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SEASONAL VARIATIONS OF MORPHOMETRIC AND HEMATOLOGICAL PARAMETERS IN DALMATIAN BARBELGUDGEON *Aulopyge huegelii* HECKEL 1843 FROM ŠATORSKO LAKE

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ABSTRACT

As an endemic species, Dalmatian barbelgudgeon was introduced into Šatorsko Lake, but it was insufficiently explored at this locality. The aim of this study was to determine seasonal variations of physicochemical parameters of the water in relation to morphometric and hematological parameters of Dalmatian barbelgudgeon from Šatorsko Lake. Physicochemical analysis of the water recorded lower values of temperature, O_2 concentration, O_2 saturation, electrolytic conductivity and suspended matter in autumn, while other analyzed parameters indicated lower values in spring. The analysis of morphometric parameters (weight, standard length, total length and Fulton's condition factor) determined higher values of all the parameters during spring, while a significant difference was noted only for Fulton's condition factor. A statistically significant difference was observed for all erythrocyte lineage parameters (RBC-red blood cells, HB-hemoglobin concentration, HCT-hematocrit, MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentrations). In white blood cells, a statistically significant difference was recorded for lymphocytes, non-segmented neutrophils and monocytes, while the proportion of segmented neutrophils and basophils was slightly increased in spring. Furthermore, the recorded values of pseudo-eosinophils were equal in spring and autumn. The results of these studies indicate a significant influence of exogenous and endogenous factors on the variability of morphometric and hematological parameters of Dalmatian barbelgudgeon from Šatorsko Lake.

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INTRODUCTION

A key requirement for developing a conservation strategy for any species is understanding its biology (Turan et al., 2006). *Aulopyge hugelli* is an endemic species of the Adriatic Basin and Dinaric karst, listed on the Red List of the Federation of BiH (Škrijelj et al., 2013) and classified as Endangered (EN) according to B1ab (iii, v) criteria. The natural habitat of Dalmatian barbelgudgeon in BiH includes the waters of Glamoč, Livno and Duvno fields, as well as Buško Lake (Mihinjač, 2018). According to Čurčić (1917), Dalmatian barbelgudgeons were introduced from Buško Lake into Blidinje Lake. In the 1970s and 1980s, Dalmatian barbelgudgeon was initially introduced to Šatorsko Lake (Delić et al., 2005). Furthermore, there is evidence that barbegudgeons were introduced to Mostarsko Blato as well (Šanda et al., 2005).

The identification of species in their native habitat depends heavily on knowledge of fish morphometric parameters, nutrition, and reproductive factors (Salhi et al., 2021). Understanding the biology and ecology of endemic fish species is necessary for their preservation, which certainly implies studies of morphometric parameters of fish (Ivanc et al., 2012). The morphological differences between fish are influenced by a number of environmental factors such as temperature, salinity, dissolved oxygen, radiation, water depth and current flow (Pollar et al., 2007; Kashei et al., 2012; Sheiner et al., 1999; Smith et al., 1966). As a result of complex influences of the external environment of a particular season, organisms develop necessary functional adaptations that are evident in a number of measurable changes (Dekić et al., 2009). Fulton's condition factor is used as an indicator of general health at either the individual or population level of fish (Houque et al., 1998), as well as to compare individuals of the same species and to indicate differences in sex, seasons, and sampling sites (Dekić, 2010).

