

Research Article

Status of endangered fish species *Aulopyge huegelii* Heckel, 1843 (Teleostei: Cyprinidae) in the Buško Blato reservoir, Bosnia and Herzegovina

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Abstract: The Dalmatian barbelgudgeon, *Aulopyge huegelii* is a stenoendemic fish species restricted to the area of west Bosnia and part of Dalmatia. Its global IUCN species status is “endangered (EN)” according to the “B1ab (iii, v)” criterion. There are not enough reliable sources containing data on this species, particularly in the recent period. As primarily a groundwater fish, it was exceptionally well adapted to environmental conditions in the Buško Blato reservoir. Based on the recent findings, the status of this species has changed over the past several years. The main objective of this study was to characterise and determine the current status of the monotype species *A. huegelii* from the Buško Blato reservoir, through the analysis of its selected morphological and ecological features. During field research, we have gathered 88 individuals of *A. huegelii*, at just four neighbouring sites. A significant decrease in its population number is evident in comparison to previous studies. The results have shown its retreat into a small part of the reservoir which is connected with the groundwater system as its primary habitat, in order to escape from predators: the four new allochthone species in this ecosystem. Comparing the results with previous studies, it could be concluded that the Dalmatian barbelgudgeon is “returning” to its original morphological form i.e. groundwater fish form. All this could in perspective have significant consequences on the survival of the population of this species in the Buško Blato reservoir.

Keywords: Conservation, Threatened species, Morphology, Ecology.

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Introduction

Changes in general physical geographical conditions, and particularly the appearance and the destruction of natural isolation barriers throughout geological history, had a very strong impact on the development and composition of the living world in its waterways and lakes. Ichthyofauna of Bosnia and Herzegovina with 118 fish species represents a unique European biological resource in terms of both the richness of total number of species and the presence of numerous

endemic forms (Sofradžija 2009). Only for the Adriatic basin, Vuković and Sofradžija (1986) enlist 12 endemic species (two salmonids and 10 cyprinids), while Guzina (2000) gives 14 endemic species for this area. However, if we take endemism in the broader sense, this number raises up to 35 species (Glamuzina et al. 2010).

One of the endemic species of Bosnia and Herzegovina is the Dalmatian barbelgudgeon, *Aulopyge huegelii* Heckel, 1843, a monophyletic

species from the family Cyprinidae. Enlisted on the Red List of the Federation of Bosnia and Herzegovina (Škrijelj et al. 2013), it is considered endangered (EN) according to the B1ab (iii, v) criteria. The species is mainly threatened by constructions of dams, habitat destruction, water pollution and introduction of allochthonous species (Crivelli 2005). No conservation measures had been implemented so far.

As one of the most unusual endemic fish species, the Dalmatian barbelgudgeon is characterized by the presence of outer fleshy cloacal canal which longitudinally fuses with the first ray of the anal fin, forming an outer extension in the form of a tub. This extension ends with the anus and urogenital opening and serves for laying eggs (greek. *aulo* - flute; *pyge* - tail). The dorsal fin is distinguished by a hard spine with a serrated inner edge. Though the body of this fish is mainly without scales, which is a common adaptation to subterranean lifestyle, a certain number of individuals had been caught in the Buško Blato reservoir with sporadically and unequally distributed large scales on the front and rear part of the body. Scales were thin and fragile, oval or square-shaped, and without a regular center (Guzina & Vuković 1987; Guzina 2000). The head is pointed and relatively large, with large eyes located just below the top of the head (Bogut et al. 2006). The body is elongated, with very unusual and wavy lateral lines (Sofradžija 2009). Body coloration is highly dependable on the environment. Primary size varies from nine to 12cm, although individuals up to 25cm long are recorded mainly in lakes and reservoirs. According to Jelić et al. (2008) this species has not changed its morphology during several millions of years.

This is a nocturnal species which hides itself behind rubbles and pebbles. The Dalmatian barbelgudgeon feeds on periphyton, plankton and benthic algae, zooplankton and invertebrates, including larvae of aquatic insects (Aganović et al. 1974; Crivelli 1996). Spawning begins at the end of April at temperatures from 13 to 17°C, and lasts until

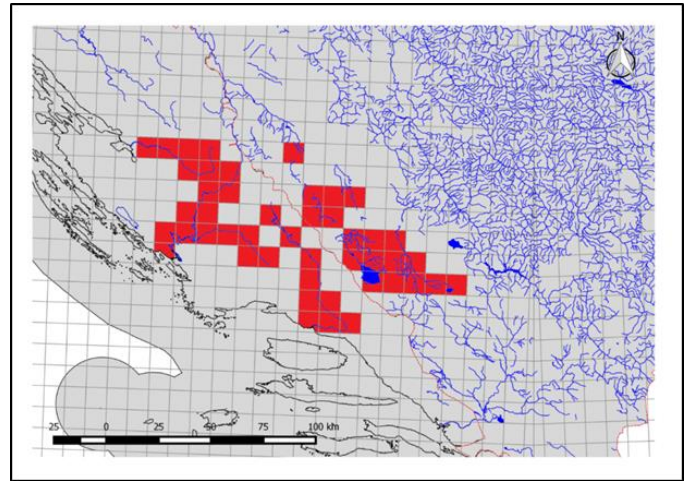


Fig.1. Distribution of the Dalmatian barbelgudgeon in Bosnia and Herzegovina and Croatia (UTM net, 10x10km).

the end of June. Males and females reach sexual maturity in the first year, although some data indicate that they sexually mature in the third or even fourth year, at the body length of 10-12cm (Sofradžija 2009). This is a phytophile species which deposits eggs in crevices and cavities among plant roots. Recent data on this species are generally very scarce: morphology (Bless & Riehl 2002; Čaleta et al. 2009), distribution (Delić et al. 2005; Sofradžija 2009), hematology (Hasković et al. 2010) and genetics (Machordom & Doadrio 2001a, b; Tsigenopoulos et al. 2002).

The Dalmatian barbelgudgeon is a stenoendemic species which is distributed in karstic rivers and lakes in western Bosnia and Herzegovina and Croatia (Guzina 2000; Crivelli 2006; Kottelat & Freyhof 2007). It lives in the Rivers of Zrmanja, Krka, Čikola and Cetina, and watercourses in the Sinjsko polje and Visovačko lake in Croatia, while in Bosnia and Herzegovina it resides in the watercourses of Glamočko, Duvanjsko, Livanjsko and Imotsko field as well as in Blidinje, Šatorsko lake and the Buško Blato reservoir (Heckel 1841, 1843; Heckel & Kner 1858; Ćurčić 1917; Taler 1949, 1953; Vuković 1963, 1977; Vuković & Ivanović 1971; Bojčić et al. 1982; Lelek 1987; Župančić 1990, 2008; European Comision 1998; Šorić & Bănărescu 1999) (Fig. 1).

