BIOTOXMET PROJECT
general overview

PI: Vlatka Filipović Marijić

Ruđer Bošković Institute

KICK-OFF MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 11th October 2021

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RB

ANDRIJA STAMPAR TEACHING INSTITUTE OF PUBLIC HEALTH

WELLFISH DIAGNOSTICS

MONTAN UNIVERSITÄT WWW.UNILEOBEN.AC.AT

FFoQSI FEED AND FOOD QUALITY SAFETY AND INNOVATION

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BIOTOXMET PROJECT 28.12.2020.-27.12.2024.

- **Ruđer Bošković Institute – Division for Marine and Environmental Research, Zagreb, Croatia**
- **Institute for Medical Research and Occupational Health, Zagreb, Croatia**
- **Andrija Stampar Teaching Institute of Public Health, Zagreb, Croatia**
- **Wellfish Diagnostics, Paisley, UK**
- **University of Leoben, Leoben, Austria**
- **Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, Tulln, Austria**

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PROJECT COLLABORATORS

Ruđer Bošković Institute – Division for Marine and Environmental Research, Zagreb, Croatia

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Dr. sc. Dušica Ivanković

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Sara Šariri, PhD student

Dr. sc. Željka Fiket

PROJECT COLLABORATORS

- **Institute for Medical Research and Occupational Health, Zagreb, Croatia**

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Dr. sc. Želimira Cvetković

- **Wellfish Diagnostics, Paisley, UK**

Dr. sc. Josip Barišić

- **University of Leoben, Leoben, Austria**

Dr. sc. Thomas Prohaska

Dr. sc. Donata Bandoniene

(Dr.nat.techn. Johanna Irrgeher)

- **Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, Tulln, Austria**

Dr. sc. Andreas Zitek

PROJECT GOALS

1. seasonal and long-term trends of metal concentrations in the water and sediments of the Krka River and its tributaries

2. biological responses of aquatic organisms to metal exposure/impact under different environmental conditions

3. bioavailable and potentially toxic fraction of dietborne metals in fish

4. active cellular processes in acanthocephalans and fish intestine under different metal exposure regimes

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1. seasonal and long-term trends of metal concentrations in the water and sediments of the Krka River and its tributaries

1.1. water: physico-chemical parameters, total and dissolved metal levels, $^{87}Sr/^{86}Sr$ isotope ratio (for the first time in the Krka River catchment);

1.2. sediment: metal concentrations, total carbonates and granulometric factors.

PROJECT GOALS

- karst and sensitive ecosystem impacted by industrial and municipal wastewaters (hot-spots of pollution)
- protected area (Krka National Park) in the Republic of Croatia
- most of the previous studies were conducted in the Krka National Park, so our study will represent the first comprehensive study on water and sediment of the the Krka River and its tributaries (Krčić, Kosovčica, Orašnica, Butišnica)

