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PRIMARY RESEARCH PAPER

Detection of morphometric differentiation between isolated up- and downstream populations of Siah Mahi (*Capoeta capoeta gracilis*) (Pisces: Cyprinidae) in the Tajan River (Iran)

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Abstract It has been postulated that the building of the Shahid-Rajaei dam on the Tajan River around 1995 has lead to the morphological divergence of Siah Mahi *Capotes capoeta gracilis* (Pisces) of up- and downstream populations due to the isolation. A 13-landmark morphometric truss network system was used to investigate the hypothesis. Univariate analysis of variance showed significant differences between the means of the two groups for 45 standardized morphometric measurements out of 78 characters studied. In linear discriminant function analysis (DFA), the overall assignment of individuals into their original groups was 87.6%. The proportion of individuals correctly classified into their original groups was 90.3% for upstream and 83.7% for downstream population. The principal component analysis (PCA) scatter plot of individual component scores between PC1 and PC2 showed 121 fish specimens grouped into two areas but with a relativity high degree of overlap between two populations. Clustering analysis based on Euclidean square distances among groups of centroids using an UPGMA resulted into two main clusters indicating two populations of *C. c. gracilis*. The present study indicated the presence of two morphologically different populations of *C. c. gracilis* in the Tajan River across the Shahid-Rajaei dam, probably due to their limited downstream dispersal and the elimination of upstream migration altogether, due the construction of the dam.

Keywords Siah Mahi · Dam · Morphometric differentiation · Truss network system · Tajan River

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Introduction

Many freshwater fish species are currently threatened by direct and indirect influences of human activities, such as habitat destruction and fragmentation (Yamamoto et al., 2006). Construction of dams across rivers in particular affects fish movements, which may restrict the gene flow and lead to differentiation of populations (Meldgaard et al., 2003). The anthropogenic disturbances are likely to alter the genetic diversity within populations and genetic differentiation between populations (Yamamoto et al., 2004). It has been suggested that fragmentation of river ecosystems may result in the alteration of migration patterns among fish populations (Horvath & Municio, 1998; Craig, 2001; Jager et al., 2001), producing ‘genetic stocks’ that are reproductively isolated units and are genetically different from other stocks (Carvalho & Hauser, 1994).

One of the most important rivers in the southern Caspian basin is the River Tajan with 19.4 m^3/s flow in this region (Nazariha & Alinezhad, 1999). The predominant fish in this river is Siah Mahi, *Capoeta capoeta gracilis* (Keyserling, 1861). The genus *Capoeta* contains potamodromous cyprinid fish, inhabiting both lotic and lentic habitats (Samaee et al., 2006) and generally occurring in lakes and streams with fast- and slow-flowing waters (Turan, 2008). They are also distributed throughout the freshwater river systems of the South Caspian Sea basin (Abdoli et al., 2008; Samaee et al., 2009). Morphologically and genetically variable populations of *C. c. gracilis* in different rivers of the southern Caspian Sea have been reported by various authors (Samaee et al., 2006, 2009). *C. c. gracilis* is an omnivorous species and feeds on detritus, ooze, some higher plants and small amounts of blue-green algae, phytoplankton, diatoms, chironomids, Ephemeroptera, mollusks, etc. (Amanov, 1970; Coad, 2008; Samaee et al., 2009). Siah Mahi fish mature by 2–4 years of age and at a length of 15–20 cm, some populations attaining maturity as early as their first year and at a size of 10 cm (Coad, 2008). This species spawns between March and September (Berg, 1949) with water temperatures ranging between 16 and 23°C (Amanov, 1970). In addition to its ecological significance, *C. c. gracilis* is an important species harvested for sport and inland water fishing (Kiabi et al., 1999). Eggs are laid on sand and stone beds and covered over by them (Coad, 2008).

The Shahid-Rajaei dam was constructed on the Tajan River in 1995 and the dam is devoid of either fishways or fish ladders (Nazariha & Alinezhad, 1999). According to McAllister et al.’s (2001a) classification of dams, Shahid-Rajaei dam on the River Tajan may be classified as a major dam, which has effectively fragmented the river Tajan into upstream and downstream parts and has probably blocked the migration of the fish between these two sections.

