https://doi.org/10.17221/215/2020-VETMED

Examination of white blood cell indicators for three different ploidy level sturgeon species reared in an indoor recirculation aquaculture system for one year

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Citation: Salkova E, Gela D, Pecherkova P, Flajshans M (2022): Examination of white blood cell indicators for three different ploidy level sturgeon species reared in an indoor recirculation aquaculture system for one year. Vet Med-Czech 67, 138–149.

Abstract: Functional diploid *Acipenser ruthenus,* functional tetraploid *Acipenser gueldenstaedtii* and functional hexaploid *Acipenser brevirostrum* juveniles were sampled monthly for one year, and the white blood cell indicators were determined. The total number of leukocytes (TL) was 40.93 ± 17.24 × 10^9/l for the diploids, 20.63 ± 11.20 × 10^9/l for the tetraploids, 14.13 ± 7.72 × 10^9/l for the hexaploids. The TL decreased with an increasing ploidy level. The highest number of leukocytes was reached during September and October for *A. ruthenus* and *A. brevirostrum*, from October to January for *A. gueldenstaedtii* (a statistically significant finding). The lymphocytes dominated (76.89–80.14%) in the differential counts and were found to be reduced in June and July in each group. Granulocytes were represented by neutrophils and eosinophils. Counting from all the leukocytes, the neutrophils represented 13.0–18.7% and eosinophils represented 5.7–6.1%. Increasing number of nuclear segments in the granulocytes was dependent on the increasing ploidy level. Nuclear segmentation in the lymphocytes was a common finding in higher ploidy level groups. The data suggest a significant effect of ploidy level on the total number of leukocytes and morphological nuclear changes in the granulocytes and lymphocytes. The seasonal variation in the differential leukocyte counts depends on the species and the influence of various external conditions rather than the ploidy level.

Keywords: *Acipenser brevirostrum*; *Acipenser gueldenstaedtii*; *Acipenser ruthenus*; differential leukocyte count; white blood cell count

The sturgeon (*Acipenseridae*) are an ancient fish species, believed to be "living fossils". Their primitive ancestral characteristics and their ability to live in both freshwater and marine environments make them interesting to study their histology and physiology. Sturgeons have to face many infectious agents and they need a well-developed immune system to be able to survive. White blood cells represent an important component of immunological defence.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic: Projects CENAKVA (No. LM2018099), Biodiversity (No. CZ.02.1.01/0.0/0.0/16_025/0007370) and by the Czech Science Foundation (Project No. 18-09323S).

https://doi.org/10.17221/215/2020-VETMED

The haematological examination of fish is a useful diagnostic tool for evaluating the physiological status and is important in the diagnosis of different pathological conditions. Basic haematological indicators are represented by red blood cell indices, white blood cell indices (white blood cell count and differential white blood cell count), and the thrombocyte count (Knowles et al. 2006; Zexia et al. 2007; Docan et al. 2012; Svobodova et al. 2012).

While performing differential white blood cell count, the lymphocytes, monocytes and granulocytes are recognised. The lymphocytes are round or spherical, with large basophilic nuclei and a basophilic cytoplasm. The size of the lymphocytes varies, small and large lymphocytes are recognised (Ellis 1977; Knowles et al. 2006; Zexia et al. 2007). Granulocytes are classified as neutrophils, eosinophils and basophils according to their ultrastructural and histochemical grounds (Ellis 1977; Ainsworth 1992). Generally, in fish, under physiological conditions, basophils seem to be a rare cell subpopulation (Palikova et al. 1999; Zexia et al. 2007). Neutrophils vary in shape, the nucleus is slightly basophilic, may occur as a band or may be two- or several-segmented. Eosinophils have easily recognisable eosinophilic cytoplasmic granules. The nuclei are basophilic, may occur as bands or may be two- or several-segmented (Ainsworth 1992; Svobodova et al. 2012). Monocytes are large cells with a prominent eccentrically located horseshoe-shaped nucleus, are slightly basophilic and have vacuolated cytoplasm (Knowles et al. 2006; Zexia et al. 2007; Svobodova et al. 2012).

The study of sturgeon genetics can provide valuable data on the mechanisms underlying the evolution of vertebrates. Sturgeons have evolved via allopolyploidisation (Gregory and Witt 2008; Smith and Gregory 2009; Crow et al. 2012) by several polyploidisation and hybridisation events. The species of the genera *Acipenser, Huso, Scaphirhynchus* and *Pseudoscaphirhynchus* are separated into different classes according to the chromosome numbers: (1) species with ca. 120 chromosomes; (2) species with ca. 250 chromosomes; (3) one species only, *Acipenser brevirostrum*, having ca. 360 chromosomes (Ludwig et al. 2001; Havelka et al. 2011; Havelka et al. 2016). Havelka et al. (2011) stated that two scales of ploidy levels are recognised at present: the evolutionary scale, which presumes tetraploid (4n) – octaploid (8n) – dodecaploid (12n) relationships, referring to ancient ploidy levels, and the functional scale, which presumes diploid (2n) – tetraploid (4n) – hexaploid (6n) relationships arising from significant functional genome re-diploidisation in the sturgeon evolution.

Sturgeons exhibit large genomes among fishes (Gregory and Witt 2008; Smith and Gregory 2009). The increasing number of chromosomes is closely associated with an increase in the DNA content in the cell nuclei. The cell and nuclear size correlate in a strongly positive mode with the genome size and with each other at each taxonomic level, independently of phylogenetics and of the ancient or neo-polyploid status (Hardie and Hebert 2003). The cytological features such as the nuclear volume, cell volume, cell surface area and nuclear surface area also correlate in a positive manner with the genome size (Palikova et al. 1999; Hardie and Hebert 2003; Flajshans et al. 2011).

