Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 11	Fasc. 1	57-67	2003

HISTOPATHOLOGICAL CHANGES IN JUVENILE CARP *CYPRINUS CARPIO* L. CONTINUOUSLY EXPOSED TO HIGH NITRITE LEVELS FROM HATCHING

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ABSTRACT. Carp were cultivated (from day 3 to 30 after hatching) in water with a nitrite concentration of 7 mg NO_2-N dm^{-3}. The results of histological studies confirmed pathological changes in the throat, gills, heart, blood, swim bladder, liver and kidneys. These changes mainly had an adverse impact on respiration and growth. The total length and weight of fish from water with nitrites was lower by 30 and 75%, respectively, and their survival rate was 62% (97% in the control group).

Key words: COMMON CARP *(CYPRINUS CARPIO)*, NITRITE, HISTOPATHOLOGICAL CHANGES, INTERNAL ORGANS

INTRODUCTION

The discharge of industrial and communal sewage to rivers and lakes, as well as the use of fertilizers and insecticides in agriculture result in the contamination of aquatic environments with toxic substances which are harmful to aquatic animals. Ammonia (NH_3) is one of the most commonly observed nitrogen compounds and is regarded as highly poisonous to fish (Arillo et al. 1981). High concentrations of ammonia can occur in water reservoirs where fish are abundant, especially when the fish are fed artificial feed. This applies to cultivation ponds, especially those with closed water systems, as well as wintering and storage ponds. Ammonia in the water oxidizes into nitrites and nitrates. These substances are not as poisonous as ammonia, nevertheless, they are not neutral to fish (especially nitrites).

The results of studies on the toxicity of nitrites for fish were presented by Eddy and Williams (1987). Numerous works indicate the variety of adverse impacts nitrites can have on fish (Jensen 1990, Hofer and Gatumu 1994, Alcaraz and Espina 1995, Knudsen and Jensen 1996, Frances et al. 1998). Wedemeyer and Yasutake (1978) confirmed that nitrite toxicity increases in acidic water and it decreases in hard, alkaline water. The tolerance of nitrites by fish is also positively influenced by Cl^- ions

(Weirich and Tomasso 1993), increased oxygen content in the water (Lloyd 1961), the presence of ascorbic acid (Wise and Tomasso 1988) and by the size of the fish (Alcaraz and Espina 1995).

The parts of the fish which are most threatened are those which come into direct contact with the poison. These include the skin, gills, throat, esophagus and the intestines. Flis (1968) conducted histological studies of carp, *Cyprinus carpio* tissues exposed to water with ammonia and described the pathological changes which occurred in the skin, muscles, gills, intestine, liver and kidneys of the studied fish. Pathological changes in the tissues of different fish species which resulted from contact with nitrites were described by Klingler (1957), Michael et al. (1987), Jensen et al. (1987), Hofer and Gatumu (1994) and others.

This work presents an evaluation of the impact of nitrites on juvenile carp. The aim is to present the histopathological changes which occurred among juvenile carp reared in water with an increased nitrite concentration.

MATERIAL AND METHODS

The study material consisted of 3-day-old carp larvae from the hatchery of the Fisheries Experimental Center of the Inland Fisheries Institute in Żabieniec. Cultivation was carried out in six aquariums with volumes of 20 dm^3 each (three each of experimental and control aquariums). Each aquarium was stocked with 600 larvae. The hatch were cultivated in water at a temperature of 22°C. The nitrite nitrogen content in the experimental aquariums was 7 mg NO_2-N dm^{-3}. Three-quarters of the volume of each aquarium was exchanged twice daily and replaced with water with the appropriate amount of NO_2-N (without the addition of nitrite). The water in the aquariums was aerated, and the oxygen saturation was maintained at 85-90%. The chemical composition of the water was as follows: Cl^- – 10 mg dm^{-3}; NH_4 – 0.006 mg dm^{-3}; NO_2 – 0.0025 mg dm^{-3}; NO_3 – 0.025, PO_4 – 0.015 mg dm^{-3}; HCO_3 – 189.2 mg dm^{-3}; pH = 8.0. The fish were fed *ad libitum* with decapsulated artemia cysts.

