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# EVALUATION OF ENVIRONMENTAL CONDITIONS BY FISH HEMATOLOGY

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**ABSTRACT**: **Evaluation of environmental conditions by fish hematology**
In the work are shown basic principles of application of the haematological status in determination of physiological status of fishes, also a review of a number of research from this area is given. One part of the research includes analysis of parametres of hematological status of the individuals in their natural habitat, while the other part is focused on the research of these parametres in experimantal conditions based on changes of some of the factors. Review include erythrocyte line parameters (erythrocyte number, hemoglobin concentration, hematocrit, MCV, MCH and MCHC) in different conditions in species: *Barbus balcanicus, Sqalius cephalus, Thymallus thymallus, Oncorhynchus mykiss, Carassius gibelio, Perca fluviatilis, Cyprinus carpio.*

**Keywords:** Hematology, fish, environmental conditions

## INTRODUCTION

Fishes make contact with environment, so the are sensite to physical and chemical changes in the environment, which can leat to changes in blood components (WILSON and TAYLOR, 1993), but also hematological parameteres represent indicators of environmental status (DEKIĆ et al., 2009).
Hematological status represents reliable means for detecting physiological state of the organism, and, indirectly, status of environment, because its parameters react to environmental changes (IVANC and MILJANOVIĆ, 2001). The quantitative characters of the red and white blood lines are used for detecting the haematological status, which provides insight in a number of processes in the organism, and based on analysis of different blood components it can be judged about changes that appears in certain systems influenced by external and internal factors. Determination of haematological parameters and blood plasma bichemisty is used for evaluation of health of wild and domestic animals. Values of this parameters are usefull in interpretation of results which are conected to various diseases and eco-environmental conditions (SEKER et al., 2005).
From there the haematological parameters are identified as very precious tool for controling fish health (TAVARES-DIAS et al., 2008) and in interpretation certain physiological response that are caused by ecological parameters (IVANC et al., 1997). Also these parameters give relevant informations during comparative study of certain species in differet habitats, and related species in the same habitat (IVANC et al., 1994). By Rowan

(2007), haematological parameters are also widely used indicators of stress in the environment beacuse the values of erythrocyte number, hemoglobin concentration, hematocrit, sedimentation rate, leukocyte number and differential blood are good indicators of presence of diseases or stressors. In the same time, by this author, changes in blood variables are caused also as a result of environmental changes, precisely changes in temperature, light, oxygen concentration and pollution (ROWAN, 2007). Importance of haematology in fish diseases diagnostics (ISHIKAWA et al., 2008), for evaluation of pollution influence and knowledge about environmental conditions (MURAD et al.,1990) is widely accepted, but for explaining blood analysis is needed knowledge about normal values of blood parameters IVANC et al., 2005) and reference range of certain parameters (HRUBEC et al., 2000).

## MATERIAL AND METHODS

This study is based on a number of previous investigations on this subject made by the authors.They are dedicated to hematology of several fish species under different environmental conditions as well as laboratory experiments conducted to analize effects of a specific environmental factor on hematological parameters. In all studies identical methods were used for blood colection and estimation of individual hematological parameters. Hematological status was estimated on the basis of the following hematological parameters: erythrocyte number (RBC), hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).
The involved 7 species chould be fisted subegvent by: List of the 7 invesligated fish species.

## RESULTS

The results of all studies are organised and listed by species, type of environmental influence or experimental factor on specefic hematological parameters and presented in table 1 with indication of significance of differences between means.
In the study of hematology of Large spot barbel (*Barbus balcanicus*) in two streams of different water quality DEKIC (2010) found out that fish from the stream with smaler saprobic index (Jakotinska River) had significantly higher values of hemoglobine concentration, values of MCV and MCH, while individuals from Suturlija River, with higher sapboric index value had significantly higher erythrocyte number. According to conducted hidrobiological analysis, both analysed watercourse belong to β-mesosaprobic category, but based on saprobity index the water from River Jakotina has better quality. Saprobity index of River Suturlija was 2.04, while River Jakotina has value 1.83, and observed by seasons and months, values in River Jakotina has often been in transit category between two classes.
Such ratio of monitored parameters is related to poor water quality from Suturlija, which in the Large spot barbel from this river cause presence of higher number of young, immature erythrocyte forms and mature erythrocytes with significant lower hemoglobin concentration, in circulation, that fish product as response to environmental conditions. Author concludes that poor water quality has two ways impact on hematopoiesis. It inhibited normal hemoglobin synthesis which rested with lower MCH. On the other hand, requirement for oxygen transport encouraged increased hematopoiesis and increase young

erythrocyte number in blood which is shown by higher RBC, and lower MCV. Such statement is also indicated by size and shape of erythrocyte nucleus which is smaller and with a slightly changed ellipsoid shape compared to erythrocyte nucleus of individuals from Jakotina river.

