Geometric Morphometric analysis of *Alosa braschnikowi* (Teleostei, Clupeidae) populations in the southern Caspian Sea

Sattari, M.¹, Mazareiy, M.H. ¹, Khataminejad, S.², Bibak, M.¹ and Imanpour Namin, J. ¹

¹Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Iran
²Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
³Department of Marine Biology, the Caspian Basin Research Center, University of Guilan, Rasht, Iran

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**Abstract**

Morphological studies are strong and instrumental for determining discreteness of the similar species and extensively used to identify differences between fish populations. A total of 216 specimens of *Alosa braschnikowi* were randomly collected by beach seine from three fishing regions along the southern Caspian Sea coasts, including some regions of Miankaleh, Sari and Anzali, in the fishing season during 2018-2019. The extracted landmark-points (body shape data) were submitted to a generalized Procrustes analysis (GPA) to remove non-shape data in PAST software. In the present study, the size effect was removed successfully by procrustes action in PAST software. Principal component analysis (PCA) was performed to summarize the variation among the specimens as few dimensions as possible. As a complement to discriminant analysis, morphometric distances among the three localities were inferred to cluster analysis by adopting the Euclidean square distance as a measure of dissimilarity method as the clustering algorithm. The dendrogram derived from cluster analysis of Euclidean square distances showed that the three populations of *A. braschnikowi* were distinct from each other in terms of morphometric characters. The Wilks’ lambda tests of discriminant analysis indicated significant differences in morphometric characters of the three populations. The results of the present study demonstrated significant morphological differences between the three populations of *A. braschnikowi*. These differences in the three studied basins were mainly related to the characteristics of head and snout, body height, caudal peduncle, dorsal and anal fin base, which could be related to the hydraulic conditions and diet of the populations.

**Key words:** *Alosa braschnikowi*, Clupeidae, Shape variation, Landmark, Caspian Sea.

**INTRODUCTION**

Morphological studies are strong and instrumental for determining the discreteness of the similar species (Mousavi-Sabet *et al.*, 2011; Mousavi-Sabet *et al.*, 2012) and extensively used to identify differences between fish populations (Mousavi-Sabet & Anvarifar, 2013). Morphometrics is a good research method that specialized in shape variation and its covariation with other variables (Bookstein, 1991). Body shape differences not only reflect the genetic characteristics of populations but also environmental parameters (Guill *et al.*, 2003). The morphological characteristics of fish are affected by genetic and environmental factors, which are an important basis for species identification and species classification. The differences between populations of a single species can indicate differences in habitat and behavioral characteristics, because the aquatic organisms, e.g., fishes require to adapt to their environmental conditions for better functioning of their biological...
systems (Webb, 1982) Therefore, morphological adaptations to environmental conditions along with geographical isolation can provide crucial information on the evolutionary trend of these organisms particularly in aquatic ecosystems. Phenotypic differences among fish populations and identifying their causes and consequences may be informative of differences in natural history across a species’ geographic range, which would have implications for both theoretical and applied researches in ecological and fishery science (Love and Chase, 2009). Morphometric population differentiation is important from various viewpoints including evolution, ecology, behavior, conservation, water resource management and stock assessment (AnvariFar et al., 2013).

For species like the Caspian shad, investigation on morphometric variations is essential as this species is widely distributed in the Caspian Sea. 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 (Coad, 2017), with eight reported species in the Caspian Sea (Esmaeili et al., 2017) and 11 species in the inland waters of Iran (Esmaeili et al. 2017). Alosa braschnicowii (Borodin, 1904) is an economically important clupeid of the Caspian Sea that widely distributed across this sea. This species is endemic to the Caspian Sea and distributes in the south in winter, moving north to spawn in spring (Coad, 2019).

