

Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

## Ph.D. thesis topics 2024/2025 DSP Ochrana vodních ekosystémů / Protection of Aquatic Ecosystems

Výzkumný ústav rybářský a hydrobiologický / Research Institute of fish Culture and Hydrobiology	. 2
Supervisor: Assoc. Prof. Paride Balzani, Ph.D	.3
Who eats whom: using stable isotopes to reveal food links in aquatic ecosystems	. 3
Supervisor: Assoc. Prof. Ing. Martin Bláha, Ph.D	.4
Urban waters: a source of hidden biodiversity of non-native species	.4
Supervisor: Ing. Bc. Kateřina Grabicová, Ph.D	. 5
Polar micropollutants and aquatic organisms – a study of fate and effects with application of targeted an non-targeted LC/HRMS analysis	וd 5.
Supervisor: Dr. Phillip J. Haubrock, Ph.D	. 6
Spatial patterns in biological invasions and impacts on biodiversity	. 6
Supervisor: Assoc. Prof. Ing. Vladimír Žlábek, Ph.D	. 7
Bioaccumulation dynamics of emerging contaminants in aquatic invertebrates	. 7
Ústav akvakultury a ochrany vod / Institute of Aquaculture and Protection of Waters	8
Supervisor: Mgr. Otakar Strunecký, Ph.D	.9
Plasmid-encoded nitrogen fixation in cyanobacteria	.9
Ústav komplexních systémů / Institute of Complex Systems1	L <b>O</b>
Supervisor: prof. RNDr. Dalibor Štys, Ph.D1	11
Detection of particles in liquids using flow light microscopy1	1
The use of human tissue cultures as rapid, sensitive and semi-specific biosensors for the detection of pollutants and bioactive compounds	of L2
Supervisor: Ing. Jan Urban, Ph.D1	13
Fish behavior as environmental markers1	13



Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

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

## Vodňany





## Supervisor: Assoc. Prof. Paride Balzani, Ph.D.

### Contact

E-mail: balzani@frov.jcu.cz

### Who eats whom: using stable isotopes to reveal food links in aquatic ecosystems

Kdo žere koho: použití stabilních izotopů k odhalení potravních vazeb ve vodních ekosystémech

### Annotation

The availability and quality of natural ecosystems' food resources vary across space and time. In aquatic ecosystems, food quantity and quality vary along changing physical and chemical conditions, among habitats, within watersheds, and across seasons. Accordingly, some food sources can be rare or absent, thus limiting consumer fitness in a given habitat and/or ecosystem.

The community composition determines the trophic relationships within food webs, which, nowadays, often contain non-native invasive species. As such, their introduction or spread can have negative impacts on native species (i.e., through competition and predation) but also the structure and stability of trophic webs. Therefore, it is crucial in trophic ecology to investigate how food sources are converted into new biomass and which food sources are most important for which species.

This PhD project will be focused on energy pathways and food webs in aquatic ecosystems under different treatments using an array of approaches, including stomach content and stable isotopes analysis. Therefore, the PhD candidate should have deep knowledge of stable isotopes and food webs in aquatic ecosystems. Specifically, this project is focused on fish, zoobenthos, and zooplankton; thus, previous experience with some of these groups is expected. Furthermore, moderate experience with R-language is essential. The candidate should be independent and familiar with field sampling. A driving license is an advantage.



## Supervisor: Assoc. Prof. Ing. Martin Bláha, Ph.D.

### Contact

E-mail: blaha@frov.jcu.cz

### Urban waters: a source of hidden biodiversity of non-native species

### Vodní plochy měst a obcí: skrytý zdroj biodiversity nepůvodních druhů

### Annotation

The rate of non-native species translocations and spreading increases mostly due to globalisation and climate changes. Many non-natives originate in the pet trade with aquarium organisms, which has been recognised as their significant source worldwide. These organisms are rarely detected in the early stages of establishment and eventual spread. Very often, the activity and negative impacts of such species are apparent when changes occur at the community and ecosystem level. At such a moment, their effective eradication is usually unfeasible.

