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SHORT COMMUNICATION

Morphological and cytometric indicators of the bream (*Abramis brama* Linnaeus, 1758) erythrocites from the Zaporizhian reservoir

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ABSTRACT

The researches were carried out in the Zaporizhian reservoir (Ukraine) in the spring-summer period of 2015 - 2016. The samples were taken in two sections of the Zaporizhian reservoir which differ in ecological and hydrological regime: the Samara Bay (characterized by high content of heavy metals and high water mineralization) and the lower section of the reservoir (conventionally "clean zone"). The four-year-old specimens of both breeds of the Bream (*Abramis brama* Linnaeus, 1758) were the objects of the research. The fish were caught using gill nests during research fishing. The following indices of red blood cells were determined: large longitudinal and small transverse diameters of mature erythrocytes and their nuclei, erythrocyte area, erythrocyte core area. The volume of the cell, including the volume of the nucleus, was determined by the Houchin equation. It was established that in the the Bream from the Samara Bay an increase in the areas and volumes of fish red blood cells was observed compared to the same age species from the lower part of the reservoir. In erythrocytes of the researched fish, the following pathologies were observed: poikilocytosis, festonchity of the membrane, hyperchromia of the nucleus, anatomic location of the nucleus, amitose, invagination of the nucleus, nuclear shadows. The most pathologies were found in the Samara Bay, which sufferes from more intensive anthropogenic influences.

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***Keywords*:** hematopathology, indicators of red blood, carp fish, reservoir

1. INTRODUCTION

At present, the research of the state of fish blood has become essential, since blood, as a self-regulating system, plays the role of a sensitive, objective indicator of fish internal and external environment state (Golovin, 2004; Kuzina, 2009). It was established that changes in hematological parameters depend on the concentration and duration of exposure to pollutants, the type of fish, their age and their health status (Adakole, 2012). Hematologic analysis is also quite successful in finding optimal conditions for breeding fish, as well as in ichthyopathology, ichthyotoxicology (Sharamok and Esipova, 2015). The research of blood morphology does not require large material costs, it allows to obtain the results that indicate the pollution of the reservoir quickly, in contrast to other long, labor-intensive and expensive methods. Erythrocytes of fish are sensible to environmental pollution and their morphological changes could be used as a bioindicator of toxicity. Morphological features of erythrocytes of fish (cell and nuclear abnormalities and frequency of immature cells) are more sensitive and reliable indicators of water pollution than the basic parameters of red blood. According to the results of researches, red cells anomalies could be caused by various organic and inorganic pollutants (Witeska, 2013).

The obtained material allowed to reveal a number of regularities concerning changes in cytometric indices of peripheral blood erythrocytes of fish different species under the influence of heavy metals (Parfenova and Soldatov, 2011) and other technological conditions of artificial cultivation, etc. But at the present there is insufficient information on changes in morpho-cytometric indices of red blood cells of freshwater fish in reservoirs with different degrees of anthropogenic influence.

Therefore, the purpose of our work was to research morphological and cytometric indices of the common Bream erythrocytes (Abramis brama Linnaeus, 1758), that habits in the Zaporizhian reservoir sites which differ in ecological state.

2. MATERIALS AND METHODS.

The samples were taken in the two parts of the Zaporizhian Reservoir (Fig. 1), which differ in the ecological-hydrological regime, and are the main spawning and industrial zones.

The lower part of the reservoir is located in the agrarian zone near the village Viys'kove (48°22'30.75"N; 35°20'80.05E) and almost does not suffer from the effects of toxic industrial wastewater. The quality of water according to the trophic-saprobiological (ecological and sanitary) criteria and the criteria for the content of specific substances of toxic and radiation action and according to the level of toxicity belongs to the II class of 3 categories and is characterized as "good" and "fairly clean". The Samara Bay (48°53'40.21"N; 35°18'73.20E) is located at the confluence of the Samara River with the reservoir, characterized by weak flow and large area of shallow water. The hydroecological state of the bay is determined by the

influence of highly mineralized sewage waters, the main contaminating components of which are fine-grained suspended particles and heavy metals, the content of which is on average 60% higher than in the lower part of the reservoir (Fedonenko, Yesipova, Sharamok, Ananieva and Yakovenko, 2012). The water of the Samara Bay belongs to the 3rd class of the 4th category and is characterized as "satisfactory" and "weakly polluted". The subjects of the research were the four-year-old specimens of the freshwater Bream (Abramis brama Linnaeus, 1758) of both sexes. Fish were caught using gill nets during research conducted in spring-summer 2015-2016. A total of 24 specimens of fish were researched.