Geometric morphometrics has been developed in the last decades as a reliable tool concerning size and shape analyses through several studies (Zelditch et al., 2004; Strauss, 2010). As the revolution of morphometrics, geometric morphometric method combined with multivariate statistical analysis could capture the overall morphological changes of shape, avoid the loss of information of specimen’s structure and consider the global anatomic context (Rohlf & Marcus, 1993; Adams et al., 2004; Slice, 2007). Geometric morphometrics (GM) is a widely used technique in determining shape variation. Instead of using linear measurements, counts, and ratios, as in traditional morphometrics (Adams et al., 2004), data in GM are recorded in the form of coordinates of landmark points (Rohlf & Marcus, 1993). Landmark-based GM uses the landmark coordinates to extract the shape data (Zelditch et al., 2004) and has been considered as a useful tool to assess phenotypic plasticity. Landmark points are defined as homologous points that bear information on the geometry of biological forms (Bookstein, 1991).

One important advantage of the GM approach to traditional ones is that GM does not need to decide a priori which measurements are likely to display differences (Rohlf and Marcus, 1993). GM also allows to point out the structures better fitted to be used to separate species, which, combined with color patterns in recently captured specimens, may allow adapting catch data to be treated at the species level (Santos et al., 2019). Due to its obvious advantages, GM has widely applied to analyze the relationship between morphology and habitat (Idaszkin et al., 2013; Foster et al., 2015), growth stages and shape morphological differences among geographic populations (Braga et al., 2017), as well as between species or subspecies (Tofilski, 2008; Stange et al., 2016). The body shape is a major component of an organism’s phenotype, and it bears important traits of its biological characters such as locomotor performance, feeding efficiency, vulnerability to predators, and reproductive success (Guill et al., 2003). Fish body shape can be the result of evolutionary adaptations to environmental pressures (Winemiller, 1991) Therefore, the body shape differences of populations are considered as essential steps in the process of speciation (Margurran, 1998). Most members of the genus Alosa Linck, 1790 in Iran exhibit relatively different body forms. This study was to investigate the body shape the interspecies, among the three populations of Alosa braschnikowii (Borodin, 1904) with visualization techniques afforded by the GM approach.

**Material and Methods**

A total of 216 specimens of A. braschnikowii were randomly collected by beach seine from three fishing regions along the southern Caspian Sea coasts, including some regions of Golestan Province (Miankaleh) (36°56'46.1"N 53°54'53.3"E; 57 individuals), Mazandaran Province (Sari)
GEOMETRIC MORPHOMETRIC ANALYSIS OF *ALOsa brachynikoit* (36°47'27.7"N 52°47'34.8"E; 64 individuals) and Guilan Province (Anzali) (37°30'01.9"N 49°26'44.2"E; 95 individuals), in the fishing season during 2018-2019 (Fig. 1). The specimens were kept in the freezer and transported to the laboratory for further examinations. After thawing, each individual was photographed for digital analysis.

![Sampling stations in the south Caspian Sea (north of Iran).](image)

**FIGURE 1.** Sampling stations in the south Caspian Sea (north of Iran).

**Laboratory works**
The left side of the specimens (with dorsal and anal fins were held erected using pins) was photographed using a digital camera (Samsung DV150F). Fifteen homologous landmark-points were defined and digitized on 2-D images using tpsDig2 software version 2.16 (Rohlf, 2004). The landmark-points were selected to the best representation of the external body shape (Fig. 2). The landmark-points were chosen at the specific points, in which a proper model of fish body shape was extracted (Bookstein, 1991).

**Data analysis**
The extracted landmark-points (body shape data) were submitted to a generalized Procrustes analysis (GPA) to remove non-shape data in PAST software. The multivariate analysis of variance/canonical variate analysis (MANOVA/CVA) was used to investigate power of distinction among groups. These morphological differences may be solely related to body shape variation and not to size effects which were successfully accounted for by the allometric transformation. On the other hand, size-related characteristics 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 Procrustes action in PAST software. Principal component analysis (PCA) was performed to summarize the variation among the specimens as few dimensions as possible (Klingenberg, 1998). As a complement to discriminant analysis, morphometric distances among the three localities were inferred to cluster analysis by adopting the Euclidean square distance as a measure of dissimilarity method as the clustering algorithm (Sneath & Sokal, 1973). The patterns of taxon’s body shape were illustrated in transformation grids relating to consensus configuration of all specimens presented, depicting relative shape differences among the populations (Khataminejad & Bani, 2018).

