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ORIGINAL ARTICLE

Geometric morphometric analysis of body shape variation in *Salmo trutta* populations from the Caspian Sea, Namak and Urmia basins, Iran

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Abstract
This study aimed to compare the body shape of five populations of *Salmo trutta* collected from the Namak Lake, Urmia Lake, and the Caspian Sea basins, using the geometric morphometric technique. To this purpose, a total of 130 specimens were sampled from Liqvan-Chai, Mardoogh, Karaj, Jajrud and Haraz rivers using electrofishing. The left side of the specimens was photographed and 16 landmark–points were defined and digitized on 2D pictures. The landmark data was superimposed using a generalized procrusts method and analyzed using PCA, CVA, and cluster analysis. The results revealed significant differences in the body shape (P<0.0001) of the studied populations, except between the Mardoogh and Liqvan-Chai populations. The differences between populations were related to the length and depth of the caudal peduncle, the position of the snout, body depth and position of the pectoral fin base. The Jajrud River population was differentiated from the others due to possessing deeper body depth, shallower head and lower anterior pectoral fin origin. The results can be useful as baseline information on the native stocks for conservational policy.

Keywords: Geometric morphometrics, Body shape, *Salmo trutta*, Phenotypic plasticity.

INTRODUCTION

Brown trout, *Salmo trutta*, is distributed in a wide area covering a significant part of Europe, the western part of Asia, and the northern part of Africa (Vatandoust et al. 2014a). This species is native to Iranian inland water found in the Caspian Sea, Namak Lake, and Urmia Lake basins (Jouladeh-Roudbar et al. 2015; Esmaeili et al. 2018). In recent years, the natural populations of *S. trutta* have been threatened by increasing human activities such as over-fishing, water pollution, destruction of natural spawning areas, and drought (Vera et al. 2011).

Basic information about fish population structure in different regions is required for their conservation programs. In this regard, the morphological differentiation of diverse populations of a single species can reflect how they have adapted to their habitats' properties and genetic differentiation (Aurelle & Berrebi 2001; Campos et al. 2007). Due to the ecological and commercial importance of brown trout, many aspects of its biological characteristics have been studied, such as morphological variation (Karakousis et al. 1991; Pakkasmaa & Piironen 2001; Maric et al. 2004; Hermida et al. 2009; Rawat et al. 2011; Vehanen & Huusko 2011; Vatandoust et al. 2014a), genetic differentiation (Charles et al. 2005; Jensen et al. 2005; Samuiloviene et al. 2009), genetic variation (Campos et al. 2007; Taghizadeh et al. 2012) and phylogenetic relationship (Aurelle & Berrebi 2001; Dudu et al. 2011; Hashemzadeh Segherloo et al. 2012a; Rezaei & Akhshabi 2012). In addition, those studies in Iran are related to their morphological aspects (Vatandoust et al. 2008; Salavatian et al. 2011a, b; Akbarzadeh et al. 2012; Vatandoust et al. 2014a), population structure (Afraei et al. 2000; Fazli et al. 2012), reproduction traits (Afraei et al. 2000), growth and mortality (Fazli et al. 2012; Kheyrandish et al. 2010), feeding habits (Rajabinezhad et al. 2011; Salavatian et al. 2011c; Eagderi et al. 2022) and habitat characteristics in tributaries of the Taleghan River (Eagderi et al. 2021).

In recent years, morphometric system is increasingly used for morphometric measurements with the purpose of species and/or stock identification

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in Iran (e.g. Heidari et al. 2013, 2014, 2019; Kohestan-Eskandari et al. 2013, 2014; Mousavi-Sabet & Anvarifar 2013; Mousavi-Sabet et al. 2018a, 2018b; Paknejad et al. 2014; Vatandoust et al. 2014a, 2014b. 2015). Morphological studies on fishes are important from various viewpoints, including evolution, ecology, behavior, conservation, water resource management and stock assessment (Mousavi-Sabet et al., 2021). Information on the population differentiation of brown trout is mainly limited to the southern Caspian Sea basin. Therefore, understanding the morphological differentiation of their populations in different basins is crucial. The phenotypic difference in shape within a species may exist because of sexual dimorphism and ecological specialization (Schluter & McPhail 1992). The study of shape variation between different populations of a fish species can be done using traditional (TM) or landmark-based methods. GM is a powerful technique (Mouludi-Saleh et al. 2019; 2020a, b; Eagderi et al. 2020; Mouludi-Saleh & Eagderi 2021) that can reveal differences in biological structures that are not easily extracted using TM methods (Bookstein 1991; Zelditch et al. 2004).

