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RESEARCH ARTICLE

Hematological and blood gas profiles of triploid Siberian sturgeon (*Acipenser baerii* Brandt)

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Abstract. The aim of the study was to determine the impact of triploidization on hematological and blood gas parameters in Siberian sturgeon, *Acipenser baerii* Brandt. The study material was comprised of juvenile Siberian sturgeon specimens with total body lengths of 22.3 ± 2.9 cm and mean body weights of 75.3 ± 40.7 g. Triploidization was confirmed to result in significant changes in the hematological profile of Siberian sturgeon. In comparison to the diploid group, the fish with higher levels of ploidy exhibited lower erythrocyte counts, increased hemoglobin concentration, and increased values of mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. A significant decrease in the percentage share of lymphocytes and an increase in the percentage share of granulocytes in the peripheral blood were also observed, which indicate disturbances in the immune response (particularly in the adaptive immune system) and greater susceptibility to stress. Triploidization did not, however, cause significant differences in the blood gas profile.

Keywords: Siberian sturgeon, triploidization, hematological profile, blood gas profile

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Introduction

Sturgeons are viewed as living fossils, because over millions of years in existence there has been relatively slow phenotype change and they retain an archaic body build (Bemis et al. 1997). In light of their valuable meat and eggs, which are used to produce caviar, sturgeons are important economically, and their production in aquaculture is on the rise annually in the face of shrinking catches in open waters (Anonymous 2012).

Triploidization is a popular method for improving the commercial value of fish and production efficiency in aquaculture (Hulata 2001). Many reports confirm the positive impact of this procedure on increased weight and immune response in fish. The advantageous traits of triploid fish stem from increasing the number of chromosomes from 2n to 3n by disrupting meiotic division. This change disrupts the development of the reproductive system resulting in female infertility and male sub-fertility (Solomon 2003). Consequently, the energy diploid specimens use for generative growth (mainly among females) is used by triploid specimens for somatic growth and this improves meat quality (Tiwary et al. 2004, Cal et al. 2005). The volume of genetic material in the nuclei of individual cells increases as ploidy increases. This is why the cells that comprise triploid organisms are larger. Despite this fact, however, this does not result in gigantism in triploid individuals (Weber et al. 2014). Increasing the volume of cells that comprise tissues results in a reduction of them. It is likely that this change can have a negative impact on various physiological processes including respiration and immunity (Benfey 1999).

Analyzing blood parameters is relatively simple and it is also a valuable method for evaluating the health of aquatic organisms. Among other things, it permits monitoring the physiological state of the body, stress response, and the impact of environment or nutrition (Akrami et al. 2013). The hematological parameters and blood gas profiles of triploid sturgeon individuals have yet to be fully investigated, as is seen in the small number of reports addressing these issues. This is why the aim of the current study was to determine the impact triploidization had on the hematological and blood gas profiles in Siberian sturgeon, *Acipenser baerii* Brandt, which is currently the most widely farmed species of sturgeon in Europe.

Materials and methods

Fish and rearing conditions

The study material was obtained from the Wąsosze Fish Farm (Poland), where triploid Siberian sturgeon specimens were created through the application of heat shock to fertilized eggs (37°C for 2 min) (Fopp-Bayat et al. 2007). Triploid and diploid specimens with a mean body weight of 3.5 g and total length Lt of 3.0 cm) were transported to the laboratory of the Department of Ichthyology, Faculty of Environmental Sciences (UWM Olsztyn, Poland). The fish were stocked into four 80 l tanks (0.31 specimen l^{-1}) that were part of a recirculating aquaculture system (RAS) in groups of either triploid or diploid (control group) specimens in which they were reared for 7 months. The fish were fed B40 feed with a 2 mm granulation manufactured by Skretting (Norway). The feed ration used was 4% of the fish biomass. Water quality parameters during rearing were measured at least once weekly at the rearing tank outflows. Oxygen concentration was 5.76-7.79 mg l^{-1}, and ammonia was 0.216-0.610 mg l^{-1}. The mean water temperature was 17.3 ± 1.5°C, pH was 8.0-8.5, overall water hardness was 665 ± 2 ppm, and mean conductivity was 729 ± 9 μS.