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KRKA RIVER

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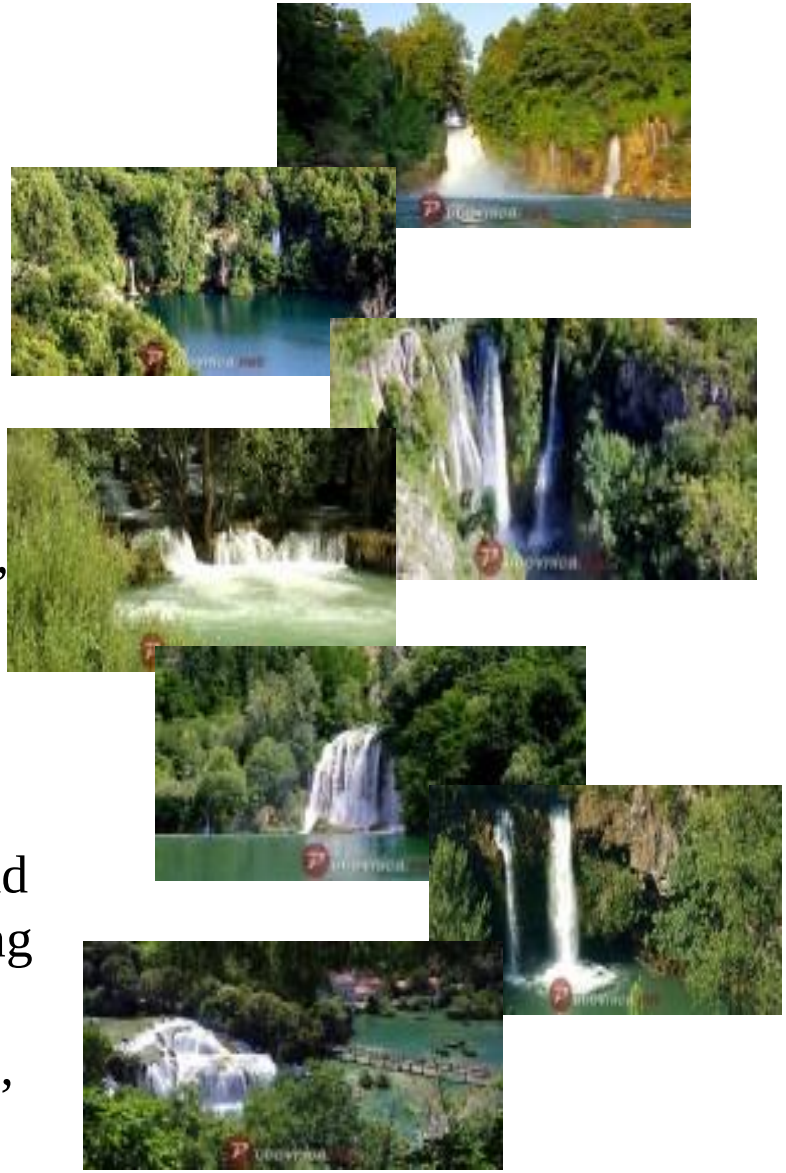
KRKA RIVER

- The present day appearance of the Krka canyon is the result of tectonic movements and surface karst-building processes in the carbonate layers.

- Thanks to the constant process of tufa-building, the Krka River is a karst phenomenon which is characterised by 7 karstic waterfalls.

- The flora and fauna of Krka National Park is very rich and diverse, with many endemic, rare and threatened species. This puts the Krka River among the most valuable natural entities in both Croatia and Europe. There are 1022 plant species recorded, as well as 20 fish, 221 bird and 18 bat species.



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KRKA RIVER- anthropogenic impact

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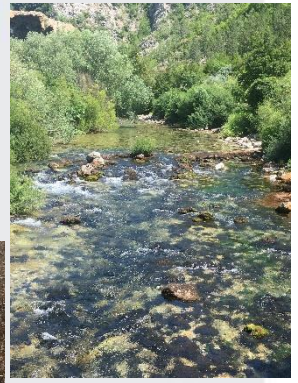
Study area



Krka Gornji Tok

Butišnica River

Brljan Lake (on the Krka River)

Krka River (near the municipal wastewater outlet)

Kninska tvrđava

Knin

Lidl

Industrial wastewaters (from the screw factory)

Orašnica River

Kosovčica River

Domovinskog rata

Kovačić

Krčić Stream

Krka River source

Krka

Ul. kralja Zvonimira

Drniška ul

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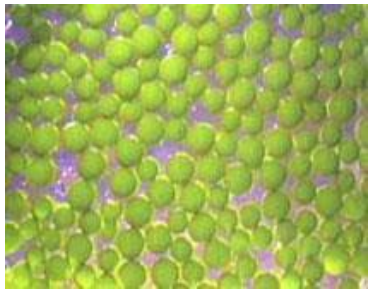
2. biological responses of aquatic organisms to metal exposure/impact under different environmental conditions

