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Histological analysis of fish gills as an indicator of water pollution in the Tamiš River

Jelena Lujić, Z. Marinović, B. Miljanović

University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Dositeja Obradovića Square 2, 21000 Novi Sad, Serbia

Abstract: Water pollution and effects of waterborne pollutants on aquatic organisms and humans has become a great concern over the last few decades. Since fish gills are in direct contact with the environment and are very sensitive to changes in water quality, the aim of this study was to evaluate the effects of waterborne pollutants from the Tamiš River on gill histology. The sample included a total of 78 individuals of the following fish species: wels catfish (*Silurus glanis*), pike (*Esox lucius*), roach (*Rutilus rutilus*), chub (*Squalius cephalus*), freshwater bream (*Abramis brama*), Prussian carp (*Carassius gibelio*) and pike-perch (*Sander lucioperca*). Histological observation showed moderate to intense histological alterations of the gill tissue. Progressive histological alterations were the most abundant in most of the analyzed species. The most common progressive alterations were hyperplasia and hypertrophy of the gill epithelium. Epithelial lifting was the most common regressive alteration and was observed in vertually all fish species. Circulatory disturbances were slightly pronounced. Obtained results show that the histological alterations in the gill tissue were most likely caused by increased concentrations of certain pollutants. Furthermore, these results represent an addition reason to proceed with a detailed monitoring of the Tamis River and the wildlife therein.
Key words: histopathology, water quality, water pollution.

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Introduction

Water pollution and effects of waterborne pollutants on aquatic organisms and humans has become a great concern over the last few decades. Untreated industrial, agricultural and domestic effluents are the most common causes of pollution since they usually contain mixtures of various pollutants potentially hazardous to aquatic organisms (Alberto et al. 2005; Camarago and Martinez 2007).

Fish, as aquatic organisms, are in a direct contact with the waterborne pollutants. They have a very thin epithelium and its total area is considerably larger than the total area of skin epithelium (Roberts, 1989). With regard to their delicate structure, various important functions and direct contact with water, gills are the first organ to be affected by pollutants and therefore are commonly used as the primary markers of water pollution (Bernet et al. 1999; Van der Oost et al. 2003).

Histopathological biomarkers are valuable indicators of the harmful effects of pollutants and potential pathogens. These markers are intermediate biomarkers in terms of ecological importance, response time and level of biological organisation, and as such are very suitable for the assessment of potentially harmful efects of various pollutants (Bernet et al., 1999; Van der Oost et al., 2003).

Antropogenic activity has had a significant impact on the hydrologic regime and water and sediment quality of river Tamiš (Marković et al., 1998). Main sources of pollution of river Tamiš are irrigation channels which are not maintained properly, sewage effluents, effluents from Sečanj city mineral industry, fish ponds and livestock farms. These waste waters in terms lead to decrease in dissolved oxygen and increase in electroconductivity, chloride anion and nitrate concentrations, hardness of water and alkalinity.

Due to the fact that in the previous studies a deterioration in the water quality was observed (Marković et al., 1998), the aim of this study was to evaluate the effects of waterborne pollutants from the Tamiš River on gill histology.

Materials and methods

Ichthyofauna samples were collected at the Sečanj, Opovo and Banatski Despotovac sites during August (summer season) and October (autumn season) 2009 with standard electrofishing device and gill nets 37 - 100 m long, 3 - 5 m deep and having a mesh diameter 45 - 100 mm.

The sample included a total of 78 adult specimens of the following fish species: wels catfish (*Silurus glanis*), pike (*Esox lucius*), roach (*Rutilus rutilus*), chub (*Squaliuus cephalus*), freshwater bream (*Abramis brama*), Prussian carp (*Carassius gibelio*) and pike-perch (*Sander lucioperca*). These fish species are common for waters of the Balkan Peninsula and include both indigenous and allochthonous species (Adrovic and Skenderovic, 2007).

