Acta Biol. Univ. Daugavp. 19 (2) 2019
ISSN 1407 - 8953

HEMATOLOGICAL INDICES OF THE PRUSSIAN CARP (*CARASSIUS GIBELIO* (BLOCH, 1782)) FROM THE ZAPORIZHIAN (DNIPRO) RESERVOIR

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Kurchenko V., Sharamok T. 2019. Hematological indices of the Prussian carp (*Carassius gibelio* (Bloch, 1782)) from the Zaporizhian (Dnipro) reservoir. *Acta Biol. Univ. Daugavp., 19 (2): 141 – 148.*

The purpose of this work was to research the hematology and cytometric indices of the Prussian carp (*Carassius gibelio* (Bloch, 1782)) in the conditions of the Zaporizhian (Dnipro) reservoir. The research was carried out in the autumn on two fishing facilities located in the Samara Bay and the lower part of the Zaporizhian (Dnipro) reservoir. Hematologic and cytometric researches were performed according to generally accepted methods. It was found that silver carp from the Samara Bay had the largest number of pathologies, twice lower hemoglobin count against the high erythrocyte sedimentation rate and more red blood cells, compared to the Prussian carp from the lower part. In addition, in the Samara Bay, there was a tendency to increase the total amount of leukocytes compared with the Prussian carp from the lower area by 14%. Index of the shift of leukocytes from the lower part of the Zaporizhian Reservoir was 0.19, and in species from the Samara Bay - 0.29, indicating a shift to the left in leukocyte formula.

Key words: Prussian carp, hematological parameters, blood, red blood cells

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INTRODUCTION

Currently, the study of fish's blood remains very relevant, since blood plays the role of a sensitive indicator of the health of fish (Kuzina 2009). It is known that changes in hematological parameters depend on the concentration and duration of exposure to many factors: type of fish, age, contaminants, and health status (Adakole 2012). Erythrocytes of fish are sensitive to environmental pollution and their morphological changes can be used as a bioindicator of toxicity (Kurchenko & Sharamok 2018). According to research results, various organic and inorganic pollutants (Witeska 2013) can cause blood cell pathology. Hematology analysis is quite successful in finding optimal conditions for fish breeding, as well as in ichthyopathology, ichthyotoxicology (Sharamok & Esipova 2015).

Hematologic studies are needed to characterize the physiological state of fish. They can indicate stress and anemic processes, as well as evidence the respiratory capacity of fish species (Tavares-Dias & Moraes 2004). They are important in determining the influence of pathophysiological conditions that may affect homeostasis (Ranzani-Paiva et al. 2013). Given the need for information on the detection and control of stressful situations and diseases, hematological analysis and evaluation of the disease become important and act as auxiliary a tool for diagnosing of fish health (Ventura et al. 2018, Shweta Maheshwari, Anish Dua 2016).

Blood is one of the main indicators of the physiological state of the body, correct and timely diagnosis of morphological changes in blood can detect imbalance or pathology in the body of fish.

Numerous researches have shown that cytometric indices of red and white blood cells (Velcheva et al. 2006, Mineev 2012) and the leukocyte fish formula (Shein 2014) may be used as indicators of the level of water pollution. The accumulated material allowed to reveal a number of regularities regarding changes in cytometric indices of peripheral blood red blood cells of different species of fish under the influence of heavy metals (Sharamok et al. 2015), hypoxia (Parfenova et al. 2011, Yesipova et al. 2015), technological conditions for artificial growth (Serpunin 2002) and others. Established the ratio of the cells of the erythroid and leukoid blood of some species of fish under different conditions of existence (V'azquez et al 2007, Gayatri et al., 2014).

However, works devoted to the research of fish blood count cannot yet be considered as numerical. This is explained by the fact that the connection between the habitat of fish and their organism, the indicators of their blood and the physiological state, age, course of pathological processes is not fully established.

The purpose of our work was to research the hematologic and cytometric indices of the Prussian carp (*Carassius gibelio* (Bloch, 1782)) in the conditions of the Zaporizhian (Dnipro) reservoir.