Hematological markers are used as general indicators of health state. The values of erythrocyte and leukocyte lineage parameters are significant indicators of fish health, and their values for a given species help determine normal values typical for that species, as well as the limits of their variation in different phases of their life cycle (Ivanc and Miljanović, 2003). Changes in the body's physiological state and a variety of environmental factors in time and space may have an impact on the blood count (Dekić, et al., 2009). Ichthyohematological studies help understand blood characteristics in relation to the habitat, as well as the adaptability of species to the environment (Acharya and Mohanty, 2019). Some hematological parameters, such as the number and size of red blood cells and the number and distribution of white blood cells, are distinctive for each species (Kekić et al., 1982, Tavares-Dias et al., 2007). There are numerous studies on changes in hematological parameters in relation to season, temperature, biotope, age, and sex (Tadić, 2016). The main limiting factor for the productivity of fish resources in aquatic ecosystems is water quality (Niyoyitungiye et al., 2019). The influence of water quality on fish changes with their size, age and condition. Water parameters such as temperature, turbidity, hardness, alkalinity and dissolved oxygen are just some of the important factors that determine the growth of living organisms in aquatic ecosystems (Smith et al., 2013). Water quality undoubtedly affects all physiological processes of organisms, which is ultimately reflected in changes in the values of hematological parameters (Dekić et al., 2009). Due to the fact that Šatorsko Lake is insufficiently explored from a biological point of view, the aim of this study was to determine the variations of morphometric and hematological parameters of Dalmatian barbelgudgeons in spring and autumn, as well as their dependence on the physicochemical parameters of the water.

MATERIAL AND METHODS

Study site

Šator is a mountain that lies in the northwest of Glamoč at a distance of about 30 km, and in the southeast of Bosansko Grahovo at a distance of about 40 km (Figure 1). It extends between 44°5'44" and 44°13'36" of north latitude and 34°7'23" and 34°23'53" east longitude with respect to the Ferro Meridian, and is named after its pyramidal shape and whiteness of the karst formations (Cvijić, 1899). The highest peak of Šator is Veliki Štor at 1,827 m, and below it lies Šatorsko Lake of glacial origin at 1,450 m above sea level. According to Delić et al. (2005), Šatorsko Lake is 337 m long, 127 m wide and 8 m deep. It flows into the Unac River, which is a tributary of the Una River, a part of the Black Sea Basin.

Water sampling and physicochemical parameters

Water samples from Šatorsko Lake were taken in accordance with the regulations on water sampling. In spring and autumn, samples of 1000 ml of water were taken from a depth of about 20-30 cm. As part of the physicochemical analysis of water, a total of 20 parameters were analyzed. Water temperature, pH, electrical conductivity and oxygen concentration were determined in the field, while other parameters were analyzed by standard methods according to APHA (1989) in the laboratories of the Biotechnical faculty.

Electrofishing

Individuals of Dalmatian barbelgudgeon from Šatorsko Lake were caught using the DC electrofisher IG 200-1 Aqua Tech with a power of 5 kW, after which they were transferred into fyke nets at the sampling site for resuscitation and adaptation after stress.

Fig 1. Sampling site (Šatorsko Lake)

Morphometric parameters

Morphometric parameters considered in this study were total and standard lengths measured with an ichthyometer and the weight (in grams) measured using digital balance. Fulton's condition factor (K) was obtained using the following formula (Šenk, 1953):

$$K = W * 100 / L^3$$

where
W - weight of fish in grams,
L - standard length of fish in centimeters.

Hematological parameters

For the study purpose, blood was collected from the Dalmatian barbelgudgeon individuals by heart puncture with a sharp and wide sterile needle (0.8-1.2 mm). Erythrocyte parameters analyzed in the study included the number of erythrocytes (RBC), hematocrit value (HCT), hemoglobin concentration (HB), while hematological index analysis included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The number of erythrocytes was determined by the standard method in a hemocytometer. Furthermore, the hematocrit value was determined by microhematocrit centrifugation, while the hemoglobin concentration was estimated by Drabkin's cyanmethemoglobin method (Blaxhall and Daisly, 1973). Hematological indices were calculated from RBC, HCT and HB values. Differentiation of elements of the leukocyte lineage was performed by preparing blood smears, fixing them in methanol, and staining the smear according to Pappenheim.

Statistical analysis

The data obtained from this study were statistically processed using Past 4.3 and Excel 2013 software. Descriptive statistics were used for the processing and analysis of the data obtained, while one-way ANOVA was used for the variance analysis with a confidence interval of 95% and an absolute precision of 5%. Multivariate statistics - principal components analysis (PCA) was also used to analyze certain parameters.

