



Ph.D. thesis topics 2023/2024

DSP Ochrana vodních ekosystémů / Protection of Aquatic Ecosystems

2nd call

Supervisor: doc. Ing. Martin Bláha, Ph.D.	2
Urban waters: a source of hidden biodiversity of non-native species.....	2
Who eats who: using biomarkers to reveal food links in aquatic ecosystems	3
Supervisor: doc. Ing. Miloš Buřič, Ph.D.	4
Fish assemblages in the Slovak part of the Danube River: recent status and possible remediation options in the future.....	4
Supervisor: doc. Mgr. Roman Grabic, Ph.D.	5
Development of LC/HRMS methods and data analysis workflows for identification of compounds with adverse effects in sample fractions selected by Effect Directed Analysis (EDA).....	5
Supervisor: Ing. Bc. Kateřina Grabicová, Ph.D.	6
Polar micropollutants and aquatic organisms – a study of fate and effects with application of targeted and non-targeted LC/HRMS analysis.....	6
Supervisor: Dr. ric. Phillip Haubrock, Ph.D.	7
Spatial patterns in biological invasions and impacts on biodiversity.....	7
Supervisor: M.Sc. Hamid Niksirat Hashjin, Ph.D	8
The implication of the immune system in molting and regeneration of crayfish.....	8
Supervisor: doc. Ing. Antonín Kouba, Ph.D	9
Ecological significance of claws in crayfish	9
Supervisor: prof. Ing. Pavel Kozák, Ph.D	10
Physiological and behavioural reaction patterns among different crayfish species with respect to environmental stressors and other conditions.....	10
Supervisor: prof. RNDr. Dalibor Štys, CSc.	11
The use of human tissue cultures as rapid, sensitive and semi-specific biosensors for the detection of pollutants and bioactive compounds.....	11
Supervisor: Ing. Jan Urban, Ph.D.	12
Fish behavior as environmental markers.....	12
Supervisor: doc. Ing. Vladimír Žlábek, Ph.D.	13
Bioaccumulation dynamics of emerging contaminants in aquatic invertebrates	13



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

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 focus on urban waters in particular.



Who eats who: using biomarkers to reveal food links in aquatic ecosystems

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.

Food quantity is the dietary energy (i.e. caloric units) available to consumers. In contrast, food quality is its nutritional value as determined by its biochemical composition (i.e. the compounds required for physiological processes such as somatic growth, survival, and fecundity). If nutritionally essential food sources are absent and cannot be substituted, the individuals' fitness can be affected, possibly altering population dynamics. However, other sources can be used just as a ballast matrix (material that fills a digestive tract and support the digestion processes or carry the food source but is not used directly as a source of energy). In contrast, others may be critically important as they are converted into body biomass and used for biochemical processes. Therefore, it is crucial in trophic ecology to investigate how food sources are converted into new biomass and which food sources are most important, i.e. which food sources support consumers at various trophic levels.

This PhD project will be focused on energy pathways and food webs in aquatic ecosystems under different treatments using stable isotopes, fatty acid analysis, and/or the faeces metabarcoding approach. Thus, a PhD candidate will be responsible for i) determining how food sources are used and incorporated into the consumer's body, ii) meta-analysis of current knowledge about energy flow in freshwater ecosystems, and iii) fractionalization of food sources in the consumer's body.

Therefore, PhD candidate should have deep knowledge about stable isotopes, fatty acids, 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 or molecular methods is essential. PhD candidate should be independent and familiar with field sampling. A driving license is an advantage.



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Fish assemblages in the Slovak part of the Danube River: recent status and possible remediation options in the future

Annotation

The PhD topic is a part of the scientific work of an international research team charged to solve LIFE project „Living Rivers“ which is focused on implementation of the „New River Basins Management Plan in Slovakia“ into practice. Based on mutual negotiation among person in charge of the project management at Faculty of Fisheries and Protection of Waters (Bořek Drozd) and a potential PhD thesis supervisor (Miloš Buřič) PhD candidate will arise a specific content of the Ph.D. study. The candidate will help to solve some of the research tasks of the project by its own scientific work as well as co-operation on more complex question with the international team members. The main task of the PhD topic are:

- I. Monitoring of the fish assemblages in the Slovak part of the Danube River and evaluation of their time development and interconnections with other measures applied within the LIFE project.
- II. Confirmation of the status of passability of the barriers built up on the three target parts of the Danube River in Slovakia (Old Danube, Danube side branch system and “New” Danube using telemetric surveys in fish from different ecological groups.
- III. Verification of sturgeons’ natural reproduction in the Slovak part of the Danube.
- IV. In situ strengthening of natural populations of Danubian sturgeons using eggs incubation in the wild and juveniles stocking.
- V. Exploration of other factors influencing the native fish assemblages and their possible conservations measures.