MATERIALS AND METHODS

The research was carried out in the autumn on two fishing facilities located in the Samara Bay and the lower part of the Zaporizhian (Dnipro) Reservoir (Fig. 1). Increased permanganate oxidation, high mineralization, and more nutrient loading, primarily phosphates in comparison with the lower section of the reservoir, characterize water in the Samara Bay. Highly mineralized shale waters containing suspended particles and heavy metals (Fedonenko & Marenkov 2018) influence the hydroecological regime of the bay. Standard shutter grids with a step of 30-45 mm carried out the fishing. The object of the study was the Prussian carp (*Carassius gibelio* (Bloch, 1782)), which is the leading industrial species in the Zaporizhian (Dnipro) reservoir (Marenkov 2016).

In total, 100 specimens of fish with an average weight of 378.98 ± 28.44 g were researched. Indices of industrial length of specimens kept at 22.64 ± 0.49 cm.

Blood was taken from the tail vein. After prepairing, it was stained with Romanovsky-Giemsa (Golovin 1989). Identification of the formed blood elements was carried out using Atlas of Ivanova N.T. and Atlas Mumford S. (Ivanova 1989, Mumford et al. 2007). Photographs of

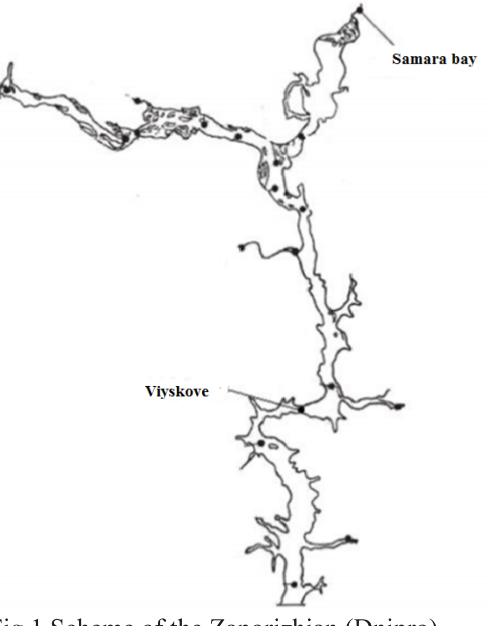

Fig.1 Scheme of the Zaporizhian (Dnipro) reservoir.

Table 1. Cytometric indices of erythrocytes of the Prussian carp from the Zaporizhian (Dnipro) reservoir

Indicator	The lower part of the reservoir	The Samara Bay
S of the cell, μm^2	87.67±0.72	87.73±0.64
D of the cell, µm	12.82±0.07	12.91±0.08
d of the cell, µm	8.75±0.09	8.67±0.05
S of the nuclei, μm^2	13.65±0.20	14.21±0.20
D of the nuclei, µm	5.13±0.05	5.18±0.06
d of the nuclei, µm	3.42±0.04	3.39±0.04

preparations were made using a digital camera "SciencelabT500 5.17M", which was connected to the microscope Ulab XY-B2TLED. The preparations looked at 200 fields of vision. The following parameters were determined: large longitudinal (D) and small transverse (d) diameters of mature erythrocytes, erythrocyte area (S), erythrocyte nucleus (s), nuclear-cytoplasmic ratio (s / S), percentage of mature erythrocytes (ME), the percentage of immature erythrocytes (IE) and their forms. The leukocyte shift index (LSI) was determined as the ratio of rodenuclear neutrophils to segmental. The calculations were performed using ScienceLabView7. Statistical processing of the obtained results was carried out using generally accepted methods of variation statistics. Hematologic researches were performed according to generally accepted methods (Davydov 2006). The following parameters were determined: the total number of erythrocytes (the method of tests in the chamber Goryaev), hemoglobin (the method for determining hemoglobin by Sali), color index, erythrocyte sedimentation rate (Punchenkov's micromethod), total number of leukocytes (direct method of counting the number of leukocytes).

RESULTS

Normally, on blood products of the Prussian carp, erythrocytes had an oval shape with clear contours, a dark purple core located in the center of the cell (Fig. 2).