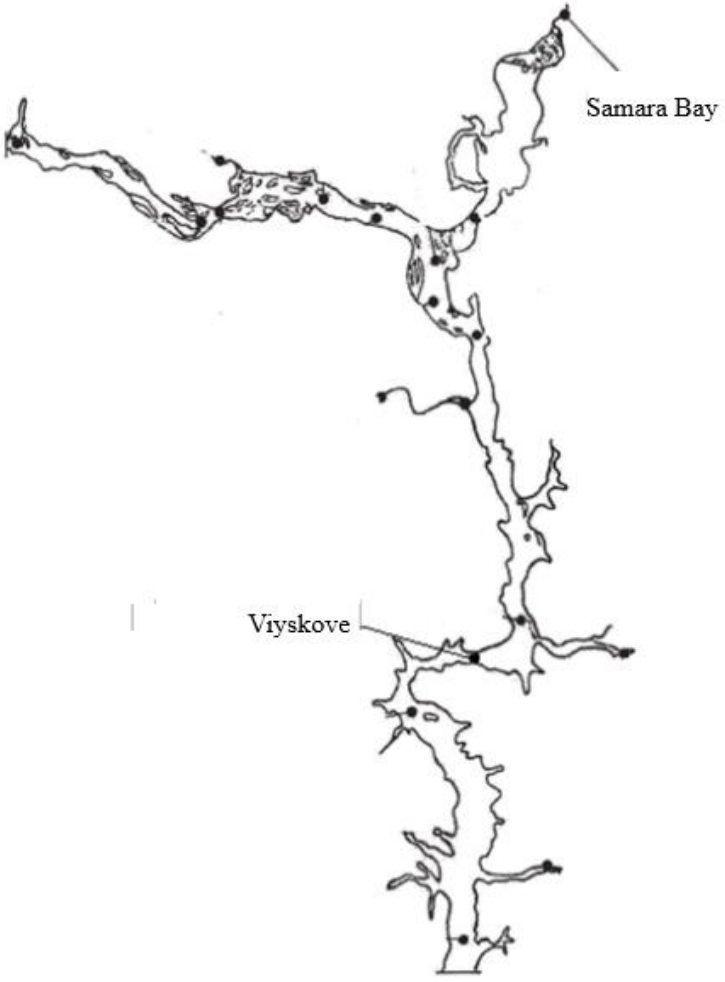


Fig.1. Scheme of the Zaporizhian reservoir.

The blood was taken from the tail vein. The morphological researches of erythrocytes were performed on blood smears that were stained by the Romanovsky-Gimza method. The blood smears were researched with increasing the 40X lens using a microscope with the Sciencelab T500 5.17 M digital camera. The preparations were looked at 100 fields of vision. In this case, the following indicators were determined: large longitudinal and small transverse diameters of mature erythrocytes and their nuclei, erythrocyte area, area of the erythrocyte nucleus.

The volume of the cell, taking into account the volume of the nucleus (Tashke, 1980), was determined by the Houchin equation (Houchin and Munn, 1958). Statistical processing of the data was carried out using generally accepted methods using the Excel 2010 program.

2. 1. The research results and their discussion

During the cytometric research of fish blood smears, it was discovered that the area of red blood cells in the Samara Bay was bigger compared to the same age species from the lower part of the reservoir by 14.6% ($p \leq 0.05$) (Table 1). The of erythrocytes volume of the Bream from the Samara Bay was 551.89 μm³ and was 69.5% larger than that of single-age individuals from the lower part of the reservoir (Viys'kove) (Fig. 2).

