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FORMATION FACTORS OF CYTOGENETIC VIOLATION OF *RUTILUS RUTILUS* (CYPRINIFORMES, CYPRINIDAE) IN TRANSFORMED RIVER ECOSYSTEMS

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Formation Factors of Cytogenetic Violation of *Rutilus rutilus* (Cypriniformes, Cyprinidae), in Transformed River Ecosystems. Klymenko, M. O., Biedunkova, O. O., Klymenko, O. M., Pryshchepa, A. M., Statnik, I. I., Kovalchuk, N. S. — For the first time, an analysis of structural damage of the peripheral blood erythrocyte nucleus in *Rutilus rutilus* (Linnaeus, 1758) was carried out for the rivers of the north-western region of Ukraine. The effect of the hydrochemical regime on the formation of cytogenetic disturbances of the representatives of this species was also clarified. It was observed that during 2013–2017, the content of phosphates, suspended solids, fluorides, iron, copper, zinc, manganese, nitrite nitrogen and nitrate nitrogen in the surface waters of rivers most significantly exceeded the current quality standards. The average frequency of nuclear disorders of peripheral blood erythrocytes *R. rutilus* from the studied hydrosystems is 5.25 ± 0.29 ‰ ($p < 0.01$), with a noticeable excess of the level of spontaneous mutations in the older age groups of fish. In all analyzed samples of *R. rutilus*, among the total number of identified structural disorders of the nucleus, the largest share is made up of red blood cells from the micronucleus (from 40.0 to 62.0 %), which is evidence of cytogenetic disturbances in the body of fish. At the same time, disturbances of erythrocyte blood of roach from small rivers are on average 1.3 times (or 21.9 %) higher compared with individuals from medium-sized rivers. The formation of cytogenetic disturbances in *R. rutilus* under the conditions of the hydrochemical regime of rivers is described by a close ($r = 0.99$ at $p < 0.003$) multifactorial regression dependence. More important regressive coefficients of the dependency are acquired by such biogenes (NH^{4+}, NO^{3-}, PO^{4-}) and toxicants (Cu^{2+}, Zu^{2+}, Mn^{2+}, F_2) in the background of oxygen regime peculiarities (COD, BOD_5, O_2).
Key words: fish, erythrocytes, structural violations.

Introduction

Negative changes of water habitat lead to reducing immune status of fish organism and outbreak of anomalous cells in their organism. That´s why the arising of cytogenetic breaking down is the indicator of worsening fish living conditions. In the world practice cytogenetic breaking down is diagnosed both on the chromosome level and with the help of micronucleus testing (Baršienė et al., 2013; Bilal et al., 2018). Then, information about fish cytogenetic breaking down is received in model experiments with the observation of some toxic substance influence on certain species of fish (ex situ), as well as, under the analysis of ichthyopopulation of natural basins in the background of complex characteristic of contamination (in situ).

It is proved that as a result of compensation of stressful processes which occur in the fish organism there appear erythrocytes with micronuclei. Then the functional overloading of individuals under sudden changes of limiting factors of habitat, starvation and cumulative toxicities can be also the cause of stress (Talapatra and Banerjee, 2007; Klimenko and Biedunkova, 2016).

Erythrocyte breaking down as a non-formed nuclear material also points out at the growth of degenerative processes in the fish organism caused by various factors, including arising of oxidative stress (Bacolod et al., 2017).

Cases when erythrocytes appear with two nuclei are sometimes considered not as cytological destruction in peripheral blood, but as the intensity of erythropoiesis growth.

However, similar explanation appears to be impartial only for young individuals with accelerated metabolism. Just because of the growth of erythrocyte quantity the transmission of oxygen under intensification of organism energy expenses is provided (Grant, 2015). In the older age groups of fish two-nucleus erythrocytes have non-specific nature and prove the arising of stressful factors in the natural basins (Obiakor et al., 2014).

As the experience of the present-day scientific works shows, such approaches are widely used to estimate the state of hydrosystems. For example, according to the results of investigations carried out in the most contaminated by radionuclides lakes of Chernobyl area of alienation, it was defined that the number of erythrocytes with nuclear breaking down in crucians (*Carassius auratus* (Linnaeus, 1758)) reached levels of 5.8 ± 3.7 ‰. At the same time the frequency of coming across micronuclei in the basin under control (Kyiv Reservoir) was considerably lower and made up 0.3 ± 0.2 ‰ (Vergolyas et al., 2010).

