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Original Article

Morphological variation of shad fish *Alosa brashnicowi* (Teleostei, Clupeidae) populations along the southern Caspian Sea coasts, using a truss system

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Abstract: A 15-landmark morphometric truss network system was used to investigate the hypothesis of population fragmentation of Shad fish *Alosa braschnicowi* Borodin, 1904 along the southern Caspian Sea. A total of 181 *A. braschnicowi* specimens were caught from six localities, respectively from the west to the east including; Astara, Rezvanshahr, Anzali, Tonekabon, Sari and Miankale. Principal component analysis, canonical variates analysis and clustering analysis were used to examine morphological differences. Univariate analysis of variance showed significant differences between the means of the six groups for 72 standardized morphometric measurements out of 105 characters studied. In canonical variates analysis, the overall assignment of individuals into their original groups was 71.46% and scatter plot of individual component scores between CV1 and CV2 showed fish specimens grouped into six areas. Clustering analysis based on Euclidean square distances among groups of centroids using UPGMA resulted into six main clusters indicating morphologically distinction populations of *A. braschnicowi* in the region. These populations of *A. braschnicowi* are distinguished especially by head shape, eye diameter, and pre-dorsal, pre-pelvic and pre-anal distances. Therefore, it is suggested considering these morphologically different populations as distinct stock in the southern Caspian Sea coasts.

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Introduction

The study of morphological characters with the aim of defining or characterizing fish stock units, has a great interest in ichthyology (Bektas and Belduz, 2009). The morphometric characters is particularly important where the differences are mostly attributed to environmental influences rather than genetic differentiation (Bektas and Belduz, 2009). Geographical isolation of populations and interbreeding can lead to morphometric variations between populations, and this morphometric variation can provide a basis for population differentiation (Bookstein, 1991).

The family Clupeidae is found in warmer marine waters with some anadromous or permanent freshwater residents. This family has about 200 species in 56 genera worldwide (Eschmeyer and Fong, 2011; Coad, 2014), with eight reported species in the Caspian Sea. *Alosa braschnicowi* is an economically important clupeids of the Caspian Sea that widely distributed across this sea. This species distributes in the south in winter, moving north to spawn in spring (Coad, 2014). The morphometric characters between male and female sexes in this species did not different (Whitehead, 1985). The study of morphometrics using the truss network system is effective in capturing information about the shape of an organism (Kocovsky et al., 2009). It covers the entire fish in a uniform network, and theoretically, increases the likelihood of extracting morphometric differences between specimens (Kocovsky et al., 2009; Cakmak and Alp, 2010).

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Figure 1. Sampling site locations of *A. braschnicowi* along the southern Caspian Sea coast.

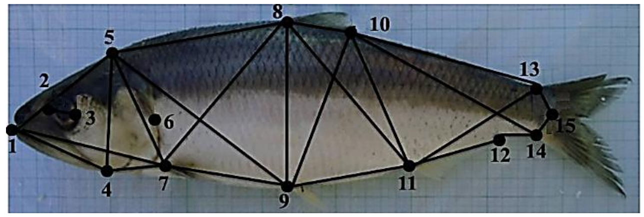

Figure 2. Locations of the 15 landmarks for constructing the truss network on *A. braschnicowi*. 1- tip of snout; 2- anterior edge of eye; 3- posterior edge of eye; 4- posterior tip of maxillary; 5- forehead (end of frontal bone); 6- end of operculum; 7- dorsal origin of pectoral fin; 8- origin of dorsal fin; 9- origin of pelvic fin; 10- termination of dorsal fin; 11- origin of anal fin; 12- termination of anal fin; 13- dorsal side of caudal peduncle, at the nadir; 14- ventral side of caudal peduncle, at the nadir; 15- end of body lateral line.