In cytometric research of red blood cells of the Prussian carp, no distinction was discovered between the indices of fish cells from the lower part of the reservoir and from the Samara Bay (Table 1).

The nuclear-cytoplasmic ratio of erythrocytes also did not differ significantly and amounted to 0.15 in the lower section and 0.16 in the Samara Bay.

Mature erythrocytes constituted the most numerous group of red blood cells in experimental fish - 86.61% in the lower part of the reservoir and 87.74% in the Samara Bay. Accordingly, the maximum number of immature erythrocytes (13.39%) was observed in the Prussian carp from the lower part of the reservoir (Table 2.). It is known that in fish, the synthesis of hemoglobin in cells of the erythroid series begins with the stage of polychromatophilic normoblast (Ivanova 1983). Young erythrocytes were represented by basophilic and polychromatophilic normoblasts (Fig. 3).

Cells with deformation were found during the morphometric investigations, namely: pear-shaped, crescent-shaped and diamond-shaped (Fig. 4). The most numerous of pathologies were nuclear shadows and fold membranes in carp from the Samara Bay - 7.66% and 4.52% respectively (Table 2).

Reducing the elasticity of the erythrocyte cell membrane indirectly indicates a change in its osmotic properties. All these changes may indicate a deterioration in the physiological state of fish, caused by the negative influence of anthropogenic factors (Sharamok & Yesipova 2015).

Table 2. Kinds of pathologies of red blood cells of the Prussian carp from Zaporizhian (Dnipro) reservoir

Kind of pathology	The lower part	The Samara Bay
Deformation of red blood cell, %	2.68	3.69
Fold membranes, %	3.44	4.52
Nuclear shadows, %	2.66	7.66
Nuclei shift to the periphery of the cell, %	-	1.0

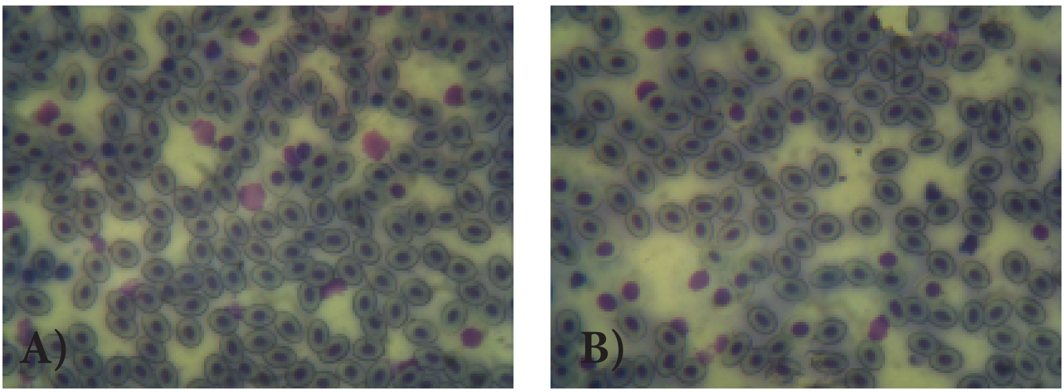

Fig.2. Erythrocytes of the Prussian carp (standard norm): A- lower section of the reservoir; B- the Samara Bay.

