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Original Article

How the body shape changes by the habitat hydrological factors in freshwater benthic fishes; case study on the genera *Cobitis* (Cobitidae) and *Ponticola* (Gobiidae)

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Abstract: Benthic fish groups with low mobility are often restricted to a narrow range of a stream course, and their morphological characters tend to be affected substantially by the environmental conditions in their habitat. Due to morphological affectability of fishes by many factors in lotic ecosystems, a landmark-based hypothesis was used to investigate the effects of habitat hydrological conditions on morphological characteristics in freshwater benthic fishes; a case study on the genera *Ponticola* and *Cobitis*. A total of 216 gobies and 128 spined loaches specimens were caught from six rivers with different hydrological conditions, along the southern Caspian Sea basin. In discriminant function analysis (DFA), the overall assignment of gobies and loaches into their original groups were 95.7% and 80.5%, respectively. Discriminant analysis for pairwise groups shows a longer snout, shallow body/head, and elongated body for populations living in the large slope channel with faster water velocity versus relatively short snout and deep body/head for those living in small slope channel with slower water velocity. The results confirm the possibility of changes in the morphological characters of the benthic gobies and loaches, which should be considered in taxonomical and biological studies.

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Introduction

The environmental control of phenotypic expression combined with variability among local environments may result in populations being distinguishable from one another in morphology, behavior, physiology and life history (Matthew, 1998; Kawecki and Ebert, 2004). Rivers, in particular, are heterogeneous environments that are stochastic and highly variable in both space and time (Matthews, 1998; Fraser et al., 2011). Fish living in such variable systems face multiple ecological constraints such as water temperature and flow regime. Environmental heterogeneity across the geographical range of the species may exert local selective pressure actings to maximize individual fitness within specific environments (Kawecki and Ebert, 2004; Fraser et al., 2011; Jalili et al., 2015).

Fish can be classified into several functional groups (e.g., pelagic, slow water and benthic fishes) on the basis of functional-morphological studies (Sagnes et al., 1997; Langerhans et al., 2003). Benthic fishes are generally considered poor swimmers, which maintain their stream position on the substratum hydrodynamically. Thus, their morphological characters should be affected by environmental conditions such as the water depth and current velocity of their habitat (Sagnes et al., 1997; Langerhans et al., 2003). Therefore, the study of morphological variation in benthic fish populations in relation to environmental gradients may hold the key to evaluate the nature of natural selection.

The analysis of morphometric characters has been widely accepted by fish biologists as a way to differentiate among different species and different populations within a species (Yakubu and Okunsebor, 2011). Morphometric measurements are widely used to identify differences between fish populations and mainly important where the differences are mostly

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attributed to environmental influences rather than genetic differentiation (Bektas and Belduz, 2009). Geographical isolation of populations and interbreeding can lead to morphometric variations between populations, and this morphometric variation can provide a basis for population differentiation (Bookstein, 1991; Cadrin and Friedland, 1999).

The genera *Cobitis* (family Cobitidae) and *Ponticola* (family Gobiidae) are small benthic freshwater fishes with a wide distribution area covering large parts of the southern Caspian Sea basin and are of particular interest from a conservation point of view in many areas of its native range (Coad, 2017; Esmaeili et al., 2018). There are several reports, indicating the existence of morphological variability on benthic fish populations in the southern Caspian Sea basin (Mousavi-Sabet et al., 2011, 2012; Mousavi-Sabet and Anvarifar, 2013; Vasil′eva et al., 2015). Despite to the recent morphology-based taxonomic studies on gobies and loaches species, there is no available data on habitat effects on morphological characters, which can help in better understanding of the real body shape differentiations between different taxa. Therefore, it should be clear that; are the morphological variations between benthic fish species caused by (i) adaptive differences that reflect genetic differences, (ii) adaptive differences that reflect phenotypic plasticity or (iii) non-adaptive differences that reflect phenotypic plasticity. On the other hand, we want to clear that; how the environmental conditions and the river hydrology can affect the body shape in freshwater benthic fish species.

Therefore, the present study examines the relationship between body shape variation and environmental conditions in freshwater benthic fishes, the genera *Cobitis* and *Ponticola*, in the southern Caspian Sea basin. We predict that fish tend to have lower body depth and longer snout under environment with faster bottom current velocities.

Materials and Methods

For the study 216 goby and 128 loach specimens were collected from November 2015 to January 2016 by electrofishing device. Fish specimens were sampled in the same month, out of the reproductive period from six rivers of the southern Caspian Sea basin, including the Babol, Sefid, Tonekabon, Siah, Talar and Gisum (Fig. 1). The hydrologic and environmental factors, including altitude, temperature, water flow, substrate and slope were estimated in each studied river (Table 1). The sampled fishes were photographed and fixed in 10% formaldehyde at the sampling site and transported to the laboratory for further studies.