**RESULTS**
The CVA of standardized morphological measurements exhibited significant differences between groups (P<0.001). PCA for all specimens explained 46.69% of shape variations by the first two PC
axes extracted from the variance-covariance matrix (PC1 = 28.91% and PC2 = 17.77%). PC1 scores were related to the curvature of the back of the body towards the bottom, the curvature of the snout and caudal peduncle upward, as well as elongation anal fin whereas PC2 scores, were related to decreased head, snout, eyes and dorsal fin size along with shorter caudal peduncle. Separation of the three examined populations showed along the first and second axis, respectively (Fig. 3).

**Figure 2.** Defined landmark points to extract body shape. 1. Anterior tip of the premaxilla; 2. Front of the eye; 3. End of the eye; 4. Beginning of the scales at the dorsal side; 5. The lower beginning of operculum; 6. End of operculum; 7. Base of the pectoral fin; 8. Base of the pelvic fin; 9 and 10. Anterior and posterior insertion of the dorsal fin; 11 and 12. Anterior and posterior insertion of the anal fin; 13. Upper margin of caudal peduncle; 14. Lower margin of caudal peduncle; 15. End of the medial region of caudal peduncle.

**Figure 3.** The results of Principal Component Analysis (PCA) of three populations of the species *A. braschnikowi* body shape
Table 1. Mahalanobis distance analysis for the three populations of A. braschnikowi

<table>
<thead>
<tr>
<th>Station</th>
<th>Golestan</th>
<th>Guilan</th>
<th>Mazandaran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guilan</td>
<td>4.1320</td>
<td></td>
<td>3.8742</td>
</tr>
<tr>
<td>Mazandaran</td>
<td>2.2054</td>
<td>3.8742</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Scatterplot of A. braschnikowi specimens according to the first two discriminant functions from Geometric morphometrics data analysis.

Mahalanobis distance analysis showed that the most differences were in terms of body shape between Guilan and Golestan populations, while the least differences among Golestan and Mazandaran ones. The Mahalanobis distances among the populations are represented in Table 1.

According to the results of the DFA analysis, the populations were divided into three groups (Fig. 4), which indicates a high degree of differentiation among the A. braschnikowi population in the study areas. Grouping related to either uniform component, Mazandaran and Golestan overlap broadly, but Guilan had less overlap with the other populations. There was a high degree of separation in body shape characters between Guilan and the other two provinces, with a slight degree of separation between Golestan and Mazandaran populations (Fig. 5).

The dendrogram derived from cluster analysis of Euclidean square distances showed that the three populations of A. braschnikowi were distinct from each other in terms of morphometric characters (Fig. 6). Longer head and snout, large body depth in the midsection, short and depth caudal peduncle, longer dorsal and anal fin bases for Guilan population, while short head and snout, shallow body depth in midsection, longer caudal peduncle, short dorsal and anal fin bases for Golestan one. Short head and snout, short dorsal and longer anal fin base, longer caudal peduncle and large body depth were observed in the Mazandaran population.

The Wilks’ lambda tests of the discriminant analysis indicated significant differences in morphometric characters of the three populations. In this test, two functions were highly significant.
Discriminant analysis (DA) on the relative warps classified 84.7% in original data and 81.9% in cross-validation of specimen into the correct groups. Medium classification success rates were obtained for Guilan (95.80%), Golestan (78.90%) and Mazandaran (64.10%) populations, indicating the first one (Guilan population) as a high correct classification of specimens into their populations (Table 3).

**Figure 5.** Histogram of discriminate analysis (DA) functions of three populations of the species *A. braschnikowi* body shapes in different locations with respect to the first two canonical variables.
GEOMETRIC MORPHOMETRIC ANALYSIS OF *ALOSA BRASCHNIKOWI*

**FIGURE 6.** Dendrogram derived from cluster analysis of morphometric variables on the basis of Euclidean distance of the three populations of *A. braschnikowi* in Iran. Mean shape of species in relation to consensus shape of the three populations of *A. braschnikowi* are represented.

