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Body shape change in Common carp, *Cyprinus carpio* var. Sazan (Teleostei: Cyprinidae), during early development using geometric morphometric method

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Abstract: This research was conducted to study the body shape changes in common carp, *Cyprinus carpio* var. Sazan during early developmental stages using landmark-based geometric morphometric method. For this purpose, a total number of 210 larvae from hatching time till 55 days post hatching (dph) were sampled. For extracting body shape data, the right side of specimens was photographed and nine landmark-points were defined and digitized on 2D pictures using tpsDig2 software. After GPA, the landmark data were analyzed using Relative Warp analysis, regression of shape on total length and cluster analysis. The results showed that change of body shape in common carp during early development includes (1) increase in the head depth, and trunk length from hatching up to 8 dph, (2) increase in the body depth, and the head and tail lengths from 8-20 dph, and (3) increase in the head length and depth from 20-55 dph. The cluster analysis was revealed that larval stages can be divided into four phases, including eleuthero-embryonic, larva, younger juvenile and juvenile. Based on our finding, the body shape changes of this species during early development is due to adjusting of vital priority.

Keywords: Morphometrics, Ontogeny, Relative Warp, Cluster analysis, Larvae.

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Introduction

Fish larvae often change very intensively during early development (Osse 1990; Osse & van den Boogaart 1995, 1999; Gisbert 1999; Loy et al. 2001). These morpho-anatomical changes are occurred in a stepwise fashion, which is regulated by gene expression and influenced by the environmental parameters (Gilbert & Bolker 2003). Changes in body shape lead to the formation of morphological characteristics 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.

Many researches have investigated the change of body shape during early developmental stages in various fish species using traditional morphometric approach but recently, geometric morphometric (GM) techniques have been applied for such a studies (Bookstein 1991; Rohlf 1998; Zedditch et al. 2004). GM method is a useful tool in the field of developmental biology to extract shape data and analyse those data using multivariate statistical tests, showing how morphological structures are generated and changed (Zelditch et al. 2004) by visualization techniques.

Common carp, *Cyprinus carpio* Linnaeus, 1758 is one of the most commercially important species of freshwater fish in the world (Balon 1995). The Caspian Sea and its basin is one of the most important

Fig.1. Defined landmark points to extract body shape data during early development of *Cyprinus carpio* var. Sazan.

natural habitats of wild common carp known as Sazan variety. Recently, this variety of common carp is endangered or disappeared in many areas of its natural range due to loss of its breeding grounds, overfishing and pollution (Kohlmann et al. 2005). Therefore, its artificial propagation in hatcheries and subsequent releasing their fingerlings into the Caspian Sea has been applied as a proper strategy for recurring its natural populations. Hence, determination of its growth pattern during early developmental stages can contribute to fisheries management and aquaculture of this species by providing a guideline to optimize hatchery production (Koumoundouros et al. 1999; Gisbert 1999; van Maaren & Daniels 2000). Thus, this study was conducted to investigate changes in the body shape of *C. carpio* var. Sazan during early developmental stage using GM technique. The results of this study can help to better understanding their priorities during early development by providing insight into its biology, behavior and ecology.

Materials and Methods

The adult specimens of *C. carpio* var. Sazan were caught in the estuary of the Gorgan River by surrounding and gill nets during spawning migration in April and May 2014. Then, they were transferred into three 250 m^2 earthen ponds in the Sijval Restocking Center (Bandar-e-Turkmen, north of Iran) with an ambient temperature. These ponds were filled three weeks prior to the introduction of the fish and fertilized to sustain primary production and benthos as natural food by adding cattle manure and 50kg urea per hectare. By semi-artificial propagation method, the broodstocks were bred, and the larvae produced. During rearing period, the larvae were fed by fertilizing ponds (using inorganic fertilizer i.e. urea plus phosphate and cattle manure according to climatic conditions) along with crushed boiled eggs from hatching up to 20 days post hatching (dph), and then with commercial food pellet (Biomar) twice a day. The water temperature, DO and pH of the ponds were 21.4-24.4°C, 6.5-8ppm and 7.6-8.4 during rearing period, respectively. Fish were reared under the natural photoperiod. In addition, the semi extensive condition was applied to provide a natural habitat and producing high-quality larvae with low anomalies (Lewis & Lall 2006).