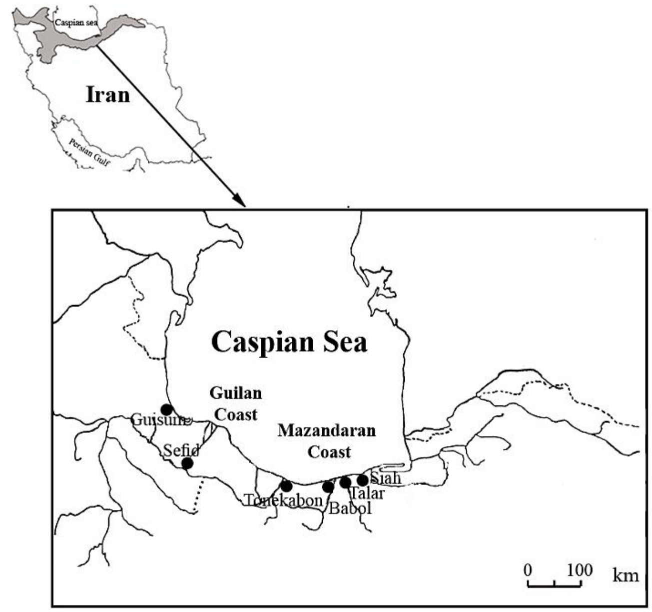

Figure 1. Map of the Iranian part of the southern Caspian Sea basin and location of sampling sites including Guisum, Sefid, Tonekabon, Babol, Talar and Siah rivers.

Standard Length (±1.0 mm) and body weight (±0.001 g) were recorded for each specimen. For goby specimens 105 distance measurements between 15 landmark points on lateral side and for loach specimens 78 distance measurements between 13 landmark points were measured using the truss network system according to Bookstein (1991) with minor modifications (Fig. 2). Also, for gobies 44 and 28 distance measurements between 10 and 8 landmark points on ventral and dorsal views, respectively, were measured for investigation ventral and dorsal faces' shape variation. The fishes were placed on a white board with dorsal and anal fins held erect by pins. The left body profile of each fish was photographed with a 300-dpi, 32-bit color digital camera (Cybershot DSC-

Table 1. The hydrologic data of the studied rivers in the southern Caspian Sea basin.

Environment conditions at sampling site				
River Name	Altitude (m)	water flow (m3/s)	substrate	Slope (%)
Babol	158	2.1	Sandy-stony	2.5
Sefid	201	5.1	Sandy-stony	5.4
Talar	943	2.2	Sandy-stony	2.0
Guisum	- 24	0.5	Sandy-muddy	1.7
Tonekabon	179	1.6	Sandy-stony	1.4
Siah	117	1.2	Sandy-muddy	1.3

