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ORIGINAL ARTICLE

Monitoring of morphological parameters of *Cyprinidae* liver

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The method of biomonitoring of natural reservoirs by diagnosing early violations in the most sensitive components of hydrobiological groups has been proved. Morphometric parameters of the liver of the family *Cyprinidae* fish are used as biotest-system for ecological analysis.Taking into account morphometric indexes of fish internal parenchymatous organs, the possibility of predicting the toxic substances influence on the aboriginal ichthyofauna state, has been determined. It has been established that protoplasmic and hemolytic toxicants violate cellular metabolism, causing dystrophy of liver cells in fish. Morphoanalysis of the parameters of the liver of the family *Cyprinidae* fish, as the most widespread object of the ichthyofauna, is used as the basis for indicating in the system of biomonitoring of the water environment quality to the action of external factors and timely prevention of existing danger.The results obtained serve as the basis for the development of environmental measures to assess and optimize the ecological conditions for the conservation of biological diversity of the ecosystem.
Keywords: anthropogenic loading; reservoir; hydroecosystem; ichthyofauna; the liver of the family *Cyprinidae* fish; liver; biomarkers; pollutants; morphological parameters

Introduction

The World ocean and land surface waters are the necessary natural resource for all living on the planet and habitat for hydrobionts. It is now difficult to find water objects that would not have anthropogenic impact. Development and exploitation of catchment, precipitation, industrial, household and household discharges, unorganized drains from catchment areas, etc. cause global changes in the geochemical cycle of elements in the system "catchment – a reservoir" (Vosylіené, 1999; Moiseenko, 2009). In modern industry, agriculture and forestry, utility companies use a huge amount of chemicals that affect the environment. The chemical substances fall into the water, which leads to changes in the water quality, biological balance, the destruction of spawning grounds and nursery grounds, the spread of fish diseases and the reduction of the fish products'quality (Handy et al., 2002; Martinez-Alvarez et al., 2005; Gutyj et al., 2017; Grynevych et al., 2018).
Fundamental research on the impact study of changes in the hydroecosystem on the ichthyofauna state has not been carried out. That's why the priority significance is a comprehensive assessment of the environmental situation. Organizing and conducting environmental monitoring includes, on the one hand, monitoring of the source of anthropogenic impact, and, observing the state of the environment components, on the other hand, in particular the reactions of the hydrobiont organism to the impact of anthropogenic factors. To assess the impact of anthropogenic pollution of the aquatic environment, fish are used both from natural reservoirs and from an aquarium (Lushchak et al., 2001; Beniarz et al., 2003).
The biomonitoring studies on using fish are relevant. A separate category of indicators is morphological markers. The subletal effects of toxic substances on fish cause changes in the histological structure of tissues and organs, which lead to various pathologies which violate their functioning. Morphological markers are direct, sensitive and reliable indicators of the influence of external stressors. The liver is the central laboratory that regulates metabolic processes in the body. There is biotransformation and detoxification of the pollutants in it. That's why it is the first to be exposed to chemical influence, which causes high sensitivity of the liver of the family *Cyprinidae* fish to the effect of various exogenous factors (Schlenk et al., 2008; Prysiazhniuk et al., 2017).

The aim of the work was to find out the morphological changes of the carp species liver of the upper and lower Bila Tserkva reservoirs during the anthropogenic load.

Materials and methods

The investigations were carried out during 2016–2017 in the upper (village Glybichka) and the lower (village Shkarivka) of Bila Tserkva reservoirs. We used seven fish specimens of the same age of fish, namely: *Cyprinus carpio* L., *Cyprinus carpio* L., *Hypophthalmichthys nobilis*, *Abramis brama, Carassius auratus* L.

The samples of the family *Cyprinidae* fish were caught with fishing nets which a stepped eyelet ranged from 18 to 36 mm. The nets were set in the evening, and their pick-up was carried out in the morning the next day after 12 hours of standing. The nets were set monotonously, parallel to the bank.

The catch was sorted out accordind to the species, measured and weighed. While using an optical device (binocular MBS-10) the scales taken above the lateral line under the spinal fin was used to determine fish age.

Before ihtiological incision, fish was pre-narcotized with a hypnodil solution (5–10 mgL^{-1}) to prevent the negative effects of the stress factor.

The pathologic and anatomical fish incision began with the abdominal cavity incision along the midline of the abdomen, later the organs of the abdominal cavity were studied. While exemining the liver externally, attention was drawn to the color, consistency, blood flow, the presence of hemorrhages, tumors. The separated liver was placed in a cuvette or in Petri dishes to measure. The organ was measured by the line to determined the length and width and then weighed on its electronic scales VLKT-500g-M. Volume was determined by the amount of displaced liquid. For this, the measuring cylinder was filled with water, the initial volume of water was recorded, the liver was immersed, the final volume of water was recorded.

