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

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Abstract: Water pollution and effects of waterborne pollutants on aquatic organisms and humans have 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 observations showed moderate to intense histological alterations of the gill tissue. Progressive histological alterations were the most abundant in most of the species analyzed. 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 virtually all fish species. Circulatory disturbances were slightly pronounced. Results show that the histological alterations in the gill tissue were most likely caused by increased concentrations of certain pollutants. Furthermore, these results are an additional reason to proceed with detailed monitoring of the Tamiš 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 have 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 direct contact with 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 effects of various pollutants (Bernet et al. 1999; Van der Oost et al. 2003).

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

Due to the fact that water quality deterioration was observed in previous studies (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 using a 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 (Adrović and Skenderović 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 the 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 species analysed. Different levels of expression in epithelial hyperplasia were observed, ranging 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.

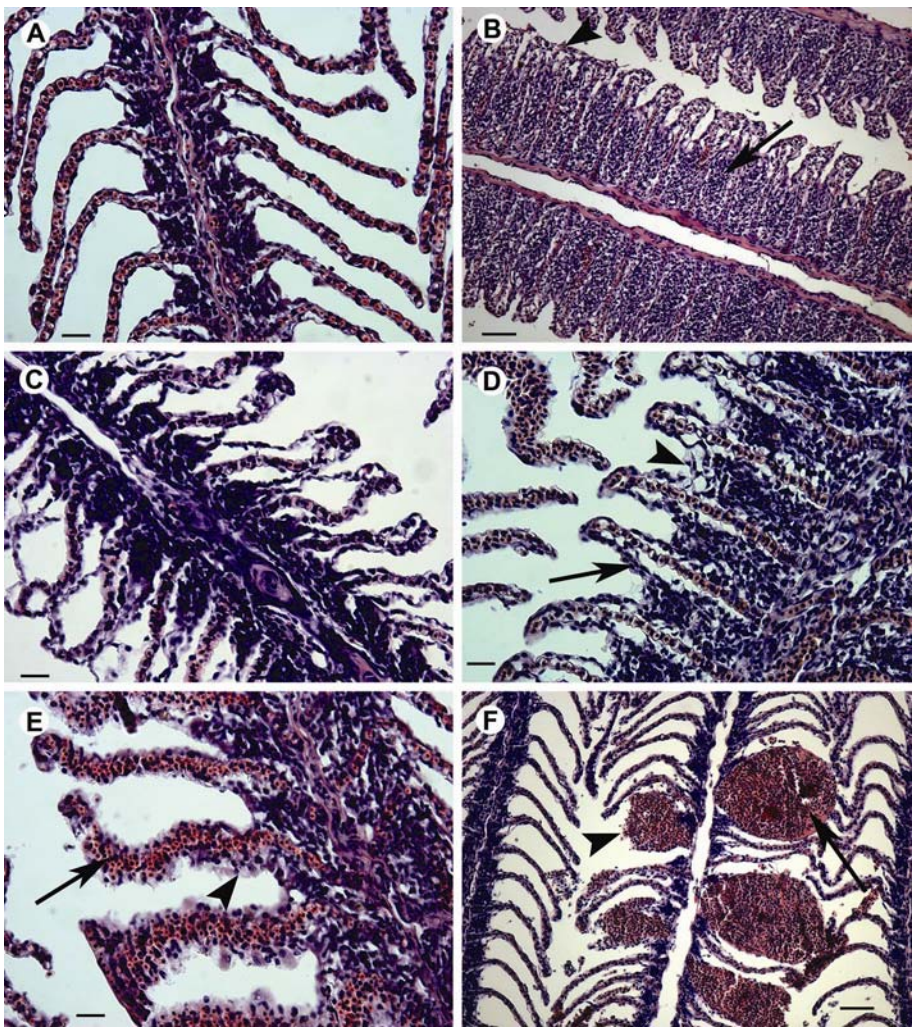

Fig. 1. Normal gill histology and histological alterations of the gill tissue in fish from the Tamiš River.

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 was 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 was the most common circulary disturbance (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 a generalised stress response (Heath 1995; Ferguson 2006). Hyperplasia which leads to a reduced respiratory surface and epithelial lifting which results in increased water-blood barrier are considered 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. Summary of 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 can 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

The results obtained 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 present in the reported concentrations have most likely led to the histopathological alterations observed. The present study is an additional reason to proceed with detailed monitoring of this river and the wildlife within it.

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References

Adrovic A., Skenderovic I. 2007. Allochthonous ichthyofauna of certain water bodies in north-eastern Bosnia. *Acta Agriculturae Serbica,* **8**: 37-46.

Alberto A., Camarago A.F.M., Verani J.R., Costa O.F.T., Fernandes M.N. 2005. Health variables and gill morphology in the tropical fish *Astyanax fasciatus* from a sewage – contaminated river. *Ecotoxicology and Environmental Safety,* **61**: 247-255.

