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# Ph.D. thesis topics 2024/2025

## DSP Ochrana vodních ekosystémů / Protection of Aquatic Ecosystems

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# Výzkumný ústav rybářský a hydrobiologický / Research Institute of fish Culture and Hydrobiology

## Vodňany





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### Third-generation sequencing: a powerful tool for studying biodiversity in freshwater ecosystems

#### Sekvenování třetí generace jako nástroj pro studium biodiversity sladkovodních ekosystémů

#### Annotation

Sequencing techniques have significantly developed over the past decade, leading to higher accuracy and lower costs. As a result, they have become more accessible to the broader scientific community and have enabled the resolution of various research questions across all fields. Nanopore sequencing is a unique method for determining the sequence of DNA or RNA from various samples without the need for PCR amplification or chemical labelling of the sample. In contrast to other techniques that sequence short fragments, it allows for long reads of single-strand DNA without losing valuable information (Wang et al., 2021; Niedringhaus et al., 2011).

The freshwater environment, one of the most threatened ecosystems on the planet, currently faces challenges from non-native organisms, which often become invasive (Reid et al., 2019; Dungeon et al., 2006). Their early detection in the ecosystem is crucial for subsequent management and protection of native biodiversity. At the same time, mapping biodiversity in various aquatic habitats using eDNA samples is a promising approach for quickly and less laboriously obtaining information compared to the often complex and time-consuming regular field sampling (Dickie et al., 2018).

The combination of nanopore sequencing and eDNA sampling sounds promising to reveal and describe the biodiversity of particular types of aquatic habitats. Additionally, nanopore sequencing can bring valuable information not only about the spread of non-native organisms but also about detecting the native, endangered species, which are, due to their protected status, very rare and difficult to detect using traditional sampling methods.

#### The main hypothesis

- Combination of eDNA sampling and application of nanopore sequencing could early detect hidden invasion in aquatic habitats.
- Non-native species spread from spots around cities and/or wholesalers of ornamental aquatic animals.
- Endangered and rare aquatic species could be detected with the eDNA sampling.

#### Aim(s) of the Ph.D. thesis

- Apply nanopore sequencing with specific bioinformatic pipelines to
  - (i) early detect the spread of non-native aquatic species,
  - (ii) describe the biodiversity of natural valuable habitats,
  - (iii) develop species-specific protocols for detecting species of interest.

#### Possible approaches to reach the aims / to verify the hypotheses

- To perform intensive eDNA sampling of (i) potential spots of non-native species near wholesalers, town parks and thermal waters, and (ii) natural valuable habitats in protected areas.



- To collect and analyse data about biodiversity and its changes in spots with non-native species presence.

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- CENAKVA VP4.



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### Application of LC/HRMS methods and data analysis workflows for identification of compounds with adverse effects on aquatic biota in passive sampler samples

**Použití LC/HRMS a postupů datové analýzy pro identifikaci sloučenin s negativním efektem na vodní organismy v pasivních vzorkovačích**

### Annotation

Constantly increasing numbers and amounts of chemicals result from development in technologies, industry, agriculture, and health care. Environmental pollution in the recent era has become highly complex. The European Chemical Agency registers more than 100000 chemicals for industrial purposes. Pharmaceuticals, metabolites, and environmental transformation products are not included and are often unknown. Conventional risk assessment following a top-down process is too slow to address emerging issues in environmental pollution. Many chemicals introduced into the environment have not been identified. In addition, the effect of most compounds on human and environmental health was not investigated (Šauer, Vrana, et al., 2023).

The proposed Ph.D. topic is focused on the bottom-up selection of compounds with biological effects. It means the identification of compounds responsible for effects in the sample (Brack, Aissa, et al., 2019). Extract the samples with a given biological effect can be fractionated to lower the complexity of the mixture. It can be performed in more than two steps in different LC systems. There are even more selective ways to achieve high selectivity, e.g., pull-down assay based on effect-specific sorption (Mikusová, Tousova, et al., 2024).

The amount of LC-HRMS data from regulatory or exploratory monitoring increases annually. The data obtained with the proper acquisition method can be used as digitally frozen samples. Besides identifying the drivers of the specific effect, developing and applying a data analysis workflow to describe complex pollution and mine information on spatial and temporal changes in surface water pollution are highly relevant. There were previously applied approaches for selecting water pollutants specific to the sources of pollution (Kiefer, Du, et al., 2021). However, the results of evaluating the growing non-targeted data are scarce.

