Morphological Variability of the *Aspius aspius taeniatus* (Eichwald, 1831) in the Southern Caspian Sea Basin

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Abstract
Traditional morphometric measurements and meristic counts were used to investigate the hypothesis of population fragmentation of *Mash mahi, Aspius aspius taeniatus* (Eichwald, 1831) among two fishing areas in southern Caspian Sea basin (Tonekabon: 32 specimens and Sari: 34 specimens). Univariate analysis of variance showed significant differences between the means of the two groups for 12 out of 26 standardized morphometric measurement and three out of nine meristic counts. In discriminant function analysis (DFA), the proportion of individuals correctly classified into their original groups was 82.1% and 61.2% for morphometric and meristic characteristics, respectively. Clustering based on Euclidean distances among groups of centroids using an UPGMA and also principal component analysis’ results (PCA) for morphometric and meristic data indicated that two samples of *Mash mahi* were distinct from each other in these regions, while there were a relatively high degree of overlap between two locations.

Keywords: *Aspius aspius taeniatus*, Morphometric, Meristic, Caspian Sea, population differentiation.

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Introduction

Mash mahi, *Aspius aspius* (Linnaeus, 1758), is a predator cyprinid fish (Kujawa et al., 1998). This freshwater fish found from the Rhine and north of the Alps in Europe to the drainages of the Black, Caspian and Aral seas but *Aspius aspius taeniatus* (Eichwald, 1831) is the subspecies found in the Caspian Sea (Coad, 2008). This species has been reported from Astara to Gorgan Bay in rivers and marshes and in the Caspian Sea of Iran (Kiabi et al., 1999). This is the only carnivorous cyprinid fish (Brylinska, 1986) that is a solitary predator on other fishes, frogs and even duckling that belong to family Cyprinidae (Coad, 2008). Kiabi et al., (1999) consider this species to be data deficient in the south Caspian Sea basin according to IUCN criteria. Also, this species classifies as vulnerable to endangered in Europe (Lelek, 1987).

Study of fishes in aquatic ecosystem is important from point of evolution, ecology, behaviour, conservation, water resource management and stock assessment (AnvariFar et al., 2011). Suitable management success of aquatic animal stock will gained by study of genetic stock of endemic species, identification of populations (Coad, 1980). To rational and effective fisheries management, determination of exploitive fish stock is too important, because each stock needs separate management to aim of optimal harvest (Erguden and Turan, 2005; Salini et al., 2004). The study of morphological characters, whether morphometric or meristic, with the aim of defining or characterizing fish stock units, has for some time been a strong interest in ichthyology (Tudela, 1999).

In addition to its ecological significance, *A. aspius* is an important taxon for sport and zoogeography (Coad, 2008). Iran is a developing country and considered as having high potential for aquaculture (Samaee et al., 2009). A notable part of the aquaculture industry in Iran is dependent on foreign fish species, and numerous attempts have been made (Ramin, 1999) or are currently underway to find propagation and rearing biotechnique norms for native species, to gradually replace foreign species. *A. aspius* is a principal candidate in Iran for reasons of brood stock availability, wide tolerance of extreme environmental conditions, food availability, high meat quality, and high consumer appreciation in northern Iran (Coad, 2008). Detailed basic knowledge of the biology, including information on population structure and variabilities at inter- and intra-population levels, will aid in establishing a reasonable breeding program for introduction of this wild species into aquaculture systems (Samaee et al., 2009). Studies carried out on Caspian Sea fishes shows that many of species possess speciation and population formation microprocess runs on, as Caspian species and black sea (Gholiev, 1997). Caspian species have formed subspecies populations in different parts of Caspian basin (Gholiev, 1997). There are several reports about southern Caspian Sea fishes that indicating the existence of morphological variability between different parts of this basin. For example Samaee et al., 2006 and 2009; Rahmani.
and Abdoli, 2008; Akbarzade et al., 2009; AnvariFar, 2011. However, the variability of this species and its spatial distribution has not been studied in Caspian Sea basin and the other parts of world. Also, there is a little information about the biology in case of this fish. Therefore, the propose of this study was to use a set of morphometric and meristic characters as a first step in analyzing potential fragmentation of *A. aspius* (Linnaeus, 1758) populations among the major fishing and spawning grounds in the southern Caspian Sea drainage, and to examine whether specific ecological constraints, due to geographic variation, could affect the formation of stock separation for this species. This study focused on identifying the differences *A. aspius taeniatus* (Linnaeus, 1758) populations inhabiting the Sari and Tonekabon casts on the basis of morphological (morphometric and meristic) polymorphisms.

