

Екол. Зашт. Живот. Сред.	Том 6	Број 1/2	стр. 19-27	Скопје 1998/99
Ekol. Zašt. Život. Sred.	Vol.	No.	p.p.	Skopje

ISSN 0354-2491

UDC: 631.862(497.17)

оригинален научен труд

LIVER LEZIONS IN OHRID MINNOW MORANEC (*Pachychilon pictus Heck. et Kner*) COLLECTED FROM SOME CONTAMINATED SITES OF LAKE OHRID. A HISTOPATHOLOGICAL EVIDENCE¹

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ABSTRACT

Roganović-Zafirova, D. and Tavčiovská-Vasileva, I. (1998/99). Liver lesions in Ohrid minnow moranec (*Pachychilon pictus Heck. et Kner*) collected from some contaminated sites of Lake Ohrid. A histopathological evidence. Ekol. Zašt. Život. Sred. Vol.6, No 1/2, 19-27, Skopje. Histopathological findings in the liver of Ohrid minnow collected from polluted areas were presented in this study. Extensive inflammatory lesions surrounding blood vessels and bile ducts accompanied by necrotic areas and occasional parasitic infestation were recognized as a consistent observation. Particular attention was paid to bile duct proliferation as a possible pollution-induced lesion.

Key words: pollution, Lake Ohrid, minnow, liver, histopathology

ИЗВОД

Рогановиќ-Зафирова, Д. & Тавчиовска-Василева, И. (1998/99). Лезии во црниот дроб на моранецот (*Pachychilon pictus Heck. et Kner*) од некои контаминирани региони на Охридското Езеро. Хистопатолошка студија. Екол. Зашт. Живот. Сред. Том 6, Бр. 1/2, 19-27, Скопје. Во оваа студија се презентирани хистопатолошките наоди во црнодробното ткиво на моранецот колекциониран од загадени региони на Охридското езеро. Регистрирани се екстензивни инфламаторни процеси околу крвните садови и жолчните канали пропратени со некротични промени и повремени паразитни инфестации. Посебно внимание беше обрнато на назначената пролиферација на жолчните канали како можна хепатоток ична лезија предизвикана од контаминираната средина.

Клучни зборови: загадување, Охридско Езеро, охридски моранец, црн дроб, хистопатологија

INTRODUCTION

The liver of various fish species living in polluted freshwater or marine environments or experimentally exposed to toxic contaminants express a number of morphological changes,

part of which may be considered histopathological biomarkers of anthropogenic stress (Meyers & Hendrick 1984; Hinton et al. 1992). Fish liver lesions like, generalized or

focal hepatocellular fatty change, hepatocytomegaly, spongiosis hepatis, bile duct proliferation, hepatocellular or cholangiocellular carcinomas and others, are seen after variety of environmental hepatotoxic insults (Kent et al. 1988, Baumann et al. 1990, Hayes et al. 1990,

Myers et al. 1987, 1990). The present study was aimed to detect histopathological changes in the liver of endemic fish Ohrid minnow moranec living in polluted littoral areas of Lake Ohrid.

MATERIAL AND METHODS

Ohrid minnow moranec individuals with total length from 90 to 165 mm, both males and females, were collected from two sites of Lake Ohrid - Grasnica (46 individuals, in August and October 1995 year) and Studencista Channel (7 individuals, in December, 1995 year). After being caught by overnight nets (various mesh dimensions) fishes were sacrificed by severing spinal cord within 24 hours. The livers were examined by hand lens for gross changes, excised, and fixed in 10% buffered formalin. Specimens containing liver pieces from 1-5 individuals were sampled for paraffin

embedding and histological processing. Blocks were sectioned at 5 μ m and stained with hematoxylin and eosin (H&E), Periodic Acid Schiff (PAS) and Feulgen nuclear reaction for DNA. About 50 liver sections were carefully screened for the presence of morphologically recognizable lesions. All observed changes were registered and described and their occurrence in the investigated material roughly estimated. Microscopical examination was performed on Reichert N° 326971 and Leitz Wetzlar microscopes.