The samples for histopathological studies (10 specimens from each of the experimental and control groups) were collected on day 27 of cultivation when the carp had completed the larval stage. The average fish total length and weight were as follows: in the control group – 17.2 mm and 79.5 mg (97% survival rate); in the water with nitrites – 11.9 mm and 19.3 mg (62% survival rate). The study material was preserved in Bouine-Holland liquid and then submerged in paraffin. A series of 5 μm thick par-

affin sections were cut with a MicroTec CUT 4050 microtome. Some fish were cut crosswise and lengthwise. The series of sections were subjected to normal histological procedures and were dyed with hematoxylin and eosin according to Delafield (Zawistowski 1983).

Pathological changes were observed in the throat, gills, heart, blood, swim bladder, liver and kidneys. The fish in waters with higher nitrite concentrations were compared to those from the control groups.

RESULTS

THROAT

Far fewer chloride cells were confirmed in the throat epithelium of carp from the water with a nitrite concentration of 7 mg NO_2-N dm^{-3} for 27 days (Photo 1A) than in that of the carp reared in control conditions (Photo 1B).

GILLS

The most adverse changes in nitrite-exposed carp were observed in the gills (Photo 2A). Clear hypertrophy and hyperplasia of the gills was confirmed. Histological studies indicated hypertrophy in the respiratory epithelial cells. Necrosis of the cell structure resulted in the creation of empty spaces between the cells. The separation of the basal membrane was also observed in the gill arches. As was the case in the throat epithelium, the number of chloride cells was decreased. Large amounts of mucus were observed on the surface of the epithelium. The gill arch epithelium

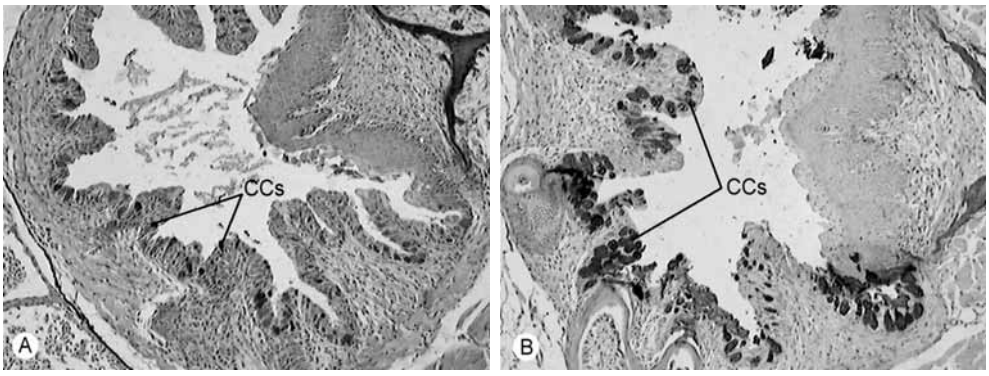

Photo 1. Cross-section of the throat epithelium; (H-E) x 25; CCs - chloride cells. A – 7 mg NO_2-N dm^{-3}. B – control.

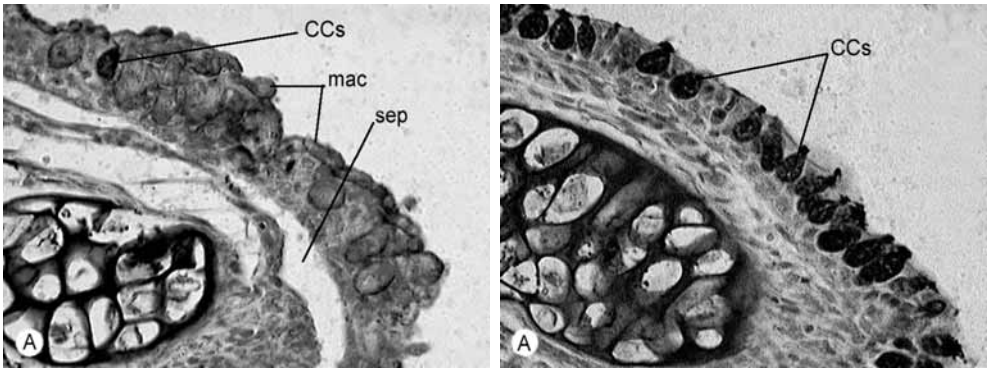

Photo 2. Cross-section of the gill arch; (H-E) x 100; CCs - chloride cells. A – 7 mg NO_2-N dm^{-3}; mac – mucus accumulation, sep – basal membrane separated from gill arch. B – control.

