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PHENOTYPIC PLASTICITY OF *RUTILUS PRESPENSIS* (KARAMAN, S., 1924) FROM LAKE PRESPA AND LAKE SKADAR

SUMMARY

The phenotypic plasticity of *Rutilus prespensis* from Lake Prespa and Lake Skadar was evaluated based on 20 morphometric measurements. Analysis of variance and covariance showed that seven morphometric characters were different between populations: maximum body height, head length, horizontal eye diameter, interorbital distance, predorsal distance, dorsal fin height and anal fin length. These seven characters did not show any overlap in size between examined populations, which indicated significant differences between populations in these characters. The present study examines the allometric growth pattern of a number of morphometric characters of *R. prespensis*. The aim of this study was to examine variability in external morphology of R. prespensis populations from Lake Prespa and Lake Skadar.

Keywords: Balkan, ancient lakes, Allometry, Inter-population variability

INTRODUCTION

Ancient lakes Prespa and Skadar are well recognized as European biodiversity hotspots (Gaston & David, 1994; Griffiths et al, 2004) and are classified into the key biodiversity areas in Mediterranean (Darwall et al, 2014). Despite the general attention that has been given to these biologically rich lacustrine systems, it is apparent that a great deal of general knowledge concerning the most basic units of biodiversity, even among fish species is still lacking. For those reasons, it is invaluable to research every biological aspect of both endemic species and those with a wider range of distribution. Taxonomic status of the species of the genus Rutilus inhabiting extant ancient lakes of Balkans was unclear for many years. Milošević et al. (2011) showed that the Lake Skadar basin is inhabited by two species from genus Rutilus: *R. prespensis* (Karaman, S., 1924) (local name yellow roach) and *R. albus* Marić, 2010 (white roach), while Lake Prespa is inhabited by *R. prespensis* (Prespa roach). Recent studies of these species have been related to taxonomic status and length-weight relationships (Marić & Radujković, 2009; Milošević et al, 2011; Milošević et al, 2012; Milošević & Talevski, 2016). Until now, there was no research dealing

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with the adaptation of the *R. prespensis* in this two, in many ways, different environments.

Differences in morphology among species and between populations of the same species can often be interpreted as adaptation to different ecological conditions. Morphometry is one of the most easily accessible means of assessing a species environmental plasticity (Kováč et al, 2009). Individuals of a species in different environments may diverge phenotypically in order to function efficiently in a given environment (McPhail 1984, Schluter & McPhail, 1992; Galis et al, 1994). In forming a phenotype, two interacting factors act on the developmental program – the genome and the environment (Scheiner, 1993). Phenotypic plasticity allows the same genotype to produce a variety of phenotypes in response to different local condition (Komers, 1997). As geographic variation in morphometry has been used to discriminate local forms of fishes (Cadrin, 2000) the aim of this study was to examine variability in external morphology of *R. prespensis* populations from Lake Prespa and Lake Skadar.

MATERIAL AND METHODS

Study area

The Lake Skadar drainage basin is located between 18º41' and 19º47' East and between 42º58' and 40º10' North in a karstic area in the outer part of the southeastern Dinaric Alps. It is the largest of the Balkan lakes and has a surface area which fluctuates seasonally from approximately 370 to 600 km2. The water level also varies seasonally from 4.7 to 9.8 m above sea level. The lake extends in the NW-SE direction, and it is approximately 44 km long. The Bojana River connects the lake with the Adriatic Sea, and the Drim River provides a link with Lake Ohrid. The exact origin of the lake is unknown but it probably originated by solution and tectonic processes during the Pleistocene (Stanković, 1960). The Southern and southwestern sides of the lake are rocky, barren and steep, having bays in which the sublacustrine springs are usually found (so called “oka” – “eye”).

Lake Prespa is the second largest lake in Macedonia after Lake Ohrid with a depth of 54 meters, and 87.5 km shoreline, maximum length of about 28 km and a maximum width of l7 km (Stilinović, 1987). Today the maximum depth is estimated at 48 m (Stavrić & Popovska, 2008). Lake Prespa consists of two lakes: Great Prespa (273km2, 853 m above sea level) and Small Prespa (41 km2, 857 meters above sea level). These two lakes are located between two mountains Galičica and Baba. Lake Prespa has no surface outflow and it is connected with Lake Ohrid by underground hydraulic connections and karst channels (Anovski et al, l991; Eftimi & Zoto, 1996; Eftimi et al, 2002; Matzinger et al, 2006).

