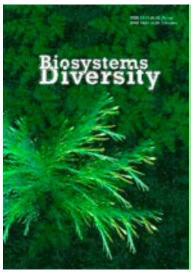

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Influence of river water quality on homeostasis characteristics of cypriniform and perciform fish

M. O. Klymenko, O. O. Biedunkova, O. M. Klymenko, I. I. Statnyk

National University of Water and Environmental Engineering, Rivne, Ukraine

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National University of Water and Environmental Engineering, Sobornaya st., 11, Rivne, 33028, Ukraine.
Tel.: +38-066-913-83-92.
E-mail: bedunkovaolga@gmail.com, kaf-ecology@nuwm.edu.ua

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Within an integrated ecosystem approach, it is preferable to evaluate the effects of pollution of surface waters through research on the organisms of fish. The aim of this study was to analyse the impact of a set of water quality indicators on the homeostasis of fish, in order to determine the response of a hydro-ecosystem to the impact of human activity. Fish samples were obtained from control catches in 16 control sites located in the rivers of Rivne Oblast which differ in intensity of anthropogenic load. The researchers observed that increased concentrations of phosphates and suspended substances, heavy metals, iron, fluorides and nitrogen compounds have violated the environmental state of the examined hydro-ecosystems. Parameters of the morphological homeostasis were assessed by the levels of the fluctuating asymmetry of the meristic signs of fish.The scientists recorded significant impairments (within IV points of body stability) in case of roach and bleak in the majority of the control sites. We carried out the analysis of cytogenetic parameters of fish homeostasis using a micronuclear test of blood erythrocytes.The investigation revealed a significant excess of spontaneous mutagenesis (1.1–1.7 times) in such species as roach, bleak and perch, and this is certainly a clear indicator of unfavourable ecological conditions of the water environment in seven areas of hydro-ecosystems. Given the results of the analysis, the authors found that different ecological groups of fish have their own complex and multifactorial processes of morphological and cytogenetic homeostasis formation. Furthermore, the regression dependences set out in the paper indicated the decisive impact of the oxygen regime of the water environment (COD, BOD_5, O_2), pollutants (Cu^{2+}, Zn^{2+}, Mn^{2+}), and substances of biogenic group (NH_4^+, NO_2^-, PO_4^-) upon fish homeostasis. differences in scope of homeostasis characteristics of different fish species were complemented by the differences in the composition of the regression equations. In particular, in case of species that had signs of homeostasis violation, the equation consisted of a greater number of members. The dependences for morphological and cytogenetic homeostasis of bleak and roach appeared to multifactorial. This finding suggests that these species are sensitive local indicators of the water environment both at early and late stages of ontogeny . Finally, as an outcome of the research we obtained prognostic forms of the relationship between water quality indicators and fish homeostasis that may form the basis of an environmental assessment method in which fish characteristics are used to assess the health of hydro-ecosystems.

Keywords: hydro-ecosystems; indicators; development instability; micronuclear violations

Introduction

Unlike conventional methods of biological indication under which experts rate surface water quality by the signs of irregularities in subsystems, the concept of ecosystem health substantiates quantitatively variable informative criteria of violations in biological systems, as well as integrated quantitative values of dose exposure that reflect the effects of complex water pollution and such water conditions in which toxic elements and compounds may act (Attri and Depledenge, 1997; Ogden et al., 2014; O'Brien et al., 2016).

Certain scientists believe that this integrated system of criteria evaluation of water quality and hydro-ecosystem health must meet the following requirements, particularly (1) reflect the details of pollution, (2) consider the number of the most sensitive indicators at the level of organism, population and ecosystem (Nunes et al., 2011), (3) consider functional reserve of the ecosystem to withstand stress (Xu et al., 2014), (4) maintain its structure and reflect the ability to restore the system after the effects of negative factors (Gilvear et al., 2013; Deng et al., 2015).

There is no single universal criterion regarding the assessments of all possible scenarios of change in hydro-ecosystems. For instance, during the assessment of water eutrophication, the changes in phytoplankton community structure allow the researchers to shape the most accurate picture (Karadžić et al., 2010), in the case of water acidification those would be changes in benthic organisms (Halsband & Kurihara, 2013; Velez et al., 2016), in the case of toxic pollution those would be disturbances in the organisms of fish (Lazorchak et al., 2003; He et al., 2012).

Within an integrated ecosystem approach, it is preferable to evaluate the environmental effects of pollution through research on the organisms of fish. In particular, such world monitoring systems as Environmental Monitoring and Assessment Program (1992), Mid-Atlantic Highlands Assessment (1997), European Environment Agency (2003), Australian and New Zealand Guidelines for Fresh and Marine Water Quality (2000), Environment Canada (2004), etc. use feedback from certain functional systems of fish in cases of long-term water pollution, especially when these are of a toxic nature.

In view of the abovementioned facts, scientists continue to refine the approaches of systems research on the state of fish at the level of individuals and offer informative hydro-ecosystem controls (Klymenko et al., 2016; Gold-Bouchot et al., 2017; Lu et al., 2017; Morán et al., 2018). Thus, in case of heavy pollution, the body resistance of fish was determined by their ability to effectively metabolize and remove toxic substances which enter their body (Boscher et al., 2010; Ramzy, 2014). Pathological changes in the organisms of fish allowed researchers to determine the toxicity of the water environment (Torres, 2014), assessing the cumulative effects (Gutiérrez et al., 2015; Jayaprakash et al., 2015), and to form an idea of the potential hazards of the substances entering the water environment, particularly for humans (Dorea, 2008; Cavas, 2011). Redistribution of toxic substances between fish tissues is used to estimate the timing of contamination of the water bodies (Albalat et al., 2002).

