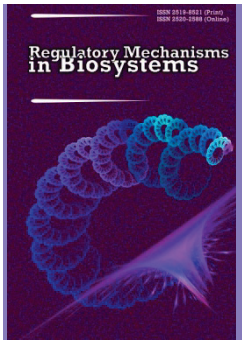

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Species specifics of morphology of the liver of the fishes of the Cyprinidae family

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Providing mankind with high-quality products of aquaculture is possible only by introduction of modern industrial technologies to fish farming, growing fish based on modern scientific achievements. Assessment of the ecotoxic situation and identification of impacts of various unfavourable factors of aquatic environment on aquatic organisms should be made through morphological studies of the organs that are first to encounter the impact. The study revealed species specifics of the morphology of the liver of the fishes of the Cyprinidae family – Prussian carp (*Carassius gibelio*), Eurasian carp (*Cyprinus carpio*), and bighead carp (*Hypophthalmichthys nobilis*), which vary by extent of the motor activity in the aquatic environment, nutrition, etc. We determined that during phylogenetic development of fish that grow in the aquatic environment, there occurs a certain restructuring of the liver: the adaptation to various living conditions were accompanied by changes in a number of parameters of macro- and microscopic architectonics of the liver. *Cyprinus carpio* and *Carassius gibelio* (omnivores) have a two-lobe liver, while *Hypophthalmichthys nobilis* (a herbivore) has a three-lobe liver. For *C. carpio* and *H. nobilis*, a characteristic feature of the liver was presence of the hepatopancreatitis (the liver and the pancreas, associated into a single organ), and in *C. gibelio*, they are differentiated into individual organs. A peculiarity of the microscopic structure of the liver of the Cyprinidae family is poorly developed interlobular connective tissue, and parenchyma of the liver lobule has a tubular structure as polyhedral, curved thick-walled tubules, the walls of which are hepatocytes. The greatest amounts of cytoplasm and karyoplasms were seen in *C. gibelio*, equaling respectively 12.98 ± 1.42 and 0.40 ± 0.02 μm³. The lowest volume of the indicated parameters was in *C. carpio*, particularly 2.97 ± 0.22 and 0.21 ± 0.01 μm³, respectively. The lowest nuclear-cytoplasmic ratio was observed in hepatocytes of *C. gibelio* (0.0316 ± 0.0024). The conducted morphological studies at the levels of organs, tissues and cells can reveal how the fishes' bodies adapt to particular living conditions and impacts of environmental factors.

Keywords: *Carassius gibelio*; *Cyprinus carpio*; *Hypophthalmichthys nobilis*; macro- and microscopic architectonics; hepatopancreas; hepatocytes.

Introduction

In vertebrates, including Osteichthyes, the liver is one of the main multifunctional digestive organs, performing vital processes in the body (Klymenko et al., 2017). It takes part in carbohydrate metabolism, providing stability in the concentration of glucose in the blood – regulates the ratio of synthesis and breakdown of glycogen, participating in all the stages of lipid metabolism, in which bile synthesizes, the salts of which emulsify fats and increase the surface of their contact with lipase. The liver is actively involved in metabolism of proteins; it is the only organ in which such important proteins as prothrombin, fibrinogen, proconvertin are synthesized, which provide blood coagulation. Moreover, it performs many metabolic functions that are important for the life of an organism (Yesipova et al., 2017; Kulyaba et al., 2019; Pepko et al., 2022; Razanova et al., 2022), participates in mineral and water metabolisms – absorbs excessive fluid, and also influences the regulation of content of mineral salts in blood and ratio of ions, regulates the activity of hormones, is a filter and source of energy for toxins (Handy et al., 2002; Melnyk et al., 2008; Pal et al., 2012; Simonov & Vlizlo, 2015), is an object for biomonitoring studies (Liavrin et al., 2014; Yancheva et al., 2016; Prysiazhniuk et al., 2019), and carries out hemostatic function (Oliinyk et al., 2017; Rabcheniuk et al., 2017). By contrast to other parenchymatous organs and digestive glands, the liver has a high regenerative ability. It has been reported that against the background of partial resection of the gland, there occurs complete recovery as a result of proliferation processes and hypertrophy of the liver lobules.

Compared with warm-blooded (mammals and birds) animals, all physiological process in the liver of fish, cold-blooded (poikilothermic) animals, depend on their environment (Prysiazhniuk et al., 2019; Honcharova et al., 2021; Hrynevych et al., 2021). Factors of the aquatic environment (temperature, light, saline composition of water, oxygen content, water density, etc.) where the fish are, undeniably affect their health (Velmurugan et al., 2007; Kofonov et al., 2020; Vodianitskyi et al., 2020; Kukhtyn et al., 2022).

Furthermore, fish have not only inter-species relations, but also relations with other aquatic organisms (invertebrates and vertebrates), plants, bacteria, viruses, which negatively affect the morphofunctional conditions of an organism, causing diseases of various genesis (Yevtushenko, 2002; Pukalo & Loboiko, 2005; Reynaud & Deschaux, 2006; Grynevych et al., 2018; Prychepa et al., 2021).

Therefore, to assess the ecological-toxicological situation and identify the impacts of various unfavourable factors of an aquatic environment on

the aquatic organisms, morphological studies of various organs which are subject to negative impact should be performed first (Velmurugan et al., 2009; Desforges et al., 2016; Bezyk et al., 2020). Only through a systematic control of organisms subject to anthropogenic impact in water bodies, is it possible to detect in time disturbances in the ecological balance and implement measures of prophylaxis of diseases and preservation of ichtyofauna (Borysevych et al., 2014).

At the same time, many studies of architectonics of the digestive organs, including the liver of Cyprinidae fishes, are not only inconsistent, but even contradict each other. This is related to the fact that the digestive organs of fish of various classes and even species of fish have certain significant differences. Therefore the objective of our studies was identification of histo- and cytometric characteristics and peculiarities of macro- and microscopic structures of the liver of the Cyprinidae family.