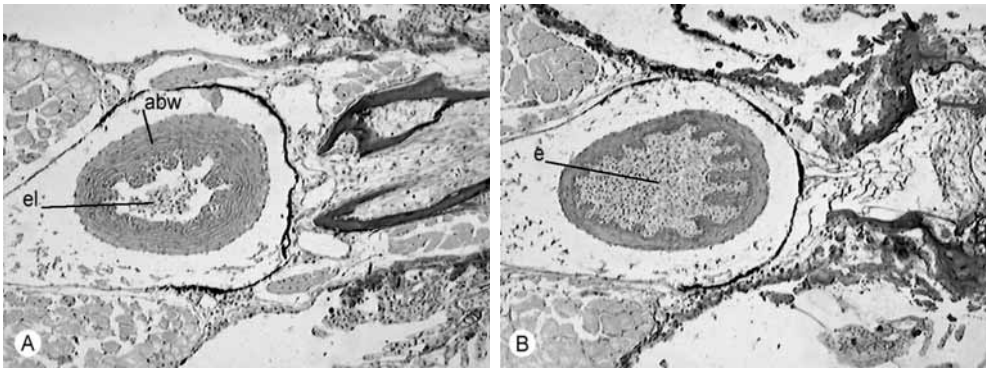

Photo 3. Cross-section of the arterial bulb; (H-E) x 25. A – 7 mg NO_2-N dm^{-3}; el – erythrocyte lysis, abw – arterial bulb walls. B – control; e – erythrocytes.

was adjacent to the basal membrane in the fish from the control group (Photo 2B). The gill epithelium of fish which developed in clean water included more chloride cells than in the nitrite-exposed carp. No excessive mucus accumulation was observed in the gill epithelium of the control group fish.

HEART

Significant thickening of the arterial bulb walls was observed in the nitrite-exposed carp specimens (Photo 3A). Histological studies indicated that there was a significant decrease in the number of erythrocytes. The arterial bulb of the control group fish had a more regular shape and was fully filled with properly developed erythrocytes (Photo 3B).

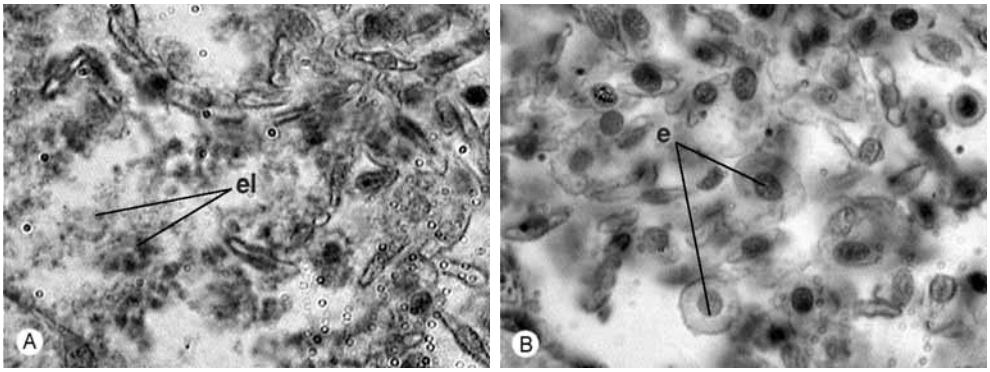

Photo 4. Erythrocytes in hepatic veins; (H-E) x 250. A – 7 mg NO_2-N dm^{-3}; el – erythrocyte lysis. B – control; e – erythrocytes.

BLOOD

Studies of the blood components of the nitrite-exposed carp indicated significant pathology (Photo 4A). Erythrocyte lysis was observed (almost complete deterioration of the cellular membrane) and red cells dysplasia. Pycnosis of the nucleus was observed in some red cells. Some red cells were necrotic. The shape of the erythrocytes from the control group fish were normal with properly developed, centrally located nuclei (Photo 4B).

SWIM BLADDER

Pathological changes were noted in the swim bladders of the nitrite-exposed carp. The filling of the anterior chamber of the swim bladder indicated that it was inflamed (Photo 5A). This was also observed in the posterior chamber of the swim bladder (Photo 6A), which also had unnaturally thick walls. The swim bladders of the control group fish were filled only with gas (Photo 5B and Photo 6B), and their walls were thinner than those found in the nitrite-exposed carp specimens.

LIVER

Histological studies of the livers of nitrite-exposed carp specimens indicated that there was partial hepatocyte necrosis (Photo 7A). Broken cell walls, lysis, necrosis and pycnosis of cellular nuclei were all observed in hepatocytes. Numerous damaged erythrocytes were visible in the hepatic veins. Damaged vascular vessels were accompanied by internal hemorrhaging.

