

Temperature-Induced Phenotypic Plasticity in *Aphanius arakensis* Teimori, Esmaeili, Gholami, Zarei, & Reichenbacher, 2012 (Teleostei: Aphaniidae)

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ABSTRACT

This study was conducted to examine the effects of different thermal conditions on the body shape of *Aphanius arakensis*, a eurythermal species in Iranian inland waters. The specimens were collected from Eshtehard Shoor River, Iran, transferred to the laboratory, and exposed to 22°C, 25°C, and 28°C for 2 months. After this period, the specimens were photographed and landmark points were digitized on the 2D pictures. The extracted coordinates of the landmark points were superimposed using the generalized procrustes analysis to remove the effects of size, rotation, and translation. Canonical variate analysis and Mahalanobis distance followed by permutation multivariate analysis of variance were used to discriminate the shapes of the specimens exposed to the three temperatures. The results indicated that the shape of *A. arakensis* was significantly affected by temperature in both males and females. The specimens exposed to 25°C and 28°C had similar shapes but dissimilar shapes compared to those exposed to 22°C. Those exposed to 22°C had shorter head and tail regions and upper position of eyes. Males and females showed similar changes to temperature variations.

Keywords: Morphometrics, phenotypic plasticity, environmental factors, tooth carp

INTRODUCTION

Phenotypic plasticity has been defined as the ability of a single genotype to produce more than one alternative morphological form in response to environmental conditions (1). This process allows organisms to adapt to environmental alternations by modification of morphological, physiological, and behavioral traits (2,3). Environmental parameters have been demonstrated to have crucial effects on the morphology of fishes (4-7); especially, temperature plays a critical function during the early development (8,9).

Temperature influences the rate of metabolism, which subsequently affects the growth and survival rates (10-12). Different fish species have various temperature requirements (13,14); increasing water temperature can increase fish growth rate, whereas in other species, this may have a negative effect (15). In addition, temperature alterations may change the morphology. The relationships between body shape and environmental temperature have been well-documented in fishes

(16-18). For example, Georgakopoulou et al. (18) studied the effects of temperature on the morphological characteristics of sea bass (*Dicentrarchus labrax*) during early developmental stages and found body shape differentiation due to temperature changes. Anatomical deformities also occur due to temperature alterations (19-21). The response of fish body to environmental parameters, particularly temperature, allows more efficient utilization of available resources, thereby improving fitness and performance.

Geometric morphometrics (GM) is a tool to study shape and size, offering powerful analytical and graphical mean for the quantification and visualization of morphological variations within and among organisms. GM analysis is performed using image processing techniques, which can be easily reanalyzed and is also inexpensive and fast (22). In landmark-based GM, landmarks are the selected points on the body by which the shape can be analyzed (23). Several studies have used GM methods in different biological fields (24,25).



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Aphanius is the only genus of Aphaniidae reported from Iran (26,27). This genus occurs in coastal (brackish) and landlocked (freshwater to saline) water bodies in the Mediterranean and Persian Gulf basins from the Iberian Peninsula as far eastward as Iran and Pakistan (28). The Arak tooth carp *Aphanius arakensis* Teimori, Esmaili, Gholami, Zarei, & Reichenbacher, 2012, is found in the endorheic Namak Lake basin of Iran with wide temperature changes (29). The wide distribution of this small-sized fish suggests its ability to adapt to different environmental conditions. This species can be easily kept in an aquarium, and it also tolerates wide ranges of temperature, which helps to understand its response to temperature changes (30). Hence, this study was conducted to assess the response of *A. arakensis* to different water temperatures in terms of body shape using visualization techniques (*i.e.*, GM) in both males and females.

MATERIALS AND METHODS

A total of 240 juvenile *A. arakensis* (120 males and 120 females) with an average length of 1.6 cm (± 0.4 [SD]) were collected in one station from Eshtehard Shoor River (Namak Lake basin, Alborz Province, Iran; 35°36'32.52"N, 50°48'36.08"E) in June 2011 using a hand net. During sampling, the salinity, temperature, and pH of water were 11-12 g.L⁻¹, 21.85°C \pm 0.22°C, and 7-8.5, respectively. The specimens were transported to the laboratory using a tank equipped with a battery-operated portable aerator and then kept in a 100L glass aquarium filled with dechlorinated tap water (mixed with marine salt to have a salinity of 12 g.L⁻¹) having constant aeration for 7 days to be acclimatized to the laboratory conditions. During the acclimation period, dissolved oxygen (DO), average water temperature, and pH were 8 mg.L⁻¹, 22°C \pm 0.4°C, and 7.27-8.24, respectively.

