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Effects of Hyperthermia on Erythrocyte Parameters of Carp *Cyprinus carpio* (Linnaeus, 1758) from Bardaca Swamp, Bosnia and Herzegovina

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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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. The hematocrit (HCT) (l/l), hemoglobin concentration (Hb) (g/l), mean corpuscular volume (MCV) (fl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/l) and red blood cells (RBC) ($x10^{12}$/L) were analyzed. Animals were grouped into two groups: control (n=10) and experimental (n=16).
The experimental fish were exposed to 28°C for 30 min. Puncture of the heart was done and the blood without anticoagulant was analyzed. During hyperthermia, an increase in hematological parameters was observed, except for MCV values that were low. Significant differences were established only for the number of erythrocytes and the hematocrit values ($p<0.05$).

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The results showed a decrease in MCV and an increase in the value of other erythrocyte parameters. Significant changes in the number of erythrocyte and hematocrit values were found. Some hematological parameters such as erythrocyte and MCV values are significant stress indicators and can serve us as important factors for physiological adaptations of fish.
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 are able to 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 increse 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. Changes in the hematological parameters can be monitored in stress reactions induced by temperature change [3]. Since they are in the long-term dynamic balance with a changing environment, fish are very sensitive to changes in physico-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 hematocrit are used as a good health indicator of the fish population [6]. The aim of this study was to determine the effect of elevated temperature (hyperthermia) on the 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. MATERIALS 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°6'6'' northern latitude and the southernmost point at 17°26'26'' of the southern latitude, and includes 11 lakes (Fig. 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. In the last 50 years, on one hand, the great anthropogenic influence has enabled the survival of this ecosystem, and on the other hand destroyed it.

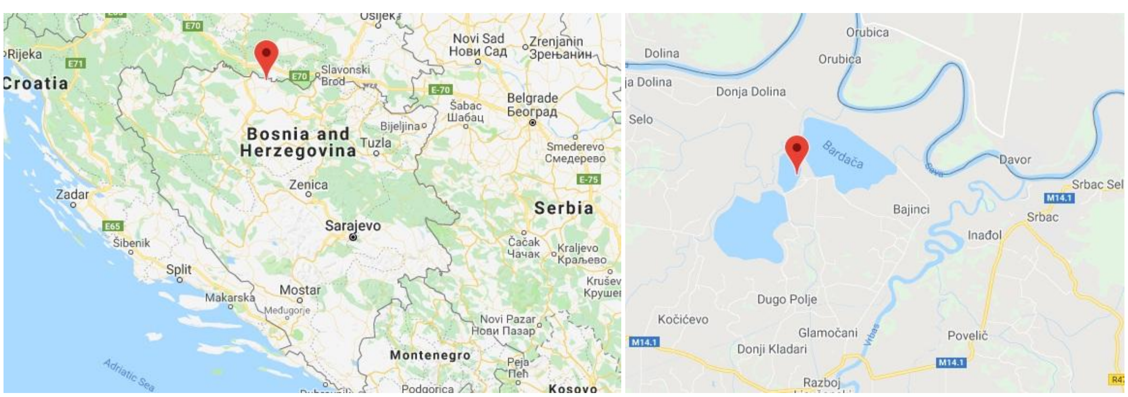


Fig. 1. Bardaca swamp

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. Changing water and filters, analysis of the concentration of oxygen and ammonium ions was done daily for the necessary adaptation of fish. After the adaptation, an aquarium with aerators and heaters was prepared for the experimental group, with the water temperature gradually increasd to 28°C and fishes were exposed in this conditions for 30 minutes.

2.3 Hematological Techniques

During the experiment, all fishes were anaesthetized using Benzoak VET 200 (Vitusapotek, Netherlands) at a concentration of 15-20 ml/10L for 15 minutes. Blood samples were taken by direct puncture of the heart without anticoagulant. The puncture was performed with the sterile needles of 1.0 mm (Medoject, Slovak Republic).

The following parameters were used for hematological analyzes: a number of erythrocytes (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 (Neubauer chamber), the values of the hematocrit were analyzed by the microhemocritic centrifuges after centrifugal blood at 15 000 rpm 10 min (Hettich Haematokrit 24 zentri-fugen, Germany), 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.