The aim of our research was to analyse the impact of a set of water quality indicators of the rivers of Rivne Oblast (Ukraine) upon the homeostasis of fish, in order to determine the response of the hydro-ecosystem to the impact of human activity. Therefore, the paper envisaged using a combination of representative and relatively simple (rapid) methods that are able to reflect the effects of the combined effects of pollution of the hydro-ecosystems and give an integrated response regarding their health.

Materials and Methods

Study areas, control catch sites and fish species. The analysis of water quality and fish homeostasis in rivers of Rivne Oblast was conducted during the low summer flows in 2013–2015. The research was conducted within the ecoregion of Europe No. 16 in small and medium rivers, right-bank tributaries of the Prypiat River.

While choosing the representative control sites we strictly followed the "Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy" or, in short, the EU Water Framework Directive (or even shorter the WFD) which is closely linked with the European Environment Agency's Monitoring and Information Network for Inland Water Resources EUROWATERNET which is designed for the analysis of a national water monitoring database and provides a clear distinction between good and poor quality water (Directive 2000/60/EC).

The fish samples were obtained from the control catches in 16 control sites located in the rivers of Rivne Oblast (Fig. 1) which differ by intensity of anthropogenic load (Table 1).

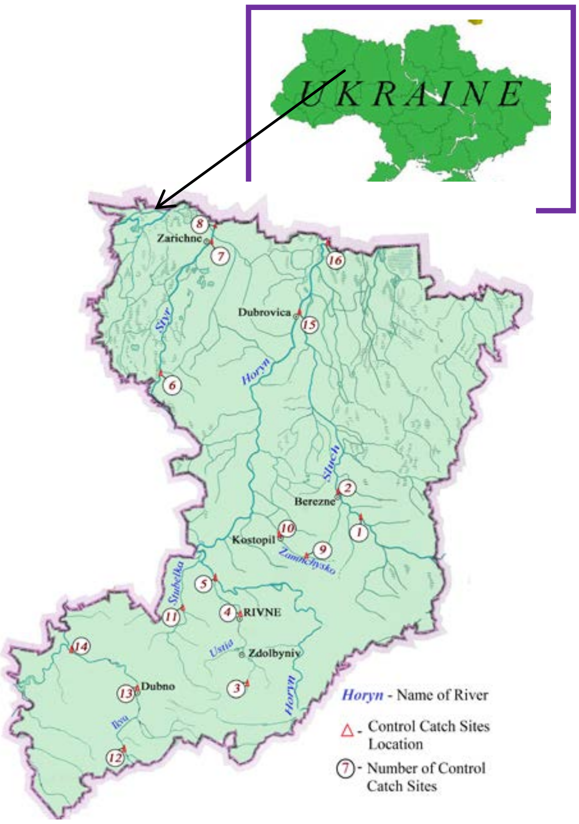


Fig. 1. Situation map displaying the layout of control catch sites along the rivers of Rivne oblast

Table 1
Control catch sites in the rivers of Rivne Oblast

Site No.	Administrative location and site description	The distance from the mouth, km	The geographical coordinates
1	Sluch River, in Bystrychi village, above the wastewater discharge	94.5	50° 52' 52.6" N 26° 55' 24.2" E
2	Sluch River, in the city of Berezne, 0.6 km below the wastewater discharge	73.4	51° 00' 50.4" N 26° 45' 35.1" E
3	Ustia River, upper part, natural background, near the village of Ivachkiv	65.0	50° 28' 49.8" N 26° 19' 06.2" E
4	Ustia River, in the city of Rivne, below the wastewater discharge	21.0	50° 36' 21.7" N 26° 15' 14.3" E
5	Ustia River, in Orzhiv village	0.7	50° 45' 21.2" N 26° 07' 37.6" E
6	Styr River, 0.5 km below the industrial wastewater discharge of the Rivne Nuclear Power Plant	167.5	5 1° 21' 33.2" N 25° 50' 39.0" E
7	Styr River, in Zarichne urban-type settlement, 0.5 km below the wastewater discharge	75.8	51° 49' 08.9" N 26° 08' 56.8" E
8	Styr River, in Ivanchytsi village, river flows to Belarus, 4 km to the border	74.0	51° 50' 36.7" N 26° 10' 00.3" E
9	Zamchysko River, Mala Liubasha village, above the wastewater discharge	21.5	50° 50' 33.4" N 26° 29' 42.7" E
10	Zamchysko River, in the city of Kostopil, below the wastewater discharge	11.9	50° 53' 50.4" N 26° 26' 24.2" E
11	Stubelka River, Klevan urban-type settlement, below the wastewater discharge	7.8	50° 46' 26.5" N 25° 58' 06.8" E
12	Ikva River, Sopanivchyk village, on the border with Ternopil Oblast	80.5	50° 10' 59.9" N 25° 43' 01.1" E
13	Ikva River, 3.2 km below the Dubno wastewater discharge	39.6	50° 26' 07.5" N 25° 44' 27.5" E
14	Ikva River, in Torhovytsia village, Mlyniv Raion (district)	1.5	50° 33' 40.0" N 25° 23' 47.5" E
15	Horyn River, in the city of Dubrovytsia, 0.5 km below the wastewater discharge	104.0	51° 34' 41.2" N 26° 35' 33.9" E
16	Horyn River, in Vysotsk village, at the Ukrainian-Belarusian border	77.5	51° 43' 28.9" N 26° 40' 18.8" E

Samples of the following commonest river fish species in the region were obtained from the control catches in representative control sites located in the rivers of Rivne Oblast in the summers of 2013–2015 . Cypriniformes were represented by the following species of fish: common bleak (*Alburnus alburnus* (Linnaeus, 1758), common roach (*Rutilus rutilus* (Linnaeus, 1758)), common rudd (*Scardinius erythrophthalmus* (Linnaeus, 1758)), silver crucian carp (*Carassius auratus gibelio* (Linnaeus, 1758)), common bream (*Abramis brama* (Linnaeus, 1758). Perciformes were represented by the European perch (*Perca fluviatilis* (Linnaeus, 1758)). The sample size for each species was from 16 to 38 fish, with age categories from 1+ to 4+.