To ascertain the mutagenic effect of pollutants of Donetsk and on the Dnipro Region of Ukraine hydrosystems, two most widespread species of fish in the given area: roach (*Rutilus rutilus* (Linnaeus, 1758)) and goldfish *Carassius auratus (Carassius auratus* (Linnaeus, 1758)) were chosen as indicators. The frequency of micronuclei in the flood cells of these species ranged in the limits from 0.14 ± 0.015 to 2.8 ± 0.31 %. It helped the authors come to the conclusion that there is difference in the mutagenous activity of the basins under investigation which had divergence in 3.7–4.1 times in some cases (Gorovaja et al., 2011).

The aim of our work was to analyze the structural breaking down of blood erythrocyte nucleus of *Rutilus rutilus* and to ascertain the influence of hydrochemical regulations of transformed river hydrosystems of north-western region of Ukraine on the formation of cytogenetic breaking down of the representatives of the given fish species.

Material and methods

The roach is euriphag, limnophytophilus reaches sexual virility at the age of 2–3, is widely spread fish in fresh basins almost all around Europe. In the rivers of north-western region of Ukraine among the rest of ichthyofaunal representatives, about 17.5 per cent comes to *R. rutilus*.

Selections of *R. rutilus,* including age categories from 1 to 4, were received as a result of control amount of bish caught (using methods of amateur fishing) in 16 representative alignments of small and medium-size rivers of north-western region of Ukraine within the after-spawning period during summer low water of 2013–2017 (table 1).

All rivers belong to the basin of the Prypiat River, the territory of which has favorably conditions of relief, relatively high damping and it forms dense and diverse system of surface waters. Representativity of the river alignments from which selection of fish was received consists of the levels of hydrosystem transformation under the impact of anthropogenic factors. Thus, sewage of chemical plant, glass factory, timber industry and communal service enterprises get into the rivers chosen for investigation. Parameters of sewer and water balance of medium-size rivers undergo substantial changes because of taking up water for technological process of nuclear power stations; small rivers because of considerable urbanization of water taking and over regulating of river-beds.

The analysis of cytogenetic damages was made using micronucleus testing of erythrocytes of peripheral blood taken from the tail artery of the living fish (Il´inskih, 1988).Coloringof smears was done just after their delivery to the laboratory, after Romarovskiy-Gimza method (L´juis, 2009). Calculation of micronuclei was done with the halp of microscope increasing 10 × 1000 with immersion. From 1000 to 1200 cell of every one individual were analyzed, taking into account all types of micronuclei and nuclear material (fig. 1). The results of calculation were given in ppm (‰).

Table 1. Representative alignments of *R. rutilus* catching in river hydrosystems in the north-western regions of Ukraine

Site #	Administrative location, representativeness substantiation and geographical coordinates	River category	The distance from the mouth, km	Sample size, fish
1	Sluch River, in Bystrychi village, above the wastewater discharge (50° 52´ 52.6″ N 26° 55´ 24.2″ E)	medium	94.5	7
2	Sluch River, in the city of Berezne, 0.6 km below the wastewater discharge (51° 00´ 50.4″ N 26° 45´ 35.1″ E)		73.4	14
3	Ustia River, upper part, natural background (50° 28´ 49.8″ N 26° 19´ 06.2″ E)	small	65	13
4	Ustia River, in the city of Rivne, below the wastewater discharge (50° 36´ 21.7″ N 26° 15´ 14.3″ E)		21.0	13
5	Ustia River, in Orzhiv village (50° 45´ 21.2″ N 26° 07´ 37.6″ E)		0.7	13
6	Styr River, 0.5 km below the industrial wastewater discharge of the Rivne Nuclear Power Plant (51° 21´ 33.2″ N 25° 50´ 39.0″ E)	medium	167.5	14
7	Styr River, in Zarichne urban-type settlement, 0.5 km below the wastewater discharge (51° 49´ 08.9″ N 26° 08´ 56.8″ E)		75.8	14
8	Styr River, in Ivanchytsi village, river flows to Belarus, 4 km to the border (51° 50´ 36.7″ N 26° 10´ 00.3″ E)		74	15
9	Zamchysko River, Mala Liubasha village, above the wastewater discharge (50° 50´ 33.4″ N 26° 29´ 42.7″ E)	small	21.5	14
10	Zamchysko River, in the city of Kostopil, below the wastewater discharge (50° 53´ 50.4″ N 26° 26´ 24.2″ E)		11.9	13
11	Stubelka River, Klevan urban-type settlement, below the wastewater discharge (50° 46´ 26.5″ N 25° 58´ 06.8″ E)	small	7.8	15
12	Ikva River, Sopanivchyk village, on the border with Ternopil Region (50° 10´ 59.9″ N 25° 43´ 01.1″ E)	small	80.5	13
13	Ikva River, 3.2 km below the Dubno wastewater discharge (50° 26´ 07.5″ N 25° 44´ 27.5″ E)		39.6	15
14	Ikva River, in Torhovytsia village, Mlyniv District (50° 33´ 40.0″ N 25° 23´ 47.5″ E)		1.5	14
15	Horyn River, in the city of Dubrovytsia, 0.5 km below the wastewater discharge (51° 34´ 41.2″ N 26° 35´ 33.9″ E)	medium	104.0	10
16	Horyn River, in Vysotsk village, at the Ukrainian-Belarusian border (51° 43´ 28.9″ N 26° 40´ 18.8″ E)		77.5	10

