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Body shape changes of hatchery-reared triploid sturgeon (*Acipenser baeri*) x (*Huso huso*) during early development using geometric morphometric technique

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Abstract. This research was conducted to study the body shape changes of a hatchery-reared triploid sturgeon (male *Acipenser baeri* × female *Huso huso*) using a landmark-based geometric morphometric method. For this purpose, larvae from hatching till 50-day post hatching (dph) were sampled, then their left sides photographed and nine landmarks-points digitized on 2D pictures to extract body shape data in the geometric morphometric teqnique. Relative Warp (RW) analysis revealed a sharp body shape change during early ontogeny on 15-dph. The results also showed that ontogenetic shape changes encompassed a pre-inflection shape changes, which included increasing the head depth and length and tail length and a post-inflection shape changes, with the elongation of the trunk. The results showed that the growths of the head and tail regions were positive allometeric and isometric before and after the inflexion point, respectively. The growth of the trunk region of the triploid larvae was negative and isometric allometric pattern at the first and second growth phases, respectively. The body shape change during the early development of triploid larvae can be associated with development of feeding apparatus, swimming, respiration and sense organs. Based on the results, the triploid larvae showed similar growth patterns with those of its parents, i.e. *A. baeri* and *H. huso*. Body shape change of the triploid larvae at the length of 15-dph, reflects full swimming ability and external feeding that are necessary for its survival.

Key Words: relative warp, allometry, growth pattern, Acipenseidae.

Introduction. The sturgeons 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; Asgari et al 2013). Hence as a strategy, their artificial propagation for aquaculture and restocking purposes has developed (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 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 diploid one (Altimiras et al 2002).

Early ontogeny of most fishes is characterized by a drastic change involving most organs, systems and very complex shape changes (Pena & Dumas 2009; Asgari et al 2013). Changes in body shape lead to the formation of characteristic morphologies and allometric growth patterns (Gisbert et al 2002), which is responsible for a progressive transformation of the recently hatched larva into a juvenile or adult form, in a relatively short time. Allometric growth pattern is a common feature during early larval development of fishes suggesting growth functionally optimized their survival by development of functional organs with their priority (Khemis et al 2013).

Determination of the growth patterns during early development can contribute to fisheries management and aquaculture by characterizing normal growth patterns under certain conditions and optimizing rearing protocols if abnormalities during larval development are detected (Pena & Dumas 2009). Furthermore, it allows the estimation of the quality of juveniles and their suitability for stocking or further rearing. Hence, this study aimed to survey the body shape change of a hatchery-reared triploid sturgeon, i.e. *Acipenser baeri* (♂) x *Huso huso* (♀) as a suitable candidate in aquaculture using the geometric morphometric technique. Information about the growth patterns of a fish may allow better understanding the patterns underlying the early life stages, their priorities during early growth and provides insight into fish biology, behavior, ecology and aquaculture (Gisbert 1999; Koumoundouros et al 1999).

Material and Method. Triploid larvae (*A. baeri* ♂) x (*H. Huso* ♀) were obtained from artificial propagation of the female beluga caught from the Caspian Sea and a reared male Siberian sturgeon in the Shahid Dadman International Institute of Sturgeon Fishes in spring of 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^{-1}) (750 gram egg per unit). After eight days of incubation, 1800 newly hatched larvae with body weight (BW) of 5.8±0.7 mg and total length (TL) of 10.17±0.2 mm (mean±SD) were introduced into 500-L circular fiberglass tanks (with depth of 20 cm) connected to a flow-through freshwater system. Water source was from the Sefidrud River filtered by a sand filtration. During the larval rearing period, water temperature, dissolved oxygen, pH and flow rate were 16.5±0.2°C, 10.7±0.3 mg L^{-1}, 7.8±0.1 and 5.7±0.4 L s^{-1}, respectively. Fish were reared under natural photoperiod. The larvae were fed with a mixture of (1:1) non-enriched *Artemia* nauplii and cladocerans (*Daphnia* sp.) from 12 to 25-dph (day post hatching) (500 nauplii larvae^{-1} day^{-1}). 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.

From hatching till 12-dph ten larvae were sampled every day, then every 5 days up to 26-dph and afterward at 31, 42 and 50-dph (n = 10). Fish were randomly sampled prior to feeding in the morning, and sacrificed with an overdose of tricaine methanosulphonate (MS-222, Sigma-Aldrich). All specimens were weighed (BW, to the nearest 0.01 mg) with an analytical microbalance and their left sides photographed immediately, with a binocular microscope (Leica EZ4 D) equipped to Canon digital camera (5 MP) from hatching until 14-dph, and afterward using a copy-stand equipped to a digital Kodak camera (6 MP) up to 50-dph.

