

## Organosomatic indices and leukocyte formula of chub (*Squalius cephalus* L) from the Suturlija River

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### Abstract

Coefficients of organs, along with haematological characteristics, represent an important means of monitoring the health and condition of fish. Organosomatic indices show the state of organ systems and individual organs. They manifest as changes in size, which are reflected through a decrease or increase, under the influence of environmental factors. Chub individuals from the Suturlija River were used for analysis. The paper analyses the Fulton's condition factor, heart, liver, and spleen coefficients as a condition factor. Furthermore, the values of total length, standard length, and body weight were determined for all individuals. Also, certain haematological parameters were analysed in the paper: total leukocyte count and differential blood count. A total of 19 chubs caught from the Suturlija River were analysed. The obtained result of Fulton's condition factor (1.53) indicates favourable habitats for chub individuals in the Suturlija River. The results of organosomatic indices are without major variations between individuals (CSI = 0.184, SSI = 0.992, HSI = 0.133). The mean leukocyte count was  $45.857 \times 10^9/l$ , with the highest proportion of lymphocytes in the differential blood count.

*Key words:* chub, organosomatic indices, leukocyte formula

## Introduction

The condition of fish, the composition of ichthyocenosis, as well as their interindividual relationships, are trustworthy indicators of a certain biotope, as well as a reflection of all the ecological factors of the area, including anthropogenic influence (Biro, 1990; Blahak and Prokeš, 1998; Treer et al., 2003; Šprem et al., 2001). Organosomatic indices and fitness parameters are used during general assessment of the health condition of fish, on the level of a single individual, and on the level of the whole population (Goede and Barton, 1990; Hoque et al., 1998). Organosomatic indices show the condition of an organ and single organs that can be manifested as changes in mass, that decreases or increases, under the influence of environmental factors. Mass of liver, spleen, heart, gonad, and other organs are connected to the total length and mass of fish and indicate their general health condition. (Dekić et al., 2016). In parallel to the organ somatic index for the examination of the health condition of fish, body fluids parameters have special importance. Therefore, examinations of blood and body fluids are of utmost importance for tracking the health and fitness of fish, in their natural habitat, as well as in aquaculture (Ivanc et al., 2005). Blood parameters are considered as diagnostic indicators of a pathological condition. Hence, they are important for the assessment of functions and overall health. Furthermore, results also help with the diagnosis of structural and functional status of organisms exposed to toxins (Atamanalap and Yanik, 2003). Haematological assessment can be useful in tracking the health conditions of fish as long as inner and outer factors affect cells traits and quantitative values (Bolasina, 2006). The goal of this research was to determine the coefficient of organs and leukocyte formula in individuals of chubs from the Suturlija River.

## Materials and Methods

The Suturlija River, with its course and catchment area, is located in the south-west area of Banja Luka. It is an estuary of the Vrbas River, as one of its left tributaries, its mouth being in the Srpske Toplice (Gornji Šeher) settlement, at the elevation of 159 m. Its spring is located near the Goleši settlement, at the elevation of 390 m, while the basis of the catchment area is Dedića točak, a hill at the elevation of 466 m (Dekić, 2006).

Electric fishing was realized in August 2014, at the location of the Suturlija River. Fishing was done with the help of an electric generator, with pulsed direct current and a possibility of output voltage adjustments, brand IG 600, power 1.2 kW, ELT62II GI HONDA GCV160, power 3.0 kW and meredov net. Caught individuals were placed into adequate tanks that were

supplied with a sufficient amount of water, and a third of water was changed in certain periods, with the constant presence of air pumps and aeration of water. The fish were transported from the tanks into mesh guards, in which a reanimation of the period of one hour was conducted, whereby the mesh guards were placed in the watercourse itself.

In all tested individuals, measurements of basic morphometric characteristics of total and standard length were determined, and as an addition to these parameters, values of body mass and mass of organs: heart, liver, and spleen. The Fulton's condition factor was analysed in all individuals as a condition factor in this work.

