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DEVELOPMENT STABILITY AND CYTOGENETIC HOMEOSTASIS OF *PERCA FLUVIATILIS* (PERCIFORMES, PERCIDAE) IN THE RIVERS OF RIVNE REGION

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Development Stability and Cytogenetic Homeostasis of *Perca fluviatilis* (Persiformes, Persidae) in the Rivers of Rivne Region. Klimenko, M. O., Biedunkova, O. O. — The article presents the results of the study on nuclear damages or injuries of red blood cells (RBCs) and asymmetry of meristic characters of the European perch (*Perca fluviatilis* Linnaeus 1758) that lives in small and medium rivers of Rivne Region. The research has found that the species representatives living in the small rivers demonstrated worse cytogenetic homeostasis indicators compared to the fish from the medium rivers: the lowest frequency of *P. fluviatilis*' nuclear damages appeared to be 4.25 times higher for the individuals living in the small rivers given the noticeable excess of spontaneous mutations by average damage frequency. Fish morphological homeostasis demonstrates a tendency to injuries in small rivers as well. The functional relationships between the studied parameters which have been established by the author were "close" for the fish from the small rivers and primary "moderate" for the fish from the medium rivers. It is concluded that the enhanced activity of the stressors existing in the water environment affects the physiological state of the *P. fluviatilis* species.
Key words: fish, nuclear damages, fluctuating asymmetry, hydro-ecosystem, stressors.

Introduction

Preserving biological diversity is one of the major challenges for the future of our planet, and therefore the problem of deviations from the norm demonstrated by living organisms in natural ecosystems has been the focus of a wealth of research worldwide.

System ichthyologic research studying mass-dimensional structure of populations, their sex ratio, species composition and taxonomic classification etc. supplements the population analysis at the level of individuals. Modern literature has a lot of evidence that such approaches allow, first of all, to get an idea of the specific fish lifestyle (Dgebuadze, 2001), and, second of all, they serve as an informative hydro-ecosystem control tool (Moiseenko, 1998).

The analysis of the issue proves that the homeostatic biota indicators may change or vary within the adaptive capacity of the body (Krysanov, 1987). Therefore, in evaluating biological hydro-ecosystems' components, the criteria applied for the deviations from the "norm" should correlate with regional natural conditions amid the influence of stressors that occur in water bodies (Moiseenko, 1998; Selezneva, 2007).

The aim of the given research was to generalize the results of the experimental and field research on morphological and cytogenetic homeostasis of European perch (*Perca fluviatilis* Linnaeus 1758) that lives in the medium and small rivers of Rivne Region which differ by specific fish lifestyles' formation and intensity of demonstrating destabilizing processes of natural and anthropogenic origin.

Material and methods

The study subject was chosen for the following reasons: 1 — *Perca fluviatilis* Linnaeus 1758, commonly known as the European perch, Eurasian river perch or common perch, is a predator found on top of the food chain of the most rivers of Rivne Region; 2 — the level of population formation in the region is about 100 % in respect of non-transformed river basins (Sondak, 2010); 3 — there is an opportunity to work with the species over the summer due to early spawning deadlines; 4 — the species is a popular quarry for amateur anglers.

Table 1. ***P. fluviatilis*** **Control Catch Sites in the Rivers of Rivne Region**

Site, No	Administrative Location and Representativeness Substantiation	River Category	The distance from the mouth, km	Sample size, fish
1	Sluch River, in Bystrychi village, above the wastewater discharge	medium	94.5	26
2	Sluch River, in the city of Berezne, 0.6 km below the wastewater discharge		73.4	21
3	Ustia River, upper part, natural background	small	65	21
4	Ustia River, in the city of Rivne, below the wastewater discharge		21.0	32
5	Ustia River, in Orzhiv village		0.7	19
6	Styr River, 0.5 km below the industrial wastewater discharge of the Rivne Nuclear Power Plant	medium	167.5	17
7	Styr River, in Zarichne urban-type settlement, 0.5 km below the wastewater discharge		75.8	19
8	Styr River, in Ivanchytsi village, river flows to Belarus, 4 km to the border		74	22
9	Zamchysko River, Mala Liubasha village, above the wastewater discharge	small	21.5	19
10	Zamchysko River, in the city of Kostopil, below the wastewater discharge		11.9	19
11	Stubelka River, Klevan urban-type settlement, below the wastewater discharge	small	7.8	16
12	Ikva River, Sopanivchyk village, on the border with Ternopil Region	small	80.5	19
13	Ikva River, 3.2 km below the Dubno wastewater discharge		39.6	17
14	Ikva River, in Torhovytsia village, Mlyniv District		1.5	18
15	Horyn River, in the city of Dubrovytsia, 0.5 km below the wastewater discharge	medium	104.0	24
16	Horyn River, in Vysotsk village, at the Ukrainian-Belarusian border		77.5	27