Research procedures

At the end of rearing, all of the specimens from the two groups were measured (total length ± 0.1 cm) and weighed (body weight ± 0.1 g). The mean total length of the triploid sturgeon was 22.3 ± 2.9 cm at a mean body weight of 75.3 ± 40.7 g. In the group of diploid specimens, the values of these parameters were 21.7 ± 3.3 cm and 72.1 ± 31.2 g, respectively. Blood samples (about 2 ml) were collected from the caudal vein of each specimen. Immediately after the blood was sampled, erythrocyte counts were performed with a cytometer using a light microscope and a Bürker chamber (Starmach 1973). The blood samples were also used to prepare smears that were used to evaluate leukograms (Stankiewicz 1973). Hematocrit (Ht) values were determined after the blood had been centrifuged at a speed of 14000 rpm for 3 min w heparinized capillaries (Pawelski 1983). Measurements of hemoglobin concentration (Hb) in the blood were performed with the cyanomethemoglobin method. Extinction was determined at a wavelength of 540 nm (Stankiewicz 1973). The results obtained were used to calculate mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Dacie and Lewis 2001).

A VetStat® Electrolyte and Blood Gas Analyzer (Idexx Laboratories Inc., USA) was used to determine the values of the following parameters: partial pressure of carbon dioxide (pCO_2); concentration of bicarbonate ions (HCO_3-); total content of carbon dioxide in the blood (tCO_2); partial pressure of oxygen (pO_2).

The test to confirm the ploidy of the fish was based on measuring the nuclei width of fifteen subsequent erythrocytes (Życzyński and Duszewska 1994, Boroń 1995), which was performed with a LEICA

DM 3000 microscope (Germany) and an image visualization, analysis, and archiving system (Leica Application Suite, Germany).

Statistical analysis

Mean values and standard deviation were determined for all parameters. The Mann-Whitney U test (a non-parametric test for two independent samples) was used to determine significant differences among the parameters determined. However, Chi^2 distribution with Yates correction for small samples was used to assess the significance of changes in the number of individual leukocytes. Differences were accepted as significant for both tests at $P \leq 0.05$.

Results

Impact of triploidization on hematological parameters

Statistically significant differences were confirmed for RBC, Hb, MCH, and MCHC ($P \leq 0.05$; Table 1). It was determined that the number of RBC in triploid sturgeon was lower in comparison to that of the control group by an average of approximately 0.18×106 mm^{-3} (26%). The opposite tendency was noted with regard to blood hemoglobin and mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. The values of these parameters were higher than those in diploid fish by 0.32 g dl^{-1} (5%), 23.25 pg (23%), and 4.31 g dl^{-1} (14%), respectively. Among the hematological parameters that did not differ statistically significantly ($P > 0.05$), the greatest changes were noted in hematocrit values, which were 8% lower in triploid sturgeon in comparison to the fish from the control group. The opposite tendency was noted for mean corpuscular volume the MCV parameter was 6.5% higher in triploid fish (Table 1).

Blood smear analysis indicated differences among groups in leukocyte percentage composition (Table 2). Statistically significant differences ($P \leq 0.05$) were noted for all forms of leukocytes with the exception of neutrophils with rod-shaped nuclei. The blood smears of triploid Siberian sturgeon confirmed a 19% lower share of lymphocytes in comparison with diploid sturgeon, while the percentages of all other leukocytes were higher in triploid fish. Among these, the greatest differences were observed in the neutrophil granulocytes with segmented nuclei (8%) followed by monocytes (5%) and acidophilic granulocytes (4.5%). The difference in the shares of neutrophil granulocytes with rod-shaped nuclei in smears of the peripheral blood of sturgeon from both groups was only 0.5%, which was not statistically significant ($P > 0.05$). The occurrence of acidophilic

Table 1
Impact of triploidization on the hematological parameters (mean values ± standard deviation; range) of Siberian sturgeon (*A. baerii*)

Parameter	Control group	Triploid group	P
Erythrocyte Mount (RBC) (10^6 mm^{-3})	0.73 ± 0.23 0.25-1.06	0.54 ± 0.12 0.31-0.70	0.021
Hematocrit (Ht) (%)	23.00 ± 1.00 22.00-25.00	21.00 ± 1.00 19.00-23.00	0.246
Hemoglobin Concentration (Hb) (g dl^{-1})	6.14 ± 0.44 5.20-6.50	6.46 ± 0.66 5.30-7.40	0.006
Mean Corpuscular Volume (MCV) (fl)	374.97 ± 240.81 207.55-960.00	399.24 ± 94.56 285.71-612.90	0.092
Mean Corpuscular Hemoglobin (MCH) (pg)	100.73 ± 65.37 61.32-260.00	123.98 ± 26.41 92.65-170.97	0.014
Mean Corpuscular Hemoglobin Concentration (MCHC) (g dl^{-1})	27.01 ± 2.79 20.80-29.55	31.32 ± 3.45 26.98-37.00	0.027

