

LEUKOCYTES OF COMMON BREAM (*ABRAMIS BRAMA*, LINNEAUS, 1758) FROM THE ARTIFICIAL MODRAC LAKE (BOSNIA & HERZEGOVINA) IN VARIOUS SEASONS

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ANALIZA LEUKOCITA DEVERIKE (*ABRAMIS BRAMA*, LINNEAUS, 1758) IZ HIDROAKUMULACIJE MODRAC (BOSNA I HERCEGOVINA) TOKOM SEZONE

Apstrakt

Analiza hematološko-biohemijskih parametara krvi riba pruža mogućnost uvida utjecaja okolinskih faktora na fiziološko stanje riba. Također, je i odličan pokazatelj kondicije i zdravstvenog stanja ovih organizama. Karakteristika ektotermnih kičmenjaka je širok raspon variranja hematoloških parametara i velike individualne varijacije, zbog izraženog uticaja okolinskih faktora. Hematološki status ribe je dobar indikator odgovora organizma ribe na navedene uticaje. Istraživanja raznih autora ukazuju na jako velike sezonske varijacije u vrijednostima pojedinih hematoloških parametara. Sezona utiče kako na broj leukocita, tako i na vrijednosti pojedinih vrsta leukocita u okviru diferencijalne krvne slike, koji su analizirani tokom sprovedenog istraživanja. Broj leukocita je manji kod zdravih riba. U dostupnoj literaturi nema podataka o pokazateljima zdravstvenog stanja deverike iz hidroakumulacije Modrac. Za potrebe istraživanja prikupljeno je ukupno 200 jedinki deverike (lat. *Abramis brama*, Linnaeus, 1758) iz akumulacije Modrac, odnosno po 50 jedinki u četiri sezone. Tokom istraživanja na izlovljenom uzorku određen je ukupan broj leukocita, kao i vrijednost diferencijalne krvne slike. Krv je uzeta punkcijom srca, te je za dalju analizu korištena nativna krv bez dodavanja antikoagulansa. Ukupan broj leukocita određen je u Bürcker-Türkovoju komorici na uvećanju 40x. Za određivanje diferencijalne krvne slike pripremljen je krvni razmaz, obojen metodom po Pappenheimu. Najniža prosječna vrijednost broja leukocita

u krvi *Abramis brama* iz hidroakumulacije Modrac utvrđena je tokom ljeta u iznosu od $1,607 \times 10^9/L$, dok je najviša vrijednost zabilježena u proljeće $3,613 \times 10^9/L$. Limfociti su leukociti koji pokazuju najveću relativnu zastupljenost u krvi *Abramis brama* iz akumulacije Modrac, slijede nesegmentirani neutrofili, heterofili, monociti, segmentirani neutrofili, te eozinofilni granulociti. Najmanju relativnu zastupljenost od svih leukocita, očekivano, pokazuju bazofilni granulociti, te nisu utvrđeni kod jedinki izlovljenih u periodu ljeta i jeseni. Korelativnom analizom je utvrđeno da monociti i nesegmentirani neutrofili pokazuju najveću signifikantnu pozitivnu korelaciju ($r = 0.4723$; $p < 0,01$). Dok limfociti negativno koreliraju sa nesegmentiranim neutrofilima ($r = - 0,8318$), heterofilima ($r = - 0,5365$) i monocitima ($r = - 0,6230$), pri čemu su ove povezanosti statistički visoko signifikantne ($p < 0,01$). Za testiranje razlika između utvrđenih vrijednosti analiziranih parametara u različitim sezonama istraživanja primjenjen je t-test. Primjenom navedenog testa utvrđeno je da su proljeće i zima sezone koje se statistički značajno razlikuju u najvećem broju analiziranih varijabli (broju leukocita, procentu segmentiranih neutrofila, nesegmentiranih neutrofila, eozinofila, heterofila, te procentu agranulocita). Proljeće i jesen su sezone između kojih su, primjenom t-testa, utvrđene signifikantne razlike u najmanjem broju analiziranih parametara (broju leukocita, procentu segmentiranih neutrofila i bazofilnih granulocita). Utvrđen je manji broj leukocita u krvi *Abramis brama* iz jezera Modrac u odnosu na broj leukocita koji su dobili različiti autori, pri analizi navedenog parametra kod istih ili sličih vrsta riba.