Materials and methods

When performing the studies, we followed the requirements of Good Laboratory Practice and the positions of the General Ethical Principles of Experiments on Animals, adopted by the first National Congress of Bioethics (Kyiv, 2001). The experimental studies were carried out according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), the Rules of Conducting Studies using Experimental Animals, according to the Order of the Ministry of Healthcare of Ukraine No. 281 as of 1 November 2000, The Measures of Further Improvement of Organizational Forms of Study using Experimental Animals and according to the Law of Ukraine On the Protection of Animals from Abuse (No. 3447-IV as of 02/21/2006, Kyiv).

The study was a collaboration between the departments of the following educational institutions: the Department of Normal and Abnormal Morphologies and Forensic Veterinary (the Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies), the Department of Normal and Pathological Morphology, Hygiene and Expertise (the Polissia National University); the Department of Zoology, Biological Monitoring and Nature Protection (the Ivan Franko Zhytomyr State University), the Laboratory of Pathomorphology of the Polissia National University.

The material for the studies was the liver from just captured clinically healthy fresh-water fishes of the Cyprinidae family: *Carassius gibelio* (Bloch, 1782), *Cyprinus carpio* L., 1758, and *Hypophthalmichthys nobilis* (J. Richardson, 1845) (Table 1).

Table 1
Characteristics of the Cyprinidae family,
from which the study material was gathered (x ± SE, n = 12)

Class	Family	Species	Number (specimens)	Age, years	Absolute weight, g
Actinopterygii	Cyprinidae	*C. gibelio*	12	2	321.1 ± 10.3
Actinopterygii	Cyprinidae	*C. carpio*	12	2	514.8 ± 42.4
Actinopterygii	Cyprinidae	*H. nobilis*	12	2	641.3 ± 2.3

The fish were caught during the summer-autumn period. Selection of the experimental animals in a comparative-anatomical order was made taking into account their age characteristics, using mature animals. Maturity was identified according to body weight of the animals.

Clinical examination of the just captured fish, evaluation of the exterior (appearance, body weight) and interior (linear parameters, absolute and relative mass of organs) parameters, after an anatomical necropsy, were carried out according to the recommendations of ichthyological and morphological guides (Horalskyi et al., 2019). Prior to necropsy, the fish were anesthetized with a hypnodil solution (5–10 mL/L) to prevent negative impacts of stress factors.

The object of the study was the liver, pieces of which for the histological studies were fixated in 10% aqueous solution of formalin and Carnoy's fluid, which after rinsing and dehydration were embedded in paraffin (Horalskyi et al., 2019).

To study the general characteristics of the liver of Cyprinidae fishes, the condition of its histo- and cytostructures in a comparative aspect, and to perform morphometry, we made paraffinic sections, which after deparaffination were stained with hematoxylin (Diapath, Italy, 2017) and eosin (Leica Geosystems, Germany, 2016) using the Van Gieson's method. To differentiate adipocytes (fatty cells) in the liver parenchyma of the experimental animals, the sections were prepared on a freezing microtome (MZ-2, Ukraine, 2004) and stained using the Kay and Whitehead's method (Horalskyi et al., 2019).

The histological sections was photographed using a CAM V-200 videocamera (Inter Med, PRC, 2017), installed in a Micros MC-50 microscope (Austria, 2010) with a system of delivering images to monitor from histological sections.

To obtain objective criteria of the structural organization of the liver, we used quantitative morphometric methods of study, in particular the nuclear-cytoplasmic ratio (NCR) (Horalskyi et al., 2019).

Results

The liver of *C. gibelio* is non-compactly located in the ventral body cavity, bordering with the pericardial sac. From the sides and caudally it is surrounded by the sexual glands, and dorsally it borders the swim bladder. The liver of *C. gibelio* has two lobes, is pink-brown, homogenous, and of loose consistency. According to organometry, the length of the liver of *C. gibelio* was 110.1 ± 3.28 mm and its width was 11.0 ± 0.72 mm. The absolute weight of the organ accounted for 9.684 ± 0.437 g, and the relative weight was 3.02%.

The liver of *C. carpio* is non-compactly located in the ventral body cavity. It is brown-red in colour, formed by two lobes of loose consistency – the left and right: the right lobe occupies the right side of the cranial part of the body cavity and is somewhat to the right of the previous section of the intestine. It has an appendage projecting along the abdominal side of the swim bladder, almost to the caudal part of the body cavity. On the left side, as a lobe, this appendage enters the hindgut and the midgut loop; the left lobe has a small appendage that is in an intestinal loop and cranially borders with the pericardial sac and is left of the previous section of the intestine.

The length and width of the organ equaled 130.3 ± 9.01 and 35.6 ± 2.1 mm, respectively. Its absolute weight was 11.875 ± 0.602 g, the relative weight or the development index was 2.31%.

The liver of *H. nobilis* is in the anterior area of the body cavity, between the intestinal loops. The front part borders with the pericardial sac, the lower part borders with the frontal chamber of the swim bladder, and caudally it borders its posterior compartment.

Unlike *C. gibelio* and *C. carpio*, the liver of *H. nobilis* is comprised of three lobes, is brown-reddish and of loose consistency. The length of the liver of *H. nobilis* measured 118.1 ± 4.3 mm and its width was 34.0 ± 1.9 mm. The absolute weight of the organ equaled 8.822 ± 0.734 g, and the relative weight was 1.38%.

Microscopically, the hepatic lobules of *C. gibelio* were of different sizes and formed the organ's parenchyma. They are polygonal and formed by hepatic glandular tubules, sinusoid hemocapillaries, and bile capillaries. Each lobule contains a central vein. As a result of weakly developed interlobular connective tissue, the boundaries between the lobules are smoothened, and thus the hepatic lobules are poorly contoured against the background of the organ's parenchyma.

