Research Article

Phenotype-environment associations in common carp, Cyprinus carpio Linnaeus 1758, in the southern coast of the Caspian Sea

Jafari O.^{1,2*}; Hedayati S.A.A.^{2*}; Poorbagher H.³; Ghorbani R.²; Abdolhay H.A.⁴; Zeinolabedini M.⁵

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Abstract

The present study was done to investigate the phenotype-environment associations of common carp in the southern coast of the Caspian Sea (Gomishan, Miankaleh, Rezvanshahr, and Anzali Wetland) during winter 2016. The morphometric data of the specimens were procured from their left sides using the Image J and TpsDig2. Both geometric and morphometric methods were applied. The two-block partial least squares was used to examine the relationship between morphological and environmental characteristics. Significant positive correlation (p<0.05) was found between two data sets of morphology and environment, indicating a high level of adaptation for C. carpio in the southern basin of the Caspian Sea. The observed correlation between geometric and environmental data (0.74) was higher than the traditional morphometricenvironment correlation (0.50). To the best of our knowledge, this is the first study trying to make a comparison between two methods of morphometric studies in relation to environment, by doing an evenness function on the raw data. The higher level of correlation obtained by geometric data with environmental parameters may imply its advantage in future morph-environment association studies. The obtained results in the present study also can provide basic required information for the wild population conservation strategies in common carp in the southern basin of the Caspian Sea.

Keywords: Body shape, Environmental adaptation, Geometric morphometric, Two-Block PLS analysis

- 1-International Sturgeon Research Institute, Agricultural Research, Education and Extension Organization, Rasht, Iran
- 2-Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
- 3-Department of Fisheries, Faculty of Natural Resources, University of Tehran, P.O. Box 4314, Karaj, Iran.
- 4-Department of Aquaculture, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran.
- 5-Department of Genomics, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.
- *Corresponding authors Email: Hedayati@gau.ac.ir; Jaafari.omid@yahoo.com; o.jafari@areeo.ac.ir

Introduction

In different habitats, organisms face too different types of challenges, which together respond by phenotypical shifts. Various factors affect the shape and including body form of fishes physicochemical parameters (Crispo and Chapman, 2010), predation pressure (Andersson et al., 2006: Langerhans, 2009). water flow (Langerhans, 2008) and feeding mode (Svanbäck and Eklöv, 2003; Andersson et al., 2006; Ruehl and DeWitt, 2007). For example, fish inhabiting environment with lentic water (e.g. wetlands) have a deep body with a welldeveloped caudal peduncle enhancing maneuverability (Webb, 1982; Webb, 1984; Robinson and Wilson, 1994; Walker, 1997; Langerhans and Reznick. 2010: Ruehl et al., 2011). while a fusiform body shape enables the fish to swim contiguously (Brönmark and Pettersson, 1994; Nilsson et al., 1995; Domenici et al., 2008). Thereby the observed phenotypes, are showing the effects of some environmental variants in addition other evolutionary factors (gene flow, genetic drift, etc.).

Physicochemical parameters are the inevitable parts of habitats, which have a main role on intra- and inter-specific variations of fishes. In fact, environmental factors may have a different magnitude of effects on organisms in different habitats and their impacts are being influenced by other factors (Crispo and Chapman, 2010). Morphological variations of a species across the gradients of environmental

parameters may indicate environmentphenotype associations (Wainwright et al., 1991). Analyzing the relationship between morphological characteristics the environmental parameters would be worthwhile to comprehend the phenotype-environment associations (Caumul and Polly, 2005; Hoverman et al., 2005; Langerhans et al., 2007). In this regard, quantification of such association may be rather difficult. Such associations mostly have an adaptive nature (Schluter, 2000, Nyboer and Chapman, 2013) and reflect the processes that make and conserve variation of traits in natural populations as the main goal of evolutionary (West-Eberhard, 1989; biology Pigliucci, 2005; Langerhans, 2008; Pfennig et al., 2010; Levis and Pfennig, 2016).

Finding and assessing these relationships are a bit difficult, for example, one environmental factor may have a direct positive effect on a specific trait but indirectly can reduce that trait by its effect on the other environmental factors (Pigliucci and Preston, 2004; Marks et al., 2005). Morphological variations induced in organisms can be assessed either by considering some morphometric traits or the variations induced in the form of the fish's body. Therefore, it is probable to miss some morphological variations if the attention goes for one of them. In this study in order to investigate that the morphological variations occurred between different locations of common carp, both methods including traditional morphometric and Geometric

morphometric (Adams et al., 2004) were applied.