Each sex was studied separately due to the presence of sexual dimorphism. After the acclimation period, each sex of fish was randomly exposed to three temperature treatments (each with 10 specimens), *i.e.*, 22°C, 25°C, and 28°C, with three replicates in 20-L glass aquaria with semi-closed water recirculating systems for 2 months (pH: 7.4 \pm 0.2; 5% water exchange rate day⁻¹; salinity: 12 g.L⁻¹). The temperature range was selected based on the minimum and maximum temperature of the natural habitat of this species during summer. The water temperature was regulated using an electrical heater. During the experiment, the specimens were fed at 2% of their body weight twice a day with 3-mm pellets of commercially available feed (Biomar®) and *Artemia* nauplii. During the experiment, the DO and pH were 8.6 \pm 0.3 mg.L⁻¹ and 7.8 \pm 0.1, respectively. The fish were kept under natural photoperiod. The bottom of the tank was siphoned every day to remove uneaten feed and feces.

After 2 months of the experimental period, the specimens were anesthetized using clove powder solution (200 mg.L⁻¹), and their left side was photographed immediately using a Kodak digital camera (with 6.2 MP resolution) coupled to a copy stand using the same magnification and focal distance. The images were stored on the hard disk for later analysis. Then, the fish were preserved in 10% buffered formalin and stored in 70% ethanol after 48 h for further examinations.

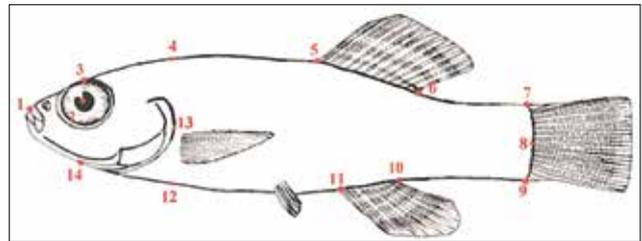


Figure 1. Defined landmark points to extract the body shape data. (1) anterior-most point of the snout tip on the upper jaw, (2) center of the eye, (3) dorsal edge of the head perpendicular to the center of the eye, (4) dorsal edge of the body perpendicular to the posterior edge of the operculum, (5)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

To extract data on shape using GM, 14 homologous landmark points were digitized using tpsDig2 software, version 2.16 (31). The landmark points were selected at specific points where a proper model of the fish body shape could be inferred (Figure 1). Correlations between the procrustes and tangent shape distances were calculated using the software tpsSmall, version 1.2, to examine whether the original data set correlates with the tangent distances to allow statistical analyses (32,33). The landmark points were submitted to a generalized procrustes analysis (GPA), which was used to remove non-shape data (including scale, direction, and position) in the MorphoJ software (34).

Statistical Analysis

Canonical variant analysis (CVA) was performed to investigate the power of distinction among the treatments by GM analysis. The p values of significant differences among the thermally treated groups were obtained using permutation tests (10,000 permutations) on the procrustes distances. Data on body shape of fish subjected to different treatments were analyzed using the MorphoJ and Past (version2 .10) softwares. The changes in body shape were illustrated in transformation grids depicting in relation to the consensus shape of fish subjected to all treatments in each sex.

RESULTS

A complete significant relationship was observed between the procrustes and tangent shape distances ($r^2=1$ and $h=0.9999$); therefore, the original data sets were used for statistical analyses. Differences were observed among the body shape of the specimens exposed to the three temperatures as well as between the sexes. The CVA discriminated three groups of male specimens exposed to the three temperatures, such that those exposed to 25°C and 28°C had almost similar shapes. The individuals exposed to 22°C showed a dif-