The hepatic parenchyma of *C. gibelio* has a tubular structure, and therefore on the transversal section of the histopeparations, hepatocytes formed polyhedral, curved thick-walled tubules, the walls of which were hepatocytes. In the center of the latter, there were bile capillaries that have no wall of their own (Fig. 1), it is formed by the surfaces of cellular membranes of bile poles of hepatocytes, which form the bile ducts, by which bile is transported. Between the hepatic tubules, there are blood-carrying inter-lobular capillaries, the wall of which is formed by vascular poles of hepatocytes. On a length-wise section of the organ, hepatocytes of the hepatic lobules were observed to not be arranged as hepatic laminae, like in mammals, but chaotically arranged in the organ's parenchyma (Fig. 1).

According to the morphometric studies, the width of the hepatic lobules of *C. gibelio* was 31.916 ± 0.861 μm, the diameter of the hepatic tubules equaled 6.984 ± 0.294 μm, the diameter of the central veins of the hepatic lobules was 9.102 ± 0.698 μm. At the same time, the average area of a hepatic lobule measured 958.5 ± 118.3 μm² (Table 2).

Hepatocytes of *C. gibelio* were generally prismatic and rounded. Their cytoplasm was light and non-uniformly coloured. In it, there was poorly expressed acidophilic granularity. The nuclei of hepatocytes were mostly in the center of cells and were stained basophilically, they were rounded and contained one, sometimes two nuclei (Fig. 1).

The cytoplasm of some hepatocytes was found to have diffuse accumulation of fatty inclusions that were differentiated using the Kay and Whitehead's technique, as a result of which nuclei of such hepatocytes shifted peripherally. The mean volume of hepatocytes equaled 12.98 ± 1.42 μm³, the volume of their nuclei was 0.398 ± 0.021 μm³, the nuclear-cytoplasmic ratio being 0.0316 ± 0.0024 (Table 2).

The microscopic structure of the liver of *C. carpio* was similar to that of *C. gibelio*. The hepatic lobules had various polyhedral shapes. The interlobular connective tissue was poorly expressed, being noticeable only around the hepatic triads (Fig. 2). From the capsule, layers of loose connective tissue branched off towards the center of the liver. They were formed of elongated fibroblasts and the extracellular matrix with collagen fibers (Fig. 2).

Because of the poor development of the interlobular tissue, the hepatic lobules were almost non-differentiated (Fig. 3). Therefore, visually, the lobular structure of the organ could have been interpreted only according to presence of central veins in the lobules.

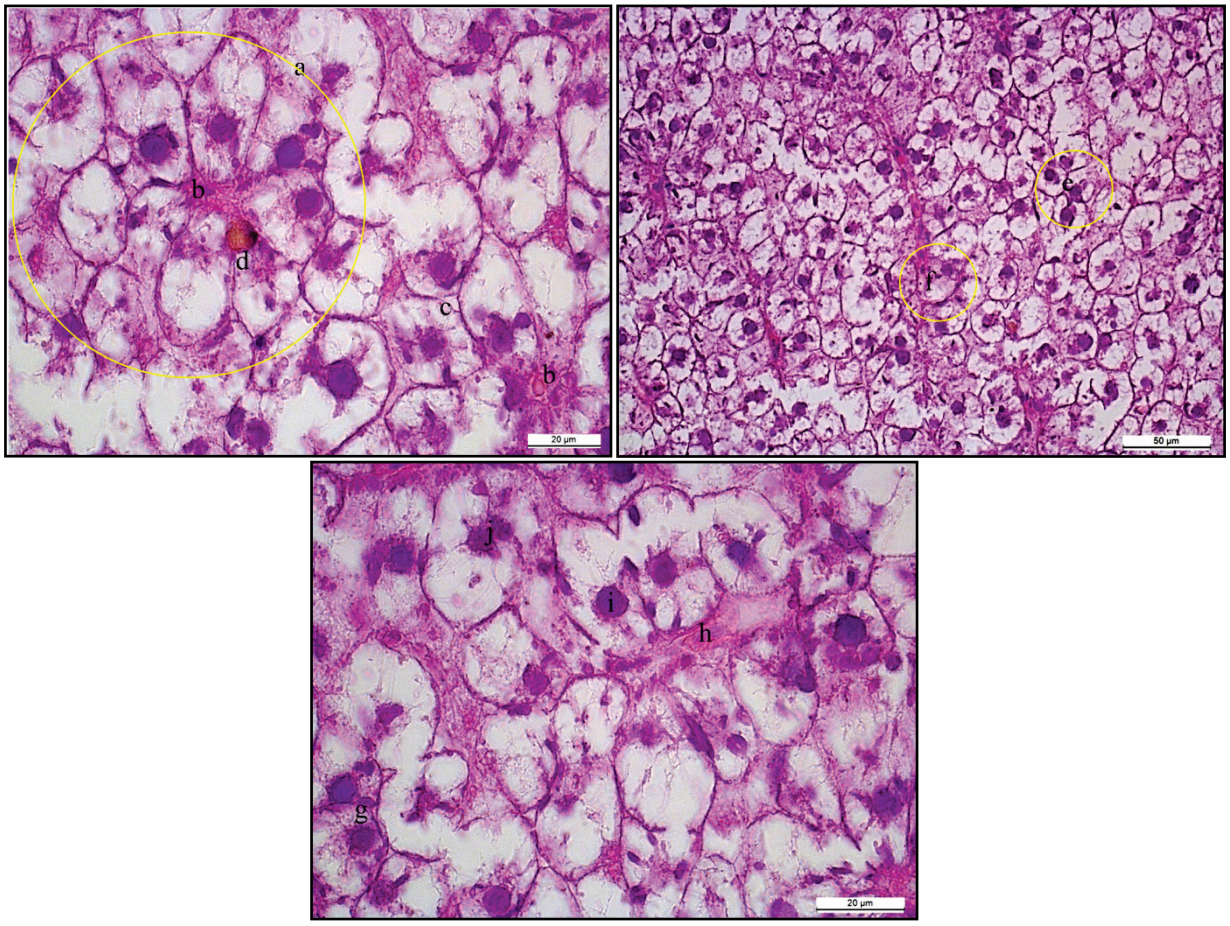

Fig. 1. Fragment of microscopic structure of the hepatic lobule of *Carassius gibelio*: *a* – secretory tubule; *b* – bile capillary; *c* – hepatocytes; *d* – bile; *e* – hepatocytes; *f* – bile ducts; *g* – hepatocytes; *h* – bile capillary; *i* – hepatocyte nucleus; *j* – two-nuclei hepatocyte; hematoxylin and eosin

Each hepatic lobule is formed by hepatocytes, which around the synusoids in *C. carpio*, formed anastomotically connected tubular histostructures – secretory tubules. This indicates that the liver of *C. carpio*, similarly to *C. gibelio*, has a tubular structure: hepatocytes form the secretory tubules, with lumens in the centers, which are intralobular bile ducts (capillaries), the wall of which is plasmolemma of bile poles of hepatocytes (Fig. 2).