The geometric morphometric method has not been used to assess body shape variation among *S. trutta* populations in Iran. Therefore, this study examined the intraspecific body shape of five populations of the *S. trutta* collected from the Caspian Sea, Namak Lake, and Urmia lake basins using the geometric morphometric technique. The results may help to a better understanding of natural history across its geographic range.

MATERIAL AND METHODS

A total of 130 specimens of brown trout were collected from the Liqvan-Chai, Mardoogh (the Urmia Lake basin), Karaj, Jajrood (the Namak Lake basin) and Haraz (the Caspian Sea basin) rivers using electrofishing in 2021, and were anesthetized using clove oil extraction 1%. Then the specimens with almost similar sizes were selected to reduce the effects of allometric growth patterns. The selected specimens had no deformity in terms of body shape. The left sides of freshly collected specimens were photographed using a copy-stand equipped with a digital camera (Canon with 18 MP resolutions). The photographed fish were returned to their habitat. Since there is no reported sexual dimorphism in *S. trutta*, therefore all data were pooled.

To extract body shape data in the landmark-based GM method, 16 homologous landmark-points were digitized on 2D photos 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 landmark data was submitted to a Generalized Procrust Analysis (GPA) to remove non-shape data, including scale, direction, and position. The landmark data after GPA superimposing were analyzed using Principal Components Analysis (PCA) to explore the patterns of variation in their body shape (Rohlf & Marcus 1993). Canonical variant analysis (CVA) was used to investigate the power of distinction of five populations with *P*-value obtained from a permutation test with 10000 replications in MorphoJ software. Finally, a cluster analysis by adapting the Euclidean square distance and selecting 100 bootstrapping was performed (Veasey et al. 2001). Mahalanobis and Procrast distances were calculated as the degree of body shape differentiation obtained from the CVA analysis.

The shape difference of each population in relation to the consensus configuration of all populations was visualized as a deformation grid in MorphoJ. All multivariate analysis were performed using PAST v2.17b (Hammer et al. 2001) and MorphoJ v 1.01 (Klingenberg 2011) softwares.

RESULTS

The results of PCA showed that all specimens explained 44.98% of shape variations by the first two PC axes extracted from the variance-covariance matrix (PC1=30.22% and PC2=14.76%). Plotting of the first and second PCs displayed a distinction between the Jajrud population, and Liqvan-Chai and

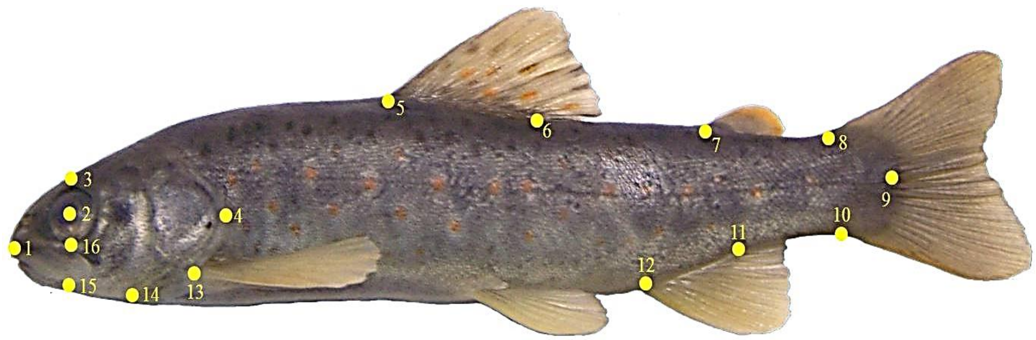

Fig.1. The 16 defined landmark points to extract body shape in *Salmo trutta*. (1) snout tip; (2) center of eye; (3) upper margin of eye; (4) posterior-most end of head; (5) anterior origin of dorsal fin; (6) posterior insertion of the dorsal fin; (7) anterior origin of adipose fin; (8) upper edge of posterior end of caudal fin base; (9) posterior body extremity; (10) lower edge of posterior end of caudal fin base; (11) posterior insertion of anal fin; (12) anterior origin of anal fin; (13) anterior origin of pectoral fin; (14) ventral junction of head and trunk; (15) ventral edge of head vertical to eye center; (16) lower margin of orbit.

