with the region where cartilaginous hyosymplectic is regressing. The region of the symplectic which articulates with the hyomandibular remains cartilaginous even in the largest specimens analyzed (Fig. 4); this result was similar to the study of Faustino & Power (2001) on sea bream. Comparing the hyomandibular with other researches on different species such as: Dutta (1980), Wagemans *et al*. (1998) , Vandewalle *et al*. (1992), Vandewalle *et al*. (2005), Ristovska *et al*. (2006), Loffler *et al*. (2008), the shape of hyomandibular and developmental period are different in various species. So, it would be as one of taxonomical characters in systematic study. Eagderi and Adriaens (2014), Jalili and Eagderi (2014), Mafakheri *et al*. (2014) showed the connection area of hyomandibula with other elements. Based on their studies, the anterior and posterior parts of the hyomandibular are connected to different areas. As a result, In *Oxynoemacheilus angorae* and *Cobitis keyvani*, the anterior facet is formed by the prootic and sphenotic and the posterior is formed by the sphenotic, prootic and pterotic bones (Jalili and Eagderi, 2014, Mafakheri *et al*., 2014). In contrast, it differs in other two species of *O. kiabii* and *O. berginaus* (Mafakheri *et al*., 2014). In addition, in *Ariosoma gilberti* which shpenotic and prootic are as anterior part and pterotic in posterior part (Eagderi and Adriaens, 2014). In our study, since in the largest specimen, 30 dph, the dermal bones were not complete; so it is not possible to decide the condyles area of hyomandibular accurately. To conclude, the developmental stages of *O.mykiss* are more similar to *Salmo letnica* (Ristovska *et al*., 2006) comparing to other species mentioned above. Generally, by using this method, it would be possible to study the skeleton development of other species, helps better understanding of their taxonomy. It also provides the opportunity to better characterize the effect of different environmental and nutritional factors on their ossification (Löffler *et al*., 2008).

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