### The main hypothesis

- Effect drivers can be identified when enough selective pre-separation of the mixtures is applied.
- Non-targeted analysis in the Czech Republic or river basin scales can identify site-specific and source-specific pollution markers.
- Back-quantification based on MS1 data can reach the trueness level suitable for risk assessment.

### Aim(s) of the Ph.D. thesis

- To develop and apply LC/HRMS methods and software workflows to identify potential candidates responsible for the activity. It will rely on cooperation within the broad environmental chemists and toxicologists team of the University of South Bohemia in České Budějovice and Masaryk University in Brno.



- To explore vast digitally frozen data from past projects to achieve qualitative information on pollution and the potential effect of the present chemical
- To develop and validate the back-quantification of digitally frozen data for more exact effect and trend information.

### **Possible approaches to reach the aims / to verify the hypotheses**

- Data from particular studies performed in cooperation with RECETOX Brno have already been collected and are ready for analysis. The fractionation and pull-down assay development are continuing, and more data for diverse effects are expected to be obtained quickly.
- Long-term monitoring of LC-HRMS data from CHMI is available for aims two and three. Digitally frozen samples from other countries and continents are also available for international comparison.
- Applications of software CompoundDiscoverer 3.3., MassFrontier 8.0 and others for the data analysis.

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### **Funding**

- QK23020018 What we don't know about organic pollution of drinking and irrigation water sources: Identification of emergent compounds through non-targeted screening
- QL24010384 Medium-term trend in the behavior of micropollutants originating from wastewater or sewage sludge in the soil environment
- CENAKVA VP2.



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### **Polar micropollutants and aquatic organisms – a study of fate and effects with application of targeted and non-targeted LC/HRMS analysis**

**Polární mikropolutanty a vodní organismy – studium osudu a působení (s využitím metod cílené a necílené analýzy LC/HRMS)**

### Annotation

Due to various anthropogenic activities, polar micropollutants (e.g., pharmaceuticals and personal care products, pesticides, per- and polyfluorinated compounds etc.) are ubiquitous in the aquatic environments. Although their concentration is relatively low, typically nanogram to lower micrograms per liter, their impact on aquatic organisms is not negligible.

Pharmaceuticals and personal care products enter the aquatic environment mainly via wastewater treatment plants (WWTPs) in developed countries. These compounds are continuously discharged from highly centralized sources with approved seasonal variability reflecting prescription patterns and removal efficiency in WWTPs or further treatment (Fedorova, Grabic, et al., 2022). On the contrary, pesticide sources are diffusive following their application in agriculture. Transformation of parent pesticides in soils leads to the leakage of pesticides and their metabolites into the aquatic environment (Kodešová, Fedorova, et al., 2023). The sources of per- and polyfluorinated compounds are also anthropogenic activities, but the pattern is unclear. All pollution pathways meet in the freshwater environment. Mentioned polar micropollutants are a broad group of chemicals with highly diverse physicochemical properties. However, pesticides and pharmaceuticals were designed to have biological activity. Based on their mode of action, these compounds could have different effects on fish, crayfish, benthos, and other aquatic organisms (Duchet, Grabicová, et al., 2024, Huben, Horky, et al., 2021). The topic aims to study the fate and impact of micropollutants under environment-mimicking (mesocosms) or environmental conditions (Grabicová, Duchet, et al., 2024, Grabicová, Vojs Staňová, et al., 2022).

We will apply liquid chromatography with high-resolution mass spectrometry (hybrid quadrupole/orbital trap instruments) to trace the pollution fate and develop a non-targeted HRMS-based approach for effects evaluation in biomarker organisms (environmental metabolomics) (Giebułtowicz, Grabicová, et al., 2024, Grabicová, Randák, et al., 2022). The applicant's background in chemistry (analytical or environmental chemistry) and toxicology is expected for the thesis.

### The main hypothesis

- The bioaccumulation differs between closed (mesocosm) and open (polluted stream) systems.
- The effect of the complex pollution differs among trophic levels.
- The fate (bioaccumulation and transformation) and effect can be traced through different approaches of nontargeted analysis.



