Aquatic Ecology
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 68 (1), 2016: 103-109

Histological Response of Fish Gills to Metal Pollution: Common Carp, *Cyprinus carpio* L., and Common Rudd, *Scardinius erythrophthalmus* L., from Topolnitsa Reservoir, Bulgaria

Vesela S. Yancheva[1], Stela G. Stoyanova[2], Iliana G. Velcheva[1], Elenka S. Georgieva[2]

[1] Department of Ecology and Environmental Conservation, Faculty of Biology, University of Plovdiv, Bulgaria;
[2] Department of Developmental Biology, Section of Histology and Embryology, Faculty of Biology, University of Plovdiv, Bulgaria; E-mail: stela.st@abv.bg

Abstract: Concentrations of six metals (As, Cd, Cu, Ni, Pb and Zn) were measured in surface waters of Topolnitsa Reservoir, Bulgaria, located in a region with intensive copper mining. The process of metal bioaccumulation in gills of two cyprinid fish species,: common carp (*Cyprinus carpio* L.) and common rudd (*Scardinius erythrophthalmus* L.), was studied in spring, summer and autumn. Higher metal concentrations in both water and fish gill samples in summer were detected. Furthermore,Histological alterations in the gill surface tissues of both fish species and the degree of expression of lesions were determined in the three seasons. Mostly pProliferative and degenerative changes in gill epithelium were observed. Changes in the blood circulatory system were presented as vasodilatation in the central venous sinus and secondary lamellae, as well as aneurysms. Overall, the extent and severity of lesions were more expressed in gills of the common carp gills than of in the common rudd. This is likely because the carp is a benthic feeder and more often has contacted with the sediments where the metal concentrations were times higher than in water. In addition, the histological alterations were more severe in summer in both fish species, which could be linked to the higher concentrations of metals in water and gills in this season.

Key words: bioaccumulation, metals, fish, gills, histological alterations, seasons

Introduction

In recent years, significant attention has being paid to the natural and anthropogenic pollution of freshwater ecosystems by a wide range of chemicals, including metals (Mendil *et al*. 2010; Aras *et al*. 2015). They pose a serious threat to aquatic life because of their toxicity, long persistence, bioaccumulation, and biomagnification in the food chain (Shah *et al*. 2010). In the case of fish, metals are characterised by different degrees of toxicity (Stanek *et al*. 2005). As fish can absorb pollutants directly from the surrounding water and sediments (waterborne exposure) or ingest metals through food chains (dietary exposure) (Murthy *et al*. 2013), they are considered one of the most indicative factors in freshwater systems for estimation of metal pollutions potential. Fish gills are organs with important multiple functions and are considered an important entry point into the organism for both essential and toxic elements. Thus, they are most frequently utilised in bioaccumulation studies and the pathological damage allows to identify the environmental toxicity (Rosseland *et al*. 2007). In addition, the histological alterations in fish gills have been proved to be a rapid and sensitive tool to detect direct effects of metals (Figueiredo-Fernandes *et al*. 2007). Metal bioaccumulation, which depends on many factors, e.g. fish species, age, tissue, season, temperature, pH, water hardness, etc., is widely studied but data on how abiotic factors in combination with metal contamination influence the degree of tissue damage of fish gills are scarce.

TopolnitsaReservoir(42°25’90’’N,23°59’38’’E) was built in the early 1960′s through damming Topolnitsa River,. It is one of the large artificial reservoirs in the country. The reservoir is located in a region, which has been under the impact of intensive copper production industry for decades because one of the biggest copper ore extraction and smelting enterprises was built simultaneously together with the reservoir. Topolnitsa River and its tributaries also suffer from untreated domestic effluents and agricultural runoff. Thus, Topolnitsa Reservoir serves as a final sink for all type of contaminants. There were no published data in the last few decades either on the water quality or on fish from Topolnitsa Reservoir, except from those presented by Georgieva *et al*. (2014a, 2014b) and Yancheva *et al*. (2014a, 2014b).

The first aim of the present study was to measure the metal concentrations (As, Cd, Cu, Ni, Pb, Zn) in surface water samples in spring, summer and autumn as well as to assess the process of bioaccumulation of metals in gills of two cyprinid fish in order to test if there were seasonal differences. The second aim was to investigate the histological structure of gills of common carp and common rudd in order to determine the degree of expression of lesions in each season and to identify which fish species was more sensitive to metal contamination.