Recently, many records originating from urban waters have been documented. Town parks with ponds, fountains or small streams offer ideal places to dump unwanted aquarium pets. A special case is thermal waters providing a suitable environment all year round in the temperate conditions of Europe. These emerging non-natives, fish and crayfish, are mostly known from the pet trade. However, there is a lack of specific markers enabling fast detection of particular species or groups of organisms using eDNA in surface water samples. The PhD candidate will screen a pet trade fish and crayfish species, which could possibly be released and detected in urban waters. The main task will be to design, test and apply in the field group/species-specific markers of non-native organisms. Thus, we will obtain a solid tool for fast screening detection of potential non-native species in the aquatic environment, with a focus on urban waters in particular.



## Supervisor: Ing. Bc. Kateřina Grabicová, Ph.D

### Contact

E-mail: grabicova@frov.jcu.cz

## 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 litre, 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. On the contrary, pesticide sources are diffusive following their application in agriculture. Transformation of parent pesticides in soils leads to the wash-off of pesticides and their metabolites to the aquatic environment. 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, all 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. The goal of the topic is to study the fate and the impact of micropollutants under environmental conditions. We will apply liquid chromatography with highresolution 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). Applicant's background in chemistry (the analytical or environmental chemistry) and toxicology fields is expected for the topic.



## Supervisor: Dr. Phillip J. Haubrock, Ph.D

### Contact

E-mail: phillip.haubrock@senckenberg.de

### Spatial patterns in biological invasions and impacts on biodiversity

### Prostorové modely v otázách biologických invazí and jejich důsledky pro biodiverzitu

### Annotation

Invasive species pose a significant threat to native biodiversity and are a leading cause of extinction. Globalization and climate change have facilitated the spread of non-native species, particularly in aquatic ecosystems where they are poorly monitored. However, recent scientific efforts have mainly focused on a few "high-profile invaders," neglecting the introductions of species and their impacts on habitats, native communities, and biodiversity trends. As a result, there is a lack of quantitative knowledge about the distribution and impacts of invaders on large scales, hindering effective management strategies. Furthermore, our understanding of how invasions interact with other anthropogenic impacts, such as climate change and pollution, is limited. This knowledge gap has led to spatial variations in the threats posed by invasive species across different areas. The establishment of invasive aquatic species is often linked to human activities like aquaculture, the pet trade, and the construction of canals.

To fully comprehend invasion rates, it is necessary to consider socio-cultural and political factors, which have been overlooked. Indicators such as governmental legislation, cultural access and participation, and people's relationship with the natural environment are important in explaining spatial variations in invasion rates. As climate change, anthropogenic stress, and globalization persist, the impacts of invasive species may vary across different locations. However, the drivers that facilitate aquatic invasions and their context-dependencies are not well understood, making predictions of future trends challenging. Despite the availability of long-term monitoring projects, which have documented invasion trends across many taxa, this information remains underutilized in invasion science.

Accordingly, this PhD program will utilize existing data and aim to study the spatial patterns and fluctuations of aquatic invasive species and their relationship with humanity. It will employ a multidisciplinary approach focusing on three key aspects: analyzing a comprehensive European dataset to understand the spatial patterns of invasive species over time, comparing invasion records from various databases to explore understudied indicators, and conducting experiments to compare the impacts of native and invasive species under different stressors and biotic contexts.



## Supervisor: Assoc. Prof. Ing. Vladimír Žlábek, Ph.D

### Contact

E-mail: vzlabek@frov.jcu.cz

### **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 models already employed for fish.



Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

# Ústav akvakultury a ochrany vod / Institute of Aquaculture and Protection of Waters

# České Budějovice





Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

## Supervisor: Mgr. Otakar Strunecký, Ph.D.

### Contact

E-mail: ostrunecky@frov.jcu.cz

### Plasmid-encoded nitrogen fixation in cyanobacteria

#### Plazmidem kódovaná fixace dusíku u sinic

### Annotation

Introduction: Nitrogen (N) fixation, a highly energy-intensive process, plays a crucial role in the global nitrogen cycles. Despite advancements in comprehending nitrogen fixation, the mechanisms governing N fixation in non-heterocytous filamentous cyanobacteria remain unresolved. This project focuses on Pseudanabaenaceae (PAs), offering a fresh perspective with plasmid-coded nitrogen fixation, positioning them as an outstanding model system for future exploration.. PAs, being the basal filamentous and phylogenetically first diazotrophic cyanobacteria having quite wide ecological valence. The topic aims to bridge knowledge gaps in N fixation, exploring the under-studied plasmid-facilitated nitrogen fixation in cyanobacteria.. This project should pave way for further research on PAs, focusing on elucidating their N fixation capacity and potential. Concurrently, the project aims to quantify the prevalence of the plasmid coded N fixation in the environment.

