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Effects of Stream Damming on Morphological Variability of Fish: Case Study on Large Spot Barbell *Barbus balcanicus*

Nataša Radojković[1,*], Zoran Marinović[2], Aleksandra Milošković[1], Milena Radenković[1], Simona Đuretanović[1], Jelena Lujić[3], Vladica Simić[1]

[1] University of Kragujevac, Institute of Biology and Ecology, Faculty of ScienceRadoja Domanovića 12, 34 000 Kragujevac, Serbia.
[2] University of Novi Sad, Department of Biology and Ecology, Faculty of Sciences, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia and Szent István University, Department of Aquaculture, Gödöllő, Páter Károly u. 1, 2100 Hungary.
[3] Szent István University, Department of Aquaculture, Gödöllő, Páter Károly u. 1, 2100 Hungary.

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Corresponding Author
Tel.: +38.165 8706295
E-mail: naca@kg.ac.rs

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Abstract

In the present study, landmark-based geometric morphometrics was applied to compare and visualize the overall size and shape variation, sexual size and shape dimorphism of the species *Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi from different fragmented localities. The body shape of the fish was significantly different both for sex and locality. Also, body size significantly differed between sexes, with females being generally larger than males. This study also proved significant differences in body shape between populations from different localities. Considering that the environmental conditions of the localities were not greatly different from each other, the morphological divergence that was observed may be partly attributed to stream damming, which acts as a stressor, permanently altering the systems and shifting them into new ecological frameworks.

Introduction

Understanding the relationship between the morphology of an organism and its environment is one of the main challenges in ecology (Gaston & Lauer, 2015; Silva *et al.*, 2016). Environmental variation, with its complex and numerous biotic and abiotic factors, has a significant influence on the body shape of individual fish, and thus populations as well. Morphological changes induced by environmental factors can help give a better understanding of the phenotypic plasticity process as result of induced factors (Mohaddasi *et al.*, 2013; Jalili *et al.*, 2015). Therefore, the body shape of fish can be expected to be of particular evolutionary and ecological relevance (Klingenberg et al., 2003).

During the last few decades, the effects of anthropogenic activity on the natural environment have been numerous. Among them, river damming is one of the most influential anthropogenic modifications to riverine ecosystems (Santos & Araújo, 2015). It affects many key aspects of the ecosystem such as hydrologic connectivity, which is crucially important to fish and other aquatic organisms (Araújo *et al.*, 2013). Impounded streams rapidly change their characteristics from being relatively shallow flowing habitats to being deeper stagnant watercourses, which native stream fishes have likely not experienced during their evolution (Baxter, 1977). Such variations in physical conditions affect the organization, structure and processes of biotic communities (Franchi *et al.*, 2014). Furthermore, construction of dams across streams and rivers affects fish movements, which may lead to the restriction of gene flow and subsequently to differentiation between populations (AnvariFar *et al.*, 2011). Morphometric variation between populations can cause long-term isolation and interbreeding, and this can provide a basis for population differentiation (Bookstein, 1991).

Every dam has unique characteristics and consequently the nature of environmental changes is highly site-specific (McCartney, 2009). In the current literature, there are a number of studies dealing with changes in the body shape of fish in rivers and lakes, or in highly lotic and lentic habitats (Gaston & Lauer, 2015; Franssen, 2011; Haas *et al.*, 2010; Santos & Araújo,

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2015), however, there is still a lack of information regarding morphological responses of fish to barriers on small rivers.

We focused our study on the large spotted barbell, *Barbus balcanicus* (Kotlik, Tsigenopoulos, Ráb & Berrebi, 2002), which belongs to the group of small-sized rheophilic species of western Palearctic barbs (Kottelat & Freyhof, 2007). This species was formally described in 2002, before which it was classified as *Barbus petenyi* (Heckel, 1852); it is widely distributed throughout the mountain regions in the Danube River basin and several adjacent drainages (Tsigenopoulos *et al.*, 2002). Similar to other barbell species, *B. balcanicus* is a demersal species that lives in fast and moderately fast streams with a gravel bottom, usually retaining in riffles and waterfalls (Žutinić *et al*., 2014).

Therefore, the purpose of this study was to use a set of morphometric characters to examine whether small river damming has an impact on the body shape of the large spotted barbell. Also, we compare and visualize the potential overall size and shape variation present in this species as well as sexual dimorphism in the body size and shape.