The specimens were sacrificed with a quick blow to the head. Immediately after the fish death, the second left gill arch from the left side of every fish was sampled and fixed in 4% formaldehyde. Samples were processed with a standard histological procedure and stained with hematoxylin and eosin technique. The sections were examined by Zeiss Primo Star light microscope and photographed by Zeiss AxioCam MRc 5 digital camera.

For description and classification of histological alterations, a slightly modified version of the protocol proposed by Bernet et al. (1999) was used (Table 1).

Table 1. Classification of histological alterations.

Reaction pattern	Alteration
Progressive changes	Epithelial hypertrophy
	Epithelial hyperplasia
	Mucous cell hyperplasia
	Chloride cell hyperplasia
	Chloride cell hypertrophy
	Fusion of secondary lamellae
Regressive changes	Atrophy
	Epithelial lifting
	Necrosis
	Other structural alterations*
Circulatory disturbances	Hyperaemia
	Haemorrhage
	Telangiectasia

*Other structural alterations include clubbing of distal parts of the lamellae, curving and branching of lamellae, rupture of epithelium; cited from Bernet et al. (1999), modified.

Results of previous water quality studies of the Tamiš River for 2009 were taken from the Study of physical, chemical and biological status of the Tamiš river (Teodorović, 2010) and Hydrological yearbook of Republic of Serbia (RHSS, 2010) and are presented in Table 2.

Results and Discussion

Gill sections from the analysed fish species showed moderate to intense histological alterations when compared to the normal gill histology (Figure 1A).

Progressive histological alterations were the most abundant in most of the analyzed species. Different levels of expression in epithelial hyperplasia was observed, from small proliferations of epithelial cells to complete fusions of secondary lamellae (Figure 1B). These changes were most evident in wels

catfish and roach caught in the autumn season. Hypertrophy (hydropic degeneration) was also a frequent alteration observed in most species, predominantly in Prussian carp and wels catfish caught in the summer and in pike and roach caught in the autumn season.