Hepatocytes had various shapes (round, polyhedral, triangular). On the histopreparations, their cytoplasm poorly responded to hematoxylin and eosin staining. Nuclei of hepatocytes were in the center of cells, or located eccentrically. As a result of good reaction to the main staining agents, they had basophilous karyoplasm, which was distinctly contoured against the background of light cytoplasm, with presence of a slight oxyphilous granularity in it (Fig. 2).

The spaces of several inter-secretory tubules were formed by sinusoid blood capillaries. The wall of the latter was formed by plasmolemma of the vascular poles of hepatocytes, etc. The centre of each lobule, sometimes eccentrically, had a central vein, into which all lobule capillaries entered. The blood vessels (sinusoids) and the connective tissue formed the stroma of the liver. According to the results of morphometric studies, the diameter of the central veins of the hepatic lobules of *C. carpio* equaled 5.105 ± 0.412 μm. The width of its hepatic lobules measured 36.98 ± 1.07 μm, and the diameter of the hepatic tubules was 3.262 ± 0.163 μm. At the same time, the mean area of one hepatic lobule occupied 1252 ± 127 μm² (Table 2). According to the cytomorphometry, the mean volume of hepatocytes equaled 2.971 ± 0.224 μm³ and the volume of nuclei was 0.212 ± 0.009 μm³. At the same time, the nuclear-cytoplasmic ratio was 0.0768 ± 0.0073 (Table 2).

As compared with *C. gibelio*, a peculiarity of the liver of *C. carpio* was presence of accumulations of pancreatic cells near the central vein of the hepatic lobules, arranged as "islets" of various configurations. Those accumulations formed structures similar to acini of exocrine part of the pancreas of mammals (Fig. 3).

According to the results of histometry, the average area of one hepatic lobule, associated with accumulation of pancreatic cells (hepatopancreas), accounted for 1294 ± 132 μm². At the same time, the area of accumulations of pancreatic cells (pancreas) in one hepatic lobule of *C. carpio* equaled 42.4 ± 2.1 μm² (3.27%), and the ratio of the area of pancreas to the area of the hepatic lobule was 1:29.6 (Table 2).

Pancreatic cells were prismatic and, unlike hepatocytes, more intensely reacted to the staining. Thus, the apical, granular (acydophilous), basal,

and hemogenic (basophilous) zones clearly differentiated in them. Nuclei of pancreatic cells were rounded and located closer to the basal zone. According to the results of cytomorphometric studies, the mean volume of pancreatic cells equaled 2.412 ± 0.264 μm³, and the volume of their nuclei was 0.052 ± 0.003 μm³. At the same time, the nuclear-cytoplasmic ratio accounted for 0.0220 ± 0.0078. Microscopically, the hepatic lobules of *H. nobilis*, similarly to *C. carpio* and *C. gibelio*, did not distinctly differentiate in the organ's parenchyma because of weak development of interlobular connective tissue. According to the results of the histological studies, the liver of *H. nobilis* was characterised by a tubular structure: the parenchyma was formed as secretory tubules with central lumen (bile capillary), which was especially noticeable on the transversal histosection of the organ (Fig. 4). Between the hepatic tubules, there were intralobular hemocapillaries of sinusoid type.

