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Body shape changes during the early development of the Beluga (*Huso huso*)

Reza Asgari[1], Soheil Eagderi *[1], Gholamreza Rafiee [1], Hadi Poorbagher[1], Naser Agh[2], Hamid Eshagh Zadeh[1]

[1] Department of Fisheries, Faculty of Natural Resources, the University of Tehran, P.O. Box: 31585-4314, Karaj, Iran.
[2] Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran.

Abstract: Early body shape changes of beluga sturgeon were studied using landmark-based geometric morphometric approach to recognize its allometric growth pattern. Sampling was done from hatching up to 50 days post hatching (DPH). Left side of specimens were photographed using digital camera and nine landmark points were digitized on two-dimensional images. Total length (Tl) was measured using the software ImageJ. To study of the body shape changes during early development, the mean procrustes distance between all specimens of same age, for all age groups, was calculated. The scores of relative warp analysis (RW) were used as descriptors for the variation in shape. RW analysis revealed a sharp body shape change during early ontogeny on 18 DPH. Growth trajectory was computed by plotting RW against TL. The inflection point of body shape corresponds to a TL of 23.3 mm (18 DPH). Results showed that ontogenetic shape changes encompassed a pre-inflection shape changes, which included the elongation of the head and tail regions i.e. positive allometric growth pattern and post-inflection shape changes, with a nearly isometric growth pattern.

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Introduction

The early development of fish larvae is accompanied with very complex shape changes. Different growth rates of various parts of the body or allometric growth is a common phenomenon during this period (Osse and van den Boogaart, 1995). During the early development, changes in body shape are related to the functions of different organs such as respiration, feeding and swimming (Simonovic et al., 1999; Russo et al., 2007). Recognition of morphogenesis process and growth pattern of fishes may lead to better understanding of biological priorities during the early developmental stages and gives insights into biological, behavioral and ecological characteristics (Gisbert, 1999).

Many studied have been carried out on change of the body shape during the early ontogeny of various fishes using traditional morphometric approaches but recently, geometric morphometric techniques have been applied (Bookstein, 1991; Rohlf, 1998; Zelditch et al., 2004). Geometric morphometric methods are useful tools in developmental biology to extract shapes data and analyze using multivariate statistical tests, explaining how morphological structures are generated (Zelditch et al., 2004). Hence, this study was conducted to study the changes of the body shape in Beluga sturgeon (Huso huso) using landmark-based a geometric morphometric method covering a period from hatching up to 50 days post hatch (DPH) that is synchronized with the transition of larvae from internal to external feeding.

Material and methods

Specimens were obtained from the Dadman International Sturgeon Research Institute (Guilan, Iran). Newly hatched larvae were stored in 500 L fiberglass tanks with a water depth of 20 cm and

* Corresponding author: Soheil Eagderi
E-mail address: soheil.eagderi@ut.ac.ir

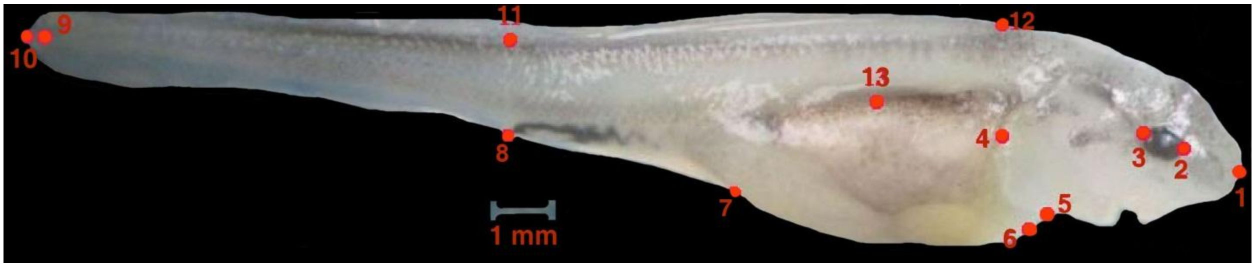

Figure 1. (1) anterior tip of the snout; (2) anterior margin of the eye ; (3) posterior margin of the eye; (4) posterior most point of the gill slit; (5) ventral point of the gill slit; (6) anterior of the yolk sac ; (7) posterior end of abdominal area ; (8) anus; (9) posterior end of vertebrate; (10) posterior most tail extremity; (11) pseudo-landmark on the dorsal edge of body in the front of the anus; (12) pseudo-landmark on the dorsal edge of body in the front of landmark 4; (13) insertion point on superior of yolk sac between landmark 7 and 12.