Therefore, it has been used in the differentiation of various populations within a species and also various species (Kocovsky et al., 2009). Various effects of the geographical isolation on fish population in the southern Caspian Sea basin have been documented in the recent past (Mousavi-Sabet et al., 2011; Mousavi-Sabet et al., 2012; Mousavi-Sabet and Anvarifar, 2013; Kohestan-Eskandari et al., 2014). However, the variability of the *A. braschnicowi* population parameters and its spatial distribution has not been studied in Iranian waters of the Caspian Sea. Therefore, propose of this study was to use a set of morphometric characters for examine whether specific ecological constraints, due to geographic variation, could affect the formation of stock separation for this species.

Materials and methods

A total of 181 specimens of *A. braschnicowi* were randomly collected by beach seine from six fishing regions along the southern Caspian Sea coasts, including the Miankale (36°54′10.89″ N, 53°48′48.33″ E; 31 individuals), Sari (36°48′04.63″ N, 53°02′07.50″ E; 30 individuals), Tonekabon (36°50′20.97″N, 50°50′17.25″E; 30 individuals), Anzali (37°29′29.86″ N, 49°27′39.59″ E; 30 individuals), Rezvanshahr (37°36′32.19″ N, 49°08′25.54″ E; 30 individuals) and Astara (38°23′47.40″ N, 48°54′10.17″ E; 30 individuals) in November 2011 (Fig. 1). The sampled fish were fixed in 10% formaldehyde at the sampling sites and transported to the laboratory.

For extracting morphometric data, the left side of fishes were photographed by a 300-dpi, 32-bit color digital camera (Cybershot DSC-F505; Sony, Japan) with dorsal and anal fins erected by pins. A total of 105 distance measurements between 15 landmarks were surveyed using the truss network system according to Bookstein (1991) with minor modifications (Fig. 2). Images were saved in jpg format, and the defined landmark points were digitized using TpsDig2 (Mustafic et al., 2008) on pictures. A box truss of 26 lines connecting the landmark points was generated for each fish to represent the basic shape of fish (Bookstein, 1991; Mustafic et al., 2008). All measurements transformed into linear distances for subsequent analysis (Mustafic et al., 2008). An allometric method was used to remove size-dependent variation in morphometric characters using following formula: $Madj = M(L_s / L_0)^b$, where M is the original measurement, $Madj$ the size adjusted measurement, L_0 the standard length of the fish, L_s the overall mean of the standard length for all fish from all samples in each analysis, and b 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 in any group. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length (Mustafic et al., 2008).

The sex of specimens was determined macroscopically, and there were no significant differences in tested variables between the sexes within the same stock. Therefore, the data for both

Table 1. The results of ANOVA for morphometric measurements of *A. braschnicowi* populations along the southern Caspian Sea.

Characters	F	P	Characters	F	P	Characters	F	P	Characters	F	P
1-2	0.000	1.000	3-4	2.801	0.019	5-9	7.800	0.000	8-11	3.636	0.004
1-3	25.690	0.000	3-5	6.842	0.000	5-10	3.547	0.005	8-12	6.034	0.000
1-4	11.691	0.000	3-6	2.720	0.022	5-11	4.875	0.000	8-13	1.980	0.085
1-5	6.637	0.000	3-7	1.169	0.327	5-12	3.665	0.004	8-14	5.013	0.000
1-6	13.629	0.000	3-8	7.566	0.000	5-13	5.467	0.000	8-15	4.246	0.001
1-7	12.917	0.000	3-9	3.790	0.003	5-14	2.905	0.015	9-10	0.517	0.763
1-8	7.118	0.000	3-10	5.837	0.000	5-15	3.658	0.004	9-11	4.800	0.000
1-9	3.654	0.004	3-11	3.430	0.006	6-7	9.633	0.000	9-12	1.729	0.131
1-10	1.298	0.267	3-12	5.928	0.000	6-8	7.476	0.000	9-13	1.807	0.114
1-11	5.886	0.000	3-13	3.609	0.004	6-9	3.316	0.007	9-14	2.028	0.078
1-12	3.354	0.007	3-14	4.244	0.001	6-10	8.110	0.000	9-15	1.606	0.161
1-13	5.519	0.000	3-15	7.471	0.000	6-11	3.348	0.007	10-11	1.960	0.088
1-14	6.359	0.000	4-5	9.823	0.000	6-12	6.974	0.000	10-12	1.401	0.227
2-3	28.648	0.000	4-6	3.275	0.008	6-13	5.541	0.000	10-13	0.443	0.818
2-4	9.007	0.000	4-7	0.086	0.994	6-14	4.937	0.000	10-14	0.822	0.536
2-5	7.263	0.000	4-8	3.720	0.003	6-15	9.226	0.000	10-15	0.425	0.831
2-6	14.608	0.000	4-9	2.135	0.064	7-8	2.386	0.041	11-12	2.907	0.015
2-7	14.325	0.000	4-10	2.523	0.032	7-9	5.660	0.000	11-13	3.253	0.008
2-8	7.073	0.000	4-11	1.837	0.109	7-10	3.010	0.012	11-14	1.164	0.330
2-9	2.917	0.000	4-12	2.746	0.021	7-11	2.020	0.079	11-15	1.951	0.089
2-10	1.472	0.202	4-13	1.659	0.148	7-12	4.070	0.002	12-13	1.746	0.127
2-11	5.020	0.000	4-14	1.945	0.090	7-13	2.921	0.015	12-14	0.410	0.842
2-12	1.167	0.328	4-15	3.272	0.008	7-14	2.026	0.078	12-15	2.963	0.014
2-13	2.895	0.016	5-6	12.284	0.000	7-15	4.412	0.001	13-14	0.837	0.525
2-14	4.422	0.001	5-7	18.966	0.000	8-9	1.209	0.307	13-15	0.389	0.856
2-15	2.595	0.028	5-8	8.181	0.000	8-10	3.924	0.002	14-15	0.998	0.421