The most thorough survey of the Dalmatian

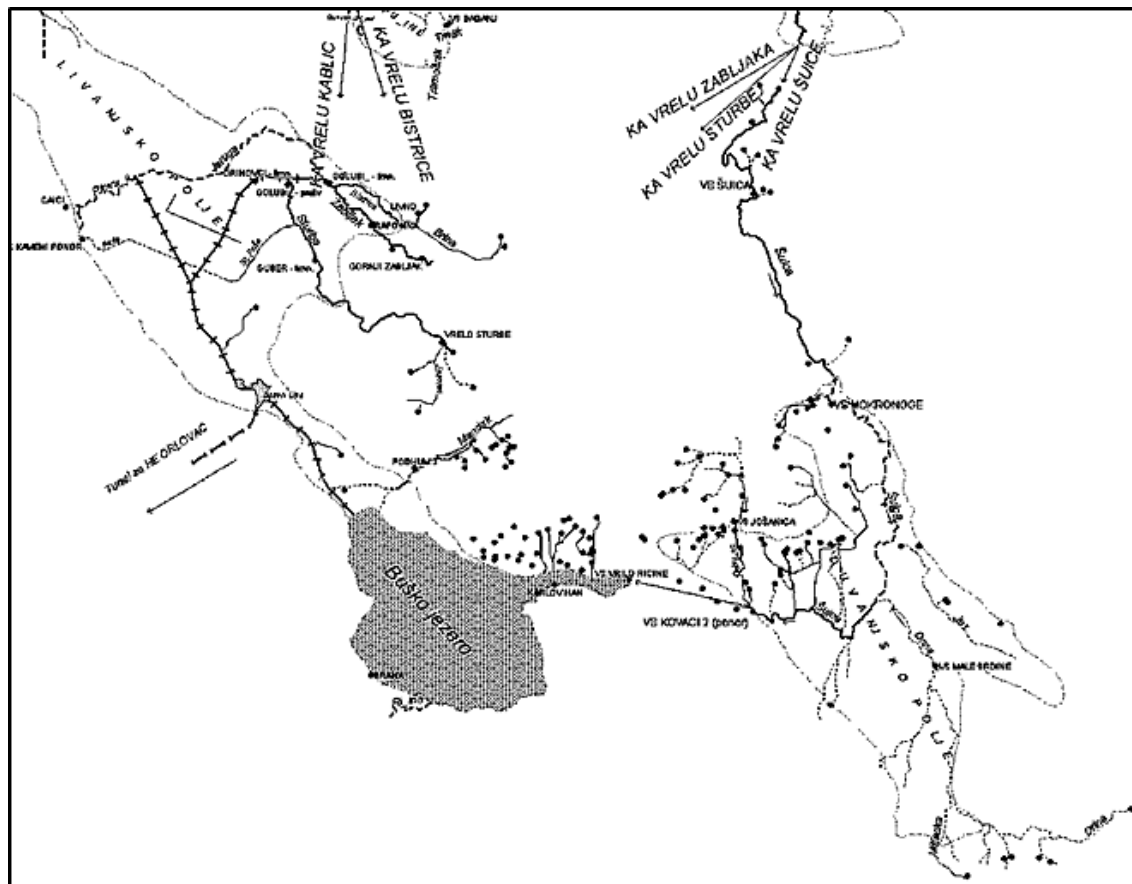


Fig.2. Hydrological network in the wider area of the Buško Blato reservoir

barbelgudgeon in Bosnia and Herzegovina was conducted during a two decades long project that lasted until the beginning of 1990 (Guzina 2000). Data from this project showed that the most abundant population was that of the Buško Blato reservoir, with significantly larger individuals in terms of length, weight and growth rate, compared to other investigated locations. Significant differences in morphometric traits between *A. huegeli* from the Buško Blato reservoir and other populations were hypothesized to be the result of its adaptation to habitat change, from subterranean rivers to a lentic ecosystem, caused by the establishment of the Buško Blato reservoir.

The Buško Blato reservoir was created for the production of the electric energy and irrigation. The project started in 1967 and was finished in 1974. The reservoir is the third largest in Europe, with an area of 57.7km². It is located in the south-eastern part of the Livanjsko karst field in Bosnia and Herzegovina

which belongs to the river Cetina basin. River Ričina and Golinjevski brook had the most important role in the natural hydrographic regime of the Buško Blato reservoir. The river bed was submerged after the creation of the reservoir. Part of the water from the reservoir drains through the subterranean system located at the southern and the south-western part of the reservoir. The largest groundwater supply is in the area where a "branch" of the Buško Blato reservoir is located, represented by the Ričina river and an underground cave system (Fig. 2).

The Buško Blato reservoir is a unique resource which enables relatively suitable conditions for various fish species. On the basis of recorded species, it could be concluded that biodiversity of fish in this artificial ecosystem is positive, although endangered due to the introduction of alien fish species, pollution and overfishing.

To investigate the current status of *A. huegeli* from the Buško Blato reservoir, we conducted a study

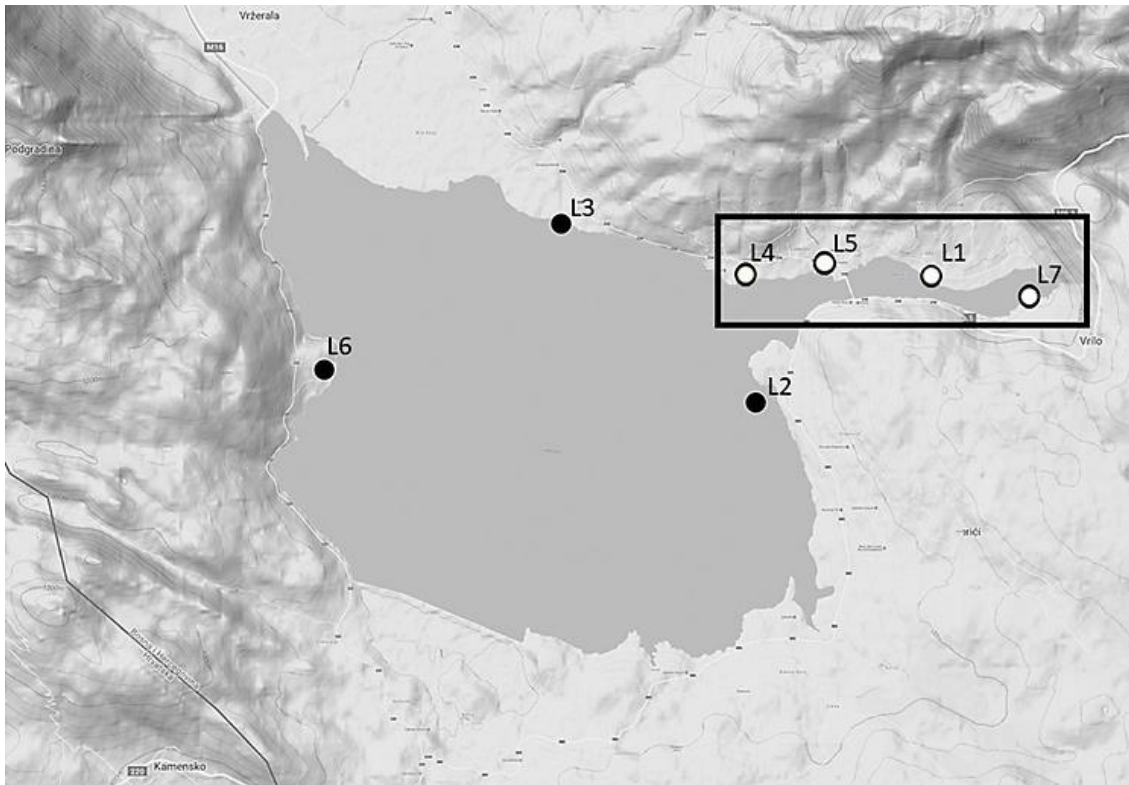


Fig.3. Sampling sites at the Buško Blato reservoir: L1 – Prisoje, L2 - Grabovica, L3 – Golinjevo, L4 – Vrbe, L5 – Bare, L6 – Kamenica, L7 – Vrilo.

whose main objective was to assess the conservation status, based on the analysis of selected morphological and ecological features and its comparison with results of previous. This will serve to assess the level of threat to this important ichthyoresource.

Materials and Methods

Field research have been carried out at seven sites in two seasons: November 2014 and September 2015. Sampling in the late spring and early summer in the Buško Blato reservoir was not recommended due to high water levels. The sampling was 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). According to the Law on nature protection, the Bosnia and Herzegovina Federal Ministry for the Environment and Tourism has been informed about research activities (permission No. 03-3-24/3-2605/14), particularly regarding sampling

of species from the Red List of the Federation of Bosnia and Herzegovina, such as Dalmatian Barbelgudgeon. Collected individuals were used for morphometric analysis and stored in 95% ethyl-alcohol. We sampled 88 individuals of *Aulopyge huegelii* in four sites (Fig. 3).

Biometric analyses: Since the sample from the first year of the research included very small, juvenile individuals (all except one from the site Vrilo), the analysis dealt only with body mass, total and standard body length. The total sample of measured individuals (51) was represented by different age groups and used all selected morphometric traits. Sixteen morphometric features of adult specimens were measured according to Pravdin (1966): body mass (B_M), total body length (T_D), fork body length (D_T_V), standard body length (S_D), head length (D_G), pre-orbital length (P_P), eye diameter (D_O), post-orbital length (Z_P), pre-dorsal length (AD_R), pre-ventral length (AV_R), pre-anal length (AA_R), dorsal fin base length (D_OLP), anal fin

basis length (D_OPP), caudal peduncle length (D_RS), maximal body height (H_H) and minimal body height (L_H). All measurements were carried out using metal vernier calipers (Mitutoyo), while total and standard body length were measured using an ichthyometer. Body mass was determined using a digital scale with the accuracy of 0.01g.