Samples of *P. fluviatilis* which included age groups from one-year-old to four-year-old perch were obtained from the control catches (amateur fishing methods) in 16 control sites located in the rivers of Rivne Region during the 2013–2015 summer period (table 1).

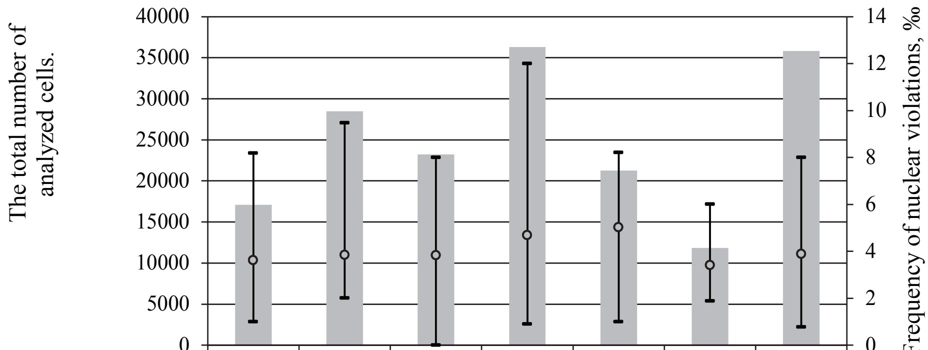
The assessment of stability was performed by levels of fluctuating asymmetry (FA) of 9 (nine) meristic bilateral symptoms. Relative frequency of asymmetries (abbreviation: RF) which describes the number of times signs that show the asymmetry has been observed to occur in relation to the total number of recorded signs was used as an asymmetry indicator. The assessment of development stability deviation from conventionally normal state was performed according to the given scale (Zaharov et al., 2000).

The author assessed cytogenetic homeostasis by erythrocyte micronucleus test with peripheral blood taken from the fishtail artery by vivo method (Il'inskih, 1988). The staining of blood smears has been performed immediately after their delivery to the laboratory in line with the Romanovsky-Himza method: the Romanovsky-type stains are neutral dyes used primarily in the staining of blood smears (L'juis, 2009). Micronuclei were examined at ×100 magnification under immersion microscopy. The results were expressed in per mille notation (‰), whereby all kinds of micronuclei and nuclear material were taken into account (Krysanov, 1987).

The statistical significance of the results was assessed in the framework of the programme Statistica 8.0. Was the analysis of indicators such as: the average value for a sample (M), standard deviation (Std. Dv.), standard error (± m), value criterion of Student (t-value), the number of degrees of freedom (df), confidence level (p). The results recognized reliable when $p \le 0.05$ (Ajvazjan, 1985).

Results

According to natural landscape division of Ukraine drainage basins of the Rivne Region rivers are located within the Volhynian Polesia (Ustia River, Zamchysko River), Volhynian Upland (Ikva River and Styr River within Rivne Region), and partially Zhytomyr Polesia (Sluch River basin in its mouth). All rivers belong to the Prypiat River basin which territory has favourable topography, relatively high moisture and forms a dense and diverse network of surface waters. Environmental assessment of water quality in medium rivers describes waters as "pure — moderately polluted", in small rivers as "moderately dirty — dirty". At the same time the largest contribution to the deterioration of surface waters is made by the substances of nitric group, phosphates, some heavy metals and phenols as a result of discharges of inadequately treated sewage (Biedunkova, 2015).