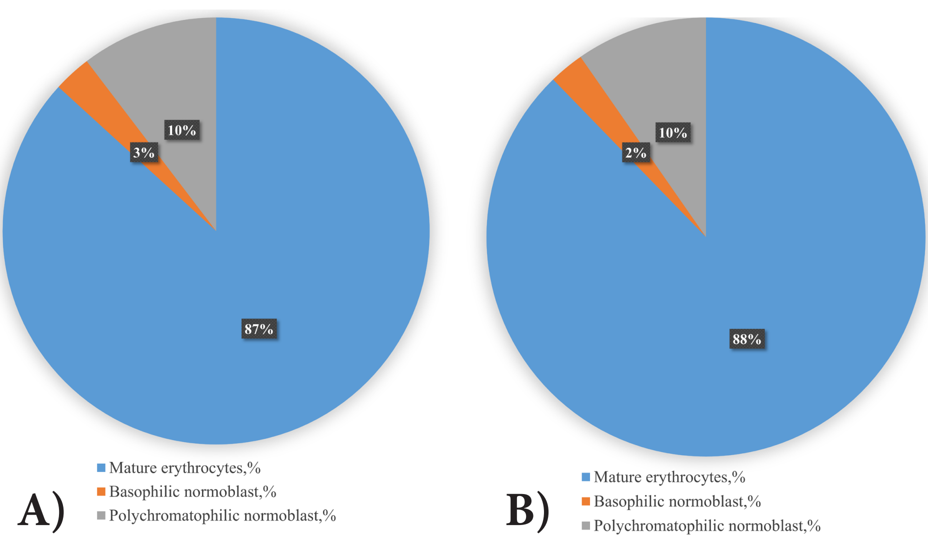

Fig. 3 Ratio of forms of erythrocytes: A- the lower section of the reservoir; B- the Samara Bay.

The amount of erythrocytes in the Prussian Carp from the Samara Bay was almost twice as high as in the lower part and was 11.7 ± 39.76 million / µl and 7.07 ± 8.59 million / µl, respectively. This difference can be caused by a variety of factors. It is known that the fish exhibit significant fluctuations in the number of erythrocytes and hemoglobin concentration, depending on the systematic position, age, activity, state of the organism, ecology of the species, season, physico-chemical factors of the environment, etc. (Witeska 2013). Increasing the salinity of the water and its various contamination leads to an increase in the number of red blood cells. For example, according to literature data, in rats, an increase in erythrocytes from 1 million / µl to 2 million / µl was observed under the influence of water pollution with copper and ammonia (Amineva & Yarzhombek 1984).

In the waters of the Samara Bay, excess copper content was detected 8 times, manganese - 1.7; lead - 1.5 and cadmium - 2 times compared with the MAC. The concentration of all investigated heavy metals in the water of the Samara Bay is higher compared with the lower section of the Zaporizhia reservoir (Sharamok et al. 2016). That is, an increase in the number of erythrocytes in fish from the Samara Bay can indicate the negative effects of heavy metals on the organism of the fish under study.

The amount of hemoglobin in the blood of the Prussian carp from the Samara Bay was lower, almost in 2 times, than in the blood of the Prussian carp from the lower part of the reservoir (Table 3). This may indicate the occurrence of anemic processes in the Samara Bay fish. It is known that the amount of hemoglobin in the blood decreases with anemia, which can be caused by metabolic diseases, with long starvation, and the violation of the function of the gills (Biktashova & Latipova 2013). The color index reflects the relative content of hemoglobin in erythrocytes, a very important diagnostic indicator for immorality, with violations of hydrochemical regimes in reservoirs and poisoning (Davydov 2006). In a hematologic study, this indicator almost did not differ from the fish from both areas and was 0.72 in the lower section and 0.82 in the Samara Bay. The rate of erythrocyte sedimentation (ESR) has a diagnostic value. According to the literary data, it is from 2 to 10 mm / h in normal fish (Amineva