After hatching, larvae were randomly sampled from hatching up to 55 dph as following: 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 15, 16, 20, 24, 26, 28, 30, 35, 45 and 55 dph (10 specimens per sampling stage from three ponds and were pooled), prior to feeding in the morning, and sacrificed by an overdose of MS 222 (Sigma-Aldrich). The right sides of the specimens were photographed using a dissecting microscope equipped with a digital camera (Cannon) with a 5 MP resolution. For better observing and contrast, the specimens were stained using Toluidine blue. Then, the specimens were preserved in 5% buffered formaldehyde and moved to 72% alcohol after 48 hours. The external morphological changes during early development were examined using a stereomicroscopy (Leica).

To extract the body shape data using GM approach, 9 landmark-points were defined and digitized on the specimens' pictures using tpsDig2 software (Rohlf 2005) as follow: (1) anterior tip of the snout at the upper jaw, (2) center of the eye, (3) dorsal edge of the eye vertical to the eye center, (4) dorsal edge of the head vertical to the eye center, (5) dorsal edge of the trunk vertical to the anus, (6) posterior end of the tail, (7) anus, (8) ventral edge of the head vertical to the eye center and (9) ventral edge of the eye vertical to the eye center. The selection of the landmark-points was due to the deficiency of anatomical structure, especially during early developmental stage (Fig. 1).

The extracted data was tested using tpsSmall software (Rohlf 2005) to confirm suitability of the procrust distance instead of the tangent distance for further analysis. The landmark data were analyzed using Generalized Progrustes Analysis (GPA), to remove non-shape data. Then, data were analyzed using Relative Warp (RW) analysis that is analogous to a Principal Component Analysis (PCA) for the landmark-based shape data (Rohlf & Marcus 1993). The relative warp scores (RW1 and RW2) were used as descriptors of the body shape variations (Bookstein 2005). Growth trajectory was computed by plotting RW1 against total length (TL). The correlation between shape descriptors and total length (TL) was tested with 1000 random permutations using tpsReg software.

A cluster analysis was performed on the procrust distance computed from nine landmark configurations of the consensus shape of each growth phase, i.e. sampling group, using Ward's algorithm, to identify clusters between age or size groups. A Multi-Response Permutation Procedure (MRPP) was performed to test differences between development intervals. MRPP is a non-parametric procedure used to test the null hypothesis of no difference between two or more groups of entities that must be created a priori (Legendre & Legendre 1998).

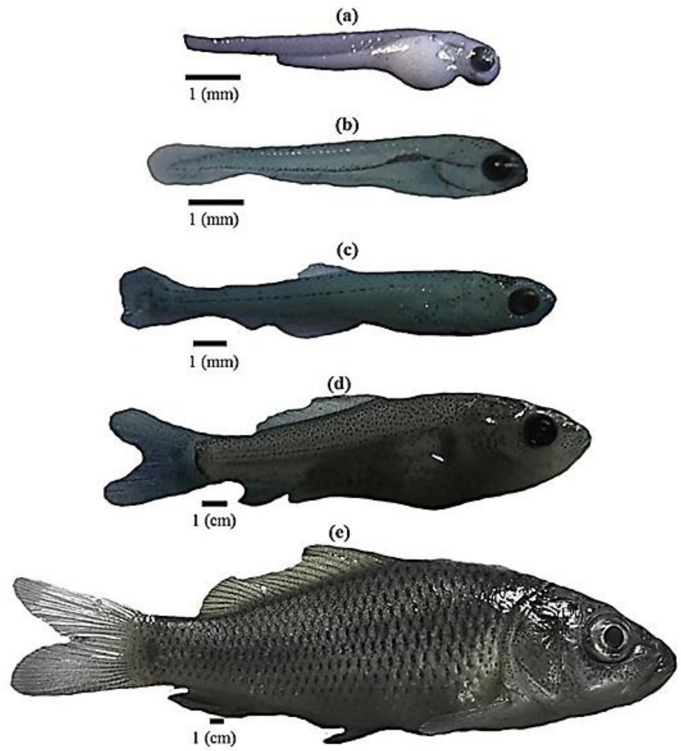

Fig.2. Early life stages of *Cyprinus carpio* var. Sazan. (a) 1 dph, (b) 6 dph, (c) 20 dph, (d) 30 dph and (e) 55 dph (scale bar=1mm).

