<u>Original Research Article</u>

Effects of hypertermy on erythrocyte parameters of carp from Bardaca swamp *Cyprinus carpio* (Linnaeus, 1758)

ABSTRACT

Thermal changes in water cause many metabolic changes that manifest themselves in physiological fish adaptations. The analysis of hematologic and biochemical blood parameters provides important information on environmental influences on the health status of fish. In this study hematological parameters of carp (*Cyprinus carpio* L.) during thermal stress were analyzed: hematocrit, hemoglobin concentration, RBC, MCV, MCH and MCHC. The fish were exposed to a half an hour of 28 °C water temperature. The results showed a decrease in MCV and an increase the value of other parameters. Significant changes for the number of erythrocyte and hematocrit values were found. The carp shows excellent ability to adjust to temperature variations that can be seen through the analysis of hematological status.

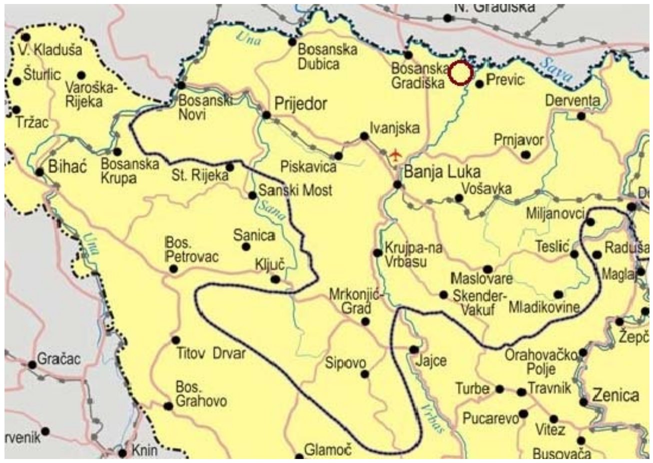
Keywords: thermal stress, erythrocyte parameters, morphometric parameters, carp

1. INTRODUCTION

Fish can bear significant variations in temperature, but in a short period of time (within 24 hours), however, if these variations last longer, serious metabolic disorders may occur, which in the end has a lethal outcome [1]. Water temperature has a significant effect on the physiological and biochemical processes in fish. It has been proven that increasement in temperature is harmful to generative processes, and has a significant effect on the nervous and endocrine system [2]. Stress reactions cause numerous changes in animal organisms, which can be adequately monitored by changes in the blood count. One of the best studied examples of stress reactions is the one which occurs due to a change in temperature (thermal stress), which can be monitored by changing the value of hematologic parameters [3]. Since they are in the long-term dynamic balance with a changing environment, fish are very sensitive to changes in physic-chemical parameters of the environment and their defense mechanisms are reflected in changes of hematological parameters in blood [4]. Regarding the changes in the erythrocyte parameters, they serve to establish the diagnosis of the existence and type of physiological adaptive mechanisms [5].The number of erythrocytes and leukocytes, the hemoglobin concentration, and the value of hematocrites are used as a good health indicator of the fish population [6]. According to documented facts, the aim of this study was to determine the effect of elevated temperature (hyperthermia) on hematological parameter of a carp, after exposure of the fish to a temperature of 28˚C, as well as mechanisms of carp adaptation in terms of hematological parameters in the state of thermal stress.

2. MATERIAL AND METHODS

2.1. Site



The swamp area of Bardaca is located in the north of Bosnia, northeast of Banja Luka, near Srbac, with the northernmost point at 45^{0}6'6''northern latitude and the southernmost point at 17^{0}26'26'' of the southern latitude, and includes 11 lakes (Figure 1) . It represents a very sensitive and important ecosystem. The main water supply of the lake is carried out mostly from the Matura river, but the tributaries of Zabljak and Brzaja are also significant. The total area of the fishpond is about 658 hectares. The complex structure of the terrain dictates the hydrological characteristics that caused the hydrological regime that is important for the development of the swap biotope. The great anthropogenical influence in the last 50 years, on the one hand, enabled the survival of the ecosystem, and on the other hand broke it the same.

