HEMATOLOGICAL STATUS OF DIFFERENT AGE CLASSES OF BARBUS BALCANICUS

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HEMATOLOŠKI STATUS RAZLIČITIH UZRASNIH KLASA *BARBUS BALCANICUS*

Apstrakt

U radu su istraživane vrijednosti osnovnih parametara crvene loze po starosnim kategorijama kod jedinki potočne mrene (*Barbus balcanicus*). Hematološki status predstavlja pouzdano sredstvo za utvrđivanje fiziološkog stanja organizma, a posredno i stanja životne sredine, jer njegovi parametri reaguju na promjene uslova sredine. Hematološki parametri su obuhvatali: broj eritrocita, koncentraciju hemoglobina, hematokrit, MCV, MCH i MCHC. Praćeni parametri su analizirani u toku dvije godine i kod populacija iz dvije rijeke (Suturlija i Jakotinska rijeka). Kod jedinki iz rijeke Suturlije u prvoj godini istraživanja bile su zastupljene jedinke starosti 1+ do 5+, dok je tokom druge godine uzorak bio predstavljen jedinkama starosti 1+ do 6+. Istovremeno su tokom 2004. godine kod riba iz Jakotinske rijeke bile zastupljene jedinke starosti 1+ do 4+, a u drugoj godini jedinke starosti 1+ do 5+. Analiza po starosnim kategorijama pokazuje postojanje značajnih razlika u vrijednostima pojedinih parametara, a razlika je izraženija kod jedinki potočne mrene iz rijeke Suturlije, kod kojih su razlike utvrđene u obje godine. Međutim, kod potočne mrene iz Jakotinske rijeke statistički značajne razlike između vrijednosti konstatovanih kod različitih starosnih klasa bile su statistički značajne samo u drugoj godini istraživanja.

Ključne riječi*: hematologija, potočna mrena, starosne klase*

INTRODUCTION

Hematological status represents reliable means for determining the physiological state of an organism and indirectly the state of its environment, because hematological parameters respond to environmental changes (Ivanc and Miljanović, 2001). The quantitative characters of red and white blood cells are used for detecting the haematological status. They provide insight in a number of processes in the organism. On this bases, analyses of different blood components give valuable information concerning changes that appear in certain systems influenced by external and internal factors. Determination of hematological parameters and blood plasma biochemistry is used to assess the health of wild and domestic animals. Values of these parameters are useful for interpretation of the results that are related to different diseases and environmental conditions (Seker et al., 2005). Prerequisite for such hematology application is satisfactory knowledge of reference range of values of fish blood parameters. Age-related values of hematological parameters are even less known and insufficient (Hrubec et al., 2001).

MATERIAL AND METHODS

Fish sampling was done by electro-shocker providing pulsed direct current and with ability to customise the output voltage. IG 600 brand, with 1.2 KW power. After appearance of galvanotaxis and galvano-narcosis, fish were collected with landing net. Fish sampling was made during two years in monthly intervals. Handbooks from Vuković and Ivanović (1971), Simonović (2001), Banaresku and Bogutavskaya (2003), Kottelat and Freyhof (2007) were used for taxonomic determination of collected fish. The fish were placed into containers with sufficient water of appropriate quality. After that were transferred in keepnets, placed in native stream and let to reanimate for one hour. River Suturlija, by its course and its basin is located in the area southwest of Banja Luka, and its mouth into Vrbas river, as a left tributary, is placed in Srpske Toplice (Gornji Šeher), at an altitude of 159 m. River Jakotina is a left tributary of river Vrbanja with length of 15 km, witch originates at an altitude of 670 m, with mouth on 260 m.

Hematological analysis

Blood for hematological analysis was collected by heart puncture using sterile needle (1.0 to 1.2 mm). Native blood, with anticoagulant is used for further analyses. Erythrocyte and leukocyte count was performed in hemocytometer using diluent by Kekić and Ivanc (1982). Hemoglobin concentration (Hb) was determined by hemiglobin cyanide method using Drabkin reagent (Blaxhall and Daisly, 1973). Hematocrit (Hct) was determined by microhematocrit centrifuge. Hematological parameters were calculated using values of hematocrit, erythrocyte number and hemoglobin concentration.

Mean corpuscular volume (MCV) $$MCV = \frac{Hct}{Br.eritrocita / l}$$

Mean corpuscular hemoglobin (MCH) $$MCH = \frac{Hb / l}{Br.eritrocita / l}$$

Mean corpuscular hemoglobin concentration (MCHC) $$MCHC = \frac{Hb / l}{Hct}$$

Age determination

Methods of scales observation and counting annual growth marks were used for age determination. Scales for age determination were collected from the shoulder area, above lateral line, and then, were placed in paper bags. Before age determination, scales were cleaned with NaCl and KOH solutions and water, and then were observed using binocular magnifier.