Materials and Methods

Study Area

This research was restricted to rivers with a similar hydrological regime in order to reduce the confounding effects of natural environmental variability. Investigations were carried out on three small rivers in the central part of Serbia: the Grošnica River, Borač River and Petrovačka River (Figure 1). On the Grošnica River, which is 17 km long, a large dam was built in 1937, creating a reservoir from which the nearby town of Kragujevac is supplied with fresh water. To slow down the effects of erosion, in 1965 a number of smaller dams along the river flow were built (Baračkov, 1973), so now the river is divided into a large number of fragmented parts with different sizes. These small dams, like waterfalls, do not typically create a large upstream reservoir and often create a plunge pool immediately below the waterfall caused by scouring of the streambed. The dams were built to retain the sediment, and consequently, due to their height the movement of fish upstream was prevented. The Grošnica River flows into the Lepenica River, as does the Petrovačka River and both belong to the Morava River Basin. The Petrovačka River is 35 km long, with no barriers and dams. Fish populations are free to move along the entire watercourse, so this river was used as a reference. The Borač River, which belongs to the basin of the West Morava is the right tributary of the Gruža reservoir. The Borač River is 8.7 km long and dammed (Čomić & Ostojić, 2005) in its middle. This barrier divides the river into two sections, and prevents individuals from migrating from downstream to upstream. Individuals were sampled from two localities in the Borač River (above and below the dam) and the Petrovačka River, while there were sampling sites on the Grošnica River (in the upper section of the river and the sections above and below the lake).

Data Collection

From each locality, approximately 30 specimens of *B. balcanicus* were collected, and all species present

Figure 1. Map of the sampling sites of *Barbus balcanicus* in central Serbia (P1 - Petrovačka River, upper section; P2 - Petrovačka River, lower section; G1 - Grošnica, upper section; G2 - Grošnica, below the lake; G3 - Grošnica, above the lake; B1- Borač, below the dam; B2 - above the dam).

were recorded. Specimens were collected during low-flow conditions in July and August 2014 using the electrofishing equipment Aquatech DC electrofisher IG 1300 (2.6 kW, 80-470 V). The size of the fish collected from the different localities varied from 65 to 176 mm Total Length (L_T). The fish were euthanized with an overdose of MS-222, preserved in 95% ethanol, returned to the laboratory and stored. The sex of specimens was determined by macroscopic observation of the gonads. The right side of each individual was captured using a NIKON 3100 bridge camera mounted on a tripod, in a fixed position.

In order to understand the role of various environmental components on the body shape besides the damming effect, we tested the effects of four hydrological parameters, six water quality parameters and the geographical distance (Table 1) on the morphological variability. Selected parameters of water quality were measured twice a year (spring and summer), during 2013 and 2014, and for further analysis we took the mean value of all measurements. At each sampling site, a HANNA HI 98130 multitester was used to measure water temperature, conductivity, pH and hardness. The water velocity was determined with an Aquatech GMH 3330 flow meter. The dissolved oxygen and oxygen saturation were measured with a Mettler Toledo, Seven Go pro. The depth and width of the riverbed were measured using a tape measure and meter ruler, and the mean value of three different points on the streams in spring and summer over a two-year period was used. The water quality parameters were then compared using ANOVA followed by Tukey's HSD to test whether there were significant differences between the localities.

Geometric Morphometrics and Statistical Analysis

On each fish specimen image a total of 14 landmarks, corresponding mostly to hard structures (fin origin and terminus), were digitized (Figure 2) using

Table 1. Hydrological parameters, water quality parameters and geographical coordinates of the localities investigated.

