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Detection of Morphometric Differentiation of *Liza aurata* (Pisces: Mugilidae) in Southeastern of the Caspian Sea, Iran

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Abstract
A 13-landmark morphometric truss network system was used for 135 specimens to investigate the hypothesis differentiation of golden grey mullet along the southeastern Caspian Sea. Univariate analysis of variance showed significant differences among the means of the three groups for 35 standardized morphometric measurements out of 78 characters studied. In linear discriminant function analysis (DFA), the overall assignment of individuals into their original groups was 66.7%. The proportions of individuals correctly classified into their original groups were 62.5%, 59.1%, 76.5% in Neka, Behshahr and Galogah populations, respectively. The Principal Component Analysis (PCA) showed that the specimens grouped into 2 areas with high degree of overlap. Clustering analysis based on Euclidean square distances among the studied groups of centroids using an UPGMA resulted segregation of the three populations into two distinct clusters. These results could be of interest for management and conservation programs of this species in the Caspian Sea.

Key words: Golden grey mullet, Truss network system, southeastern Caspian Sea

Introduction
During the years 1930-1934, about three millions juveniles of Black Sea grey mullet were successfully introduced from the Black Sea into the Caspian Sea (Zablotski, 1966). That including grey mullet (*Mugil cephalus*), leaping grey mullet (*Liza saliens*), and golden mullet (*L. aurata*) but only the two last species have successfully acclimated, adapted and propagated in the Caspian Sea. These species are currently of high economic importance (Fazli and Ghaninejad, 2004; Nematzadeh *et al.*, 2013) as in industrial capturing of Mugilidae family ration of each species that including 30% *L. saliens* and 70% *L. aurata* (Fazli and Ghaninezhad, 2004).

The golden grey mullet, *Liza aurata* is an euryhaline (1-38 ppt), eurythermic (3-35°C) (Amini, 1989) and pelagic coastal marine species which usually lives in inshore waters, entering lagoons and estuaries. It rarely enters freshwater and prefers a muddy bottom (Jardas, 1996) and feed on periphyton, detritus and small invertebrates (Fazli *et al.*, 2008). Golden grey mullet in the Caspian Sea spend spring in the north and autumn in the south (Probatov and Tereshchenko, 1951). This species is a target of a commercial fishery and appeared in the catches which by the middle of the 1950s reached up to 3000 tons for the USSR and Iran (Fazli *et al*., 2008).

Because of their economic importance, Golden grey mullet has been broadly studied, in terms of biological characteristics (Fazli, 1998), age and growth (Andaloro, 1983; Illkyaz *et al*., 2006; Mehanna, 2006; Fazli *et al*., 2008; Kraljević *et al*., 2011), reproduction (Hotos *et al*., 2000), systematic status (Turan *et al*., 2011), distribution and migration (Mićković *et al*., 2010), genetic diversity (Ghodsi *et al*., 2011) and phylogenetic relationships (Turan *et al*., 2005). However, information on population differentiation of adult specimens in the Southeastern Caspian Sea is still rather limited. In addition, it is important to understand that this unit population had morphological differentiation.

Morphometric population differentiation is important from various viewpoints including evolution, ecology, behaviour, conservation, water resource management and stock assessment (AnvariFar *et al*., 2011; 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 for some time been a strong interest in ichthyology (Tudela, 1999).

In morphological studies, conventional and Truss network system are normally used to describe morphological variations between different populations of a species. The study of morphometrics using truss network system (Strauss and 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 (Cardin and Friedland, 1999; Turan, 1999; Akbarzadeh *et al*., 2009; Kocovsky *et al*., 2009; Mousavi-Sabet and AnvariFar, 2013).

Despite the biodiversity and commercial importance of golden grey mullet as one of the major commercial and introduced species in the Caspian Sea, unfortunately there is no any study available on population differentiation of this species in the southern coast of the Caspian Sea. Considering the above mentioned facts, the present study was aimed to obtain information about morphometric variation and differentiation of *L. aurata* in southern coasts of the Caspian Sea runs to be employed in the future enhancement programs to maintain this valuable species in the Sea.

Figure 1. Location of sampling sites including Neka, Behshahr and Galogah in the southeastern Caspian Sea.

Materials and methods

Sampling

A total of 135 adult individuals of golden grey mullet were collected from three sampling sites, during March-April period of 2012 that comprising 40 individuals from Neka (36°49'N, 53°9'E), 44 individuals from Behshahr (36°52'N, 53°27'E) and 51 individuals from Galogah (36°54'N, 53°48'E), along the southeastern Caspian Sea (Fig. 1). The specimens captured by beach seine.