Various effects of the dams on fish population have been documented in the recent past. Dakin et al. (2007) showed that the Morgan-Falls dam on Chattahoochee River has reduced the genetic diversity in the population of shoal bass, *Micropterus cataractae*, especially in the upstream population compared to the downstream population, and the morphological differences between the two populations were highly significant. Yamamoto et al. (2006) also noted similar results in white-spotted charr, *Salvelinus leucomaenis*, populations after 20 years from the construction of the dam.

The long-term isolation of populations and interbreeding can lead to morphometric variations between populations, and this morphometric variation can provide a basis for population differentiation. The study of morphometrics using the truss network system (Strauss & Bookstein, 1982) is a landmark based on geometric morphometrics, which poses no restriction on the directions of variation and localization of shape changes, and is very effective in capturing information about the shape of an organism (Cavalcanti et al., 1999). It covers the entire fish in a uniform network, and theoretically, it increases the likelihood of extracting morphometric differences between specimens (Turan, 1999; Akbarzadeh et al., 2009; Kocovsky et al., 2009). Therefore, it has been increasingly used for the differentiation of various populations within a species and also various species (Strauss & Bookstein, 1982; Bookstein et al., 1985).

Siah Mahi is an important fish species of the Tajan River; however, its spatial distribution has not been studied and also, to the best of our knowledge, there are no reports with regard to the impact of dams on morphometric differentiation of fish populations in the Caspian Sea basin.

Considering the above-mentioned facts, the present study was aimed at assessing the impact of construction of the Shahid-Rajaei dam on the morphometric traits of *C. c. gracilis* and in examining whether specific ecological constraints, due to geographic variation, have influenced the population of *C. c. gracilis* to differentiate into various stocks.

Materials and methods

Sampling

A total of 121 individuals of *C. c. gracilis* were collected from two sampling sites, one each from upstream (36°11′24.91″N, 53°19′32.13″E; 72 individuals) and downstream (36°16′15.36″N, 53°12′51.44″E; 49 individuals) of the Shahid-Rajaei dam on the Tajan River (Fig. 1), in February 2010 by electroshocking with 200–300 V. The sampled fish were fixed in 10% formaldehyde at the sampling site and transported to the Fisheries Laboratory of Department of Fisheries, University of Babol, for further studies.

Laboratory work

A total of 78 distance measurements between 13 landmarks were surveyed using the truss network system according to Bookstein (1991) and Strauss & Bookstein (1982) with minor modifications for this species (Fig. 2). Truss network measurements are a series of measurements calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form (Turan, 1999). In this system, for example, the distance between landmarks 3 and 6 is a truss character in first quadrilateral or cell (landmarks 3, 6, 5, 7) (Fig. 1). Measurements of specimens are made by collecting X–Y coordinate data for relevant morphological features, and followed the three-step process as described below (Turan, 1999).

The fish were placed on a white board with dorsal and anal fins held erect by pins. The right body profile of each fish was photographed with a 300-dpi, 32-bit color digital camera (Cybershot DSC-F505; Sony, Japan). Images were saved in jpg format and analyzed with TPSdig (v.2.04; Rohlf, 2005) to coordinates of 13 landmarks. A box truss of 26 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish (Cardin & Friedland, 1999). All measurements were transferred to a spreadsheet file (Excel 2007), and the X–Y coordinate data transformed into linear distances by computer (using the Pythagorean Theorem) for subsequent analysis (Turan, 1999).

After image capture, each 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 males and females of *C. c. gracilis*.

Data analysis

Size dependent variation was corrected by adapting an allometric method as suggested by (Elliott et al., 1995)

$$M_{\mathrm{adj}} = M\,(L_s/L_0)^{\mathrm{b}},$$

where M is original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of

Fig. 1 Location of sampling sites upstream and downstream of the Shahid-Rajaei dam on the Tajan River, Iran

