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Histological and ultramicroscopic features of the liver of *Hypophthalmichthys nobilis*

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To determine the ecological and toxicological situation and assess the impact of various adverse factors in the aquatic environment on aquatic organisms, a morphological analysis of specific organs, which are primarily subjected to adverse effects, should be conducted. Histological and ultramicroscopic features of the liver structure in the fish of the carp family, specifically the bighead carp – *Hypophthalmichthys nobilis* (Eschmeyer, 2003), are considered in this paper. An analysis of the microstructure of liver tissue was carried out using light microscopy and ultramicroscopic methods, which enabled us to identify the organ's key morphological and functional features. It was established that during the phylogenetic development of fish that grow in aquatic environments, specific structural rearrangements of the liver occur: adaptations to various living conditions were accompanied by changes in several parameters of liver morphology. In the bighead carp (herbivorous), the liver is trilobed. A characteristic feature of the bighead carp liver is the presence of a hepatopancreas (liver and pancreas associated as a single organ). A distinctive feature of the microscopic structure of the liver in the carp family is the poorly developed interlobular connective tissue. In contrast, the parenchyma of the liver lobule has a tubular structure consisting of polygonal, irregular, thick-walled tubules whose walls are formed by hepatocytes. These tubules are represented by sinusoidal capillaries, which ensure efficient exchange of substances between the blood and liver cells, aiding in the detoxification and processing of nutrients. Large glycogen reserves are also observed in these tubules, indicating a high level of metabolic activity and the ability of the carp liver to accumulate energy resources. The state of hepatocytes, the liver's vascular system, and structural changes resulting from various ecological and physiological factors were investigated. The main adaptive mechanisms of liver tissue, which ensure its functional activity in bighead carp, were identified. The results of this study expand and complement the knowledge of the macro- and microscopic structure of the liver concerning the species characteristics of the bighead carp, contributing to our understanding of its anatomy, histology, comparative anatomy, zoology, and more.

Keywords: vertebrate animals; comparative anatomy; microscopic structure; organ research; bony fish; hepatocytes; organelles.

Introduction

The agricultural sector of Ukraine is characterized by significant potential due to the presence of fertile soils, favorable climatic conditions, and water resources, creating all the necessary conditions for the production of a wide range of agricultural products (Makhyboroda, 2019; Grynevych et al., 2021).

Given the growing global population, aquaculture is one of the most promising directions in the food industry, capable of ensuring the need for high-quality animal protein without harming natural fish resources (Bigarré et al., 2017; Rud et al., 2021). Meeting the population's food requirements, specifically through aquaculture, is only possible by developing and implementing cutting-edge technologies in animal husbandry, including fisheries (Irm et al., 2020; Dyudyaeva & Rutta, 2021). Innovative approaches and modern fish farming methods help increase production efficiency, ensure the sustainability and ecological safety of processes, and reduce resource consumption while maintaining high product quality (Jannathulla et al., 2019; Radchenko et al., 2020; Laktuka et al., 2023).

There is no doubt that the application of modern technologies in the field of fish farming, concerning fish cultivation and disease prevention measures of various origins, can cause morphofunctional changes, disrupt homeostasis in fish organisms, leading to the emergence of new diseases or atypical courses of existing ones (Matras et al., 2019). Moreover, the influence of various technological factors through the use of new technologies in fish farming causes metabolic disorders in fish, negatively affecting their productivity and immune system development (Glavatchuk, 2024).

The production processes directly related to fish feeding are important factors shaping productive qualities: feed consumption establishes a connection between fish organisms and the surrounding environment (Korzhenevska et al., 2019; Hu et al., 2020). It is undeniable that natural feed in natural conditions is rational feeding for fish (Abhijith et al., 2016; Ali et al., 2022) while adjusting feeding in industrial conditions by using various compound feeds balanced in macro- and microelements significantly influences the productive qualities of industrial fish, depending on their species characteristics, physiological digestion processes, and affecting the macro- and microscopic structure of digestive organs, particularly the liver (Taddesse et al., 2014; Glencross et al., 2024; Maulu et al., 2024). Additionally, the conditions of fish feeding, the quality of feed, and its composition directly affect the morphofunctional condition of fish, their somatic characteristics, metabolism, etc. (Mashkova & Sharamok, 2023; Cui et al., 2024). It has also been noted that a crucial issue in ensuring and improving the productive qualities of fish is the anatomical features of the digestive system, including physiological processes occurring in fish organs, particularly in digestive glands such as the liver and pancreas (Klymenko et al., 2017; Kovacheva et al., 2022; Chanet et al., 2023). Therefore, the adaptation of different fish species to consuming specific feeds and their digestion depends on the morphophysiological features of the digestive system, including the liver, which performs essential functions for the organism: it synthesizes bile, blood plasma proteins, serves as a depot for glycogen, lipids, and vitamins, detoxifies endogenous and exogenous toxic substances, and participates in hematopoiesis (Georgieva et al., 2016; Oliinyk et al., 2017; Balakrishna, 2024). All physiological processes in the liver of