Results

Newly hatched larvae were 5.72±0.06mm, in total length (TL) and increased to 55.07±4mm at 55 dph (Fig. 2). The first two relative warps explained 74.69% of the body shape variations (RW1=53.08% and RW2=21.61%). Figure 3 displays the morphospace defined by RW1 and RW2 based on the consensus shape data of each sampling group. The specimens are spread along RW1 according to age (youngest specimens on the left side of the graph, and the older ones on the right side). Based on the RW analysis, the major changes of body shape along with increasing age reflects three stages, including (1) from hatching up to 8 dph (from +RW2 to -RW2) reflecting increase in the head depth, and trunk length, (2) from 8-20 dph (from -RW1 to +RW1) reflecting increase in the body depth (head, trunk and tail), and the head and tail lengths, and (3) from 20-55 dph (from -RW2 to +RW2) reflecting increase in

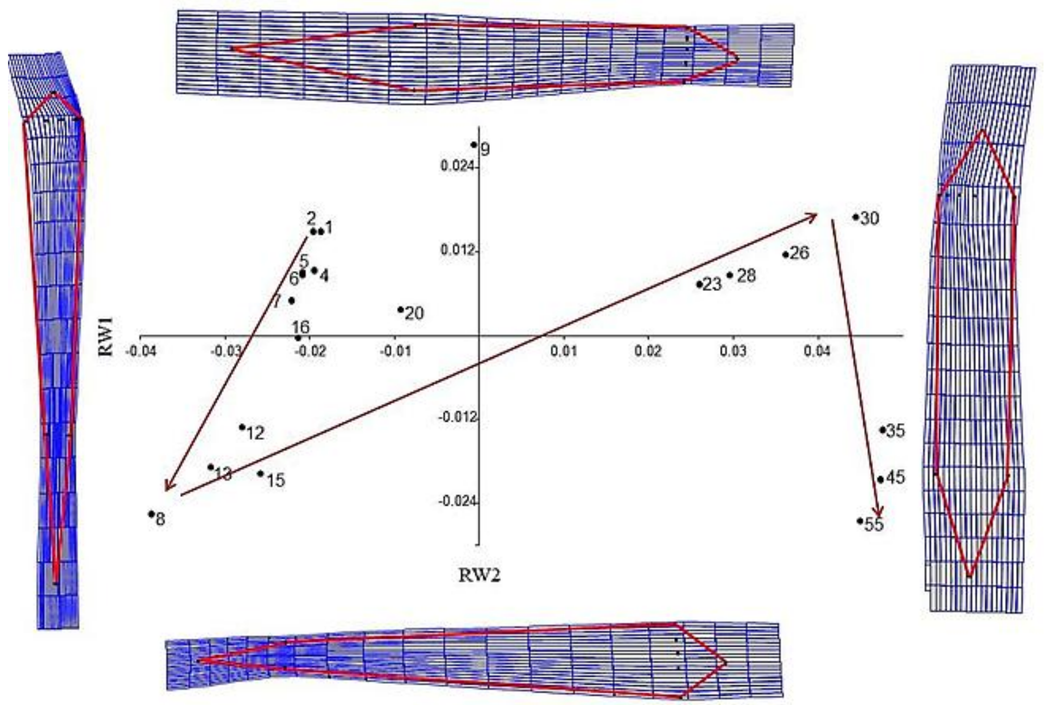

Fig.3. Scatterplot depicting the ordination of *Cyprinus carpio* var. Sazan specimens on RW1 and RW2 from 1 dph up to 55 dph (vectors show directions of the body shape change along the axis of the ordination plot).

