Primary signs of internal organ diseases in carp in case of standard condition violations in fish farming ponds

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Abstract. Hematological and histopathological study results of adult carp from a private fish farm are presented. Abnormalities in the main hematological parameters (blood elements ratio, leukocyte formula, erythropoiesis level, possible pathologies of erythrocytes) were not revealed. One third of the examined fish were found to have abnormalities in the tissues of gills, liver, and heart muscle in the form of minor focal formations (detachment of the gill epithelium, dissection of myofibrils, disorders of glycogen metabolism, foci of necrosis and infiltration) which indicates the initial stage of the pathological process. Kidneys, gonads, and somatic muscle fibers showed no signs of pathology. The main factors determining the primary signs of internal organ diseases are water quality (water saturated with magnesium, calcium, and iron salts, increased hardness) and an unbalanced diet (primarily, an insufficient amount of raw protein) that does not meet the standards adopted in fish farming. The registered initial signs of the pathological process in the gills, liver and heart muscle can be leveled by improving the carp keeping conditions.

1. Introduction

The development of aquaculture in modern economic conditions is one of the most important goals in the agricultural sector aimed at improving the quality of domestic fish products and ensuring the food security of the country. In the central and southern areas of Russia, one of the main objects of pond aquaculture is the common carp (domesticated carp) *Cyprinus carpio* Linnaeus, 1758. This unpretentious to environmental conditions, omnivorous, fast-growing species is successfully grown in many private pond farms of Samara region.

The deterioration of conditions for keeping and breeding (anthropogenic pollution of water, violation of the hydrological, thermal and hydrochemical regimes of the water reservoir, violation of the biotechnology of growing fish, the presence of pathogens of dangerous infections and infestations) can provoke the development of fish diseases and reduction of fish productivity in water reservoirs [1, 2].

The aim of this work was the study of internal organ histopathology of common carp under the conditions of a fish-breeding pond.

2. Material and Methods

Ichthyological studies were conducted in June 2020 in a private pond farm (Samara region, Mordovovo village, 53°17'15" N, 49°45'70" E) on a cascade system containing two artificial fish ponds measuring
30×25 m and 20×40 m, with a level difference of 3.5 m. Reservoirs were constantly filled with artesian water, oversaturated with salts of magnesium, calcium, iron, fluorine, and manganese. Ten uneven-aged (from 2 to 5 years old) carp species without external signs of any diseases and pathologies were studied.

At the fishing site, peripheral blood was taken from the fish and preparations were performed. The smears were fixed with ethanol and stained with azur-eosin according to Romanovsky-Giemsa [3]. Differential counts for leukocytes and erythrocytes were performed on each preparation. Standard hematological parameters (blood elements ratio, leukocyte formula, erythropoiesis level) were studied; possible pathologies of erythrocytes were recorded.

The internal organs condition of the carp was assessed by visual pathomorphological analysis followed by fragments fixation and making the histological preparations of gills, liver, kidneys, gonads, somatic muscles and myocardium. Organs were removed from live fish immediately after the catch. Making and staining of histological preparations (paraffin sections, 8 microns) corresponded to standard methods [4].

Pictures of histological preparations were taken using the Levenhuk C-Series C510 NG ocular digital microphotometer. Adobe Photoshop CS6 and Paint were used to graphically illustrate the results.

Statistical processing of the data obtained was carried out using the Microsoft Excel program.

3. Results and Discussion

Numerous studies in the field of ichthyopathology have established that fish under adverse external influences of different nature have a variety of abnormalities in internal organs at the tissue and cellular levels. The disease severity directly depends on the duration and intensity of aggressive environmental factors [5]. Histopathologies of internal organs are often observed in fish under the influence of pollutants both in natural populations [6, 7] and in artificial breeding [8–10].