Materials and Methods

RBoth studied fish species are common in Bulgaria and in Topolnitsa Reservoir. Common carp (*Cyprinus carpio* L.) is a species, which has economic importance and wide geographical distribution (Europe and Asia), and has also been proposed as a test organism in toxicological assays because it can survive and accumulate contaminants even at heavily polluted sites (Snyder *et al*. 2004). Common rudd (*Scardinius erythrophthalmus* L.) is used in recreational fishing. Few reports have investigated the metal bioaccumulation in various common rudd organs (Carassco *et al*. 2011, Djedjibegovic *et al*. 2012) but there are no comprehensive reports on the effects of metal pollutions on the common rudd.

Water samples for metal analysis were collected (iso 5667-4:1987) in 2012 near the reservoir wall in three seasons (spring summer and autumn). Temperature (°C), pH, dissolved oxygen (mg/l) and conductivity (μS/cm) were recorded simultaneously, in situ, using a combined field meter (Multi 340i, WTW). Water from the national Institute of Fisheries and Aquaculture in Plovdiv, Bulgaria, was used as reference. Water analysis was performed according to iso 17294-2:2003. Metal content in the water was analysed using ICP-MS (Agilent 7500ce, Japan) and reported as mg/l. The detection limits of the instrument were: As < 0.0005 mg/l, Cd < 0.00005 mg/l, Cu < 0.0005 mg/l, Ni < 0.0005 mg/l, Pb < 0.0001 mg/l, Zn < 0.0001 mg/l. ,sSimultaneously with the water sampling in each seasones, 15 fish individuals of each species were caught using fishing nets and a boat. Totally, 90 fish individuals specimenswere collected, i.e. (45 common carps and 45 common rudds;,. Common carps (15.4 ± 1.5 cm; 65.2 ± 10.3 g) and common rudds (12 ± 2.5 cm; 45 ± 5.5 g) were of the same size group. All fish samples were collected following international standard procedures for determination of metal accumulation as given in the Emerge EMERGE Protocol (Rosseland *et al*. 2003). Fifteen healthy common carps and 15 common rudds were provided by the national Institute of Fisheries and Aquaculture in Plovdiv, Bulgaria, which were used as reference fish. Fish were reared under strict controlled conditions, which do not allow the presence of any toxicants, including metals, pesticides or hormones in the water. All experiments were conducted in accordance with the national legislation and international guidelines of the European Parliament and the Council on the protection of animals used for scientific purposes (Directive 2010/63/EU).

Prior to the examination, c. 1 g of each gill sample was mineralised wet using a microwave digestion system (Milestone Ethos Plus, Italy). Digestion solution was prepared with 6 ml of 65% HNO_3 and 2 ml of 30% H_2O_2 at 200°C. Upon mineralisation, the samples were brought up to 25 ml by adding ultra-pure water, and analysed for metals using an ICP-MS (Agilent 7500ce, Japan). The results are reported as mg/kg wet weight. The detection limits of the instrument were: As – 0.01 mg/kg; Cd – 0.001 mg/kg; Cu – 0.01 mg/kg; Ni – 0.01 mg/kg; Pb – 0.03 mg/kg; and Zn – 0.03 mg/kg. All metal analyses were carried out at the accredited regional laboratory of the Executive Environment Agency in Plovdiv, Bulgaria. Accuracy of the applied analytical procedures and obtained results was checked using certified reference materials for metals in waters: SRM 1643e (National Institute of Standards and Technology, USA) and fish – DORM-3 (National Research Council Canada, Ottawa, Ontario, Canada).