INDICATOR ORGANISMS

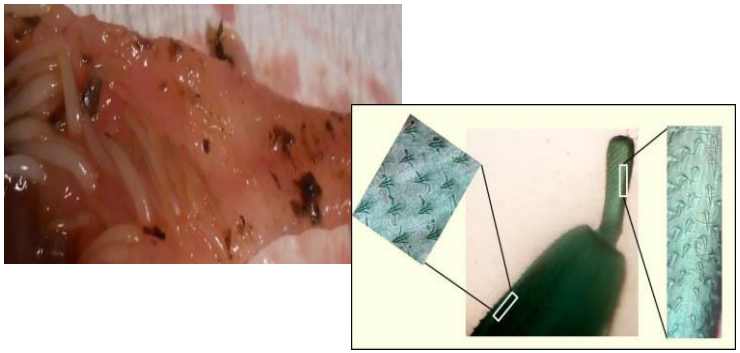
PROJECT GOALS



Green algae (*Selenastrum capricornutum* Printz, 1914)

Water flea (*Daphnia magna* Straus, 1820)

Brown trout *(Salmo trutta* Linnaeus, 1758)



Acanthocephala *(Dentitruncus truttae* Sinzar, 1955)

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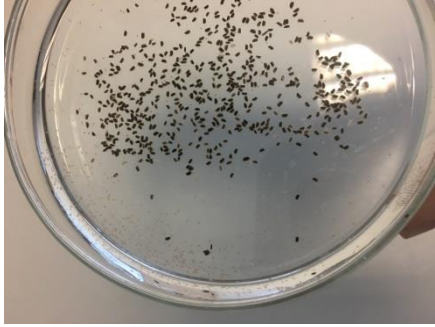
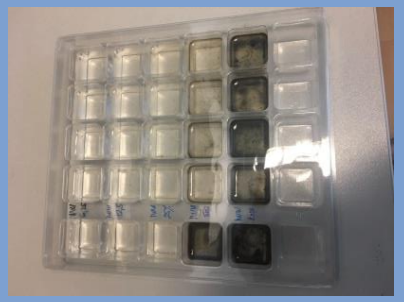
2. biological responses of aquatic organisms to metal exposure/impact under different environmental conditions

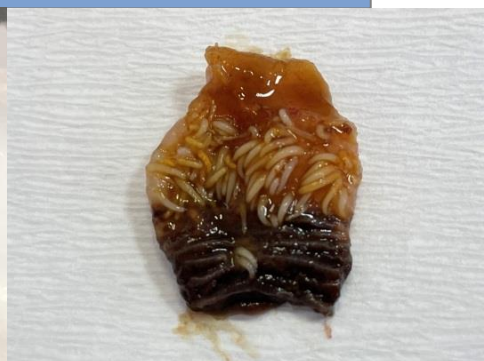


PROJECT GOALS

2.1. river- and waste-water acute effects on experimental organisms (algae, *Daphnia* - zootoxicity and phytotoxicity tests) of different trophic levels and sensitivity to pollution than fish- novel trends in the LBEM, new knowledge on toxicity testing;



2.2. biomarker responses in the intestine of native fish and metallothioneins in acanthocephalans- the first data of this kind; literature data on biomarker and metal levels in intestine of native fish are rare;



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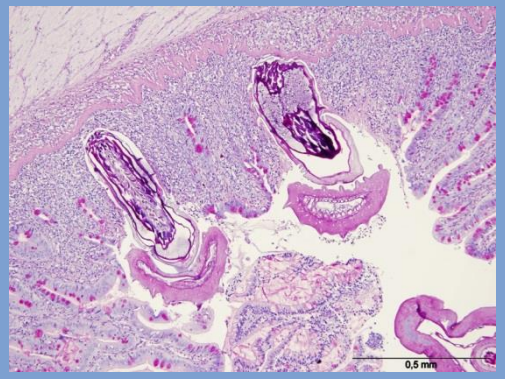
2. biological responses of aquatic organisms to metal exposure/impact under different environmental conditions

2.3. histopathological alterations, especially quantitative and qualitative changes of fish intestinal mucous cells- novel data on mucosal mapping;



