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A morphology-based hypothesis for homeward migration success and population differentiation in the anadromous kutum *Rutilus kutum* (Pisces: Cyprinidae) along the southern Caspian Sea, Iran

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Abstract. The anadromous *Rutilus kutum* Kamenskii, 1901 is the most important fish in north of Iran with the highest economic value. Ignoring the natural process of reproduction due artificial propagation, catching some migrating broodstocks from the sea (not from the estuaries) and mixing the produced fingerlings in culture ponds within restocking programs in the last 30 years have caused an uncertainty about homing success and population differentiation in the fish. A 13-landmark morphometric truss network system was used to investigate the hypothesis of homeward migration success and population differentiation, while the previous studies revealed low genetic variability among the fish populations. A total of 504 adult specimens were caught from four localities along the southern Caspian Sea in the reproductive period. Univariate analysis of variance showed significant differences among the four groups for 78 standardized morphometric measurements. In linear discriminant function analysis (DFA), the overall assignments of individuals into their original groups were 73.3 % and 80.3 % in males and females, respectively. The principal component analysis (PCA) and clustering analysis based on Euclidean square distances showed that the 504 examined specimens grouped into distinct areas. The results showed that each sampling site represents independent population which confirmed the success of homeward migration. The high grouping ranges suggests that almost all populations return to their birthplace river to breed, which results in high inbreeding. The results can be interesting for management and conservation programs of this valuable species in the Caspian Sea.

Key words: homing, population structure, truss network system, sexual dimorphism

Introduction

Kutum or mahisefid, *Rutilus kutum* (Kamenskii, 1901), is one of the most important fishes for fisheries and stocking programs in south of the Caspian Sea (Abdolhay et al. 2012a). This species is endemic to the Caspian Sea and considered as an economically high value species. It is mainly distributed along the southern coasts of the Caspian Sea (Valipour & Khanipour 2008). Nearly 60 % of the catch of bony fishes in this region goes to this species (Rezvani et al. 2012). This species is reported as a candidate species of least concern in the southern Caspian Sea basin according to IUCN criteria (Abdolhay et al. 2012b). Natural reproduction of kutum has been limited because of destruction of natural spawning areas and other factors (Ghaninejad

& Abdulmaleki 2007). As a result, the Iranian fisheries organization launched a restocking project from 1984 (Ebrahimi & Ouraji 2012). This project seems to be successful in increasing kutum biomass in the Caspian Sea, and the annual landing of kutum increased from 563 t in 1982 to 16118 t in 2006 (Salehi 2008). To restock this valuable species, the Iranian fisheries organization annually produces and releases more than 200 million fingerlings into rivers, which carry them towards the Caspian Sea (Heyrati et al. 2007). Ignoring the natural process of reproduction due artificial propagation, catching some migrating broodstocks from the sea (not from the estuaries), mixing the produces fingerlings in the culture ponds within restocking programs, and inattention to releasing the produced fingerling into the

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rivers where the broodstocks were caught in the last 30 years have caused an uncertainty about homing success and population differentiation in the fish. To this purpose, Kavan et al. (2009), Chakmehdouz Ghasemi et al. (2009), Rezaei et al. (2010), Abdolhay et al. (2012a, b) and Rezvani et al. (2012) studied genetic diversity of kutum's populations in south of the Caspian Sea, and revealed low genetic variability among fish populations.

These results suggest that due to the long term artificial propagation and growing in the same habitat (Caspian Sea) without any physical barrier, populations of the fish have been mixed and homing behaviour has been hampered. So, the morphometric analysis was planned to investigate the hypothesis of homeward migration success and population differentiation in the valuable fish.

Morphological studies on fishes are important from various viewpoints including evolution, ecology, behaviour, conservation, water resource management and stock assessment (AnvariFar et al. 2011, Mousavi-Sabet & AnvariFar 2013). Suitable and successful management of aquatic organisms stock will be gained by study of genetic stocks of endemic species and identification of populations (Coad 1980). The study of morphological characters with the aim of defining or characterizing fish stock units has been of a strong interest in ichthyology (Tudela 1999). There are some evidences of morphological differences among geographically different populations of kutum (Abdolhay et al. 2010). Conventional and truss morphometrics are normally used to describe morphological variations between different populations of a species. The study of morphometrics using 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 much 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, Cardin & Friedland 1999, Akbarzadeh et al. 2009, Kocovsky et al. 2009).