fish, which are cold-blooded (poikilothermic) animals, as opposed to warm-blooded ones (mammals and birds), have a direct correlation with the environmental conditions in which they live (Prysiazhnyuk & Onyshchenko, 2016; Honcharova et al., 2021). Water temperature, its chemical composition, oxygen levels, and other ecological factors directly impact metabolic processes in the liver of fish, such as the synthesis and distribution of nutrients, detoxification, and energy exchange. Specifically, changes in water temperature affect the speed of enzymatic reactions in the liver, reflecting the organism's ability to adapt to new environmental conditions (Al-Ghanim, 2014; Vodianitskyi et al., 2020; Kukhtyn et al., 2022). Thus, the physiological activity of the liver in fish is not only the result of internal processes in the organism but is also directly influenced by changes occurring in the environment where these animals live.

Materials and methods

During the scientific research, the established laboratory practice norms GLP (1981) and the provisions of the "General Ethical Principles of Animal Experiments," adopted at the First National Congress on Bioethics (Kyiv, 2001), were adhered to. The experiments were conducted following international standards, including the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), "Rules for Conducting Work Involving Experimental Animals", according to the Ministry of Health Order No. 281, dated November 1, 2000, "On Measures for Improving the Organization of Work Involving Experimental Animals", and following the Law of Ukraine "On the Protection of Animals from Cruel Treatment". The work was carried out in collaboration with the departments of higher education institutions: the Department of Normal and Pathological Morphology and Forensic Veterinary Medicine at Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Hzhytsky; the Department of Zoology, Biological Monitoring, and Nature Conservation at Zhytomyr Ivan Franko State University; and the Department of Normal and Pathological Morphology, Hygiene, and Expertise at Polissia National University.

Animal selection was conducted during the summer-autumn period, considering their age. In the comparative anatomical series, sexually mature animals of two years of age, weighing 641.3 ± 2.3 g, were used. Sexual maturity was assessed based on the animal's weight (Horalskyi et al., 2019). A clinical examination of the fish and evaluation of exterior (external appearance, body mass) and interior (linear parameters, absolute and relative organ mass) characteristics after anatomical dissection was performed according to the recommendations of morphological guides (Horalskyi et al., 2019). To prevent the negative impact of stress factors, the fish were anesthetized with a hypnotic solution (6–10 mL/L) before dissection.

The object of the study was the liver, with pieces for histological studies fixed in 10% aqueous neutral formalin solution and in Carnoy's fluid, followed by rapid paraffin embedding (Horalskyi et al., 2019). For the general characterization of the liver, the state of its structures, and conducting morphometric studies, serial paraffin sections were prepared and stained with hematoxylin and eosin (Horalskyi et al., 2019). Quantitative morphometric methods were used to obtain objective criteria for the structural organization of the liver (Horalskyi et al., 2019).

For electron microscopy, liver material was selected according to the recommendations outlined in the guide (Horalskyi et al., 2019). Small pieces of the organ, up to 1 mm³ in size, were cut and immersed in a fixative mixture – a 1% osmium tetroxide solution in a Collifield buffer for 2 hours. Then, the material was washed with a 0.1 M phosphate buffer (3 times for 30 minutes each) and dehydrated in increasing concentrations of alcohol (50º, 70º, 90º, 100º for 10 minutes with three changes in each alcohol portion), after which the tissue was contrasted with a 2% uranyl acetate solution prepared in 70% alcohol. The material was then passed through absolute alcohol with acetone (10 minutes) and through acetone (10 minutes). After that, the tissue was placed in a mixture of acetone and epoxy resins (3:1 for 30 minutes; 1:1 for 1 hour) and pure resin for 1 hour. The samples were embedded in gelatin capsules, filled with epoxy resins mixed with a catalyst, and placed in a thermostat (t +56 ºC) for polymerization for 24 hours. Ultra-thin sections (50–60 nm) were prepared using an LKB ultramicrotome (Sweden) Tesla BS-490A, mounted on copper grids (d = 1 mm), contrasted with uranyl acetate and lead citrate according to Reynolds, and examined with a PEM-125K electron microscope (Ukraine) with a DX 2 KAPPA video camera, followed by photography at magnifications of 6,400, 8,000, and 10,000 times.

To ensure the accuracy and detail of the microstructural characteristics of the tissues, microphotography of histological preparations was performed using the CAM V200 video camera (InterMed, Ukraine) mounted on the Micros MC-50 microscope (Micros, Austria) and a digital camera. The obtained digital images were organized and subjected to statistical analysis. The mathematical processing of the research results was statistically developed using the Statistica 7.0 software package (StatSoft, Tulsa, USA). Differences between the values in the control and experimental groups were determined using ANOVA, with differences considered significant at $P < 0.05$ (with Bonferroni error adjustment).

