

Habitat effects on morphological variation of *Garra rufa* (Heckel, 1843) populations in Iran

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Received: March 2021

Accepted: September 2021

Abstract

Understanding the processes involved in evolution of organisms is a principal topic that has received much attention from the research community. A common way to achieve this is to study the ecological conditions and morphological relationships of taxa. Fish are widely regarded as notable animal models in studies of environmental influence on morphological features given their high environmental adaptability and morphological flexibility. Here, we investigated the effects of environmental factors on 40 morphological traits of *Garra rufa* in four basins/subbasins of Karkheh, Karun, Zohreh, and the Persian Gulf. Our findings indicated that the morphological changes of the studied populations exhibited an east-west cline. It appears that a combination of two regional factors, i.e., elevation and temperature, and three local factors, i.e., substrate type, water current velocity, and water depth could account for this pattern. The effect of these variables was evident in almost all the characteristics we measured. Among the local factors, river width made the smallest contribution to inducing morphological variations.

Keywords: Ecological factor, Cline, *Garra rufa*, Fish, Trait

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Introduction

The high environmental adaptability of fishes due to their morphological plasticity is one of the primary factors that allows fish populations to occupy different aquatic habitats (Hutchings, 1996; Svanbäck and Eklöv, 2006), thus improving their chances of survival (Chapman *et al.*, 2008). This morphological plasticity can be used to distinguish between different populations of one species (Wootton, 1999; Torres-Dowdall *et al.*, 2012). Also, differences in ecological and environmental parameters of organisms can be inferred from variations in their morphological traits (Ghalenoei *et al.*, 2010). Therefore, fishes generally offer one of the best models for studying the interactions between environmental factors and morphological features (Svanbäck and Eklöv, 2006).

Morphological differences can result from either genetic or environmental factors or their mutual interaction (Cadrin, 2000). Studies suggest that morphological differences often fail to reflect genetic distinctiveness among fish groups. In addition, morphological differences may arise solely from environmental, not genetic, factors, which stresses the central role of environment in shaping morphological variations (Akbarzadeh *et al.*, 2007).

Morphological parameters are commonly used in fish biology to measure distinctiveness and investigate the relationship between different taxa (Turan, 1999). Moreover, understanding the mechanisms leading to evolution is possible by examining the

morphological characteristics and ecological conditions which have contributed to adaptation (Siemers and Schnitzler, 2004). Thus, numerous studies have applied this approach to investigate fish species' relationships. These studies have focused on the relationship between ecological factors (e.g., water current velocity (Rajput *et al.*, 2013; Çiçek *et al.*, 2016), river slope, substrate type, depth, water temperature (Rajput *et al.*, 2013), and predation (Brönmark and Miner, 1992)) and morphological, behavioral and physiological variations of freshwater fish populations (Weigensberg and Roff, 1996; Hoffmann, 2000). However, it is worth noting that species behave differently in response to environmental factors based on the varying intensity of factors and their interactions. For instance, studies on the effects of environmental factors (nutrition and elevation) on functional morphology of various fish reported different results (Albouy *et al.*, 2011; Shuai *et al.*, 2018).

Similar to many fish genera, *Garra* is comprised of numerous morphologically diverse species that are widely dispersed throughout the world. Out of a total of 146 species in this genus, 11 species are known to occur in Iran (Froese and Pauly, 2020). Among the Iranian species, *G. rufa* is more abundant and has a wider distribution range worldwide. It is considered an endemic species in rivers of Ceyhan, Euphrates, Tigris, the Eastern Mediterranean basin, Kuwait, Jordan, southern Iran and Syria (Demirci *et al.*, 2016). Although the morphological variations of *G. rufa* have

been previously studied within its Iranian and Turkish ranges (Ghalenoey *et al.*, 2010; Keivany *et al.*, 2015; Çiçek *et al.*, 2016), the effects of ecological factors on the species' morphological variability are yet to be investigated. Here, we aimed to assess changes in morphology of *G. rufa* in four basins/subbasins throughout its entire distribution range in Iran, and evaluate the effects of several habitat factors on the species' morphological change. The findings of this study, as the first attempt conducted at this scale, will reveal the effect of environmental, particularly regional, variables on morphological variability of *G. rufa* in this region.

