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Inter-population variability in morphology and reproduction of *Alburnus alburnus* (Linnaeus, 1758) from Bosnia and Herzegovina

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Abstract

Inter-population variability in morphological traits and fecundity of *Alburnus alburnus* (*Linnaeus*, 1758) from Bosnia and Herzegovina was analysed in this study. The results of the analyses based on 21 standard measurements and six meristic characters have shown the highest variation between distance between pelvic (ventral) fins and anal aperture. Analysis of variance (ANOVA) indicate statistically significant differences between six populations. Differences among meristic characters have been tested by Multiple Comparisons Kruskal-Wallas. Obtained results indicate statistically significant difference among samples for number of branched rays for caudal fin (KA/Ca), lateral line scales (Le) and a number of gill rackers (Rac). Discriminant function analysis indicate characters that had the strongest effect on the geographical variation between *A. alburnus* samples from Bosnia and Herzegovina. Characters with the strongest discriminant power are: preanal distance, length of pectoral fin, length of the ventral (pelvic) fin, minimum body height, caudal fins branched rays, and pelvic fins branched rays.Values for weight of the gonads and gonadosomatic index (GSI) have not shown any statistically significant difference among observed bleak populations. Regression analysis has been used to estimate the relations between GSI and standard length, body, and gonads weight. The gonadosomatic index has shown positive, non substational, relationship to gonads weight.

Introduction

The genus *Alburnus* Rafinesque, 1820 belongs to the family Cyprinidae (Romero, 2002). So far, 44 species from this genus have been described in the world, with 20 from Europe (Kottelat and Freyhof, 2007). In Bosnia and Herzegovina genus *Alburnus* is represented with four species: *Alburnus albidus* (Costa, 1838), *A. arborella* (Bonaparte, 1841), *A. scoranza* Bonaparte, 1845, and *A. alburnus* (Linnaeus, 1758) (Buj et al.,2010). *A. albidus* and *A. scoranza* live in the Adriatic basin while *A. alburnus* and *A. arborela* are distributed in the Sava river basin. Two newly described species *Alburnus neretvae* (Buj et al., 2010) and *A. save* (Bogutskaya et al., 2017) probably also live in the waters in

Bosnia and Herzegovina. New data on the distribution of *A. save* indicate its presence in the rivers Bosna and Drina (Bogutskaya et al., 2017).

Bleak is distinguished by its elongated, narrow and flattened body, pointed head and a relatively small mouth with a lower jaw oriented strongly upward, as well as a large number of rays in the anal fin (16-20). Body length is 15-20 cm, although some specimens could reach 25 cm (Billard, 1997). Its relatively small size is the main reason why it is of little interest to commercial or sport fisheries (Vinyoles et al., 2007; Kompowskiet al., 2010). Its spawning begins in May and lasts until the beginning of July. Bleak reach sexual maturity in the second or even the third year with a body length of 8 to 10 cm. Life expectancy is usually up to eight years (Sofradžija, 2009).

Alburnus alburnus is distributed almost in the whole Europe, including European part of Russia. It is not naturally present in the southern countries and it is considered as an allochthonous and invasive species in Spain, Portugal, and Italy (Kottelat & Freyhof, 2007; Vinyoles et al., 2007). In Bosnia and Herzegovina, bleak is common in the waters of the Black Sea basin, especially the middle parts of the rivers Drina and Bosna as well as in other tributaries of the Sava river (Sofradžija, 2009).

Existing data on distribution of this species in Bosnia and Herzegovina are rather inconsistent due to the use of homonyms and the numerous described subspecies and varieties (Vuković & Ivanović, 1971; Šorić, 1980; Buj et al., 2010). Recently described new species *Alburnus neretvae* Buj, Sanda and Perea, 2010 confirms thesis that the eastern Adriatic Basin is a hotspot for several fish genera from the family Cyprinidae (Kottelat & Freyhof, 2007; Buj et al., 2010).

Although species from the genus *Alburnus* are widespread, especially in the waters of the Adriatic Basin, their taxonomy, systematics, and actual distribution are still not sufficiently clarified (Buj et al., 2010).

Growth and reproduction are key aspects for assessing phenotypic variability in fish populations (Copp & Fox, 2007). Investigation of reproductive biology of fish species is of crucial importance for objective assessment of commercial potentials of the stock, cultural practice and actual management of its fishery. It is also, essential for understanding of life history of given species (Dopeikar et al., 2015; Mousavi-Sabet et al., 2017; Pilić et al., 2018).

Environmental factors also have significant impact on gonad maturation during the reproductive cycle in fish (Joy et al.,1992; Rinchard & Kestemont, 1996; Pilić et al., 2017).

Studies of variations of Bleak population traits are scarce in Europe, in general, particularly in the Mediterranean region (Latorre et al., 2018). There are several studies about the growth, morphometric and meristic traits, reproduction and parasite fauna of the Bleak in Turkish waters (Ozuluğ et al., 2005; Koyunand Karadavut, 2010). Munoz-Mas et al. (2016) have presented data about habitat preferences of this species. Several population traits restricted to local scales are investigated by Almeida et al. (2014) and Maso´ et al. (2016).

Data regarding the biology of *Alburnus* populations from Bosnia and Herzegovina, even for *Alburnus alburnus*, are very scarce. Inter-population variability of bleak populations from Bosnia and Herzegovina has not been analysed yet.

The aim of this study is to assess the inter-population variability of morphological traits and fecundity of *Alburnus alburnus* from different rivers/sites from Bosnia and Herzegovina. For this purpose, a detailed analyses of 21 morphometric, six meristic traits and gonadosomatic index (GSI) have been carried out. The population morphological differentiation and classical morphological analyses have been performed in order to determine the level of intraspecific variation of *Alburnus alburnus.* Obtained results have been compared with newly described *Alburnus* species, *Alburnus neretvae* and *A. save.* Interpopulation variability is more relevant when more parameters are included, such as GSI in our study, because wide inter-population variability of fecundity is related to the high adaptability of species to different habitat conditions.