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 10.50-11 cm, and a body mass between 25- 30 g.

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 between 8.50 - 10 cm, and a body mass between 10 -15 g. In control fishes, higher values of morphometric parameters in relation to experimental fishes were recorded.

Table 1 presents the values of hematological parameters of control and experimental fishes. As a statistical parameter, mean, standard deviation and range are presented. One-Way ANOVA was used to analyze intergroup differences (Sig.)

In the experimental fish group, there was an increase for most parameters, except for MCV value. Significantly high values in the experimental group were determined for HCT and RBC in relation to the control group.

Today, the response to stress in the aquatic habitat is more and more present. The study of hematological parameters, as an indicator of the effects of negative stress factors, is very important from the aspect of physiological dynamics within thermal stress. According to available literature data, there is not much available data for blood values of these species, 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 different from the reference values of some studies for cyprinid species [10]. The data obtained by this research largely deviate from the results of large numbers of authors [11-13]. Hematological parameters in *Carassisus carassius* L. and *Cyprinus carpio* L. [14] 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. The fish used in this study 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 [15]. 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 [16], no significant increase in RBC and HB values was found. RBC values of control and experimental group of carp were lower than in *Aulopyge hugeli, Leuciscus turskyi, Chondrostoma phoxinus, Leuciscus turskyi, Paraphoxinus alepidotus, Phoxinus phoxinus,* and the values of HB of these species and the control group were quite similar, while the value of the HB experimental group was considerably lower in relation to these species [10]. Some other species of cyprinides, such as *Leuciscus cephalus* had almost the same vaule of RBC as our experimental group of carp [17].

In this research, although there was an increase in all parameters, only significant differences were found for RBC and HCT. HCT increased 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 [18] 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. [19]. The key event in the hematopoietic response to temperature elevation is the replacement of aged and ripe erythrocyte with new, young cells that are metabolically more competent in terms of gas transport [20]. The reference values of RBC carp vary between 1.8-2.2 x 10^{12} [21], while this research has significantly lower values in fish in the state of thermal stress.

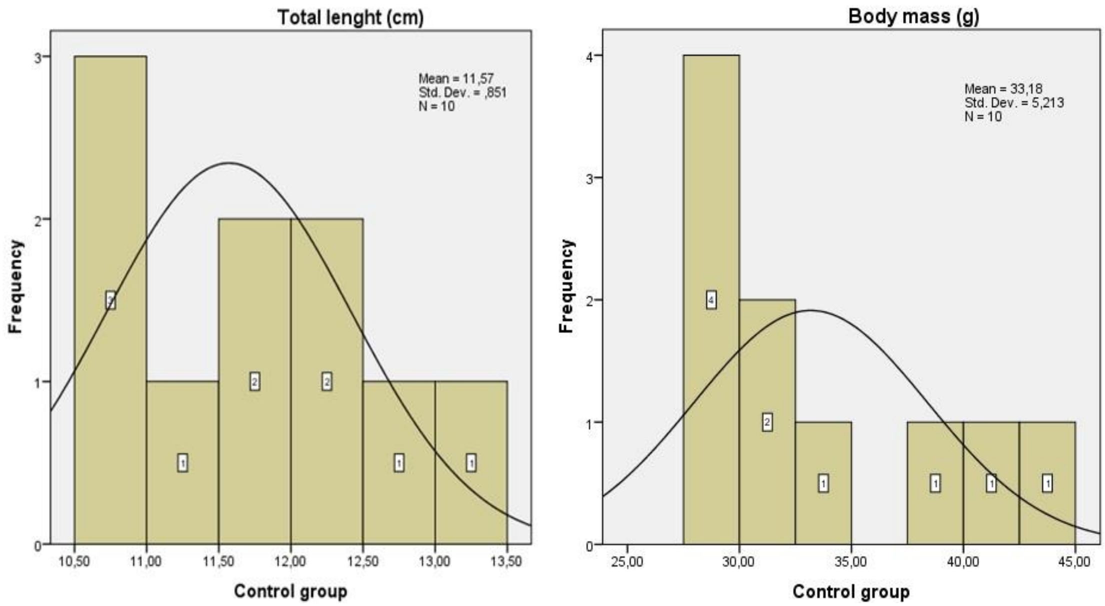
The concentration of hemoglobin depends on the ecological conditions [22] and the physiological state of the fish [23]. Elevated hemoglobin values in the blood of our experimental fish can be attributed to a rapidly adaptive response to acute hypoxia, with available hemoglobin increasing the amount of oxygen in the blood of fish [24]. HB values of our experimental group are similar in other research, like those of *Delminichthys gethaldii, Crassius crassius* [8,14].

