Int. J. Aquat. Biol. (2018) 6(6): 330-339
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

How the selective breeding in aquaculture programs can change the body shape of cyprinids; a case study on the native *Cyprinus carpio* and a cultured stock

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Abstract: Decades since restocking program of the vulnerable native *Cyprinus carpio* in the southern Caspian Sea, the cultured stocks in hatcheries have created new challenge to protect the native population. Releasing the cultured common carp in natural water-bodies caused an uncertainty about originality of the carp broodstocks within the restocking program. To clarify that how the selective breeding with aquaculture purpose could change the body shape with aiming to prepare an identification key for the indigenous and cultured stocks, a landmark based morphological characteristics of these stocks from the Anzali Wetland and a hatchery were analyzed. Univariate analysis of variance of 100 adult specimens collected during the non-reproductive season were observed in 62 morphometric characters out of 78 (P<0.05). Principle component analyze (PCA) of morphometric characteristic showed a high differentiation between these stocks. In morphometric traits, linear discriminate function analysis (DFA), the overall assignments of individuals into their original groups between stocks were 100%. The PCA and DFA showed a morphological segregation of the studied stocks based on the characters head shape, pre-dorsal, pre-pelvic and pre-anal distances, caudal peduncle depth, dorsal fin and ventral fin origins, body depth and caudal fin origin. The results showed stocks represent two distinct morphological forms of *C. carpio* that had high morphometric differentiation. The results can be useful as baseline information on the native stock for conservational policy. To protect the vulnerable population, using wild native broodstocks in the restocking program is strongly recommended.

Article history:
Received 11 September 2018
Accepted 19 December 2018
Available online 25 December 2018

Keywords:
Morphology
Stock Structure
Caspian Sea
Restocking program

Introduction

Information on the biology and population structure of any fish species is a prerequisite for developing management and conservation strategies (Tudela, 1999) and maybe applicable for studying short-term and environmentally induced variations. Morphological studies on fishes are important from various viewpoints, including evolution, ecology, behavior, conservation, water resource management and stock assessment (Mousavi-Sabet and Anvarifar, 2013; Kohestan-Eskandari et al., 2013; Heidari et al., 2013; Natsumeda et al., 2014; Jalili et al., 2015; Vatandoust et al., 2015). The study of morphological characters with the aim of defining or characterizing fish stock units has been of a strong interest in ichthyology (Tudela, 1999).

Traditional and truss morphometrics are often used to describe morphological variations between different species. The study of morphometrics using truss network system is a landmark-based on geometric morphometrics, which poses no restriction on the directions of variation and localization of shape changes, and much effective in capturing information on the shape of an organism (Bookstein, 1991; Cardin and Fried, 1999; Kocovsky 2009; Bagherian and Rahmani, 2009). It covers the entire fish body shape in a uniform network, and theoretically, it increases the likelihood of extracting morphometric differences between specimens (Cardin and Fried, 1999; Turan, 2004a; Kocovsky, 2009).

The common carp (*Cyprinus carpio*) is native to the Caspian Sea, which is a widespread of eutrophic waters in lakes and large rivers in Europe and Asia (Esmaeili et al., 2018). The wild stocks are considered

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DOI: https://doi.org/10.22034/ijab.v6i6.599

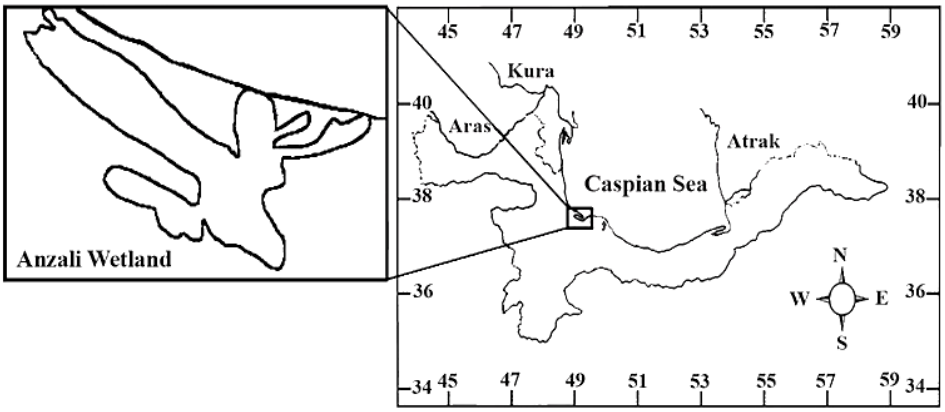

Figure 1. Map of Iranian parts of the Caspian Sea basin showing sampling point.

