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Short communication:

Morphological analysis of *Alburnoides samiii* from Toolkhone river, Guilan province, south Caspian Sea basin

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Introduction

Morphological variations within and among populations routinely occur in response to environmental and genetic factors, or as a consequence of differences in behavioral and physiological conditions (West–Eberhard, 1989; Schwander and Leimar 2011). In a general overview, variations in hydrological conditions of rivers may cause differences in habitat structure and hydrological conditions, inducing shape changes in fishes (Colihueque *et al*., 2017). It is well known that genetic factors induce morphological variations in natural populations of several fish species (Svanbäck and Eklöv, 2006; Taylor *et al*., 2011). In addition, the effects of environmental factors and phenotypic plasticity in the morphological variations of fish populations have also been documented by several researchers (West–Eberhard, 1989; Bagherian and Rahmani, 2009).

Human interference and man-made structures in natural aquatic environments could cause variations in habitat condition. Flood dams are constructions that used to control river floods. It has been reported that dams caused significant environmental impact on fish populations due to isolation and destruction of spawning grounds as well as alteration in the physico-chemical properties of the river (Dynesius and Nilsson, 1994; Daniels *et al*., 2005). Therefore, population intraspecific interactions in separated populations would be disrupted and the populations would be subjected to different environmental regimes in downstream and upstream of the dam. These variable conditions are responsible for variations in the fish population phenotypes (Vehanen and Huusko, 2011). It has been documented that phenotypic plasticity in body shape of fish populations is a typical response in fish gene expression to the various environmental conditions (Vehanen and Huusko, 2011), such as temperature (Beacham, 1990) and flow regulation (Jackson and Marmula, 2001),

Based on Mousavi-Sabet *et al*. (2015), at least eight *Alburnoides* species were considered to occur in Iranian inland waters. They prefer fast-flowing waters of streams and rivers (Vajargah *et al*., 2013; Varjagh and Hedayati, 2014) *Alburnoides samiii* Mousavi-Sabet *et al*. (2015) is a newly described small endemic cyprinid inhabiting in the southern Caspian Sea basin, Iran (Mousavi-Sabet *et al*., 2015). The aim of the present study was to assess whether the newly - found species in upstream and downstream of a river with a dam separation exhibits any morphological variations or not. The other aim of this study was to find which morphological features have contributed more to dissimilarities between two separated populations.

Materials and methods

Specimens (N=100) were collected from the Toolkhone River, in the Southern Caspian Sea basin, Iran (36° 99' N, 50° 43' E) in 2016. Samples were collected form upstream and downstream of the river that was separated by a flood dam. The Geographical coordinates were as follows: Flood dam: 36° 99' 01" E 50° 43' 49" N, Upstream: 36° 96' 41" E 50° 43' 56" N and Downstream 37° 00' 79" E 50° 43' 32" N. The presence of the dam has divided the *A. samiii* population into two sections. Fish samples were collected using a backpack portable electrofishing device (Hans Grassl Direct Pulse Current Electrofishing Device IG200/2) with three replicates in each sampling site. Samplings were repeated until 50 specimens were collected at each site (Vajargah and Hedayati, 2015). All the specimens were preserved in 4% formaldehyde for an equal period prior to the analyses and transferred to the laboratory for further measurements. Measurements were taken between 22 morphometric distances to the nearest 0.01 mm with a digital caliper (Table 1).

Table 1: Morphometric characteristics analyzed for the species in the current study.

Code	Description
FL	Fork length
TL	Total length
SL	Standard length
B_M	Distance from the beginning of anal fin to the end of head length
Posh_P	Predorsal distance
B_P	Distance from the beginning of dorsal fin to the end of caudal fin
B_M_B	Distance from the beginning of anal fin to the end of caudal fin
BH	Body depth
DFH	Dorsal fin depth
BW	Body width
Sin_P	Prepectoral distance (Distance from snout to operculum)
PFH	Depth of pectoral fin
AFL	Length of anal fin base
HL	Head length

Table 1 continued:

CL	Length of caudal fin
ABFH	Depth of Pelvic fin
D.Fbase.L	Depth of anal fin base
FNL	Length of dorsal fin base
PFL	Length of pectoral fin
H.D	Head depth
CH	Depth of caudal fin
Pel.F.L	Length of Pelvic fin