reared up to 50 DPH. Larvae were stocked in tanks with a density of 1800 tank 1. The water was supplied from a mixture of ground and river water with a discharge of 400 and 250 mL 1 min 1, respectively. The water temperature (°C), DO (mg L^{-1}) and pH of were 15.9, 7.7 and 7.8 during experiment, respectively. Due to asynchrony in external feeding of pre-larvae and to avoid starving and cannibalism, feeding was started from 8 DPH using artemia nauplii (500 nauplii larvae 1) which continued till 12 DPH. The larvae were then fed using a mixture of artemia nauplii and daphnia till 25 DPH. Afterwards the larvae were fed using a mixture of commercial food pellet (Biomar) and mashed chironomid larvae in a rate of 30% bw d 1 (4-6 times per day).

Ten specimens were sampled every day from each tank, anaesthetized with MS222, preserved in 10% buffered formalin and stored in 70% ethanol after 24 hours. Before fixation, the total length (TL) was measured (in mm) as an independent data using the software ImageJ (version 1.240). The left sides of specimens were photographed using a stereomicroscope equipped with a Cannon camera with a 5 MP resolution and nine landmarks points were digitized on two-dimensional images using the software tpsDig2 (Fig. 1).

The landmark data was tested using tpsSmall which confirmed the suitability for further analysis and the

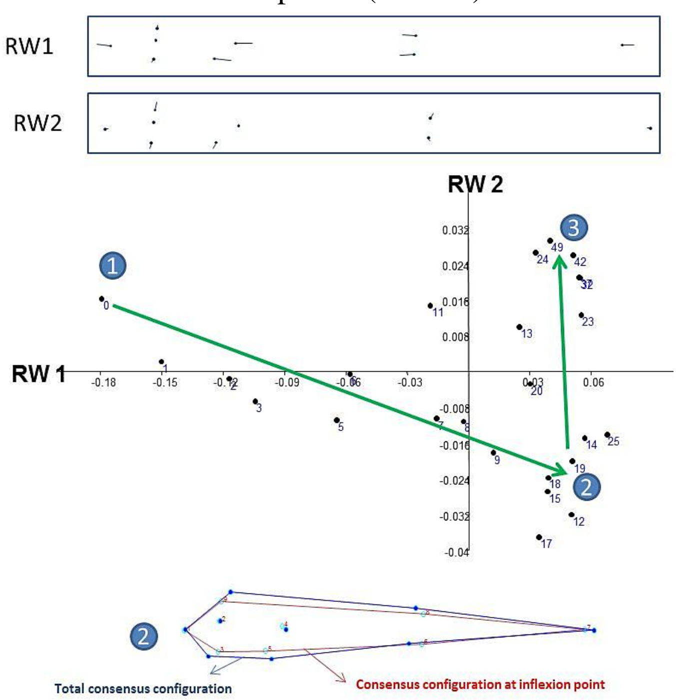

Figure 2. Scatter plot of relative warp analysis, depicting RW1 and RW2. The youngest specimens labeled from 1, the older ones have higher numbers.