Figure 1. Bardaca swamp

2.2. Experimental design

Ten control and 16 experimental fish (*Cyprinus carpio* L.) were used in this study. The fish were fished on the Bardaca area, and then transferred to a container with water that was permanently enriched with oxygen by using aerators with a pump. The fish were transported to the laboratory and adapted for 20 days. Fish adaptation included daily monitoring that included: water change, filter change, oxygen control, and ammonia control. After the adaptation, an aquarium with aerators and heaters was prepared for the experimental group, with the water temperature gradually increasing to 28 ˚C. After reaching the temperature, the fish were kept under these conditions for 30 minutes.

2.3. Hematological techniques

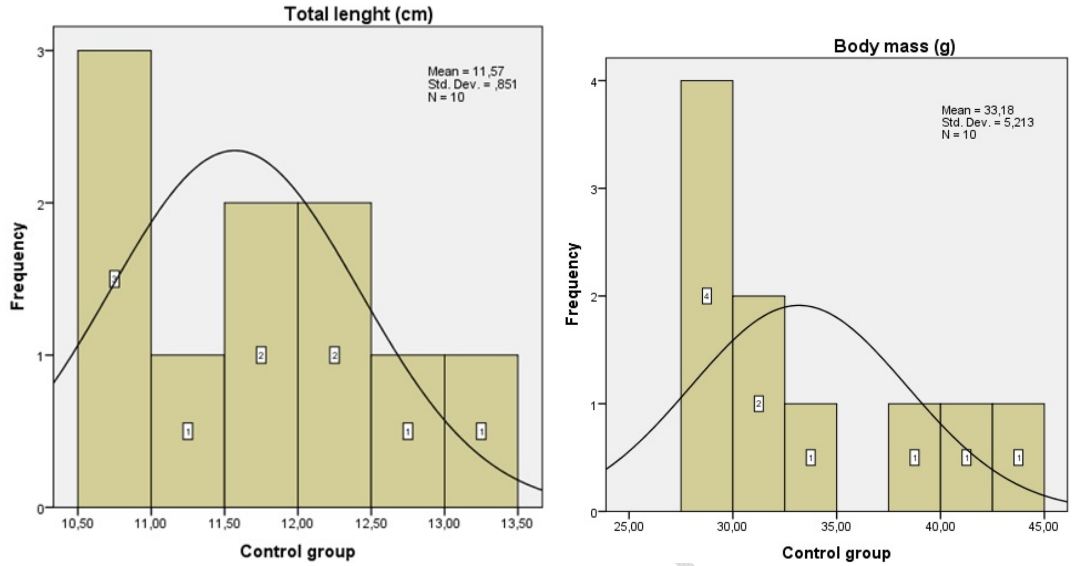
On control and experimental fish were performed direct puncture (using needles of 1 mm, Semikem, B & H) of the heart without anticoagulant in order to collect blood. The following parameters were used for hematological analyzes: number of erythrocyte (RBC), hematocrit value (HCT), hemoglobin concentration (HB) and hematology indexes: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The analysis of the number of erythrocytes was performed using the standard method in the hemocytometer, the values of the hematocrit were analyzed by the microhemocritic method after centrifugal blood at 15 000 rpm 10 min (Hettich zentifugen), and the concentration of hemoglobin was determined by Drabkin's hemoglobin cyanide method [7]. Hematological indexes are obtained as a calculational value from RBC, HCT and HB.

2.4. Statistical analysis

The data were analyzed using the IBM SPSS Statistics version 20 software for determining the average value and range. The 95% confidence interval and 5% absolute precision were used for the analysis of variance test (One Way ANOVA).