Due to the destruction of rivers, natural spawning of the fish has reduced significantly, on the other hand, populations of this species are subject of artificial propagation. Then it is important to understand that this unit population shows morphological differentiation. On the other hand despite the biodiversity and commercial importance of kutum, there is not any study available on population differentiation of the fish

in southern coasts of the Caspian Sea. Considering the above mentioned facts, the present study was aimed to obtain information about homeward migration success, population differentiation and morphometric sexual dimorphism of kutum in the southern Caspian Sea, and it can be employed in the future enhancement programs of this species in the region.

Material and Methods

Sampling

A total of 504 adult individuals of kutum were collected from four sampling sites, during the March-April period of 2012 – 40 individuals from Behshahr (36°52' N, 53°33' E), 153 individuals from Sari (36°45' N, 52°55' E), 170 individuals from Mahmod-Abad (36°37' N, 52°13' E) and 141 individuals from Ramsar (36°56' N, 50°38' E) (Fig. 1). The specimens were captured by beach seine.

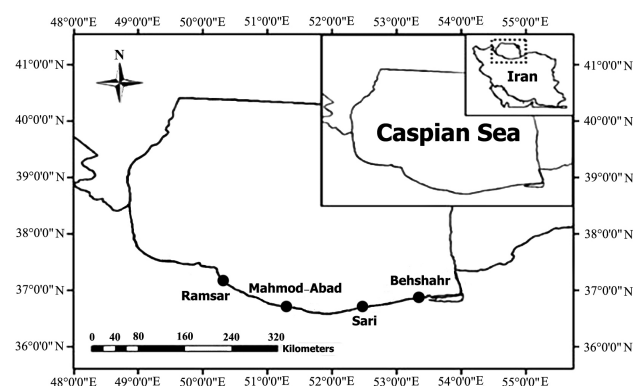


Fig. 1. Location of sampling sites including Behshahr, Sari, Mahmod-Abad and Ramsar in the southern Caspian Sea.

Laboratory work

A total of 78 distance measurements among 13 landmarks were surveyed using truss network system according to Bookstein (1991) and Strauss & Bookstein (1982) with minor modification for this species (Fig. 2). Fish specimens were placed on a white board with dorsal and anal fins erected by pinning. The left body profile of each fish was photographed in 300-dpi, 32-bit colour digital camera (Sony Cybershot DSC-F505, Sony, Japan). Images were saved in jpg format and analyzed by TPSdig (Version 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 2010), and X-Y coordinate data was transformed into linear distances by computer (using the Pythagorean Theorem) for subsequent analysis (Turan 1999).

