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# Osteological development of the head in a triploid sturgeon (*Acipenser baeri* × *Huso huso*)

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**Abstract**. Understanding the normal osteological development of fishes is essential for diagnosis of any malformation which caused by ill management in aquaculture. Hence, this study was conducted to provide ontogeny of the cephalic skeleton of a triploid sturgeon (*i.e.* male *Acipenser baeri* × female *Huso huso*) as a suitable candidate for aquaculture. For this purpose, specimens of this triploid sturgeon were collected from 0-dph (days post hatch) till 50-dph. The specimens were cleared and for examination of osteological development and a detailed description of the head skeleton ontogeny were provided. The results showed that there were no cartilaginous or bony elements up to 2-dph larvae indicating development of the cephalic skeleton after hatching in this triploid fish. The present study also showed that ontogeny of the head skeleton of the triploid larvae has many similarities with those of *H. huso* and other sturgeons during the early developmental stage. These similarities can suggest possibility to apply those feeding protocols that is used for others sturgeons especially its parents for this triploid sturgeon during early developmental stage in hatcheries.
**Key Words**: Sturgeon, hybrid, bone, ontogeny, cephalic skeleton.

**Introduction**. Sturgeon fishes are widely distributed in the northern hemisphere and adapted to a wide range of the environmental characteristics (Choudhury & Dick 1998). They are a group of slow-growing fishes that mature very late and consequently, vulnerable to overfishing and other threats such as pollution and destruction of their spawning grounds (Gisbert et al 2002; Gisbert 1999). Hence as a strategy, their artificial propagation for aquaculture and restocking purposes has been developed in recent years (Bronzi et al 1999).

Following development of sturgeon aquaculture, farmers have paid attention to introduce new species (Bronzi et al 1999). The use of hybridogenesis among the sturgeon species is an ordinary task, as the hybrid species display better growth in compare to their parents (Bronzi et al 1999; Williot et al 2001). In this regard, triploid fishes have been special potential due to being sterile and therefore, not be considered as a threat to endemic species (Altimiras et al 2002). In addition, their cell size is almost 50 percent larger than a diploid one (Altimiras et al 2002).

Hatchery reared fishes have a malformation frequency higher than those of wild counterparts, therefore, understanding the development of bony elements not only provide additional characters for their identification, but also help us to interpret the osteological characters in adults (Fritzsche & Johnson 1980). In addition, knowledge on the normal osteological development is critical in addressing when and where abnormal development occurs under culture conditions. Hence, it can be used as an early bio-indicator of non-optimal rearing conditions (Lewis & Lall 2006), determination of the proper diet and the action of food components (Cahu et al 2003).

Since there is no information available regarding the ontogeny of the skeletal structures in the sturgeon fishes, therefore this study tries to provide ontogeny of the skeletal structure in a triploid sturgeon (*i.e*. male *Acipenser baeri* × female *Huso huso*) as a suitable candidate for aquaculture.

**Material and Method**. Triploid larvae (*A. baeri* ♂ x *H. huso* ♀) were obtained from artificial propagation of female beluga caught from the Caspian Sea and a reared male Siberian sturgeon in the Caspian Sea International Institute of Sturgeon Fishes (Rasht, Iran) in 2013. Cold shock was applied to induce the triploidy and then their triploidy were confirmed. The eggs were incubated in Yushchenko incubators at 11-12°C in a closed freshwater recirculation system (with 10 cm water depth and 0.4-0.5 L/s) (750 g egg/unit). After eight days of incubation, 1800 newly hatched larvae were introduced into 500-L circular fiberglass tanks (filled up to 20 cm depth) connected to a flow-through freshwater system. Water source was from from Sefidroud River filtered by a sand filtration. During the larval rearing period, water temperature, dissolved oxygen, pH and flow rate were 16.5 ± 0.2ºC, 10.7 ± 0.3 mg $L^{-1}$, 7.8 ± 0.1 and 5.7 ± 0.4 L/s, respectively. Fish were reared under natural photoperiod. The larvae were fed with a mixture of non-enriched *Artemia* nauplii and cladocerans (*Daphnia* sp.) (1:1) from 12 to 25 dph (500 nauplii/larvae/day). Then, they were fed by cladocerans and inert diet (Biomar, Denmark; D2 - particle size = 0.8 mm) from 25 to 30 dph at the rate of 20% of stocked fish biomass 4 to 6 times a day and particle size were progressively adjusted according to the fish size.