Parameters	Units	Grošnica upper section	Grošnica above the lake	Grošnica below the lake	Borač above the dam	Borač below the dam	Petrovačka upper section	Petrovačka lower section
Hydrological parameters (HB)								
Width of the riverbed	m	2.3	2.7	3.2	2.1	2.2	2.3	3.1
Depth of the riverbed	m	0.5	0.7	0.7	0.4	0.7	0.7	0.8
Velocity	m/s	0.9	0.8	0.7	0.9	0.8	0.8	0.7
Water quality parameters (WQP)								
t	°C	17.15±2.65	19.07±2.64	17.25±1.23	14.12±0.29	15.27±0.49	17.75±2.08	17.9±1.98
pH	-	8.38±0.07	8.79±0.13	8.65±0.17	8.54±0.63	8.45±0.52	8.69±0.41	8.82±0.23
Conductivity	µS/cm^3	399±16.14	348±11.87	714.5±78.87	137.6±26.21	143±23.73	746±44.88	974±14.42
Water hardness	Mg/l	201±15.55	172.33±8.73	341.25±50.64	69±5.44	67±4.96	357.33±31.03	407±58.83
Dissolved oxygen	Mg/l	9.44±0.77	7.87±0.21	6.705±1.09	8.46±0.19	8.93±0.28	8.977±0.24	7.53±0.49
Oxygen saturation	%	104.1±15.39	93.56±2.86	82.57±15.17	96.76±2.26	95.5±0.86	96.46±6.05	80.5±8.63
Geographical coordinates (GC)								
N	-	43.897051	43.917350	43.959483	43.960894	43.961393	44.088957	44.052649
E		20.913093	20.897309	20.875088	20.583605	20.592229	20.802140	20.881309

*Values of water quality parameters are presented as mean±SD

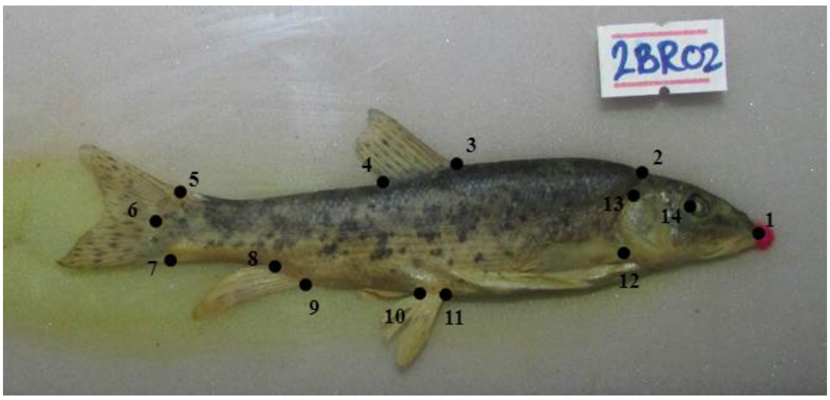

Figure 2. Landmarks used for the morphometric analysis of *Barbus balcanicus*: 1. Anterion snout tip; 2. Boundary between head and trunk; 3. 4. Boundaries of the dorsal fin; 5. 7. Boundaries of the caudal fin; 6. Base of the caudal fin at the lateral line; 8. 9. Boundaries of the anal fin; 10. 11. Boundaries of the abdominal fin; 12. Base of the pectoral fin; 13. Top point of the operculum; 14. The last edge of the eye.

TpsDig2 software (Rohfl, 2005). As a measure of size, the centroid size (CS) was computed, presenting the square root of the summed squared distances between all landmarks and their centroids (Mitteroecker *et al*., 2013). General Procrustes analysis (GPA) was applied in order to superimpose raw landmark coordinates, derive a consensus shape and remove variation unrelated to shape including scaling, positioning and orientation (Rohfl & Slice, 1990).

Differences in size between the sexes and localities were tested by two-factor analysis of variance (ANOVA). Multivariate regression was used to test the amount of shape variation explained by size, sex and locality. For this aim, three separate multivariate regressions were conducted in which the Procrustes (shape) coordinates were set as dependent variables, and the centroid size (log CS), dummy sex (coded as 1 and 2) or dummy locality (coded as 1 to 7) variables were set as independent variables. The statistical significance of these tests was assessed using a permutation test with 10000 iterations against the null hypothesis of independence of these variables (Good, 1994). Additionally, the effects of sex and locality on the fish body shape were tested using two-factor ANOVA. Since in some localities an uneven number of either males or females was ascertained (in the Grošnica River, below the lake, only 5/28 males, and in the upper section of the Grošnica River, only 5/20 females), there is a possibility that the sex-related variation influenced the locality-related variation. In order to test this, the residuals from the multivariate regression of shape variables on the dummy locality variable were used, whereby the residuals represented the locality-adjusted shape variation. Thereafter, a one-way ANOVA and multivariate regression of locality-adjusted shape coordinates were performed on the dummy sex variable.