**Materials and methods**

**Sampling**

Because of the conservation status of *Mash mahi* in Iran, a total of 67 mature *Aspius aspius taeniatus* (Eichwald, 1831) during the Spawning season in mid March from two sampling sites, including Sari (36° 47' 34.42" N, 53°01' 35.18" E) 35 individuals and Tonekabon (36° 51' 05.30" N, 50°49' 30.37" E) 32 individuals on Mazandaran coast in southern Caspian Sea (Fig. 1), were collected in 2010, by beach seine with 8 mm mesh. The distance between two stations is about 250 km. In order to morphological studies, the sampled fish were fixed in 10% formaldehyde and sent to the fisheries laboratory of Department of Fisheries in Azad University of Babol.

![Figure 1: Location of sampling sites including Sari (1) and Tonekabon (2) on Mazandaran coasts in southern Caspian Sea (modified from Samaee et al. 2009)](image-url)
Laboratory work

A total of 26 traditional morphometric characters (Fig. 2) were measured using electronic digital calipers to the nearest 0.001 mm. Morphometric parameters investigated in this study selected based on studies of Holcik et al., (1989); Coad (2008) and Samaee et al., (2009). To keep the morphological error constant, all measurements were applied by the same investigator. Based on Holcik et al., (1989), nine meristic variables were count in each specimen by direct observation. Abbreviations used for meristic characteristics are: ll, Lateral line scales; squ.sup, Scales above lateral line; squ.inf, Scales below lateral line; D1, Dorsal fin soft rays; D2, Dorsal fin spines; A1, Anal fin soft rays; A2, Anal fin spines; Gr1, posterior gill rocker; Gr2, Anterior Gill rocker. After measurement, the fishes were determined by macroscopic examination of their gonads, and this subset was used to statistically ANOVA test for sexual dimorphism in morphometric characters of *Mash mahi*.

Data analysis

As variation should be attributable to body shape differences, and not related to the relative size of the fish, an allometric method (Elliott et al., 1995) was used to remove size-dependent variation in morphometric characters:

\[ M_{adj} = M \left( \frac{L_s}{L_o} \right)^b \]

where \( M \) is original measurement, \( M_{adj} \) is the size adjusted measurement, \( L_o \) 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 \( \log L_o \) 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.

Univariate Analysis of Variance (ANOVA) was performed for each morphometric character to evaluate the significant difference between the two locations (Zar, 1984) and the morphometric characters that were significant were used for function analyses (DFA), principal component analysis (PCA). As a complement to discriminant analysis, morphometric and meristic distances between the individuals of two groups were inferred to Cluster analysis (CA) (Veasey et al., 2001) by adopting the Euclidean 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 morphological data were performed using the SPSS version 11.5 software package, Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) (Rohlf, 1990) and Excel 2007.

Results

Descriptive data for the sex ratio, range (Minimum-Maximum), mean, standard deviation (SD) of length and weight in case of sampled specimens is shown in Table 1. The age of the samples ranged from 2+-10+. Age composition of *Mash mahi* depending on sex in Sari and Tonekabon is shown in Table 2.
Table 1: Descriptive data of *Aspius aspius taeniatus* (Eichwald, 1831) in the Sari and Tonekabon samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sex</th>
<th>N</th>
<th>Min-Max (length)</th>
<th>Mean± S.D. (length)</th>
<th>Min-Max (weight)</th>
<th>Mean± S.D. (weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sari</strong></td>
<td>Male</td>
<td>23</td>
<td>317.3-399.1</td>
<td>376.4±23.7</td>
<td>730-3090</td>
<td>1097.7±470.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>11</td>
<td>400.7-583.6</td>
<td>434.7±50.4</td>
<td>700-1550</td>
<td>1250.0±255.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>34</td>
<td>317.3-583.6</td>
<td>401.0±46.9</td>
<td>700-3090</td>
<td>1154.2±406.7</td>
</tr>
<tr>
<td><strong>Tonekabon</strong></td>
<td>Male</td>
<td>22</td>
<td>334.8-358.1</td>
<td>366.7±13.4</td>
<td>500-1400</td>
<td>942.1±223.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
<td>387.4-433.9</td>
<td>403.1±16.0</td>
<td>650-1450</td>
<td>1023.7±235.9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>32</td>
<td>334.8-433.9</td>
<td>381.5±23.1</td>
<td>500-1450</td>
<td>975.0±228.6</td>
</tr>
</tbody>
</table>