	1	2	3	4	5	6	7
number of cells analyzed	17100	28460	23240	36300	21250	11850	35830
the highest frequency of violations	8,18	9,47	8	12	8,2	6	8
the lowest frequency of violations	1	2	0	0,9	1	1,88	0,77
average frequency of violations	3,62	3,84	3,84	4,69	5,02	3,41	3,89

Fig. 1. Frequency of Nuclear Damages of *P. fluviatilis* Peripheral Blood Erythrocytes in the Rivers in Rivne Region: 1 — Styr River; 2 — Sluch River; 3 — Horyn River; 4 — Ustia River; 5 — Zamchysko River; 6 — Stubelka River; 7 — Ikva River.

The impact of stressors on the fish fauna of the studied rivers is related not only to hydrochemical changes and fodder deterioration, but also to the growing pressure of alien species. The analysis of native fish fauna mortality shows that over the last 30 years its value is 86.0 % for the waters of the region; in particular 66.7 % belongs to the preserved estuaries of the rivers (Sondak, 2010).

In conducting our research, we evaluated cytogenetic homeostasis of *P. fluviatilis* as a stress indicator, reflecting favourable environment during the fishing time (fig. 1).

The comparison of the results by river category allows to note that the average frequency of nuclear damages in fish red blood cells was 1.13 times higher in the individuals from the small rivers (Ustia River, Zamchysko River, Stubelka River, Ikva River) compared to the individuals from the medium rivers (Sluch River, Styr River, Horyn River) of Rivne Region.

Assessment of the highest frequency of injuries, which was recorded during the erythrocyte micronucleus test, reflects completely equal average indices for the fish from both river categories. The average of the lowest nuclear damages frequency for *P. fluviatilis* appeared to be 4.25 times higher for the individuals from the small rivers, compared with the species' representatives from the medium rivers.

Statistical validation of the reliability of the results triggered for all analyzed samples of *P. fluviatilis* (table 2).

Table 2. The statistical validation of data about Nuclear Damages of *P. fluviatilis* Peripheral Blood Erythrocytes in the Rivers in Rivne Region

River	Statistical indicators					
	M	Std. Dv.	± m	t-value	df	p
Styr	3.62143	1.05041	0.31073	12.89992	13	0.000089
Sluch	3.84167	1.80006	0.55001	12.80512	35	0.000001
Horyn	3.83545	1.03857	0.24085	13.99365	21	0.000047
Ustia	4.68889	1.80662	0.58110	15.57236	35	0.000003
Zamchysko	5.01905	1.14862	0.51253	12.46879	20	0.000038
Stubelka	3.41300	1.20694	0.38167	8.94230	9	0.000083
Ikva	3.89265	1.36734	0.52345	16.60002	33	0.000001

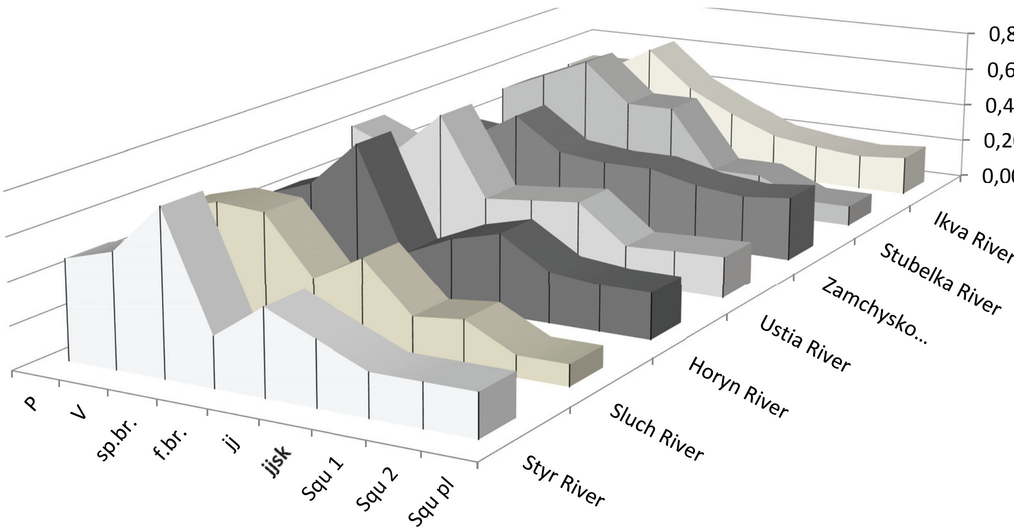


Fig. 2. Fluctuating Asymmetry of *P. fluviatilis* Bilateral Meristic Features in the Rivers of Rivne Region: *P* — the number of rays in the pectoral and *V* — pelvic fins; *sp.br.* — the number of gill rakers on the first gill arch; *f.br.*— the number of petals in branchiostegal membrane; *jj* — number of scales in the lateral line; *jj.sk* — number of scales with touch tubules; *squ.1* — the number of scales' rows above and; *squ.2* — under the lateral line; *squ. pl* — the number of scales on the side of the caudal fin.