Sample collection

Samples were collected in Lake Prespa and Lake Skadar with multi mesh size gill nets (EU standard EN 14757). The specimens were sampled from several different, primary littoral sites. Fish were removed from the catch and immediately deep-frozen for later analysis. A total of 20 morphometric characters were analyzed: TL - Total length, SL - Standard length, H - Maximum body height, h - Minimum body height, hc – Head height, lc - Head length, Oh - Horizontal eye diameter, iO - Interorbital distance, prO - Preorbital distance, poO - Postorbital distance, Lpc- Length of caudal peduncle, pV – Preventral distance, pD - Predorsal distance, poD - Postdorsal distance, lD - dorsal fin length, hD - dorsal fin height, lA - Anal fin length, hA - Anal fin height, lP - Pectoral fin length, lV - Ventral fin length. Data were only collected from the left side of the fish. Morphometric measurements were taken to the nearest 0.02 mm using calipers according to and in accordance with Holčik (1989). Measurements were performed on 70 fish (35 of which were from Lake Skadar and 35 from Lake Prespa).

Statistical analyses

The resulting data were statistically analyzed using Statistica 7.0. and Minitab 16.0. All measurements were plotted against TL, in order to eliminate variability that could occur as a result of allometric growth. ANOVA was used to examine interpopulation variability, and statistical significance was determined by Tukey HSD Post-hoc test. ANCOVA was used to examine differences in body shape between populations. The allometric growth relative to TL was calculated from the function $Y = aTL^b$, where *a* and *b* are constants and Y is the morphometric variable (Minos et al, 1995). The 95% confidence intervals (Cls) of the parameters and the statistical significance of the regression relationship (r^2) were estimated. The morphometric variables were then divided into three categories: positive allometry (+ A), when the slope (*b*, allometry coefficient) was higher than 1 and the variable increased relatively to TL; negative allometry (- A), when the slope was lower than 1, indicating direct proportionality between the variable and TL. The significance of the slope was tested by means of a t-test (Zar, 1999).

RESULTS

Data obtained from the measurements of morphometric characters of R. prespensis from Lake Prespa and Lake Skadar are provided in Table 1. Analysis of variance (ANOVA) revealed differences between populations for seven morphometric characters (Table 2). Characters H, lc, Oh, iO, pD and hD showed extremely high significant differences ($P<0,001$) while lA showed high significant differences ($P<0,01$) (Table 2). Characters H, lc, oH, iO, pD, hD, lA showed no value overlapping, which indicated significant differences between populations in these characters (Figure 1-7).

The parameters of the equation of each morphometric variable versus total length (TL) of *R prespensis* from Lake Prespa and Lake Skadar are presented in Table 3. In roach from Lake Skadar, two dimensions (pO and lD) revealed a positive allometric relationship, and ten (h, hc, Oh,iO, lpc, hD, lA, hA, lP and lV) had a negative allometric relationship. Six dimensions (H, lc, prO, pD, poD and PV) had an isometric relationship with TL. In roach from Lake Prespa all characters, except lA that showed positive allometric relationship, revealed negative allometric relationship.

Table 1: Summary of variation found in morphological characters in roach, *R. prespensis* from Lake Prespa and Lake Skadar. Shown are the: M - arithmetic means, SD – standard deviation Min - minimum and Max - maximum values:

Morphometric character	Lake Skadar				
	N	**Mean**	**Min**	**Max**	**SD**
TL	35	141.2	130.5	154.1	5.9
SL	35	119.8	110.5	129.7	5.8
H	35	32.4	29.9	37.8	1.8
h	35	12.2	10.7	13.7	0.7
hc	35	22.3	20.2	24.5	1.3
lc	35	28.3	26.0	32.0	1.5
Oh	35	7.2	6.3	7.9	0.6
iO	35	10.5	9.4	11.9	0.6
prO	35	8.1	6.5	10.0	0.8
poO	35	13.1	9.4	14.9	1.1
Lpc	35	22.3	20.1	25.1	1.3
pD	35	59.9	55.2	65.6	2.9
poD	35	43.2	38.6	49.1	2.5
lD	35	17.0	14.4	19.9	1.2
hD	35	21.7	20.0	23.7	0.9
lA	35	11.2	9.1	12.8	0.9
hA	35	16.7	14.5	19.0	1.1
lP	35	19.3	11.3	22.3	3.6
pV	35	57.5	51.3	63.6	3.2
lV	35	19.0	17.0	21.3	1.0
Morphometric character	**Lake Prespa**				
	N	**Mean**	**Min**	**Max**	**SD**
TL	35	169.9	157.9	195.3	10.6
SL	35	140.8	130.5	161.8	7.3
H	35	42.9	38.1	48.1	7.3
h	35	14.7	13.2	16.8	1.2
hc	35	26.2	22.2	29.6	1.7
lc	35	32.2	29.0	36.0	1.8
Oh	35	7.5	6.3	8.8	0.6
iO	35	11.5	10.0	13.9	0.8
prO	35	9.2	7.2	10.9	1.1
poO	35	16.2	14.2	19.9	1.6
Lpc	35	26.4	23.5	30.8	1.9
pD	35	67.2	60.8	75.0	3.7
poD	35	52.5	46.8	60.0	3.4
lD	35	19.4	16.7	23.1	1.7
hD	35	29.8	24.0	39.8	3.5
lA	35	12.2	9.3	15.6	1.4
hA	35	20.3	17.6	22.7	1.3
lP	35	24.1	22.1	26.8	1.5
pV	35	68.0	60.6	73.8	4.1
lV	35	23.3	21.4	26.6	1.4

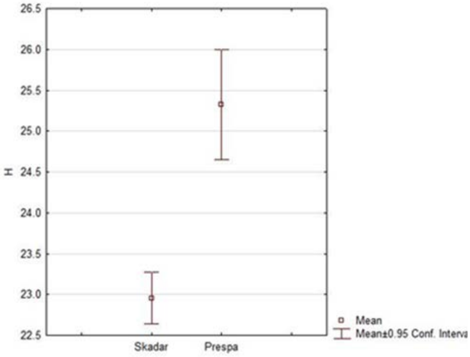

Figure 1. Box & Whisker diagram of morphometric character H of *R.prespensis* in relation to examines lakes

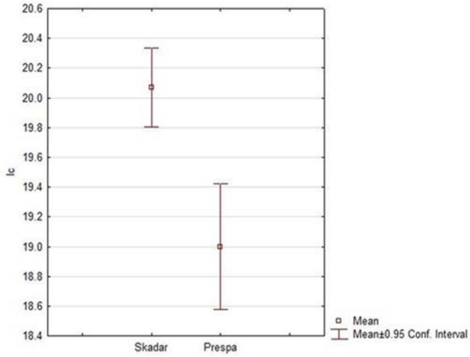

Figure 2. Box & Whisker diagram of morphometric character lc of *R.prespensis* in relation to examines lakes

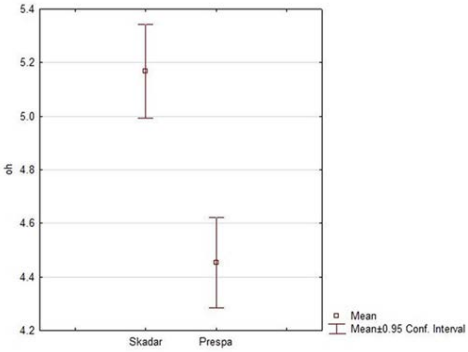

Figure 3. Box & Whisker diagram of morphometric character oh of *R. prespensis* in relation to examines lakes

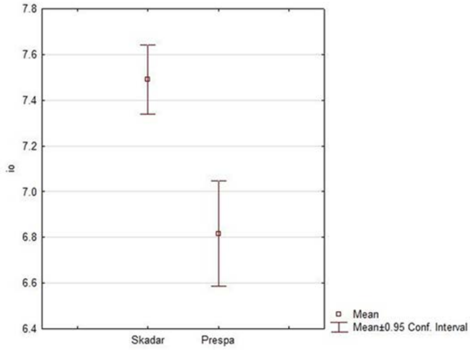

Figure 4. Box & Whisker diagram of morphometric character io of *R. prespensis* in relation to examines lakes