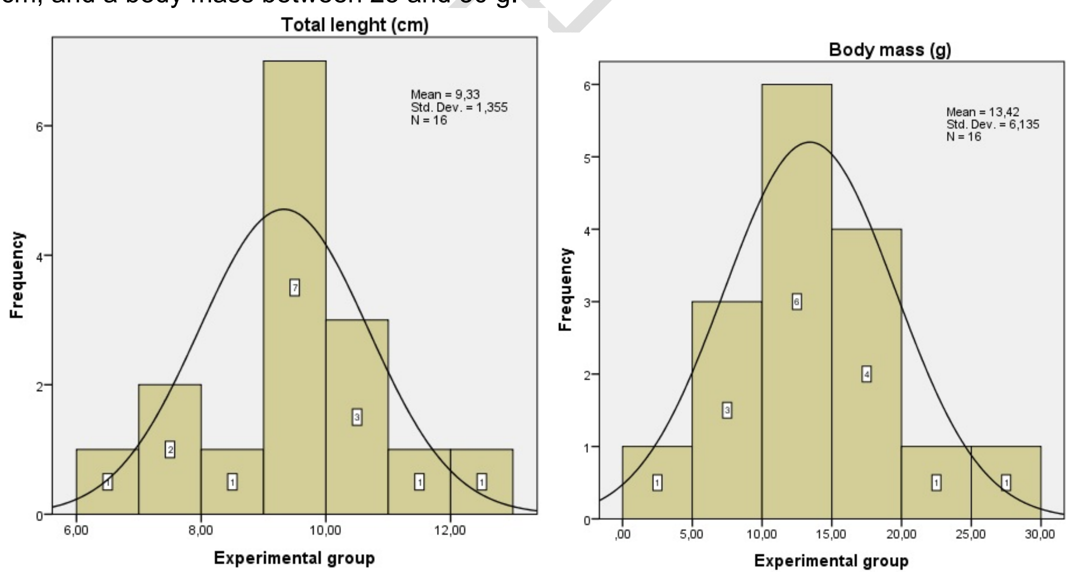
3. RESULTS AND DISCUSSION

Graphs 1 and 2 represent the values of morphometric parameters (total length and body mass) of control and experimental fish.



Graph 1. Total length and body mass in control fish

The average body length of the control fish was 11.57 ± 085 cm, and the body mass was 33.18 ± 5.21 g. The largest number of individuals had a body length between 10.50 and 11 cm, and a body mass between 25 and 30 g.



Graph 2. Total length and body mass in experimental fish

The average body length of the experimental fish was 9.33 ± 1.35 cm, and the body mass was 13.42 ± 6.13 g. The largest number of individuals had a body length of between 8.50 and 10 cm, and a body mass between 10 and 15 g. In control fish, higher values of morphometric parameters in relation to experimental fish were recorded.

Table 1 presents the values of hematological parameters of control and experimental fish. As a statistical indicator, mean, standard deviation and range are presented. One Way ANOVA was used to analyze intergroups differences.

Table 1. Hematological parameters of control and experimental fish

	Control group (10)		*Experimental group (16)*		*Sig.*
	Mean ± stdv	**Range**	**Mean ±s tdv**	**Range**	
HCT (l/l)	0,25±0,04	0,19-0,30	0,29±0,04	0,22-0,39	**0,022***
HB (g/l)	62,22±16,00	41,80-96,14	76,99±22,48	49,40-112,48	0,062
RBC (10^{12}/l)	1,191±0,155	1,010-1,460	1,409±0,131	1,130-1,590	**0,001***
MCV (fl)	209,90±48,12	130,14-267,86	205,81±34,02	148,00-273,43	0,752
MCH (pg)	52,24±17,91	30,94-85,84	54,64±16,24	33,19-82,24	0,868
MCHC (g/l)	248,88±56,13	137,50-320,47	265,48±81,67	177,24-410,51	0,578

**significant values at 0.05*

In the experimental fish group there was an increase in most parameters, except for MCV values, while significantly higher values were characteristic of HCT and RBC.

Nowadays, the stress reaction in the aquatic habitat is more and more present. The study of hematological parameters, as an indicator of the effects of negative stress factors, are very important from the aspect of physiological dynamics within thermal stress. According to available literature data, research has been carried out only in some species within the family of Cyprinidae, which makes this research even more important.

In order to use blood parameters as biomarkers, it is necessary to know their standard values and the reference interval [8]. Since the reference values are difficult to determine for a family, it is necessary to analyze each type individually [9].