The average frequency of the nuclear damages of peripheral blood erythrocytes was the highest for the *P. fluviatilis* individuals that have been caught in Ustia River and Zamchysko River and appeared to be 4.69 ± 0.58 ‰ and 5.02 ± 0.51 ‰ respectively. The values for the fish caught in Ikva River were somewhat lower (3.89 ± 0.52 ‰), as well as for the individuals caught in Sluch and Horyn Rivers (3.84 ± 0.55 ‰ and 3.84 ± 0.24 ‰, respectively). The nuclear damages of fish from Styr River (3.62 ± 0.31 ‰) and Stubelka River (3.41 ± 0.38 ‰) appeared to demonstrate the lowest average levels.

We'd have to note that the cytological changes in peripheral blood cells of fish occur both at the different stages of ontogeny (Krysanov, 1987) and at different periods throughout a year (Lugas'kova, 2003). However, the number of micronuclei in red blood cells substantially depends on the activity of existing contamination in the aquatic environment. Thus, according to some authors (Krysanov, 1987; Il'inskih, 1988; Lugas'kova, 2003) the frequency of spontaneous mutations in the nuclei of red blood cells in peripheral blood of healthy fish ranges from 0.05 to 0.4 % or from 0.5 to 4 ‰ respectively.

It is absolutely clear that the nuclear damages of *P. fluviatilis* living in the rivers of Rivne Region is quite high, furthermore, first of all, there is a significant excess of induced mutagenesis in the species of small rivers. We assume that fish cytogenetic homeostasis is disturbed due to the presence of mutagenic factors in the studied hydro-ecosystems.

The manifestation of fluctuating asymmetry of morphological characters is another non-specific reaction of fish to stress factors. In particular, the RF rate assesses the stability of individuals and provides a picture of the conditions in which the body was at the early stages of ontogeny when the studied traits were formed (Zaharov et al., 2000). Thus, the results of our research state that among nine signs of *P. fluviatilis* bilateral meristic characteristics, the highest levels of fluctuating asymmetry were typical for the number of gill rakers on the first gill arch which ranged in size from 0.56±0,08 (Zamchysko River) to 0.79 ± 0.05 (Sluch River) (fig. 2).

The FA levels for the number of rays in the *P. fluviatilis* pectoral and pelvic fins were somewhat lower, with RF values ranging from 0.42±0,02 (t-value = 59.56; df = 50; p ≤ 0.05) in Horyn River to 0.59 ± 0.03 (t-value = 173.21; df = 51; p ≤ 0.05) in Ustia River and from

0.42 ± 0.03 (t-value = 54.33; df = 37; p ≤ 0.05) in Zamchysko River to 0.60 ± 0.05 (t-value = 123.05; df = 46; p ≤ 0.05) in Sluch River, respectively. The FA of the number of scales in the lateral line was the least severe, ranging from 0.10 ± 0.02 (t-value = 22.36; df = 15; p ≤ 0.05) in Stubelka River to 0.29 ± 0.03 (t-value = 89.87; df = 37; p ≤ 0.05) in Zamchysko River; the same tendency can be noted regarding the number of scales on the side of the *P. fluviatilis* caudal fin, ranging from 0.10 ± 0.02 (t-value = 16.95; df = 15; p ≤ 0.05) in Stubelka River to 0.32 ± 0.04 (t-value = 91.24; df = 37; p ≤ 0.05) in Zamchysko River.

In general, the values of the RF of meristic characteristics of *P. fluviatilis* living in the rivers of Rivne Region made the following descending series: *sp.br. > V > R > jj > f.br. > Jj.sk > squ.1 > squ.2 = squ.pl.*

The calculation of the average RF values for the studied meristic characteristics of fish samples allowed determining the development stability of *P. fluviatilis* in the rivers of the region demonstrated mostly average level of irregularities (deviations from the norm), ranging from 0.36 ± 0.04 (Ikva River) to 0.39 ± 0.03 (Zamchysko River). Only the Sluch River sample showed average RF values of 0.33 ± 0.02 and, thus, provided evidence of initial (minor) deviations from normal development stability of *P. fluviatilis*. Statistical validation proves the authenticity of the received values of the RF in the samples of *P. fluviatilis* in the rivers of Rivne Region (table 3).