To extract body shape data using the geometric morphometric method, nine landmark-points were digitized on the 2D pictures using TpsDig2 software (Rohlf 2006) (Figure 1). The landmark data was tested using tpsSmall which confirmed the suitability for further analysis and the consensus configurations for each age class were computed (Rohlf 2005) and used for further analysis. The data were analyzed using Generalized Procrustes Analysis (GPA) to remove all non-shape related information and relative warp analysis using PAST software (Version 2.10). Relative warp analysis is analogous to a principal component analysis (PCA) for these sorts of data (Rohlf 1993).

The relative warp scores (RW1 and RW2) were used as descriptors for the variation in shape (Bookstein 1991). The shape changes during early developmental stage were visualized by generating deformation grids using MorphoJ software (Klingenberg 2011). Growth trajectory was computed by plotting RW1 and RW2 (as dependent variable) against TL (as independent variable) by permutation tests with 1000 random permutations using regression analysis in TpsReg software (Rohlf 2005). The inflexion point was computed based on Fuiman (1983) and Van Snik et al (1997). Inflexion point and regression analyses were extracted and performed using MS-Excel 2007 (Microsoft Corporation).

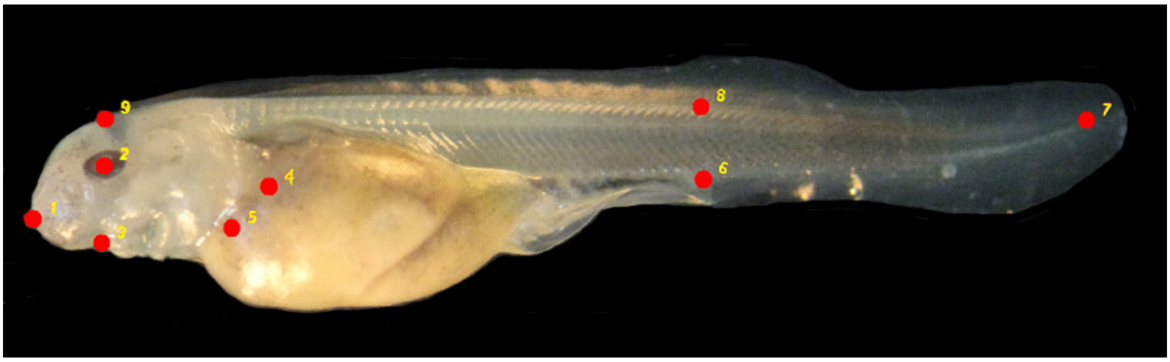

Figure 1. Defined landmark points to extract body shape during early development of the triploid sturgeon. (1) anterior tip of the snout, (2) center of the eye, (3) pseudo-landmark on the ventral part of the head perpendicular to the center of the eye, (4) posterior end of the opercula, (5) ventral point of the opercular slit, (6) anus, (7) posterior end of the notochord, (8) pseudo-landmark on the dorsal part of the body perpendicular to the anus and (9) pseudo-landmark on the dorsal part of the head perpendicular to the center of the eye.

Results. The first two relative warps explained 84.23% of the body shape changes during early development (RW1 = 66.07% and RW2 = 18.16%). Figure 2 displays the morphospace defined by RW1 and RW2 spreading along RW1 according to age (youngest specimens on the right side of the graph; the older ones on the left side). Body shape changes according to RW1 (from +RW1 to -RW1) reflect increasing the head depth and length and tail length and that of RW2 (from +RW1 to -RW1) showed the elongation of the trunk i.e. the head depth and length and tail length were shorter at 0-dph and they grow up to 15-dph as the inflexion point (beginning of the exogenous feeding). During the post inflexion point, the length of the trunk was increased more than head and tail region.