Our data are very different from the reference values of some studies for cyprinid species. The data obtained by this research largely deviate from the results of large numbers of authors [10-12]. Hematological parameters in *Carassisus carassius* L. and *Cyprinus carpio* L. [13] show similar values for erythrocytes only, while other values are very different. Because of this, there are such differences in the same species, probably due to different age groups. Our fish were very young, which can be seen on the basis of their morphometric parameters.

It is very important in fish to analyze hematologic parameters by age and other external influences. However, in the mentioned studies, there is also an increase in hematological parameters during thermal stress and hypoxia. The metabolic response to hypoxia may vary from the physiological state of the animal, activity and temperature [14]. Increasing the water temperature affects the oxygen solubility, so it also results in decreased oxygen concentration, which causes hypoxia. Hypoxia causes greater erythrocyte production, which affects the increase in the value of all hematological parameters. However, in the [15], no significant increase in RBC and HB values was found. In our research, although there was an increase in all parameters, only significant differences were found for RBC and HCT. HCT increases when the blood cell production increases, in this case RBC. Since under such conditions there was a constant inflow of oxygen, hypoxia can't be considered a key stressor, but it is attributed to increased temperature. Such temperatures lead to greater agility and muscle contraction, which increases the metabolic rate, which requires higher amounts of oxygen.

However, the fish contain reserve depletion of erythroblasts that can be rapidly excreted into the bloodstream [16] but their production is oxygen dependent and it is indispensable for a longer time to synthesis, which means no significant increase in hemoglobin concentration. The increased number of RBCs is a primary hematologic response to hypoxia, as documented in *Carassius auratus* L. [17]. The key event in the hematopoietic response to temperature elevation is replacement of aged and ripe erythrocyte with new, young cells that are metabolically more competent in terms of gas transport [18]. The reference values of RBC carp vary between 1.8-2.2 x 1012 [19], while our research has significantly lower values in fish in the state of thermal stress.

The concentration of hemoglobin depends on the ecological conditions [20] and the physiological state of the fish [21]. Elevated hemoglobin values in the blood of our experimental fish can be attributed to a rapidly adaptive response to acute hypoxia, with available hemoglobin increasing the amount of oxygen in the blood of fish [22].

Observing the total value of hematological indices, elevated mean values of MCH and MCHC were observed during thermal stress. The hematologic indexes in our study are lower than the value of the same parameters observed in certain cultures of carp raised in an intensive system [23]. The research carried out by [24] indicates that MCV, MCH and MCHC in carp are temperature independent, while in our study higher values have been recorded during hyperthermia. Higher hematological index values were recorded in *Crassius gibelio* L. in the state of thermal stress [1].

4. CONCLUSION

Thermic stress in *Cyprinus carpio* L. causes increase in the value of all hematological parameters, and significant differences have been noted for RBC and HCT. As an adaptation mechanism in the state of hyperthermia and thermal stress, the number of erythrocytes in carp is primarily increased, which results in elevation in other hematological parameters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

All authors read and approved the final manuscript.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

REFERENCES

1. Dekić R, Ivanica A, Mandić M, Lozić SB, Bakrač-Bećiraj A. Utjecaj promjena temperature vode na eritrocitni profil *Carassius gibelio. Croatian Journal of Fisheri.* 2013; 71: 32-36.

2. Modrić T. Socijalni stres jegulje. *Ribar. Jugosl.* 1988; 43: 30-32.

3. Suljević D, Islamagić E, Fočak M. The effects of high temperature level on electrolytes and glucose concentration in tench (tinca tinca Linnaeus, 1758) serum. *Veterinaria.* 2009; 60 (2): 60-64.

4. Wilson RW, Taylor EV. The physiological responses of fresh-water rainbow trout, *Oncorhynchus mykiss* during acutely lethal copper exposure. *Journal of Comparative Physiology*. 1993; 163: 38-47.

5. Ivanc A, Maletin S, Đukić N, Miljanović B. Comparative haematology of some European Percidae species.Percis II, Second International Percid Fish Symposium, Vaasa, Finland, Abstracts. 1995; 37.

6. Omoregie E, Oyebanji SM. Oxytetracycline-induced blood disorder in juvenile nile tilapia oreoshromis ničoticus (Trewavas). *J. World Aquac. Soc*. 2002; 33: 337-382.