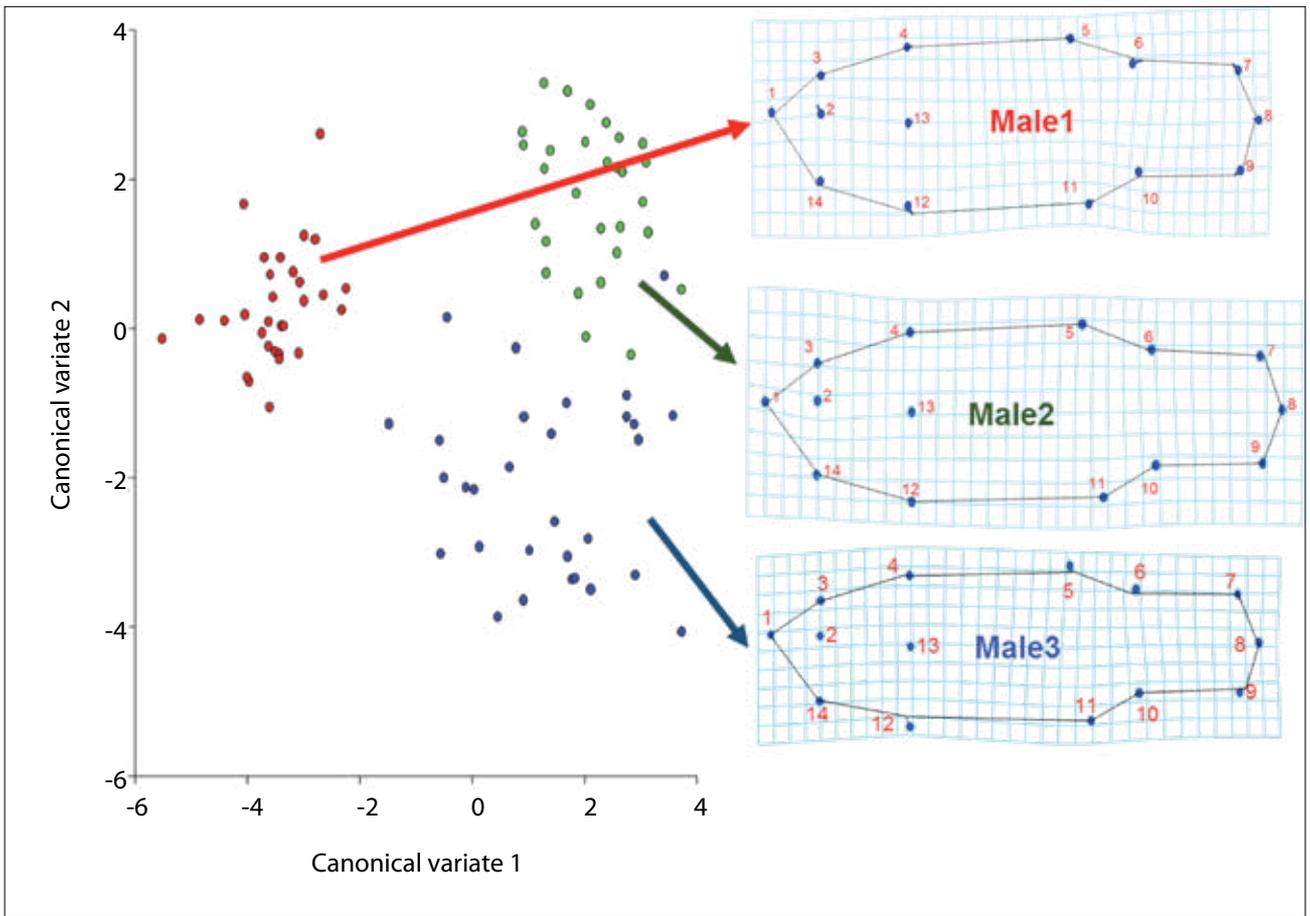


Figure 2. Scatterplot of the CVA for the morphometric characters in the three groups of males (male1 = 22°C, male2 = 28°C, and male3 = 25°C). Shape deformations associated with change in centroid size.

Table 1. Mahalanobis distances and permutation tests (10,000 permutations) for procrustes distances among thermally treated male groups. The p values are shown in parenthesis.

	Male1	Male2
Male2	5.8472 (<0.0001)	
Male3	5.388 (<0.0001)	3.9423 (=0.0016)

Table 2. Mahalanobis distances and permutation tests (10,000 permutations) for procrustes distances among thermally treated female groups. The p values are shown in parenthesis.