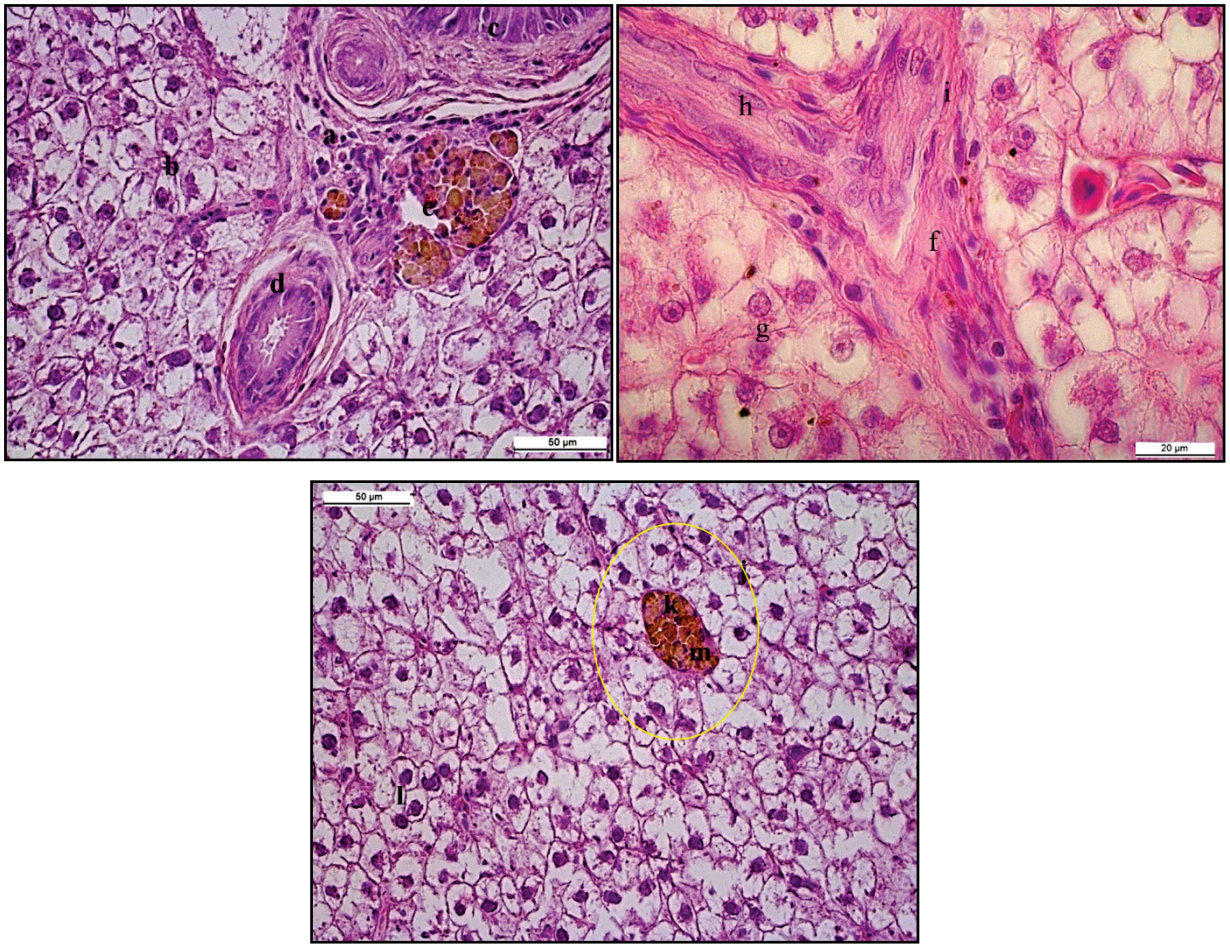

Fig. 2. Fragment of microscopic structure of the hepatic lobule of *Cyprinus carpio*: *a* – interlobular connective tissue; *b* – hepatocytes; *c* – artery; *d* – vein; *e* – bile duct (hematoxylin and eosin); *f* – interlobular connective tissue; *g* – hepatocytes; *h* – fibroblasts; *i* – collagen fibers (Van Gieson); *j* – secretory tubule; *k* – bile capillary; *l* – hepatocytes; *m* – bile (hematoxylin and eosin)

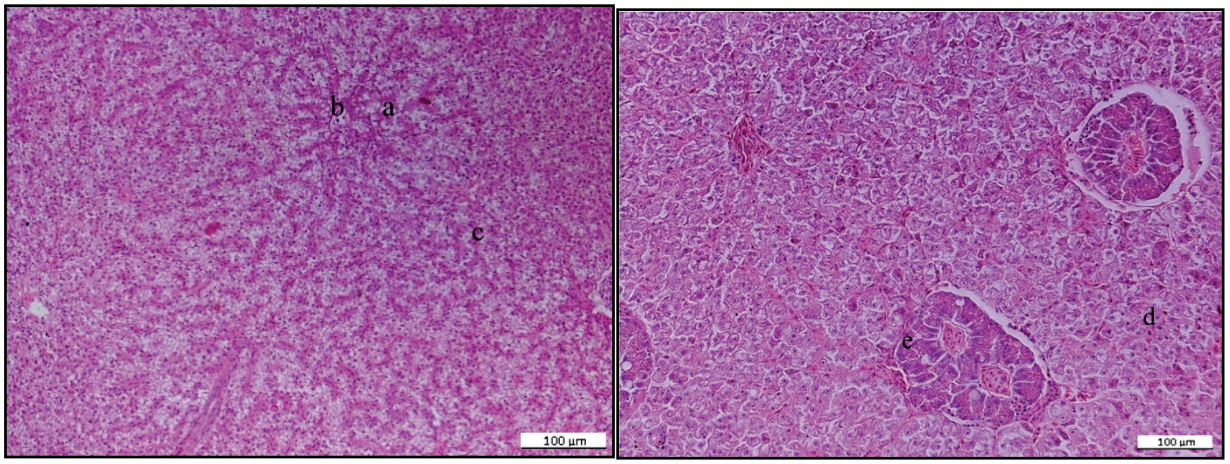

Fig. 3. Microscopic structure of the liver of *Cyprinus carpio*: *a* – lobule; *b* – central vein; *c* – hepatocytes; *d* – hepatocytes; *e* – pancreatic cells; hematoxylin and eosin

They had an expressed lumen, and their wall was formed by endotheliocytes of a quite dense form. The histometric studies revealed that the width of the lobules of liver parenchyma of *H. nobilis* equaled 24.00 ± 1.05 μm, the diameter of the hepatic tubules was 4.73 ± 0.31 μm, and the diameter of the central vein was 5.97 ± 0.40 μm. At the same time, the average area of the hepatic lobule accounted for 477 ± 82 μm² (Table 2).

Hepatocytes of *H. nobilis* were mainly irregular, polygonal, and distinctly contoured against the background of the lobular parenchyma. On the histopreparations, their cytoplasm reacted to hematoxylin and eosin staining to various extent and contained fine-granular acidophilic-colored non-uniform mass. As a result of diffuse accumulation of fatty inclusions in cytoplasm, nuclei of the cells shifted towards the cytoplasm periphery, closer to the sinusoid edge. Hepatocyte nuclei were rounded, located in the center or eccentrically in the cytoplasm of cells. They had denser consistency, and somewhat small sizes, and therefore clearly differentiated in the structure of cells against the background of non-stained cytoplasm (Fig. 4). The cytomorphometry we performed revealed that the average volume of hepatocytes was 4.964 ± 0.302 μm³, the mean volume of their nuclei was 0.302 ± 0.026 μm³, and the nuclear-cytoplasmic ratio equaled 0.0648 ± 0.0064 (Table 2).