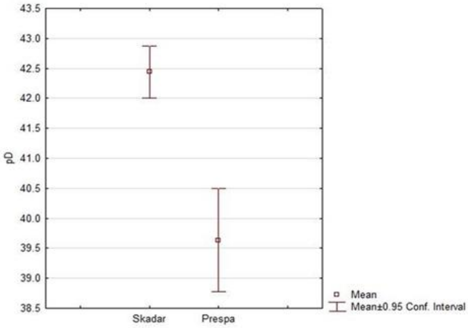

Figure 5. Box & Whisker diagram of morphometric character pD of *R.prespensis* in relation to examines lakes

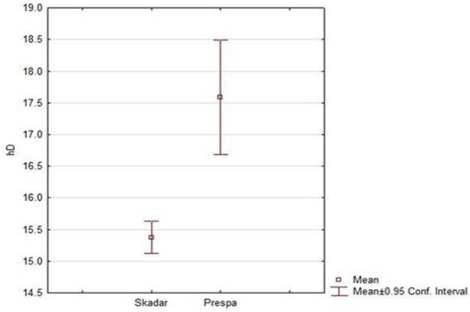

Figure 6. Box & Whisker diagram of morphometric character hD of *R.prespensis* in relation to examines lakes

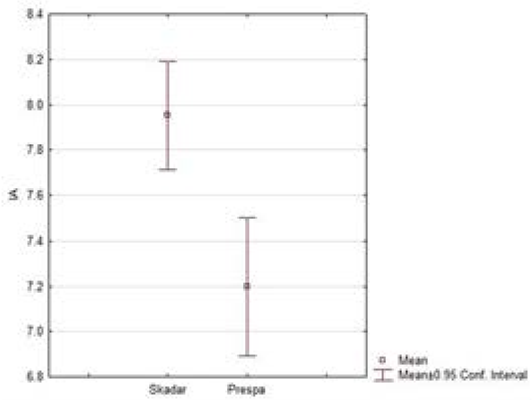

Figure 7. Box & Whisker diagram of morphometric character lA of *R.prespensis* in relation to examines lakes

Table 2. Analysis of variance and covariance (covariable is standard length SL) of morphometric characters of *R.prespensis* from Lake Prespa and Lake Skadar

Morphometric character	**ANOVA Prespa/Skadar**		**ANCOVA Prespa/Skadar**	
	F	*P*	F	*P*
SL	10.18	0.002**		
H	53.55	0.0001***	88.27	0.000***
h	0.25	0.618	3.66	0.062
hc	2.56	0.116	0.04	0.848
lc	21.95	0.0001***	9.67	0.003**
Oh	33.46	0.0001***	22.78	0.000***
iO	27.91	0.0001***	18.63	0.000***
prO	3.22	0.079	0.75	0.392
poO	1.423	0.238	4.38	0.042
Lpc	1.05	0.311	0.33	0.566
pD	0.51	0.0001***	28.10	0.000***
poD	2.32	0.477	4.74	0.034*
lD	8.51	0.005**	3.95	0.053
hD	33.15	0.0001***	41.26	0.000***
lA	16.34	0.0002**	10.42	0.002**
hA	0.22	0.638	0.09	0.767
lP	0.83	0.366	1.33	0.255
pV	1.50	0.226	0.01	0.915
lV	2.14	0.150	3.83	0.056

Table 3. Morphometric variables versus Total length (TL) of *R. prespensis* from Lake Prespa and Lake Skadar