RESULTS

The results of the physicochemical analysis of the water from Šatorsko Lake are shown in Table 2. The recorded values of physicochemical parameters indicated slightly lower values of certain water parameters such as temperature, O_2 concentration, O_2 saturation, electrolytic conductivity and suspended matter in autumn compared to spring, while other analyzed parameters had lower values during spring. According to the results of physicochemical parameters of the water from Šatorsko Lake and the Decision on the characterization of surface and groundwater, reference conditions and parameters for water assessment and water monitoring (2014), the analyzed parameters indicate a high or good ecological status, which indicates the absence of anthropogenic influence or very low influence of anthropogenic action, where these conditions do not have a negative impact on the biological world.

Table 1. Physicochemical parameters of the water

Parameters	Measuring unit	Measured values	
		Spring	Autumn
Water temperature	°C	15.8	12
pH		8.61	9.24
O_2 concentration	mg/l	10.71	9.12
O_2 saturation	%	128.7	98.8
Electrolytic conductivity	µS /cm	204	162.7
Suspended matter	mg/l	9.8	7.8
Turbidity	NTU	1.61	5.4
BOD_5	mg O_2/l	2.25	4.15
COD	mg O_2/l	>10	28
Nitrates	mg/l	1.498	5.824
Chlorides	mg/l	25	47.221
Fluorides	mg/l	0.296	0.321
Nitrites	mg/l	0.016	0.036
Phosphorus	mg/l	0.27	2.46
Phosphates	mg/l	0.875	7.653
Ammonia	mg/l	0.023	0.028
Total nitrogen	mg/l	0.044	1.7
Ash	mg/l	14	28
Dry matter	mg/l	196	294
Sulphates	mg/l	29.341	37.241

The values of the morphometric parameters of the Dalmatian barbelgudgeon individuals are shown in Figure 2.

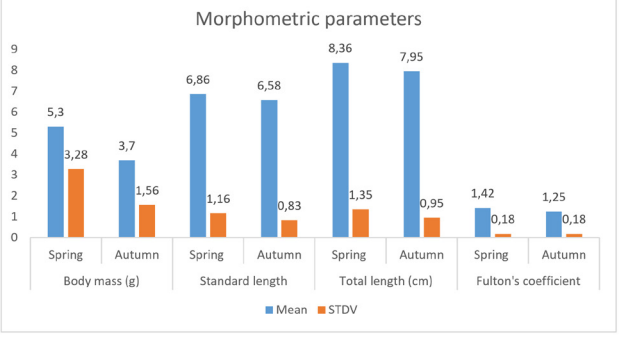

Fig 2. Morphometric parameters of Dalmatian barbelgudgeon

The average weight, standard length and total length of the Dalmatian barbelgudgeon individuals in spring were 5.3±3.28 g, 6.86±1.56 cm, 8.36±1.35 cm, respectively, and Fulton's condition factor was 1.42±0.18. The largest number of individuals weighed between 2.00-14.00 g, had a standard length of 5.30-9.50 cm, total length of 6.50-11.50 cm, and Fulton's condition factor of 1.09-1.80. The recorded average weight of the Dalmatian barbelgudgeon individuals in autumn was 3.70±1.56 g, standard length 6.58±0.83 cm, total length 7.95±0.95 and Fulton's condition factor of 1.25±0.18. The variation intervals for weight, standard length, total length and Fulton's condition factor were 2.00-8.00 g, 5.20-8.40 cm, 6.90-10.10 cm, 0.97-1.68, respectively. Only the values of Fulton's condition factor showed a statistically significant difference, with larger values observed in the spring, which was expected since this is the period before spawning. The values of the other analyzed morphometric parameters were also higher in the individuals of Dalmatian barbelgudgeon in early spring than in autumn. Table 2 shows the results of the hematological parameters of Dalmatian barbelgudgeon in spring and autumn.

Lower values for the parameters of HCT and MCV were observed in autumn, while HB, RBC, MCH, and MCHC indicated lower values in spring. A statistically significant difference was recorded for all the mentioned parameters. Figure 3 shows the grouping and correlation of erythrocyte parameters in a total of 40 individuals of Dalmatian barbelgudgeon in spring and autumn. PCA analysis shows a negative correlation between MCV and MCHC values in both spring and autumn since elevated MCV values in spring resulted in lower MCHC values. However, during autumn, much lower values of MCV were recorded in the Dalmatian barbelgudgeon individuals, which resulted in higher MCHC values.