Figure 2: Morphological parameters investigated in *Aspius aspius taeniatus* (Eichwald, 1831) samples. Landmarks selected based on studies of Holcik et al., 1989; Coad, 2008 and Samaee et al., 2009. H, body depth; h, minimum body depth; hA, height of anal fin; hD, height of dorsal fin; io, inter-orbital distance; hA, anal fin length; lac, head width; hc, head length; ID, length of dorsal fin base; PI, pectoral fin length; IV, ventral fin length; Oh, eye diameter; pA, preanal distance; pD, predorsal distance; poD, postdorsal distance; poO, postorbital length; prO, preorbital length (=snout length); P-V, pectoral-pelvic length; ; poA, Postanal length; hh, head high; IA, length of anal fin base; A-C, anal-caudal length; pV, preanal distanceV-A: ventral-anal length; SI, Standard length [straight line distance from most anterior point of snout (upper lip) to base of caudal fin (basis of central rays)]; TI, Total length; Fl, Fork length.
Table 2: Age composition of *Aspius aspius taeniatus* (Eichwald, 1831) depending on sex in the Sari and Tonekabon

<table>
<thead>
<tr>
<th>Age(Year)</th>
<th>Sari Males</th>
<th>Sari Females</th>
<th>Tonekabon Males</th>
<th>Tonekabon Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>3</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Interaction between morphometric characteristics used in this study and sex were not significant (*p*>0.05), demonstrating a negligible effect of sex on observed variations (Table 3). Data for both sexes were pooled for all subsequent analyses. There was no significant correlation between any of the transformed measured morphometric variables and standard length (*p*>0.05) indicating that the size effect was accounted for. Descriptive data for mean length and standard deviation (S.D.) and length range of sampled specimens is shown in Table 4. Differences (*p*<0.05) between the Sari and Tonekabon coast Sea’ Mash mahi was observed for 12 out of 26 of the morphometric characters and 3 out of 9 meristic counts (Table 4). The traits that were significant were used for DFA, PCA and CA. For decades authors of theoretical works on PCA and DFA recommended that the ratio of number of organisms (N) measured to the parameters (P) included in the analysis be at least 3:1. In recent work, Kocovsky et al., (2009) demonstrated that ratios between 3.5:1 and 8:1 were required for stability of eigenvalues and eigenvectors of PCA of morphometrics for three species of fish in the Laurentian Great Lakes in North America. Therefore, for this study N:P ratio in case of morphometric characters was 2.9:1 for the Sari site, 2.6:1 for the Tonekabon site and 5.5:1 overall. The appropriateness of factor analysis is dependent upon the sample size (Nimalathasan, 2009). In this connection, Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy is still another useful method to show the appropriateness of data for factor analysis. Being KMO coefficients approximately more than 0.6 indicate that PCA method will suitable for our data (Kasier, 1974). In this study the KMO coefficient were obtained 0.715 and 0.530 for morphometric and meristic characteristics, respectively that is explaining of appropriation of this test at good and medial level about these traits, respectively.
Table 3: The results of ANOVA for morphological characteristics between two sexes of *Aspius aspius taeniatus* (Eichwald, 1831) in the Sari and Tonekabon on Mazandaran coast in southern Caspian Sea basin.