The comparison of the fluctuating asymmetry indices that reflect morphological homeostasis of *P. fluviatilis* and micronucleus test which makes it possible to estimate the level of cytogenetic homeostasis is presented in a diagram (fig. 3).

Figure 3 shows some synchronicity changes in both indices, except Sluch River where RF index is the best among medium rivers taking into account similar levels of nuclear damages in fish. Definitive explanation of this fact causes some difficulties. In this case we can consider both an experimental error and different intensity of destabilizing factors manifestation in hydro-ecosystems at the time of the research.

For a more objective understanding of the relationship between the homeostasis of fish in the small and medium rivers, we used simple regression analysis. While establishing a linear relationship, we accepted RF as a variable x and the frequency of the nuclear damages in *P. fluviatilis* samples from the relevant observation sites of each river as a variable y (fig. 4).

We have observed the most notable functional relationship between the morphological and cytogenetic homeostasis in the sample *P. fluviatilis* from Ustia River that had an approximation factor $r^2 = 0.9306$ and could be described by the equation $y = 9.9094\ x + 0.6536$. It also should be noted that there was a "close" relationship between the studied parameters for the fish from other small rivers. Thus, for Zamchysko River the dependence was $r^2 = 0.8924$ with the corresponding equation $y = 13.964\ x -0.4294$; for Stubelka River it was $r^2 = 0.7927$ with the equation $y = 4.9273\ x +1.4864$; for Ikva River it was $r^2 = 0.7535$ with $y = 6.6991\ x +2.3923$.

Table 3. The statistical validation of data about Fluctuating Asymmetry of *P. fluviatilis* Bilateral Meristic Features in the Rivers of Rivne Region

River	Statistical indicators					
	M	Std. Dv.	± m	t-value	df	p
Styr	0.36666	0.20000	0.03849	9.52628	26	0.000001
Sluch	0.33277	0.19981	0.02479	7.06572	17	0.000002
Horyn	0.30944	0.17086	0.04027	7.68376	17	0.000001
Ustia	0.37851	0.20500	0.03945	9.59394	26	0.000100
Zamchysko	0.38611	0.10170	0.02927	16.10754	17	0.000010
Stubelka	0.37777	0.22791	0.07597	4.97265	8	0.001089
Ikva	0.35518	0.17115	0.03923	10.78298	26	0.000010

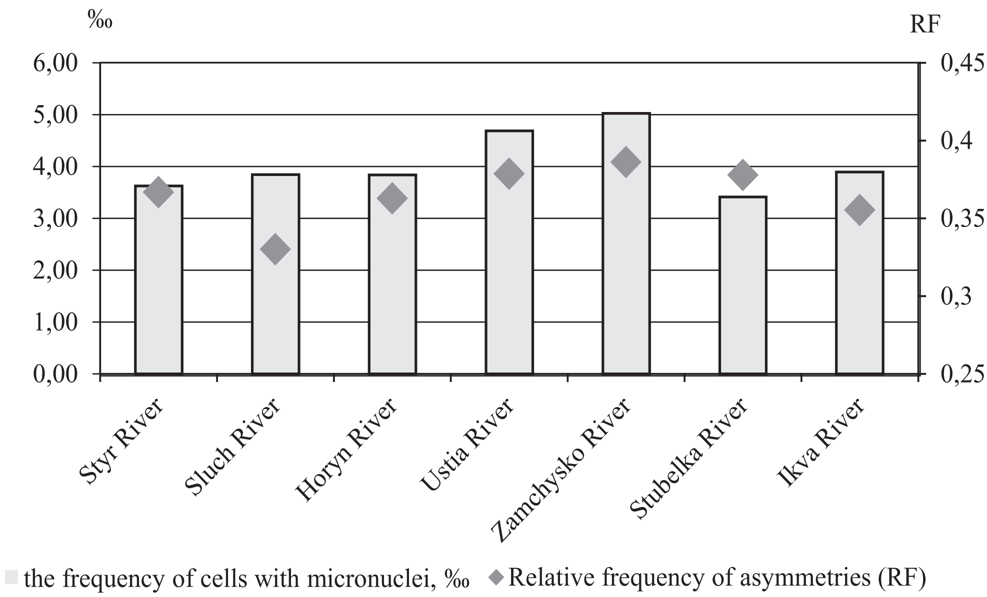


Fig. 3. Comparison of Morphological and Cytogenetic Homeostasis Samples of *P. fluviatilis* in the Rivers of Rivne Region.