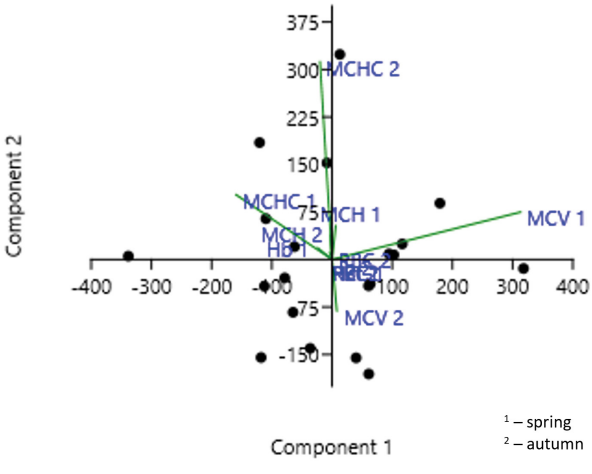

Fig 3. Grouping and interrelations of erythrocyte parameters

Regarding the representation of elements in the white bloodline, higher values of non-segmented neutrophils, segmented neutrophils, monocytes and basophils were observed in spring, while in autumn higher values were

Table 2. Hematological parameters of Dalmatian barbelgudgeon in spring and autumn

Šatorsko Lake	Spring		Autumn		Sig.
	Mean ± stdv	Range	Mean ± stdv	Range	
Hct (l/l)	0.45 ± 0.10	0.26 - 0.61	0.33 ± 0.07	0.20 - 0.44	**0.000**
HB (g/l)	84.98 ± 20.27	51.85 - 148.15	143.86 ± 19.18	111.11 - 185.19	**0.000**
RBC (x10^{12}/l)	0.98 ± 0.29	0.60 - 1.46	1.27 ± 0.27	0.84 - 2.07	**0.003**
MCV (fl)	472.61 ± 133.23	273.68 - 789.47	264.52 ± 60.31	168.07 - 377.36	**0.000**
MCH (pg)	97.95 ± 36.71	56.19 - 176.37	118.11 ± 31.02	67.99 - 202.82	**0.034**
MCHC (g/l)	208.01 ± 102.05	98.77 - 569.81	456.28 ± 117.32	303.02 - 740.75	**0.000**
Number of leukocytes (x10^{9}/l)	36.00 ± 18.49	18.00 - 78.00	34.58 ± 13.79	18.00 - 58.00	0.849
Lymphocytes	76.80 ± 7.05	61.00 - 87.00	86.10 ± 3.45	80.00 - 93.00	**0.000**
Non-segmented neutrophils	12.05 ± 7.27	1.00 - 32.00	5.35 ± 3.33	0.00 - 12.00	**0.000**
Segmented neutrophils	3.45±2.37	0.00-10.00	3.20±1.79	0.00-6.00	0.901
Monocytes	6.90±3.54	2.00-13.00	4.35±1.76	1.00-8.00	**0.025**
Basophils	0.45±0.76	0.00-2.00	0.40±0.60	0.00-2.00	0.934
Pseudo-eosinophils	0.20±0.41	0.00-1.00	0.20±0.52	0.00-2.00	0.742

*significant values at 0.05

recorded only for lymphocytes, and a statistically significant difference was recorded for lymphocytes, non-segmented neutrophils and monocytes. PCA analysis of white blood cell elements indicates a negative correlation between lymphocytes and segmented neutrophils, whereby in spring a lower value of lymphocytes is accompanied by a higher value of segmented neutrophils, while in autumn, a decrease in the value of non-segmented neutrophils and other white blood cell elements is accompanied by an increase in the value of lymphocytes (Figure 4).