Biostatistical analysis: The total sample of 51 individuals from all age groups was used for statistical analysis of all morphometric traits. The statistical significance was tested using a one way ANOVA. Principal Component Analysis (PCA) was carried out to determine variables which most significantly contribute to discrimination between sexes and between various age groups. All statistical analyses were completed in PAST2 (Hammer et al. 2001) and Statistica 10.

Further statistical analyses have been carried out on a sample that included only 38 adult individuals (3+, 4+, 5+ and 6+). The younger individuals were excluded from these analyses. Minimal and maximal values, arithmetic mean with standard error and standard deviation, as well as variance were calculated in order to describe parameters and assess variations. A coefficient of variability within the group was used to assess variability. T-test with the statistical significance level of $P < 0.05$ was carried out for morphometric traits in order to estimate potential statistically significant differences among males and females.

Normal distribution within the sample was tested using the Kolmogorov-Smirnov test. Data without normal distribution have been transformed into ranks, and a non-parametric analysis - using the Mann-Whitney test, has been carried out at the significance level of $P < 0.05$. The results of biometric analyses from this study and previous research were compared in PAST2, using one sample t-test (Hammer et al. 2001).

Ecological analyses: Ecological characterisation of the Dalmatian barbelgudgeon in the Buško Blato reservoir included several stages and analyses. In the first stage, hydromorphological and physical-

chemical water analyses, relevant for biological quality elements, were carried out. Water temperature, pH and electric conductivity were determined in the field, using the WTW Multi 3420 SET C, while oxygen concentration and saturation with O_2 were determined using Oxi 3310 IDS Set 1. Data on the presence of certain important elements (phosphorus, nitrogen, cuprum, chrome, zinc, etc.) were taken from the Agency for Water Management of the Adriatic Sea Basin, in Mostar. The analysis of biological quality elements (BQE) was carried out in accordance with guidelines from the Water Framework Directive (Champ et al. 2009). It included an analysis of phyto- and zooplankton, and zoobenthos as components important for the nutrition of the Dalmatian barbelgudgeon. The identification of these biotic components was made according to reference keys for each group.

The structure of the fish fauna was the most important element for the analyses of interspecies relationships that include predation and competition. The determination of samples was carried out using taxonomic keys (Vuković & Ivanović 1971; Vuković 1977; Kottelat & Freyhof 2007).

Sex and age of each individual were determined in the laboratory. Sex was determined by an analysis of gonads. Age was determined by an analysis of otoliths, since the Dalmatian barbelgudgeon do not have scales. Age of the Dalmatian barbelgudgeon could also be based on sections of the third unbranched ray of the dorsal fin.

Natural population growth was also analysed for all recorded fish species. The results of the analyses have enabled an estimation of the realized niche of the Dalmatian barbelgudgeon, in comparison to the species' fundamental niche.

Results

During field research of the Buško Blato reservoir, we sampled 88 individuals of *A. huegelii* Heckel, 1843, at four sites: Prisoje, Vrbe, Bare and Vrilo. The largest number of individuals (77) was gathered at the site Prisoje in both sampling years.

Table 1. Statistical analysis of morphometric data regarding 51 individuals of *Aulopyge huegelii* from the Buško Blato reservoir (AV – average, SD – standard deviation, CV – coefficient of variability, SE – standard error).

Morphometric trait	Males N = 23			Females N = 28		
	AV±SD (min; max)	CV	SE	AV±SD (min; max)	CV	SE
Body mass (B_M)	9.39±2.52 (5.8; 16)	26.80	0.54	10.3±6.3 (6.4; 41.2)	61.30	1.20
Total body length (T_D)	104±7.18 (91; 121)	6.90	1.53	106.6±11 (96; 155)	10.30	2.10
Fork body length (D_T_V)	91.34±9.7 (81; 109)	7.48	1.46	93.8±9.7 (85; 133)	10.40	1.80
Standard body length (S_D)	85.45±9.6.5 (75; 102)	7.61	1.39	87.5±9.5 (78; 128)	10.90	1.80
Pre-dorsal length (AD_R)	45.68±3.56 (75; 102)	7.80	1.39	46.3±5 (41.5; 68.2)	10.70	0.90
Dorsal fin base length (D_OLP)	9.87±0.76 (9; 11)	7.72	0.16	10.1±1.1 (9; 13)	10.80	0.20
Head length (D_G)	23.21±1.88 (20.77; 29.09)	8.09	0.40	23.7±2.4 (20.3; 34.2)	10.10	0.50
Pre-orbital length (P_P)	9.17±0.89 (7.99; 12)	9.72	0.19	9.1±1.7 (3.8; 15.4)	18.50	0.30
Eye diameter (D_O)	4.39±0.33 (3.88; 5.31)	7.54	0.07	4.6±0.8 (3.9; 8.3)	17.70	0.20
Post-orbital length (Z_P)	9.7±0.87 (8.48; 12.27)	8.94	0.18	9.9±0.9 (8.3; 13)	8.80	0.20
Pre-ventral length (AV_R)	44.27±3.16 (39.85; 52.82)	7.14	0.67	45.1±5.4 (38.6; 69.5)	12.00	1.00
Pre-anal length (AA_R)	59.84±4.61 (52.64; 70.83)	7.71	0.98	60.6±7.6 (53.3; 95.1)	12.50	1.40
Anal fin basis length (D_OPP)	6.69±0.77 (5; 8.1)	11.56	0.16	6.7±0.9 (5; 9)	13.40	0.20
Caudal peduncle length (D_RS)	18.77±1.67 (15.54; 21.61)	8.91	0.36	18.7±2.1 (16.4; 25.7)	11.20	0.40
Maximal body height (H_H)	19.1±1.71 (16.56; 22.82)	8.97	0.37	19.2±2.6 (16.5; 30.5)	13.30	0.50
Minimal body height (L_H)	6.95±0.82 (5.38; 9.03)	11.82	0.17	7±1 (5.9; 11.1)	13.80	1.00

The results of the analyses of morphological traits:

Since the specimens in the first year of sampling were quite small, i.e. in the juvenile stage (except one individual from the site Vrilo), the sample of 51 individuals (50 from 2015 and one from 2014) was sufficiently representative for the analysis. The comparison of morphometric characteristics between males and females in the total sample of measured individuals from all age groups was made using methods of descriptive statistics (Table 1). The highest variability in *A. huegelii* males was recorded for body mass, maximal body height (H_H) and anal fin basis length (D_OPP). In females, the highest variability was recorded for body mass (B_M), pre-orbital length (P_P) and minimal body height (L_H) (Table 1). Average body mass (B_M), standard and

total body length (T_D, S_D) values were higher in *A. huegelii* females (Table 1). An absence of statistically important differences, tested by two-tailed t-tests between males and females, at the level of statistical significance of $P < 0.05$, was the result of a high level of variability within the groups.

The Principal Component Analysis (PCA) of morphometric parameters in the Dalmatian barbelgudgeon separated 16 components, where the first two components describe 96% of the total sample variability (Table 2). The most important variables in the construction of the first component were total body length (T_D), pre-dorsal length (AD_R) and standard body length (S_D).

A high correlation between fish age and total body length affects the distribution and individual

Table 2. Eigenvalues and percentage of variability for the first two components (PC) of the species *Aulopyge heugelii* from the Buško Blato reservoir.

PC	Eigenvalue	% of variance
1	329.715	94.01
2	6.94649	1.98

Table 3. Results of statistical analysis of morphometric traits on adult Dalmatian barbelgudgeon from the Buško Blato reservoir (AV – average, SD – standard deviation, CV – coefficient of variability, SE – standard error).