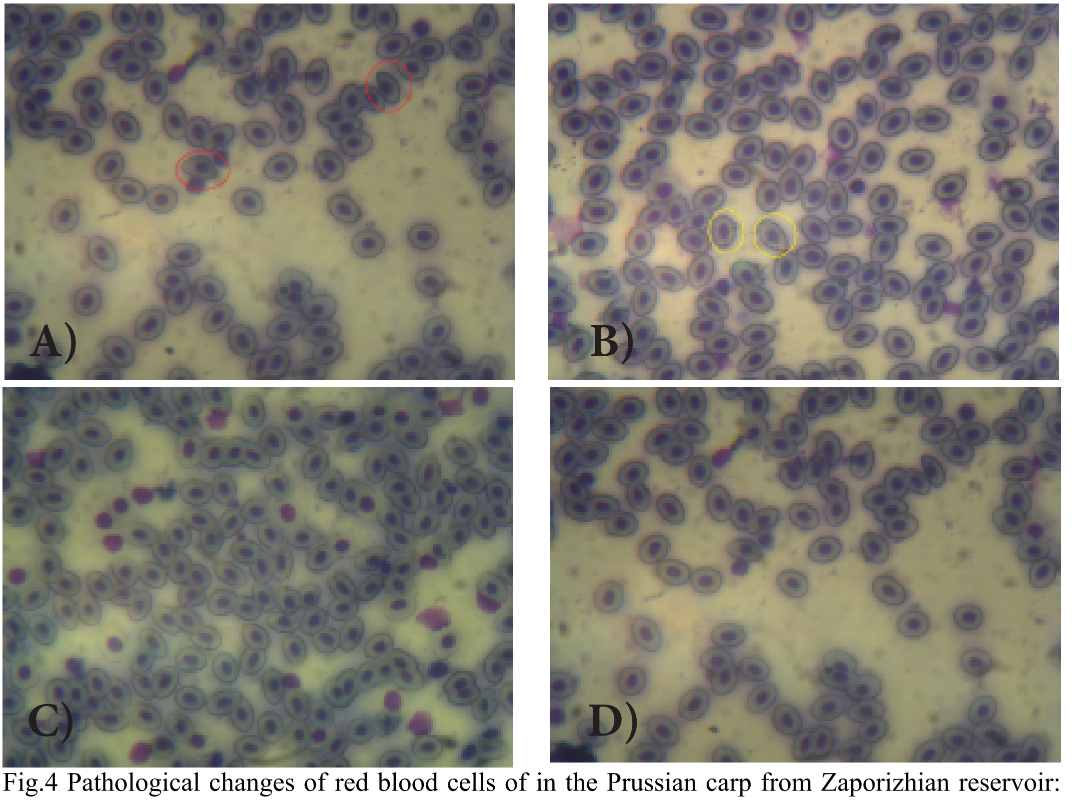

Fig.4 Pathological changes of red blood cells of in the Prussian carp from Zaporizhian reservoir: A-deformation of the cell; B- fold membranes; C-nuclear shadows; D- nuclei shift to the periphery of the cell.

Table 3. Hematological indicators of the Prussian carp from the Zaporizhian (Dnipro) reservoir

Indicator	The lower part	The Samara Bay
Erythrocytes, million / µl	7.07±8.59*	11.7±39.76*
Hemoglobin, g / l	4.3±1.23*	2.2±0.11*
Color indicator	0.72±0.03	0.82±0.03
Leukocytes, thousand / µl	37.5±0.14	39.05±3.1
Reduction rate of erythrocytes mm / h	3.5±0.64*	11.0±0.57*

Remark. * - the difference between the indicators is probable, p≤0,05.

Table 4 Indicators of leukocyte blood formula of Prussian carp from Zaporizhian (Dnipro) reservoir

Indicator,%	The lower part	The Samara Bay
Segmented neutrophils	16.75±6.20	16.0±6.22
Rodenuclear neutrophils	3.25±0.28	4.75±2.47
Lymphocytes	76.75±0.03*	70.25±81.25*
Monocytes	3.25±0.28*	9.0±1.0*

Remark * - the difference between the indicators is probable, p≤0.05.

& Yarzhombek 1984). During hematological research copper in the Prussian carp from the Samara Bay, the ESR was higher by 68.18% than in the carp from the lower part of the reservoir. Increasing the rate of erythrocyte sedimentation may indicate an inflammatory process in the body.

The predominant cells of the white blood of the fish were lymphocytes, which comprised 81% in the lower part and 70% of all leukocytes in the Samara Bay. In the carp from the Samara Bay there was a tendency for increasing the total number of leukocytes in comparison with the carp from the lower part by 14% (p≥0.05) (Table 4). This may indicate an increased protective function of the blood in the body under the influence of chemical toxicants (Fedonenko E. et al. 2016).

The role of the immune system in the adaptive reactions of animals to change the conditions of existence is universally accepted. Objective indicators of the influence on the immune status of an animal's organism are leukocyte indices that reflect the correlation of blood cells and allow to evaluate the work of the immune system effector mechanisms, as well as the level of immunological reactivity of the organism (Mustafin et al. 1999, Tkachenko & Derham 2014).