After fixation in formaldehyde (pH7) for 24 h, gills were rinsed with tap water, dehydrated in a graded series of ethanol concentrations, cleared in xylene, embedded in paraffin wax with melting point of 54-56°C, sectioned to a thickness of 5-7 μm using a semi-rotary microtome and mounted on sterilised glass slides. Sections were then deparaffinised and stained with haematoxylin and eosin (H&E) for histological examinations (Bancroft, Stevens, 1996). Histological changes of gills were studied using a light microscope and photographed. All specimens were appraised individually and semi-quantitatively, using the grading system of Mishra, Mohanty (2008), which was slightly modified for the purposes of the present study. The evaluation of histological changes was presented as an average value in percentages. Mean prevalence of each histological lesion in the gill surface was categorised as follows: none – (-); mild – 10-20% (+/-); moderate – 30-50% (+); severe – 60-80% (++); and very severe – above 80% (+++).

Raw data on the metal concentrations in the water and fish gills, representing different seasons were statistically analysed using the STATISTICA (version 7.0 for Windows, StatSoft, 2004). Comparison between the metal concentrations was tested using Student's t-test at significance level of 0.05. Correlation analysis between the fish size and metal concentration in the gills, as well as between the metal concentration in the water and fish gills was also performed.

Results and Discussion

According to unpublished data from the East Aegean Sea River Basin Directorate, Bulgaria, the freshwater chemistry data (pH 8.46 ± 0.5; temperature 11.26 ± 10.3°C; dissolved oxygen 7.96 ± 0.81 mg/l and conductivity 530 ± 190 µS/cm) for Topolnitsa Reservoir showed that the reservoir water has good physico-chemical status. The analysis of the water samples the indicated variations in the metal concentrations in spring, summer and autumn (Table 1). Lead concentrations were below the detection limit (Pb < 0.0001 mg/l) in the three seasons while copper concentrations were determined in each season. Statistical analysis showed a significant difference between arsenic concentrations in summer and autumn, and copper concentrations in spring and summer ($p < 0.05$). Metal concentrations in the reference water samples were smaller than the detection limits. According to the maximum permissible levels set by the Bulgarian legislation (Regulation Norms, 2010, 2013) based on Directive 2000/60/EC. Arsenic concentration in summer and Cu concentrations in the three seasons in Topolonitsa Reservoir were above the allowable limit. We associate the presence of all metals in the reservoir, and particularly that of Cu and As, with the continuous input of these toxicants from the activities of mining and processing of copper ores in the region. Furthermore, more factors for the presence of metals can also include complex interactions between the water, sediment and biota, or other anthropogenic activities, such as fluctuations of discharged amounts of domestic sewage, agricultural runoff and input of industrial waste waters in the reservoir. In our opinion, this is an indication for chronic contamination of the reservoir with these metals, which may lead to negative consequences (alterations at tissue or cellular level) for wildlife, particularly for the fish inhabiting Topolnitsa Reservoir.

Metal concentrations in the reference fish gills were less than the detection limits while in the fish gills from Topolnitsa Reservoir were higher (Table 2). In the present study, Cu concentrations in common carp gills in spring were significantly different from those in summer, as well as Cd concentrations in common rudd gills in spring compared to those in summer ($p < 0.05$). Overall, both cyprinid fish species showed a similar mode of metal deposition in the gills. However, we found a significant difference between cadmium concentrations in the common carp gills and common rudd gills in spring; lead concentrations in the common carp gills and common rudd gills in spring and summer; as well as zinc concentrations between the two fish species in all three seasons ($p < 0.05$). According to Heier *et al*. (2009), metal concentrations in the fish gills can reflect levels of pollution more accurately than the indices of contaminant content in the water. Furthermore, the gill surface serves as a metal-binding ligand and metal bioaccumulation can occur due to positively charged metal ions in the water to negatively charged sites on the gills (Teien *et al*. 2006). Our results confirmed that the gills could be used in toxicological research as they accurately reflected arsenic and copper concentrations in the reservoir water. In addition, our findings confirmed that fish gills could be successfully used in the assessment of the process of metal bioaccumulation because Pb concentrations could not be detected in the reservoir water but they were significantly higher in both common carp and common rudd gills.