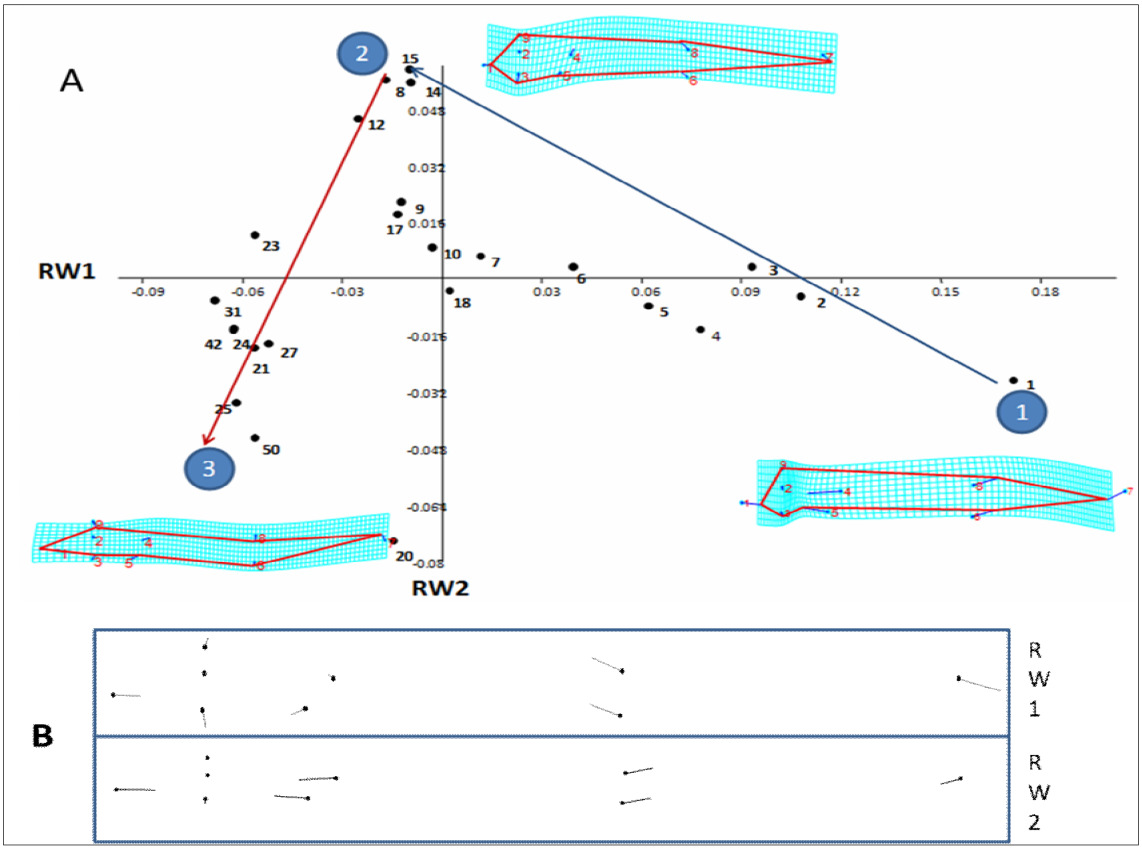

Figure 2. (A) Scatter plot of relative warp analysis, depicting RW1 and RW2 (the youngest specimens labeled from 1, the older ones have higher numbers; deformation grids show body shape of specimens at 1, 15 and 50-dph in relation to the consensus configuration of all age classes) and (B) lollipop graphs show the body shape changes related to RW1 and RW2.

A weak correlation was found between RW1 and TL (R^2 = 0.32, and $p < 0.05$) (Figure 3A). However, the regression model showed that RW1 scores were strongly correlated to TL during early ontogeny up to 15-dph (R^2 = 0.935) but a weak correlation onwards (R^2 = 0.275) (Figure 3A). In addition, a low correlation was found between RW2 and TL prior (R^2 = 0.476) and post inflexion point (R^2 = 0.189) (Figure 3B).