Results

The liver in the bighead carp is located in the central part of the body cavity, between the loops of the intestine. Cranially, it borders the pericardial sac; ventrally, it is limited by the anterior chamber of the swim bladder. And caudally, it is limited by its posterior chamber. Macroscopically, the liver of the bighead carp consists of three lobes, has a soft consistency, and a brownish-red color. According to organometric studies, the absolute liver mass is 8.82 ± 0.73 g, its relative mass is 1.38%, its length is 11.81 ± 0.43 cm, and its width is 3.40 ± 0.19 cm.

Histological studies have shown that the liver lobules in the bighead carp have a polygonal shape. The interlobular connective tissue of the liver is weakly expressed and is only noticeable around the hepatic triads, where fibroblasts of elongated form and collagen fibers are differentiated (Fig. 1). Hepatic glandular tubules, bile capillaries, and sinusoidal blood capillaries form the liver lobules. In the center of each lobule is the central vein. The secretory tubules of the liver parenchyma are composed of 6–8 or more hepatocytes connected by desmosomes. Each of these tubules, in cross-section, has the appearance of a polygonal, broken, thick-walled structure, the walls of which consist of hepatocytes, with a visible central lumen (bile capillary) in the center. The bile capillaries do not have their wall; the cell membranes of the biliary poles of the hepatocytes form the wall. Between the hepatic tubules, there are blood capillaries, the walls of which are formed by the vascular poles of the hepatocytes. The biliary pole of the hepatocytes, directed towards the lumen of the bile capillary, secretes bile.

In contrast, the vascular pole, directed towards the blood capillary of the intra-lobular capillaries, secretes glucose and urea. The hepatocytes of the liver lobules, in longitudinal sections, are randomly arranged in the organ's parenchyma. This structural feature is associated with the fact that the liver lobules in the bighead carp have a tubular structure, unlike the plate-like structure in animals of the "mammal" class.

Hepatocytes in the bighead carp have a polygonal shape and are clearly outlined against the background of the parenchyma of the lobules (Fig. 1). Their cytoplasm, when stained with hematoxylin and eosin, absorbs the dye in various ways and contains slight acidophilic inclusions in the form of fine granularity. This gives the cytoplasm of individual hepatocytes a foamy appearance. The nuclei of the hepatocytes are round and have clearly defined nucleoli located centrally or eccentrically. Alongside the mononuclear hepatocytes, occasional binucleated cell forms are observed. The nuclei generally have a dense consistency and are somewhat reduced in size, so they are clearly differentiated in the cell structure against the background of the unstained cytoplasm. Due to the diffuse accumulation of lipid inclusions in many hepatocytes in their cytoplasm, the nuclei are often displaced to the periphery of the cytoplasm, closer to its sinusoidal edge.