Cyprinus carpio is a species with a worldwide range of distribution and is naturally well dispersed in the Caspian Sea (Mazaheri Kouhestani et al., 2020). This species is present throughout the southern parts of the Caspian Sea and several population genetic studies using DNA markers have proposed different populations of common carp across the southern basin (Laloei et al., 2013; Ghelichpour et al., 2010). The southern parts of the Caspian Sea exhibit different environmental conditions annual mean temperature, regarding depth and shore flow type (Mehdipour et al., 2015; Nouri et al., 2008). Therefore, the extended distribution range of common carp in southern coast of the Caspian Sea can reflect its adaptation capability to the specific environmental conditions in these regions. Furthermore, the strain existing in the Anzali Wetland is accepted to be the permanent resident of the lagoon (the resident form), while the sea-form (migratory population) moves towards the shallow freshwater rivers to spawn between winter and spring (Vazirzadeh Yelghi, and 2015). Considering facts, C. these carpio can be a suitable model in phenotype-environment association studies. Hence, based on the current knowledge of the authors, the present study has been conducted for the first time to investigate the relationship between environmental factors and phenotypic traits of common carp in the southern coasts of the Caspian Sea

using both geometric and traditional morphometric approaches.

Materials and methods

Sampling

100 specimens were collected from four locations in the Southern Caspian Sea during winter 2016 (Fig. 1): Gomishan (GW; E: 53°29', N: 37°51'), Miankaleh 36°52'). (MK: E: 53°30'. N: Rezvanshahr (RS; E: 49° 8', N: 37°34) and the Anzali wetland (AW; E: 49°26', 37°25'). The specimens were anesthetized using tricaine mesylate (MS-222, Syndel USA), and their left sides were photographed for morphometric analyses. Seven water samples were collected by random in each location to measure pH, total dissolved solids (TDS), total suspended (TSS). alkalinity. electrical solids conductivity (EC), salinity, PO₄, NO₃, NO₂, NH₃, temperature, and turbidity. Temperature, salinity, EC, pH, and turbidity were measured using an electronic device (®Wag tech, England). NO₃, PO₄, NO₂, NH₃, and alkalinity were measured using a photometer (Palin Test® photometer 7100). TSS was measured using a standard method (Rieger et al., 2004).

Morphometric analysis
Traditional morphometrics

Sixteen morphometric distances were measured using Image J version 1.45s (Fig. 2). The size component from multivariate data sets of measured distances was removed based on the method described by Elliott *et al.* (1995) using the software PAST version 3 (Hammer *et al.*, 2001).

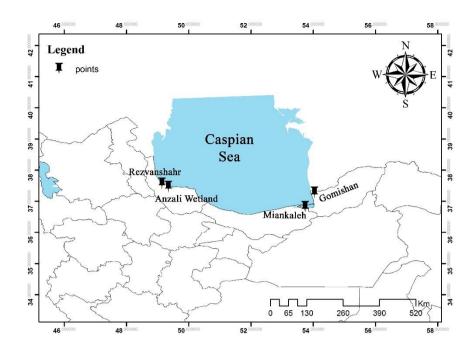


Figure 1: The investigated locations of common carp in the southern coast of the Caspian Sea.

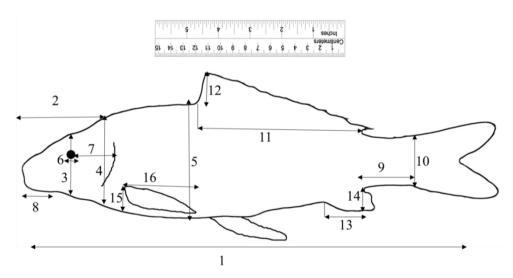


Figure 2: Morphometric characteristics of the Caspian common carp; 1(FL): fork length, 2(HL): head length, 3(HH1): head height on eye area, 4(HH2): maximum head height, 5(BD): body depth, 6(ED): eye diameter, 7(POL): post orbital length, 8(ML): mouth width length, 9(CPL): caudal peduncle length, 10(CPH): caudal peduncle height, 11(DBL): dorsal base length, 12(DH): dorsal fin height, 13(ABL): anal base length, 14(AH): anal fin height, 15(PBL): pectoral base length, 16(PH): pectoral fin height.

Geometric morphometrics

Fourteen landmark points were digitized on the images using the software TpsDig2 version 2.10 (Fig. 3). A generalized Procrustes analysis was used to remove the effects of size,

rotation, and translation from the landmark data; (Rohlf and Slice, 1990; Rohlf, 2001; Klingenberg, 2011). The environmental parameters were standardized to have a range of 0-1 (Zuur *et al.*, 2007) because they had

different dimensions. A two-block partial least squares (PLS) was used to examine the covariation between morphometric data and environmental parameters using the package Geomorph in R (Rohlf and Corti, 2000).