Presence of lower MCV cause lower MCH, and also lower values of total hemoglobin. Hematological responce to environmental conditions is different in different fish species. In hematology research of European chub (*Squalius cephalus*) from the same Jakotina River and more polluted Dragocaj River. Based on categorisation by Kol River Jakotina belongs to II class waters, while River Dragočaj belongs to III class waters. DJURDJEVIC et al., (2005) is concluded that this species in low quality streams also has higher values of hemoglobin concentration, but lower MCV, and unlike of large spot barbel chub has significantly higher MCHC but lower PCV in low quality water.

In Grayling, *Thymallus thymallus* (order Salmoniformes) hematology is monitored in fishes from open water stream Krusnica and farm conditions (DEKIC et al., 2009). It is very indicative that Grayling inhabiting Krusnica River and those farmed at Martin brod differed only in values of RBC and PCV which were significantly higher in fish living under natural conditions of river habitat. Authors are of the opinion that this was in correlation with higher activity level, which is well known to greatly influence hematological parameters of fish (IVANC et al., 1997a).

Beside parameters of hematological status which were recorded in individuals from two habitats with different water quality, experimantal researches were also performed, where during the experiment occured changes in some of the environmental factors. Such researches are performed on carp and perch at different exposure to water oxygen saturation, also on Rainbow trout at different growing temperatures, or on Prussian carp with sharp changes in ambient temperature (Table 1).

Hematological parameters of Rainbow trout are monitored on individuals from the pond and after that on individuals from the same population after thirty day at different ambiental temperatures, 9°C and 14°C. Comparation of recorded results from three gropus of individuals of Rainbow trout in different ambiental conditions shows presence of significant difference in most parameters. So, the highest values of erythrocyte number are recorded in individuals that were analysed directly on the fishpond and were significantly different compared to values of individuals grown at 14°C but not compared to individuals at 9°C. Authors concluded that fish transport and experimental treatment did not effect on erythrocyte number, while increasing water temperature caused increase of MCV and decrease of erythrocyte number. This also resulted with increase in MCH. Opposite of the erythrocyte number values of MCV shows significant increase with temperature increase, and the difference is significant compare to individuals from the fishpond, while in comparison, values in experimental individuals shows difference on the border of statistical significance.

Looking at total results of erythrocyte line parameters rof Rainbow trout from the fishpond and experimental conditions can conclude that there are slighty difference between values in individuals from fishponds and individuals grown at 9°C. Significantly higher difference were recorded in comparison to values of the individuals form fishponds and individuals grown at 14°C. Based on the results it is evident that in fishes grown at 14°C comes to increase in MCV, which also cause higher hematocrit values although has lower erythrocyte number. Also in this individualas are recorded slightly higher values of hemoglobin concentration, and related to that, values of MCH in individuals grown at 14°C is higher, which probably represents adaptation on temperature increase and rate of physiological processes. Influence of ambiental temperature increase on erythrocyte profile was done in experimental conditions on Prussian carp, Carassius gibelio. Fish were kept in aquaria at 10°C, for three weeks, and after that the temperature in one the aquaria was raised gradually to 20°C in three days period (DEKIĆ ET AL., 2011). Comparison of results of erythrocyte parameters in Prussian carp which was exposed to different ambiental temperatures shows presence of significant difference in most of analysed parameters.