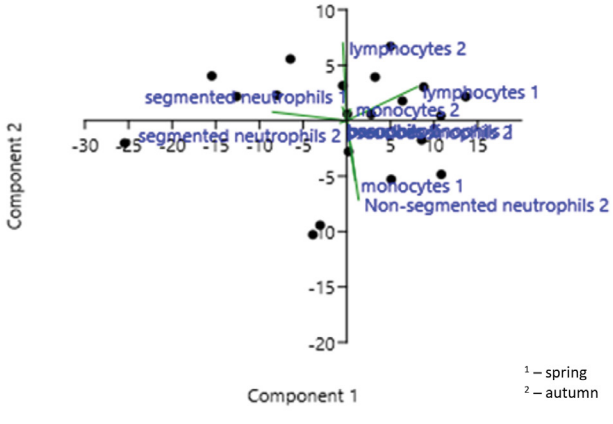

Fig 4. Grouping and interrelations of white bloodline elements

DISCUSSION

In our study, standard and total length values were marginally lower than those found in the literature. This is probably the result of specific environmental conditions, such as altitude and the fact that the species of Dalmatian barbelgudgeon was introduced into Šatorsko Lake. According to Mušović et al. (2018), the total length of the Dalmatian barbelgudgeon individuals from Buško Lake was 108.26±9.22 mm, and the average length of the Dalmatian barbelgudgeon individuals from Buško Lake was 88.91±8.32 mm. However, Mihinjač (2018) recorded values for the total length (TL=80.20±18.86 mm) of the female Dalmatian barbelgudgeon from the Čikola River which were very similar to the values of the total length of the individuals in spring from our study. In the study conducted by Dekić et al. (2015), the determined weight of Dalmatian barbelgudgeon from Šatorsko Lake was higher compared to the results of our study, both in spring and autumn, and it was 9.73±5.20 g. The individuals of Dalmatian barbelgudgeon from our study had a much lower weight compared to the results of Dekić et al. (2015) and Mušović et al. (2018). In our study, a lower value of Fulton's condition factor was recorded in both seasons compared to the results for individuals of Dalmatian barbelgudgeon (K=2.05±0.89) reported by Dekić and Ivanc (2021).

Since Dalmatian barbelgudgeon is the only species in the genus *Aulopyg* and there are scarce studies on hematological factors, the results of our study were compared with the species from the same family, Cyprindae.

Vuković and Žnidaršić-Krzyk (1969) reported significantly higher values of the number of erythrocytes for Dalmatian barbelgudgeon (RBC=1.743x 1012/l) compared to our results in spring and autumn. In the study conducted by Dekić and Ivanc (2021), the recorded values of the erythrocyte lineage of the Dalmatian barbelgudgeon individuals did not differ much compared to the results of our study. In *Capoeta capoeta umbla* individuals from Karakaj accumulation, higher values of the number of erythrocytes, hemoglobin and hematocrit were recorded during spring and summer (Orun & Erdemll, 2003), which is in accordance with the results of our study on hematocrit values. However, our study recorded significantly higher values of the number of erythrocytes and hemoglobin in autumn. Moreover, many abiotic factors of the aquatic environment show an immediate effect on the blood characteristics, and thus reduced partial oxygen pressure in the aquatic environment leads to adaptive changes in the red bloodline, such as an increase in the number of erythrocytes and hemoglobin concentrations (Ivanc et al, 1996; Ivanc et al., 1997), which is evident in our study. The lowest value of hemoglobin in individuals of *Acanthobrama marmid* was recorded in spring (Basusta and Sen, 2004), which was also confirmed in our study on Dalmatian barbelgudgeon. In the study by Aras et al. (2008), a negative correlation of temperature according to the values of hemoglobin, MCH and MCHC of *Leuciscus cephalus* species was determined, which is in accordance with the results of our studies. This is the result of higher water temperatures in spring and higher values of the aforementioned parameters. The results of our study could be explained as a kind of adaptation of Dalmatian barbelgudgeon to other physical and chemical parameters of the water, such as much higher pH values in autumn compared to spring. The reason for the much higher pH values in autumn could be an increased amount of precipitation that can dilute alkaline substances, and the dissolution of atmospheric carbon dioxide (Sheikh and Yaregi, 2003).