Characters	Males N = 16			Females N = 22			Total sample N = 38		
	AV±SD (min; max)	CV	SE	AV±SD (min; max)	CV	SE	AV±SD (min; max)	CV	SE
B_M (g)	10.26±2.30 (8.10; 16.00)	5.31	0.57	11.23±6.90 (6.90; 41.20)	47.55	1.47	10.82±5.42 (6.90; 41.20)	50.07	0.88
T_D (mm)	107.34±5.39 (101.00; 121.00)	29.00	1.35	108.93±11.31 (97.00; 155.00)	127.82	2.41	108.26±9.22 (95.00; 155.00)	8.51	1.50
D_T_V (mm)	94.19±6.02 (82.00; 109.00)	36.29	1.51	95.77±10.06 (85.00; 133.00)	101.14	2.14	95.11±8.53 (82.00; 133.00)	8.96	1.38
S_D (mm)	88.13±5.88 (81.00; 102.00)	34.55	1.47	89.48±9.83 (81.00; 128.00)	96.56	2.10	88.91±8.32 (81.00; 128.00)	9.36	1.35
AD_R (mm)	47.16±2.88 (44.34; 55.56)	8.30	0.72	47.34±5.16 (41.99; 68.15)	26.62	1.10	47.26±4.29 (41.99; 68.15)	9.10	0.69
D_OLP (mm)	9.89±0.79 (9.00; 11.00)	0.62	0.20	10.22±1.15 (9.00; 13.00)	1.32	0.24	10.08±1.01 (9.00; 13.00)	10.07	0.16
D_G (mm)	23.94±1.73 (22.01; 29.09)	2.98	0.43	24.2±2.42 (21.95; 34.15)	5.87	0.52	24.09±2.13 (21.95; 34.15)	8.86	0.35
P_P (mm)	9.46±0.86 (8.57; 12.00)	0.74	0.21	9.50±1.44 (8.24; 15.44)	2.09	0.31	9.48±1.22 (8.24; 15.44)	12.85	0.19
D_O (mm)	4.56±0.38 (4.10; 5.50)	0.15	0.10	4.48±0.43 (3.92; 5.69)	0.18	0.09	4.51±0.40 (3.92; 5.69)	8.93	0.06
Z_P (mm)	9.98±0.83 (9.00; 12.27)	0.68	0.20	10.23±0.77 (9.36; 13.02)	0.59	0.16	10.11±0.79 (9.00; 13.02)	7.83	0.13
AV_R (mm)	45.71±2.53 (43.22; 52.82)	6.39	0.63	46.18±5.62 (40.67; 69.53)	31.48	1.19	45.97±4.53 (40.67; 69.53)	9.85	0.74
AA_R (mm)	61.82±3.86 (55.74; 70.83)	14.89	0.96	62.08±7.88 (56.03; 95.11)	62.12	1.68	61.97±6.48 (55.74; 95.11)	10.37	1.04
D_OPP (mm)	6.82±0.64 (6.00; 8.00)	0.41	0.16	6.92±0.89 (5.00; 9.00)	0.79	0.19	6.88±0.79 (5.00; 9.00)	11.44	0.13
D_RS (mm)	19.42±1.37 (16.83; 21.61)	1.86	0.34	19.09±2.19 (16.41; 25.71)	4.83	0.47	19.23±1.88 (16.41; 25.71)	9.77	0.30
H_H (mm)	19.74±1.45 (17.76; 22.82)	2.10	0.36	19.71±2.67 (17.17; 30.54)	7.11	0.57	19.73±2.21 (17.17; 30.54)	11.21	0.36
L_H (mm)	7.23±0.71 (6.44; 9.03)	0.49	0.17	7.25±0.99 (6.34; 11.12)	0.98	0.21	7.24±0.87 (6.34; 11.12)	12.31	0.14

grouping along the first PCA component axis (Fig. 4).

The ANOVA analyses of the Dalmatian barbelgudgeon carried out on 38 adults of ages 3+, 4+, 5+ and 6+ have shown no statistically significant differences between males and females from the Buško Blato reservoir. In the total sample, body mass (B_M) had the highest variance. In males, the highest

variability was detected for fork body length (D_T_V) and in females, the total body length (T_D) (Table 3.).

The Kolmogorov-Smirnov test was used for testing normal distribution. Absolute value (D) on the total sample of adult individuals was from 0.075 for the caudal peduncle length (D_RS), to 0.28 for body mass (B_M). Statistical significance at the level of

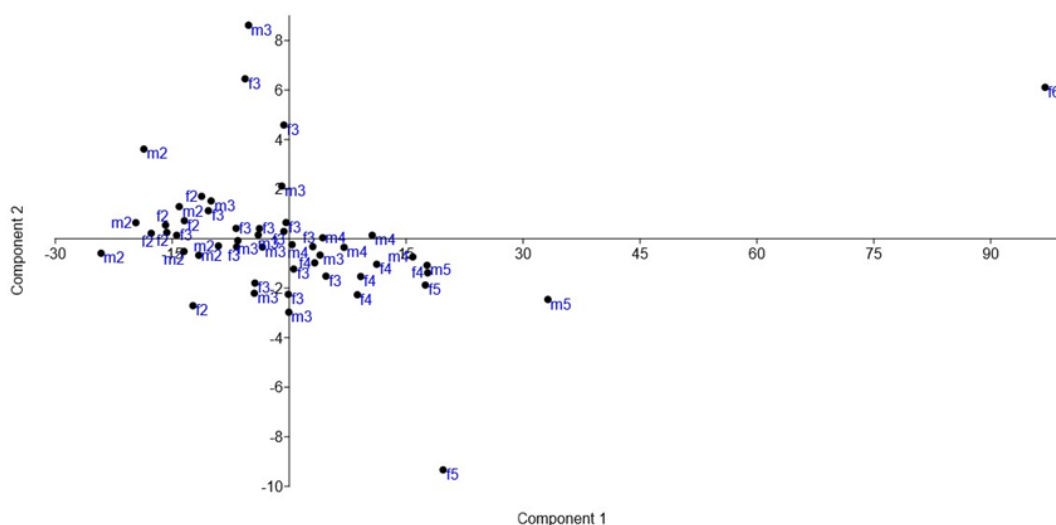


Fig.4. Biplot for the first two PCA components, samples of *Aulopyge huegelii* from the Buško Blato reservoir: f – female, m – male, numbers 2-6 represent age of the individuals.

Table 4. Results of One-Sample Kolmogorov-Smirnov test on the sample of adult individuals of the species *Aulopyge huegelii* from the Buško Blato reservoir (N = 38).

Morphological parameters	Absolute D value	p value
B_M	0.283	0.005
D_G	0.233	0.032
P_P	0.234	0.031
AV_R	0.232	0.034
AA_R	0.223	0.046
D_RS	0.075	0.984

$P < 0.05$ was recorded for body mass (B_M), head length (D_G), pre-orbital length (P_P), pre-ventral length (AV_R) and pre-anal length (AA_R) (Table 4).

The results of One-Sample Kolmogorov-Smirnov test on the sample of male individuals of the Dalmatian barbelgudgeon (16) have shown the absence of a statistically significant difference for all analysed parameters. One-Sample Kolmogorov-Smirnov test D value was in the range from 0.120 (pre-anal length - AA_R) to 0.299 (anal fin basis length - D_OPP). The sample of female individuals of *A. huegelii* (22 individuals) showed a statistically significant difference for the following parameters: body mass (B_M), pre-anal length (AA_R) and the maximal body height (H_H). The smallest Kolmogorov-Smirnov test D value was shown for the caudal peduncle length (D_RS) (0.120), and the

largest for body mass (B_M) (0.382). The results of the t-test have not indicated any statistically significant difference in the analysed morphometric traits between males and females.

Since the results of the Kolmogorov-Smirnov test have shown a statistically significant difference for certain morphological parameters, continued data have been transformed into ranks, and non-parametric analysis, using the Mann-Whitney test, was carried out on adults in accordance with the results of the t-test. The obtained results were in the rank 0.198-1.000, and they indicated no statistically significant difference for $P < 0.05$. The highest values in the Mann-Whitney test were for body mass (B_M) (176.0), and the lowest for post-orbital length (Z_P) (132.5).

A comparison of previous morphological data from Guzina (2000) and our research indicated a

Table 5. Comparative analysis of morphological parameters in *Aulopyge huegeli* 3+ year old individuals; average values according to Guzina, 2000 and studies conducted in 2015.