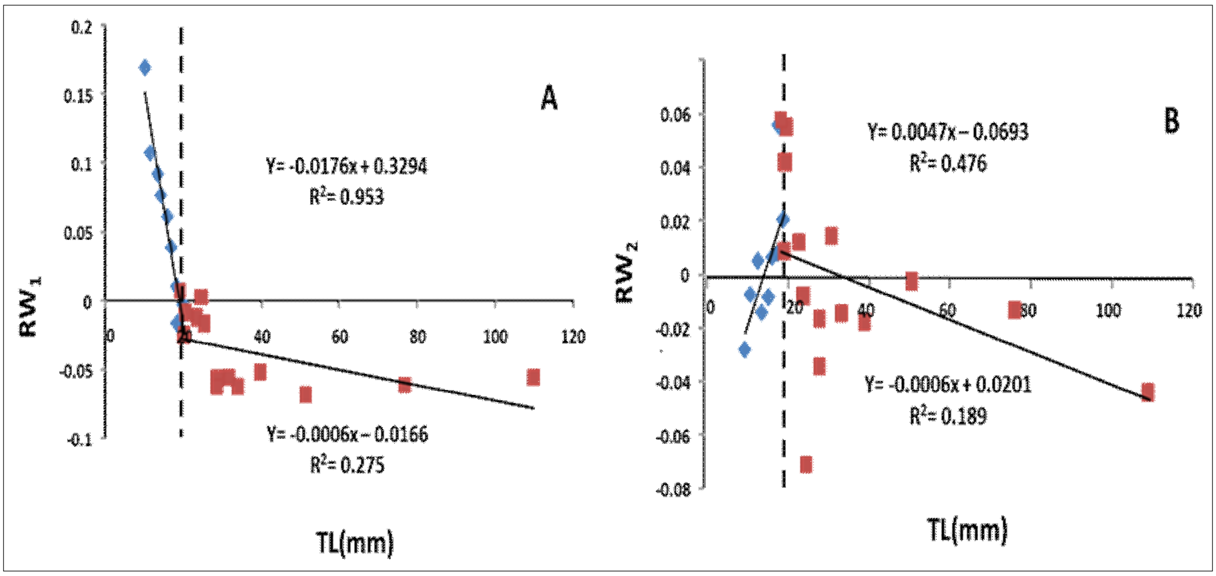

Figure 3. The growth trajectory diagram of the triploid sturgeon from hatching till 50-dph. (A) Regression diagram between RW1 and TL and (B) Regression diagram between RW2 and TL (the dash line represents inflexion point of growth trajectory).

The inflection point of body shape was coincided with the age of 15-dph (18.89 mm TL). Ontogenetic body shape of triploid fish was encompassed two phases, including (1) pre-inflection shape changes, which included relative increasing the head depth and length and tail length (positive allometric growth pattern) along RW1 axis and (2) post-inflection shape changes, including a little increasing of the trunk length, with a nearly isometric growth pattern along RW2. In summary, main change of the body shape was occurred during the absorption of the yolk sac.

Discussion. Developments of organs and changes in morpho-anatomical characters are occurred in a stepwise fashion, which is regulated by gene expression and influenced by environmental parameters (Gilbert & Bolker 2003; Osse & Van den Boogart 1995). Based on the results, the growth pattern of the head in the triploid fish was positive allometeric prior to inflexion point but nearly isometric during post inflexion. This is similar to that of the parents of the triploid fish, i.e. Siberian sturgeon (Gisbert 1999) and Beluga (Asgari 2012). Positive allometric growth of the head is a common phenomenon during early ontogeny of many fish species e.g. sturgeons (Van Snik et al 1997), that is concomitant with the development of the brain, sensory, respiratory organs and feeding system (Gisbert & Doroshov 2006). The early differentiation and development of the nervous, sensory and feeding systems contribute to help prey detection and obtaining larger food items (Pena & Dumas 2009).

Furthermore, the results showed increasing the tail length prior inflexion point revealing its importance and priority during early developmental stage. This growth pattern is similar to those of its parents, i.e. the Siberian (Gisbert 1999) and beluga sturgeons (Asgari 2012; Bahrami-Ziarani et al 2012) that can be related to increasing swimming ability. Since predation and starvation are two main causes of mortality in fish larvae, therefore, the early development of feeding apparatus and swimming organs (the positive allometric growth of anterior and posterior regions of the body) appear to be important priorities during early ontogeny (Osse et al 1997).

Inflexion point of the triploid fish that was similar to that of Siberian sturgeon (Gisbert 1999), is in accordance with beginning of the exogenous feeding and this confirmed the hypothesis that growth patterns during early life stages closely match to their priorities during early growth (Gisbert 1999). After the inflexion point, the growth pattern of the head and tail of the triploid fish declined and became isometric and that of the trunk increased and changed from negative allometric to an isometric pattern (Bahrami-Ziarani et al 2012) similar to green sturgeon and beluga (Gisbert 1999), showing less importance of the trunk growth compared to those of the head and tail regions.

Conclusions. The present study showed the importance of body shape changes during the early development of triploid larvae (*A. baeri* ♂) x (*H. huso* ♀) larvae, which are associated with development of feeding apparatus, swimming, respiration and sense organs. Based on the pattern of body shape changes, the triploid larvae showed similar growth patterns with those of its parents, i.e. the Siberian and beluga sturgeon. Body shape changes of the triploid larvae at the length of 15-dph reflects full swimming ability and external feeding that are necessary for its survival.