Statistic value of divergence among selections was estimated according to Student´s *t*-test and presented as the average data indicating mean squared error (Ajvazjan, 1985). The influence of hydrochemical parameters of the river on *R. rutilus* cytogenetic violations was analyzed by means of multi-factor (multiplicated) regression using a computer software package *Statistica 8.0.*

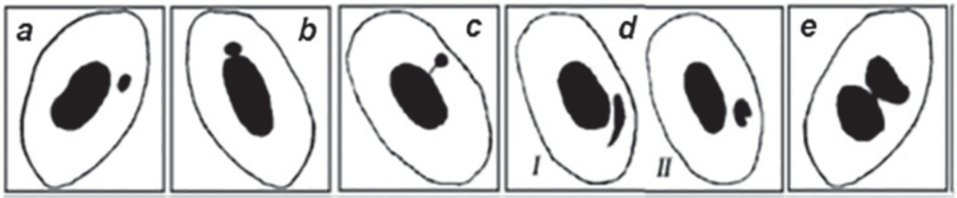

Fig. 1. Possible versions of micronuclei and nuclear material in erythrocytes of peripheral fish blood: *a* — micronuclei of "standart" type; *b* — "attached"; *c* — "linked to the nucleus with chromatine thread"; *d*-I — non-formed nuclear material shaped as small sticks; *d*-II — non-formed nuclear material in the form of clews; *e* — rounded formations of nuclear material of big enough sizes.

Results and discussion

The indices of water quality in representative alignments are given as medium values which were done within the years of observations (table 2).

The data introduced were checked up according to correspondence to the Water Quality Standards of Fishery, 1999 that are functioning in Ukraine as official documents for ecological assessment of surface water quality. It is worth remarking that regulation of such parameters of basin oxygen regime as chemical consumption of oxygen (CCO) is not envisaged by fishery standards.

Table 2. Indices of water quality in river hydro systems under research in the north-western regions of Ukraine (average ones of the years 2013–2017)