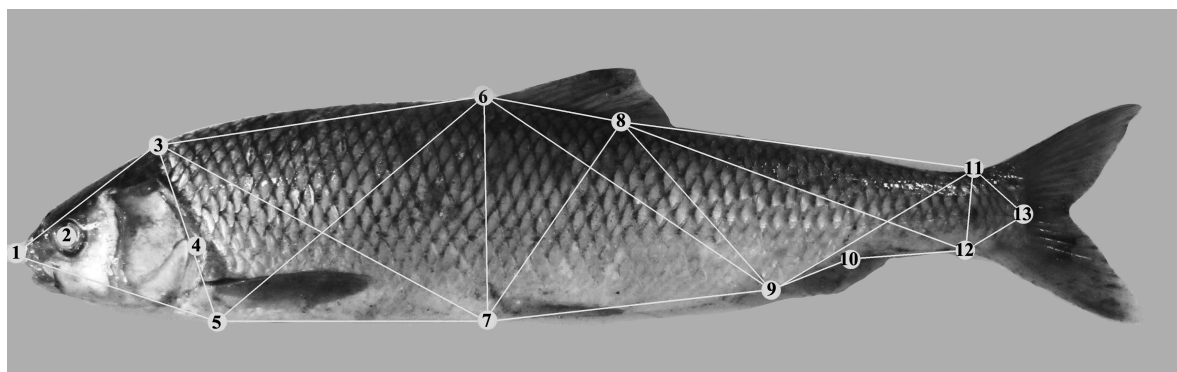


Fig. 2. Digital image of a specimen depicting the thirteen landmarks and associated box truss used to infer morphological differences among populations. 1. Tip of snout 2. Center of eye 3. Forehead (end of frontal bone) 4. End of operculum 5. Dorsal origin of pectoral fin 6. Origin of dorsal fin 7. Origin 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 (modified from Bookstein, 1991 and Strauss & Bookstein, 1982).

After image capture, the fish was dissected to identify the sex of the specimen by macroscopic examination of the gonads and breeding tubercles (evident in males). 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 kutum.

Data analysis

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

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

where M is original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of 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 among the four locations (Zar 1984). In the present study linear discriminant function analyses (DFA), principal component analysis (PCA) and cluster analysis (CA) were employed to discriminate the four populations. Principal component analysis helps in morphometric data reduction (Veasey et al. 2001) in decreasing the redundancy among the variables (Samaee et al. 2006, AnvariFar et al. 2013) and extracting a number of independent variables for population differentiation. The Wilks' lambda was used to compare the difference among 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 among the individuals of four groups were inferred to cluster analysis (Veasey

Table 1. Descriptive data (Mean \pm S.D. and range) of kutum, caught from four sampling sites including Behshahr, Sari, Mahmood-Abad and Ramsar in the southern Caspian Sea.

Station	Sex	N	Min-Max (length mm)	Mean \pm S.D. (length)	Min-Max (weight g)	Mean \pm S.D. (weight)
Behshahr	Male	24	370-573	456 \pm 34.77	440-1700	844 \pm 226.03
	Female	16	288-600	469 \pm 48.19	175-2000	972 \pm 291.42
Sari	Male	83	330-555	455 \pm 46.87	200-2000	978 \pm 381.27
	Female	70	320-600	476 \pm 60.92	250-2318	1115 \pm 460.30
Mahmood-Abad	Male	87	380-535	427 \pm 73.25	320-1420	480 \pm 229.00
	Female	83	300-590	450 \pm 67.19	230-2120	1070 \pm 515.76
Ramsar	Male	73	303-655	428 \pm 48.38	220-2061	767 \pm 274.06
	Female	68	325-600	502 \pm 61.46	331-2318	1353 \pm 471.65

N – Number of specimens.

Table 2. Results of ANOVA for sex dimorphism of morphometric characters of kutum, caught from four sampling sites including Behshahr, Sari, Mahmud-Abad and Ramsar in the southern Caspian Sea.

Morphometric measurements	F value	P value	Morphometric measurements	F value	P value	Morphometric measurements	F value	P value
1-2	22.90	0.00	3-7	66.99	0.00	6-9	50.96	0.00
1-3	1.77	0.18	3-8	44.57	0.00	6-10	21.07	0.00
1-4	1.37	0.24	3-9	63.97	0.00	6-11	13.99	0.00
1-5	0.15	0.70	3-10	44.75	0.00	6-12	17.82	0.00
1-6	53.56	0.00	3-11	39.42	0.00	6-13	11.32	0.00
1-7	41.86	0.00	3-12	40.62	0.00	7-8	73.61	0.00
1-8	35.15	0.00	3-13	34.55	0.00	7-9	77.88	0.00
1-9	52.89	0.00	4-5	30.85	0.00	7-10	38.45	0.00
1-10	38.12	0.00	4-6	87.50	0.00	7-11	33.27	0.00
1-11	34.14	0.00	4-7	74.62	0.00	7-12	33.71	0.00
1-12	35.78	0.00	4-8	50.16	0.00	7-13	24.19	0.00
1-13	7.33	0.01	4-9	73.40	0.00	8-9	70.39	0.00
2-3	3.77	0.05	4-10	50.01	0.00	8-10	31.52	0.00
2-4	17.83	0.00	4-11	43.17	0.00	8-11	21.99	0.00
2-5	65.12	0.00	4-12	45.52	0.00	8-12	24.70	0.00
2-6	56.68	0.00	4-13	37.94	0.00	8-13	15.49	0.00
2-7	42.07	0.00	5-6	84.57	0.00	9-10	3.11	0.08
2-8	61.12	0.00	5-7	67.65	0.00	9-11	0.00	0.96
2-9	43.76	0.00	5-8	51.80	0.00	9-12	0.00	0.99
2-10	38.60	0.00	5-9	76.32	0.00	9-13	0.45	0.50
2-11	40.48	0.00	5-10	51.79	0.00	10-11	0.95	0.33
2-12	34.65	0.00	5-11	44.74	0.00	10-12	3.06	0.08
2-13	2.84	0.09	5-12	47.56	0.00	10-13	0.07	0.79
3-4	28.87	0.00	5-13	39.39	0.00	11-12	0.12	0.73
3-5	75.68	0.00	6-7	119.74	0.00	11-13	0.17	0.68
3-6	22.90	0.00	6-8	0.00	0.95	12-13	1.67	0.20