vulnerable to extinction, but the species has also been domesticated and introduced into environments worldwide, and often considered a very destructive invasive species, being included in the List of the world's 100 worst invasive species (Radkhah et al., 2016). *Cyprinus carpio* is one of the most important fishes for fisheries and stocking programs in south of the Caspian Sea. Native bony fishes to the Caspian Sea are economically high value species, which are mainly distributed along the southern coasts of the Caspian Sea (Kohestan-Eskandari et al., 2013). Due to limited natural reproduction of these species, caused by destruction of natural spawning areas and other factors (Kohestan-Eskandari et al., 2013), Iranian Fisheries Organization has launched its restocking program from last decade (Ebrahimi and Ouraji, 2012).

Despite the biodiversity and commercial importance of *C. carpio*, there is no any available study on stock differentiation of the fish in the southern Caspian Sea. Considering the above-mentioned facts, the present study was aimed to obtain information about morphological differentiation between two wild and cultured stocks, from the Anzali Wetland and a hatchery in the southern Caspian Sea, respectively, which can be employed in the future enhancement programs of this species. The present study deals with the stock structure of *C. carpio* from a phenotypical point of view to determine the morphometric difference between the stocks.

Materials and Methods

During September to October 2014, 100 *C. carpio* were collected from 2 sources: the Anzali Wetland (50 specimens) in the southern Caspian Sea basin (Fig. 1) and a hatchery (50 specimens). In order to investigate the body shape of the specimens, 13 homologous landmark-points were used. A total of 78 distance measurements between these 13 landmark-points were extracted using the truss network system according to Strauss and Bookstein (1982) with minor modifications (Fig. 2). Measurements of specimens were made by collecting X–Y coordinate data for relevant morphological features, followed the three-step process as described below (Turan, 2004a). The fish were placed on a white board with dorsal and anal fins held erect by pins. The left body profile of each fish was photographed with a 300-dpi, 32-bit color digital camera (Cybershot DSC-F505; Sony, Japan). Images were saved in jpg format and digitized with TPSdig to coordinates of 13 landmark points. A box truss of 26 lines connecting these landmark points was generated for each fish to represent the basic shape of the fish (Cardin and Fried, 1999). All measurements were transferred to a spreadsheet file (Excel 2010), and the X–Y coordinate data transformed into linear distances by computer for subsequent analysis (Turan, 2004a).

The extracted landmark-points were submitted to a generalized Procrustes analysis (GPA) to remove non-shape data in PAST software for visualization purpose. In respect of truss network measurements, as variation should be attributable to body shape differences and not related to the relative size of the fish, an allometric method was used to remove size