<table>
<thead>
<tr>
<th>Morphometric characters</th>
<th>F value</th>
<th>P value</th>
<th>Meristic characters</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sl</td>
<td>0.41</td>
<td>0.52</td>
<td>L1</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>Tl</td>
<td>1.53</td>
<td>0.22</td>
<td>squ.sup</td>
<td>0.87</td>
<td>0.35</td>
</tr>
<tr>
<td>Fl</td>
<td>3.09</td>
<td>0.08</td>
<td>squ.inf</td>
<td>0.82</td>
<td>0.37</td>
</tr>
<tr>
<td>Hc</td>
<td>1.75</td>
<td>0.19</td>
<td>D2</td>
<td>0.04</td>
<td>0.85</td>
</tr>
<tr>
<td>Lac</td>
<td>0.04</td>
<td>0.84</td>
<td>D1</td>
<td>1.32</td>
<td>0.25</td>
</tr>
<tr>
<td>Hh</td>
<td>0.39</td>
<td>0.53</td>
<td>A2</td>
<td>0.15</td>
<td>0.70</td>
</tr>
<tr>
<td>H</td>
<td>3.06</td>
<td>0.08</td>
<td>A1</td>
<td>0.16</td>
<td>0.69</td>
</tr>
<tr>
<td>Hh</td>
<td>0.09</td>
<td>0.76</td>
<td>Gr1</td>
<td>3.48</td>
<td>0.06</td>
</tr>
<tr>
<td>prO</td>
<td>0.21</td>
<td>0.65</td>
<td>Gr2</td>
<td>1.85</td>
<td>0.18</td>
</tr>
<tr>
<td>Oh</td>
<td>0.31</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Io</td>
<td>0.27</td>
<td>0.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>poO</td>
<td>1.19</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lD</td>
<td>0.65</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hD</td>
<td>0.47</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD</td>
<td>0.17</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>poD</td>
<td>1.94</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lA</td>
<td>0.01</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hA</td>
<td>0.49</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pA</td>
<td>0.00</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-C</td>
<td>0.01</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>1.72</td>
<td>0.19</td>
<td></td>
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<tr>
<td>lV</td>
<td>0.02</td>
<td>0.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pV</td>
<td>1.15</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>poV</td>
<td>1.25</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-V</td>
<td>0.90</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-A</td>
<td>0.45</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this analysis the characteristics with an eigenvalues of 1 were included and others discarded (Table 4, 5). Principal component analysis of 12 morphometric characters and 9 meristic counts showed that PC I accounts for 38.84% and 18.42% of the variation and PC II for 18.19% and 15.54%, respectively (Table 5, 6) and that the most significant weightings on PC I were from Tl, Fl, Hc, Io, Pl, D2, D1, A2, A1, Gr1, Gr2 and on PC II were from Sl, poO, V-A, lI (Table 7, 8). The rotated (Varimax) component loadings for the four components (factors) are presented in Table 7, 8. For parsimony, in this study only those factors with loadings above 0.40 were considered significant. Visual examination of plotted PC I and PC II scores for each sample (Fig. 3) revealed that two samples of *Mash mahi* from the Sari and Tonekabon on Mazandaran coasts in southern Caspian Sea were distinct from each other in these regions, while there were a relatively high degree of overlap between two locations when using morphometric and meristic characters.
Table 4: Descriptive data and the results of ANOVA for morphometric measurements and meristic counts of *Aspius aspius taeniatus* (Eichwald, 1831) in the Sari and Tonekabon