Indices of water quality. The analysis of surface water quality in the studied hydro-ecosystems was conducted according to the following hydro-chemical parameters: sulphates (SO_4^{2-}), chlorides (Cl^-), ammonia nitrogen (NH_4^+), nitrate-nitrogen (NO_3^-), nitrogen dioxide (NO_2^-), phosphorus in the form of phosphates (PO_4^-), the rate of chemical oxygen demand (COD), the rate of biochemical oxygen demand in five days (BOD_5), suspended solids (SS), dissolved oxygen (O_2), pH, iron (Fe^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), manganese (Mn^{2+}), fluorides (F_2). The data on these substances in the studied rivers of Rivne Oblast was obtained as a result of systematic analytical observations made in the State Surface Water Monitoring Laboratory in Rivne Oblast. The data were compared with the permissible concentrations of substances in fisheries (Water Quality Standards of Fishery. Resolution of the Cabinet of Ministers of Ukraine, 1999).

Indices of morphological fish homeostasis. The assessment of morphological fish homeostasis was performed by levels of fluctuating asymmetry (FA) of meristic bilateral symptoms values according to the measurements of the right and left sides ($|R–L|$) of fish: the number of bony spines in the pectoral and pelvic fins; the number of gill rakers on the first gill arch; the number of petals in gill membranes; number of scales in the lateral line; number of scales with sensory tubules; number of scale rows above and below the lateral line; the number of scales on the side of the tail fin. The study used relative frequency of asymmetries (RF) as an asymmetry indicator, which was calculated as the ratio of the number of signs that show asymmetry to the total number of recorded signs (Zaharov & Chubinishvili, 2000):

$$RF = \frac{\sum_{i=1}^{k} Ai}{n \cdot k},$$

where Ai is the number of asymmetrical manifestations of the i sign (number of individuals, asymmetric on the basis); n is the sample size; k is the number of signs.

The assessment of development instability deviation from conventionally normal state was performed according to the Zakharov-Chubinishvili scale (Table 2).

Table 2
Scale for assessing fish deviations from normal state

No. of points	Development Instability Indicator, RF	Quality of Environment
1	< 0.30	relatively normal
2	0.30–0.34	initial (minor) deviations from the norm
3	0.35–0.39	average abnormalities
4	0.40–0.44	substantial (significant) deviations from the norm
5	0.45 and >	critical condition

Indices of morphological fish homeostasis. Evaluation of cytogenetic homeostasis was performed by erythrocyte micronucleus test with peripheral blood. The blood was taken from the fishtail artery and the samples included age groups from one-year-old to four-year-old fish. The staining of blood smears was 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) (Il'inskih, 1988). Micronuclei were examined at 10 ×100 magnification under immersion microscopy. All kinds of micronuclei and nuclear material were taken into account during the cell calculation (Ledebur & Schmid, 1973). From 1000 to 2500 cells of each individual were analysed. The results were expressed in per mille notation (‰).The research considered that 4‰ shall be the level of spontaneous mutagenesis, which is also substantiated by a number of authors (Ledebur & Schmid 1973).

Data analysis. All statistical analyses (ANOVA) were performed in the software package Statistica 8.0 (Statsoft Inc., USA). The data of research results were presented as the mean value for the sample (M) and the standard deviation (± SD).

In order to understand the mechanism of complex impact of hydro-ecosystem quality parameter indicators upon fish homeostasis, the authors conducted multivariate regression analysis The results of multivariate regression analysis were assessed with the use of the pleiades correlation method. Apart from the pleiades analysis we have also taken into account the following parameters: G – pleiade capacity (number of pleiade members); r – total correlation coefficient of a pleiade; F – Fisher criterion value for the pleiade; p – p-level of statistical significance for the pleiade; B – intercept term; b – regression coefficient of individual pleiade members (Hoaglin et al., 2000).

Results

The water quality indicators in the control sites are represented as average values for particular river hydro-ecosystems by the maximum and average concentrations of substances that occurred during the years of observation (Table 3).

Parameters of salt water composition, the content of dissolved oxygen and medium reaction (pH) were within the norm. At the same time, the surface waters of the rivers of Rivne Oblast are polluted with phosphates and suspended solids, heavy metal ions (Cu^{2+}, Zn^{2+}, Mn^{2+}) and iron, fluorides and a group of nitrogenous substances. It is important to bear in mind that the regulations for fisheries do not foresee the valuation of such parameters of water oxygen regime as COD and BOD_5.

Table 3
Indices of water quality in river hydro-ecosystems of Rivne Oblast, average for 2013–2015
(data of observation in the State Surface Water Monitoring Laboratory in Rivne region; Repeatability of each indicator, n = 5)

Substance	Control Sites #															
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
SO_4^{2-}, mg/dm^3	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
Cl^-, mg/dm^3	23.4	23.9	21.3	27.4	26.6	13.3	16.3	13.7	1.8	14.1	21.3	20.6	13.9	30.8	18.4	19.8
NH_4^+, mg/dm^3	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
NO_3^-, mg/dm^3	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
NO_2^-, mg/dm^3	0.02	0.05	0.20*	0.20*	0.10*	0.10*	0.10*	0.10*	0.04	0.20*	0.10*	0.30*	0.20*	0.10*	0.10*	0.10*
PO_4^-, mg/dm^3	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*
Suspended solids, mg/dm^3	10.9*	13.5*	11.3*	12.3*	14.0*	11.7*	10.9*	7.9*	9.2*	13.1*	2.2*	151.0*	10.1*	10.4*	9.0*	9.9*
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
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
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/dm^3	258*	299*	284*	155*	125*	124*	216*	182*	937*	478*	180*	196*	269*	166*	380*	181*
Cu^{2+}, mkg/dm^3	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*
Zn^{2+}, mkg/dm^3	8.3	19.2*	19.7*	40.9*	58.1*	17.0*	13.5*	17.9*	25.3*	14.0*	21.0*	87.3*	84.0*	19.0*	26.0*	24.4*
Mn^{2+}, mkg/dm^3	36.0*	42.0*	37.0*	42.7*	10.0*	17.8*	29.0*	25.9*	38.3*	42.3*	12.0	51.7*	23.5*	22.3*	12.0	35.0*
F_2, mkg/dm^3	27	130*	190*	315*	300*	217*	150*	70*	277*	289*	255*	110*	137*	416*	597*	249*

Note: the noted data were checked for compliance with 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.