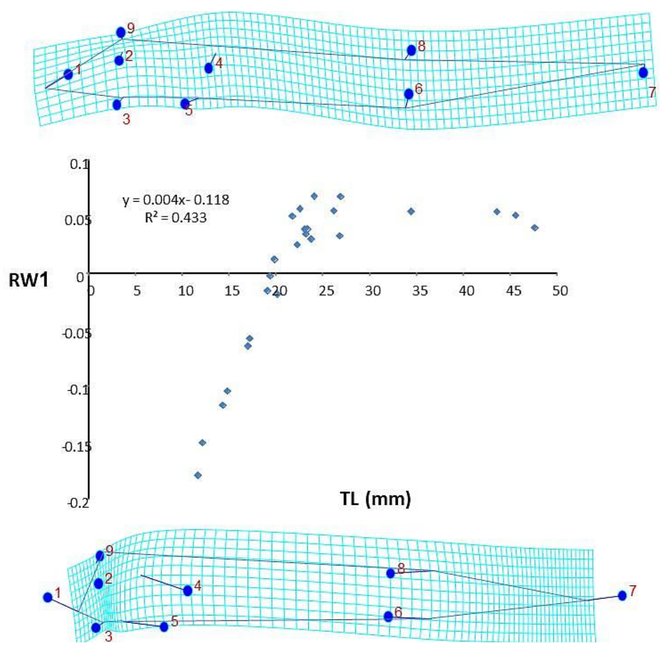

Figure 3. Growth trajectory from 1 to 50 DPH: RW1 and TL. The splines represent the shape at the extremes of the growth trajectory: the bottom one at the beginning and the upper at the end of development, respectively.

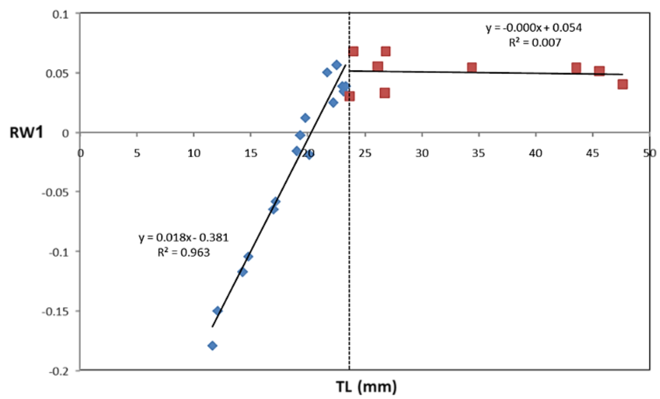

Figure 4. RW1 versus TL. The dotted line represents the inflexion point of growth.

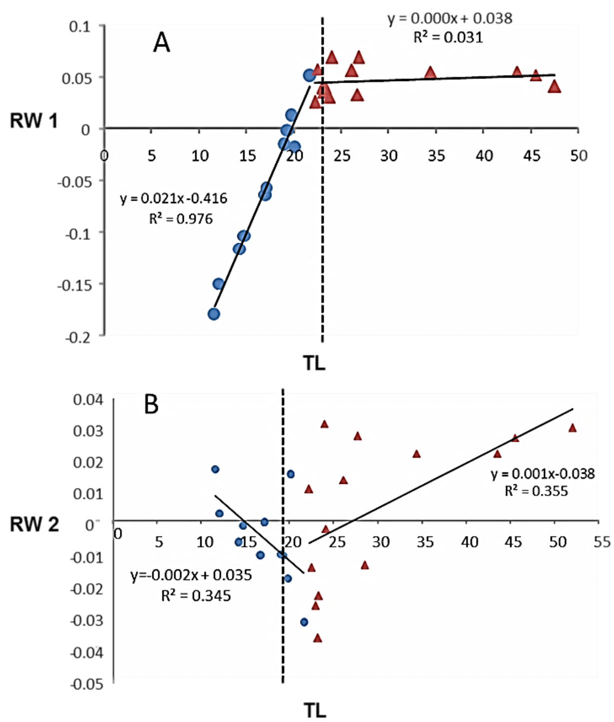

Figure 5. (A) RW1 versus TL, (2) RW2 versus TL. ● = before yolk sac absorption and ▲ = after yolk sac absorption. The dotted line represents the inflexion point of growth.

consensus configurations were computed for each age sample (Rohlf, 2005). The data were analyzed using generalised procrustes analysis (GPA), in order to remove all non-shape related information and relative warp analysis using PAST soft (version 2.10). Relative warp analysis is analogous to a principal component analysis (PCA) for these sorts of data (Rohlf and Marcus, 1993).