Morphological parameter	Mean value by Guzina (2000)	Mean value of recent study	Difference	One sample t-test	p-value
Body mass	115.04	10.82	104.22	118.53	P<0.0001
T_D	215.31	108.26	107.05	71.60	P<0.0001
S_D	183.94	88.91	95.03	70.39	P<0.0001
D_G	51.36	24.09	27.27	78.74	P<0.0001
P_P	22.80	9.49	13.32	67.32	P<0.0001
D_O	6.51	4.52	1.99	30.47	P<0.0001
Z_P	22.63	10.11	12.52	97.39	P<0.0001
AD_R	98.99	47.26	51.73	74.17	P<0.0001
AV_R	102.30	45.98	56.32	76.65	P<0.0001
AA_R	135.44	61.97	73.47	70.46	P<0.0001
D_RS	41.45	19.23	22.22	72.92	P<0.0001
D_OLP	20.65	10.08	10.57	64.24	P<0.0001
D_OPP	12.05	6.88	5.17	40.52	P<0.0001
H_H	48.62	19.73	28.89	80.56	P<0.0001
L_H	16.69	7.24	9.45	66.81	P<0.0001

statistically significant decline in body size for 14 analysed parameters. In adult individuals, the difference between morphometric data according to Guzina (2000) and our measurement was significant by all morphometric parameters. The differences in body mass, total body length and standard body length are important for understanding the adaptive response of *A. huegeli* in the Buško Blato reservoir. One sample t-test was significant for all compared characteristics, with the highest p-value for body mass. Individuals of the most dominant age group (3+) were compared, in order to evaluate the trend in the variation of morphological parameters (Table 5). The highest differences were recorded for the same parameters as in the sample of adult individuals.

The results of a paired samples t-test of average values of analysed morphological parameters in adults have shown a statistically significant reduction of nominal value in our study (average difference – 36.41; $t=-3.985$; $P=0.0011$). According to the analyzed study there is a high difference in average values of all analysed parameters, in both adult individuals and the age group 3+.

The results of the analyses of ecological traits: Basic physical and chemical parameters in the Buško Blato reservoir (temperature, pH, dissolved oxygen concentration, saturation with O₂, electric

conductivity) were measured during field research in 2014 and 2015. During the research in November 2014, all measured parameters were within standard values, except electric conductivity, which was very high, probably due to the connection with groundwaters. Water temperature in the Buško Blato reservoir in September 2015 varied from 10 to 16.4°C, pH value was 8.4-9.1, dissolved oxygen 8.6-9.9mg/l, oxygen saturation 101.2-106.2% and electric conductivity 122-227µS/cm. These basic physical and chemical parameters indicate waters of the first class in the Buško Blato reservoir. However, based on the concentration of other parameters (KMnO₄, nitrates, phosphates, ammonia, chromium, copper and chlorophyll “a”) waters of the Buško Blato reservoir belong to the second class. Ammonium and nitrate have not been recorded, while concentrations of both nitrates and phosphates varied within the range which is ideal for the life of cyprinids.

Analysis of BQE showed satisfactory results. Diatoms (*Bacillariophyta*) had the highest percentage (68%) in phytoplankton from the Buško Blato reservoir. The dominant role in zooplankton was played by Copepoda (43%), Rotatoria (30%) and Cladocera (21%). These organisms indicate beta mezosaprobic waters, while in zoobenthos, the



Fig.5. The Dalmatian barbelgudgeon - *Aulopyge huegeli* with visible marks of predator bites on its tail (September 2015, site: Prisoje).

Table 6. Absolute values of the number of the individuals according to sex and age.

Sex structure		All gathered individuals (88)					
		Age structure					
males	females	1+	2+	3+	4+	5+	6+
42	46	27	16	30	10	4	1
		(15M, 12F)	(8M, 8F)	(12M, 18F)	(5M, 5F)	(2M, 2F)	(F)

dominant role was played by species from the Diptera order, family Chironomidae (32%).

The results have shown a presence of 11 fish species from four families. It is necessary to stress that seven fish species are allochthone ones: Carp, Tench, Prussian Carp, False Harlequin, Pumpkinseed, Pikeperch and Wells Catfish. The most abundant species was the Prussian carp (38.83%), while the Dalmatian barbelgudgeon was present with 17.48%. Although the percentage participation of this species in the whole sample is not small, it is necessary to take into account that it was caught at just few neighbouring sites, in one secluded part of the Buško Blato reservoir. The sampling intensity was oriented towards catching the Dalmatian barbelgudgeon required for this research. Thus, we have biased the frequency of the Dalmatian barbelgudgeon within the sample.

The annual natural growth for all fish species was estimated at 239 individuals per hectare of water area. The Dalmatian barbelgudgeon is the second most abundant fish species in this ecosystem, with 154 individuals/ha, and its expected growth rate is 47 individuals/ha. From eleven fish species registered in

our study, only four (36.63%) are autochthone. Four new allochthone species (Pike-perch, Pumpkinseed, False Harlequin, and Tench) were introduced into this ecosystem, as competitors and potential predators of the Dalmatian barbelgudgeon (Fig. 5).

The dynamics of fish populations were determined on the basis of the analysis of sex and age structure on 88 individuals of the Dalmatian barbelgudgeon (42 males and 46 females). The age of individuals ranged from 1+ to 6+ years old, as follows: 27 (1+), 16 (2+), 30 (3+), 10 (4+), four (5+), and only one individual was 6+ (Table 6.). In the age structure, younger individuals have a dominant role, with sexually mature individuals of age 3+ (34%) as the most dominant group. The sex ratio slightly deviates from the theoretically expected one, with the domination of female individuals (52%). It is important to stress that females have a dominant role in the most abundant age group (3+).

Discussion

During field research we have gathered 88 individuals of *A. huegeli* Heckel, 1843, at four neighbouring sites in a small and isolated part of the

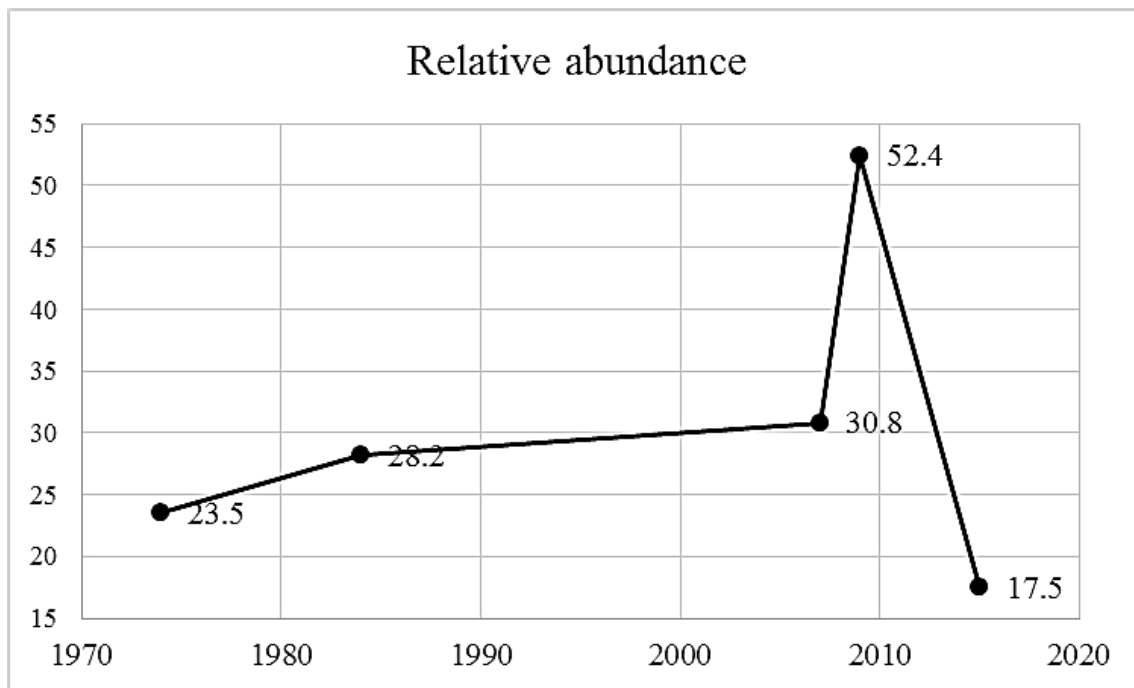


Fig.6. Relative abundance of *Aulopyge huegelii* in Buško Blato since 1975.