Thus, the main reasons that contribute to the deterioration in water quality in regional rivers include the flow of inadequately treated wastewater, as well as significant levels of anthropogenic load due to the urbanization of the fishing areas adjacent to the channel. Without a doubt, this situation causes certain hazards in hydro-ecosystems, which in their combination and interaction with natural hydro-chemical parameters of natural freshwaters can negatively affect the local biota, including the representatives of the studied fish fauna.

In order to assess the morphological characters of fish homeostasis in the river hydro-ecosystems of Rivne Oblast, the authors evaluated samples of 6 fish species for 9 bilateral symptoms. Based on the received results, the researchers calculated relative frequency of asymmetries (RF) which, in its turn, verifies the levels of fluctuating asymmetry of morphological characters and indicates the development instability of fish fauna representatives. Therefore, among the 6 analysed fish species of the studied region, in most cases, roach and bleak demonstrated the highest levels of fluctuating asymmetry (average rate of IV points along the control sites). The morphological homeostasis of perch and rudd was slightly better, with the average development instability in the analysed samples of nearly III points (Table 4).

FA averages of bream samples indicated II points for the stability of the species' development in regional river hydro-ecosystems, while the silver crucian carp sample showed I point respectively. Apparently, different representatives of fish fauna have different sensitivities to environmental conditions.

Comparison of morphological fish homeostasis in control sites indicates that the representatives of fish fauna in sites No. 4, 10 and 13 demonstrate the highest RF values, and therefore the worst development instability. This fish reaction shows the enhanced effect of unfavourable factors in the water environment.

Roach and perch indicated the highest average frequency of nuclear violations in all control sites (5.22 ± 0.29‰ and 4.10 ± 0.21‰ respectively). In case of bleak, its average value for the control sites was 3.76 ± 0.25‰ (Table 5).

Table 4
Results of development instability assessment for studied fish species in the rivers of Rivne Oblast

Control Sites No.	Fish species and sample size for each species*					
	common bleak n = 21–27	common roach n = 17–32	common rudd n = 17–35	European perch n = 16–32	silver crucian carp n = 17–38	common bream n = 17–22
1	0.38 ± 0.27	0.42 ± 0.18	0.33 ± 0.13	0.32 ± 0.16	0.13 ± 0.05	0.34 ± 0.21
2	0.34 ± 0.19	0.45 ± 0.18	0.36 ± 0.14	0.35 ± 0.25	0.15 ± 0.08	0.39 ± 0.17
3	0.38 ± 0.23	0.36 ± 0.19	0.40 ± 0.17	0.34 ± 0.18	0.19 ± 0.13	0.34 ± 0.23
4	0.46 ± 0.22	0.53 ± 0.19	0.40 ± 0.21	0.42 ± 0.21	0.28 ± 0.13	0.45 ± 0.15
5	0.48 ± 0.26	0.46 ± 0.21	0.38 ± 0.21	0.38 ± 0.24	0.24 ± 0.14	0.23 ± 0.12
6	0.39 ± 0.07	0.37 ± 0.07	0.34 ± 0.06	0.30 ± 0.08	0.20 ± 0.04	0.34 ± 0.09
7	0.46 ± 0.10	0.44 ± 0.06	0.34 ± 0.08	0.38 ± 0.08	0.24 ± 0.06	0.30 ± 0.05
8	0.33 ± 0.06	0.37 ± 0.04	0.24 ± 0.05	0.42 ± 0.06	0.19 ± 0.05	0.33 ± 0.06
9	0.46 ± 0.05	0.45 ± 0.12	0.30 ± 0.14	0.33 ± 0.11	0.17 ± 0.13	0.35 ± 0.13
10	0.47 ± 0.18	0.51 ± 0.14	0.37 ± 0.14	0.44 ± 0.06	0.32 ± 0.09	0.42 ± 0.12
11	0.41 ± 0.07	0.46 ± 0.10	0.39 ± 0.09	0.38 ± 0.08	–	0.31 ± 0.06
12	0.44 ± 0.05	0.45 ± 0.07	0.47 ± 0.08	0.33 ± 0.04	0.26 ± 0.02	0.27 ± 0.05
13	0.49 ± 0.06	0.47 ± 0.04	0.47 ± 0.06	0.38 ± 0.07	0.22 ± 0.04	0.39 ± 0.08
14	0.48 ± 0.06	0.42 ± 0.09	0.42 ± 0.08	0.36 ± 0.07	0.24 ± 0.07	0.34 ± 0.05
15	0.26 ± 0.05	0.31 ± 0.06	0.28 ± 0.05	0.32 ± 0.05	0.20 ± 0.04	0.40 ± 0.05
16	0.28 ± 0.06	0.37 ± 0.07	0.30 ± 0.07	0.31 ± 0.07	–	0.27 ± 0.06

Note: the sample size for each species varied depending on the number of fish in the catches in control sites; dash indicates that the indicated species was absent from the catches.

