HAEMATOLOGICAL RESPONSE OF *ACIPENSER STELLATUS* JUVENILES IN COLD SHOCK STRESS

Iulia Grecu[1], Angelica Docan[1*], Lorena Dediu[2], Alina Antache[1], Mirela Crețu[1], Marilena Maereanu[3]

[1]Faculty of Food Science and Engineering, University of Galati, Romania
[2]Cross Border Faculty, "Dunarea de Jos" University of Galati, Romania
[3]S.C. Danube Research-Consulting S.R.L., Isaccea, Romania

Abstract

During the intensive growth of the sturgeons, different stressful factors may occur which could act isolated or in association, influencing their physiological state. In these conditions, the organism tries to cope through a series of biochemical and physiological changes as a result of the activation, in cascade, of some mechanisms of the neuroendocrine response to these stressors. This paper presents the results of the haematological investigations in a controlled experiment that followed the effect of the cold shock stress of water temperature, in the range 12.8 ºC ÷ 7 ºC (initially, after 1 hour, respectively after 3 hours) on the physiology of the Acipenser stellatus species (stellate sturgeon), a very valuable species for sturgeon farming in Romania. This experiment was part of a series of experiments applied to intensive sturgeon breeding with the aim of selecting specific molecular and biochemical markers (gene and protein expressions) to allow the analysis of the response mechanisms to stress factors in aquaculture. Haematological indices and erythrocyte constants were determined using standard methods for veterinary haematology and the data obtained were statistically processed. The determined values of the haematological parameters ranged near the lower limit of the optimum interval for Acipenseridae. In the context of our study, although there was a constant increasing tendency of haematocrit (Ht) values with the raise of the exposure to cold (14.46% - initially, 16.39% - after 1 hour, respectively 17.51% - after 3 hours), in parallel with a less uniform reaction of the other haematological parameters, the statistical analysis did not reveal significant differences between the three experimental groups. The obtained data show the evolution of the haematological profile of the stellate sturgeon and it is useful in completing the database with reference values for research in the field of sturgeon culture

Key words: *Acipenser stellatus*, cold shock, acute stress, haematology

INTRODUCTION

In fishes, like in other ectothermic organisms, water temperature influences, significantly, the metabolic and physiological processes, being considered the "ecological master factor" [5], [12] with control on growth, reproduction, behaviour etc. In the natural aquatic ecosystems, the biogeographic distribution of fish species is phylogenetically related to its requirements in terms of temperature and oxygen. In the anthropogenic ecosystems, like aquaculture production systems, the fish species could suffer, intentionally or not, a wider exposure, with larger variation limits of temperature. In fact, till now, many commercially farmed fish species have been domesticated and breeding improved by manipulating the thermal and/or photoperiod regimes during growth or gonads' maturation (rainbow trout, European seabass, common carp, common perch, tilapia, Atlantic cod etc.).

Nowadays, sturgeon farming is considered one of the most profitable activity in aquaculture with the clear target of obtaining the fish in the commercial weight and caviar as soon as possible [22]. So, manipulating the thermal regime during the prespawning and spawning season in domestic sturgeon females could have a positive impact on the ovarian development [14], [23]. As a result of the specific rearing requirements, most production

*Corresponding author: adocan@ugal.ro
The manuscript was received: 14.10.2019
Accepted for publication: 01.11.2019

facilities need to deal with wide anthropogenic variations in temperature, during different technological activities, in order to cover the temperature range from 8 to 30 °C (natural variations in water temperature) and, sometimes, sturgeons must face it to acute acclimation episodes. In response to these disturbances, fish initiate adaptive response to thermal stress that includes a large number of biochemical, physiological and behavioural changes [6]. To understand the effects of water temperature on the physiology of juvenile Danube sturgeon within the lowest range of "normal" temperatures, we performed a teamwork experiment to monitor the acclimation response of young stellate sturgeon in cold shock exposure from 12,8 °C to 7 °C, after 1 hour and, respectively, 3 hours. In fact, our study was part of a series of experiments applied to intensive sturgeon breeding with the aim of selecting specific molecular and biochemical markers (gene, protein expressions, haematological and growth patterns) to allow the analysis of the response mechanisms to stress factors in aquaculture.

