



Some hematological parameters for genitors of the sterlet (*Acipenser ruthenus*) from Isaccea region of the Danube River

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Abstract. The objective of the study was to analyzing the hematological parameters in the blood of genitors of the sterlet, *Acipenser ruthenus* from Danube River for assessing the physiological health status during reproductive period. In this study, *A. ruthenus* captured in May 2014 from Isaccea region of the Danube River were used. *A. ruthenus* individuals were anesthetized with MS222 and 5 mL of blood was sampling from each fish by caudal venous puncture. Blood was analysed with routine method used in fish hematology. The following haematological parameters were determined: red blood cell counts (RBCc, $\times 10^6 \mu\text{L}^{-1}$), the hematocrit (PCV, %) and hemoglobin concentrations (g dL^{-1}). Using standard formulas the red blood indices were computed: the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). Hematological reference interval were as follows: RBC count $0.68\text{-}1.2 \times 10^6$ per mm^3 , hemoglobin concentration $7.02\text{-}8.94 \text{ g dL}^{-1}$, hematocrit concentration 20-25%, MCV 219.78-330.88 fL, MCH 67.86-131.49 pg, MCHC 28.47-39.74 g dL^{-1} , for *A. ruthenus* males and RBC count $0.65\text{-}1.25 \times 10^6$ per mm^3 , hemoglobin concentration $6.02\text{-}10.58 \text{ g dL}^{-1}$, hematocrit concentration 17-29 %, MCV 208.51-261.54 fL, MCH 71.33-95.77 pg, MCHC 31.60-40.70 g dL^{-1} , for *A. ruthenus* females, respectively. Our findings showed that there is a similarity between haematologic indices in male and female of *A. ruthenus*. Hematological values reported here will be used as reference for the early identification and monitoring of physiological health status of *A. ruthenus* from the Danube River.

Key Words: *A. ruthenus*, sturgeon, red blood cell counts, hematocrit, hemoglobin concentration, red blood indices.

Introduction. All sturgeon species worldwide are covered under the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Of the six species, living in the Danube River (Bacalbasa-Dobrovici 1997), four of them (*Huso huso*, *Acipenser gueldenstaedtii*, *Acipenser stellatus*, *Acipenser nudiiventris*) are critically endangered and *Acipenser ruthenus* is vulnerable (www.iucnredlist.org). Every year the stocks decrease because of over fishing, loss of habitat and decrease of water quality (Pikitch et al 2005). The sterlet (*A. ruthenus*) is currently the most widespread species in the Danube River basin which, unlike the other sturgeon species, does not migrate from the Black Sea (Bacalbasa-Dobrovici 1997). *A. ruthenus* is a potamodromous freshwater fish with the shortest life span in the genus *Acipenser*, inhabiting the lowland and foothill zones of Danube Rivers. When the water level rises in spring, *A. ruthenus* migrates onto the flooded lowlands to feed. After spawning, the spent fishes (= fishes after the release of their gametes during spawning) move slowly downstream to bays, sandy shoals or channels with muddy bottoms, where they feed intensively (Reinartz 2002).

Haematological evaluations are gradually becoming a routine practice for determining health status in fish (Tavares-Dias et al 2003; Ranzaini-Paiva et al 2003; Hrubec et al 2000). Haematological parameters are recognized as secondary stress indicators (Wedemeyer et al 1990). The study of piscine haematology has contributed significantly to our understanding of the comparative physiology, phylogenetic relationships, habitat, food selection, and other significant ecological parameters of

fishes. Since blood parameters are influenced by a variety of environmental stressors, they have the potential to be used as biomarkers.

In the last years, there has been increased interest in determining "normal" blood values from sturgeons for evaluating the fishes health status. Several studies provide normal hematological values from captive or wild fish including *A. stellatus* (Palíková et al 1999; Shahsavani et al 2010), *Acipenser baerii* (Docan et al 2011), *H. huso* (Akrami et al 2013). Hematological parameters in fish are influenced by different factors including: environmental (temperature, photoperiod) (Sadati et al 2011; Hrubec et al 1997; Bani et al 2009), biological (age, sex, maturity and activity levels) (Golemi et al 2013; Akrami et al 2013; Khadjeh et al 2010; Webb et al 2002) and anthropogenic (pollution or hypoxia) (Witeska 1998; Huertas et al 2002; Scott & Rogers 1981).

Unfortunately we could not find any information about blood parameters of *A. ruthenus* from the Danube River. So in this case study an attempt was made within the present study to have an aspect of some blood parameters of this endangered sturgeon species.

Material and Method. Fish used in this study were represented by genitors of *A. ruthenus*; they were captured from the Danube River near Isaccea region. Fish specimens were collected in the May 2014. Upon capture, fish, including 10 female and 10 male, were examined, measured and weighed. Fish were anesthetized with tricaine methanesulfonate (MS-222). Under anesthesia, approximately 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 (Docan 2014). The red blood cell counts (RBCc, $\times 10^6 \mu\text{L}^{-1}$) 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 was used for determination of the hemoglobin concentration (Hb, g dL^{-1}) and read the absorbance values at 540 nm. Using standard formulas 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 ($p \leq 0.05$). Means and standard deviation (SD) were calculated for each parameter measured.