**Table 1. Hematological parameters of several fish species under different environmental and experimental conditions**

| Environment | **Parameter** | **Hb g/l** | **PCV l/l** | **RBC $x10^{12}/l$** | **MCV fl** | **MCH pg** | **MCHC g/l eryt.** |
|---|---|---|---|---|---|---|---|
| ***Barbus balcanicus*** from an unpoluted (Jakotina) and poluted stream (Suturlija) | | | | | | | |
| River Jakotina (**A**) | AVG | **74.55$^b$** | **0.437** | **1.099$^b$** | **398.55$^b$** | **67.95$^b$** | **171.498** |
| | STD | 8.96 | 0.046 | 0.061 | 46.740 | 8.551 | 20.734 |
| River Suturlija (**B**) | AVG | **67.48$^a$** | **0.411** | **1.148$^a$** | **359.08$^a$** | **58.80$^a$** | **165.79** |
| | STD | 9.83 | 0.06 | 0.052 | 54.73 | 8.19 | 24.94 |
| ***Sqalius cephalus*** from an unpoluted (Jakotina) and poluted stream (Dragočaj) | | | | | | | |
| River Jakotina (**A**) | AVG | **75.02$^b$** | **0.423$^b$** | **1.464** | **289.59$^b$** | **51.39** | **182.93$^b$** |
| | STD | 7.78 | 0.068 | 1.59 | 43.37 | 6.36 | 39.51 |
| River Dragočaj (**B**) | AVG | **78.70$^a$** | **0.393$^a$** | **1.484** | **265.36$^a$** | **53.67** | **205.34$^a$** |
| | STD | 7.97 | 0.068 | 2.04 | 37.44 | 7.01 | 35.64 |
| ***Thymallus thymallus*** from river Krušnica and a fish farm | | | | | | | |
| River Krušnica (**A**) | AVG | **69.21** | **0.477$^b$** | **1.561$^b$** | **306.92** | **44.43** | **145.40** |
| | STD | 7.73 | 0.030 | 1.068 | 25.83 | 4.86 | 17.93 |
| Farm Martin Brod (**B**) | AVG | **61.81** | **0.429$^a$** | **1.387$^a$** | **308.77** | **44.33** | **143.15** |
| | STD | 14.40 | 0.067 | 1.636 | 31.71 | 8.46 | 20.54 |
| ***Oncorhynchus mykiss*** from a fish farm and aquaria with two ambient temperatures | | | | | | | |
| Farm (**A**) | AVG | **41.20** | **0.334** | **0.894$^c$** | **364.58$^{b, c}$** | **45.66$^c$** | **125.24** |
| | STD | 12.52 | 0.058 | 0.144 | 60.65 | 12.27 | 33.84 |
| Aquarium 9°C (**B**) | AVG | **42.52** | **0.311$^c$** | **0.872** | **373.66$^{a, c}$** | **51.57** | **137.43** |
| | STD | 4.91 | 0.044 | 0.177 | 96.56 | 16.13 | 18.04 |
| Aquarium 14ºC (**C**) | AVG | **44.11** | **0.344$^b$** | **0.823$^a$** | **431.67$^{a, b}$** | **55.24$^a$** | **129.35** |
| | STD | 7.22 | 0.043 | 0.151 | 98.79 | 13.78 | 22.19 |
| ***Carassius gibelio*** control fish and fish exposed to short term temperature increase for 10°C | | | | | | | |
| Aquarium 10°C | AVG | **74.66** | **0.450$^b$** | **1.046$^b$** | **438.60** | **77.16$^b$** | **172.79** |
| | STD | 11.13 | 0.085 | 0.196 | 91.49 | 18.02 | 49.52 |
| Aquarium 20°C | AVG | **76.73** | **0.545$^a$** | **1.234$^a$** | **448.16** | **63.31$^a$** | **144.35** |
| | STD | 1.47 | 0.076 | 0.161 | 85.13 | 12.28 | 35.69 |
| ***Perca fluviatilis*** from low (30 %) and high (90 %) $O_2$ saturated water | | | | | | | |
| High saturation (**A**) | AVG | **67.76$^b$** | **0.326** | **1.597** | **212.55** | **43.12** | **206.45** |
| | STD | 12.64 | 0.034 | 0.347 | 39.70 | 5.03 | 25.76 |
| Low saturation 30% (**B**) | AVG | **78.48$^a$** | **0.357** | **1.787** | **200.67** | **44.35** | **221.24** |
| | STD | 6.79 | 0.040 | 0.207 | 15.77 | 5.08 | 22.10 |
| ***Cyprinus carpio*** in water with normal (**A**) and low (**B**) oxygen concentration | | | | | | | |
| **A** | AVG | **49.08** | **0.234$^b$** | **1.040$^b$** | **237.61** | **46.99** | **201.94$^b$** |
| | STD | 20.00 | 0.061 | 0.374 | 45.62 | 6.37 | 33.66 |
| **B** | AVG | **56.62** | **0.329$^a$** | **1.314$^a$** | **251.38** | **42.91** | **173.34$^a$** |
| | STD | 15.46 | 0.084 | 0.266 | 40.20 | 6.07 | 27.82 |
| ***Cyprinus carpio*** from three cage fams with different environmental conditions | | | | | | | |
| Novi Becej **(A)** | AVG | **63.52** | **0.366** | **1.422** | **252.50** | **43.82** | **174.85$^b$** |
| | STD | 4.66 | 0.039 | 0.066 | 28.02 | 2.68 | 13.20 |
| Vrbas (**B**) | AVG | **63.40** | **0.335** | **1.353** | **246.80** | **46.72** | **189.65$^a$** |
| | STD | 13.48 | 0.070 | 0.243 | 29.26 | 5.83 | 13.27 |
| Kovilj **(C)** | AVG | **81.11$^b$** | **0.420$^{a,b}$** | **1.679$^{a,b}$** | **251.09** | **48.79$^a$** | **194.18$^a$** |
| | STD | 11.21 | 0.050 | 0.206 | 24.41 | 7.85 | 23.56 |

Mean values (AVG) of one parameter in the same study are marked with different letters in superscript when they are significantly different (significance level at least 0.05).