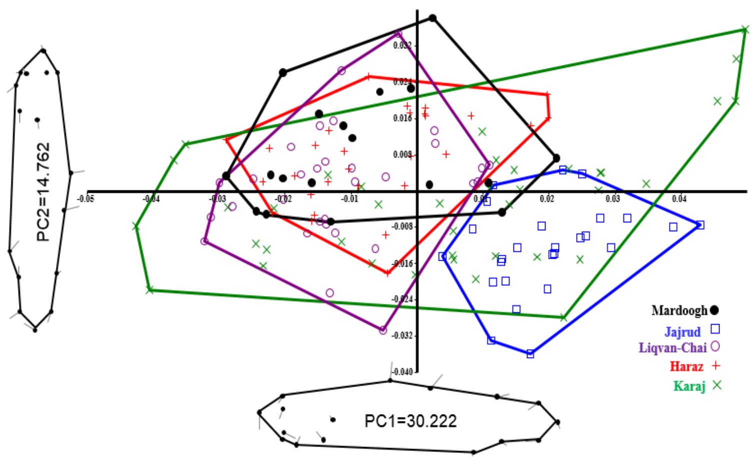

Fig.2. Scatter of individuals' body shape of studied populations of *Salmo trutta* on PC1 and PC2 plot (the wireframe graphs showing mean shape as wireframe and vectors pointing in the direction of the axis loading).

Haraz populations (Fig. 2). PC1 based on the wireframe plot, showed the shifts of landmark positions of 1, 5, and 6 along the positive direction, explaining the elongation of the length and body depth, whereas PC2 shows the shifts of landmark positions of 1, 4, and 13 towards its positive direction that explain the elongation of the head. The differences between populations were related to the length and depth of the caudal peduncle, position of snout head, body depth, and pectoral-fin base.

The CVA analysis revealed significant differences in the body shape among the studied species ($P<0.05$). The CVA plot displayed a clear distinction of the Jajrud and Haraz populations from others. The population of the Liqvan-Chai had a low degree of overlapping with the Karaj, Mardoogh and Haraz populations (Fig. 3). The results revealed significant differences between studied populations in terms of body shape ($P<0.0001$), except between Haraz and Liqvan-Chai populations. The observed differences between populations were related to the length and depth of the caudal peduncle, the position of the snout head, body depth, and pectoral-fin base (Fig. 3). Mahahlanobis and procrustes distances are presented in Table 1. Maximum and minimum distances were calculated between Mardoogh-Jajrud and Mardoogh-Liqvan-Chai populations, respectively.

The dendogram derived from cluster analysis among groups showed that the five *S. trutta* populations were partly distinct based on their morphometric characters (Fig. 4). With regard to this analysis, by 100% of possible trees, the Jajrud

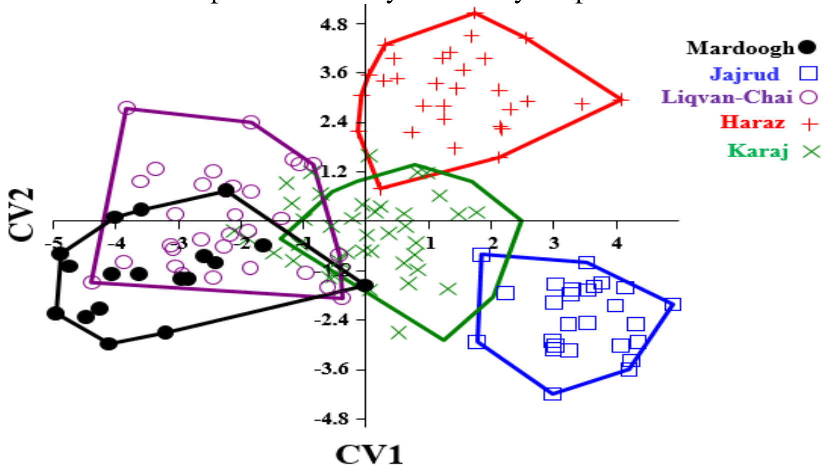

Fig.3. The results of Canonical discrimination analysis (CVA) of the five population of *Salmo trutta* body shape with respect to the first two canonical variables.