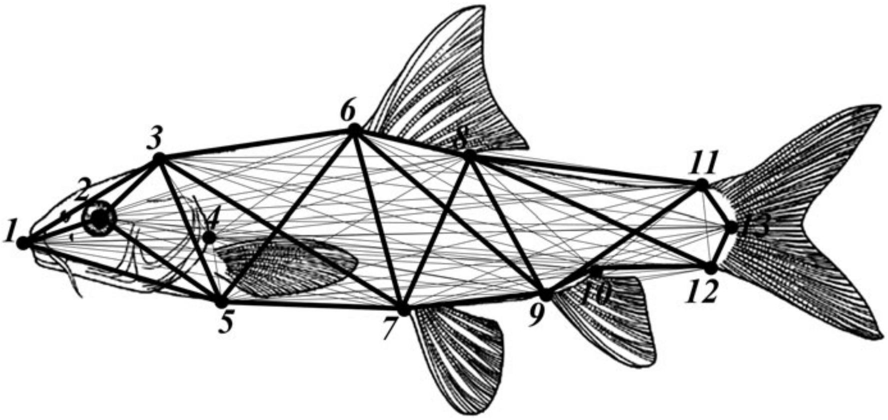

Fig. 2 Schematic image of a *C. c. gracilis* depicting the 13 landmarks and associated box truss used to infer morphological differences among populations. *1* Tip of snout; *2* center of eye; *3* forehead (end of frontal bone); *4* end of operculum; *5* dorsal origin of pectoral fin; *6* origin of dorsal fin; *7* origin of pelvic fin; *8* termination of dorsal fin; *9* origin of anal fin; *10* termination of anal fin; *11* dorsal side of caudal peduncle, at the nadir; *12* ventral side of caudal peduncle, at the nadir; *13* end of lateral line (Adapted from truss box, after Strauss & Bookstein, 1982 and Bookstein, 1991)

the fish, L_s the overall mean of standard length for all fish from all samples in each analysis, and b was estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish from both the groups. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length (Turan, 1999).

Univariate analysis of variance (ANOVA) was performed for each morphometric character to evaluate the significant difference between the two locations (Zar, 1984), and those morphometric characters which showed highly significant variations ($P < 0.01$) 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 (Johnson, 1981; Kocovsky et al., 2009), in order to obtain a stable outcome from multivariate analysis. In the present study, linear discriminant function analyses (DFA), principal component analysis (PCA) and cluster analysis (CA) were employed to discriminate the two populations.

Principal component analysis helps in morphometric data reduction (Veasey et al., 2001), in decreasing the redundancy among the variables (Samaee et al., 2006) and in extracting a number of independent variables for population differentiation (Samaee et al., 2009). The Wilks' λ was used to compare the difference between all groups. The DFA was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using PCC was done to estimate the expected actual error rates of the classification functions. As a complement to discriminant analysis, morphometric distances between the individuals of two groups were inferred to CA (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 the clustering algorithm (Sneath & Sokal, 1973).

Statistical analyses for morphometric data were performed using the SPSS version 11.5 software package, Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) (Rohlf, 1990) and Excel (Microsoft Office, 2007).

Results

Descriptive data for the sex ratio, range (minimum–maximum), mean, standard deviation (SD) of length and weight in case of sampled specimens are shown in Table 1. The age of the samples upstream and downstream of the Shahid-Rajaei dam on the Tajan River ranged from 1+ to 5+ years with an average of 2 years indicating that 6+ generations may have developed after the construction of the dam. Age composition of *C. c. gracilis* depending on sex upstream and downstream is shown in Table 2.

The correlation between transformed morphometric variables and standard length was non-significant ($P > 0.05$) which confirms that size or allometric signature on the basic morphological data was accounted for. Statistically significant differences among upstream and downstream populations of Siah Mahi of Shahid-Rajaei dam was observed in 45 morphometric characters out of 78 studied (Table 2). Of these 45 characters, 28 were found to be highly significant ($P < 0.01$) and were used further for multivariate analysis. The morphometric characters between two sexes did not diffed significantly ($P > 0.05$) (Tables 3 and 4); hence, the data for both sexes were pooled for all subsequent analyses.