### **Aim(s) of the Ph.D. thesis**

- To quantify the bioaccumulation of pollutants at different trophic levels in mesocosm with a focus on low levels (biofilm, invertebrates).
- To identify possible transformation products or unknown pollutants (in stream experiment).
- To identify and describe the changes caused by pollution at different trophic levels.

### **Possible approaches to reach the aims / to verify the hypotheses**

- We will use mesocosm and real-stream experiments with clearly defined unpolluted (control) and polluted (exposed) conditions. These experiments were already performed or are planned for the ongoing project.
- The differences between the experimental groups allow for the application of nontargeted LC-HRMS methods, which can identify the effect of present pollutants at the metabolome level.
- The developed targeted method will allow quantification of the pollution. The workflow of the non-targeted method will be optimized and applied to identify potential metabolites or metabolic responses of the organisms to pollution.

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### **Funding**

- 24-11779S Effects of warming and pollutants on nutrient flows and lower trophic levels in freshwater communities: from microbes to Daphnia, CENAKVA VP2.





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### Spatial Patterns in Biological Invasions and Impacts on Biodiversity

#### Prostorové vzorce biologických invazích a dopady na biodiverzitu

#### Annotation

Invasive species are a major threat to native biodiversity and a leading cause of extinction, exacerbated by globalization and climate change. Aquatic ecosystems are especially vulnerable due to their high connectivity and insufficient monitoring. Despite increasing species introduction rates, research has predominantly focused on a few high-profile invaders, neglecting less recognized species and broader spatiotemporal trends. This gap in understanding hinders effective management efforts to mitigate invasions, as there is insufficient quantitative knowledge about the impacts of these less prominent invaders.

The interactions between invasive species and other anthropogenic impacts, such as climate change and pollution, remain poorly understood, particularly regarding spatial dynamics. This lack of insight has resulted in varied threats posed by invasive species across different regions. While human activities like aquaculture, the pet trade, and ballast water are known pathways for introducing invasive species, the role of socio-cultural and political factors in driving invasion rates has not been adequately explored. Common indicators like GDP per capita, international trade, population density, and education are often used, but important socio-cultural and political factors such as governmental legislation stringency and cultural openness have been overlooked.

Addressing these knowledge gaps requires a multidisciplinary approach that leverages long-term data, socio-cultural and political indicators, and experimental research. By integrating these elements, we can enhance our understanding of invasive species dynamics and develop effective management strategies to mitigate their impacts on biodiversity. This comprehensive approach is essential for predicting future trends and implementing measures to conserve biodiversity in the face of ongoing globalization and climate change.

#### The main hypothesis

- Invasive species show distinct spatial and temporal distribution patterns in European aquatic ecosystems, influenced by environmental and human factors.
- Socio-cultural, political, and economic indicators (e.g., legislation stringency, cultural openness, GDP, population density) significantly affect invasion rates.
- Invasive species impact native communities differently under various environmental stressors (e.g., temperature, pollution) and biotic contexts (e.g., predator density).
- Interactions between invasive species and anthropogenic impacts (e.g., climate change, pollution) cause spatial variations in invasive species threats.
- Functional response and behavioral experiments will reveal differences in the feeding behavior and efficiency of invasive versus native species.



### **Aim(s) of the Ph.D. thesis**

- Investigate the spatial and temporal patterns of invasive species in European aquatic ecosystems using a long-term dataset.
- Analyze the influence of socio-cultural, political, and economic indicators on invasion rates and species spread.
- Assess the ecological impacts of invasive species on native communities under varying environmental stressors and biotic contexts.
- Understand the interactions between invasive species and other anthropogenic impacts, such as climate change and pollution.
- Conduct functional response and behavioral experiments to compare the impacts of invasive and native species, informing management strategies.

### **Possible approaches to reach the aims / to verify the hypotheses**

- Utilizing a comprehensive European long-term dataset of aquatic organisms to analyze spatial and temporal patterns of invasive species.
- Comparing invasion records from databases (GBIF, GISD, InvaCost) with socio-cultural, political, and economic indicators.
- Employing statistical models (GLM, GAM, species distribution models) to explain spatial patterns of invasive species.
- Conducting functional response and behavioral experiments to assess impacts of invasive and native species under different environmental stressors and biotic contexts.
- Integrating long-term monitoring data with experimental findings to understand interactions between invasive species and anthropogenic impacts.