MATERIAL AND METHODS

The experiment was conducted in the Horia Research Station of the S.C. Danube Research Consulting S.R.L. on one-year old juveniles of *Acipenser stellatus* (Pallas, 1771). 21 healthy fish were randomly assigned into three experimental groups: control, 1h_cold stress, 3h_cold stress, namely after the period time of the cold stress application. The fish were weighed (g), measured (cm) and the somatic data are presented in the Table 1.

Table 1 Somatic features of stellate juveniles in the experimental groups (M±SD)

Group	Total length (cm)	Total weight (g)
Control (no stress applied)	28.286 ± 0.756	84.429 ± 12.246
After **1 h**_cold stress	27.929 ± 1.718	81.429 ± 18.026
After **3 h**_cold stress	27.857 ± 1.215	77.429 ± 8.772

Blood samples (1 ml) were collected, in situ, from the caudal vein, transferred into tubes containing lithium heparin as anticoagulant and transported (4÷6 °C) to the Research Centre MoRAS-UDJ Galati (http://moras.ugal.ro) for haematological investigations. Haematological analyses were performed by routine methods used in fish haematology: the red blood cell counts (RBCc, x 10^6 /μL) were determined by the erythrocytes counting on Neubauer haemocytometer using Vulpian diluting solution. The cells counting (×400) and other observations were performed in classic microscopy using the AXIO-Imager A1 (ZEISS) microscope and the photomicrographs were performed with the Canon PowerShot A640 digital camera.

The haematocrit (Ht, %) was determined using microcapillary tubes centrifuged for 5 minutes at 12000 rpm. The haemoglobin concentration (Hb, g/dL) was spectrophotometrically determined at a 540 nm wavelength (UV/VIS Specord 210 AnalytikJena) applying cyanmethaemoglobin method. The erythrocyte indices: mean corpuscular volume (MCV, fL), the mean corpuscular haemoglobin (MCH, pg) and the mean corpuscular haemoglobin concentration (MCHC, g/dL). were calculated by standard formulas.

The obtained values were statistically computed using SPSS Statistics 17.0 program and analyzed for the normality of distribution with the Kolmogorov-Smirnov Z test, for the homogeneity of variances with Levene's test, for the comparison of the means with one-way ANOVA. Significance was accepted at $p<0.05$. The data is presented as mean ± standard deviation (M±SD).

RESULTS AND DISCUSSIONS

Due to its functions, blood analysis can highlight, through its parameter dynamics, the ability of the organism to cope, operatively, with environmental factors variations in order to keep within normal limits the integrity, structure and functionality of the fish body [3], [15].

In our study, the haematological reaction was reflected by the variations of the haematocrit, the haemoglobin concentration,

the number of red blood cells, as well as of the erythrocyte's indices, recorded during the experiment and presented in fig. 1. The blood profile showed values considered normal for sturgeon species: [1], [9], [10], [11], [13], [17], [25].

The *red blood cells count* (RBCc) showed no significant differences between those 3 groups (ANOVA, $p > 0.05$) with the values' variation range between 0.555÷1.235 x 10^6 cells/µL. A slight decrease in erythrocytes number was observed, after 1h_cold stress exposure (from 0.830±0.146 x 10^6/µL to 0.806±0.196 x 10^6/µL), same pattern as observed in *Oreochromis niloticus* [2] cited by [24], *Ameiurus nebulosus* [8], *Cyprinus carpio* [4], followed by an insignificant increase after 3h_cold stress exposure. The RBCc's rise could results from an erythropoiesis process indicating a stress reaction, like a hypoxic condition, developed under the longer period of applying the cold stimulus [18], [21].

