

# Histopathological Analysis of Liver in Fish Population of Reservoir 'Tikves'

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**Abstract:** Liver pieces of 12 fish individuals collected from reservoir 'Tikves' during 2010, were excised and processed for standard histopathological analysis. The obtained results revealed pathological changes in the liver tissue including an inflammatory processes, fibrosis and necrosis on a level of hepatocellular parenchyme, bile duct proliferation and bile duct epithelium necrosis on a level of hepatic bile tract. Estimation of water quality of reservoir 'Tikves' on the basis of physico-chemical and microbiological parameters indicate that waste water have distinct impact to the natural fish population.

**Key words:** liver, histopathology, inflammation, necrosis, Tikves

## Introduction

Tikves is one of the largest artificial reservoir, which has been build with constraining of Crna River near the town of Kavadarci. This reservoir is a hydroenergetic and irrigation system that is exploited for sports and economic fishery (LOKOSKA *et al.* 2001).

In the accumulation Tikves (a.k.a. Lake Tikves) there are to be found 20 different species of fish, representatives of 7 families. Twelve of them are representatives of the family Cyprinidae, two of them are representatives of both Percidae and Acipenceridae and there is one representative for each, Esocidae, Siluridae, Ictaluridae and Centrarrhidae. Moreover, this is the first place in the Republic of Macedonia where is detected presence of two representatives of the family Acipenceridae, i.e. Russian sturgeon – *Acipenser gueidenstaedtii* and starlet – *Acipenser ruthenus* (Official gazette of Republic of Macedonia No. 66/2011). Ako tova e durzavniaat vestnik – to se kazva State Gazette – a ne taka

The uncontrolled stocking and releasing of any kind of fish, without prior consultation with competent individuals and experts, have resulted in the

present condition of the reservoir Tikves whereby there is an uncontrolled increase of the population of the brown bullhead (*Ameiurus nebulosus*), which is one of the reason for the decreased populations of the other autochthonous species of fish. In addition, there have been released another invasive species of fish into the reservoir, i.e. *Acerina cernua*. On the other hand, the populations of the autochthonous fish followed a trend of a decrease. There have been recorded a decrease in the quantity of carp (*Cyprinus carpio*), the Macedonian vimba or malamida (*Vimba melanops*), the roaches (*Rutilus rutilus*) and the tench (*Tinka tinka*) (Official gazette of Republic of Macedonia No. 66/2011).

## Material and Methods

During the summer period of 2011, in the reservoir Tikves, from the locality Resava Bay, there have been caught total of 12 pieces of fish, which were classified in 6 different species (*Carasius gibelio*, *Rutilus rutilus*, *Ameirus nebulosus*, *Perca fluviatilis*,

*Vimba malanops*, *Silurus glans*). Out of each specimen there have been dissected a piece of the liver for histological analysis, which, thereafter has been processed on the standard procedure for development of histological preparation. They have been analyzed under a microscope and the registered tissue lesion has been photographed.

During the same period there have been collected samples of water from the reservoir Tikves, at two different localities from a vertical column with different depths, i.e.:

At Resava Bay (near the cages) at depth points of 0.5 m, 7 m and 15 m;

Near the dam at depth points of 0.5 m, 7 m, 15 m and 40 m.

The conducted microbiological and physico-chemical analysis are in accordance to the world's known and recognized standard methods, which are applied during the research of waters, as well as in accordance to the Macedonian Decree for Classification of the Waters (OFFICIAL GAZETTE OF REPUBLIC OF MACEDONIA No.18/1999).

## Results and Discussion

Histology offers a powerful tool in the study of detailed structures of different tissues in fishes. It is used for sex verification, identifying stages of development, presence of parasites, tumors diagnosis, other abnormalities and changes in the arrangement of tissue layers including the digestive tract (MONSEFIL *et al.* 2010).

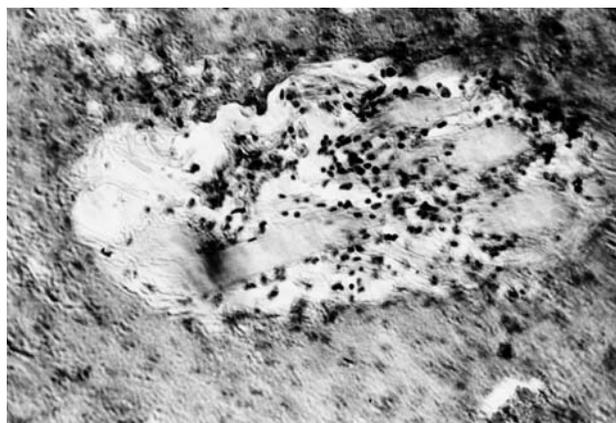
There has been conducted a histological analysis of the samples of liver from the natural population of fish, which inhabits the reservoir Tikves. The caught specimens of fish were with satisfactory external look, active and without any external changes. Only in the case with the brown bullhead, there have been recorded cases of samples with disorientation in the movement, low activity and manifestation of hypoxia. The reason for such condition presumably is the nonsufficient adaptation of this introduced species of fish to the circumstances of the accumulation. Since this species usually inhabits the layers of water near the bottom, where during the summer period the processes of pollution are manifested earliest, as well as the insufficient amount of oxygen and in some cases the formation of layers without any oxygen, there have been noticed some specimens characterized with dizziness and some of the regis-

tered samples were even dead (MALINS *et al.* 1987; WINSTON, DI GIULIO 1991; VELKOVA-JORDANOSKA, KOSTOSKI 2005).