Materials and methods

Study area

Sampling of *G. rufa* was conducted in

36 rivers across four basins/subbasins of Karkheh, Karun, Zohreh, and the Persian Gulf distributed from west and south-west of Iran to the south. In total, 36 stations were selected across the sampling area based on the species' distribution range and river conditions (Fig. 1). The location of each station was recorded using a handheld Garmin eTrex 20 GPS. The selection of sampling sites was done in such a way as to ensure each station could efficiently represent the biological characteristics of the river and that sampling activities at one station would not influence sampling at other sites. Sampling was conducted under the natural environment and biodiversity regulations of Iran's Department of Environment (permit number 97/3345). None of the sampling stations were located within sensitive habitats or non-hunting reserves.

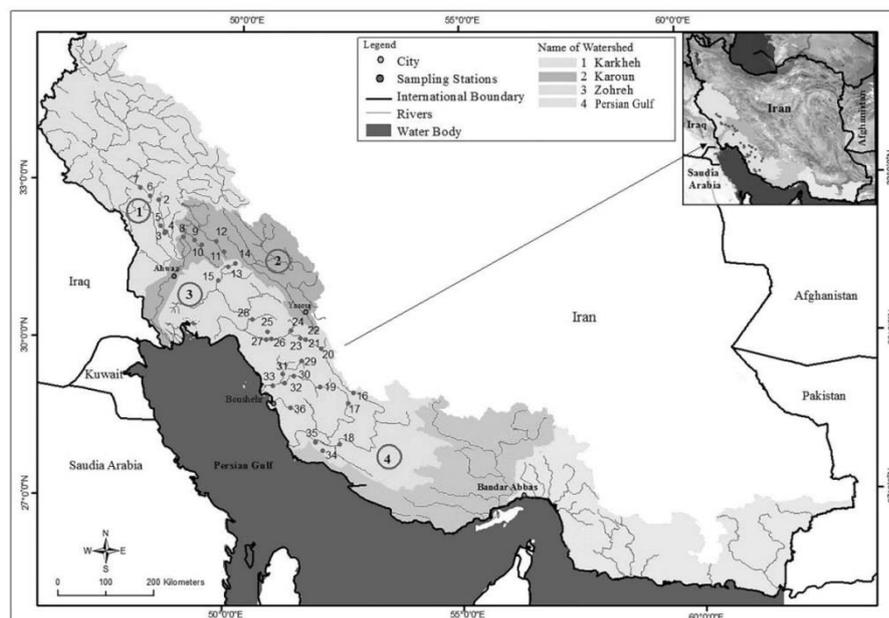


Figure 1: Location of sampling stations in the study area: (1) Karkheh, (2) Karun, (3) Zohreh, and (4) the Persian Gulf.

Field sampling

Sampling was carried out in mid-autumn 2018 and mid-autumn 2019 (Fig. 2). At the time of sampling, team members were accompanied by rangers of Iran's Department of Environment. Each sampling site was electrofished by a team of at least four people during daylight hours only. For each stream, a section with 100 m² was electrofished.

A total of 514 samples were collected using a hand net (5 mm mesh), fixed in 10% formalin and delivered to the lab. Of these, 388 *Garra rufa* specimens whose populations were confirmed by genetic data were used in the analysis. Despite suitable ecological conditions, no samples were collected from S1, S7, S8, S10, S13, and S23 stations.