Last but not least, we consider that metal bioaccumulation in the gills of both fish species are mainly due to entering of the metal in the fish body from the water as the fish in this study were of the same size and age group. Statistical analysis showed a strong positive correlation between arsenic concentration in the water and common carp gills in summer ($r = 0.9$; $p < 0.05$), copper concentrations in the water and common carp concentrations in spring ($r = 0.8$;

$p < 0.05$) and summer ($r = 0.9$; $p < 0.05$); as well as between arsenic concentrations in the water and common rudd gills in summer ($r = 0.7$; $p < 0.05$). We found that the fish size correlated positively and significantly only with zinc concentrations in the common carp gills and copper concentrations in the common rudd gills in summer. We do not think that in this case the fish diet is important for the metal concentrations which we determined in the fish gills. Furthermore, according to Čihař (1976) and Karapetkova, Zhivkov (2010), the two studied fish species have a similar diet (juvenile common carps and rudds feed on zooplankton, mainly crustaceans, and adult fish – on plankton).

Overall, the metal concentrations in the cyprinid fish gills from Topolnitsa Reservoir were similar and higher in summer for both species. We associated this result with the metabolic fish activity, which is more intense during summer. Furthermore, Sokolova, Lanning (2009) state that the toxicant bioaccumulation increases with increasing the water temperature in ectothermic organisms due to a higher energy demand. On the other hand, we consider that the differences in the higher concentrations of some metals in the common carp gills, i.e. zinc, are mainly due to the fact that it lives in the lower water layers, where bioavalability of the metals released from the sediment is higher. Hence, the metal bioaccumulation can lead to disturbances in the cell metabolism and negative alterations in the morphological structure of organs such as the gills can occur.

Table 1. Metal concentrations in water column of Topolnitsa Reservoir, mg/l

	Spring	**Summer**	**Autumn**
Element	water	water	water
As	**0.007****±0.001	**0.03****±0.005	<0.0005*
Cd	0.0005±0.0001	<0.00005*	<0.00005*
Cu	**0.02****±0.005	**0.05****±0.01	**0.009****±0.001
Ni	<0.0005*	<0.0005*	0.001±0.0005
Pb	<0.001*	<0.001*	<0.001*
Zn	0.006±0.001	<0.001*	<0.001*

***** smaller than the detection limit of the instrument.
******above Bulgarian maximum permissible levels set for metals in surface waters.
(As – 0.0025 mg/l, Cd – 0.00045 mg/l, Cu – 0.001 mg/l, Ni – 0.02 mg/l, Pb – 0.00072 mg/l, and Zn – 0.008 mg/l).

Table 2. Metal concentrations in common carp and common rudd gills from Topolnitsa Reservoir, mg/kg (wet weight)

	Spring	**Summer**	**Autumn**
Element	Carp	Carp	Carp
As	0.06±0.01	0.09±0.05	0.07±0.03
Cd	**0.7±0.2**	**0.8±0.3**	**0.2±0.01**
Cu	0.9±0.3	**2.85±0.5**	**1.9±0.5**
Ni	0.3±0.05	0.25±0.05	0.36±0.05
Pb	0.4±0.2	0.35±0.1	0.25±0.1
Zn	**126.2±11.5**	152±15.5	124±10.5
	Spring	Summer	Autumn
Element	Rudd	Rudd	Rudd
As	**0.15±0.5**	**0.14±0.5**	**0.15±0.1**
Cd	0.08±0.1	0.3±0.3	0.12±0.5
Cu	1.3±0.3	1.3±0.2	1.8±0.3
Ni	0.15±0.05	**0.8±0.05**	0.2±0.05
Pb	**1.1±0.3**	**1.2±0.5**	0.4±0.03
Zn	40±5.5	40±3.5	50±7.4

Bold – higheer metal concentration (comparing both fish species).