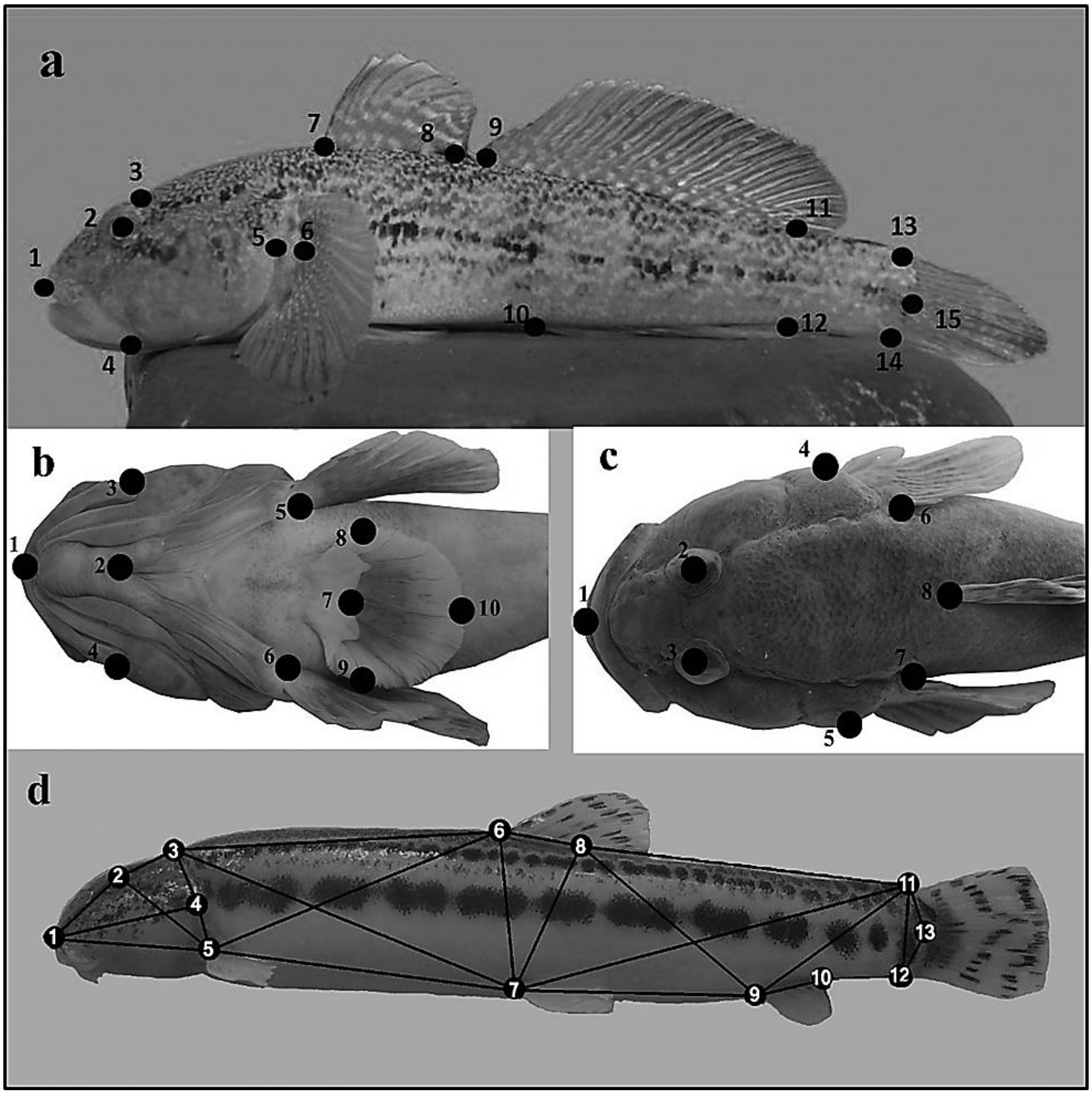

Figure 2. Locations of the landmark points on lateral (a), ventral (b) and dorsal (c) sides of the goby and on lateral side of the loach (d).

F505; Sony, Japan). Images were saved in jpg format and analyzed with TPSdig2 to digitize landmark points (Mustafic et al., 2008). All measurements transformed into linear distances by computer for subsequent analysis. After image capture, the fish was dissected to identify the sex of the specimen by macroscopic examination of the gonads. Gender was used as the class variable in ANOVA to test for the significant differences in the morphometric characters if any, between male and female specimens. An allometric method (Elliott et al., 1995) was used to remove size-dependent data in morphometric characters using following formula:

$$Madj = M\,(L_s / L_0)^b$$

Table 2. Descriptive data [mean ±SD standard length (mm) and body weight (g)] of goby and loach specimens collected from the studied rivers of the southern Caspian Sea basin.

Genus	Locality	*n*	Standard length		Body weight	
			min–max	mean ± SD	Min–Max	Mean ± SD
Ponticola	Tonekabon	32	35.71-112.87	70.55±21.85	1.15-34.90	10.25±9.30
	Babol	55	53.33-139.92	74.23±23.64	3.77-51.61	12.86±11.85
	Guisum	41	20.80-111.95	55.30±20.80	1.00-33.05	5.81±8.11
	Sefid	88	44.54-72.89	55.63±6.84	2.18-8.16	3.99±1.40
Cobitis	Talar	39	22.66-99.15	55.41 ± 13.59	0.3-6.1	1.6±0.5
	Siah	40	22.47-98.80	53.61 ± 11.74	0.4-5.9	1.5±0.4
	Babol	49	17.65-95.73	50.01 ± 16.26	0.3-5.6	1.5±0.5

Where M is the original measurement, $Madj$ the size adjusted measurement, L_0 the standard length of the fish, L_s the overall mean of the standard length for all fish from all samples in each analysis, and b is estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish in any group. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length (Mustafic et al., 2008).

Univariate analysis of variance (ANOVA) was performed for each morphometric character to evaluate the significant difference among the fish specimens (Bookstein, 1991). To identify morphological differences of the loaches and gobies between rivers, discriminant function analysis (DFA) performed. This analysis will allow an identification of whether the differences between the rivers are significant and define the discriminant functions that account for these differences (Bookstein, 1991; Mustafic et al., 2008). The resultant discriminant function was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using the leaving one-out procedure was done to estimate the expected actual error rates of the classification functions. Principal component analysis (PCA) and canonical variates analysis (CVA) were employed to discriminate these fishes from studied rivers. Statistical analyses for morphometric data were performed using the SPSS version 16 software package, PAST ver. 1.36, MorphoJ and Excel (Microsoft Office 2010).