Laboratory work

A total of 78 distance measurements between 13 landmarks were surveyed using truss network system (Strauss and Bookstein, 1982; Bookstein, 1991) with minor modification for this species (Fig. 2). Fishes 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 color digital camera (Sony Cybershot DSC-F505, Sony, Japan). Images were saved in jpg format and analyzed with TPSdig (Ver. 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 and 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).

After the photography, 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 males and females of golden grey mullet.

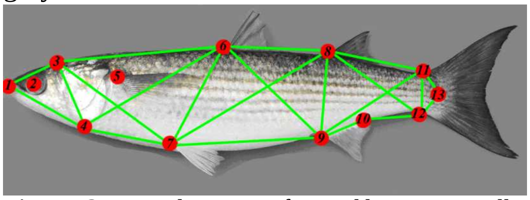

Figure 2. Digital image of a golden grey mullet depicting the thirteen landmarks and associated box truss used to infer morphological differences among populations. 1. Tip of snout 2. Center of eye 3. Forehead (end of frontal bone) 4. End of operculum 5. Dorsal origin of pectoral fin 6. Origin of first dorsal fin 7. Origin of pelvic fin 8. Origin of second 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. (Strauss and Bookstein, 1982; Bookstein, 1991)

Data analysis

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

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

Where, M is original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of the fish, Ls 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 statistical significance of individual morphometric characters among the three groups (Zar, 1984). The morphometric characters which showed significant variation ($P<0.05$) only were used for obtaining the 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 three studied 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*., 2011) and to extract a number of independent variables for population differentiation. The Wilks' lambda 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 among the individuals of the three groups were inferred to cluster analysis (Veasey *et al*., 2001) by adopting the Euclidean square distance as a measure of dissimilarity and the UPGMA (Unweighed Pair Group Method with Arithmetical average) method as the clustering algorithm (Sneath and 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 range (Minimum-Maximum), mean, standard deviation (SD) of length and weight in case of 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. Differences ($P<0.05$) among the three populations of golden grey mullet in the Neka, Behshahr and Galogah stations in the southern Caspian Sea were observed for 35 out of 78 morphometric characters (Tab. 2) and these variables were used further for multivariate analysis (PCA, DFA and CA). The ANOVA for differences in morphometric characters between female and male of golden grey mullet did not differ significantly ($p<0.05$); hence, the data for both sexes were pooled for all subsequent analyses.

In order to determine which morphometric measurement (MM) most effectively differentiates populations, the contributions of variables to principal components (PC) were examined. 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 value of KMO for overall matrix was 0.569. The Bartlett's Test of Sphericity was significant ($P\leq0.01$). Principal component analysis of 35 MMs extracted eight factors with eigenvalues >1, explaining 89.63% of the variance (Tab. 3). The first principal component (PC1) accounted for 27.88%, the second principal component (PC2) for 23.11% and the third principal component (PC3) for 13.69% (Tab. 3). The most significant loadings on PC1 were 1-8, 2-8, 3-8, 4-8, 5-8, 6-8, 8-11, 8-12, 8-13, on PC2 were 1-2, 1-5, 2-3, 2-5, 2-13, 4-13, 5-13, and on PC3 were 1-7, 2-7, 3-7, 4-7, 5-7, 6-7. Visual examination of plots of PC2 and PC3 scores revealed that

Table 1. Length (mm) and weight (gr) of golden grey mullet from sampling sites.

Station	Sex	N	Min-Max (length)	Mean± S.D. (length)	Min-Max (weight)	Mean± S.D. (weight)
Neka	Male	21	230-355	288.67±20.50	151-395	248.92±59.25
	Female	19	250-420	296.35±39.14	144-760	283.16±150.60
Behshahr	Male	18	280-440	341.9±41.04	195-980	468.5±193.13
	Female	26	230-460	333.9±54.09	114-1250	403.71±203.62
Galogah	Male	23	230-450	282.81±57.58	122-842	250.23±174.12
	Female	28	230-450	291.45±63.33	89-842	264.75±178.98

Table 2. ANOVA of morphometric characters of golden grey mullet samples.