Table 3. Eigenvalues, percentage of variance and percentage of cumulative variance for the five and four principal components in male and female, respectively for different sexes of kutum specimens in the southern Caspian Sea.

Male				Female		
Factor	Eigenvalues	Percentage of variance	Percentage of cumulative variance	Eigenvalues	Percentage of variance	Percentage of cumulative variance
1	62.92	80.66	80.66	65.40	83.84	83.84
2	3.39	4.35	85.01	3.19	4.08	87.93
3	2.48	3.18	88.19	2.02	2.59	90.52
4	1.62	2.07	90.26	1.39	1.78	92.30
5	1.23	1.58	91.84	-	-	-

Table 4. Result of Wilks' lambda test for verifying difference among four kutum populations in different sexes when morphological measurements are separately compared using discriminant function analysis.

	Test of functions	Wilks' lambda	Chi-square	Df	Sig.
Male	1 through 3	0.07	655.34	66.00	0.00
	2 through 3	0.37	254.48	42.00	0.00
	3	0.68	95.91	20.00	0.00
Female	1 through 3	0.07	804.53	48.00	0.00
	2 through 3	0.36	306.65	30.00	0.00
	3	0.81	62.94	14.00	0.00

Table 5. Percentage of specimens classified in each group and after cross validation for morphometric data in male specimens of kutum populations in the southern Caspian Sea.

Male		Station	Predicted Group Membership				Total
			Behshahr	Sari	Mahmod-Abad	Ramsar	
Original	Count	Behshahr	21	2	0	1	24
		Sari	1	78	2	2	83
		Mahmod-Abad	1	2	68	16	87
		Ramsar	1	3	17	52	73
	%	Behshahr	87.50	8.33	0.00	4.17	100
		Sari	1.20	93.98	2.41	2.41	100
		Mahmod-Abad	1.15	2.30	78.16	18.39	100
		Ramsar	1.37	4.11	23.29	71.23	100
Cross-validated ^a	Count	Behshahr	15	5	1	3	24
		Sari	3	75	3	2	83
		Mahmod-Abad	2	2	63	20	87
		Ramsar	1	3	21	48	73
	%	Behshahr	62.50	20.83	4.17	12.50	100
		Sari	3.61	90.36	3.61	2.41	100
		Mahmod-Abad	2.30	2.30	72.41	22.99	100
		Ramsar	1.37	4.11	28.77	65.75	100

^a Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

Table 6. Percentage of specimens classified in each group and after cross validation for morphometric data in female specimens of kutum populations in the southern Caspian Sea.

