



International scientific workshop

INFLUENCE OF ACTIVE MINES ON FRESHWATER ECOSYSTEMS

May 12-16, 2014

International scientific workshop

**INFLUENCE OF ACTIVE MINES ON FRESHWATER
ECOSYSTEMS**

May 12-16, 2014

Ruđer Bošković Institute
Zagreb, Croatia

Publisher: Ruđer Bošković Institute, Zagreb, Croatia

Editor: Dr. Zrinka Dragun

ISBN: 978-953-7941-00-0

A CIP catalogue record for this book is available in the Online Catalogue of the National and University Library in Zagreb as 877292.

Photo: Dr. Zrinka Dragun

Zagreb, 2014

RUĐER BOŠKOVIĆ INSTITUTE
Division for Marine and Environmental Research
Laboratory for Biological Effects of Metals
Laboratory for Aquaculture and Pathology of Aquatic Organisms
Zagreb, Croatia

SS. CYRIL AND METHODIUS UNIVERSITY IN SKOPJE
Faculty of Natural Sciences and Mathematics
Faculty of Veterinary Medicine
Skopje, Macedonia

INSTITUTE OF ANIMAL SCIENCES
Skopje, Macedonia

UNIVERSITÉ DE PAU ET DES PAYS DE L'ADOUR/CNRS
Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux
Pau, France

organize

International scientific workshop

INFLUENCE OF ACTIVE MINES ON FRESHWATER

ECOSYSTEMS

May 12-16, 2014

within activities of the Projects:

1. The assessment of availability and effects of metals on fish in the rivers under the impact of mining activities (project leaders: Dr. Zrinka Dragun and Dr. Maja Jordanova)
2. Bacterial and parasitological communities of chub as indicators of the status of environment exposed to mining activities (project leaders: Dr. Damir Kapetanović and Dr. Rodne Nastova)
3. Intracellular mapping of essential and nonessential trace elements in the organs of indigenous fish by NanoSIMS (project leaders: Dr. Zrinka Dragun and Dr. Dirk Schaumlöffel)

Workshop organizers:

Dr. Zrinka Dragun and Dr. Vlatka Filipović Marijić
Ruđer Bošković Institute, Zagreb, Croatia
Division for Marine and Environmental Research
Laboratory for Biological Effects of Metals

Dr. Damir Kapetanović and Dr. Damir Valić
Ruđer Bošković Institute, Zagreb, Croatia
Division for Marine and Environmental Research
Laboratory for Aquaculture and Pathology of Aquatic Organisms

PROGRAMME

Monday, May 12, 2014

Arrival and registration

Tuesday, May 13, 2014

- 10:00-10:15 Zrinka Dragun, Damir Kapetanović: Welcome address and introduction to the workshop
- 10:15-10:35 Zrinka Dragun: Water quality of mining impacted rivers in the north-eastern Macedonia: I. Physico-chemical parameters and concentrations of dissolved metals/metalloids
- 10:35-10:50 Damir Kapetanović: Water quality of mining impacted rivers in the north-eastern Macedonia: II. Microbiological water quality of rivers Bregalnica, Zletovska and Kriva - Preliminary results
- 10:50-11:20 Coffee break
- 11:20-11:40 Katerina Rebok: Morphometric data of Vardar chub (*Squalius vardarensis*) in the rivers under the impact of mining activity
- 11:40-12:00 Sheriban Ramani: Accumulation of metals and metalloids in the liver and gills of Vardar chub (*Squalius vardarensis*) from three mining impacted rivers in north eastern Macedonia
- 12:00-14:00 Lunch break
- 14:00-14:20 Vlatka Filipović Marijić: Evaluation of dietary metal exposure of *Squalius vardarensis* dwelling in mining impacted rivers in the north-eastern Macedonia
- 14:20-14:40 Nesrete Krasnići: Cytosolic distribution of Cd, Co, Cu, Fe, Pb, V and Zn in liver, gills and intestine of Vardar chub (*Squalius vardarensis*) from mining impacted rivers in Macedonia
- 14:40-14:50 Irena Vardić Smrzlić: Molecular characterisation of the metazoan parasites of Vardar chub (*Squalius vardariensis*) from three rivers in north eastern Macedonia
- 14:50-15:20 Coffee break
- 15:20-15:40 Vlatka Filipović Marijić: Acanthocephalans, fish intestinal parasites, as bioindicators of metal exposure in rivers impacted by mining waste
- 15:40-16:00 Nesrete Krasnići: Metallothionein and total protein concentrations in gills and liver of Vardar chub (*Squalius vardarensis*) as biomarkers of water contamination in three rivers in Macedonia