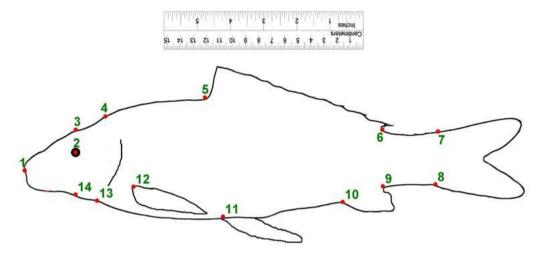


Figure 3: The digitized landmark points on the Caspian common carp.

Results

Environmental and morphometric data are presented in Tables 1 and 2. The

highest mean (\pm SE) temperature (14.56 \pm 0.24) and nitrate concentration (0.37 \pm 0.09) were found in RS (Table 1).

Table 1: The mean (± SE) of the environmental parameters measured in the Southern Caspian Sea.

Location*	pН	$\begin{array}{c} \textbf{Temperature} \\ (^{\circ}\textbf{C}) \end{array}$	salinity (ppt)	EC (µs)	Turbidity (NTU)	NH ₃ (mg/L)	NO ₃ (mg/l)	NO ₂ (mg/l)	Alkalinity (mg/L CaCO ₃)	PO ₄ (mg/L)	TSS (mg/L)
MK	8.42±0.01	13.29 ± 0.25	11.94± 0.31	547.22± 2.31	5.84 ± 1.07	5.40± 2.84	0.08 ± 0.04	0.46 ± 0.20	177.5± 10.30	$3\!\pm0.83$	0.05 ± 0.01
GW	$8.40{\pm}\ 0.003$	12.71 ± 0.09	20.50± 2.07	594.75± 4.53	173.6± 63.59	$3.38{\pm}\ 1.78$	$0.31{\pm}~0.10$	0.00	152.5 ± 4.78	$0.5{\pm}~0.07$	0.65± 0.18
AW	8.34± 0.11	14.46 ± 0.46	$0.45 \!\pm 0.08$	$128.87\!\pm 6.05$	3.42 ± 0.92	$2.95\!\pm1.28$	$0.27{\pm}~0.04$	0.02 ± 0.003	123.33±11.81	$0.22{\pm}~0.13$	0.10± 0.02
RS	8.46 ± 0.01	14.56 ± 0.24	5.87± 0.39	426.71±54.64	10.12 ± 3.84	$1.50 {\pm}~0.88$	0.37 ± 0.09	$0.01 {\pm}~0.006$	171 ± 6.78	$0.82 {\pm}~0.28$	0.19 ± 0.08

*MK: Miankaleh, GW: Gomishan, AW: Anzali Wetland, RS: Rezvanshahr

For morphometric data, the highest (46.75±2.26) and lowest (28.07±0.94) mean values of the FL were found in RS and AW locations, respectively (Table 2). Three dimensions were extracted from the two-block PLS using the singular value and the relevant scree plot (Figure 4). There was a significant positive correlation between scores of the projected values on the first singular vectors of the traditional morphometric

data (morphometric distances) and the water parameters (r=0.498, P=0.01).

Nitrate was correlated with HH1, HL, pH, BD, POL, ED, and PBL. pH was correlated with HL and CPH. The temperature was highly correlated to the morphometric parameters FL, POL, DH, ABL, and pH. The attributes ED, FL, ML, and ABL, displayed high correlation with salinity. Ammonia, turbidity, and phosphate were correlated with the parameters HH2, HH1, and

BD, respectively. Alkalinity was correlated to ABL, BD, and PBL and the environmental parameter, nitrogen

dioxide, had a positive correlation with CPL and DBL (Fig. 5).

Table 2: The mean (cm) ± SE of morphometric parameters and weight measurements in *Cyprinus carpio* in the Southern Caspian Sea.