	Female1	Female2
Female2	7.3528 (<0.0001)	
Female3	7.6914 (<0.0001)	3.8131 (0.0055)

ferent shape pattern (Figure 2). The P values obtained from the permutation tests among the groups indicated significant differences among male specimens exposed to the three thermal treatments. Based on the Mahalanobis distances (Table 1), the greatest distance was found between those exposed to 22°C and 28°C.

The CVA indicated that the females exposed to 25°C and 28°C had almost similar shapes, whereas the body shape of

the specimens exposed to 22°C differed from those of others (Figure 3). Furthermore, the Mahalanobis distance confirmed the CVA results and classified the specimens of the three treatments into two groups (Table 2). The P values from the permutation tests among the groups showed significant differences among the thermally treated female group. The largest difference was found between those exposed to 22°C and 25°C.

In males, the body shape in the three groups of *A. arakensis* showed differences that were primarily related to the landmark positions on the caudal peduncle, dorsal fin base, and eye position (interpreted from changes in the forms of the deformation grid and shifting of landmark points), such that the specimens exposed to 25°C and 28°C were characterized by a slightly longer tail region. However, those exposed to 22°C had a deeper body at the posterior region of the head, a slightly shorter and deeper caudal peduncle, and an upper (dorsal) position of the eye (Figure 2). In females, those exposed to both 25°C and 28°C had longer caudal peduncles. In addition, those exposed to 28°C showed a longer head and anterior position of dorsal and anal fins compared with those exposed to other temperatures (Figure 3).