Prior to the statistical analysis Kolmogorov–Smirnov (K-S) test was applied to analyze normal distribution of the data. Differences among morphometric parameters were tested with an analysis of variance (one-way ANOVA). The Analysis of Similarity (ANOSIM) was applied in order to assess the differences between the stations in terms of Bray Curtis dissimilarity ($R=0.54$, $p>0.001$), then the similarity percentage (SIMPER) analysis was employed to assess the contribution of each factor in dissimilarity (Clarke and Warwick, 1994). The data were log-transformed and then standardized prior to the analysis. Thereafter, non-multi-dimensional scaling (NMDS) was used to classify the pattern of dissimilarity in Euclidean space (Kruskal and Wish, 1978). Linear discriminant analysis (LDA) was employed to evaluate any phenotypic differences among populations. All analyses were conducted in R version 3.3.2 (R Development Core Team, 2016) using vegan (Oksanen *et al*., 2016), MASS (Venables and Ripley, 2002).

Results and discussion

Table 2 shows the average values of measured morphometric parameters. No significant differences were found based on ANOVA test. However, minor differences were observed between morphometric parameters (Table 2).

Table 2: Mean and standard deviation (SD) of measured morphometric parameters in this study.

Parameters	Upstream		Downstream	
	Mean	SD	Mean	SD
TL	73.01	7.93	76.65	9.2
SL	57.72	7.16	61.62	7.97
FL	64.61	8.01	68.24	8.93
HL	14.11	1.86	14.52	1.68
HH	13.29	9.96	12.6	1.85
H.D	8.32	1.68	8.25	1.1
BH	16.35	2.79	16.22	2.82
BW	5.75	1.53	7.62	1.84
CL	8.36	1.96	8.81	1.57
CH	6.43	1.43	6.63	1.08
FNL	6.05	1.42	6.96	1.96
DFH	11.62	3	13.06	2.28
AFL	8.57	2	8.86	1.7
D.Fbase.L	8.17	1.41	9.06	1.84
PFL	1.31	0.61	2.78	3.66
PFH	10.94	1.55	11.34	2.28
Pel.F.L	1.49	2.11	1.68	2.54
ABFH	7.94	1.37	8.67	2.08

Table 2 continued:				
Sin_P	14.7	2.14	15.47	2.15
Posh_P	32.6	5.25	33.22	4.06
B_P	32.93	4.53	33.78	4.5
B_M	39.04	5.18	41	6.17
B_M_B	23.68	3.63	24.89	3.14

Results of ANOSIM analysis revealed that upstream sampling stations were significantly different from the downstream ones in terms of. (R=0.54, p>0.001). SIMPER analysis showed that FL, TL, DFH, BW, Sin_P, CL, ABFH, D.Fbase.L, FNL and PFL were significantly contributed in the dissimilarity (Table 3). Among these parameters, FL, TL and SL had more rates (%) of contribution. However, averaged dissimilarity between upstream and downstream samples was low (8%). The results of the NMDS showed that samples taken in the two sampling sites were distinct from each other and have dissimilar dispersion in the Euclidean distance (Fig. 1). The results of this study revealed that there is a significant dissimilarity between the upstream and downstream *A. samiii* populations in the Toolkhone River. Both similarity and discriminant analyses suggested distinct variations in matrix of morphometric measurements. Significant contributing factors in dissimilarity were FL, TL, DFH, BW, Sin_P, CL, ABFH, D.Fbase.L, FNL and PFL. However, TL, FL, SL, DFH and BW had high rate (%) of contribution. On the other hand, BW had the most correlation with LD1 in discriminant analysis. It could be suggested that in the present study, environmental conditions had the most effect on the length of specimens and dorsal fin depth. As shown in Table 2, Downstream samples exhibited higher values of TL, FL, SL, BW and DFH. As a result, the downstream fish had higher length and width as well as higher dorsal fin. On the other hand, upstream fish displayed lower values of the above -mentioned characteristics and thus a more hydrodynamic shape. Indeed, upstream water current is much higher than downstream, which might be one of the reasons for different body shape. Many studies have demonstrated that water current can impel the development of morphological differentiation (Bhagat *et al*., 2006; Hendry *et al*., 2006). McLaughlin and Grant (1994), Brinsmead and Fox (2002) and Kerfoot and Schaefer (2006) concluded that fishes inhabiting ecosystems with a high water velocity, such as streams, tend to be more hydrodynamic and have a more fusiform body shape. Webb (1984) stated that a more fusiform shape helps the fish to reduce the drag caused by the current, a suggestion that is also supported by McLaughlin and Grant (1994) and Yavno *et al*. (2013). Generally *et al*. (1996) and Yavno *et al*. (2013) suggested phenotypic plasticity of the species, in an effort to adapt to a novel environment. In the case of Toolkhone River, *A. samiii* species had to adapt to their conditions (Upstream and downstream of the river) and their populations are separated by a flood dam as well.

