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Morphometric variation among four populations of Shemaya (*Alburnus chalcoides*) in the south of Caspian Sea using truss network

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Abstract To analyze the morphometric differentiation among four groups of Shemaya, *Alburnus chalcoides* (Guldenstadf, 1772), sited in four habitats i.e.: Lisar, Shiroud, Babolroud rivers and Anzali region (southern part of the Caspian Sea), truss network analysis was employed to take multivariate analysis. Truss characters between fourteen selected landmarks on 357 specimens were measured, then allometric method was used to obtain size-adjustment shape data. Multi and univariate analysis of variance revealed significant differences between means of four populations and sexes ($P < 0.01$). In principal component analysis of the shape variations among the populations, the first two components accounted for 50% and 56.6%, of males and females, respectively. The loadings of the first and the second principal components and discriminant functions showed that the differences were located mainly on abdominal, caudal regions and length of fins, indicating to be important in description of the population characteristics. Results indicated that samples of Anzali region were clearly distinct and diverged from other three populations probably because of difference in the habitat condition. Generally female samples showed more morphometric differences than male samples did, but their influence on this issue remained unknown.

Introduction

Stock identification, as a field of fishery science, employs many tools such as genetics and morphometric to discriminate stocks (Cadrin, 2000). The effects of genetic and environmental parameters on different growth and developmental processes would create shape variation among stocks (Garrod and Horwood, 1984). Therefore some morphometric methods have been developed and applied to discriminate stocks such as

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univariate comparisons, bivariate analyses of relative growth pattern and a series of multivariate methods (Cadrin, 2000).

In traditional morphometric, the analysis of distant features obtained by image processing techniques is used for identification (Cavalcanti et al., 1999). Traditional techniques have recently been improved for more comprehensive and precise data collection, suitable quantification of shape and modern landmark-based techniques of geometric morphometric (Rohlf, 1990; Bookstein, 1991; Rohlf and Marcus, 1993). Homologous landmarks as end points are common features among specimens (Bookstein, 1990) and are used as points of correspondence on an object that matches between and within populations (Barlow, 1961; Swain and Foote, 1999). As an alternative to traditional morphometric, a box-truss network between landmarks has been proposed by Strauss and Bookstein (1982) as a more comprehensive representation of landmarks. Truss network systems constructed with the help of landmark points are also a powerful tool for stock identification. Image analysis systems played a major role in the development of morphometric techniques, boosting the utility of morphometric research (Cadrin and Friedland, 1999). The characteristics may be more applicable for studying short-term, environmentally induced disparities, stock identification (Bronte and Moore, 2007; Shao et al., 2007), species differences (Palma and Andrade, 2002), ontogeny (Hard et al., 1999), practical morphology (Dean et al., 2006), and improved fisheries management (Ihssen et al., 1981; Templeman, 1983; Smith and Jamieson, 1986; Turan, 2004; Turan et al., 2004a,b).

Shemaya (*Alburnus chalcoides*) is a cyprinid fish with wide distribution in the river systems of the Black, Caspian and Aral Sea basins (Bogutskala, 1997). Though there have been numerous variant views of synonymy for *Alburnus* (Coad, 2012), *Alburnus* is more often regarded as *Chalcalburnus* (Berg, 1933). This benthopelagic species live in fresh as well as brackish waters. The populations that live in lakes move upstream for spawning from early May to end of July (Slastenenko, 1959). Shemaya has an increasingly commercial importance in southern regions of the Caspian Sea. Thus, a basic knowledge on its biology, including information on population structure could be a privilege. Few studies have been carried out on the morphological traits of different populations of the Shemaya in the southern parts of the Caspian Sea (Abdurakhmanov, 1975; Coad, 1999). Hence present study has been carried out to investigate the morphometric variation sexes within the four populations of *A. chalcoides* using truss network.