Material and methods

Field investigations have been carried out in different seasons from 2015 to 2017 (Table 1, Figure 1). The sampling has been conducted in accordance with European standards for fish sampling with multi-mesh gillnets - EN 14757:2005 (CEN, 2005)

and with electricity – EN 14011:2003 (CEN, 2003). Collected individuals were stored in 4% solution of formaldehyde. Total number of 62 individuals of *Alburnus alburnus* was gathered at six sites. Determination of samples has been carried out using taxonomical keys (Vuković & Ivanović, 1971; Vuković, 1977; Kottelat & Freyhof, 2007). Sex of each individual has been determined in the laboratory by analysis of gonads.

Table 1. List of investigated sites with dates of sampling and coordinates

Sites	Dates	Coordinates	
		Latitude	Longitude
Mala Spreča	08.09.2015.	44.422302°	18.797518°
Zelinska river	09.08.2015.	44.803818°	18.439162°
Jajce,Piva, HE2	24.10.2016.	44.378150°	17.281376°
Salakovac - Salmon 1	15.03.2016.	43.626287°	17.755760°
Jablanica, 5th beach	08.09.2015.	43.681066°	17.774398°
Drina Ustiprača	April 2017.	43.690139°	19.086886°

Figure 1. Sampling sites: 1 – Zelinska river, 2 – Mala Spreča, 3 – Piva, 4 – Drina-Ustiprača, 5 – Jablanica, 6 – Salakovac

Meristic characters included a number of branched and unbranched fin rays in dorsal (Do), anal (An), pelvic (ventral) (Pe), pectoral (Pl) and caudal fin (Ca). The number of gill rackers (Rac) was counted on the first gill arch. The number of scales in the lateral line was determined under a stereozoom microscope in full lateral line from the operculum to the caudal fin.

The following 21 morphometric characters have been analysed: total length (TL), standard length (SL), head length (c), distance between head tip and anal aperture (pan), preanal distance (aA), preventral distance (aV), prepectoral distance (aP), predorsal distance (aD), caudal peduncle length (lpc), length of dorsal (lD), anal (lA), caudal (lC), pectoral (lP) and pelvic (ventral) fin (lV), distance between pelvic (ventral) fins and anal aperture (Van), head depth (hc), maximum body depth (H) and the caudal peduncle depth (or the minimum body height – h), eye diameter (o), preorbital distance (prO), postorbital distance (poO). All measurements follow standard ichthyological measurements (Kottelat & Freyhof, 2007). Morphomeristic measurements have been carried out using electronic digital calliper to the nearest 0.10 mm, while total and standard body length have been measured using ichthyometer. Body weight and gonads weight have been determined using digital scale (Tehtnica ET 1111) with an accuracy of 0.01 g. They have been determined for two populations from the Drina river and Salakovac reservoir. After morphological analysis, fish were dissected in order to identify the sex of specimens. The gonadosomatic index (GSI) has been calculated using the formula after Bolger and Connolly (1989):

GSI=gonad weight (g)÷total fish weight (g) × 100.

Descriptive statistics has been used to describe local populations (mean, standard deviation, minimal and maximal value, standard error, coefficient of variation). Differences among populations have been tested by one-way ANOVA test, post hoc Kruskal-Wallace, and Kolmogorov-Smirnov test.

Sexual dimorphism based on metric traits has been tested using students t-test with the statistical significance of $p<0.05$. The statistical significance level of $p<0.05$ has been used for all metric traits in order to estimate potential statistically significant differences between males and females. Linear discriminant function analysis (LDF) has been used for identification of the most crucial characters responsible for the differentiation among populations. Size-dependent variation has been

corrected by calculating body ratios. Populations have been compared using ratios of morphometric characters in relation to standard length (SL), head length (c) and maximum body depth (H). For GSI power regression model was calculated. All statistical analyses were performed using PAST3 (Hammer et al., 2001) and Statistica 10 softwares.

Results and Discussion

A total of 62 individuals of *Alburnus alburnus* have been gathered from six sites in the Sava river basin and the Neretva river basin. Individuals from the Piva river and Zelinska river had the smallest body measurements. Average total body length (TL) of *Alburnus alburnus* individuals from the Piva river was 73.77±5.77 (68.00, 79.54 mm). The largest individuals are from the Jablanica reservoir populations, with average total body length of 132.80±15.51 (115.00, 143.40 mm). Average total body length for the whole sample (N = 62) was 120.69±23.58 (68.00, 158.00 mm). The linear body measures show high variability affected by a difference in standard body length of individuals in the samples CV = 5.13%-12.85%. Differences between morphometric data and morphometric ratios have been tested by ANOVA at 5% significance (Table 2). The significant phenotypic difference has been observed for the samples: Zelinska river-Piva river-Mala Spreča, and Drina-Salakovac-Jablanica reservoir samples. The interspecific variation was significant for almost all variables for the samples Zelinska river, Piva, and Mala Spreča. The results of *post hoc* Newman-Keules test indicate differences for 16 morphometric indices among samples with the most significant differences between Piva river and other investigated populations (Table 3).

The discriminatory power of different meristic characters has been tested by Discriminant function analysis. Discriminant function analysis of morphometric data Wilks' Lambda: 0.0003244; approx. F (135.152) = 4.643491; p < 0.0000, indicates that in the model crucial discriminatory power has the following: length of pelvic (ventral) fin (lV) (Partial Wilks' Lambda: 0.43; p: 0.000096), preanal distance (aA) (Partial Wilks' Lambda: 0.58; p: 0.004449), length of pectoral (lP) (Partial Wilks' Lambda: 0.59; p: 0.005944), minimum body height (h) (Partial Wilks' Lambda: 0.69; p: 0.044997), caudal fin branched ray number (Ca) (Partial Wilks' Lambda: 0.43; p: 0.000083), pelvic fin raynumber (Pe) (Partial Wilks' Lambda: 0.69; p: 0.044520). In the model, length of pelvic (ventral) fin and the caudal fin branched ray number contribute most to the discrimination among samples. The most significant distance was between Piva river sample and all other localities (Table 4). Biplot of each individual was plotted on the basis of the canonical scores (Figure 2).