Table 5
Average frequency of micronuclei in peripheral blood erythrocytes of various fish species in the control catch sites in the rivers of Rivne Oblast (‰)

Control Sites No.	Fish species*					
	common bleak	common roach	common rudd	European perch	silver crucian carp	common bream
1	2.86 ± 0.26	4.57 ± 0.42	2.38 ± 0.11	3.40 ± 0.29	1.45 ± 1.21	1.83 ± 0.29
2	3.56 ± 0.23	6.02 ± 0.19	3.21 ± 0.17	3.84 ± 0.15	1.47 ± 0.13	1.60 ± 0.18
3	2.45 ± 0.41	3.99 ± 0.56	3.58 ± 0.32	4.25 ± 0.48	1.61 ± 0.26	2.18 ± 0.23
4	5.52 ± 0.45	6.92 ± 0.89	3.98 ± 0.46	5.63 ± 0.62	1.40 ± 0.19	4.83 ± 0.37
5	4.93 ± 0.55	5.96 ± 0.29	3.01 ± 0.26	4.18 ± 0.44	1.45 ± 0.18	1.57 ± 0.22
6	2.66 ± 0.31	4.57 ± 0.34	2.40 ± 0.17	3.43 ± 0.23	1.50 ± 0.18	3.32 ± 0.32
7	3.52 ± 0.32	5.62 ± 0.59	3.86 ± 0.44	4.73 ± 0.78	1.89 ± 0.59	4.23 ± 0.65
8	2.57 ± 0.35	3.47 ± 0.45	2.92 ± 0.24	3.36 ± 0.51	1.16 ± 0.17	2.23 ± 0.16
9	4.41 ± 0.44	5.91 ± 0.52	3.27 ± 0.41	4.21 ± 0.48	1.46 ± 0.27	3.43 ± 0.40
10	4.80 ± 0.49	6.78 ± 0.66	3.69 ± 0.58	5.83 ± 0.67	1.81 ± 0.26	4.83 ± 0.54
11	3.21 ± 0.62	5.14 ± 0,39	2.75 ± 0.41	3.41 ± 0.40	–	2.99 ± 0.22
12	3.79 ± 0.40	5.59 ± 0.49	3.74 ± 0.40	3.04 ± 0.39	1.50 ± 0.28	2.24 ± 0.29
13	4.70 ± 0.58	6.19 ± 0.36	3.87 ± 0.45	4.37 ± 0.41	1.64 ± 0.23	3.83 ± 0.47
14	4.81 ± 0.61	5.72 ± 0.73	3.28 ± 0.49	4.26 ± 0.37	1.62 ± 0.26	2.01 ± 0.25
15	2.75 ± 0.32	3.24 ± 0.23	2.60 ± 0.19	4.57 ± 0.34	1.20 ± 0.24	2.90 ± 0.28
16	3.54 ± 0.38	3.82 ± 0.44	2.13 ± 0.41	3.10 ± 0.33	–	2.92 ± 0.34

Note: From 1000 to 2500 blood erythrocytes were analyzed for each fish species; dash indicates that indicated species of fish was absent from the catches.

The average frequency of nuclear violations appeared to be even lower for rudd (3.17 ± 0.15‰) and bream (2.93 ± 0.28‰). The lowest average levels of nuclear disorders in the control sites along the rivers of Rivne Oblast were typical for silver crucian carp (1.51 ± 0.06‰).

The provided values confirm that there is some excess of spontaneous mutagenesis for roach and a slight excess for perch. In addition, in certain control sites average values for bleak and bream samples showed excess levels of spontaneous mutagenesis red blood cells (RBCs) of peripheral blood.

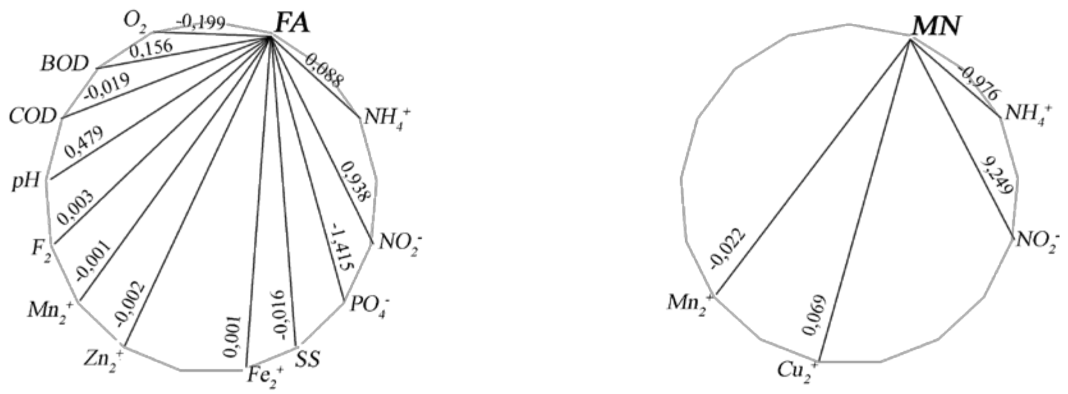
Nonetheless, our results with regard to conducted micronucleus test of the RBCs of the fish of Rivne Oblast suggest that fish fauna representatives in seven areas of the studied hydro-ecosystems (sites No. 1, 4, 5, 7, 9, 10, 13) out of 16 control sites demonstrated clear signs of nuclear violations of erythrocytes. In particular, four of the six studied species (bleak, roach, perch and bream) in the sites No. 4 and 10 showed excess levels of spontaneous mutagenesis (1.2–1.7 times). We observed excess levels (1.1–1.5 times) in the control sites No. 5, 9 and 13 in the samples of three species: bleak, roach and perch. The control site No. 7 appeared to be one more area where we recorded excess levels of spontaneous mutagenesis (1.1–1.4 times) for three fish species (roach, perch, and bream).