For fixation, the liver fragments of 0.3–0.5 cm thick were selected. Sample fixation was carried out in a 10% aqueous solution of neutral formalin for 24 hours at room temperature. After fixation, the material was washed with running water, dehydrated with increasing concentration alcohols and poured into paraffin. Cuttings of 10 $m10^{-6}$ thick were made with a microtome MPS-2 and painted with hematoxylin and eosin. The prepared histological preparations were studied using Axiostar plus microscopes. The ocular micrometer screw MOV-1-16h of Biolam Lomo light microscope was used to perform calculations (Goralsky et al., 2011).

Using histological preparations the number of hepatocytes in the field of view of the microscope (lens 40, eyepiece 10) was calculated. For statistical data, the number of cells in 10 fields of view of the microscope was calculated. The number of nuclei in hepatocyte nuclei was calculated, the cell area, their cytoplasm and nuclei were measured, as well as the distance between the adjacent hepatocyte nuclei. All cytomorphometric measurements were performed with an increase 400 times (Yanovich et al., 2017). An increase in the nuclear and cytoplasmic growing in the number of nucleoli in hepatocyte nuclei and the number of dual-nucleic hepatocytes, etc. were considered to be the morphological criterion for the activation of physiological regeneration and functional activity of the liver parenchyma. Statistical processing of the received cytomorphometric data was carried out by Excel (Microsoft, USA). The probability of the difference between the control and experimental groups was estimated according to the Stjudent T-criterion.

Results and Discussion

The external review of fish corresponds to the physiological norm (there is no change in the color of the body cover, fins and their damage). The liver of investigated fish had certain changes. The morphometry of the fish live, which was caught within the upper and lower reservoirs of the Ros river was evaluated by absolute weight, liver and somatic index and the central veins' diameter, as presented in Table 1. Significant changes in the hepatic and somatic index (%) should be noted. It has increased in *Cyprinus carpio* L., *Hypophthalmichthys nobilis*, *Abramis brama*, *Carassius auratus* L. and was 3.53,1.94, 2.54, and 4.57 respectively. It concerns fish, caught in the lower reservoir. The hepatic and somatic index (%) reflected the physiological state of the fish, which may be the result of adaptive mechanisms under anthropogenic influence.

Table 1. Morphometric indexes of the liver of the family Cyprinidae fish, caught in the upper and lower reservoirs of the Ros River, (n = 7)

Indexes	Fish of the upper reservoir				Fish of the lower reservoir			
	Cyprinus carpio L.	*Hypophthalmichthys nobilis*	*Abramis brama*	*Carassius auratus* L.	*Cyprinus carpio L.*	*Hypophthalm ichthys nobilis*	*Abramis brama*	*Carassius auratus* L.
Absolute mass, g	12.01±0.55***	8.73±0.12***	4.81±0.67***	9.41±0.32***	14.82±0.12***	10.40±0.16***	5.84±0.48***	12.11±0,09***
The hepatic and somatic index, %	2.56±0.12***	1.43±0.24***	1,92±0.55***	3.20±0.01***	3.53±0.18***	1.94±0.47***	2.54±0.13***	4.57±0.49***
Diameter of central veins, $m \times 10^{-6}$	5.21±0.87***	6.28±0.05***	4,96±0.72***	10,70±0.50***	5.98±0.13***	7.0±±0.64***	5.68±0.24***	12.43±0,37***

Note: *** – P < 0.001

In fish caught within the lower reservoir, large sizes and a slightly yellowish color of the liver were observed. The weight of the liver of *Cyprinus carpio* L., *Hypophthalmichthys nobili*, *Abramis brama*, *Carassius auratus* L. in the lower reservoir was larger by 23.4%, 19.2%, 21.4%, and 28.7% respectively, as compared to the organ weight of fish caught within the upper Ros river reservoir (Fig. 1).