A common problem with many fish morphology studies that use multivariate analysis is potentially inadequate sample size. For decades, authors of theoretical works on PCA and DFA recommended that the ratio of the number of organisms measured (N) relative to the parameters included (P) in the analysis be at least 3–3.5 (Johnson, 1981; Kocovsky et al., 2009). Small N alues may fail to adequately capture covariance or morphological variation, which may lead to false conclusions regarding differences among groups (McGarigal et al., 2000). In this study, for multivariate analysis we used only from morphometric characters that were significant at a high level ($P < 0.01$), and under these circumstances the N:P ratio was 4.32 (121/28) for this traits including 1–2, 1–5, 1–6, 1–12, 2–3, 2–7, 2–9, 2–10, 2–11, 2–12, 2–13, 3–4, 3–5, 3–12, 3–13, 4–9, 4–11, 4–12, 4–13, 5–11, 5–12, 5–13, 6–11, 6–12, 6–13, 8–12, 8–13 and 10–12.

In order to determine which morphometric measurement most effectively differentiates populations, the contributions of variables to principal components (PC) were examined. To examine the suitability of the data for PCA, Bartlett's Test of sphericity and the Kaiser–Meyer–Olkin (KMO) measure was performed. The Bartlett's Test of sphericity tests the hypothesis that the values of the correlation matrix equal zero (small significance levels support the hypotheses that there are real correlations between the variables) and the KMO measure of sampling adequacy tests whether the partial correlation among variables is sufficiently high (Nimalathasan, 2009). The KMO statistics varies between 0 and 1. Kasier (1974) recommends that values greater than 0.5 are acceptable. Between 0.5 and 0.7 are mediocre, between 0.7 and 0.8 are good, between 0.8 and 0.9 are superb (Field, 2000). In this study, the value of KMO for overall matrix is 0.653 and the Bartlett's Test of sphericity is significant ($P \leq 0.01$). The results (KMO and Bartlett's) suggest that the sampled data is appropriate to proceed with a factor analysis procedure.

Principal component analysis of 28 morphometric measurements extracted seven factors with eigenvalues >1, explaining 86.91% of the variance (Table 5). The first principal component (PC1) accounted for 37.29% of the variation and the second principal component (PC2) for 14.07% (Table 5), and the most significant loadings on PC1 were 1–12, 2–12,

Table 2 Age composition of *C. c. gracilis* depending on sex upstream and downstream of the Shahid-Rajaei dam on the Tajan River

Age (years)	Upstream		Downstream	
	Males	Females	Males	Females
1+	10	8	5	8
2+	14	16	9	16
3+	5	11	3	5
4+	0	3	0	2
5+	1	2	0	1
Total	30	42	17	32

Table 1 Descriptive data [mean ± SD and range of standard length (mm) and weight (g)] of *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River

Sample	Sex	*n*	Length		Weight	
			Min–max	Mean ± SD	Min–max	Mean ± SD
Upstream	Males	30	53.90–144.90	102.78 ± 21.98	4.06–67.21	21.07 ± 10.02
	Females	42	46.70–180.20	115.91 ± 32.16	6.67–111.39	32.11 ± 26.95
	Total	72	46.70–180.20	110.44 ± 28.93	4.06–111.39	26.59 ± 21.76
Downstream	Males	17	49.40–169.90	99.93 ± 31.90	3.04–97.62	19.79 ± 10.48
	Females	32	45.40–196.10	84.05 ± 39.98	2.41–113.63	13.24 ± 6.37
	Total	49	45.40–196.10	89.56 ± 37.81	2.41–113.63	16.51 ± 7.53

Table 3 The results of ANOVA for morphometric measurements of *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River

