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The red blood cells cytometric characteristics of young fresh-water fish of various families

T.S. Sharamok, N.B. Yesipova, V.O. Kurchenko

The morphometric indices of red blood cells of young fish of various species inhabiting coastal zones of the water bodies were studied. The subjects of the research were the fish of four families: Carp (*Alburnus alburnus*, *Carassius gibelio*, *Rhodeus amarus*, *Abramis brama*, *Rutilus rutilus*), Needles (*Syngnathus abaster nigro lineatus*), Centrarchidae (*Lepomis gibbosus*), Loaches (*Cobitis taenia taenia*), Gobies (*Neogobius fluviatilis*). The hydroecological conditions were characterized by an intense oxygen regime, high content of phosphates, and heavy metals (zinc). The fish peripheral blood was taken from the tail vein; smears were made according to the classical Romanowsky-Giemsa method. Our research showed that the red blood cells of young fish belonging to the ecological group of inactive and unpretentious species (*Neogobius fluviatilis*, *Carassius gibelio*) had the largest cross-sectional area and a high index of nuclear-cytoplasmic ratio. The indicators of erythrocyte eccentricity were the highest in the active fish with high energy costs (*Alburnus alburnus*, *Lepomis gibbosus*). The largest number of erythrocytes with pathological events (cytolysis, karyolysis, pyknosis, poikilocytosis) was observed in the young *Alburnus alburnus* (14%), and the smallest one in *Rhodeus amarus* and *Lepomis gibbosus* (2–4%). In the individuals of *Syngnathus abaster nigro lineatus* affected by parasitic nematodes of the genus *Ascaris*, the number of erythrocytes with pathologies increased to 81%. Under the toxic load, destabilization of the fish circulatory system begins with the appearance of the young forms of erythrocytes as compensation for depleted mature erythrocytes and ends with the mass destruction of mature erythrocytes. Given these patterns, as well as the relatively low number of young ballast forms of erythrocytes and mature erythrocytes with pathological features, we can assume that the state of the red blood cells in the studied young fishes meets the conditional norm, with the exception of the blood of *Syngnathus abaster nigro lineatus* infected with parasites. In our opinion, the main characteristics of the fish red blood that reflect the fishes' adaptive capacities are as follows: eccentricity ratio of erythrocytes, the nuclear-cytoplasmic ratio, ratio of the young ballast forms of erythrocytes, and the relative number of erythrocytes with pathology.

Key words: *erythrocytes, cytometric indices, pathological changes, Zaporizhzhia Reservoir.*

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About the authors:
T.S. Sharamok – Oles Honchar Dnipro National University, Gagarin Ave., 72, Dnipro, 49050, Ukraine, sharamok@i.ua, https://orcid.org/0000-0003-3523-5283
N.B. Yesipova – Oles Honchar Dnipro National University, Gagarin Ave., 72, Dnipro, 49050, Ukraine, yesipova.natalia@gmail.com, https://orcid.org/0000-0003-1924-2547
V.O. Kurchenko – Oles Honchar Dnipro National University, Gagarin Ave., 72, Dnipro, 49050, Ukraine, kurchenko.viktoriia.3@gmail.com, https://orcid.org/0000-0002-1199-3760

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Introduction

In modern aquatic ecosystems, significant changes occur under the impact of natural (climate change) and human (economic activity) factors. As a result, the structure of populations and the physiological status of fish are changing (Gamito et al., 2015; Zeng et al., 2017; Năstase, Oţel, 2017; Fedonenko et al., 2018). The younger age groups of fish are hypersensitive to environmental changes; firstly, they have not formed the mechanism of physiological adaptation yet, and secondly, most of the young fish live in the reservoirs' coastal zones, where the influence of external factors is the strongest. Therefore, the studies on young fish's adaptogenic potential are very important for forecasting the further development of industrial fish populations and assessing their strategic stocks, as well as for the environmental introductions and the artificial formation of ichthyofauna in inland water bodies.

The self-regulating blood system plays a major role in adapting to changing conditions. Blood, as an internal part of an organism, responds quickly to environmental changes and always reflects accurately the physiological state of an organism. Therefore, in the ecological monitoring system, hematological indicators are often used as a benchmark for the adaptive capacity of fish to anthropogenic impact (Ahmed et al., 2020; Fazio, 2019; Fazio et al., 2020; Yilmaz, 2015; Belokon et al., 2013; Fedonenko et al., 2019).

When analyzing formed blood elements, special attention is paid to the cells of the erythroid series. Many authors indicate the expediency of using erythrocytes as indicators of hypoxic conditions. Thus, during the research of *Neogobius melanostomus* (Pallas, 1814) adult specimens' blood that was kept for 10 days under experimental hypoxia conditions (1.7–1.8 mg O_2 / l), a probable increase in the volume of the mature erythrocyte was detected as a result of their swelling, the ellipsoidal form of the erythrocytes changed to a circular, and there was a nearly two-fold increase in the number of the immature erythroid cells (Parfenova, Soldatov, 2011). After six-hour experimental hypoxia (2 mg O_2 / l), the number of red blood cells with chromatolysis, karyolysis, and poikilocytosis in the three-year-old pikes and breams increased significantly. In the silver carp, 1.8 times increased the number of erythrocytes with amitosis (Moroz, Yesipova, 2011).