The aim of our study was the sampling of different ploidy level representatives (sterlet *Acipenser ruthenus,* the Russian sturgeon *Acipenser gueldenstaedtii* and the shortnose sturgeon *A. brevirostrum*) over a year and evaluating the white blood cell indicators in context of the ploidy level and season.

MATERIAL AND METHODS

In this study, ten juvenile functional diploid specimens (2n) *A. ruthenus* [initial age 8 months, total body length (TBL) of 16 cm, body weight of 31 g], ten juvenile functional tetraploid specimens (4n) *A. gueldenstaedtii* (initial age of 6 months, TBL of 12 cm, body weight of 24 g) and ten juvenile functional hexaploid specimens (6n) *A. brevirostrum* (initial age of 2 months, TBL of 11 cm, body weight of 6 g) were examined. The tested fish, representing specimens of the particular ploidy level group, originated from a population of identical age and similar size representatives.

All the fish originated from the Genetic Fisheries Centre of the Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Czech Republic. The fish were kept in an indoor recirculation aquaculture system (RAS) at a density of 10 kg/m^3 in 3.2 m^3 tanks at 16–18 °C, with a daily feeding rate of 4% of the fish biomass using a commercial diet (Coppens® Supreme-10; Coppens International B.V., Helmond, The Netherlands) containing 49% protein, 10% fat, 0.8% crude fibre and 7.9% ash.

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The water temperature, dissolved oxygen, pH and water chemistry were monitored daily. The water temperature was maintained between 16–18 °C, but occasionally during the summer reached 20 °C. Generally, the temperature of the inflowing water reached 16 °C. The dissolved oxygen in the outflowing water ranged between 60–75%. The water pH was kept between 7 and 8. The nitrite concentration was kept under 0.1 mg NO_2-N. The daily water volume exchange in the RAS was 10% of the total water volume.

The tested fish were visually healthy. Sampling was performed each month in a period of one year, starting in May and continuing until April. Sampling was performed during the first half of the month, always within one day for all the tested groups, thus the interval between the samplings was at least three weeks. Ten fish specimens for each ploidy level group were sampled at each time point.

Blood samples were taken from the caudal blood vessel with a heparin-coated syringe and the needle was adjusted for the individual fish. After sampling, the fish were put back into the tank. According to our previous experience with rapid blood clotting, blood smears were taken immediately, dried at room temperature, fixed with methanol and stained using a Hemacolor® Rapid Staining Set (Merck KGaG, Darmstadt, Germany). The slides obtained were inspected under an Olympus BHS microscope using × 1 000 magnification. While performing the leukocyte differential count, two hundred leukocytes were determined and counted for each specimen. The cellular size, nuclear morphology, and cytoplasmic staining patterns were the criteria for the cells' identification and determination (Ellis 1977; Ainsworth 1992; Knowles et al. 2006; Zexia et al. 2007; Svobodova et al. 2012). The granulocytes were further classified as neutrophils and eosinophils, and the bands and segments were counted in each subgroup. The number of nuclear segments in the neutrophils and eosinophils was also established for each ploidy level group. The differential leukocyte count was recorded as a percentage of a particular cell type (Knowles et al. 2006; Svobodova et al. 2012). Only mature cells were involved in the leukogram, otherwise blasts and vanishing cells were excluded.

The blood samples for the determination of the total number of leukocytes were stored in heparinised tubes and placed on ice until further analysis, but no longer than one hour. The heparinised blood was diluted 200 times with a solution containing NaCl, Na_2SO_4, $Na_2HPO_4.12H_2O$, KH_2PO_4, formaldehyde and 1% cresyl violet diluted in distilled water (Svobodova et al. 2012). The total number of leukocytes was performed by counting the white blood cells in a Bürker chamber and was recorded in 10^9/l (Knowles et al. 2006; Svobodova et al. 2012). The thrombocytes and thrombocyte-like cells were not included in the white blood cell parameters, since they represent a distinct blood cell component (Khandekar et al. 2012).

The study was carried out in accordance with Czech Law No. 246/1992 "Animal welfare". The Institutional Animal Care and Use Committee (IACUC) of the University of South Bohemia (USB), Faculty of Fisheries and Protection of Waters (FFPW) in Vodňany supervised the protocols. The USB FFPW has the approval of the Ministry of Agriculture of the Czech Republic for handling and usage of experimental animals (Ref. No. 16OZ15759/2013-17214).

Statistical analysis

The analyses of the haematological data were performed every month for one year. The mean was used as an indicator of central tendency. For the analysis of the different blood components, an analysis of variance (ANOVA) test with Tukey's honestly significant difference test and *t*-test were used. The level of significance was 0.05. All *P*-values were interpreted descriptively, and no adjustment was applied to the *P*-values or significance levels. The statistical analyses were performed using SPSS v23 (SPSS; Chicago, IL, USA) and Matlab R2018b software (The MathWorks, Inc.; Natick, MA, USA).

RESULTS

Nuclear changes to the granulocytes (multinucleated nuclei, atypical nuclear shapes and increasing number of nuclear segments) were common in the higher ploidy level groups (see Figure 1) (statistically significant finding). The changes were related to the increasing genome size and DNA folding. The increasing ploidy level in the lymphocytes was associated with the tendency of the nuclei to divide, and the nucleus revealed a "budding" appearance (see Figure 2).