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 [25]. Higher hematological index values were recorded in comparative physiological assessment of *Cyprinus carpio* and *Carassius carassius* compared to our research [14]. The research carried out by [26] 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].

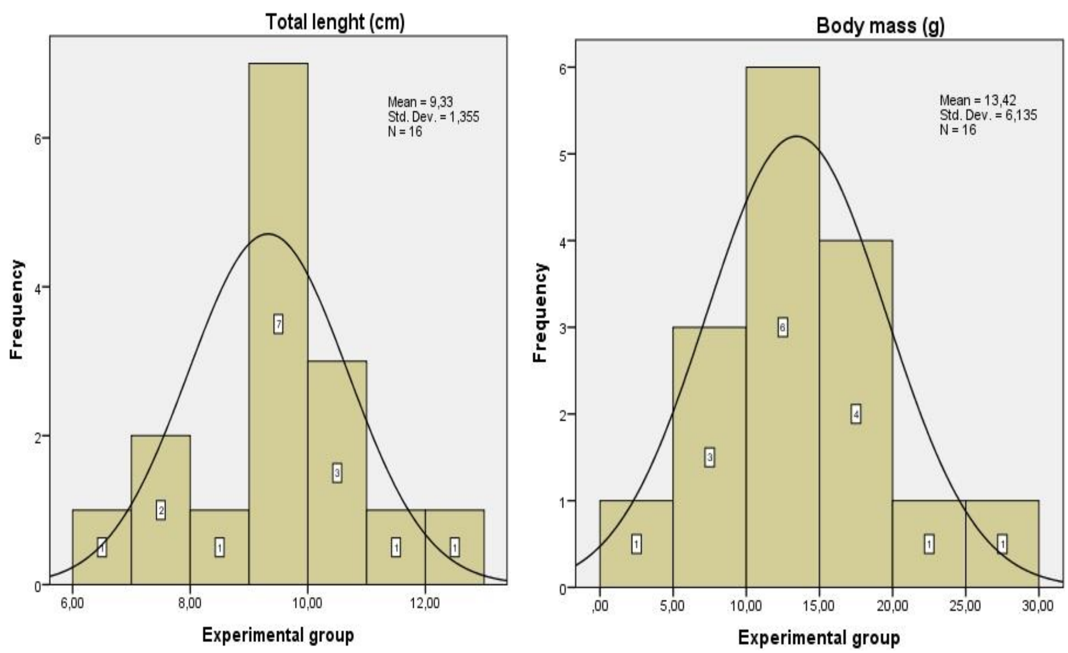
Table 1. Hematological parameters of control and experimental fish

	Control group (10)		**Experimental group (16)**		**Sig.**
	Mean ± SD	**Range**	**Mean ±SD**	**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*
SD – standard deviation



Graph 1. Total length and body mass in control fish



Graph 2. Total length and body mass in experimental fish

4. CONCLUSION

Hematological evaluation is significant in the early detection of health problems and very important in physiological adaptations to stress factors. Energy metabolic requirements are very pronounced during hyperthermia, and as the best indicators for fish adaptation are erythrocyte and MCV values. The fact is that fish adaptations depend on the type and age, as well as other parameters. Therefore the blood parameters analysis must be very careful and the results must be clarified for each species individually. The present work has shown that *Cyprinus* *carpio* possesses very good physiological adjustments to environmental stressors such as water temperatures.

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.

DISCLAIMER

This manuscript was presented in a Conference.
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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