sexes were pooled for all subsequent analyses.

Univariate analysis of variance (ANOVA) was performed for each morphometric character to evaluate the significant difference between the six populations (Rodriguez et al., 2010). Those morphometric characters which showed highly significant variations ($P \leq 0.01$) 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 (Bookstein, 1991) to obtain a stable outcome from multivariate analysis. The principal component analysis (PCA), canonical variates analysis (CVA) and cluster analysis (CA) by adopting the Euclidean square distance as a measure of dissimilarity and UPGMA (Unweighted Pair Group Method with Arithmetical average) as the clustering algorithm (Yakubu et al., 2011) were employed to discriminate the six populations. Statistical analyses for morphometric data were performed using the SPSS version 16 software package.

To determine the most effective morphometric

Table 2. Eigen values, percentage of variance and percentage of cumulative variance of principal component analysis of morphometric measurements for *A. braschnicowi* populations along the southern Caspian Sea.

Factor	Eigenvalues	Percentage of variance	Percentage of cumulative variance
1	15.567	25.945	25.945
2	9.203	15.338	41.283
3	7.332	12.220	53.503
4	6.235	10.392	63.895
5	3.961	6.601	70.496
6	3.198	5.330	75.826
7	2.662	4.437	80.263
8	2.609	4.348	84.611
9	1.907	3.179	87.789
10	1.771	2.952	90.741
11	1.178	1.963	92.704
12	1.094	1.823	94.527

measurement to differentiate studied 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) measures were performed. The Bartlett's Test of sphericity, tests the hypothesis that the values of the correlation matrix equal zero and the KMO measure of sampling adequacy tests, whether the partial correlation among variables is sufficiently high (Yakubu et al., 2011). The KMO statistics vary between 0 and 1 and the values greater than 0.5 are acceptable (Nimalathasan, 2009; Yakubu et al., 2011).

Results

The correlation between transformed morphometric variables and standard length was not significant (P>0.05) which confirms that size or allometric signature on the basic morphological data was accounted. Significant differences between six populations of *A. braschnicowi* were observed in terms of 72 morphometric characters out of 105 studied (Table 1). Of these 72 characters, 60 characters were found to be highly significant (P≤0.01) and were used for further multivariate analysis. In this study N:P ratio was 3.01 (181/60) that revealed samples size were adequate.

The value of KMO for overall matrix is 0.695, and the Bartlett's Test of sphericity is significant (P≤0.01). The results of KMO and Bartlett's suggest that the sampled data is appropriate to proceed with a factor analysis procedure.