MM	FV	PV	MM	FV	PV	MM	FV	PV
1-2	3.19	0.04	3-7	9.77	0.00	6-9	0.49	0.62
1-3	2.41	0.09	3-8	4.96	0.01	6-10	1.45	0.24
1-4	2.40	0.09	3-9	0.05	0.95	6-11	0.31	0.74
1-5	3.58	0.03	3-10	1.76	0.18	6-12	0.65	0.52
1-6	1.43	0.24	3-11	0.79	0.46	6-13	1.53	0.22
1-7	16.30	0.00	3-12	0.07	0.94	7-8	0.70	0.50
1-8	8.39	0.00	3-13	2.31	0.10	7-9	4.15	0.02
1-9	0.83	0.44	4-5	2.76	0.07	7-10	0.59	0.56
1-10	3.79	0.03	4-6	0.88	0.42	7-11	6.51	0.00
1-11	6.95	0.00	4-7	9.90	0.00	7-12	6.42	0.00
1-12	4.82	0.01	4-8	4.02	0.02	7-13	14.42	0.00
1-13	16.11	0.00	4-9	0.20	0.82	8-9	1.53	0.22
2-3	3.66	0.03	4-10	0.57	0.57	8-10	0.15	0.86
2-4	1.76	0.18	4-11	0.16	0.85	8-11	4.59	0.01
2-5	5.11	0.01	4-12	0.84	0.44	8-12	4.24	0.02
2-6	1.50	0.23	4-13	3.90	0.02	8-13	7.64	0.00
2-7	17.25	0.00	5-6	6.12	0.00	9-10	0.49	0.61
2-8	6.29	0.00	5-7	10.43	0.00	9-11	1.01	0.37
2-9	0.21	0.81	5-8	3.54	0.03	9-12	1.40	0.25
2-10	1.60	0.21	5-9	0.54	0.59	9-13	2.09	0.13
2-11	1.12	0.33	5-10	1.01	0.37	10-11	2.58	0.08
2-12	0.58	0.56	5-11	1.69	0.19	10-12	2.55	0.08
2-13	3.49	0.03	5-12	0.45	0.64	10-13	7.19	0.00
3-4	1.95	0.15	5-13	4.74	0.01	11-12	0.39	0.68
3-5	0.70	0.50	6-7	16.54	0.00	11-13	8.46	0.00
3-6	9.70	0.00	6-8	5.26	0.01	12-13	6.46	0.00

MM = Morphometric measurement, FV = F value, PV = P value

Table 3. Eigenvalues, % of variance and % of cumulative variance for thirteen principal components for different sexes of golden grey mullet specimens.

Factor	Eigen values	% of variance	% of Cum. var.
PC1	9.76	27.88	27.88
PC2	8.09	23.11	50.99
PC3	4.79	13.69	64.67
PC4	3.09	8.83	73.51
PC5	2.10	5.99	79.50
PC6	1.34	3.83	83.33
PC7	1.20	3.43	86.76
PC8	1.00	2.86	89.63

the 135 fish specimens grouped into 2 areas (Behshahr and Galogah) with low degree of overlap among the populations (Fig. 3). However, Neka specimens showed high degree of overlap with two other populations. In this analysis the characterristics with an eigenvalues exceeding 1 were included and others were discarded. It is worth mentioning out here that factor loading greater than 0.70 are considered significant, 0.40 are considered more important and 0.50 or greater are considered very significant (Nimalathasan, 2009). For parsimony, in this study 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 three populations. In this test, two functions were highly significant ($P \leq 0.01$) (Tab. 4). The linear discriminant analysis in male gave an average PCC was 66.7%. Medium classification success rates were obtained for Neka (62.50%), Behshahr (59.09%) and Galogah (76.47%) that indicated a high correct classification of specimens into their original populations (Tab. 5). In both of male and female the cross-validation testing procedure were exactly the same as PCC results. Figure 4 indicates the coordinates of three populations in the two first axes of DFA. In this analysis there was a high degree of separation among golden grey mullet specimens in the southern Caspian Sea. In order to illustrate which morphometric characters are playing role to differentiate species contribution of each variable to the canonical functions were examined, and high contribution from measurements 1-13, 11-13, 12-13, 2-5, 2-7, 7-9, 8-13 were observed (Tab. 6).

Clustering analysis based on Euclidean square distances among the groups of centroids using an UPGMA populations were clustered into two distinct clads Neka and Behshahr in one group and the second clad include Galogah populations (Fig. 5), although they are far apart geographically.