A reliable criterion for evaluating the status of individual individuals is the deviation in the ratio of different forms of leukocytes (Krylov 1974, Balabanova & Mikryakov 2002), in particular, the leukocyte shift index (LSI). Increasing the relative content of immature neutrophilic cells in the peripheral blood is called a shift to the left, and the decrease of the particle share of nuclear neutrophils in the presence of hyper-segmented nuclei is defined as a shift to the right (Zhitieneva et al. 1997). Changing the LSI in one way or another from the conditional norm is a sign of the disease or increased negative pressure from the environment. The LSI of the Prussian carp from the lower part of the Zaporizhian reservoir was 0.19, and in the species from Samara Bay it was 0.29, indicating a shift to the left in the leukocyte formula. Neutrophilic leukocytosis with shift to the left is observed, as a rule, when the inflammatory processes and various intoxications are performed (Moiseenko 2000).

The number of monocytes in fish from the Samara Bay was more than three times larger than in the lower part, which could be an answer to the adverse conditions of existence or the course of the disease (Davydov 2006).

CONCLUSIONS

Thus, with morphometric research of red blood cells of the Prussian carp, there were pathologies, the most numerous of which were nuclear shadows and festonsity of the membrane in the carp from the Samara Bay. In a hematological study, it was discovered that the Prussian carp from the Samara Bay had twice the lower amount of hemoglobin on the background of a high rate of erythrocyte sedimentation and almost twice the number of red blood cells compared to the lower part.

Also, in the Samara Bay, there was a tendency to increase the total amount of leukocytes compared with the carp from the lower part by 14% ($p \geq 0.05$). LSI of the Prussian carp from the lower part of the Zaporizhian reservoir was 0.19, and in species from the Samara Bay it was 0.29, indicating a shift to the left in the leukocyte formula.

All this suggests that the Prussian carp from the Samara Bay suffers from inflammatory and anemic processes in the body, and therefore, is under strong anthropogenic pressure, which manifests itself in a number of pathological and inflammatory processes.

REFERENCES

Adakole J. A. 2012. Changes in some haematological parameters of the African catfish (*Clarias gariepinus*) exposed to a metal finishing company effluent. *Indian Journal of Science and Technology*, 5: 2510-2514.

Amineva V.A., Yarzhombek A.A. 1984. Fish physiology. Moscow, 153.

Balabanova L.V., Mikryakov V.R. 2002. Comparative characteristics of the effect of naphthalene and phenol on the indicators of white blood carp *Carassius carassius* (L.). *Biology of inland waters*, 2: 100-102.

Biktasheva F.H., Latypova G.F. 2013. Hematological indicators of representatives of predatory fish in the natural park of lake Asylykul. *Proceedings of the Orenburg State Agrarian University*, 1 (39): 72-73.

Davydov ON, Temnikhanov Yu.D., Kurovskaya L.Ya. 2006. Fish blood pathology, Kiev, Pp. 61-66.

Fedonenko E.V., Sharamok TS, Ananyeva T.V. 2017. Features of the leukocyte formula in some carp fish from the Zaporizhian reservoir. *Proceedings of VNIRO: commercial species and their biology*, 167: 59-65.

Fedonenko O.V., Marenkov O.M. 2018. The Zaporizhian (Dnipro) reservoir Ichthyofauna Industrial development. Ukraine. Pp. 26-27.

Gayatri A., Prafulla M. 2014.The morphometrical characterisation of normal blood cells of two air breathing fishes, *Clarias batrachus* and *Anabas testudineus*. *International Research Journal of Biological Sciences*, 3(11): 37–41.

Golovina.N. A., Trombitsky I.D. 1989. The Hematology of the pond fish. Kishenev: Stiz. Pp. 156.

Ivanova N. T. 1989. Atlas of the fish blood cells. M .: Leg. food industry. Pp. 184.

Krylov O.N. 1974. Guidelines for hematological examination of fish in aquatic toxicology. Pp. 39.