Buško Blato reservoir. Although Žujo Zekić (2009) registered 331 individuals of this species at the site Golinjevo, which is the largest number compared to all the sites analysed in this study, we have not found any individuals here.

A comparison with results of previous investigations, when the Dalmatian barbelgudgeon was the dominant species, revealed a significant decrease of its population (Guzina 2000; Bogut et al. 2007; Žujo-Zekić 2009). *Aulopyge huegelii* was not only one of the leading species (in terms of number and density), but was distributed along the entire beneficial water surface of the Buško Blato reservoir, at all sites and in all annual seasons. Since 1974 an increase in the relative abundance of *A. huegelii* in the Buško Blato reservoir was recorded. The first data that can be tracked and compared are the fishing management plans for the Buško Blato reservoir from 1974, 1984, 2007 and 2015. The increase in *A. huegelii* abundance in 1974 was 23.5% of individuals in the total sample. In 1984 it was 28.2%. Recent investigations of the Dalmatian barbellgudgeon population in the Buško Blato reservoir are limited to two surveys conducted by Bogut et al.

(2007) and Žujo Zekić (2009), when it was represented with 30.8% and 52.4%, respectively. In our study, the relative abundance of *A. huegelii* was 17.5% of the total sampled fish individuals from the Buško Blato reservoir, which was a significant decrease and the first sign of population decline and population structure change (Fig. 6).

Morphological methods of phenotype analysis have been used for comparison of individuals exposed to various factors and analysis of population diversity, and they are often the sources for investigations of phylogenetic relationships among certain fish species (Klykanov 1975; Shaposhnikova 1975). Aganović & Vuković (1968) have concluded that changes in morphological traits in the Dalmatian barbelgudgeon from waterways in Livanjsko polje occur with age. They also stress that this fact should be taken into account particularly when analysing morphometric traits, which are very useful in detection of potential changes. The results of the analyses of morphometric traits of *A. huegelii* have shown neither statistically significant variations within the population of this species in the Buško Blato reservoir, nor differences between sexes, which

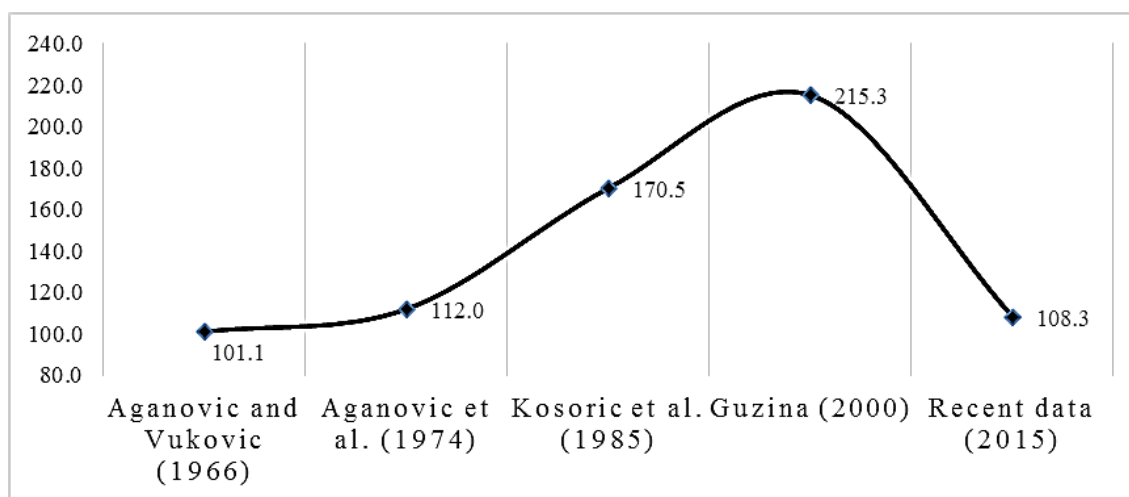


Fig.7. Comparison of average total body length of *Aulopyge huegelii* from investigated sites at the Buško Blato reservoir in period 1966-2015.

would indicate a homogenization of the population.

Recent morphological data on the Dalmatian barbelgudgeon from the Buško Blato reservoir have been compared to earlier data. Morphological data on population structure indicated significant changes in body mass, standard and total body length. The changes were directed toward body mass and general body size decline. The morphological changes in population structure occurred in a short period of time. Earlier morphological data on population structure indicated an average *A. huegelii* body size to be statistically larger than in the recent Buško Blato population (Guzina 2000). The said changes in population structure, showing a decline in the population number and individual body size, indicate recent poor conditions in the Buško Blato reservoir.

Aganović & Vuković (1966, 1868) investigated the *A. huegelii* populations from the Livanjsko field (before the Buško Blato reservoir was formed) and the Glamočko field. They recorded *A. huegelii* with an average standard body length between 70.15 and 94.60 mm. Aganović et al. (1974) analysed the ichthyopopulation structure of Buško Blato after the reservoir was formed, and they provided data indicating average standard body lengths for different fish age groups: 1+ 68.9mm; 2+ 96.06mm; 3+ 115.15mm; 4+ 124.45mm; 5+ 131.16mm; 6+ 135.8mm, and total body lengths: 1+ 57.44mm; 2+

93.63mm; 3+ 101.05mm; 4+ 110.15mm; 5+115.75mm; 6+ 155mm. Maximal standard body length of *A. huegelii* from Aganović & Vuković (1966) and Aganović et al. (1974) studies indicated similar average standard body lengths.

Ten years after the Buško Blato reservoir was formed, Kosorić et al. (1985) investigated it, and the results of these investigations indicated significant changes in the Dalmatian barbelgudgeon growth. Individuals with body length of 201-250mm formed the largest group, while in our investigations, the total body length varies from 91 to 155mm. In previous investigations body mass was in the range from 51 to 100 g, while in our investigations it varied from 5.8 to 41.2g. Therefore, the Dalmatian barbelgudgeon was much bigger and with larger body mass in the previous period. A comparison of the results of morphological parameters according to Guzina (2000) and our investigations indicate statistically significant differences for all analysed parameters (Fig. 7).

From the first morphometric data on *A. huegelii* populations from the Buško Blato reservoir, we can track a rapid increase in body length and mass (Aganović & Vuković 1966; Aganović et al. 1974; Kosorić et al. 1985; Guzina 2000). The increase in body size was due to the new favourable ecological conditions created by the reservoir construction. The

new ecological conditions were favourable to the extent that they induced an occurrence of scales on *A. huegeli*; recorded individuals were treated as lake ecomorphs (Guzina 2000).

Until recent years *A. huegeli* was thriving in the Buško Blato reservoir, but our research showed a decline in population number and body size. According to our data the population of *A. huegeli* in the Buško Blato reservoir is now by its morphological structure more similar to the population recorded by Aganović & Vuković (1966). These findings support the theory on the retreat of the Dalmatian barbelgudgeon into groundwaters and its “return” to its original morphological form i.e. a groundwater fish form, with smaller body dimensions. The main causes could be: competition with allochthone species, frequent retreat into groundwaters in order to avoid predators and potential lack of food in this secluded part of the reservoir.

Water quality is the most important abiotic factor for the general state of the fish community in open waters, because it has been established that in the waters of poorer physico-chemical composition, we find fewer individuals than in waters of satisfactory quality (Kosorić et al. 1984). Although the Dalmatian barbelgudgeon as a groundwater fish species is adapted to the lack of food and dissolved oxygen in its habitat, as an endemic species it has limited capabilities to adjust to changes in abiotic environmental factors (Glamuzina et al. 2010). For fishes as poikilotherms, water temperature is a limiting factor in the control of the metabolism, biological activity, nutrition, sexual activity, etc. Spawning of the Dalmatian barbelgudgeon begins in April, at water temperatures of 13-17°C, and it lasts until the end of June; and according to Bless & Riehl (2002), in laboratory conditions the spawning begins at 20°C. Measured temperatures at the time of spawning had the indicated values. During the sampling period, no significant deviations from the average/mean or allowed values were recorded in any of the measured parameters. Based on the analysis of

all reliable data from previous studies, it could be concluded that there were no significant changes in environmental conditions in the Buško Blato reservoir (Žujo Zekić 2009; Kosorić et al. 1985). Therefore, physical and chemical characteristics of this aquatic ecosystem are suitable for living communities, including the Dalmatian barbelgudgeon.