RESULTS

Histological inspection of liver tissue revealed metabolically active hepatocytes with large euchromatic nuclei, prominent nucleoli and relatively wide basophilic cytoplasm. Hydropic degeneration of hepatocytes and lipid droplets accumulation in hepatic cells as a general or regional finding was more or less uncommon. A small area located near a central vein with a group of vacuolated hepatocytes, which may be interpreted as a focus of cellular alteration (FCA) was registered in one case (Fig. 1). Dilatation of central veins and hepatic sinuses was observed in approximately 20% of analyzed sections. Fibroid degeneration in the walls of medium and larger vessels was noticed in several liver sections (Fig. 2).

Foci of hepatic cell coagulative necroses mostly accompanied with inflammation were demonstrated in parenchyma or around larger blood vessels and bile ducts. Individuals caught in Studencista Channel showed stronger evidence of this finding. Even though small part of these necrotic lesions were clearly associated with parasitic infestations most of them showed no recognizable causative agent.

The presence of wide inflammatory areas in hepatic tissue was one of the most prominent histopathologic findings. Enormous granulomas containing lymphocytes, macrophages and plasma cells encompassing bigger and smaller vessels, and bile ducts, with no recognizable causative agent present (Fig. 3) was observed in most of the sections. Part of inflammatory infiltration areas showed typical features of fibrous granulomata (Fig. 4). Each contained a central zone of necrotic cellular debris, parasite remnants, or intact parasite cysts surrounded by a layer of epithelioid cells (macrophages resembling epithelial cells), and an external layer of other inflammatory cells. The entire focus was encapsulated by fibrous connective tissue. Induction of melanomacrophage centers in hepatic tissue was registered with remarkable prevalence.

Bile duct proliferation was detected as an imposing frequent finding. In some sections, relatively wide areas of multiplied bile ducts surrounded by stromal tissue (cholangiofibrosis) were observed (Fig. 5). The process was sometime accompanied with massive karyopyknosis of bile duct epithelium, which was

occasionally followed by complete lysis of duct walls (Fig. 6).

Scarce evidence of parasitic infestation included findings of encapsulated nematode larvae regularly surrounded by wide

granulomas, mixosporean cysts causing mild lymphocytic infiltration, and an unidentified protistan parasite in blood vessels. Histopathological findings are summarized in Tab. 1.

DISCUSSION

Microscopical survey of liver specimens from Ohrid minnow moranec inhabiting contaminated Lake Ohrid areas demonstrated histopathologically detectable lesions, part of which may be with certainty related to the impact of environmental toxicants.

Proliferation of bile ducts may be considered the most specific lesion registered in the investigated material. Profiles of bile ducts are normally infrequent in fish liver tissue except near the vena porta hepatis (Hayes et al. 1990). In some liver sections investigated in this study, the profiles of these passageways were numerous, contacting and with abundant branching and coiling. This lesion is of a chronic duration and has been reported as a consistent finding in wild fish from contaminated sites (Murchelano & Wolke 1985; Hayes et al. 1990). In western Lake Ontario, proliferative biliary diseases (cholangiohepatitis and cholangiofibrosis) of white suckers (*Catostomus commersoni*) were associated with bile duct neoplasms in polluted harbors (Hayes et al. 1990). These authors suggested that proliferative bile duct epithelial changes could predispose fish to initiation and promotion of bile duct neoplasia. Further investigation of this lesion in Ohrid minnow moranec from polluted and unpolluted areas in Lake Ohrid and surrounding tributaries may give better insight into this lesion and its eventual relation with carcinogenesis.

A single lesion - interpreted as a focus of cell alteration (FCA) - a small round area of fat vacuolated hepatocytes, was noticed in entire analyzed material. Focal fatty vacuolization of hepatocytes was reported after exposure of fish to a variety of different carcinogenic agents (Hendrick et al. 1984). This lesion is supposed to precede other changes in the development of neoplasia (Hinton et al. 1992). Liver lesions that appear as "ground glass cell" foci of cellular alteration were registered in bleak

inhabiting the same collecting sites (Roganovic-Zafirova & Jordanova 1998) which indicate the notability of this single evidence.