To analyze the shape variation between different localities, canonical variate analysis (CVA) was used (Klingenberg, 2011). Variations along the first and second CV were visualized using outline illustrations. Differences were observed in the mean shapes of locality pairs by analyzing the Mahalanobis distances, and the statistical significance for all pairwise comparisons was estimated using a permutation test against the null hypothesis of no mean difference between groups. Additionally, in order to assess similarity among the different localities analyzed, the pairwise Mahalanobis distances were plotted using the unweighted pair-group method and arithmetic averages (UPGMA).

Additionally, in order to test whether morphological distances are caused by habitat-specific parameters rather than damming, we assessed the association between the morphological distances and distances obtained for key hydrological (width and depth of the riverbed and flow speed) and water quality parameters (pH, conductivity, water hardness, O_2 concentration and O_2 saturation) (Table 1) using the Mantel test (Mantel, 1967). In addition, the association between morphological distances and geographical distances between the localities was tested. Both the Procrustes and Mahalanobis distances were used as morphological distances, while the distances between the localities in key hydrological and water quality parameters and geographical distances were generated in R software (https://www.r-project.org/). The Mantel test was conducted for each parameter separately as well as for the combined hydrological parameters and water quality parameters.

ANOVA and the construction of UPGMA phenograms was conducted in STATISTICA v12 software (Statsoft Inc., Tulsa, OK, USA), and the Mantel test was conducted in R software while all other analysis were carried out in MorphoJ v1.06a software (Klingenberg, 2011).

Results

Overall Size and Shape Variation

Sex had a statistically significant effect on fish size (CS) ($F_{(1, 174)} = 52.8$, $p < 0.001$), while the locality effect and interaction were non-significant ($F_{(6, 174)} = 1.2$, $p = 0.32$ and $F_{(6, 174)} = 1.6$, $p = 0.15$, respectively). Size predicted 1.22% of the shape variation, but the regression did not significant follow the permutation test ($p = 0.07$), indicating that allometry had no effect on shape. Therefore, subsequent analysis on the overall shape variation was performed.

Body shape was significantly affected by both sex and locality ($F_{(24, 151)} = 5$, $p < 0.001$ and $F_{(144, 890)} = 6$, $p < 0.001$, respectively). Furthermore, a significant interaction between sex and localities ($F_{(144, 890)} = 1$, $p < 0.01$) was also observed. Localities explained the largest amount of shape variation among the three factors with 7.50% of the total variance ($p < 0.001$).

Sexual Size and Shape Dimorphism

As indicated above, body size was significantly affected by the sex of the fish since the females were generally larger than the males. The overall shape variation was also significantly influenced by the sex (two-factor ANOVA) since it predicted 2.16% of the total shape variation (multivariate regression; $p = 0.01$). Following locality adjustment, sex predicted 1.89% of the total shape variation ($p = 0.017$) and it had a significant effect on the locality-adjusted shape (one-way ANOVA; $F_{(24, 163)} = 6$, $p < 0.001$). The main differences in shape variation between the sexes were observed in the head region whereby females had a more pointy tip of the snout and the dorsal fin was positioned further back while the caudal and pectoral fins were positioned slightly further forward compared to the consensus shape (Figure 3). Based on these results, all subsequent analyses were conducted separately for each sex.

Shape Variation Between the Localities

In both females and males, the body shape was significantly affected by the locality ($F_{(144,\ 399)} = 5$, $p < 0.01$ and $F_{(144,\ 364)} = 4$, $p < 0.01$, respectively). In the plot of the first two CVs, similar separation patterns can be observed in both sexes. In both graphs, three major groups can be observed: (1) the Borač River above and below the dam, (2) both localities on the Petrovačka River and the section of Grošnica River located below the dam, and (3) two localities from the Grošnica River above the dam. The shape outline graphs displayed shape variation in CV 1 and 2 for both sexes. The main differences occurred in the head region, whereby the tip of the snout was positioned lower or higher, and in the dorsal region, whereby the dorsal fin was positioned lower or higher (Figure 4 and Figure 5).

Based on body shape, UPGMA analysis displayed three clusters in females: (1) the Borač River above and below the dam and Grošnica River located below the lake; (2) the upper section of the Grošnica River and above the lake; and (3) the upper and lower sections of the Petrovačka River. Three clusters were also displayed in males: (1) the Grošnica River below the lake, the upper section of the Petrovačka River and the Borač River above and below the dam; (2) the upper section of the Grošnica River and the lower section of the Petrovačka River; and (3) the Grošnica River above the lake (Figure 6).