Substance	Representative alignments#															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
SO_4^{2-}, mg/dm³	55.4	65.2	23.9	52.5	51.4	49.5	59.3	47.3	40.5	33.0	24.8	27.3	50.8	55.5	46.4	56.0
	0.55	0.65	0.24	0.53	0.51	0.50	0.59	0.47	0.41	0.33	0.25	0.27	0.51	0.56	0.46	0.56
Cl^-, mg/dm³	23.4	23.9	21.3	27.4	26.6	13.3	16.3	13.7	1.8	14.9	21.3	20.6	13.9	30.8	18.4	19.8
	0.08	0.08	0.07	0.09	0.09	0.04	0.05	0.05	0.01	0.05	0.07	0.07	0.05	0.10	0.06	0.07
NH_4^+, mg/dm³	0.2	0.3	2.6	1.1	0.4	0.3	3.1	0.3	1.0	4.1	0.7	0.6	0.3	0.2	0.2	0.5
	0.4	0.6	5.2	2.2	0.8	0.6	6.2	0.6	2.0	8.2	1.4	1.2	0.6	0.4	0.4	1.0
NO_3^-, mg/dm³	0.5	2.9	3.1	9.1	7.2	2.5	0.1	2.9	1.9	1.1	2.8	6.6	4.0	3.3	3.2	6.2
	0.01	0.07	0.08	0.23	0.18	0.06	0.00	0.07	0.05	0.03	0.07	0.17	0.10	0.08	0.08	0.16
NO_2^-, mg/dm³	0.02	0.05	0.2	0.2	0.1	0.1	0.1	0.1	0.04	0.2	0.1	0.3	0.2	0.1	0.1	0.1
	0.25	0.63	2.5	2.5	1.25	1.25	1.25	1.25	0.5	2.5	1.25	3.75	2.5	1.25	1.25	1.25
PO_4^-, mg/dm³	0.1	0.2	26.0	0.6	0.3	0.2	0.1	9.2	0.2	1.2	0.2	0.2	0.4	0.5	1.1	0.4
	2.0	4.0	520	12.0	6.0	4.0	2.0	184	4.0	24.0	4.0	4.0	8.0	10.0	22.0	8.0
Suspended solids, mg/dm³	10.9	13.5	11.3	12.3	14.0	11.7	10.9	7.9	9.2	13.1	2.2	151	10.1	10.4	9.0	9.9
	14.5	18.0	15.1	16.4	18.7	15.6	14.5	10.5	12.3	17.5	2.9	201	13.5	13.9	12.0	13.2
Dissolved oxygen(DO), mgO_2/dm^3	10.6	10.1	10.4	10.1	9.8	7.9	8.8	6.9	9.5	7.5	10.7	6.7	7.1	10.0	10.9	10.0
	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.6	0.4	0.5	0.4	0.6	0.6	0.4	0.4	0.4
pH	8.2	7.9	8.2	8.0	8.3	8.1	7.8	7.8	7.6	7.6	8.0	8.0	7.6	7.9	8.3	7.9
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
COD, mgO_2/dm^3	26.6	31.9	21.6	31.9	33.4	38.7	41.4	28.2	35.0	39.6	32.3	43.2	39.4	35.8	33.6	30.5
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BOD_5, mgO_2/dm^3	4.5	5.7	1.2	4.7	3.4	2.7	4.1	2.2	3.0	6.3	3.1	4.2	3.7	3.3	3.3	3.5
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fe^{2+}, mkg/dm³	258	299	284	155	125	124	216	182	937	478	180	196	269	166	380	181
	2.58	2.99	2.84	1.55	1.25	1.24	2.16	1.82	9.37	4.78	1.8	1.96	2.69	1.66	3.8	1.81
Cu^{2+}, mkg/dm³	14.7	17.0	22.3	34.9	31.0	29.5	21.7	15.6	49.0	83.5	25.5	9.3	10.0	10.0	5.0	15.9
	1.47	1.7	2.23	3.49	3.1	2.95	2.17	1.56	4.9	8.35	2.55	0.93	1	1	0.5	1.59
Zn^{2+}, mkg/dm³	8.3	19.2	19.7	40.9	58.5	17.0	13.5	17.9	25.3	14.0	21.0	87.3	84.0	19	26.0	24.4
	0.83	1.92	1.97	4.09	5.85	1.7	1.35	1.79	2.53	1.4	2.1	8.73	8.4	1.9	2.6	2.44
Mn^{2+}, mkg/dm³	36.0	42.0	37.0	42.7	10.5	17.8	29.0	25.9	38.3	42.3	12.0	51.7	23.5	22.3	12	35.0
	3.6	4.2	3.7	4.27	1.05	1.78	2.9	2.59	3.83	4.23	1.2	5.17	2.35	2.23	1.2	3.5
F_2, mkg/dm³	26.7	130	190	315	300	217	150	70.2	277	289	255	110	137	416	597	249
	0.5	2.6	3.8	6.3	6.0	4.3	3.0	1.4	5.5	5.8	5.1	2.2	2.7	8.3	11.9	5.0

Note. In the numerator — the actual value; in the denominator — the multiplicity of exceeding the standards of the regulations governing water use by fisheries (Water Quality Standards of Fishery, 1999) operating in Ukraine as an official document in the environmental assessment of surface water quality.

In representative alignments of the rivers under investigation the indices of salt components of water, the content of oxygen dissolved in the water and environment reaction (pH) are in the limits of rate. Their surface waters are simultaneously contaminated by phosphates ($PO4^-$) with aliquot exceeding of permissible values from 2.0 to 520.0 times and suspended substances (SS) from 2.9 to 201.0 times. Heavy metal ions are characterized by aliquot exceeding of permissible concentrations in water from 1.5 to 8.4 times for capper (Cu^{2+}), from 1.4 to 8.3 times for zink (Zn^{2+}) and 1.2–4.3 times for manganese (Mn^{2+}). Aliquot parts of exceeding iron concentration (Fe^{2+}) in representative alignments make up 1.6–9.4 times, fluorine (F_2) — 1.4–11.9 times. In the group of nitrogenous substances for most alignments the aliquot part of exceeding permissible value is characterized in 1.3–3.8 times for nitrite nitrogen (NO_2^-) and in 2.0–8.2 times for ammonium nitrogen (NH_2^+) in some alignments. In all cases, exceeding of hydrochemical parameter standards affirms unsatisfactory water quality, reducing river self-purifying ability and breaking of balance in their ecosystems. Meanwhile, the aliquot part of exceeding is higher in representative alignments of small rivers.

It is known that nuclear breaking down of erythrocytes of peripheral fish blood reflects the favorableness of water habitat during the period of fish catching (Sopinka et al., 2016). It is interesting that when the stressful factor is removed the given physiological reaction of the organism can turn back to the rate (Omar et al., 2012).

The results of the micronucleus test conducted for *R. rutilus* selection in representative alignments are shown in fig. 2.