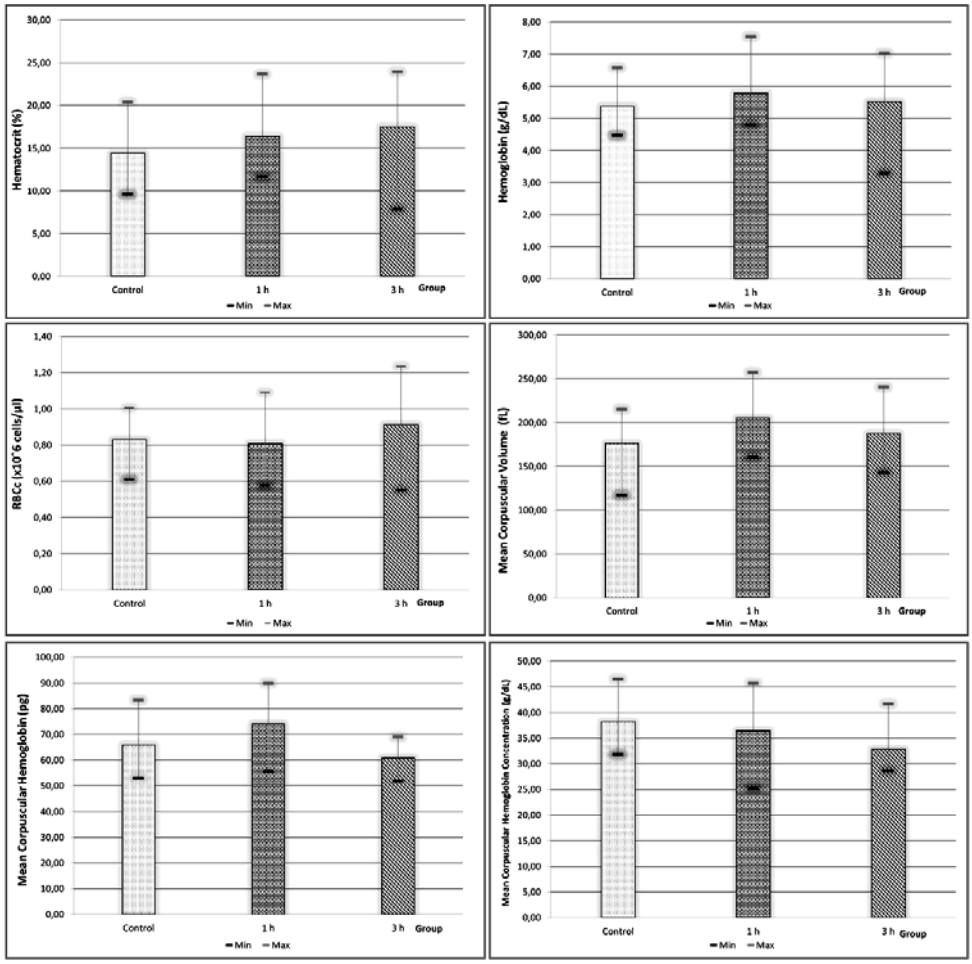

Fig. 1 Blood profiles of stellate sturgeon juveniles during the experiment of cold shock stress

The *haemoglobin* concentration (Hb) showed a specific pattern without statistic difference between groups: after an hour, the mean value of the 1h_cold stress group (5.794±0.888 g/dL) was higher than in the group 3 h_cold stress (5.520±1.399 g/dL), followed by the mean value of the control group (5.371±0.692 g/dL). In correlation

with the anterior values of RBCc, this shift suggests, for the 1h_cold stress group, that the increasing oxygen affinity of fish haemoglobins could be explained by adrenergic swelling of erythrocytes [6], [24]. The other oxygen transport variables, the *haematocrit*, highlight the same assumption of compensatory potential due to increase in volume of the erythrocyte: the 3 h_cold stress group showed the higher mean value (17.514±6.049 %) through the experimental lots (16.390±4.126 % for 1 h_cold stress group and 14.461±3.695 % for control) that can be associated with an increase in oxygen demand [6], [20]. In the experiment performed on Atlantic sturgeon juveniles regarding the effects of temperature and hypoxia, Kieffer et al. [16] found that the haematocrit was dependent on both hypoxia and temperature treatment, but within each acclimation temperature (5 °C and 15 °C), haematocrit levels were not significantly different between the control (normoxia) and following 1h of hypoxia.

The *mean corpuscular volume* (MCV) follows the patern of the haemoglobin concentrations: the mean value of the 1h_cold stress group (205.418±33.128 fL) was higher than in the group 3 h_cold stress (188.012±31.807 fL), followed by the mean value of the control group (176.218±40.039 fL), with no statistical differences determined.

The *mean corpuscular haemoglobin* (MCH) indicates the erythrocyte loading with hemoglobin and the maximum range of variation is observed in the 1h_cold stressed group. Fig. 1 presents the value distribution of this parameter among the experimental groups.