Female		Station	Predicted Group Membership				Total
			Behshahr	Sari	Mahmod-Abad	Ramsar	
Original	Count	Behshahr	13	3	0	0	16
		Sari	0	69	1	0	70
		Mahmod-Abad	0	1	64	18	83
		Ramsar	0	1	29	111	141
	%	Behshahr	81.25	18.75	0.00	0.00	100
		Sari	0.00	98.57	1.43	0.00	100
		Mahmod-Abad	0.00	1.20	77.11	21.69	100
		Ramsar	0.00	0.71	20.57	78.72	100
Cross-validated ^a	Count	Behshahr	13	3	0	0	16
		Sari	0	68	2	0	70
		Mahmod-Abad	0	2	62	19	83
		Ramsar	0	1	34	106	141
	%	Behshahr	81.25	18.75	0.00	0.00	100
		Sari	0.00	97.14	2.86	0.00	100
		Mahmod-Abad	0.00	2.41	74.70	22.89	100
		Ramsar	0.00	0.71	24.11	75.18	100

^a Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

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 16 software package, Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) (Rohlf 1990) and Excel (Microsoft office 2010).

Results

Descriptive data for the sex ratio, range (minimum-maximum), mean and standard deviation (SD) of length and weight in sampled specimens are shown in Table 1. The correlation between transformed morphometric variables and standard length was non-significant ($p > 0.05$) that confirmed size or allometric signature on the basic morphological data was accounted. Univariate statistics (ANOVA) showed that all the morphometric measurements were significantly different among the samples ($p < 0.001$). The ANOVA for differences in morphometric characters between female and male kutum in reproductive season (Table 2) revealed sexual dimorphism ($p < 0.05$) in 63 of the 78 studied measurements. Therefore, the analyses of morphometric characters were conducted with the sexes separated.

To examine the suitability of the data for principal component analysis, Bartlett's test of sphericity and Kaiser-Meyer-Olkin (KMO) measure were performed. In this study, the values of KMO for overall matrix are 0.953 and 0.952 in males and females, respectively. The Bartlett's test of sphericity is significant ($p \leq 0.01$). The results suggest that the sampled data is appropriate to proceed with a factor analysis procedure. In order to determine which morphometric measurement most effectively differentiates among the populations, the contributions of variables to principal components (PC) were examined. Principal component analysis of 78 morphometric measurements extracted five factors with eigenvalues > 1 , explaining 91.83 % of the variance in male and four factors with eigenvalues > 1 , explaining 92.29 % of the variance in female (Table 3). The first principal component (PC1) accounted for 80.66 % and 83.84 % of the variation and the second

principal component (PC2) for 4.37 % and 4.08 % in males and females, respectively (Table 3). The most significant loadings on PC1 in males were 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-13, 2-6, 2-7, 2-8, 2-9, 2-10, 2-11, 2-12, 2-13, 3-6, 3-7, 3-8, 3-9, 3-10, 3-11, 3-12, 3-13, 4-6, 4-7, 4-8, 4-9, 4-10, 4-11, 4-12, 4-13, 5-6, 5-7, 5-8, 5-9, 5-10, 5-11, 5-12, 5-13, 7-8 and in females were 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-13, 2-6, 2-7, 2-8, 2-9, 2-10, 2-11, 2-12, 2-13, 3-6, 3-7, 3-8, 3-9, 3-10, 3-11, 3-12, 3-13, 4-6, 4-7, 4-8, 4-9, 4-10, 4-11, 4-12, 4-13, 5-6, 5-7, 5-8, 5-9, 5-10, 5-11, 5-12, 5-13, 6-7, 6-9, 6-10, 6-11, 6-12, 6-13, 7-8, 7-9, 7-10, 7-11, 7-12, 7-13, 8-9, 8-10, 8-11, 8-12, 8-13. Visual examination of plots of PC1 and PC2 scores revealed that the 504 specimens grouped into four areas with low degree of overlap among the four populations (Fig. 3). In this analysis the characteristics with an eigenvalues exceeding 1 were included and others discarded. It is worth mentioning that factor loading greater than 0.30 are considered significant, 0.40 are considered more important and 0.50 or greater are considered very significant (Nimalathasan 2009). For parsimony, only those factors with loadings above 0.7 were considered significant. The Wilks' lambda tests of discriminant analysis indicated significant differences in morphometric characters of the four populations. In this test, three functions were highly significant ($p \leq 0.01$) (Table 4). The linear discriminant analysis in male gave an average PCC was 73.3 %. Medium classification success rates were obtained for Behshahr (62.5 %), Sari (90.4 %), Mahmod-Abad (72.4 %) and Ramsar (65.8 %) that indicated correct classification of specimens into their original populations (Table 5). The discriminant analysis in female showed that the average of PCC was 80.3 % for morphometric