In recent years, more and more researchers have been using the parameters of erythrocytes to assess the status of fish populations living in areas with anthropogenic pollution. Thus, 22 erythrocyte pathologies were detected in the adult roach, bream, perch, and gobies from the Saratov Reservoir (Russia) under conditions of the high content of phenols, petroleum products, copper, and sulphates in the water. The most widespread was the deformation of the cell and nucleus, carriolysis, chromatolysis, agentric location of the nucleus, and amitozes (Mineev, 2013). In the roach from the Zaporizhzhia Reservoir (Ukraine), inhabiting areas with heavy metal and organic contamination, there were detected a decrease in the relative proportion of mature erythrocytes and an increase in young blast forms of erythrocytes, changes in the shape of erythrocyte nuclei, anisocytosis, hypochromia, cytolysis, cell membrane destruction, karyolysis and karyopyknosis (Sharamok et al., 2016).

Despite the popularity of ichthyological research on formed blood elements, information on the red blood cell cytometric indices is limited. In particular, in the early stages of ontogenesis. A small number of researches in this direction suggest that the morphological features of erythrocytes and their functional properties in young fish have certain differences from that in adult fish. Thus, comparing the morphology of fingerlings and adult erythrocytes of roach and crucian carp, it was found that with age in fish, the ratio of the nucleus area to the erythrocytes' area is likely to decrease, and the number of mature forms of erythrocytes increases by almost 20% (Fedonenko et al., 2016). It is known that mature erythrocytes of fish, compared to young cells, have a higher concentration of hemoglobin protein, the main carrier of the blood respiratory function (Soldatov, 2005). Therefore, the determination of the ratio of various forms of erythrocytes could indirectly characterize the hematopoietic function of the blood activity. It was also established that in conditions of environmental pollution, the number of erythrocytes with various pathologies (vacuolation of the cytoplasm, nucleus hemolysis, carioerexis, amitosis) in the young bream *Abramis brama* (L., 1758) reaches 44.4% of the total number of red blood cells in an organism (Konkova, Fedorova, 2016). Thus, the existing data prove the expediency of using erythropoiesis indicators in young fish to assess the impact of the aquatic environment on ichthyofauna and to understand the mechanism of fish adaptation to changing hydroecological conditions.

Our research aimed to study the cytomorphological indices of young fish red blood cells from the Zaporizhzhian reservoir coastal populations.

Materials and methods

The selection of fish for hematological research was carried out in the summer of 2016–2017 in the coastal zone of the central part of the Zaporizhzhia (Dnipro) Reservoir (Figure 1). The water reservoir was created on the Dnipro River in 1932; it has an area of 420 km^2. It is located in the southeast of Ukraine, in the territory of agrarian-industrial zones under heavy anthropogenic influence.

The young fish were taken in a small 10 m long frying net. The young fish were divided by species and counted. Nine fish species of different families were researched. The carp family was represented by four species: *Alburnus alburnus* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Rhodeus amarus* (Bloch, 1782), *Abramis brama* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758); Family of Needles – a *Syngnathus abaster nigro lineatus* (Eichwald, 1831); Family Centrarchidae (Sunfish) – *Lepomis gibbosus* (Linnaeus, 1758); Family Cobitidae (Loaches) – *Cobitis taenia taenia* (Linnaeus, 1758); Family Gobiidae (Gobies) – *Neogobius fluviatilis* (Pallas,1814). All experimental fish, except the *Syngnathus abaster nigro lineatus*, were the fingerlilngs (0+); *Syngnathus abaster nigro lineatus* were represented by two-year-olds (1+).

Blood from the fish was taken from the tail vein. The smears were made using the generally accepted method and stained by the Romanowsky-Giemsa method. The smears were studied with a 40^X objective using a microscope with the Sciencelab T500 5.17 M digital camera. Atlases of blood cells were used to identify cells of the erythroid population (Ivanova, 1983; Sharon, Zilberg, 2012). The preparations were looked at 100 fields of vision. The following parameters were determined: large longitudinal (D) and small transverse (d) diameters of mature erythrocytes, the ratio of small and large erythrocytes diameters (d / D), erythrocyte separation area (S), area of erythrocyte nucleus (s), nuclear-cytoplasmic ratio (s / S), the ratio of mature and young forms of erythrocytes. The eccentricity coefficient (E), which characterizes the degree of the cell deviation from the round shape, was determined as the square root of the square difference (1 – d / D^2). Measurements of cytometric erythrocytes indices were performed using the Science Lab View 7 program.

Fig. 1. Study area on the scheme of the Zaporizhzhia Reservoir

The data were analyzed as Mean ± S.E.M. at reliability of 95% and significant level of $P < 0.05$. To test the significance of the test, the *t*-test method was used.

Ethical Statements

Bioethical standards were not violated during the research. It was performed under the Regulations on the Ethics Committee (Bioethics) in Ukraine (Regulations on the Ethics Committee (Bioethics), 2019).

Results

The central part of the Zaporizhzhia reservoir, where the selection of fish was carried out, is characterized by weak flow and a large shallow water area. The coastal populations of young fish are concentrated. The hydroecological regime of the area is affected by the household and domestic water of Dnipro city and agrarian enterprises.