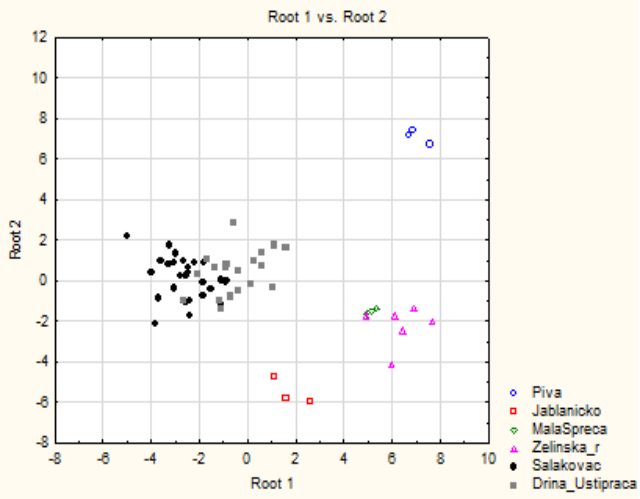

Figure 2. Canonical scores biplot based on linear morphometric characters for six local populations of *Alburnus alburnus* from Bosnia and Herzegovina: Piva river (circle), Jablaničko reservoir (hollow square), Mala Spreča river (diamond), Zelinska river (triangle), Salakovac reservoir (dot), Drina river near Ustiprača (full square)

Table 4. Distances between *Alburnus alburnus* populations from Bosnia and Herzegovina based on p-values

	Piva river	Jablaničko reservoir	Mala Spreča river	Zelinska river	Salakovac reservoir	Drina river near Ustiprača
Piva river		0.000004	0.000521	0.000081	0.000000	0.000001
Jablaničko reservoir	0.000004		0.007065	0.002586	0.000238	0.000166
Mala Spreča river	0.000521	0.007065		0.044637	0.000034	0.000307
Zelinska river	0.000081	0.002586	0.044637		0.000000	0.000001
Salakovac reservoir	0.000000	0.000238	0.000034	0.000000		0.000008
Drina river near Ustiprača	0.000001	0.000166	0.000307	0.000001	0.000008	

Table 2. Morphometric data regarding six populations of *Alburnus alburnus* from Bosnia and Herzegovina: Piva river, Jablanica reservoir, Mala Spreča river, Zelinska river, Salakovac reservoir, Drina River near Ustiprača; data in table: mean±standard deviation (SD), (minimal, maximal value), coefficient of variation (CV%), standard error (SE)