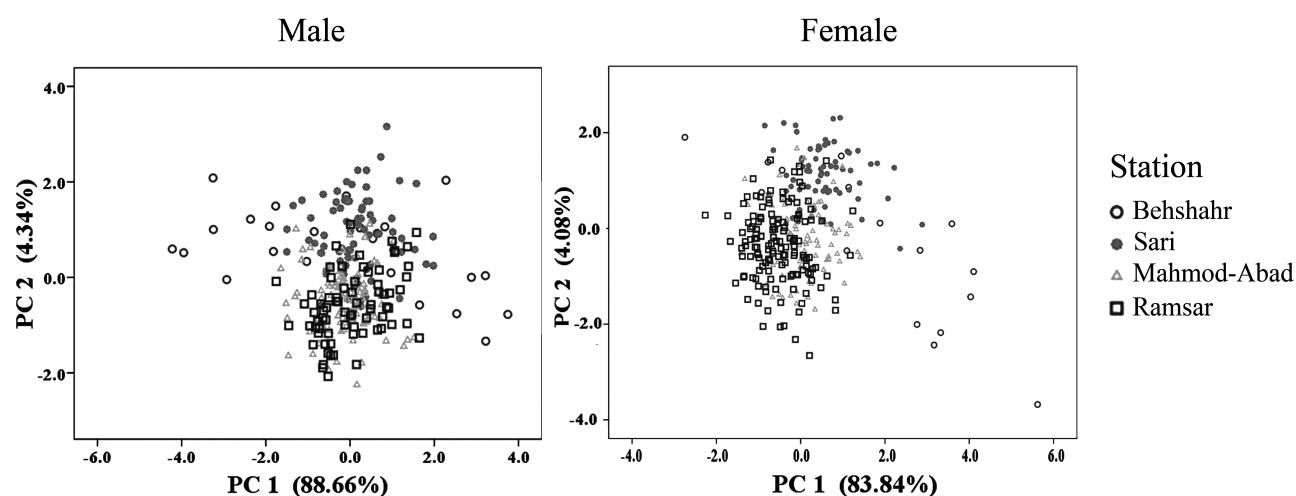


Fig. 3. Plot of the factor scores for PC1 and PC2 of all morphometric measurements for different sexes of kutum populations in the southern Caspian Sea.