After hatching, larvae were randomly sampled from hatching up to 50 dph prior to feeding in the morning, sacrificed with an overdose of MS 222 (Sigma-Aldrich) (n=10) and preserved in 4% buffered formalin. The specimens were moved to 72% alcohol after 48 hours. Then, the specimens were photographed using a dissecting microscope equipped with a Cannon camera with a 5 MP resolution and their Total Length (TL) was measured using ImageJ software (version 1.240).

For osteological examinations, the specimens were cleared and stained with alizarin red S and alcian blue according to Hanken & Wassersug (1981) and Asgari (2012). Then, the specimens were studied using a stereomicroscope (Leica MC5); and their skeletal elements were dissected and scanned by a scanner equipped with a glycerol bath (Epson v700). Drawings were made using CorelDrawX6 software. The nomenclature of the skeletal elements was followed Hilton et al (2011).

## Results

***1-dph - TL: 11.80 mm***. After hatching no cartilaginous or ossified structures were noticeable. The eye was not pigmented. The gill slits and mouth were closed (Figure 1).

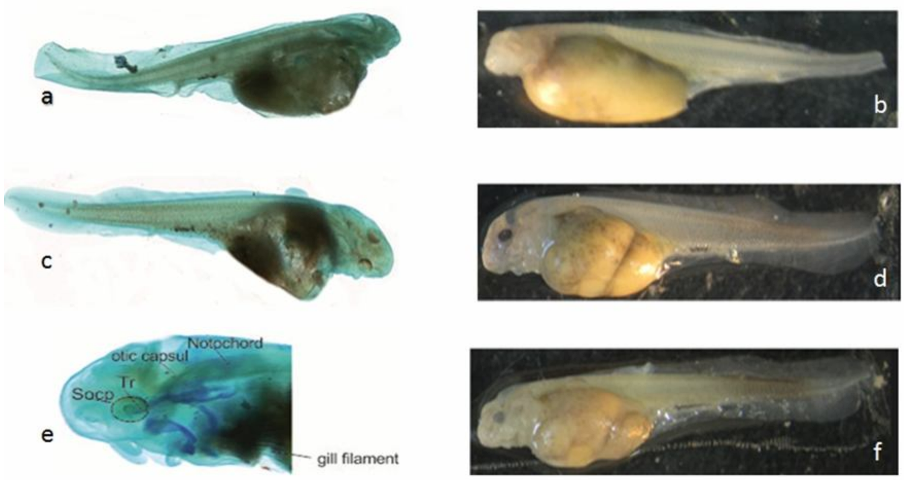


Figure 1. Lateral side of the triploid sturgeon (*Huso huso* × *Acipenser baeri*). A & B: 1-dph, C & D: 3-dph, E: 4-dph, F: 2-dph. Tr - trabicular, Socp - sclerotic capsule.

***2-dph - TL: 12.50 mm***. The mouth opening and eyes' pigment were observed; the barbels and pectoral fins were appeared (Figure 1).
***3-dph - TL: 13.50 mm***. The larvae showed no ossification, however, as the first sign of the creation of the neurocranium, the trabecular bar between two eyes was appeared. The thin sclerotic cartilage enclosing the eye ball was formed (Figure 1).
***4-dph - TL: 14.40 mm***. Both sclerotic capsule and trabecular bar were developed and distinct. Unlike the anterior region of the neurocranium, its posterior part was formed somewhat, nevertheless, the otic capsule was imperceptible. Formation of the jaws was initiated by Meckels cartilage and platoquadrate which joins Meckel's cartilage at its ventral tip. The barbel's chrysalises were clear under a binuclear. Development of the first branchial arch was evident in the ventro-lateral region of the orbital. The rudiments of the gill filaments were appeared. The notochord was extended behind the eyes. The first signs of the opercular apparatus formation were distinct (Figure 1).