The number of leukocytes in males and females of *Tinca tinca* species is significantly lower in spring and winter, compared to summer and autumn (Collazos et al., 1998), while in our study, higher values of the number of leukocytes were recorded in spring in the Dalmatian barbelgudgeon individuals. Dekić and Ivanc (2021) recorded results similar to our study regarding the proportion of elements in white blood cells, except that in our study the lowest proportion of pseudoeosinophils was determined, unlike the aforementioned researchers who reported that basophils were the least represented. Like other blood parameters, the elements of the white bloodline are also subject to seasonal changes, which was confirmed in our study. In individuals of *Acanthobrama marmid* species, a dominance of lymphocytes was observed in all seasons with the highest proportion in autumn, which is in accordance with our study (Basusta and Sen, 2004). In the *Barbus rajanorum mystaceus* individuals, the highest value of monocytes was observed in autumn (Yilayaz and Bitmis, 2002), which is in contrast to our study, considering that in the Dalmatian barbelgudgeon individuals, a lower value of monocytes was recorded in autumn. In the study by Orun and Erdemll (2003), an increase in the proportion of neutrophils and monocytes of *Capoeta umbla* species was noted in relation to the age, length and weight of the individuals, whereby it could be concluded that the individuals of Dalmatian barbelgudgeon in our study had a higher proportion of monocytes and neutrophils in spring, considering that they had greater weight and length.

CONCLUSION

Based on the results of our research, it has been confirmed that seasonal variations significantly affect the physical and chemical parameters of the water, which directly affects the hematological parameters of fish. In addition to exogenous factors, the values of hematological parameters are also significantly influenced by endogenous factors, such as spawning, since the studies were conducted in spring before spawning and in autumn. Significant differences in a large number of analyzed hematological parameters of the Dalmatian barbelgudgeon individuals from Šatorsko Lake in spring and autumn are the result of the adaptation of the Dalmatian barbelgudgeon individuals to changing exogenous factors, physicochemical parameters of the water as well as endogenous factors, such as the spawning of the Dalmatian barbelgudgeon individuals.

SEZONSKA VARIJACIJA MORFOMETRIJSKIH I HEMATOLOŠKIH PARAMETARA OŠTRULJE *Aulopyge huegelii* HECKEL 1843 IZ ŠATORSKOG JEZERA

SAŽETAK

Oštrulj, kao endemska vrsta introducirana je u Šatorsko jezero, međutim nedovoljno je istražena na ovom lokalitetu. Cilj ovog istraživanja bio je utvrditi sezonske varijacije fizikalno-kemijskih parametara vode u odnosu na morfometrijske i hematološke parametre oštrulje iz Šatorskog jezera. Fizikalno-kemijskom analizom vode zabilježene su niže vrijednosti temperature, koncentracije O_2, zasićenja O_2, elektrolitičke vodljivosti i suspendiranih tvari u jesen, dok su ostali analizirani parametri ukazivali na niže vrijednosti u proljeće. Analizom morfometrijskih parametara (težina, standardna duljina, totalna dužina i Fultonov faktor kondicije) utvrđene su veće vrijednosti

svih parametara tijekom proljeća, dok je značajna razlika zabilježena samo za Fultonov faktor kondicije. Uočena je statistički značajna razlika za sve parametre eritrocitne loze (RBC-eritrociti, koncentracija HB-hemoglobina, HCT-hematokrit, MCV-srednji korpuskularni volumen, MCH-srednji korpuskularni hemoglobin, MCHC-srednje koncentracije korpuskularnog hemoglobina). U bijeloj krvnoj lozi statistički značajna razlika zabilježena je za limfocite, nesegmentirane neutrofile i monocite, dok je udio segmentiranih neutrofila i bazofila blago povećan u proljeće. Nadalje, zabilježene vrijednosti pseudoeozinofila bile su jednake u proljeće i jesen. Rezultati ovih istraživanja ukazuju na značajan utjecaj egzogenih i endogenih čimbenika na varijabilnost morfometrijskih i hematoloških parametara oštrulje iz Šatorskog jezera.

Ključne riječi: *Aulopyge huegelii*, fizikalno-kemijski parametri vode, morfometrijski parametri, hematološki parametri

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