According to long-term monitoring, the average annual mineralization of water in the site is 334 mg / l, hardness – 3.2 mg equ / l, pH 7.5–8.6. The content of ammoniacal nitrogen in water during the experimental period varied from 0.22 to 0.97 mg / dm^3 at different points (average 0.54 mg / dm^3 in the reservoir); nitrites – from 0.01 to 0.14 mg / dm^3 (0.019 mg / dm^3), nitrates – 0.11–2.8 mg / dm^3 (0.47 mg / dm^3), phosphates – from 0.25 to 0.62 mg / dm^3 (0.39 mg / dm^3). The content of dissolved oxygen in summer varies between 2,2–6,8 mg O_2 / dm^3 (Fedonenko et al., 2018).

The most of the Zaporizhzhia reservoir toxicants are heavy metals. The content of toxic metals in the water of the reservoir's central part corresponded to the existing Ukrainian norms for the water of fish-water reservoirs. There was a high concentration of zinc, which was 2.7 MPCs (0.027 ± 0.007 mg / l) and copper – 12 MPCs (0.012 ± 0.006 mg / l). Persistent contamination with copper may be a result of sewage emissions from enterprises of the chemical, metallurgical industry, mine waters, and aldehyde reagents

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used for the destruction of algae. Zinc enters the natural waters as a result of natural processes of destruction and dissolution of rocks and minerals. A considerable amount of zinc gets into water bodies with anthropogenic pollution, namely the wastewater from ore dressing factories and galvanic shops, parchment paper production, and mineral plants. The most important external source of zinc in the Zaporizhzhia reservoir is a runoff that changes zinc distribution in the littoral.

The obtained data made it possible to calculate the chemical index of water quality, where the index of quality according to the trophic and saprobiological indicators was 4.4 and for indicators of specific toxic effect was 3.8. Thus, the weighted chemical index was 4.1, which characterizes the water quality of the Zaporizhzhia reservoir as "satisfactory" and "poorly polluted".

In the research of species composition of fish coastal populations, the domination of the short-cycle species as the *R. amarus* was identified. Its number was 40–45% of the total from the fish in catches. In the second place was the *R. rutilus* – 24–28%, followed by *A. alburnus* and *N. fluviatilis* – 6–10%. The number of other fish species varied from 1.5% to 4%. The prevalence of a small population of low-value species with a short growing cycle has been observed in the reservoir during the last 10 years and it indicates the degradation changes in the ichthyotsenoses of the Zaporizhzhia reservoir. The rapid increase in the number of invasive species of the self-settler Sunfish was another negative feature of the coastal population structure (Fedonenko et al., 2018).

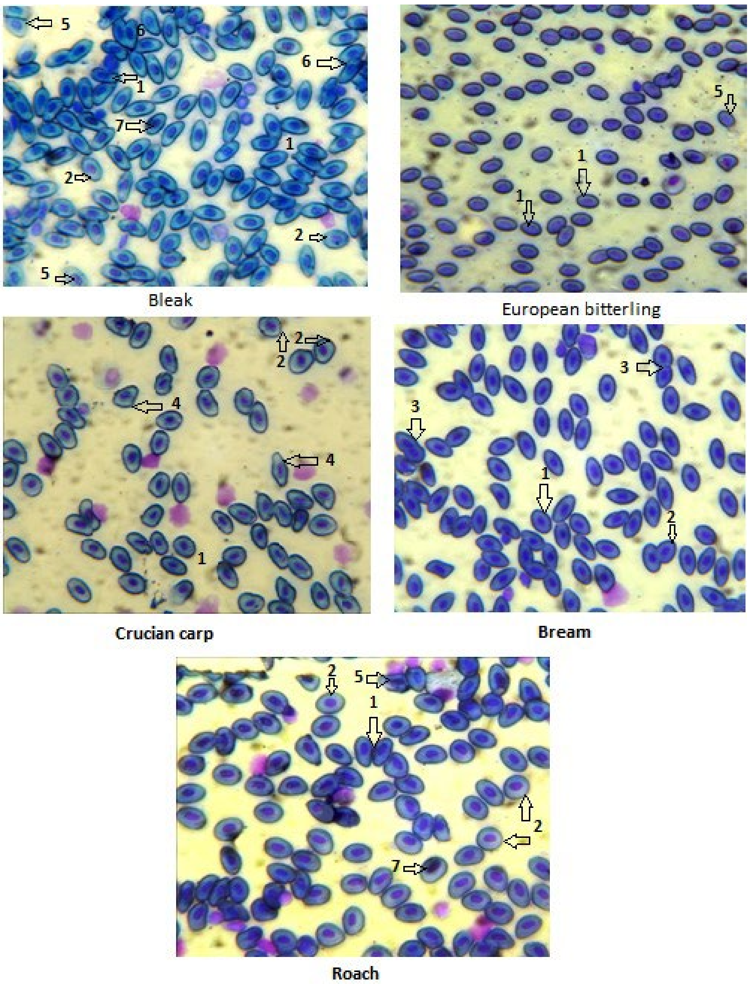

Fig. 2. The picture of the Carp family young fish peripheral blood:
1 – mature erythrocytes; 2 – young red blood cells; 3 – amitosis; 4 – poikilocytosis; 5 – karyolysis; 6 – adhesion of red blood cells; 7 – pyknosis (40^x)

The picture of the carp fish fingerlings' peripheral blood is presented in Figure 2. In all the fish, mature erythrocytes had a clear ellipsoidal form. The nuclei are well-expressed, dark purple, and have a central location. The cytoplasm is transparent and homogeneous.