Results

Descriptive data were examined for the mean and standard deviation (SD) of length and weight in the sampled specimens (Table 2). Although it is well known that the female and male specimens of the gobies and loaches have some morphological differences (Mousavi-Sabet, 2012; Mousavi-Sabet et al., 2012, 2015; Vasil′eva et al., 2015), but the interaction between morphometric measurements used in this study by truss network system and sexes were not significant ($P>0.05$) demonstrating a negligible effect of sex on observed variations; hence, the data for both sexes were pooled for all subsequent analyses. There was no significant correlation between any of the transformed measured morphometric variables and standard length ($P>0.05$) indicating that size or allometric signature on the basic morphological data was accounted.

Statistically significant differences in goby specimens were observed in 79 morphometric characters out of 105 studied. Of these 79 characters, 72 characters were found to be highly significant ($P\leq0.01$) and used further for multivariate analysis. Also, statistically significant differences for loach specimens were observed in 54 morphometric characters out of 78 studied which of these 54 characters, 41 characters were found to be highly significant ($P\leq0.01$). Those morphometric characters which showed highly significant variations ($P\leq0.01$)

Table 3. Eigen values, percentage of variance and percentage of cumulative variance of morphometric measurements for goby and loach specimens collected from the studied rivers in the southern Caspian Sea basin.

Genus	Factor	Eigenvalues	Percentage of variance	Percentage of cumulative variance
Ponticola	1	20.141	28.367	28.367
	2	11.606	16.347	44.714
	3	8.061	11.354	56.068
	4	6.906	9.727	65.795
	5	4.550	6.408	72.203
	6	4.242	5.974	78.178
	7	2.754	3.879	82.056
	8	2.447	3.447	85.503
	9	2.097	2.953	88.456
	10	1.478	2.082	90.538
	11	1.350	1.901	92.439
	12	1.041	1.466	93.905
Cobitis	1	12.780	31.170	31.170
	2	7.389	18.021	49.191
	3	5.715	13.938	63.129
	4	2.930	7.146	70.275
	5	2.372	5.785	76.061
	6	1.929	4.705	80.766
	7	1.335	3.256	84.022
	8	1.189	2.899	86.921
	9	1.120	2.732	89.653

were used to achieve the recommended ratio of the number of organisms measured (N) to the parameters included (P) in the analysis of at least 3-3.5 (Bookstein, 1991), to obtain a stable outcome from multivariate analysis. In this study N: P ratio for goby and loach specimens were 3.00 (216/72) and 3.12 (128/41), respectively, that revealed samples size were adequate. The value of Kaiser-Meier-Olkin coefficient (KMO) for overall matrix are 0.716 and 0.630 for goby and loach specimens, respectively and the Bartlett's Test of sphericity is significant ($P\leq 0.01$). The results of KMO and Bartlett's suggest that the sampled data is appropriate to proceed with a factor analysis procedure.

In order to determine which morphometric measurement affected populations differentiates mostly, the contributions of variables to principal components (PC) were examined. The PCA of 72 morphometric measurements for goby specimens extracted 12 factors with eigenvalues >1, explaining 93.91% of the variance (Table 3). Of these, the first explained 28.37% and the second 16.35% of the variance. The most significant weightings on PC1 were from 1-5, 1-6, 5-15, 2-6, 2-5, 6-15, 6-9, 4-6, 4-5, 6-13, 6-14, 5-13, 1-14, 5-14, 1-4, 1-13, 1-11, 1-12, 1-2, 5-12, 1-10, 1-8, 1-3, 1-7, 1-9 and on PC2 were from 1-12, 12-15, 9-12, 12-13, 12-14, 3-12, 2-12, 10-12, 5-12, 4-12 and 3-14. The PCA of 41 morphometric measurements for loach specimens extracted 9 factors with eigenvalues >1, explaining 89.65% of the variance (Table 3). Of these, the first explained 31.17%, the second 18.02% and the most significant weightings on PC1 were from 1-2, 1-4, 2-4, 4-11, 4-13 and on PC2 were from 1-10, 2-10, 3-10, 4-10, 10-11, 10-12 and 10-13.

Canonical variates analysis confirmed the significant difference among the goby and loach specimens. The scores of the two canonical variables for each river revealed that goby specimens grouped into four distinct areas while there was a relativity low degree of overlap between Tonekabon and Babol populations and for loaches revealed that specimens grouped into three distinct groups while there was a relativity high degree of overlap among these populations (Fig. 3).

The Wilks' lambda tests indicated differences between fish when their morphometric measurements were compared by means of discriminant analysis. In

Table 4. Percentage of specimens classified in each group and after cross validation for morphometric data of gobies and loaches specimens collected from the studied rivers along the southern Caspian Sea basin.