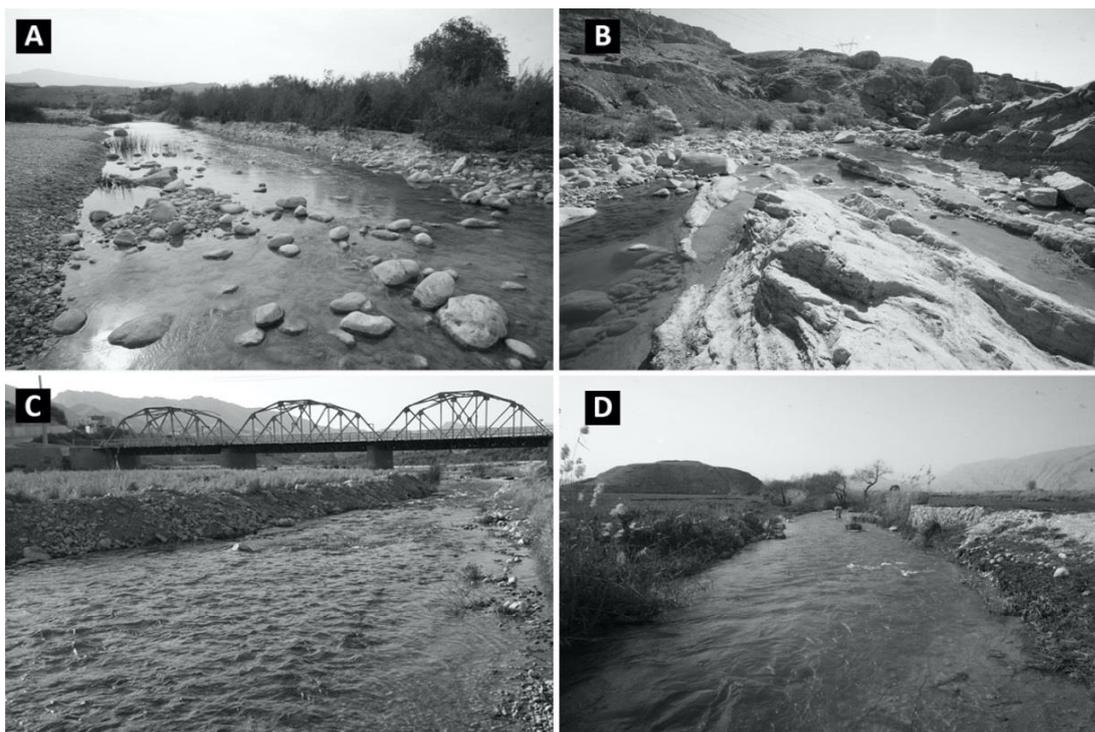


Figure 2: Habitat of *Garra rufa* and sampling sites in A: Baghan River; B: Northern Balarood, C: Bagh Malek River, D: Kopen River.

Collection of morphometric data

Following standard protocols, 40 morphological traits (Fig. 3) were measured using a digital caliper with an accuracy of 0.01 mm. All morphometric measurements were conducted by the same person. To avoid the effect of allometric growth on variability, trait measurements were standardized using the following formula (Elliott *et al.*, 1995):

$$M_s = M_o \left(\frac{L_s}{L_o} \right)^b$$

Where M_s =standardized measurement, M_o =original length of the measured trait, L_s =average standard length of all the samples, L_o =standard length of the sample, b =slope of the regression line between $\log M_o$ on $\log L_o$ for each trait.