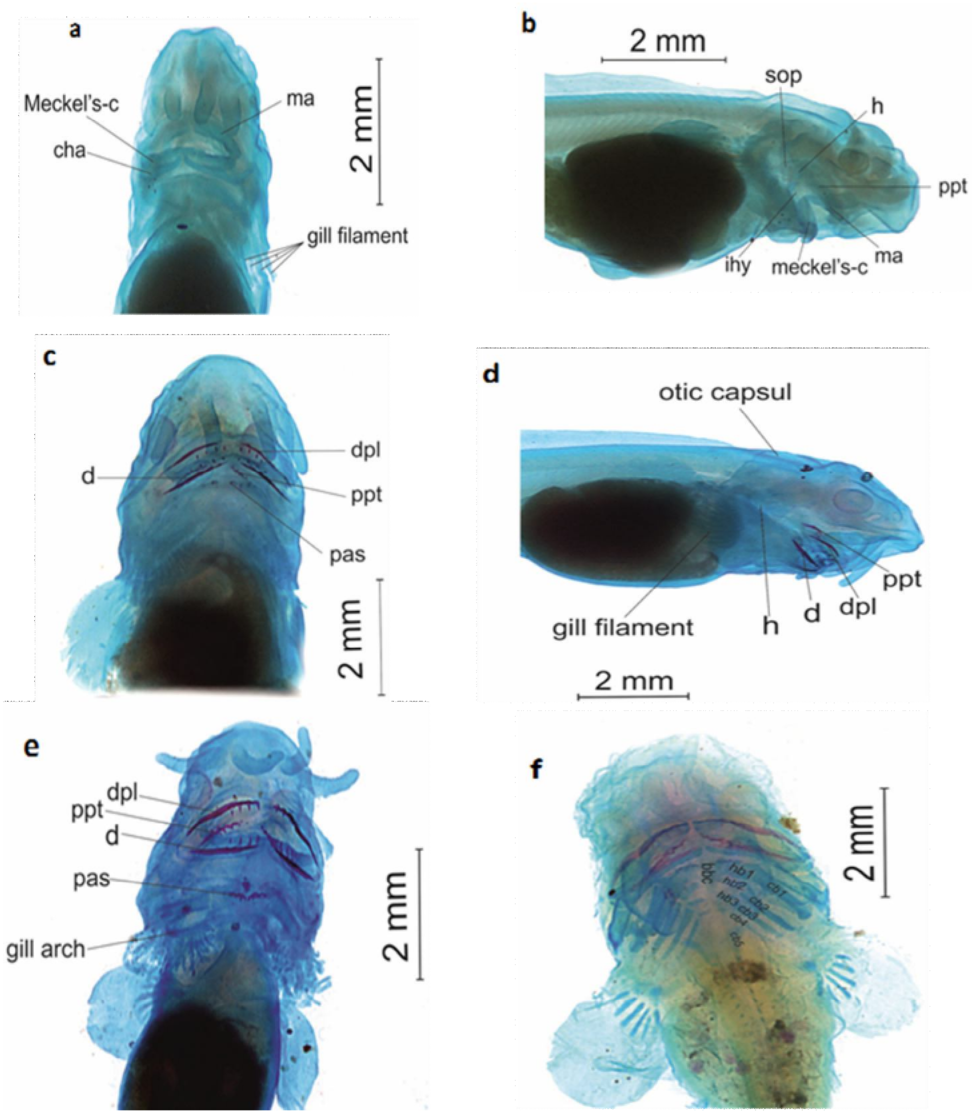


Figure 2. 6-dph (A - ventral view, B - lateral view), 8-dph (C - ventral view, D - lateral view), 10-dph (E - ventral view), 12-dph (F - ventral view) of the triploid sturgeon. ppt – palatopterygoid, dpl – dermopalatine, d – dentary, cha - anterior ceratohyal, pas - parasphenoid; h- hyomandibula, sop – subopercle, ma - dermopalatine.

***6-dph - TL: 16.80 mm***. The neurocranium was little developed up to the olfactory region. The suspensorium was first represented by the hyomandibula which appeared as a thin bar of the cartilage in the anterior region of the opercular series. The interhyal cartilage started to form on the ventro-lateral margin of the lower jaw and the anterior of the hyomandibula. The interhyal was articulated to the hyomandibula posteriorly, the posterior part of the lower jaw anteriorly and the anterior part of the ceratohyal ventrally. Six teeth in one row were observed in each jaw. The platopterigoid cartilage was formed in the roof of the mouth. The nostrils were differentiating into two sections. The barbels developed in terms of its length (Figure 2).

***8-dph - TL: 18.15 mm***. Teeth's numbers increased to eight in both lower and upper jaws. Osteogenesis of the dermatopalatine and dentary were begun at the border of the jaws. The snout was developing (Figure 2).

***10-dph - TL: 19.69 mm***. Ossification of the parasphenoid started in the ventral face of the neurocranium. The gill filaments were observable (Figure 2).

***14-dph - TL: 20.34 mm***. Osteogenesis of the subopercular and supracliethrum were initiated. The anterior part of the opercle was as a thin bone, while its posterior region was broad with a super-facial sculpture. The supracliethrum was as a wide plate. The cartilaginous quadrate was completely formed and anteriorly articulated to the platoquadrate (Figure 3).