So, in individulas which were exposed to temperature increase were recorded significantly higher values of hematocrit and erythrocyte number, while values of MCH in this fishes is singnificantly lower. Based on this results authors conclude that gradual temperature increase encouraged hematopoiesis, which resulted to higher proportion of young erythrocyte in which hemoglobin synthesis is not finished. Looking at parameters of erythrocyte line in total in control and heat-treated fishes it can be detected that in fishes that were exposed to longterm termic stress values of erythrocyte number and hemtocrit are increased. In the same time in this group of individualas are also recorded a slightly higher values of hemoglobin concentration and MCV, while in control fishes are recorded significantly higher values of MCH and slightly values of MCHC.
Changes of hematological status are also recorded in timie of acclimation of carp to the water with low oxygen concetration (IVANC et al., 1996). Researches were performed on fished kept in cages in natural habitats for two months at three locality with different oxygen regime, and in experiment, after thirty days exposure to hipoxyc environment. In carp blood are recorded changes common to both types of exposure (increase in RBC, Hb concentration and PCV), also changes characteristic for certain exposure type. After three days stay in water with low oxygen concentration occures increase in MCV, and after two months of exposure to low oxygen concentration, increase of MCH and MCHC. Effort spent for additinal erithropoiesis is probably one of the cause of poor growth is carp grown in water with the most unforable oxygen regime.
These physiologic adaptation are recorded also in fishes from other families. Researches of IVANCA et al., (1997) show that in perch, in conditions when the most part of the day in the water is decreased oxygen concentration, occures changes in blood which improve oxygen transport efficiency. They goes in two ways: by increasing the number of circulatory erythrocytes and by increasing the total hemoglobin concentration. These conditions do not cause increase of MCH and MCHC.
Same authors showed (IVANC et al. 1994; 1995; 1997; 1997a) that in perch, also, chronic exposure to conditions of worse oxygen regime cause increase in MCV and MCHC values, which was also noticed in carp in similar ambiental conditions.
Thereby it can be noticed that a short exposure to unforable conditions does not cause increase in MCH ans MCHC, while longer exposure cause increase. SPECKNER et al (1989) founded that in carps mature erythrocytes also can sythesize hemoglobin. Results with short-term hypoxy show that primarily erythrocite number increase, and then hemoglobin concentration. Certain increase of MCV shows, however, a possibilty that synthesis of hemoglobin can be occured in mature erythrocyte.

## CONCLUSIONS

Hematological status reflects good reaction of organism on cahnges in environmental conditions. They are manifested by increasing the number of circulatory erythrocytes, increasing total hemoglobin concentration in blood and mean corpuscular volume. In short-termed expositon to unforable conditions MCH anad MCHS is not increasing, while in long-term exposition they do increase.

## REFERENCES

DEKIĆ, R. (2010): Cirkanualna istraživanja hematološkog statusa Barbus peloponnesius u fiunkciji staništa. Doktorska disertacija, Univerzitet u Banjoj Luci.

DEKIĆ, R., IVANC, A., BAKRAČ-BEĆIRAJ AZRA, BOŠKOVIĆ JELENA (2009): Normalne hematološke vrijednosti gajenog lipljena, IV međunarodna konferencija i Sajam tehničkih i tehnoloških dostignuća "Ribarstvo" 27. – 29. Maj, 2009. Beograd. 358-364.

DEKIĆ, R., IVANC, A., BAKRAČ-BEĆIRAJ AZRA, BOŠKOVIĆ JELENA, LOLIĆ SVJETLANA (2009): Hematološki parametri riba kao indikatori stanja životne sredine, IV međunarodna konferencija i Sajam tehničkih i tehnoloških dostignuća "Ribarstvo" 27. – 29. Maj, 2009.Beograd. 204-210.

DEKIĆ, R., IVANC, A., MIROŠLJEVIĆ, D. (2010): Eritrogram kalifornijske pastrmke *(Oncorhynchus mykiss*) iz ribnjačkih i eksperimentalnih uslova. II simpozijum biologa Republike Srpske, 4-6. 11. 2010. Banja Luka.