Genus			Rivers				
			Tonekabon	Babol	Guisum	Sefid	Total
Ponticola	Orginal	Tonekabon	100.0	0.0	0.0	0.0	100.0
		Babol	0.0	97.6	2.4	0.0	100.0
		Guisum	0.0	2.7	97.3	0.0	100.0
		Sefid	0.0	2.0	6.1	91.8	100.0
	Cross-validation	Tonekabon	81.8	18.2	0.0	0.0	100.0
		Babol	4.9	89.2	4.9	7.3	100.0
		Guisum	0.0	10.8	83.8	5.4	100.0
		Sefid	0.0	6.1	14.3	79.6	100.0
			Rivers				
			Babol	Siah	Talar		Total
Cobitis	Orginal	Babol	79.6	14.3	6.1		100.0
		Siah	7.5	75.0	17.5		100.0
		Talar	5.1	7.7	87.2		100.0
	Cross-validation	Babol	75.5	16.3	8.2		100.0
		Siah	7.5	75.0	17.5		100.0
		Talar	7.7	7.7	84.6		100.0

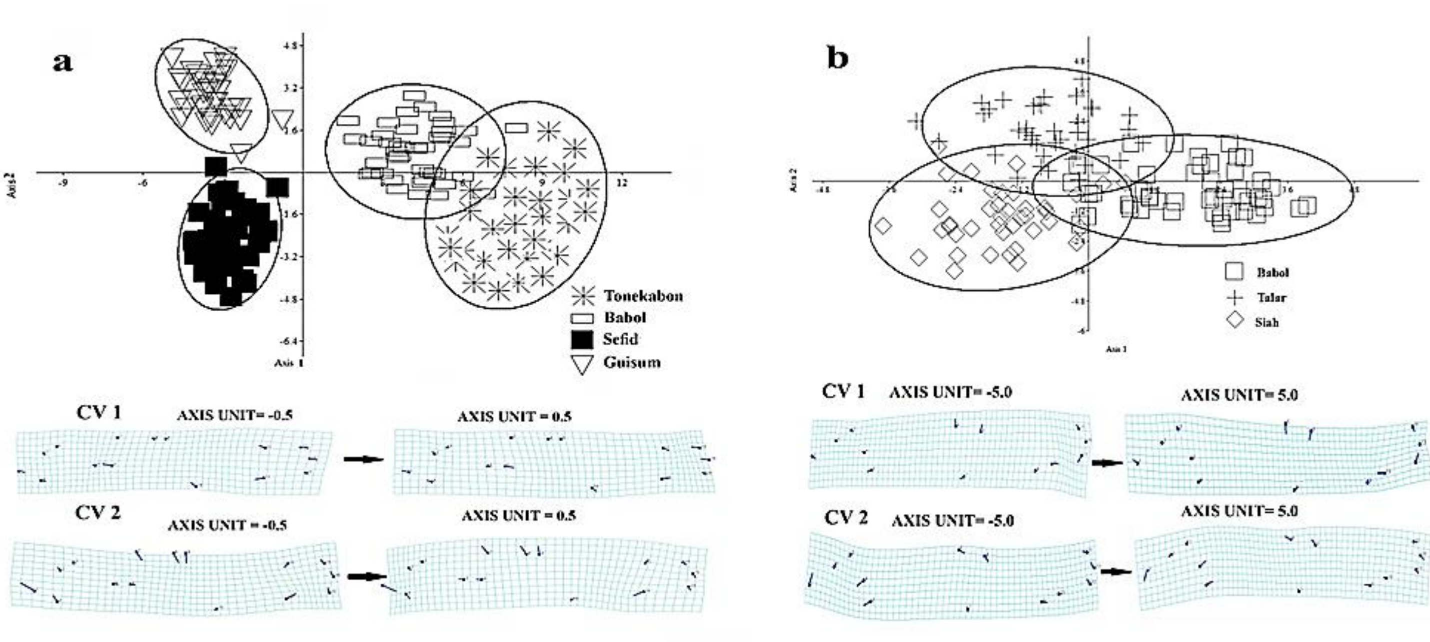

Figure 3. Scatterplot of standardized canonical variates functions 1 (CV1) and 2 (CV2) for morphometric characteristics of goby specimens (a) and loach specimens (b) collected from the studied rivers along the southern Caspian Sea basin. Axis units for deformation grids are also shown.

this test, all functions were highly significant ($P \leq 0.01$). For the discriminant analysis, the average of PCC for gobies and loaches morphometric characters were 95.7% and 80.5%, respectively, indicating a correct classification of specimens into their original populations. In this analysis, there was a high degree of separation in goby and a slight degree of separation in loach specimens in different rivers. The cross-validation testing procedure was exactly the same as the PCC results (Table 4).