Discussion

The aim of the present study was to investigate variability and differentiation of morphometric characters among golden grey mullet populations using truss network system. In this study for the first time, the results revealed that there are at least two distinct populations of golden grey mullet in the southeastern Caspian Sea. The analysis of variance revealed significant phenotypic variation among the three populations (35 out of 78 morphometric characters). Discriminant Function Analysis could be a useful method to distinguish different stocks of a same species (Karakousis *et al.*, 1991). In the present study, individuals were correctly classified into three respective groups by DFA (Fig. 4) indicating a high differentiation among the populations of golden grey mullet in the studied areas and this segregation shown in coordinate Plot according to the first two discriminant functions. This segregation was partly confirmed by PCA, where the graphs of PC1 and PC2 scores for each sample (Fig. 3) revealed that the populations were clearly distinct from each other. CA resulted there is two distinct populations of golden grey mullet in the southeastern Caspian Sea. According to our results, the most important measures to take into account for discrimination purposes by populations were the 1-13, 2-5, 2-7, 2-9 and 8-13 that these characters were common between measurements that used in DFA and PCA.

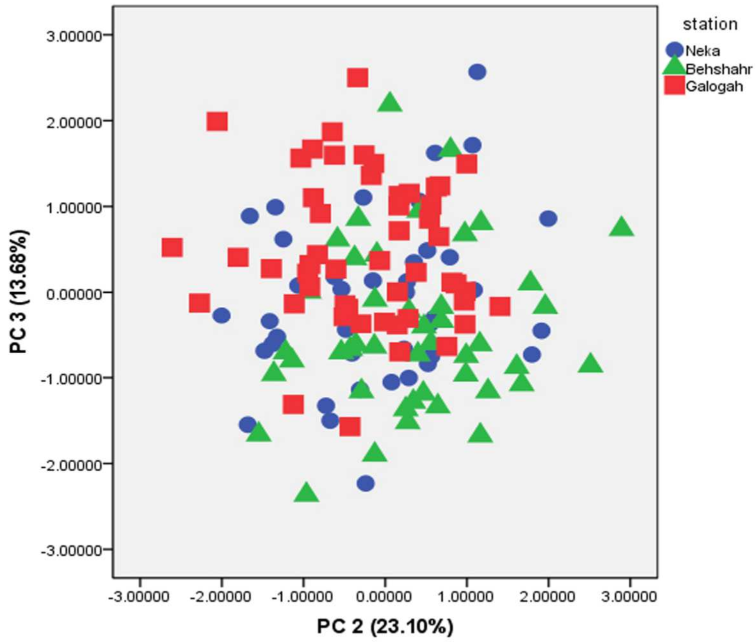

Figure 3. Plot of the factor scores for PC1 and PC2 of all MMs of golden grey mullet populations.

Table 4. Wilks' lambda test for verifying difference among three populations of golden grey mullet populations when morphological measurements are separately compared using discriminant Function analysis.

Test of Functions	Wilks' Lambda	Chi-square	Df	sig
1 through 2	0.37	128.75	14	0.00
2	0.78	32.03	6	0.00

Table 5. Percentage of specimens classified in each group and after cross validation for morphometric data of golden grey mullet populations.

		Locality	Predicted group membership			Total
			Neka	Behshahr	Galogah	
Original	Count	Neka	29	8	3	40
		Behshahr	13	28	3	44
		Galogah	4	6	41	51
	%	Neka	72.50	20.00	7.50	100.0
		Behshahr	29.55	63.64	6.82	100.0
		Galogah	7.84	11.76	80.39	100.0
Cross-validated[a]	Count	Neka	25	11	4	40
		Behshahr	14	26	4	44
		Galogah	4	8	39	51
	%	Neka	62.50	27.50	10.00	100.0
		Behshahr	31.82	59.09	9.09	100.0
		Galogah	7.84	15.69	76.47	100.0

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.

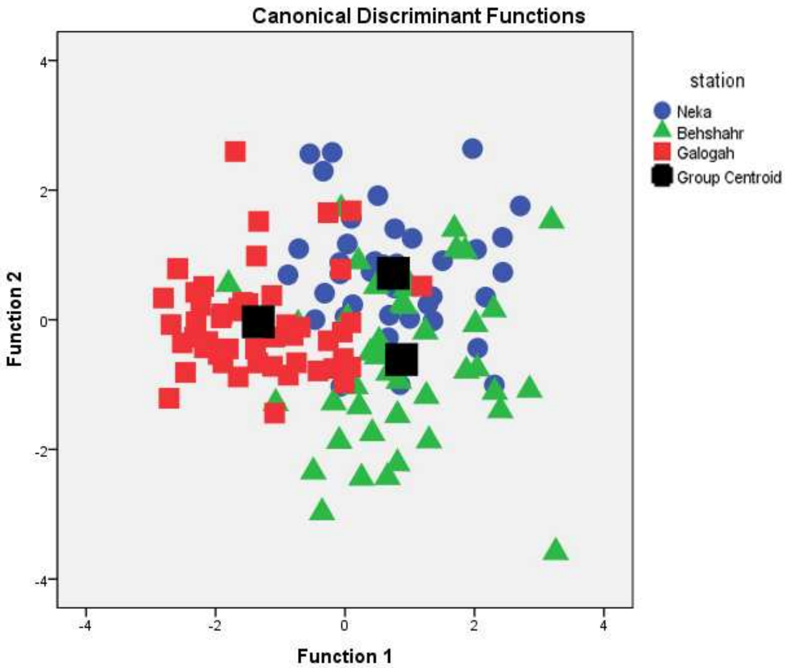

Figure 4. Coordinate plot of golden grey mullet specimens according to the first two discriminant functions from morphometric data analysis.