RESULTS AND DISCUSSION

Values of monitored hematological parameters were correlated with age groups. Mean values of erythrocyte parameters for different age classes, rivers and years of researches are shown in table 1. Blood values of Large-spot barbel (*Barbus balcanicus*) from River Suturlija in 2004. showed significant age-related differences. Erythrocyte number was the highest in age class 5+, and the lowest in class 2+. Significant difference was recorded between values of age classes 2+ and 4+ (0.021), 2+ and 5+ (0.020), as well as between 1+ and 5+ (0.029). The highest value of hemoglobin concentration was recorded in age class 4+, and the lowest in 1+ class.

Table 1. Mean values of erythrocyte parameters of *Barbus balcanicus* from Rivers Suturlija and Jakotina by age classes and years of research

River	Year	Age class	Mean value	Erythrocyte number x 10^{12}/l	Hb (g/l)	Hct (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l eryt.)
Suturlija	2004	1+	x	1.254	69.02	0.398	318.750	55.241	175.272
		2+		1.246	72.13	0.422	341.563	58.393	172.825
		3+		1.294	71.64	0.431	333.855	55.609	167.768
		4+		1.310	73.61	0.411	315.757	56.490	180.797
		5+		1.390	69.44	0.416	301.609	50.139	169.562
	2005	1+	x	1.270	67.64	0.389	308.489	53.603	175.528
		2+		1.300	70.44	0.417	323.889	54.652	170.758
		3+		1.297	74.26	0.442	342.574	57.791	172.104
		4+		1.366	74.49	0.445	329.548	55.159	169.074
		5+		1.343	75.34	0.438	329.086	56.846	173.660
		6+		1.430	80.56	0.440	322.938	59.423	187.237
Jakotina	2004	1+	x	1.225	73.25	0.443	362.869	60.329	168.150
		2+		1.207	74.75	0.451	374.768	62.316	168.443
		3+		1.250	76.30	0.451	376.408	63.896	170.751
		4+		1.355	77.77	0.457	345.420	58.155	171.695
	2005	1+	x	1.258	72.49	0.437	349.536	58.081	168.989
		2+		1.240	75.10	0.453	375.276	61.671	168.131
		3+		1.273	75.87	0.466	371.632	60.594	165.223
		4+		1.282	73.56	0.451	360.319	58.366	164.954
		5+		1.269	71.91	0.450	358.376	57.126	161.549
		6+		1.245	70.37	0.317	256.317	56.585	223.562

Significant differences were established comparing values of class 1+ with values of age classes 2+ (0.014) and 4+ (0.013). Hematocrit values also were related with fish age. Significant differences in hematocrit values existed between class 1+ and 2+ (0.005) and 1+ and 3+ class (0.004). The highest value was in age class 3+, and the lowest in 1+. Values of mean corpuscular volume (MCV) ranged from 301.608 fl (age class

5+) to 341.563 fl (class 2+). Significant differences were between age class 2+ and 1+ (0.003) and 2+ and 4+(0.014). Values of MCH showed significant differences by comparing class 2+ with class 1+ (0.007) and 5+ (0.022). The highest value of this parameter was in age class 2+, and the lowest in 5+. In the same time, significant difference was recorded in MCHC values between age classes 3+ and 4+ (0.039).

In 2005 in research of *Barbus balcanicus* from River Suturlija, the individuals from age 1+ to 6+ were found. Analysis by age classes showed presence of significant difference in most of monitored parameters. The values of erythrocyte number ranged from 1.270 x 10^{12}/l (in age class 1+) to 1.430 x 10^{12}/l (class 6+). Relation of this parameter with fish age showed significant difference between age class 1+ on one side, and classes 4+ (0.000), 5+ (0.035) and 6+ (0.016) on the other side. Comparing values of class 2+ with values of 4+ (0.010) and 6+ (0.050). Differences were significant between values from class 3+ and 4+ (0.006) and 3+ and 6+ (0.044). Hemoglobin concentration was constantly increasing from classes 1+ to 6+. Significant differences were recorded comparing values from age class 1+ with classes 3+ (0.000), 4+ (0.000), 5+ (0.001) and 6+ (0.001). Values of class 2+ were significantly different from those in classes 3+ (0.012), 4+ (0.013), 5+ (0.024) and 6+ (0.017). The lowest hematocrit value was in age class 1+, and the highest in class 4+. Values from age class 1+ were significantly different from those in classes 2+ (0.015), 3+ (0.000), 4+ (0.000) and 5+ (0.003). Also, statistically significant difference was recorded comparing values from age class 2+ with values from classes 3+(0.030) and 4+ (0.021). Age class 3+ had signifaicantly higher values than class 1+ (0.002). This is also true rom MCH (0.013). MCHC values did not show significant difference by age classes. Correlation between hematological parameters and age was less expressed in barbel from River Jakotina.