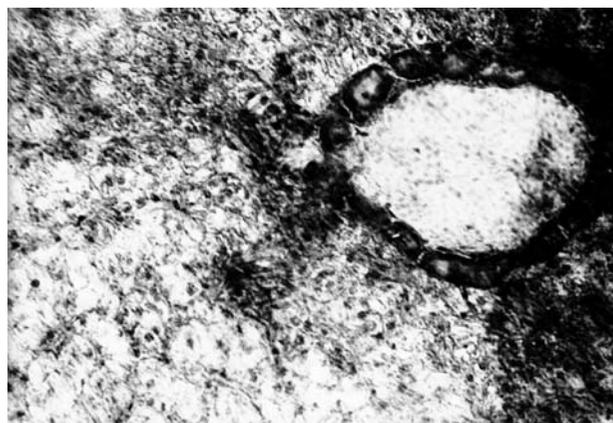
The analysis of the histological preparations of liver from the investigated specimens indicated presence of many types of tissue lesions. The obtained results revealed pathological changes in the liver tissue including an inflammatory processes, fibrosis and necrosis on a level of hepatocellular parenchyme, bile duct proliferation and bile duct epithelium necrosis on a level of hepatic bile tract (Fig. 1, 2, 3, 4). Approximately 75 % of all fish collected at reservoir Tikves showed evidence of steatosis, 66% showed the presence of inflammation, 41% showed presence of necrotic lesions and in 41% was detected fibrosis. Prevalence of parasite infestation was only 16%.

Almost in all cases in all investigated specimens of all researches species there has been registered presence of macrovesicular steatosis, which indicates mainly high quantity of organic material in the water (LEE 1994). In the long term this results into decrease of the quality of the water, increased presence of organic matter and decrease of the levels of oxygen in the water. In the liver of the investigated specimens there has been recorded presence of inflammatory processes, necrosis, as well as presence of parasites.

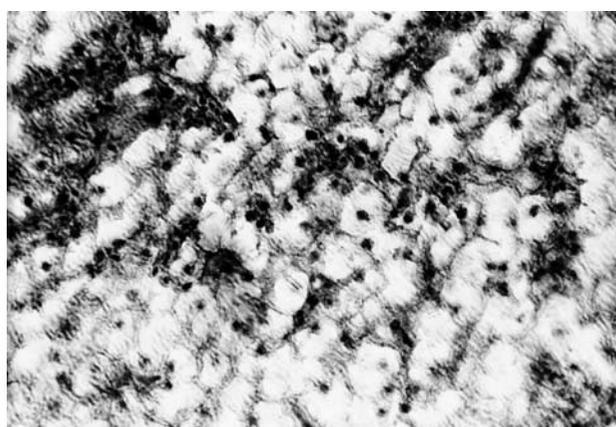
Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the mechanism of action of more xenobiotics could initiate formation of a specific enzyme that causes changes in metabolism, which further leads to cellular intoxication and death, on a cellular level, whereas that manifests as necrosis, i. e. histopathological biomarker on a tissue level (HINTON *et al.* 1992; VELKOVA-JORDANOSKA 2005). Chemicals provoke necrotic and degenerative alterations to with an inflammatory, defensive reaction (FOLMAR *et al.* 1993; ROGANOVIC-ZAFIROVA *et al.* 2003). A number of other studies have reported changes in the liver of fishes collected from polluted localities. Bile duct proliferation and bile duct epithelium necrosis has been found in the liver of Ohrid roach (*Rutilus rubilio ochridanus*) collected from polluted regions in Lake Ohrid (ROGANOVIC-ZAFIROVA, JORDANOVA 1998). Microskope analysis of histological preparation of barbell hepatic tissue (*Barbus peloponnesius*) at five



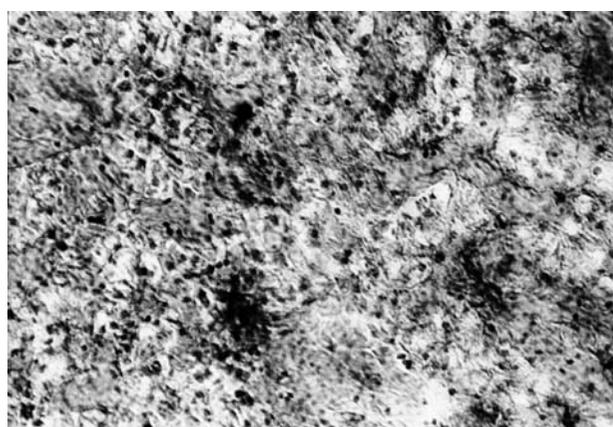
**Fig. 1.** The penetrated parasites into the liver of *Carasius gibelio*, H&E x100.