Table 2

Histo- and cytometric parameters of the liver of the Cyprinidae fishes (x ± SE, n = 12)

Parameters	*Carassius gibelio*	*Cyprinus carpio*	*Hypophthalmichthys nobilis*
Area of the hepatic lobule, associated with accumulation of pancreatic cells (hepato-pancreas), μm²	958 ± 118	1294 ± 132	552 ± 97
Area of accumulations of pancreatic cells (pancreas), μm²	–	42.4 ± 2.1	74.9 ± 4.5
Area of the hepatic lobule, μm²	958 ± 118	1252 ± 127	477 ± 82
Ratio of the area of the pancreas to the area of the hepatic lobule	–	1:29.6	1:6.4
Width of the hepatic lobules, μm	31.92 ± 0.86	36.98 ± 1.07	24.00 ± 1.05
Diameter of the hepatic tubules, μm	6.98 ± 0.29	3.26 ± 0.16	4.73 ± 0.31
Diameter of the central veins, μm	9.10 ± 0.70	5.11 ± 0.41	5.97 ± 0.40
Volume of hepatocytes, μm³	12.98 ± 1.42	2.97 ± 0.22	4.96 ± 0.30
Volume of nuclei of hepatocytes, μm³	0.398 ± 0.021	0.212 ± 0.009	0.302 ± 0.026
Nuclear/cytoplasmic ratio of hepatocytes, conventional units	0.0316 ± 0.0024	0.0768 ± 0.0073	0.0648 ± 0.0064
Volume of pancreatic cells, μm³	–	2.41 ± 0.26	2.00 ± 0.20
Volume of nuclei of pancreatic cells, μm³	–	0.052 ± 0.003	0.036 ± 0.005
nuclear/cytoplasmic ratio of pancreatic cells, conventional units	–	0.0220 ± 0.0078	0.0183 ± 0.0062

Note: "–" – in the liver parenchyma of *Carassius gibelio*, pancreas (accumulations of pancreatic cells) was not found.

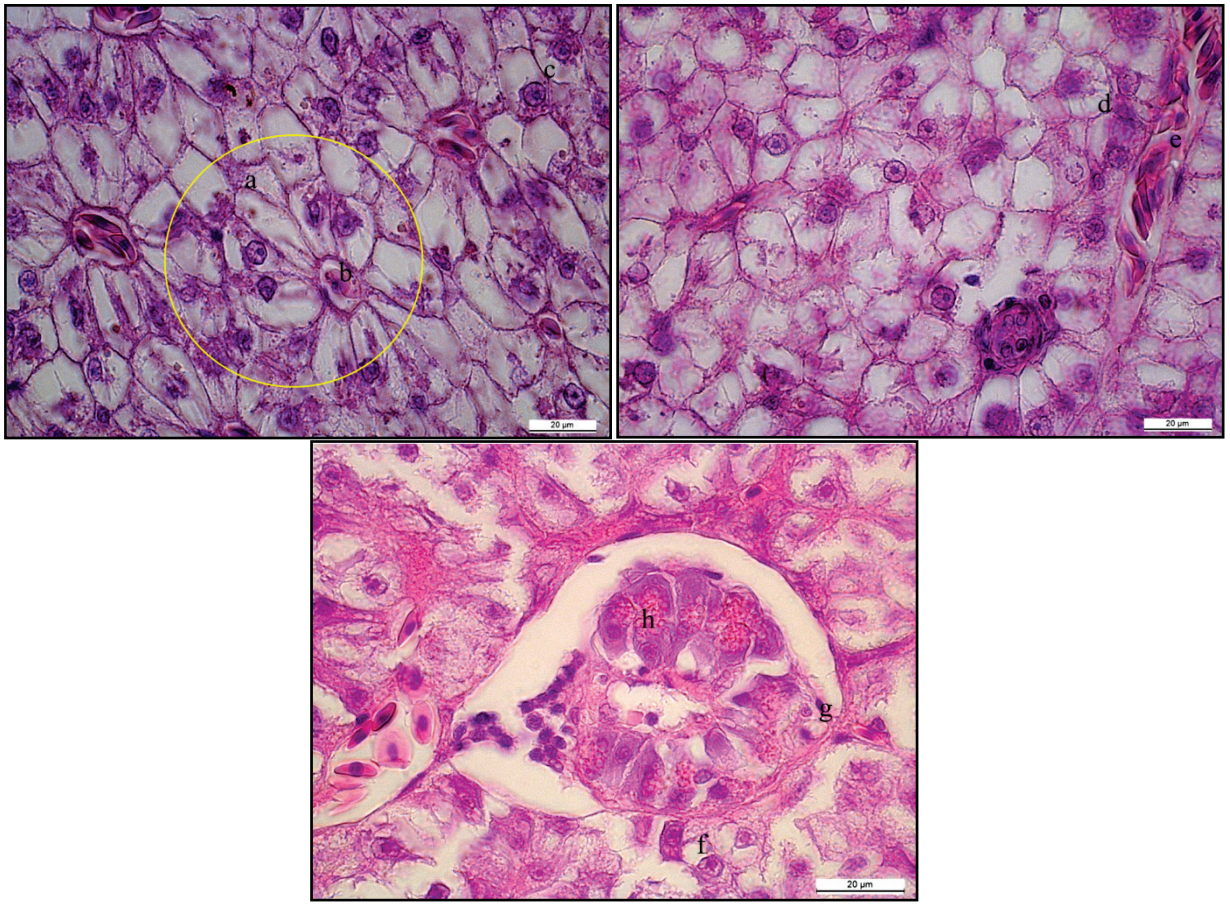

Fig. 4. Fragment of microscopic structure of the hepatic lobule of *Hypophthalmichthys nobilis*: *a* – secretory tubule; *b* – bile capillary; *c* – hepatocytes; *d* – hepatocytes; *e* – hemocapillary; *f* – hepatocytes; *g* – pancreatic cells; *h* – acidophilic granularity of cytoplasm of pancreatic cells; hematoxylin and eosin

The liver parenchyma of *H. nobilis*, similarly to *C. carpio*, had individual accumulations of pancreatic cells (Fig. 4), formed by 15–35 and more cells, often located in different regions of the hepatic lobules, compared with such of *C. carpio*. Those cells were prismatic, and their cytoplasm, according to hematoxilin and eosin staining, was non-uniform in colour: basophilous near the nucleus, and acidophilic farther from the nucleus, with large amount of acidophilic granularity (Fig. 4).