The erythrocytes with pathological events were found in all the carp fish. The disturbances were in the form of cytolysis, karyolysis, pyknosis, poikilocytosis, and amitosis. The most common cells with variable forms (poikilocytosis) are pear-shaped and crescent-shaped. The relative number of such cells ranged from 26 to 68% of the total number of cells with pathologies. Cytolysis was detected in 15–32% of erythrocytes in the form of partial lysis of cells. Nuclear pathologies were found in the form of karyopyknosis (shrinkage of the nucleus) and karyolysis (partial or complete dissolution of the nucleus) and occurred in 28–54% of variable erythrocytes. Erythrocytes in the state of amitotic division were much less common – in 8–14% of the total number of cells with pathologies. The largest indicator of erythrocyte pathologies is noted in *A. alburnus* – 14% of the red blood cells total number, then in *A. brama* – 12%, the *R. rutilus* – 9%, *C. gibelio* – 7%, and the smallest number of red blood cells with pathologies was in the *R. amarus* – 2%.

The relative number of mature erythrocytes in all the carp fishes significantly exceeded the number of young red blood cells and ranged from 93% to 98%. The young forms of erythrocytes were represented mainly by blast forms – early erythrocytes (EE) and i polychromatophilic erythroblasts (PE). They had a well-defined perinuclear zone, a basophilic cytoplasm, and a large nucleus with prominent chromatin granules. PE differed in the absence of the perinuclear zone and in the presence of oxyphilic properties in the cytoplasm.

In our research of the ratio PE : EE of the carp fish was as follows: *Abramis brama* and *A. alburnus* – 0.8–1 : 1, *R. rutilus* – 2.5 : 1, *C. gibelio* – 3 : 1, *R. amarus* – 4 : 1. Thus, among the carp fish, the highest percentage of functionally active erythrocytes forms in blood was in *R. amarus* and *C. gibelio* fingerlings.

The morphological signs of erythrocytes in the young fish of other families had their peculiarities. The red blood cells of *L. gibbosus* and *C. taenia taenia* had a classic ellipsoid form, whereas, in *N. fluviatilis* and *S. abaster nigro lineatus*, mature erythrocytes had a more rounded shape. The nuclei and shells of erythroid cells were clearly expressed in the majority, the cytoplasm was homogeneously colored (Figure 3).

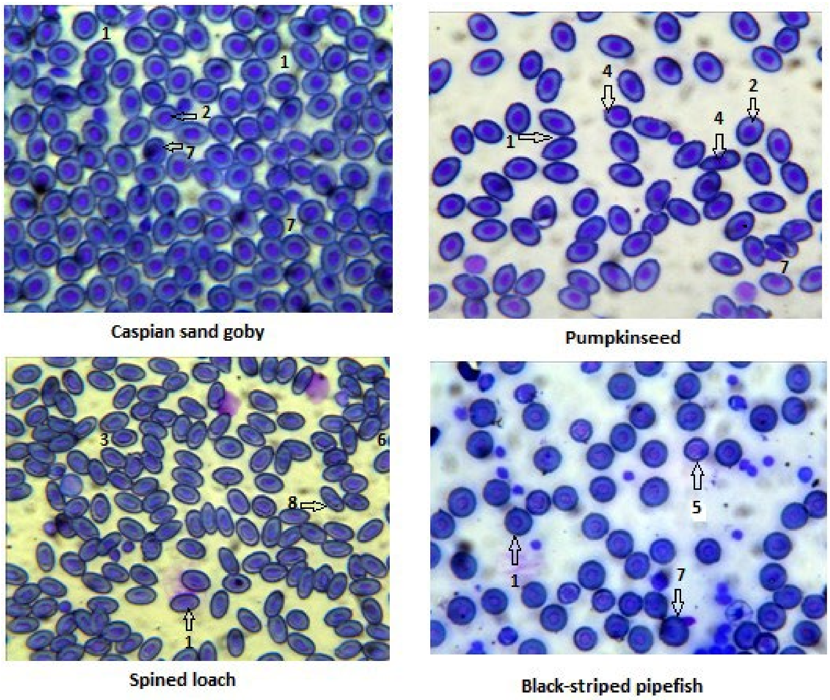

Fig. 3. Picture of the peripheral blood of different families of young fish:
1 – mature erythrocytes; 2 – young red blood cells; 3 – amitosis; 4 – poikilocytosis; 5 – karyolysis; 6 – adhesion of red blood cells; 7 – pyknosis; 8 – micronuclei (40^x)

The relative number of mature erythrocytes in *L. gibbosus* and *C. taenia taenia* was 98 and 97% respectively, *N. fluviatilis* – 72%, *S. abaster nigro lineatus* – 68%. The ratio of young forms of erythrocytes (PE : EE) in the first two fish species was 1 : 1, in *N. fluviatilis*, polychromatophilic erythroblasts (1 : 2) predominated, but in *S. abaster nigro lineatus*, on the contrary, it was early erythrocytes (1 : 1,5).