The scientific work will be financially supported by the LIFE project „Living Rivers“.



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Development of LC/HRMS methods and data analysis workflows for identification of compounds with adverse effects in sample fractions selected by Effect Directed Analysis (EDA)

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 not known. Conventional risk assessment following a top-down process is too slow to address emerging issues in environmental pollution. Too many compounds in the environment are not identified, and even more with unknown effects on human and environmental health.

The proposed Ph.D. topic is focused on bottom-up selection of the compounds with biological effects. It means the identification of compounds responsible for effects in the sample. Extract of the samples with given biological effect (e.g. antiestrogenic) will be fractionated to lower the complexity of the mixture. It can be performed in more than two steps in different LC systems. The study aims to develop 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 based on the Czech Science Foundation project EXPRO.



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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

Annotation

Polar micropollutants (e.g., pharmaceuticals and personal care products, pesticides, etc.) are ubiquitous in the aquatic environment due to continual anthropogenic activities. 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. Contrary, pesticides 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. Both 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 high-resolution mass spectrometry (QExactive 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.



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Spatial patterns in biological invasions and impacts on biodiversity

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.



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The implication of the immune system in molting and regeneration of crayfish

Annotation

The body of decapod crustaceans is covered by a hard exoskeleton that protects them against dangers in the aquatic environment. However, the hard exoskeleton is considered a hindrance to the further growth of such animals. In order to facilitate the growth, they must shed older exoskeletons and develop a new one called molting. On the other hand, decapod crustaceans are able to regrow their lost limb in the complicated process of limb regeneration. Our recent findings in our laboratory showed that the immune system plays an important role in both regeneration and molting processes in decapod crustaceans. We have found that granules of immune cells are the main sources of the new organelles in the regenerated nerve of crayfish. We will apply cellular and molecular techniques such as electron microscopy and proteomics to investigate further the potential roles of the immune system in tissues such as skeletal muscle, epidermis, etc. The other purpose of this study is to investigate the interconnection between molting and regeneration. Practical experience in the measurement of gene expression will be useful for candidate. For further information, read this paper: - Kor, G., Mengal, K., Buřič, M., Kozák, P. and Niksirat, H., 2023. Granules of immune cells are the source of organelles in the regenerated nerves of crayfish antennae. *Fish & Shellfish Immunology*, 137, 108787. <https://doi.org/10.1016/j.fsi.2023.108787>



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Ecological significance of claws in crayfish

Annotation

Crayfish claws hold great ecological significance, playing vital roles in survival and interactions within aquatic ecosystems. Firstly, crayfish claws are instrumental in procuring food. Thanks to them, crayfish can grasp and manipulate food sources, including plants, detritus, macroinvertebrates, and even small fish, allowing them to occupy various trophic levels. This ability to consume various food items helps maintain ecosystem stability by controlling the population sizes of prey species and participating in nutrient cycling. Second, claws are used directly in defending from predators. Third, claws are used in construction of burrows, which provides habitat for other organisms. Lastly, crayfish claws facilitate reproductive success, as males often use their enlarged claws in courtship of females and to establish dominance over males. Overall, the ecological significance of claws in crayfish extends far beyond their physical attributes, playing crucial roles in foraging, defence, reproduction, and overall ecosystem functioning. Still, how crayfish and their claws influence behavior and ecosystem function remains an under researched area.



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Physiological and behavioural reaction patterns among different crayfish species with respect to environmental stressors and other conditions

Annotation

The aim of the dissertation will be to evaluate the vulnerability of selected species of European native crayfish in comparison to non-native crayfish through physiological and behavioural responses to environmental changes related to anthropogenic effects such as climate change, siltation of streams by fine sediment, eutrophication of waters, and especially their combination. To quantify the physiological response of crayfish, respiratory analysis (Loligo systems) and heart rate analysis will be used in the laboratory. Behavioural patterns will be assessed using Noldus Ethovision software. The physiological limits of non-native crayfish species will be determined to predict their possible adaptation to these ongoing environmental changes.



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

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.

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 rybnářství a ochrany vod Jihočeské univerzity v Českých Budějovicích, 2023.



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Fish behavior as environmental markers

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.



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Bioaccumulation dynamics of emerging contaminants in aquatic invertebrates

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.