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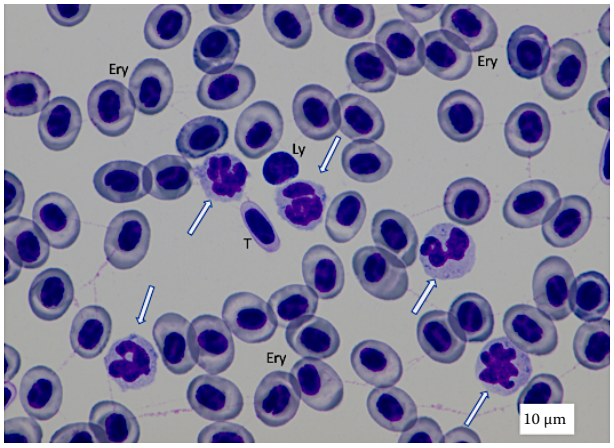

Figure 1. Blood smear, *Acipenser brevirostrum* (6n), Hemacolor® Rapid staining set, (magnification 1 000 ×, scale bar 10 µm)

Erythrocytes (Ery) dominate in the blood smear. Thrombocytes (T) are spindle or fusiform, with elongated nucleus. Lymphocytes (Ly) are round or spherical, with large basophilic nuclei and have a thin rim of basophilic cytoplasm. Nuclear changes of granulocytes including atypical nuclear shapes and increasing number of nuclear segments (indicated by arrows) are common findings in higher ploidy level groups

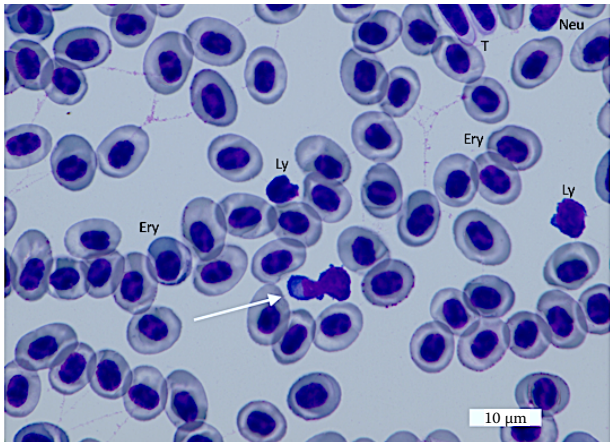

Figure 2. Blood smear, *Acipenser brevirostrum* (6n), Hemacolor® Rapid staining set (magnification 1 000 ×, scale bar 10 µm)

Lymphocytes (Ly) are round or spherical, with basophilic nuclei and have a thin rim of basophilic cytoplasm. The increasing ploidy level in lymphocytes is associated with the tendency of the nuclei to divide. Arrow points to the "budding" appearance of the nucleus in the lymphocyte. Neutrophils (Neu) represent the granulocytic group, different developmental stages are present (bands or segments). Erythrocytes (Ery) dominate in the blood smear. Thrombocytes (T) are oval, spindle or fusiform and occur as single cells or may constitute clusters or small groups

Total number of leukocytes

The total number of leukocytes over the year ranged between 40.93 ± 17.24 × 10^9/l for *A. ruthenus*, 20.63 ± 11.20 × 10^9/l for *A. gueldenstaedtii* and 14.13 ± 7.72 × 10^9/l for *A. brevirostrum* (summary in Table 1). Statistically significant differences ($P < 0.001$) in the total number of leukocytes were found for each group within the year, with highest number of leukocytes reached during autumn (September and October) for *A. ruthenus* (45.5 × 10^9/l and 63.0 × 10^9/l) and for *A. brevirostrum* (22.0 × 10^9/l and 20.5 × 10^9/l), and during autumn and winter (October until January) for *A. gueldenstaedtii* (from 21.0 × 10^9/l to 27.0 × 10^9/l). The lowest value was reached in November for *A. ruthenus* (28.0 × 10^9/l), in July for *A. gueldenstaedtii* (13.0 × 10^9/l), and in February for *A. brevirostrum* (7.5 × 10^9/l). The seasonal variation in the total number of leukocytes is given in Figure 3.

Table 1. Summary of the white blood cell (WBC) parameters within the different ploidy level groups (*Acipenseridae* species)

Species	Ploidy level, functional scale	WBC (10^9/l)	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)	Granulocytes bands (%)	Granulocytes segments (%)
A. ruthenus	2n	40.93 ± 17.24	77.59 ± 8.45	0.85 ± 0.66^{NS}	21.63 ± 8.20	15.64 ± 5.32	5.83 ± 1.77
A. gueldenstaedtii	4n	20.63 ± 11.20	80.14 ± 8.13	1.00 ± 0.81^{NS}	18.85 ± 7.75	10.14 ± 3.37	7.37 ± 2.62
A. brevirostrum	6n	14.13 ± 7.72	76.89 ± 7.82	1.06 ± 0.94^{NS}	22.04 ± 7.47	10.10 ± 3.07	10.5 ± 4.27

Total number of leukocytes (10^9/l), differential leukocyte counts comprised of lymphocyte, monocyte and granulocyte counts recorded as percentages. Granulocytic content within the total number of leukocytes and proportion of the developmental stages recorded as bands and segments are presented. Values are recorded as mean ± SD

*Statistically significant difference at $P = 0.001$; **Statistically significant difference at $P < 0.000\ 1$

NS = non-specific

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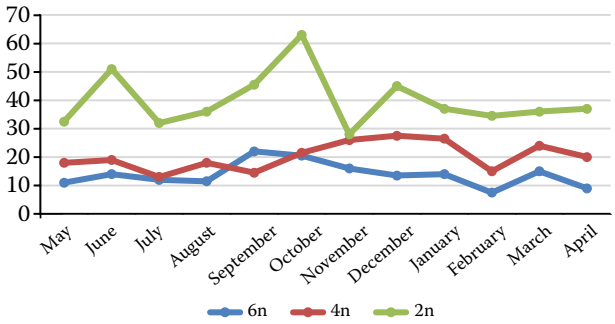

Figure 3. Total number of leukocytes
The monthly variation in the total number of leukocytes (TLs) within the different ploidy level groups is presented. Axis x represents the time schedule as months in the year, the study ran from May till April. Axis y marks the mean of the TLs recorded in 10^9/l. The increasing ploidy level is associated with a decreasing TL. Seasonal changes are evident: The highest number of leukocytes was reached during September and October for *A. ruthenus* (2n) and *A. brevirostrum* (6n), from October to January for *A. gueldenstaedtii* (4n). The seasonal changes in the TL within each ploidy level group are statistically significant

Lymphocytes

The lymphocytes dominated in the differential leukocyte counts regardless of the species or ploidy level. The lymphocytes constituted 77.59 ± 8.45% in *A. ruthenus*, 80.14 ± 8.13% in *A. gueldenstaedtii* and 76.89 ± 7.82% in *A. brevirostrum*. Statistically significant differences were found between *A. gueldenstaedtii* and *A. ruthenus*, and between *A. gueldenstaedtii* and *A. brevirostrum* (Table 1).