The smallest number of erythrocytes with pathological features was observed in the *L. gibbosus* – from 2 to 4%, mostly these were cells with poikilocytosis, and nuclei were more common in a state of piknosis (Fig. 3). In *N. fluviatilis*, the relative number of erythrocytes with pathology ranged from 4 to 7%, with more frequent lysis and nucleus picnosis. In *C. taenia taenia*, the number of erythrocytes with pathology was 10% on average. The pathological phenomena appeared in infractions associated with cell division (amitosis), the appearance of several nuclei (micronuclei) in cells, and the adhesion of red blood cells. The largest number of erythrocytes with pathological changes was detected in blood smears of the *S. abaster nigro lineatus* – from 16 to 27%. There were the erythrocytes with picnosis and asymmetry of nuclei.

In the course of small-scale fishing, we found specimens of the *S. abaster nigro lineatus*, damaged by *Ascaris (Contracoecum)* larvae. The one parasite localized in the abdominal cavity of the fish. The infection incidence of *S. abaster nigro lineatus* population was 20%. It was interesting to see how the picture of the fish's blood changes in the presence of parasitic invasion. In the Figure 4, we could see that pathological changes affect almost all erythroid cells. The total number of erythrocytes with pathology reached 77–81%. Most often, there were erythrocytes with a displacement of the nucleus, karyolysis, and karyopiknosis. In many cells, the membrane had a festooned edge, or it was completely disturbed. There were poikilocytosis and cytolysis.

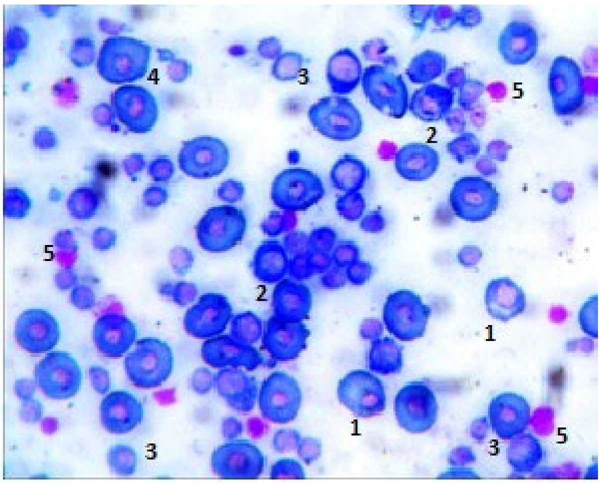

Fig. 4. The picture of the female Black-striped pipefish peripheral blood, damaged by parasitic larvae of the genus *Ascaris (Contracoecum)*:
1 – displacement and lysis of the nucleus, vacuolation of the cytoplasm, infraction of the cell membrane; 2 – karyorrhexis; 3 – cytolysis; 4 – piknosis, poikilocytosis; lymphocytes (40^x)

The large number of lymphocytes that are concentrated near the affected red blood cells, which indicates the active course of phagocytic processes is drawing attention.

The research on erythrocytes cytometric indices in different species of the fish allowed to reveal the following features. In the carp fish, longitudinal diameters of erythrocytes (D) ranged from 10,27 ± 0,08 μm, in the *R. amarus* to 13,4 ± 0,1 μm, in *A. brama* (Table 1). The difference between the indicators reached 23% and was significant ($p \leq 0.05$).

The largest cross-sectional diameters were in red blood cells of *C. gibelio*, *A. brama,* and *R. rutilus* (8.1–8.7 μm). In *R. amarus* and *A. alburnus*, they were on average less for 20% (6.2–6.6 μm).

In other fish families, longitudinal diameter indices ranged from 11.7 ± 0.06 μm in the *S. abaster nigro lineatus*, to 13.5 ± 0.08 μm in *C. taenia taenia*. The difference between the indicators did not exceed 6–10% ($p \geq 0.05$). The values of the cells' transversal diameter, on the contrary, had significant differences. The largest cross-sectional diameter was in *S. abaster nigro lineatus* erythrocytes (9.7 ± 0.04 μm), and the

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smallest – *L. gibbosus* erythrocytes (6.7 ± 0.02 µm). The difference was probable ($p \leq 0.05$) and reached 30%.

The largest area of erythrocytes (S) among the carp fish was in *C. gibelio* and *A. brama* and was 88.9 ± 0.47 and 89.04 ± 0.58 mkm^2, respectively. The area of erythrocytes of *R. rutilus* was less by 16%, *A. alburnus* by 22%, and *R. amarus* by 45%. A similar dependence was observed in the indices of the area of erythrocyte nucleus division (s). In *C. gibelio,* it was 17.4 ± 0.16 µm^2, in *A. brama* was less by 9%, *R. rutilus* by 26%, *A. alburnus* by 37%, and *R. amarus* by 43%.

In the case of other families of fish, the highest rates of S and s had the erythrocytes of the young *N. fluviatilis* – respectively 98.9 ± 0.9 and 21.7 ± 0.3 µm^2. Compared to carp fish (*A. brama*), they were higher to S by 10%, and to s – by 27%. In *S. abaster nigro lineatus*, the area of erythrocytes and their nuclei occupied an intermediate position among the indicators of other species of fish. The *C. taenia taenia* had rather large area of the cores of erythrocytes – 17.7 ± 0.33 µm^2, which exceeded the value of this index in carp fish by 10–44%. In *L. gibbosus*, S and s did not differ much from *A. alburnus*.

