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Original Article

Allometric body shape changes and morphological differentiation of Shemaya, *Alburnus chalcoides* (Guldenstadf, 1772), populations in the southern part of Caspian Sea using Elliptic Fourier analysis

Mohammad Mohadasi[1], Soheil Eagderi*[2], Nader Shabanipour[1], Mahboube Sadat Hosseinzadeh[3], Hossein AnvariFar[4], Roozbahan Khaefi[5]

[1]Department of Biology, Faculty of Science, Guilan University, Rasht, Iran.
[2]Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran.
[3]Department of Biology, Faculty of Science, Ferdowsi University, Mashhad, Iran.
[4]Department of fisheries, University Applied Science and Technology, Gorgan, Iran.
[5]Department of Biology, Faculty of Science, Shiraz University, Shiraz, Iran.

Abstract: Study of phenotypic diversity among populations can help better understanding of diversification of species within ecosystems and intraspecific diversification in fishes. A geometric morphometric study was carried out using the Elliptic Fourier analysis to demonstrate the effect of habitat type on morphological features of shemaya (*Alburnus chalcoides*) populations. Populations were sampled from three rivers and one lagoon, from the southern part of Caspian Sea. Significant differences in body shape were found among the populations. Differences in shapes of the riverine populations were minute compared to those of lagoon one in terms of size and shape. Shemaya is an anaderemus fish and its populations have a common origin, therefore, observed differences could be as result of environmental factors. In addition, this study suggest that the amount of curvature i.e. fusiform body shape of this species could be independent form environmental condition.

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Introduction

The general body shape of an organism is determined not only by its genetics, but also by its ecology and environment (Sara et al., 1999). Different selective environmental pressures can generate and maintain different phenotypes. However, some geographical variations such as morphology, reproductive patterns, growth rates and mortality, are not always consistent with genetic variation and then can be related to phenotypic plasticity as a result of different environmental conditions (Orensanz et al., 1991; Cadrin, 2000).

Fish are among the most diverse aquatic organism in terms of morphology and ecology (Helfman et al., 2009) and their intra-specific diversification is well-documented (Robinson and Wilson, 1994; Jonsson and Jonsson, 2001; Robinson and Wilson, 1994). Fishes' morphological characters provide information about their ecological niches (Winemiller, 1991), allow more efficient use of available resources and improving fitness and performance (Pianka, 1994).

Morphological characters are essential for identifying discrete phenotypic stocks (Booke, 1999), and the historical development of stock identification methods has paralleled the advancement of morphometric techniques (Cadrin, 2000). Morphometric variation has been used as a method of stock identification for many fishery resources (Palmer et al., 2004; Cadrin and Friedland, 2005). Therewith, significant advances in morphometric analysis have occurred in the last two decades, offering more efficient and powerful techniques, such as image analysis (Cadrin and

* Corresponding author: Soheil Eagderi
E-mail address: soheil.eagderi@ut.ac.ir

Friedland, 1999) and geometric morphometrics methods (Rohlf and Marcus, 1993) for detecting differences among groups.

Traditional morphometrics apply a variety of lengths, widths, angles, and ratios to obtain information about shape, whereas geometric morphometric approaches focus on complete, uniform measurement of shape, retaining all geometric information throughout its analysis. Within this context, measurement of curves or outlines poses some challenges, since mathematically curves are infinite sets of points. In this way, fish outline data is collected automatically from images (Ruff et al., 1994; Russ, 1995) and analyzed using Elliptic Fourier analysis (EFA) (Kuhl and Giardina, 1982; Rohlf and Archie, 1984; Lestrel, 1997) to compute growth trajectories and visualizing shape changes.

Shemaya, *Alburnus chalcoides* (Guldenstadf, 1772), is widely distributed in the river systems of Black, Caspian and Aral Seas (Bogutskaya, 1997). This species is benthoplagic and anadromous and found in fresh and brackish water of the southern Caspian Sea basin (Slastenenko, 1959). Therefore, this study aimed to study allometric body shape changes and morphological differentiation of Shemaya populations in the southern part of Caspian Sea using Elliptic Fourier analysis.

