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

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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 (Wootten, 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 (Ghalenoei 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.

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 m$^2$ 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.
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 | Karkheh       | Karun         | Zohreh      | Persian Gulf |
|------------------|---------------|---------------|-------------|--------------|
| Width (m)        | 21.30 ± 20.63 | 7.33 ± 6.80   | 10.80 ± 6.61| 12.08 ± 7.50 |
| Depth (cm)       | 88.00 ± 58.48 | 30.00 ± 10.00 | 37.50 ± 4.25| 29.58 ± 10.96|
| Elevation (m)    | 173.40 ± 155.82| 409.34 ± 309.10| 750.60 ± 369.95| 423.58 ± 544.04|
| Water temperature (°C) | 22.70 ± 2.22 | 23.00 ± 3.90 | 20.15 ± 3.15 | 23.04 ± 3.40 |
| Current velocity (m/s) | 0.58 ± 0.33 | 0.16 ± 0.14 | 0.82 ± 0.31 | 0.61 ± 0.42 |
| Substrate type   | 4.92 ± 1.76   | 4.46 ± 0.44   | 4.28 ± 0.50  | 4.44 ± 0.54   |

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          | 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 |
Shirzad et al., Habitat effects on morphological variation of *Garra rufa* (Heckel, 1843) populations …

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

| Basins/Subbasins | Karkheh F | Karun p | Zohreh |
|------------------|-----------|---------|--------|
| Karun            | 0.8672    | 0.6585  |        |
| Zohreh           | 0.0011    | 0.0064  |        |
| Persian Gulf     | 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*<0.054). With slight differences (*P*<0.060), 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 (*π*=0.71, *p*<0.001) by depth, elevation, water temperature, and substrate index variables.

Table 4: Harmonic rank correlations (*P*<0.05) between *Garra rufa* and environmental similarity matrices.

| Number of variables | Best variable combinations | Correlation (*P*<0.05) |
|---------------------|----------------------------|------------------------|
| 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   | p    | 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 (>0.0534) |     |      |     |       |

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 morphologicalcline 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.

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