The Fulton's condition factor was calculated by the formula

$$K = W \times 100 / L^3$$

W - The mass of the fish in grams

L - Standard length of fish in centimetres (Akombo et al., 2013)

After determining the main morphometric characteristics of total and standard length, the liver, spleen, and heart were carefully removed and weighed. Organosomatic indices for liver (HSI), heart (CSI), and spleen (SSI) are calculated in the following way:

$$OSI = (\text{mass of organs (g)} / \text{body mass of the fish (g)}) \times 100$$

Blood samples for haematological analysis were taken by puncturing the heart, with a sharp and wide sterile needle (1.0-1.2 mm) with the application of correct sterile work, and blood without anticoagulant was used for further analysis. The following parameters were used for haematological analysis: the total number of leukocytes, the proportion of neutrophils, lymphocytes, monocytes, pseudoeosinophils, and basophils. To determine the number of leukocytes, blood was taken with mélange, to the 0.5 mark and put into a test tube with a solution, whereas the solution by Kekić and Ivanc (1982) was used for dilution and counting. Leukocytes were defined as the following forms: lymphocytes, monocytes, neutrophils, basophils, and pseudoeosinophils. When it comes to leukocyte forms found in fish, eosinophils and pseudosinophils are represented, and in this working form of pseudoeosinophils are represented. Statistical processing of analysed data was conducted with the use of the statistical program, Microsoft Excel 2007 - Statistical Analysis Tools.

## Results and Discussion

Table 1 shows the results of the statistical processing of the standard length, total length, mass, and the Fulton's condition factor. The mentioned characteristics have been followed on 19 individuals from the Suturlija River.

Tab. 1. Morphometric characteristic of chub individuals from the Suturlija River

N=19	Standard length (cm)	Total length (cm)	Mass (g)	Fulton's condition factor
Mean	16.87	14.21	49.11	1.53
Standard deviation	2.87	2.47	31.88	0.12
Minimum	12.40	10.10	15.08	1.28
Maximum	24.70	20.90	161.63	1.76
Coefficient of variation	17.30	17.43	64.92	8.35

Morphometric characteristics of chub individuals from the Suturlija River did not differ much, except for body mass, whereby a great range of variation between an individual with minimum and an individual with maximum body mass was found, which is confirmed by the high coefficient of variation. The value of the Fulton's condition factor ranges from 1.29 to 1.76 with a mean of 1.53.

Tab. 2. Organ index of chub individuals from the Suturlija River

N=19	Heart index (%)	Liver index (%)	Spleen index (%)
Mean	0.184	0.992	0.133
Standard deviation	0.054	0.306	0.055
Minimum	0.109	0.612	0.033
Maximum	0.291	1.719	0.248
Coefficient of variation	29.571	30.890	41.476

Statistical analysis of the data for organosomatic indices has shown no major deviations between the chub individuals from the Suturlija River. Statistical processing of the analysed data was performed using the statistical program Microsoft Office Excel 2007 and SPSS 15. The ANOVA and LSD test were used to compare the values.

Tab. 3. Leukocyte parameters of chub individuals from the Suturlija River

	Total number of leukocytes (x 10 <sup>9</sup> /l)	Proportions of certain form of leukocytes				
		Neutrophils	Lymphocytes	Mono-cytes	Pseudo-oenophiles	Basophiles
Mean	45.857	0.293	0.556	0.120	0.018	0.014
Standard deviation	6.374	0.031	0.054	0.030	0.014	0.011
Minimum	34.000	0.230	0.460	0.060	0.000	0.010
Maximum	54.000	0.340	0.710	0.170	0.010	0.030
Coefficient of variation	13.906	10.57	9.71	25.50	78.51	104.60

When it comes to differential blood count in the conducted research, the following leukocytes were differentiated: lymphocytes, neutrophils, monocytes, basophils, and pseudoenophiles, as well as the highest number of lymphocytes in the chub individuals from the Suturlija River was recorded. The condition factor varies depending on the nutrition, season, and most likely as a response to food source changes, metabolism, or reproductive status.

It can vary from place to place within the species (Doyon et al., 1988; Fisher et al., 1996). According to Bromage (1996), the value of condition factor less than 1.00 indicates unfavourable conditions of living and malnutrition of an individual, whereby it can be stated that the conducted studies are actually about favourable conditions of living since a value of 1.53 of the Fulton's condition factor was measured. Certain variations in the values of the Fulton's condition factor are listed in the studies of Balik and Ustaoglu (2006), who list average value of condition factor of an analysed population of carp in Lake Karamuk (Turkey) 2.022, which represents higher values compared to other lakes: Golhisar FK=1.579, (Alp and Balik, 2000); Aksehir FK=1.668, (Alp et al., 1999); Hafik FK=1.96, Cengizler and Erdem (1989), and all listed values are higher compared to values found in the present study. Singh and Canario (2004) point out that hepatosomatic index (HSI) represents one of the most important tested biomarkers, because of the crucial role of the liver in detoxication.