Material and methods

Sampling: A total 120 individuals of the Shemaya from Lisar, Shiroud and Babolroud Rivers and Anzali Lagoon were sampled during April and May, 2008 (Table 1). The specimens were caught by handy net, cast net, and electrofishing and then fixed into 10% formalin solution. The specimens were photographed (Canon G12, 2,304×1,704 pixel dimensions) and digitization was performed to obtain the body shape data for Elliptic Fourier analysis (EFA) analysis. Outline data were automatically collected as 150 profile point coordinates by tpsDig2 software version 2.16 (Rohlf, 2004), excluding fins (Fig. 1).

Elliptical Fourier analysis: The applied method in this study was explained by Kuhl and Giardinia (1982) and were derived as a parametric formulation from conventional Fourier analysis, i.e. as a pair of equations that represent the variation in x and y coordinates as a function of a third variable t, along the body outline (Kuhl and Giardina, 1982; Lestrel, 1989). The EFA includes description of the outline of a specific shape with several components (harmonics) with an ellipse as the first approximation step. Each harmonic is characterized by four coefficients (FDs), come from the sine and cosine part of the variation in the x and y coordinates (Lestrel, 1997). For this study, first thirteen harmonics were selected as statistical variables and were forwarded to EFW software (Rohlf and Ferson, 1992) using the GMTP software (Taravati, 2010) for further analysis. Before subsequence analysis, data were normalized and invariant to size, location, and rotation.

Figure 1. Manual curve tracing of the fish (*A. chalcoides*).

Size and multivariate analysis: After transformation, the centroid size (CS) was calculated (by GMTP software) for each specimen. In addition, the body length and width were measured using TpsDig2. For visualizing purpose, the size variation among groups, a 95% confidence interval error bar graph was plotted. To determine significant differences among groups Multivariate analysis of variance (MANOVA) was performed. For analyzing size, three variables including centroid size, area, and perimeter were used. These variables were obtained from GMTP software. For variables with normal distribution and similar variances, one-way ANOVA was employed, and for others, with PAST program, the Kruskal-Wallis test was used. Canonical Variate Analysis (CVA) was performed to analyze the data and for classification functions and to assign individual specimens to putative populations, the stepwise discriminant function analysis (DFA) was performed. The classification success rate was evaluated based on percentage of individuals correctly assigned into

Table 1. Brevity of sampling site and sample size of Shemaya (*A. chalcoides*) from southern of the Caspian Sea.

Locality	Brevity	GPS	Sample size
Lisar River	LR	N: 37°58', E:48°56'	33
Anzali Lagoon	AL	N: 37°28', E: 49°27'	38
Shiroud River	SHR	N: 36°49', E: 50°52'	36
Babolroud River	BR	N: 36°42', E: 52°39'	35

Table 2. Tukey's pairwise comparisons for centroid Size (over diagonal) and perimeter (under diagonal) showing the P (same) value. Asterisks (*) indicate significant differences.

	Lisar	Anzali	Shiroud	Babolroud
Lisar	---	7.72E-06*	7.72E-06*	1.179E-5*
Anzali	7.72E-06*	---	0.01664*	0.03918*
Shiroud	0.8926	7.721E-06*	---	7.814E-06*
Babolroud	0.0865	7.721E-06*	0.01174*	---

Table 3. Mann-Whitney pairwise comparisons (Bonferroni corrected) of body area. Asterisks (*) indicate significant differences.

	Lisar	Anzali	Shiroud	Babolroud
Lisar	---	1.47E-09*	5.91E-13*	1.24E-07*
Anzali	8.81E-09*	---	0.002723*	0.09694
Shiroud	3.55E-12*	0.01634*	---	0.01634*
Babolroud	7.44E-07*	0.5816	0.000141*	---

original sample. As a complement to discriminant analysis, morphometric distances among the individuals of four groups were inferred to cluster analysis (Veasey et al., 2001) by adopting the Euclidean square distance as a measure of dissimilarity and the UPGMA (Unweighted Pair Group Method with Arithmetical average) method as clustering algorithm (Sneath and Sokal, 1973).

Allometry: The relationship between shape variables and CS was evaluated by multivariate regression analysis (Rohlf and Marcus, 1993), to investigate the allometric patterns associated with size. Therefore, a principal component analysis (PCA) was performed for each new set of variables. The correlation test was used between CS and PCA scores using the Pearson product-moment correlation coefficient and the PCA with the highest correlation which was plotted against CS.