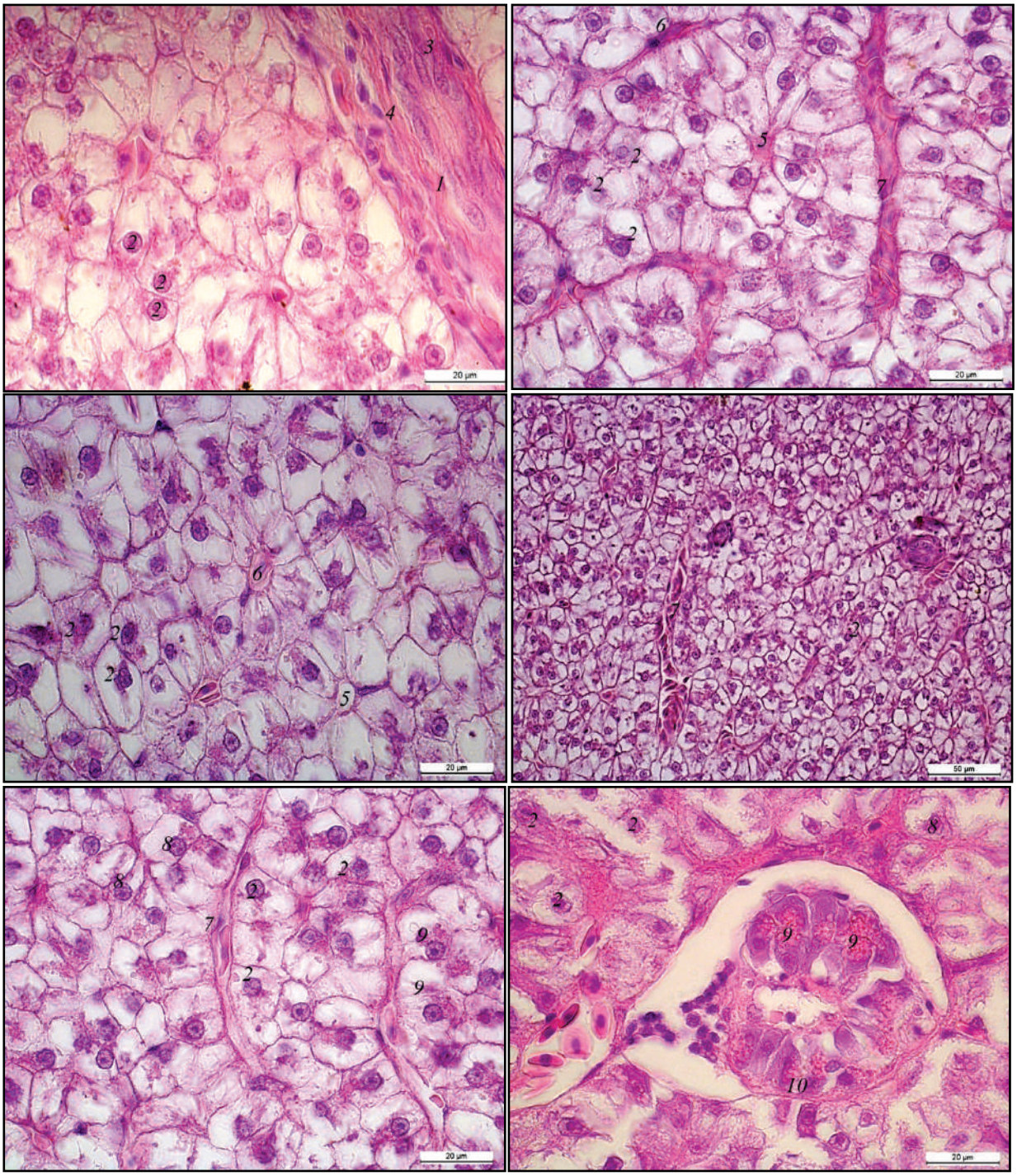

Fig. 1. Fragment of the microscopic structure of a liver lobule in the bighead carp: *1* – interlobular connective tissue; *2* – hepatocytes; *3* – fibroblasts; *4* – collagen fibers; *5* – secretory tubule; *6* – bile capillary; *7* – hemocapillary; *8* – nuclei of hepatocytes; *9* – acidophilic granular cytoplasm; *10* – hepatopancreas; hematoxylin and eosin

According to cytomorphometric studies, the average volume of hepatocytes in the liver of the bighead carp is 4.964 ± 0.302 μm³, the average volume of their nuclei is 0.302 ± 0.026 μm³, and the nuclear-cytoplasmic ratio is 0.0648 ± 0.0064 (Table 1). A characteristic feature of the liver of the bighead carp is the presence of clusters of pancreatic cells (Fig. 1) in various regions of the liver lobules. These clusters consist of 15–35 or more cells and form structures similar to the acini of the exocrine part of the pancreas in mammals.

Pancreatic cells have a prismatic shape, and their cytoplasm, when stained with hematoxylin and eosin, reacts unevenly to the dye: it is basophilic near the nucleus and acidophilic farther from the nucleus, with a significant amount of acidophilic granularity (Fig. 1). According to histometric studies, the average area of a liver lobule associated with clusters of pancreatic cells in the bighead carp is 552 ± 97 μm². The area of the pancreas in one liver lobule of bighead carp occupies 74.9 ± 4.5 μm² (13.6%), and the ratio of the pancreas area to the total area of the liver lobule is 1:6.4 (Table 1). According to histomorphometry, the average volume of pancreatic cells is 2.004 ± 0.198 μm³, the volume of their nuclei is 0.036 ± 0.005 μm³, and the nuclear-cytoplasmic ratio is 0.018 ± 0.006.

According to ultramicroscopic studies, hepatocytes in the liver of the bighead carp have a polygonal shape, which may be multilateral, round, or oval. They are clearly outlined by the plasma membrane, which consists of three layers: an external and an internal dark layer, with a light layer in between. The plasma membrane of the hepatocytes is generally uneven, with a convoluted form, and often forms separate protrusions and invaginations. Between the hepatocytes that form glandular tubules, there is a narrow, sometimes expanded inter-

cellular space (Fig. 2). Hepatocytes have multiple bile poles, with the plasma membrane of these poles forming short microvilli. The plasma membranes of adjacent hepatocytes tightly contact each other through plasma membrane projections in the form of microvilli, which are directed towards the hemocapillary sinusoids (Fig. 2). These intralobular capillaries, of the sinusoidal type, have a distinctly wide lumen, and endotheliocytes of a highly condensed form their walls. The hepatocytes have large, often two, round or oval-shaped nuclei surrounded by a dense nuclear membrane. The nuclear membrane consists of two layers (internal and external), separated by a perinuclear space. According to ultramicroscopic studies, the perinuclear space is unevenly expanded (Fig. 2). Canal-like pores are observed in the two-layered nuclear membrane. The outer layer of the nuclear membrane sometimes contacts the membranes of the granular endoplasmic reticulum. The perinuclear space without a clear boundary transitions into the cavities of the granular endoplasmic reticulum. The nucleoplasm is light in color and contains an electron-transparent matrix with evenly distributed euchromatin. At the periphery of the nucleoplasm, heterochromatin is sometimes observed (Fig. 2). In the center or eccentrically located within the karyoplasm, the nucleoli are usually large and consist of fibrous and granular components.