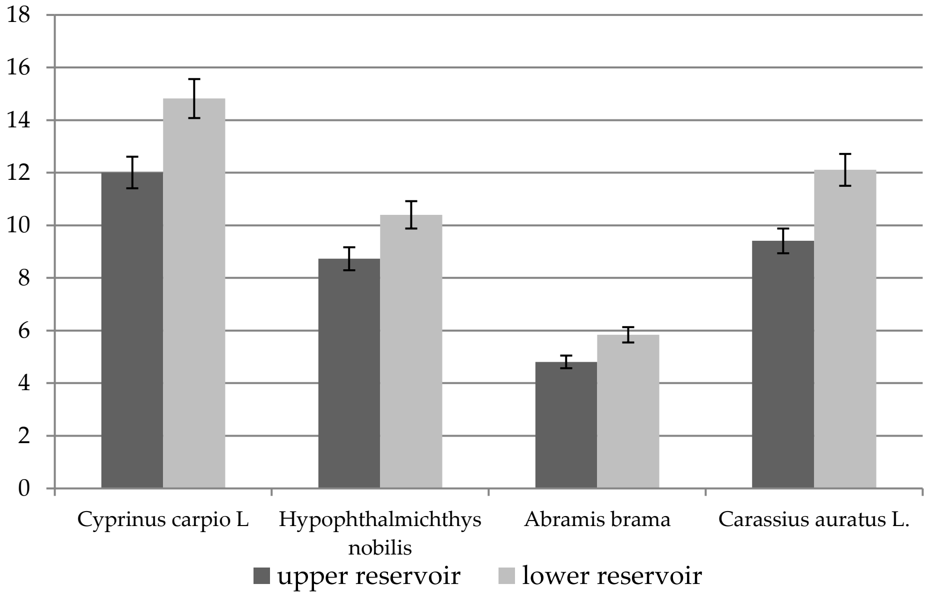

Fig. 1. Mass of fish liver of upper and lower reservoirs

The hepatic and somatic index of *Cyprinus carpio* L., *Hypophthalmichthys nobili*, *Abramis brama*, *Carassius auratus* L. of the lower reservoir has increased by 38%, 35%, 32%, and 43%, respectively, as compared to the index of fish of the upper reservoir (Fig. 2).

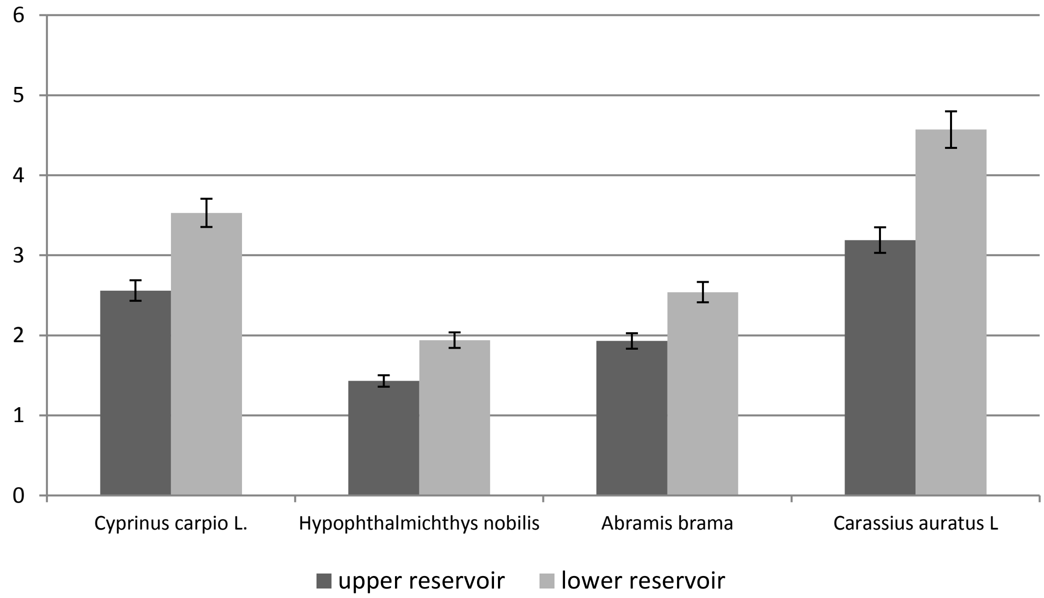

Fig. 2. Hepatic and somatic index of the fish of the upper and lower Bila Tserkva reservoirs

Therefore, in order to find out the features of morphological changes in the liver, histological structure, morphometric indexes of the organ and cells were studied. When conducting microscopy of the liver of *Cyprinus carpio* L., *Hypophthalmichthys nobili*, *Abramis brama*, *Carassius auratus* L., it should be noted the presence in the parenchyma of the pancreas structural elements. Microscopically, the liver of investigated fish was constructed from individual hepatic particles. The fish liver of the lower reservoir was observed significantly developed intercellular connective tissue.