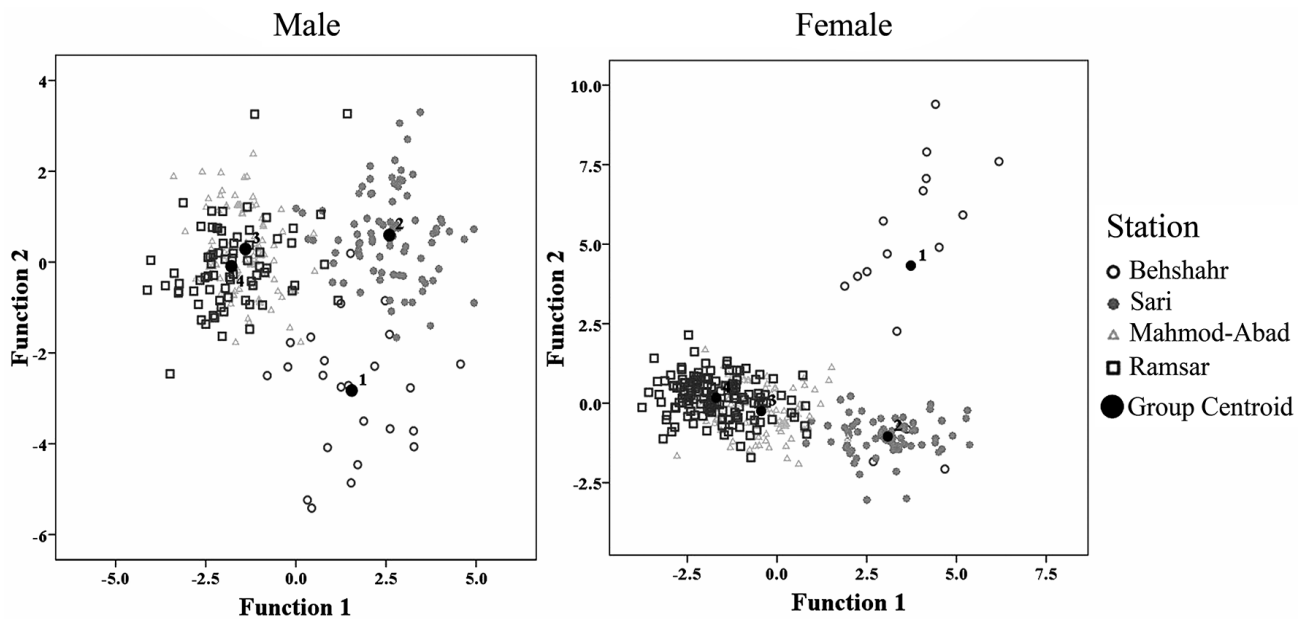


Fig. 4. Coordinate plot for different sexes of kutum specimens according to the first two discriminant functions from morphometric data analysis in the southern Caspian Sea.

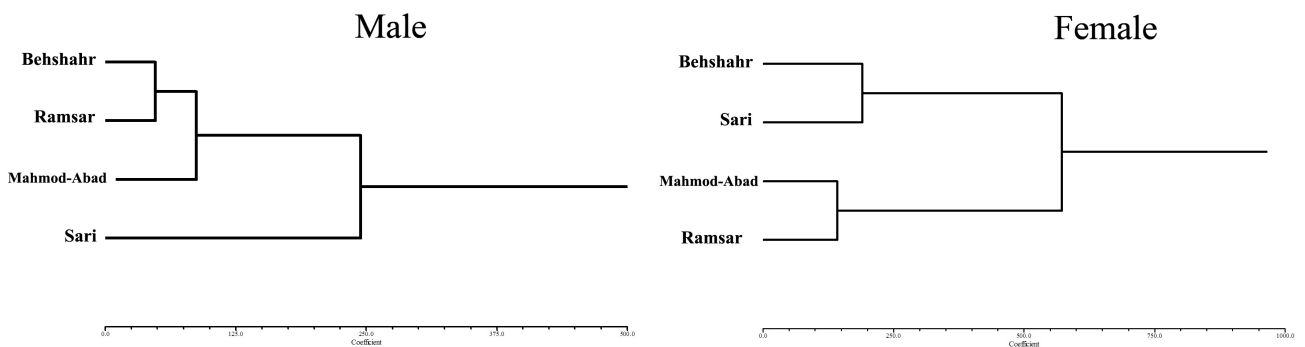


Fig. 5. Dendrogram derived from cluster analyses of 78 morphometric measurements on the basis of Euclidean distance for different sexes of kutum specimens in the southern Caspian Sea.

characters. The proportion of individuals correctly classified into their original groups were 81.3 %, 97.1 %, 74.7 % and 75.2 % in Behshahr, Sari, Mahmod-Abad and Ramsar, respectively (Table 6). In both males and females the cross-validation testing procedure was exactly the same as PCC results. Fig. 4 indicates the coordinates of four populations in the two first axes of DFA. In this analysis there was a high degree of separation among kutum specimens in the southern Caspian Sea. The measurements used in this analysis for males included 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-13, 2-3, 2-4, 2-5, 2-6, 3-5, 3-7, 6-7, 6-8, 7-9, 10-11 and for females 1-4, 1-9, 2-11, 3-5, 3-6, 3-12, 4-5, 5-7, 5-12, 6-12, 7-9, 7-11, 7-13, 9-13, 11-12, 11-13.