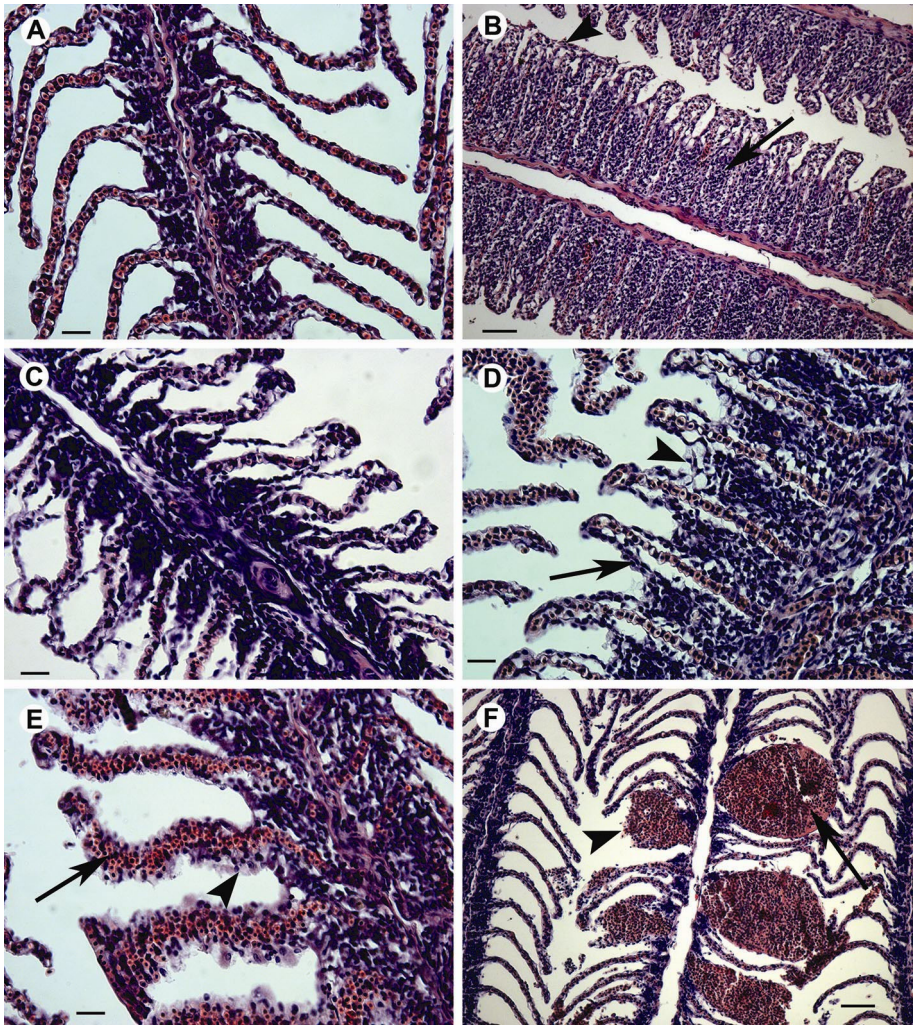


Fig. 1. Normal gill histology and histological alterations of the gill tissue in fish from the Tamiš River.

A) Normal gill histology. B) Hyperplasia of the interlamellar epithelium (arrow) which may lead to complete fusion of the lamellae (arrowhead). C) Epithelial lifting. D) Epithelial lifting (arrow) and hyperplasia of the mucous cells (arrowhead). E) Hyperaemia (arrow) and epithelial hypertrophy (arrowhead). F) Telangiectasia (arrow) which may lead to haemorrhage (arrowhead). H&E. Scale bars: A, C, D, E = 20 μm; B, F = 50 μm.

Hyperplasia of the mucous and chloride cells (Figure 1D) were registered in chub and roach in autumn season.

Epithelial lifting (Figure 1C and 1D) was the most common regressive alteration and was observed in all fish species. Atrophies were registered in wels catfish caught in the summer season. Light necrosis were observed in roach, pike and chub caught in the autumn and in Prussian carp and pike-perch caught in the summer season.

Circulatory disturbances were slightly pronounced. Hyperaemia were the most common circulary disturbances (Figure 1E), mostly observed in roach caught in the autumn and in pike-perch and wels catfish caught during the summer. Furthermore, telangiectasia and hemorrhages were also registered.

Most studies report that fish gills react non-specifically, implying that various pollutants can cause similar reactions and alterations of the gill tissue and thus these gill alterations can be regarded as reflections of generalized stress response (Heath, 1995; Ferguson, 2006). It is considered that hyperplasia which leads to reduction in the respiratory surface and epithelial lifting which leads to the increase in the water-blood barrier present defensive mechanisms of gills when exposed to pollutants. However, these alterations may lead to respiratory impairments. Degenerative changes may be the result of the direct effects of high concentrations of xenobiotics on the gills (Koca et al., 2005).

Results of previous water quality studies of this river (Table 2) may further help in interpreting the observed histopathological alterations. When compared with maximum admissible values proposed by Svobodova et al. (1993), concentrations of pollutants from the Tamiš River have showed elevated levels of iron, copper and chlorine.

Table 2. Summarized water quality data for 2009 with respective references.