Results

ANOVA assumes data normality and homogeneity of variances. The Centroid Size (CS) and perimeter data were checked for normality (P>0.05) for each population, and the homogeneity of variances were tested by Levene's test (P>0.05). The P value for ANOVA of CS was 1.586E^{-21} (< 0.05) and 4.75E^{-21} for perimeter, showing CS and perimeter of populations are significantly different (Fig. 2). The MANOVA (Wilks' Lambda) indicated a significant difference for mean vectors among four populations (Λ= 0.031, F= 2.78, P= 3.082E-15). Tukey's test was also used to determine which populations were different from each another (Table 2).

Table 3 shows the Bonferroni corrected Mann–Whitney pairwise comparison for all groups. Confidence interval is another way of visualizing the difference among means of three or more populations. Means and 95% confidence intervals for CS and body length are presented in Figure 2. For assessing the power of multivariate analysis, the length/width ratio was calculated for each specimen, and its confidence interval was obtained for comparing the results of multi-width univariate analysis (Fig. 2). 76.6% of individuals were correctly classified into their respective groups by discriminant function analysis (Table 4), indicating a high differentiation between the studied populations.

The CVA scatter plot of Elliptic Fourier coefficients

Table 4. Classification matrix showing the percentage of individuals that were correctly classified. (Bold values indicate correct classifications).

	Lisar	Anzali	Shiroud	Babolroud	Total
Original (%)					
Lisar	**70.7**	2.8	2.8	5.6	100
Anzali	.0	**94.9**	.0	2.8	100
Shiroud	2.9	2.9	**67.5**	2.9	100
Babolroud	11.1	11.1	5.6	**72.5**	100

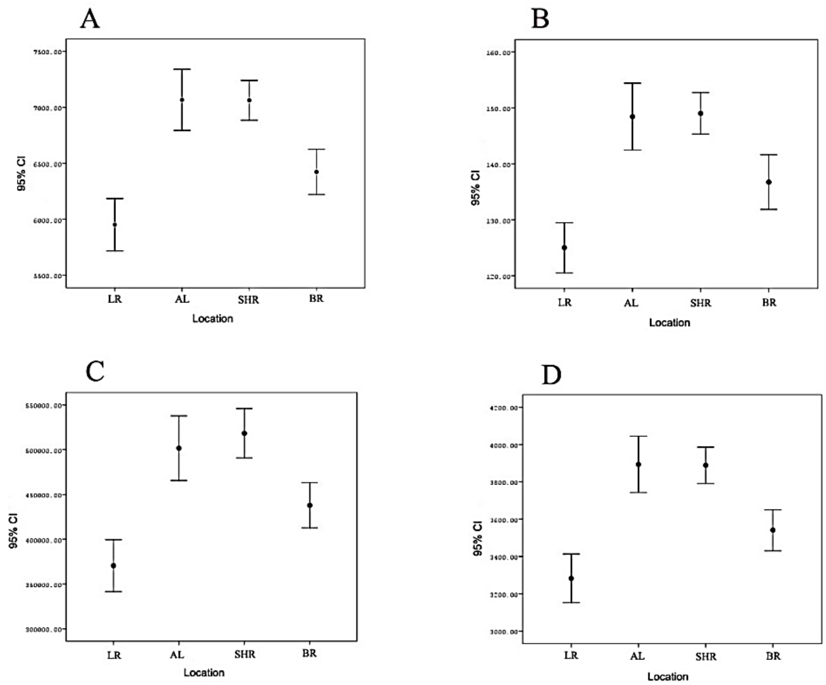

Figure 2. Four size variables: A: centroid size, B: body length, C: area, D: perimeter. (LR: Lisar River, AL: Anzali Lagoon: SHR: Shiroud River, BR: Babolroud River).