The histogram of discriminant functions for pairwise groups in goby specimens is shown in Figure 4. Body shape differences shows a longer snout, shallow body and head, and elongated body for Sefid and Babol groups versus relatively short snout, and deeper body and head for Tonekabon and Guisum

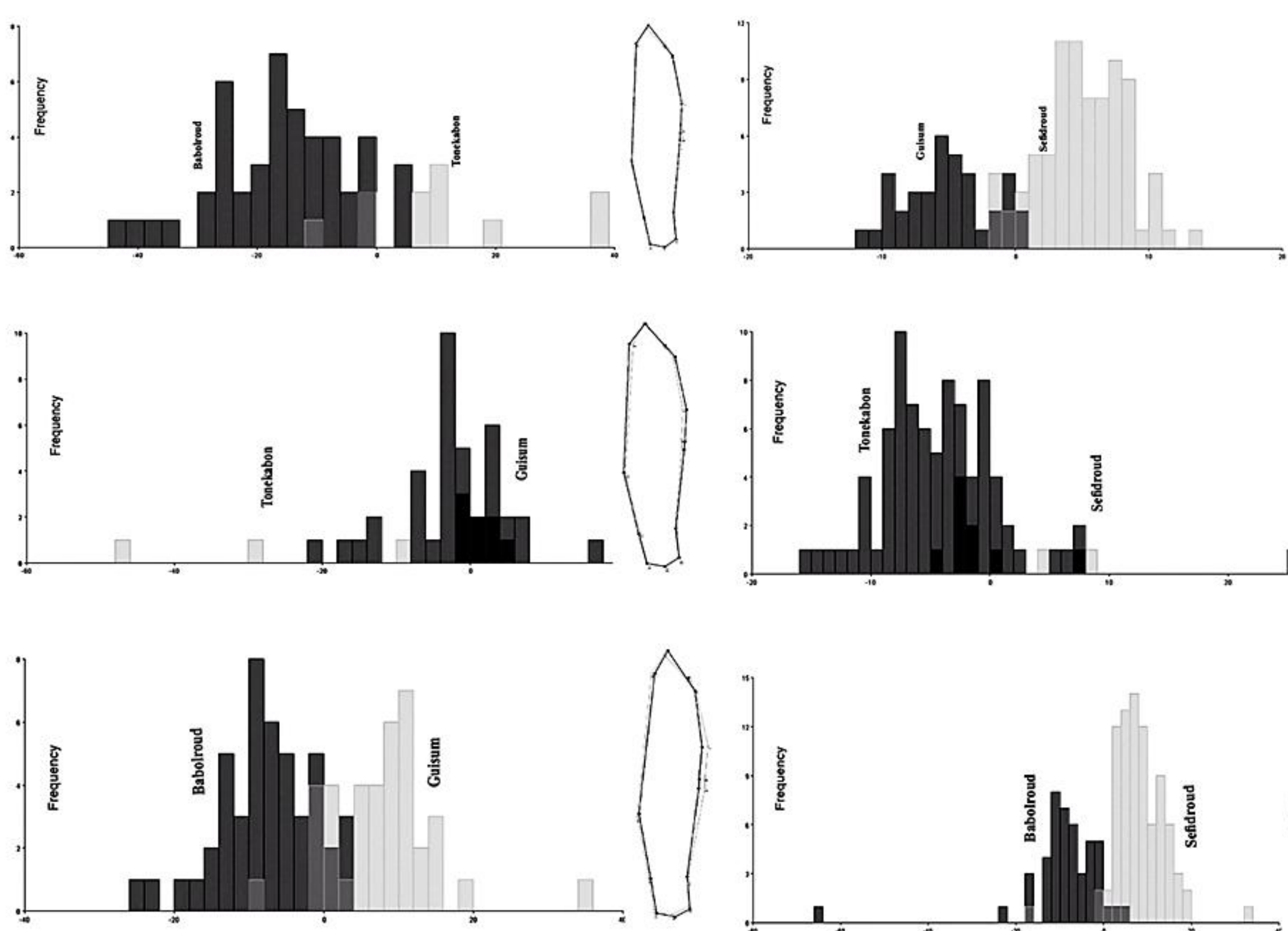

Figure 4. Histogram of discriminate analysis (DA) functions for pair wise competitions' of gobies (left). Shape differences on the extremities of each river (right).

populations. Also, the histogram of discriminant functions for pairwise groups in loach specimens shows a longer snout, shallow body and head, and elongated body for Talar and Babol populations versus relatively short snout, and high body and head depths Siah river group (Fig. 5). Also, the ventral and dorsal shape variation analysis showed a longer snout and bigger disc for Sefid and Babol populations versus relatively short snout and smaller disc for Tonekabon and Guisum groups (Fig. 6).