The next stage of our research was the elucidation of the combinations of water quality parameters that affect the asymmetry of morphological characteristics and levels of nuclear violations of various fish species. Thus, in case of bleak the pleiade capacity of the combined impact of hydro-chemical parameters on the formation of morphological structures' asymmetry (FA) had 12 members with statistically significant correlation coefficient r = 0.99. The formation of bleak micronucleus violations (MN) could be described by a less powerful pleiade (G = 4) with r = 0.85 (Fig. 2). Therefore, the formation of morphological and cytogenetic characteristics of bleak in the region, with a sufficiently high probability, can be described by the following regression equations:

$$FA = -1.624 + 0.088(NH_4^+) + 0.938(NO_2^-) - 1.415(PO_4^-) - 0.016(SS) + 0.001(Fe^{2+}) - 0.002(Zn^{2+}) - 0.001(Mn^{2+}) + 0.003(F_2) + 0.479(pH) - 0.019(COD) + 0.156(BOD_5) - 0.199(O_2)$$

$$MN = 6.34 - 0.976(NH_4^+) + 9.249(NO_2^-) + 0.069(Cu^{2+}) - 0.022(Mn^{2+})$$

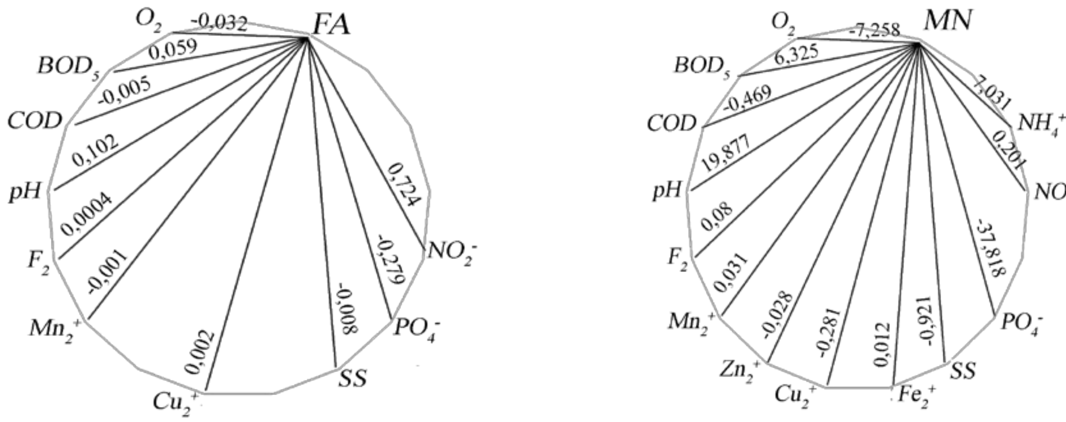
In case of roach, the correlation pleiade of the complex impact of hydro-chemical parameters on fluctuating asymmetry had 10 members (G = 10) with a statistically significant correlation coefficient r = 0.99. The micronucleus violations of this species depended on the presence of more substances in the water (G = 13), with an overall r = 0.99 (Fig. 3).



G = 12; r = 0.990; F = 14.52; P = 0,046; B = –1.62

G = 4; r = 0.851; F = 6.34; P = 0,008; B = –2.60

Fig. 2. Correlation pleiades for the impact of hydro-chemical parameters of the rivers of Rivne Oblast on bleak homeostasis: G – pleiade capacity (number of pleiade members); r – total correlation coefficient of a pleiade; F – Fisher criterion value for the pleiade; P – p-level of statistical significance for the pleiade; B – intercept term; b – regression coefficient of individual pleiade members; ——— regression coefficients with a P-level of $P < 0.05$; - - - - - regression coefficients with a P-level of $P > 0.05$



G = 10; r = 0.99; F = 33.55; P < 0.002; B = –0.13

G = 13; r = 0.99; F = 416.32; P = 0.003; B = –88.89

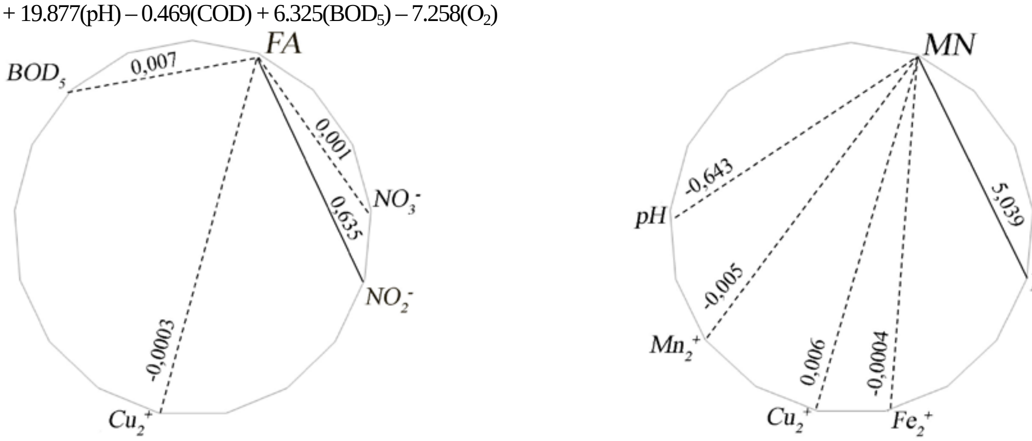
Fig. 3. Correlation pleiades for the impact of hydro-chemical parameters of the rivers of Rivne Oblast on roach homeostasis: the designation corresponds to Fig. 2

Consequently, the formation of roach homeostasis characteristics in the regional rivers, with sufficiently high probability, can be described by the following regression equations:

$$FA = -0.134 + 0.724(NH_4^+) - 0.279(PO_4^-) - 0.008(SS) + 0.002(Cu^{2+}) - 0.001(Mn^{2+}) + 0.0004(F_2) + 0.102(pH) - 0.005(COD) + 0.059(BOD_5) - 0.032(O_2)$$

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

The correlation pleiade of the impact of substances present in the water on rudd fluctuating asymmetry level consisted of four members ($G = 4$ with $r = 0.77$), but for three of them (NH_4^+, Cu^{2+}, BOD_5) the confidence probability was insufficient for the equation. The formation of micronucleus violations for this species of fish was shown by the pleiade of five members ($G = 5$ with $r = 0.79$). However, for four of them (Fe^{2+}, Cu^{2+}, Mn^{2+}, pH) the confidence probability proved to be unacceptable (Fig. 4).



