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Effects of rearing temperature on the body shape of swordtail (*Xiphophorus hellerii*) during the early development using geometric morphometrics

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Abstract. Temperature, as an environmental factor, plays an important role in life of fishes. This study was conducted to investigate effects of temperature on body shape of swordtail (*Xiphophorus hellerii*) during the early development using geometric morphometrics. A total of 60 newly-born fries were reared in two temperature treatments (17 and 26°C) for two months. The left side of the specimens were then photographed, 16 landmarks were defined and digitized using the software tpsDig2 to extract the body shape data. The extracted data were superimposed using generalized procreates analysis, analyzed using the discriminant function analysis and Hoteling's T2. There was a significant difference between body shape of the specimens exposed to the temperature treatments with those experienced higher temperature had a deeper head at the level of the operculum, deeper body depth and caudal peduncle, and shorter tail length. The results indicated that temperature is an important environmental parameter impacting the body shape of green swordtail during the early developmental stage.
Key Words: morphology, phenotype plasticity, ontogeny, geometric morphometrics.

Introduction. The environmental parameters may affect fish performance and survival directly or indirectly via environmentally induced phenotypic variation (Hare & Cowen 1997; Fuiman & Batty 1997; Brander et al 2001; McCormick 2003; Eagderi et al 2013, 2014). Phenotypic plasticity is an important mechanism of phenotypic adaptation and defined as the ability of a single genotype to produce more than one alternative phenotype in response to environmental conditions (West-Eberhard 1989; Schlichting & Pigliucci 1998; Robinson & Parsons 2002; Relyea & Hoverman 2003). In fishes, the environmentally induced phenotypic plasticity have been proved during the early developmental stages (Pechenic et al 1998; Relyea & Hoverman 2003).

Water temperature affects the phenotype of fish species and thermally induced phenotypic plasticity can be stated as modifications of the relative timing of development of different organs during ontogeny (Stickland et al 1988; Fuiman & Batty 1997; Koumoundouros et al 2001; Johnston & Temple 2002; Campinho et al 2004; Sfakianakis et al 2004; Jordaan et al 2006), or changes of features like gender (Pavlidis et al 2000; Koumoundouros et al 2002; Piferrer et al 2005) and morphometric and meristic counts (Lindsey 1988). With the exception of studies in gender and meristic characters, most studies on phenotypic plasticity in fishes are focused on developmental stages.

The poecilids are the popular ornamental fishes across the world. The genus *Xiphophorus* with 25 species (Nelson 2006) is native to areas of Belize, Guatemala, Honduras and Mexico. The green swordtail, *Xiphophorus hellerii* Heckel, 1848 is a small viviparous species and one of the most popular aquarium fish species being introduced to at least 33 countries (Sterba 1989; Tamaru et al 2001; Webb et al 2007). This species tolerates a wide range of temperature, salinity and dissolved oxygen, consuming a wide range of food items that are available in its habitat (Goodwin 2003). This exotic species has been reported in two natural water bodies of Iran i.e. Persis and Namak Lake basins of Iran (Esmaeili et al 2010; Mousavi-Sabet & Eagderi 2014).

This study was conducted to investigate whether water temperature experienced by *X. hellerii* during the early larval stage influences its phenotype, namely, body shape. Hence, effects of water temperature during the early life stage of *X. hellerii* on phenotype were investigated. At the present study, this species was exposed to temperatures lower and higher than its threshold tolerance, i.e. 17°C and 26°C, then the phenotypic responses of such exposure were studied before the onset of sexual dimorphism using geometric morphometric techniques.

Material and Method. A total of 60 one-month old juveniles of *X. hellerii* were obtained from a local ornamental fish farm in September 2013 and transferred to two 100 L rearing fiberglass tanks at the fisheries laboratory of University of Tehran (Karaj, Iran). During rearing period, the animals were fed by a mixture of the *Artemia* nauplii and a commercial food pellet (Biomar, Denmark; 58% protein, 15% lipid) at 5% of their body weight twice a day. Throughout this period, water temperature, dissolved oxygen and pH were 24-26°C, 7.5±0.6 mg L^{-1} and 7.2±0.1, respectively.

After three months, a total of 20 ripe females were randomly sampled and transferred to a 100-L breeding glass aquarium. After giving birth, a total of 60 newly born fish were collected and exposed to two temperature treatments for two months, which were lower and higher than the threshold tolerance of *X. hellerii*, i.e. 17°C and 26°C. Three 30-L glass aquariums were devoted for each temperature treatment equipped to continuous aeration having 30 fish (each had 10). The natural photoperiod was applied for both treatments. During the rearing period, water temperature, dissolved oxygen and pH were similar to those mentioned above and fries were fed by *Artemia* nauplii and micro-worms (*Panagrellus redivivus*). Later, a mixture of the *Artemia* nauplii and Biomar commercial food pellet were also added to their diet.

