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Original Article

Effect of turbidity on fish morphology: A case study of swordtail, *Xiphophurus helleri*, during early ontogeny

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Abstract: This work is aimed to study the effect of turbidity on the swordtail's body shape during its early developmental stages. For this purpose, two treatments were designed as turbid and clear water, each with three replicates for 60 days. The body shape data was extracted by digitizing 16 landmark points on 2D pictures to analysis using the geometric morphometric technique. The results showed a significant difference between the two treatments in terms of body shape. Fish under turbid conditions had shorter eye diameters, lower and shorter heads, more dorsal position snout, and deeper caudal peduncles. The priorities in the new environment i.e. turbid water needs to be changed to decrease the adverse effect of the resulting pressures of this environment and increase the survival rate leading to increasing the ability of the developing fish to occupy a wider range of habitats.

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Introduction

Phenotypic plasticity induced by environmental factors has been proved in fishes, particularly during their early development (Pechenic et al., 1998; Relyea and Hoverman, 2003; Jalili et al., 2015; Poorbagher et al., 2017; Moshayedi et al., 2017; Eagderi et al., 2015, 2017). Among environmental factors, turbidity is one of the important factors, formed by various factors such as natural events (heavy rainfall, eutrophication, etc.) or anthropogenic changes (agriculture, deforestation, etc.) (Utne-Palm, 2002; Wolanski et al., 2004; IPCC, 2013). It can affect fishes by reducing the penetration of light and decreasing the visual range of sighted organisms (Vogel and Beauchamp, 1999) which in turn may affect foraging, predator response, and mate selection (Vogel and Beauchamp, 1999; Gregory, 1993; Seehausen et al., 1997).

In this study, we investigated the effect of turbidity on the body shape of the swordtail, *Xiphophorus helleri*, during early development using the geometric morphometric technique. This method is a proper developmental biology tool to extract and analyze the shape data by multivariate statistics, showing how biological structures are generated and changed (Zelditch et al., 2004; Mouludi Saleh et al., 2019). The swordtail is a viviparous ornamental fish species of the Poeciliidae, which inhabits fresh and brackish waters of North and Central America stretching from Veracruz, Mexico, to northwestern Honduras (Dawes, 1991; Jacobs, 1969; Nelson et al., 2016). We expect alternation of the body shape in this species to adapt to a new environment, but types of morphological changes can help us to a better understanding of fish response to overcome such effects due to turbidity.

Materials and Methods

Sixty-one-month-old swordtails were purchased from an ornamental fish farm and reared in two one-hundred-liter tanks in the laboratory. During this period, fish were fed by a mixture of *Artemia* nauplii and a commercial food pellet based on Eagderi et al. (2015). During rearing, the water temperature was 24-26°C, DO = 7.5±0.6 mg L^{-1}, and pH = 7.2±0.1. After 90 days, twenty ready females were transferred to a one-hundred liters' breeding aquarium. Sixty newly born fish were collected and transferred into treatment

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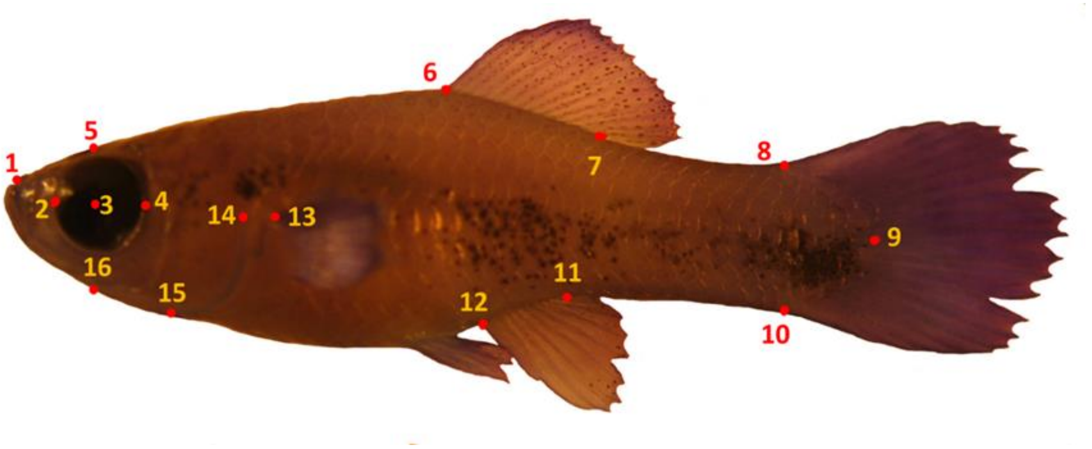