In 2004, significant differences between age classes were not recorded. However, it should be pointed out that the highest values of erythrocyte number, hemoglobin concentration, hematocrit and MCHC were recorded in age class 4+, while the highest values of MCV and MCH were recorded in age class 3+. Lack of significant differencess in 2004 could be explained by different sample sizes. So, more than 50% of total sample belonged to age class 1+, followed by age class 2+, while the rest of age classes were presented by fewer individuals. Slightly different correlation between hematological parameters from barbel inhabiting River Suturlija was recorded in 2005. During that year, significant differences of hemoglobin concentration values were determined between age class 1+ on one side, and age classes 2+ (0.044) and 3+ (0.044) on the other side. The highest value of this parameter was in age class 3+, and the lowest in class 6+. The lowest hematocrit value was recorded in age class 6+, and it was significantly different from age classes 1+ (0.007), 2+ (0.002), 3+ (0.001), 4+ (0.003) and 5+ (0.005). Statistically significant difference was also recorded between age classes 1+ and 3+ (0.037).

Erythrocyte number did not differ significantly with age, while MCV values were different between age class 6+ (in which were recorded the lowest values) and classes 2+ (0.020), 3+ (0.027) and 4+ (0.046). Also, significant difference was recorded between age classes 1+ and 2+ (0.036). MCH differed significantly between classes 1+ and 2+ (0.030). MCHC values had highest values in class 6+ which was significantly different from classes 1+ (0.002), 2+ (0.002), 3+ (0.001), 4+ (0.001) and 5+ (0.001).

The other researches also showed presence of significant differences in values of hematological parameters between different age classes. Age-related analysis of blood parameters of hybrid *Morone chrysops* and *Morone saxatilis* showed presence of differ-

ence. In the research, the individuals from 4 to 19 month of age were presented, divided into age classes: 4 months, 6 months, 9 months, 15 months and 19 months. Values of hematocrit and MCV were significantly lower in the youngest individuals in comparison with other age classes. Individuals aged 6 months had significantly lower values of hemoglobin concentration, MCH and MCHC in comparison with other age groups. The values of erythrocyte number were the highest in individuals aged 9 and 15 months (Hrubec et al., 2001). Also, research regarding to correlation between hematological parameters and fish biology in *Silurus triostegus* showed that hemoglobin concentration and hematocrit values were growing with age, until the age of four, and then were decreasing. Research in correlation between hemoglobin concentration and hematocrit value showed high correlation coefficient (Abood, 1992).

According to Chanchal et al., (Chanchal et al., 1979), young fishes usually show high physical activity and intense feeding during growth period which explains high recorded values of hemoglobin concentration and hematocrit. However, the older fishes show slow metabolic activity after certain age (Joshi and Tandon, 1977). Research of erythrocyte parameters in species *Rhamdia quelen* showed that hematological parameters were also correlated with body length. Individuals of this species were divided in two groups, based on difference in standard body length. Standard body length values of fishes from the first group were ranged from 20.5 to 30.0 cm, and the second group from 30.5 to 40.0 cm. Analysis of hematological parameters showed significantly higher erythrocyte number in the first group, while the values of hemoglobin concentration, hematocrit and MCHC were approximately the same in the both groups. Higher values of MCV and MCH were recorded in fishes from the second group (Camargo and Pouey, 2004).

Results of haematological parameters in *Capoeta capoeta umbla* showed increased values of erythrocyte number, hemoglobin concentration and hematocrit with age (Örün and Erdemli, 2003).

Based on all presented hematological parameters it can be noted that they are different in different age classes, but specific for every fish species.

CONCLUSIONS

Erythrocyte parameters of Large-spot barbel (*Barbus balcanicus*) from researched rivers show significant difference with age.

Major differences of monitored parameters correlated with age groups were recorded in individuals from River Suturlija.

Differences were not recorded between barbel individuals from River Jakotina in the first year of research, while they were recorded in the second year.

It can be noted that all presented haematological parameters are different for different age classes, but specific for every fish species.

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