Ključne riječi: leukociti, Abramis brama, hidroakumulacija Modrac
Keywords: white blood cells, Abramis brama, artificial Modrac Lake

INTRODUCTION

A characteristic feature of all ectothermic vertebrates is a wide physiological range of blood composition and a large individual variation, resulting among others from the fact that they are under a great effect of environment. Their homeostatic system and mechanisms of its control are much less specialised compared to mammals, and thus maintaining some parameters within a narrow range of the so called physiological standard is very difficult (Homątowska et al., 2002). The physiological status of fish body is directly related to inner and outer factors, biotic and abiotic influences which acting on the organism (Stosik et al., 2002). Fish blood cells (RBC, WBC counts) are good indicators of systemic response to external stimulus and any changes are therefore reflected in their morphology and distribution in the blood (Srivastava and Choudhary, 2010). Count of leukocyte depends on the age of the fish, sex, sexual activity, nutritional status, season, temperature changes of water and health of fish (Bogut et al., 2006). It is known that leukocyte cells are normally lower in healthy fishes and could be used as a significant indicator for infectious diseases (Jamalzadeh et al., 2009). Research of many authors suggest a large variation in the values of some haematological parameters during different seasons. The artificial Modrac Lake is built in year 1964, and it is the largest water management facility of this kind in Bosnia and Herzegovina (Arnautić, 2006). In the recent literature there is no data of the health status indicators of common bream from the accumulation Modrac.

MATERIALS AND METHODS

For research purposes, we collected a total of 200 samples of common bream (*Abramis brama*, Linnaeus, 1758) from the accumulation Modrac: 50 individuals in each of four seasons. Samples of blood were taken by puncture of the heart, using a sterile needle wide diameter 1.1 mm, with a place that was previously disinfected. For further analysis we used native blood without adding anticoagulant. The total count of leukocytes was determined in 20 μ l of blood and added to a test tube with 4 ml Natt Herrick's solution. Content is homogenized and poured into Bürcker-Türck chamber for leukocytes counting. Leukocytes were counted at magnification of 40x. Differential blood picture is a relative representation of certain types of white blood cells. To determine parameters of differential blood picture prepared blood smear, stained according to the Pappenheim. For statistical analysis, we used the value of the relevant software programs: Statistica 8, SPSS and Microsoft Excel. Seasonal difference between the arithmetic mean of the analyzed variables we tested by t-test. Correlation for all analyzed parameters according to the seasons is determined.

RESULTS AND DISCUSSION

Table 1. presents the mean count of leukocytes and the mean relative abundance of granulocytes in the blood smear of common bream identified during the different seasons. The lowest average value of the number of leukocytes in the blood of *Abramis brama* from the reservoir Modrac is determined during the summer, while the highest value was recorded in the spring. Basophils shows lowest relative number of all leukocytes. Basophils were not observed in the blood of individuals who have been caught during the summer and fall.

Table 1. The total count of leukocytes and the relative distribution of granulocytes in the blood of common bream

Variable	Season	Number of fish	Mean	SD	Min	Max	Coefficient of variation	Kurtosis	Skewness
Leukocyte number (10 ⁹ /L)	Winter	50	2,330	0,997	0,600	4,350	42,769	-0,785	0,286
	Spring	50	3,613	1,341	1,450	7,700	37,119	1,087	0,896
	Summer	50	1,607	0,542	0,650	3,050	33,699	-0,339	0,451
	Autumn	50	1,804	0,960	0,600	4,000	52,239	-0,599	0,724
Segmented neutrophils (%)	Winter	50	4,060	4,177	0,000	20,000	102,876	3,932	1,752
	Spring	50	1,340	2,125	0,000	12,000	158,566	12,125	2,937
	Summer	50	0,780	1,075	0,000	4,000	137,765	2,070	1,591
	Autumn	50	2,300	2,597	0,000	12,000	112,917	3,811	1,796
Nonsegmented neutrophils (%)	Winter	50	3,260	4,222	0,000	17,000	129,523	2,553	1,763
	Spring	50	15,700	10,708	0,000	40,000	68,204	-0,599	0,618
	Summer	50	12,000	10,753	0,000	42,000	89,611	0,338	1,046
	Autumn	50	19,760	11,644	0,000	47,000	58,925	-0,209	0,431
Eosinophils (%)	Winter	50	0,140	0,351	0,000	1,000	250,364	2,684	2,14
	Spring	50	0,480	0,886	0,000	4,000	184,620	4,951	2,171
	Summer	50	0,380	2,547	0,000	18,000	670,165	49,673	7,038
	Autumn	50	0,320	0,621	0,000	2,000	193,978	2,086	1,799
Basophils (%)	Winter	50	0,020	0,141	0,000	1,000	707,107	50	7,071
	Spring	50	0,160	0,510	0,000	2,000	318,438	8,929	3,165
	Summer	50	0,000	0,000	0,000	0,000	0,000		
	Autumn	50	0,000	0,000	0,000	0,000	0,000		
Heterophils (%)	Winter	50	2,620	3,392	0,000	16,000	129,466	5,589	2,227
	Spring	50	9,320	6,678	0,000	29,000	71,647	0,803	1,064
	Summer	50	17,560	9,719	0,000	40,000	55,346	-0,219	0,548
	Autumn	50	7,140	5,421	0,000	21,000	75,926	0,168	0,841

Besides granular leukocytes in blood smears stained according to Pappenheim, we determined the relative number of agranular leukocytes: monocytes and lymphocytes (Table 2). In contrast to basophils, lymphocytes showed the highest prevalence levels in blood of *Abramis brama*. In some instances values of lymphocytes in common bream amounted to 100%.