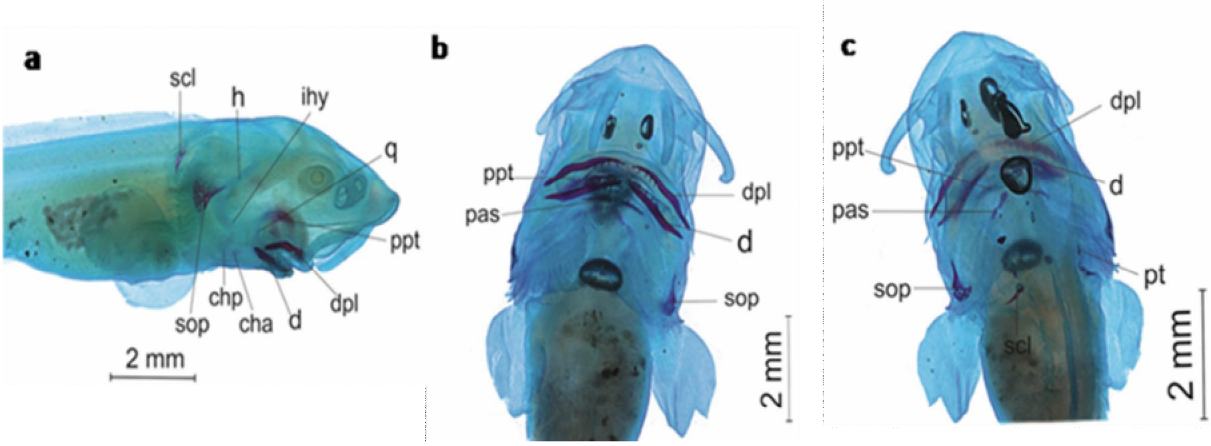


Figure 3. 14-dph (A - lateral view, B - ventral side, C - dorsal view), of the triploid sturgeon. ppt – palatopterygoid, dpl – dermopalatine, d – dentary, cha - anterior ceratohyal, pas – parasphenoid, h – hyomandibula, sop – subopercle, ma – dermopalatine, ihy – interhyal, scl – supracleithrum, q – quadrate, chp - posterior ceratohyal.

***20-dph - TL: 25 mm***. The quadrate began to ossify ventrally and developed ventrally while its median and posterior parts were ossified as a slender shaped band. The parietal was extended anteriorly and posteriorly and its median part was wider (Figure 4). Ossification of the first branchiostegal ray, ectopterigoid, opisthotic, clavicle and dermopterotic were observed. The subopercle is further developed. The first branchiostegal ray was rectangular in shape, positioned ventral to the subopercle. The ectopterigoid is a narrow bone forming a bridge between dermatopalatine and the anterior margin of the platopterigoid. The platopterigoid was partially ossified. The clavicle was the largest bony element of the pectoral girdle.

***23-dph - TL: 31.44 mm***. Osteogenesis of the frontal and the dermosphenotic were commenced. Like other sturgeons, the dermosphenotic was merged into the roof of the skull. At the most posterior region of the neurocranium, the first dorsal scute with first signs of ossification was observed (Figure 4).

***27-dph - TL: 39.71 mm***. First signs of osteogenesis in the dorsal part of the rostral canal, ventral part of the jugal, median anterior process of the parasphenoid and pectoral fin spine were observable. The pectoral fin spine was slender in shape (Figure 5).