We did not record any excess in main groups of pollutants in the studied fisheries ponds where artesian springs were used for filling and subsequent maintaining the water level. However, water saturated with magnesium, calcium, and iron salts was characterized by increased hardness (pH = 9.0–10.5). Another adverse factor for fish living in the studied ponds was non-compliance with the biotechnology of breeding. The high density of carp held in a small isolated reservoir leads to a decrease in the concentration of dissolved oxygen and, as a result, respiratory depression in fish. A physiologically inadequate fish diet (primarily, an insufficient amount of raw protein) can cause alimentary diseases (for example, injuries of the liver structure and functioning). We also registered that studied reservoirs do not meet the veterinary and sanitary standards, namely non-compliance with the procedures and terms of preventive measures.

Hematological studies that preceded the histological analysis of internal organs did not reveal any abnormalities in the cellular composition of red and white blood and other hematological parameters. However, even with short-term stabilization of environmental factors, blood parameters can return to the normal state due to increased reactivity of the hematopoietic system.

In contrast to hematological indicators, abnormalities of internal organs at the cellular level are stable. Complete tissue regeneration under conditions of external factor normalization occurs only in exceptional cases, provided that the manifestations of the pathological process were insignificant.

In the study of gills, 3 out of 10 carp showed foci of lamella epithelium detachment (gill filaments of the second order) (figure 1(b)–1), dysplasia of the apical part of isolated lamellae (figure 1(c)–1), and erosion marks in the blood vessel of the filament (gill filaments of the first order) (figure 1(c)–2). Compared to the picture of healthy gill tissue (figure 1(a)–1,2), the manifestations of the detected histopathologies are insignificant. The size of the registered foci abnormalities was no more than 0.5–1.0% of the surface of individual filaments, which characterizes the severity of these abnormalities as the initial stage of the pathological process.
Figure 1. Histological condition of carp gills (staining with Weigert’s iron hematoxylin followed by azur-eosin staining according to Romanovsky). (a) (×400) – normal gills: 1 – vessels and tissue of the filament (gill filament of the first order) without signs of dysplasia and deformation; 2 – lamellae (gill filaments of the second order) are not curved, the epithelium is smooth, no detachments, capillaries without signs of erosion; (b) (×400): 1 – detachment of the lamellae epithelium; (c) (×200): 1 – dysplasia (necrosis) of the lamellae, 2 – accumulation of melanin – initial signs of erosion of the filament vessel.

It is known that similar pathologies are observed in fish in reservoirs characterized by a high level of pollution. Gills, as a rule, are the first to be negatively affected by the environment, since they are in direct contact with water. Thus, the increased content of heavy metals, petroleum products and synthetic surfactants in the water provokes acute toxicosis in cyprinid fish, accompanied by pronounced foci of lamellae and filaments necrosis, infiltration, hyperemia, fusion and dissection of the gill filament epithelium [5, 9].

Histopathologies of gills in carp recorded in our study are associated with a violation of the oxygen regime in fish-breeding ponds and increased water mineralization. In the absence of anthropogenic toxic pollution of water reservoirs, abnormalities of the structure of the gill epithelium of lamella and filaments, recorded in a third of the examined fish, were mild.
The most important histophysiological marker of the body condition and its response to the environmental background is the liver, which performs many metabolic functions including detoxification [6]. National and foreign researchers have described various histopathological changes that have different degrees of significance for the functional state of the organ [11-17].

Histological analysis of liver preparations in 40% of the studied carp revealed multiple pigmented granules (figure 2(a)-1), foci of necrosis of isolated hepatocytes (figure 2(a)-2) and areas of infiltration of blood cells into the organ tissue (figure 2(a)-3).

Figure 2. Histological state of carp liver and myocardium (staining with Weigert iron hematoxylin with additional staining with azur-eosin according to Romanovsky). (a) (×400) – multiple pigmented granules are results of impaired glycogen metabolism; 1 – pigmented granules, 2 – areas of hepatocyte dysplasia around pigmented granules, 3 – foci of infiltration (penetration) of blood cells into liver tissue; (b) (×400) – heart muscle (myocardium) of a healthy individual: myocardial tissue without signs of pathology: muscle fibers are uniform, not curved, without signs of dissection, cavities, necrosis, 1 – transverse section of myofibrils, 2 – longitudinal section of myofibrils; (c) (×100) – initial signs of myocardial dystrophy: 1 – curvature of myofibrils, 2 – extraneous cavities formed as a result of dissection of muscle fibers.
Pigment deposition in the liver parenchyma is considered to be a very characteristic sign of its toxicity [18]. However, no pollutants were registered in the studied reservoirs. Multiple pigmented granules observed on serial sections of the liver of four carp are the result of disorders of glycogen metabolism due to the unbalanced diet of fish.