<table>
<thead>
<tr>
<th>Morphometric characters</th>
<th>Sari (1)</th>
<th>Tonekabon (2)</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.D. Range</td>
<td>Mean ± S.D. Range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>410.57±43.44 317-583</td>
<td>381.56±23.11 334-433</td>
<td>11.327 0.001</td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>451.60±35.71 385.36-491.36</td>
<td>475.19±11.02 451.24-507.12</td>
<td>12.836 0.001</td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>408.59±29.78 349.15-439.31</td>
<td>431.95±4.77 423.64-445.08</td>
<td>19.205 0.000</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>87.24±8.02 63.63-95.57</td>
<td>90.18±3.73 81.51-99.07</td>
<td>3.590 0.048</td>
<td></td>
</tr>
<tr>
<td>Lac</td>
<td>43.50±4.09 37.01-56.87</td>
<td>43.57±4.46 34.8-53.02</td>
<td>0.044 0.947</td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td>53.51±7.99 38.36-85.34</td>
<td>53.37±2.79 47.28-59.01</td>
<td>0.008 0.928</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>92.93±8.94 71.66-113.11</td>
<td>92.27±5.96 72.86-101.94</td>
<td>0.124 0.726</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>37.59±5.36 30.73-56.53</td>
<td>37.29±2.42 31.23-41.10</td>
<td>0.079 0.780</td>
<td></td>
</tr>
<tr>
<td>prO</td>
<td>25.92±8.68 19.50-61.15</td>
<td>25.22±2.47 20.60-29.62</td>
<td>0.192 0.662</td>
<td></td>
</tr>
<tr>
<td>Oh</td>
<td>13.52±1.97 11.22-20.29</td>
<td>12.71±1.09 10.48-15.00</td>
<td>4.286 0.042</td>
<td></td>
</tr>
<tr>
<td>Io</td>
<td>31.18±2.50 25.87-36.77</td>
<td>33.26±2.44 28.37-39.37</td>
<td>11.780 0.001</td>
<td></td>
</tr>
<tr>
<td>poO</td>
<td>1.68±0.13 1.34-2.01</td>
<td>1.78±0.07 1.65-1.90</td>
<td>15.046 0.000</td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>50.04±6.95 37.85-68.75</td>
<td>48.36±2.41 44.32-53.16</td>
<td>1.674 0.200</td>
<td></td>
</tr>
<tr>
<td>hD</td>
<td>59.24±9.59 33.32-75.39</td>
<td>63.56±3.77 51.68-69.53</td>
<td>5.677 0.020</td>
<td></td>
</tr>
<tr>
<td>pD</td>
<td>193.75±14.63 152.30-210.97</td>
<td>197.28±6.19 187.60-210.07</td>
<td>1.605 0.210</td>
<td></td>
</tr>
<tr>
<td>poD</td>
<td>145.48±11.09 117.36-175.63</td>
<td>139.37±6.84 119.39-159.63</td>
<td>7.199 0.009</td>
<td></td>
</tr>
<tr>
<td>lA</td>
<td>57.68±7.24 46.25-81.22</td>
<td>57.23±3.94 49.88-65.28</td>
<td>0.096 0.758</td>
<td></td>
</tr>
<tr>
<td>hA</td>
<td>55.95±14.66 44.36-113.80</td>
<td>52.58±3.64 44.31-58.88</td>
<td>1.600 0.210</td>
<td></td>
</tr>
<tr>
<td>pA</td>
<td>257.71±17.01 232.15-324.50</td>
<td>262.81±7.12 249.31-283.26</td>
<td>2.479 0.120</td>
<td></td>
</tr>
<tr>
<td>A-C</td>
<td>79.75±18.83 52.73-139.92</td>
<td>70.12±3.90 62.46-76.01</td>
<td>8.047 0.006</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>63.07±4.28 54.54-72.18</td>
<td>67.05±3.17 62.07-75.84</td>
<td>18.424 0.000</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>51.44±10.39 40.33-107.33</td>
<td>53.17±3.56 47.37-61.95</td>
<td>0.795 0.376</td>
<td></td>
</tr>
<tr>
<td>pV</td>
<td>181.58±16.46 157.22-247.93</td>
<td>180.17±5.25 169.90-190.80</td>
<td>0.215 0.644</td>
<td></td>
</tr>
<tr>
<td>poV</td>
<td>195.00±10.73 166.96-215.93</td>
<td>194.10±9.19 171.46-212.88</td>
<td>0.132 0.717</td>
<td></td>
</tr>
<tr>
<td>P-V</td>
<td>98.30±6.64 81.90-111.16</td>
<td>99.55±4.77 87.50-110.83</td>
<td>0.777 0.381</td>
<td></td>
</tr>
<tr>
<td>V-A</td>
<td>1.87±0.14 1.45-2.25</td>
<td>1.99±0.09 1.82-2.18</td>
<td>15.676 0.000</td>
<td></td>
</tr>
<tr>
<td>LI</td>
<td>77.49±3.25 71-82</td>
<td>78.47±3.63 71-85</td>
<td>1.370 0.246</td>
<td></td>
</tr>
<tr>
<td>squ.sup</td>
<td>12.71±0.89 10-15</td>
<td>12.50±0.80 11-15</td>
<td>1.058 0.307</td>
<td></td>
</tr>
<tr>
<td>squ.inf</td>
<td>7.29±0.96 6-9</td>
<td>8.19±1.28 6-10</td>
<td>10.773 0.002</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>3.06±0.24 3-4</td>
<td>2.94±0.25 2-3</td>
<td>4.136 0.046</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>8.03±0.79 5-9</td>
<td>8.13±0.34 8-9</td>
<td>0.413 0.523</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>2.94±0.42 2-4</td>
<td>2.91±0.30 2-3</td>
<td>0.169 0.682</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>12.29±0.89 11-14</td>
<td>12.09±0.86 11-14</td>
<td>0.803 0.373</td>
<td></td>
</tr>
<tr>
<td>Gr1</td>
<td>9.37±0.84 8-11</td>
<td>9.75±0.67 9-12</td>
<td>4.080 0.048</td>
<td></td>
</tr>
<tr>
<td>Gr2</td>
<td>10.17±1.40 7-12</td>
<td>9.91±1.15 7-12</td>
<td>0.710 0.403</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3: Plot of the factor scores for PC1 and PC2 of 12 morphometric (A) and all meristic (B) characters for *Aspius aspius taeniatus* (Eichwald, 1831)