A reduced numbers of lymphocytes were documented in June and July for each ploidy group (statistically significant). The seasonal variation in the lymphocyte differential counts in given in Figure 4.

Granulocytes

Three types of granulocytes were determined in the sturgeons, i.e., neutrophils, eosinophils and basophils. No basophils were recognised in any specimens. From all the leukocytes, the granulocytes represented 21.63 ± 8.2% in *A. ruthenus*, 18.85 ± 7.75% in *A. gueldenstaedtii* and 22.04 ± 7.47% in *A. brevirostrum* (Table 1). Statistically significant differences were found between *A. brevirostrum* (6n) and *A. gueldenstaedtii* (4n), and between *A. brevirostrum* (6n) and *A. ruthenus* (2n) (Table 1).

In contrast to the lymphocytes, the highest number of granulocytes was reached during April and June in *A. gueldenstaedtii* and during June and July in *A. ruthenus* and *A. brevirostrum*. Evaluating the blood smears, different developmental stages

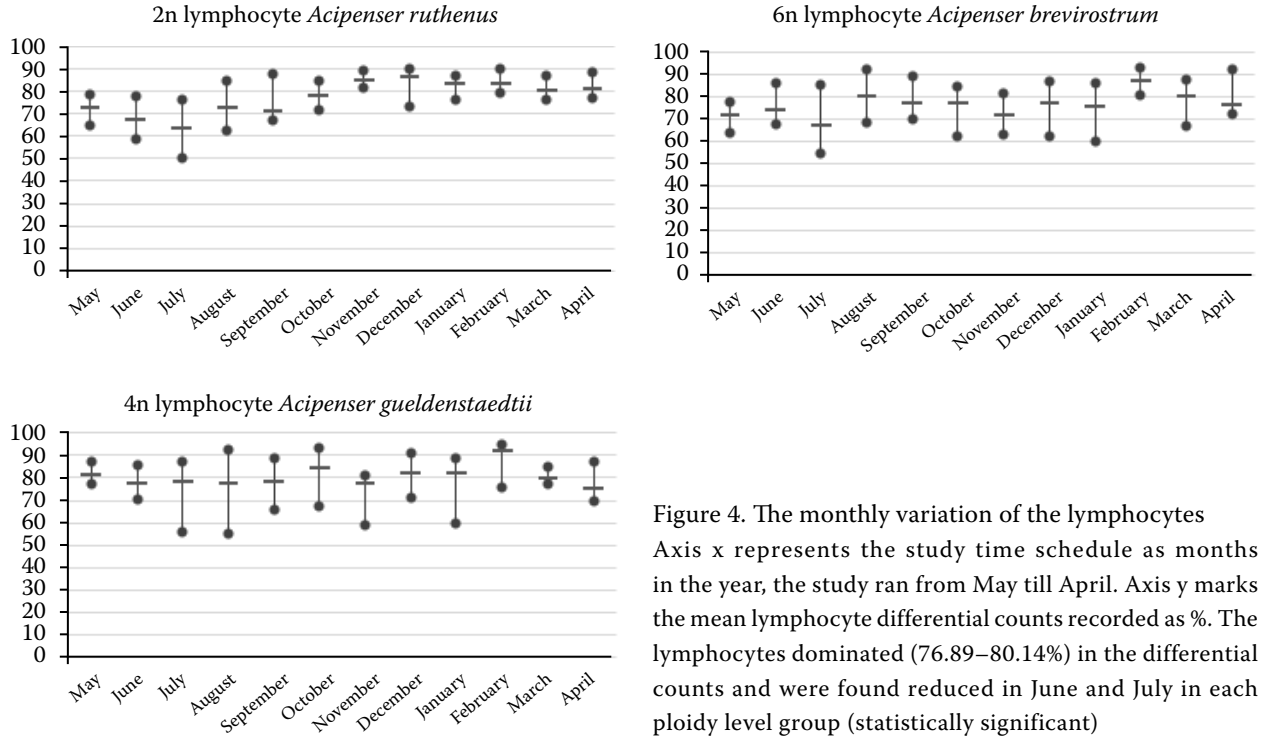

Figure 4. The monthly variation of the lymphocytes
Axis x represents the study time schedule as months in the year, the study ran from May till April. Axis y marks the mean lymphocyte differential counts recorded as %. The lymphocytes dominated (76.89–80.14%) in the differential counts and were found reduced in June and July in each ploidy level group (statistically significant)

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Table 2. Granulocytic parameters within the different ploidy level groups in details

Species	Ploidy level, functional scale	Neutrophils (%)	Neutrophils: Number of nuclear segments	Eosinophils (%)	Eosinophils: Number of nuclear segments
A. ruthenus	2n	18.7 ± 9^{NS}	2.6 **	5.8 ± 3.2^{NS}	2.05 **
A. gueldenstaedtii	4n	13.1 ± 7^{NS}	3.1 **	5.6 ± 2.8^{NS}	2.6 *
A. brevirostrum	6n	15.5 ± 9^{NS}	4.1	6 ± 4.9^{NS}	3.3

Granulocytes are classified as neutrophils and eosinophils, number of nuclear segments in each granulocytic subgroup is recorded. Values are recorded as mean ± SD

*Statistically significant difference at $P < 0.01$; **Statistically significant difference at $P < 0.000\ 1$

NS = non-specific

of granulocytes were evident, granulocytic bands and segments were present: the bands constituted 10.1–15.6 % and the segments comprised 5.8–10.5% (Table 1).