**Fig. 2.** Liver tissue of *Ameiurus nebulosus* with fibrous connective tissue around debris from parasite, H&E x 100.



**Fig. 3.** Liver tissue of *Perca fluviatilis* from the reservoir Tikves with excessive fat storage, H&E x 100.



**Fig. 4.** Necrotic lesion in liver tissue of *Perca fluviatilis*, H&E x 100.

**Table 1.** Categorization (by class) of water from reservoir Tikves according to total coliform and heterotrophic bacteria (Official gazette of Republic of Macedonia No.18/1999: Decree for Categorization of Waters)

Parameter	Resava Bay			Near to the dam			
	0.5 m	7 m	15 m	0.5 m	7 m	15 m	40 m
Coliform bacteria (bact/100 mL)	III	II	II	II	III	III	II
Heterotrophic bacteria (bact/mL)	III	III	II-III	II-III	II-III	II-III	II-III

**Table 2.** Categorization (by class) of water from reservoir Tikves according to physicochemical parameters (Official gazette of Republic of Macedonia No.18/1999: Decree for Categorization of the Waters)

Parameter	Resava Bay			Near to the dam			
	0.5 m	7 m	0.5 m	7 m	0.5 m	7 m	0.5 m
Dissolved O <sub>2</sub> (mg/l)	I	V	V	I	V	V	V
BPK5 (mg/l O <sub>2</sub> )	V	V	I	V	V	I	I
Organic matters- Consumption of KMnO <sub>4</sub> (mg/l)	V	V	IV	V	V	V	IV
N-NH <sub>3</sub> (µg/l)	III-IV	III-IV	I-II	III-IV	III-IV	I-II	III-IV
Total phosphorus (µg/l)	IV	IV	IV	IV	IV	V	V

localities in Ohrid Lake displayed a dominant presence of two types of histopathological lesions: granulomatous inflammation on a level of hepatocellular parenchyma and cholangiofibrosis on a level of hepatic bile duct (VELKOVA-JORDANOSKA 2002).

The liver is the central metabolic organ of fishes and has numerous anabolic and catabolic functions. This is an organ that stores carbohydrates as glycogen and, especially before spawning, fats. The hepatocytes, which normally appear compact, may give the impression of being more or less vacuolated under the light microscope, according to the degree of fat storage. A storage of a rich fat supply in the liver is scarcely ever encountered among juveniles or mature fishes after spawning (PETERS *et al.* 1987)

The phenomenon of fatty liver, or better defined, the storage of large quantities of fats in the fish liver, is quite normal at certain times, including periods of sexual maturation, when fish store considerable amounts of fat in the hepatocytes (KRANZ, PETERS 1985). A dietary imbalance is another especially common cause of fatty livers in cultured fishes (HILLE *et al.* 1980). However, when the diet is normal, when the fishes are beyond the spawning season, and when a concurrent combination with other degeneration phenomena is observed, fatty livers must undoubtedly be considered pathological (ROBERTS 1978). The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds, and could subsequently result in structural damage (BRUSLE *et al.* 1996).

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The obtained results of the conducted analysis during the summer period of 2011 indicate to a substantially decreased quality of the water, that is, specially increased development of the investigated bacteria (according KOHL 1974), as well as increased values for all related physicochemical parameters (according to Decree for Classification of the Waters – Official Gazette of Republic of Macedonia No. 18/99).

The analyzed samples of water from all investigated localities indicate to a relatively high level of organic load, which in accordance to the obtained values for the presence of the organic matter (represented as consumption of KMnO<sub>4</sub>) indicate that the water is mainly with quality of IV and V class. These obtained results indicate to an increased nutrient loading of the water from the accumulation.

## Conclusions

Liver lesions of certain character, macrovesicular steatosis, inflammatory processes, fibrosis and necrosis on a level of hepatocellular parenchyme, bile duct proliferation and bile duct epithelium necrosis on a level of hepatic bile tract were registered in fish population from reservoir Tikves. The hepatic-toxic impact of the pollutants in aquatic environment upon fish liver represents one of the possible reasons.

In order to be increased the quality of the water, there should be undertaken some measures for prevention the alohtone pollution, as well as the continuous monitoring of the quality of the water in the reservoir Tikves.

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