Effect of salinity on the body shape of sword tail, *Xiphophurus helleri*, during early developmental stage

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Received: March 2018 Accepted: June 2018

Abstract

This study aimed to investigate the effect of an isosmotic point of salinity i.e. 12 ppt on the body shape of sword tail, *Xiphophurus helleri*, during the early development using geometric morphometric approach. In total sixty newly hatched larvae reared in two salinity treatments (0.5 and 12 ppt) for a period of two months. Then the left side of the specimens were photographed and sixteen landmark-points defined and digitized on 2D picture using tpsDig2 software to extract body shape data. The extracted data after GPA analysis, were analyzed using DFA/T-test Hoteling. The results revealed significant different between body shape of two treatments showing shorter snout, head and caudal peduncle, lower head and body depth, anterior position of gill and longer dorsal fin in the group exposed to higher salinity. The results confirmed that salinity as important environmental parameter impacts and regulate the body shape of swordtail during early ontogenic development.

Keywords: Morphology, Phenotype plasticity, Geometric Morphometrics.

[DOI: 10.18331/SFS2019.5.2.2]

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[DOI: 10.18331/SFS2019.5.2.2]

Introduction

The environmental factors affect fish performance and survival rate via their direct action, or indirectly via environmentally induced phenotypic variation (Brander *et al*., 2001; McCormick 2003, Eagderi *et al*., 2015). Phenotypic plasticity plays an important role in phenotypic adaptation of organisms and defined as the ability of a single genotype to produce more than one alternative phenotype in response to environmental conditions (Robinson and Parsons 2002; Relyea and Hoverman, 2003). In fishes, the environmentally induced phenotypic plasticity have been proved during the early developmental stages (Pechenic *et al*., 1998; Relyea and Hoverman, 2003). Salinity is one of important environmental factors and every fish species have a specific range of tolerance (Boyed, 1982). Teleost fish are able to maintain the ionic and osmotic homeostasis of their body fluids across environmental salinities by using osmoregulatory mechanisms, which are energy demanding processes (Sampaio and Bianchini, 2002). Hence understanding the effect of different salinity condition is crucial in aquaculture that seeks to provide an ideal and healthy environment to maximize economic gain. There is little studies regarding the effect of salinity on phenotypic plasticity of fishes especially during developmental stages of live-breeder species e.g. poecilid family.

Among the ornamental fishes across the world, the member of poecilid family are very popular. The poeciliid genus *Xiphophorus* with 25 species (Nelson, 2006) is native to areas of Belize, Guatemala, Honduras and Mexico. The swordtail, *Xiphophorus hellerii* Heckel, 1848 is a small viviparous species and one of the most popular aquarium fish species and has been introduced into at least 33 countries (Sterba, 1989; Tamaru *et al*., 2001; Webb *et al*., 2007). This species can tolerance a wide range of temperature, salinity and dissolved oxygen, and can consume any food that is available in its habitat (Goodwin, 2003).

In light of above mentioned background, this study was conducted to investigate the effect of salinity on body shape of *X. helleri* during early life stage. For this purpose, this species was exposed to the 12 ppt as isosmotic point in teleosts (Herrera *et al*., 2009; Nordlie, 2009) and compared its phenotypic consequences in terms of body shape with those reared in freshwater i.e. 0.5 ppt before appearing its sexual dimorphism using geometric morphometric technique.

Materials and methods

A total of twenty ripe females along with 10 adult males were obtained from a local ornamental fish farm in September 2013 and transferred to 100 L breeding aquarium. After giving birth, a total of 60 newly born fish were collected and exposed to two salinity treatments of 0.5 and 12 ppt for two months experimental period. For this purpose, 60 collected fry were divided randomly into six 30-L aquariums with continuous aeration at a stocking density of 10 fish per aquarium (0.5 and 12 ppt treatments each with three replicates). The natural photoperiod was applied for both treatments. During the rearing period, water temperature, dissolved oxygen and pH were 24-26°C, 7.5±0.6 and 7.2±0.1, respectively. Fry were fed using *Artemia* nauplii and micro-worms (*Panagrellus redivivus*) and along with their growth with a mixture of the *Artemia* nauplii and commercial food pellet (Biomar, Denmark; 58% protein, 15% lipid) at 5% of their body weight twice a day.

At the end of rearing period, fish were collected by a scoop net and anesthetized using 1% clove oil solution. The left side of fish were photographed by a stereomicroscope equipped with a digital Cannon camera with a 5 MP 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 fixed specimens were examined under a stereomicroscope to determine their sex that was not detectable at 60 day after birth in specimens of 0.5 ppt treatment, whereas few specimens of 12 ppt showed developed gonads that discarded from study; then all specimens were pooled and analyzed. To extract body shape data, 16 landmark-points were digitized using tpsDig2 software (version 2.16). The landmark-points were selected at the specific points, in which a proper model of fish body shape was extracted (Fig. 1). The digitization error was estimated according to Adriaens (2017). The obtained error based on a sub-sample was about 4.2% that is low enough to be ignored. The landmark data was submitted to a generalized procrustes analysis (GPA) to remove non-shape data including scale, direction and position.