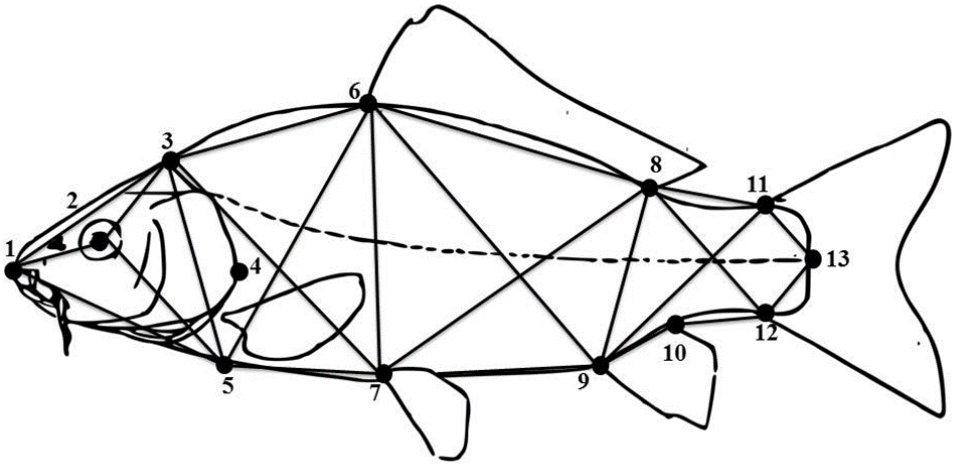

Figure 2. Position of 13 digitized landmark points for constructing the truss network on *Cyprinus carpio*.1. Tip of snout; 2. center of the eye; 3. forehead (end of frontal bone); 4. end of the operculum; 5 ventral edge of the body appendicular to origin of the pectoral fin; 6 origin of the dorsal fin; 7 origin of the pelvic fin; 8 insertion of the dorsal fin; 9 origin of the anal fin; 10 insertion of the anal fin; 11 dorsal side of caudal peduncle, at the nadir; 12 ventral side of caudal peduncle, at the nadir; 13 end of the lateral line.

dependent variation using following formula:

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

Where, M is original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of the fish, Ls is the overall mean of standard length for all fish from all samples in each analysis, and b was estimated for each character from the observed data as the slope of the regression of log M on $log\ L_0$ using all fish from both the groups. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length (Turan, 2004a; Kocovsky, 2009).

Univariate Analysis of Variance (ANOVA) was performed for each morphometric character to evaluate the significant difference between the two stocks. Discriminant function analyses (DFA) and principal component analysis (PCA) were employed to discriminate the two stocks. The analysis of variance revealed significant phenotypic variation between the two stocks. In order to determine which morphometric measurement made most effectively differentiates between the stocks, 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) measure was 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 (Nimalathasan, 2009). A cross-validation using percentage of correctly classified (PCC) was done to estimate the expected actual error rates of the classification functions. A dendrogram of the stocks based on the morphometric and landmark distance data was drawn using the unweighted pair group method analysis (UPGMA).

Statistical analyses for morphometric data were performed using the SPSS version 16 software package, Past software ver. 1.36, taxonomy and multivariate analysis system (NTSYSpc), MorphoJ and Excel (Microsoft office, 2010).

Results

The correlation between transformed morphometric variables and standard length was non-significant (P>0.05) confirming size or allometric signature on the basic morphological data was accounted. Statistically significant differences between the wild and hatchery stocks were found in 62 morphometric characters out of 78 characters (Table 1). Of these 62 characters, 54 characters were found significantly different (P<0.01) and were used for multivariate analysis. This traits include 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 2-4, 2-6, 2-11, 3-4, 3-6, 3-7,

Table 1. The results of ANOVA for morphometric measurements of *Cyprinus carpio* stocks from the Anzali Wetland and a hatchery.

Characters	F	P	Characters	F	P	Characters	F	P
1-2	1.510	0.201	3-7	52.417	0.000	6-9	975.587	0.000
1-3	103.450	0.000	3-8	41.105	0.000	6-10	45.755	0.000
1-4	105.441	0.000	3-9	77.673	0.000	6-11	66.571	0.000
1-5	135.297	0.000	3-10	5.219	0.042	6-12	47.067	0.000
1-6	54.318	0.000	3-11	9.091	0.005	6-13	162.295	0.000
1-7	21.285	0.000	3-12	1.625	0.206	7-8	1.552	0.188
1-8	109.632	0.000	3-13	1.115	0.295	7-9	4.899	0.029
1-9	1.417	0.227	4-5	3.158	0.078	7-10	231.981	0.000
1-10	96.384	0.000	4-6	85.726	0.000	7-11	0.630	0.421
1-11	34.453	0.000	4-7	3.221	0.075	7-12	35.524	0.000
1-12	76.432	0.000	4-8	237.274	0.000	7-13	22.779	0.000
1-13	33.453	0.000	4-9	5.789	0.017	8-9	53.055	0.000
2-3	0.905	0.334	4-10	438.500	0.000	8-10	23.320	0.000
2-4	85.638	0.000	4-11	3.688	0.058	8-11	46.074	0.000
2-5	0.224	0.634	4-12	414.887	0.000	8-12	17.327	0.000
2-6	24.456	0.000	4-13	33.226	0.000	8-13	34.216	0.000
2-7	4.973	0.020	5-6	4.422	0.065	9-10	8.891	0.002
2-8	0.047	0.858	5-7	599.238	0.000	9-11	145.985	0.000
2-9	0.318	0.567	5-8	61.678	0.000	9-12	56.852	0.000
2-10	0.402	0.526	5-9	10.312	0.003	9-13	97.355	0.000
2-11	87.337	0.000	5-10	70.619	0.000	10-11	122.384	0.000
2-12	3.755	0.054	5-11	6.008	0.015	10-12	64.940	0.000
2-13	6.386	0.013	5-12	98.391	0.000	10-13	41.320	0.000
3-4	222.642	0.000	5-13	37.424	0.000	11-12	257.389	0.000
3-5	6.313	0.021	6-7	779.005	0.000	11-13	247.291	0.000
3-6	146.901	0.000	6-8	110.912	0.000	12-13	32.757	0.000