Dimension	Regression parameters (*R.prespensis* from Lake Skadar)					
	b	**95% Cl of *b***	***a***	**95% Cl of *a***	r^2	**Slope (*b*)**
H	1.066	0.743-1.389	0.166	-0.100-0.432	0.773	I
h	0.434	-0.069-0.937	1.420	-2.1178-4.9578	0.304	-A*
hc	0.854	0.418-1.289	0.326	-0.377-1.029	0.345	-A*
lc	1.022	0.708-1.335	0.180	-0.100-0.460	0.769	I
Oh	0.186	-0.557-0.928	2.896	-7.766-13.558	0.092	-A*
iO	0.568	0.117-1.018	0.634	-0.781-2.049	0.423	-A*
prO	1.058	0.266-1.849	0.043	-0.125-0.211	0.443	I
poO	1.249	0.586-1.911	0.027	-0.061-0.115	0.572	+A*
pD	0.973	0.728-1.218	0.489	-0.103-1.075	0.826	I
poD	0.957	0.578-1.335	0.379	-0.331-1.088	0.683	I
pV	1.073	0.788-1.363	0.283	-0.125-0.691	0.807	I
Lpc	0.419	-0.098-0.924	2.801	-4.282-9.884	0.291	-A*
lD	1.202	0.737-1.666	0.044	-0.057-0.145	0.692	+A*
hD	0.434	0.091-0.777	2.526	-1.770-6.822	0.424	-A*
lA	0.523	-0.177-1.222	0.840	-2.071-3.751	0.267	-A*
hA	0.181	-0.401-0.763	6.780	-12.727-26.287	0.115	-A*
lP	0.746	-0.569-2.679	1.325	-1.324-2.985	0.721	-A*
lV	0.591	0.152-1.030	1.018	-1.197-3.233	0.446	-A*

Dimension	**Regression parameters (Lake Prespa)**					
	b	**95% Cl of *b***	***a***	**95% Cl of *a***	r^2	**Slope (*b*)**
H	0.698	0.292-1.103	1.194	-1.287-3.675	0.623	-A*
h	0.436	-.0174-1.026	1.653	-3.437-6.743	0.312	-A*
hc	0.585	0.154-1.016	1.299	-1.579-4.174	0.531	-A*
lc	0.606	0.285-0.927	1.431	-0.928-3.791	0.657	-A*
Oh	0.579	-0.005-1.163	0.385	-0.771-1.541	0.416	-A*
iO	0.449	-0.048-0.946	1.149	-1.785-4.083	0.385	-A*
prO	0.306	-0.611-1.227	1.899	-7.060-10.858	0.152	-A*
poO	0.770	0.152-1.387	0.310	-0.67-1.29	0.500	-A*
pD	0.618	0.310-0.925	2.818	-1.644-7.281	0.679	-A*
poD	0.861	0.568-1.153	0.631	-0.316-1.577	0.806	-A*
pV	0.594	0.214-0.974	3.222	-3.069-9.514	0.585	-A*
Lpc	0.603	0.121-1.085	1.192	-1.765-4.149	0.499	-A*
lD	0.710	0.155-1.264	0.506	-0.932-1.944	0.510	-A*
hD	0.559	-0.274-1.392	1.678	-5.503-7.181	0.296	-A*
lA	1.168	0.468-1.867	0.030	-1.048-1.108	0.611	+A*
hA	0.762	0.393-1.130	0.404	-0.362-1.170	0.690	-A*
lP	0.649	-0.105-1.404	0.859	-0.742-2.454	0.637	-A*
lV	0.701	0.407-0.995	0.638	-0.326-1.602	0.740	-A*

Slope patterns are: +A, positive allometry; -A, negative allometry; I, isometry.
*indicates significant difference of *b* value from 3 (t-test; $P<0.05$)

DISCUSSION

Rutilus spp. from the extant ancient Balkan lakes (Prespa, Ohrid and Skadar) share a relatively recent (within the Pleistocene) common ancestry. This ancestral haplotype was found in Lake Prespa and in Lake Skadar individuals of *R. prespensis* (Milošević et al, 2011). Genetic variation for a fixed phenotype has been hypothesized in stable environments (Smith, 1993). Besides genetic variation for a canalized phenotype, phenotypic plasticity, an environmental-induced phenotypic change that occurs within an organism's lifetime (Stearns, 1989), is also likely to play an important role in the process of diversification (West-Eberhard, 1989). Moreover, phenotypic plasticity is regarded to be more beneficial in variable environments, where a single optimal phenotype may be favored instead of maintaining plasticity in traits (Schlichting & Pigliucci, 1998). Bearing in mind the fact that *R. prespensis* presumably represents the allochthonous species of the Lake Skadar, the subject of this paper was an analysis of morphological variability, in two significantly different lakes, in order to determine morphological adaptation in a new environment. Phenotypic plasticity allows the same genotype to produce a variety of phenotypes in response to different local condition (Komers, 1997). Indeed, body shape in fishes can be influenced by various factors such as temperature (Martin, 1949; Beacham, 1990; Šumer et al., 2005), food ratio (Currens et al, 1989) and type of food or feeding mode (Day et al, 1994; Robinson & Wilson, 1996).