2.4. metal concentrations in acanthocephalans and fish soft tissues (intestine, muscle) and calcified structures (scales, otoliths- the first data for fish from the aquatic ecosystem in Croatia); considering the difference in biomagnification of most metals and Hg, measurement of Hg concentrations in fish intestine and muscle as Hg content increases over food chains

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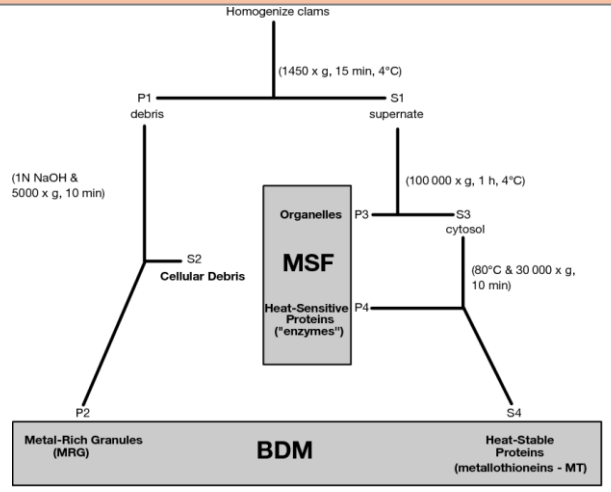
3. evaluation of bioavailability and toxicity of dietborne metals in fish (determination of metal levels in different intestinal subcellular fractions)

PROJECT GOALS

3.1. metabolically available fraction (which might be potentially toxic due to binding to biologically important molecules, MSF- metal sensitive fraction);
3.2. detoxified metal fraction (without toxic effects, BDM- biologically detoxified metals);
3.3. trophically available metal fraction (available to predators, TAM- trophically available metals);
3.4. metal levels in the gut content under different metal exposure regimes (estimation of metal uptake from prey). It is known that metals bound to granules are not bioavailable, while those bound to metallothioneins will be detoxified, so total metal concentrations only partially represent potentially toxic metal fraction



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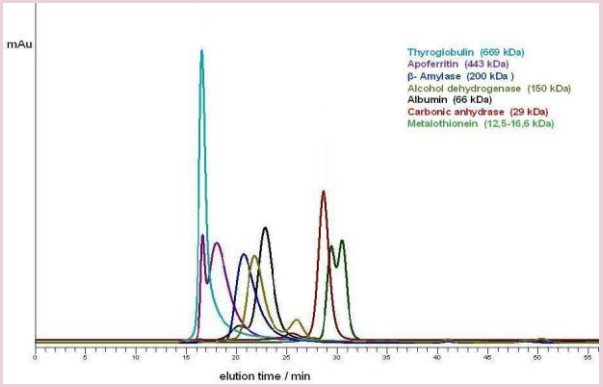
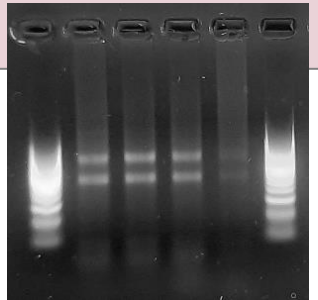
4. estimation of active cellular processes in acanthocephalans and fish intestine under different metal exposure regimes

PROJECT GOALS

4.1. estimation of metal distribution among cytosolic proteins of different molecular masses in acanthocephalans and fish intestine (HPLC)- novel findings;



4.2. transcriptome and gene expression- analyse *de novo* sequencing of transcriptome of acanthocephalans and transcriptome profiling of fish intestine. Gene expression in acantocephalans and fish intestine will be compared between the control and higher metal impacted site and the results will be confirmed by qRT-PCR reactions- novel findings in the scientific community.



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New equipment

- UV-VIS spectrophotometer
- boat for the fish sampling
- incubator
- light table
- centrifuge
- freezer +4°C/-20°C
- ice maker
- laboratory digestor
- computers
- spare parts for potentiostatic instrument Computrace
- spare parts for tissue homogenizer
- adaptors for spectrophotometer

TOTAL BUDGET
1.222.500,00 kn
164.447,10 EUR

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THANK YOU FOR YOUR ATTENTION

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