Substance	Republic Hydrometeorological Service of Serbia (2010)						Dalmacija et al. (2010)	
	Jan. 2009	Mar. 2009	May 2009	July 2009	Sep. 2009	Nov. 2009	Aug. 2009	Oct. 2009
O_2 (mg/l)	13.2	8.4	7.8	8.2	10.4	11.9	6.5	7.1
Ammonia (mg/l)	0.08	0.04	0.07	0.03	0.2	0.12	0.1	0.05
Orthophosphates (mg/l)	0.035	0.034	0.042	0.012	0.028	0.037	2.75	2.25
Fe (mg/l)	2.2	2.37	0.65	0.79	1.24	0.83	-	-
Mn (mg/l)	0.08	0.17	0.08	0.05	0.07	0.02	-	-
Zn (µg/l)	34	43	51	44.6	44.1	17.2	-	-
Cu (µg/l)	12	23	34	19.1	19.8	6.6	-	-
Cr (µg/l)	3	4	2	1.5	2	1.8	-	-
Pb (µg/l)	2	5	2	3.9	9.2	<0.5	40	0.5
Cd (µg/l)	<0.2	3.7	0.2	<0.2	0.2	0.15	1.4	0.5
Hg (µg/l)	-	-	-	-	-	-	0.5	0.5

Although the observed alterations are not pollutant-specific, they could be related to previously published articles concerning gill alterations caused by experimental exposure to certain pollutants. Iron concentrations close to the

values observed in the Tamiš River, in experimental conditions, induced gill damage in the form of hypertrophy, hyperplasia of epithelium, fusions, lifting and rupture of epithelium, haemorrhages and necrosis (Peuranen et al., 1994; Dalzell and Macfarlane, 1999; Pandey et al., 2008; Wu et al., 2012). Epithelial lifting and swelling, hyperplasia with lamellar fusions, hyperaemia, telangiectasia and alterations in chloride cells were common lesions of gills from fish experimentally exposed to copper (Karan et al., 1998, Arellano et al., 1999; Liu et al., 2010). Middaugh et al. (1980) observed that chlorine induced epithelial lifting, fusion of lamellae and telangiectasia.

Conclusion

Obtained results show that the histological alterations in the gill tissue are most likely caused by increased concentrations of certain pollutants. According to the previous water quality studies of the Tamiš River, concentrations of iron, copper and chlorine were elevated. These substances in reported concentrations have most likely lead to the observed histopathological alterations. Present study represents an additional reason to proceed with a detailed monitoring of this river and the wildlife within it.

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Histološka analiza škrga riba kao indikator zagađenja reke Tamiš

- originalni naučni rad -

Jelena Lujić, Z. Marinović, B. Miljanović

Univerzitet u Novom Sadu, Prirodno-matematički fakultet, Departman za biologiju i ekologiju, Trg Dositeja Obradovića 2, 21000 Novi Sad, Srbija

Rezime

Zagađenje vode i uticaj zagađivača rastvorenih u vodi na akvatične organizme i ljude su postali velika briga u poslednjih nekoliko decenija. S obzirom da su škrge riba u direktnom kontaktu sa vodom i da su veoma osetljive na promene u kvalitetu vode, cilj ovog istraživanja je bio da se ispita uticaj zagađivača iz reke Tamiš na histologiju škrga riba iz ove reke. Uzorak se sastojao od 78 jedinki sledećih ribljih vrsta: som (*Silurus glanis*), štuka (*Esox lucius*), bodorka (*Rutilus rutilus*), klen (*Squalius cephalus*), deverika (*Abramis brama*), babuška (*Carassius gibelio*) i smuđ (*Sander lucioperca*). Histološka analiza je pokazale srednje do intenzivne histološke promene epitela škrga. Progresivne promene su bile najzastupljenije kod većine analiziranih riba. Najčešće progresivne promene su bile hiperplazije i hipertrofije epitela. Odlubljivanje epitela je bila najčešća regresivna promena i uočena je kod gotovo svih vrsta. Cirkulatorne promene su bile slabo izražene. Dobijeni rezultati pokazuju da su histološke promene škrga najverovatnije uzrokovane povećanim koncentracijama određenih zagađivača. Ovi podaci predstavljaju dodatan razlog da se nastavi sa detaljnim praćenjem kvaliteta vode reke Tamiš i živog sveta u njoj.

Ključne reči: histopatologija, kvalitet vode, zagađenost vode.