Principal component analysis of 60 morphometric measurements extracted 12 factors with Eigen values>1, explaining 94.52% of the variance (Table 2). The first principal component (PC1) accounted for 25.94% of the variation and the second principal component (PC2) for 15.33% (Table 2), and the most significant loadings on PC1 were 1-3, 1-4, 1-6, 1-7, 1-9, 1-11, 1-12, 1-13, 1-14, 2-3, 2-6, 2-7, 2-11, 3-13, 3-14, 3-15, 4-6, 5-6, 5-7, 5-9, 6-8, 6-10, 6-11, 6-12, 6-13 6-14, 6-15, 7-15 and on PC2 were 1-8, 2-8, 3-8, 4-8, 5-8, 6-8, 8-10, 8-11, 8-12, 8-14, 8-15. In this analysis, the characteristics with an Eigen value exceeding 1 were included, and others discarded.

The CV1 and CV2 were plotted to allow visual examination of the distribution of each locality sample along the CVs (Fig. 3). In the scatter plot, the Miankale, Astara and Anzali specimens were grouped together in the same quadrant with high value for CV1 and low value for CV2 (quadrate IV), Tonekabon in a quadrant with low value for both CVs (quadrate III), Rezvanshahr in a quadrant with

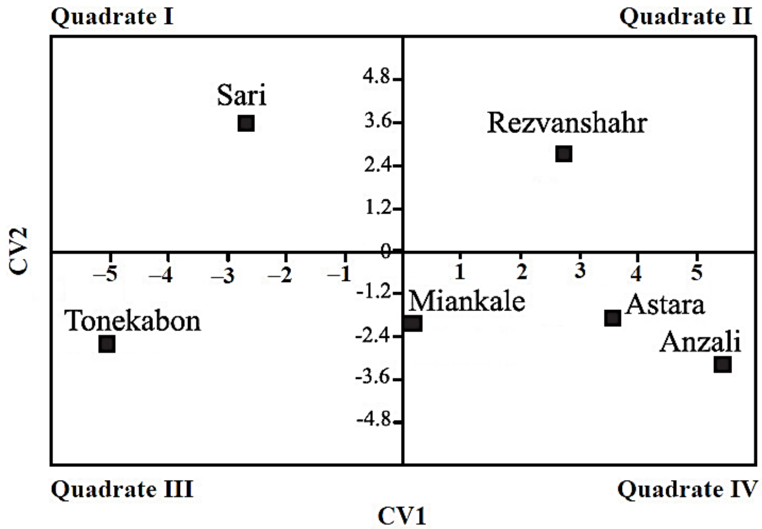

Figure 3. Scatterplot of group centroids of standardized canonical variates functions 1 (CV1) and 2 (CV2) for morphometric characteristics of six populations of *A. braschnicowi* along the southern Caspian Sea.