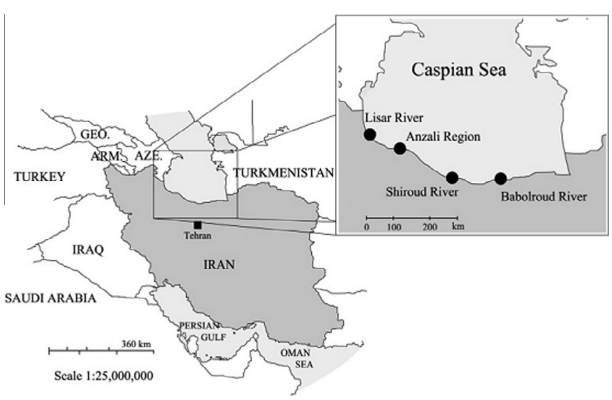

Figure 1 Map showing the sites (filled circle) in south of the Caspian Sea where *A. chalcoides* were collected.

Materials and methods

A total of 357 specimens were collected from the four populations of Shemaya between March and June (spring) in four locations i.e.: Lisar river (LR), Anzali region (ARE), Shiroud river (SHR), and Babolroud river (BR) (Fig. 1) by cast net and electrofishing.

Only healthy adult specimens were selected. Descriptive locality, sex ratio and range of standard length (SL) are shown in Table 1. The left side of specimens was photographed using a Canon G12 camera with 3648 × 2736 pixel dimension

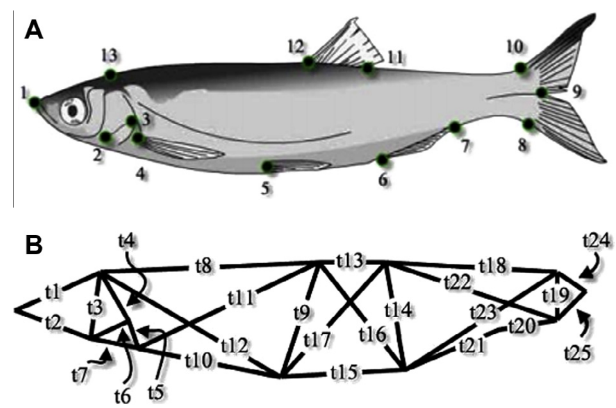

Figure 2 (A) Location of 13 anatomic landmark points and truss network (contain 25 truss characters) designed on the left view of the *A. chalcoides*, Landmarks points, 1: Tip of snout, 2: down of operculum, 3: end of operculum, 4: origin of pectoral fin, 5: origin of pelvic fin, 6: origin of anal fin, 7: ending of anal fin, 8: ventral origin of caudal fin, 9: end of caudal peduncle, 10: dorsal origin of caudal fin, 11: ending of dorsal fin, 12: origin of dorsal fin, 13: forehead (end of frontal bone). (B) 25 truss characters (body distances t1–t25) making a truss network.

Table 1 Brevity of sampling area, sample size and mean, standard deviation (SD) and range of standard length (SL, cm) of Shemaya (*A. chalcoides*) in south of Caspian Sea.

Locality	Brevity	GPS	Sample size	Sex Ratio (F/M)	Range of SL	Mean of SL ± SD
Lisar river	LR	N: 37″58, E:48″56	83	1.7	10.6–15.7	13.3 ± 0.84
Anzali region	ARE	N: 37″28, E: 49″27	94	1.8	13.0–19.9	15.6 ± 1.27
shiroud river	SHR	N: 36″49, E: 50″52	92	2.1	13.2–18.5	15.4 ± 1.21
Babolroud river	BR	N: 36″42, E: 52″39	88	2.3	11.8–16.6	14.3 ± 0.93