Also, the gradual decresing of the *mean corpuscular haemoglobin concentration* (MCHC) values could be observed, which emphazises that the affinity of haemoglobin to oxygen in the 1 h_cold stress group is due to an increasing in erythrocite volume, followed by an activation of erythrocytes releasing in the 3 h_cold stress group.

Thermal tolerance of the ectothermes from the natural cold waters (Antarctic) is explained by the low metabolic rates as a uniform character of life in those conditions [19]. It is considered [20] that in cartilaginous fish the spleen seems to have the major role in erythropoiesis [20]. Differents authors [7], [19],]24] described that thermal changes lead to increased oxygen demand and production of reactive oxygen species which can determine cellular hypoxia and oxidative stress.

The presented data showed that oxygen capacity is a labile parameter that reflects the organism's acclimation to cold shock stress, even if no statistical differences between the experimental groups of stellate sturgeon juveniles were determined.

CONCLUSIONS

Among the Danube sturgeon species, the stellate sturgeon is considered a promising and valuable species for domestication, due to its caviar quality and that is why we wanted to know more about the temperature variations in aquatic systems as well as the general principles of thermal tolerance and the adaptive response of this sturgeon during growth period.

Is it true that, in addition to our haematological data, that related to the molecular expression (gene and protein expression – data unpublished) must be integrated in order to illustrate the entire systemic levels of biological organization (among haematological and blood chemistry responses, fish condition, growth, disease resistance etc.) for an integrative view. Understanding the way in which sturgeons respond to environmental stress is essential in order to improve the sustainability of both natural and farmed fish populations which are facing increased environmental pressures through habitat conditions and aquaculture intensification.

This study enhances the knowledge about the physiological values of haematological indices in one-year old juveniles of farmed stellate sturgeon exposed to cold shock exposure, from 12.8 °C to 7 °C, considered to be the lowest range of "normal" temperatures to which Danube sturgeons might be currently exposed in aquaculture and in natural conditions.

ACKNOWLEDGMENTS

This work was supported by the project "EXPERT", financed by the Romanian Ministry of Research and Innovation, Contract no. 14 PFE/17.10.2018. The authors are grateful for the technical support offered by MoRAS infrastructure through the Grant POS CCE ID 1815, cod SMIS 48745 (www.moras.ugal.ro) and the project PN II no 116/2012 AQUASTUR.

REFERENCES

[1] Andrei (Guriencu) R. L., Cristea V., Dediu L., Creţu M., Docan A. I., Grecu I. R., Coadă M. T., Simionov (Chihaia) I. A., 2016: The influence of different stocking densities on growth performances of hybrid bester (*Huso huso* ♂ x *Acipenser ruthenus* ♀) in a recirculating aquaculture system. AACL Bioflux, 9(2), p. 541-549.

[2] Barros M.M., Tavares Ranzani-Paiva M.J., Pezzato L.E., Falcon D.R., Guimaraes I.G., 2009: Haematological response and growth performance of Nile tilapia (*Oreochromis niloticus* L.) fed diets containing folic acid. Aquaculture Research, 40(8), p. 895–903, DOI: 10.1111/j.1365-2109.2009.02175.x

[3] Barton B. A., 2002: Stress in Fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integ. and Comp. Biol., 42, p 517–525

[4] Bozorgnia A., Hosseinifard M., Alimohammadi R., 2011: Acute effects of different temperature in the blood parameters of common carp (*Cyprinus carpio*). 2nd International Conference on Environmental Science and Technology, IPCBEE vol.6, IACSIT Press, Singapore, p. V2-52÷55

[5] Brett J. R., 1971: Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). American Zoology 11, p. 99–113.

[6] Burgos-Aceves M. A., Lionetti L., Faggio C., 2019: Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Science of the Total Environment, 670, p.1170–1183

[7] Clauss T. M., Dove A. D. M., Arnold J. E., 2008: Hematologic Disorders of Fish. Vet Clin Exot Anim, 11, p. 445–462

[8] Cocan D., Popescu F., Laţiu C., Uiuiu P., Coroian A., Răducu C., Coroian C. O., Mireşan V., Kokkinakis A. Constantinescu R., 2018. Effects of thermal stress on hematological and metabolic profiles in brown bullhead, *Ameiurus nebulosus* (Lesueur, 1819). AgroLife Scientific Journal - Volume 7, Number 1, p. 33-41.