Table 2. Relative representation monocytes and lymphocytes in the blood *Abramis brama*

Variable	Season	Number fish	Mean	SD	Min	Max	Coefficient of variation	Kurtosis	Skewness
Monocytes (%)	Winter	50	1,440	1,248	0,000	4,000	86,668	-1,386	0,208
	Spring	50	7,640	7,136	0,000	28,000	93,409	0,725	1,306
	Summer	50	3,900	3,786	0,000	18,000	97,087	3,422	1,685
	Autumn	50	7,640	4,952	1,000	21,000	64,815	-0,261	0,662
Lymphocytes (%)	Winter	50	88,460	8,885	55,000	98,000	10,044	4,169	-1,794
	Spring	50	65,280	16,054	32,000	93,000	25,592	-0,836	-0,312
	Summer	50	65,380	16,638	18,000	100,000	25,448	0,192	-0,376
	Autumn	50	62,840	14,947	32,000	88,000	23,785	-0,881	-0,228

To test the difference between the determined values of the research parameters analyzed in different seasons we applied t-test. Those results are shown in Table 3. The biggest difference is found between the spring and winter seasons, contrary to difference between spring and autumn, which was smallest.

Table 3. T-test values between different seasons

	Seasons	Leu	Segm. neutrophils	Nonsegm. neutrophils	Eosinophils	Basophils	Heterophils	Monocytes	Lymphocytes
t-test Sig. (2-tailed)	Spring / Summer	0.000	0.099	0.088	0.794	0.029	0.000	0.001	0.976
	Summer / Autumn	0.209	0.000	0.001	0.320	-	0.000	0.000	0.424
	Summer / Winter	0.000	0.000	0.000	0.511	0.320	0.000	0.000	0.000
	Spring / Autumn	0.000	0.046	0.073	0.298	0.029	0.076	1.000	0.433
	Spring / Winter	0.000	0.000	0.000	0.013	0.064	0.000	0.000	0.000
	Autumn / Winter	0.008	0.013	0.000	0.077	0.320	0.000	0.000	0.000

Correlation analysis of between different type of leukocytes in total sample is presented in Table 4. At the same shows the values of the coefficient correlation (r) and statistical significance of the correlation (p). Monocytes and nonsegmented neutrophils showed the highest positive correlation.

The number and relative distribution of white blood cells is an important parameter that indicates the health of the fish. The average value of the number of leukocytes of *Squalius cephalus* from the river Krupica is higher than that of common bream and

Squalius cephalus from the River Željeznica (Mitrašinović, Suljević, 2009). Also, Stosik et al., 2002 found higher values of leukocytes in common bream (*Abramis brama*) from Lake Dąbie and Szczecin in relation to the common bream from reservoir Modrac. These authors found that *Abramis brama* from Lake Dąbie and Szczecin has the highest value of lymphocytes, in the analysis of differential blood picture, which is consistent to the results of our research.

Table 4. The coefficients correlation (r) between different types of leukocytes in the total sample

Variable	Leu	Segm. neutrophils	Nonsegm. neutrophils	Eosinophils	Basophils	Heterophiles	Monocytes	Lymphocytes
Leukocytes	1.0000	.0120	-.0196	-.0217	.0660	-.1442*	.1416*	.0383
Segmented neutrophils		1.0000	-.0238	-.0690	.0554	-.1916**	-.1203	-.0190
Nonsegmented neutrophils			1.0000	-.0242	.0822	.0939	.4723**	-.8318**
Eosinophils				1.0000	.0271	.0305	.0525	-.0828
Basophils					1.0000	.0228	-.0561	-.0736
Heterophiles						1.0000	.0615	-.5365**
Monocytes							1.0000	-.6230**
Lymphocytes								1.0000

* $p < 0,05$; ** $p < 0,01$

The negative correlation is established between lymphocytes and nonsegmented neutrophils, heterophiles and monocytes ($p < 0.01$).

CONCLUSIONS

White blood cells in the blood of common bream from the accumulation Modrac is showing the lowest average values during the summer, while the highest values were recorded in spring;

Basophils showed lowest relative number of all cells determined in this study and were not noticed in animals collected during the summer and fall;

Lymphocytes are the most common white blood cells in the blood of common bream;

Spring and winter are the seasons that show statistically significant differences in the values of the majority of the analyzed parameters;

Variables that are positively correlated to nonsegmented neutrophils were monocytes, while lymphocytes were significantly negatively correlated to nonsegmented neutrophils, heterophiles and monocytes.

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