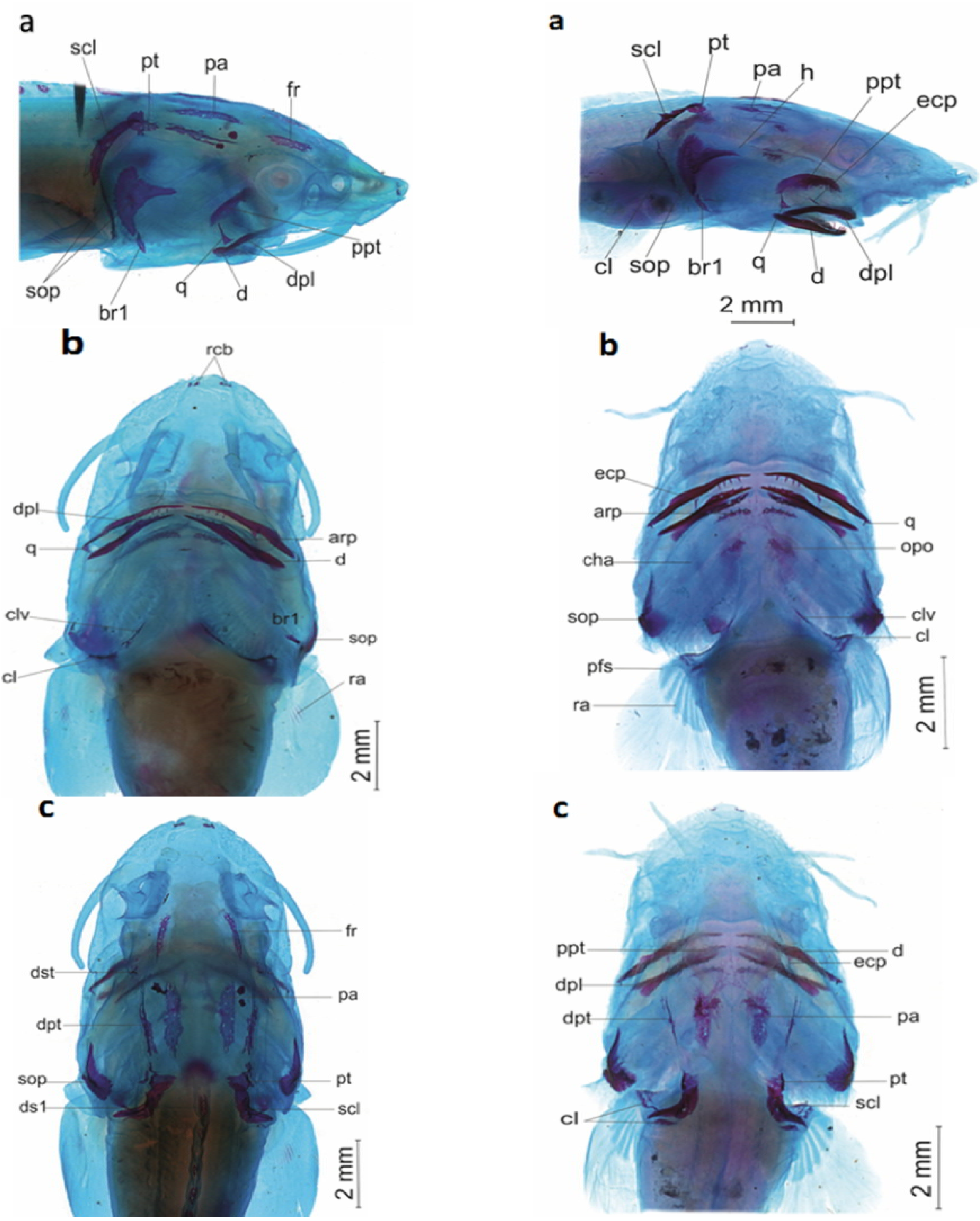


Figure 4. A - lateral view (left - 23-dph, right - 20-dph), B - ventral view (left - 23-dph, right - 20-dph), C - dorsal (left - 23-dph, right - 20-dph) of the triploid sturgeon. d – dentary, h – hyomandibula, scl – supracleithrum, q – quadrate, ppt – palatopterygoid, sop- subopercle, pt – posttemporal, pa – parietal, ecp – ectopterygoid, br1 – branchiostegal, dpt – dermopterotic, clv – clavicle, ra – radials, pfs - pectoral fin spine, fr – frontal, cl – cleithrum, dsp – dermosphenotic, dpl – dermopalatine, cha - anterior ceratohyal, rcb - rostral canal bones, arp - ascending ramus of the parasphenoid.