Thickening the liver capsule as a result growth of the connective tissue and microcirculation disorders was observed in fish of the lower reservoir. The liver vessels of the were filled with blood, sometimes the vascular walls are impregnated with plasmocides, there is an obliteration of individual vessels. In the central part of the liver lobules were enlarged sinusoids and perivascular spaces. The diameter of the central veins of *Cyprinus carpio* L., *Hypophthalmichthys nobili*, *Abramis brama*, *Carassius auratus* L. was higher in fish of the lower reservoir by 14.8%, 11.4%, 14.6% and 16.2% respectively (Fig. 3).

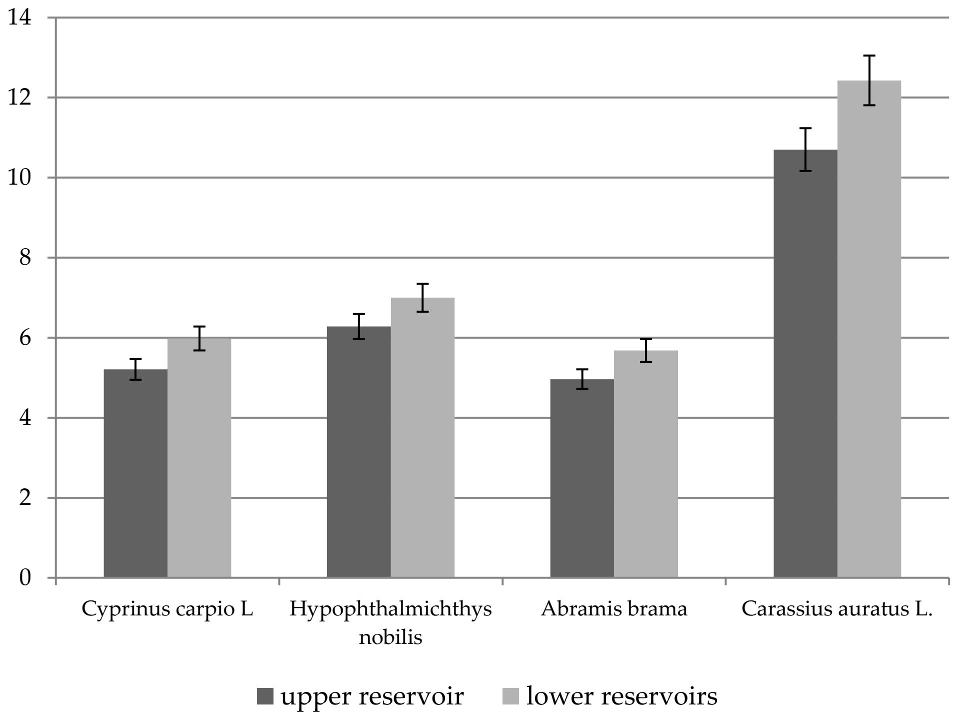

Fig. 3. Diameter of central veins of the liver of fishes of the upper and lower reservoirs

Around the blood vessels of the liver revealed small infiltrates, hepatocytes in each hepatic lobe form more or less ordered ranks – liver plates, where most of the hepatocytes have finely dipped lipid inclusion in the cytoplasm. In the center of the liver lobe, hepatocellular cells were observed with large vacuoles and a round, medium and large nucleus, occupy a central position in the cell, sometimes located and eccentric. In the subcapsular zone of the liver there was swelling of hepatocytes, chromatolysis and nuclei lysis.

The number of hepatocytes per 100 fields of vision in fish of the lower reservoir is almost the same as the fish of the upper reservoir.

Alongside with this, manifestations of compensatory and adaptive processes were observed. That is hypertrophy of nuclei and nucleoli of hepatocytes, vascular endothelial hyperplasia, increase in the number of dual-core hepatocytes in *Cyprinus carpio* L., *Hypophthalmichthys nobili*, *Abramis brama*, *Carassius auratus* L. by 18.6%, 17.1%, 15.2%, and 16.8% respectively; the nuclear-cytoplasmic ratio of *Cyprinus carpio* L., *Hypophthalmichthys nobili*, *Abramis brama*, *Carassius auratus* L. were 13.9%, 12.7%, 13.4%, and 13.8%, respectively.

Most authors believe (Storey, 1996; Bols et al., 2001; Regoli et al., 2002; Schlenk et al., 2008) that an increase in the number of dual-core hepatocytes suggests an increase in the intensity of regeneration of liver parenchyma at the intracellular level. This is a manifestation of the compensatory and adaptive reaction to dystrophic changes in the fish liver.