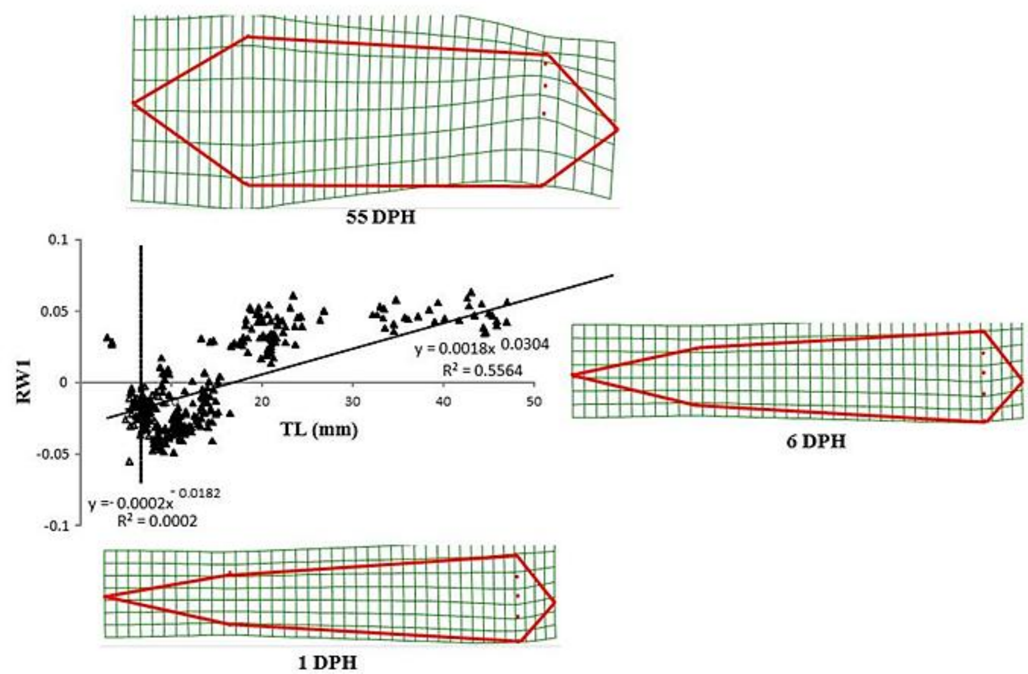

Fig.4. Growth trajectory from hatching up to 55 dph in *Cyprinus carpio* var. Sazan..

the head length and depth especially in orobranchial area (Fig. 3).

The regression model account 75.81% of the external shape, and the Goodal F test (P<0.0001) showed a good relationship between body shape variable i.e. RW1 and TL during early developmental stages. The RW1-scores relatively correlated to the total length (P<0.0001) (Fig. 4). The inflection point

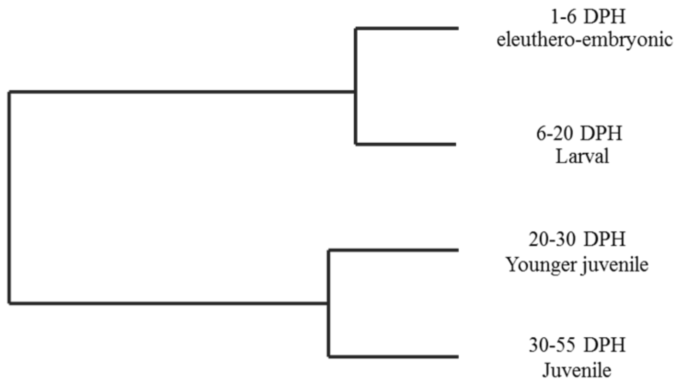

Fig.5. Dendrogram of the cluster analysis performed on nine landmark configurations of the consensus shape of each sampling group in *Cyprinus carpio* var. Sazan.

corresponds to an age of 6 dph (5.54mm TL) (Fig. 4). Based on the inflection point, the body shape change of *C. carpio* var. Sazan during early developmental stages can be divided into two phases, including (1) pre-inflection body shape change showing relative increasing of the head depth, and (2) post-inflection body shape change that shows increasing of the body depth (head, trunk and tail), and the head length, especially in orobranchial region (Fig. 4).

The cluster analysis discriminates four groups (Figs. 2 and 5), which are recognized and named as following based on their important morphological characteristics; (1) eleuthero-embryonic phase consists of specimens with yolk sac, first observation of the pectoral fin formation and pigmented eyes, (2) larva or propterygiolarval phase characterized by lacking yolk sac and formation of the caudal, pelvic and dorsal fin rays, (3) younger juvenile or pterygiolarval phase characterized by the complete formation of the fin rays, and (4) juvenile phase referred to specimens with miniature form of the adults.

Discussion

At the hatching, the majority of structures and functional systems of *C. carpio* var. Sazan, including mouth, gills, fins and scales had not yet fully differentiated. Development of the functional systems in the recently hatched larvae are occurred in a relatively short period, suggesting that growth functionally optimizes the survival as a common feature among teleost fish (Gisbert 1999). The growth trajectories of *C. carpio* var. Sazan obtained by GM method showed a very intensive body shape changes during early ontogeny similar to many teleost fishes (Osse & van den Boogaart 1995; Klingenberg 1996; Loy et al. 1998).