Clustering analysis based on Euclidean square distances among the groups of centroids using an UPGMA in females resulted into two main clusters

Behshahr and Sari in one group and Mahmod-Abad and Ramsar in the other group (Fig. 5). Also males were clustered in two distinct clades, the first one consists of Behshahr, Mahmod-Abad and Ramsar populations and the second clade includes Sari population, although they are far apart geographically (Fig. 5).

Discussion

The aims of the present study were to investigate the hypothesis of homeward migration success, population differentiation and morphologic sexual dimorphism in *Rutilus kutum* using truss network system. Previous studies revealed low genetic variability among the fish populations (Kavan et al. 2009, Chakmehdouz Ghasemi et al. 2009, Rezaei et al. 2010, Abdolhay et al. 2012a, b, Rezvani et al. 2012). The results suggested that due to a long

term artificial propagation, and growing in the same habitat (the Caspian Sea) without any physical barrier, populations of the fish have been mixed and homing behaviour has been hampered in kutum. The obtained results of our morphologic analysis of variance revealed significant phenotypic variation among the four studied populations, including sexual dimorphism. Discriminant Function Analysis could be a useful method to distinguish different stocks of a same species (Karakousis et al. 1991). In the present study, 73.3 % and 80.3 % of males and females were correctly classified in to their respective groups by DFA (Fig. 4) indicating a high differentiation among the populations of kutum in the studied areas. This segregation was partly confirmed by PCA, where the PC1 and PC2 scores for each sample (Fig. 3) revealed that these populations were clearly distinct from each other. Also, this survey indicated that population differentiation was more distinct in females than in males. This study reveals that high inbreeding resulting from artificial propagation probably created morphologically distinct populations of kutum. Based on different stocking programs of kutum, each river had different broodstocks and larvae that belong to different populations; in this reason fingerling should be released to the same river where the broodstocks are caught (e.g. Abdolhay et al. 2010). Holčík (1999) stated that dramatic declines in migratory species such as lampreys, sturgeons, salmon and clupeids were well known in European which requires attention. AnvariFar et al. (2011) had studied dam effects on morphometric differentiation of *Capoeta capoeta gracilis* and stated that dams obstruct migration of fishes, especially of the migratory species, resulting in an ecological trap that ascend the fish passages. Abdolhay et al. (2010) used conventional and truss morphometrics to cluster kutum into four independent populations in different rivers and assumed that differences among populations could be based on physical characteristics of each habitat, such as water temperature, environment because the varied climate of rivers. Some conventional morphometric data for *Rutilus kutum* from different rivers have been recorded and analyzed previously by Razavi Sayad (1993) for stock assessment of the fish. According to Razavi Sayad (1993), there was no significant difference ($p > 0.05$) among kutum geographic populations. Abdolhay et al. (2012a) showed the high inbreeding happened in

kutum populations that is possibly related to artificial production of fingerling in last 30 years and there was a low genetic variability in four studied populations of kutum in the southern Caspian Sea. Abdolhay et al. (2012b) explained the low genetic variability by inbreeding at artificial propagation, in four rivers in the southern Caspian Sea basin, while there is no physical barrier among the sampling sites in the sea. Similarly, the study by Kitada et al. (2009) on red sea bream showed that artificial propagation and stocking reduced genetic diversity.

The morphological differences may be solely related to body shape variation and not to size effect which was successfully accounted 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 had been removed successfully by allometric transformation, and the significant differences among the populations are due to the body shape variation according to 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 background, environment and the interaction between them (Swain & Foote 1999, Poulet et al. 2004, Pinheiro et al. 2005). The environmental factors are prevailing during the early development stages, when individual's phenotype is more amenable to environmental influence is of particular importance (Pinheiro et al. 2005). The influences of environmental parameters on morphometric characters are well discussed by several authors (e.g. Swain & Foote 1999).

In conclusion, the present study showed that each sampling site represents distinct population, which suggests that populations of the fish were able to maintain their structure despite ignoring the natural process of reproduction, mixing the caught broodstocks and the production of fingerlings due artificial propagation within restocking programs in last 30 years. Based on the obtained results it can be concluded that the homing behaviour had not been hampered in the fish, which is reported previously on different anadromous fish species. The results can be interesting for management and conservation programs of this valuable species in this region.

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