Table 2
Impact of triploidization on the leukograms (mean values ± standard deviation; range) of Siberian sturgeon (*A. baerii*)

Parameter	Control group	Triploid group	P
Lymphocytes (%)	86.44 ± 9.38 69.00-98.00	67.80 ± 16.21 39.00-93.50	0.000
Monocytes (%)	5.19 ± 5.55 0.50-17.50	10.50 ± 5.93 1.00-20.50	0.000
Total neutrophils (%)	5.88 ± 4.12 0.50-11.50	14.50 ± 12.30 1.50-34.50	0.000
neutrophils with rod-shaped nuclei (%)	1.31 ± 1.00 0.00-3.00	1.95 ± 2.27 0.00-7.00	0.176
neutrophils with segmented nuclei (%)	4.56 ± 3.46 0.00-8.50	12.55 ± 10.59 1.50-31.00	0.000
Eosinophil (%)	2.50 ± 1.85 1.00-6.00	7.20 ± 3.18 2.50-12.00	0.000

granulocytes or juvenile forms of neutrophil granulocytes were not confirmed in the blood smears from either of the sturgeon groups.

Impact of triploidization on blood gas parameters

Statistical analysis of the blood gas profiles did not indicate any statistically significant differences between the diploid and triploid fish ($P > 0.05$; Table 3). Blood pH in all fish examined was similar at approximately 7.6. The greatest differences among groups, although not statistically significant, were noted in the partial pressure of oxygen. The mean pO_2 in the peripheral blood of triploid fish was higher by nearly 10 mm Hg (24.9%) in comparison to diploid fish ($P > 0.05$; Table 3). Substantial differences were also in the concentration of bicarbonate ions and the total content of carbon dioxide in the blood ($P > 0.05$; Table 3). The values of these parameters were lower in triploid specimens 1.07 (13%) and 1.09 mmol l^{-1} (12%), respectively. Similar tendencies also occurred in the case of CO_2 with mean intra-group differences of 0.33 mm Hg, which was 5% (Table 3).

Table 3
Impact of triploidization on pH and blood gas parameters (mean values ± standard deviation; range) of Siberian sturgeon (*A. baerii*)

Parameter	Control group	Triploid group	P
pH	7.63 ± 0.08 7.55-7.80	7.60 ± 0.07 7.45-7.68	0.846
Partial pressure of oxygen (pO_2) (mm Hg)	39.38 ± 11.15 26.00-62.00	49.17 ± 10.53 35.00-70.00	0.064
Partial pressure of carbon dioxide (pCO_2) (mm Hg)	7.00 ± 0.53 6.00-8.00	6.67 ± 0.65 6.00-8.00	0.232
Total content of carbon dioxide (tCO_2) (mmol l^{-1})	9.00 ± 1.64 7.70-12.70	7.91 ± 0.77 6.50-9.30	0.122
Bicarbonate ions (HCO_3-) (mmol l^{-1})	8.50 ± 1.61 7.30-12.10	7.43 ± 0.77 6.00-8.80	0.153

Discussion

This study is one a few that addresses hematological and blood gas profile changes stemming from the induction of triploidy in sturgeons. Based on the results obtained, it can be postulated that increased ploidy in Siberian sturgeon does not result in the occurrence, under optimal conditions, of disadvantageous physiological pathology. It does appear, however, that triploid specimens have increased susceptibility to stress, oxygen deficiency, and pathogens. Among the parameters examined, the most substantial changes were observed in the hematological profile. In most instances, these changes were a consequence of the increased quantity of genetic material (from 2n to 3n) located in the erythrocyte nuclei. The literature describing this phenomenon in other fish species subjected to triploidization includes information regarding increased nucleus size by as much as 50% (Ballarin et al. 2004, Peruzzi et al. 2005). Authors report that despite the growth in nucleus volume, the changes in erythrocyte volume is not as pronounced. In the sturgeon with triple sets of chromosomes, the volume of individual red blood cells only increased on average by barely 7%. The increased volume of individual red blood cells was offset by the smaller total number of them in the peripheral blood. Similarly, the hematocrit values remained nearly the same as those in the diploid sturgeon. Lowering RBC values justified the absence of gigantism, which is the result of larger cell volume. Similar observations are documented and described in several species of fish (mainly salmonids) subjected to triploidization (Graham et al. 1985, Small and Randall 1989, Sezaki et al. 1991, Parsons 1993, Biron and Benfey 1994, Yamamoto and Iida 1994a).