[9] Dicu (Stroe) M. D., Cristea V., Docan A., Grecu I. R., Dediu L., Coadă M. T., 2013: The influence of feeding frequency on the hematological profile of *A. stellatus* (Pallas 1771), reared in a recirculating aquaculture system. Lucrări Ştiinţifice – Seria Zootehnie Iasi, 59, p. 242-246.

[10] Dicu (Stroe) M. D., Cristea V., Maereanu M., Dediu L., Petrea S. M. 2013: The effect of stocking density on growth performance and hematological profile of stellate sturgeon (*A. stellatus* Pallas, 1771) Fingerlings Reared in an Industrial "Flow-through" Aquaculture System. Bulletin UASVM Animal Science and Biotechnologies, 70(2), p. 244-254

[11] Docan, A., Cristea, V., Dediu, L., 2011 Effect of feeding with different dietary protein level on hematological indices of juvenile Siberian sturgeon, *Acipenser baeri* reared under recirculating systems condition, AACL Bioflux 4(2), p. 180-186.

[12] Donaldson M. R., Cooke S. J., Patterson D. A., Macdonald, J. S., 2008: Cold shock and fish. J. Fish Biol., 73, p. 1491–1530.

[13] Falahatkar B., Barton B.A., 2007: Preliminary observations of physiological responses to acute handling and confinement in juvenile beluga *Huso huso* L. Aquaculture Research 38:1786-1789.

[14] Ghomi M. R., Nazari R. M., Sohrabnejad M., Ovissipour M., Zarei M., Esmaili.Mola A, Makhdoomi C., Rahimian A., Noori H., Naghavi A., 2010: Manipulation of photoperiod in growth factors of beluga sturgeon *Huso huso*. African Journal of Biotechnology, 9(13), p. 1978-1981

[15] Ivanc A., Haskovic E., Jeremic S., Dekic R., 2005: Hematological evaluation of welfare and health of fish. Praxis Veterinaria, 53 (3), p. 191-202

[16] Kieffer J. D., Baker D. W., Wood A. M., Papadopoulos C. N., 2011: The effects of temperature on the physiological response to low oxygen in Atlantic sturgeon. Fish Physiol Biochem., 37, p. 809–819, DOI 10.1007/s10695-011-9479-y

[17] Knowles S., Hrubec TC., Smith SA., Bakal RS., 2006: Hematology and plasma chemistry reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*). Veterinary Clinical Pathology (35) 4: pp 434 - 440.

[18] Munteanu G., Bogatu D., 2005: Treaty of Ichthyopathology. Excelsior Art, Timisoara, p. 591-593 (*in Romanian*)

[19] Pörtner H. O., Peck L., Somero G., 2007: Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. Phil. Trans. R.

Soc. B, 362, p. 2233–2258, doi:10.1098/rstb.2006.1947
[20] Soldatov A. A., 2005: Peculiarities of organization and functioning of the fish red blood system. Journal of Evolutionary Biochemistry and Physiology, 41(3), p. 272-281, DOI: 10.1007/s10893-005-0060-0
[21] Sopinka N. M., Donaldson M. R., O'Connor C. M., Suski C. D., Cooke S. J., 2016: Stress indicators in fish. Chap. 11. *In*: Biology of stress in fish: Volume 35. Fish Physiology. Elsevier Inc. p. 405-462, http://dx.doi.org/10.1016/B978-0-12-802728-8.00011-4
[22] Van Eenenaam J. P., Chapman F., Jarvis P., 2004: Aquaculture. *In* Le Breton et al. (eds.) Sturgeons and paddlefish of North America. Kluwer Academic Publishers, Dordrecht/Boston/London, p. 277-311
[23] Webb M. A. H., Van Eenennaam J.P., Doroshov S. I., Moberg G. P., 1999: Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. Aquaculture, 176 (3–4), p. 315-329, https://doi.org/10.1016/S0044-8486(99)00108-8.
[24] Witeska M., 2015: Anemia in teleost fish (review). Bull. Eur. Ass. Fish Pathol., 35(4), p. 148-160
[25] Zarejabad AM, Sudagar M, Pouralimotlagh S, Bastami K.D., 2009: Effects of rearing temperature on hematological and biochemical parameters of great sturgeon (*Huso huso* Linnaeus, 1758) juvenile. Comp Clin Path19: 367-371.