Morphometric				
and weight characteristics	AW	MK	GW	RS
FL	28.07 ± 0.94	36.25 ± 1.53	32.93 ± 1.54	46.75 ± 2.26
HL	4.88 ± 0.31	5.76 ± 0.28	5.96 ± 0.23	9.09 ± 0.44
HH1	5.97 ± 0.22	6.99 ± 0.27	7.03 ± 0.31	9.88 ± 0.59
HH2	3.49 ± 0.13	4.28 ± 0.17	3.88 ± 0.20	5.28 ± 0.29
BD	7.86 ± 0.30	9.97 ± 0.41	9.35 ± 0.34	12.94 ± 0.58
ED	1.33 ± 0.04	1.35 ± 0.08	1.45 ± 0.05	1.88 ± 0.12
POL	3.32 ± 0.13	3.82 ± 0.18	3.66 ± 0.16	5.93 ± 0.30
ML	1.26 ± 0.07	1.36 ± 0.07	1.33 ± 0.08	1.89 ± 0.08
CPL	3.21 ± 0.18	4.56 ± 0.27	4.12 ± 0.24	5.90 ± 0.40
CPH	3.21 ± 0.11	4.17 ± 0.14	3.83 ± 0.15	5.68 ± 0.26
DBL	9.35 ± 0.31	12.47 ± 0.57	10.97 ± 0.49	14.70 ± 0.83
DH	2.91 ± 0.18	3.09 ± 0.27	2.45 ± 0.20	4.38 ± 0.27
ABL	2.10 ± 0.07	2.60 ± 0.10	2.34 ± 0.11	3.37 ± 0.28
AH	1.44 ± 0.17	1.76 ± 0.10	1.86 ± 0.09	2.82 ± 0.27
PBL	1.02 ± 0.07	1.43 ± 0.07	1.20 ± 0.06	1.70 ± 0.13
pН	4.56 ± 0.26	5.73 ± 0.26	5.34 ± 0.25	7.77 ± 0.42
W (gr)	344.78 ± 38.08	841.57 ± 112.44	694.44± 186.77	1653.8 ± 230.9

FL: Fork Length, HL: Head Length, HH1: Head Height in optical area, HH2: Maximum Head Height, BD: Body Depth, ED: Eye Diameter, POL: Post Orbital Length, ML: Mouth Length, CPL: Caudal Peduncle Length, CPH: Caudal Peduncle Height, DBL: Dorsal fin Base Length, DH: Dorsal Fin Height, ABL: Anal fin Base Length, AH: Anal fin Height, PBL: Pectoral fin Base Length, pH: Pectoral fin Height, W: Weight (gr).

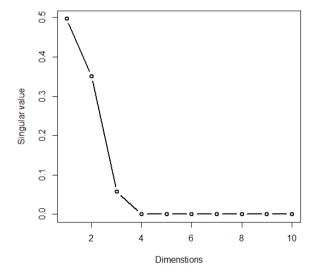
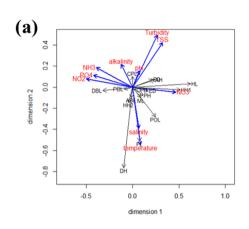
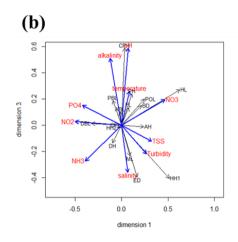


Figure 4: The scree plot of the singular values from the two-block PLS calculated using the morphometric distance data (traditional morphometric).





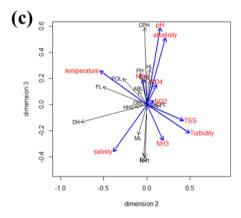


Figure 5: The biplots of morphometric and environmental (water) data for the first three dimensions.

Based on 1000 random permutation test two-block **PLS** through analysis (r=0.745,p=0.001), there was significant positive correlation between scores of the projected values on the first singular vectors of the distance of the landmarks from the centroid and the water parameters. Three dimensions were extracted from the two-block PLS using the singular value and the relevant scree plot (Figure 6). Turbidity and Total Suspended Solids (TSS) had the highest correlation with the distance of the landmark 11 from the centroid (the position of the pelvic fin). Nitrate had the maximal correlation with the centroid distance of the landmark 4 the head (related to position). Temperature was highly correlated with the distances of the landmarks of 1, 2, 3, 10, and 14 from the centroid and the highest correlation was seen with the centroid distance of the first landmark. In addition, salinity and TDS displayed high correlation with the distance of the landmark point 9 from the centroid (related to the anal fin). The relationships between these two sets of (landmark data points and environmental parameters) are available at a biplots under Fig. 7.

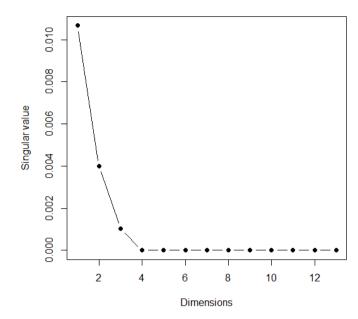


Figure 6: The scree plot of the singular values of the two-block PLS calculated from the distance between the landmark coordinate and the centroid (geometric morphometric).