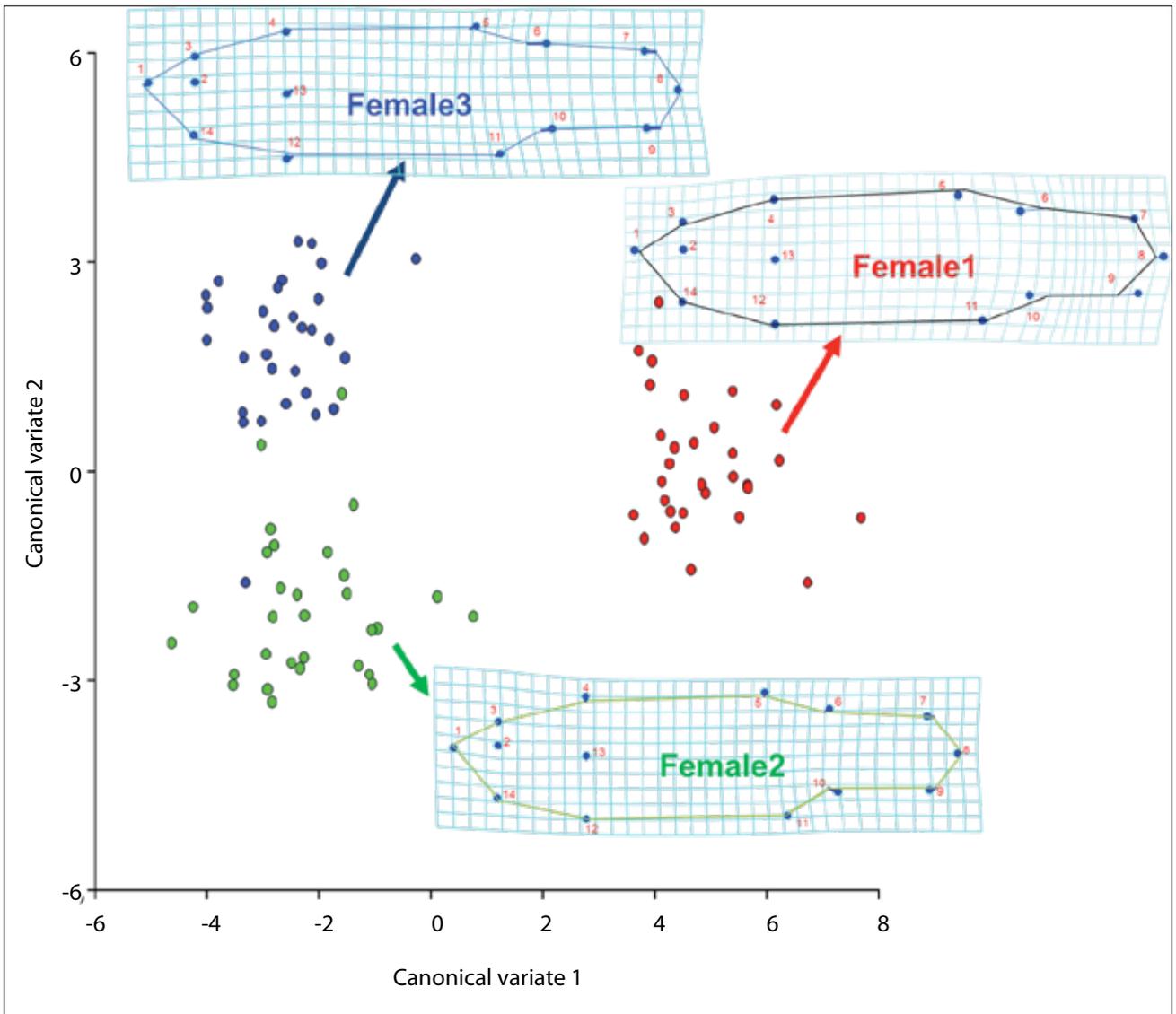


Figure 3. Scatterplot of the CVA for the morphometric characters in the three groups of females (female1 = 22°C, female2 = 28°C, and female3 = 25°C). Shape deformations associated with change in centroid size.

DISCUSSION

Most of the environmental parameters such as temperature, salinity, and food availability may influence the body shape of fishes (17). However, environmentally induced morphological variations are among the most widespread changes in fishes (35,36). Morphology is a useful tool to study the feeding ecology, the swimming mode, and even the habitat use (37-39). Several studies have assessed the body shape and the effects of external factors; however, only a few investigations have been conducted on the influences of temperature on fish morphology (40).

Our results indicated that the body shape of *A. arakensis* was significantly affected by temperature in both males and females, with a clear variation between the three thermally treated groups. Those exposed to 25°C and 28°C had almost similar body shape but different from those exposed to 22°C. Some

morphometric studies have provided evidence for the effects of temperature on fishes (8,17). For example, Sfakianakis et al. (8) used 22°C, 25°C, 28°C, and 31°C to study the effects of temperature on zebrafish (*Danio rerio*). They found significant variations among the specimens exposed to various temperatures. The changes were attributed to the general morphological plasticity in the different environments, being reflected in alterations of muscle and bone developmental patterns. Ayala et al. (41) studied the effects of temperature on muscle growth in two populations (Atlantic and Mediterranean) of sea bass, *D. labrax*. The temperatures were cultivation condition, 15°C, natural, 15°C/19°C, 17°C/natural, and 17°C/19°C. Their study showed that 17°C accelerated embryonic development (hatching), and following the hatching, a higher cultivation temperature (19°C) accelerated prelarval (mouth opening) and larval development (metamorphosis, scaling).

The results of the present study showed a longer head and caudal peduncle in the groups exposed to higher temperatures. Alterations in muscle and bone developmental patterns were the response of the specimens to various thermal conditions. As addressed by others (42,43), a deeper and longer caudal peduncle provides an adaptation for maneuverability and rapid acceleration. Several studies have stated that muscle development is extremely dependent on temperature (41,44). In our study, the possible explanations for the changes in the caudal peduncle structure could be better feeding conditions in those temperatures. A fusiform shape of the caudal peduncle in the fish exposed to 28°C decreases the energetic cost of swimming for longer periods (45). Moreover, higher average temperatures increase the fish activity because the rate of metabolism in warm waters is high, which may cause a shallower caudal peduncle.

Compared to the specimens exposed to 25°C and 28°C, those exposed to 22°C had eyes in the dorsal position of the head. Viscosity is a function of temperature and an influencing factor on the sinking rate of particles (8). In 25°C, a part of the food materials may not have been sunk and remained below the water surface. Therefore, the fish need their eyes in the dorsal part of the body to catch the food materials. Since we had no data on particle concentration and sinking rates, another experiment is required to scrutinize this speculation.

In conclusion, our study indicated that small changes in water temperature can have significant effects on the shape of *A. arakensis*. It is thus possible to use morphological characteristics to differentiate *A. arakensis* populations experiencing different environmental parameters. Meanwhile, our results showed that males and females showed similar changes to thermal variations. Further studies with additional factors to investigate the interaction of ambient parameters are suggested.

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