At the end of experiment, fish were collected using a scoop net and anesthetized using 1% clove oil. The left side of fish was photographed using a stereomicroscope equipped to a digital Cannon camera with a 5 megapixel resolution. Then, the larvae were preserved in 5% buffered formalin and stored in 70% ethanol after 24 hours for further examinations. The visceral contents of the specimens were examined under a stereomicroscope to find their sex that was not detectable 60 days after birth. To extract body shape data, 16 landmark-points were digitized using tpsDig2 software (version 2.16). The landmark-points were selected at specific points where a proper model of fish body shape could be inferred (Figure 1).

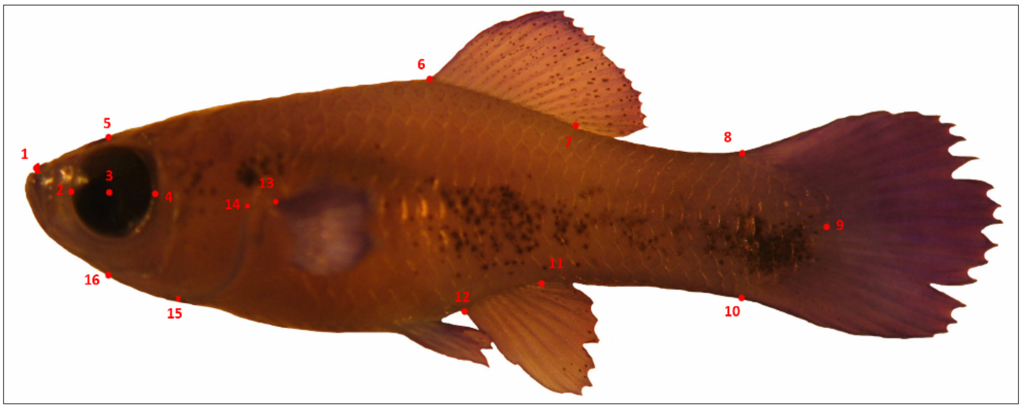

Figure 1. Defined landmark points to extract the body shape data of *Xiphophorus hellerii*. (1) anterior-most point of the snout tip on the upper jaw, (2) anterior margin of the eye, (3) center of the eye, (4) posterior margin of the eye, (5) dorsal edge of the head perpendicular to the center of the eye, (6) anterior and (7) posterior end of the dorsal fin base, (8) posterodorsal end of caudal peduncle at its connection to caudal fin, (9) posterior end of caudal peduncle, (10) posteroventral end of caudal peduncle at its connection to caudal fin, (11) posterior and (12) anterior ends of the anal fin base, (13) dorsal origin of pectoral fin, (14) posterior edge of operculum, (15) ventral end of the gill slit, and (16) ventral edge of the head perpendicular to the center of eye.

The digitization error was estimated according to Adriaens (2015). The estimated error was about 6.8% based on a sub-sample, which was low enough to be ignored. The landmark data was subjected to a generalized procrustes analysis (GPA) to remove non-shape data including scale, direction and position.

The landmark data were superimposed using GPA and were then analyzed using Discriminate Functional Analysis (DFA) and Hotelling's T2 to examine a significant difference between the temperatures treatments. All multivariate analyses were performed using the software PAST (Hammer et al 2001). The consensus configurations of two populations were visualized using the wireframe graphs in MorphoJ (version 1.01) (Klingenberg 2011) to compare their shape difference.

Results and Discussion. Both DFA and Hoteling's T2 indicated that there was a significant difference in body shape of the two treatments ($p < 0.0001$, Figure 2). Comparison of the body shape of the two temperature groups using wireframe diagram showed that they differ in the position of the snout (mouth), head depth at the level of the operculum, the position of the eye and pectoral fin, depth of the caudal peduncle and tail length (Figure 3). The results showed a dorsal position of the mouth and eye, deeper head at the level of the operculum, deeper body depth and caudal peduncle, and shorter tail length in those exposed to the higher temperature.

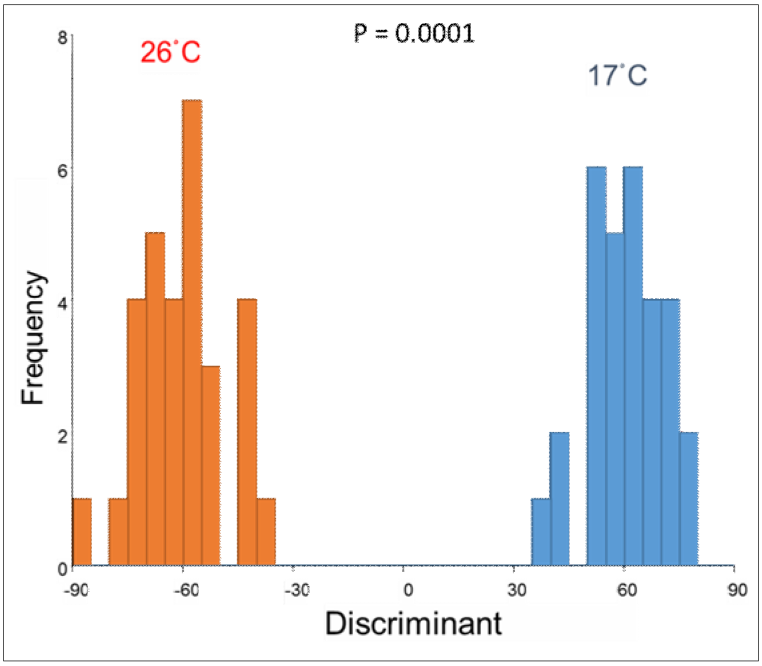

Figure 2. The discriminant function analysis of *Xiphophorus hellerii* exposed to two temperature treatments during the early development.