Regarding the particular granulocytes, neutrophils dominated and constituted 70.0–75.0% of the granulocytes and 13.1–18.7% of all the leukocytes. Eosinophils represented 25.0–32.0% of the granulocytes and 5.6–6.0% of all the leukocytes. The ploidy level did not influence the percentage of neutrophils and eosinophils in the granulocytic subgroup.

An increasing number of nuclear segments in the granulocytes was evident and dependent on the increasing ploidy level status. Statistically significant differences were found between *A. brevirostrum* (6n) and *A. gueldenstaedtii* (4n) as well as between *A. brevirostrum* (6n) and *A. ruthenus* (2n). The white blood cell parameters are summarised in Table 1. The granulocytic parameters are presented in Table 2.

Monocytes

The monocytes were easily recognised in the blood smears due to their size, nuclear shape and cytoplasmic tincture. From all the leukocytes, the monocytes represented 0.85 ± 0.66% in *A. ruthenus*, 1.00 ± 0.81% in *A. gueldenstaedtii* and 1.06 ± 0.94% in *A. brevirostrum.* No statistically significant differences were found between the ploidy level groups. The highest number of monocytes was reached in May for *A. ruthenus*, in June for *A. gueldenstaedtii* and in November for *A. brevirostrum*. The seasonal variation in the monocyte differential counts is given in Figure 5.

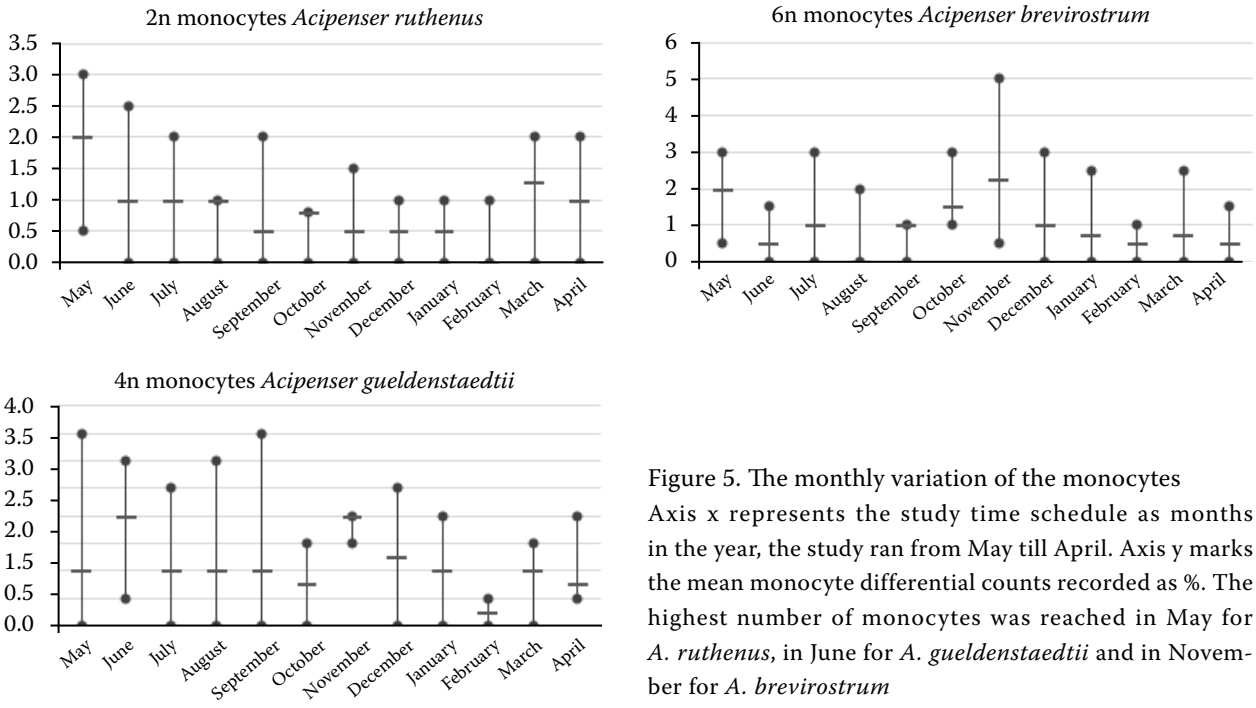

Figure 5. The monthly variation of the monocytes

Axis x represents the study time schedule as months in the year, the study ran from May till April. Axis y marks the mean monocyte differential counts recorded as %. The highest number of monocytes was reached in May for *A. ruthenus*, in June for *A. gueldenstaedtii* and in November for *A. brevirostrum*