Dogan and Can (2011) found that organosomatic indices can be used as bioindicators for endocrine disorders in the body that arise under exposure to disruptors. Similar to our study, significant differences in the values of the index of organs of five groups of individuals of *Oncorhynchus mykiss* that come from different parent flock were found. The hepatosomatic index represents a good indicator of the status of energy. Therefore, in fish with scarce habitat conditions the liver is smaller than in fish that lives in feed-rich habitats, which, in the conducted studies, points out that the habitat is rich with feed that chub consume (Dekić et al., 2016). Regarding the values of the spleen coefficient, endogenous and exogenous factors have an impact on the spleen, and it varies depending on the taxonomic affiliation of the individual (Anderson et al., 1982; Ruklov, 1979) and through the population of the same species (Lipiskaya and Selekhova, 1980; Ruklov, 1979). Differences in the number of leukocytes were spotted in the studies of blood count of chub from two lakes in Turkey as well, with different biochemical oxygen consumption. In the lake where greater consumption of oxygen and a lower concentration of it was found, activation of the immune system that can be seen in the higher number of leukocytes, leukocytosis, was observed. Variation of numbers of leukocytes of some cyprinid species, in the work by Vuković and Žnidarić

(1969), was in the range from 44.7 to 59.46 x 10<sup>9</sup>/L, and the value of numbers of leukocytes, in an individual chub, in this research, was found in a range of 36 to 54 × 10<sup>9</sup>/L. According to McCarthy et al. (1973), the total number of leukocytes is a haematological parameter with great individual variations in the number that increased during the summer when the quality of water decreases (Guijarro et al., 2003).

In the work by Mitrašinović and Suljević (2009) it was determined that the values of the total number of leukocytes show significant growth from spring to winter. In our study, lymphocytes prevail in the differential blood count. Such results are in accordance with other studies where these forms of leukocytes take the greatest part in the blood count (Dekić, 2006; Dekić, 2010.). Lymphocytes have represented the largest share of leukocytes in the study by Adams et al. (1985) as well, where it was established that in the species *Leucaspis delinatus* in the time of spawn there is an increase in the number of leukocytes with phagocytic properties. Additionally, the study of the differential blood count in three cyprinid species of fish: *Leuciscus turskyi*, *Chondrostoma toxostoma*, and *Aulopyge hugeli*, showed that in all three species, the participation of lymphocytes is the largest, followed by neutrophils and eosinophils, and in all the species presence of younger forms was found, even blast Adams et al. (1992).

The high coefficient of variations refers to pseudoeosinophiles and basophils, which points to very expressed individual variations of blood parameters. Individual variations of the mentioned parameters were recorded in the work by Mitrašinović and Suljević, (2009). Higher values of pseudoeosinophiles and lymphocytes are attributed to presence of stress (Ellis, 1977), which is also found in tench (*Tinca tinca*) that is exposed to thermal stress (Hasković et al., 2013) while investigating differential blood count.

## Conclusion

Based on the recorded values of all the parameters and the comparison with the data found in literature, it can be concluded that the individuals of chub from the Suturlija River are in good health condition and live in favourable environment conditions. All analysed parameters, like the Fulton's condition factor that was 1.53 are an indicator that the conditions of the Suturlija River, in the period while the research lasted, are satisfactory for chub individuals.

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# Органосоматски индекси и леукоцитна формула код клена (*Squalius cephalus* L.) из ријеке Сутурлије

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## Сажетак

Коефицијенти органа упоредо са хематолошким карактеристикама представљају значајно средство за праћење здравља и кондиције риба. Органосоматски индекси приказују стање система органа и појединачних органа. Манифестују се као промјене у величини, које се огледају кроз смањење или повећавање, под утицајем фактора средине. За анализу су кориштене јединке клена из ријеке Сутурлије. У раду је као кондициони фактор анализиран Фултонов коефицијент кондиције, коефицијенти срца, јетре и слезене, а код свих јединки одређене су вриједности: тоталне дужине, стандардне дужине и масе тијела. Такођер у раду су анализирани одређени хематолошки параметри: укупан број леукоцита и диференцијална крвна слика. Укупно је анализирано 19 јединки клена које су изловљене из ријеке Сутурлије. Добивени резултат Фултоновог коефицијента кондиције (1,53) указује на повољно станишта за јединке клена у ријечи Сутурији. Резултати органосоматских индекса су без већих варирања између јединки (CSI=0,184, SSI=0,992, HSI=0,133). Средња вриједност броја леукоцита износила је  $45,857 \times 10^9$ , са највећим удјелом лимфоцита у диференцијалној крвној слици.

*Кључне ријечи:* тешки метали, вода, риба, хемијске анализе.

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*Received:* 27.10.2020  
*Accepted:* 21.05.2021