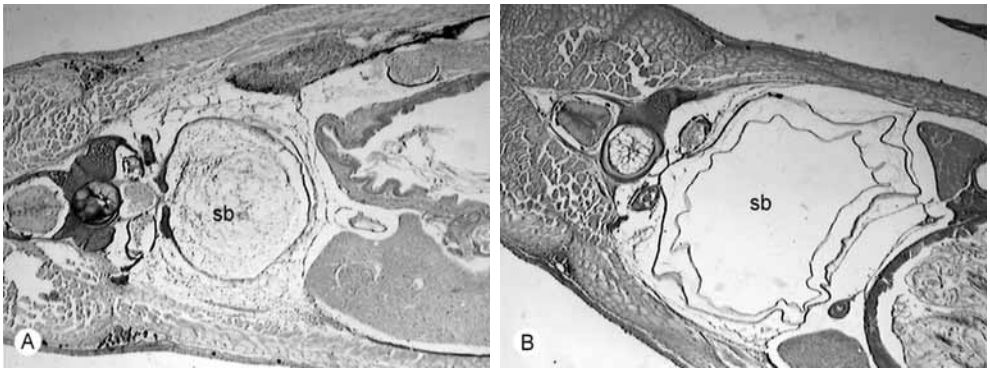

Photo 5. Cross-section of the anterior chamber of the swim bladder. (H-E) x 10; sb – swim bladder. A - 7 mg NO_2-N dm^{-3}. B – control.

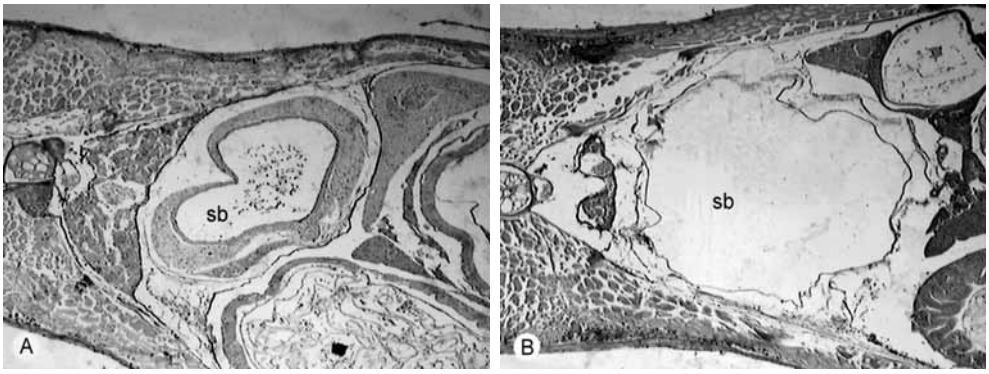

Photo 6. Cross-section of the posterior chamber of the swim bladder; (H-E) x 10; sb – swim bladder. A - 7 mg NO_2-N dm^{-3}. B – control.

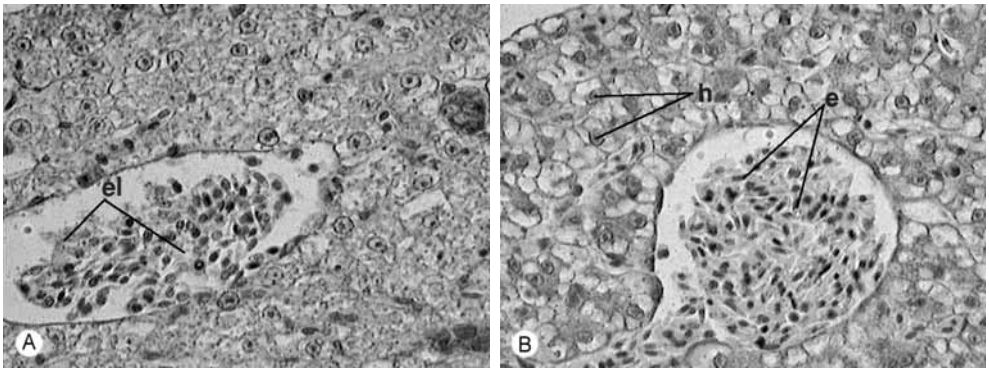

Photo 7. Cross-section of the liver, (H-E) x 100; A - 7 mg NO_2-N dm^{-3}; Hepatocyte necrosis, el – erythrocyte lysis. B – control; h – well developed hepatocytes, e – erythrocytes.