Figure 1. Digitize landmarks on the body of *Xiphophorus hellerii*. (1) the anterior-most point of the snout tip on the upper jaw, (2) the eye in the anterior margin, (3) the center, (4) and posterior end, (5) The head in the dorsal edge perpendicular to the eye center, (6) anterior and (7) posterior end of the dorsal fin base, (8) posterior dorsal end of the caudal peduncle, (9) posterior end of the caudal peduncle, (10) posterior ventral end of the caudal peduncle, (11) posterior and (12) anterior ends of the anal fin base, (13) dorsal origin of the pectoral fin, (14) posterior edge of the opercle, (15) ventral end of the gill slit, and (16) ventral edge of the head perpendicular to the center of the eye (Eagderi et al., 2015).

glass aquaria. Two treatments viz. turbid and clear water, each with three replicates (each aquarium containing 10 specimens) kept for 60 days. For turbid treatment, 88 gr clay powder was added to turbid aquaria; then the turbid condition was verified using a Secchi disk i.e. it was invisible at 30 cm depth. Furthermore, a water pump was installed at the bottom of the aquarium to suspend clay constantly. The applied photoperiod for both treatments was natural, and water parameters viz. temperature, DO and pH were the same as the broodstock rearing period. Fry fed *Artemia* nauplii and micro-worms (*Panagrellus redivivus*) during the rearing period of up to 4 weeks, and later, they were supplemented with a mixture of the *Artemia* nauplii and commercial feed (Eagderi et al., 2015).

The reared fish of treatments were collected by scoop net and anesthetized using 1% clove oil after a 60-day experimental period. The left side of the fresh fish was photographed by a stereomicroscope equipped with a digital camera (Cannon 5 MP), and then they were fixed in 5% buffered formalin for further examinations after anesthesia using MS222. In addition, their visceral contents were examined to distinguish their sex. The examined specimens' maturity was not detectable until 60 days after birth. A total of sixteen landmark points were selected and digitized using tpsDig2 software (version 2.16) on two-dimensional pictures to extract their body shape data. The selected landmark points were those to extract a proper fish body shape (Fig. 1).

The extracted data were analyzed using Generalized Procrustes Analysis (GPA) to eliminate non-shape data, viz. scale, direction, and position, and then analyzed using multivariate techniques of Discriminate Functional Analysis and Hoteling's T-test to examine a significant difference between the body shapes between treatments. The statistical analyses were done in PAST software (Hammer et al., 2001). The consensus configurations of the two studied treatments were visualized in MorphoJ software (version 1.01) (Klingenberg, 2011).

Results and Discussions

The results of DFA displayed the separation of two groups (Fig. 2), and Hoteling's T-test showed a significant difference in terms of the body shape between the two treatments (P<0.0001). Based on the wireframe graph (Fig. 3), those specimens of the turbid treatment had shorter eye diameters, lower and shorter heads, a more dorsal position of the snout, and a deeper caudal peduncle. As seen in Figure 3, most landmark point displacements i.e. morphological changes, have occurred in the head region (landmark-points of 1, 5, 15, and 16). Changes in head dimensions can be associated with sensory organs such as vision (Fuiman, 1983; Koumoundouros et al., 1999; Arnold, 1974). Moshayedi et al. (2015) reported