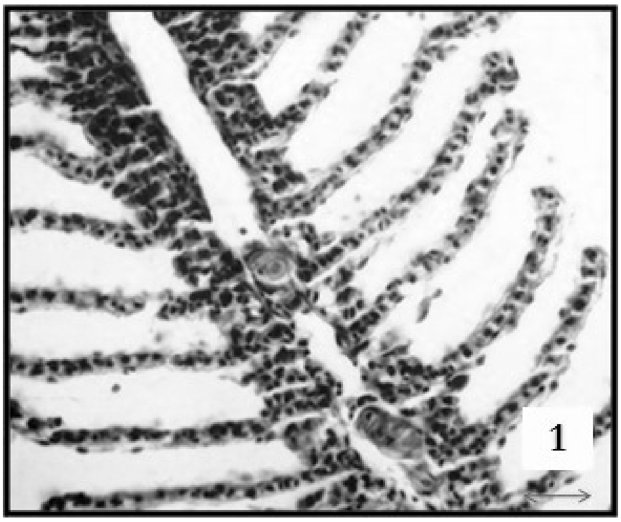

Fig. 1. Histological structure of reference fish gills, H&E, x400

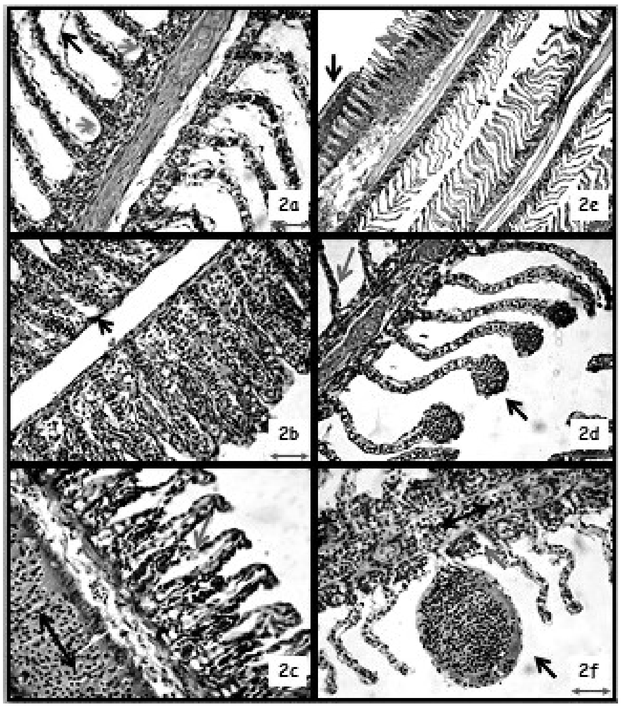

Fig. 2. Histological alterations in common carp gills from Topolnitsa Reservoir, H&E: 1a – lamellar lifting (black arrow), vasodilatation in secondary lamellae (grey arrow), x400; 1b – oedema (black arrow), proliferation of gill epithelium (white arrow), x400; 1c – vasodilatation in central venous sinus (double-headed arrow); lamellar lifting (grey arrow), x400; 1d – proliferation of gill epithelium (grey arrow), lamellar fusion (black arrow), x200; 1e – aneurysms (black arrow); degeneration of lamellae (grey arrow), x400; 1f – aneurysm (black arrow), oedema (grey arrow), vasodilatation in central venous sinus (double-headed arrow), x400

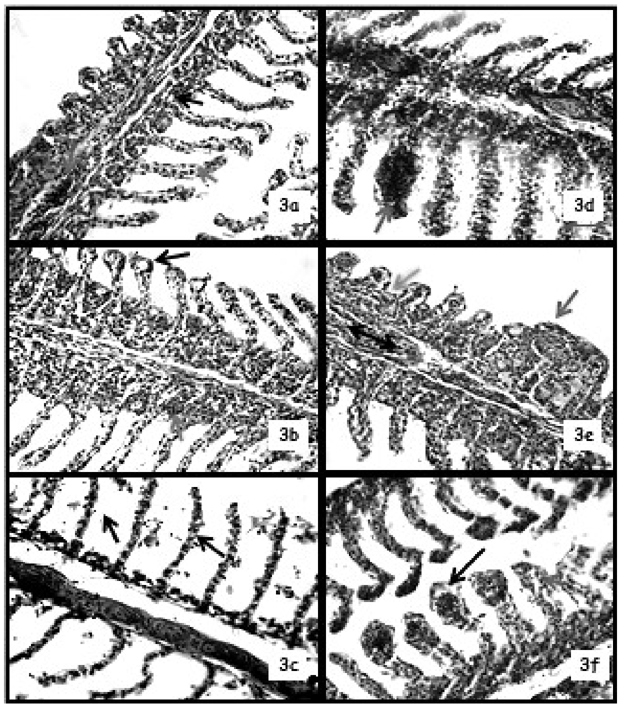

Fig. 3. Histological alterations in common rudd gills from Topolnitsa Reservoir, (H&E): 2a – vasodilatation in central venous sinus (grey arrow), lamellar lifting (black arrow), oedema (black arrow), x200; 2b – aneurysm (black arrow), proliferation of gill epithelium (grey arrow) , x200; 2c – degeneration of lamellae (black arrows), x200; 2d – aneurysm (grey arrow), x400; 2e – vasodilatation in central venous sinus (double-headed arrow), proliferation of gill epithelium (grey arrow), lamellar fusion (black arrow) , x200; 2f – aneurysm (black arrow), lamellar lifting (grey arrow), x400