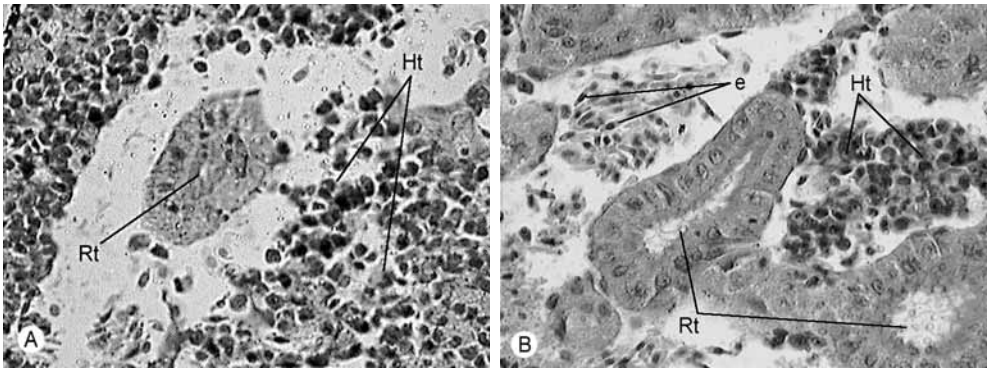

Photo 8. Cross-section of the kidney; (H-E) x 100; Rt – Renal tubuli, Ht – Hematopoietic tissue. A - 7 mg NO_2-N dm^{-3}; Renal tubuli partly occluded, necrosis of hematopoietic tissue. B – control, e – erythrocytes.

The liver cellular structure in the control group carp specimens was clearly formed with properly developed hepatocytes (Photo 7B). No pathology was observed in the build of the vascular vessels or in the erythrocytes of this group of fish.

KIDNEY

The kidneys of the nitrite-exposed carp specimens had a more relaxed cellular structure in comparison with the control group fish (Photo 8A). Hematopoietic tissue lysis and necrosis were both observed. Some of the renal tubuli were occluded, and lysis was observed in the surrounding cells. The structure of the hematopoietic tissue cells of the kidney were normal in the control group fish (Photo 8B). The renal tubuli were formed of a single layer of hexagonal cells with centered nuclei and were not occluded.

DISCUSSION

The choice of the water nitrite concentration of 7 mg NO_2-N dm^{-3} was made based on results of the authors' earlier studies (Korwin-Kossakowski and Myszkowski 1995). This concentration clearly impeded the growth rate in comparison with that of fish cultivated under controlled conditions, and nitrite concentrations of 1, 2, 3 and 5 mg NO_2-N dm^{-3} also significantly increased carp larvae mortality. The nitrite-exposed carp specimens in the present experiment were smaller than those from the control group; the latter were almost 50% longer and four times heavier than the former. The slower

growth rate and lower survival rate (62 versus 97%) meant that pathology in the experimental fish was to be expected.

Not only do gills perform respiratory functions, they are also responsible for osmoregulation (Frances et al. 1998). They are directly exposed to poisons occurring in the external environment which often cause pathology in fish (Mallatt 1985). The hypertrophy and hyperplasia of the gill epithelium and the separation of the basal membrane observed in the nitrite-exposed fish from the current study concur with earlier observations made by Wedemeyer and Yasutake (1978) in *Oncorhynchus mykiss*, Michael et al. (1987) in *Clarias lazera* and Frances et al. (1998) in *Bidyanus bidyanus*. Gill hyperplasia was also observed in fish cultivated in water contaminated with ammonia (Larmouex and Piper 1973). Thurston et al. (1978) confirmed hypertrophy in the plates of the gill epithelium, necrosis in the epithelial cells and the separation of the epithelium from the gill arch in trout fry reared for 29 days in water with elevated ammonia levels.