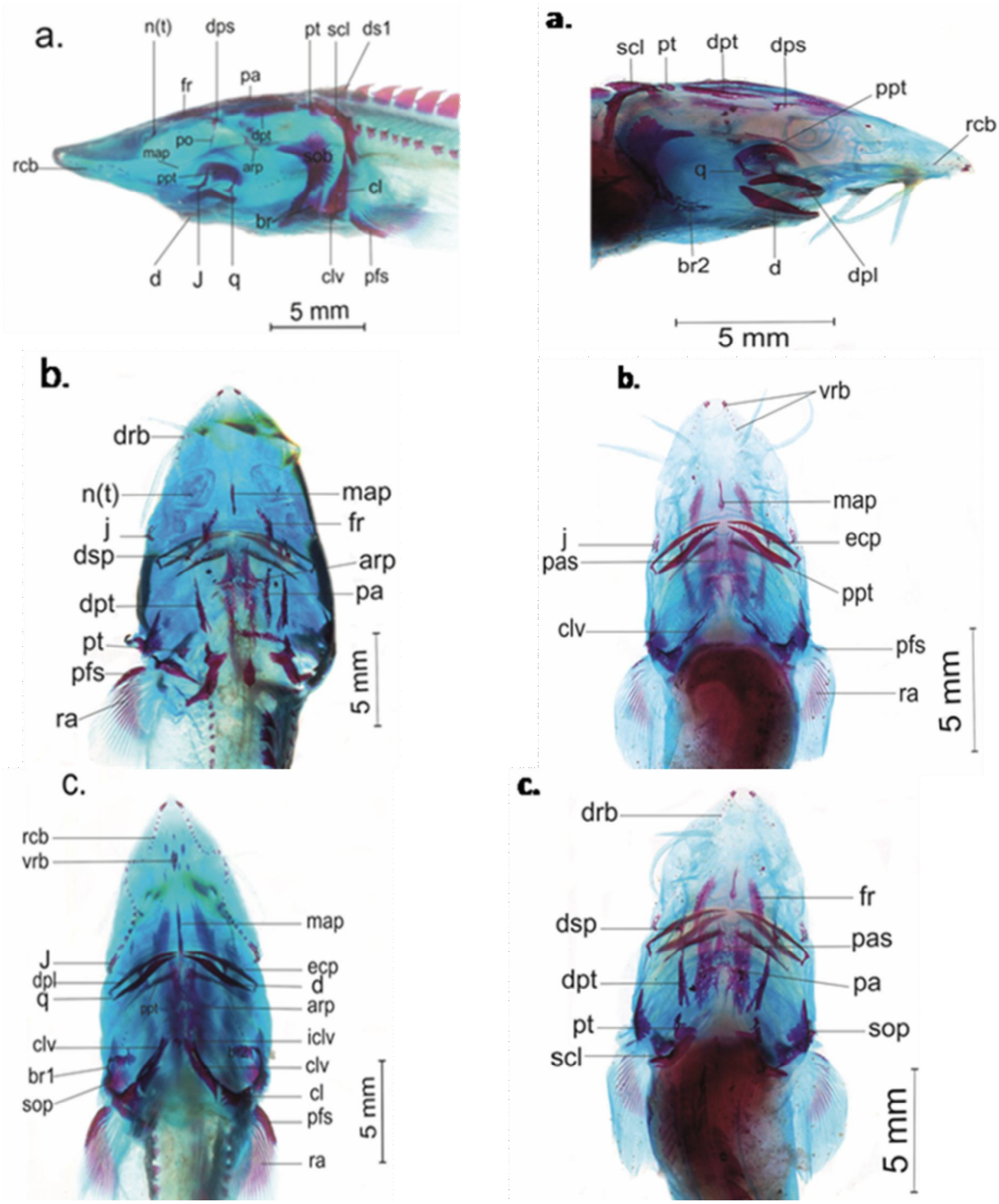


Figure 5. A - lateral view (left - 31-dph, right - 27-dph), B - ventral view (left - 31-dph, right 27-dph), C - dorsal view (left 31-dph, right - 27-dph) of the triploid sturgeon. scl – supracleithrum, q – quadrate, ppt – palatopterygoid, sop – subopercle, pt – posttemporal, pa – parietal, ecp – ectopterygoid, br2 – branchiostegal, dpt – dermopterotic, clv – clavicle, ra – radials, pfs - pectoral fin spine, fr – frontal, j – jugal, cl – cleithrum, vrb - ventral rostral bone, drb - dorsal rostral bones, dsp – dermosphenotic, map - median anterior process of the parasphenoid, n(t) - tubular bone anterior of the nasal, po – postorbital, rcb - rostral canal bones, ds1 - first dorsal scute, pfs - pectoral fin spine rcb - rostral canal bones, dpl – dermopalatine, d – dentary, arp - ascending ramus of the parasphenoid, iclv - interclavicle.