Table 1
Morphometric indicators of the liver structures in the bighead carp (x ± SD, n = 6)

Indicators	Numerical indicators
Area of the liver lobule associated with the accumulation of pancreatic cells (hepatopancreas), μm²	552 ± 97
Area of pancreatic cell accumulations (pancreas), μm²	74.9 ± 4.5
Area of the liver lobule, μm²	477 ± 83
Diameter of central veins, μm	5.974 ± 0.398
The volume of hepatocytes, μm³	4.964 ± 0.302
The volume of hepatocyte nuclei, μm³	0.302 ± 0.026
Nucleus-to-cytoplasm ratio of hepatocytes, arbitrary units	0.065 ± 0.006
The volume of pancreatic cells, μm³	2.004 ± 0.198
The volume of pancreatic cell nuclei, μm³	0.036 ± 0.005
Nucleus-to-cytoplasm ratio of pancreatic cells, arbitrary units	0.018 ± 0.006

In the cytoplasm of hepatocytes, formed organelles are present: ribosomes, granular and agranular endoplasmic reticulum, mitochondria, lysosomes, and the Golgi complex.

According to our electron microscopy studies, a significant portion of the cytoplasm of hepatocytes in fish is occupied by basophilic (chromatophilic) material formed by ribosomes, polysomes, and cisterns of the granular endoplasmic reticulum, which vary in size. The granular endoplasmic reticulum is most often located in the perinuclear and central zones of the cytoplasm (Fig. 2) and also at the periphery of the hepatocyte. It consists of wide cisterns, almost always parallel and separated by nearly equal intervals. These cisterns often connect through anastomoses.

The cytoplasm of hepatocytes contains many free ribosomes and polysomes. The free ribosomes are typically located in the cytoplasmic matrix between the cisterns of the endoplasmic reticulum or attached to the outer surface of the endoplasmic reticulum membranes. Around the nucleus of hepatocytes, or in other areas of the cytoplasm, is the Golgi complex, which consists of parallel cisterns and micro- and macropinocytotic vesicles. The cisterns of the Golgi complex are usually narrowed, though some are expanded. Occasionally, the cisterns exhibit branching, with some branches joining through anastomoses. Lysosomes of various shapes and sizes, typically round or oval, are found near the Golgi complex and are surrounded by a single-layered membrane.

Around the nucleus, in the condensed cytoplasm, there are many mitochondria of various sizes (small, medium, large) and shapes (round, oval, spherical, elongated, thread-like, rod-like, or dumbbell-shaped). Round or oval-shaped mitochondria dominate (Fig. 2). Cisterns of the endoplasmic reticulum often surround these mitochondria and are often found around the endoplasmic reticulum. The membranes of the mitochondria have clearly defined contours and resemble the surface membrane of cells, consisting of dark external and internal layers, with a light layer separating them. Inside the mitochondria are short, parallel cristae formed by the internal membrane folds. The arrangement of cristae in the mitochondria is quite varied. In some mitochondria, the cristae are perpendicular, while in others, they are parallel to the long axis of the mitochondria. The mitochondrial matrix is electron-transparent (Fig. 2).

In the cytoplasm of hepatocytes, besides the general organelles (granular and agranular endoplasmic reticulum, Golgi complex, mitochondria, lysosomes), various inclusions (glycogen, lipids, vitamins) are also found.

According to the results of our electron microscopy studies, in hepatocytes containing a significant amount of lipid inclusions, their nuclei are displaced to the periphery of the cytoplasm due to the diffuse arrangement of lipid droplets. As a result, they most often take an eccentric position. Their size and shape noticeably differ from most hepatocytes; they are condensed and have a lobed shape (Fig. 2). Moreover, there is a reduction in the perinuclear space and the canaliculi of the endoplasmic reticulum, which indicates the suppression of the protein-synthesizing function of the hepatocytes.

Discussion

In the phylogenetic series of vertebrate animals, bony fishes, including the bighead carp, belong to taxonomic groups of lower organizational levels. These species differ in their degree of locomotor activity (aquatic environment) and appropriate feeding habits, which undoubtedly influence the morphofunctional characteristics of the gastrointestinal tract, specifically the liver. In this regard, we studied the macro-, micro-, and ultrastructural characteristics of the liver in clinically healthy freshwater fish, class Osteichthyes, family Cyprinidae, species bighead carp (Prysiazhniuk et al., 2019).