Blood cell infiltration refers to inflammatory types of changes associated with immune processes. This pathology usually precedes necrosis of hepatic tissues, since some types of white blood cells (neutrophils and eosinophils) perform a phagocytic function [6].

The most serious pathology is necrosis (dysplasia) of hepatocytes recorded in some fish. The presence of necrosis indicates a severe, usually progressive pathological process caused by water polluted with heavy metals, or parasitic infestations [19-21]. Microscopic signs of necrosis are changes in both the cell nucleus (karyopyknosis, karyorhexis, karyolysis) and the cytoplasm [18].

The study of the fish heart muscle showed that only one out of ten examined individuals has initial signs of myocardial dystrophy: dissection of myofibrils (figure 2(c)–1) followed by the formation of extraneous cavities (figure 2(c)–2). However, the overall histological picture of the heart smooth muscles state differs significantly from that of healthy carp (figure 2(b)–1, 2).

In dystrophy, the myocardial fibers have a loose structure and are often curved, while healthy muscle fibers are localized in the form of dense bundles and strands forming certain specific anatomical structures. Such abnormalities can be localized in the myocardium in the form of foci of various sizes, or can cover large areas of the heart. In the affected individual, the foci of myofibril dystrophy were isolated and compactly localized, which also indicates the initial stage of the pathological process.

Histological pathologies of the myocardium are not a direct consequence of the impact of adverse environmental factors on the body, such as, for example, pathologies of the gills and liver. Perhaps the presence of such abnormalities in the tissue structures of the heart is the result of the general physiological state of the fish body, which due to a number of factors (intoxication, exhaustion, stress, etc.) may not correspond to the norm [22]. However, the very fact that such pathologies are found in the tissue of the organ, which is most physiologically protected from adverse external influences and does not directly perform the detoxification function, indicates the chronic impact of negative environmental factors on the body [22].

It is known that destructive processes in the myocardium and cardiocytes develop by the type of autoimmune reactions, just as in the striated skeletal muscles. However, no histopathologies were recorded in the latter.

4. Conclusion
Histopathology of internal organs in the carp in fish farming ponds occurs due to various reasons and have different degrees of severity and depth of the pathological process. In the absence of a systemic toxic load on the system of fish-breeding reservoirs, the main adverse factors of the aquatic environment can be violations of hydrological, thermal and hydrochemical regimes, as well as violations of the fish breeding biotechnology. In this case, the histopathology of animals’ internal organs is less pronounced than in the case of toxic effects of pollutants.

Thus, only a third of the examined carp have minor focal abnormalities in the tissues of the gill apparatus, which may be due to a lack of oxygen and increased water hardness. Liver tissue in some individuals has signs of metabolic disorders, in particular, glycogen metabolism. Improving the composition and quality of the feed used will help to eliminate this pathology of the organ which is characterized by a high regenerative ability. A disturbance in the morphology of the myocardium myofibrils, recorded only once, may be a consequence of chronic stress caused by the complex of the above factors.