G = 4; r = 0.77; F = 3.74; P = 0.004; B = 0.27

G = 5; r = 0.79; F = 3.48; p = 0,004; B = 7.76

Fig. 4. Correlation pleiades for the impact of hydro-chemical parameters of the rivers of Rivne Oblast on rudd homeostasis: the designation corresponds to Fig. 2

In this case, the regression equation may be as follows:

$$FA = 0.271 + 0.635(NO_2^-);$$

$$MN = 7.760 + 5.039(NO_2^-).$$

The formation of perch fluctuating asymmetry, under the influence of water quality in the river hydro-ecosystems of the region, was described by the pleiade of two members (G = 2 with r = 0.75). The levels of perch micronucleus violations demonstrated dependence on three water quality indicators (G = 3 with r = 0.71), but two of them (Zn^{2+} and BOD_5) had poor confidence probability (Fig. 5).

Therefore, the regression equations for the formation of perch homeostasis characteristics in the rivers of Rivne Oblast may look as follows:

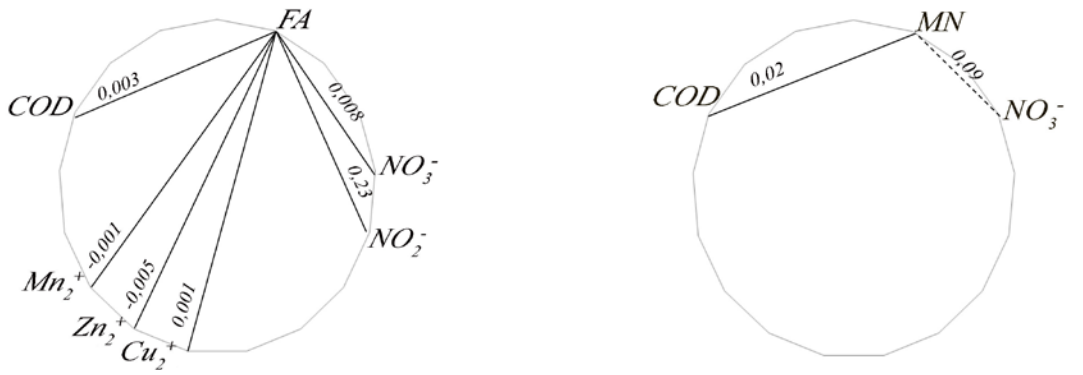
$$FA = 0.35 + 0.002(Cu^{2+}) – 0.001(Mn^{2+})$$

$$MN = 2.86 + 1.42(PO_4^-)$$

The correlation pleiades which described the impact of river water quality on the fluctuating asymmetry of silver crucian carp had six members (G = 6 with r = 0.98), a pleiade that described the effects on nuclear violations had only two members (G = 2 with r = 0.63), with sufficiently high probability of regression coefficients (Fig. 6).

G = 2; r = 0.75; F = 7.81; P = 0.006; B = 0.35 G = 3; r = 0.71; F = 3.81; P = 0.04; B = 2.86

Fig. 5. Correlation pleiades for the impact of hydro-chemical parameters of the rivers of Rivne Oblast on perch homeostasis: the designation corresponds to Fig. 2



G = 6; r = 0.98; F = 38.76; P = 0.0002; B = 0.04 G = 2; r = 0.63; F = 3.91; P = 0.04; B = 0.71

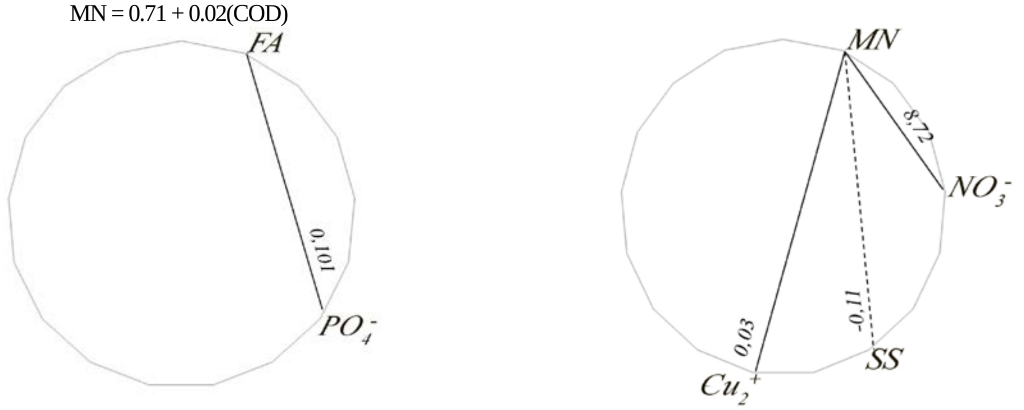
Fig. 6. Correlation pleiades for the impact of hydro-chemical parameters of the rivers of Rivne Oblast on silver crucian carp homeostasis: the designation corresponds to Fig. 2

Consequently, the regression equations for the formation of silver crucian carp homeostasis characteristics in the rivers of Rivne Oblast may look as follows:

$$FA = 0.004 + 0.008(NO_3^-) + 0.23(NO_2^-) + 0.001(Cu^{2+}) – 0.005(Zn^{2+}) – 0.001(Mn^{2+}) + 0.003(COD)$$

$$MN = 0.71 + 0.02(COD)$$

The correlation pleiade which described the impact of river water quality on bream fluctuating asymmetry had only one member (G = 1 with r = 0.55), a pleiade that described the effects on nuclear violations had three members (G = 3 with r = 0.72), with sufficiently high mathematical probability of regression coefficients (Fig. 7).