The liver's most important functions are homeostatic, depurative, excretory, and metabolic (Carvalho et al., 2021; Rekha et al., 2021). The liver detoxifies endogenous and exogenous toxic substances, produces bile necessary for emulsifying fats, and participates in anabolic and catabolic protein metabolism. It also produces amino acids required for synthesizing tissue proteins and most plasma proteins, phospholipids, and cholesterol. The liver plays a leading role in the intermediate metabolism of carbohydrates, lipids, biologically active substances (hormones, vitamins), and minerals (Payuta & Flerova, 2020; Payuta & Flerova, 2021; Pulido-Rodriguez, 2024).

Therefore, studying the macro-, micro-, and ultrastructural characteristics of the liver in clinically healthy animals and during experiments has both fundamental and applied significance. Fish, such as the bighead carp, consume plankton, vegetation, and detritus in small portions at short intervals, which makes their liver crucial for digestion. It primarily produces all the digestive enzymes (Zhu et al., 2020; Dias et al., 2021).

An important factor influencing the structure and location of the liver within the body cavity of fish is the ratio of intestinal length to body length, which is 2–3 in omnivorous fish (common carp and crucian carp) and 6–15 in herbivorous fish (such as the silver carp). Therefore, the liver of freshwater fish, class Osteichthyes, family Cyprinidae, is branched and located between the loops of the intestine. Most bony fish are bilobed, but they may have one lobe (in the case of carp, pike, and perch) or three lobes (in many cyprinids). Its mass can range from 10 to 20% of the body mass (Božidar et al., 2011; Horalskyi et al., 2023).

According to our research, the liver of the bighead carp is located in the ventral body cavity between the loops of the intestine. Three lobes form it, which have a brownish-red color and loose consistency due to its feeding habits (Horalskyi et al., 2023). These macroscopic features of the liver and its location in cyprinid fish are directly related to the body shape of fish, which evolved over their life cycle. Therefore, the liver structure in cyprinid fish, its placement, and its relation to the digestive system type (without a separate stomach) and body shape are interconnected: the body of cyprinids (less mobile fish) is dense, oval, with a relatively weak dorsal curvature and almost a straight ventral line.