Results. All analyzed fish were apparently healthy and there were no indications of infectious and parasitic diseases. Summaries and comparison of hematological values in male and female of *A. ruthenus* are shown in the following. The average data of the hematological parameters such as RBC, hemoglobin, PCV, MCV, MCH and MCHC were depicted for both the sexes in spring season.

Hemoglobin content of *A. ruthenus* oscillated between 7.92 ± 0.75 and $8.86 \pm 1.72 \text{ g dL}^{-1}$ during spring season. In male hemoglobin (Hb), concentration varied from 7.02 to 8.94 dL^{-1} , with mean of $7.92 \pm 0.75 \text{ dL}^{-1}$. In the female specimen, value was within the range of 6.02 - 10.58 dL^{-1} with mean of $8.86 \pm 1.72 \text{ dL}^{-1}$. There are significant differences ($p < 0.05$) between sexes. The total number of erythrocyte ranged from 0.68×10^6 to $1.07 \times 10^6/\text{mm}^3$ blood in male fish and 0.65×10^6 to $1.285 \times 10^6/\text{mm}^3$ blood in female fish, during spring study period. Female possessed higher number of RBC than male. The average of RBC in male and female was $0.96 \pm 0.17 \times 10^6$ and $1.05 \pm 0.24 \times 10^6/\text{mm}^3$ blood, respectively, but differences were insignificant ($p > 0.05$). The PCV value ranged from 20 to 28% in male fish, where as for female fish it varied between 17 to 26%. The mean value of the male and the female fish was 24%. The MCV value of male fish ranged between 219.78 and 330.32 fl and for female fish 208.51 and 261.54 fl. The mean value of the male and the female fish was 254.14 fl and 232.76 fl, respectively, with insignificant differences ($p > 0.05$) between sexes. The value of MCH throughout the experimental period was found to be varied between 67.86 and 131.49 pg in male fish

and for female fish it was between 71.33 and 95.77 pg. The MCH value was higher in female fish (85.53 pg) than that of male fish (85.46 pg). There was no major difference in male and female fish. The MCHC value ranged from 28.47 to 39.74% in male fish where as for female fish it varied between 31.60 % and 40.70%. The mean value indicated as slightly higher in female fish (36.81%) in comparison with male fish (33.26%), with significant differences ($p < 0.05$) between sexes.

Discussion. Throughout its life cycle, *A. ruthenus* is directly exposed to contaminants through water, sediments and benthic prey. Therefore, it could be a good species for assessing the overall quality of their aquatic habitat. However, little is known about the seasonal changes of some hematological parameters that could be used as biomarkers in *A. ruthenus* and other sturgeon species. The analysis of hematological parameters is one of the most valuable diagnostic tools to understand fish health status. Recently, many researchers established that the physiological values of hematological parameters are species specific and sex dependent (Knowles et al 2006; Yousefzadeh & Khara 2014; Charoo et al 2013; Akrami et al 2013; Mazandarani et al 2013). Some hematological reports are available in species of Acipenseridae family by different researchers in different approaches, but the present study basically focused on reference ranges for hematological information in genitors species of *A. ruthenus*. In our study significant reduction of some haemoglobin indices (Hb and MCHC) in the peripheral blood of male fish suggested oxygenation capacity of the blood depletion. The higher hematological values in favour of female fish may be attributed to physiologically activeness than the male fish or may be related to differential oxygen demand by sex, which in turn can be linked to reproductive activity (Knowles et al 2006). Mazandarani et al (2013) reported that level of hemoglobin, MCHC and RBCc in female of *A. nudiventris* is higher than male; it could be due to macrocytic or microcytic anemia but is not possible to conclude because of lack of normal data available in this endangered species. Tatina et al (2010) have studied the effect of diet with vitamin C and E on hematological parameters on *A. ruthenus* and values of PCV and RBCc similar to those in our study were found.

Bahmani et al (2001) reported that there were significant differences in Hb, RBCc, WBC (white blood cells count) between male and female of *Huso huso*, possibly due to methodological differences or different responses of males and females to stress. Some articles reported by different authors that quantitative variations in hematological parameters including the red blood corpuscles (RBC), the amount of hemoglobin and the size of RBC are the most significant findings as regards diagnosis (Patra et al 2014; Tatina et al 2010). PCV, erythrocytes count and hemoglobin concentrations are the most readily determined hematological parameters under the field conditions (Bhaskar & Rao 1990). Among different hematological parameters, PCV value is not easily altered as other parameters and should be used in conjunction with RBC and WBC count, hemoglobin content, osmotic fragility, and differential leucocytes count (Wedemeyer et al 1983). Raizada et al (1983) considered that the differences in haematocrit between the two sexes might be due to the higher metabolic rate of males compared to females.

Conclusions. This study indicates that some hematological parameters, such as the RBCc, Hb concentration, MCHC in *A. ruthenus* are related to endogenous factors such as gender and reproductive state. Hemoglobin and MCHC values in female fish were significantly higher than of male fish that might be potentially caused by intense physiological and metabolic activity of female than male 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 *A. ruthenus* from Danube River during spring season. The hematological values reported in this paper will be used as reference for monitoring of physiological and health status of *A. ruthenus* from the Danube River.

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