Based on the results, early development of common carp was divided into four phases, including (1) eleuthero-embryonic, (2) larva, (3) younger juvenile and (4) juvenile. During the first phase, the body shape was elongated and many structures of the feeding apparatus such as mouth and anus were observed. The most important change of eleuthero-embryonic stage was the absorption of the yolk sac that gradually happened up to the end of this phase. The increasing of the head depth during this stage i.e. 1-8 dph can be resulted a larger orobranchial cavity to start exogenous feeding. Along with the absorption of yolk sac, the larvae must switch to exogenous feeding, and thus they need a functional food intake apparatus (van Snik et al. 1997). In addition, the head growth is a common feature during early development of teleost fishes associating with ontogeny of the brain, sensing, feeding and respiratory organs (Fuiman 1983; Osse & van der Boogart,1995; van Snik et al. 1997; Osse et al. 1997; Gisbert 1999; Loy et al. 2001; Gisbert & Doroshov 2006).

During two next phases i.e. larvae and younger juvenile stages, increasing of the body depth (head, trunk and tail), and the head and tail lengths were the most changes that were coincided with the complete formation of the fins at the end of younger juvenile stage. Increasing body depth can be associated with development of internal organs (Pena & Dumas 2009) and a rapid turning and maneuvering ability (van Snik et al. 1997; Ontario 2011). In nature, common carp prefers slow flowing rivers and stagnant water bodies such as lake (e.g. the Caspian Sea) that this deeper body shape is in accordance with its functional requirement. Also, the growth of head particularly orobranchial region and tail (length and depth) in these two stages can reflect their priority that are related to vital functions such as feeding, improving swimming ability and predator avoidance (Houde & Zastrow 1993; Osse & van den Boogaart 2004).

In conclusion, the present study showed the importance of morphological modifications occurred in *C. carpio* var. Sazan during early life stages, showing that these changes are in agreements with functional demands throughout ontogeny and its adaptation to adult life style.

Acknowledgments

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تغییرات شکل بدن کپور معمولی واریته سازان (*Cyprinus carpio* var. Sazan) (Cyprinidae) در طی مراحل اولیه تکوین با استفاده از روش ریخت‌سنجی هندسی

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چکیده: این تحقیق با هدف مطالعه تغییرات شکل بدن ماهی کپور معمولی واریته سازان (*Cyprinus carpio* var. Sazan)، در طی مراحل اولیه تکوین با استفاده از روش ریخت‌سنجی هندسی لندمارک پایه به اجرا درآمد. برای این منظور، در مجموع تعداد ۲۱۰ لارو از زمان تفریخ تا ۵۵ روز بعد از آن نمونه‌برداری شد. برای استخراج داده‌های شکل بدن از سمت راست نمونه‌ها عکس‌برداری شد و در مجموع تعداد ۹ لندمارک هم‌ساخت تعریف و با استفاده از نرم‌افزار TpsDig2 بر روی تصاویر دوبعدی رقومی شدند. داده‌های لندمارک پس از روی هم‌گذاری براساس آنالیز پروکراست با استفاده از تحلیل Relative Warp، رگرسیون شکل نسبت به طول کل و آنالیز خوشه‌ای مورد تحلیل قرار گرفتند. نتایج نشان داد که روند تغییر شکل بدن در کپور معمولی در طی مراحل اولیه تکوین شامل (۱) افزایش عمق سر و طول تنه از زمان تفریخ تا روز ۸ بعد از تخم‌گشایی، (۲) افزایش عمق بدن و طول سر و دم از روز ۸ تا ۲۰ بعد از تخم‌گشایی و (۳) افزایش طول و عمق سر از روز ۲۰ تا ۵۵ بعد از تخم‌گشایی بود. براساس آنالیز خوشه‌ای، مراحل لاروی این گونه را می‌تواند به چهار مرحله شامل Eleuthero-embryonic، Larva، Younger juvenile و Juvenile تقسیم کرد. براساس یافته-های بدست آمده، تغییرات شکل بدن در این گونه در طول مراحل اولیه مطابق با اولویت‌های حیاتی آن است.

کلمات‌کلیدی: ریخت‌سنجی، فردزایی، وارپ نسبی، آنالیز خوشه‌ای، لارو.