Discussions

The results based indicated significant morphological differences in the studied benthic fishes between six different rivers system. Which for gobies and loaches 95.7% and 80.5%, respectively, of individuals were classified correctly in each river. This study found the presence of the body shape variation of the benthic fishes at a macro spatial scale between different riverine systems along the southern Caspian Sea basin. The results also revealed no significant phenotypic variation between male and female specimens. The results showed that 79 out of 105 transformed morphometric measurements and 54 out of 78 transformed morphometric measurements were significantly different in these groups of goby and loach specimens living in the southern Caspian Sea basin, showing a high phenotypic variation among them.

Also, ddifferentiation between samples from adjacent rivers, such as those from Tonekabon, Babol, Siah and Talar and those from Sefid and Guisum may

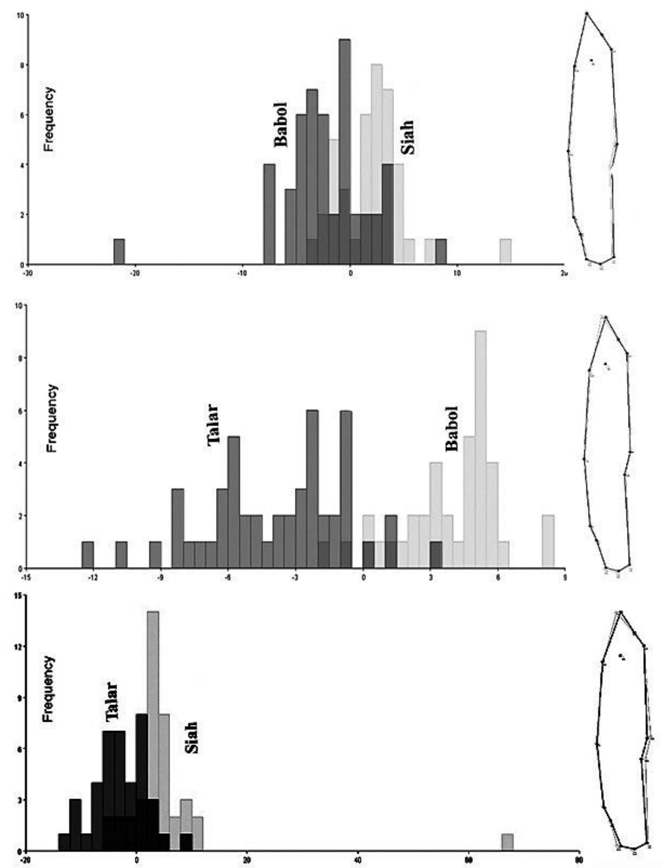

Figure 5. Histogram of discriminate analysis (DA) functions for pair wise competitions of loaches (left). Shape differences on the extremities of each river (right).

be due to the geographic isolation allowing morphological differentiation to proceed independently in each river. The Tonkabon, Siah and Guisum are short rivers considering as cold rivers with high slope, whereas Babol, Talar and Sefid are long and warm rivers with low slope. It is well-known that morphological characteristics can show high plasticity in response to differences in environmental conditions (Mousavi-Sabet and Anvarifar, 2013; Heidari et al., 2013, 2014).

The PCA showed a morphological segregation of the studied fishes based on the characters, i.e. head shape, pre-dorsal, pre-anal distances, pre-orbital and post-orbital distances, body length, body depth, caudal peduncle depth, caudal peduncle length, dorsal and anal fins origin and caudal fin origin positions for gobies and head length, pre-orbital and post-orbital distances, body length and caudal peduncle length for loaches. Body shape differences for these benthic fishes shows a longer snout, shallow body and head depths, and elongated body for the Sefid, Talar and Babol populations versus relatively short snout, and deep body and head for Tonekabon, Siah and Guisum groups. These fishes tend to have a lower caudal peduncle depth in environments with faster water current in the Sefid, Talar and Babol rivers. Similar trends have been observed in other fish species inhabiting stream environments (McLaughlin and Grant, 1994; Pakkasmaa and Piironen, 2000; Paknejad et al., 2014). Empirical study has indicated that the cost of deviating from the optimal current velocity for swimming increased markedly in such high-drag fishes with a bottom-dwelling body shape (Pakkasmaa and Piironen, 2000). Gobies are typical benthic fishes in that they hold their large disc out ventrally when in a current and use them to hydrodynamically press onto the substratum (Vasil'yeva and Vasil'yev, 1995). For such benthic fishes, body shape with a lower caudal peduncle depth would be expected to reduce the influence of shear stress related to positioning energetics even under high water velocity (McLaughlin and Grant, 1994; Pakkasmaa and Piironen, 2000). Some empirical studies have indicated that the deterioration of body condition could influence the fitness-related attributes of individuals, such as fecundity, competitive ability and predator avoidance (e.g. Hoey and McCormick, 2004).