The fish communities in these localities did not vary greatly in terms of species diversity, since all of the species belong to the family Cyprinidae. In both localities of the Petrovačka River, *Rhodeus sericeus* (Pallas, 1776) and *Gobio gobio* (Linnaeus, 1758) were present. *G*. *gobio* was also caught in the Grošnica River below the lake, in the community with *B*. *balcanicus* and *Barbatula barbatula* (Linnaeus, 1758). In the upper section of the Grošnica River no other species except *B*. *balcanicus* was found, and in the Grošnica River above the lake, *B*. *barbatula* was also caught. At both localities in the Borač River *Phoxinus phoxinus* (Linnaeus, 1758) was present, and *B. barbatula* was also present above the dam.

According to the Mantel test, none of the

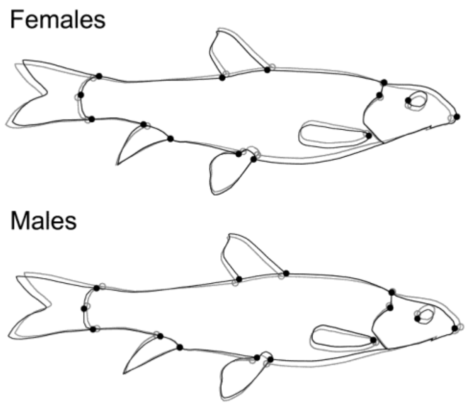

Figure 3. Outline diagram visualization of body-shape changes occurring between female and male *Barbus balcanicus* individuals (gray outlines present the consensus shape while the black lines depict variation from the consensus; enlarged by a factor of 5)

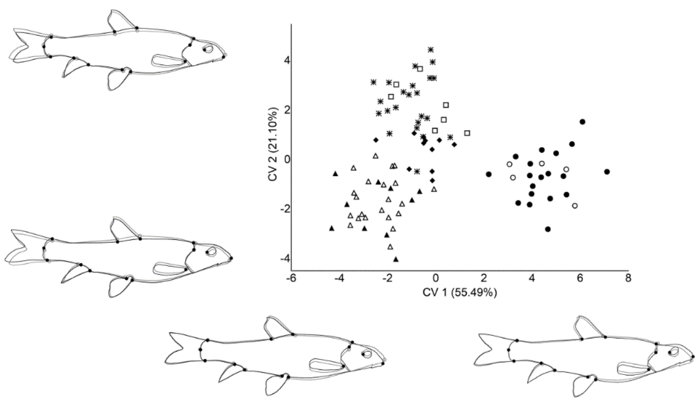

Figure 4. Scatter diagram of the first two canonical variates of the *Barbus balcanicus* female body-shape variation caused by damming. Localities: Borač below the dam (▲), Grošnica above the lake (●), Petrovačka, upper section (□), Borač below the dam (Δ), Grošnica below the lake (*), Petrovačka, lower section (♦), Grošnica, upper section (○). Shape variation was visualized by outline diagrams (gray outlines present consensus shape while the black lines depict variation from the consensus along the canonical variates; enlarged by a factor of 5)

hydrological or water quality parameters tested had a significant effect on the morphological variability in either males or females (Table 2). Furthermore, Tukey's HSD displayed no significant differences in water quality between the localities ($p > 0.05$). Additionally, geographical distance between the localities had no significant effect on the Mantel test either.

Discussion

In the present study, landmark-based geometric morphometrics was applied to compare and visualize the overall size and shape variation and sexual dimorphism in the body size and shape. Also, we examined shape variation in the body of the fish between localities affected by the fragmentation of the watercourse and similar localities without barriers. It has been suggested in previous studies that freshwater fish commonly exhibit sexual dimorphism for both body size and shape (Caldecutt *et al*., 2001; McGuigan *et al*., 2003; Hendry *et al*., 2006). This study pointed out that sexual dimorphism in size was present in populations of *B. balcanicus* since the females were larger than the males. This result is in accordance with the study by Žutinić *et al*. (2014), which has so far been the only study to provide data on the existence of dimorphism in terms of body size in *B. balcanicus*. The same results were observed by Vasiliou & Economidis (2005) in similar species: *B. peloponesius* (Valenciennes, 1842) and *B. cyclolepis* (Heckel, 1837). The larger body and greater abdominal region are likely a result of selection for increased fecundity in females (Jacquemin & Pyron, 2014). Sexual dimorphism may also arise due to natural selection operating differentially on the sexes (Lostrom *et al*., 2015).