Table 1. Mature erythrocytes cytometric indices of fish fingerlings of different families

Species of fish	Diameter of erythrocyte, µm		Eccentricity (E)	Area of erythrocyte division, (S, µm^2)	Area of erythrocyte nucleus division (s, µm^2)	s / S
	longitudinal (D)	transversal (d)				
Alburnus alburnus	13.03 ± 0.09	6.6 ± 0.07	0.86	69.6 ± 0.62	10.9 ± 0.13	0.16
Rhodeus amarus	10.27 ± 0.08	6.2 ± 0.05	0.80	49.3 ± 0.29	9.9 ± 0.12	0.20
Carassius gibelio	12.8 ± 0.08	8.7 ± 0.07	0.73	88.9 ± 0.47	17.4 ± 0.16	0.20
Abramis brama	13.4 ± 0.08	8.3 ± 0.06	0.79	89.04 ± 0.58	15.9 ± 0.15	0.18
Rutilus rutilus	11.9 ± 0.06	8.1 ± 0.07	0.73	75.4 ± 0.57	12.8 ± 0.15	0.17
Neogobius fluviatilis	12.7 ± 0.07	9.6 ± 0.08	0.65	98.9 ± 0.9	21.7 ± 0.3	0.22
Lepomis gibbosus	12.4 ± 0.06	6.7 ± 0.02	0.84	66.8 ± 0.5	10.8 ± 0.12	0.16
Cobitis taenia taenia	13.5 ± 0.08	7.7 ± 0.06	0.82	80.4 ± 0.65	17.7 ± 0.33	0.22
Syngnathus abaster nigro lineatus	11.7 ± 0.06	9.1 ± 0.04	0.62	89.9 ± 0.38	14.7 ± 0.18	0.16

To evaluate the functional state of erythrocytes, the nuclear-cytoplasmic ratio (s / S) is often used. According to our calculations among carp fish, the highest s / S was in *R. amarus* and *C. gibelio* – 0.20; the lowest – was in *A. alburnus* – 0.16. Among the fish of other families, the maximum values were in *N. fluviatilis* and *C. taenia taenia* – 0.22; the least – in *L. gibbosus* – 0.16. As the s / S value decreases, the researched fish were constructed in the following series: *N. fluviatilis* = *C. taenia taenia* > *R. amarus* = *C. gibelio* > *A. brama* > *C. gibelio* > *A. alburnus* = *L. gibbosus* = *S. abaster nigro lineatus*.

Discussion

In the conditions of the Zaporizhzhia reservoir, several features concerning the morphology and ratio of red blood cells, nuclear-cytoplasmic ratio, and pathological abnormalities in erythrocytes were detected in the young fish blood of different families.

According to our research, the mature erythrocytes of the young fish of all the researched fish species had an ellipsoidal shape, but the coefficient of eccentricity (ellipsoidity) of the cells differed significantly.

The highest elevation of erythrocytes (E) among the carp fish was in *A. alburnus* – 0.86. In other carp fish, it was less than 7–15%. Compared to other families, the highest values of E were at *L. gibbosus* –

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0.84, and the smallest – in *S. abaster nigro lineatus*. – 0.62. In species of the Black-striped pipefish was noted a decline in E index to 0.56 in infection by parasitic nematodes. The dynamic reduction of E by the species of fish is as follows:

A. alburnus > *L. gibbosus* > *C. taenia taenia* > *R. amarus* > *A. brama* > *C. gibelio* = *R. rutilus* > *N. fluviatilis* > *S. abaster nigro lineatus*.

The coefficient of eccentricity (ellipsoidity) of erythrocytes characterizes not only morphological but also functional features of cells. It is known that in warm-blooded – for example, humans and higher animals (mammals) common erythrocytes have a round shape. The appearance of ellipsoid cells with an eccentricity index E > 0.62 is regarded as a pathological symptom and is observed in hemolytic anemias. On the contrary, in cold-blooded animals – fish, and frogs, the ellipsoid form of erythrocytes is normal, and an increase in the number of red blood cells with a round form is regarded as a deviation from the norm. Thus, in the round goby in the conditions of experimental hypoxia, red blood cells became larger, acquired a rounded form, and increased the volume of their nuclei (Parfenova, Soldatov, 2011). In the research of erythropoiesis in the frog *Rana ridibunda* L., three groups of red blood cells were noted according to morphological features: normal ellipsoid cells (eliptocytes) with E = 0.74 ± 0.004; strong ellipsoid cells (magnulocytes) – E = 0.81 ± 0.004; rounded cells (teretiocytes) – E = 0.63 ± 0.012 (Zelentsova, Skorkina, 2004).

If we classify erythrocytes of fish by the E index in analogy with red blood cells, then in the group of fish with normal ellipsoid erythrocytes (E = 0.73 – 0.80) there are *A. brama*, *C. gibelio*, *R. rutilus*, *R. amarus*; with strongly ellipsoid eritrocytes (E = 0.81–0.86) – *A. alburnus*, *L. gibbosus*, *C. taenia taenia*; with rounded erythrocytes (E = 0.62 – 0.72) – *N. fluviatilis*, *S. abaster nigro lineatus*.

Thus, the eccentricity coefficients of erythrocytes of the different species of young fish within the same family had high variability and depended not so much on the systematic affiliation of the fish but the biological and physiological features of the species. So, the active, mobile fish (*A. alburnus*, *L. gibbosus*) with high energy costs had erythrocytes with a high ellipsoid index, and the sedentary fish (*N. fluviatilis*, *S. abaster nigro lineatus*) had erythrocytes with a more rounded excentric form. Besides, the infection of the *S. abaster nigro lineatus* by parasitic nematodes (*Contracoecum*) resulted in a 10% decrease in this index and an increase in the number of red blood cells with a rounded form. A similar phenomenon was observed in *N. melanostomus* under conditions of experimental hypoxia (Parfenova, Soldatov, 2011), as well as in *Siganus rivulatus* under conditions of lead and cadmium intoxication (Ezzat et al., 2013).