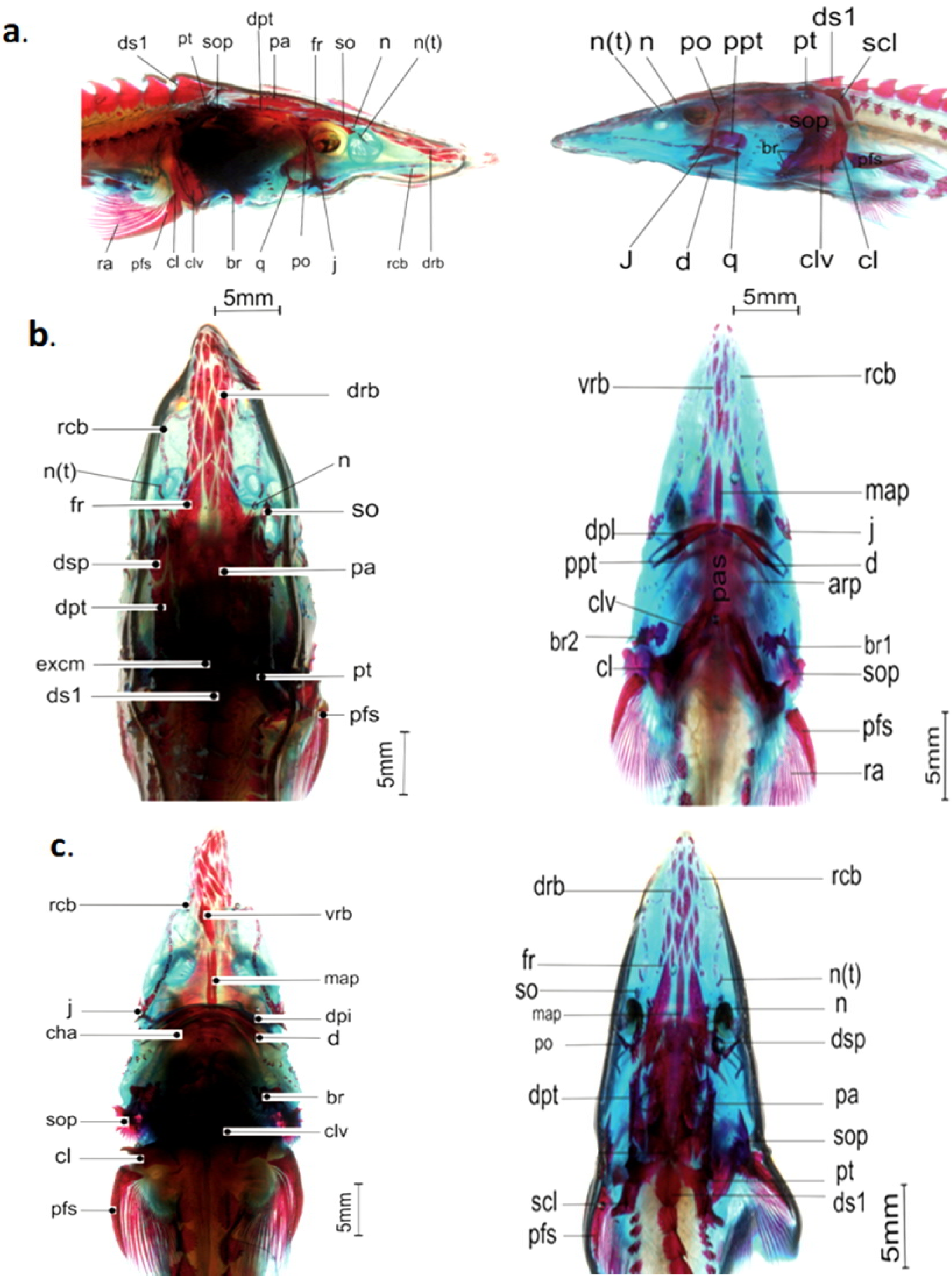


Figure 6. A - lateral view (left - 50-dph, right - 42-dph), B - ventral view (left - 50-dph, right 42-dph), C - dorsal view (left - 50-dph, right - 42-dph) of the triploid sturgeon. scl – supracleithrum, q – quadrate, ppt – palatopterygoid, sop – subopercle, pt – posttemporal, pa – parietal, ecp – ectopterygoid, br2 – branchiostegal, dpt – dermopterotic, clv – clavicle, ra – radials, pfs - pectoral fin spine, fr – frontal, j – jugal, cl – cleithrum, vrb - ventral rostral bone, drb - dorsal rostral bones, dsp – dermosphenotic, map - median anterior process of the parasphenoid, n(t) - tubular bone anterior of the nasal, excm - median extrascapular, po – postorbital, rcb - rostral canal bones, drb - dorsal rostral bones, ds1 - first dorsal scute, n – nasal, so – supraorbital, pfs - pectoral fin spine, dpl – dermopalatine, d – dentary, cha - anterior ceratohyal, rcb - rostral canal bones, arp - ascending ramus of the parasphenoid.