Wednesday, May 14, 2014

- 10:00-10:15 Damir Kapetanović: Bacterial community of Vardar chub (*Squalius vardarensis*): Preliminary results
- 10:15-10:30 Damir Valić: Hematological assessment of Vardar chub (*Squalius vardarensis*) from three rivers in north-eastern Macedonia
- 10:30-11:00 Coffee break
- 11:00-11:20 Josip Barišić: Spatial and seasonal variability of histopathological alterations on the gills of Vardar chub (*Squalius vardarensis*) from mining impacted rivers in the north-eastern Macedonia
- 11:20-11:40 Maja Jordanova: Toxicopathic changes in Vardar chub (*Squalius vardarensis*) in rivers under the impact of mining activities
- 12:00-14:00 Lunch break
- 14:00-14:30 Dirk Schaumlöffel: Potential and challenges of NanoSIMS for element imaging in biological cells
- 14:30-14:45 Zehra Hajrulai-Musliu: Fatty acid composition in some river fish species in Republic of Macedonia
- 14:45-15:00 Risto Uzunov: Detection of methyltestosterone with ELISA method in fish

Thursday, May 15, 2014

- 08:00-21:00 Visit to Research marine station "Martinska" near Šibenik and National Park "Krka"

Friday, May 16, 2014

Departure

Hematological assessment of Vardar chub (*Squalius vardarensis*) from three rivers in the north-eastern Macedonia

Damir Valić¹, Damir Kapetanović¹, Irena Vardić Smrzlić¹, Maja Jordanova², Katerina Rebok², Sheriban Ramani³, Zrinka Dragun⁴, Vlatka Filipović Marijić⁴, Nesrete Krasnići⁴, Risto Uzunov⁵, Aleksandar Cvetkovikj⁵, Zehra Hajrulai-Musliu⁵, Stojmir Stojanovski⁶, Rodne Nastova⁷, Vasil Kostov⁷

¹Ruđer Bošković Institute, Division for Marine and Environmental Research, LAPAO, Bijenička 54, 10000 Zagreb, Croatia, dvalic@irb.hr

²Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje, Gazi Baba bb, 1000 Skopje, Macedonia

³National Hydrometeorological Service, Hydrology and Ecology Department, Skupi 28, 1000 Skopje, Macedonia

⁴Ruđer Bošković Institute, Division for Marine and Environmental Research, LBEM, Bijenička 54, 10000 Zagreb, Croatia

⁵Faculty of Veterinary Medicine, Ss. Cyril and Methodius University in Skopje, Gazi Baba bb, 1000 Skopje, Macedonia

⁶Hydrobiological Institute, Naum Ohridski 50, 6000 Ohrid, Macedonia

⁷Institute of Animal Sciences, Ile Ilievski 92a, 1000 Skopje, Macedonia

Pollution of surface water presents the major ecological problem in developed industrial world. Various human activities lead to the deterioration of water quality and mining presents one of those impacts. This need for resources has been proven to have extremely negative influence on all biological as well as physico-chemical characteristics of water. Animals that inhabit waters under severe mining activity diversely respond to environmental changes. The changes of hematological parameters in fish can present the consequences of mining influence on the river water.

The objective of this study was to determine how mining activity influence hematological parameters in Vardar chub (*Squalius vardarensis*). Samples from three rivers in the north-eastern Macedonia were obtained: two under mining influence, Zletovska and Kriva River and Bregalnica River as a non-impacted one.

Comparison of the values of hematological parameters (hematocrit (HCT), total serum protein (TSP), refractive index (RI) and serum specific gravity (SG)) of Vardar chub captured in three differently polluted rivers showed significant differences. All four parameters had the lowest values in Zletovska River (HCT 30%, TSP 3.6 g/100 mL, RI 1345, SG 1028), which is characterized by the most prominent water contamination with metals. Hematocrit was also reduced in Kriva River (HCT 35%), where metal contamination and poor microbiological quality of the water was found. Hematocrit has the highest value in Bregalnica River (HCT 39.5%), which was selected for sampling as a reference station. Serum proteins, refractive index and specific gravity were elevated in Vardar chub from the Kriva River (TSP 6.75 g/100 mL, RI 1348, SG 1045).

Except of the degree of water pollution with metals in three investigated rivers, there were physiological and health differences between examined Vardar chubs. Consequently correlation of hematological parameters and several physiological parameters was analyzed (total weight, condition factor and sex). None of hematological parameters showed a clear correlation with the weight and condition factor. In all three investigated rivers hematocrit

values were observed to be somewhat higher in males than in females (about 5% higher median), but the differences were not statistically significant. The other three parameters had higher values in males only in Kriva River, and the differences were statistically significant only for serum proteins ($p < 0.05$).

The association between hematological parameters and parasitic infections of Vardar chub was also investigated. Intestinal parasites were found only in Vardar chub from Bregalnica River and comparison between infected (70%) and uninfected (30%) specimens was made. Although the differences were not significant, there was an indication of higher values of serum proteins, the refractive index and specific gravity in uninfected specimens, while hematocrit showed no association with parasitic infection.

Although connections between hematological parameters and some physiological and health indicators of Vardar chub were observed, the differences between the investigated rivers were much more pronounced and suggested the possibility of using hematological parameters as indicators of pollution of river water.