Table 1. Cytometric indices of carp fish erythrocytes.

Indicator	The Samara Bay	The lower part of the reservoir (Viys'kove)
Area of erythrocyte, μm^2	85.01±0.55*	74.17±0.67*
Big longitudinal diameter of erythrocyte, μm	11.81±0.07	10.89±0.07
Small transversal diameter of erythrocyte, μm	7.52±0.06	7.32±0.06
Nuclei area, μm^2	11.01±0.11	12.59±0.19
Big longitudinal diameter of nuclei, μm	4.37±0.04	4.02±0.04
Small transversal diameter of nuclei, μm	2.83±0.03	2.55±0.03

* - The difference between the indicators is statistically significant

The probable differences in the volume of the erythrocyte nuclei from the lower part of the reservoir were not detected in comparison with the Samara Bay, but there was a tendency of increase in the volume of the erythrocyte nuclei in the Samara Bay Bream by 25%.

It is known that erythrocytes take on a number of characteristic reactions in conditions of hypoxia, the most significant of which is the change in cell volume (Andreeva, 2014). The growth of red blood cells is the most common and described reaction of red blood cells in fish (Jensen, 1995). It occurs in conditions of hypoxia due to the entrance to the Na^+ cell through Na^+ - H^+ - antioport. The most powerful stimulus for the activation of transport is catecholamines that enters blood when the available oxygen is reduced and it interacts with erythrocytes β-adrenergic receptors (Borgese Garcia-Romeu and Motais, 1987). The reason for an increase in cell volume could also be seen in the activation of HCO_3^- - Cl^- - antiportation, which allows Cl^- to get into the cell under hypoxia conditions (Wood and Simmons, 1994).