Part of the inflammatory lesions seen in the surveyed Ohrid minnow moranec livers were clearly associated with the presence of some helminths and mixosporeans. The rest of perivascular and peribiliary infiltration of lymphocytes and other leukocytes were not related with any visible etiological factor. Toxicant induced injury, as possible inflammation-provoking agent may not be excluded neither proved with certainty. The liver as a tissue in which lymphocytic type of inflammation commonly occurs may normally have resident populations of lymphocytes in portal triads, which vary in number according to animal species (Meyers & Hendrich 1984). Induced melanomacrophage centers in fish liver indicate an immune response provocation in various pathological states including anthropogenic stress (Hinton et al. 1992). In the collected Ohrid minnow moranec, it can not be taken as confident biomarker because the parasitism can not be excluded. From the same reasons, registered wide necrotic areas in liver parenchyma mostly associated with lymphocytic infiltration have to be interpreted with caution.

The congestion of central veins and sinuses was demonstrated with relatively high prevalence although with much less incidence than in Ohrid bleak collected from the same polluted areas in Lake Ohrid (Roganovic-Zafirova & Jordanova 1998). From the similar reasons that were considered in cited bleak liver study this finding could not be considered hepatotoxic lesion with certainty. The finding of fibroid degeneration in the walls of medium and larger vessels registered in few investigated liver sections is probably result of a local antigen-antibody reaction (Meyers & Hendrich 1984).

Tab. 1 Histopathological survey of liver lesions registered in Ohrid minnow moranec *Pachychilon pictus* (Heck et Kner) collected in Grasnica and Studencista Channel at various dates during 1995 year.

Таб. 1 Хистопатолошки преглед на лезиите во црниот дроб на единки од моранец *Pachychilon pictus* (Heck et Kner) изловени во регионот на Грашница и Каналот Студенчишта во различни периоди од 1995 година.

SPECIMEN	CON	XPT	DEGENER.		FCA	NEKROSES				INFLAMMATION				BP	TUMR	PARASITES		
			FCD	HD		IP	PV	PB/IB	IP	PV	PB/IB	MC	NP			MXS	HLM	
G-15.08.95 8 individuals				+++			++		++++	++	++++				++	++	++	
G-17.08.95 27 individuals	++	+	+	+	+	+	++	++	++++	++++	+	++++			+		+	
G-10.10.95 4 individuals	++++								++	++	++++							
K - 6.12.95 7 individuals	+++	+				+++	+++		+	++	++	++	+++					

Legend: G -Grasnica, K -Studencista Channel, CON - congestion, HRG -hemorrhage, FD - fatty degeneration, HD - hydropic degeneration, FCA - foci of cell alteration, IP - intraparenchymal, PV -perivascular, PB -peribiliar, IB -intrabiliar, MC-macrophage centers, BP - bile duct proliferation, TUMR - tumors, NP- non identified protistan, MXS - mixosporean, HLM -helminths, Finding observed in percent of analyzed sections: 1-10% +; 10-20% ++; 20-30% +++; over 30% ++++

Легенда: G -Грашница, K - Канал Студенчишта, CON - конгестива, HRG - хеморагија, FD - масна дегенерација, HD - хидропска дегенерација, FCA - фокуси на клеточна алтерација, IP - интрапаренхимално, PV - периваскуларно, PB - перибилиарно, IB - интрабилијарно, MC-макрофажни центри, BP - билијарна пролиферација, TUMR - тумори, NP- неидентификувани протисти, MXS - микоспоровоз, HLM -хелминти. Лезии видени во процент на анализирани пресеци: 1-10% +; 10-20% ++; 20-30% +++; преку 30% ++++