According to the histometric studies, the average area of the hepatic lobule, associated with accumulations of pancreatic cells of *H. nobilis*, was 552 ± 97 μm². At the same time, the area of pancreas in one hepatic lobule of *H. nobilis* occupied 74.9 ± 4.5 μm² (13.56%), and the ratio of the pancreas to the general area of the hepatic lobule equaled 1:6.4 (Table 2). According to the results of cytomorphometry, the mean volume of pancreatic cells was 2.004 ± 0.198 μm³, the volume of their nuclei was

0.036 ± 0.005 μm³, and the nuclear-cytoplasmic ratio equaled 0.0183 ± 0.0062.

Discussion

Provision of mankind with proper products of aquaculture is possible only by introduction of modern industrial technologies, based on modern scientific achievements, into fishery (Lushchak et al., 2001; Hrytsyniak & Tretiak, 2007; Sharamok et al., 2017; Prysiazhniuk et al., 2019; Kurchenko & Sharamok, 2020; Yesipova et al., 2022). At the same time, technogenic factors impact the fishery, causing metabolism impairments that are negative for the fish productivity (Bols et al., 2001; Silkina & Mikrjakov, 2017). Morphometric parameters of parenchymatous digestive organs of fish were found to be helpful in predicting the impact of toxic compounds on the condition of ichthyofauna (Prysiazhniuk et al., 2019).

Therefore, the morphological studies we performed allowed us to identify the adaptation of the animal organism to particular living conditions and identify the impact of the environmental factors on the fish organism. Osteichthyes, in the phylogenetic taxon of vertebrates, belong to the taxonomical groups, the representatives of lower level of organization, differing by the extent of locomotor activity (aquatic environment), nutrition, etc, which undeniably affect the morphofunctional characteristics of the gastrointestinal tract, including the liver. Therefore we carried out the studies of the structural organization of the liver at the organ, tissue, and cellular levels in the species aspect on the clinically healthy fresh-water Cyprinidae fish: *C. gibelio*, *C. carpio*, and *H. nobilis*.

Stomachless fishes, to which Cyprinidae belong, consume plankton, vegetation, detritus in small portions after short time periods, and therefore their liver plays an important role in digestion (Prysiazhniuk et al., 2013). All digestive enzymes form in it, and the ability of the organism to survive depends on its activity (Esypova et al., 2017), it is ramified and located between the intestinal loops. The ratio of length of intestine to body length in the omnivore fishes – *C. carpio* and *C. gibelio* – was 2–3. In herbivores (*H. nobilis*), it was 6–15. This was reflected in the structure and location of the liver in body cavity. As with anatomical peculiarities, according to the literature sources, the liver in most Osteichthyes has two lobes, but can be comprised of only one lobe (*C. carpio*, *Esox lucius* L., 1758, *Perca fluviatilis* L., 1758) or three lobes (in many Cyprinidae fishes). Their weight may account for 10% to 20% of the body weight (Prysiazhniuk et al., 2019).

According to results we obtained, the liver of *C. gibelio* has two lobes, is pink-brown, homogenous and of loose consistency. It is located non-compactly in the dorsal part of the body cavity, borders with the pericardial sac cranially and with the sex glands caudally on the sides. The liver of *C. carpio* is of loose consistency, brown-red in colour. It is located non-compactly in the lower cavity of the body of the fish, and consists of two lobes. The liver of *H. nobilis* is brown-red, has loose consistency, formed of three lobes. The organ is in the ventral cavity of the body between the intestinal loops.

Thus, according to our studies, the liver in the experimental animals had loose consistency, was located non-compactly in the ventral part of the body cavity between the intestinal loops, which was due to their nutrition type (Prisyazhnyuk, 2011). Such specifics of its macroscopic structure and peculiarities of location of the liver in Cyprinidae fishes directly correlate with their body shape, formed over the process of their life cycle. Such a structure and location, etc, of the liver in Cyprinidae fishes are directly related to the type of the digestive system (stomachless) and the body shape of fishes: the body of Cyprinidae fishes (low-mobile fish) is dense, elongated, with relatively poor curve dorsally and almost straight line ventrally.

Histo- and cytomorphometric methods of histological study allow us to identify processes of development of the organs and systems, individual body parts, and monitor the growth and development of cells, their differentiation in comparative and species aspects (Horalskyi et al., 2019). Such a systemic quantitative analysis of the restructuring of the tissue elements, their compositions and relations give a convincing material for confirming characteristic morphological changes and functional deviations related to them in the tissues and systems during onto- and philogenesis. According to the results of the literature review, a distinctive feature of some representatives of families Esocidae, Siluridae, Salmonidae, etc. is presence of separate digestion glands – the liver and pancreas. A trait of a large number of Cyprinidae and Percidae fishes is presence of a hepatopancreas: the liver and pancreas, associated into a single organ (Morhun & Soroka, 2017). At the same time, the liver and the pancreas of *C. gibelio* (family Cyprinidae) are separated one from another into individual organs, as indicated in our studies. Accumulations of pancreatic cells in *C. carpio* were found around the central veins of the lobules, in *H. nobilis* – in various areas of the hepatic lobules. According to the histometric studies, the average area of the hepatic lobule, associated with accumulations of pancreatic cells (hepatopancreas) in *C. carpio* was 1294 ± 132 μm², and the area of accumulations of pancreatic cells in the hepatic lobule was 42.4 ± 2.1 μm² (3.27%), and the ratio of the area of pancreas to the area of the hepatic lobule equaled 1:29.6. Similar results were observed for *H. nobilis*. At the same time, the area of the hepatic lobule, associated with the accumulations of pancreatic cells, in *H. nobilis* – as compared with *C. carpio* – was 2.35 times lower, equaling respectively 552 ± 97 μm². At the same time, the area of pancreas in one hepatic lobule of *H. nobilis* was 13.56%, and the ratio of pancreas area to the overall area of hepatic lobule was 1:6.4. Such a structure of the liver in Cyprinidae is likely associated with specifics of its vascularisation by secretion type, etc.