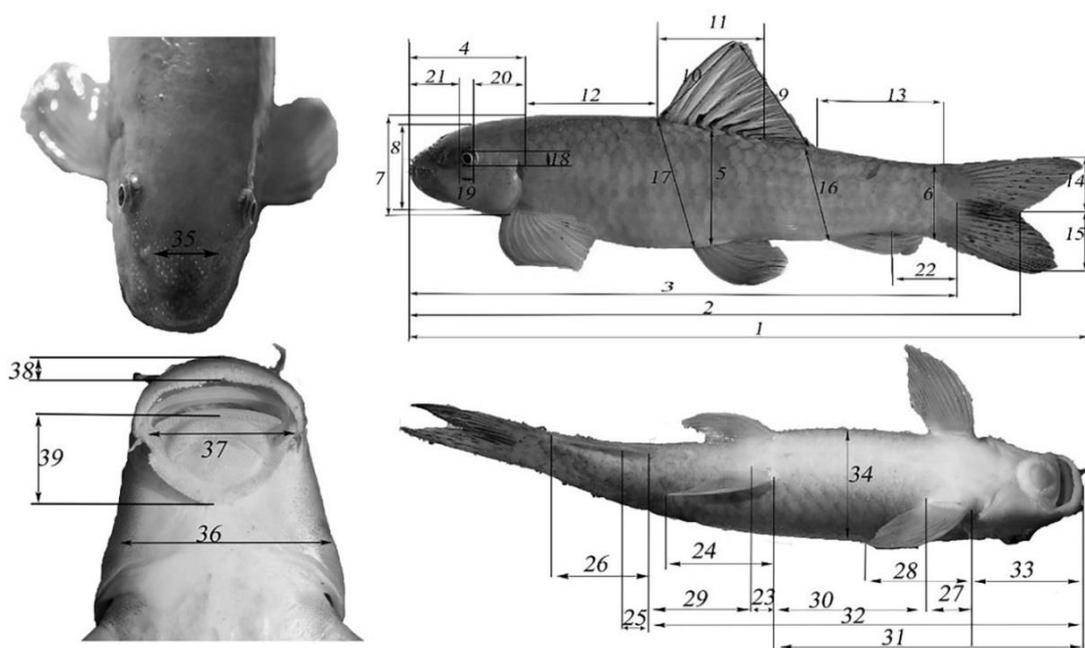


Figure 3: Measurements of external morphology traits. (1) Total length (TL); (2) Standard length (SL); (3) Fork length (FL); (4) Head length (HL); (5) Body depth (BD); (6) Minimum body depth (MBD); (7) Body depth at the end of the opercle (BDO); (8) Head depth (HD); (9) Depth of the posterior part of the dorsal fin (DPD); (10) Depth of the dorsal fin (DDF); (11) Length of dorsal fin base (LDF); (12) Distance between the end of the imaginary line of the gill and the beginning of dorsal fin base (DIGDF); (13) Distance between the end of dorsal fin base and beginning of caudal fin base (DDFCF); (14) Length of the upper lobe of the caudal fin (LUCF); (15) Length of the lower lobe of the caudal fin (LLCF); (16) Distance between the end of dorsal fin base and the beginning of anal fin base (DDFAF); (17) Distance between the beginning of dorsal fin base and the beginning of ventral fin base (DDFVF); (18) Vertical diameter of eye (VDE); (19) Horizontal diameter of eye (HDE); (20) Distance between end of eye and opercle (DEO); (21) Preorbital distance (POD); (22) Distance between end of anal fin base and beginning of caudal fin base (DAFCF); (23) Length of ventral fin base (LVFB); (24) Length of ventral fin (LVF); (25) Length of anal fin base (LAFB); (26) Length of anal fin (LAF); (27) Length of pectoral fin base (LPB); (28) Length of pectoral fin (LPF); (29) Distance between end of ventral fin base and beginning of anal fin base (DVFAF); (30) Distance between end of pectoral fin base and beginning of ventral fin base (DPFVF); (31) Pre-ventral distance (PVD); (32) Preanal distance (PAD); (33) Pre-pectoral distance (PPD); (34) Body width (BW); (35) Distance between the two nostrils (DBN); (36) Head width (HW); (37) Distance between two barbels (DBB); (38) Depth of upper jaw (DUJ); (39) Depth of lower jaw (DLJ); (40) Body depth at caudal fin base (BDCF).

Habitat data

To determine the effects of environmental factors on morphometric characteristics of *G. rufa*, the following six habitat variables were assessed:

1. Elevation from sea-level (m): at each sampling station, elevation from sea level was recorded using a Garmin GPS3.
2. Water current velocity (m/s): A current meter with 0.03 m/s accuracy was used to measure water current velocity.
3. River width (m): river width was measured at the beginning, middle, and end of each sampling station using a meter tape and their average was considered as width of the river.

4. Water depth (cm): at each sampling station, three line transects were randomly set across the river and at each transect, depth was measured using a grade rod. Four points were selected and their average was considered as the river's depth at each station.
5. Water temperature (°C): A digital thermometer with 0.1°C accuracy was used to measure water temperature.
6. Index of substrate: the index of substrate was calculated by examining the texture components of substrate according to the classification description of Platts *et al.* (1983) in a 1 m² quadrat using four random iterations. Finally, the average of each group was analyzed using Jowett and Davey's method (2007).

Data regarding water temperature (°C) and current velocity (m/s) for some of the stations were obtained from regional water authorities.

Statistical analyses

ANOVA test was used to analyze changes in environmental factors in four basins/subbasins. CLUSTER analysis as well as Similarity Profile Analysis (SIMPROF), Discriminant Function Analysis (DFA) and the PERMANOVA tests were performed to evaluate the overall morphological changes of *G. rufa* populations. The BEST and LINKTREE analyses were used to determine the most influential environmental variables in shaping patterns of morphological variation and

evaluate the relationship between environmental variables and morphological changes (Clarke *et al.*, 2014). For all analyses, statistical significance was accepted at $p < 0.05$ level. ANOVA and PERMANOVA analyses were carried out using SPSS and PAST, respectively. PRIMER-E was used to perform the remaining analyses.