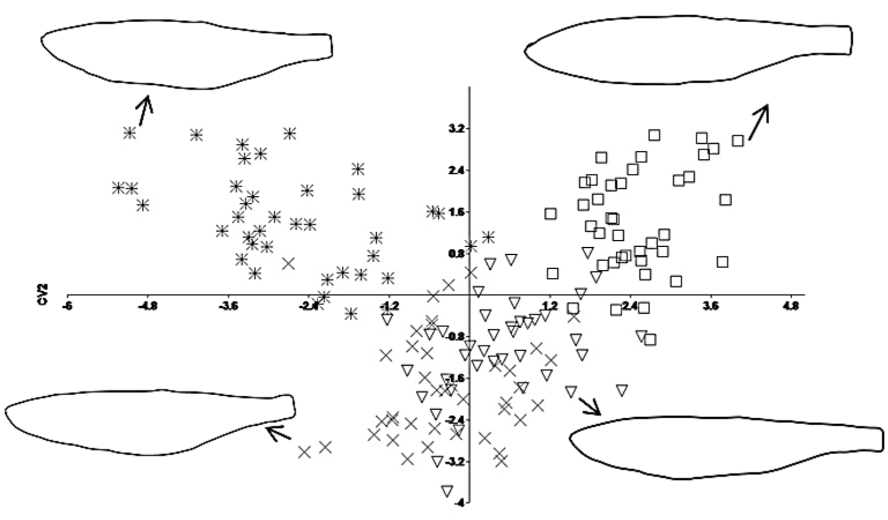

Figure 3. Canonical variate analysis scatter plot of four samples of *A. chalcoides*. Species are shown with different shape.

showed differences among studied populations. In CVA analysis, the two first components were responsible more than 80% of all variation (CV1= 53.3 and CV2= 32). Figure 3 shows the projection of the specimens on the first two Canonical functions and the changes in shape associated with them showing

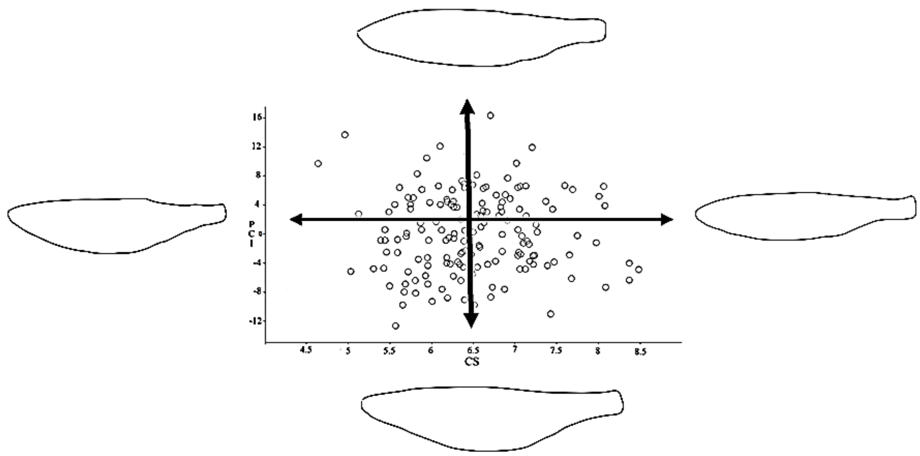

Figure 4. PC1 versus centroid size (CS). Graphical outline representations are reported for extreme values of principal components.

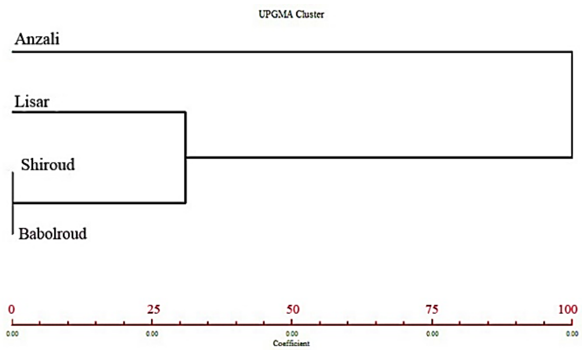

Figure 5. Dendogram resulting from cluster analysis based on Euclidean distances among the mean shapes of the species.

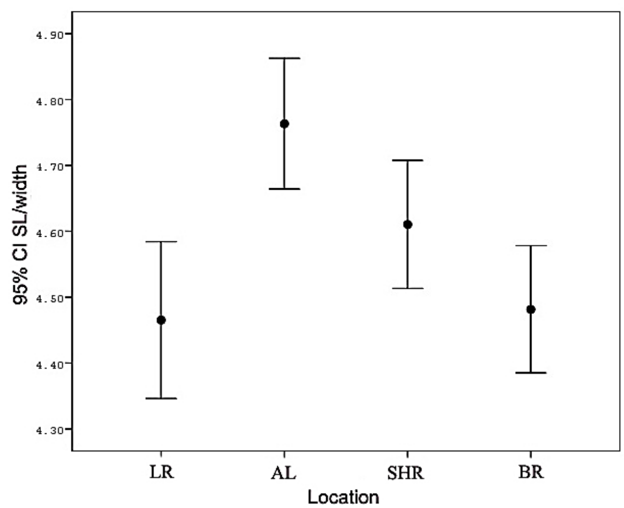

Figure 6. The 95% confidence intervals of body length/width ratio of four *A. chalcoides* Populations (LR: Lisar River, AL: Anzali Lagoon: SHR: Shiroud River, BR: Babolroud River).