**TABLE 2.** Wilks' lambda test for verifying difference among three populations

<table>
<thead>
<tr>
<th>Test of Function(s)</th>
<th>Wilks' Lambda</th>
<th>Chi-square</th>
<th>Df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 through 2</td>
<td>0.18</td>
<td>353.69</td>
<td>22</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.71</td>
<td>69.38</td>
<td>10</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**TABLE 3.** Classification matrix showing the number and percentage of individuals that were correctly classified

<table>
<thead>
<tr>
<th></th>
<th>Guilan</th>
<th>Mazandaran</th>
<th>Golestan</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Original (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guilan</td>
<td>96.8</td>
<td>2.1</td>
<td>1.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Mazandaran</td>
<td>7.8</td>
<td><strong>68.8</strong></td>
<td>23.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Golestan</td>
<td>3.5</td>
<td>14.0</td>
<td><strong>82.5</strong></td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Cross-validated (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guilan</td>
<td>95.8</td>
<td>3.2</td>
<td>1.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Mazandaran</td>
<td>7.8</td>
<td><strong>64.1</strong></td>
<td>28.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Golestan</td>
<td>3.5</td>
<td>17.5</td>
<td><strong>78.9</strong></td>
<td>100.0</td>
</tr>
</tbody>
</table>

The causes of morphological differences between populations are often quite difficult to explain (Poulet *et al.*, 2004) but it has been suggested that the morphological characteristics of fish are determined by genetic, environment and the interaction between them (Poulet *et al.*, 2004; Pinheiro *et al.*, 2005). Prevailing environmental factors are important in the early stages of development because larvae are very effective at these stages (Pinheiro *et al.*, 2005). Body shape differences between two habitats often reflect variations in the swimming and feeding of fishes (Langerhans *et al.*, 2003; Tahmasebi *et al.*, 2014; Haghighy *et al.*, 2015). Different prey resources for larvae and juveniles may lead to different adult body shapes, suggesting that there is a phenotypic plastic response to resource availability (Wimberger, 1990). Morphological changes induced by environmental factors may help a better understanding of the phenotypic plasticity process as a result of induced factors (Mohaddasi *et al.*, 2013; Jalili *et al.*, 2015).
DISCUSSION

The results of the present study demonstrated significant morphological differences between the three populations of *A. braschnikowi*. These differences in the three studied basins were mainly related to the characteristics of head and snout, body height, caudal peduncle, dorsal and anal fin base, which could be related to the hydraulic conditions and diet of the populations (Chapman et al., 2008). Differences in dorsal and anal fins and caudal peduncle are considered as pivotal variations associated with motion. Differences in body height and dimensional dimensions can be considered as adaptations related to hydrodynamic or nutritional conditions (Fisher & Hogan, 2007). The *A. braschnikowi* population in Guilan had a longer head and snout, short and depth caudal peduncle, longer dorsal fin base that was different from *A. braschnikowi* populations in Mazandaran and Golestan. However, in the population of Guilan and Mazandaran, large body depth in the midsection and longer anal fin base were observed, and the population of Golestan was shallow body depth and short anal fin base. Essentially, the large body depth suggests adaptability for quick maneuverability and can help find food (Langerhans et al., 2003). Inland populations, must cope with long and energetically demanding migrations, thus selection should favor a more fusiform body shape that minimizes energy expenditure. Differences in head and snout shapes of Guilan population of *A. braschnikowi* with other populations of *A. braschnikowi*, can be considered as reflective of differences in feeding resources, selection of food items and direction of feeding (Langerhans et al., 2003). Environmental factors through natural selection can increase the efficiency of a phenotype among the members of a population and thus is led to morphological isolation in different habitats (Keeley et al., 2007).