Arellano J.M., Storch V., Sarasquete C. 1999. Histological changes and copper accumulation in liver and gills of the Senegales sole, *Solea senegalensis*. *Ecotoxicology and Environmental Safety,* **44**: 62-72.

Bernet D., Schmidt H., Meier W., Burkhardt-Holm P., Wahli T. 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases,* **22**: 25-34.

Camarago M.M.P. & Martinez C.B.R. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology,* **5**: 327-336.

Dalmacija B., Tričković J., Agbaba J., Krčmar D., Pešić V., Maletić S., Rončević S., Bečelić Tomin M., Kragulj M., Rajić Lj., Dalmacija M., Molnar J., Tubić A., Watson M.A., Ugarčina Perović S., Leovac A., Kerkez Đ. 2010. Rezultati hemijskog ispitivanja kvaliteta vode i sedimenta. In Teodorović I. (ed), *Studija fizičkog, hemijskog i biološkog statusa reke Tamiš*. Pančevo, 19-42.

Dalzell D.J.B. & Macfarlane N.A.A. 1999. The toxicity of iron to brown trout and effects on the gills: a comparison of two grades of iron sulphate. *Journal of Fish Biology,* **55**: 301-315.

Ferguson H.W., editor. 2006. *Systemic Pathology of Fish: A Text and Atlas of Normal Tissues in Teleosts and their Responses in Disease. 2nd edition*. Scotian press, London.

Heath A.G. 1995. *Water pollution and fish physiology*. Lewis publishers, Boca Ration.

Koca Y.B., Koca S., Yildiz Ş., Gürcü B., Osanç E., Tunçbaş O., Aksoy G. 2005. Investigation of histopathological and cytogenetic effects on *Lepomis gibbosus* (Pisces: Perciformes) in the Çine stream (Aydin/Turkey) with determination of water pollution. *Environmental Toxicology,* **20**: 560-571.

Liu X.J., Luo Z., Xiong B.X., Liu X., Zhao Y.H., Hu G.F., Lv G.J. 2010. Effect of waterborne copper exposure on growth, hepatic enzymatic activities and histology in *Synechogobius hasta*. *Ecotoxicology and Environmental Safety,* **73**: 1286-1291.

Marković S., Tomić P., Bogdanović Ž., Svirčev Z., Lazić L., Jovanović M., Pavić D. 1998. Pokazatelji i uzroci smanjenja kvaliteta vode u jugoslovenskom delu toka reke Tamiš. In: Marković S., Svirčev Z. (eds) *Naš Tamiš*. Novi Sad (SRB): Univerzitet u Novom Sadu, Novi Sad, 11-18.

Middaugh D.P., Burnett L.E., Couch J.A. 1980. Toxicological and physiological responses of the fish, *Leiostomus xanthurus*, exposed to chlorine produced oxidants. *Estuaries,* **3**: 132-141.

Pandey S., Parvez S., Ansari R.A., Ali M., Kaur M., Hayat F., Ahmad F., Raisuddin S. 2008. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. *Chemico-Biological Interactions,* **174**: 183-192.

Peuranen S., Vuorinen P.J., Vourinen M., Hollendar A. 1994. The effects of iron, humic acid and low pH on gills and physiology of Brown Trout (*Salmo trutta*). *Annales Zoologici Fennici,* **31**: 389-396.

[RHSS] Republic Hydrometeorological Service of Serbia 2010. *Hydrological yearbook, 3. water quality*. [Internet]. Belgrade (Serbia); [cited December 13th 2012]; Available at: http://www.dgt.uns.ac.rs/download/glshg2009.pdf.

Roberts R.J. 1989. *Fish pathology. 2nd edition*. Baillière Tindall, London.

Svobodova Z., Lloyd R., Machova J., Vykusova B. 1993. *Water quality and fish health. Eifac technical paper 54*. FAO, Rome.

Van der Oost R., Beyer J., Vermeulen N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology,* **13**: 57-149.

Wu Z., You F., Liu H., Liu M., Li J., Zhang P. 2012. Effects of waterborne Fe(II) on juvenile turbot *Scophthalmus maximus*: analysis of respiratory rate, hematology and gill histology. *Chinese Journal of Oceanology and Limnology,* **30**: 193-199.

HISTOLOŠKA ANALIZA ŠKRGA RIBA KAO INDIKATOR ZAGAĐENJA REKE TAMIŠ

- originalni naučni rad -

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Rezime

Zagađenje vode i uticaj zagađivača rastvorenih u vodi na akvatične organizme i ljude su postali velika briga u poslednjih nekoliko decenija. S obzirom na to 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 pokazala 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.