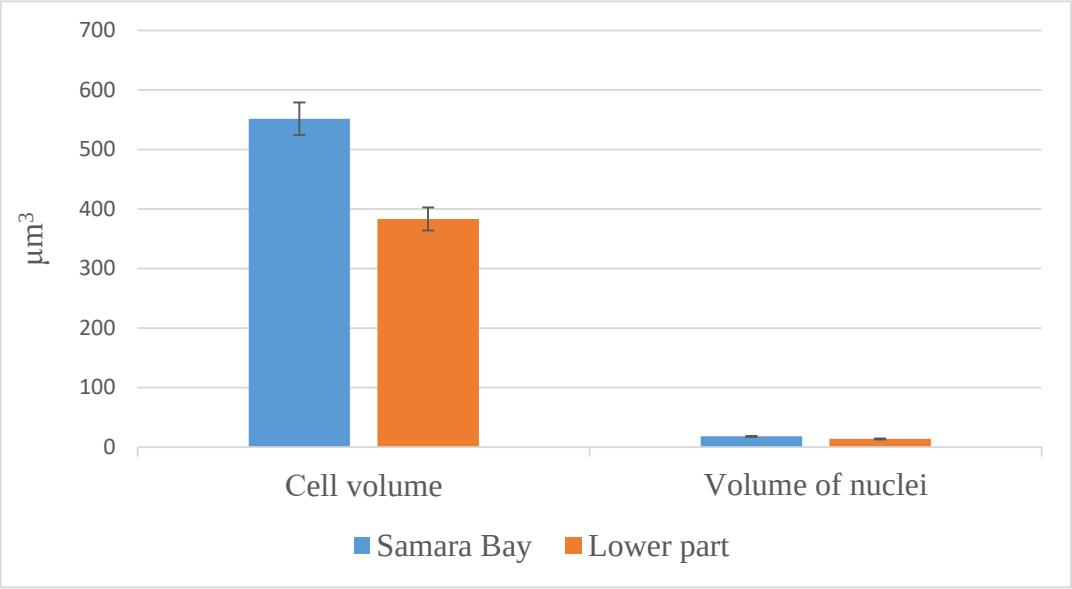


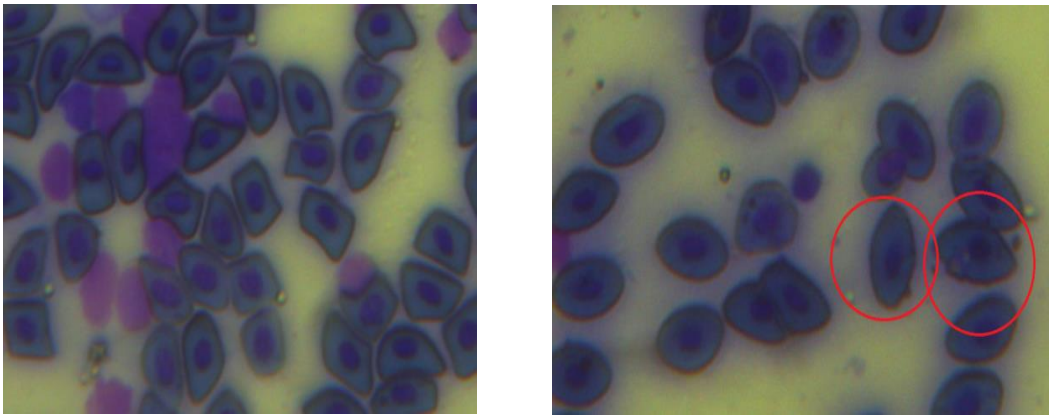
Fig. 2. Indicators of the Bream erythrocyte volume and their nuclei.

During the research of the blood smears it was seen that along with healthy erythrocytes that had an elliptical shape, the core of the red-violet color, transparent cytoplasm of pink color, there were cells with pathologies. In the researched Bream from the Samara Bay, a greater number of pathological changes were observed compared to the lower section of the reservoir (Table 2).

Table 2. Pathology of the Bream erythrocytes from Zaporizhian reservoir.

The Bream		
Kind of pathology	The Samara Bay, % of the total number of erythrocytes	The lower part (Vyis'kove), % of the total number of erythrocytes
Poikilocytosis	19.9	3.9
Scalloped membrane	2.0	1.6
Hyperchromia of the nucleus	3.0	8.0
Accentric location of the nuclei	4.9	1.9
Amitose	0.6	-
Invagination of the nucleus	0.2	-
Nuclear shadows	13.58	-

The number of erythrocytes that were altered in the Samara Bay was 44% of the total number of red blood cells, and in the fish of the lower part of the reservoir (Vyis'kove) - 15.4%. The most commonly observed is poikilocytosis, which is characterized by a change in the shape of cells (Fig. 3-A). Pear-shaped, diamond-shaped, triangular shaped cells were marked. In general, poikilocytosis is a degenerative phenomenon and shows a functional insufficiency of the hematopoietic organs, as well as in severe anemia (Konyakova and Fedorova, 2016). The violation of the osmotic resistance of red blood cells is evidenced by the scalloped membrane of the cell (Fig. 3-B).



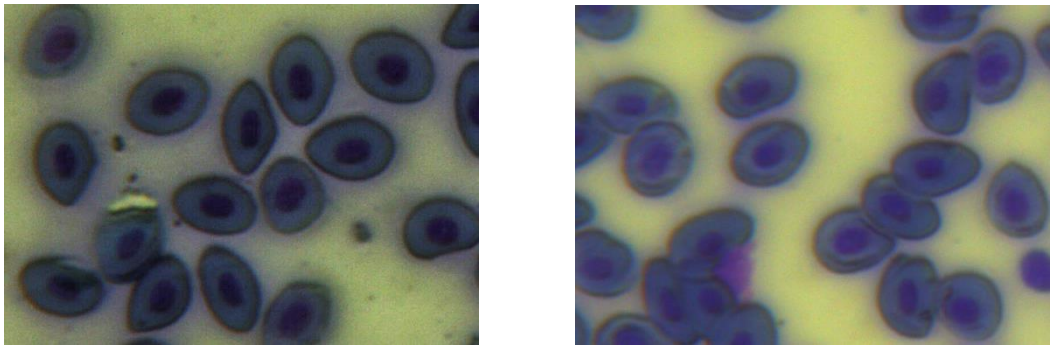
A B

Fig. 3. A-poikilocytosis; B- scalloped edge of red blood cells.