The results of Mahalanobis distances and cluster analysis showed a closer distance between the *A. braschnikowi* populations of Mazandaran and Golestan, which could indicate a greater similarity in their body shape compared to the *A. braschnikowi* population of Guilan. The results revealed the relationship between the geographical distance and the differentiation of various populations of the *A. braschnikowi* region (Bibak et al., 2013). Mazandaran is located in the southern part of the Caspian Sea, Golestan in the southeastern part of the Sea and Guilan in the southwest of the Sea. Different environmental conditions of Mazandaran, Golestan and Guilan may affect morphological differentiation among these three populations. Geographical separation can affect the growth and reproduction strategy of fish, the importance of these factors in morphological differentiation is known in fish species (Yamamoto et al., 2006). Langerhans et al. (2003) found that the distance between habitats correlated positively with the level of divergence in body shape among conspecific populations of two Neotropical fish species. According to Cadrin & Silva (2005), the geographic variation in adult morphology for *Limanda ferruginea* may be explained by differences in ontogenetic rates among local populations if morphology is a product of ontogenetic history. Turan et al. (2011) examined systematic relationships among four genera and nine species of the Mugilidae family in the Mediterranean Sea, and in all species except *L. chelon* and *L. oedalechilus*, a significant degree of morphological differentiation was detected. Costa & Cataudella (2007) found that shape differences were related to trophic ecology for several species of the family *Sparidae*, thus indicating local adaptation and possibly ecological radiations (Langerhans et al., 2003). If the shape is related to either environmental influences on larval development (Cadrin & Silva, 2005) or diversifying selection and ecological adaptation at a trophic level (Costa & Cataudella, 2007), then spatially or latitudinal different environmental factors (e.g., temperature and resource availability) may explain the variations in body shape among the studied species.

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 inter-population structuring (Mousavi-Sabet and Anvarifar, 2013). Abdolhay et al. (2012) showed the high inbreeding that happened in *Rutilus kutum* population, can lead to low genetic variability in four populations of...
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*Rutilus kutum* in southern shores of the Caspian Sea. Franssen *et al.* (2013) evaluated shape differences between stream and reservoir populations of emerald shiners and also found inconsistent patterns in divergence. Morphometric studies of Turan & Basusta (2011) showed that there is significant heterogeneity among the populations of *Alosa fallax* in Turkish waters. Meng *et al.* (2018) analyzed geometric morphometry among three Lenoks of *Brachymystax* in China. Their results showed that the shape variation of specimens between high- and low-altitude habitat is a significant difference (P<0.0001), in other words, they belonged to three different categories. The results of Paknejad *et al.* (2014) on the morphological diversity of the *A. braschnikowi* population using the truss system showed that there were various morphological populations on the southern Caspian coast. In general, the morphological characteristics of fish are more intra- and inter-species variation and are more sensitive to environmental changes, so the effects of some environmental factors such as temperature, salinity, food availability, or migration distance can potentially be effective on morphological distinctions (Turan *et al.*, 2006). Temperature plays a vital role during the early development of a green swordtail by the alternation of its body shape to provide its biological requirements to survive and decrease the adverse effect of resulted pressures (Bibak *et al.*, 2012; Bibak *et al.*, 2013).

The existence of morphology differences among populations of a species is a common phenomenon due to differences in the characteristics of habitat on the path to their evolutionary history (Antonucci *et al.*, 2009; Bertrand *et al.*, 2008), which is achieved through the directional selection in the habitats and can increase the chance of sustainability and survival in their habitat (Torres-Dowdall *et al.*, 2012). Morphological analysis could improve our knowledge of species stock structure, as the stock is considered as a group with unique phenotypic attributes (Paramo and Saint-Paul, 2010). The present study shows that there is a difference in the shape of the body of the *A. braschnikowi* in the south of the Caspian Sea, which it is suggested to use biochemical and molecular genetic methods in future studies to confirm the morphological. Specifications obtained based on the shape body of the *A. braschnikowi* can provide an identification key that is useful for distinguishing species, also can be considered for the programs of exploitation, management, and conservation programs in the Caspian Sea.

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