Proposed topic has two main goals:

Goal 1: To measure the Biological Nitrogen Fixation (BNF) in PAs and quantify the extent of BNF in PAs fixation. Determine if plasmid-bearing Pseudanabaenales exhibit sustained BNF even in nitrogen-rich environments as suggested by preliminary results. The hypothesis suggests BNF remains consistently active in the earliest phylogenetic cyanobacterial nitrogen fixers and potentially in other uncomplicated filamentous species, suggesting an evolutionary adaptation to environments characterized by ongoing nitrogen scarcity.

Goal 2: Explore the distribution of cyanobacteria having BNF bearing plasmid in environment and evaluate whether the environment favors plasmid-bearing BNF strains. This goal is designed to provide a deeper understanding of the prevalence or absence of PAs and other cyanobacteria carrying BNF-coding plasmids in the natural environment. Furthermore, it aims to suggest potential alterations in the genetic makeup of cyanobacteria, influenced by prolonged human activities that impact freshwater ecosystems.

Significance: The outcomes of this research will significantly contribute to our understanding of global nitrogen dynamics. By researching the intricacies of plasmid-coded nitrogen fixation, particularly in Pseudanabaenales, this project lays the foundation for a potential paradigm shift in our comprehension of nitrogen cycling even on planetary scale.



Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

# Ústav komplexních systémů / Institute of Complex Systems

# Nové Hrady





## Supervisor: prof. RNDr. Dalibor Štys, Ph.D.

### Contact

E-mail: stys@frov.jcu.cz

### Detection of particles in liquids using flow light microscopy

### Detekce částic v kapalinách pomocí průtokové světelné mikroskopie

### Annotation

Water in nature contains a large number of microscopic particles, whether desirable (e.g. plankton) or undesirable (e.g. microplastics). Weight analysis by which the particles are captured on a filter with a given pore size and subsequently dried and weighed is currently most often used for the analysis of particles in liquids. This method is indirect, very time- and volume-consuming, and can lead to large errors in the analyzed liquids [1]. The method has a lower particle size detection limit of 20  $\mu$ m, but acceptable measurement accuracy can only be achieved up to 50  $\mu$ m. Particle analysis in liquids by direct observation or in situ is required. Despite the real-time light microscopy method providing the size and shape distribution of particles in the size range of 1  $\mu$ m to 1 mm is commercially available [2-5], the analysis of some atypical objects (e.g., bubbles) must be performed visually from the detected images.

The Laboratory of Experimental Complex Systems is being developing a flow optical device that is technically similar to the device in [2-5], but with simpler optics. The main difference lies in the control and evaluation software that distinguishes bubbles from the rest of the particles, allows measuring very dilute solutions compared with commonly used "inaccurate" turbidimetric analysis and provides a large statistical set of size and quality distribution of particles in water.

The expected output of the thesis will be progress in the development of the flow microscopy device for the analysis of particles in water and other liquids and the determination of the possibilities of its applicability in practice. The student will participate in the development of the entire device and its application for quantitative/qualitative evaluation of water sample pollution. The student will design experiments suitable for optimization the method, define the types of particles and their appropriate parameterizations, and create a statistical evaluation of the results of detection and classification suitable for a simple interpretation of the device outputs for the task of analyzing the quality of polluted waters.

### References

1. Pfeiffer T.J., Osborn A., Davis M. (2008) Particle sieve analysis for determining solids removal efficiency of water treatment components in a recirculating aquaculture system. Aquac. Eng. 39, 24-29.

2. Chen Y.T., Wang Q., Xue J.Z., Yang Y.F., Wu H.X. (2023) Applicability of flow imaging microscopy (FlowCAM) as ballast water investigation tool. Reg. Stud. Mar. Sci. 60, Article No. 102821

3. Chen Y.T., Wang Q., Xue J.Z., Yang Y.F., Wu H.X. (2023) Ballast water management systems (BWMS) type approval: A new application for flow imaging microscopy (FlowCAM). Reg. Stud. Mar. Sci. 65, Article No. 103060

4. Romero-Martínez L., van Slooten C., Nebot E., Acevedo-Merino A., Peperzak, L. (2017) Assessment of imagingin-flow system (FlowCAM) for systematic ballast water management. Sci. Total Environ. 603, 550-561