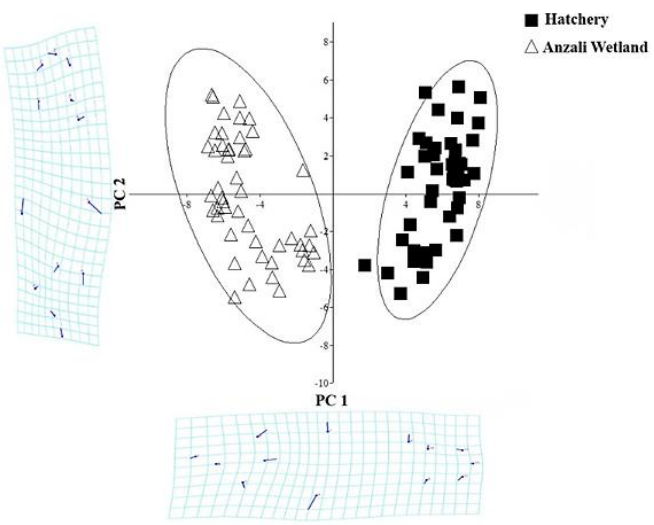

Figure 2. Plot of the factor scores for PC1 and PC2 of all morphometric measurements between *Cyprinus carpio* stocks from the Anzali Wetland and a hatchery.

3-8, 3-9, 3-11, 4-6, 4-8, 4-10, 4-12, 4-13, 5-7, 5-8, 5-9, 5-10, 5-12, 5-13, 6-7, 6-8, 6-9, 6-10, 6-11, 6-12, 6-13, 7-10, 7-12, 7-13, 8-9, 8-10, 8-11, 8-12, 8-13, 9-10, 9-11, 9-12, 9-13, 10-11, 10-12, 11-12, 11-13 and 12-13. The morphometric characters between two sexes (out of reproductive season) did not differ significantly (P>0.05) (Table 2); hence, the data for both sexes were pooled for all subsequent analyses.

The value of KMO for overall matrix was 0.773 and the Bartlett's Test of sphericity is significant (P<0.01). The KMO statistics varies between 0 and 1. Kaiser recommends that values greater than 0.5 are acceptable, 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 54 morphometric measurements extracted 8 factors with eigenvalues >1, explaining 93.925 % of the variance (Table 3). The first principal component (PC1) accounted for 46.92% of the variation and the second principal component (PC2) for 12.67%, respectively (Table 3). The most significant loadings on PC1 were 1-3, 1-4, 1-6, 1-7, 1-8, 1-10, 1-12, 2-4, 2-6, 3-4, 3-6, 3-7, 4-6, 4-8, 4-10, 4-12, 5-7, 5-8, 5-10, 5-12, 6-9, 6-11, 6-13, 7-9, 7-10, 8-9, 8-13, 11-12 and

Table 2. The results of ANOVA for morphometric measurements between two sexes of *Cyprinus carpio* in the studied stocks from the Anzali Wetland and a hatchery.