Results

Fluctuations of environmental variables

Due to the large size of the study area, considerable fluctuations were observed in the environmental variables (Table 1). With the exception of river width (one-way ANOVA, $F_{3,26}=1.533$, $p=0.230$) and the index of substrate (one-way ANOVA, $F_{3,26}=0.667$, $p=0.580$), the remaining variables showed significant changes.

Intraspecific variations

Based on the cluster analysis with SIMPROF test, the morphological variations of *G. rufa* populations in Iran followed a specific pattern in which any two adjacent basins/subbasins showed similarity and formed a single cluster (Fig. 4). In other words, this analysis revealed the morphological diversity between geographically distant populations. Also, the DFA indicated that 84.0 percent of fish in each basin/subbasin were categorized within a single group (Table 2); however, this percentage decreased as the distance between basins/subbasins increased. Cluster analysis (Fig. 4) and PERMANOVA (Table 3) tests found

significant morphological variations between populations of non-adjacent basins/subbasins. Accordingly, populations of Karkheh and Karun, and also Zohreh and the Persian Gulf were not significantly different from each

other, while outside this grouping, each basin was significantly morphologically distinct from the other basins. Morphological variability was detected in all traits.

Table 1: Environmental variable changes in four drainage basins/subbasins based on ANOVA analysis.

Basins/subbasins	$\bar{x} \pm SD$				F	p<
	Karkheh	Karun	Zohreh	Persian Gulf		
Width (m)	21.30 ± 20.63	7.33 ± 6.80	10.80 ± 6.61	12.08 ± 7.50	1.533	0.230
Depth (cm)	88.00 ± 58.48	30.00 ± 10.00	37.50 ± 4.25	29.58 ± 10.96	7.369	0.001
Elevation (m)	173.40 ± 155.82	409.34 ± 309.10	750.60 ± 369.95	423.58 ± 544.04	2.773	0.041
Water temperature (°C)	22.70 ± 2.22	23.00 ± 3.90	20.15 ± 3.15	23.04 ± 3.40	2.855	0.039
Current velocity (m/s)	0.58 ± 0.33	0.16 ± 0.14	0.82 ± 0.31	0.61 ± 0.42	2.986	0.035
Substrate type	4.92 ± 1.76	4.46 ± 0.44	4.28 ± 0.50	4.44 ± 0.54	0.667	0.580

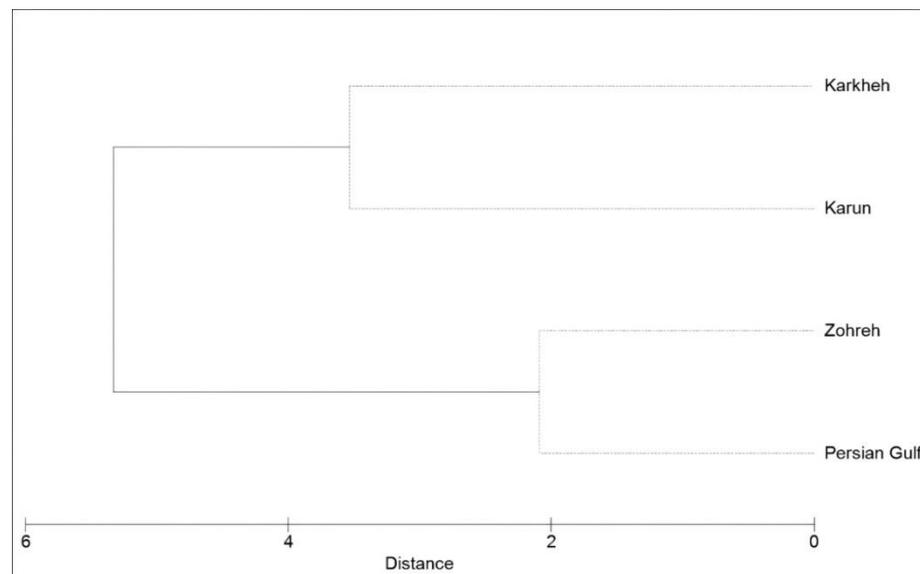


Figure 4: Morphological analogy of *Garra rufa* populations across the study area (the grey dashed line shows a lack of significant difference between populations, and the black lines exhibit a significant difference between populations based on the SIMPROF test).