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### **Funding**

- CENAKVA VP4.



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### Bioaccumulation dynamics of emerging contaminants in aquatic invertebrates

#### Bioakumulační dynamika emergentních kontaminantů ve vodních bezobratlých organismech

#### Annotation

Pharmaceuticals are designed to be highly bioactive at low doses in human or animals, but their bioaccumulation dynamics is less studied in invertebrates. Freshwater crayfish have a prominent role in aquatic ecosystems and pose important segments of the trophic web. Crayfish pose a unique biological feature with potential as a alternative experimental model to advance the 3Rs strategy of animal welfare. The primary aim of the present study is to describe a predictive crayfish bioaccumulation model based on empirical measurements of representative pharmaceutical kinetics using advanced analytical methods. Specifically, the experimental design will estimate key bioconcentration parameters in crayfish exposed to a model acid, a model base and a model neutral pharmaceutical using first order kinetics. Simultaneously, toxicological responses, such as biochemical and behavioral effects will be determined in exposed organisms. The study will conclude with comparative analysis of crayfish bioaccumulation model dynamics with models already employed for fish. All toxicological responses evaluated in a pilot experiment have indicated lower bioactivity of ionizable pharmaceuticals in crayfish than fish. Its evident, that the first indications of significant differences of uptake, biotransformation and elimination in invertebrates deserve full investigation under the proposed experiments.

#### The main hypothesis

- Ionizable pharmaceuticals exhibit specific patterns of behavior during the uptake and depuration phases in crayfish.
- The empirically revealed bioconcentration parameters for selected model pharmaceuticals can be generalized to develop an invertebrate bioaccumulation model.
- Various concentrations of pharmaceuticals significantly influence the toxicological biomarkers and detoxification enzymes in crayfish during both acute and chronic exposure.
- Selected pharmaceuticals are associated with behavioral changes in crayfish.

#### Aim(s) of the Ph.D. thesis

- Assessing the uptake and elimination rates, bioconcentration factor, body burden, apparent volume of distribution, hemolymph-water partition coefficient
- Comparison of the suggested 'crayfish model' with the previously established fish bioaccumulation model for emerging ionizable compounds
- Assessing the toxicity and neurotoxicity indicators (lipid peroxidation, antioxidant enzymes and acetylcholinesterase activity) in crayfish exposed to model pharmaceuticals
- Investigating the substrate-specific cytochromes P450 involved in detoxification in crayfish
- Behavioural changes in crayfish exposed to tested pharmaceuticals.



## Possible approaches to reach the aims / to verify the hypotheses

- Pharmaceuticals of different properties and therapeutic classes were selected as model chemical compounds.
- Description of bioaccumulation dynamics based on first-order kinetics in marbled crayfish exposed to three model ionizable pharmaceuticals
  - a) Bioconcentration experiments
  - b) Chemical analysis
  - c) Bioconcentration parameters calculation
- Investigation of toxicological biomarker responses and behavioral effects in marbled crayfish exposed to different doses of selected pharmaceuticals in acute and subchronic tests
- Biochemical and behavioural end-points will elucidate the real effects of absorbed, distributed, and biotransformed pharmaceuticals in crayfish tissues.
  - a) Behavioral end-point of toxicity tests
  - b) Biochemical end-points from toxicity tests
- Activities will be based on several previous pilot experiments that eliminates unpredictable phenomena during the experimental phase. Biochemical and chemical methods are being optimised for application on crayfish tissues. The project's milestones are three toxicological and three bioconcentration experiments derived from three used model pharmaceuticals. The individual experiments will be timed within three years in respect to the availability of sufficient crayfish specimens. Around 200 crayfish specimens will be used in each bioaccumulative laboratory experiment. The advantage of the crayfish species is the season independency with continuous culturing under laboratory conditions. Crayfish culturing and rearing capacity together with experimental conditions allow only reasonable testing of three model pharmaceuticals instead of a wide range of individual pharmaceuticals of environmental concern.

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## Funding

- Project of GAČR 23-07274S Bioaccumulation dynamics of emerging contaminants in aquatic invertebrates using marbled crayfish (2023–2025, responsible leader: Assoc. Prof. Vladimír Žlábek)
- CENAKVA VP2.