However, it is necessary to point out that this was probably not the case in the previous period. Has-Schön et al. (2008) have measured elevated concentrations of cadmium in kidneys and livers of four fish species, among them the Dalmatian barbelgudgeon. This metal had probably been washed out from the agricultural areas near the reservoir. There is also documented data from 2011 showing a massive recorded fish die-off in the Buško Blato reservoir as well as high measured concentrations of organic compounds, especially nitrites, nitrates, phosphates and ammonia. The mass fish die-off could be one of the reasons for population decline and structural change in the Dalmatian barbelgudgeon.

Key biological quality elements imposed by the Water Framework Directive are phytoplankton, macrophytes, macroinvertebrates, and ichthyofauna. Due to its short life cycle and capability to absorb nutrients from water column, phytoplankton is the most direct and very important indicator of impacts of changes in lake ecosystems. Diatoms, which were the most represented in phytoplankton from the Buško Blato reservoir, are the main plant component in the nutrition of the Dalmatian barbelgudgeon (Aganović et al. 1967; Kačanski et al. 1978; Crivelli 1996; Guzina 2000). According to Guzina (2000), larvae of Chironomidae (Diptera), as the most abundant in the zoobenthos (as recorded in our investigation), were the only representatives of animal components in the nutrition of the Dalmatian barbelgudgeon in the Buško Blato reservoir, while results of previous investigations indicate a nutrition with copepods (*Aseillus*), family Gammaridae, and larvae from the order Trichoptera (Aganović et al.

1967, 1974; Kačanski et al. 1978).

The structure of the ichthyofauna was the most important element for the analyses of interspecies relationships, including primarily predation and competition. Compared to earlier data, the presence of 11 fish species is the largest number of fish species ever recorded in the Buško Blato reservoir. Some species were registered for the first time in this ecosystem, the domination of allochthone species (seven) was recorded as well as the absence of some previously recorded species.

During the last five years, there have been repeated reports from the local anglers' associations on the rapid decrease in the abundance of this endemic species in the reservoir, probably due to severe anthropogenic pressure and competition with invasive species such as Carp, Tench, Prussian Carp and Pikeperch. Aganović et al. (1974) have recorded a presence of seven fish species from two families: Cyprinidae: *Aulopyge huegelii* Heckel, 1843, *Phoxinellus alepidotus* Heckel, 1843, *Chondrostoma phoxinus* Heckel, 1843, *Telestes turskyi* Heckel, 1843, *Scardinius dergle* Heckel & Kner 1858, and Salmonidae: *Salmo trutta lacustris* Linnaeus, 1758, and *Salmo dentex* Heckel, 1852. Cvijović and Kosorić (1985) have recorded five fish species (one salmonid and four cyprinid). After the construction, Prussian Carp - *Carassius gibelio* (Bloch, 1782), Common carp - *Cyprinus carpio* Linnaeus, 1758 and Catfish - *Silurus glanis* Linnaeus, 1758 were introduced in the reservoir. The results of recent investigations (Žujo Zekić 2009) have shown a presence of nine fish species from four families, Cyprinidae: *C. phoxinus* (Hackel, 1843), *T. turskyi* (Heckel, 1843), *A. huegelii* (Heckel, 1843), *C. carpio* (Linnaeus, 1758), *C. a. gibelio* (Bloch, 1782), *Hypophthalmichthys molitrix* (Valenciennes, 1844); Salmonidae: *S. trutta* (Linnaeus, 1758) (*S. t. lacustris*); Siluridae: *S. glanis* (Linnaeus, 1758), and family Ameiuridae (Ictaluridae), with *Ameiurus nebulosus* (Le Sueur, 1819).

Investigations from 2009 indicate that autochthonous fish species have a dominant role, and

78% of the total caught individuals were endemic fish species. Only 22% were allochthone fish species (Carp, Prussian Carp, Wels Catfish, Silver Carp and Brown Bullhead), which have been introduced either by sport fishermen or for controlled growth. In our study, only four fish species (36.63%) are autochthone. When compared to previous research (Bogut et al. 2007; Žujo Zekić 2009), recent investigations indicate an introduction of four new allochthone species in this ecosystem. As competitors and potential predators, they represent a significant threat to the population of the Dalmatian barbelgudgeon. The results of these activities are indicating significant changes in both the structure and composition of the ichthyofauna in this ecosystem. We are witnessing the daily and unplanned introduction of allochthone fish species. Introduced allochthone species are in many cases more resistant and aggressive, and they gradually suppress the native populations, so it is not rare that indigenous populations disappear in this way.

Niche is a special form of survival of species in a certain position in the general "economy" of nature and food nets (Škrijelj & Đug 2009). More than 80 years after Grinnell's definition of niche, researchers have enabled the measurement of the so called niche „space“ in given conditions for a selected group of fish species (Nagelkerke & Rossberg 2014). A multidimensional or fundamental niche is different from a realized niche. A realized niche is limited in space due to the resilience of the environment and it usually represents a small part of the fundamental niche. Dimensions include all ecological factors and resources, which are defined by the values of ecological valence and changes in the abundance and growth rate of given species (Stevanović and Janković 2001). Most attempts which focused on researching ecological niches analysed the trophic composition and compared spatial distribution between target species (Sampaio et al. 2013). Competition leads to specialisation of individuals, who then use a part of a subset of resources, enabling them to avoid competition with other species as much

as possible. Predation narrows the fundamental niche. Changes in niche also happen when organisms retreat into a zone which has the smallest overlap with predators. The most important competitors are the introduced alien species. The main problem with invasive aquatic species is that it is almost impossible to eradicate them after their adaptation (Chen et al. 2007).

After the formation of the Buško Blato reservoir, Aganović et al. (1974) have concluded that, on the basis of its hydrological regime, this is one of the rare reservoirs which creates and enables suitable conditions for life and further growth of various fish species. Ichthyological investigations conducted after 1974 have shown that population density have been increased. The results of our investigation have indicated that there is enough food in the reservoir. However, it is very doubtful that this amount of food will suffice in this small and secluded part of the reservoir and that the Dalmatian barbelgudgeon would be able to use it, due to its retreat into groundwaters. Also, it is necessary to stress that competitors also reduce the amount of available food.

Therefore, the fundamental and previously realized niche is narrowed to a very small area as a result of interspecific competition and predation. A comparison with results of previous investigations has shown that the niche of the Dalmatian barbelgudgeon has rapidly decreased. Žujo Zekić (2009) collected the species at all sites (10) during two years of investigations. In the total sample of 2714 individuals, *A. huegelii* was the most abundant species, with 1422 individuals (52.4%). All this leads to the process of enclosure of the population, which could in perspective have significant consequences on the survival of the population of this species in the Buško Blato reservoir. On the other hand, the presence of predators such as Pike perch and Catfish in a non-adequate niche for them, could be explained by their chase for food, like the Dalmatian barbelgudgeon, for example, leading to a reduction of its abundance and absence of larger individuals. Finally, this could also cause a complete retreat of the

species into groundwaters.

Previous investigations have also emphasized that the largest impact on fish communities in the Adriatic Sea basin in Bosnia and Herzegovina was caused by the introduction of alien species (Mrakovčić et al. 2006; Tutman et al. 2008; Piria et al. 2018). The results of our study have shown that the impact of allochthone species could lead in the potentially worst but a very realistic scenario, to the eradication of the Dalmatian barbelgudgeon from the Buško Blato reservoir.

The sex ratio indicates suitable reproductive capabilities of the population of this species in the Buško Blato reservoir, as it has also been shown in previous studies (Aganović et al. 1974; Kosorić et al. 1985; Guzina 2000), when females have played an even more dominant role. The age structure is also in accordance with results of previous researches (Aganović et al. 1974; Guzina 2000). The results of the analysis of both sex and age structure indicate a positive growth rate dynamics of the Dalmatian barbelgudgeon population.