In this study both ANOVA and ACOVA, showed differences in body morphology (form) of examined populations, indicated by differences in H, lc, oH, iO, pD, hD, and lA. These seven characters did not show any overlap in size between examined populations (Figure 1-7). Roach from Lake Prespa had a deeper body and deeper dorsal fin, while roach from Lake Skadar had larger head, bigger horizontal eye diameter, bigger interorbital distance, bigger predorsal distance and longer anal fin. The obtained results are in agreement with the results of Marić, 1989. Autor has highlighted significant differences between populations from Lake Prespa and Skadar and describes a new subspecies *Rutilus prespensis vukovici ssp.* new.

Roach from Lake Prespa had a deeper body than roach from Lake Skadar. This result explains the fact that *R. prespensis* is a long period of time assigned as an endemic species for Lake Prespa, because the phenotypically observed body shape can indicate differences from other taxa. The body form is also related to habitat use. A deeper body is thought to be better for maneuvering in structured habitats, whereas a streamlined body is thought to be adapted for minimizing drag while searching for food in open water (Webb, 1984). Study on perch (Svanbäck & Eklöv, 2002; 2006) indicated that perch caught in the littoral habitat, independent of size, had a deeper body, larger head, and mouth and longer fins than perch caught in the pelagic zone. Bearing in mind that the Lake Skadar is generally shallow and subject to large surface area fluctuations obtained results are in correlation with general littoral distribution of *R. prespensis* (Milošević et al, 2011).

The analysis of morphometric variables demonstrated a substantial degree of differences between examined populations of *R. prespensis* concerning the growth pattern. Variability in growth, development and maturation creates a variety of body shapes within a species (Cadrin, 2000) that, along with ecological interactions of organism are directly or indirectly influenced by environmental conditions (Norton et al, 1995). In *R. prespensis* from Lake Skadar, two characteristics (pO and lD) revealed a positive allometric relationship, and ten (h, hc,Oh, iO, lpc, hD, lA, hA, lP and lV) had a negative allometric relationship. Six dimensions (H, lc, prO, pD, poD and PV) had an isometric relationship with TL. In roach from Lake Prespa all characters, except lA that showed positive allometric relationship, revealed negative allometric relationship. However, comparative analysis of available literature data Simonović, 1995, showed that some characters have relatively constant variability, such as horizontal eye diameter, which often exhibits negative values of correlation coefficient in available literature, and it is ontogenetically determined. The obtained differences in body shape are in correlation with different growth models based on length-weight relationships. The relationship of body weight versus length showed negative allometric growth for *R. prespensis* from Lake Prespa (Milošević & Talevski, 2016). On the contrary, positive allometric growth has been reported for *R. prespensis* from Lake Skadar (Milošević et al, 2012). This variation for the same species is obviously attributed to differences in age and stage of growth increment, food, as well as environmental conditions (Weatherley & Gill 1987).

Differences in morphology among species and between populations of the same species can often be interpreted as adaptation to different ecological conditions. The specificity of the morphometric characters variable nature often creates difficulties in the precise determination of morphological adaptivity in a new environment. In this study, we showed substantial differences in body morphology (form) of examined populations which are obviously result of ecological conditions in the littoral zones of studied lakes such as water level, temperature, macrophyte vegetation (Talevska et al, 2009), as well as different lakes altitudes, lakes surfaces and lakes depths. Also, the present study examines the allometric growth pattern of a number of morphometric characters of *R. prespensis*. Bearing in mind the fact that *R. prespensis* presumably represents the allochthonous species, these findings represent a basis for further research in the area that will contribute in the determination of its distribution in Adriatic drainages and adaptations in different environments.

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