Hyperchromia of the nucleus (Fig. 4-A) most often was found in the the Bream from the lower part, while in the the Bream from the Samara Bay the index of the nucleus hyperchromia reached only 3%. Hyperchromia is an enhanced coloring of erythrocytes or their nuclei due to the increasing of hemoglobin content in them, characterized by an increase in color index and observed in some hemolytic anemias (Lyutinsky, 2005).

The shift of the nucleus to the periphery of red blood cells was revealed (Fig. 4-B). According to the researches of many scientists, the reasons for the manifestation of such changes could be of different nature, first and foremost, it is the result of toxicosis that manifests itself in fish in conditions of chronic contamination (Zhitneva, Poltavetseva and Rudnitskaya, 1989; Moiseyenko 1998; Kaniev and Vorobyev, 1960; Lukin, Sharova and Bilicheva, 2010; Izergina, Izergin and Izergin, 2014; Korolyova, 2015). In the the Bream from the Samara Bay, there were some cases of invagination of the nucleus, which are the result of violations of nuclear-plasma relations and resistances of the nuclear shell (Konyakova and Fedorova, 2016) and amitozes. Also, we revealed nuclear shadows in the Samara Bay fishes (Fig. 5), which are cells with a destroyed nucleus and an uneven edge.

Nuclear shadows appear after the complete collapse of the cytoplasm and nucleus and are pathological structures (Konyakova and Fedorova, 2016). Thus, the greatest pathologies in the blood were found in the fish from the Samara Bay, that suffered from a more severe anthropogenic loading.



A B

Fig. 4. A- hyperchromia of the erythrocyte nucleus; B - shift of the nucleus to the periphery of red blood cells.

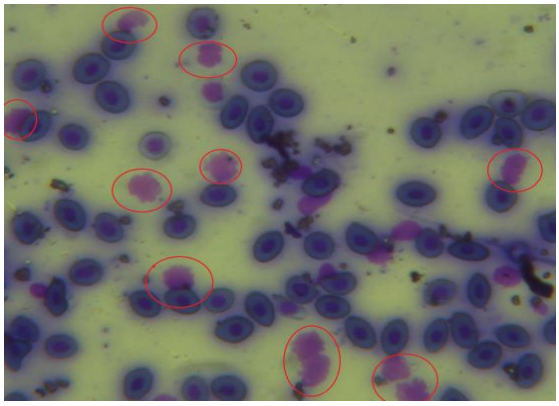


Fig. 5. Nuclear shadows

3. CONCLUSIONS

During the cytometric research of the blood smears of the fish from the Samara Bay, an increase in the area of red blood cells was observed compared to the same age individuals from the lower part of the reservoir by 14.6% and in the volume - by 69.5%. One of the reasons for this might be the hypoxia. In the erythrocytes of the researched fish, the following pathologies were observed: poikilocytosis, scalloped membrane, hyperchromia of the nucleus, accentric location of the nucleus, amitose, invagination of the nucleus, nuclear shadows, which were more developed in the Samara Bay fish. The revealed pathologies of red blood cells of the Bream might be the result of the complex influence of adverse factors, namely the unsatisfactory toxicological conditions of the bay.

Biography

This work is devoted to the study and research of cytometric and pathomorphological indices of red blood cells in a common the Bream from Zaporizhian reservoir. The research was conducted in the Zaporizhian Reservoir (Ukraine), in two sections, which differ in ecological and hydrological conditions, in the spring-summer period

2015-2016. The objects of the research were four-year-old specimens of both breeds of the Bream (Abramis brama Linnaeus, 1758). During the cytometric research, it was found that in the the Bream from the Samara Bay there was an increase in the areas and volumes of red blood cells compared to the same age individuals from the lower part of the reservoir. In the erythrocytes of the researched fish, the following pathologies were observed: poikilocytosis, dentate membranes, hyperchromia of the nucleus, accenric location of the nucleus, amitose, nucleus invagination, nuclear shadows. The most pathologies were found in the fish from the Samara Bay, which suffered from more intensive anthropogenic influences.

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