Hatchery stock						Wild stock					
Characters	F	P	Characters	F	P	Characters	F	P	Characters	F	P
1-3	1.003	0.322	5-10	0.986	0.326	1-3	1.357	0.250	5-10	0.421	0.518
1-4	0.069	0.794	5-12	0.380	0.541	1-4	0.744	0.393	5-12	0.226	0.636
1-5	0.814	0.371	5-13	3.598	0.064	1-5	0.525	0.472	5-13	0.144	0.706
1-6	3.374	0.072	6-7	0.000	0.989	1-6	0.865	0.357	6-7	1.384	0.197
1-7	0.362	0.550	6-8	0.005	0.947	1-7	1.585	0.214	6-8	2.981	0.087
1-8	3.100	0.085	6-9	1.174	0.284	1-8	0.665	0.419	6-9	0.881	0.351
1-9	1.247	0.265	6-10	0.928	0.340	1-9	3.113	0.084	6-10	1.545	0.217
1-10	3.636	0.063	6-11	2.853	0.098	1-10	0.713	0.403	6-11	1.952	0.166
1-11	2.810	0.100	6-12	1.123	0.294	1-11	3.191	0.080	6-12	2.031	0.157
1-12	0.500	0.483	6-13	0.029	0.865	1-12	2.874	0.097	6-13	0.840	0.361
2-4	0.003	0.955	7-10	1.054	0.310	2-4	0.691	0.410	7-10	0.299	0.586
2-6	0.649	0.424	7-12	3.297	0.076	2-6	0.137	0.713	7-12	3.569	0.062
2-11	0.738	0.394	7-13	0.023	0.881	2-11	0.628	0.432	7-13	0.186	0.663
3-4	2.397	0.128	8-9	0.230	0.633	3-4	1.224	0.274	8-9	0.518	0.475
3-6	2.344	0.132	8-10	0.015	0.903	3-6	0.605	0.440	8-10	2.081	0.155
3-7	2.216	0.143	8-11	2.978	0.088	3-7	2.833	0.092	8-11	0.781	0.385
3-8	0.449	0.506	8-12	0.786	0.388	3-8	0.217	0.644	8-12	2.341	0.135
3-9	0.062	0.805	8-13	0.034	0.855	3-9	3.466	0.069	8-13	2.345	0.133
3-11	0.001	0.974	9-10	2.086	0.152	3-11	3.103	0.085	9-10	0.692	0.411
4-6	3.715	0.060	9-11	1.426	0.235	4-6	1.605	0.203	9-11	0.851	0.365
4-8	0.001	0.974	9-12	1.786	0.377	4-8	0.722	0.400	9-12	0.695	0.415
4-10	0.247	0.622	9-13	0.043	0.837	4-10	0.517	0.476	9-13	0.288	0.578
4-12	0.028	0.867	10-11	2.426	0.123	4-12	2.603	0.108	10-11	2.067	0.105
4-13	3.204	0.080	10-12	0.518	0.473	4-13	0.004	0.951	10-12	1.611	0.204
5-7	0.426	0.517	11-12	0.187	0.666	5-7	0.998	0.320	11-12	0.035	0.851
5-8	0.690	0.410	11-13	3.579	0.361	5-8	0.016	0.901	11-13	3.106	0.089
5-9	0.849	0.361	12-13	0.299	0.586	5-9	0.230	0.370	12-13	1.425	0.234

Table 3. Eigenvalues, percentage of variance and percentage of cumulative variance for *Cyprinus carpio* stocks from the Anzali Wetland and a hatchery.

Factors	Eigenvalues	Percentage of variance	Percentage of cumulative variance
PC1	25.401	46.921	46.921
PC2	6.851	12.671	59.592
PC3	6.267	11.639	71.231
PC4	4.679	8.676	79.907
PC5	3.010	5.498	85.405
PC6	2.181	4.036	89.441
PC7	1.411	2.591	92.032
PC8	1.029	1.893	93.925

11-13. The plot of PC1 and PC2 scores revealed that the 100 specimens grouped into two stocks (Fig. 3). The Wilks' λ tests of discriminant analysis indicated significant differences in morphometric characters of the two stocks. In this test, one function was highly significant (P<0.01) (Table 4).

The histogram of discriminant functions for pairwise groups is shown in Figure 4. There was a slight degree of separation between two stocks. The linear discriminant analysis gave an average PCC (Percentage of specimens classified) of 100% for morphometric characters indicating a high rate of correct classification of individuals into their original stocks (Table 5).