	Piva N = 3			Jablaničko lake N = 3			Mala Spreča N = 3			Zelinska river N = 6			Salakovac N = 27			Drina river near Ustiprača N = 20		
	Mean±SD (MIN, MAX)	CV%	SE	Mean±SD (MIN, MAX)	CV%	SE	Mean±SD (MIN, MAX)	CV%	SE	Mean±SD (MIN, MAX)	CV%	SE	Mean±SD (MIN, MAX)	CV%	SE	Mean±SD (MIN, MAX)	CV%	SE
c	13.31±1.15 (12.16, 14.46)	8.64	0.66	24.60±0.40 (24.20, 25.00)	1.63	0.23	19.75±1.35 (18.40, 21.10)	6.84	0.78	15.51±1.04 (13.66, 16.50)	6.70	0.42	22.75±3.20 (15.95, 31.00)	14.05	0.62	23.85±2.80 (18.92, 28.70)	11.75	0.63
o	4.19±0.30 (3.89,4.49)	7.16	0.17	6.20±0.35 (6.00, 6.60)	5.59	0.20	6.08±0.08 (6.00, 6.15)	1.24	0.04	4.59±0.36 (3.98, 4.93)	7.75	0.15	6.57±1.01 (4.92, 10.50)	15.33	0.19	6.76±0.68 (5.38, 8.12)	10.02	0.15
prO	2.85±0.37 (2.48, 3.22)	12.98	0.21	6.07±0.21 (5.90, 6.30)	3.43	0.12	4.70±0.30 (4.40, 5.00)	6.38	0.17	3.96±0.35 (3.47, 4.40)	8.91	0.14	6.14±1.20 (4.49, 10.50)	19.62	0.23	6.53±0.72 (5.60, 8.30)	11.01	0.16
poP	6.17±0.27 (5.90, 6.44)	4.38	0.16	12.00±0.30 (11.70, 12.30)	2.50	0.17	9.15±1.05 (8.10, 10.20)	11.48	0.61	7.31±0.54 (6.48, 8.10)	7.39	0.22	10.89±1.65 (7.06, 13.80)	15.15	0.32	11.52±1.47 (8.26, 13.50)	12.73	0.33
hc	8.23±0.43 (7.80, 8.65)	5.17	0.25	14.30±0.30 (14.00, 14.60)	2.10	0.17	11.35±0.85 (10.50, 12.20)	7.49	0.49	8.46±0.49 (7.67, 9.00)	5.79	0.20	13.21±1.80 (9.00, 16.09)	13.66	0.35	13.78±1.74 (10.71, 17.12)	12.66	0.39
aA	37.19±0.29 (36.90, 37.47)	0.77	0.16	74.38±3.13 (71.35, 77.60)	4.21	1.81	56.00±4.70 (51.30, 60.70)	8.39	2.71	42.30±1.83 (39.71, 45.20)	4.33	0.75	67.74±9.68 (46.76, 86.30)	14.29	1.86	67.47±9.82 (51.57, 85.22)	14.56	2.17
aV	26.99±0.79 (26.20, 27.77)	2.91	0.45	50.10±1.71 (48.70, 52.00)	3.40	0.98	39.40±2.90 (36.50, 42.30)	7.36	1.67	29.74±1.98 (27.51, 32.50)	6.66	0.81	46.16±6.20 (31.70, 58.20)	13.44	1.19	47.45±5.74 (37.02, 56.31)	12.11	1.28
aP	12.85±1.55 (11.30, 14.40)	12.06	0.89	25.67±0.29 (25.50, 26.00)	1.12	0.17	19.80±1.00 (18.80, 20.80)	5.05	0.58	15.79±0.79 (14.39, 16.60)	5.01	0.32	23.95±2.76 (17.85, 29.20)	11.51	0.53	24.85±3.04 (19.32, 30.10)	12.23	0.68
aD	32.50±0.73 (31.77, 33.23)	2.25	0.42	65.40±2.33 (63.50, 68.00)	3.56	1.35	49.60±3.10 (46.50, 52.70)	6.25	1.79	37.60±1.49 (35.55, 40.10)	3.96	0.61	58.93±7.79 (41.17, 73.80)	13.22	1.50	60.14±7.79 (46.05, 73.72)	12.96	1.74
lpc	13.10±0.20 (12.90, 13.30)	1.53	0.12	25.13±1.33 (24.00, 26.60)	5.30	0.77	16.65±0.35 (16.30, 17.00)	2.10	0.20	13.15±0.92 (12.30, 14.70)	6.96	0.37	21.50±2.89 (14.50, 26.56)	13.43	0.56	22.01±3.37 (16.58, 31.00)	15.33	0.75
lD	10.22±1.11 (9.11, 11.33)	10.86	0.64	11.53±0.85 (10.90, 12.50)	7.37	0.49	8.25±0.35 (7.90, 8.60)	4.24	0.20	7.43±2.35 (5.57, 12.10)	31.58	0.96	11.06±2.09 (7.51, 17.60)	18.92	0.40	10.16±1.39 (7.13, 12.74)	13.72	0.31
lA	10.98±0.06 (10.92,11.04)	0.55	0.03	21.33±0.57 (20.70, 21.80)	2.67	0.33	16.40±1.70 (14.70, 18.10)	10.37	0.98	11.57±0.98 (9.88, 12.40)	8.48	0.40	19.36±2.59 (14.47, 24.70)	13.36	0.50	19.88±2.82 (14.00, 26.79)	14.17	0.63
lC	15.17±0.48 (14.69, 15.65)	3.16	0.28	26.97±0.91 (26.30, 28.00)	3.36	0.52	20.20±4.30 (15.90, 24.50)	21.29	2.48	15.33±1.67 (13.00, 17.50)	10.86	0.68	26.35±5.23 (8.56, 32.78)	19.86	1.01	24.57±3.42 (18.43, 31.75)	13.92	0.76
lP	13.25±0.35 (12.90, 13.60)	2.64	0.20	22.72±1.33 (21.45, 24.10)	5.85	0.77	21.60±6.00 (15.60, 27.60)	27.78	3.46	13.24±0.64 (12.08, 13.70)	4.80	0.26	21.75±2.76 (15.34, 26.20)	12.69	0.53	20.65±2.59 (15.73, 24.98)	12.56	0.58
lV	6.36±0.22 (6.14, 6.57)	3.38	0.12	17.23±0.85 (16.40, 18.10)	4.94	0.49	12.25±0.55 (11.70, 12.80)	4.49	0.32	9.82±0.73 (8.87, 10.70)	7.44	0.30	15.86±1.94 (10.96, 18.50)	12.23	0.37	14.87±1.50 (11.27, 17.33)	10.10	0.34
Van	9.26±0.46 (8.80, 9.72)	4.97	0.27	22.00±0.40 (21.60, 22.40)	1.82	0.23	17.65±2.35 (15.30, 20.00)	13.31	1.36	13.15±0.93 (12.20, 14.70)	7.05	0.38	20.82±3.98 (12.42, 30.00)	19.12	0.77	21.24±3.81 (14.92, 30.72)	17.91	0.85
H	12.72±0.58 (12.14, 13.29)	4.52	0.33	25.73±0.64 (25.00, 26.20)	2.50	0.37	17.60±2.00 (15.60, 19.60)	11.36	1.15	14.44±0.92 (12.93, 15.70)	6.36	0.38	24.51±4.41 (15.07, 32.57)	18.00	0.85	24.98±3.89 (17.74, 32.14)	15.58	0.87
h	5.40±0.26 (5.14, 5.65)	4.73	0.15	10.10±0.46 (9.70, 10.60)	4.54	0.26	7.05±0.55 (6.50, 7.60)	7.80	0.32	5.84±0.38 (5.21, 6.40)	6.55	0.16	9.50±1.42 (6.26, 12.00)	14.96	0.27	9.23±1.16 (7.08, 10.84)	12.56	0.26
pan	37.20±0.81 (36.39,38.00)	2.16	0.46	72.17±2.40 (69.40, 73.60)	3.32	1.38	56.15±4.85 (51.30, 61.00)	8.64	2.80	40.92±2.26 (37.84, 43.80)	5.53	0.92	66.86±11.17 (44.96, 96.36)	16.71	2.15	65.55±8.99 (48.87, 81.67)	13.72	2.01
SL	63.41±2.22 (61.19, 65.63)	3.50	1.28	109.67±11.86 (97.00, 120.50)	10.81	6.85	87.80±6.60 (81.20, 94.40)	7.52	3.81	66.29±3.02 (60.98, 70.00)	4.56	1.23	108.66±15.46 (74.00, 134.00)	14.22	2.97	107.25±12.95 (84.00, 130.00)	12.08	2.90
TL	73.77±5.77 (68.00, 79.54)	7.82	3.33	132.80±15.51 (115.00, 143.40)	11.68	8.95	104.05±6.75 (97.30, 110.80)	6.49	3.90	80.08±4.11 (73.02, 84.50)	5.13	1.68	129.98±16.70 (92.00, 156.00)	12.85	3.21	128.05±15.83 (99.00, 158.00)	12.36	3.54

Table 3. Morphometric indeces data regarding six populations of *Alburnus alburnus* from Bosnia and Herzegovina: Piva river, Jablanica reservoir, Mala Spreča river, Zelinska river, Salakovac reservoir, Drina river near Ustiprača; data in table: mean ± standard deviation (SD), (minimal, maximal value), coefficient of variation (CV%), variables tested by one-way ANOVA with statistically significant difference ($p < 0.05$) between samples are marked with (*)