Usually the first reaction of the external tissues of fish to the presence of toxic compounds in water is increased mucus production, mainly in delicate areas such as the gill epithelium. This phenomenon was observed in fish which inhabited acidic (Ultsch and Gros 1979) and alkaline waters (Daye and Garside 1976, Jezierska 1988, Ostaszewska et al. 1999) as well as those with increased levels of ammonia (Flis 1968, Smart 1976) and nitrites (Michael et al. 1987). An increase in the number of mucus cells and greater mucus production are regarded by some authors as defense mechanisms (Michael et al. 1987, Laurent et al. 1995). Pathological changes in the gills always have an adverse impact on respiration. Diminished respiration ability in nitrite-exposed carp was reported by Jensen et al. (1987) and Alcaraz and Espina (1997), by Murthy et al. (1981) for fish in alkaline waters and by Korwin-Kossakowski and Jezierska (1985) for fish in acidic waters. Chloride cells participate in ion transport through the gills and the body surface (Henrikson and Matoltsy 1968, Fosket and Scheffey 1982, van der Heijden et al. 1999). The number of these cells depends on the species. Williams and Eddy (1988) reported that there were nearly two-fold fewer of them in carp gills than in trout gills. They also observed an increased number of chloride cells in the gill epithelium of nitrite-exposed trout. The results of the present authors' own studies indicate the reverse reaction, i.e. the number of chloride cells decreases in nitrite-exposed fish.

No data regarding cardiac pathology in carp larvae which are exposed to nitrites for an extended period was found in the available literature. Changes in the arterial

bulb walls of juvenile carp specimens held in alkaline water with a pH 10.3 were described by Ostaszewska et al. (1999). However, Ostaszewska reported decreases in wall thickness, while they were clearly thicker in fish from water with increased nitrite concentrations.

Hematological pathology in carp caused by higher nitrite contents in water was described by Jensen et al. (1987) and Williams et al. (1993). They reported the shrinkage of erythrocytes, decreased hematocrit values, methaemoglobinaemia and a distorted electrolyte balance.

Data regarding histopathological changes in the swim bladder caused by nitrites are scarce. Korwin-Kossakowski et al. (2001) confirmed swim bladder pathology in, nitrite-exposed carp which was manifested by the disappearance of gas from the bladder. This state was reversible; when the fish were moved into clean water their swim bladders refilled with gas, they fed and continued growing. Deformations in the swim bladder were also observed in fish cultivated in alkaline water (Ostaszewska et al. 1999).

Michael et al. (1987) observed concentrations of fat around the hepatic vein and the formation of cell vacuoles in *Clarias lazera* exposed to nitrites, and increased transaminase activity related to hepatic pathology. The histopathological studies of the kidneys of *Clarias lazera* held in water with elevated nitrite concentrations did not indicate any pathology. The liver changes observed in the current study are similar to those described by Flis (1968), who studied the livers of 16 month-old carp specimens which had been held in water with elevated ammonia levels.

SUMMARY

The nitrite content in water at a level of 7 mg NO_2-N dm^{-3}, although not lethal, still has a significant, adverse impact on the health of fish. Histopathological changes which are observed in nitrite-exposed carp specimens mainly have an adverse impact on respiration and growth. This is due to decreased respiratory potential caused by the destruction of gill epithelium cells and increased mucus excretion and by the impaired ability of the blood to absorb and transport oxygen (destruction of erythrocytes, decreased hematocrit, methaemoglobinaemia, distorted ion equilibrium and osmoregulation), thus, the metabolic rate is decreased. Hepatic and renal pathologies also have an adverse impact on the fish.

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STRESZCZENIE

ZMIANY HISTOPATOLOGICZNE U MŁODOCIANEGO KARPIA, *CYPRINUS CARPIO* L., PODDANEGO DZIAŁANIU AZOTYNÓW

Na podstawie badań histologicznych stwierdzić można, że obecność azotynów w wodzie w okresie rozwoju larwalnego karpia może powodować znaczące patologiczne zmiany w narządach i tkankach ryb młodocianych. Obserwowane zmiany dotyczyły: nabłonka gardzieli (fot. 1) - redukcja komórek chlorkowych; skrzeli (fot. 2) - rozrost i przerost skrzeli, hipertrofia komórek nabłonka oddechowego, oddzielanie się błony podstawnej nabłonka łuków skrzelowych, redukcja komórek chlorkowych w nabłonku skrzeli; serca (fot. 3) - pogrubienie ścian opuszki tętniczej; krwi (fot. 4) - zredukowana liczba erytrocytów, liza erytrocytów, ich dysplazja i nekroza, a także pyknoza jąder komórkowych; pęcherza pławnego (fot. 5, 6) - stan zapalny przedniej i tylnej komory, pogrubienie ścianek przedniej komory; wątroby (fot. 7) - zniszczenie struktury komórkowej, liza hepatocytów oraz pyknoza ich jąder, pękanie naczyń krwionośnych; nerki (fot. 8) - liza i dysplazja komórek tworzących zręb nerki, częściowo niedrożne kanaliki nerkowe oraz liza komórek je otaczających.

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