DJURDJEVIC SVJETLANA, DEKIĆ, R., VUKOVIĆ DRAGOJLA., IVANC, A. (2005): Kvalitet vode morfološke, merističke i hematološke karakteristike klena (*Leuciscus cephalus*), I Simpozijum biologa Republike Srpske, Zbornik radova, 231-244.

HRUBEC, T.C., CARDINALE, J. L. AND SMITH, S. A.(2000): Hematology and Plasma Chemistry Reference Intervals for Cultured Tilapia (*Oreochromis* Hybrid). *Veterinary* Clinical Pathology, 29, 1: 7-12.

ISHIKAWA, N.M., RANZANI-PAIVA, M.J.T. and LOMBARDI, J.V.(2008): Total leukocyte counts methods in fish, *Oreochromis niloticus*. Archives of Vet. Science,13,1,54-63.

IVANC, A., S. MALETIN, N. ĐUKIĆ, B. MILJANOVIĆ (1994):Ecophysiological interpretation of the hematology of different species of *Percidae* in the Tisa river. Tiscia, 28 : 53-57.

IVANC, A., MALETIN, S., N. ĐUKIĆ, V. PUJIN, V. MILJANOVIĆ B. (1995): Haematology of Percidae from the Yugoslav section of the Danube river system. First International Symposium "The Ecology of Large Rivers", Abstracts: 49, Krems, Lower Austria.

IVANC, A., MALETIN, S., DJUKIĆ, N., PUJIN, V., MILJANOVIĆ, B., BUGARSKI, R. (1996): Adaptation der Karpfen während periodischen Verschlechterungen der Sauerstoffverhältnisse auf dem Überschwemungsgebiet der Donau. I.A.D. Internationale Arbeitsgemeinschaft Donauforschung der Societas Internationalis Limnologiae S.I.L. Limnologische Berichte Donau, Band I, Wissenschaftliche Referate, 40 Jahre I.A.D. Göd/Vàcràtót. 31. Konferenz der IAD, Baja-Ungarn, 335-340.

IVANC, A., ETINSKI, M., MALETIN, S., ĐUKIĆ, NADA., MILJANOVIĆ, B., PUJIN VLASTA (1997): Grgeč kao test organizam u proceni kvaliteta vode. Zaštita voda, 253-257.

IVANC, A., MALETIN, S., DJUKIC, N., MILJANOVIC, B. (1997a): Haematology of fish species inhabiting running and stagnant waters in Danube river system. 32. Konferenz der Internationale Arbeitsgemeinschaft Donauforschung der S.I.L. Limnologische Berichte Donau 1997, Band I, Wissenschaftliche Referate, Wien, 377-380.

IVANC, A., MILJANOVIĆ, B. (2001): Hematološki status u dijagnostici fiziološkog stanja riba i uslova staništa. Eko-konferencija 2001. Ekološki pokret grada Novog Sada.

IVANC, A., HASKOVIC, E., JEREMIC, S., DEKIĆ, R. (2005): Hematological Evaluation of welfare and health of fish, *Praxis veterinaria* 53, 3: 191-202.

MURAD, A., HOUSTON, A.H., AND SAMSON, L. (1990): Haematological response to reduced oxygen-carrying capacity, increased temperature and hypoxia in goldfish, Carassius auratus L. *Journal of Fish Biology*. 36: 289-305.

ROWAN, M. W. (2007): Use of blood parameters as biomarkers in brown bullheads (*Ameiurus* NEBULOSUS) from lake erie tributaries and cape cod ponds. Dissertation. The Ohio State University 2007.

SEKER, Y., KARATAS, M. AND SEZER, M. (2005): Determination of Biochemical Parameter Values of Chub (*Leuciscus cephalus*) Population in Almus Dam Lake, Turkey. *Journal of Animal Andveterinary Advanses* 4, 11: 927 – 929.

TAVARES-DIAS, M., AFFONSO, G.E., OLIVEIRA, R.S., MARCON, L.J. AND EGAMI, I.M. (2008): Comparative study on hematological parameters of farmed matrinxa, *Brycon amazonicus* Spix and Agassiz, 1829 (Characidae: Bryconinae) with others Bryconinae species. Acta amazonica. 38, 4: 799-806.

WILSON, R.W. AND TAYLOR, E.W.(1993). The physiological responses of freshwater rainbow trout, *Onchorynchus mykiss* , during acute exposure. *J. Co.. Phy*. 163b: 38- 47