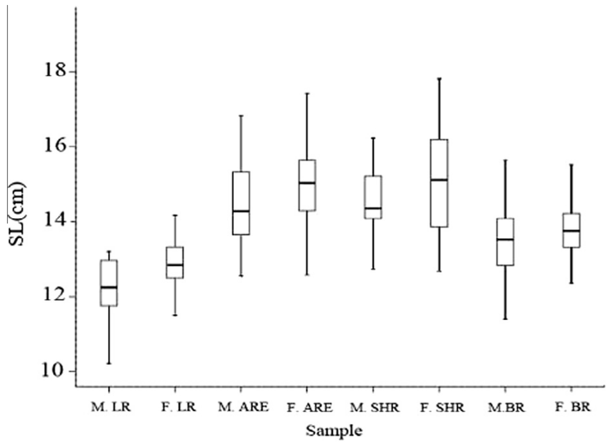

Figure 3 Box plot graph of mean Standard Length = SL (cm) of Shemaya (*A. chalcoides*) populations and sexes with 95% confidence intervals. (M. refers to male F. refers to female specimens).

images. Then the samples were fixed in 10% formalin solution. 13 landmark points were defined and digitized on two dimensional images using tpsDig (Version 2.12-omittes reference) as a two dimensional coordinate (Strauss and Bookstein, 1982) (Fig. 2A). The landmark points were selected to provide a homogeneous coverage of the whole shape, but their homology and clarity in each fish were also taken into account. 25 truss characters (body distances t1–t25) made a truss network used in multivariate methods (Strauss and Bookstein, 1982; Bookstein, 1991) (Fig. 2B).

An allometric formula given by Elliott et al. (1995) was used to remove the size effect from the dataset: $M_{adj} = M(L_s/L_o)$, where M is the original measurement, M_{adj} is the size-adjusted measurement, L_o is the SL (standard length) of the fish, and L_s is the overall mean of the SL for all fish from all samples. Since variations should be imputable to body shape differences,

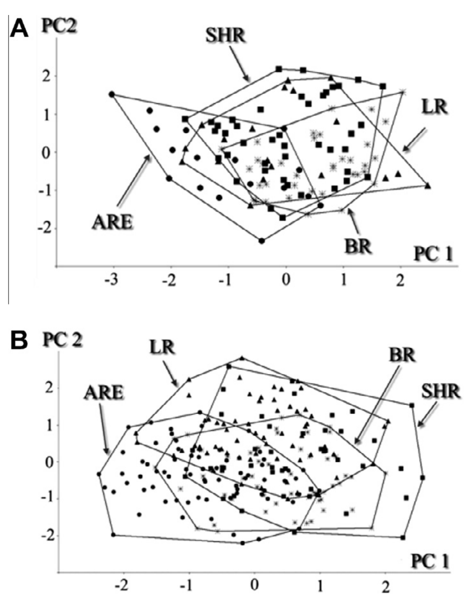

Figure 4 Scatter plots of the individual scores on the principal components of truss characters for Shemaya (*A. chalcoides*). A: male samples, B: female samples. (Triangles: LR = Lisar river, circles: ARE = Anzali region, squares: SHR = Shiroud river, stars: BR = Babolroud river).

and they should not be related to the relative size of fish (Reist, 1985) hence, the standardized data were examined using bivariate plots against SL to see whether the size relation had been removed or not. Multivariate analysis of variances (MANOVA) was performed to examine for significant differences between the populations and sexes. In order to determine which morphometric measurement most effectively differentiates populations, the contributions of variables to principal component analysis (PCA) were examined. Kruskal–Wallis test was used to compare means or medians among the groups and sexes.

The effects of sample and sex differences on the standardized characters were examined by One-way analysis of variances (ANOVA) and to assess the statistical significance of each character among stock identification. All 25 truss characters were combined to perform stepwise discriminant function analysis (DFA). Classification functions were derived from DFA to assign individual specimens to putative stocks. The classification success rate was evaluated based on percentage of individuals correctly assigned into original sample. To investigate the phenotypic relationships among populations, a dendrogram was constructed based on Euclidean distances using Unweighted Pair Group Method (UPGMA) with arithmetical average Cluster Analysis (CA) of arithmetic averages (Sneath and Sokal, 1973). All data and statistical analysis were carried out using the SPSS (version 16), PAST (version 2.06) and Excel (Microsoft Office, 2010).