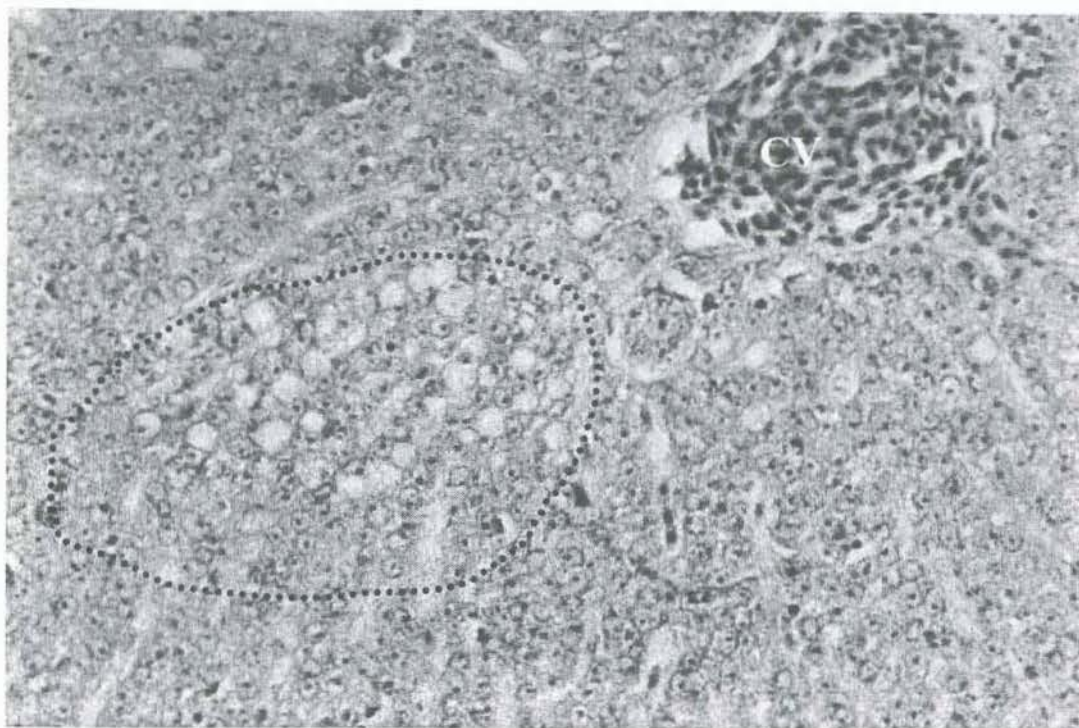


Fig. 1 A small area of focal alteration containing vacuolated hepatocytes (doted circle) near a central vein (CV) H&E x400

Сл. 1 Мал фокус на клеточна алтерација со вакуолизирани хепатоцити (точкеста линија) во близина на централна вена (CV). H&E x400