Reference fish gills presented normal distribution of the cellular constituents and organisation pattern of the primary and secondary lamellae, and blood vessels (Fig. 1). In contrast, the fish collected from the studied site showed serious alterations in the gill structure during all three investigated seasons (Table 3). We observed in all individuals of both fish species from Topolnitsa Reservoir histological gill lesions, which were classified in three main groups: 1) proliferative changes – lamellar lifting, oedema, proliferation of epithelial cells, and lamellar fusion; 2) degenerative changes of gill lamellae, and 3) changes in the blood vessels – vasodilatation in the main blood sinus, secondary lamellae, and aneurysms (Figs. 2 and 3). However, the extent and severity of each particular lesion varied among the two studied species in all three seasons (Table 3). In general, the gill alterations were presented in more severe expression in common carp than in common rudd. In addition, the proliferative changes were more pronounced in summer, and dominated the degenerative and blood circulatory changes. Such trend towards increasing the degree of expression of all histological changes in the fish gills in summer can be due to the higher metal content in the reservoir water and fish gills in this particular season.

Table 3. Histological alterations in gills of common carp and common rudd gills from Topolnitsa Reservoir.

Histological alteration	Spring		Summer		Autumn	
	Carp	Rudd	Carp	Rudd	Carp	Rudd
Lamellar lifting	+++	+/-	+++	+	++	+/-
Oedema	++	+/-	++	+	+	+/-
Proliferation of gill epithelium	+	+/-	++	+	+	+/-
Lamellar fusion	+	+/-	++	+	++	+/-
Degeneration of lamellae	+/-	-	+/-	+/-	-	-
Vasodilatation in central venous sinus	+/-	+/-	+	+/-	-	-
Vasodilatation in the secondary lamellae	+/-	+	+	+	+	+/-
Aneurysms	-	-	+	+	-	+/-

none – (-); mild – 10-20% – (+/-); moderate – 30-50% – (+); severe – 60-80% – (++); and very severe – above 80% – (+++).

Presence of oedema along with detachment of the lamellar epithelium is the first sign of pathology in fish and one of the more frequent lesions observed in gills of fish exposed to heavy metals (Santos *et al.* 2011). Similarly to Camargo, Martinez (2007) we consider that these alterations could serve as an adaptive and protective mechanism of the fish organism against the metal-contaminated metals water of Topolnitsa Reservoir. Proliferative changes can increase the water blood distance and reduce the absorption of metals but, in turn, decrease the respiratory surface area, which reduces the effectiveness of gas exchange ion uptake (De Boeck *et al.* 2007). We could speculate that the proliferative alterations in the gill epithelium of both investigated species in our study were one of the dominant lesion types owing to the fact that the fish protective mechanisms most likely work towards forcing cell division instead of cell death. Aneurysms result from extended vasodilatation with the collapse of pillar cells and the breakdown of vascular integrity (Martinez *et al*. 2004). Garcia-Santos *et al*. (2007) observed an increased frequency of aneurysms in fish from contaminated areas, and affirmed that they can be associated with the presence of metals in the water.

Overall, we found more severe gill alterations in common carp than common rudd, and this result shows that carp is more sensitive than common rudd in terms of histological changes induced by metals. We consider that this fact may be due to the specific biology of this species, e.g. behaviour, different preferences to the depth of the water layer, and not that that much the diet itself. For example, common rudd searches for food mainly in the surface water layers, whereas common carp is a benthic fish, which is relatively sedentary and it searches for food and spawn on sediments. Furthermore, we assume that the polluted sediments from Topolnitsa Reservoir can have even more negative effects than the water on the fish gills, their morphology and functions. Georgieva *et al.* (2014) already showed results from a preliminary study, which investigated how the metal-contaminated sediments from Topolnitsa Reservoir reflected the gill epithelium of common carp. Thus, we think that further investigations need to be carried out in this particular area in order to better understand how this altered freshwater ecosystem impacts the morphology and physiology of native fish.