Result

Shemaya (*A. chalcoides*) of Lisar river (LR) had the smallest size (with an average of 11.5 and 13.5 cm, for male and female,

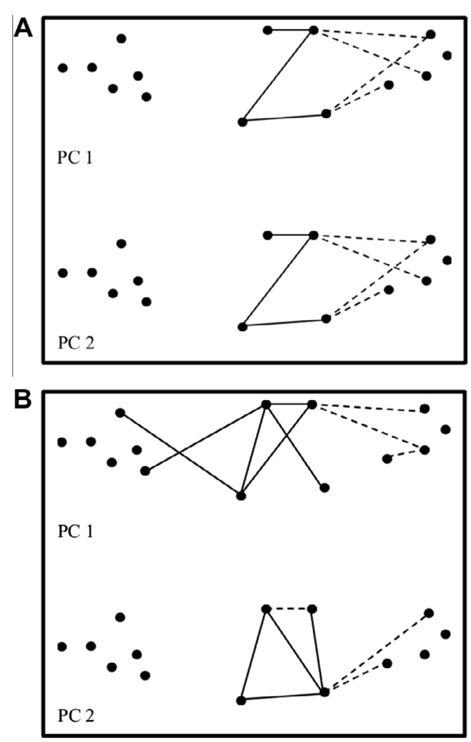

Figure 5 Principal component loading for first two component analysis for Shemaya (*A. chalcoides*). (A: male, B: female). Continuous lines indicate characters with high positive loading and dashed lines indicate negative loading.

Table 2 Result of discriminant analysis of the four groups of Shemaya (*A. chalcoides*), based on size-adjusted shape data. (Bold values indicate appropriate classifications).

Area	LR (%)	ARE (%)	SHR (%)	BR (%)
Male				
LR	**56.3**	12.5	18.5	12.5
ARE	20.0	**66.7**	6.7	6.7
SHR	11.4	13.6	**52.3**	22.7
BR	8.3	19.4	22.2	**50.0**
Female				
LR	**71.2**	15.2	6.1	7.6
ARE	10.0	**73.8**	12.5	3.8
SHR	18.8	14.6	**43.8**	22.9
BR	5.7	5.7	15.1	**73.6**

respectively), whereas the largest Shemaya belonged to the Anzali region (ARE) (with an average of 15.1 and 15.7 cm, for male and female, respectively) (Fig. 3).

There was no significant correlation ($P > 0.05$) between standardized truss measurements and standard length, indicating that the effect of size was successfully removed with allometric transformation. The MANOVA (Wilk's test) indicated a significant difference for mean vectors between populations ($\Lambda = 0.036$, $F = 6.2$, $P = 5.854\text{E}^{-90} < 0.001$) and sexes ($\Lambda = 0.387$, $F = 4.74$, $P = 2.171\text{E}^{-23} < 0.001$). All subsequent analysis was performed separately for each sex.

Univariate analysis of variance (ANOVA) revealed significant differences ($P < 0.05$) among means of the four groups for 13 and 23 (for male and female, respectively) out of 25 standardized truss characters. (For females, only t8, t19 and t25 were not significant). Eleven components accounted for most of the 95.9% of the total variations (PC1 = 33%, PC2 17% and PC3 13.5%), for male, and for female twelve components accounted with 96.3% of the total variations (PC1 = 37.5%, PC2 19% and PC3 14.7%).

Scatter plots of specimens relating the first and the second principal components (Fig. 4) revealed a visual definition of groups (separation of sexes). PCA dispersion showed a vast divergence between samples of LR and ARE compared to others (SHR and BR) that differ marginally (for both sexes). PCA loading showed differences in the abdominal and peduncle part of the body. The first three PCA altogether contained 70% of differences in males and 75% in females. Truss characters that had more correlation with the first two components are explained in Fig. 5 (separately for each sex).