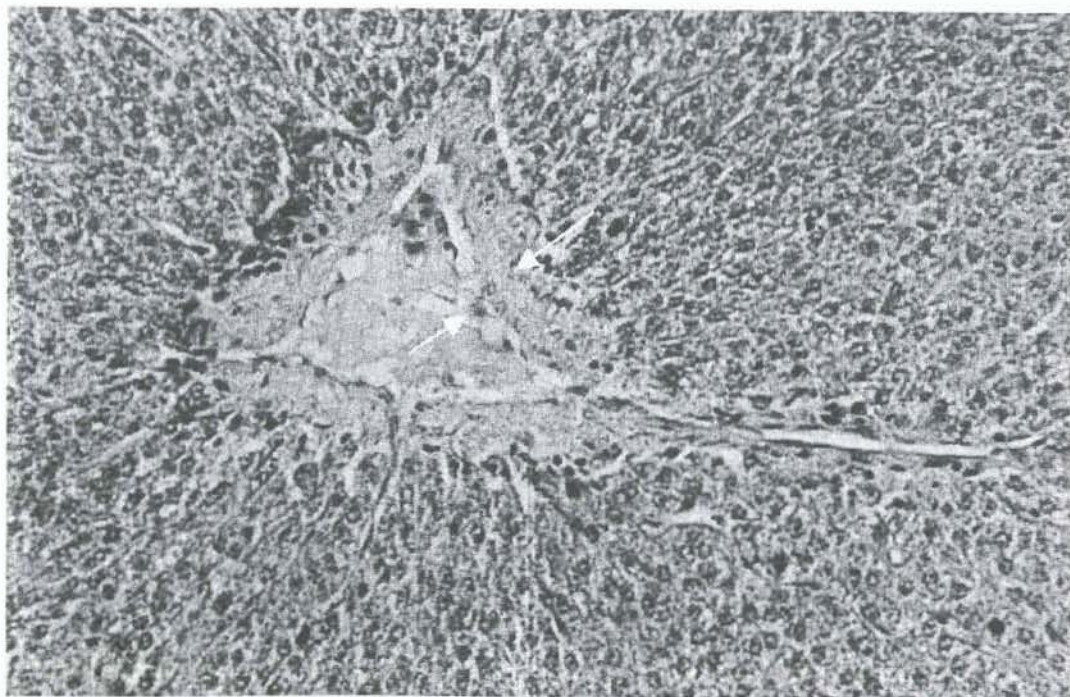


Fig. 2 A medium vessel in liver parenchyma showing thickened walls with fibroid degeneration (arrows) H&E x400

Сл. 2 Среден крвен сад во хепаталниот паренхим со фибриноидна дегенерација на сидот (стрелки) H&E x400

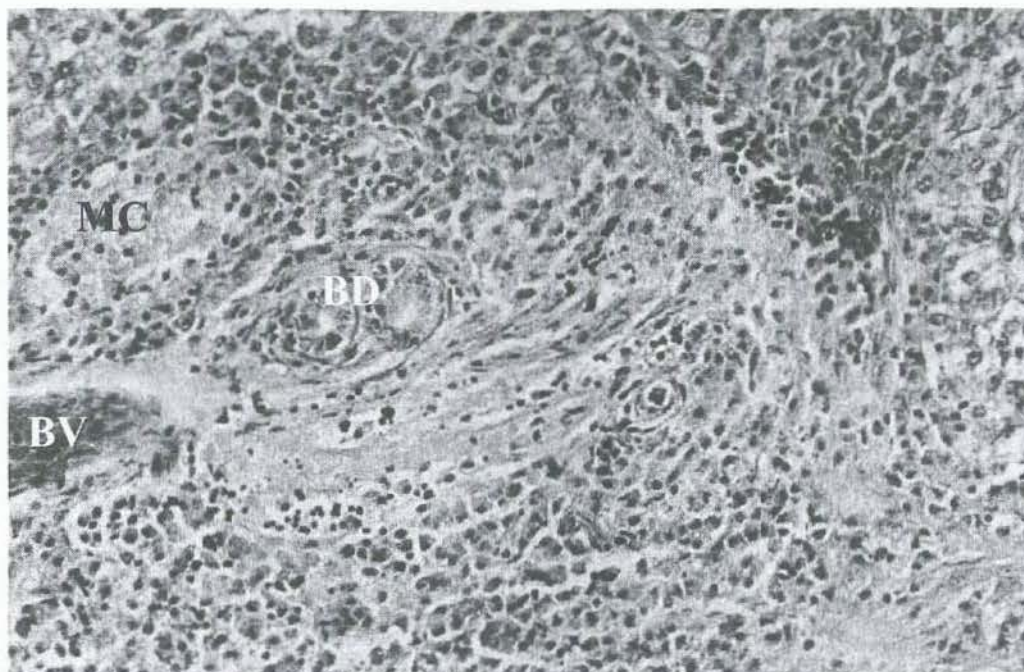


Fig. 3 Section through a wide inflamed area in liver parenchyma surrounding two bile ducts (BD) and a blood vessel (BV). Note the presence of a melanomacrophage center (MC) H&E x400