Statistic control of the data affirms the authenticity ($p \leq 0.01$) of the received results as for the frequency of nuclear breaking down of fish erythrocytes in founds of some selections (table 3).

In general within representative alignments the frequency of *R. rutilus* nuclear breaking was ranging from 3.24 ± 0.22 ‰ (alignment No. 15 — the Goryn River) to 6.92 ± 0.89 ‰ (alignment No. 4 — the Ustia River) with the average value of indices at the level of 5.25 ± 0.29 ‰.

According to literary data, the level of spontaneous mutation of erythrocyte nucleus of ailing fish makes up from 0.05 to 0.4 ‰, or 0.5–4 ‰ correspondingly (Krysanov, 1987; Sopinka et al., 2016). Thus, fixed exceeding of this quantity according to average frequency of *R. rutilus* in small and medium-size rivers of north-western region of Ukraine can affirm the existence of stressful factors for the given species of fish in hydrosystems of the area under research.

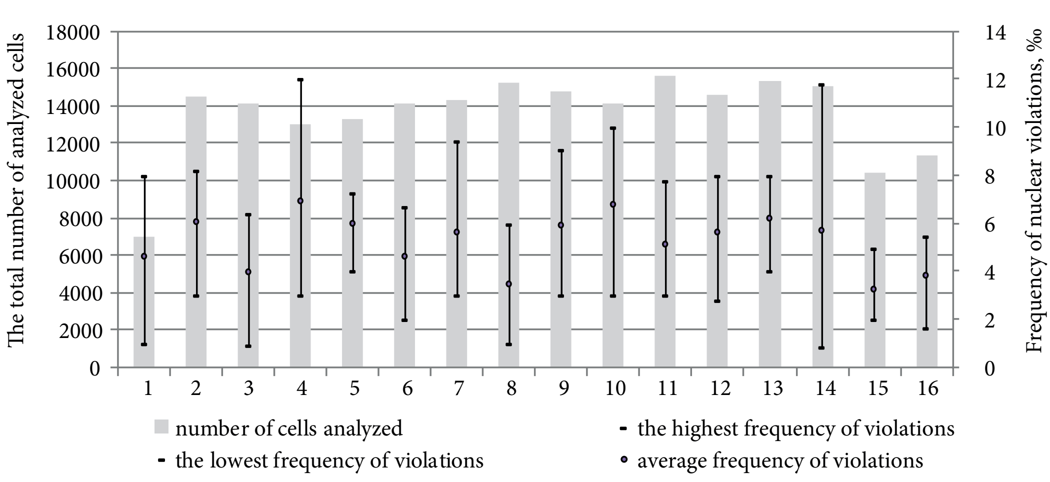

Fig. 2. Frequency of nuclear violations of *R. rutilus* peripheral blood erythrocytes in representative alignments of river hydrosystems in the north-western region of Ukraine.

Table 3. Results of statistic control of the data concerning nuclear violations (M ± m) of *R. rutilus* peripheral blood erythrocytes in representative alignments of river hydrosystems

Representative alignments#	Statistical indicators						
	N	M	Std.Dv.	±m	t-value	df	p
1	7	5.000	2.2361	0.845	5.91608	6	0.001038387
2	14	6.016	2.0359	0.544	11.05619	13	0.000000055
3	13	3.992	1.9350	0.537	7.43912	12	0.000007849
4	13	6.923	3.0947	0.858	8.06599	12	0.000003456
5	13	5.962	1.0021	0.278	21.44931	12	0.000000000
6	14	4.574	1.2119	0.324	14.12073	13	0.000000003
7	14	5.621	2.1380	0.571	9.83778	13	0.000000217
8	15	3.470	1.6701	0.431	8.04700	14	0.000001279
9	14	5.914	1.8907	0.505	11.70261	13	0.000000028
10	13	6.777	2.3011	0.638	10.61844	12	0.000000187
11	15	5.139	1.4778	0.382	13.46904	14	0.000000002
12	13	5.595	1.7231	0.478	11.70668	12	0.000000064
13	15	6.189	1.3580	0.351	17.65163	14	0.000000000
14	14	5.717	2.6413	0.706	8.09890	13	0.000001954
15	10	3.235	0.6916	0.219	14.79130	9	0.000000127
16	10	3.820	1.3359	0.422	9.04225	9	0.000008217

Comparison as for the river categories reveals that the average frequency of nuclear breaking down of fish blood erythrocytes in small rivers (the Ustia River, the Zamchysko River, the Stubelka River, the Ikva River) made up 5.8 ± 0.31 ‰ and in fish of medium — size rivers (the Sluch River, the Styr River, the Goryn River) made up 4.53 ± 0.44 ‰ with the divergence by 1.3 times or by 21.9 ‰.