***31-dph - TL: 51.31 mm***. The rostral bones and jugal were completely ossified. The ossification of the postorbital, median extra scapular and nasal were started. Nearly all elements of the opercle series, pectoral fin and girdle were ossified. The bones' of the skull roof were formed. Utmost change in the formation of the dermal bones of the pectoral girdle was related to the median clavicle (Figure 5).
***42-dph - TL: 76.69 mm*.** The nasal and supraorbital were started to ossify. The rostral bones and pectoral fin spines were mostly ossified (Figure 6).
***50-dphv - TL: 109.78 mm***. Most of the dermal bones of the neurocranium were completely formed and ossified. Ossification of the jaws was completed. The components of the hyoid arch were cartilaginous. The pectoral girdle was completely formed and its spine was ossified. The fin rays were cartilaginous (Figure 6).

**Discussion**. Osteological development of the cephalic skeleton in triploid sturgeon (male *A. baeri* × female *H. huso*) was examined in this study. Based on the results, there was no bony or cartilaginous structure up to 2-dph. This indicates that the development of the skull is started after hatching, like some teleost *e.g. Sparus aurata* (Saka et al 2008). Whereas, this process is commenced before hatching in other bony fishes such as *Barbus barbus* (Vandewalle et al 1992).

The platoquadrate and Meckels cartilage are formed at 4-dph in triploid sturgeon. These two bony elements are formed at 3-dph in beluga sturgeon (Asgari 2012). Teeth formation were observed at 6-dph in triploid sturgeon similar to that of *H. huso* (Asgari 2012). The dermopalatine and dentary are started to ossify at 8-dph in triploid larvae. Osteogenesis of these cartilages and palatopterigoid were observed at 9-dph in beluga sturgeon (Asgari 2012). Also, osteogenesis of the dermopalatine is started at 18.8 mm of TL in *A. brevirostrum* (Hilton et al 2011). Development of the hyomandibular in the triploid sturgeon was similar to those of other sturgeons such as *A. brevirostrum* (Hilton et al 2011) and *H. huso* (Asgari 2012). The hyomandibular cartilage was formed at 6-dph in triploid larvae and remained cartilaginous until 50-dph. The first sign of the hyomandibular appearance was at 4-dph beluga larvae (Asgari 2012). The quadrate is completely developed at 14-dph that accordance with beginning of exogenous feeding of triploid larvae at 15-dph. This shows the role of the quadrate in feeding, since by developing the quadrate, mouth opening is became larger to obtain larger food items.

Dermal bones of the ventral face of the head and rostrum in the triploid sturgeon are the parasphenoid, ventral rostral bones and lateral rostral canal bones like other sturgeons (Hilton et al 2011). The ventral rostral bones situate on the ventral face of the snout form a bony complex. The most anterior, ventral rostral bone is the longest bone of this complex as seen in the triploid sturgeon and is similar to that of *A. brevirostrum* and other sturgeons (Hilton et al 2011). Polyodontidae and the members of the genus *Huso* have one pair of the ventral rostral bone at dorsal part of the complex, whereas, only a single ventral rostral bone is found in the dorsal part of this complex in the triploid sturgeon and *A. brevirostrum* (Hilton et al 2011). The dorsal rostral bones are positioned randomly on the snout and there is not any intra specific accordance in sturgeons (Hilton & Bemis 1999).

The parietal is appeared earlier than the frontal in the triploid sturgeon, whereas the parietal and frontal are synchronously formed in *A. brevirostrum* (Hilton et al 2011). The supraorbital is formed the antero-posterior part of the eye and appeared in 51.31 mm and 51.6 mm TL in the triploid sturgeon and *A. brevirostrum* (Hilton et al 2011), respectively. The most anterior bone enclosing the supraorbital sensory canal of the sturgeons is the nasal which, is formed by the nasal, and a series of the anterior tubular bones surrounding the supraorbital sensory canal (Hilton et al 2011) as observed in triploid sturgeon. The nasal is elliptical to oblong in shape in the triploid sturgeon while, this is elliptical to triangle in *A. brevirostrum*. The post temporal is ossified at 24.49 mm and 20.5 mm of TL in the triploid and *A. brevirostrum*, respectively.

**Conclusions**. The present study showed that ontogeny of the head skeleton of the triploid larvae (*A. Baeri* ♂ x *H. huso* ♀) has many similarities with those of *H. huso* and other sturgeons *e.g. A. brevirostrum* during the early developmental stages. These may be associated with its vital functions such as feeding and respiration that are necessary for its survival. Also, these similarities can suggest possibility to apply those feeding protocols that is used for others sturgeons especially its parents for this triploid sturgeon during early developmental stage.

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