Сл. 3 Пресек низ широк регион на инфламаторна реакција околу два жолчни канали (BD) и крвен сад (BV) со присуство на меланомакрофаген центар (MC). H&E x400

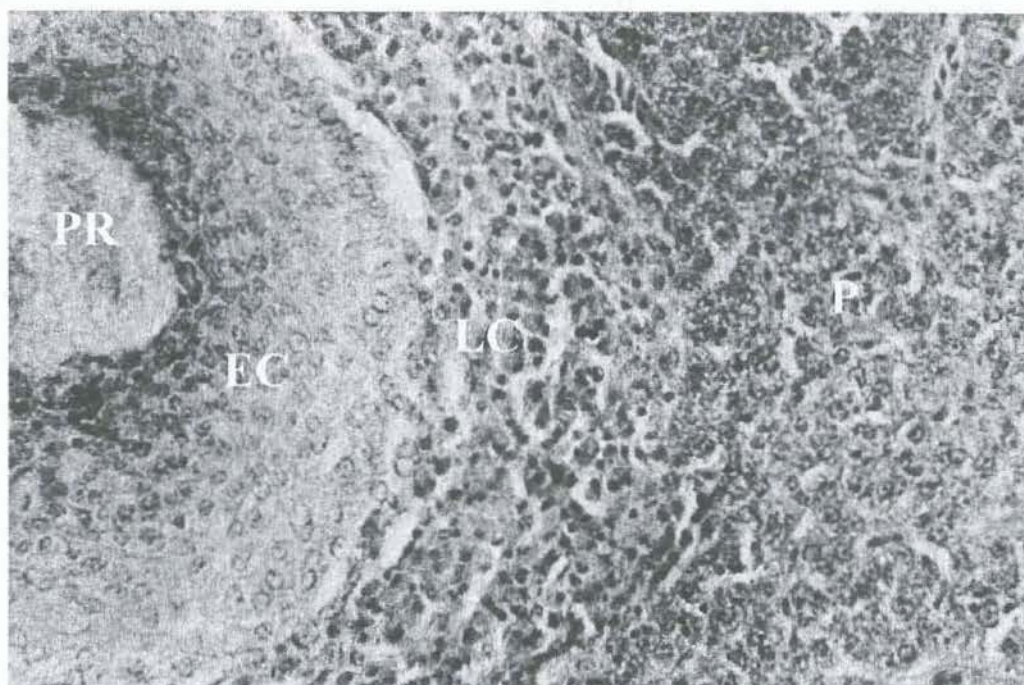


Fig.4 Section of a fibrous granulomata around a parasite remnant (PR). EC - epithelioid cells, LC - leukocytes, P- parenchyma. H&E x400

Сл. 4 Пресек на фиброиден гранулом околу остаток на паразит (PR). EC – епителоидни клетки, LC – леукоцити, P – паренхим. H&E x400