Acknowledgements: We thank everyone at the accredited regional laboratory of the Executive Environment Agency in Plovdiv, Bulgaria, who have been helping with the metal analyses and the local fisherman who is always eager to catch all fish samples which are needed for our research.

References

Bancroft J.D., A. Stevens 1996. Theory and practice of histological techniques, fourth ed. Hong Kong: Churchill–Livingstone, 766 p.

Aras S., N. Arslan, Ö. Emiroğlu, T. Arslan 2015. Cadmium and nickel accumulation in water, sediment and tissues of *Esox lucius* in Lake Büyük Akgöl, Turkey. – *Journal of Applied Biological Sciences,* **9**: 64-68.

Camargo M.M.P., C.B.R. Martinez 2005. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. – *Neotropical Ichthyology*, **5**: 327-336.

Carrasco L., L. Benejam, J. Benito, J.M. Bayona, S. Díez 2011. Methylmercury levels and bioaccumulation in the aquatic food web of a highly mercury-contaminated reservoir. – *Environment International*, **37**: 1213-1218.

Čihař J. 1976. A colour guide to familiar freshwater fishes. Octopus books limited, 183 p.

De Boeck G., K. Van der Ven, W. Meeus, R. Blust 2007. Sublethal copper exposure induces respiratory stress in common and gibel carp but not in rainbow trout. – *Compararative Biochemistry and Physiology*, **144**: 380-390.

Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

Directive 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. http://ec.europa.eu/environment/water/water–dangersub/lib_pri_substances.htm

Djedjibegovic J., T. Larssen, A. Skrbo, A. Marjanović, M. Sober 2012. Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP–MS). – *Food Chemistry*, **131**: 469-476.

Figueiredo–Fernandes A., J. V. Ferreira–Cardoso, S. Garcia–Santos, S. M. Monteiro, J. Carrola, P. Matos, A. Fontaínhas–Fernandes 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. – *Pesquisa Veterinária Brasileira*, **27** (3): 103-109.

Garcia–Santos S., M. Monteiro, J. Carrola, A. Fontaínhas–Fernandes 2007. Histopathological lesions of tilapia *Oreochromis niloticus* gills caused by cadmium, – *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **59**: 376-381.

Georgieva E., I. Velcheva, V. Yancheva, S. Stoyanova 2014a. Trace metal effects on gill epithelium of common carp, *Cyprinus carpio* L. (Cyprinidae). – *Acta Zoologica Bulgarica*, **66** (2): 277-282.

Georgieva E., S. Stoyanova, I. Velcheva, T. Vasileva, V. Bivolarski, I. Iliev, V. Yancheva 2014b. Metal effects on histological and biochemical parameters of common rudd (*Scardinius erythrophthalmus* L.). – *Archives of Polish Fisheries*, **22**:197-206.

Heier L.S., I.B. Lien, A.E. Strømseng, M. Ljønes, B.O. Rosseland, K.E. Tollefsen, B Salbu. 2009. Speciation of lead, copper, zinc and antimony in water draining a shooting range – time dependant metal accumulation and biomarker responses in brown trout (*Salmo trutta* L.). – *Science of the Total Environment*, **407**: 4047-4055.

ISO 5667–4 1987. Water quality – Sampling. Part 4: Guidance on sampling from lakes, natural and man–made. http://www.iso.org/iso/catalogue_detail.htm?csnumber=11767

ISO 17294–2 2003. Water Quality – Application of Inductively Coupled Plasma Mass Spectrometry (ICP–MS) – Part 2: Determination of 62 Elements. http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=36127

Karapetkova M., M. Zhivkov 2010. Fishes in Bulgaria. Geya Libris, Sofia, 215 p. (In Bulgarian).