Morphometric measurements	*F* value	*P* value	Morphometric measurements	*F* value	*P* value	Morphometric measurements	*F* value	*P* value
1–2	39.994	0.000	3–7	1.679	0.198	6–9	6.109	0.015
1–3	4.578	0.034	3–8	0.142	0.707	6–10	6.451	0.012
1–4	6.472	0.012	3–9	3.304	0.072	6–11	12.889	0.000
1–5	11.028	0.001	3–10	2.197	0.141	6–12	22.718	0.000
1–6	11.197	0.001	3–11	6.126	0.015	6–13	22.527	0.000
1–7	0.311	0.578	3–12	16.270	0.000	7–8	2.006	0.159
1–8	2.956	0.088	3–13	13.180	0.000	7–9	0.096	0.757
1–9	1.172	0.281	4–5	6.160	0.014	7–10	0.522	0.471
1–10	0.306	0.581	4–6	1.506	0.222	7–11	0.016	0.900
1–11	3.712	0.050	4–7	4.734	0.032	7–12	2.798	0.097
1–12	16.012	0.000	4–8	0.003	0.953	7–13	0.883	0.349
1–13	0.000	0.988	4–9	7.928	0.006	8–9	4.016	0.047
2–3	7.521	0.007	4–10	5.790	0.018	8–10	6.065	0.015
2–4	1.360	0.246	4–11	10.567	0.001	8–11	6.131	0.015
2–5	5.368	0.022	4–12	23.777	0.000	8–12	17.973	0.000
2–6	0.788	0.376	4–13	31.936	0.000	8–13	14.175	0.000
2–7	7.105	0.009	5–6	5.543	0.020	9–10	0.213	0.645
2–8	2.139	0.146	5–7	2.436	0.121	9–11	0.007	0.932
2–9	13.437	0.000	5–8	0.321	0.572	9–12	3.397	0.068
2–10	11.050	0.001	5–9	6.620	0.011	9–13	1.736	0.190
2–11	16.307	0.000	5–10	4.520	0.036	10–11	1.328	0.252
2–12	41.269	0.000	5–11	8.196	0.005	10–12	7.026	0.009
2–13	63.270	0.000	5–12	22.046	0.000	10–13	6.542	0.012
3–4	7.178	0.008	5–13	26.297	0.000	11–12	1.729	0.191
3–5	24.451	0.000	6–7	2.664	0.105	11–13	0.235	0.629
3–6	1.950	0.165	6–8	0.000	0.996	12–13	0.587	0.445

3–12, 4–12, 5–12, 6–12, 8–12, 10–12 and on PC2 were 1–2, 1–5, 3–4, 3–5, 4–9, 4–12, 4–13, 5–12, 5–13 (Table 6). Visual examination of plots of PC1 and PC2 scores revealed that specimens were grouped into two areas, but with a some degree of overlap between two populations (Fig. 3). In this analysis, the characteristics with an eigenvalues exceeding 1 were included and others discarded (Table 6).

One method to reduce the number of factors to something below that found by using the "eigen value greater than unity" rule is to apply the scree test (Cattell, 1966). In this test, eigenvalues are plotted against the factors arranged in descending order along the X-axis. The number of factors that correspond to the point at which the function so produced appears to change slope is deemed to be the number of useful factors extracted. This is a somewhat arbitrary procedure. Its application to this dataset led to the conclusion that the first seven factors should be accepted (Fig. 4). It is worth mentioning here that factor loading greater than 0.30 is considered significant, 0.40 more important and 0.50 or greater very significant (Nimalathasan, 2009). The rotated (Varimax) component loadings for the eight components (factors) are presented in Table 6. For parsimony, in this study only those factors with loadings above 0.40 were considered significant.

The Wilks' λ tests of discriminant analysis indicated significant differences in morphometric characters of the two populations. In this test, one function was highly significant ($P \leq 0.01$) (Table 7).

Table 4 The results of ANOVA for morphometric measurements of between two sexes of *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River

Morphometric measurements	*F* value	*P* value	Morphometric measurements	*F* value	*P* value
1–2	0.015	0.903	3–13	2.630	0.108
1–5	2.978	0.088	4–9	0.004	0.951
1–6	0.786	0.378	4–11	0.998	0.320
1–12	0.034	0.855	4–12	0.016	0.901
2–3	2.086	0.152	4–13	1.230	0.270
2–7	1.426	0.235	5–11	0.421	0.518
2–9	0.786	0.377	5–12	0.226	0.636
2–10	0.043	0.837	5–13	0.144	0.706
2–11	2.426	0.123	6–11	1.684	0.197
2–12	0.518	0.473	6–12	2.981	0.087
2–13	0.187	0.666	6–13	0.881	0.350
3–4	3.579	0.061	8–12	1.545	0.217
3–5	0.299	0.586	8–13	1.952	0.166
3–12	0.840	0.362	10–12	2.031	0.157

Table 5 Eigenvalues, percentage of variance and percentage of cumulative variance for the 15 PC in case of morphometric measurements for *C. c. gracilis*