Average frequency of nuclear violations of blood erythrocytes of *R. rutilus* dufferent age categories by which selections under research were represented are given in fig. 3.

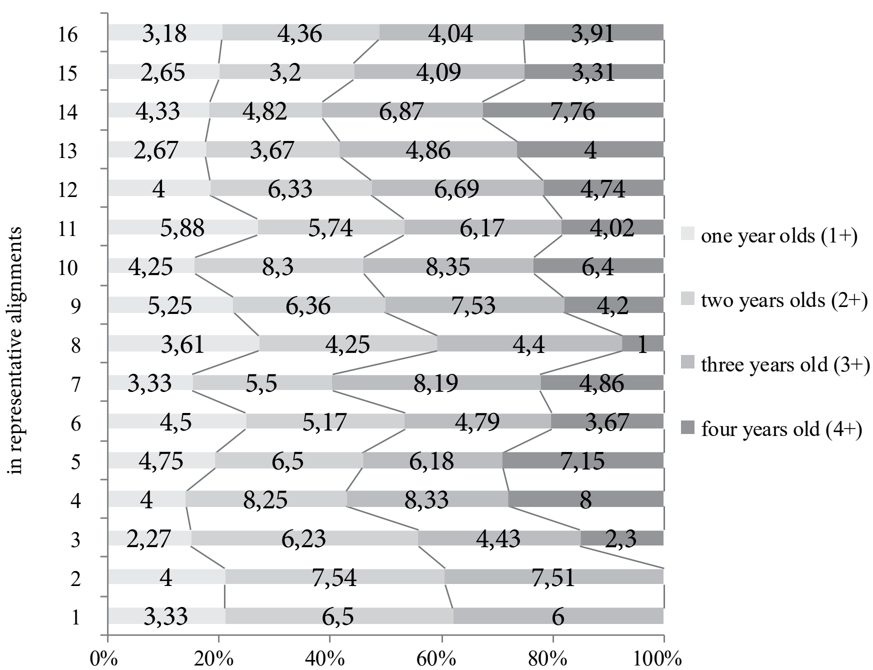

Fig. 3. Medium rates of nuclear violations of blood erythrocytes of *R. rutilus* various age categories.

Thus, average frequency of nuclear violations of analyzed roach individuals aged a year (1+) made up 3.88 ± 0.25 ‰. Average frequency of nuclear breaking down of those aged 2 years (2+) made up 5.8 ± 0.37 ‰, of those aged 3 years (3+) 6.15 ± 0.38 ‰, of those aged 4 years (4+) 4.67 ± 0.52 ‰. So, frequency of nuclear breaking down of 2 year old *R. rutilus* in comparison with those one-year-old ones was increasing by 1.5 times or by 9.4 %. For the individuals aged 3 this exceeding was on the level of 1.6 times or by 11.1 %, for individuals aged 4+ it was on the level of 1.2 times or 3.86 %. The highest values of frequency of nuclear breaking down of erythrocytes are registered for two years old roach in alignments No. 2 (7.54 ± 0.77 ‰), No. 4 (8.25 ± 2.18 ‰), No. 10 (8.3 ± 1.56 ‰), for three years old ones — in alignments No. 2 (7.51 ± 0.76 ‰), No. 4 (8.33 ± 1.78 ‰), No. 7 (8.19 ± 0.79 ‰) and No. 10 (8.35 ± 0.83 ‰), and also for four years old individuals — in alignments No. 4 (8.0 ± 2.83 ‰), No. 5 (7.15 ± 0.21 ‰) and No. 14 (7.76 ± 0.86 ‰). It´s worth remarking that most of these alignments are located in the places after dumping drained waters of communal and industrial section of the region being researched.

In our opinion the exceeding of the spontaneous mutation level of the nucleus of blood erythrocytes in older aged groups of *R. rutilus* can be explained by fish reaction to the unfavorable conditions of water habitat.

As it has been pointed out before, the analyses of structural violations of nuclear apparatus of fish peripheral blood is considered to be more unbiased criterion of water habitat quality evaluation. Thus, the biggest part in general amount of revealed nuclear violations in *R. rutilus* selections made erythrocytes with micronucleus (fig. 4).

From 40.0 to 62.0 % belonged to them. Erythrocytes with doubled nucleus took the intermediate place (from 24.0 to 42.0 %) and non-formed nuclear material, from 9.0 to 20.0 % was revealed to be the smallest part.

Either in small or medium-size rivers a part of erythrocytes with micronuclei which are evidence of stressful effects on fish organism, including toxicity of water habitat, had approximately equal values and made up correspondingly 51.2 and 54.6 % among all the kinds of discovered violations.

In the division of structural breaking types of blood erythrocytes of *R. rutilus* different age individuals certain regularity was observed (fig. 5).