The research on the ratio of erythrocytes in different forms showed that in all fish species the majority of red blood cells were mature erythrocytes – 93–98%. This fact is emphasized by many authors on different fish species (Parfenova, Soldatov, 2011; Mineev, 2013; Kurchenko, Sharamok, 2020) and because mature erythrocytes have more hemoglobin and are able to more actively bind oxygen compared to young erythrocyte species (Speckner et al., 1989).

Concerning the ratio of young erythroid cells, different species of fish were distinguished by their peculiarities. The following regularities could be observed: in active, mobile fish species, blood was more actively rejuvenated by young ballast forms of erythrocytes – early erythrocytes (EE) and therefore in relation to more mature ballast forms – polychromatophilic erythroblasts (0.8 – 1 : 1). We noticed this pattern in the *A. brama*, *A. alburnus*, *L. gibbosus*, *C. taenia taenia*, *R. rutilus*. On the contrary, in the blood of the sedentary species – *N. fluviatilis*, *C. gibelio*, and *S. abaster nigro lineatus* early erythrocytes were much smaller, and the ratio of PE: EE was as follows – 2.5–4 : 1.

We also noted the fact that in *S. abaster nigro lineatus*, when infected with parasitic nematodes, the number of early erythrocytes increased several times, exceeding the number of polychromatophilic erythroblasts (PE: EE = 1: 1.5). Apparently, the appearance of young ballast forms of erythrocytes was a protective reaction of the body to parasite intoxication.

According to the cytometric data, the largest area of erythrocyte segregation was in the Caspian sand goby, *S. abaster nigro lineatus*, and *A. brama,* and the *C. gibelio*, and the smallest in the *R. amarus*, the *L. gibbosus,* and the *A. alburnus*. It is thought that in smaller cells, a shorter diffusion path and faster oxygen transfer occur. Our data, in general, coincide with this conclusion – namely, in the bleak, the perch, and the bitter, which were fast-moving and therefore actively needed oxygen for respiratory processes, the erythrocytes' area was the smallest among other types of fish. While in the slow-moving fish (*N. fluviatilis*, *C. gibelio*), which are unpretentious to oxygen conditions, the area of erythrocytes was 30–50% higher.

The indicators of the nuclear-cytoplasmic ratio differed in fish with varying agility to environmental conditions. The highest were in the *N. fluviatilis*, the *C. taenia taenia*, and the *C. gibelio* (0.20–0.22) – that

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is fish that was resistant to oxygen conditions and pollution. In more sensitive fish (*A. alburnus*) they were 20–25% lower. High indices of the nuclear-cytoplasmic ratio can indicate the ability of erythrocytes to rapidly accumulate nuclear mass and switch to an amyotrophic division, which may be one of the adaptive reactions of fish to adverse conditions.

The erythrocytes with pathological abnormalities were found in all the fish species studied, but their relative numbers differed. Among the carp fish family, the largest number of erythrocytes with pathology (cytolysis, karyolysis, piknosis, poikilocytosis, amitosis) was in the *S. abaster nigro lineatus* (14%), the lowest was in the *R. amarus* (2%); then in descending order followed by the *A. brama*, *Rutilus rutilus*, *Carassius gibelio*. In the fish of other families, the proportion of damaged erythrocytes ranged from 2% (*L. gibbosus*, *N. fluviatilis*) to 10% (*C. taenia taenia*). In the *S. abaster nigro lineatus* only, the number of erythrocytes with pathological features reached 27%. In the *S. abaster nigro lineatus* individuals infected with parasitic nematodes, the number of erythrocytes with pathological features (nucleus displacement, karyolysis, karyopiknosis, poikilocytosis, and cytolysis) increased to 81%.

Unfortunately, we did not have the opportunity to compare the data obtained with the literature data for all the fish species researched. In the literature available to us, we did not find information on the status of red blood cells in the *R. amarus*, *A. alburnus*, *L. gibbosus*, *C. taenia taenia*, and *S. abaster nigro lineatus*. That is, we can assume that these researches were conducted for the first time and need to be continued. The most researched erythrocyte hematopathies in the *A. brama*, *R. rutilus*, and *C. gibelio*, which are common and common species in reservoirs. Thus, according to Russian scientists in the contaminated areas of the Kuibyshev reservoir in the *A. brama* and the *R. rutilus* the number of red blood cells with pathological signs increased from 26.4% to 40.6%. In this case, the most common cell pathologies were: vacuolation of the cytoplasm, acentric location of the nucleus, cytolysis, karyolysis, and karyopiknosis (Mineev, 2016). In the young *A. brama*, contaminated sites of the Volga delta with kernel displacement, chromatinolysis, anisakidosis, and other pathologies reached 32–42% (Konkova, Fedorova, 2016). We saw a similar pattern in the *R. rutilus* from the Zaporizhzhian reservoir. The number of damaged erythrocytes in the fish from the contaminated area of the reservoir increased from 8% to 33% compared to the conditionally clear area (Sharamok et al., 2016).