Table 3: SIMPER analysis of dissimilarity among upstream and downstream samples based on morphometric measurements.

Parameters	Contribution (%)
FL	0.956*
TL	0.9512*
SL	0.8657**
B_M	0.605
Posh_P	0.5177
B_P	0.5024
B_M_B	0.3617
BH	0.3103
DFH	0.3075**
BW	0.2559***
Sin_P	0.2462*
PFH	0.2044
AFL	0.2
HL	0.1965
CL	0.1962*
ABFH	0.1937*
D.Fbase.L	0.1904*
FNL	0.1832*
PFL	0.1755***
H.D	0.1537
CH	0.1494
Pel.F.L	0.1352
Average	**8.1**

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

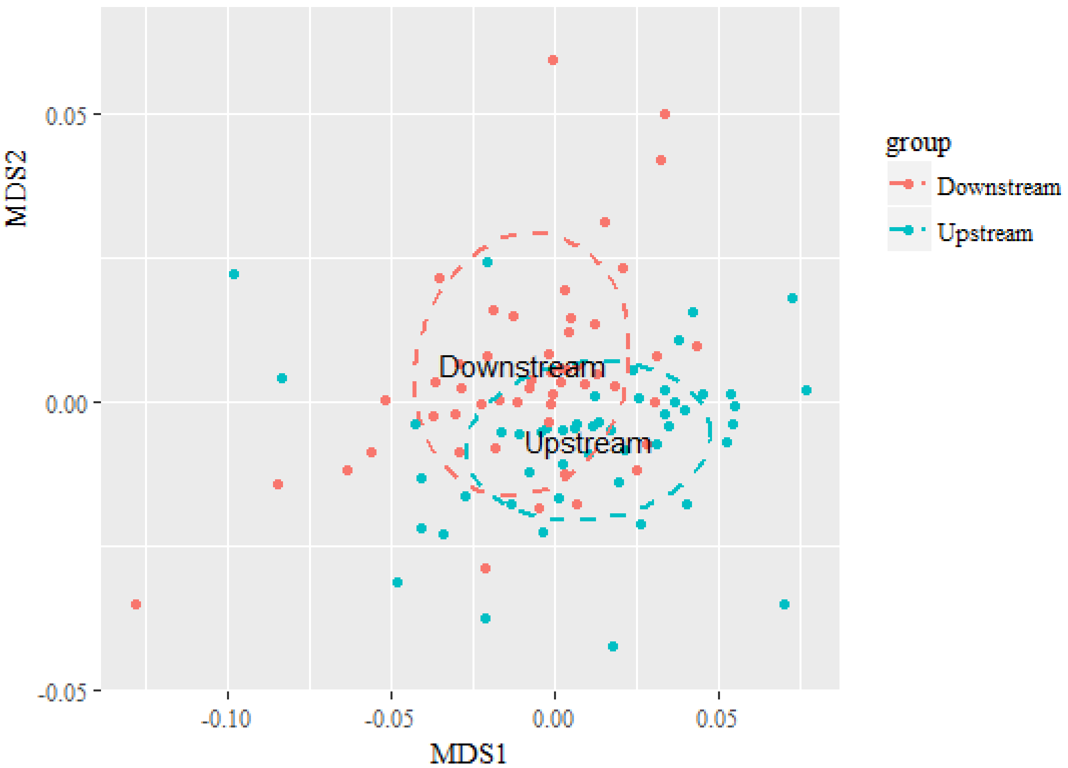

Figure 1: NMDS analysis between upstream and downstream populations showing the effect of ordination dispersion in population differentiation.

The results of LDA are represented in Fig. 2 and Table 4. Three discriminant functions were generated, and a Kappa test showed that they were highly significant ($p<0.001$). LD1 accounted for 100% between-group variability.

According to Table 4, BW and H.D had maximum indirect and direct correlation with LD1, respectively.