The relative warp scores (RW1 and RW2) were used as descriptors for the variation in shape (Bookstein, 2005). The shape changes during growth were visualized as generating wireframe graph and deformation grids (splines) using the software MorphoJ. The correlation between shape descriptors and TL was tested with permutation tests with 1000 random permutations. Growth trajectory was computed by plotting RW1 and RW2 against TL. Allometric growth was calculated as a power function of total length using non-transformed data: Y = axb where Y was the independent variable, x the dependent variable, a the intercept and b the growth coefficient. Isometric growth, positive and negative allometric growth are indicated by b=1, b>1 and b<1, respectively (Zelditch et al., 2004). The Inflexion points of growth curves were determined according to Fuiman (1983) and Van Snik et al. (1997). Inflexion point and regression analyses were extracted and performed using MS-Excel 2007 (Microsoft Corporation).

Results

The first two relative warps explained 91.06% of the body shape changes during ontogeny (RW1 63.86% and RW2 27.20%). 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) (Fig. 2). RW1 reflects (1) elongation of the head (including the snout and post-orbital region) and (2) elongation of caudal area *i.e.* allometric growth pattern of the trunk region, whereas RW2 indicated an increase in depth of the head and trunk (Fig. 2). There was a weak correlation between RW1 scores and TL (r^2=0.433) (Fig. 3). However, the regression model showed that RW1 scores were strongly correlated to TL during early ontogeny upto 18 DPH (r^2=0.963) but no correlation onwards (r^2=0.007; Fig. 4). Similar results were found before and after the absorption of the yolk sac (r^2=0.345 and r^2=0.355, respectively; Fig. 5).

The inflection point of body shape corresponds to a TL of 23.3 mm, which was coincided with the age of 18 DPH. Ontogenetic shape changes encompassed two phases: (1) pre-inflection shape changes, which included the elongation of the head and tail regions (positive allometric growth) and (2) post-inflection shape changes, with a nearly isometric growth pattern. In summary, main changes of the shape occurred during the absorption of yolk sac when a period started that the body shape became a miniature form of the adults.

Discussion

The present study indicated that most important changes in shape of beluga larvae occur during early development up to 18 DPH and involve the elongation of head and caudal area along with the increase of the body depth. During this period, the trunk had a negative allometric growth. As the first study of allometric growth in sturgeons using geometric morphometric approach, our study is in agreement with other researches that used traditional methods (Gisbert, 1999; Russo et al., 2007; Fuiman, 1983; Osse, 1990; Osse and Boogart, 1995; Van snik et al., 1997) confirming that there is a correlation between changes of body shape and growth pattern, which is according to their functional importance. Since predation and starvation are the main causes of mortality in fish larvae, development of feeding and swimming organs appears to be two important priorities during the early ontogeny (Osse et al., 1997). The positive allometic growth of anterior and posterior regions of body in beluga larva is similar to other fishes indicating the importance of the organs that are related to respiration, feeding and movement (Osse and van den Boogart, 1995; Gisbert et al., 2002).

Changes in body shape pattern of the beluga larvae at the length of 23 mm (18 DPH), reflects full swimming ability that is necessary for external feeding. It has been suggested that ability to escape from predation and prey is synchronous with fully development of fins (Hale, 1999, Gibb et al, 2006). Formation of fins in beluga is occurred at 15 DPH being coincided with stiffening of fin rays (Asgari, 2012). Also the cephalic lateral line canals are completed at 18 DPH (Asgari, 2012). The neoromasts of these canals play a vital role in reception of environmental stimuli during feeding and swimming (Omori et al., 1996). The present study show the importance of body shape changes during the early development of beluga larvae, which are associated with development of feeding apparatus, swimming, respiration and sense organs. Based on the pattern of body shape changes, beluga larva can be considered fully developed or fry after 18 DPH. Since these body shape changes are associated with skeletal changes, the main structures of beluga skeleton are probably formed during this period. Further studies on the ontogeny of muscklo-skeletal and digestive systems are recommended.

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