This study reveals that geographical separation by distance and high inbreeding probably created morphologically different populations of golden grey mullet, because of their limited dispersal and migration. Discrimination between regions can be explained by the life history of the mullet, as they migrate to the ocean to spawn (Ibáñez and Gutiérrez-Benítez, 2004) that migrate to ocean is impossible in the Caspian Sea. González-Castro *et al*. (2012) explained non-contact populations Mugilidae species, reflected broad shape differentiation. Holcík (1999) stated that dramatic declines in migratory species such as lampreys, sturgeons, salmons 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 dams obstruct migration of fishes especially that of the migratory species resulting in an ecological trap for migratory fishes that ascend the fish passages. Abdolhay *et al*. (2012) showed the high inbreeding happened in Mahisefid *Rutilus kutum* population, which is another economic species in the Caspian Sea, can lead to low genetic variability in four populations of Mahisefid in southern shores of the Caspian Sea. Ghodsi *et al*. (2011) investigated the level of genetic variation of *Liza aurata* in the southern Caspian Sea using microsatellite marker and they results showed compulsory inbreeding of golden grey mullet lead to no conspicuous genetic variations and accordingly a relatively high level of gene flow was found among populations. Also, they stated in result of irregular capturing, short time after

Table 6. Morphometric variables for the canonical functions. Variables ordered by size of correlation within the functions*, indicate largest correlation between each variable and any discriminant function

Morphometric	Function	
	df 1	df 2
1-2	-0.025	0.224*
1-5	-0.018	0.447*
1-7	-0.438*	0.33
1-8	-0.314	0.366*
1-10	-0.078	0.098*
1-11	-0.315	0.051
1-12	-0.199	0.247*
1-13	0.445*	0.288
2-3	-0.071	0.291*
2-5	-0.02	0.522*
2-7	-0.460*	0.298
2-8a	-0.294	0.314*
2-13	0.037	-0.201*
3-6	-0.025	0.281*
3-7	-0.404*	0.107
3-8	-0.260*	0.187
4-7	-0.316*	0.115
4-8	-0.229*	0.181
4-13	0.097	-0.353*
5-6	-0.011	0.100*
5-7	-0.387*	0.097
5-8	-0.264*	0.175
5-13	0.036	-0.406*
6-7	-0.377*	0.187
6-8	-0.255*	0.224
7-9	-0.006	0.472*
7-11	0.216	-0.250*
7-12	0.254*	-0.148
7-13	0.369*	-0.277
8-11	0.191	-0.337*
8-12	0.203	-0.272*
8-13	0.272	-0.344*
10-13	0.125	-0.138*
11-13	0.339*	0.007
12-13	0.24	-0.344*

introducing to the Caspian Sea, closed environment and no connection with ocean waters can lead to decrease of genetic variation and increase gen flow among populations. Also, several studies have questioned the systematic status family of the Mugilidae. Antovic and Simonovic (2006) surveyed interspecific variability and phenetic relationships in six southern Adriatic mullet species and declared they were clearly separated from the other species. Turan *et al.* (2011) investigated systematic relationships among four genera and nine species of the Mugilidae family living in the Mediterranean Sea and stated all species except *L. chelon* and *L. oedalechilus* detected appreciable degree of morphologic differentiation.

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 and Foote, 1999; Poulet *et al*., 2004; Pinheiro *et al*., 2005). The environmental factors 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 in the course of fish population segregation (Swain and Foote, 1999).

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 had been removed successfully by allometric transformation, and the significant differences among the populations were due to the body shape variation when it tested by ANOVA and multivariate analysis.

In conclusion, the present study suggests that there are two independent populations in the southeastern Caspian Sea that could be of interest for commercial exploitation, management and conservation programs of this species in the sea. A detailed study involving the molecular genetics and environmental aspects may further confirm the present findings unambiguously. However, in order to have better conservational policy and restocking programs, further studies are recommended on determining other possible populations of this species in other regions of the Caspian Sea.

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