Furthermore, evidence for sexual dimorphism in shape was also found. The biggest differences were observed in the snout and fin positions. These sexual shape differences can possibly have implications regarding the differential ecology of these fish, primarily in feeding. According to Lenhardt *et al*. (1996) and Piria *et al*. (2005), *B. balcanicus* feeds mainly on bottom fauna and the main food for this species is the larvae of Chironomidae and Trichoptera. Sapounidisa *et al*. (2015) investigated the diet of *B. strumicae* Karaman, 1955, a

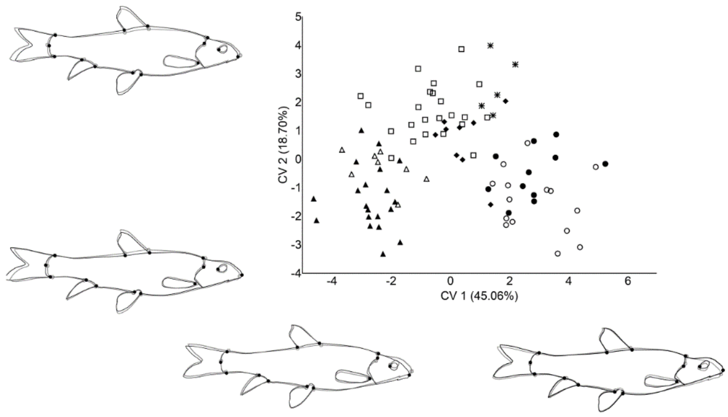

Figure 5. Scatter diagram of the first two canonical variates of the *Barbus balcanicus* male body-shape variation caused by damming. Localities: Borač below the dam (▲), Grošnica above the lake (●), Petrovačka, upper section (□), Borač below the dam (Δ), Grošnica below the lake (*), Petrovačka, lower section (♦), Grošnica, upper section (○). Shape variation was visualized by outline diagrams (gray outlines present consensus shape while the black lines depict variation from the consensus along the canonical variates; enlarged by a factor of 5).

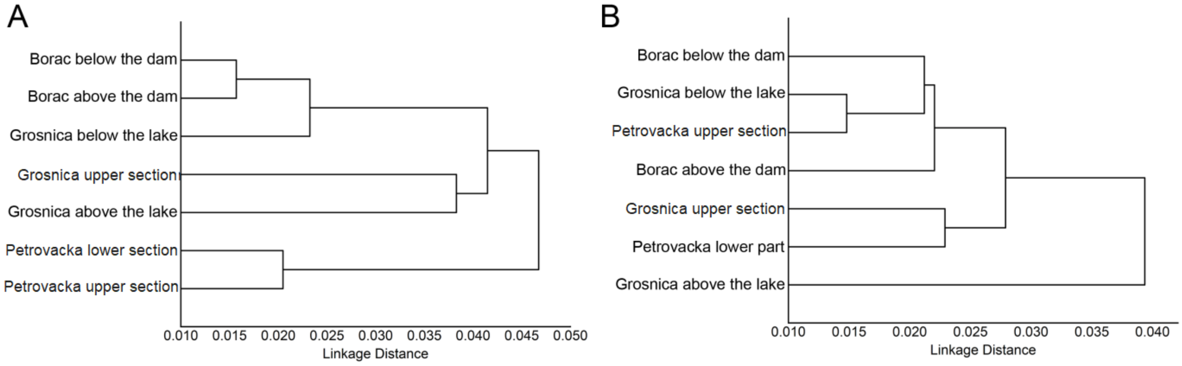

Figure 6. The UPGMA graph for seven populations of *Barbus balcanicus* for A) females and B) males.

Table 2. Results of the Mantel test conducted for each parameter separately as well as for the combined hydrological parameters and water quality parameters, and for geographical distance

Trait	Females		Males	
	Mahalanobis distance	Procrustes distance	Mahalanobis distance	Procrustes distance
Hydrological parameters (HP)				
Width of the riverbed	0.916	0.923	0.151	0.563
Depth of the riverbed	0.542	0.776	0.697	0.554
Flow speed	0.820	0.921	0.379	0.815
HPs combined	0.933	0.978	0.151	0.632
Water quality parameters (WQP)				
pH	0.389	0.661	0.509	0.365
Conductivity	0.330	0.180	0.515	0.589
Water hardness	0.240	0.124	0.235	0.560
O2 concentration	0.741	0.906	0.141	0.774
O2 saturation	0.678	0.827	0.408	0.721
WQPs combined	0.325	0.170	0.487	0.586
Geographical distance				
	0.069	0.094	0.166	0.183