Potentially, the concentration of hemoglobin in polyploid fish should increase along with increased erythrocyte volume. In the sturgeon examined, the mean hemoglobin concentration in red blood cells increased over 23%, while the mean corpuscular hemoglobin concentration increased 14% in comparison to the diploid sturgeon. Consequently, the hemoglobin concentration in sturgeon peripheral blood also increased. In turn, despite the increased volume of individual red blood cells, Barker et al. (1983), Aliah et al. (1991), or Sezaki et al. (1991) did not report increased hemoglobin concentrations in the blood of triploid fish. However, Benfey and Sutterlin (1984), Graham et al. (1985), Parsons (1993), and Beyea et al. (2005) even reported decreases in the levels of this respiratory dye.

Benfey (1999) confirmed that the greater erythrocyte volume and their decreased number in the blood of triploid fish could have resulted in a smaller total red blood cell surface area. Consequently, this could have a negative impact on the quantity of bound and transported oxygen and carbon dioxide. It follows that triploid fish ought to exhibit greater susceptibility to dissolved oxygen deficits in water. This thesis appears to be confirmed by the observations of the occurrence of increased mortality among triploid salmonids in comparison to diploid fish in higher temperature waters in which there is less dissolved oxygen and requirements for this gas are higher (Quillet and Gaignon 1990, Aliah et al. 1991, Simon et al. 1993, Ojolick et al. 1995, Johnstone 1996). Large decreases in the numbers of erythrocytes and disproportional increases in their hemoglobin concentrations could have decreased oxygen transport capabilities. MCH increased in the case of triploid Siberian sturgeon on average by 23%. Thus, it is plausible that the decrease in the total number of red blood cells in these fish, by an average of 26% did not disrupt oxygen transport to tissues and cells. This hypothesis is confirmed by the results of blood gas profile tests. Although no statistically significant differences were noted, the partial pressure of oxygen in the blood of triploid fish was 25% higher than in the sturgeon from the control group. Additionally, it was confirmed that in triploids the concentration of bicarbonate ions and the total concentration and partial pressure of carbon dioxide was lower. It is noteworthy that the proportions of HCO_3- and tCO_2 were similar in both groups of fish. This is evidence of efficient gas exchange and the appropriate buffering properties of the blood (acid-base balance) in triploid Siberian sturgeon. Graham et al. (1985) reported quite different observations in that the quantity of oxygen transport through a defined quantity of hemoglobin was lower in triploid Atlantic salmon, *Salmo salar* L. by almost one quarter. Therefore, it can be concluded that in some species of fish triploidization can cause adverse morphological and physiological changes.

Ranzani-Paiva et al. (1998) noted that the main leukocytes in the peripheral blood of both triploid and diploid fish are lymphocytes. In the current study, a dominant share of these white blood cells were also confirmed in the peripheral blood in both groups of sturgeon specimens. However, the percentage share of lymphocytes in the pool of all leukocytes in sturgeon with a triple set of chromosomes decreased from 86.44 to 67.80%. A similar dependence was noted by Svobodová et al. (1998) in a comparison of the constituents of peripheral blood between diploid and triploid tench. Angelidis et al. (1987), Schwaiger et al. (1996), and Benfey and Biron (2000) reported a decrease in the number of lymphocytes in the peripheral blood of fish with a simultaneous increase in the number of granulocytes often occurred when the fish were stressed and also during viral, bacterial, or fungal infection. Benfey (1999) noted that because of the lower total surface area of erythrocytes, which negatively impacts gas exchange and oxygen transport, triploid fish are continually exposed to stress, which disrupts their immune systems (disruption in the composition and number of leukocytes). Likewise, triploid fish can be more susceptible to disease. For example, Yamamoto and Iida (1994b) and Ojolick et al. (1995) revealed that triploid rainbow trout are more prone to bacterial gill infection that causes increased mortality. Furthermore, Ojolick et al. (1995) noted mass mortality of triploid rainbow trout infected with vibriosis in stressful conditions caused by exposure to high temperature.

In conclusion, the values of the hematological and gas profile parameters presented do not indicate that negative changes occur as a result of increasing ploidy to 3n in Siberian sturgeon. Triploid Siberian sturgeon can be more resistant to stress and more sensitive to oxygen deficit and disease factors, which can lead to increased mortality.

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Author contributions. K.D.Z. designed the experiment and methods, all of the authors did all the experiment procedures, M.R. statistically analyzed the data, prepared the manuscript and conducted the literature review, K.D.Z. and D.F.B reviewed the article.

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