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DISCUSSION

Polyploidy in sturgeons and number of leukocytes

Sturgeons are well known for their polyploidy (Flajshans and Vajcova 2000; Ludwig et al. 2001; Havelka et al. 2011; Crow et al. 2012; Havelka et al. 2016). It is now believed, that polyploidisation might represent an important phenomenon in the evolution of fish (Hardie and Hebert 2003; Havelka et al. 2011). The cell and nuclear size both correlate strongly with the genome size and with each other in ray-finned and cartilaginous fish. These relationships remain significant at each taxonomic level and exist independently of the phylogeny (Hardie and Hebert 2003). The chromosome number positively correlates with the DNA content among fish (Smith and Gregory 2009). The increased cellular and nuclear volume due to the additional chromosomal set or sets is balanced by reduced cell numbers (Benfey 1999; Maxime 2008). The same observations were made in studies documenting changes in the physiology and, especially, haematology of sturgeons with different ploidy levels [*Acipenser baerii*, *Acipenser stellatus*, *Huso huso* in Palikova et al. (1999); *A. ruthenus, A. gueldenstaedtii, A. baerii* in Flajshans and Vajcova (2002); *A. persicus* (Borodin, 1897), *H. huso* in Bahmani et al. (2001); *A. baerii* in Havelka et al. (2016)]. Similar results were obtained from studies comparing the haematological profiles between diploids and induced triploids [*A. brevirostrum* in Beyea et al. (2005); *A. baerii* in Wlasow and Fopp-Bayat (2011); *A. baerii* in Fopp-Bayat et al. (2012); *A. baerii* in Rozynski et al. (2015)].

Our results showed a dependence between the total number of leukocytes and the ploidy level. The increasing ploidy level associated with the decreasing total number of leukocytes was statistically significant. Our results were, thus, in agreement with other studies, especially with Hardie and Hebert (2003). The total number of leukocytes revealed a ploidy specificity and dependence.

Seasonal changes in blood cells parameters, general considerations

Available haematological data of annual and seasonal changes in blood cell indicators are scarce, especially for sturgeons, and have concentrated mostly on red blood cells. Haematological data on white blood cell indicators were collected from specimens reared in indoor recirculation aquaculture systems [*H. huso* in Zarejabad et al. (2010), Zarejabad et al. (2009); Akrami et al. (2013); *A. baerii* in Fopp-Bayat et al. (2013) and Rozynski et al. (2015); *A. ruthenus, A. baerii, A. gueldenstaedtii* in Flajshans and Vajcova (2002); *A. brevirostrum* in Knowles et al. (2006)], or obtained from wild caught fish [*A. stellatus* in Docan et al. (2014); *H. huso* in Mazandarani et al. (2015); *A. persicus, A. stellatus* in Pourgholam and Saeidi (2000); *A. brevirostrum* in Matsche and Gibbons (2012) and Matsche et al. (2013)]. However, the sturgeons investigated were of a different age (juveniles, adults or unknown age of wild caught fishes), gender, ploidy level, number of samples, and from different environmental conditions. The continuous blood analysis of red or white blood cell indicators rarely exceeded three months. Data for selected periods of the year (spring, autumn) were documented for *A. stellatus* (Docan et al. 2014), *A. brevirostrum* (Matsche and Gibbons 2012; Matsche et al. 2013) and *A. persicus* (Pourgholam and Saeidi 2000).

In our study, we provided periodical assessment of the white blood cell indicators, the tested fish were sampled each month over one year. Seasonal changes were evident: the highest number of leukocytes was reached during September and October for *A. ruthenus* and *A. brevirostrum*, from October to January for *A. gueldenstaedtii*. Lymphocytes were found to be reduced in June and July in each ploidy level group. The highest number of granulocytes was reached during April and June in *A. gueldenstaedtii* and during June and July in *A. ruthenus* and *A. brevirostrum.* The highest number of monocytes was reached in May for *A. ruthenus*, in June for *A. gueldenstaedtii* and in November for *A. brevirostrum.* The seasonal variation in differential leukocyte counts depended on the species rather than the ploidy level, and the influence of various external factors and conditions was evident.

Lymphocytes

Generally, the differential white blood cell counts in sturgeons were of lymphocytic origin as documented in studies listed further. Exceptionally,

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thrombocytes and thrombocyte-like cells were encountered in two differential counts (Knowles et al. 2006; Zexia et al. 2007). Nowadays, thrombocytes are believed to belong to an individual blood cell category (Khandekar et al. 2012) with unique function and origin.

The lymphocytes ranged from 51% to 98% (Matsche and Gibbons 2012; Rozynski et al. 2015; Adel et al. 2016). In our study, the lymphocytes ranged between 76.89–80.14%, and thus, the results were in accordance with other studies. The increasing ploidy level in the lymphocytes was associated with the tendency for the nuclei to divide, the nucleus revealed a "budding" appearance.

In our study, the "budding" appearance in the lymphocytes was first seen in the tetraploids (*A. gueldenstaedtii*). This finding seemed to be analogous to the erythrocytic nuclei divisions reported by Beyea et al. (2005) in the triploid *A. brevirostrum* and Wlasow and Fopp-Bayat (2011) in the triploid *A. baerii.*

Our observation of the lymphocyte nuclear division, however, was in contrast with findings made by Wlasow and Fopp-Bayat (2011) in the lymphocytes.

Granulocytes

Generally, the neutrophils dominated, while the eosinophils occurred in each study, always with less frequency compared to neutrophils. Interestingly, Gharaei et al. (2016) reported only basophils as the granulocytic representatives in *H. huso*. In our study, the neutrophils dominated in each ploidy level group, and constituted 13.1–18.7% from all the leukocytes. The eosinophils were present in lower percentages and constituted 5.6–6.0% from all the leukocytes. Our findings on the granulocytes were in accordance with other studies (Palikova et al. 1999; Pourgholam and Saeidi 2000; Flajshans and Vajcova 2002; Ruchin 2007; Zexia et al. 2007; Zarejabad et al. 2009; Docan et al. 2012; Matsche and Gibbons 2012; Akrami et al. 2013; Fopp-Bayat et al. 2013; Mazandarani et al. 2015; Rozynski et al. 2015; Adel et al. 2016). The dependence between the increasing number of nuclear segments both in neutrophils and eosinophils and increasing ploidy level was statistically significant. Similar findings were documented by Wlasow and Fopp-Bayat (2011) in *A. baerii*.