References

Altimiras J., Axelsoon M., Claireaux G., Lefrançois C., Mercier C., Farrell A. P., 2002 Cardiorespiratory status of triploid brown trout during swimming at two acclimation temperatures. Journal of Fish Biology 60(1):102-116.

Asgari R., 2012 Early development of *Huso huso* (Acipenseridae): a case study for ontogeny of feeding apparatus and digestive enzyme (trypsin, amylase and lipase) activity up to 50 DPH. PhD thesis, Department of Fisheries, University of Tehran, 170 pp.

Asgari R., Eagderi S., Rafiee G., Poorbagher H., Agh N., Eshagh Zadeh H., 2013 Body shape changes during the early development of the Beluga (*Huso huso*). International Journal of Aquatic Biology 1:1-5.

Bahrami-Ziarani M., Eagderi S., Pourbagher H., Farahmand H., 2012 Allometric growth patterns of a triploid sturgeon (*Acipenser baeri*) x (*Huso huso*) during early development. First International Larviculture Conference in Iran, 11-12, December, 2012, Karaj, Tehran University, pp. 124-128.

Bookstein F. L., 1991 Morphometric tools for landmark data: geometry and biology., Cambridge University Press, New York, 435 pp.

Bronzi P., Rosenthal H., Arlati G., Williot P., 1999 A brief overview on the status and prospects of sturgeon farming in western and Central Europe. Journal of Applied Ichthyology 15:224-227.

Fuiman L. A., 1983 Growth gradients in fish larvae. Journal of Fish Biology 23:117-123.

Gilbert S. F., Bolker J. A., 2003 Ecological developmental biology: preface to the symposium. Evolution and Development 5(1):3-8.

Gisbert E., 1999 Early development and allometric growth patterns in Siberian sturgeon and their ecological significance. Journal of Fish Biology 54:852-862.

Gisbert E., Doroshov S. I., 2006 Allometric growth in green sturgeon larve. Journal of Applied Ichthyology 22:202-207.

Gisbert E., Merino G., Muguet J. B., Bush D., Piedrahita R. H., Conklin D. E., 2002 Morphological development and allometric growth patterns in hatchery-reared California halibut larvae. Journal of Fish Biology 61:1217-1229.

Khemis I. B., Gisbert E., Alcaraz C., Zouiten D., Besbes R., Zouiten A., Slaheddine Masmoudi A., Cahu C., 2013 Allometric growth patterns and development in larvae and juveniles of thick-lipped grey mullet *Chelon labrosus* reared in mesocosm conditions. Aquaculture Research 44(12):1872–1888.

Klingenberg C. P., 2011 MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11:353-357.

Koumoundouros G., Divanach P., Kentouri M., 1999 Ontogeny and allometric plasticity of *Dentex dentex* (Osteichthyes: Sparidae) in rearing conditions. Marine Biology 135:561-572.

Pena R., Dumas S., 2009 Development and allometric growth patterns during early larval stages of the spotted sand bass *Paralabrax maculatofasciatus* (Percoidei: Serranidae). Scientia Marina 73:183-189.

Osse J. W. M., Van den Boogaart J. G. M., 1995 Fish larvae, development, allometric growth, and the aquatic environment. ICES Marine Science Symposium 201:21-34.

Osse J. W. M., Van den Boogaart J. G. M., Van Snik G. M. J., Van der Sluys L., 1997 Priorities during early growth of fish larvae. Aquaculture 155:249-258.

Rohlf F. J., 1993 Relative warp analysis and an example of its application to mosquito wings. In: Contributions to morphometrics. Marcus L. F., Bello E., García-Valdecasas A. (eds). Madrid: C.S.I.C, pp. 131-159.

Rohlf F. J., 2005 Software by F. James Rohlf. Available at: http://life.bio.sunysb.edu/ee/rohlf/software.html.

Rohlf F. J., 2006 A comment on phylogenetic correction. Evolution 60:1509-1515.

Van Snik G. M. J., Van den Boogaart J. G. M., Osse J. W. M., 1997 Larval growth patterns in *Cyprinus carpio* and *Clarias gariepinus* with attention to the finfold. Journal of Fish Biology 50:1339-1352.

Williot P., Sabeau L., Gessner J., Arlati G., Bronzi P., Gulyas T., Berni P., 2001 Sturgeon farming in Western Europe: recent developments and perspective. Aquatic Living Resources 14:367-374.

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