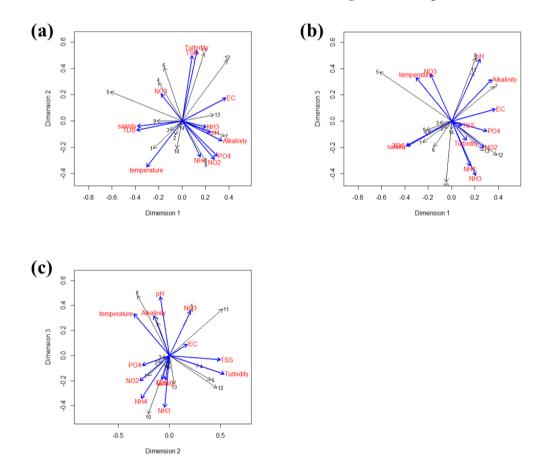


Figure 7: The biplots of the landmark coordinates and environmental (water) data for the first three dimensions.

Discussion

This study investigated the morphology association (traditional and geometric) data belonged to Cyprinus carpio with environmental parameters southern coast of the Caspian Sea. The two-block PLS has indicated that there was a significant correlation between morphological and environmental parameters as examined using both traditional and geometric data. In comparison with a traditional morphology, which is focused on linear distances, the geometric morphometric mostly considers the form of body landmark's coordinates. based on Adams et al. (2004) concluded that morphological variations through geometric approach is better than traditional approach, as we took out higher correlation and significance from this approach in this study (Zelditch et al., 2012). A two-Block PLS on traditional data indicated that the caudal peduncle form (CPL and CPH) had positive correlations with alkalinity, nitrate, and nitrogen dioxide. These environmental variables except nitrate had shown their correlations by variations in landmark points of caudal peduncle (7 and 8) through geometric approach (Figs. 5 and 7). The head morphology (head length and head height) showed high correlation with Similarly, nitrate. the geometric approach showed correlation between nitrate and landmarks as related to the head shape. Bouton et al. (2002), found that eight ecological variables influenced the head shape of haplochromines in the Lake Victoria.

Also, Crispo and Chapman (2010) investigated the effects of hypoxia on body shape variations of cichlid and found big heads in waters with low oxygen levels.

We found a high correlation between temperature and head morphology through two morphological methods, traditional and geometric methods. High temperature increases the oxygen demand of fishes. Dissolved oxygen was not measured in this study, however, the variations in the head size might have been related to the direct effect of oxygen demand which is in agreement with Crispo and Chapman (2010).

Phenotypic traits may show different correlations in different size and age classes (Nyboer and Chapman, 2013). In the study on Nile perch, Lates niloticus, the different size classes (small, medium, and large size classes) of L. niloticus were investigated the oxygen level in each location and by this approach; they could remove the effect of size and allometric growth on the observed variations. In this study, morphological (geometric traditional based) and ecological data were first standardized, and then the associations were investigated. So the observed associations were not affected by the size of the variants.

The maximum value of nitrate was found in the RS (Guilan province, southwest of the Caspian Sea) which had the specimens with heads bigger than those of other specimens (higher values for the head length and height in eye area). Furthermore, the water

temperature at this location was higher than other locations increasing the oxygen demand. The role of nitrate in fish adaptation to hypoxia has been well identified (Dolomatov *et al.*, 2016), so the higher amount of nitrate may be one of the more important factors in fish adaptation in the southern coast of the Caspian Sea.

A distinctive negative correlation was found between salinity and the gill shape in both morphometric approaches (Figs. 5 and 7). Having a smaller gill in salt waters may be a function of adaptation for osmoregulation so that the fish can keep more salts and so keep their osmotic pressure close to the environment. Ecological factors can show their effects on phenotype directly and indirectly. Langerhans et al. (2007) found that water flow and dissolved oxygen have direct or indirect effects on gill size, body form and the shape of caudal fin on Barbus neumayeri. Also, temperature, as an important ecological variable, showed both positive negative correlation with dorsal fin (in morphometric) traditional and position of pectoral fin (In geometric morphometric) respectively, which is somehow in agreement by the results obtained from 88 samples of **Schizothorax** richardsonii. The observed difference between two methods of morphology is probably due to the difference of their views and visualizing of traits.

The results of this study show somewhat convergence between relationship of geometric and traditional morphometric and the association of environmental parameters with the higher level of assurance for geometric morphometric approach. The findings of this study present new information about morphoenvironmental association across the southern coasts of the Caspian Sea for C. Carpio as a native species. It is therefore suggested that evaluating effects of ecological parameters may provide valuable information on stocks reestablishment and thus vearly investigation is recommended. This is the first investigation of phenotype environment association with two morphological methods performed in the Caspian Sea.

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