5. Kim S., Hyeon Y., Park C. (2023) Microplastics' shape and morphology analysis in the presence of natural organic matter using flow imaging microscopy. Molecules 28(19), Article No. 6913



Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

## The use of human tissue cultures as rapid, sensitive and semi-specific biosensors for the detection of pollutants and bioactive compounds

Využití lidských tkáňových kultur jako rychlých, citlivých a polospecifických biosenzorů pro detekci znečišťujících látek a bioaktivních sloučenin

### Annotation

Human tissue cultures, such as monolayers of wild-type cells isolated from human or mammalian tissues, are one of the best approximations of whole organ function. The Laboratory of Experimental Complex Systems of the ICS FFPW USB has developed microscopes and software that simultaneously monitor and automatically evaluate large numbers of cells 1) traditionally by counting the rate of increase in the number of cells and their divisions at different stages and 2) rapidly by measuring the intracellular dynamics, both in unprecedentedly large numbers or with sufficient statistics to cover cell differentiation. This opens up the possibility of using tissue cultures as biosensors.

Research on so-called "new" pollutants in water is becoming a specific branch of pharmacology because most of the contaminants affecting the behaviour of aquatic organisms and water-consuming organisms, including humans, are pharmaceuticals and their metabolites. Pharmaceuticals are specifically synthesised to affect a particular organ. Organs can be simulated by tissue culture. The decontamination of substances occurs in the liver. The most general such culture is hepatocytes, although specific cell cultures are available and can be selected according to the studied substance. The Laboratory of Experimental Complex Systems FFPW USB has extensive experience with this, including the growth of these cells on synthetic and natural, isolated, scaffolds.

This knowledge can be next used to develop semi-specific and rapid bioassays. Pilot experiments are described in Lonhus et al., EPJ-ST (2020) and Jan Kosek's bachelor thesis (2023). The latter hypothesises that tissue culture can rapidly and sensitively detect substances dissolved in water at concentrations relevant to their occurrence in water. For this purpose, cultures growing on a Petri dish, natural scaffolds (amniotic membranes) or synthetic scaffolds (3D-printed transparent structures) will be used. Cells will be selected according to the type of tested compounds. A secondary outcome of the dissertation will be to speed up, simplify, and increase the accuracy and precision of primary pharmacological testing.

#### References

Lonhus et al. 2021, Estimation of rheological parameters for unstained living cells, Eur. Phys. J. Spec. Top. (2021) 230: 1105-1112.

Jan Košek, 2023, Studium dynamiky živých lidských buněk v odezvě na běžné kontaminanty vod s cílem vývoje citlivého specifického analytického postupu. Bakalářská práce. Fakulta rybářství a ochrany vod Jihočeské univerzity v Českých Budějovicích, 2023.



Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

### Supervisor: Ing. Jan Urban, Ph.D.

### Contact

E-mail: urbanj@frov.jcu.cz

### Fish behavior as environmental markers

### Chování ryb jako environmentální markery

### Annotation

One of the important ecosystems research areas is focused on the relations between inputs and outputs of its subsystems. The fish shoal in aquaria or tanks represents the ideal example of subsystem with relatively high level of complexity, yet offering the possibility to partially control the ecosystem environment, namely water parameters (and contaminants or pollutants).

To parametrize also the fish behavior, there were already introduced and developed several approaches including digital imaging and machine learning methods for analyzing visual fish shoal information, its hierarchy, and dynamics. The conditionality of the fish shoal behavior parameters on the given water parameters in causal description is an open question. In other words, is there a functional dependency to describe (in statistical meaning), how the fish react?

Student will run the series of experiments to acquire relevant datasets of water parameters and visual fish behavior information using in house developed methods. To parametrize the behavior will be necessary to involve neural networks approach for detection, segmentation, classification, and tracking. With processed data, student will focus on the estimation of the functional relation between system inputs (water parameters) and subsystem outputs (fish behavior parameters). The complementary task is on the solution of the inverse problem: is there statistically relevant estimation of the water parameters from the fish behavior itself?

Such model could serve as a more general descriptive tool for prediction of the situation in aquaria or tanks and therefore serve also as an early warnings system within the limits of such semi-closed ecosystems. Further generalization should make steps on the transferability of the principle for the ponds and rivers monitoring.