Thus, more than 50 % makes up erythrocytes with micronuclei including all age categories. A part of erythrocytes with double nuclei makes up more them 30 % and a part of non-formed nucleus material makes up less than 14 %.

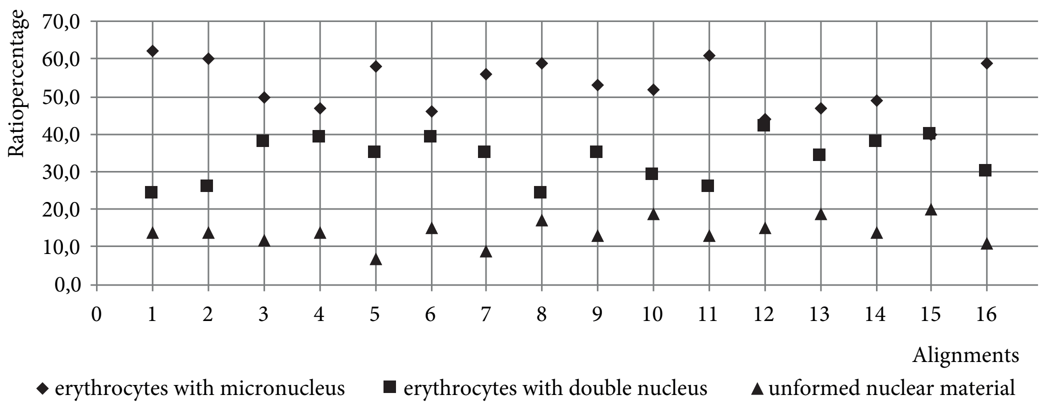

Fig. 4. Distribution of revealed structural breaking down of nucleus in *R. rutilus* selections from representative alignments of transformed river hydrosystems in the north-western region of Ukraine.

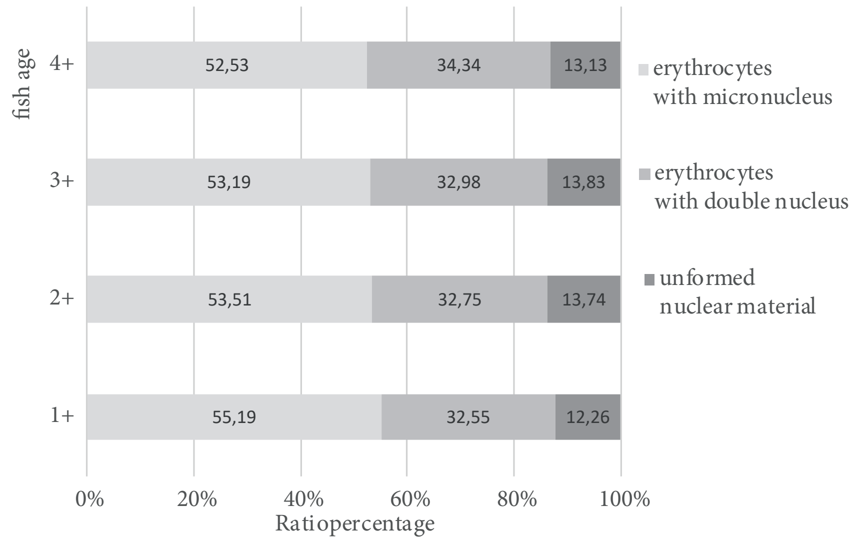

Fig. 5. Distribution of revealed nucleus structural breaking down in *R. rutilus* individuals of different age: (1+) — one-year-old; (2+) — two-year-old; (3+) –three-year-old; (4+) — four-year-old.

Since the frequency of nucleus breaking down of fish erythrocytes mainly depends on the impact of existing pollution in the water habitat, so that to understand this action in the conditions of river hydroecosystems of the region under research there was performed a many-factored regression analysis. Than hydrochemical parameters of small and medium-size rivers (table 2) and frequency of nucleus breaking down of blood erythrocytes of all age categories, representatives of *R. rutilus* type that were analyzed, were taken into account.

Statistic parameters, according to which mathematic control of many-factored regression was made included: G — capacity of regressive dependency (the number of individuals); r — general correlation coefficient of regressive dependency; F — the value of Fisher criterion for regressive dependency; p — statistical significance of regressive dependency; B — free term of regression; b — regressive coefficient of separate term of dependency (table 4).

