

## HEMATOLOGICAL PROFILES OF MATURE *ACIPENSER STELLATUS* FROM DANUBE RIVER DURING SPRING SEASON

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### Abstract

In the Danube River, the stellate sturgeon population has declined dramatically from a numeric perspective. The screening of hematological parameters is regarded as a useful tool for assessing the health status of fish species. The objective of the study was to analyzing the parameters in the blood of genitors of the *A. stellatus* fishing for assessing the health status. Individual *A. stellatus* were anesthetized with MS222 and 3 ml of blood was sampling and analyzed with routine method used in fish hematology. Was determined: red blood cell counts (RBCc,  $\times 10^6/\mu\text{l}$ ), the hematocrit (PCV, %), the leukocrit (LK) and hemoglobin concentrations (g/dl). Using standard formulas the red blood indices were computed: the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). According to the present results, hematological values in males and females were: PVC 35,80 and 31,73 %, RBC count 1,36 and 1,05  $\times 10^6/L$ , hemoglobin 9.97 and 9.41 g  $\text{dl}^{-1}$ , MCV 266,93.76 and 305.47 fL, MCH 74,33 and 89.98 pg, MCHC 27,83 and 30.55 g  $\text{dl}^{-1}$ . The hematological values reported in this paper will be used as reference for monitoring of physiological and health status of *A. stellatus* from the Danube river.

**Key words:** *Acipenser stellatus*, mature sturgeon, hematological parameters, Danube River

### INTRODUCTION

The Danube River and the Black Sea were originally inhabited by six sturgeon species [2]: the beluga sturgeon (*Huso huso*), the Russian sturgeon (*Acipenser gueldenstaedtii*), the stellate sturgeon (*A. stellatus*), the ship sturgeon (*A. nudiventris*), the freshwater sterlet (*A. ruthenus*) and the European sturgeon (*A. sturio*). In the Danube River, the stellate sturgeon population has declined dramatically from a numeric perspective. Overfishing, loss of critical habitat, loss of access to spawning grounds as a result of dam construction, and pollution have contributed to declines in sturgeon populations worldwide [7]. Hematologic reference interval can be important tools to diagnose disease and monitor the effects of environmental change, anthropogenic impacts or the effectiveness of management actions. In the last years, there

has been increased interest in determining “normal” blood values from sturgeon for evaluating the fish health status. Several studies provide normal blood values from captive or wild fish including *Acipenser stellatus* [16] [19], *Acipenser baerii* [6], *Huso huso* [1]. Hematological parameters in fish is influenced by different factors including: environmental (temperature, photoperiod) [17], [12], [3], biological (age, sex, maturity and activity levels) [10], [1], [14], [21] and anthropogenic (pollution or hypoxia) [22], [13], [18]. The objective of the this study was to determine the hematological parameters of genitors of the *A. stellatus* from the Danube river during spring season and to compare the blood indices between sexes.

### MATERIAL AND METHODS

Fish used in this study were represented by stellate sturgeon; they were captured from the Danube River near Isaccea. Sampling was conducted in the May 2013. Upon capture, fish, including 10 female and 10 male, were

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examined, measured and weighed. Fish were anesthetized in tricaine methanesulfonate (MS-222). While under anesthesia, cca 3 ml blood samples were collected from the caudal vein of each fish and transferred to blood collection tubes containing lithium heparin. Blood was analysed with routine method used in fish hematology [5]. The red blood cell counts (RBCc,  $\times 10^6/\mu\text{l}$ ) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer, slide at a magnification of  $\times 400$ , using Vulpian diluting solution. The hematocrit (PCV, %) was determined by the microhematocrit method using heparinised capillary tubes centrifuged for 5 minutes at 12000 rpm and expressed as percentages. The spectrophotometric cyanohaemoglobin method [20] was used for determination of the hemoglobin concentration (Hb, g/dl) and read the absorbance values at 540 nm. Using standard formulas according Svobodova Z., [20] the red blood indices were calculated: the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC).

Differences in haematological parameters between males and females fish were statistically analyzed by Student's test ( $p < 0.05$ ). Means and standard deviation (SD) were calculated for each parameters measured.