Monocytes

The reports about the monocytes in the differential counts were often conflicting; some authors did not mention any monocytes in the differential counts at all (Wlasow and Fopp-Bayat 2011; Fopp-Bayat et al. 2013), other authors pointed out the sporadic occurrence of monocytes (Palikova et al. 1999).

Based on our results, the monocytes constituted 0.85–1.06% from all the leukocytes, and were in accordance with published results.

Ploidy level representatives' assessment and comparison

DIPLOID GROUP

The results of white blood cell indicators measured between the representatives of the particular ploidy level groups were of interest. A comparison of species was undertaken upon the functional ploidy level scale (Havelka et al. 2011). The white blood cell indicators found for the *A. ruthenus* under study were compared to published data for sturgeons of the same ploidy level, i.e., *A. ruthenus, H. huso* and *A. stellatus* (summary in Table 3). In our study, the total number of leukocytes for th diploid *A. ruthenus* was $40.93 \pm 17.24 \times 10^9$/l. The lymphocytes dominated and constituted 77.59 ± 8.45%, followed by granulocytes with 21.63 ± 8.20% and monocytes with 0.85 ± 0.66%.

Palikova et al. (1999) evaluated the white blood cell profile of *A. stellatus* which corresponded to the results for *A. ruthenus* in our study. Pourgholam and Saeidi (2000) performed evaluations from wild adults of *A. stellatus* with a lower total leukocytes and lymphocytes compared to our data and with an increased number of granulocytes.

Flajshans and Vajcova (2000) reported a lower total leukocyte level in *A. ruthenus* compared to our results, as well as slightly decreased eosinophils and slightly elevated monocytes. Bahmani et al. (2001), Bani et al. (2009), Akrami et al. (2013) and Mazandarani et al. (2015) presented a corresponding total leukocyte for *H. huso* as a diploid representative which compared well to our data from *A. ruthenus*. In contrast, Gharaei et al. (2016) and Zarejabad et al. (2010), Zarejabad et al. (2009) reported a significantly lower total leuko-

https://doi.org/10.17221/215/2020-VETMED

Table 3. White blood cell parameters measured between the representatives of the diploid group

Parameter	Ploidy level (2n)												
	Acipenser ruthenus		*Huso huso*									*Acipenser stellatus*	
	our study	Flajshans and Vajcova (2002)	Akrami et al. (2013)	Palikova et al. (1999)	Bani et al. (2009)	Shahsavani and Mohri (2004)	Mazandarani et al. (2015)	Bahmani et al. (2001)	Zarejabad et al. (2009)	Gharaei et al. (2016)*	Zarejabad et al. (2010)	Palikova et al. (1999)	Pourgholam and Saeidi (2000)
WBC (10^9/l)	40.93 ± 17.24	10.6 ± 4.6	23–25	NA	37–52	4	23 ♀ 24.9 ♂	31–57.7	18–22	6.8	18	NA	16.5 ± 4
Lym (%)	77.59 ± 8.45	76.6 ± 9	68–72	73	NA	75	68.4 ♀ 72.4 ♂	54.5–67.5	56–69	74	58	73	52
Neu (%)	18.7 ± 9	17.2 ± 8.5	18–20.5	22	NA	20.56	21.4 ♀ 18.6 ♂	22–33	24–56	no	28	21	36
Eos (%)	5.8 ± 2.8	1.2 ± 1.8	5.5–6.5	5	NA	2.2	7 ♀ 4.6 ♂	6.6–13.7	6–14	no	13	4	11.9
Mono (%)	0.85 ± 0.66	2.6 ± 2.2	3.3–4.2	NA	NA	0.342	3.2 ♀ 4 ♂	0.6–2.25	0.3–0.6	4	1	NA	0.1

*Gharaei, basophils 0.6%, no neutrophils or eosinophils recorded

Eos = eosinophils; Lym = lymphocytes; Mono = monocytes; NA = data not available; Neu = neutrophils; WBC = white blood cell

cyte in *H. huso*. The lymphocytes dominated and reached 54–75%.

The majority of the sturgeons examined in the abovementioned studies originated from the hatchery and juveniles dominated. Pourgholam and Saeidi (2000) examined wild adults of *A. stellatus*, and Akrami et al. (2013) performed haematological examination of *H. huso* of different ages reared in ponds.

TETRAPLOID GROUP

Published data on the white blood cell indicators in *A. baerii, A. persicus* and *A. sinensis* were used for comparative purposes in the tetraploid group (summary in Table 4). In our study, the total number of leukocytes for the tetraploid *A. gueldenstaedtii* was 20.63 ± 11.20 × 10^9/l. The lymphocytes constituted 80.14 ± 8.13%, followed by the granulocytes with 18.85 ± 7.75 % and monocytes 1.00 ± 0.81%.

Comparing results from *A. gueldenstaedtii* in our study, a corresponding total leukocyte and differential counts were reported by Flajshans and Vajcova (2000), Docan et al. (2012) and Rozynski et al. (2015) in *A. baerii.* In contrast, Ruchin (2007) documented a higher total leukocyte in *A. baerii.* Changes in the lymphocyte percentages in *A. baerii* decreased in the studies provided by Palikova et al. (1999), Wlasow and Fopp-Bayat (2011), Fopp-Bayat et al. (2013). Increased lymphocytes were documented in the study of Ruchin (2007). Bahmani et al. (2001) provided an evaluation of *A. persicus* which corresponded to our results, Pourgholam and Saeidi (2000) and Adel et al. (2016) documented a decreased total count of leukocytes compared to our results. Decreased lymphocytes were the most prominent in the study of Adel et al. (2016) and reached up to 51%. The most confusing results were found in the study of Zexia et al. (2007) on *A. sinensis*; thrombocytes were encountered in the white blood cell count, and monocytes represented the majority in the differential leukocyte count followed by lymphocytes and neutrophils. The majority of sturgeons studied originated from hatcheries and juveniles dominated. Adel et al. (2016) presented a comparison of haematological indices between *A. persicus* adult males and females.