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 eye, (3) center of eye, (4) posterior margin of eye, (5) dorsal edge of the head perpendicular to the center of 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 most 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.

[DOI: 10.18331/SFS2019.5.2.2]

The landmark data after GPA superimposing were analyzed using Discriminate Functional Analysis (DFA) and T-test hotelling (Hotelling's T-test) to investigate power of distinction among the treatments. All multivariate analysis were computed using PAST software (Hammer *et al*., 2001). The consensus configuration of two groups were visualized using wireframe graphs in MorphoJ (version 1.01) (Klingenberg, 2011) to compare the shape difference.

Results and discussion

The survival rate of 12 ppt treatment was about 22% that shows many fry could not adapt to higher salinity. Osmoregulatory mechanism is an energy demanding processes (Sampaio and Bianchini, 2002); therefore it is reasonable to expect many swordtail fry cannot survive during early developmental stage due to shortage of energy i.e. they consumed their energy for osmoregulation instead of growth (Boyed, 1982). However, the plasma isosmotic point in teleosts generally corresponds to the water salinity of 12 ppt (Herrera *et al*., 2009; Nordlie, 2009). In addition, optimal salinities for growth rate is depend on species and developmental stages (Morgan and Iwama, 1991). The results of the presents study revealed that swordtail cannot tolerate higher salinity even that of isosmotic point during early ontogeny. Hence, it is suggested that in a saline environment, different feeding regime should be provide to overcome this shortage of energy in swordtail fry.

DFA/T-test Hoteling analysis showed that the body shape of two treatments are significantly different ($p<0.0001$, and Fig. 2).

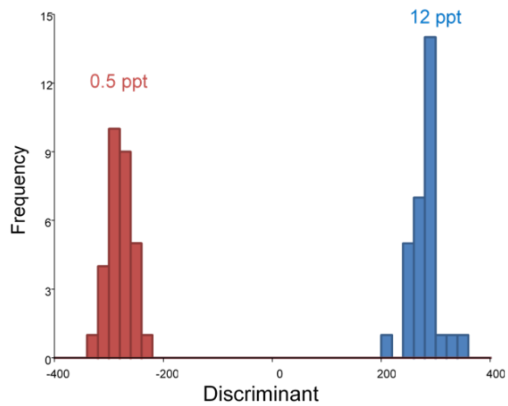

Figure 2: Graphs of the discriminant function analysis of *Xiphophorus hellerii* exposed to two salinity treatments during early development.

Comparison of the body shape of two groups using wireframe diagram revealed shorter snout, head and caudal peduncle, lower head and body depth, anterior position of gill and longer dorsal fin in the group exposed to higher salinity (Fig. 3).

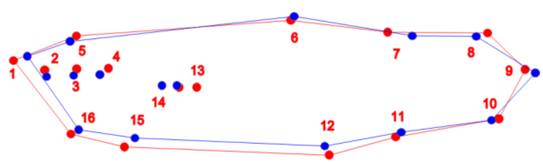

Figure 3: Wireframe graph showing the consensus body shape differences of *Xiphophorus hellerii* exposed to two salinity treatments during early development.

The body shape difference of swordtails exposed to two different salinities indicate the effect of the salinity on the phenotype of this species during early developmental stage. Body shape

[DOI: 10.18331/SFS2019.5.2.2]

differences not only reflect genetic characteristics of populations but also environmental parameters (Guill *et al*., 2003). Morphological changes induced by environmental factors can help to better understanding of the phenotypic plasticity process as result of induced factors (Mohadasi *et al*., 2013). Various environmental factors can effect on the biological features such as morphological characters of fishes (Peres-Neto and Magnan, 2004). They create new ecological and evolutionary challenge for fishes (Baxter, 1977); subsequently, this new conditions can cause some variations in their body shape, because they have to respond to this new environment to survive and decrease the adverse effect of resulted pressures (Fuiman and Batty, 1997) or increase their fitness for better exploitation of new environmental conditions (Chapman and Riddle, 2005).

The results showed that swordtail obtains shorter head and caudal peduncle, lower head depth and pointed snout forming a fusiform body shape in higher water salinity. Increasing water salinity can increases water viscosity and density and consequently, fish changes its body shape to more fusiform in order to decrease dragging cost for swimming in salty water (Wimberger, 1992) i.e. a fusiform body shape makes it easier to glide through the water. Hence, physicochemical features of the aquatic environment are effected by environmental factors and therefore, fish need to respond to this new condition by changing its body shape (Chapman and Riddle, 2005; Sfakianakis, 2011).

Shorter snout cause a dorsal position of mouth in fish exposed to higher salinity. The higher water salinity decrease dissolved oxygen (Mortimer, 1971). Therefore, this pelagic species probably obtains an adaptation as upper position of the mouth to compensate this problem. Since, upper mouth position can help to effectively use of the surface water that contains higher dissolved oxygen (Kramer and McClure, 1982).

As conclusion, the results of the present study showed that temperature play a vital role during 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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[DOI: 10.18331/SFS2019.5.2.2]