Factor	Eigenvalues	Percentage of variance	Percentage of cumulative variance
PC1	10.443	37.296	37.296
PC2	3.941	14.075	51.371
PC3	3.239	11.567	62.938
PC4	2.685	9.590	72.528
PC5	1.528	5.458	77.986
PC6	1.440	5.144	83.130
PC7	1.059	3.781	86.911

The linear discriminant analysis gave an average PCC of 87.6% for morphometric characters indicating a high rate of correct classification of individuals into their original populations. The PCC (90.3%) for the downstream population was higher compared to the PCC (83.7%) for the upstream population. The cross-validation test results were comparable to the results obtained from PCC (Table 8). Correlations between the measured morphometric variables and the discriminant functions are presented in Table 9. Correlations between the measured morphometric variables and the discriminant functions for *C. c. gracilis* specimens showed in Table 8 and the measurements used in this analysis included 2–3, 2–11, 3–5, 4–11 and 8–12.

The dendogram derived from CA of Euclidean square distances among groups of centroids showed two clusters indicating the presence of two populations of Siah Mahi (Fig. 5).

Discussion

The Siah Mahi in Tajan River are limited to a very short stretch of appropriate habitat, with limited genetic diversity and high differentiation between the upstream and downstream populations, and are probably suffering from the effects of genetic drift and inbreeding. The present study reveals that the Shahid-Rajaei dam on the Tajan River has probably created two morphologically different populations of *C. c. gracilis* upstream and downstream of the dam, because of limited downstream dispersal of fish, and elimination of upstream migration altogether (Dakin et al., 2007). The dams obstruct the upward migration of fish especially that of the migratory species resulting in an ecological trap for migratory fish that ascend the fish passages (Pelicice & Agostinho, 2008). Fragmentation of the river converts a free-flowing river into reservoir habitat, affecting the

Table 6 Results of factors extraction in PCA after Varimax normalized rotation

Morphometric measurements	Component						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
1–2		−0.5996	−0.4022				
1–5		−0.8392					
1–6					−0.7513		
1–12	0.9202						
2–3							0.9001
2–7				0.8347			
2–9				0.8185			
2–10			0.5342	0.6399			
2–11			−0.7708				
2–12	0.8236						
2–13			0.7508				
3–4		−0.6978					
3–5		−0.8337					
3–12	0.7295		0.5124				
3–13			0.8184				
4–9		0.4251		0.7546			
4–11						0.7967	
4–12	0.7202	0.4554					
4–13		0.6613					
5–11						0.7689	
5–12	0.7396	0.4985					
5–13		0.7118					
6–11					0.6506	0.6502	
6–12	0.5166				0.6063		
6–13			0.5405		0.7630		
8–12	0.5980				0.6799		
8–13					0.8468		
10–12	0.6904						

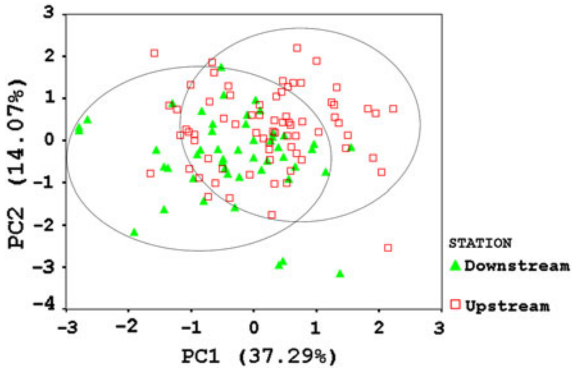

Fig. 3 Plot of the factor scores for PC1 and PC2 of morphometric measurements for *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River

ecosystem. This phenomenon in turn affects the migratory pattern of fish populations (Jager et al., 2001). In the downstream area of large dams, most of the impacts are negative, while above the dam the effects are not much different. The effects are very severe particularly in the tropics, where the number of species present has increased (Craig, 2001). The construction of dams has led to loss of many stocks of *Salmonidae* and *Clupeidae* (Craig, 2001). In the Columbia River, USA, more than 200 stocks of anadromous, Pacific salmonids became extinct (McAllister et al., 2001b). Sturgeon populations in the Caspian Sea especially in Iran are mainly recouped by hatchery-bred stocks, since Russian dams have blocked natural spawning migrations. Holcík (1999)