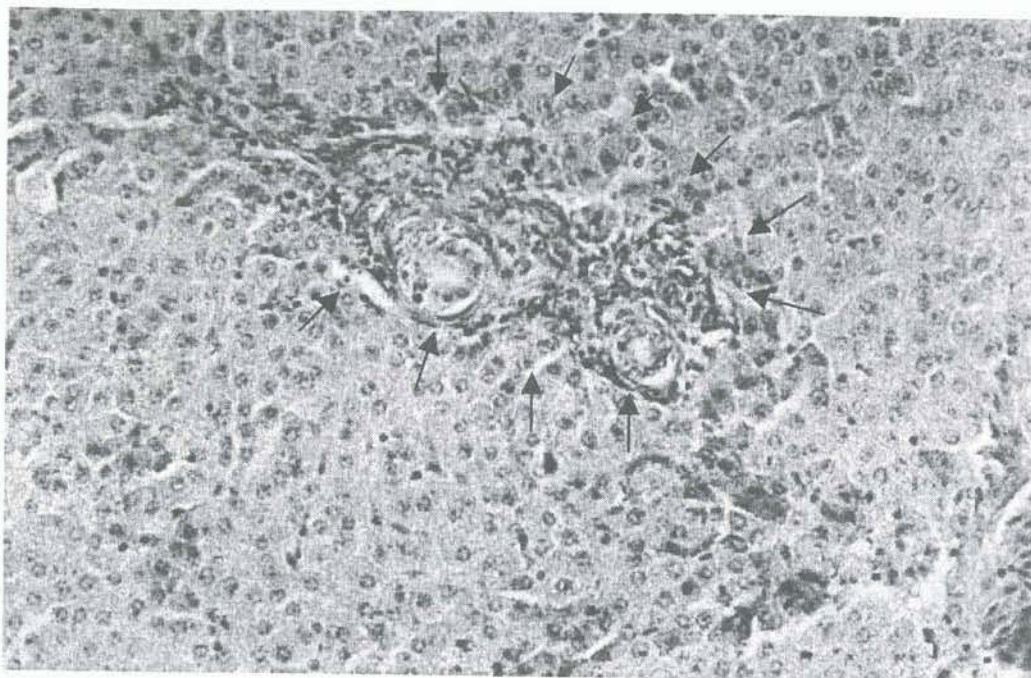


Fig. 5 A group of proliferated bile ducts with various sizes surrounded by stromal tissue in an otherwise normal parenchyma (arrows) H&E x400

Сл. 5 Група на пролиферирани жолчни канали со различни димензии опкружени со фиброзни клетки во нормален паренхим (стрелки). H&E x400



Fig. 6 Bile duct proliferation associated occasionally by karyopyknotic necrosis and complete wall lysis (arrow) H&E x400

Сл. 6 Пролиферација на жолчни канали асоцирана со кариопикнотична некроза и комплетно лизирање на билијарните сидови кај некои од нив (стрелка). H&E x400

CONCLUSION

Ohrid minnow moranec individuals collected from polluted areas in Lake Ohrid showed various liver lesions. The extensive granulomata and leukocytic inflammations surrounding blood vessels and bile ducts were registered as a prominent histopathological finding. Occasionally associated with parasitic infestation this finding is not obviously

correlated with contamination effects. Bile duct proliferation may be considered a specific chronic response to the pollution. The quality and the quantity of registered liver lesions indicated toxicopathic susceptibility of this endemic fish species to present contaminants in Lake Ohrid, which need to be verified in further investigations.

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**ЛЕЗИИ ВО ЦРНИОТ ДРОБ НА МОРАНЕЦОТ
(*Pachychilon pictus* Heck. et Kner) ОД НЕКОИ КОНТАМИНИРАНИ РЕГИОНИ НА
ОХРИДСКОТО ЕЗЕРО. ХИСТОПАТОЛОШКА СТУДИЈА**

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РЕЗИМЕ

Во тек на 1995 година беа колекционирани 53 единки на Охридски моранец во регионот на Грашница и Каналот Студенчишта при што беше извршена хистопатолошка анализа на повеќе витални органи кај овој ендемичен вид од Охридското езеро. Во овој труд се презентирани наодите регистрирани во црниот дроб на испитуваните единки (Табела 1) и дискутирано е значењето на добиените резултати за евалуација на биолошкиот одговор на моранецот на присутната полуција во Охридското езеро. Од овој аспект посебно внимание заслужува појавата на пролиферација на жолчните канали и холангиофиброза кај некои од испитуваните единки (Сл. 5 и 6), што може да се смета за специфичен хроничен одговор на загадувањето. Инфламаторни реакции и појава на грануломи во црнодробниот паренхим, во дел од пресеците асоциран со паразитни инфестации, беше зачестен и екстензивно присутен наод, (Сл. 3 и 4). Кај една од испитуваните единки регистриран е фокус на клеточна алтерација (Сл. 1) што може да укажува на пренеопластичен процес. Регистрираните хепатални лезии кај моранецот сугерираат осетливост на овој ендемичен вид на контаминантите присутни во загадените региони од Охридското езеро што бара верификација во понатамошни поопсежни истражувања.