Table 2: The categorization percentage of morphological traits in *G. rufa* populations based on the DFA test in the four studied basins/subbasins

Basins/Subbasins	Predicted Group Membership (%)				Total
	Karkheh	Karun	Zohreh	Persian Gulf	
Karkheh	82.4	13.7	3.9	0.0	100.0
Karun	5.3	94.7	0.0	0.0	100.0
Zohreh	2.7	3.5	78.8	15.0	100.0
Persian Gulf	0.0	2.0	11.7	86.3	100.0

Table 3: Morphological differences of *Garra rufa* in four basins/subbasins based on PERMANOVA.

Basins/ Subbasins		Karkheh	Karun	Zohreh
Karun	F	0.8672		
	<i>p</i>	0.6585		
Zohreh	F	9.32	6.87	
	<i>p</i>	0.0011	0.0064	
Persian Gulf	F	7.321	4.993	1.178
	<i>p</i>	0.0002	0.0026	0.2519

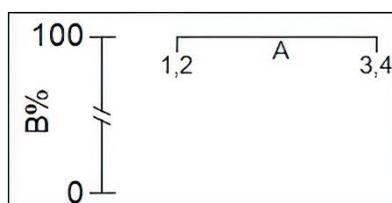
Effects of environmental factors on intraspecific morphology

In this section, the variables that affect the morphology of *G. rufa* populations are discussed. Based on the BEST test (Table 4), the variables of depth,

elevation, current velocity, and substrate index showed the highest correlation with the morphological variation of *G. rufa* populations ($P_w=0.654$). With slight differences ($P_w=0.600$), the three variables of depth, elevation, and substrate index can be regarded as the most influential variables. Also, LINKTREE analysis (Fig. 5 and Table 5) shows that Karkheh and Karun are separated from Zohreh and the Persian Gulf basins/subbasins ($\pi=0.71$, $p<0.001$) by depth, elevation, water temperature, and substrate index variables.

Table 4: Harmonic rank correlations (P_w) between *Garra rufa* and environmental similarity matrices.

Number of variables	Best variable combinations	Correlation (P_w)
4	Depth-Elevation-Current velocity-Substrate	0.654
3	Depth-Elevation-Substrate	0.600
1	Depth	0.429
1	Substrate	0.429

**Figure 5: Separation of (1) Karkheh, (2) Karun, (3) Zohreh, and (4) the Persian Gulf basins/subbasins in one class based on: (A) depth, substrate type, elevation or water temperature.****Table 5. LINKTREE analysis of *Garra rufa* populations in IRAN.**

Node/station split	Variable	LHS (RHS) split	π	<i>p</i>	R	B (%)
A	Depth	<-0.289 (>0.036)	0.71	0.001	1	100
	or Substrate index	or >0.445 (<-0.039)				
	or Elevation	or <-0.044 (>0.383)				
	or Water temperature	or <-0.0182 (>0.0334)				

Discussion

Geographic isolation can generally lead to morphological separation of fish populations. Also, it must be noted that

anthropogenic activities could induce morphological differentiation by altering the physical and chemical properties of rivers (Ferreira, 2007).

Morphological variation affects fish physiological functions in an ecosystem (Shuai *et al.*, 2018), which consequently leads to adaptation of species to environmental conditions for reproduction and survival. Such adaptations are not necessarily arisen from genetic changes (Turan, 1999).

The notable pattern of morphological cline observed in Iranian *G. rufa* populations is likely a result of geographic isolation and adaptation. For instance, the correlation between geographic conditions and morphological characteristics in *G. variabilis*, that is to say high morphological similarity between geographically-adjacent populations, is induced by the occurrence of similar ecological and habitat conditions (Çiçek *et al.*, 2016). Such a pattern is also observed in Iranian *G. rufa* populations in which an inverse correlation exists between geographical distance and morphological similarity. Therefore, populations of Karkheh and Karun, and also Zohreh and the Persian Gulf were morphologically similar in pairs. On the other hand, comparing populations of Karkheh with the Persian Gulf, which are farthest apart from each other, showed the lowest similarity. This pattern has likely resulted as an adaptive response to regional (elevation and temperature) and local factors (current velocity, substrate type, and water depth).