Table 1. Mahalanobis and Procrustes distances obtained from CVA analysis among the studied populations of *Salmo trutta*.

Mahalanobis					Procrustes				
	Haraz	Jajrud	Karaj	Liqvan-Chai		Haraz	Jajrud	Karaj	Liqvan-Chai
Jajrud	5.614				Jajrud	0.0338			
Karaj	4.204	4.7934			Karaj	0.0212	0.0227		
Liqvan-Chai	4.9003	5.9814	3.9108		Liqvan-Chai	0.0207	0.0368	0.0208	
Mradoogh	5.8206	6.258	4.0514	2.8528	Mradoogh	0.0218	0.0368	0.0222	0.0131

population separated from others due to possessing greater body depth, lower head depth, and anterior position of the pectoral-fin base.

DISCUSSION

The present study on the five populations of the brown trout shape variation revealed significant morphological variations. Differences between the studied populations can be divided into two categories; (1) deeper body depth, shallower head, and short caudal peduncle as seen in Jajrud population, and, (2) shallow body and longer caudal peduncle in the other studied populations. One of the main factors that may be responsible is the size of the habitat reflecting several traits of brown trout that are strongly influenced by habitat variables (Jonsson et al. 2001). In addition, the five studied *S. trutta* populations were distinct from each other based on cluster analysis. Mardoogh and Liqvan-Chai populations were most similar morphologically, since they belong to the same basin i.e. the Urmia Lake. Haraz and Karaj were separated by having deeper body from the Urmia lake populations.

Observed differences in head shape of the studied populations can be related to their feeding behaviors (Andersson et al. 2005). Changes in head and mouth shapes can be considered as reflective of differences in the selection of food items and the direction of feeding (Langerhans et al. 2003). This also can be related to trophic ecology indicates local adaptation and possibly ecological radiations (Schluter & McPhail 1992; Langerhans et al. 2003). The reasons of morphological differences between populations are often quite difficult to explain (Poulet et al. 2004), but it is well-known that morphometric characteristics can show a 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 can reflect their phenotypic plasticity and the genetic constitution (Adams & Funk 1997; Orr & Smith 1998).

In Salmonids, in response to environmental factors (i.e. water clarity and bottom structure), some

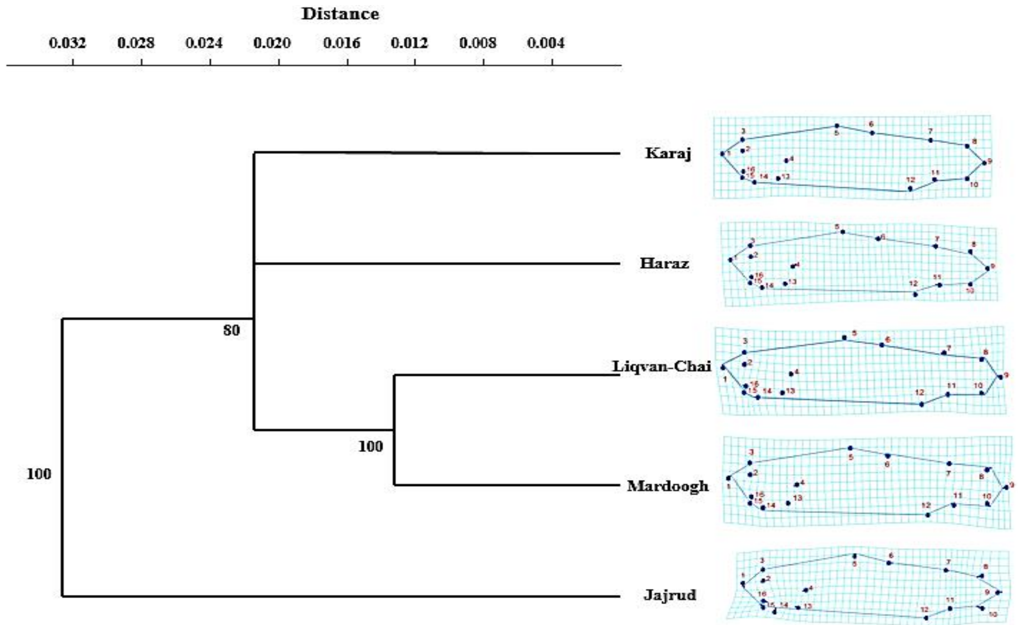

Fig.4. Dendrogram derived from cluster analysis of morphometric variable on the basis of Euclidean distance of five populations of *Salmo trutta*. Mean shape of species in relation of consensus shape for each population are represented.

