



International scientific workshop

# INFLUENCE OF ACTIVE MINES ON FRESHWATER ECOSYSTEMS

May 12-16, 2014

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Ruđer Bošković Institute  
Zagreb, Croatia

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Zagreb, 2014

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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  
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# 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

## Detection of methyltestosterone with ELISA method in fish

Risto Uzunov<sup>1</sup>, Zehra Hajrulai-Musliu<sup>1</sup>, Biljana Stojanovska-Dimzoska<sup>1</sup>,  
Elizabeta Dimitrieska-Stojkovic<sup>1</sup>, Aleksandra Todorovic<sup>1</sup>, Velimir Stojkovski<sup>2</sup>

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Synthetic hormone like substances anabolic steroids (trenbolone, 19 nortestosterone, methyltestosterone etc.) and stilbenes (diethylstilbestrol, hexestrol etc.) are often used for therapy in farm animals (Shaoa et al., 2005). Moreover, in livestock farming they have been used as growth promoters (Regal et al., 2010). In *Cyprinus carpio*, methyltestosterone induces faster growth in three different ways: activation of secretion of other androgenic anabolic hormones, increased food conversion and direct effect of methyltestosterone on the gene expression in the muscle cells (Felix, 1989). Due to potential carcinogenic effect and human health risk of their residues hormonal growth promoter are prohibited in the European Union since 1988 (Shaoa et al., 2005; Regal et al., 2010; Uzunov et al., 2013). The aim of this study is detection of methyltestosterone in fish with ELISA method.

Forty five fish samples, *Salmo trutta fario*, which are included in Macedonian monitoring plan, were analysed for detection of methyltestosterone in fish. Fish samples were taken from the Macedonian fish farm. Analyses were performed with ELISA kit (R-Biopharm, Germany, Art. no. R3603). Prior the analyses of the fish samples, ELISA method were validated according to European Commission Decision 2002/657/EC. During the validation procedure linearity, limit of detection (LOD), detection capability (CC $\beta$ ), precision and recovery were investigated.

Validation procedure showed that the ELISA kit for detection of methyltestosterone has a good linearity ( $r^2 = 0.9927$ ), good precision (coefficient of variation was from 0.2 – 11.5%) and good recovery from 82.4 to 97.4%. The limit of detection of the method was 0.14  $\mu\text{g}/\text{kg}$  and the CC $\beta$  was found to be 0.56  $\mu\text{g}/\text{kg}$ . Determined CC $\beta$  was less than minimum required performance limit (MRPL) for the methyltestosterone (1.0  $\mu\text{g}/\text{kg}$ ). Because of these performances the method is applicable as a screening method. All fish samples were analysed in period of two years and the results were lower than the established CC $\beta$ .

Fish and fish products play an important role in human nutrition, for this reason they should not contain any factors or substances harmful for human health. In our study fish samples did not contain residue of methyltestosterone. In the future, detection of anabolic steroids in the fish will be continued in order to prevent abuses of these substances.

### References

- Shaoa, B., Zhao, R., Meng, J., Xue, Y., Wu, G., Hu, J., Tu, X. 2005. Simultaneous determination of residual hormonal chemicals in meat, kidney, liver tissues and milk by liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta* **548**:41–50.
- Regal, P., Nebot, C., Vázquez, I.B., Cepeda A., Fente, A., F. 2010. Determination of 17 $\alpha$ -methyltestosterone in bovine serum using liquid chromatography tandem mass spectrometry. *Journal of Food and Drug Analysis* **18**:51-57.

Felix, S. 1989. Effect of 17  $\alpha$  methyltestosterone on the growth ornamental fish, *Xiphophorus maculatus*. *Indian Journal of Fisheries* **36**:263-265.

Uzunov, R., Hajrulai-Musliu, Z., Stojkovic, D.E., Dimzoska, S.B., Sekulovski, P., Stojkovski, V. 2013. Use of ELISA for preliminary screening of 19 nortestosterone anabolic steroid in cattle meat in Republic of Macedonia. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* **19**:173-177.

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