The most prominent differences in separate morphometric features were observed for four characters. The snout length, body and head depths and body length. Snout and body lengths were smaller in short rivers as compared to longer rivers specimens and body and head depths were longer in short rivers specimens. In addition, these benthic fish have longer disc in rivers with faster water currents, including Sefid, Talar and Babol in compare with Tonekabon, Siah and Guisum rivers with slower water velocity. The influences of environmental parameters on morphometric characters are considered by several authors in the course of fish population segregation already (e.g. Tura, 2004; Yamamoto et al., 2006; Pollar et al., 2007; Eschmeyer and Fong, 2011;

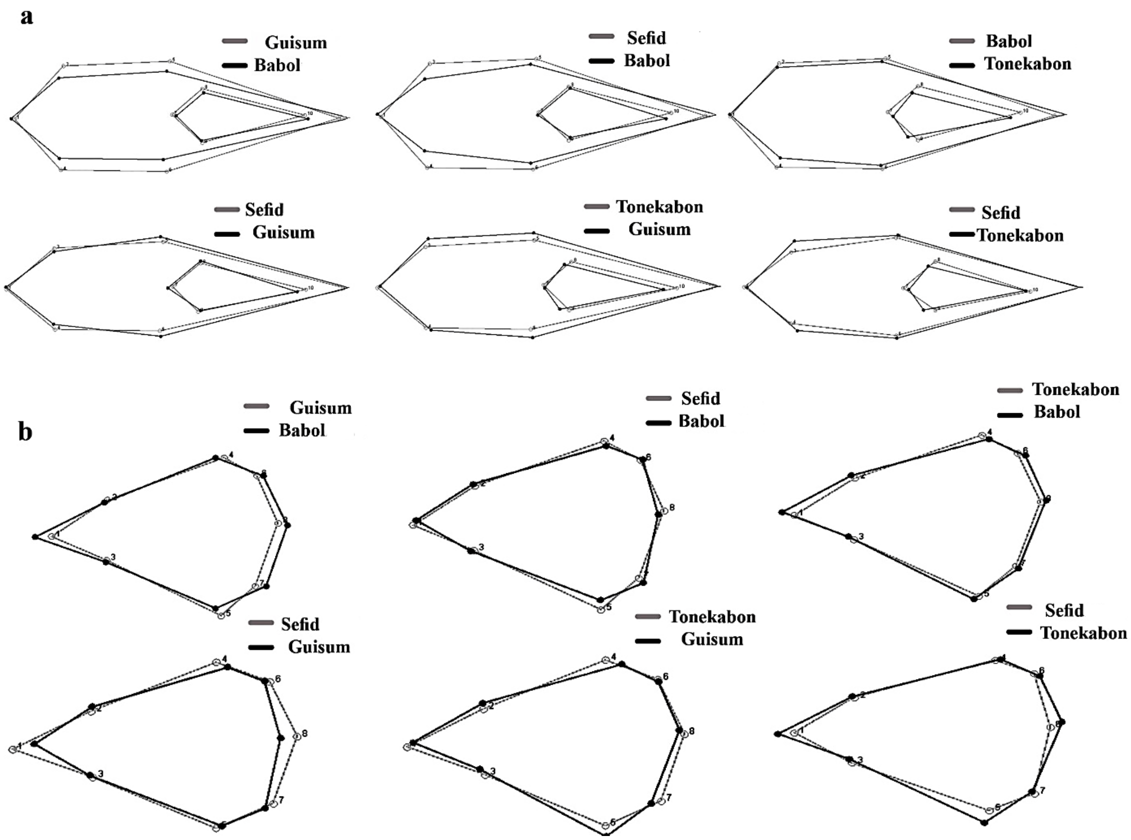

Figure 6. Ventral and dorsal shape variation analysis of gobies collected from the studied rivers along the southern Caspian Sea basin. (a) Ventral view (b) dorsal view.

Vatandoust et al., 2014a). Different rivers in the same basin have various conditions that can change the feeding habits and food items, growth pattern and reproductive strategy of individual species (Mousavi-Sabet and Anvarifar, 2013; Kohestan-Eskandari et al., 2014; Vatandoust et al., 2014b, 2015; Mousavi-Sabet et al., 2016). The phenotypic plasticity of fish is very high and quickly adapt themselves by their physiology and behavior to environmental changes. These modifications ultimately change their morphology (Turan et al., 2004). Along the southern Caspian Sea basin there are probably very small environmental changes from place to place. Therefore, each river system can possess a unique environment that differs from other rivers of along the southern Caspian Sea basin.

The present study provides basic information about the body shape variation of these fishes in relation to different environmental condition in the southern Caspian Sea basin rivers. To accurately delineate units for conservation, multiple characters, such as ecological and genetic features, must be examined and the processes that influence those characters must be understood (Yamamoto et al., 2006; Eschmeyer and Fong, 2011). Therefore, to investigation of morphological variation between rivers of along the southern Caspian Sea basin, further exploration is necessary using DNA techniques under different environmental conditions.

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