**p* values are indicated in Table

closely related species to *B. balcanicus*, and observed that it was highly specialized in its diet of chironomidae larvae, which appears to be its main food source throughout the year. Haas *et al*. (2010) suggested that shifts in the type of diet and availability of food may give rise to morphological differences related to feeding performance, such as the changes observed in the head region. Therefore, the preference of males and females towards different food type may have influenced the observed differences in mouth position in this study. Since there are very little data regarding the diet specifics of *B. balcanicus*, additional studies are needed to confirm these claims.

The causes of morphological differences among populations from different habitats with similar ecological conditions are often difficult to explain (Poulet *et al*., 2004). Previous studies have revealed that morphological characteristics can show high plasticity in response to differences in environmental conditions (Swain *et al*., 1991; Pompei *et al*., 2016). Strong selective pressures, related to fitness in some cases, may produce rapid genetic divergence in morphology between populations of fish before there has been time for the accumulation of neutral genetic differences between groups (Sequeira *et al*., 2011).

An environment with high predator pressure may result in morphological changes, such as a deeper posterior body and larger caudal peduncle in order to increase burst swimming (Brönmark & Pettersson, 1994; Langerhans, 2008; Santos & Araujo, 2015). The fish community in the localities investigated here is typical for small streams in this part of the Balkan Peninsula, and it is related to the main environmental variables (Giannetto *et al*., 2013). The presence of only four small sized species was recorded that feed mostly on invertebrates, and to which *B. balcanicus* is not prey. Based on these facts, we presumed that the presence of predators could not be a source of shape variability.

Stream habitat heterogeneity could potentially create spatial and temporal variation in selection pressures or facilitate variable plastic morphological responses in stream fish (Franssen, 2011). The specimens from the Borač and Grošnica upper flows showed lower body depth, a sharper snout and a more spindle-shaped body than specimens from the Petrovačka and Grošnica lower flows. This is specific to lotic environments since they tend to select a body shape that reduces drag because a fusiform shape reduces resistance in aquatic environments, allowing effective propulsion and maintenance of velocity at a lower energy cost (Webb, 1984; Langerhans & Reznick, 2009; Foster *et al*., 2015). A certain degree of differentiation between two localities from the Grošnica River, above the dam and below the lake, was also detected. This is probably due to the dam on the river flow making a major barrier to the passage of fish. The blockage of fish motions, especially in the upper flow, can have a very significant impact on fish stocks due to the obstruction of genetic exchange with fish from the lower segments. Recent studies have supported the statement that river stretches above dams are poor migration corridors, thus reducing gene flow and leading to phenotypic differences (Franssen, 2011; Hudman & Gido, 2013).

As expected, the fewest morphological divergences were observed between the two localities on the Petrovačka River and Grošnica River below the dam. This was probably caused by the highest hydrological connectivity between these three habitats and undisturbed contact between individuals.

The differences in the morphometry of *B. balcanicus* between the Borač River and Petrovačka River could be due to their belonging to different river basins and their lack of connectivity. Differences in prey type and abundance between different habitats may have given rise to morphological character diversification, such as mouth position and head size (Foster *et al*., 2015), which is noticeable among groups of both sexes.

Considering that environmental conditions between the localities were not greatly different (Tukey's HSD, $p > 0.05$), the observed morphological divergence may be partly attributed to stream damming, which acts as a stressor and permanently alters the systems, shifting them into a new ecological medium.

Physical factors within habitats, such as water velocity and dissolved oxygen, can cause changes in the phenotype (Crispo & Chapman, 2010; Cureton & Broughton, 2014). Our studies based on the Mantel test confirmed that the role of hydrological and water quality parameters does not influence morphological variation. In addition, the Mantel test showed no significant correlation between geographical distances and morphological divergence, which can be explained by the fact that the ecosystems investigated are not located very close to each other. This suggests that the body shape is influenced by other factors, probably as a consequence of impoundments.

Body shape variation is completely related to many aspects of fish ecology, including locomotion, space resource limitations and foraging tactics, and it can indicate the functional diversity of fish assemblages (Ingam, 2015). To determine the contribution of genetics in differences in morphological findings, further analyses based on DNA techniques such as microsatellite markers are necessary.

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