It is well acknowledged that regional factors have a stronger impact on species' morphology than local factors (Shuai *et al.*, 2018). Additionally, the

role of elevation and temperature variables in separation of the morphologically-distinct basins/subbasins is clearly seen from the results of the LINKTREE analysis. The high correlation between elevational changes and the observed morphological pattern is also confirmed by the BEST test. Therefore, it could be concluded that the two regional factors of elevation and temperature are most influential in *G. rufa*'s morphology. Nonetheless, the effect of local variables must not be disregarded. In an aquatic ecosystem, factors such as substrate type (Rajput *et al.*, 2013); water current velocity and depth (Mahon, 1984) can profoundly affect the morphological parameters of fish species. Using tests that reveal the relationship between biotic and abiotic variables, it was shown that the three local factors of substrate type, current velocity, and depth have a considerable impact on the morphological variation of *G. rufa* populations. As *G. rufa* sticks to the bottom of riverbeds using its sucker-like mouth, different individuals of the species tend to select similar substrate types. Thus, in our study, no significant difference was observed regarding substrate index between different basins/subbasins.

Differences and similarities in the overall morphology of populations of each basin/subbasin were evident in every studied trait. As body, fin and mouth shapes change under the effect of elevation (Boisclair and Tang, 1993; Shuai *et al.*, 2018), temperature (Rajput *et al.*, 2013), water current velocity (Gatz, 1979; Bourke *et al.*, 1997;

Langerhans *et al.*, 2003; Rajput *et al.*, 2013), and substrate type (Rajput *et al.*, 2013; Çiçek *et al.*, 2016), changes in every trait were likely to be expected, particularly because all the traits measured in this study were related to body, fin, and mouth sizes. Overall, even traits with no key functional roles may exhibit variations under the influence of environmental factors (Kerfoot and Schaefer, 2006); however, the synergy between environmental variables with different impact rates leads to changes in traits that affect swimming and feeding functions in *G. rufa*.

Acknowledgements

We thank Dr. K. R. Clarke for his instructions in performing the tests. We are particularly grateful to Dr. Hamid Zohrabi, deputy director of the natural environment and biodiversity bureau of Iran's department of environment for issuing sampling permits. Finally, we thank all the park rangers and officials at regional offices of environment in each province for their contribution to sampling.

References

- Akbarzadeh, A., Karami, M., Nezami, S. A., Igdari, S., Bakhtiari, M. and Khara, H., 2007.** Analysis of population structure of pikeperch (*Sander lucioperca*), in Iranian waters of Caspian Sea and Anzali wetland using truss system. *Iranian Journal of Natural Resources Research*, 60, 127-139.
- Albouy, C., Guilhaumon, F., Villéger, S., Mouchet, M., Mercier, L., Culioli, J., Tomasini J. and Mouillot, D., 2011.** Predicting trophic guild and diet overlap from functional traits: statistics, opportunities and limitations for marine ecology. *Marine Ecology Progress Series*, 436, 17-28.
- Boisclair, D. and Tang, M., 1993.** Empirical analysis of the influence of swimming pattern on the net energetic cost of swimming in fishes. *Journal of Fish Biology*, 42(2), 169-183.
- Bourke, P., Magnan, P. and Rodriguez, M., 1997.** Individual variations in habitat use and morphology in brook charr. *Journal of Fish Biology*, 51(4), 783-794.
- Brönmark, C. and Miner, J.G., 1992.** Predator-induced phenotypical change in body morphology in crucian carp. *Science*, 258(5086), 1348-1350.
- Cadrin, S.X., 2000.** Advances in morphometric identification of fishery stocks. *Reviews in Fish Biology and Fisheries*, 10(1), 91-112.
- Chapman, L.J., Albert, J. and Galis, F., 2008.** Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. *The Open Evolution Journal*, 2(1), 75-88.
- Çiçek, T., Ünlü, E., Bilici, S. and Uysal, E., 2016.** Morphological differences among the *Garra variabilis* populations (Cyprinidae) in Tigris River system of South East Turkey. *Journal of Survey in Fisheries Sciences*, 3(1), 9-20.