It is known that in conditions of toxic loads on fish, destabilization of the circulatory system begins with the appearance in the blood of young forms of erythrocytes as compensation for depleted mature erythrocytes, and ends with the mass destruction of mature erythrocytes.

Given these patterns, as well as the relatively low number of young ballast forms of erythrocytes and mature erythrocytes with pathological features, we can assume that the state of red blood cells in the young fish species researched by us meets the conditional norm, except for the picture of the parasite-infected *S. abaster nigro lineatus*.

Conclusion

The hematological research of the fish was carried out at the central section of the Zaporizhzhia (Dnipro) reservoir, which is characterized as “satisfactory” and “poorly contaminated” by toxicity indicators. The morphometric indices of erythroid cells were used to assess the adaptive capacity of the young fish populations. According to the results of research, the young fish which belong to the ecological group of sedentary and oxygen-less fish species (*Neogobius fluviatilis*, *Carassius gibelio*) had the largest erythrocytes area and a high nuclear-cytoplasmic ratio. Indicators of eccentricity (ellipticity) of erythrocytes had the highest values in the mobile fish (*Alburnus alburnus*, *Lepomis gibbosus*) with high energy costs. The mobile fish also had a high content of young ballast forms of early erythrocytes, indicating active rejuvenation of red blood cells. The relative number of erythrocytes with pathological features (nucleus displacement, karyolysis, karyopiknosis, poikilocytosis, and cytolysis) was not high in all fish species (2–10%). Only in the *Syngnathus abaster nigro lineatus*, the percentage of erythrocytes with pathology ranged from 16–27%, and in individuals affected by *Ascaris* parasitic nematodes, it reached 81%.

Thus, the main indicators of the red and blood of fish, which, in our opinion, reflect the adaptive capacity of fish, are the eccentricity ratio of erythrocytes, the ratio of young ballast forms of erythrocytes, nuclear-cytoplasmic ratio, and the relative number of erythrocytes with pathology.

In the less mobile fish, the physiological adaptation of red blood cells to the environmental conditions was more pronounced than in the actively moving fish.

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Цитометрична характеристика еритроцитів молоді прісноводних риб різних родин

Т.С. Шарамок, Н.Б. Єсіпова, В.О. Курченко

Досліджено морфометричні показники еритроцитів молоді різних видів риб, що мешкають у прибережних зонах водойм. Об'єктом дослідження були риби чотирьох родин: коропові (верховодка, карась сріблястий, гірчак звичайний, лящ, плітка), іглицеві (голка пухлощока чорноморська), центрархові (сонячний окунь), в'юнові (щиповка звичайна), бички (бичок пісочник). Гідроекологічні умови характеризувались напруженим кисневим режимом, високим вмістом фосфатів і важких металів (цинку). Периферичну кров риби відбирали з хвостової вени; мазки виготовляли за класичною методикою та фарбували за методом Романовського-Гімзи. За результатами досліджень було виявлено, що у молоді риб, які належать до екологічної групи малоактивних і невибагливих видів (бичок пісочник, карась сріблястий), еритроцити мають найбільшу площу поперечного перерізу та високий показник ядерно-цитоплазматичного співвідношення. Показники ексцентричності еритроцитів були найвищими у активних риб з високими енергетичними витратами (верховодка, сонячний окунь). Найбільшу кількість еритроцитів з патологічними явищами (цитоліз, каріолізис, пікноз, пойкілоцитоз) спостерігали у молоді верховодки (14%), а найменшу – у гірчака звичайного та сонячного окуня (2–4%). У особин голки пухлощокої чорноморської, уражених паразитичними нематодами р. *Ascaris*, кількість еритроцитів з патологією зросла до 81%. В умовах токсичного навантаження дестабілізація кровоносної системи у риб починається з появи в крові молодих форм еритроцитів як компенсація руйнування зрілих еритроцитів, а закінчується масовим руйнуванням зрілих еритроцитів. Враховуючи ці закономірності, а також відносно низьку кількість молодих форм еритроцитів і зрілих еритроцитів з патологічними ознаками, можна вважати, що стан еритроцитів у досліджених нами видів молоді риб відповідає умовній нормі, за винятком крові зараженої паразитами голки пухлощокої чорноморської. Таким чином, основними показниками червоної крові риб, які, на нашу думку, відображають адаптаційні можливості риб, є: коефіцієнт ексцентричності еритроцитів, співвідношення молодих баластних форм еритроцитів, ядерно-цитоплазматичне співвідношення та відносна кількість еритроцитів з патологією.

Ключові слова: *еритроцити, цитометричні індекси, патологічні зміни, Запорізьке (Дніпровське) водосховище.*

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Про авторів:
Т.С. Шарамок – Дніпровський національний університет імені Олеся Гончара, проспект Гагаріна, 72, Дніпро, 49050, Україна, sharamok@i.ua, https://orcid.org/0000-0003-3523-5283
Н.Б. Єсіпова – Дніпровський національний університет імені Олеся Гончара, проспект Гагаріна, 72, Дніпро, 49050, Україна, yesipova.natalia@gmail.com, https://orcid.org/0000-0003-1924-2547
В.О. Курченко – Дніпровський національний університет імені Олеся Гончара, проспект Гагаріна, 72, Дніпро, 49050, Україна, kurchenko.viktoriia.3@gmail.com, https://orcid.org/0000-0002-1199-3760

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