## RESULT AND DISCUSSION

All fish analyzed were apparently healthy and there were no indications of infectious and parasitic diseases. Summaries and comparison of hematological values in male and female of *Acipenser stellatus* shown in table 1. So, the mean amounts of RBC were recorded  $1.05 \times 10^6 \pm 0.21 \text{ cel}/\text{mm}^3$  and  $1.36 \times 10^6 \pm 0.37 \text{ cel}/\text{mm}^3$  for female and male respectively. The hematocrit (PVC) values were  $31.73 \pm 3.27 \%$  and  $35.80 \pm 2.87\%$  for female and male respectively. Amounts of hemoglobin (Hb) were  $9.41 \pm 0.35 \text{ g dL}^{-1}$  for female and  $9.97 \pm 0.54 \text{ g dL}^{-1}$  for male. Also, the records for female and male for MCV amounts were  $305.47 \pm 12.03 \text{ fl}$  and  $266.93 \pm 14.21 \text{ fl}$  respectively, the amounts of MCH for female and male were measured  $89.98 \pm 4.12 \text{ pg}$  and  $74.33 \pm 5.06 \text{ pg}$  respectively. MCHC amounts for female and male were  $30.55 \pm 2.08 \text{ g l}^{-1}$  and  $27.83 \pm 1.78 \text{ g l}^{-1}$ , respectively. The results showed that there is no significant difference in relation with gender, except in RBCc and PVC values.

Reference intervals as a diagnostic tool are more useful when partitioned by physiologically important subcategories, such as gender, maturity and season.

Table 1 Hematological profile of *Acipenser stellatus* genitors (mean  $\pm$  SD)

Hematological parameters <sup>a</sup>	Female	Male
RBCc ( $\times 10^6 \text{ cel}/\text{mm}^3$ )	$1.05 \pm 0.21$	$1.36 \pm 0.37^a$
Hb ( $\text{g dL}^{-1}$ )	$9.41 \pm 0.35$	$9.97 \pm 0.54^b$
PCV (%)	$31.73 \pm 3.27$	$35.80 \pm 2.87^a$
MCV (fl)	$305.47 \pm 12.03$	$266.93 \pm 14.21^b$
MCH (pg)	$89.98 \pm 4.12$	$74.33 \pm 5.06^b$
MCHC ( $\text{g dL}^{-1}$ )	$30.55 \pm 2.08$	$27.83 \pm 1.78^b$

<sup>a</sup>,"a"- significant differences ( $p < 0.05$ ); „b"- insignificant differences ( $p > 0.05$ )

<sup>b</sup>RBCc – red blood cell count; Hb – hemoglobin; PVC – hematocrit; MCV - mean corpuscular volume; MCH - mean corpuscular haemoglobin; MCHC - mean corpuscular hemoglobin concentration  
SD - standard deviation

In this study the male *A. Stellatus* showed higher haematocrit values and RBCc than females. PCV is a useful indicator of physiological status and health condition; it is

probably the most widely reported hematological parameters in fishes [11]. The usually ranges are between 20 to 45%, with higher values more commonly found in active

species. Our results is in agreement with results obtained by different authors from other fish species [9], [10], [8]. High level of PVC and RBCc are resulte of higher metabolism in male than female; these defferences on hematological parameters are influenced by different needs of oxygen in male and female, especially in breeding period. Higher values of some hematological indices for males than females of most fish species are caused by varying activity of erythropietin [4]. Some authors mention that the value of PVC can be influenced by several biological factors as sex, age or maturity state [1], [10]. The other authors have highlighted an increase of PVC and red blood cell counts as a result of hemodilution of the blood in mature males [5] or stressors that causes erythrocyte swelling [15]. Diverse environmental conditions encountered in the wild and physiological changes that accompany the reproductive cycle can have a large impact on the hematology in fish. In summary the results of our research provide a contribution to the knowledge of profile on blood values of the wild population of mature stellate sturgeon from Danube River durin spring season. The hematological values reported in this paper will be used as reference for monitoring of physiological and health status of *A. stellatus* from the Danube river.

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