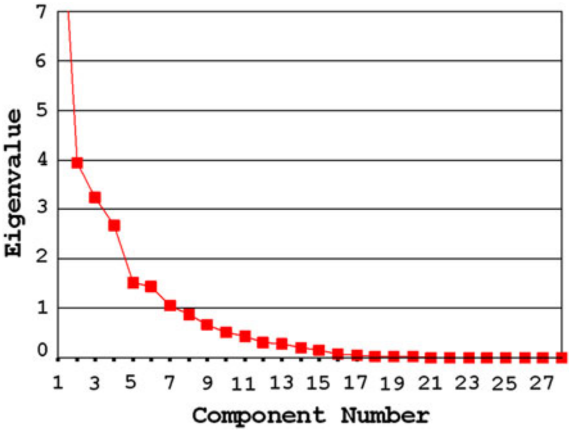

Fig. 4 Scree plot of principal component in case of morphometric measurements for *C. c. gracilis*upstream and downstream of the Shahid-Rajaei dam on the Tajan River

Table 7 Result of Wilks' λ test for verifying difference among two populations when morphometric measurements are separately compared using DFA

Test of functions	Wilks' λ	χ^2	*df*	Signif.
1	0.426	99.384	5	0.000

Table 8 Percentage of specimens classified in each group and after cross validation for morphometric measurements for *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River

Area	Upstream (%)	Downstream (%)
Original		
Upstream	91.7	8.3
Downstream	6.1	93.9
Cross-validated		
Upstream	91.7	8.3
Downstream	6.1	93.9

stated that while dramatic declines in migratory species such as lampreys, sturgeons, salmons and clupeids are well known in European rivers, other fish, the so-called resident or nonmigratory fish which perform in-stream movements, require attention.

The analysis of variance revealed significant phenotypic variation between the two populations (Tables 3 and 4). Discriminant function analysis (DFA) could be a useful method to distinguish different stocks of the same species (Karakousis et al., 1991). In the present study, 87.6% of individuals were correctly classified into their respective groups by DFA (Table 8), indicating a high differentiation between the populations of Siah Mahi in the study area. This segregation was partly confirmed by PCA, where the graphs of PC1 and PC2 scores for each sample (Fig. 3) revealed that the two populations were clearly distinct from each other.

Table 9 Correlations between the measured morphometric variables and the linear discriminant functions for *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River

Morphometric measurements	DF1	Morphometric measurements	DF1
1–2	−0.4346	3–13	0.2419
1–5	−0.2930	4–9	0.1739
1–6	−0.1009	4–11	0.2568
1–12	0.2151	4–12	0.3142
2–3	0.2166	4–13	0.4426
2–7	0.2414	5–11	0.2136
2–9	0.2299	5–12	0.2857
2–10	0.2329	5–13	0.3891
2–11	−0.3190	6–11	0.2376
2–12	0.4265	6–12	0.3502
2–13	0.5788	6–13	0.3505
3–4	−0.0950	8–12	0.3349
3–5	−0.3906	8–13	0.3154
3–12	0.2296	10–12	0.1325

These morphological differences may be solely related to body shape variation and not to size effects which were successfully accounted for by allometric transformation. On the other hand, size-related traits play a predominant role in morphometric analysis and the results may be erroneous if not adjusted for statistical analyses of data (Tzeng, 2004). In the present study, the size effect was removed successfully by allometric transformation, and the significant differences between the populations are due to the body shape variation when tested using ANOVA and multivariate analysis.