Kurchenko V., Sharamok T. 2018. Morphological and cytometric indicators of the bream (*Abramis brama* Linnaeus, 1758) erythrocites from the Zaporizhian reservoir. *World Scientific News,* 112: 228-236.

Kuzina, T.V. 2009. Analysis of hematological parameters of the pike perch from the Volga Caspian canal. *Natural Sciences*, 4 (29): 96-100.

Marenkov O.M. 2016. Reproductive features of roach, bream and common carp of

Zaporozhian (Dnipro) Reservoir in contemporary environmental conditions. *International Letters of Natural Sciences*, 57: 26–40.

Mineev A.K., Kalinin E.A. 2012. Features of the leukocyte formula in roach (*Rutilus rutilus* Linnaeus, 1758) from reservoirs of different types (on the example of the Saratov reservoir and small rivers of the Republic of Udmurtia). *Proceedings of the Samara Scientific Center of the Russian Academy of Sciences*, 14(1): 213-217.

Moiseenko T.I. 2000. Morphological rearrangements of the fish organism under the influence of pollution (in S.Schwartz's theory). Ecology, 6: 463– 472.

Mustafina Zh. G., Kramarenko Yu. S., Kobtseva V. Yu. 1999. Integral hematological parameters in the assessment of the immunological reactivity of the organism in patients with ophthalmopathology. *Clinical laboratory diagnosis*, 5: 47 - 48.

Parfenova, IA, Soldatov, A.A. 2011. Functional morphology of circulating erythrocytes of the round goby under conditions of experimental hypoxia. *Marine Ecological Journal*, 2: 59–67.

Ranzani-Paiva, M. J. T., Pádua, S. B., Tavares-Dias, M., Egami. 2013. Métodos para análise hematológica empeixes. Maringá: EDUEM.

Rey G. V´azquez G. 2007. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes. Guerrero Tissue and Cell, 39:151–160.

Serpunin G. G. 2002. Hematological parameters of fish adaptations. Diss. Dr. Biol. sciences. Kaliningrad. Pp. 482.

Sharamok T.S., Yesipova N.B., Fedonenko O.V., Biletska O.V. 2016. Ecological-hematological characteristic of the roach (*Rutilus rutilus* Linnaus, 1758) from Zaporizhian reservoir. *Biological Journal of Bogdan Khmelnitsky Melitopol State Pedagogical University,* 6 (2): 303-310.

Sharamok T., Esipova N. 2015. Influence of anthropogenic factors on fish hematological parameters. *Scientific notes Ternopil National Pedagogical University*, 3/4 (64): 722-726.

Sheina T.A. 2014. Composition of blood and the content of heavy metals in the organs and tissues of three species of fish in the Kama River basin. Author. PhD Thesis. Perm, Pp. 22.

Tavares-Dias M., Moraes F. R. 2004. Hematologia de peixes Teleósteos. Loading and transport stress in juvenile matrinxã (Bryconcephalus) at various densities. *Aquaculture*, 229(1-4), 389-400.

Tkachenko E. A., Derho M. A. 2014. Leukocyte indices in experimental cadmium intoxication of mice. *Proc., Of Orenb., state Agricultural University*, 3 (47): 81-83.

Velcheva I., Arnaudov A., Hadžiablahovic S. 2006. A study on some physiological parameters of three hydrobiotic species under the influence of copper. *Proceedings of II International Symposium of Ecologists of Montenegro. Kotor*, 155–160.

Ventura A. S., Ishikawa M. M., Gabriel A. M. D. A., Silbiger, H. L. N., Cavichiolo, F., Takemoto, R. M. 2016. Histopathology from liver of tuvira (*Gymnotus* spp.) parasitized by larvae of nematodes. *Ciência Rural*, 46(7), 1233-1239.

Witeska M. 2013. Erythrocytes in teleost fishe. *Zoology and Ecology*, 23(4), 1-4.

Zhiteneva LD, Rudnitskaya O.A., Kalyuzhnaya T.I. 1997. Ecological and hematological characteristics of some species of fish. Directory. Rostov. Pp. 149.

Received: 01.06.2019.
Accepted: 01.11.2019.