Clustering analysis based on Euclidean square distances between the groups of centroids using an UPGMA resulted two main clusters, including the Anzali Wetland and hatchery groups in separate clades (Fig. 5).

Discussion

The aim of the present study was to investigate the hypothesis; morphological differentiation between native wild and cultured *C. carpio* stocks using

Table 4. Result of Wilks' test for verifying difference between two stocks when morphometric measurements are separately compared using DFA.

Test of functions	Wilks' λ	X^2	*df*	Significance
1	0.006	467.966	11	0.000

Table 5. Percentage of specimens classified in each group and after cross validation for morphometric data between *Cyprinus carpio* stocks from the Anzali Wetland and a hatchery.

		Stocks	Hatchery	Anzali Wetland	Total
Original	Count	Hatchery	50	0	50
		Anzali Wetland	0	50	50
	%	Hatchery	100.0	0.0	100.0
		Anzali Wetland	0.0	100.0	100.0
Cross-validated	Count	Hatchery	50	0	50
		Anzali Wetland	0	50	50
	%	Hatchery	100.0	0.0	100.0
		Anzali Wetland	0.0	100.0	100.0

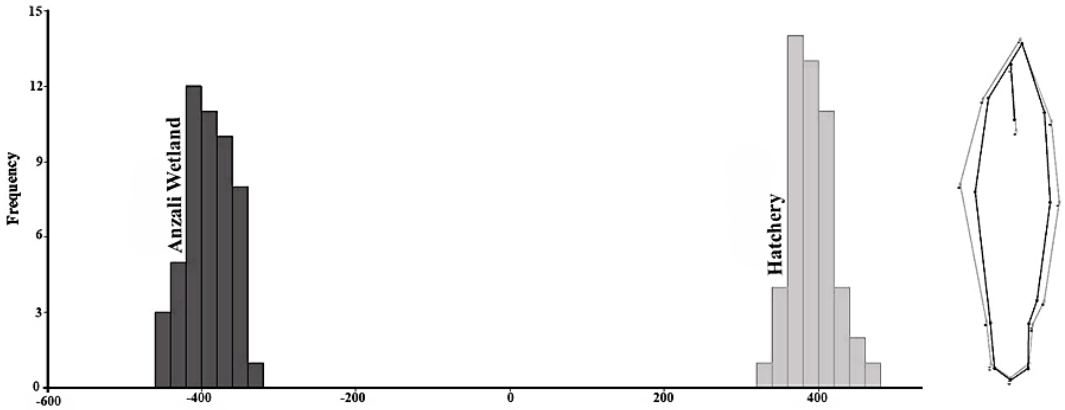

Figure 4. Histogram of discriminate analysis (DA) functions for pairwise competition' between *Cyprinus carpio* stocks of the Anzali Wetland and a hatchery (left). Shape differences on the extremities of each stock (right).

landmark-based methods. The landmark-based morphometric method is recently used to investigate various hypothesis in freshwater and marine species in the region (Kohestan-Eskandari, 2013; Heidari et al., 2013, 2014; Paknejad et al., 2014; Vatandoust, 2014a, b, 2015; Mohadasi et al., 2014). The result of the present study also demonstrate there is significant phenotypic variation between the two studied stocks.

Many natural populations of fish species have decreased drastically in number, mainly because of the effects of over-exploitation, habitat alterations, including physiography, abiotic, and biotic features, the release and introduction of exotic fish species, etc. Over fishing, especially when directed against a specific size or class age, can reduce the size of the population to a level where inbreeding and loss of genetic diversity may be a serious problem or may lead to extinction of local fishes (Mostafa, 2010).