The present study showed that there was a significant difference in the body shape of *X. hellerii* specimens exposed to different temperatures. A difference in the body shape not only reflects genetic characteristics of populations but also environmental parameters (Guill et al 2003). Morphological changes induced by environmental factors may help better understanding of the phenotypic plasticity process as result of induced factors (Mohaddasi et al 2013; Jalili et al 2015). Various environmental factors affect biological features such as morphological characters of fishes (Peres-Neto & Magnan 2004). They create new ecological and evolutionary challenge for fishes (Baxter 1977) causing some variations in their body shape because they have to respond to new environmental condition to survive and decrease the adverse effect of resulted pressures (Fuiman & Batty 1997). The water temperature is one of the important abiotic factors influencing fishes (Georgakopoulou et al 2007; Sfakianakis et al 2011). Sfakianakis et al (2011) studied the effect of four different water temperature (22, 25, 28, and 31°C) on phenotype of *Danio rerio* during the early developmental stages and showed that their morphometric and meristic characters were influenced by temperature. Therefore, temperature as an important abiotic factor, may influence fish growth and metabolism through alternation of the physical parameters of the water and dissolved oxygen (Wimberger 1992).

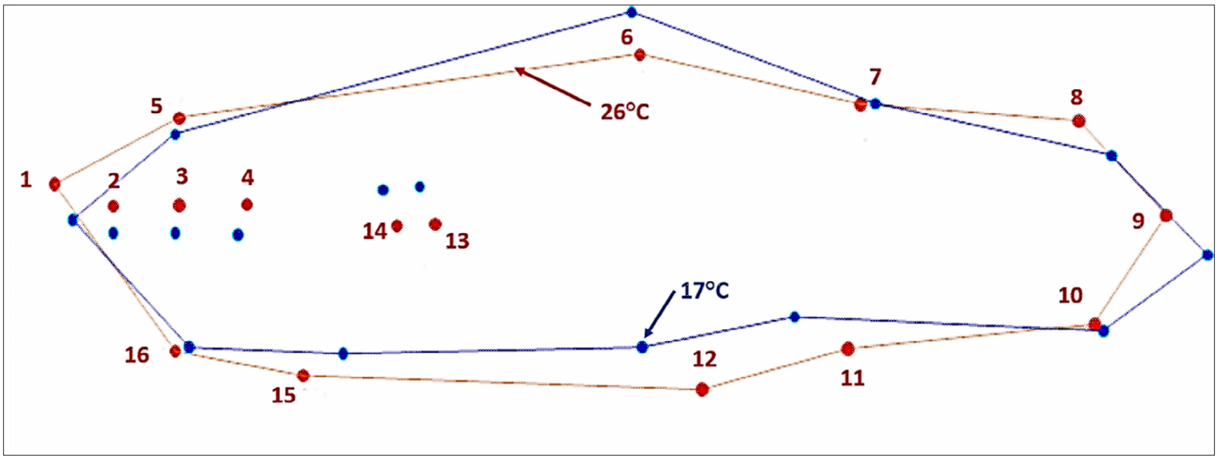

Figure 3. Wireframe graph showing the consensus body shape differences of *Xiphophorus hellerii* exposed to two temperature treatments during early development.

Our results showed that *X. hellerii* has deeper head and body shape in higher water temperatures. The results also showed that lower temperatures lead to a pointy head, longer tail and a dorsal position of the pectoral fin forming a fusiform body shape. Georgakopoulou et al (2007) studied the effect of the temperature on the body shape of *Dicentrarchus labrax* during the early developmental stages and showed that the fish were more fusiform in lower temperatures. Decreasing water temperature increases water viscosity and density and, thus, changes of body shape towards more fusiform figure to decrease the dragging cost of swimming in cold water (Wimberger 1992). Hence, physicochemical features of aquatic ecosystems change with environmental factors especially water temperature and, therefore, fish respond to new variation using the body shape (Sfakianakis 2011).

A deeper head at the level of the operculum and a dorsal position of the mouth were observed in the specimens exposed to higher temperatures. A high water temperature decreases dissolved oxygen and increases the oxygen demand in fishes (Mortimer 1971). Therefore, fishes adapt to these changes through developing larger gill cavity and the upper position of the mouth. Since, a larger gill cavity and an upper mouth may help to effectively use of the surface water that contains higher dissolved oxygen (Kramer & McClure 1982).

Conclusions. Phenotypic plasticity is an important mechanism of phenotypic adaptation in response to environmental conditions. The results of the present study showed that temperature play a vital role during the early development of green swordtail by alternation of its body shape to provide its biological requirements to survive and decrease the adverse effect of resulted pressures.

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