Structural-functional unit of the liver in the experimental fish, similarly to mammals and birds, was a lobule, with a central vein in the center, from which hepatic laminae project radially towards the periphery, usually consisting of two rows of cells – hepatocytes (Borysevych et al., 2014). However, it has to be noted that the connective tissue in Cyprinidae is developed weakly, and therefore the hepatic lobules poorly differentiate into individual morphofunctional structures. Of the representatives of Cyprinidae, according to the results of histometry, the largest area of hepatic lobules was in *C. carpio*, accounting for 1252 ± 127 μm², then in *C. gibelio* – 958 ± 118 μm², and the lowest was in *H. nobilis* – 477 ± 82 μ².

Such varying morphometric parameters of the hepatic lobules are perhaps related to the fact that *C. carpio* and *C. gibelio* are omnivores, while *H. nobilis* is a herbivore. Furthermore, the ratio of intestine length to body length in them varied: 2:30 in the omnivores, 6:15 in the herbivores, which in our opinion conditions a characteristic shape, topographic-anatomic peculiarities of the liver, its microscopic structure, including histo-, cytometric parameters, because of various physiological load on this organ.

According to the results of microscopic studies, the hepatic lobule of mammals and Osteichthyes, the Cyprinidae family (Prysiazhniuk et al., 2019), is formed by hepatocytes, which form the hepatic laminae (Prysiazhniuk et al., 2013; Kozij & Sherman, 2018).

The results of our studies revealed that the parenchyma of the liver of the Cyprinidae fishes has a tubular structure, similar to vertebrates, the birds, in particular. At the same time, hepatocytes were polygonal and mostly contained one nucleus, located eccentrically, in karyoplasms of which, there were distinctly seen heterochromatin grains. In hepatocytes that form hepatic tubules, two surfaces differentiate : the first is oriented towards the bile capillary (bile) and the other towards sinusoidal hemocapillary (vessel). According to the morphometric studies, the diameter of the hepatic tubules in the Cyprinidae fishes varied: the largest was in *C. gibelio* (3.262 ± 0.163 μm), the lowest in *C. carpio* (3.262 ± 0.163 μm), and in *H. nobilis* this parameter was average (4.731 ± 0.312 μm).

Cytoplasm and karyoplasm of cells are closely integrated with each other and comprise a single morphofunctional system. Therefore, isolated study of morphometric characteristics of nucleus or cytoplasm alone gives only a limited knowledge of the structure of cells. Currently, it is confirmed that measurements of cell volume (cytometry), volume of their nuclei (cartometry), and especially identification of nuclear-cytoplasmic ratio are the most informative indicators of morphofunctional condition of the cells (Evsikov et al., 1990; Maniotis et al., 1997). According to the results of our morphometric studies, hepatocytes of the experimental fish had various sizes of cytoplasm and karyplasm and different nuclear-cytoplasmic ratio. Therefore, the largest volume of cytoplasm and karyoplasms of hepatocytes were found in *C. gibelio*, respectively 12.98 ± 1.42 and 0.398 ± 0.021 μm³, and the lowest in *C. carpio*, measuring 2.97 ± 0.22 and 0.212 ± 0.009 μm³ respectively. At the same time, the lowest nuclear-cytoplasmatic ratio was in hepatocytes of *C. gibelio* (0.0316 ± 0.0024), indicating high level of morphofunctional condition of hepatocytes and their metabolic activity.

Thus, the varying specifics of the morphological structure and morphometric parameters of cyto- and histostructures of the liver of the Cyprinidae fishes, are perhaps due to various ecological factors of the aquatic environment in which the fish live, and also the characteristic type of their nutrition: *C. carpio* and *C. gibelio* are omnivores, while *H. nobilis* is a herbivore.

Conclusion

The liver of the Cyprinidae fishes is dark-red, loose in consistency, and non-compactly located in the lower part of the body between the intestinal loops. Depending on the nutrition type, *C. carpio* and *C. gibelio* (omnivore) have a two-lobe liver, and *H. nobilis* (herbivore) has a three-lobe liver. A characteristic feature of the liver of *C. gibelio* and *H. nobilis* is presence of a hepatopancreas (the liver and pancreas, associated into a single organ). In *C. gibelio*, they were differentiated between each other into individual organs. The average area of the hepatic lobule, associated with accumulation of pancreatic cells in *C. carpio*, was 1294.4 ± 132.2 μm², the area of accumulations of pancreatic cells in the hepatic lobule was 3.27%. The ratio of area of pancreas to area of hepatic lobule equaled 1:29.6. Such parameters in *H. nobilis* equaled respectively 552.0 ± 96.9 μm² and 13.56%, and the ratio of pancreas to overall area of hepatic lobule was 1:6.4.

A feature of microscopic structure of the liver of the Cyprinidae fishes was a poorly developed interlobular connective tissue, which manifested more only in the area of the hepatic triads. The parenchyma of hepatic lobule of the Cyprinidae fishes had a tubular structure, as polyhedral, curved thick-walled tubules, walls of which were hepatocytes.

In the experimental fish, hepatocytes had different sizes of cytoplasm and karyoplasms, and usually different nuclear-cytoplasmic ratio: the greatest volumes of cytoplasm and karyoplasms were found in *C. gibelio*, respectively 12.98 ± 1.42 and 0.398 ± 0.021 μm³, and the lowest in *C. carpio*, measuring 2.97 ± 0.22 and 0.212 ± 0.009 μm³, respectively. At the same time, the lowest nuclear-cytoplasmic ratio was found for hepatocytes in *C. gibelio* (0.0316 ± 0.0024), indicating a high level of morphofunctional condition of hepatocytes and their metabolic activity.

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