	Piva river N = 3		Jablanica reservoir N = 3		Mala Spreča river N = 3		Zelinska river N = 6		Salakovac reservoir N = 27		Drina river near Ustiprača N = 20	
	Mean±SD (MIN, MAX)	CV%	Mean±SD (MIN, MAX)	CV%	Mean±SD (MIN, MAX)	CV%	Mean±SD (MIN, MAX)	CV%	Mean±SD (MIN, MAX)	CV%	Mean±SD (MIN, MAX)	CV%
*C/SL	21.0±2.6 (18.5, 23.6)	12.1	22.6±2.4 (20.7, 25.4)	10.8	22.5±0.2 (22.4, 22.7)	0.7	23.4±1.1 (21.9, 24.4)	4.6	21.0±1.5 (17.2, 24.6)	7.1	22.3±0.7 (20.8, 23.7)	2.9
*O/SL	6.6±0.7 (5.9, 7.3)	10.6	5.7±1.0 (5.0, 6.8)	16.8	6.9±0.4 (6.5, 7.4)	6.3	6.9±0.5 (6.3, 7.5)	6.6	6.1±0.6 (5.1, 8.3)	10.5	6.3±0.4 (5.7, 7.0)	5.7
*PRO/SL	4.5±0.7 (3.8, 5.3)	16.4	5.6±0.8 (4.9, 6.5)	14.7	5.4±0.1 (5.3, 5.4)	1.1	6.0±0.5 (5.4, 6.6)	8.0	5.7±0.7 (4.7, 8.3)	12.8	6.1±0.4 (5.2, 7.2)	7.3
*POP/SL	9.7±0.8 (9.0, 10.5)	7.9	11.0±1.4 (10.0, 12.7)	13.1	10.4±0.4 (10.0, 10.8)	4.0	11.0±0.3 (10.6, 11.6)	3.1	10.0±0.8 (7.7, 11.3)	7.8	10.7±0.5 (9.8, 11.4)	4.4
HC/SL	13.0±1.1 (11.9, 14.1)	8.7	13.1±1.4 (12.1, 14.7)	10.7	12.9±0.0 (12.9, 12.9)	0.0	12.8±0.3 (12.2, 13.1)	2.5	12.2±0.8 (10.4, 14.1)	6.4	12.8±0.4 (12.1, 13.6)	3.2
aA/SL	58.7±2.5 (56.2, 61.2)	4.3	68.2±4.8 (64.4, 73.6)	7.0	63.8±0.6 (63.2, 64.3)	0.9	63.8±1.0 (62.5, 65.1)	1.5	62.5±3.5 (51.3, 67.5)	5.6	62.8±3.4 (50.0, 66.9)	5.5
*aV/SL	42.6±2.7 (39.9, 45.4)	6.4	45.9±3.7 (43.2, 50.2)	8.2	44.9±0.1 (44.8, 45.0)	0.2	44.8±1.8 (43.2, 48.1)	4.1	42.6±2.1 (36.6, 46.2)	5.0	44.2±1.0 (42.5, 46.1)	2.2
*aP/SL	20.3±3.2 (17.2, 23.5)	15.5	23.6±2.4 (21.6, 26.3)	10.3	22.6±0.6 (22.0, 23.2)	2.5	23.8±0.4 (23.4, 24.4)	1.5	22.2±1.2 (18.6, 24.1)	5.6	23.2±0.6 (21.8, 24.0)	2.5
*aD/SL	51.3±2.9 (48.4, 54.3)	5.7	60.0±4.8 (56.4, 65.5)	8.0	56.5±0.7 (55.8, 57.3)	1.3	56.7±1.0 (55.4, 58.3)	1.7	54.4±3.4 (44.7, 59.8)	6.2	56.0±1.8 (52.5, 60.1)	3.2
lpC/SL	20.7±1.0 (19.7, 21.7)	5.0	23.2±3.8 (19.9, 27.4)	16.6	19.0±1.0 (18.0, 20.1)	5.4	19.9±1.5 (17.6, 21.7)	7.3	19.9±2.0 (15.8, 24.0)	9.9	20.6±2.9 (17.3, 31.6)	13.9
*lD/SL	16.2±2.3 (13.9, 18.5)	14.3	10.6±0.9 (9.8, 11.5)	8.5	9.4±0.3 (9.1, 9.7)	3.3	11.2±3.4 (9.0, 17.9)	30.0	10.2±1.2 (7.3, 13.5)	12.1	9.5±0.7 (8.1, 11.0)	7.8
lA/SL	17.3±0.5 (16.8, 17.8)	3.0	19.6±2.5 (17.8, 22.5)	12.7	18.7±0.5 (18.1, 19.2)	2.9	17.4±0.9 (16.2, 18.7)	5.2	17.9±1.5 (15.3, 21.5)	8.4	18.5±1.3 (16.0, 20.6)	6.9
lC/SL	24.0±1.6 (22.4, 25.6)	6.7	24.8±2.5 (22.1, 27.1)	10.2	22.8±3.2 (19.6, 26.0)	14.0	23.1±2.1 (19.6, 25.0)	8.9	24.2±3.6 (8.8, 28.4)	14.9	23.0±2.0 (18.6, 26.3)	8.7
*lP/SL	20.9±1.3 (19.7, 22.2)	6.1	20.9±2.8 (17.8, 23.3)	13.5	24.4±5.0 (19.2, 29.2)	20.6	20.0±0.5 (19.5, 20.8)	2.4	20.1±1.5 (15.3, 22.7)	7.5	19.3±1.0 (17.6, 20.8)	5.2
*lV/SL	10.0±0.0 (10.0, 10.0)	0.1	15.8±1.4 (14.3, 16.9)	8.7	14.0±0.4 (13.6, 14.4)	3.0	14.8±0.6 (13.9, 15.5)	4.3	14.7±1.1 (11.8, 16.5)	7.4	13.9±1.1 (11.2, 15.8)	7.7
*Van/SL	14.6±0.2 (14.4, 14.8)	1.5	20.2±2.6 (17.9, 23.1)	13.0	20.0±1.2 (18.8, 21.2)	5.9	19.8±0.9 (18.4, 21.0)	4.4	19.2±2.7 (12.8, 24.2)	13.9	19.7±1.8 (17.0, 23.6)	9.1
H/SL	20.1±1.6 (18.5, 21.7)	8.0	23.6±2.1 (21.6, 25.8)	8.9	20.0±0.8 (19.2, 20.8)	3.9	21.8±0.6 (21.1, 22.5)	2.7	22.5±2.2 (16.8, 26.6)	9.7	23.2±1.1 (21.1, 25.4)	4.8
*h/SL	8.5±0.7 (7.8, 9.2)	8.2	9.3±0.9 (8.3, 10.0)	9.4	8.0±0.0 (8.0, 8.1)	0.3	8.8±0.3 (8.5, 9.1)	2.9	8.8±0.6 (7.3, 10.3)	7.1	8.6±0.3 (8.1, 9.1)	3.0
*PAN/SL	58.7±0.8 (57.9, 59.5)	1.3	66.2±5.3 (61.0, 71.5)	8.0	63.9±0.7 (63.2, 64.6)	1.1	61.7±2.2 (58.3, 64.8)	3.5	60.7±3.0 (53.1, 66.3)	5.0	61.0±1.8 (57.9, 64.9)	3.0
SL/TL	86.2±3.7 (82.5, 90.0)	4.3	82.7±2.6 (79.6, 84.3)	3.2	84.3±0.9 (83.5, 85.2)	1.0	82.8±1.1 (81.1, 84.0)	1.4	83.5±3.2 (80.0, 95.7)	3.9	83.8±2.1 (77.2, 88.8)	2.5
*prO/c	21.4±0.9 (20.4, 22.3)	4.4	24.7±1.0 (23.6, 25.6)	4.1	23.8±0.1 (23.7, 23.9)	0.5	25.5±1.8 (23.0, 28.4)	6.9	26.9±2.1 (24.1, 33.9)	7.8	27.5±1.8 (23.3, 31.7)	6.4
poP/c	46.5±2.0 (44.5, 48.5)	4.3	48.8±1.1 (48.0, 50.0)	2.2	46.2±2.2 (44.0, 48.3)	4.7	47.2±2.9 (44.8, 52.9)	6.2	47.8±1.9 (44.3, 51.3)	4.1	48.3±2.2 (43.7, 52.2)	4.5
*hc/c	61.9±2.2 (59.8, 64.1)	3.5	58.1±0.3 (57.9, 58.4)	0.5	57.5±0.4 (57.1, 57.8)	0.7	54.6±2.5 (52.3, 58.8)	4.7	58.1±2.9 (49.7, 64.8)	4.9	57.8±2.0 (55.1, 63.0)	3.5
*hc/H	64.7±0.4 (64.3, 65.1)	0.6	55.6±1.9 (53.4, 57.2)	3.5	64.7±2.5 (62.2, 67.3)	3.9	58.6±1.0 (57.3, 59.9)	1.7	54.5±4.2 (49.3, 67.6)	7.7	55.5±3.0 (50.5, 60.9)	5.4
h/H	39.3±5.3 (33.2, 42.4)	13.4	39.2±1.1 (38.5, 40.5)	2.7	40.2±1.4 (38.8, 41.7)	3.6	40.5±0.9 (39.3, 42.1)	2.3	39.1±2.7 (33.4, 45.7)	7.0	37.1±1.8 (33.7, 40.9)	4.8