In discriminant function analysis (DFA) the first two DF accounted for 88.5% and 93.8% of variance for male and female populations, respectively. But the overall random assignment of individuals into their original samples was low (male: 54.1%, female: 67.2%) (Table 2). The proportion of correctly classified individuals into their original samples revealed high inter-mixing among the males rather than females. Correlations between the truss characters and the discriminant functions are presented in Table 3. The classification functions of the four groups are listed in the Table 4.

Table 3 Contribution of truss characters to the canonical function for Shemaya (*A. chalcoides*), south of the Caspian Sea.

Truss distances of male	Function			Truss distances of female	Function		
	DF1	DF2	DF3		DF1	DF2	DF3
t1	0.916[a]	0.232	0.327	t14	0.566[a]	0.156	−0.235
t2	0.465[a]	−0.126	0.042	t9	0.439[a]	0.323	0.062
t8	−0.365[a]	0.034	−0.141	t16	0.426[a]	0.349	0.126
t3	0.251[a]	0.177	0.13	t3	0.415[a]	0.082	0.213
t9	0.202[a]	0.087	−0.198	t7	0.322[a]	−0.309	0.032
t12	−0.157[a]	0.067	0.008	t19	0.268[a]	−0.076	0.121
t24	0.106[a]	−0.026	0.093	t4	0.264[a]	0.068	−0.064
t10	−0.073[a]	−0.015	−0.059	t6	0.212[a]	−0.045	0.171
t4	0.045[a]	0.029	−0.022	t20	−0.173[a]	0.154	−0.003
t21	0.051	0.879[a]	−0.475	t5	0.159[a]	0.095	0.015
t15	−0.085	−0.588[a]	0.081	t21	0.21	−0.643[a]	−0.204
t20	−0.016	−0.568[a]	0.151	t23	0.144	−0.600[a]	−0.219
t23	0.002	0.539[a]	−0.375	t10	0.328	0.558[a]	−0.322
t19	0.095	0.300[a]	0.023	t12	0.505	0.525[a]	−0.402
t16	0.101	−0.201[a]	0.073	t15	−0.235	0.434[a]	0.383
t5	−0.018	0.196[a]	0.167	t11	0.157	0.299[a]	−0.187
t13	0.025	0.137[a]	−0.131	t2	0.165	0.177[a]	0.162
t22	0.012	0.132[a]	0.011	t25	−0.138	−0.177[a]	0.002
t18	−0.068	−0.113[a]	0.026	t24	0.037	−0.156[a]	−0.062
t7	−0.27	0.478	0.836[a]	t13	0.241	−0.196	0.675[a]
t6	0.041	0.296	0.513[a]	t1	0.236	0.08	0.553[a]
t17	0.063	0.088	−0.291[a]	t22	−0.208	0.045	−0.519[a]
t11	−0.142	0.043	−0.256[a]	t18	−0.285	0.042	−0.489[a]
t25	−0.134	−0.036	0.213[a]	t17	0.355	0.093	0.433[a]
t14	0.068	0.115	−0.115[a]	t8	0.047	0.128	−0.373[a]
Eigen value	0.303	0.258	0.073	Eigen value	0.846	0.448	0.086

Variables ordered by absolute size of correlations within function.

[a] Largest absolute between each variable and any discriminant function.

Table 4 Classification functions for the four groups of Shemaya (*A. chalcoides*).

BR	SHR	ARE	LR	
Male				
1.903	1.951	1.825	1.846	t1
2.038	1.996	2.142	1.948	t7
0.341	0.309	0.316	0.300	t21
−441.747	−439.746	−424.714	−402.596	Constant
Female				
3.208	3.223	3.132	3.200	t1
3.131	3.130	3.076	3.165	t12
1.950	1.906	1.871	1.819	t7
1.471	1.513	1.520	1.527	t15
0.217	0.221	0.180	0.177	t13
0.268	0.214	0.145	0.184	t14
1.070	1.050	1.060	1.038	t21
−2107.457	−2097.679	−2009.458	−2089.004	Constant

Character t1 (frontal length) was dominantly contributed to DF1 for both sexes and the common important character to DF2 was t21 (length of anal fin) for both sexes.