Table 2. Indicators of the fish liver morphometry of the upper and lower reservoirs of the Ros river

Indexes	Fish of the upper reservoir				Fish of the lower reservoir			
	Cyprinus carpio L	*Hypophthalmichthys nobili*	*Abramis brama*	*Carassius auratus* L.	*Cyprinus carpio* L	*Hypophthalmichthys nobili*	*Abramis brama*	*Carassius auratus* L.
Length, $m \times 10^{-3}$	12.71±0.47***	11.64±0.32***	5.06±0.45***	10.65±0.22***	14,60±0.52***	13.44±0.37***	6.93±0.37***	12.50±0.03***
Width, $m \times 10^{-3}$	3.38±0.12***	3.54±0.07***	2,03±0.05***	1.20±0.06***	3.99±0.10***	4.11±0.27***	2.59±0.25***	1.54±0.40***
Number of hepatocytes per 100 field of vision	1231.70±0.78***	1248.60±0.06***	1234.70±0.25***	1231.90±0.08***	1241.80±0.13***	1261.10±0.11***	1245.40±0.87***	1244.30±0.63***
Number of dual-core hepatocytes, %	12.40±0.13***	16.7±0.11***	15.70±0.11***	15.40±0.14***	14.71±0.001***	19.56±0.001***	18.09±0.001***	17.99±0.001***

Note: *** – P < 0.001

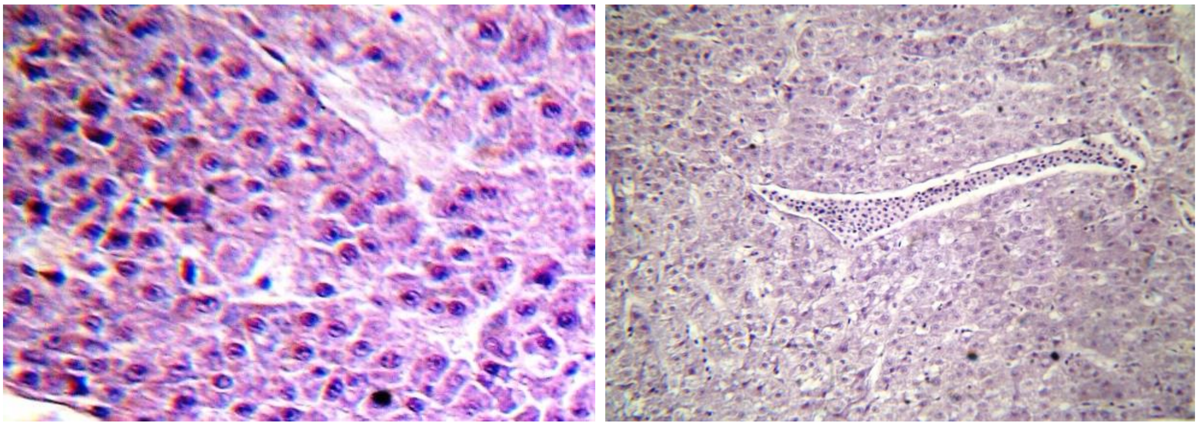

Fig. 4. Microscopic structure of the liver Hypophthalmichthys nobilis and Abramis brama: a – hepatocytes; c – nucleus of hepatocytes

The increase in the number of nuclei and the nuclear and cytoplasmic ratio in the hepatocyte nuclei of the lower Ros river reservoir indicates the activation of the latent nucleus forming regions of the chromosomes, that is, their transition to a more active functional state – the activation of the protein's activity of the cells. Increasing protein synthesis leads to the accumulation of plastic material, increased activity of enzymes. Such intensification of metabolic processes promotes increase of cell resistance and optimal development of cellular and intracellular mechanisms of regeneration (Khariv et al., 2017; Sobolev et al., 2017; Gutyj et al., 2018).

The obtained data made it possible to determine that the insignificant influence of toxic substances in fish intensifies the processes of physiological regeneration and functional activity of hepatocytes. This is evidenced by an increase in the number of nuclei in the nuclei of hepatocytes and the number of dual core hepatocytes, an increase in the nuclear and cytoplasmic ratio.

Conclusion

Biomonitoring of the Ros river reservoirs by diagnosing early violations in the most sensitive components of the hydrobiological groups enables to predict the influence of toxic substances on the state of aboriginal ichthyofauna.

The morphometric analysis of the fish liver is the basis of an indicative study of reproducing and breeding fish in natural and artificial conditions.

The results of this study informatively reflect the impact of biotic and abiotic factors on the fish body, and can, therefore, be used as a test object for biomonitoring studies to normalize the anthropogenic load on aquatic ecosystems.

Prospects for further research. It is advisable to way of continue the study of the problem of water pollution by pollutants of varios origin and influence on of living organisms from the subcellular to the population level of the organization of living matter.

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