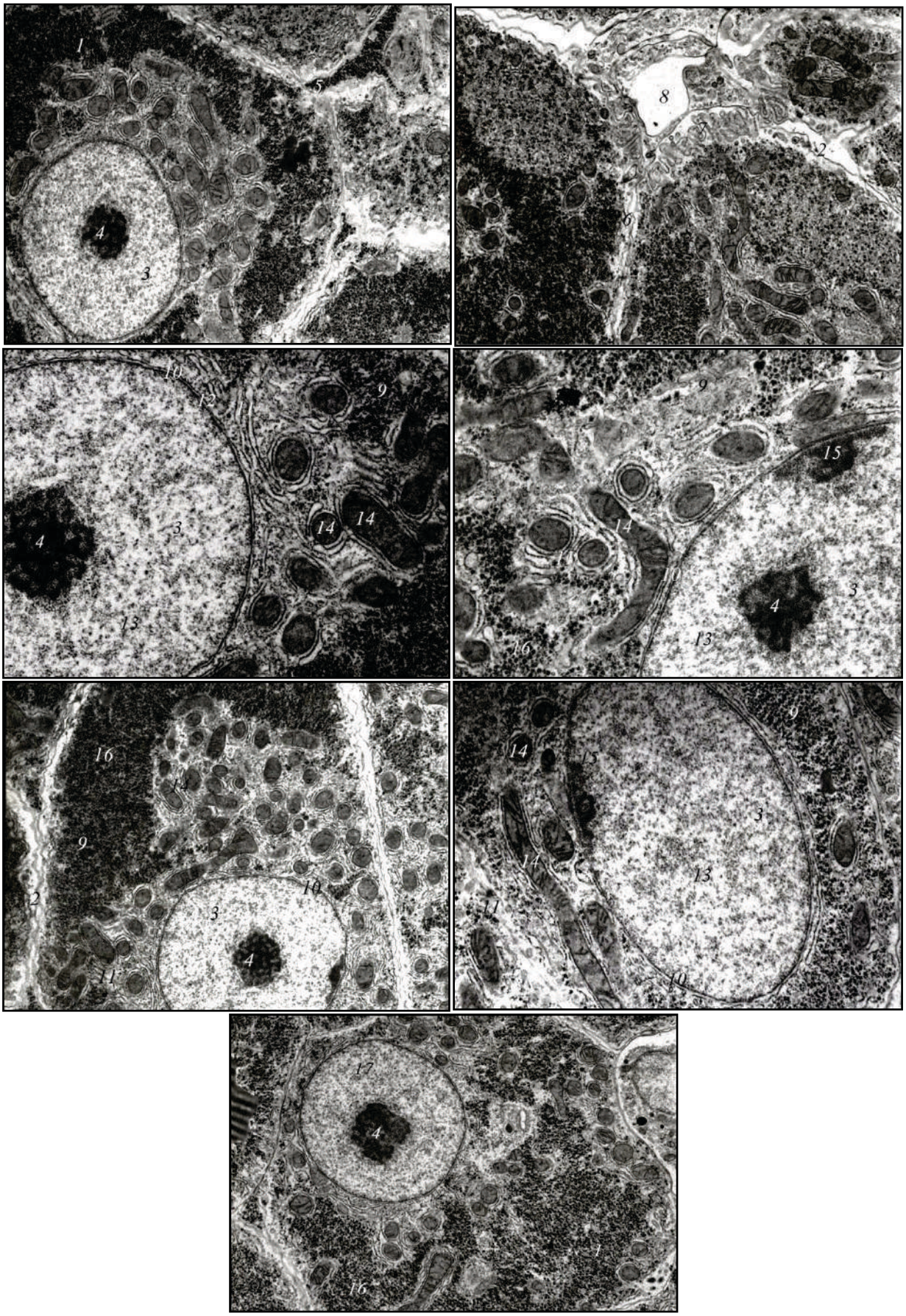

Fig. 2. Fragment of the ultrastructural organization of the liver in the bighead carp: *1* – hepatocyte; *2* – cytoplasmic membrane; *3* – nucleus; *4* – nucleolus; *5* – intercellular space; *6* – hepatocyte contacts; *7* – microvilli; *8* – lumen of the hepatic sinusoidal capillary and intercellular space; *9* – cytoplasm; *10* – nuclear membrane; *11* – granular endoplasmic reticulum; *12* – contact of the outer layer of the nuclear membrane with the membranes of the granular endoplasmic reticulum; *13* – euchromatin; *14* – mitochondria; *15* – heterochromatin; *16* – lipid inclusions; *17* – eccentric nuclear positioning

According to the literature review, for some species in the fish family – pike, catfish, salmonids, etc., it is characteristic to have separate digestive glands – the liver and pancreas. However, in most cyprinid and perch species, a characteristic feature is that the hepatopancreas: the liver and pancreas are associated as a single organ (Szarek et al., 2010; Mokhtar et al., 2018). According to the results of our microscopic studies, the liver of the bighead carp, together with the pancreas, is associated as a single organ (hepatopancreas), where the clusters of pancreatic cells are located in various regions of the liver lobules, possibly due to the peculiarities of its vascularization and secretion type. The average volume of pancreatic cells is 2.004 ± 0.198 μm³, the volume of their nuclei is 0.036 ± 0.005 μm³, and the nuclear-cytoplasmic ratio is 0.018 ± 0.006.

The liver of the bighead carp demonstrates a structure typical for fish, including a high organization of hepatocytes, various organelles, and vascular structures. According to the results of histometric studies, the average area of the liver lobule associated with pancreatic cell clusters (hepatopancreas) in the bighead carp is 552 ± 97 μm². At the same time, the area of the pancreas in a single liver lobule is 74.9 ± 4.5 μm² (13.6%), and the ratio of the pancreas area to the total area of the liver lobule is 1:6.4.

The structural-functional unit of the liver in the studied fish, like in mammals and birds, is the lobule, with interlobular connective tissue located between them (Abusrer & Shtewi, 2023). However, in the bighead carp, the connective tissue stroma is poorly developed, which results in the lobules of the liver being poorly differentiated into separate morphofunctional structures. According to histometric studies, the average area of the liver lobule in the bighead carp is 477 ± 83 μm².

According to microscopic studies, the liver lobule in mammals and bony fish of the cyprinid family (Campos et al., 2017) is formed by hepatocytes that form liver plates. At the same time, according to our research, the parenchyma of the liver in the bighead carp has a tubular structure similar to that in the vertebrates, class Aves According to histometric studies, the diameter of the liver tubules in the bighead carp is 4.731 ± 0.312 μm. Hepatocytes are polygonal in shape, and their cytoplasm contains a nucleus, which is either centrally or eccentrically located. Our analysis indicates that binucleate cell forms are observed along with mononuclear hepatocytes, which may indicate their increased synthetic activity (Sun et al., 2019).

In the hepatocytes that form the liver tubules, two surfaces differentiate: one facing the bile capillary (biliary) and the other facing the sinusoidal hemocapillary (vascular). Their cytoplasm stains differently and contains slight acidophilic inclusions in fine granularity, often making it foamy when stained with hematoxylin and eosin. This may be because the liver's main functions as a multifunctional organ are performed by hepatocytes, which make up 60% of all cellular elements of the organ. Therefore, we believe the weakly stained hepatocytes perform the liver functions while some intensely stained cells rest.

The cytoplasm and karyoplasm of the cells are closely interconnected and form a single morphofunctional system. Thus, isolated study of the morphometric characteristics of the nucleus or cytoplasm alone gives a one-sided view of the cell structure. It has been proven that measurements of cell volume (cytometry), the volume of their nuclei (karyometry), and especially the determination of the nuclear-cytoplasmic ratio are the most informative indicators of the morphofunctional state of cells (Atta, 2013; Sokulskyi et al., 2021).