Due to high intrapopulation variability, body indices have been calculated using ratios of standard length (SL), head length (c) and maximum body depth (H). Differences based on body indices have been analysed by ANOVA test. The results have indicated statistically significant differences at $p<0.01$, while the results of *post hoc* Newman-Keuls test have indicated differences between Piva river and other samples (Table 5).

Discriminant function analysis conducted on 25 morphometric body ratios indicate that 11 characters have statistically significant discriminant power (Table 5). The strongest difference has been observed between Piva river sample and other samples (Table 6). In the model, discriminant roots were statistically significant (Table 7).

Based on the canonical scores biplot of each individual was plotted (Figure 3). The multivariate analysis (LDF) separated from other samples individuals collected from Piva river and Zelinska river (Figure 3).

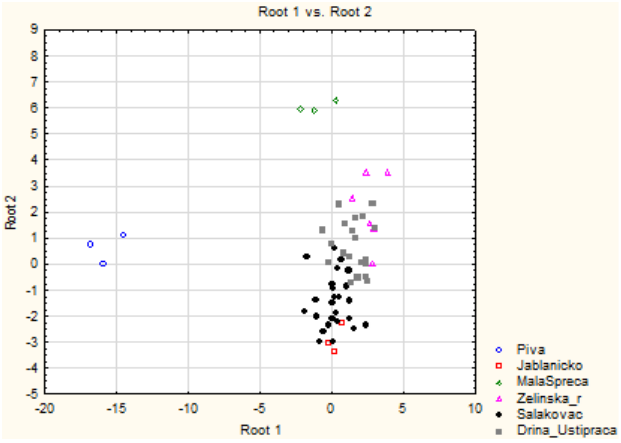

Figure 3. Canonical scores biplot based on morphometric indices for six local populations of *Alburnus alburnus* from Bosnia and Herzegovina: Piva river (circle), Jablaničko reservoir (hollow square), Mala Spreča river (diamond), Zelinska river (triangle), Salakovac reservoir (dot), Drina river near Ustiprača (full square)

The differences among meristic characters have been tested by Multiple Comparisons p-values two-tailed Kruskal-Wallis test. The results of the test have indicated statistically significant differences for a number of branched rays in caudal fin between the Drina river near Ustiprača and Jablaničko reservoir - Zelinska river; Salakovac reservoir and Zelinska river. A number of branched rays in caudal fin tested by Kruskal-Wallis test was significant at 1% between Drina Ustiprača and Mala Spreča river; Salakovac reservoir and Zelinska river – Mala Spreča river. The stability of meristic taxonomic characters was suggested by a number of authors, thus meristic data are used for a taxonomy of species from genus *Alburnus* (Dimovski & Grupče, 1975, Šorić, 1980, Kottelat, 1997, Kottelat and Freyhof, 2007, Freyhof & Kottelat, 2008, Buj et al., 2010).

For the total sample, number of rays in dorsal fin was in range: 7 – 9, mode: 8, frequency of mode: 38; number of rays in anal fin (14 – 21, mode = 17, f = 18), number of branched rays in caudal fin (16 – 22, mode = 20, f = 25), number of rays in pelvic fin (7 – 9, mode = 8, f = 48), number of scales in lateral line (42 – 54, mode = 49, f = 16) number of gill rackers (13 – 22, mode = 16, f = 21). Significant differences in number of gill rakers have been observed between Mala Spreča river and Jablaničko reservoir.

The highest standard deviation has been observed in the number of lateral line scales (1.98), number of gill rakers (1.43) and the lowest deviation was in both pelvic fin ray (0.27) and dorsal fin ray (0.48). The meristic characters are in accordance with the results of previous studies (Kottelat & Freyhof, 2007, Buj et al., 2010). Observed values for the most important meristic taxonomic characters for genus *Alburnus*, such as lateral line scales, gill rakers, branched anal-fin rays are within published morphometric range for *Alburnus alburnus* (Kottelat & Freyhof, 2007).