7. Blaxhall PC, Daisley KW. Routinehaematological Methods for Use with Fish Blood. Journal of Fish Biology. 1973; 5: 771-781.

8. Dekić R, Ivanc A, Erić Ž, Gnjato R, Trbić G, Lolić S, Manojlović M, Janić N. Hematological characteristics of Delminichthys ghetaldii (Steindachner 1882) in habiting the Karst region of Eastern Herzegovina. Archive of Biological Science Belgrade. 2014; 66 (4): 1423–1430.

9. Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research. Third edition. W. H. Freeman and Company, San Francisco. 1995; 859.

10. Chernyavskikh SD, Kuet DH, Trikula NL, Buslovskaya LK, Kovtunenko AY, Makarova YA. Hematologic profile for Cyprinus carpio. Indo American Journal Pharmaceutical Sciences. 2017; 4 (9), 3155-3161.

11. Niraj KT, Kenneth SL, Victoria VB. Hematologic reference intervals for koi (Cyprinus carpio), including blood cell morphology, cytochemistry, and ultrastructure. Veterinary Clinical Pathology. 2004; 33(2): 74-83.

12. Groff JM, Zinkl JG. Hematology and clinical chemistry of Cyprinid fish. Common carp and goldfish. Veterinary Clinicas of North America: Exotic Animal Practice. 1999; 2 (3): 741–746.

13. Suljević D, Alijagić A, Mitrašinovic-Brulić M, Fočak M, Islamagić E. Comparative physiological assessment of common carp (Cyprinus carpio) and crucian carp (Carassius carassius) based on electrolyte and hematological analysis. *Macedonian Journal of Animal Science*. 2016a; 6 (2): 95–100.

14. Burggren W, Roberts J. In: Environmental and Metabolic Animal Physiology, 4th edition, edited by C.L. Prosser. Wiley-Liss, New York; 1991; 353-435.

15. Ghittino P.. Tecnologia e Patologia in Aquacoltura. Vol I: tecnologia. E.Bono. 1983; 53219.

16. Suljević D, Islamagić E, Alijagić A, Fočak M, Mitrašinovic-Brulić M**.** Morphological identification of haematopoietic cells in pronephros of common carp **(***Cyprinus carpio* Linnaeus, 1758)**.** *Acta Biol Szeged 2016.* 2016b; *60(2):113-118.*

17. Houston AH, Murad A. Erythrodynamics in goldfish, Carassius auratus L : Temperature effects. Phys. Zool. 1992; 65: 55-76.

18. Houston AH, Dobric N, Kahurananga R. The nature of hematological response in fish. Studies on rainbow trout Onorynchus mykssis exposed to simulated winter, spring and summer conditions. Fish Physiology and Biochemistry. 1996; 15 (4): 339-347.

19. Bogut I, Novosilević D, Pavličević J. Biologija riba. Sveučilište J. J. Strosmajer u Osijeku, Sveučilište u Mostaru; 2006.

20. Zhiteneva LD, Rudnitskaya OA, Kalyuzhnaya TI. Ecology-hematologic characteristics of some species of fish: Reference book. Rostov-on-Don: Hammer. 1997; 149.

21. Golovina NA. Morpho-functional characteristics of the blood of fish as objects of aquaculture. PhD Thesis, Moscow. 1996; 53.

22. Speckner W, Schindler JF, Albers C. Age-dependent changes in volume and haemoglobin content oferythrocytes in the carp (*Cyprinus carpio* L.). J. Exp. Biol. 1989; 141: 133–149.

23. Radu d, Oprea L, Bucur C, Costache M, Oprea D. Characteristics of Haematological Parameters for Carp Culture and Koi (Cyprinus carpio Linneaus, 1758) Reared in an Intensive System. Bulletin UASVM Animal Science and Biotechnologies. 2009; 66 (1-2).
24. Smith GL, Hattingh J, Ferreria JT. The physiological responses of blood durnig thermal adaptation in three freshwater fish species. J. Fish Biol. 1981; 19: 147-160.