morphological and coloration traits have been developed (Bourke et al. 1997). Sanz et al. (2002) in Mediterranean brown trout populations showed large local genetic divergences could be related to the morphological variation. Populations of this species have a high morphological diversity (Ferguson & Taggart 1991), which has recently challenged the high morphological flexibility in *S. trutta*. Hence in Iran, based on studies, inhabiting populations in the Liqvan-Cahi River, due to the number of red spots on the body probably belong to a special subspecies of *S. trutta* (Abdoli 2000).

Based on Vatandoust et al. (2014a), 31 morphometric and 7 meristic characteristics in five populations of *S. trutta* from the Caspian Sea basin were studied and their results showed a high morphological differentiation. Vatandoust et al. (2008) pointed out meristic traits are more important than morphometric to distinguish populations. Comparison of morphological variations of *S. trutta* in Chesli and Khorma Rivers in Guilan province using 39 morphometric and 13 meristic traits showed no significant differences between males and females, i.e. no sexual dimorphism in morphological characteristics (Akbarzadeh et al. 2012). Hashemzadeh Segherloo et al. (2012b) showed no significant difference in *S. trutta* populations of Mardoogh and Liqvan-Chai rivers using microsatellite i.e. showing similarity of them. Therefore, the geometric morphometric method showed similar results as a suitable method for the variations of the morphological pattern in *S. trutta*.

Our results showed significant body shape differences among the populations of the *S. trutta* in Iranian inland waters, showing the variation of their body shape based on their inhabiting basin. These results provide a morphological insight, which can be used for further taxonomic studies, stock management, and conservation programs.

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مقاله کامل

آنالیز ریخت‌سنجی هندسی تنوع ریختی جمعیت‌های *Salmo trutta* در حوضه‌های دریای خزر و دریاچه‌های نمک و ارومیه، ایران

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چکیده: این مطالعه به‌منظور مقایسه شکل بدن پنج جمعیت *Salmo trutta* جمع‌آوری شده از حوضه‌های دریاچه نمک، ارومیه و حوضه دریای خزر با استفاده از روش ریخت‌سنجی هندسی انجام شد. بدین منظور تعداد ۱۳۰ نمونه از رودخانه‌های لیقوان‌چای، مردوق، کرج، جاجرود و هراز با استفاده از دستگاه الکترو شوکر صید و نمونه‌برداری شد. از سمت چپ نمونه‌ها عکسبرداری شد و تعداد ۱۶ نقطه لندمارک تعریف و بر روی تصاویر دو بعدی رقومی سازی شد. روی هم‌گذاری داده‌های لندمارک با استفاده از روش پروکراست انجام شد و با استفاده از آنالیزهای PCA، CVA و تحلیل خوشه‌ای مورد تجزیه و تحلیل قرار گرفتند. نتایج، تفاوت معنی‌داری را در شکل بدن ($P<۰/۰۰۰۱$) جمعیت‌های مورد مطالعه به‌جز جمعیت‌های مردوق و لیقوان‌چای نشان داد. تفاوت بین جمعیت‌ها مربوط به طول و عمق ساقه دمی، موقعیت پوزه، عمق بدن و موقعیت پایه باله سینه‌ای بود. جمعیت رودخانه جاجرود به دلیل داشتن عمق بدن بیشتر، عمق سر کمتر و موقعیت قدامی قاعده باله سینه‌ای، از سایرین متمایز شد. نتایج می‌تواند به عنوان اطلاعات پایه در مورد ذخایر بومی در راستای سیاست حفاظتی مفید باشد.

کلمات‌کلیدی: ریخت‌سنجی هندسی، شکل بدن، قزل‌آلای خال قرمز، انعطاف‌پذیری ریختی.