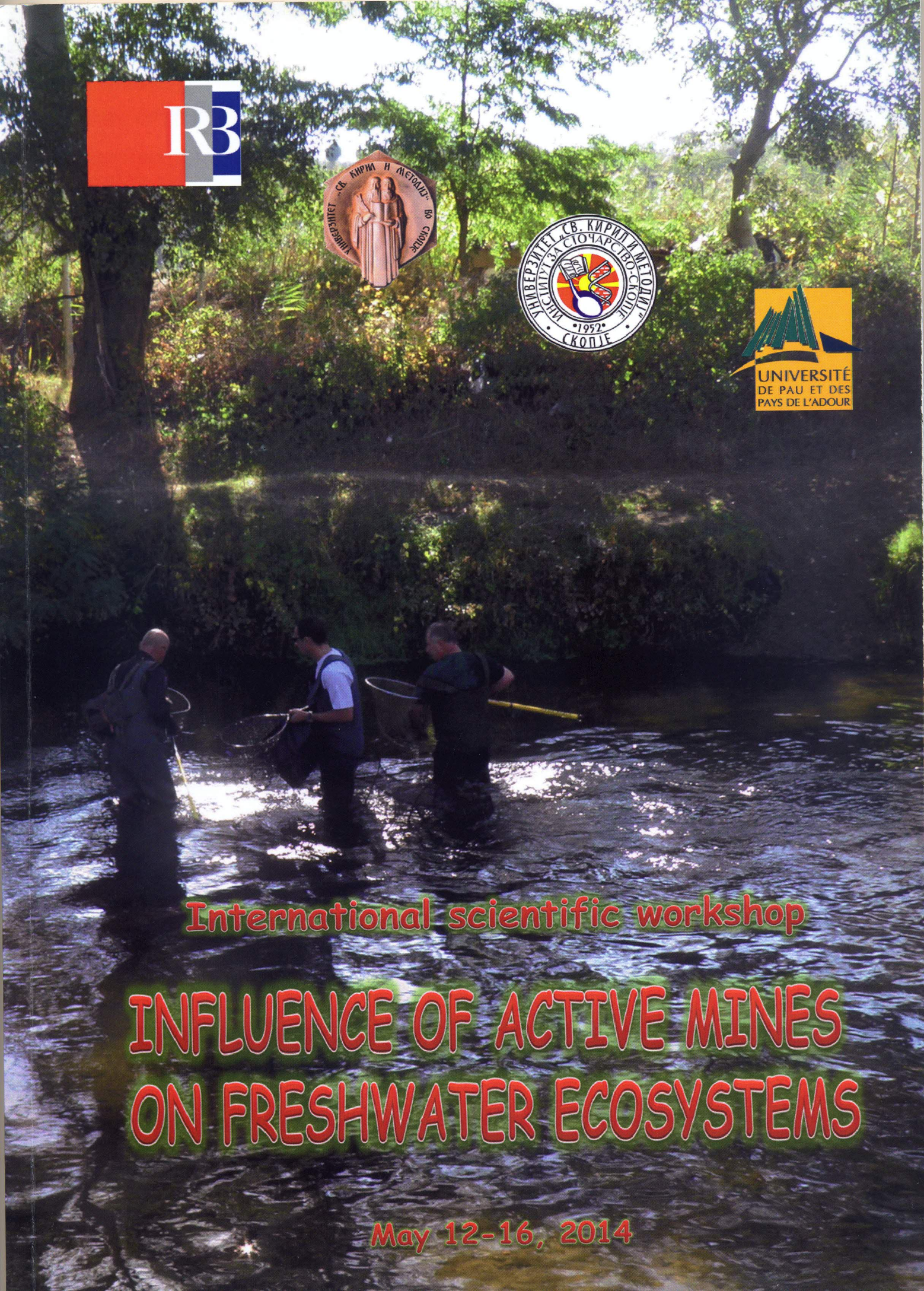

International scientific workshop

INFLUENCE OF ACTIVE MINES ON FRESHWATER ECOSYSTEMS

May 12-16, 2014

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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

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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 parasitical 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

Molecular characterisation of the metazoan parasites of Vardar chub (*Squalius vardarensis*) from three rivers in north eastern Macedonia

Irena Vardić Smrzlić[1], Damir Kapetanović[1], Damir Valić[1], Zrinka Dragun[2], Vlatka Filipović Marijić[2], Nesrete Krasnići[2], Emil Gjurčević[3], Maja Jordanova[4], Katerina Rebok[4], Sheriban Ramani[5], Riste Uzunov[6], Aleksandar Cvetkovikj[6], Zehra Hajrulai-Musliu[6], Stojmir Stojanovski[7], Rodne Nastova[8], Vasil Kostov[8]

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Fish parasites with a complex life cycle, including intermediate and paratenic hosts, can be used as indicators of aquatic environmental stress. Their identification is however often difficult, due to the high level of intraspecific variability. The aim of the present study was to identify metazoan parasites found in Vardar chub (*Squalius vardarensis*) from three rivers in northeastern Macedonia under different minning impact.

Parasite specimens were collected from the dissected Vardar chub (*S. vardarensis*) sampled in spring and autumn from three rivers in northeastern Macedonia: Zletovska River and Kriva River under mining activity impact and Bregalnica River which is non-impacted. Abdominal cavity cysts were observed in the fish from Bregalnica (spring and autumn) and Kriva River (spring) and cysts from two fish specimens were used for the identification. Intestinal parasites were observed in the fish from Bregalnica (spring and autumn) and Zletovska River (spring) and specimens from four different fish were used for identification. Morphological analysis of the parasitic cysts content was done by light microscope, while molecular identification was performed by sequence analysis of 18S rRNA region. Molecular identification of intestinal parasites was done by sequence analyses of three different DNA regions: 18S rRNA, ITS and COI gene. For the phylogenetic analysis of intestinal parasites based on partial COI sequence data, maximum likelihood as well as maximum parsimony method was applied by MEGA 6 software.

Members of two different parasitic phyla were determined from the examined Vardar chub: Myxozoa (Cnidaria) in the abdominal cavity and Acanthocephala in the intestine. Morphological analysis of myxozoan cysts indicated presence of *Myxobolus* sp., while molecular analysis based on 18S rRNA analysis confirmed this genus. Molecular analysis of acanthocephalans based on the 18S rRNA and ITS region confirmed two different species:

Pomphorhynchus laevis and *Acanthocephalus* sp. most closely related to the *A. anguillae* (99.8% identity to the sequence from the GenBank). Phylogenetic analysis of *P. laevis* based on COI sequence data indicated separated clustering of Macedonian specimens in relation to the other European specimens available from the GenBank.

Identification of *Myxobolus* sp. in Vardar chub is important as members of this genus are potentially dangerous to their fish host. Determination of acanthocephalans is important not just for their further phylogeographic studies, but also for the water contamination studies, as these parasites were suggested as a sensitive biological indicator of metal bioavailability in the river water.