The causes of morphological differences between populations are often quite difficult to explain (Poulet et al., 2004). It has been suggested that the morphological characteristics of fish are determined by genetic, environment and the interaction between them (Swain & Foote, 1999; Poulet et al., 2004; Pinheiro et al., 2005). The environmental factors prevailing during the early development stages, when

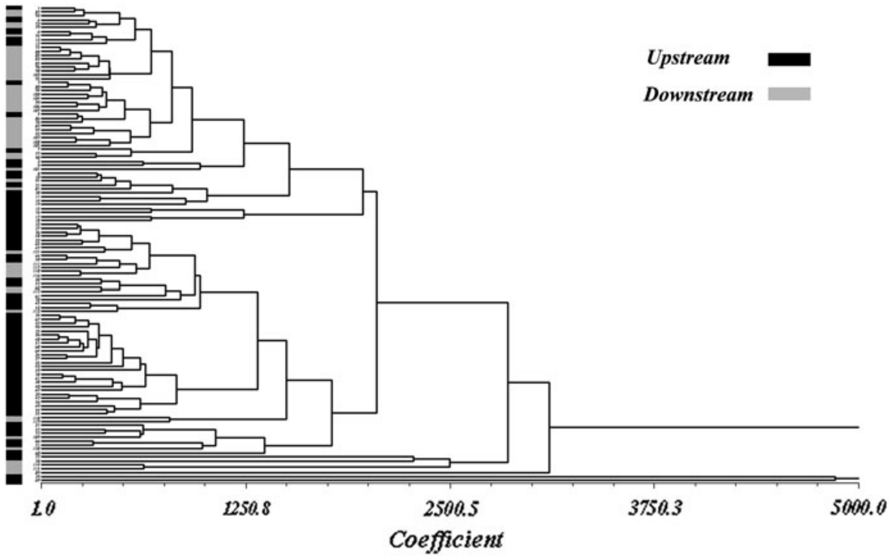

Fig. 5 Dendrogram derived from cluster analyses of morphometric measurements on the basis of Euclidean distance for *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River

the individual's phenotype is more amenable to environmental influence is of particular importance (Pinheiro et al., 2005). The phenotypic variability may not necessarily reflect population differentiation at the molecular level (Ihssen et al., 1981). Apparently, the fragmentation of river impoundments can lead to an enhancement of pre-existing genetic differences, providing a high interpopulational structuring (Esguicero & Arcifa, 2010). Such a kind of morphological and genetic discrimination has been reported among six populations of *C. c. gracilis* located in the Aras, Sefidrud, Shirud, Tonekabon, Haraz and Gorganrud River systems in Iran (Samaee et al., 2006).

Construction of a dam on a river can affect upward and downward migration of species. The blockage of fish movements, especially the upstream movement, can have a very significant impact on fish stocks by obstructing the genetic exchange with downstream segments (McAllister et al., 2001a). Also, the construction of a dam can lead to dramatic changes in the environment of a river and particularly affect fish communities. The influences of environmental parameters on morphometric characters have been well discussed by several authors in the course of fish population segregation (e.g., Swain & Foote, 1999). The quantity of water discharged, when it is discharged during dial and seasonal cycles, relative to the river's natural flow pattern, and abiotic characteristics of the discharge such as temperature, oxygen, turbidity, and water quality, significantly affect downstream biodiversity (McAllister et al., 2001b).

It is well known that morphological characteristics can show high plasticity in response to differences in environmental conditions (Wimberger, 1992). Therefore, the distinctive environmental conditions of the upstream and downstream regions relative to the other study areas may underline the morphological differentiation between these two sites. Differentiation between samples from adjacent stations may be due to the geographic isolation of stations by artificial obstacles from each other allowing morphological differentiation to proceed independently at each station (Samaee et al., 2006). Dams can also alter the feeding habits of the species, availability of food items, growth pattern and reproductive strategy of fish species living upstream and downstream of a river. The importance of such factors on producing morphological differentiation in fish species is well known (Akbarzadeh et al., 2009). Also, fragmentation of rivers by dams further biases colonization rates by blocking upstream, more than downstream, movement (Jager et al., 2001).

The present study indicates that there exist two distinct populations of *C. c. gracilis* upstream and downstream of the Shahid-Rajaei dam on the Tajan River, and the probable reason for the differentiation of the population is the construction of the dam. A detailed study involving the molecular genetics and environmental aspects may further confirm the present findings unambiguously. In conclusion, the present study provides basic information about the morphometric differentiation of *C. c. gracilis* populations in the upstream and downstream sections of the Tajan River and suggests that morphological variations observed in *C. c. gracilis* should be considered in fisheries management and commercial exploitation of this species.

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