that Lisar and Anzali populations are separated and Shiroud and Babolroud groups have overlapped to some extent. The Lisar population with positive scores of CV1, was characterized by a stout-shaped body, whereas the Anzali population, with negative scores on CV2, presented a slender-shaped body.

The UPMGA graph shows two major distinct clads that first one includes Anzali population and the rest positioned in the second one. The second clad is divided to Lizar and Shiroud-Babolroud branchs (Fig. 5).

For distinguishing correlation between size and shape, the pearson product-moment correlation was used to find the correlation between the first three PC axes scores and CS. The scores of PC1 had the highest correlation ($r = 0.72$; $P<0.001$). The growth trajectory related in PC1 clarifies high shape variability in small specimens followed by a better defined pattern of shape change in larger specimens. Figure 4 shows the plot of PC1 versus CS and shapes related in the extreme values of the axis, and it appears as a saturating curve. Gradually, the shape of larger fish is more fusiform, the anterior region sharpens and the caudal peduncle is longer and slimmer as they are growing.

Discussion

The results showed a significant difference in size of studied groups. The population of Anzali Lagoon has a larger and more curved body shape than others and their body curvature increases with increase of fish size. It is known that physical conditions of lagoons differ from rivers/streams systems (e.g. Hendry et al., 2000; Brinsmead and Fox, 2002). Lagoons have lentic physical conditions, lower water transparency, higher water temperature, and greater leaf litter accumulation on the substratum (Haas et al., 2011). It is commonly known that growth of lagoon fish is greater than that of riverine populations (Warburton, 1979; Marian et al., 2002). Coban et al. (2008) also reported that there is no significant shape variation between cultured fish (that are always fed well) and lagoon caught. Lisar is a river and characterized due to less depth, muddy bottom and high turbidity, fast-running water and less nutritious. Meanwhile, the Shiroud and Babolroud Rivers characterized due to more water clarity (particularly the Shiroud River has a sandy bottom), lower turbidity, higher depth and more nutritious having a better environmental conditions than Lisar River. Therefore, it seems that hard environmental conditions of the Lizar specimens could be led a smaller size (Boily and Magnan, 2002).

The CVA showed that the shape of four groups are significantly different. Bagherian and Rahmani (2007) reported a morphometric separation of Shemaya populations from two geographical regions of the southern Caspian Sea. The reasons of morphological differences between populations are often quite difficult to explain (Poulet et al., 2004; Anvarifar et al., 2011), but it is well-known that morphometric characters can show high degree of the plasticity in response to environmental conditions (Wimberger, 1994), such as food availability, water depth and flow, temperature and turbidity (Allendorf, 1988; Wimberger, 1994).

It is generally considered that variation in size between populations depends largely on environmental conditions, whereas a variation in shape reflects in the genetic constitution (Adams and Funk 1997; Orr and Smith, 1998; Schluter, 2000). Since, the studied Shemaya population are anadromous and have a common origin population. Therefore, the difference in shape can therefore be considered to be a result of environmental affection.

Many fish species are famous to show distinct morphologies between lotic and lentic habitats (Robinson and Wilson, 1994; Taylor et al., 1997; Hendry et al., 2000; Pakkasmaa and Piironen, 2000; Brinsmead and Fox, 2002). Hydrodynamic theory proves that a fusiform body shape decrease drag, and hence reduces energy consumption to maintain position in flowing water (Keast and Webb, 1966; Blake, 1983; Webb, 1984; Videler, 1993; Vogel, 1994), Therefore, it is expected that in the same ages, river samples (particularly Lizar specimens) show more fusiform body shape, but our results showed that the size of body is more effective, and river water current does not have any important role forming a more fusiform body shape (Mohadasi et al., 2013). The Anzali population (having better food condition with relatively bigger size) that lives in a lentic environment, shows more fusiform body shape than other populations. Therefore, this study suggest that the amount of curvature could be as result of genetic and independent form water current.

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