- Clarke, K.R., Gorley, R., Somerfield, P.J. and Warwick, R., 2014.** *Change in marine communities: an approach to statistical analysis and interpretation*. Primer-E Ltd.
- Demirci, S., Ozdilek, S.Y. and Simsek, E., 2016.** Study on nutrition characteristics of *Garra rufa* on the river asi. *Fresenius Environmental Bulletin*, 25(12 A), 5999-6004.
- Elliott, N., Haskard, K. and Koslow, J., 1995.** Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *Journal of Fish Biology*, 46(2), 202-220.
- Ferreira, K.M., 2007.** Biology and ecomorphology of stream fishes from the rio Mogi-Guaçu basin, Southeastern Brazil. *Neotropical Ichthyology*, 5(3), 311-326.
- Froese, R. and Pauly, D., (Editors), 2020.** FishBase. World Wide Web electronic publication. www.fishbase.org, version (07/2020).
- Gatz, A.J., 1979.** Ecological morphology of freshwater stream fishes. *Tulane Studies in Zoology and Botany*, 21, 91-124.
- Ghalenoei, M., Pazooki, J., Abdoli, A., Hassanzadeh Kiabi, B. and Golzarian, K., 2010.** Morphometric and meristic study of *Garra rufa* populations in Tigris and Persian Gulf basins. *Iranian Scientific Fisheries Journal*, 19(3), 107-118.
- Hoffmann, A.A., 2000.** Laboratory and Field Experiments: Some Lessons from *Drosophila*. In T. A. Mousseau, B. Sinervo and J. A. Endler (Eds.), *Adaptive Genetic Variation in the wild* (pp. 200-218). Oxford: Oxford University Press.
- Hutchings, J.A., 1996.** Adaptive phenotypic plasticity in brook trout, *Salvelinus fontinalis*, life histories. *Ecoscience*, 3(1), 25-32.
- Jowett, I.G. and Davey, A.J.H., 2007.** A comparison of composite habitat suitability indices and generalized additive models of invertebrate abundance and fish presence-habitat availability. *Transactions of the American Fisheries Society*, 136(2), 428-444.
- Keivany, Y., Nezamoleslami, A. and Dorafshan, S., 2015.** Morphological diversity of *Garra rufa* (Heckel, 1843) populations in Iran. *Iranian Journal of Ichthyology*, 2(3), 148-154.
- Kerfoot, J.R. and Schaefer, J.F., 2006.** Ecomorphology and habitat utilization of Cottus species. *Environmental Biology of Fishes*, 76(1), 1-13.
- Langerhans, R.B., Layman, C.A., Langerhans, A.K. and Dewitt, T.J., 2003.** Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society*, 80(4), 689-698.
- Mahon, R., 1984.** Divergent structure in fish taxocenes of north temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 41(2), 330-350.
- Platts, W.S., Megahan, W.F. and Minshall, G.W., 1983.** Methods for evaluating stream, riparian, and biotic

- conditions. US Department of Agriculture, Forest Service, Intermountain Forest and Range, Washington, DC.
- Rajput, V., Johnson, J. and Sivakumar, K., 2013.** Environmental effects on the morphology of the Snow Trout *Schizothorax richardsonii* (Gray, 1832). *TAPROBANICA: The Journal of Asian Biodiversity*, 5(2), 102-110.
- Shuai, F., Yu, S., Lek, S. and Li, X., 2018.** Habitat effects on intra-species variation in functional morphology: Evidence from freshwater fish. *Ecology and evolution*, 8(22), 10902-10913.
- Siemers, B.M. and Schnitzler, H.U., 2004.** Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature*, 429(6992), 657-661.
- Svanbäck, R. and Eklöv, P., 2006.** Genetic variation and phenotypic plasticity: causes of morphological variation in Eurasian perch. *Evolutionary Ecology Research*, 8(1), 37-49.
- Torres-Dowdall, J., Handelsman, C.A., Reznick, D.N. and Ghalambor, C.K., 2012.** Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*). *Evolution: International Journal of Organic Evolution*, 66(11), 3432-3443.
- Turan, C., 1999.** A note on the examination of morphometric differentiation among fish populations: the truss system. *Turkish Journal of Zoology*, 23(3), 259-264.
- Weigensberg, I. and Roff, D.A., 1996.** Natural heritabilities: can they be reliably estimated in the laboratory? *Evolution*, 50(6), 2149-2157.
- Wootton, R.J., 1999.** Ecology of teleost fishes (Vol. 2). Netherlands: Springer.