Table 6. Distance between *Alburnus alburnus* samples based on p-values

	Piva river	Jablaničko reservoir	Mala Spreča river	Zelinska river	Salakovac reservoir	Drina river near Ustiprača
Piva river		0.000000	0.000000	0.000000	0.000000	0.000000
Jablaničko reservoir	0.000000		0.000375	0.004846	0.123414	0.010984
Mala Spreča river	0.000000	0.000375		0.001715	0.000075	0.000122
Zelinska river	0.000000	0.004846	0.001715		0.000635	0.005629
Salakovac reservoir	0.000000	0.123414	0.000075	0.000635		0.000119
Drina river near Ustiprača	0.000000	0.010984	0.000122	0.005629	0.000119	

Lateral line scales number for the total sample was in range (42-54). The Zelinska river sample had the smallest number of scales in lateral line (42-46). For samples Salakovac reservoir and Drina river number of scales in lateral line were in the range (45-54).

The number of gill rakers was in range (13-21) with the lowest values in Mala Spreča and Zelinska river. Phenotypes of *A. alburnus* with lower number of gill rakers have been observed in Salakovac and Drina, Ustiprača. According to Kottelat and Freyhof (2007) number of gill rakers in *A. sava* is in range 23-27 (mode 24-26), which is much higher than in our investigated populations from the Sava river basin (samples Mala Spreča, Zelinjska Rijeka, Piva river, and Drina Ustiprača). Branched anal-fin rays were in range (14-21), for samples from Piva the number of soft rays in anal-fin was in range (14-17) which is lower than expected for *Alburnus alburnus*. Besides the fact that some individuals in the sample have values lower or higher than expected ratios, it was not possible to determine combination of characters that would indicate the presence of *A. neretvae* or *A. save* in the analysed samples (Table 8).

Obtained results have shown that there is no significant difference in average values of total body length and total body weight between males and females from the Drina river and those from Salakovac reservoir. The average weight of testes and gonadosomatic index of males from Drina river has not shown any significant difference as compared to samples from the Salakovac reservoir. Weight of ovary and gonadosomatic index of females from the Drina river have not shown any significant difference compared to the individuals from Salakovac reservoir (Table 9).

There was a positive relationship between the GSI and gonad weight in males from the Drina river and Salakovac reservoir, but the correlation was not statistically significant at 5%. In females from the Drina river and Salakovac reservoir, there was also a positive, non-significant, relationship between the GSI and the total length, total body weight and gonad weight (Table 10-11).

Environmental factors affect fecundity, which is different among fish species (Pilić et al., 2018). The results indicate a small level of variation in the observed parameters, which can be related to similar microclimate conditions of the habitat. Yıldırım et al. (2007) examined fish species *Chalcalburnus mossulensis* in Karasu River (Turkey) and they found significant correlations between fecundity, total weight, and gonad weight. Various ecological conditions in both stagnant and running waters have significant effects on the spawning period (Bennett, 1970). Temperature is important in maturation gonads and spawning, providing the capacity for reproductive cycles (Pankhurst & King, 2010). According to Latorre et al. (2018), a wide inter-population variability is related to the high adaptability of this species to different habitat conditions. Similar results were recorded by Mousavi-Sabet et al. (2017).

Table 7. Chi-Square Tests with Successive Roots Removed

Roots removed	Eigen-value	Canonical R	Wilks' Lambda	Chi-Sqr.	df	p-value
0	14.98977	0.968225	0.000635	334.9884	125	0.000000
1	4.04485	0.895421	0.010148	208.8647	96	0.000000
2	2.68914	0.853776	0.051198	135.2289	69	0.000003
3	1.48799	0.773349	0.188875	75.8335	44	0.002019
4	1.12803	0.728067	0.469919	34.3614	21	0.033138

Table 10. The power regression equation for the gonadosomatic index (GSI) of males from the Drina river and Salakovac reservoir, a linear relationship in parameters: total body length (cm), total body weight (g) and testis weight (g)

Male	Drina river	Salakovac reservoir
Total body length (cm)	GSI=0.009x + 2.9436 (R^2= 0.0018. P>0.05)	GSI=-0.1261x + 17.203 (R^2= 0.696. P>0.05)
Total body weight (g)	GSI=0.0414x + 3.2238 (R^2= 0.0069. P>0.05)	GSI=-0.4521x + 10.045 (R^2= 0.7282. P>0.05)
Testes weight (g)	GSI=4.305x + 1.7626 (R^2= 0.382. P>0.05)	GSI=4.2305x + 2.8365 (R^2= 0.102. P>0.05)

Table 11. The power regression equation for the gonadosomatic index (GSI) of females from the Drina river and Salakovac reservoir, a linear relationship in parameters: total body length (cm), total body weight (g) and ovary weight (g)

Female	Drina river	Salakovac reservoir
Total body length (cm)	GSI=0.153x -11.724 (r^2= 0.3149. P>0.05)	GSI=0.024x +1.8303 (r^2= 0.0322. P>0.05)
Total body weight (g)	GSI=0.3212x - 1.1708 (r^2= 0.2716. P>0.05)	GSI=0.0915x + 2.9263 (r^2= 0.087. P>0.05)
Ovary weight (g)	GSI=3.4929x + 1.2317 (r^2= 0.9269 P>0.05)	GSI=0.1667x + 4.8248 (r^2= 0.0008. P>0.05)

Table 8. Meristic characters assessed for six samples of *Alburnus alburnus* from Bosnia and Herzegovina with frequency for different categories: dorsal fin rays (Do), anal fin rays (An), caudal fin rays (Ca), pelvic fin rays (Pe), number of lateral line scales (Ll), number of gill rakers (Rac)