However, the registered initial signs of the pathological process in the gills, liver and heart muscle can be leveled by improving the fish keeping conditions.
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References
[1] Noga E J 1996 Fish diseases: diagnosis and treatment (St Louis, MO: Mosby-Year Book)
[2] Grischenko L I, Akbaev M S and Vasil’kov G V 1999 Fish diseases and the basics of fish farming (Moscow: Kolos Publ.)
[3] Ivanova N T 1983 Atlas of fish blood cells (Comparative morphology and classification of fish blood elements) (Moscow: Legkaya i pishchevaya promyshlennost’ Publ.)
[4] Mikodina E V, Sedova M A, Chmilevsky D A, Mikulin A E, Pyanova S V and Poluektova O G 2009 Histology for ichthyologists: Experience and advice (Moscow: VNIRO)
[5] Fomina A S 2019 Histopathological studies of fish from Bratsk reservoir Transactions of IBIW 87(90) 41-50.
[6] Mineev A K and Mineeva O V 2019 Histopathology of liver of fish in the Saratov reservoir Theoretical and Applied Ecology 3 109-114.
[7] Fedorova N N, Grushko M P and Kanieva N A 2019 Pathomorphological changes in vital organs of the Volga fishes Vestnik of Astrakhan State Technical University. Series: Fishing Industry 4 104-109
[8] Gavurseva T V 2007 Pathomorphological changes from alimentary toxicosis in juveniles of Pacific Salmon in Kamchatka Research of water biological resources of Kamchatka and of the northwest part of Pacific Ocean: Selected Papers 9 170-184.
[9] Donnik I M and Prokkoeva Zh A 2013 Pathomorphological state fish farm on the waters of power stroke Agrarian bulletin of the Urals 9(115) 29-32.
[10] Adyrbeko K B, Isbekov K B, Assylbekova S Zh, Kobegenova S S and Koishybayeva S K 2017 Histomorphological estimation of some internal organs of rainbow trout Oncorhynchus mykiss (Walbaum, 1792) cultivated on experimental feed Vestnik of Astrakhan State Technical University. Series: Fishing industry 4 144-154
[11] Selyukov A G 2007 Morphological and functional status of fishes of the Ob-Irtshy watershed in the modern world (Tyumen: Publishing house of Tyumen State University)
[12] Fish pathology 1989 (London; Philadelphia; Sydney; Tokyo; Toronto: Stirling Univ.)
[13] Pedlar R M, Ptashinski M D, Evans R and Klaverkamp J F 2002 Toxicological effects of dietary arsenic exposure in lake whitefish (Coregonus clupeaformis) Aquatic toxicology 57(3) 167-189.
[14] Thophon S, Kruatrachue M, Upathan E S, Poketheityook P, Sahaphong S and Jarikhuan S 2003 Histopathological alterations of white seabass (Lates calcarifer) in acute and subchronic cadmium exposure Environmental pollution 121 307-320
[15] Agamy E 2012 Histopathological changes in the livers of Rabbit Fish (Siganus canaliculatus) following exposure to crude oil and dispersed oil Toxicologic pathology 40 1128-1140.
[16] Grushko M P, Fedorova N N and Nasikhanova M N 2013 State of vital organs of pike perch in the Volgo-Caspian basin Vestnik of Astrakhan State Technical University. Series: Fishing industry 3 108-112.
[17] Schuman L A, Nekrasov I S and Selyukov A G. 2013 Morphofunctional correlations of the perch Perca fluviatilis in polluted lakes of the Middle Ob region Bulletin of Tyumen State University . Ecology 12 128-139.
[18] Kruchkov V N, Dubovskaya A F and Fomin I V 2006 Peculiarities of pathologic morphology of fish liver in modern conditions Vestnik of Astrakhan State Technical University. Series: Fishing industry 3(32) 94-100
[19] Tramp B F, Jones R T and Sahaphong S 1975 Cellular effects of mercury on fish kidney tubules *The pathology of fishes* ed W E Ribelin and M Migan (Wis. Univ., Wisconsin Press) pp 585-612

[20] Kent M L, Myers M S, Hinton D E, Eaton W D and Elston R A 1988 Suspected toxicopathic hepatic necrosis and megalocytosis in pen-reared Atlantic salmon *Salmo salar* in Puget Sound, Washington, USA *Diseases of aquatic organisms* 4 91-100.

[21] Hibiya E T 1996 *An atlas of fish histology: normal and pathological features* (N.Y.: Kodansha Ltd)

[22] Mineev A K 2013 Nonspecific reactions in fish from waters Middle and Lower Volga *Bulletin of the Samara Scientific Center of the RAS* 15(3(7)) 2301-2318