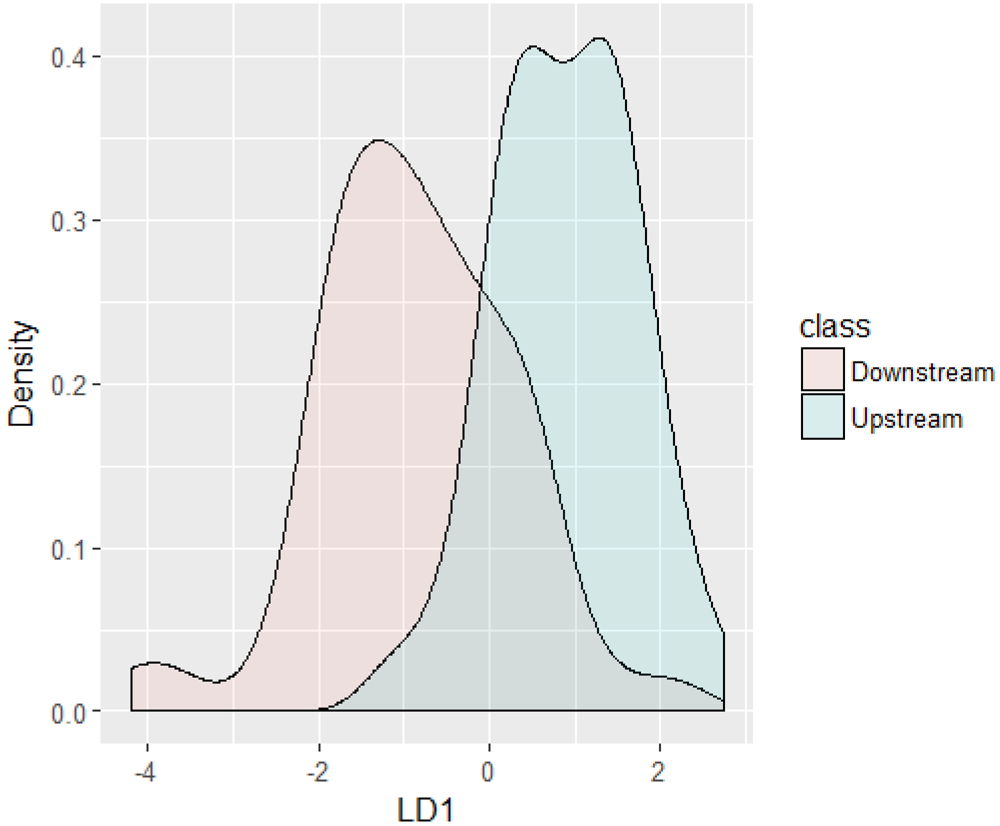

Figure 2: Results of the linear discriminant analysis (LDA) showing the distribution and overlap of upstream and downstream groups in LD1 dimensions.

Table 4: Correlation ratio of morphometric parameters with LD1 in the linear discriminant analysis (LDA) in this study.

Parameters	LD1
TL	0.153
SL	-0.098
FL	-0.002
HL	-0.062
H.D	0.440
BH	0.044
BW	-0.668
CL	-0.198
CH	-0.052
FNL	-0.050
DFH	-0.193
AFL	0.121
D.Fbase.L	-0.168
PFL	-0.104
PFH	-0.101
Pel.F.L	-0.024
ABFH	0.134
Sin_P	0.013
Posh_P	0.104
B_P	-0.033
B_M	0.010
B_M_B	-0.035

A. samiii samples in upstream, developed a more streamlined body with smaller body width and total length. As Kerfoot and Schaefer (2006) noted about the *Cottus* species, it developed a more streamlined body in habitats with higher water velocities as a mean to increase their ability to maintain their position in these conditions. This morphometric characteristic allows the fish to reduce the energy loss and the drag produced. Based on these findings, the morphological variation observed between the upstream and downstream specimens of *A. samiii* could relate to their need to maintain their position during increased water flow.

In conclusion, despite the isolation of groups due to the construction of the dams being relatively short (approximately 10 years), the upstream and downstream groups of two of the *A. samiii*, exhibit significant variations in morphological variables associated with their hydrodynamic balance and swimming abilities. These differences are assumed to reflect an adaptation to optimize their hydrodynamic characteristics to the different hydrological conditions, which are due to the changes in water flow. However, a more thorough investigation of the factors influencing these morpho-anatomical changes in these two species is needed, since there are various habitat parameters that could affect the fish morphology.

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