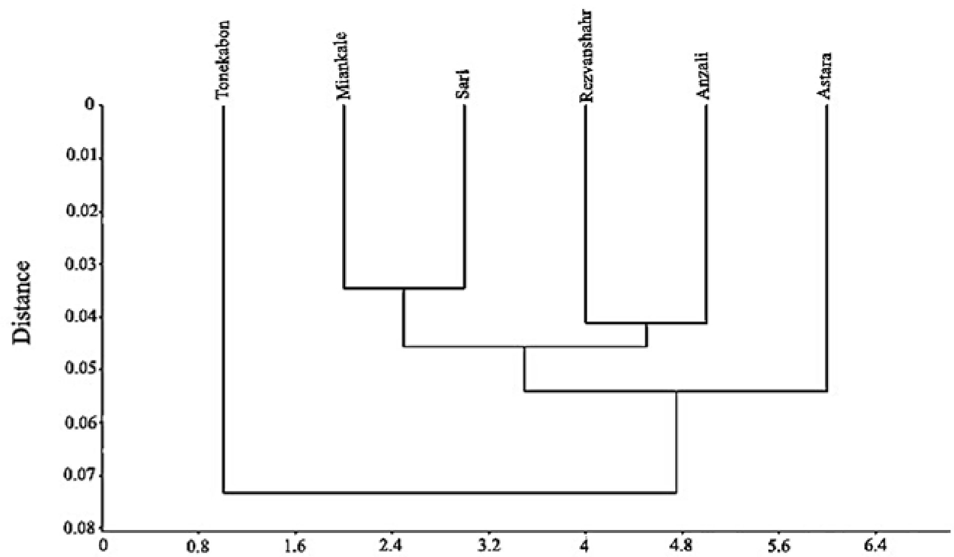

Figure 4. Dendrogram derived from cluster analyses of morphometric measurements on the basis of Euclidean distance for *A. braschnicowi* populations along the southern Caspian Sea.

high value for both CVs (quadrate II) and finally Sari in a quadrant with low value for CV1 and high value for CV2 (quadrate I). The overall random assignment of individuals into their original groups was high (71.46%), indicating that these samples are probably divergent from each other (Fig. 3).

The dendrogram derived from CA of Euclidean square distances among groups of centroids showed six main clusters based on morphometric characteristics. Despite the geographical distance between the Miankale and Sari regions and Rezvanshahr and Anzali regions, morphometric clustering revealed that the individuals of these locations form the same clade with great homogeneity, while Tonekabon and Astara exhibited higher heterogeneity, confirming the results obtained from discriminant analysis for this species (Fig. 4).

Discussion

The multivariate analysis of morphometric characteristics classified the *A. braschnicowi* populations along the southern Caspian Sea coasts into six distinct groups. The results demonstrate that there are morphologically distinct populations of

A. braschnicowi particularly for Tonekabon region. These morphological differences is solely related to body shape variation and not to size effects which were successfully accounted by allometric transformation. Size-related traits play a predominant role in morphometric analysis and the results may be erroneous if not removed from data (Bookstein, 1991; Buj et al., 2008; Torres et al., 2010).

The analysis of variance also revealed significant phenotypic variation among the six populations. CVA could be a useful method to distinguish different stocks of the same species (Yakubu and Okunsebor, 2011). The present study showed a high differentiation among the populations of *A. braschnicowi* in the studied areas. This segregation was partly confirmed by PCA, where revealed that the populations were distinct from each other.

The causes of morphological differences among populations are often quite difficult to explain (Bookstein, 1991). It has been suggested that the morphological characteristics of fishes are determined by genetic, environment and the interaction between them (Heidari et al., 2013; Kohestan-Eskandari et al., 2014). The environmental factors prevailing during the early development stages, when the individual's phenotype is more amenable to environmental influence is of particular importance (Eschmeyer and Fong, 2011). The phenotypic variability may not necessarily reflect population differentiation at the molecular level (Bookstein, 1991). Apparently, different environmental conditions can lead to an enhancement of pre-existing genetic differences, providing a high interpopulation structuring (Eschmeyer and Fong, 2011; Heidari et al., 2013; Mousavi-Sabet and Anvarifar, 2013).

The Tonekabon (in the south-central part of the Caspian Sea), Miankale (in the southeast part of the sea) and Astara (in the southwest part of the sea) specimens are more distinct from the others. The distinctive environmental conditions of the Tonekabon, Miankale and Astara relative to the other studied areas may underlie the morphological differentiation among these three populations. The studied populations are distinguished from each other by morphologic differences especially in head shape, eye diameter, and pre-dorsal, pre-pelvic and pre-anal distances.

Geographical isolation can also affect growth pattern and reproductive strategy of fish species. The importance of such factors on producing morphological differentiation in fish species is well-known (Yamamoto et al., 2006; Pollar et al., 2007; Heidari et al., 2013).

As conclusion, the present study proposes high morphological differentiation among *A. braschnicowi* populations along the southern Caspian Sea coasts. The results also suggest these morphologically different populations should be considered as distinct stock in the southern Caspian Sea in fisheries management and commercial exploitation of this species and any stock enhancement program. Nevertheless, future studies on determination of population structure will be elucidated using biochemical and molecular genetics methods.

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