However, a very small number of older individuals (5+ and 6+), five in total, could be an indicator of a decrease of the population of the Dalmatian barbelgudgeon. The lifespan of this species is around eight years (Kačanski et al. 1978), maximum 15 (Sofradžija 2009), and they were not found during our study. The age group 6+ has not shown to be more abundant in previous studies carried out in the Buško Blato reservoir (Aganović et al. 1974; Guzina 2000), while in 1974 the age group 5+ was represented with 14.13% in the total sample. A realistic assumption is that older, and therefore larger individuals, are more attractive as a food source for predators.

The results of the comparison of age structure of the Dalmatian barbelgudgeon from the Buško Blato reservoir with previous findings by Guzina (2000) indicate certain similarities (Fig. 8). The age group 3+ is dominant in both studies, but it was more abundant in the past. The highest level of differences is present in the youngest age group (1+), where

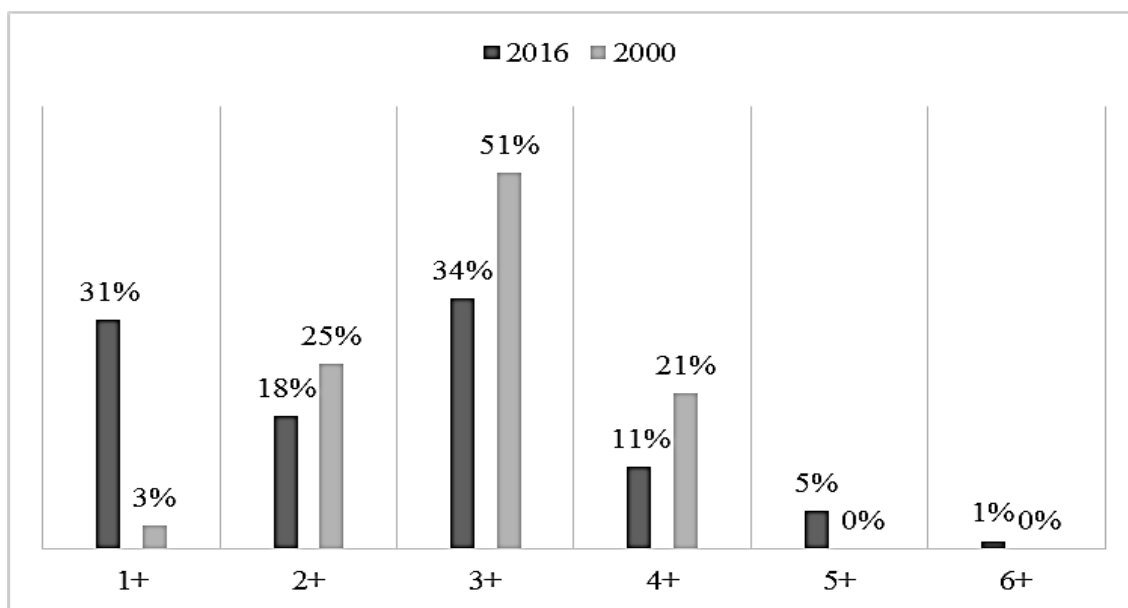


Fig.8. Comparison of the relative values of the age structure with the earlier research of the Dalmatian barbelgudgeon from the Buško Blato reservoir (Guzina, 2000).

significantly more individuals from this group were present in our study compared to Guzina (2000). The results of previous investigations have shown that the youngest individuals were represented with just 3.19% (Aganović et al. 1974). This would be a satisfactory result if other age groups followed this percentage, which was not the case.

Results of the analyses of morphometric traits of *A. huegeli* Heckel, 1843 have not shown either statistically significant variations within the population of this species in the Buško Blato reservoir, or differences between sexes, which would indicate a homogenization of the population. A comparison of the results of the analyses of morphometric traits with the results of several previous investigations leads to the conclusion that the Dalmatian barbelgudgeon is returning to its original morphological form, which is the form of a typical groundwater fish with smaller body size.

Based on the analysed water quality parameters, we have concluded that the Buško Blato reservoir belongs to the second class water quality and the zooplankton indicate a beta-mezosaprobic water. Therefore, this aquatic ecosystem is suitable for living fish communities including the Dalmatian barbelgudgeon. Since the main nutrient components

of the Dalmatian barbelgudgeon are very abundant (the *Bacillariophyta* in the phytoplankton and larvae of Diptera from the family Chironomidae in the zooplankton), this represents a solid basis for the survival of the Dalmatian barbelgudgeon in this ecosystem.

The results of our investigations have shown a presence of 11 fish species from four families, which is the largest number of fish species ever recorded in this ecosystem. Pike-perch, Pumpkinseed, False Harlequin, and Tench, as competitors and potential predators of the Dalmatian barbelgudgeon, have been recorded for the first time in this reservoir. The ecological niche of *A. huegeli* has been narrowed and changed, which could be caused by the introduction of four allochthone fish species.

Although both sex and age structure indicate a positive rate of population dynamics, a small number of individuals from the age groups 5+ and 6+ is troublesome, since it could indicate that an insufficient number of individuals reach older age because they become prey for allochthone fish or they simply retreat into groundwaters.

The results of both aspects of the research coincide and are complementary. They show a specific spatial isolation of the Dalmatian

barbelgudgeon population in a small part of the Buško Blato reservoir where the groundwater supply is at the highest level.

This study is one of the ways to point to the adverse consequences that unconscious and unplanned introduction of fish species may have on the Dalmatian barbelgudgeon as a very important stenoendemic species of Bosnia and Herzegovina. Due to a very questionable status of this species in the Buško Blato ecosystem, which could present some sort of a reservation for this species, and since there is still a certain realistic level of environmental basis for the revitalization, it is necessary to take urgent action. This action should include various protection measures through joint activities of state structures, public and private sector and research institutions.

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مقاله پژوهشی

وضعیت گونه در خطر انقراض *Aulopyge huegelii* Heckel, 1843 (ماهیان استخوانی عالی):

کیورماهیان) در سد مخزنی بوسکو بلاتو بوسنی و هرزگوین

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چکیده: کیورماهی دالماتی *Aulopyge huegelii* یک گونه بومزاد با پراکنش محدود است که در ناحیه غرب بوسنی و قسمتی از دالماتی یافت می‌شود. از نظر اتحادیه بین‌المللی حفاظت از محیط زیست (IUCN) این ماهی به‌عنوان یک گونه در خطر انقراض در نظر گرفته شده است. منابع قابل اطمینان که حاوی اطلاعات مربوط به این گونه، به‌ویژه در دوره اخیر باشد در دسترس نمی‌باشد. گرچه اساساً به‌عنوان یک گونه سازش یافته با آب‌های زیر زمینی در نظر گرفته می‌شود، اما به‌طور استثنایی با شرایط زیست محیطی سد مخزنی بوسکو بلاتو نیز سازش پیدا کرده بود. بر اساس یافته‌های اخیر، وضعیت کیورماهی دالماتی در چند سال گذشته تغییر کرده است. هدف اصلی این مطالعه مشخص نمودن وضعیت کنونی جنس تک گونه‌ای کیورماهی دالماتی در سد مخزنی بوسکو بلاتو از طریق آنالیز شماری از ویژگی‌های ریخت‌شناختی و اکولوژیکی انتخابی بوده است. در طی عملیات میدانی پژوهشی، ۸۸ نمونه از این ماهی از ۴ سایت مجاور هم جمع‌آوری گردید. کاهش معنی‌داری در اندازه جمعیت این ماهی در مقایسه با مطالعات گذشته مشهود بود. نتایج نشان می‌دهد که هم‌اکنون دامنه پراکنش آن به بخش کوچکی از سد مخزنی محدود است که به عنوان زیستگاه اولیه آن به سیستم آب‌های زیرزمینی متصل است که علت آن فرار از چهار گونه شکارگر جدید غیربومی معرفی شده به این اکوسیستم است. با مقایسه نتایج این مطالعه با مطالعات قبلی می‌توان چنین نتیجه گرفت که کیورماهی دالماتی در حال برگشت به فرم ریخت‌شناسی اصلی یعنی فرم وابسته به سیستم آب‌های زیرزمینی است. این موضوع می‌تواند پیامدهای قابل توجهی بر بقای جمعیت این گونه ماهی در سد مخزنی بوسکو بلاتو داشته باشد.

کلمات کلیدی: کیورماهی دالماتی، حفاظت، گونه در معرض تهدید، ریخت‌شناسی، اکولوژی.