Table 5: Eigenvalues, percentage of variance and percentage of cumulative variance for the seven principal components in case of morphometric variables for *Aspius aspius taeniatus* (Eichwald, 1831) specimens

<table>
<thead>
<tr>
<th>Factor</th>
<th>Eigenvalues</th>
<th>Percentage of Variance</th>
<th>Percentage of Cumulative variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC 1</td>
<td>4.661</td>
<td>38.843</td>
<td>38.843</td>
</tr>
<tr>
<td>PC 2</td>
<td>2.210</td>
<td>18.420</td>
<td>57.263</td>
</tr>
<tr>
<td>PC 3</td>
<td>1.641</td>
<td>13.678</td>
<td>70.941</td>
</tr>
<tr>
<td>PC 4</td>
<td>1.008</td>
<td>8.404</td>
<td>79.345</td>
</tr>
</tbody>
</table>

Table 6: Eigenvalues, percentage of variance and percentage of cumulative variance for the seven principal components in case of meristic characters for *Aspius aspius taeniatus* (Eichwald, 1831) specimens

<table>
<thead>
<tr>
<th>Factor</th>
<th>Eigenvalues</th>
<th>Percentage of Variance</th>
<th>Percentage of Cumulative variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC 1</td>
<td>2.095</td>
<td>23.281</td>
<td>23.281</td>
</tr>
<tr>
<td>PC 2</td>
<td>1.399</td>
<td>15.547</td>
<td>38.828</td>
</tr>
<tr>
<td>PC 3</td>
<td>1.180</td>
<td>13.110</td>
<td>51.937</td>
</tr>
<tr>
<td>PC 4</td>
<td>1.139</td>
<td>12.656</td>
<td>64.593</td>
</tr>
</tbody>
</table>
Table 7: Factor loadings for the seven principal components and correlations between the measured morphometric variables and the discriminant functions for *Aspius aspius* taeniatus (Eichwald, 1831) specimens.