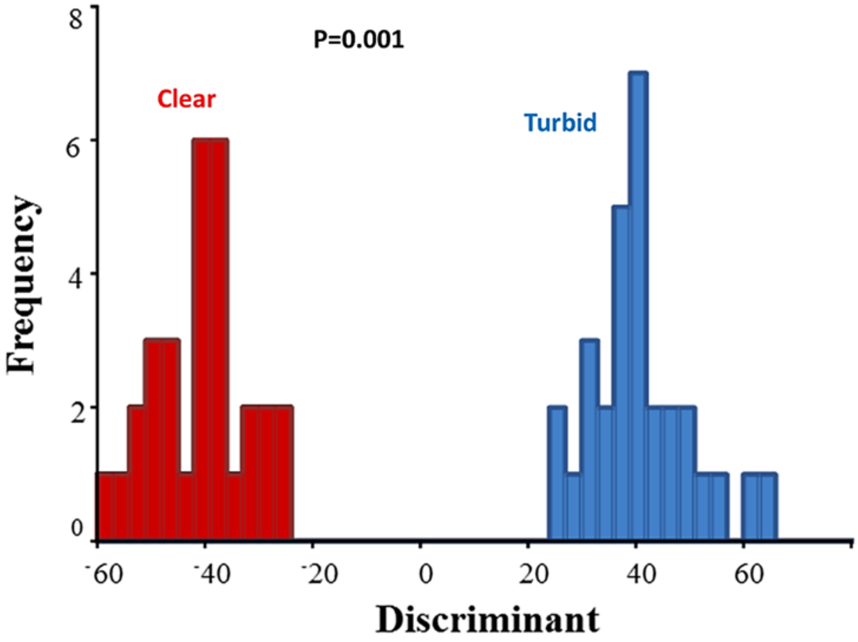

Figure 2. The discriminant function analysis of *Xiphophorus hellerii* exposed to two turbidity groups during the early development.

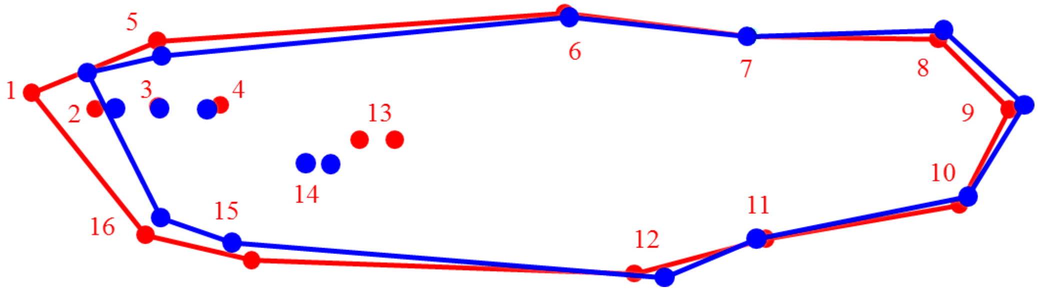

Figure 3. The consensus body shape differences of *Xiphophorus hellerii* treated to clear (red line) and turbid (blue line) treatments during early development based on Wireframe graph.

a positive allometric growth pattern in the head of *X. helleri*, showing its importance to survive during early development, but in those in the turbid environment, the priority of the head growth has decreased, and therefore, the head growth was relatively slower than normal conditions i.e. in the clear water. The differences in the growth of the body parts with those of normal conditions during early developmental stages are related to various development priorities of essential organs, which are thought to create the best conditions for survival (Osse et al., 1997; Moshayedi et al., 2016; Saemi-Komsari et al., 2018). In addition, the reduced eye diameter occurred in the turbid treatment fish and it can be an adaptation to protect from damaging eyes by suspended particles (Nikolsky, 1991; Wootton, 1999).

A dorsal position of the snout in *X. hellerii* in turbid water can be an adaptive strategy to feed on surface water, where light is proper to detect prey. In turbid water, little penetration of the light is caused, reducing the visual range of the sighted organisms (Gregory, 1993; Seehausen et al., 1997; Vogel and Beauchamp, 1999); hence the surface water in specimens such as *X. hellerii* with upper mouth position can be a proper strategy for finding food. Deeper anterior and posterior sections of the body parts associated with locomotor and swimming capability enable fish to swim properly (Fuiman, 1983). A deeper caudal peduncle occurred during the early life stage of *X. hellerii* can be agreed with its survival priority. However, this priority in the new environmental condition is more important than the normal condition that had a positive allometric pattern (Moshayedi et al., 2015), due to limiting visual range to avoid predators and faster swimming (Fuiman, 1983).

In this study, turbidity had a significant effect on the body shape of the swordtail during early life. Alternating the body shape due to adaptation to new environmental conditions can decrease the adverse effect of the resulting pressures and increase the survival rate (Fuiman and Batty, 1997). The results of our findings showed types of morphological changes as decreasing head and increasing tail growth patterns during the early development of swordtail as a response to overcoming the adverse effects of turbidity. During early development, fish needs to invest its little energy budget in those organs to improve behavioral and physiological capabilities (Peña and Dumas, 2009), which contributes to the increasing ability of the developing fish to occupy a wider range of habitats, which in turn influences their distribution, recruitment, and survival (Gisbert, 1999).

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