Martinez C.B.R., M.Y. Nagae, C.T.B.V. Zaia, D.A.M. Zaia 2004. Morphological and physiological acute effects of lead in the neotropical fish *Prochilodus lineatus*. – *Brazilian Journal of Biology*, **64**: 797-807.

Mendil D., Z. Demirci, M. Tuzen, M. Soylak 2010. Seasonal investigation of trace element contents in commercially valuable fish species from the Black sea, Turkey. – *Food Chemistry and Toxicology*, **48**: 865-870.

Murthy K.S., B.R. Kiran, M. Venkateshwarlu. 2013. A review on toxicity of pesticides in Fish. – *International Journal of Open Scientific Research*, **1** (1): 15-36.

Mishra A.K., B. Mohanty 2008. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). – *Environmental Toxicology and Pharmacology*, **26** (2): 136-141.

Regulation Norm 2010. Regulation norm of the priority substances in surface waters (in Bulgarian). http://www3.moew.government.bg/files/file/Legislation/Naredbi/vodi/N_standarti_priorit_ve6t_zamyrsiteli.pdf

Regulation Norm 2013. Regulation norm – H4 of the quality and characterization of surface waters, in Bulgarian). http://eea.government.bg/bg/legislation/water/Naredba13.pdf

Rosseland B.O., J.C. Massabuau, J. Grimalt, R. Hofer, R. Lackner, G. Raddum, S. Rognerud, I. Vives 2003. EMERGE, European Mountain Lake Ecosystems: Regionalisation, Diagnostic & Socio–economic Evaluation. Workpackage 5: Fish Ecotoxicology, Fish Sampling Manual for Live Fish. Norwegian Institute for Water Research (NIVA), Oslo, Norway, 7 p.

Rosseland B.O., S. Rognerund, P. Collen, J.O. Grimalt, I. Vives, J.C. Massabau 2007. Brown trout in Lochnagar: population and contamination by metals and organic micropollutants. – *Developments in Paleoenvironmental Research*, **12**: 253-285.

Santos D.C.M, S.L.P. Matta, J.A. Oliveira, J.A.D. Santos 2011. Histological alterations in gills of *Astyanax* aff. *Bimaculatus* caused by acute exposition to zinc. – *Experimental Toxicology and Pathology*, **64** (7-8): 861-866.

Shah A.Q., T.G. Kazi, J.A. Baig, H.I. Afridi, G.A. Kandhro, M.B. Arain, S.K.D. Wadhwa, N.F. Kolachi 2010. Determination of inorganic arsenic species (As^{3+} and As^{5+}) in muscle tissues of fish species by electrothermal atomic absorption spectrometry (ETAAS). – *Food Chemistry*, **119**: 840-844.

Snyder E.M., S.A. Snyder, K.L. Kelly, T.S. Gross, D.L. Villeneuve, S.D. Fitzgerald, S.A. Villalobos, J.P. Giesy 2004. Reproductive responses of common carp (*Cyprinus carpio*) exposed in cages to influent of the Las Vegas Wash in Lake Mead, Nevada, from late winter to early spring. – *Environmental Science and Technology*, **38**: 6385-6395.

Sokolova I.M., G. Lannig 2008. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. – *Climate Research*, **37**: 181-201.

Stanek M., B. Janicki, B. Kupcewicz 2005. Content of selected heavy metals in the organs of fish from Żnin Duże Lake. – *Folia biologica (Kraków)*, **53**: 115-119.

Teien H.C., F. Kroglund, B. Salbu, B.O. Rosseland 2006. Gill reactivity of aluminium species following liming. – *Science of the Total Environment*, **358** (1-3): 206-220.

Yancheva V., S. Stoyanova, I. Velcheva, S. Petrova, E. Georgieva 2014a. Metal bioaccumulation in common carp and rudd from the Topolnitsa reservoir, Bulgaria. – *Archives of Industrial Hygiene and Toxicology*, **65**: 57-66.

Yancheva V.S., E.S. Georgieva, I.G. Velcheva, I.N. Iliev, T.A. Vasileva, S.T. Petrova, S.G. Stoyanova 2014b. Biomarkers in European perch (*Perca fluviatilis*) liver from a metal–contaminated dam lake. – Biologia, 69(11): 1515-1624.

Received: 26.02.2015
Accepted: 02.10.2015