<table>
<thead>
<tr>
<th>Morphometric characters</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>PC 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sl</td>
<td></td>
<td>-0.974</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tl</td>
<td>0.843</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fl</td>
<td>0.829</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hc</td>
<td>0.671</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oh</td>
<td></td>
<td></td>
<td></td>
<td>0.969</td>
</tr>
<tr>
<td>Io</td>
<td></td>
<td></td>
<td></td>
<td>0.706</td>
</tr>
<tr>
<td>poO</td>
<td></td>
<td>0.925</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hD</td>
<td></td>
<td></td>
<td>-0.816</td>
<td></td>
</tr>
<tr>
<td>poD</td>
<td></td>
<td></td>
<td>0.758</td>
<td></td>
</tr>
<tr>
<td>A-C</td>
<td></td>
<td></td>
<td>0.906</td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>0.671</td>
<td></td>
<td>-0.468</td>
<td></td>
</tr>
<tr>
<td>V-A</td>
<td></td>
<td></td>
<td></td>
<td>0.962</td>
</tr>
</tbody>
</table>

Table 8: Factor loadings for the four principal components and correlations between the measured meristic variables and the discriminant functions for *Aspius aspius* taeniatus (Eichwald, 1831) specimens.

<table>
<thead>
<tr>
<th>Meristic characters</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>PC 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>-0.689</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>squ.sup</td>
<td></td>
<td>0.501</td>
<td>-0.587</td>
<td></td>
</tr>
<tr>
<td>squ.inf</td>
<td></td>
<td>0.770</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>-0.434</td>
<td>0.730</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td></td>
<td>0.830</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.439</td>
<td>0.525</td>
<td>0.477</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>-0.687</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr1</td>
<td>0.629</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr2</td>
<td>0.795</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 9: Percentage of specimens classified in each group and after cross validation for morphometric and meristic data.

<table>
<thead>
<tr>
<th>Area</th>
<th>Morphometric</th>
<th>Meristic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sari (%)</td>
<td>Tonekabon (%)</td>
</tr>
<tr>
<td><strong>Original</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sari</td>
<td>77.1</td>
<td>22.9</td>
</tr>
<tr>
<td>Tonekabon</td>
<td>12.5</td>
<td>87.5</td>
</tr>
<tr>
<td><strong>Cross Validated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sari</td>
<td>77.1</td>
<td>22.9</td>
</tr>
<tr>
<td>Tonekabon</td>
<td>12.5</td>
<td>87.5</td>
</tr>
</tbody>
</table>
For the discriminant analysis, the averages of percentage of correctly classified (PCC) were 82.1% and 61.2% for morphometric and meristic characters, respectively. High classification success rates were obtained for the Sari (77.1%) and Tonekabon (87.5%) stocks indicating a high correct classification of individuals into their original populations with respect to morphometric characters. This was 60% (Sari) and 62.5% (Tonekabon) in case of meristic characters (Table 9). The dendogram derived from cluster analysis of Euclidean distances among groups of centroids showed that the populations of *Mash mahi* from the Sari and Tonekabon on Mazandaran coasts in southern Caspian Sea were partly distinct from each other with respect on morphometric characters (Fig. 4). However the clustering result of meristic characters shown that the individuals of these locations had same clade with great homogeneity that confirming the results obtained from PCA and DFA in case of meristic counts (Fig. 5).

**Figure 4: Dendrogram derived from cluster analyses of 12 morphometric variables on the basis of Euclidean distance for *Aspius aspius taeniatus* (Eichwald, 1831)**
Discussion

This is the first report on differentiations between the Mash mahi populations in the south Caspian Sea basin. Discriminant Function Analysis could be a useful method to distinguish different stocks of a same species, concern to stock management programs (Karakousis et al., 1991). The results of DFA obtained in this study, demonstrated a high differentiation among the populations of Mash mahi in the studied areas. It is well known that morphological characteristics can show high plasticity in response to differences in environmental conditions (Swain & Foote, 1999). Therefore, the distinctive environmental conditions of the Sari and Tonekabon may underlie the morphological differentiation between these two locations.

This segregation was confirmed by another multivariate analysis, PCA, where the visual examination of plotted PC I and PC II scores for each sample revealed that two samples with high degree of overlap between two locations were distinct from each other with respect on morphometric characters. Also, The results of visual examination of plotted PC I and PC II scores for each sample in case of meristic characters revealed that two samples have high degree of overlap between two locations. The overlapping distribution of these samples may be attributable to extensive migration in these waters. There is no significant literature on the spawning distribution and migration pattern of Mash mahi in Caspian Sea basin waters. It has been suggested that the morphological characteristics of fish are determined by an interaction between genetic and environmental factors. The environmental characteristics prevailing during the early
development stages, when individuals are more phenotypically influenced by the environment, are of particular importance (Tudela, 1999; Pinheiro et al., 2005) especially in case of meristic characters (Lindsey, 1988).