Table 4. Results of statistical verification of data of multivariate regressive dependence of cytogenetic violations of *R. rutilus* on hydrochemical parameters of surface water of rivers in the conditions of northwestern region of Ukraine

Substance	Statistical indicators					
	Beta	Std. Err.	B	Std. Err.	t(1)	p-level
Intercept			–88.8964	3.823123	–23.2523	0.027362
NH_4^+	6.5550	0.245254	7.0305	0.263043	26.7275	0.023808
NO_3^-	0.4080	0.025314	0.2008	0.012460	16.1196	0.039443
PO_4^-	–10.5893	0.334907	–37.8177	1.196062	–31.6186	0.020128
Suspended solids	–4.0944	0.162371	–0.9206	0.036510	–25.2164	0.025233
Fe^{2+}	1.0730	0.062382	0.0115	0.000670	17.2003	0.036971
Cu^{2+}	–4.9395	0.223259	–0.2812	0.012711	–22.1244	0.028755
Zn^{2+}	–0.7631	0.042740	–0.0278	0.001556	–17.8539	0.035620
Mn^{2+}	1.1873	0.065510	0.0308	0.001698	18.1246	0.035089
F_2	9.9607	0.328118	0.0801	0.002639	30.3569	0.020964
pH	3.8995	0.151986	19.8770	0.774726	25.6569	0.024800
COD	–2.3802	0.092959	–0.4687	0.018306	–25.6044	0.024851
BOD_5	6.9562	0.239241	6.3252	0.217536	29.0764	0.021886
O_2	–9.4473	0.336423	–7.2577	0.258450	–28.0817	0.022661

Thus, many-factored regressive dependency of complex impact of hydrochemical parameters on the formation of cytogeneses violations (X) of roach in the conditions of river hydrosystemsof the region under research is characterized by general statistic parameters: q = 13; r = 0.99; F = 416.32; p < 0.003; B = –88.896 and is a following:

$$X = -88.896+7.031(NH_4^+) +0.201(NO_3^-)-37.818(PO_4^-)-0.921(3P)+ 0.012(Fe^{2+})-0.281(Cu^{2+})-0.028(Zn^{2+})+0.031(Mn^{2+})+0.08(F_2)+19.877(pH)- 0.469(COD) +6.325(BOD_5)-7.258(O_2).$$

The revealed combination of substances affirms the complication and multy-factors of the formation process of cytogenetic *R. rutilus* breaking down in the conditions of river hydrosystems in the north-western regions of Ukraine. More important regressive coefficients of the dependency are acquired by such biogenic as nitrogen ammonium (NH_4^+), nitrogen nitrate (NO_3^-) and phosphates (PO_4^-). It´s likely to be explained by the fact that hydrobiological regime of the hydrosystems under research is characterized by quite a long period of eutrophication of surface water. Such specific substances of toxic nature as copper (Cu^{2+}), zinc (Zu^{2+}), manganese (Mn^{2+}) and fluorites (F_2) which have either natural or anthropogenic origin in the background of oxygen regime peculiarities (COD, BOD_5, O_2) of water habitat, affect the formation of *R. rutilus* cytogenetic violations. It´s obvious that exceeding of spontaneous nucleus mutation levels of erythrocytes in the analyzed selections of roach is receptive and demonstrative reaction of ecological state of the transformed river hydrosystems.

Similar mathematical analysis and comparison of parameters of surface water quality and levels of *R. rutilus* cytogenetic damage can be useful in monitoring the status of rivers. Ideally, it is necessary to conduct similar studies for other representatives of the local ichthyofauna, which will make it possible to forecast the ecological situation and monitor the state of the biotic component of hydroecosystems.

Conclusion

In the surface waters of the transformed river hydroecosystems of the north-western region of Ukraine, the phosphate content exceeds the current standards up to 520 times, and suspended solids up to 201 times. The exceeding reaches almost 12 times for fluorides, for iron ions — 9 times, for copper, zink and nitrogen nitrite — 8 times, for manganese and nitrogen nitrate — about 4 times.

Average frequency of disturbances of the peripheral blood erythrocyte nuclei of *R. rutilus* from the studied hydroecosystems is 5.25 ± 0.29 ‰, with a noticeable excess of the level of spontaneous mutations in the older age groups of fish.

In all analyzed samples of *R. rutilus*, among the total number of identified structural disturbances of the nucleus, the largest share is erythrocytes with micronuclei (from 40.0 to 62.0 %), which is evidence of cytogenetic disorders in the fish body. At the same time, the disturbance of erythrocyte structure in roach from small rivers is on average 1.3 times higher (or 21.9 %) than in individuals from medium-sized rivers.

The formation of cytogenetic disorders in *R. rutilus* under the conditions of the hydrochemical regime of rivers in the north-western region of Ukraine is determined by tight (r = 0.99 with p < 0.003) multifactorial regressive dependency. The value of the regression coefficients of individual dependencies indicate that disturbances of roach erythrocyte nuclei are a sensitive indicator, primarily for the content of ammonium phosphate and nitrogen in the water, water pH and its oxygen regime.

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