Category		Piva N = 3		Jablaničko Jezero N = 3		Mala Spreča N = 3		Zelinska river N = 6		Salakovac N = 27		Drina, Ustiprača N = 20	
Do	7			1	33.33%			1	16.67%	1	3.70%		
	8			2	66.67%	3	100.00%	5	83.33%	16	55.55%	13	65.00%
	9	3	100.00%							27	40.74%	7	35.00%
An	14	1	33.33%										
	15									1	3.70%		
	16	1	33.33%	2	66.67%			1	16.67%	4	14.81%	2	10.00%
	17	1	33.33%			3	100.00%	4	66.67%	6	22.22%	4	20.00%
	18							1	16.67%	6	22.22%	6	30.00%
	19			1	33.33%					10	37.04%	5	25.00%
	20											1	5.00%
	21											2	10.00%
Ca	16			2	66.67%								
	17							4	66.67%				
	18			1	33.33%	3	100.00%	2	33.33%				
	19	2	66.67%							4	14.81%		
	20	1	33.33%							1	3.70%	12	60.00%
	21									12	44.45%	8	40.00%
	22									9	33.34%		
Pe	7									4	14.81%	1	5.00%
	8			3	100.00%	3	100.00%	6	100.00%	22	81.48%	14	70.00%
	9	3	100.00%							1	3.70%	5	25.00%
Pl	42							1	16.67%				
	43					3	100.00%						
	44							3	50.00%				
	45									1	3.70%		
	46			1	33.33%			2	33.33%			1	5.00%
	47									1	3.70%		
	48									6	22.23%	1	5.00%
	49	2	66.67%	1	33.33%					6	22.23%	7	35.00%
	50	1	33.33%							4	14.81%	6	30.00%
	51			1	33.33%					4	14.81%	2	10.00%
	52									2	7.41%	2	10.00%
	53									2	7.41%	1	5.00%
	54									1	3.70%		
Rac	13							1	16.67%	1	3.70%		
	15							1	16.67%				
	16					3	100.00%			12	44.45%	5	25.00%
	17	3	100.00%					2	33.33%	3	11.12%	4	20.00%
	18							2	33.33%	4	14.81%	6	30.00%
	19									2	7.41%	4	20.00%
	20									3	11.12%	1	5.00%
	21									2	7.41%		

Table 9. Morphometric data and values gonadosomatic index: mean ± standard deviation (SD), (minimal, maximal value)

Category	Drina river (male) N=6	Salakovac reservoir (male) N=8	Drina river (female) N=11	Salakovac reservoir (female) N=7
	Mean±SD (MIN; MAX)	Mean±SD (MIN; MAX)	Mean±SD (MIN; MAX)	Mean±SD (MIN; MAX)
Total body length (cm)	99.17±9.17 (84.00; 108.00)	96.75±11.89 (74.00; 110.00)	113.91±10.16 (98.00; 130.00)	115.52±11.19 (97.00; 134.00)
Total body weight (g)	14.87±3.88 (8.30; 18.00)	11.15±3.39 (5.10-15.10)	21.42±4.50 (14.00; 27.00)	18.271±4.81 (12.10; 27.20)
Gonads weight (g)	0.48±0.28 (0.30; 1.00)	0.5±0.14 (0.40; 0.80)	1.28±0.76 (0.30; 3.00)	1.07±0.34 (0.70; 1.70)
Gonadosomatic index (GSI)	3.22±1.38 (2.01; 5.71)	5.00±1.80 (2.65; 7.84)	5.71±2.77 (2.14; 11.11)	5.84±0.61 (4.62; 6.37)

Table 5. Discriminant Function Analysis Wilks' Lambda: 0.00063 approx. F (125.162)=4.5009 p<0,001

	Wilks' Lambda	Partial Lambda	F-remove (5.32)	p-value	Toler.	1-Toler. (R-Sqr.)
*lV/SL	0.001141	0.556030	5.110166	0.001483	0.310897	0.689103
*lD/SL	0.001232	0.514971	6.027890	0.000488	0.313076	0.686924
*C/SL	0.001329	0.477718	6.997020	0.000162	0.000259	0.999741
lP/SL	0.000877	0.724004	2.439733	0.055543	0.481939	0.518061
O/SL	0.000859	0.739085	2.259350	0.072201	0.175942	0.824058
*prO/c	0.000899	0.705741	2.668484	0.039900	0.002804	0.997196
*h/H	0.000923	0.687790	2.905170	0.028420	0.063233	0.936767
HC/SL	0.000842	0.753336	2.095547	0.091680	0.001907	0.998093
aA/SL	0.000866	0.732826	2.333313	0.064831	0.198844	0.801156
aV/SL	0.000808	0.785959	1.742916	0.153233	0.127257	0.872743
*PRO/SL	0.000924	0.687071	2.914905	0.028028	0.001219	0.998781
*hc/c	0.000899	0.706138	2.663380	0.040194	0.003992	0.996008
aP/SL	0.000863	0.735256	2.304450	0.067612	0.079623	0.920377
*poP/c	0.001055	0.601597	4.238348	0.004541	0.000913	0.999087
*POP/SL	0.001055	0.601361	4.242533	0.004516	0.000414	0.999586
*h/SL	0.001224	0.518464	5.944165	0.000539	0.059026	0.940974
hc/H	0.000737	0.860977	1.033416	0.414926	0.009306	0.990694
*SL/TL	0.000884	0.717994	2.513717	0.049894	0.259774	0.740226
lC/SL	0.000809	0.784255	1.760606	0.149350	0.444463	0.555537
H/SL	0.000747	0.849902	1.130276	0.364495	0.005347	0.994653
lpC/SL	0.000716	0.886523	0.819218	0.545095	0.631287	0.368713
Van/SL	0.000780	0.813331	1.468871	0.227350	0.337158	0.662842
lA/SL	0.000761	0.833789	1.275799	0.298459	0.484743	0.515257
PAN/SL	0.000724	0.876240	0.903932	0.490629	0.151966	0.848034
aD/SL	0.000661	0.960245	0.264964	0.928883	0.377572	0.622428

Conclusion

Inter-population variability in 21 standard measurements and six meristic morphological traits of *Alburnus alburnus*, have shown statistically significant differences among investigated sites. The exception is populations from Jablanicko reservoir and Salakovac reservoir. The results indicate that some characters had a strong effect on the geographical variation among *Alburnus alburnus* samples from Bosnia and Herzegovina. The characters with the strongest discriminant power are: preanal distance (aA), length of pectoral fin (lP), length of ventral (pelvic) fin (lV), minimum body height (h), caudal fins branched rays (Ka/Ca), and pelvic fins branched rays (Pe). Morphometric data indicate that the introduced *Alburnus alburnus* in the Neretva River reservoirs - Salakovac is well adapted and by body dimensions it is similar to Drina samples. Weight of the gonads and gonadosomatic index did not show a statistically substantial difference between these samples. On the other hand, gonadosomatic index showed positive, although not significant, relationship to gonads weight.

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Conflict of Interest

The authors state there is no conflict of interest.

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