UPGMA drawing base on Euclidian Distance coefficient in case of morphometric characters showed that populations of Mash mahi in two stations fragmented from each other in spite of meristic counts. Hence results of DFA, PCA and dendrogram of UPGMA indicate in two phenotypically fragmented in Sari and Tonekabon in southern Caspian Sea basin in case of morphometric measurements more than meristic characters. It has seems isolation by distance seems to be the mechanism responsible for population differentiation of Mash mahi. When a species has a more or less continuous distribution across a range, the balance between gene flow and the forces responsible for population differentiation, such as genetic drift or differential selection, may result in clines, whereby genetic differentiation increases with geographic distance (Borsa et al., 1997; Pinheiro et al., 2005).

These morphological differences may be solely related to body shape variation and not to size effects which were successfully accounted for by allometric transformation. Literature shows that factor of size account more than 80% of variation among a set of variables in morphometric studies. In the other hand, factor of size play a predominant role in morphometric analysis and make result in erroneous status if it cannot be removed in statistical analyses of data (Tzeng, 2004). In present study, the size effect had been removed successfully by the allometric transformation, so any significant differences represented the body shape variation when it tested using ANOVA and multivariate analysis.

In general, fishes demonstrate greater variance in morphological traits both within and between populations than other vertebrates, and are more susceptible to environmentally induced morphological variation (Thompson, 1991; Wimberger, 1992; Turan et al., 2006), which might reflect different feeding environment, prey types, food availability or other features. Thus, environmental factors such as temperature, salinity, food availability, or prolonged swimming may determine the phenotypic differentiation in the Mash mahi.

Results obtained from ANOVA analysis showed that 12 out of 26 transformed morphometric data were significantly different in two groups Mash mahi living in the south Caspian Sea basin that demonstrates a high phenotypic variation among these two populations. The causes of morphological differences between populations are often quite difficult to explain (Poulet et al., 2004). As morphology is especially dependent on environmental conditions during early life history stages (Lindsey, 1988), phenotypic differentiation may indicate that the majority of fish spend their entire lives in separate locations (Turan et al., 2006). It has been suggested that the morphological characteristics of fish are determined by an interaction between genetic and environmental factors (Swain and Foote, 1999; Poulet et al., 2004; Salini et al.,
2004; Pinheiro et al., 2005). The phenotypic variability may not necessarily reflect population differentiation at the molecular level (Tudela, 1999). Such kind of discrimination has been reported among six populations of *C. c. gracilis* located in the Aras, Sefidrud, Shirud, Tonekabon, Haraz and Gorganrud river systems in Iran (Samaee et al., 2006).

The influences of environmental parameters on morphometric characters are well discussed by several authors in the course of fish population segregation already (e.g. Swain, and Foote, 1999; Smith, 1966). The importance of such factors on producing morphological differentiation in fish species is well known (Akbarzadeh et al., 2009). Hence effects of some environmental factors such as temperature, salinity, food availability and migration distance can potentially determine morphometric segregation of fish (Smith, 1966; Lindsey, 1988; Turan et al., 2004, Turan et al., 2006). Samaee et al. (2006) stated different environmental and habitat conditions, such as temperature, turbidity, food availability, and water depth and flow in different river are caused differentiation in *C. capoeta gracilis* population.

In conclusion, the present study provides basic information about the differentiation of *Aspius aspius taeniatus* (Eichwald, 1831) populations in southern Caspian Sea basin and it suggests that morphological and meristic variations observed in *Mash mahi* should be considered in fisheries management and commercial exploitation of this species. These were analyzed using multivariate methods to establish the value of conducting deeper and more detailed morphological and molecular analyses in the future, although the preliminary investigation was not looking for the differentiation mechanisms (environmental parameters or genetics). To determine the contribution of genetics in the differentiations in the morphological findings, further exploration is necessary using DNA techniques. It suggests that use molecular markers with a high polymorphism, such as microsatellite, which have been able to detect population differentiation.

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