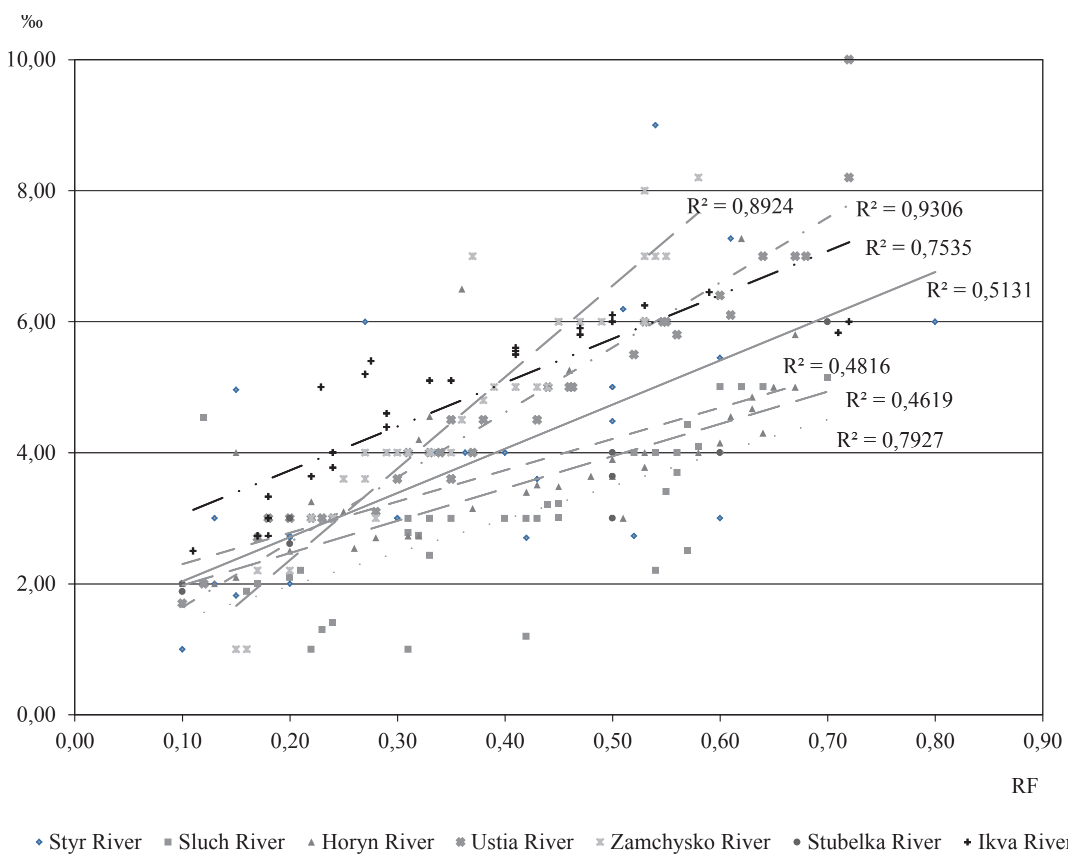


Fig. 4. The Linear Dependence between the Relative Frequency of Asymmetries of Morphological Characters (RF) and Levels of Nuclear Damages (‰) for *P. fluviatilis* in the Rivers of Rivne Region.

The studied parameters of *P. fluviatilis* demonstrated "moderate" relationship in medium rivers, in particular in the samples from Sluch River: $r^2 = 0.4619$ at $y = 5.0791x + 0.953$; and Horyn River: $r^2 = 0.4816$ with $y = 4.7795x + 1.8237$. The relationship between the homeostasis indices of the fish from Styr River was "medium", as testified by the approximation factor $r^2 = 0.5131$ with the linear equation $y = 6.7395x + 1.3654$.