https://doi.org/10.17221/215/2020-VETMED

Table 4. White blood cell parameters measured between the representatives of the tetraploid group

Parameter	Ploidy level (4n)													
	Acipenser gueldenstaedtii	*Acipenser baerii*							*Acipenser persicus*				*Acipenser sinensis*	
	our study	Docan et al. (2012)	Fopp-Bayat et al. (2013)	Wlasow and Fopp-Bayat (2011)	Palikova et al. (1999)	Flajshans and Vajcova (2002)	Ruchin (2007)	Rozynski et al. (2015)	Pourgholam and Saeidi (2000)	Bahmani et al. (2001)	Adel et al. (2016)	Padash-Barmchi et al. (2010)	Zexia et al. (2007)**	
WBC (10^9/l)	20.63 ± 11.20	20	NA	NA	NA	20.1 ± 11	30–43.7	NA	10 ± 4	13–46	10–14	1.25	22.4	
Lym (%)	80.14 ± 8.13	70–77	60	69	68	74 ± 15	83–90	86 (69–98)	70	73–82	44.5–51	82.6	12	
Neu (%)	13.1 ± 7	15–25	35*	28*	25	12.9 ± 9	3–7	14.5*	22	12–20	45–50	15*	11	
Eos (%)	5.6 ± 2.8	2.9–4	0.6	2.2	3	8.8 ± 8.3	0.5–1	2.5	7	2–6.5	0.23–0.69	2*	4	
Mono (%)	1.00 ± 0.81	1–2	NA	NA	NA	1.8 ± 1.6	–	5	1	0.2–2.5	0.7–1.1	0	13	

*Authors presented developmental stages of neutrophils or eosinophils, respectively; number represents total count of different neutrophilic/eosinophilic subtypes; **Zexia et al. included thrombocytes into the white blood cell differential count, thrombocytes constituted 60% of the white blood cells

Eos = eosinophils; Lym = lymphocytes; Mono = monocytes; NA = data not available; Neu = neutrophils; WBC = white blood cell

HEXAPLOID GROUP

Haematological data on white blood cell indicators from *A. brevirostrum* are rare. In our study, the total number of leukocytes for the hexaploid *A. brevirostrum* was 14.13 ± 7.72 × 10^9/l. The lymphocytes constituted 76.89 ± 7.82%, followed by the granulocytes with 22.04 ± 7.47% and monocytes 1.06 ± 0.94%. Comparing our results (summarised in Table 5), the corresponding data for the total number of leukocytes were documented by Matsche and Gibbons (2012) and Matsche et al. (2013), while Knowles et al. (2006) presented a significantly higher total leukocyte count. Neutrophils dominated in the study of Matsche and Gibbons (2012) in most of the examined fish owing to the long-lasting stress. Knowles et al. (2006) encountered thrombocyte-like cells in the differential counts, but despite this, the lymphocytes dominated in the differential count. The results obtained from Matsche and Gibbons (2012) and Matsche et al. (2013) reflected the haematological profiles of adult fish during spawning activity, while the data obtained from Knowles et al. (2006) were based on immature cultured fish.

The results obtained from a variety of studies documented changes in the haematological and biochemical indicators under different influences and conditions, and thus represented useful knowledge about the sturgeons' physiology and their ability to adapt. The results have often been provided for comparative purposes and have been reported as descriptive summaries.

To our current knowledge, there has not been any standardised guidelines published for sampling a sturgeon's blood profile. This is not surprising since fish have revealed high variability in blood cell indicators among species and under different environmental and anthropogenic conditions.

The periodical examination of fish populations, as described in our study, appear to be unique. The data obtained contributed to the basic knowledge on the sturgeon's physiology, especially the haematology. The total number of leukocytes was dependent on the ploidy level, while the variations in the differential counts during the periodical examinations were species specific. Thus, knowledge of seasonal changes in the white blood cell parameters should be considered while performing haematological examinations.

https://doi.org/10.17221/215/2020-VETMED

Table 5. White blood cell parameters measured between representatives of the hexaploid group

Parameter	Ploidy level (6n)		
	Acipenser brevirostrum		
	our study	Knowles et al. (2006)*	Matsche and Gibbons (2012), Matsche et al. (2013)*
WBC (10^9/l)	14.13 ± 7.72	57 (28–90)	2.08–8.74
Lym (%)	76.89 ± 7.82	39–74**	30
Neu (%)	15.5 ± 9	13–37	51
Eos (%)	6 ± 4.1	0–1.7	2
Mono (%)	1.06 ± 0.94	0–7.8	3

*Authors presented differential leukocyte count as number of particular cell type and reference interval; **Lymphocytes were originally subdivided as small and large lymphocytes, the number represents the total count of lymphocytes
Eos = eosinophils; Lym = lymphocytes; Mono = monocytes; Neu = neutrophils; WBC = white blood cell

In sturgeons, the higher ploidy level was associated with the granulocytic and lymphocytic nuclear changes became apparent when performing the blood smear assessment. Multisegmented nuclei and atypical nuclear shapes, changes related to the increasing genome size and DNA folding were common findings in the granulocytes. The increasing ploidy level in the lymphocytes was associated with the tendency of the nuclei to divide, and the nucleus revealed a "budding" appearance.

Acknowledgement

The authors wish to acknowledge the technical assistance of M. Pecena and M. Kahanec from the South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice. The authors wish to acknowledge the language help of Dr. John F. Craig from Craig Consulltancy, Whiteside, Dunscore, Dunfries, Scotland.

Conflict of interest

The authors declare no conflict of interest.

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Received: November 7, 2020
Accepted: September 9, 2021
Published online: January 6, 2022