The Euclidean distance values between the four populations are shown in Table 5 and the dendrogram obtained by UPGMA cluster analysis (CA) among centroids revealed three major groups (Fig. 6).

Discussion

Present study revealed considerable distinction between populations of Shemaya (*A. chalcoides*) in the south of the Caspian Sea in terms of size and body shape for both sexes. The standard length (SL) of *A. chalcoides* was significantly different among populations (Fig. 3). Females showed a significant longer standard length than males in all populations indicating a sexual dimorphism when size was considered. Bagherian and Rahmani (2009) had already pointed out such sexual dimorphism for *Chalcalburnus chalcoides*. From a different point of view, size related characteristics strongly seem to have a conquering role in morphometric analysis and the outcome might be fallacious if not adjusted for statistical analysis of data (Tzeng, 2004).

The causes of morphological differences between populations are often quite difficult to explain (Poulet et al., 2004), but it is well known that morphometric characters can show a high degree of plasticity in response to environmental conditions (Wimberger, 2008). Lisar (LR) and Anzali (ARE) populations are geographically very close to each other, but they displayed different body shapes. Such morphological differences among different populations of a species may be related to differences in habitat factors such as temperature, turbidity, food availability, water depth and flow (Allendorf, 1988; Swain et al., 1991; Wimberger, 2008). Anzali population of *A. chalcoides* lives in water with gentle current and dense aquatic vegetation, whereas that of Lisar (LR) population lives in fast running shallow water with little vegetation on river bank and Shiroud (SHR) population is found in faster water current and high turbidity of Shiroud river. The taller frontal of the head and smaller anal fin of SHR and LR populations might be acclimations to repel the agitated water. The discriminant function analysis (DFA) displayed the length of frontals (t1) and anal fin (t21) differences (for both sexes) (Table 4).

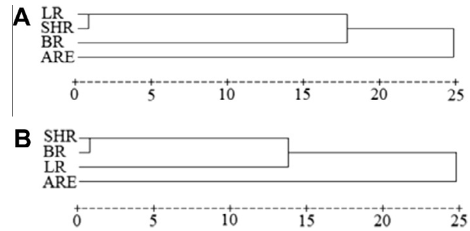

Figure 6 Cluster dendrogram of the four populations of Shemaya (*A. chalcoides*) derived from Euclidean distances. A: male samples, B: female samples. (LR: Lisar river, ARE: Anzali Region, SHR: Shiroud river, BR: Babolroud river).

Also cluster Analysis (CA) using UPGMA revealed (Fig. 6) that populations which have been studied could be divided into two groups (clusters) showing ARE as one side and the remaining three in the other side based on the type of water body i.e. lentic versus flowing water. The lentic water of Anzali region (ARE) has provided secured environment with plenty of food causing an average larger size of ARE population and to be remarked as a separate population.

Bagherian and Rahmani (2007) have considered the variation in size among populations to be largely dependent on environmental parameters, whereas the shape variation may reflect genetic constitution. The present study suggests morphologic differences among Shemaya populations in the southern Caspian Sea were the results of different evolutionary patterns due to different environmental conditions such as water depth, water current, and food availability. As Hossain et al. (2010) have stated these populations or stocks seem to be

Table 5 The Euclidean distances between the four groups of Shemaya (*A. chalcoides*) derived from size-free shape matrix. (A: male, B: female).

Area	LR	ARE	SHR	BR
A				
LR	0.00	45.2	36.1	54.2
ARE		0.00	51.1	54.2
SHR			0.00	38.2
BR				0.00
B				
LR	0.00	56.6	39.8	55.8
ARE		0.00	53.9	60.0
SHR			0.00	38.0
BR				0.00

reproductively isolated and therefore need to be considered as unique evolutionary taxa or evolutionary significance for conservation purposes.

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