It is a common fact that fish living in small rivers undergo more significant stress factors, owing to such features of a hydro-ecosystem as: slow flow rate, low depth, low water usage and poor self-cleaning ability from natural and human-caused pollution. Jointly, this affects on the physiological characteristics of fish (Demchenko, 2011) which has adaptive value in stressful conditions (Lugas'kova, 2003). We should also mention that the intensity of destabilizing processes manifestation depends on the strength and origin of the impact factors. It certainly requires further study of the issue, taking into account the direct and indirect conditions of the environmental changes in the rivers of Rivne Region.

Conclusions

The results of the study suggest that the representatives of the *P. fluviatilis* species in all investigated rivers of Rivne Region have signs of the violation of morphological and cytogenetic homeostasis of their organisms.

Were statistically significant levels of Nuclear Damages of Peripheral Blood Erythrocytes the studied fish — within 3.41 ± 0.38 ‰ — 5.02 ± 0.51 ‰ ($p \leq 0.05$). This indicates cases exceedances of spontaneous mutagenesis. There were also statistically significant the value of the Fluctuating Asymmetry Bilateral Meristic Features — within 0.33 ± 0.02 — 0.39 ± 0.03 ($p \leq 0.05$). In most cases, this is regarded as a middle tier of the development stability *P. fluviatilis.*

Thus, such violation is typical for the representatives of the species living in small rivers to a great extent.

The research has also established a "close" functional relationship between the studied homeostasis indicators in the samples from small rivers; the author has regarded this fact as a deterioration of living conditions of *P. fluviatilis* due to enhanced activity of stressors that exist in the hydro-ecosystem today.

References

Ajvazjan, S. A. 1985. *Applied Statistics*. Finance and Statistics, Moskow, 1–487 [In Russian].

Biedunkova, O. A. 2015. On the question of eco-toxicological assessment of surface water, *Bulletin of the University of Brest: Chemistry, Biology, Earth Sciences*, **1**, 5–13 [In Russian].

Demchenko, V. O. 2011. Theoretical and practical aspects of the use of fish as indicators of hidroekosystem (for example, the Azov Sea). *Nauk. zap. Ternop. nac. ped. un-tu. Ser. Biol.* **2** (47), 26–31 [In Ukraine].

Dgebuadze, Ju. Ju. 2001. *Environmental variability patterns of fishgrowth*. Nauka,Moskow, 1–276 [In Russian].

Il'inskih, N. N. 1988. Using the micronucleus test in screening and monitoring of mutagens using the micronucleus test in screening and monitoring of mutagens. *Cytology and Genetics*, **22**, 67–71 [In Russian].

Krysanov, E. Ju. 1987. *Aneuploidy and chromosomal mosaicism in fish (for example, representative sof Cyprinidontidaeand Synbranchidae)*. Ph.D thesis, A. N. Severtsov *Institute of Evolutionary Morphology* and *Animal Ecology,* USSR Moscow, 1–20 [In Russian].

L'juis, S. M., Bjejn, B., Bjejts, I. 2009. *Practical and laboratory hematology*. GJeOTAR-Media, Moskow, 1–672 [In Russian].

Lugas'kova, N. V.2003. Species specificity of cytogenetic instability fish in a eutrophic reservoir. *Ecology*, **3**, 235–240 [In Russian].

Moiseenko, T. I. 1998. Hematological parameters in fish in assessing of their toxicosis (example white fish (*Coregonus lavaretus*). *Voprosy ihtiologii*, **38**, 371–380 [InRussian].

Pravdin, I. F. 1966.*Guide to the study of fish*. Pishhevaja promyshlennost', Moskow, 1–374 [InRussian].

Selezneva, A. V. 2007. *Environmental regulation of anthropogenic load on waterbodies*. Samar. NCRAN, Samara, 1–107 [In Russian].

Sondak, V. V. 2010. Preservation of species diversity, natural conditions of reproduction and conservation of fishery resources in the river network in western Polissya Ukraine. *Rybogospodars`ka nauka Ukrayiny*, **2**, 99–110 [In Ukraine].
Zaharov, V. M., Chubinishvili, A. T., Dmitriev, S. G. et al. 2000. *Environmental Health: Practice evaluation*. Center for environmental health, Moskow, 1–320 [InRussian].

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