Therefore, an objective and important indicator of the morphofunctional state of the liver and at the cellular level is cytophotometric research (Vicentini et al., 2005; Wilson & Castro, 2010; Kim et al., 2019), which not only qualitatively but also quantitatively reveals the parameters of the liver cytostructures in the bighead carp. Thus, according to our cytometric studies, the average volume of hepatocytes in the liver of the bighead carp is 4.964 ± 0.302 μm³, the average volume of their nuclei is 0.302 ± 0.026 μm³, and the nuclear-cytoplasmic ratio is 0.065 ± 0.006, which indicates a high level of the morphofunctional state of hepatocytes and their metabolic activity.

Electron microscopy studies are vital marker signs of the morpho-functional state of organs and tissues in animals, as they allow us to examine their structure at the ultrastructural level and confirm or refute the microarchitectonics of cells and tissues observed under light microscopy (Al-Zahaby et al., 2024).

Therefore, electron microscopy is widely used in scientific research, primarily examining the same objects as light microscopy. However, it has significant advantages, as it allows us to determine the features of the microscopic structure of cells and tissues at various levels of their structural organization, both in normal conditions and in pathology, which cannot be detected by light microscopy (Horalskyi et al., 2019). This justified the application of this method for performing a morphofunctional assessment of the liver in the bighead carp.

Thus, the observations made at the light microscopy level were confirmed based on our electron microscopic studies of the liver in clinically healthy freshwater fish (class Osteichthyes, family Cyprinidae, species bighead carp). The ultrastructural structure of hepatocytes contains a significant number of cellular organelles: granular and agranular endoplasmic reticulum, mitochondria, lysosomes, ribosomes, and the Golgi complex. Many lysosomes and mitochondria indicate high metabolic activity, including protein and lipid metabolism. The hepatocytes had light nucleoplasm and large nucleoli. The nuclei were clearly outlined due to a well-defined nuclear membrane. Sometimes, hepatocytes had two nuclei. The perinuclear space was unevenly expanded. The nuclear chromatin was evenly distributed in the karyoplasm, resulting in nuclei with medium electron density. Many polymorphic mitochondria were concentrated around the nucleus, tightly surrounded by cisternae of the endoplasmic reticulum.

At the same time, the synthetic and depurative functions of hepatocytes were reflected in the structure and number of nuclei and cellular organelles and in the presence of cellular inclusions (Rejane & Vildes, 2008). Their morphological structure reflects the complex biochemical processes occurring in the cell. Thus, according to the results of our electron microscopic studies, in hepatocytes containing a significant number of lipid inclusions, their nuclei were displaced to the periphery of the cytoplasm due to the diffuse arrangement of lipid droplets. In such cases, they took an eccentric position. Their size and shape differed significantly from most hepatocytes, as they were compacted and had a lobed shape. This led to a reduction in the perinuclear space and the canaliculi of the granular endoplasmic reticulum, which indicated the suppression of the protein-synthesizing function of hepatocytes. Therefore, the structural features of hepatocytes, including the number of organelles and intracellular inclusions, are important indicators of their synthetic and depurative activity, which is crucial for maintaining the organism's viability and adaptation to environmental changes.

Conclusion

The liver of the bighead carp is composed of three lobes, has a brownish-red color and loose consistency, and is not compactly arranged in the ventral part of the body between the loops of the intestine. Cranially, it borders the pericardial sac; ventrally, it is limited by the anterior chamber of the swim bladder. And caudally, it is limited by its posterior chamber. The absolute mass of the liver in the bighead carp is 8.822 ± 0.734 g, while its relative mass is 1.375%. Its length is 11.808 ± 0.432 cm, and its width is 3.402 ± 0.194 cm.

The bighead carp is characterized by a tubular liver structure and poorly developed interlobular connective tissue. The average volume of the hepatocytes that form the thick-walled tubules of the liver lobules is 4.964 ± 0.302 μm³, with the volume of their nuclei being 0.302 ± 0.026 μm³. As a result, they have a low nuclear-cytoplasmic ratio (0.065 ± 0.006), which indicates their high morphofunctional state and metabolic activity.

Ultrastructurally, the liver's hepatocytes in the bighead carp are clearly outlined by the plasma membrane, have a polygonal shape, and formed nuclei with well-defined nucleoli. The vascular poles of the closely located hepatocytes, which are oriented toward the direction of the hemosinusoids, tightly contact each other via microvilli of the plasmalemma. In the cytoplasm of the hepatocytes, general-purpose organelles are found, including ribosomes, granular (usually located in the perinuclear and central zones of the cytoplasm), and

agranular endoplasmic reticulum, mitochondria, lysosomes, the Golgi complex, and inclusions such as glycogen, lipids, and vitamins.

The prospects for further research are focused on conducting histochemical studies of the liver in bony fish of the carp family.

The authors claim no conflict of interest.

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