G = 1; *r* = 0.55; *F* = 5.53; *p* <0.04; *B* = 0.29 *G* = 3; *r* = 0.72; *F* = 3.98; *p* <0.04; *B* = 2.49

Fig. 7. Correlation pleiades for the impact of hydro-chemical parameters of the rivers of Rivne Oblast on bream homeostasis: the designation corresponds to Fig. 2

Therefore, the regression equations for the formation of bream homeostasis characteristics in the river water bodies of Rivne Oblast might look as follows:

$$FA = 0.29 + 0.101(PO_4^-)$$
$$MN = 2.49 + 8.72(NO_3^-) + 0.03(Cu^{2+})$$

Thus, the obtained regression dependences suggest that different species have a complex and multifactorial process of morphological and cytogenetic homeostasis formation. However, the presence of such factors as the oxygen regime of the water environment (COD, BOD_5, O_2), pollutants (Cu^{2+}, Zn^{2+}, Mn^{2+}) and biogenic substances (NH_4^+, NO_2^-, PO_4^-) in the vast majority of regression equations is outstanding. None of the pleiades has established interrelation between fish homeostasis parameters and salt block substances (SO_4^{2-}, Cl^-), and it clearly proves the safety of the concentrations of these substances present in the river water bodies of the region and the lack of synergistic or additive action with other quality indicators of hydro-ecosystems.

Discussion

The most relevant finding of the present study is that the research and analysis of the impact of river water quality parameters in Rivne Oblast on the homeostasis characteristics of fish fauna representatives was conducted for the first time. The results of the research lead to the following conclusions.

Surface river waters are polluted with phosphates, suspended solids, heavy metal and iron ions, fluorides and nitrogen compounds. This situation has been observed over the past decades (Statnyk, 1999; Biedunkova, 2015).

Different representatives of fish fauna have different sensitivity to environmental conditions. In particular, in our studies, such fish species as common roach and common bleak have demonstrated morphological violations (within the rate of IV points of the body development instability); in the present study, we have recorded the development instability of European perch and common rudd at the level of mainly III points, common bream indicated II points, and silver crucian carp carp showed I point respectively. The cytogenetic violations were recorded for such species as roach, bleak and perch; excess levels of spontaneous mutagenesis ranged from 1.1–1.7 times. Similar results have been obtained by other researchers (Rodriguez-Cea et al., 2003; Porto et al., 2005).

Accordingly, morphological homeostasis violations were recorded in four control sites, cytogenetic homeostasis violations – in seven sites. Perhaps, if we consider the features of the approaches taken, there is a chance to receive an explanation. Hence, the rate of cytogenetic homeostasis is an indicator of stress reflecting the favourable environment at the time of fishing (Al-Sabti & Metcalfe, 1995; Minissi et al., 1996; Gutiérrez et al., 2015).

The indicators of development sustainability by the FA levels provide an idea about the conditions in which an organism existed in the early stages of ontogeny, when the studied traits were formed (Parsons 1990; Swaddle, 2003; Vinohradov et al., 2012).

To the best of our knowledge, the difference between the values of the studied characteristics for different fish species also draws substantial attention. Thus, homeostasis violations were mainly recorded for such species as roach, bleak and perch. In some control sites rudd and bream also demonstrated violations. The homeostasis characteristics of silver crucian carp were not recorded in any of the control sites. In certain sites rudd and bream also demonstrated violations. These research results correspond well to other prominent studies and proves that the analysis of the characteristics of the homeostasis of various species of fish is suitable for elucidating the most sensitive local species (Almeida et al., 2008; Rochetta et al., 2014).

In our studies, morphological homeostasis violations were recorded in four control sites, cytogenetic homeostasis violations – in seven sites. This confirmed the unfavourable environmental conditions of the water environment in the corresponding areas of the studied hydro-ecosystems at different ontogenetic stages of the analysed fish species. Many modern scientists believe that this is the effect of combined ratings of water pollution and the background conditions in which harmful elements and compounds act (Arenas-Sánchez et al., 2016; Kalogianni et al., 2017; Zargar et al., 2017; Hussain et al., 2018).

The multivariate regression analysis of the impact of water quality on fish homeostasis confirmed that different species have a complex and multifactorial process of morphological and cytogenetic homeostasis formation. Nevertheless, the obtained regression dependence evidenced decisive influence of such factors as the oxygen regime of the water environment (COD, BOD_5, O_2), pollutants (Cu^{2+}, Zn^{2+}, Mn^{2+}) and biogenic substances (NH_4^+, NO_2^-, PO_4^-) on the formation of fish homeostasis. In other regions, similar studies are conducted. At the same time, the general physiological characteristics of fish (Lemos et al., 2009; Schinegger et al., 2018) or histological characteristics (Ballesteros et al., 2017; Dalzochio et al., 2017) are used as indicators. But, we have obtained regression models for the formation of cytogenetic and morphological homeostasis of river fish in the region under study, for the first time. We believe that fairly simple mathematical models based on a comprehensive comparison of surface water quality parameters and indices of fish homeostasis may be useful for monitoring the river environment, in particular for forecasting the ecological situation and for the control of the biotic component of hydro-ecosystems.

Conclusions

The most relevant finding of the present study is that the research and analysis of the impact of river water quality parameters in Rivne Oblast on the homeostasis characteristics of fish fauna representatives leads to the following conclusions: it is worth mentioning that differentces in scope of homeostasis characteristics of different fish species were complemented by the differences in the composition of the regression equations. In particular, in cases of species that had signs of homeostasis violations, the equation consisted of a greater number of members. The dependences for morphological and cytogenetic homeostasis of bleak and roach appeared to multifactorial. This finding suggests that these species are sensitive local indicators of the water environment both at early and late ontogenetic stages. Finally, as an outcome of the research we obtained prognostic forms of the relationship between water quality indicators and fish homeostasis that may become the basis for an environmental assessment method in which fish characteristics are used practically to assess the health of hydro-ecosystems.

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