Discriminant function analysis could be a useful method to distinguish different stocks of a same species (Karakousis, 1991). In the present study, achieved high classification of individuals that were correctly classified in to their respective groups by DFA, confirmed by PCA. Mostafa et al. (2010) compared morphometric characteristics among three groups of *Labeo calbasu*, from stocks of two isolated Rivers and a hatchery and reported high isolation of these stocks. The PCA and DFA showed a

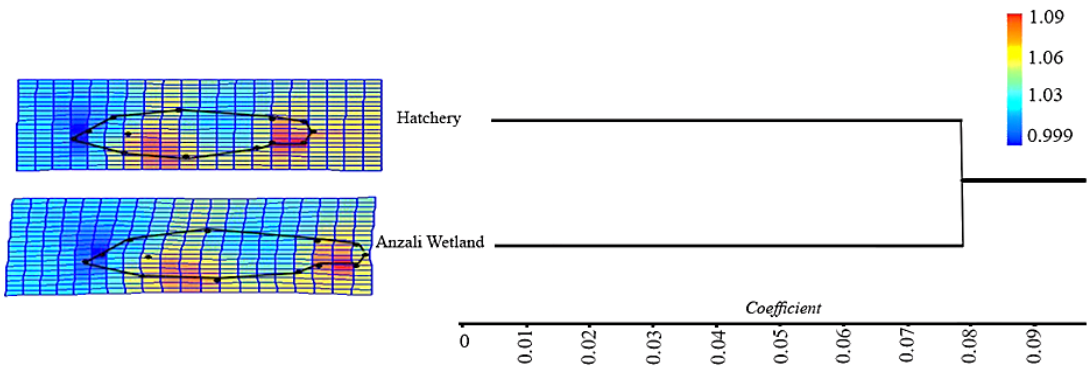

Figure 5. Dendrogram derived from cluster analyses of morphometric characteristics for *Cyprinus carpio* stocks from the Anzali Wetland and a hatchery. Mean shape of species in relation of consensus shape of the populations are also represented.

Figure 6. *Cyprinus carpio* from the Anzali Wetland (above) and a hatchery (below), in the southern Caspian Sea basin.

morphological segregation of the studied stocks based on the characters, including head shape, pre-dorsal, pre-pelvic and pre-anal distances, caudal peduncle depth, dorsal fin and ventral fins origins, body depth and caudal fin origin position (Fig. 6). These morphological differences are solely related to body shape variation and not to size effect which was successfully accounted by allometric transformation. On the other hand, size related traits play a predominant role in morphometric analysis and the results may be erroneous if not removed for statistical analyses (Tzeng, 2004). In the present study, the size effect had been removed successfully by allometric transformation, and the significant differences between the body shape of stocks are due to the body shape variation. The causes of morphological differences between stocks are often quite difficult to explain (Poulet, 2004). It has been suggested that the

morphological characteristics of fish are determined by genetic, environment and the interaction between them (Swain and Foote, 1999; Poulet, 2004; Vatandoust et al., 2015). The environmental factors prevailing during the early development stages, when individual's phenotype is more amenable to environmental influence (Pinheiro, 2005). The influences of environmental parameters on morphometric characters are well-discussed by several authors in the course of fish segregation (e.g., Swain and Foote, 1999). In general, fish demonstrate greater variances in morphological traits both within and between populations than any other vertebrates and are more susceptible to environmentally induced morphological variations (Wimberger, 1992; Mostafa, 2010; Mohadasi et al., 2014; Vatandoust et al., 2015). In this study, PCA of morphometric characteristic showed a high differentiation between the stocks of *C. carpio*. The dendrogram resulted in two distinct clade i.e. the Anzali Wetland wild and hatchery reared stocks. The differences between the hatchery and wild stocks may have been due to environmental conditions as well as genetic variations (which needs further studies to confirm).

The phenotypic plasticity of fish is very high. They adapt quickly by modifying their physiology and behavior to environmental changes. These modifications ultimately change their morphology (Wimberger, 1992).

The present study showed that each stock represents different body shape. The results also provide useful baseline information of *C. carpio* stocks for further studies and conservation programs. In both aquaculture and open-water management, it is essential to select genetically superior stocks along with better features. More research especially genetic studies and impacts of environmental factors are need for conservation and mass seed production of selected stocks to pave the way to protect this vulnerable species. The results also present a key to identify the native and cultured stocks, which can be useful for selecting broodstocks to the restocking program. To protect the vulnerable native population, using wild native broodstocks in the restocking program is strongly recommended.

Acknowledgements

We express our sincere thanks to A. Habibi for his help in specimen collection, and thank to H. Mojdekanlou for helping us in fixing and taking the photograph.

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